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DEPARTMENT OF NATURAL RESOURCES

AND ENVIRONMENTAL CONTROL

DIVISION OF WATERSHED STEWARDSHIP

WATERSHED ASSESSMENT SECTION

SHELLFISH AND RECREATIONAL WATER PROGRAMS

MARINE BIOTOXIN CONTINGENCY PLAN
2014 UPDATE

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DELAWARE MARINE BIOTOXIN CONTINGENCY PLAN - 2014

I. General Plan Specifications

A. Introduction& Background

Delaware is a member of the Interstate Shellfish Sanitation Conference (ISSC), the administrative body of the National Shellfish Sanitation Program (NSSP), a tripartite organization made of State, Federal, and Industry representatives. NSSP guidance documents provide the public health principles supporting major components of the NSSP and its Model Ordinance (MO) pursuant to regulating the human health-protection elements associated with the growing, harvesting, processing, and interstate transport of molluscan shellfish.

The specifications contained herein provide for the monitoring of marine and estuarine shellfish growing waters for phytoplankton typically associated with the production of toxins. In addition, a total inventory of phytoplankton – including non-toxin-producing species measured as present, common, abundant, actual concentrations, and/or relative abundance – shall be taken both in efforts relating to routine surveillance and in response to blooms. Baseline numeric or cell density data may be used to assess potential toxicological effects. Emergency-closure protocol may be invoked as per Title 7 of the Delaware Annotated Code, as the Model Ordinance was adopted by reference in the "Delaware Shellfish Sanitation Regulations."

Shellfish are filter feeders, and have the ability to concentrate toxigenic dinoflagellates from the water column when present in shellfish growing waters. The toxins produced by these dinoflagellates can cause illness and death in humans. Toxins are accumulated in the viscera and/or other tissues of shellfish, and are transferred to humans when the shellfish are eaten (Gordan et al, 1973).These toxins are not normally destroyed by cooking or processing, and cannot be detected by taste. Most of these toxins are detected through animal testing. However, some involve the use of instrument based or biochemical analyses for detection. Since the dinoflagellates are naturally occurring, their presence in the water column, or traces of their toxin in shellfish meat, does not necessarily constitute a health risk, as toxicity is dependent on various environmental "triggers" for toxin production, and concentration (dose) in the shellfish.

There are three types of shellfish poisonings which are specifically addressed in the NSSP Model Ordinance: paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), and amnesic shellfish poisoning (ASP), also known as

domoic acid poisoning. All three are dangerous toxins. PSP and ASP (or domoic acid) can cause death at sufficiently high concentrations. In addition, ASP can cause lasting neurological damage. PSP is caused by dinoflagellates of the genus *Alexandrium* (formerly *Gonyaulax*).NSP is caused by brevetoxins produced by the dinoflagellates of the genus *Karenia* (formerly *Gymnodinium*).Both of these dinoflagellates can produce "red tides", i.e. discolorations of seawater caused by blooms of the algae. Toxic blooms of these dinoflagellates can occur unexpectedly, or follow predictable patterns. The unpredictably in occurrence of toxic blooms was demonstrated in New England in 1972 when shellfish suddenly became toxic in a previously unaffected portion of the coastline, which resulted in many illnesses (Schwalm.1973).Historically, *Alexandrium* blooms have occurred between April and October along the Pacific coasts, from Alaska to California, and in the Northeast, from the Canadian Provinces to Long Island Sound (U.S. Public Health Service, 1958). The blooms generally last only a few weeks, and most shellfish (with the exception of clams which retain the toxin for longer periods) clear themselves rapidly of the toxin once the bloom dissipates. Global warming may be playing a role with regard to distribution of potentially toxic algae species outside of their traditional range, and with regard to duration of blooms.

