Virginia Department of Health
Office of Environmental Health Services Shellfish Division
Marine Biotoxin Control Plan

2017

Introduction

Phytoplankton are microscopic algae that are common members of freshwater and marine habitats. They represent the major source of food and oxygen for many of the organisms present in lakes, rivers, estuaries, and oceans. Among the several thousand species of phytoplankton that exist worldwide, approximately 70 to 80 of these are known toxin producers. These toxins are potentially harmful to humans through direct ingestion, inhalation of aerosolized toxins, or through consuming fish or shellfish that have accumulated toxins from their environment and/or food sources. Toxins produced by phytoplankton can also be potentially harmful to birds, fish, and other inhabitants of aquatic habitats.

Usually, toxin-producing algae are absent or present in low concentrations that pose no environmental or human health threat. However, with certain environmental conditions, toxin-producing algae may proliferate to form dense concentrations of cells on water surfaces referred to as "harmful algal blooms" (HABs). HABs have been documented in the coastal waters of both the eastern and western United States, as well as other coastal areas throughout the world. In Virginia, HABs may occur throughout the year, with the majority occurring from early spring through the fall months. To date there are at least 38 potential toxin producing species that have been recorded in Virginia waters and the lower Chesapeake Bay, with the possibility of other toxic species becoming established (Marshall et al. 2008, Marshall and Egerton 2012). Algal blooms by non-harmful algae are also common seasonally, with discolored water (green, red, brown, etc.) that do not pose health concerns.

The heat-stable toxins produced by some HAB species may accumulate in certain shellfish and finfish, causing illness when ingested. Human illness is usually associated with the presence of a bloom, although shellfish and finfish may become toxic and cause human illness in the absence of a bloom. To address the issue of biotoxins and protect the public from shellfish poisonings, the National Shellfish Sanitation Program (NSSP) requires a Marine Biotoxin Contingency Plan, and has established standards concerning the closing of growing areas based on toxin concentrations in shellfish meats during HAB events (NSSP Model Ordinance @.04). Growing areas are to be closed based on the following criteria:

a) PSP (Paralytic shellfish poisoning): 80µg saxitoxin/100g shellfish meat
b) NSP (Neurotoxic shellfish poisoning): 5,000 cells *Karenia brevis*/L or 0.8mg brevetoxin/kg
c) AZP (Azaspiracid shellfish poisoning): 0.16mg azaspiracid/kg
d) DSP (Diarrhetic shellfish poisoning): 0.16mg okadaic acid/kg
e) ASP (Amnesic shellfish poisoning): 2 mg domoic acid/100g

Contingency Plan

Virginia has developed and implemented a multi-agency effort for monitoring , response, and research on estuarine HAB organisms. This effort includes extensive participation by VDH, DEQ, ODU and VIMS in performing sampling and analyses. These groups are members of the Virginia HAB Taskforce, whom share current bloom status and data throughout the year.

Monitoring:

A total of 75 stations are monitored throughout the growing areas, with monthly water samples collected and analyzed for the presence/abundance of potentially toxin-forming algal species. This includes collections from a partnership of contributing organizations (Figure 1). Six stations within the Chesapeake Bay mainstem are sampled 12 times per year from Jan-December as part of the Chesapeake Bay Monitoring Program (CBMP). 10 additional stations are sampled in the James, York and Rappahannock River from March-October as part of the CBMP. 62 stations within classified shellfish growing areas are sampled by VDH DSS monthly from March-October.

In addition to monthly routine fixed station sampling, reactionary post hoc bloom samples are collected by multiple agencies throughout the year based on visual sighting of blooms, fish kill events, etc. and processed for phytoplankton analysis. Additional higher frequency (daily-weekly) sampling occurs at a limited number of locations as part of ongoing research programs (ODU and VIMS), with HAB data shared between taskforce members.

Sampling protocols:

Two surface (<1m) water samples (100-1000ml) are collected from blooms and fixed stations. One water sample is preserved with Lugol's solution (ca. 3-4 ml/1000ml); with no preservative added to the second water sample. Each water sample should be labeled with station number, collection date and time, indicated. The unpreserved water samples should be stored in a cooler, but not on direct ice, with little jostling, and separated from the preserved samples and the Lugol's solution.

