Considerations for Benthic Harmful Algal Bloom Detection and Monitoring in Virginia Free-flowing Freshwater Rivers

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Name
AU	Assessment units
BGA	Blue-green algae (Cyanobacteria)
BPJ	Best professional judgement
Ca^{2+}	Calcium

DEQ	Virginia Department of Environmental Quality
DO	Dissolved oxygen
DP	Dissolved phosphorus
ELISA	Enzyme-linked Immunosorbent Assay
EPA/OW	United States Environmental Protection Agency, Office of Water
FGA	Filamentous green algae (Chlorophyta)
GIS	Geographic information system
GPS	Global positioning system
HAB	Harmful Algal Bloom
HPLC	High-performance liquid chromatography
ICPRB	Interstate Commission on the Potomac River Basin
ID	identification
ITRC	Interstate Technology Regulatory Council
LC-MS	liquid chromatography mass spectrometry
Mg^{2+}	Magnesium
MVS	Multi-view stereo
NO_3^-, NO_2^-, N	Nitrate, nitrite, and nitrogen
NZ MfE/MH	New Zealand Ministry for the Environment, Ministry of Health
ORP	Orthophosphate
PPIA	Protein phosphatase inhibition assays
PTOX	Potentially toxigenic
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
SfM	Structure-from-Motion
SPATT	Solid phase adsorption toxin tracking
TALK	Total alkalinity
TKN	Total Kjeldahl nitrogen
TP	Total phosphorus
TSS	Total suspended solids
UAV	Unmanned aerial vehicle
VDH	Virginia Department of Health
WHO	World Health Organization
WVDEP	West Virginia Department of Environmental Protection

Considerations for Benthic Harmful Algal Bloom Detection and Monitoring in Virginia Free-flowing Freshwater Rivers

June 2022

1. Introduction

The Commonwealth of Virginia currently does not have an active Harmful Algal Bloom (HAB) surveillance program for benthic algae. Rather, it has a response-based program triggered by reports of suspected benthic HABs from the public and/or field observations made by state agency staff. The Virginia Department of Health (VDH) coordinates the Commonwealth's responses to suspected benthic HAB events. Virginia Department of Environmental Quality (DEQ) normally conducts the initial response to any potential HAB, which may include visits to the HAB site for visual observations and collection of water column samples above or near the benthic algal mats. DEQ does not collect algal material from solid mats, benthic or floating, and has limited resources to commit beyond the initial response investigation of reported potential HABs. VDH is charged with the responsibility to weigh the available evidence and determine whether there is sufficient information to issue an advisory or alert notifying the public of possible risk due to the presence of harmful algae.

Advisories may be issued based on confirmed, quantitative data such as an exceedance of a toxin threshold measured in the water column. Alerts may be based, partially or fully, on qualitative information such as the widespread presence, or suspected presence, and extent of solid floating/benthic mats or scums. The subsequent benthic HAB response monitoring program must therefore consider protocols to be implemented in both circumstances, i.e., an advisory based on confirmed, quantitative measurements versus a qualitative "abundance of caution" alert informing the public of a possible health risk.

This project report describes systematic protocols that could be implemented if an advisory or alert is issued by VDH for a benthic HAB event. The report identifies the information needed to issue an advisory or alert, the recommended actions, an effective schedule of activities, and the resources needed to characterize the nature and extent of the HAB and implement the protocols. The suggested monitoring program considers the conditions and information that led to the HAB advisory or alert. The report describes how decision-makers are informed of the health risks associated with recreational swimming, fishing, and other water contact activities.

2. What are Harmful Algal Blooms?

Algal blooms occur when water quality conditions promote excessive growth of algae. Recreational uses and municipal sources of water can be compromised if the blooms contain toxigenic algae. Most of the species of toxigenic algae found in freshwater systems are cyanobacteria ("blue-green algae"). Cyanobacteria cells grow singularly or in colonies and filaments, as plankton in the water column (planktonic) or attached to submerged surfaces in the form of mats, periphyton, or biofilm (benthic). Toxigenic species of cyanobacteria can actively secrete toxins into the water column and will release their intracellular toxins upon death and decomposition. If ingested in sufficient amounts, either by drinking contaminated water or recreating in a contaminated waterbody, *cyanotoxins* can cause severe illness and death in humans, pets, livestock, and aquatic life. Harmful algal blooms therefore represent a major threat to public health, and an economic burden to the public due to lost revenue when recreational areas are closed.

Many U.S. states have developed methods to detect and monitor planktonic HABs. Thresholds for responsive actions typically are based on concentrations of cyanobacteria and toxins in the water column. This focus on planktonic cyanobacteria has produced a large gap in our understanding and management of HABs caused by benthic cyanobacteria. Only a few U.S. states have developed response strategies based on analysis of benthic HABs (ITRC 2022). These strategies rely on identifying the presence or absence of a benthic HAB because thresholds based on any defined metric are unknown. Since benthic HABs can be just as harmful as planktonic HABs, and are common in many inland rivers, streams, and lakes, there is an urgent need for development of response strategies and identification of safe thresholds for HABs caused by benthic cyanobacteria.

Benthic algae include diatom (Bacillariophyta), filamentous "green" (Chlorophyta), and other taxa as well as cyanobacteria. Benthic algal mats and biofilms naturally occur in inland freshwater systems and can form on most river and lake surfaces, including deeper benthic regions as long as water clarity allows for sufficient light penetration to these areas (Vadeboncoeur and Power 2017). Growth is regulated by a variety of stimuli including nutrient availability, water clarity and light intensity, temperature, and flow (Wood et al. 2020). Planktonic HABs are generally limited by nutrients in the water column and are thus often found in eutrophic systems where nitrogen and phosphorous in the water are high. Benthic cyanobacteria differ from their planktonic counterparts in this respect, since they have access to nutrients from additional sources such as groundwater and leaked nutrients from macroalgae and other microorganisms (Wood et al. 2015, Vadeboncoeur et al. 2021). Access to these additional sources of nutrients allows benthic cyanobacteria and HABs to occur in oligotrophic systems without high water column nutrients, including otherwise healthy streams or rivers and oligotrophic alpine lakes (Vadeboncoeur and Steinman 2021).

Benthic cyanobacteria are most likely to cause harm to humans, pets, and livestock when mats break free from their substrate, accumulate in masses, and start to die at the surface. This detachment occurs due to mechanical disturbance from trampling, high flow rates, or the buoyancy of accumulated oxygen bubbles in the mat matrix. Although the harmful cyanotoxins produced by cyanobacteria in these blooms are mostly intracellular when the cells are alive, direct ingestion of toxic mat material by children and animals can occur and lead to severe poisoning and death. Once the mat material has broken off and is floating in the water column, senescence of the cyanobacteria cells also begins and cyanotoxins are released to the water column upon cell death. Once these toxins in the water column approach thresholds they are an additional threat to humans or animals that drink from contaminated water, during recreation when accidental ingestion of water occurs, and when animals groom themselves after bathing in contaminated water. Finally, the toxins produced by benthic HABs, above certain thresholds, can also result in poisoning when contaminated fish or shellfish are eaten.

The toxins produced by cyanobacteria are diverse and include hepatotoxins (toxic to the liver), neurotoxins (toxic to the nervous system), and dermatoxins (toxic to the skin). Benthic and planktonic HABs are caused by different species of cyanobacteria yet produce many of the same toxins. These toxins are equally harmful regardless of source, although the route and amount of toxin exposure is different between benthic and planktonic HABs. The most common and well-understood cyanotoxins include microcystins, anatoxin-a, cylindrospermopsin, nodularin, and saxitoxin (Table 1). Threshold concentrations of these toxins have been developed for drinking water, above which negative health impacts are expected. These thresholds are calculated based on toxicity reference values generated in human or animal toxicity studies. Recreational thresholds have also been adopted by many states, yet these are limited by a lack of recreational toxicity data, especially for anatoxin-a and saxitoxin. For microcystins and cylindrospermopsins, many states use EPA-developed recreational threshold values of 8 µg/L and 15 µg/L, respectively (EPA 2019). Recreational thresholds for anatoxin-a and saxitoxin are less common across states and have no nationally recognized guidance values. States with thresholds for anatoxin-a and saxitoxin base their estimates on limited recreational human and animal toxicity studies. Only ten states have recreational thresholds for four of the major classes of cyanotoxins (Mehinto et al. 2021). Development of these thresholds has also been mostly limited to planktonic HABs, and current recreational thresholds for these toxins based on water column analysis do not translate to benthic HABs due to differences in exposure routes.

Table 1. *Cyanotoxins produced by common cyanobacteria and associated health effects (Adapted from EPA 2021 and ITRC 2022).*

Cyanotoxin or Toxin Group	**Type of Toxin**	**Health Effects**
Anatoxin-a	Neurotoxin	Muscle twitching, burning, tingling, numbness, drowsiness, salivation, incoherent speech, respiratory paralysis leading to death
Cylindrospermopsin	Hepatotoxin	Diarrhea, vomiting, nausea, gastroenteritis, liver inflammation, liver hemorrhage, pneumonia, dermatitis, kidney damage, headache
Microcystin-LR	Hepatotoxin	Diarrhea, vomiting, nausea, abdominal pain, headache, weakness, liver inflammation, liver hemorrhage, pneumonia, sore throat, dry cough, dermatitis
Nodularin	Hepatotoxin	Diarrhea, vomiting, weakness, liver inflammation, liver hemorrhage, pneumonia, dermatitis
Saxitoxins	Neurotoxin	Muscle twitching, burning, numbness, drowsiness, headache, vertigo, respiratory paralysis leading to death

3. Responses to HAB Events

Only some U.S. states, including Virginia and California, have implemented HAB response strategies that issue advisories or alerts based on analysis of benthic HABs (ITRC 2022). Due to our limited ability to quantitatively define when benthic HABs become a significant threat, both Maryland and Virginia (VDH 2021) implement one-tiered response strategies based on presence or absence of cyanotoxins in benthic HAB mats. The VDH does not issue guidance based on cyanobacterial mat analysis because thresholds are unknown. Instead, VDH suggests posting warning signs to alert the public if benthic HABs are suspected. The only exceptions are when water column toxin concentrations or cell counts exceed VDH thresholds, in which case advisories may be issued. Interagency guidance from California's response strategy states that if potentially toxigenic cyanobacteria are observed in algal mat samples, signage will be posted warning the public of the danger from HABs (CWQMC 2022; Figure 1). To allow for more stringent advisories in response to benthic HABs, California also will post more restrictive signage based on their planktonic HAB strategy if water column toxins exceed thresholds in a waterbody with a benthic HAB. A flexible response strategy such as this may be helpful in protecting public health while more quantitative methods of benthic HAB risk assessment are being developed by state agencies.