The minimum concentration of PSP toxin that will cause intoxication in susceptible persons is not known. Epidemiological investigations of PSP in Canada; however, have indicated 200 to 600 micrograms of PSP toxin will produce symptoms in susceptible persons. A death has been attributed to the ingestion of a probable 480 micrograms of PSP toxin. Investigations indicate that lesser amounts of the toxin have no deleterious effects on humans. Shellfish growing areas should be closed at a PSP toxin level, which provides an adequate margin of safety, since in many instances PSP toxicity levels can change rapidly. The NSSP Model Ordinance requires that growing areas be placed in the closed status when the PSP toxin concentration is equal to or exceeds the action level of 80 micrograms per 100 grams of edible portion of raw shellfish (FDA, 1977; FDA, 1985).

In shellfish growing areas where low levels of PSP routinely occur, harvesting for thermal processing purposes may be an alternative to consider. Thermal processing as defined by applicable FDA regulations (21 CFR 113) will reduce but not entirely destroy the PSP content in the shellfish. If thermal processing is practiced, a state's shellfish program authority must develop and implement procedures to control the harvesting and transportation of the affected shellfish to the processing plant.

NSP, which is less common, had been documented to have occurred near Ocracoke Inlet in 1987; however, more commonly further south, particularly in Florida and throughout the Gulf Coast. Delaware experienced a *Karenia spp.* bloom (including K. papilionacea and *K. brevis*) in August and September, 2007, with confirmed maximum coastal concentrations of 14,000 cells / liter (Whereat,

2007). The transport mechanism is believed to be an eddy from the Gulf Stream. At those concentrations, no discolored water or other physical signs of the bloom were detected. The bloom was detected via routine surveillance. Bloom-related elevated chlorophyll levels are generally recognized to be visible via satellite at concentrations ranging from 50,000 to 100,000 cells / L. (K.p. is not proven to produce brevetoxin under laboratory conditions. However, New Zealand has a K.p. shellfish bed closure threshold >100K/L.)

In Gulf coast areas, toxicity in shellfish has been associated with red tide outbreaks caused by massive blooms of *K. brevis*. The most common public health problem associated with such blooms is respiratory irritation; however, neurotoxic shellfish poisonings associated with K. *brevis* blooms have been reported in Florida (Center for Disease Control, 1973 [a] and [b]). Uncooked clams from a batch eaten by a patient with neurotoxic symptoms were found to contain 118 mouse units per 100 grams of shellfish meat. However, concentrations of >20 MU/100 grams of shellfish meat were confirmed by Karen Steidinger (Heil, 2007). The NSSP Model Ordinance mandates that growing areas be placed in the closed status when any NSP toxin is found in shellfish meat, or when the cell counts for *K. Brevis* in the water column exceed 5,000 cells / liter (cells / L). However, as blooms are often transient in nature, and/or not uniform in concentration and distribution, toxin levels in molluscan shellfish meats, as determined by approved methods, shall take precedence in determining the need for, and/or extent of shellfish growing area closures precipitated by the presence of *K. brevis* in Delaware waters.

ASP is caused by domoic acid, which is produced by diatoms of the genus *Pseudo-nitzschia*. Blooms of *Pseudo-nitzschia spp*. are of relatively short duration. However, during the 1991-1992 incidents in Washington, high toxin levels persisted for several months (Liston, 1994).The NSSP Model Ordinance requires that growing areas be placed in the closed status when the domoic acid concentration is equal to or exceeds 20 parts per million in the edible portion of raw shellfish.

B. Characterization of Delaware Waters Relative to Algae Blooms

The presence of the above causative organisms has been documented in Delaware waters; however, not typically in sufficient concentrations to cause toxicological effects. Typically, on the East Coast of North America, the presence of the above organisms in concentrations sufficient to cause toxicological effects in humans occur only in waters to the north of, or to the south of Delaware. This is due to an accident of geography, latitude, and Ocean currents. The Gulf Stream laps at the shore of the Outer Banks, coming within twenty to thirty miles of Cape Hatteras. Despite periodic eddies, frequent, routine surveillance for algae – both potentially toxic and non-toxin-producing species – reveals that the Gulf Stream is not generally a conduit for southern species of potentially toxin-producing phytoplankton entering into Delaware's near-shore environment, despite its movement at approximately three