Phytoplankton analysis:

Lugol's preserved samples are analyzed by DSS marine science staff and/or the ODU phytoplankton analysis laboratory. A settling chamber method (Palmer-Maloney/ Sedgwick-Rafter/ Utermohl chamber or equivalent) is used to examine an adequate volume of water via light microscopy (100-400X) to characterize the presence of HAB species. All bloom forming or potentially toxic species are quantified in each sample. Densities of particular HAB species that exceed guidance thresholds can lead to additional monitoring/management actions as described below and illustrated in the flow chart (Figure 2). These thresholds are based on collaboration between Virginia (DEQ, VDH, ODU, VIMS) and Maryland (MDE, DNR, DHMH), and are summarized in the *Harmful Algal Bloom Management in the Chesapeake and Coastal Bays* (MD Sea Grant 2014).

Table 1: Working guidance thresholds for estuarine/marine algal species potentially associated with shellfish poisoning in Virginia waters.

Algal species	**Shellfish Related Illness**	**Main Toxin**	**NSSP shellfish growing area closure level (toxin w/in meat)**	**Virginia/Maryland level of concern (cell density in water column)**
Alexandrium tamarense species complex	PSP	saxitoxin	80µg /100g	presence
Karenia brevis	NSP	brevetoxin	0.8mg /kg	presence
Dinophysis spp.	DSP	okadaic acid	0.16 mg/kg	$\geq$ 10 cells/ml
Pseudo-nitzschia spp.	ASP	domoic acid	2mg/100g	$\geq$ 1,000 cells/ml

Toxin analyses and growing area closure/reopening protocols:

Samples with phytoplankton densities exceeding the above thresholds are analyzed for toxin concentration within the water column using ELISA/PP2A analyses. Unpreserved water samples (fresh <24hrs, frozen, filtered) are analyzed using appropriate toxin kits/methods. In addition, shellfish

samples from the growing area can be collected when phytoplankton analyses indicate significant bloom concerns. The following toxin analyses are conducted by DSS on water and/or shellfish samples:

Table 2: Algal toxin analyses (ELISA) associated with HAB species potentially present in Virginia shellfish growing areas.

Toxin	Test	Limits of test
Saxitoxin	Abraxis Saxitoxin (PSP) ELISA, Microtiter Plate #52255B	0.02-0.4 ng/ml (water) 2-40 µg/100g (shellfish)
Brevetoxin	Abraxis Brevetoxin (NSP) ELISA, Microtiter Plate #520026	0.01-2 ng/ml (water) 4.5-900 µg/100g (shellfish)
Okadaic acid	Abraxis Okadaic Acid (DSP) ELISA, Microtiter Plate #520021	0.1-5 ng/ml (water) 100-5000 µg/100g (shellfish)
Domoic acid	Abraxis Domoic Acid (ASP) ELISA, Microtiter Plate #ON0021	0.5-10 ng/ml (water) 100-2000 µg/100g (shellfish)

ELISA tests of seawater/phytoplankton samples can be conducted quickly (within 24hrs when needed) by DSS/ODU staff and provide guidance for management decisions, including the need to collect shellfish samples, and when appropriate, may lead to a precautionary closure of the growing area directly. **Toxin concentrations in meat samples that exceed the NSSP thresholds will result in immediate closure of the growing area.** Guidance to the size of the closure will be based on phytoplankton and/or seawater toxin analyses. Shellfish recalls, if necessary, will be established following NSSP guidelines and established VDH policies. Results from ELISA tests with concentrations in meat samples below the NSSP thresholds can also trigger additional sampling and analysis of toxins by LCMS-MS.

LCMS-MS (or equivalent) analysis of shellfish samples will be conducted when initial ELISA results indicate that biotoxins are present in significant concentrations. Shellfish samples ($\geq$ 12 individuals) will be collected from the growing area and analyzed for the appropriate toxin identified by initial phytoplankton analysis/ELISA results. NSSP approved methods will be used to verify reopening status. Growing areas can be reopened once concentrations **below** the NSSP closure threshold are measured from consecutive shellfish collections $\geq$7 days apart.