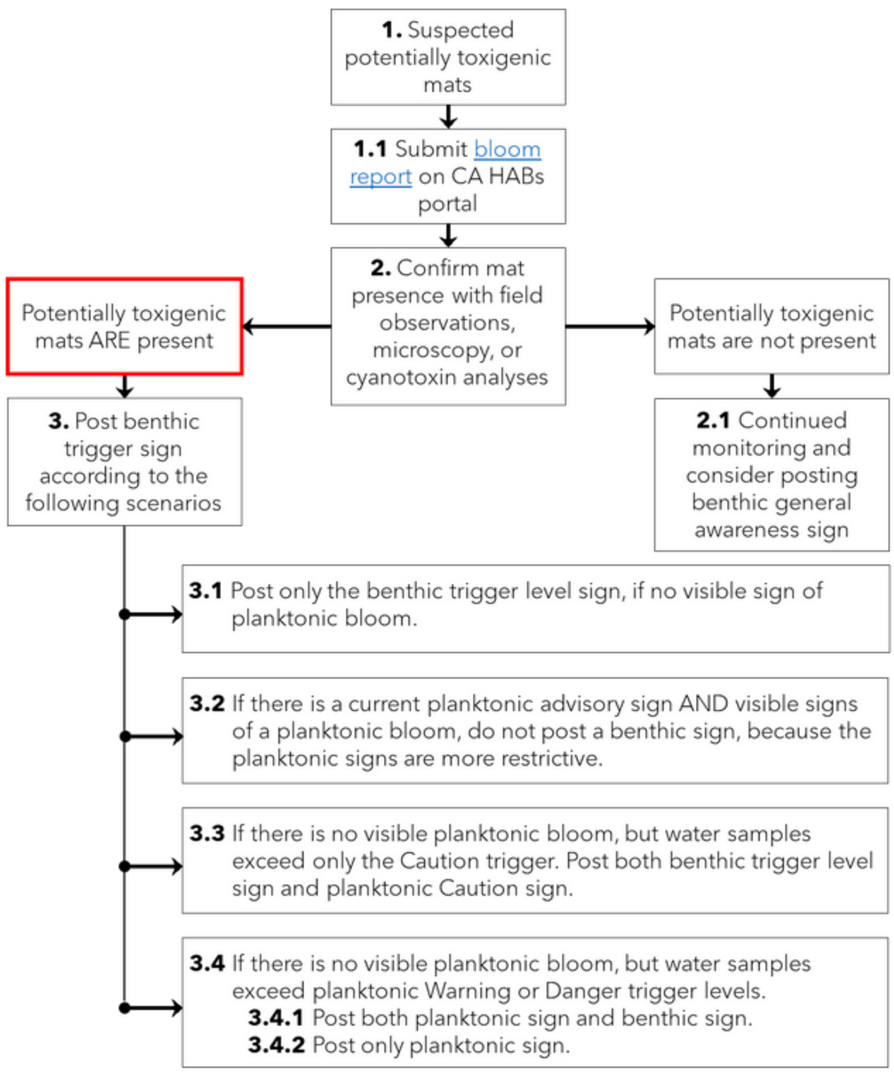

Figure 1. *Interagency benthic HAB response decision tree used by the state of California (CWQMC 2022).*

Globally, several countries have national benthic HAB response plans, including New Zealand. New Zealand's Ministry for the Environment and Ministry of Health (NZ MfE/MH) has developed a response strategy that is unique in its multi-tiered response to benthic HABs (NZ MfE/MH 2009). This strategy has three alert levels specifying guidance to be provided to the public and waterbody managers when benthic cyanobacteria have been identified (Figure 2). Thresholds for each alert level are primarily based on estimated percent coverage by potentially toxigenic cyanobacteria, but also allow for flexibility based on other data. Alert levels can also be escalated based on cyanotoxin analysis of the water column or knowledge of other factors that indicate an increased risk from benthic cyanobacteria. The percent coverage thresholds are based on preliminary knowledge of the relationship between percent coverage and the likelihood of observing floating cyanobacteria mats or scum at the surface, which is believed to occur at 50%.

Decision Chart 2: Alert-level framework for benthic cyanobacteria

Alert level[a]	Actions (See section 2.4 for the recommended framework for roles and responsibilities relating to actions, and the text box at the beginning of Section 3 for advice on interpreting the guidance in this table.)
Surveillance (green mode) Up to 20% coverage[b] of potentially toxigenic cyanobacteria (see Table 1) attached to substrate.	• Undertake fortnightly surveys between spring and autumn at representative locations in the water body where known mat proliferations occur and where there is recreational use.
Alert (amber mode) 20–50% coverage of potentially toxigenic cyanobacteria (see Table 1) attached to substrate.	• Notify the public health unit. • Increase sampling to weekly. • Recommend erecting an information sign that provides the public with information on the appearance of mats and the potential risks. • Consider increasing the number of survey sites to enable risks to recreational users to be more accurately assessed. • If toxigenic cyanobacteria (see Table 2) dominate the samples, testing for cyanotoxins is advised. If cyanotoxins are detected in mats or water samples, consult the testing laboratory to determine if levels are hazardous.
Action (red mode) *Situation 1:* Greater than 50% coverage of potentially toxigenic cyanobacteria (see Table 1) attached to substrate; **or** *Situation 2:* up to 50% where potentially toxigenic cyanobacteria are visibly detaching from the substrate, accumulating as scums along the river's edge or becoming exposed on the river's edge as the river level drops.	• Immediately notify the public health unit. • If potentially toxic taxa are present (see Table 2) then consider testing samples for cyanotoxins.. • Notify the public of the potential risk to health.

a The alert-level framework is based on an assessment of the percentage of river bed that a cyanobacterial mat covers at each site. However, local knowledge of other factors that indicate an increased risk of toxic cyanobacteria (eg, human health effects, animal illnesses, prolonged low flows) should be taken into account when assessing a site status and may, in some cases, lead to an elevation of site status (eg, from surveillance to action), irrespective of mat coverage.

b This should be assessed by undertaking a site survey as documented in Section 4.4.

Figure 2. *New Zealand Ministry for the Environment threat evaluation for benthic HABs, based primarily on percent coverage estimates (NZ MfE/MH 2009).*

In addition to percent coverage, thresholds used for benthic HAB response may be based on identification of toxigenic cyanobacteria and cyanotoxin concentrations in the water column. The same methods used for analysis of planktonic HABs can generally be utilized for benthic HAB analysis yet differ in collection and sample processing. Species composition of mat samples or periphyton scrapes may be determined by microscopy or genetic analysis such as quantitative reverse transcription polymerase chain reaction (RT-qPCR). It is important that the final analysis metric be potentially toxigenic (PTOX) cyanobacteria since not all cyanobacteria produce cyanotoxins. Toxin concentrations from mat cell lysates or water column samples may be analyzed by enzyme-linked immunosorbent assay (ELISA) or liquid chromatography mass spectrometry (LC-MS). The benefits and limitations of each of these methods are discussed below in Section 4.3 (*HAB Verification*).

4. Developing a Response Plan

The framework of a benthic HAB response plan is outlined in Figure 3. The implementation of such a framework will require dedicated resources in terms of personnel, analytical capacity and program administration. The main steps of this response plan consist of 1) *detection* of a suspected HAB; 2) *site assessment* of suspected HAB by trained individuals; 3) *HAB verification;* 4) *threat assessment* to recreational users and water supplies; 5) *public notification*: and 6) *end response monitoring and/or notification.* For most of these steps there are multiple options to acquire the required data, both in terms of methods used and parties responsible for the effort. Each

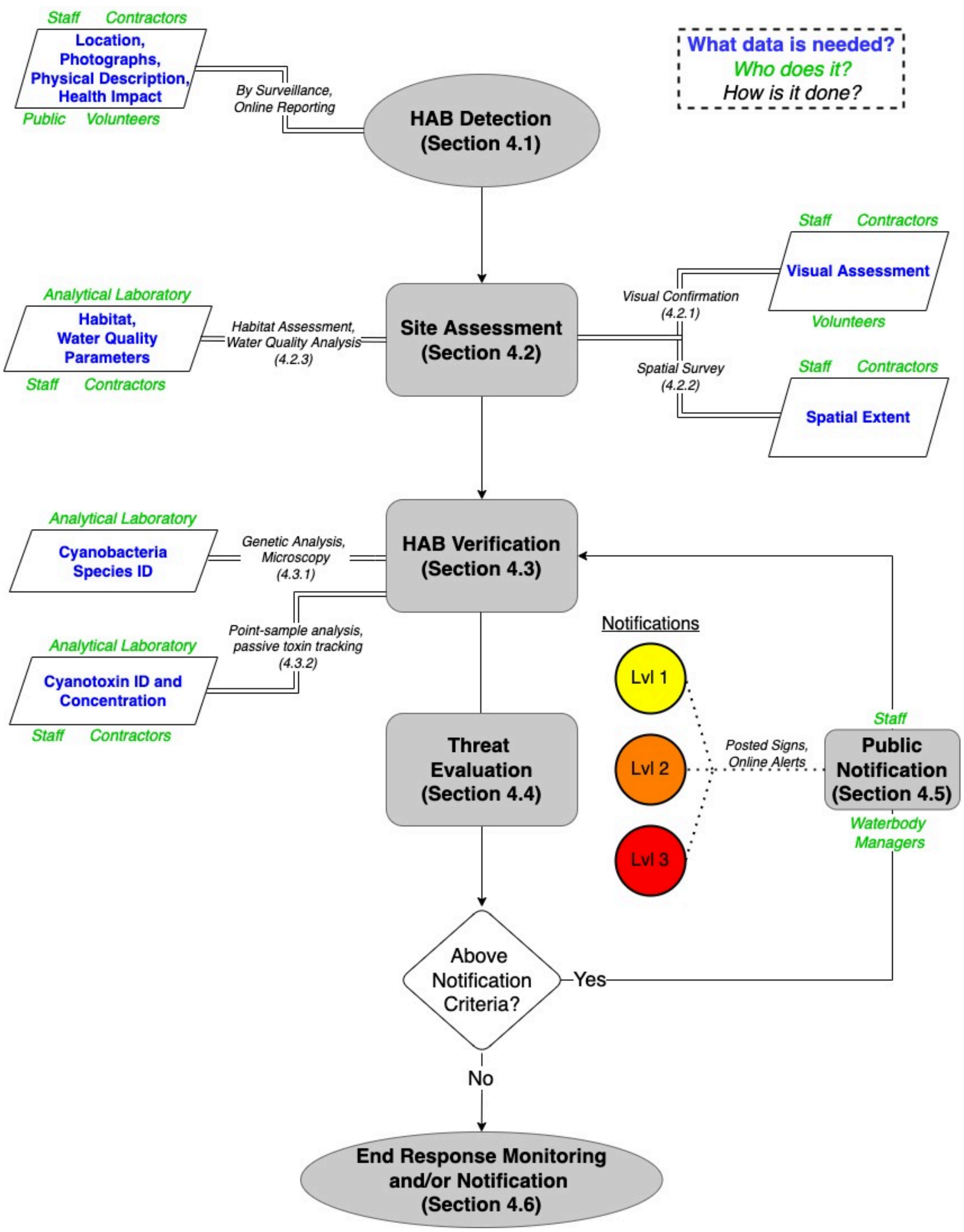

Figure 3*. A benthic HAB response plan framework specifying data required, methods, and responsible parties at each step.*

step is expanded upon in detail below.