feet per second. However, there are exceptions, such as the 2007 Karenia spp. bloom. The late-season propensity for northeast winds increases the probability for development of a Gulf Stream eddy developing – the probable transport mechanism for the Karenia bloom. (However, Karenia spp. prefer low-nutrient water – not a characteristic of the Delaware near-shore environment. As such, such blooms are potentially relegated to a short duration once in near-shore Delaware waters.) The general / net movement of Ocean water to Delaware is south due to the Labrador Current. However, water temperatures become too warmed at these latitudes to support northern species of potentially toxic phytoplankton at bloom-like concentrations.

Alexandrium sp. is not known to exist in bloom-like concentrations south of Cape Cod. In the U.S. it is primarily a problem on the West Coast, and in George's Bank, on the East Coast. *Pseudo-nitzschia spp*. are primarily, also, a problem in cooler waters. *Karenia brevis* is primarily a warm-water species / phenomenon.

Of particular concern in Delaware are *Dinophysis spp*., and *Prorocentrum spp*. during the cooler months, with a caution about *Pseudo-nitzschia spp*. primarily, also, during the cooler months. Both routine and emergency-response sampling have revealed *Dinophysis spp*. (including *D. norvegica*) and *Prorocentrum spp*. at significant levels; however, not at bloom-like concentrations. *Pseudo-nitzschiapungens* and *seriata*, as well as *Chatonella spp*., and *K. mikimotoi*, believed to produce brevetoxin, have been found in Delaware waters. In addition, *Pfiesteria spp*. are also common to abundant.

Other potentially toxic species commonly found in Delaware waters include; but are not limited to: *Chaetoceros spp*., *Karlodiniumveneficum*, *Ditylum spp*., *Rhizosolenia spp*., *Anabaena spp*., *Amphidinium spp*., *Ceratium spp*., *Heterosigma akashiwo*, *Chatonella spp*.

Non-toxin-producing species most commonly found in Delaware waters include; but are not limited to:*Heterocapsa rotundata*, *Kryptoperidinium foliaceum*, *Scrippsiella spp*., As well as *Chaetoceros spp*. and *Skeletonema costatum* (diatoms). Other species are also present at background or periodic bloom-like concentrations, with new discoveries occurring at a high frequency.

C. Biotoxin Control Plan Specifications

These procedures are instituted to provide constructive notice to the public regarding toxic or potentially-toxic algae blooms (early warning system), and to define the severity and extent of the occurrence pursuant to minimizing illness. Protocol for algae identification, administrative procedures, laboratory support, sample collection procedures, and patrol procedures, are contained herein, including adequate surveillance, public notification, including both a characterization of the severity of occurrences, and contingencies to minimize illness, including shellfish bed closures, and returning shellfish beds to the

Approved / open status. Parameter organisms shall include; but are not limited to all those listed herein.

1. Sample Observation, Analyses, and Speciation

Observations of toxic and non-toxic phytoplankton (genus profiles, and species identification when such expertise allows it) shall be made to augment existing data regarding the presence of potentially toxic phytoplankton, and shall constitute baseline information as to typical and normal concentrations of said organisms.

Field observation (sample collection and analyses / speciation) and satellite and/or aerial surveillance shall take place whenever practicable, and at a minimum of two times per month during warmer months, and whenever practicable during cooler months. Observations shall be made to augment existing data regarding the presence of potentially toxic phytoplankton, and shall constitute baseline information as to typical and normal concentrations of said organisms. Additionally, excursions in response to possible blooms shall be conducted for the purpose of making observations as to the concentration of these organisms relative to baseline concentrations, and any possible effects that may have occurred or may be occurring at the time of the excursion. Effects may include: dead or dying fish; behavioral anomalies in fish; dead, dying, or behavioral anomalies in fish predators; discolored water (may be mahogany or milky colored); an "off-odor" to the water (may be a "sweet" smell); or neurological effects in humans which may include disorientation, a tingling sensation, asthma-like symptoms, or neuromuscular effects. These effects may be induced by direct water contact, consumption of contaminated shellfish, or due to the aerosolizing of the toxins.