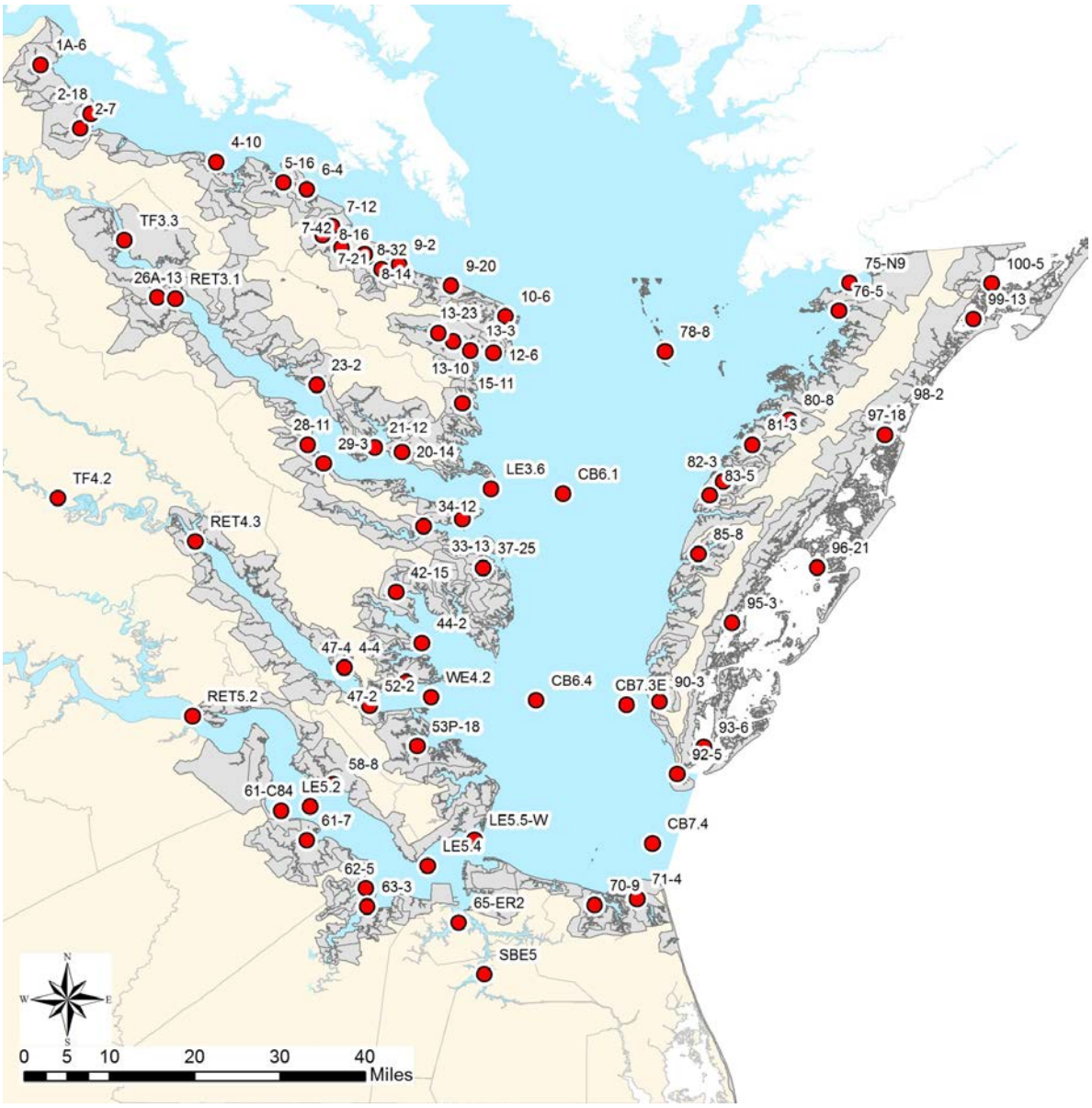

Figure 1: Phytoplankton monitoring stations in Virginia monitored for potential biotoxin producing algal species.