4.1 Detection

Benthic HABs may be detected several ways including reports of suspected HABs by citizens and routine visual assessment of at-risk areas by volunteers, staff, or third-party contractors. As shown in Figure 4, suspected HABs that are reported by the public or volunteers should undergo an initial review of the provided information. If a HAB is still suspected after review, the affected reach should be designated as "critical" or "non-critical" to determine if further response is necessary in light of available resources. A site may be deemed "critical" due to its high level of recreational activities, proximity to drinking water sources, utility in understanding cyanobacterial bloom ecology, or if adverse health effects are reported. In the case of adverse health effects observed in humans, pets, or livestock, a reported HAB site should be deemed critical and investigated further even if the site was designated as "non-critical" previously.

4.1.1 Citizen Reporting

Citizens recreating in public waterbodies should be informed of benthic HABs and how to spot them through posted signage. The signage should ideally provide exemplary pictures that represent the multiple ways benthic HABs present including attached and floating mats, and accumulated scum at the surface. A photograph of the suspected HAB is one of the most valuable pieces of data that citizens can provide, and the sign should provide clear instructions on how to submit an online report through the VDH reporting page:

https://www.vdh.virginia.gov/waterborne-hazards-control/harmful-algal-bloom-online-report-form/.

Additional information submitted by the public should include date, time, and location of the observation ideally with latitude-longitude. Citizens may also be informed of the jar and/or stick test to aid in distinguishing cyanobacterial blooms from other submerged aquatic vegetation. These simple tests are based on the tendency of cyanobacteria to float at the surface of a collected vessel of water, or when a stick is passed through them, their appearance as a non-filamentous coating that appears paint-like. This route of detection can be incredibly valuable since visual monitoring of every recreation area during the summer months will generally not be feasible for DEQ, VDH or local government or other agency staff.

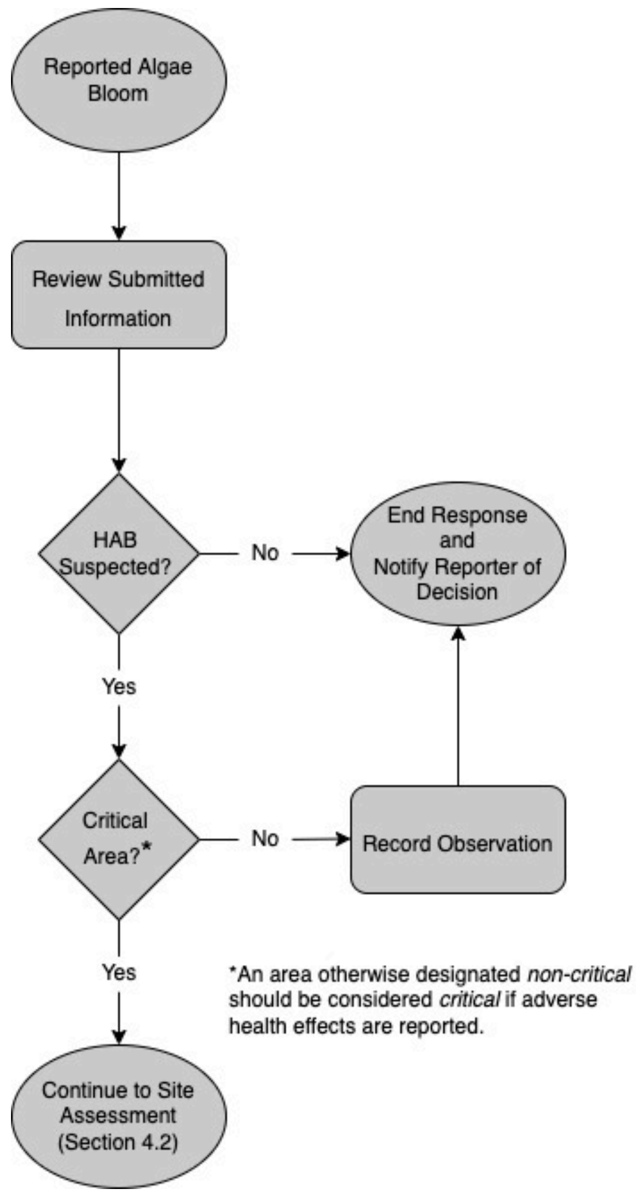

Figure 4. *Initial response to a suspected HAB during the Detection step.*

4.1.2 Volunteer Reporting

Additional detection of suspected benthic HABs should come from trained volunteers or staff. Volunteers from public groups such as River Keepers will need to receive hands-on training to properly identify cyanobacteria in its various forms *in situ*. After this training volunteers may be assigned sites within their local watersheds to visually monitor routinely. As resources allow, DEQ and VDH staff may be assigned as contacts for direct reporting of suspected HABs, as well as confirmation of monitoring tasks regardless of HAB presence.

4.1.3 Staff or Contractor Surveillance

Depending on available resources, staff or third-party contractors may dedicate time to perform drive-by assessments of sites that are of particular interest, either due to frequent high recreational use, value for understanding the ecology of benthic HABs, or proximity to sources of drinking water. However, most effort in terms of available staff personnel hours should be dedicated to more comprehensive routine monitoring of at-risk sites, regardless of the presence of a suspected HAB, to gain a better understanding of what conditions will likely result in the appearance of a benthic HAB.

4.2 Site Assessment

Screening of suspected benthic HABs should be performed by dedicated DEQ/VDH staff or contracted professionals in response to citizen reports. Response efforts may vary widely based on what is deemed necessary and/or appropriate given resource availability and circumstance. In regions defined in this report as "critical areas," additional effort conducting site assessments will be needed to describe the HAB as well as communicate to the public when the HAB event has passed. Site assessments should include inference of bloom spatial and temporal extent, collection of HAB samples to determine species composition and cyanotoxin concentrations, a rapid water quality profile to describe the HAB environment, and a survey to determine HAB spatial coverage (Figure 5.). Available resources, environmental factors, and potential for harm to human health all drive the level of response.

4.2.1 Determination of Scope

Before the severity of the bloom can be described, duration and the spatial extent of the bloom should be investigated based on the time of reporting (4.1). In many cases, response to a cyanobacterial bloom begins when an issue is first reported, however, presence of a HAB likely began weeks prior to the growth phase.

4.2.2.1 Site Description

When responding to HABs that can be readily traversed from end-to-end, Global Positioning System (GPS) coordinates should be taken at the river entry point, and the downstream/upstream points that mark the current extent of the bloom. These GPS coordinates should be collected at the first response to a HAB bloom and each subsequent field visit. If for any reason the sampling location was moved, or the bloom shifted due to environmental conditions, the recorded GPS coordinates should reflect that change. If the bloom extent is too large to define during an initial site visit (i.e. > 1 km or at multiple locations), these GPS points should define the section of reach that will be assessed, and the true extent of the bloom can be assessed by visiting additional downstream/upstream sites or by aerial survey. The nearest USGS gaging station(s) to a HAB event should be identified to provide flow information incident to the bloom site. In cases where there are no proximal gaging stations, flow calculations should be conducted near the bloom site but far enough away that the flow measure is not impacted by the bloom.

Narrative field descriptions of periphyton, aquatic moss, aquatic vascular plants, filamentous green algae (FGA) and cyanobacteria/blue-green algae (BGA) abundance should be recorded during each site visit. Although a lab is needed to identify and quantify PTOX species, field scale IDs by a trained professional are still important contributions to a response. Non-HAB producers should also be described as total primary production can manifest in diverse forms throughout the year.