2.Emergency Observation, Analyses, and Speciation

In addition to routine monitoring, waters shall be monitored for toxic phytoplanktonic forms in response to observations which may indicate their presence, such as those listed above.

See Appendix 2 analyses parameters

II. Emergency Shellfish Sampling, Assay, Public Notification and Re-Opening of Closed Shellfish Beds

The following is specifically written to address *Karenia brevis* blooms; however, shall constitute a template to address any and all toxic or potentially toxic algae blooms that occur in Delaware waters.

A. Background / Contingency Sampling & Testing

The phenomenon known as “red tide” occurs when K. brevis concentrations increase above normal background levels of 1,000 cells/liter. Concentrations equal to or greater than 250,000 cells/liter can cause fish kills; however, much lower concentrations (greater than or equal to 5,000 cells/liter) may cause shellfish to become toxic if exposure is sustained. Shellfish become toxic by filter-feeding on the dinoflagellates, and absorbing the toxin into their digestive tissues. Toxic shellfish meats, when ingested, may cause illness to humans and animals. The period of exposure required to elevate NSP levels in shellfish to levels toxic to humans depends on environmental factors. Since this is a warm-water phenomenon, shellfish are assumed to be pumping during blooms. However, hydrographic conditions may limit exposure. In the field, shellfish may retain toxicity for 2 to 4 weeks.

The NSSP Model Ordinance mandates that growing areas be placed in the closed status when any NSP toxin is found in shellfish meat, or when the cell counts for *K. brevis* in the water column exceed 5,000 cells / L. However, as blooms are often transient in nature, and/or not uniform in concentration and distribution, toxin levels in shellfish meats, as determined by approved methods, shall take precedence in determining the need for, and/or extent of shellfish growing area closures precipitated by the presence of K. brevis in Delaware waters. Shellfish meats shall be tested for toxicity by an approved procedure during and after a *K. brevis* bloom. If a shellfish growing area is closed (for either exceeding 5,000 *K. brevis* cells / L, or due to toxicity in shellfish meats), the area shall only be re-opened / returned to the Approved status pending testing / negative (<MDL) results for brevetoxin from shellfish meats from shellfish harvested therein.

B. Early Warning System

Delaware’s early warning system shall include; but is not limited to routine surveillance, as described earlier in this Plan, observations by DNREC staff, satellite and aerial surveillance.

1. Defining Severity and Extent of Occurrences

When elevated concentrations (>1,000 cells/liter) of *K. brevis* are found, the initial sampling program shall continue, and be expanded. Sampling shall be conducted at predetermined stations, which shall include both Citizen Monitor sites and sites in proximity to observed concentrations >1,000 cells / L, and or where effects are observed, including; but not limited to dead or dying fish; behavioral anomalies in fish; dead, dying, or behavioral anomalies in fish predators; discolored water (may be mahogany or milky colored); an "off-odor" to the water (may be a "sweet" smell); or neurological effects in humans which may include disorientation, a tingling sensation, asthma-like symptoms, or neuromuscular effects.

Closure of an area will occur as follows:

Due to the possible dynamic nature of phytoplankton blooms, including non-uniformity in concentration and distribution, and not necessarily occurring in association with discolored water, odor, or obvious effects - shellfish growing area closure boundaries shall be large enough to accommodate a significant margin of safety. A reasonable and significant margin of safety shall be determined on a case-by-case basis; however, may include all of Rehoboth Bay (growing area 2) and/or Indian River Bay (growing area 3). The Delaware Bay may be divided into zones of closure on a case-by-case basis. The area(s) of closure shall be established using well defined and clearly visible points of land, navigation markers, and/or other easily-recognizable landmarks.