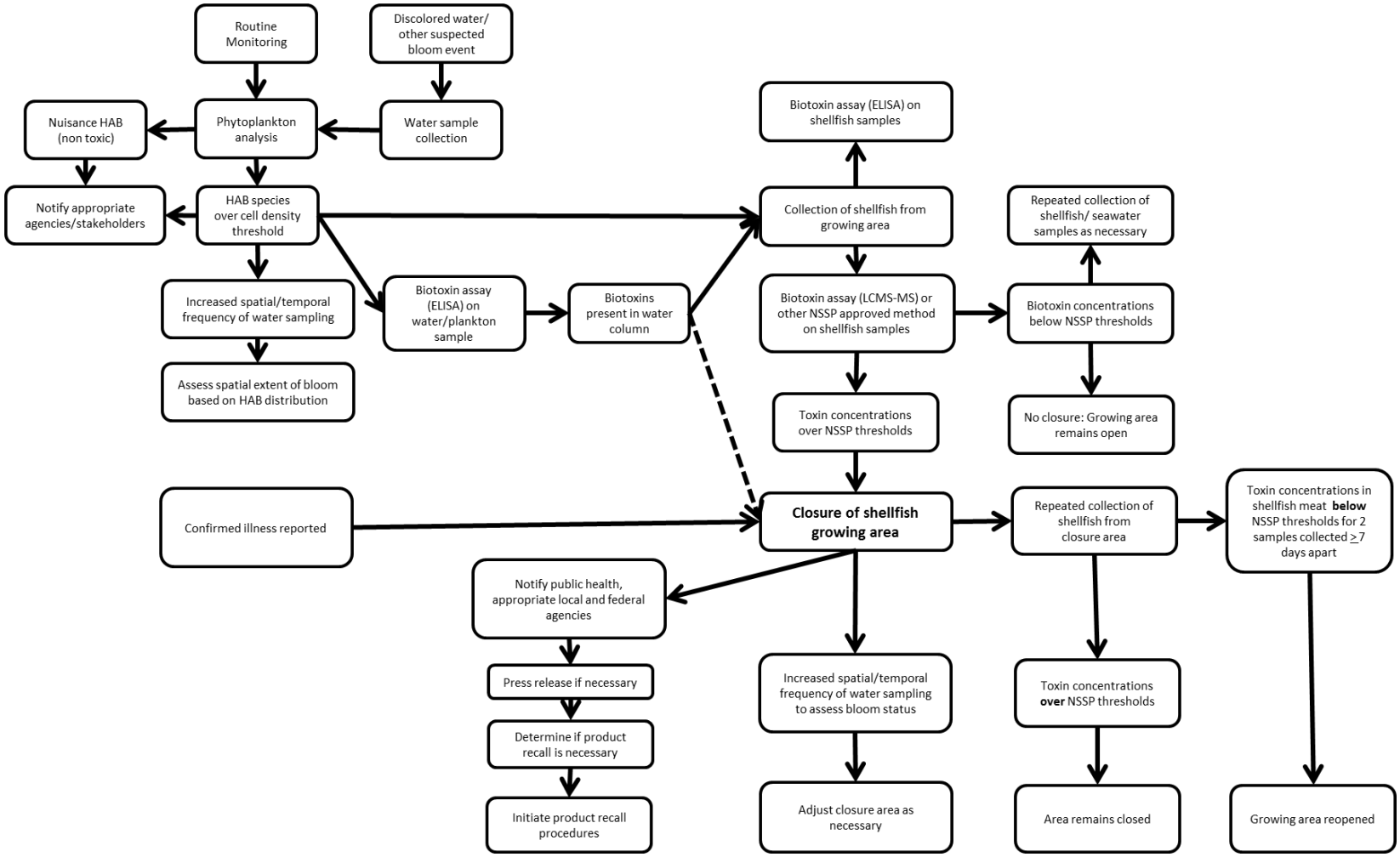

Figure 2: Virginia Biotoxin Contingency Plan flow chart and decision tree. Dashed line indicates water column biotoxins can be used to establish precautionary closure by VDH prior to collection/analysis of shellfish samples.