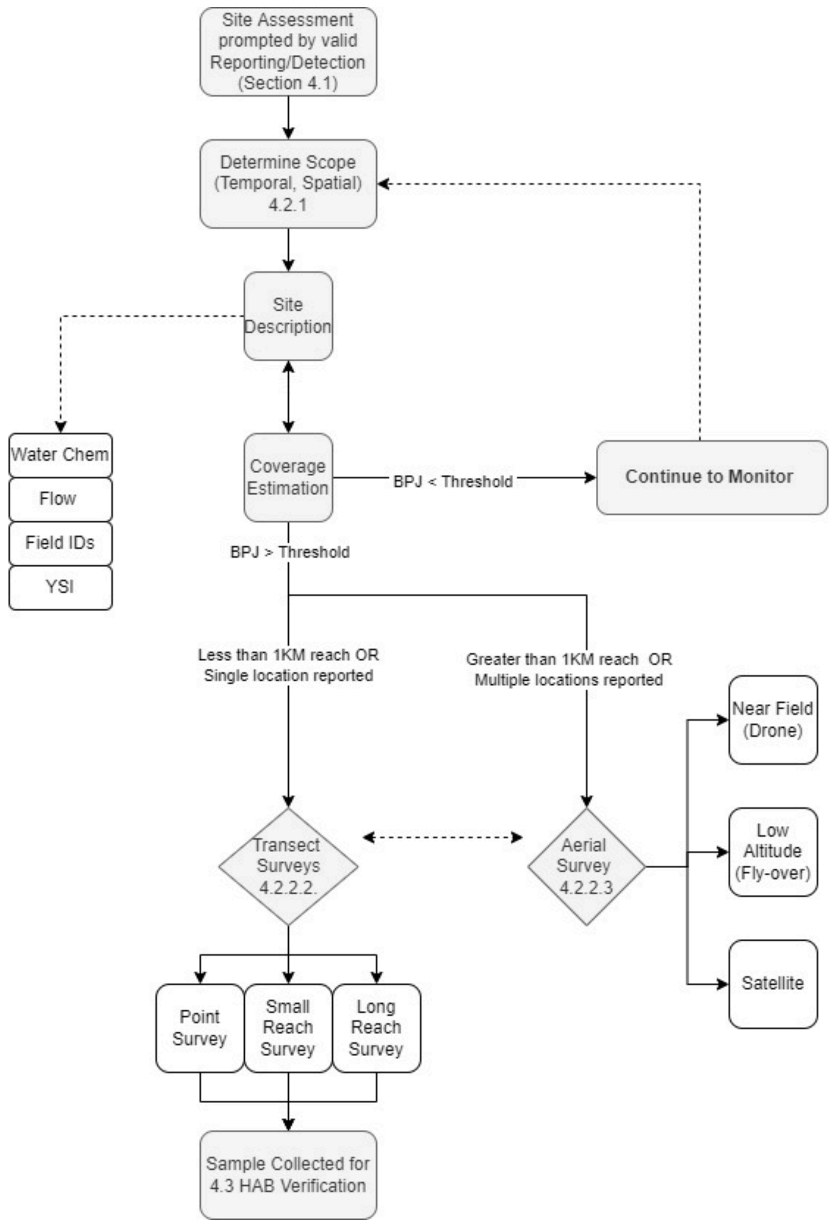

Figure 5*. Framework for creating a site assessment following HAB detection.*

If resources allow, in-situ water quality data should be collected at every site with a multi-parameter sonde throughout the season. Standard probes such as water temperature, dissolved oxygen (DO), pH, specific conductance and total dissolved solids (TSS) should be standard for all responses. If additional probes/ports are available, chlorophyll *a* and/or phycocyanin probes should be considered.

When water chemistry is included in a field HAB response, nine parameters should be included to define the growing environment of the cyanobacteria: 1.) total phosphorous (TP), 2.) dissolved phosphorous (DP), 3.) orthophosphate (ORP) 4.) total Kjeldahl nitrogen (TKN), 5.) nitrate-nitrite-N (NO_3^-, NO_2^-, N), 6.) total alkalinity (TALK), 7.) calcium (Ca^{2+}), 8.) magnesium (Mg^{2+}), and 9.) total suspended solids (TSS). This suite of parameters captures nutrient species and non-nutrient cofactors that best explain hyper-productive reaches in the Mid-Atlantic.

4.2.2. Benthic Spatial Coverage Estimation

4.2.2.1 Best Professional Judgement (BPJ) point confirmation: Defining a low-end threshold

The simplest, fastest, but least informative site assessment technique is a rapid point site visit using best professional judgement (BPJ). A rapid site visit consists of a trained individual visiting a field site to 1) identify potentially toxigenic algae species and 2) rapidly assess abundance and/or coverage of the affected area. As there are no guidelines for quantitatively assessing spatial coverage of benthic cyanobacteria at the time of this study, methods such as the Virginia's or West Virginia's nuisance algae monitoring strategy could be considered. BPJ is not sufficient as a quantitative measure of algal abundance, so it should be considered a screening tool to reduce effort and time spent responding to a report. Virginia's method proposes benthic chlorophyll a thresholds that correspond to significant amounts of algae filling the water column across a transect that correspond to a nuisance condition. West Virginia's method identifies low-end and high-end visual thresholds where less than 10% algal coverage no further action is needed at the site and above 60% the site is immediately flagged as recreationally impaired. Research by West Virginia DEP and ICPRB independently assessed automated image analysis and study participants' ability to visually estimate benthic algae coverage in digital photography. Although coverage estimates become more variable between 20%-60% coverage, there is a high degree of agreement between the individuals' ability to define less than 10% coverage and greater than 60% coverage, even with little training. Defining acceptable upper and lower bound thresholds from the onset of study design could dramatically reduce effort required from responding agencies.

4.2.2.2 Transect Surveys and Representative Quadrats

Transect surveys and representative quadrats are utilized to quantitatively describe the benthic environments at a single location. Scale and sampling frequency reflect the objectives of the researchers/responders. These methods cannot realistically (or statistically) describe any bloom site at the reach scale due to the patchy distribution of benthically attached organisms (ie. cyanobacteria). This deficiency limits responders to describing conditional states via thresholds, much like BPJ. A single transect at a HAB affected site is the least descriptive and lowest statistical power assessment of benthic coverage but may be sufficient to provide adequate response in situations where response time or resources are limited. Similar to how the BPJ approach may be adequate in defining sufficiently low or high levels of HAB benthic coverage, a single transect may be appropriate in describing the single *most impacted* part of a river during a bloom event. As is observed in the both Virginia and West Virginia's Filamentous Algae Methodology, a single transect indicating a bloom of sufficient intensity can cross a threshold, prompting response. In the same way, multiple transects within a defined reach over a particular threshold can also prompt a response.

4.2.2.3 UAVs, drones, helicopters, and other forms of aerial digital photogrammetry

Photogrammetry is the science of extracting information from photographs. The process involves taking overlapping photographs of an object, structure, or space, and converting them into 2D or 3D digital models capable of describing the subject beyond what a single image or simple mosaic can provide. In the past, film and digital photography at ground level provided a means to archive the condition of an environment but due to diminishing horizons, glare, field of view, depth of field, and photographer biases, these images largely could not be considered quantitative. This limitation led to state and federal agencies favoring semi-quantitative approaches such as transects, quadrant griding, and narrative descriptions from professionally trained personnel (described in the previous section). All of these methodologies are labor intensive, training intensive, and lack a quantitative means of measuring reach scale spatial coverage.

Orthophotography and photogrammetry can be considered stand-alone methodologies or valuable supplements to already existing semi-quantitative transect techniques. Recent advancements in georeferenced photography, digital image post-processing, and affordability make reach scale quantitative aerial analyses a feasible option (see Appendix B). Aerial photogrammetry is also scalable as costs to deploy cameras and sensors on a helicopter capable of high-speed capture or satellite reconnaissance is far more expensive than Unmanned Autonomous Vehicles (UAVs, Table 2.). One of the most significant modern developments in documenting and quantitatively analyzing spatial information is the technique Structure-from-Motion (SfM) with multi-view stereo (MVS) imaging. This method uses multiple images overlapped (~65% overlap) to triangulate points within the area of interest at a rapid speed. A dense cloud made up of high-resolution images allows for scientists to archive river conditions as well as perform more than just targeted coverage calculations. For example, Woodget et al. (2017) used near field (UAV/drones) to define river habitat and hydromorphology and Wiboro et al (2021) characterized fluvial sedimentology, topographic mapping, and surface water velocity. Orthophotographic images are enhanced by multi-spectral or hyper-spectral sensors that can detect vegetation beyond the influence of surface glare on the water. Photopigment data (chlorophyll a, chloropyll b, and phycocyanin) collected from spectral imaging can be used for identification of submerged primary production instream. In some cases, the unique composition of photopigments can be used to taxonomically ID full river reaches (Terrence et. Al. 2018). Some study of satellite spectral data has already started in estuarine waters of Virginia (see Tish Robertson, DEQ).

Table 2. Summary of photo acquisition methodologies (*For cost breakdown see Appendix C.).

Photo Acquisition Methodology	Speed	Area Covered	Cost of Data*	Image Resolution	Temporal Availability
Ground level	+	+	$	4k, 1080p	At time of field visit
UAV/Drone	++	++	$	~1.5" per pixel	At time of field visit
Fly-Over (Helicopter/Plane)	+++	+++	$$$	~6" per pixel	Within 24hrs
Satellite (Commercial)	++++	++++	$$$$	2m x 2m to 5m x 5m	Variable, Daily - Annual

4.3 HAB Verification

The primary goal of the HAB verification step is to confirm the identity of the bloom as harmful cyanobacteria through PTOX species identification and cyanotoxin analysis (Figure 6). Multiple laboratories in the U.S. can identify cyanobacterial species and cyanotoxins in water column and biomatrix samples, and can be found on the EPA's webpage:

https://www.epa.gov/cyanohabs/laboratories-analyze-cyanobacteria-and-cyanotoxins

4.3.1 Cyanobacterial Species Identification

Because potential HABs may contain toxin-producing cyanobacteria, samplers should wear appropriate personal protective equipment, such as gloves, eye protection, and waders, during sample collection. Grab samples of mat material and accumulated scum should be analyzed for the presence of PTOX cyanobacteria to verify that a risk to human health is present. A highly accurate and informative method by which to analyze PTOX is RT-qPCR, which is best performed on unpreserved samples, but quantitative estimates can also be achieved using microcopy on preserved samples. It is important to consider that unpreserved samples can be split and analyzed for both PTOX by RT-qPCR and toxin concentration by multiple methods, and therefore allows for a more streamlined collection and shipping process compared to having separate preserved samples for microscopy. Both RT-qPCR and microscopy can yield quantitative estimates of PTOX and are generally expressed in amount of genetic material per gram of mat material, or cells per gram of mat material, respectively. For quantitative estimates of PTOX per area, staff should collect mat samples from defined quadrats or other area-based sampling equipment. Additional methods to quantify the amount of cyanobacteria at a site include pigment analysis of phycocyanin either by handheld devices or remotely with UAVs or aircraft. These pigment-based methods may be desirable for rapid point sampling or sampling across large areas for cyanobacterial presence and quantification but cannot identify PTOX cyanobacteria. A summary of the analytical methods to determine species composition can be found in Table 3.