C. Embargo of Potentially Toxic Shellfish

Potentially toxic shellfish constitute a deviation from a critical limit. This requires that a certified dealer take corrective action either by following a corrective action that is appropriate for the particular deviation, or by segregating and holding the affected product until a review can determine the acceptability of the affected product for distribution. Corrective actions include, when necessary, reconditioning, seizure, or destruction of affected product to ensure that no product enters commerce that is either injurious to health or is otherwise adulterated as a result of the deviation.

Stop Sale Action shall be immediate, and destruction of shellstock determined on a case-by-case basis.

D. Patrol Program

The Delaware Department of Natural Resources and Environmental Control (DNREC) Division of Fish and Wildlife Enforcement Section, along with assistance from State Parks personnel in shellfish growing waters adjacent to state parks, under the DNREC Division of Parks and Recreation, shall enforce emergency harvest restrictions, as specified under Title 7 of the Delaware Annotated Code.

E. Procedures to Disseminate Information

Procedures for disseminating information regarding emergency closure of Delaware shellfish growing areas due to a toxic phytoplankton bloom shall include; but not be limited to direct notification of harvesters and processors, direct notification to the Delaware Department of Health and Social Services / Division of Public Health, direct notification to other states, ISSC, and U.S. FDA, a press release, emergency signage, and enhanced patrols, as referenced above.

F. Procedures to Re-Open Closed Shellfish Harvest Areas:

Following area or zone closure, water samples are collected at key and/or representative sampling stations for *K. brevis* identification and cell counts. Shellfish shall be collected concurrently for toxicity analyses using approved methods. Negative (<MDL) results shall result in immediate re-opening of the potentially affected / affected area(s).

III. References

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APPENDIX 1

PROJECT ORGANIZATION AND RESPONSIBILITY
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APPENDIX 2

UD CITIZEN MONITORING PROGRAM
PHYTOPLANKTON LABORATORY ANALYTICAL PROCEDURES
(Detection Limits and Quality Assurance Objectives)

Edward Whereat, Program Coordinator, January 2012

note: These are laboratory procedures, and do not necessarily constitute field observation procedures.

Purpose:
The purpose is to identify and enumerate phytoplankton species that have the potential to form Harmful Algae Blooms (HABs) and to generally define the phytoplankton community. Highest priority and effort is given to HABs that may affect human health via the production of toxins that could be eaten (bio-accumulated in shellfish tissue), inhaled (aerosolized and dispersed beyond water body), or absorbed through skin during recreational contact (dermatitis). Moderate priority is given to HABs that effect aquatic resources via the production of toxins (kills fish and/or invertebrates) or the reduction of dissolved oxygen as a consequence of high biomass. Lowest priority is given to other members of the phytoplankton community.

Field Collection and Sample Custody:
Grab samples are collected in 250 ml to 2.0 L bottles depending on the purpose, and the location of sample sites. Routine estuary samples are collected in 250 ml bottles. Samples that are to be forwarded to Dr. Coyne's Lab (UD CEOE) for identification and/or enumeration of phytoplankton by DNA probes are collected in 1.0 L bottles. For the past few years, these included all routine samples collected from the ocean beaches and the Indian River Inlet, and a few routine samples collected from residential canals. If there is reason to expect the need for outsourcing of samples to other laboratories for phytoplankton identification, enumeration or toxin tests, 2.0 L bottles are used.

All samples are stored and transported to the lab in coolers to keep samples close to the ambient water temperature at collection. Small amounts of air space are left in bottles to facilitate thorough mixing prior to any analysis or splitting of samples.

Analysts:
Laboratory procedures are performed by trained staff members. Trained volunteers are involved in sample collection. In addition, a limited number of volunteers have been trained to utilize field microscopes to screen samples for HABs in a more qualitative fashion designed to spot blooms at home or in the field. The "field" methods used by volunteers are described briefly.

Microscopic analysis of live and preserved samples:
The method and extent of effort involved in microscopic analysis depends on the desired detection limit of cell density, which depends on the total volume of water screened. We utilize the following microscopes in combination with the following counting chambers.

Microscopes	Chambers and volumes
Nikon TMS Inverted Microscope	Lab-Tek tissue culture chambers (155380), 2 x 3.0 ml
American Optical Corporation, model 50	Sedgewick-Rafter chamber (gridded), 1.0 ml
	Palmer-Maloney chamber, 0.1 ml

Dropletson standard slides with coverslips, 2 x 0.04 ml

Swift Field Microscope, model FM-31 * ——— VitroCom Inc. Capillary tubes (3530-100), 0.09 ml *

(* restricted to use in field) (* effective volume about 0.07 ml)

Lab-Tek chambers are used to detect low cell densities in preserved samples collected from the ocean and Indian River Inlet. We use a modified Utermöhl sedimentation technique to settle preserved cells from 6.0 ml of sample in 2-paired 3.0 ml chambers which are then viewed with the Nikon Inverted microscope, mostly at 100X, but with cell dimensions measured at 400X. The entire bottom surfaces of both chambers are screened after an over-night settling period resulting in a detection limit of 167 cells/L.

Sedgewick-Rafter chambers are used to detect intermediate cell densities in 1.0ml of live or preserved samples that are generally collected from the ocean or the Indian River Inlet. S-R chambers can be viewed with the Nikon Inverted scope at all magnifications, or the American Optical Corp. microscope at magnification of 100X or 40X. The entire chamber is screened, resulting in a detection limit of 1,000 cells/L. The chamber has 1000 1 mm^2 grids in a 20 x 50 array, which permits counting a fraction of the chamber if a higher detection limit is permissible (i.e. cell density is high, or species is of little concern). The chamber depth is 0.1 mm.

Palmer-Maloney chambers are used to detect high cell densities in 0.1 ml of live samples collected from the estuaries using the American Optical Corp. microscope. The entire chamber is screened, resulting in a detection limit of 10,000 cells/L. For species at very high cell densities, cell density is estimated by averaging the number of cells seen per field of view at 100X across at least 10 fields of view which are chosen in a blind array. The chamber depth is 0.4 mm, so considerable adjustments in focus may be needed.

Droplets on standard slides with coverslips are used to detect high cell densities in live samples collected from the estuaries. Typically, two 0.04 ml drops are screened with the American Optical Corp. microscope, resulting in a detection limit of12,500 cells/L. A third drop may be analyzed if species diversity is high, or if the variance in counts for a particular species is high between the first two drops. For species at very high cell densities, cell density is estimated by averaging the number of cells seen per field of view at 100X across at least 10 fields of view from each drop which are chosen in a blind array. The depth of the water film under the coverslip is about 0.08 mm, and minimal focus adjustments are needed, however slides must be analyzed quickly since some phytoplankton may be subject to desiccation or heat stress (within about 10 min).Given the low cost, the ease of cleaning, and the minimal focus adjustments, standard slides and cover slips are used for most high cell density analyses.

Capillary tubes are used to detect high cell densities in live samples from the estuaries using the field scopes, usually in the field or at a volunteer's home. Capillary tubes contain an effective volume of about 0.07 ml. If the entire tube is screened, the resulting detection limit is14,300 cells/L. For species at very high cell densities, cell density is estimated by averaging the number of cells seen per field of view at 100X across at least 10 fields of view which are chosen in a blind array. The depth of the tube is 0.3 mm, so considerable adjustments in focus may be needed. However, an advantage of the capillary tubes is that the length of time that phytoplankton survive is considerably extended. Field scopes can also accommodate the use of standard slides and cover slips as described above, but since they lack a mechanical stage, complete coverage of the slide/drop can be difficult, so cell counts are generally based on field of view estimates.