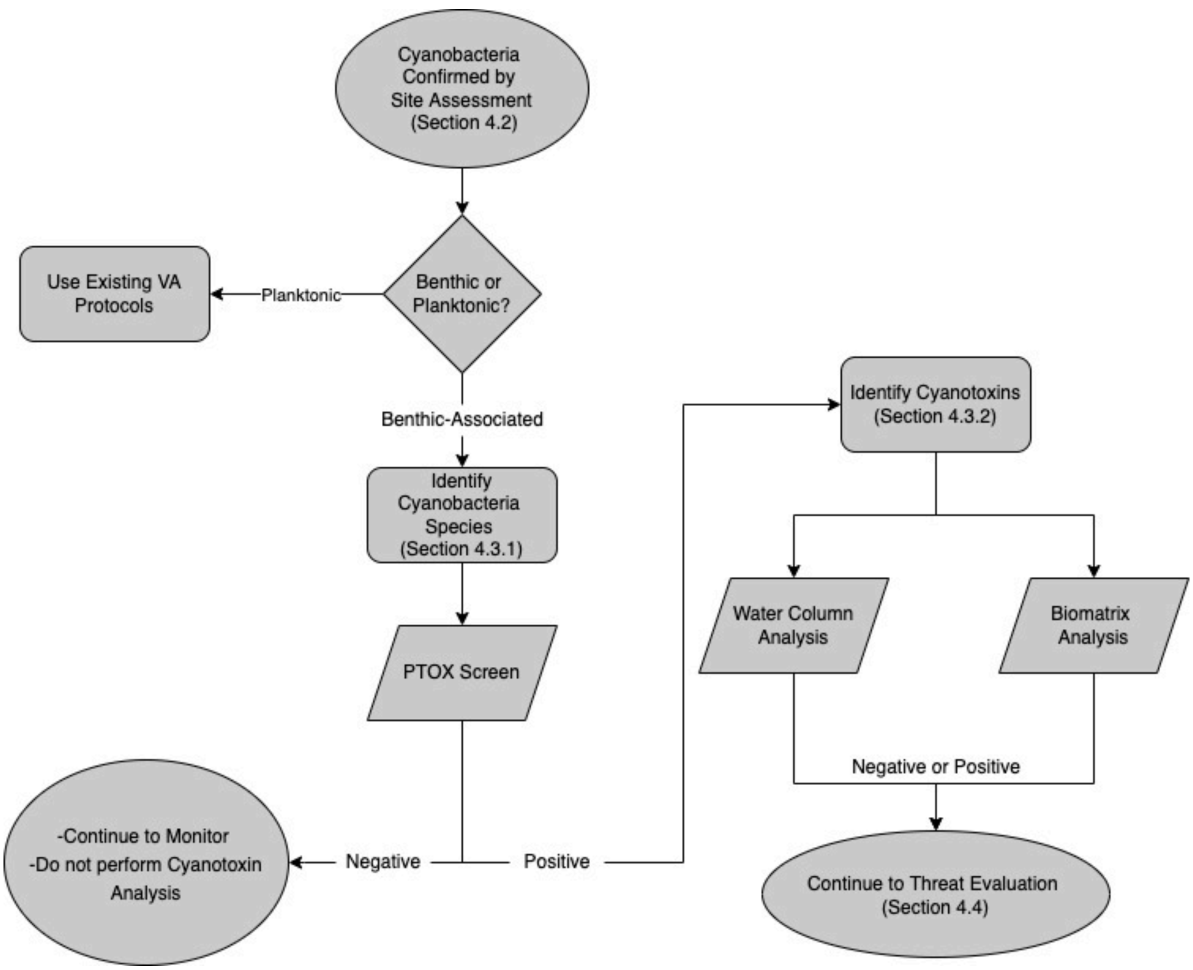

Figure 6. *Decision tree for the HAB Verification step.*

4.3.2 Cyanotoxin Analysis

Cyanotoxins can be analyzed by a number of techniques, summarized in Table 4. Liquid chromatography (LC) or high-performance liquid chromatography (HPLC) combined with mass spectrometry (MS) offers high precision and accuracy, and can resolve individual congeners of toxins if required standards are available. Congener-specific analysis is useful because different forms of toxins may differ in their risk to human health, yet this information is not essential to inform advisories and other responses. High performance liquid chromatography is an advanced form of LC that uses high pressure injection of a solvent through the chromatography column rather than relying on gravity as in standard LC. For the purposes of this report LC-MS will refer to both HPLC and regular LC. There are also multiple forms of detection by mass spectrometry including ion trap, time-of flight, and tandem mass spectrometry. Differences between the various forms of MS include sensitivity, resolution, and ability to produce quantitative results. Tandem mass spectrometry is one of the most common detection methods used with LC, and suitable for quantitative analysis of cyanotoxins in unpreserved water and complex biomatrix samples. However, because any form of LC-MS requires expensive equipment as well as extensive training and education, DEQ and VDH staff should consult with experts from the chosen analytical laboratory for selection of the most appropriate chromatography and detection methods.

Table 3. *Methods to verify toxigenic cyanobacteria in benthic HAB mat or scum samples (adapted from ITRC 2022).*

Method	Presence/Absence	PTOX	Result Type	Cost*	Turn-around Time#	Training Required
Visual Assessment	yes	*x*	Qual.	$ - $$	< 1 day	Novice to Expert
Remote Sensing	yes	*x*	Qual./ Quant.	$**	< 1 day	Intermediate
Pigment Analysis (bentho-torch, phycocycanin field pigment sensors, etc)	yes	*x*	Qual./ Quant.	$$	< 1 day	Intermediate to Expert
Microscopy	yes	yes	Qual./ Quant.	$$	1-3 days	Intermediate to Expert
Genetic Analysis (primarily RT-qPCR)	yes	yes	Qual./ Quant.	$$	1-3 days	Intermediate

**Cost is based upon a per sample approach. $ = <$10; $$ = $10-$100*
***Assumes remote sensing images are freely available. Cost of results is a function of number of samples and cost of acquiring images.*
#Does not include shipping time if transport to lab is required.

4.3.2.1 ELISA Screening

Enzyme-linked immunosorbent assays (ELISA) rely on antibodies that are specific to classes of cyanotoxins and quantify concentration by detecting toxin molecules directly or indirectly with a spectrophotometer. The presence of toxins in ELISAs is generally reported by production of a colored molecule. Production of this colored molecule is either proportional or inversely proportional to toxin concentration, This assay allows toxin concentration in samples to be determined when run side-by-side with standards that are used to model optical density as a function of toxin concentration (Qian et al. 2015). These assays are available commercially and generally come in 96-well microplate kits allowing many samples to be processed at once. This assay also requires less expensive equipment and less expertise than LC-MS. Although ELISAs are less sensitive than LC-MS they are sensitive enough for detection of cyanotoxins from water and mat material in most scenarios. However, ELISAs are also less specific, require a different assay for each class of cyanotoxin, and do not provide congener-specific data. Therefore, a response plan may initially perform cyanotoxin analysis by ELISA and reserve LC-MS for when larger classes of cyanotoxins or PTOX species have already been confirmed.

4.3.2.2 SPATT Samplers

Cyanotoxin analysis for benthic HABs requires careful consideration of the routes through which toxins from benthic cyanobacteria cause harm. These toxins usually come in contact with humans or pets by trampling and contact with the mat, or through contact with dying cells and water near detached mats and scum. Water column grab samples for analysis by LC-MS or an ELISA may miss cyanotoxins in the waterbody if they are not close enough to a cyanobacterial mat, are taken in fast flowing water, or collected during a time when cyanotoxins have not been released or able to accumulate. Passive sampling by solid phase adsorption toxin tracking (SPATT) provides in situ, continuous, qualitative detection of cyanotoxins over periods from 24 hours to one month (Mackenzie et al. 2004, Howard et al. 2017). In SPATT, toxins bind and accumulate on a porous resin contained in SPATT bags anchored in the waterbody. The SPATT bags are then collected and analyzed in the laboratory for bound toxins. This method allows detection of cyanotoxin presence when their release into the waterbody is not constant, or toxin concentrations are very low. A response plan may thus include deployment and collection of SPATT sample bags weekly in addition to standard grab samples for LC-MS and ELISAs during site assessment. It is important to note, however, that SPATT sampling does not estimate toxin concentration and should not be used alone as a basis to issue advisories or other guidance. Additionally, since accumulation of toxin on the resin is a function of both toxin concentration and time, SPATT samplers should be deployed and collected consistently when used.

4.3.2.3 Other Methods

Other methods to detect cyanotoxins include strip tests and protein phosphatase inhibition assays (PPIA). Strip tests can be used to rapidly detect (< 1hr) microcystins, anatoxin-a, and cylindrospermopsin. Results must be interpreted carefully since they can be influenced by contaminants in the waterbody, have limited sensitivity, and provide only qualitative results (LeDuc et al., 2020). However, ongoing development of more sensitive strip test assays may yield more reliable and effective tests in the future (Eurofins Abraxis, Inc., personal communication). Protein phosphatase inhibition assays can quantitatively test for microcystins and nodularins based on their affinity for phosphatase enzymes. This assay is similar in sensitivity to ELISAs, but since both toxins are measured at once and they have the same effect, PPIA cannot distinguish between the two and further testing would be required to identify specific toxin concentrations.

Table 4. *Methods to analyze cyanotoxins in benthic cyanobacteria or water column samples (adapted from ITRC 2022).*

Method	**Presence/Absence***	**Toxins Identified***	**Result Type**	**Sample Type**	**Relative Cost****	**Training Required**	**Turn-around Time#**
LC-MS	yes	A,C,M,N,S	Qual./Quant	point	$$$	Expert	1-3 days
ELISA	yes	A,C,M,N,S	Quant	point	$$	Intermediate	<1 day
SPATT	yes	A,C,M,N,S	Qual.	passive, continuous	$$	Novice to Intermediate	1-3 days
Strip Tests	yes	A,C,M	Qual.	point	$$	Novice	<1 day

PPIA	combined M/N	M,N	Quant.	point	$$	Intermediate	<1 day

**A = anatoxin-a; C = cylindrospermopsin; M = microcystins; N = nodularin; S = saxitoxin*
***Cost is based upon a per sample approach. $ = <$10; $$ = $10-$100; $$$ = >$100*
[#]Does not include shipping time if transport to lab if required.

4.4 Threat Evaluation

Data from the *Site Assessment* and *HAB Verification* steps will be used to determine the threat HABs pose to public health. This *Threat Evaluation* step will assess whether the identified HAB exceeds thresholds that are known indicators of a significant threat to human health. Although thresholds for benthic HABs are not well-defined, several metrics may be considered as guidelines when formulating a threat evaluation plan, including the presence of PTOX cyanobacteria, established recreational guidelines for water column cyanotoxins, and spatial extent of the HAB. In the sub-sections below "notifications" refers to either advisories or alerts, as described in section 1.

4.4.1 Potentially Toxigenic Cyanobacteria as an Indicator

The presence of PTOX cyanobacteria in benthic mats or in the water column is a defining criterion of HABs, and thus should be a primary metric by which a benthic HAB is evaluated for threat to human health. Presence or absence of PTOX species may inform a binary indicator system, whereby PTOX presence or other metrics such as exceeding recreational cyanotoxin thresholds in the water column trigger a warning notification. If a two-tiered or three-tiered notification system is being developed, PTOX presence would be an ideal metric to trigger the lowest notification level, and should also be used as a qualifier for elevated notifications since PTOX species define a HAB. In these two-tiered or three-tiered notification systems, PTOX presence may be combined with recreational cyanotoxin guidelines and/or percent coverage estimates as indicators to trigger the higher-level notifications.