Precision of cell counts by microscopy:
Microscopic cell counts are time-consuming, so there is a compromise between the precision and the cost/time for the analysis of a single sample. Collecting and analyzing replicate field samples from a single site is generally not done due to cost/time constraints. There may be high spatial and temporal variability in field samples, and according to our professional judgment, more useful information can be obtained by resampling sites following significant samples rather than achieving what may be unrealistically high precision on a single sample.

The precision of cell counts depends on how many cells are counted. In the case of colonial or filamentous forms, natural units, based on estimates of the number of cells per colony or filament, may be counted and are later converted to individual cell counts. Assuming the distribution of cells/units is random, and that the population fits a Poisson distribution, the approximate 95% confidence intervals for a count can be estimated from the following formula: $\pm (2/\sqrt{n}) \times (100\%)$, where n is the numbers of cells/units counted. The approximate 95% confidence limits for a range of counted cells/units are given below.

Count of cells/units	95% confidence limits (%)
4	±100%
25	±40%
100	±20%
400	±10%

Most of our counts for a given taxa fall within the range of 4 to 100 cells/units. Precision is low for rare cells, but sufficient volumes of water are screened to achieve adequate precision when cell densities reach levels of concern for particular taxa.

The context for the Delaware Beaches and Inland Bays:
In September 2007, we detected and intensively monitored a mixed bloom of *Karenia brevis* and *K. papilionacea* along the DE coast over a two week period. There were limited incursions into the Inland Bays and the DE Bay as well. We primarily used the droplet method on live samples, and outsourced live and preserved samples to expert microscopists for verification of identification and cell counts.

Karenia brevis can produce Brevetoxins, which are potent neurotoxins. Even when cell densities of *K. brevis* in the water are low, the toxins could bioaccumulate in shellfish tissue, leading to a risk of Neurotoxic Shellfish Poisoning (NSP) if those shellfish were consumed. At higher cell densities, the toxin can cause fish kills or become aerosolized in the surf and cause respiratory irritation to beach-goers. Brevetoxin production in *K. papilionacea* remains equivocal. In 2008, we implemented a routine monitoring program for the ocean beaches and Indian River Inlet, and adopted protocols used to detect lower cell densities from the microscopic analysis of preserved samples. In addition, we began forwarding these samples to Dr. Coyne for the development of molecular probes for *Karenia* species. This turned out to be particularly important since *K. papilionacea* exhibits variation in size and morphology that may overlap with that of *K. brevis*, which makes microscopic identifications uncertain in some cases.

NSP is a periodic risk in the Gulf of Mexico and the Atlantic coast of Florida, and isolated cases have occurred as far north as North Carolina. In states where NSP is a risk, the detection of a cell density of *K. brevis* $\geq$ 5,000/L in the waters of a shellfish harvesting area induces harvesting restrictions and toxicity tests on shellfish meat (by mouse bioassay). According to our protocols, if we reported a cell density of *K. brevis* to be 5,000/L, it would be based on counting 30 cells in 6 ml, and the approximate 95% confidence limits would be ± 36.5%, or 5000 ± 1825 cells/L.

Respiratory irritation in the general public and fish kills might be expected to occur if cell densities of *K. brevis* are ≥ 100,000/L. If we reported this density based on the analysis of 6.0ml in Lab-Tek chambers, the approximate 95% confidence intervals would be 100,000 ± 8,200 cells/L. However, we would probably find the precision from the analysis of 1.0 ml in a Sedgewick-Rafter chamber to be adequate (100,000 ± 20,000cells/L)and devote the time saved to additional sample collection and analysis.

Historically, there have been no documented cases of shellfish toxicity or shellfish poisoning in humans due to HABs in the mid-Atlantic region, however there are also other potentially toxic phytoplankton that could conceivably impact DE waters at similarly low to moderate cell densities as *K .brevis*. In Long Island, there have been shellfish bed closures due to *Alexandrium spp.* which could cause Paralytic Shellfish Poisoning. In Delaware waters, toxic species of *Pseudo-nitzchia*, and low levels of the toxin Domoic acid have been detected which could cause Amnesic Shellfish Poisoning. *Dinophysis acuminata*, a species with potential to cause Diarrhetic Shellfish Poisoning, has been detected in Delaware waters as well.