4.4.2 Recreational Guidelines for Cyanotoxins in the Water Column as an Indicator

The EPA Office of Water has established national recreational guidelines for microcystins (8 µg/L) and cylindrospermopsins (15 µg/L) in the water column, which many states use as indicators to issue advisories in response to planktonic HABs (EPA/OW 2019, Mehinto et al. 2021). For toxins such as anatoxin-a and saxitoxin, states generally derive their own planktonic HAB indicators based on literature reference values. In response to planktonic HABs, VDH uses EPA guidelines as microcystin and cylindrospermopsin indicators and has calculated conservative thresholds for anatoxin-a at 8 µg/L and saxitoxin at 4 µg/L based on literature reference values and thresholds used by other states (VDH 2021). The VDH calculation for determining advisory thresholds is as follows:

$$Advisory\ Level = \frac{(dose\ x\ safety\ margin\ x\ body\ mass)}{ingestion\ volume}$$

The World Health Organization (WHO) additionally has issued recreational guidance values for microcystin-LR at 24 µg/L, cylindrospermopsin at 6 µg/L, anatoxin-a at 60 µg/L, and saxitoxin at 30µg/L (Chorus and Whelker 2021). Established water column guidance values used as thresholds for planktonic HABs should ideally be included as thresholds for any benthic HAB threat evaluation since cyanotoxins from benthic HABs can be released into the water column. However, benthic cyanobacteria can pose a threat to public health even when the cyanotoxins are intracellular, through direct contact with mat material or surface scum, and threat evaluation for benthic HABs could thus include thresholds based on percent coverage or PTOX presence as well. California has implemented a benthic HAB response system that combines PTOX presence and recreational cyanotoxins guidelines as thresholds in this way for issuing advisories (Figure 1, CA/SWAMP 2020).

4.4.3 Percent Coverage as an Indicator

As discussed in Section 3, New Zealand has implemented a benthic HAB advisory system which relies on a combination of PTOX presence and percent coverage estimates (Figure 2, NZ MfE/MH 2009). Thresholds are set at 20% and 50% based on knowledge of the likelihood of cyanobacterial mats to detach and pose a greater threat due to accumulation at the surface. These thresholds may be included in an advisory or alert strategy, or modified percent coverage thresholds may be used based on expert knowledge of Virginia watersheds. New Zealand continues to investigate how percent coverage relates to cyanotoxin toxicity and human health during recreation, however a clear relationship between these two variables has yet to be determined (Wood et al. 2018, Wood and Puddick 2018). Therefore, percent coverage thresholds could be carefully considered when developing a threat evaluation plan and not be the sole determining factor for issuance of an advisory or alert. A key knowledge gap identified by New Zealand is that percent coverage does not always indicate risk to human health. For instance, low percent coverage blooms may still result in accumulation of large amounts of scum and mats at the surface. A threat evaluation system could ideally include the flexibility to elevate a benthic HAB notification level when percent coverage thresholds are not met but floating mats and/or scum cannot be reasonably avoided during recreation, such as when pooled at access areas.

4.4.4 Notifications

Notifications should alert the public of the dangers associated with a benthic HAB, and what has been observed. Associated signage can specify what groups are most at-risk, including pregnant people, children, people with medical issues, and pets. In a two-tier or three-tier system these at-risk groups may be targeted in lower-level advisories and advised to avoid contact with the water. Elevated notifications may then expand to include all recreational users. Public notification plans may be designed to increase restrictions as they are elevated in other ways as well, such as advancing from a general notification to avoid cyanobacterial mats and scum, to a more severe notification to avoid the waterbody all-together. Signage may additionally include information on how to protect children and pets, such as monitoring their activities near the waterbody and washing off pets with clean water after any contact with the waterbody. It is also important that the public are notified to the risk of consuming fish and shellfish in areas with cyanobacterial HABs.

4.4.5 Example Three-tiered Public Notification System

The following three-tier system serves as an example of a plan with specific thresholds and increasingly restrictive notifications (Table 5). This plan is based on PTOX cyanobacteria presence, EPA and VDH cyanotoxin thresholds, and the percent coverage estimates used by New Zealand MfE/MH. The implementation of such a system will require dedicated resources in terms of personnel, analytical capacity and program administration.

Table 5. *An example three-tier system to inform the public of risks associated with a benthic HAB event.*

Notification Level	Criteria	Recommendations
Low	Positive for PTOX Cyanobacteria with <20% Coverage	-Repeat Site Assessment and HAB Verification at least bi-weekly -Notify waterbody managers -Public Notification: Warn public to avoid contact with mats or scum. Advise that at-risk groups avoid contact with water.
Medium	Positive for PTOX Cyanobacteria with 20-50% Coverage	-Repeat Site Assessment and HAB Verification at least bi-weekly -Notify waterbody managers -Public Notification: Warn public to avoid activities that may involve disturbance of mat material, splashing, or accidental ingestion (e.g. swimming not advised, boating still ok). Advise that at-risk groups avoid contact with water.
High	Water Column Toxin Thresholds Reached ***OR*** Positive for PTOX Cyanobacteria With > 50% Coverage*	-Repeat Site Assessment and HAB Verification at least bi-weekly -Notify waterbody managers -Public Notification: Advise that all public avoid use of the waterbody for recreation. Recommend closure of access areas by waterbody managers.

**High level notifications may also be considered if transect sampling estimates <50% coverage but PTOX species are confirmed and scum or mats are unavoidable during recreation.*

4.5 Public Notification

Public notification can occur through online notices and posted signage at waterbody access areas. When the Threat Evaluation step exceeds notification level criteria, waterbody managers should be contacted, and staff or waterbody managers should post signs at all access points to the affected reach. These signs should specify what has been observed, communicate the level of risk to the public and at-risk groups, provide guidance on public use of the waterbody, and specify what efforts are ongoing to monitor the HAB. The same web page used by VDH Waterborne Hazards Control for planktonic HABs may be expanded to include benthic HABs (https://www.vdh.virginia.gov/waterborne-hazards-control/harmful-algal-blooms/). The algal bloom surveillance map will be particularly useful to communicate where suspected or active HABs are currently under investigation. General awareness can also be provided through this website that is specific to benthic HABs, including brochures that detail the ways benthic cyanobacteria can appear and the specific risks associated with benthic HABs. Benthic HAB awareness signs may be posted at recreational access points, regardless of HAB presence or history, and should include directions to report suspected benthic and planktonic HABs through the HAB online report form.

4.6 Ending a Public Notification Period

VDH currently lifts advisories when two consecutive sampling events at least ten days apart indicate metric values below all notification thresholds. This convention agrees with many other state responses to HAB events and can generally be followed for benthic HABs, since follow-up sampling efforts may necessarily be limited to this time interval due to resource limitations. It will also be useful to identify necessary exceptions to this convention. Exceptions may include lifting the notification without a second acceptable sampling result, such as at the end of a recreational season or when benthic HABs in the area are known to quickly resolve after high-flow flushing events.

5. Data Management/Information Sharing

Management of a HAB event's data for the purpose of preserving and sharing the information is often an afterthought unless a consistent plan is established in advance and then followed. Locational data, site photos, associated water quality data, and algal taxonomic identifications, cell counts, and toxicity data are typically collected during an event. With relatively little effort, these data can be secured in a protected location for easy retrieval when needed for investigative analyses and comparisons to other HAB events.

Agency staff will need to establish who is responsible for managing the collected data and information after a HAB event, and how accessible the data will be to other agencies and the public. At the time of publication, Amani Bassyouni (amani.bassyouni@vdh.virginia.gov) manages a HAB contact list that identifies organizations, divisions/sub-agencies, names and contacts of individuals, and classifies primary and secondary support members.

Data and information can be stored multiple ways, including on hard disk drives, flash drives, and the internet (through a "cloud" computing provider), and should follow appropriate VDH and DEQ guidelines. Each has its strengths and weaknesses, and archived copies on multiple platforms can ensure security. Formats of the archived data and information should be universal or forward compatible. For example, data initially recorded in Microsoft Excel spreadsheets can be archived as text files (e.g., *.csv), and locational data (i.e., coordinates of HABs, assessment transects, bloom extent) can be converted to a shapefile using GIS software and stored in a geodatabase. The choice of storage method and format will determine the data/information's accessibility to others as well as its longevity. Metadata, either in a standardize structure or a short summary report, is essential for accurate use of the data and information by others.

6. Virginia HAB Response Plans/ Resource Limitations

Two possible benthic HAB response scenarios are diagramed below. Scenario 1 (Figure 8) represents a response plan that is comprehensive yet has high cost and effort requirements. The high effort requirement of scenario 1 is mostly due to weekly reconnaissance of at-risk sites, a highly comprehensive site assessment and verification step, and repeated site assessment and verification weekly after HAB confirmation. The high cost of this scenario is due to personnel hours required, number of samples analyzed, and analysis methods used. For instance, testing mat and water column samples for PTOX species and toxins will require many samples and moderately expensive analysis. Imaging by UAV is also expensive upfront, but it is important to note that required effort is much lower than transect sampling, and cost per datum over time is very low for this method.

Scenario 2 (Figure 9) represents a response plan that is lower in effort and cost than scenario 1. Effort is saved in this scenario by relying on volunteers to perform reconnaissance of at-risk sites, performing a less comprehensive site assessment and validation step, and less frequent follow-up monitoring. Cost is saved by fewer required personnel hours, only testing benthic mat samples for PTOX species and the water column for toxins, and by performing a simple visual transect during site assessment.