Details on the multi-tiered analysis of samples from the ocean beaches or the Indian River Inlet:
Upon arrival at the lab, a 60 ml portion of the well-mixed1.0 L sample is preserved with Lugol's solution (I_2KI, acidified with acetic acid), with a final concentration in samples being 1% Lugol's. Samples are stored in a refrigerator in 60 ml amber bottles or in 60 ml or 15 ml centrifuge tubes. This provides ample volume for our microscopic analysis, for an archive, and to outsource the sample to expert taxonomists, if needed.

To determine Chlorophyll levels, a 50 ml portion of the well-mixed sample is filtered through 2.5 cm Whatman Glass Fiber Filters which are placed in 20 ml glass scintillation vials and promptly frozen (-20 C). At a later time, Chlorophyll is extracted by the addition of 10 ml of a 90% acetone/water mixture to the vials, which are then returned to the freezer for 24 hours before analysis on a Turner AU-10 Fluorometer, which is part of a shared analytical equipment pool at UD CEOE.

An immediate live screening of a 1.0 ml portion of well-mixed sample in a Sedgewick-Rafter chamber is performed to ensure that blooms at levels of concern are identified and acted upon the same day if needed. An initial rapid screening at 40Xpermits counts of mobile, medium-sized dinoflagellates, including *Karenia spp*., which may have a limited survival time in the chamber. A more thorough screening at 100X permits identifications and counts for the rest of the phytoplankton community. Within hours, the remaining portion of the 1.0 L sample is forwarded to Dr. Coyne's lab for analysis by DNA probes. On the following day, a 6.0 ml portion of the preserved sample is analyzed more thoroughly for known HABs after an over-night settling period in the Lab-Tek chambers.

Early history of our HAB monitoring program:
We originally implemented a HAB monitoring program in 2001 in response to the discovery of a potentially ichthyotoxic HAB, *Chattonella cf. verruculosa*, in the midst of a fish kill of juvenile Menhaden in the Inland Bays in 2000.Our initial focus was monitoring the estuaries for several potentially toxic HABs that have been implicated in fish kills around the world, as well as for other non-toxic HABs whose dense blooms may negatively impact dissolved oxygen dynamics. Most of these HAB events result from blooms at high cell densities(≥1,000,000 cells/L), and coarse quantification methods (Palmer-Maloney, droplet, capillary tube) were adequate for this purpose. In the early years, we maintained a significant effort to train volunteers to screen samples with field microscopes, but due to the steep learning curve, few became proficient, particularly at cell counts, so we increasingly depended on analysis by staff members. Volunteers also collect samples with

plankton nets(10µm mesh), which can concentrate cells by at least 2 orders of magnitude. This provides an easy and rapid method to detect rare HAB cells although actual cell densities cannot be estimated. Net samples are great tools for teaching identifications, and they can provide estimates of the relative abundance of taxa. Net samples may discriminate against certain taxa, particularly small and soft-bodied cells. Nevertheless, net samples have been valuable, either alone or in combination with a coarse quantification of a paired grab sample, in detecting the presence of HABs that could impact shellfish safety at low cell densities.

For several years, we have provided estuarine field samples, identifications and cell counts to researchers at UD CEOE (Drs. Hutchins and Coyne) to complement their research on HABs (ecology, physiology and the development of molecular techniques for identification and enumeration). We have established contacts with experts in other states (MD, VA, NC, SC, FL) to verify our identifications and counts, perform toxin tests, and to utilize samples for their own research. We issue HAB reports on a routine basis from May to October (http://citizen-monitoring.udel.edu/reports/HAB.shtml), and have made significant contributions to the HAB monitoring efforts of the DE DNREC.

Analytical References

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