Example Scenarios:

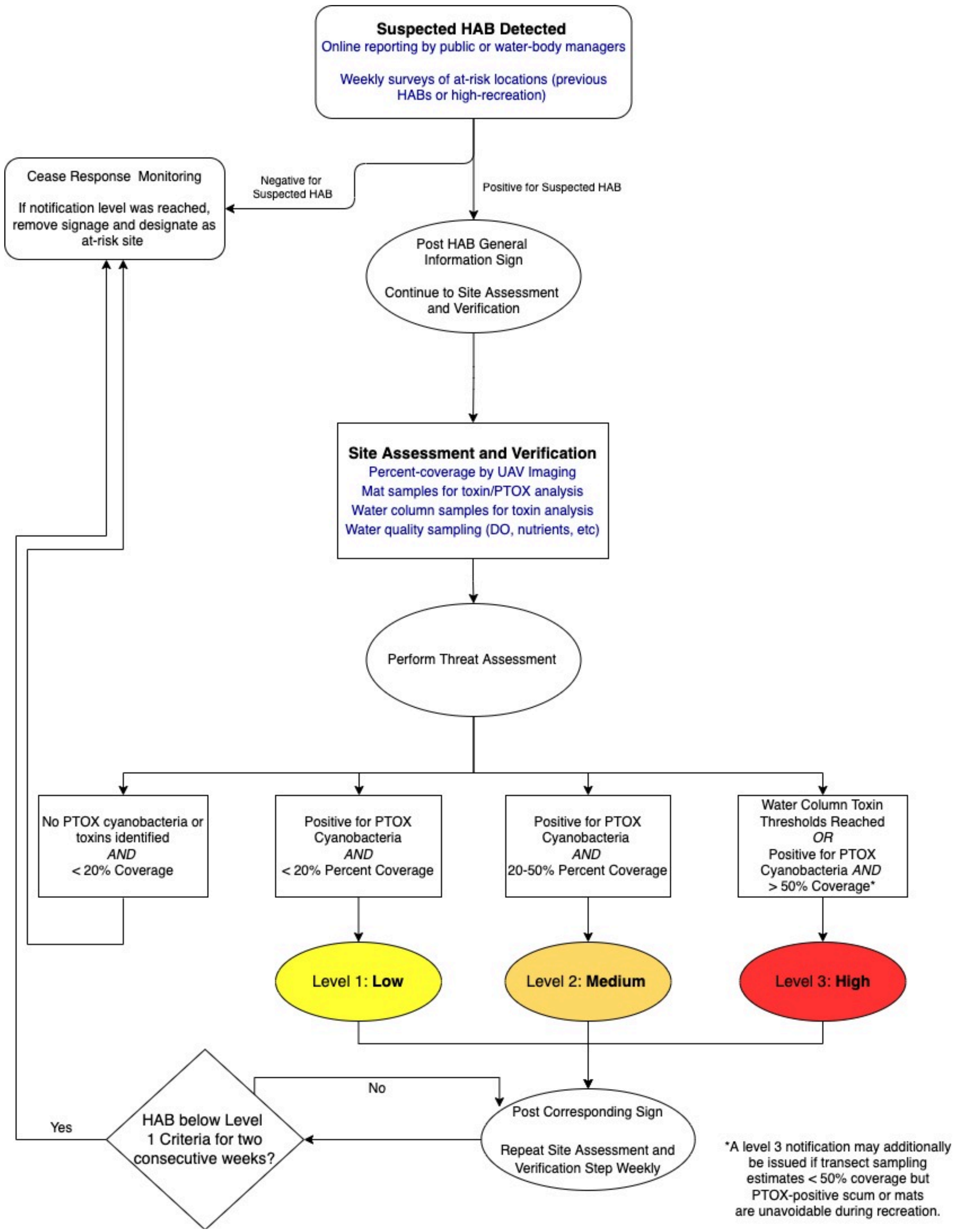

***Figure** 7. Scenario 1: High-effort, high-cost response to a benthic harmful algal bloom by DEQ.*

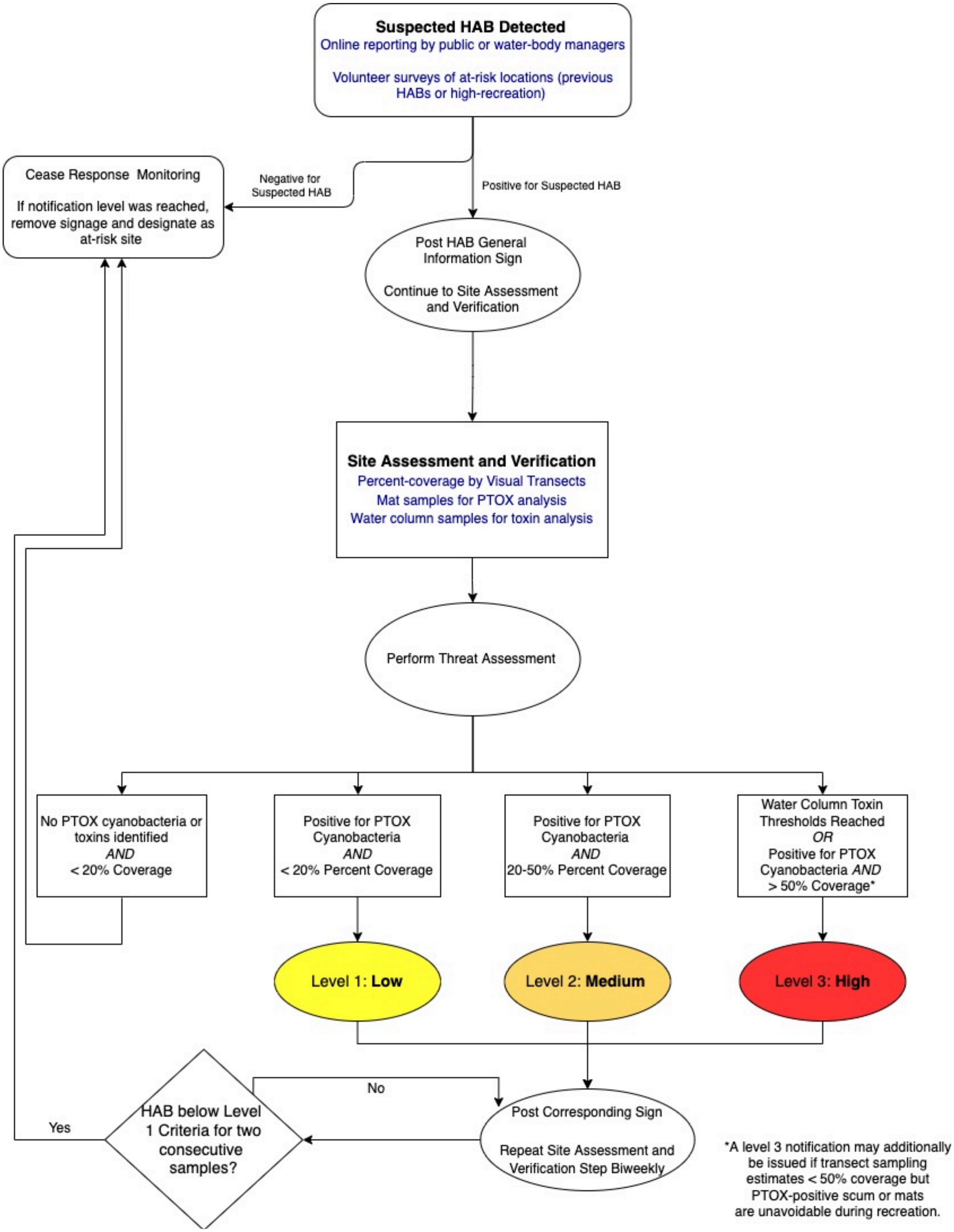

Figure 8. *Scenario 2: Low-effort, low-cost response to a benthic harmful algal bloom by DEQ.*

7. Developing a Monitoring Strategy

Monitoring benthic as well as planktonic HAB blooms comes with costs but should be as thorough as deemed necessary to meet agency objectives. Two common monitoring objectives in monitoring algal blooms are to determine 1) the spatial extent and duration of a bloom and 2) the toxicity of the bloom. Funding available for personnel, travel, equipment and supplies, laboratory analyses, and data management typically decides how intensively to monitor for both these objectives. Seasonal or weather-related changes in the blooms will also influence monitoring intensity. Leveraging or sharing existing resources usually reduces overall costs. Investing in newer technologies (e.g., drones) can also reduce costs while providing significantly more information.

Timing of Sampling

Cyanobacteria are present throughout the year. Different species have different environmental niches, preferred flow regimes, and stressor responses. Despite this complexity, toxigenic species of greatest concern and probability of human contact increase in late spring, peak throughout the summer months, and decrease by early fall. A response (*at-ready*) season between May 1st and October 31st should be adequate for responding to HAB reports in most Mid-Atlantic rivers.[1] Benthically attached cyanobacteria are vulnerable to scour by flushing events, so persistent high flows in spring or a late summer storm may temporarily suppress or wipe out algal growth from known problematic reaches. Field surveys should ideally avoid responding to events after a moderate to major precipitation event. When the objective of a monitoring program is to respond and quantify the size of a reported bloom event, sampling can be considered a reactive strategy and follow up considered relative to periods of peak algal growth and abundance and time of the year.

The same May 1st through October 31st period should also be considered for proactive strategies. Probabilistic and targeted (regions with historical HAB events) monitoring strategies could begin as soon as water temperatures begin to rise after winter. Sampling frequency and intensity during this time period is a function of available resources. Proactive strategies that capture both HAB and non-HAB sites prior to bloom events may provide comparisons beyond what a reactionary/response strategy could provide.

Site Selection

Site selection may be based on a number of different variables depending on the objectives of the responders. Subject to available resources, critical areas could be defined and then identified spatially prior to the summer bloom response season. Examples of critical areas may include sites proximal to public water intakes, recreational zones where there is high likelihood of human contact, agricultural regions where there is a high likelihood of livestock contact, or aesthetic reaches. In some cases, identifying nutrient enriched river sites such as wastewater treatment facilities and golf courses may also be a good starting point and predictors of HAB blooms.

Another consideration for site selection is cyanobacteria life history in the reported region. Benthic cyanobacteria can manifest in locally fixed forms such as benthic mat matrices, isolated colonies, or interspersed among other streambed associated vegetation. These benthic forms could be considered separately of their free-floating forms, despite being of the same colony. Free-floating cyanobacteria manifest in productive reaches and break free or shift in response to flow and high photic periods into collection zones. In regions that are recreated more intensely, collections of free-floating cyanobacteria can amass within root balls, log jams (strainers), eddies, emergent vegetation, and/or sandbars and may act as false positives for identifying the source of a HAB sites.

A low cost means to passively define hotspots in streams and rivers statewide is the implementation of an observer database (See Section 5). Volunteers, state biologists, and river users can opportunistically collect field observations over time when resources for a dedicated monitoring plan are absent. As critical regions emerge, resources can be more efficiently and economically disbursed in regions of need. This approach has two

[1] Regions where waters are artificially influenced by dams or where water is returned to a river may alter bloom trends. Dams and returns are shown to flatten flow peaks and artificially influence instream temperatures, two of the most significant variables in defining bloom presence.

significant weaknesses: 1) The time to develop hotspots will require multiple years and is inherently reactionary to HABs and 2) the approach will not capture underrepresented reaches (low population, lack of dedicated river user groups). If resources are available, a monitoring program to track HAB recurrence can take two forms: 1) a statewide probabilistic monitoring approach or 2) fixed stations established at the locations where cyanobacteria have historically occurred. These programs will be much more resource intensive but will provide information prior to and after HAB bloom events. Sampling frequency and intensity are a function of available resources and should be considered prior to the HAB season (May-October).

Wadable vs Non-Wadable Reaches

The methods employed to spatially measure benthic production (filamentous green algae, cyano bacteria, submerged aquatic vegetation) will depend on the water's width and depth at the monitoring/response site. Certain sites may be wadable during summer low flows and non-wadable other times of the year. Precipitation events may cause conditions hazardous for wading, and wadable surveys are never appropriate under flood conditions. Use of UAV/drone technology gets around this limitation, however, periods of increased flow often coincide with increased turbidity so aerial surveillance may be limited as well.

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Appendix A. Virginia HAB Contact List (6/3/2022)

In-state only toll free HAB Hotlineà Illnesses only	888-238-6154
ODU Phytoplankton Analysis Laboratory	757-683-4994 phytolab@odu.edu
VIMS Kim Reece Lab	804-684-7873
VMRC Emergency contact	800-541-4646
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Appendix B. Examples of Near-Field Drone based image analysis and resolution

Example 1: Balmaceda Rio NDVI, Chile

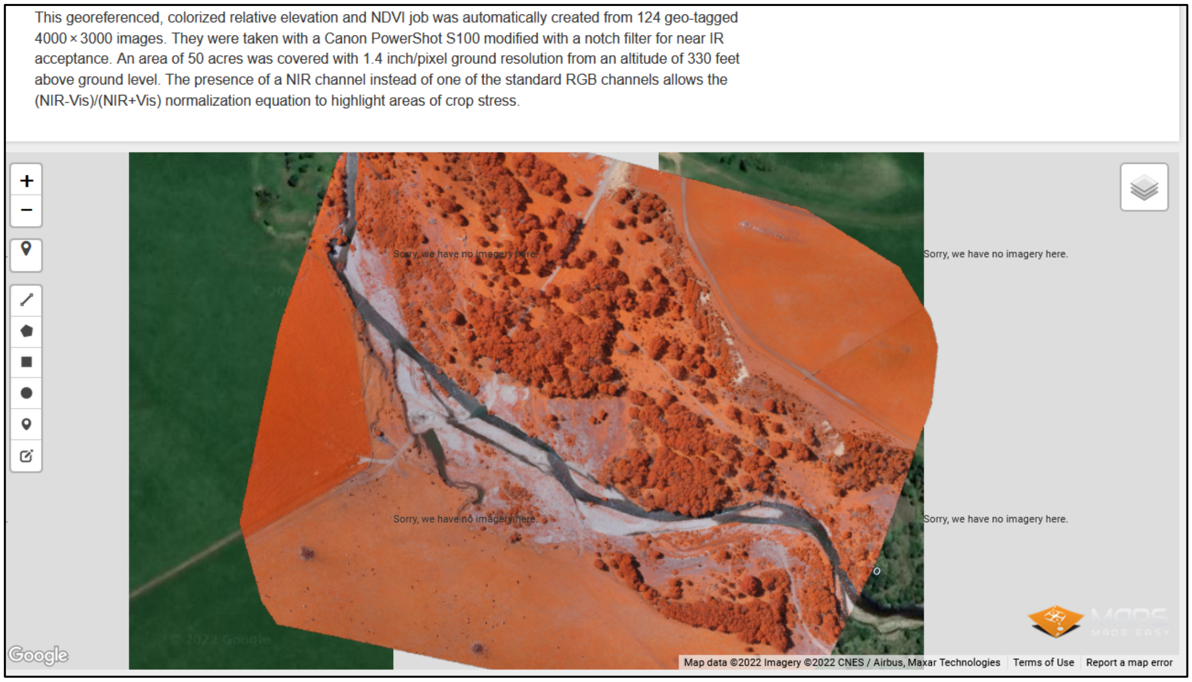

Figure 9*. Example of digital mosaic using near-surface survey drones. 124 geo-referenced images were used to make a detailed (1.4inch/pixel) description of a 50 acre parcel. This example also shows a drones ability to be mounted with other sensors/optics.*

Example 2: San Pedro River, Chile

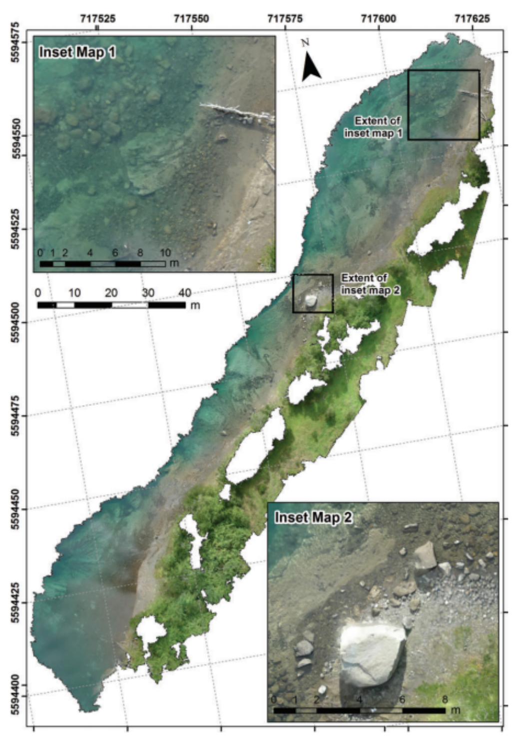

Figure 10*. Orthophoto of the Piedra Blanca site on the San Pedro River, Chile, obtained using the drone-structure-from-motion (SfM)*

Appendix C. Example Cost Breakdowns of Aerial Imagery

UAVs and Drones

Important considerations in drone selection include flight time, camera suitability, size, range, and cost. Flight time is currently limited to around 30-60 minutes for most models but survey time is easily extended with extra batteries. There are a number of commercially available drones that are suitable for high resolution digital photography, however, fewer exist that can be fitted with accessory imaging sensors such as multispectral and thermal imaging cameras. Drones can cost between $1,500 to $15,000 dollars and imaging sensor cost can range from included on the drone at purchase to stand-alone multi-spectral units around $10,000. Despite initial upfront cost, the low cost per data point, multiple sensor applicability, and reduction of field crew survey time begins to return on initial investment quickly.

Model (Manufacturer)	**Flight Time**	**On-board Camera**	**Size (portability)**	**Range**	**Cost (USD)**	**Other Features**
Nano+ (Evo) 	28 mins	3axis-stabilized, 50MP, 4K, 30 FPS, RYYB sensor	0.249 kg, 149x94 mm folded	10 km	$999.00 (without video screen)	Obstacle avoidance, 2.5 hr controller battery life and 90 min charge time
Phantom 4 RTK (DJI) 	30 mins	3axis-stabilized, 20MP, 4K, 60 FPS, RGB sensor	1.4 kg, 0.35m diagonal size w/o propellers	7 km	$6,500.00	All features of Pro model with cm-scale positioning accuracy and linked metadata for surveying.
P4 Multispectral (DJI) 	27 mins	5 monochrome and one RGB camera	1.48kg 0.35m diagonal size w/o propellers	7 km	$9,100.00 ($5,500.00 refurbished)	Multispectral imaging
Matrice 300 (DJI) 	55 mins	Modular Multi sensor payload	3.6kg .895m diagonal size w/o propellers	15 km	$13,500.00 (not including accessory sensors)	Modularity of gimbles and sensors

Large Fixed Wing/Rotary Aircraft

Unlike UAVs, large aircrafts are prohibitively expensive and therefore could be considered on a rental/per need basis. In 2021 ICPRB requested quotes from commercial aerial photographers for visualization of the Shenandoah River, Virginia HAB bloom event. A high speed orthoimage of a ~60 mile bloom can be captured in about 2 hours of flight time at a cost of $7970.00 for the image and $2,500.00 (1,250.00/hour) flight time. An additional cost to prepare the vehicle for a flight before any data is collected was quoted at $10,000. Not provided in this quote was use of advanced sensors/imaging. If a multispectral/hyperspectral camera were affixed to the aircraft as well, one could expect a rental fee for the camera to be an additional $1,500- $5,000 (depending on technology and duration).

FAIR LIFTS

QUOTE

Company Address	2601 Summers Street Ste 300 Kennesaw, GA 30144 United States	Created Date	8/23/2021
		Expiration Date	9/22/2021
		Quote Number	00050542
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Bill To Name	ICPRB		

Product	Line Item Description	Quantity	Sales Price	Total Price
Aerial DP	Color Digital Ortho 6 Inch Resolution	1.00	$7,970.00	$7,970.00
Mobilization & Demobilization	Helicopter Repositioning	4.00	$2,500.00	$10,000.00
On Site Time	2 Hours On Site	2.00	$2,500.00	$5,000.00
		Grand Total		$22,970.00

Satellite Imaging

The cost of satellite imaging can vary widely based company, image the resolution, scale, and temporal ability to acquire images. Low resolution images (< 10 m resolution), such as google earth, are free and updated every few years. This quality of data is not adequate for any kind of quantitative analysis and is often not captured during a bloom event. Satellite imaging at this time (Spring, 2022) is an emerging technology, worthy of mention, but not realistically applicable at this time. Sub-meter accuracy and images at agency request should be minimum requirements for HAB response. In theory if conditions were favorable (no cloud cover and satellite was in position) a newly acquired satellite image of a 100km long by 1km wide reach, similar scale to the Shenandoah Bloom of 2021, would cost ~5,000.00 per capture.

ULTRA HIGH RESOLUTION < 0.3 m	SUPER-HIGH RESOLUTION 0.31-0.55 m	VERY HIGH RESOLUTION 0.56-0.99 m	HIGH RESOLUTION 1-5 m	MEDIUM RESOLUTION 5.1-10 m	LOW RESOLUTION > 10 m
Archive from **$16*** min 25 sq.km	Archive from **$8*** min 25 sq.km	Archive from **$3***	Archive from **$1*** (5.0 m data)	Archive from **$0.6*** min 500 sq.km	Archive **Free** (when self-accessed)
New collection from **$48*** min 100 sq.km	New collection from **$15*** min 100 sq.km	New collection from **$6*** min 100 sq.km	New collection from **$2*** (5.0 m data)	New collection from **$1,2*** min 1000 sq.km	New collection N/A

*price per sq. km