Overview

In 1995-1996 and again in 2000 and 2005, Alabama experienced *Karenia brevis* red tide events. Shellfish harvest areas were closed. Tourist and resident populations were affected by the aerosols, which caused respiratory irritation. Fish kills caused by the dinoflagellates, *Alexandrium monilatum* and *Karlodinium veneficum* have been documented at Gulf Shores and Weeks Bay National Estuarine Research Reserve; and a hypoxia-driven fish-kill occurred in eastern Mobile Bay during a bloom of the dinoflagellate *Heterocapsa triquetra*. A fish-kill caused by the raphidophyte *Chattonella subsalsa* has been documented in the Theodore Industrial Canal. The diatom, *Pseudo-nitzchia*, has been identified in blooms along the Gulf in the Little Lagoon area. This event in 2005 showed low levels of the toxin, domoic acid, the agent of amnesic shellfish poisoning.

In 2006 there was a report of sewage at the site of a moored barge in the Intercoastal Canal in Baldwin County, Alabama. Black, stinking, floating material was reported by a citizen and investigated by health department environmentalists. *Lyngbya*, a cyanobacterium, was identified. The cyanobacteria are included in the Alabama HAB surveillance because of these potential public health impacts.

The recounting of these events and the toxin details are not meant to alarm, but to promote awareness and preparedness for events. The *Karenia brevis* blooms required state, federal, and local agencies to implement plans to reduce impact to public health; provide information about the blooms and their duration; and assess long-term effects. This document will attempt to codify these procedures for reporting a HAB event, collection and analyses of samples, public health response, and pathways of communication.

Goals

The Alabama HAB program is a multi-agency cooperation between the Departments of Public Health (ADPH), Environmental Management (ADEM), Conservation and Natural Resources (ADCNR), Baldwin County Health Department (BCHD), Dauphin Island Sea Lab (DISL) and the Alabama Volunteer Microalgal Monitoring Network (AVMMN).

The primary goals of the Alabama HAB Response Plan are:
1. Provide accurate information regarding HAB to local, state, federal and academic agencies in Alabama
2. Provide timely health advisories associated with HAB and human health.
3. Assure regulation of the shellfish harvest as required by the Marine Biotoxin Control Plan within the National Shellfish Sanitation Program.
4. Define contacts and roles within county, state and federal agencies that respond to HAB events
5. Provide, in a timely manner, accurate analyses of toxins and organisms using standardized methodologies for identification and quantification

Management of HABs in Alabama is an integrated effort conducted by a number of state, federal and academic agencies. Sample sites are shown below.
Figure 1. Coastal Alabama, showing sites routinely monitored for HABs by the agencies involved in the Alabama HAB Management Plan.
Known and Potentially-Harmful Algal Bloom Organisms

Several potentially toxic organisms have been identified in Alabama waters. Toxicity is not always expressed. The principle organism of interest is *Karenia brevis*, which affects shellfish harvest, fish populations, and beach areas. Below are listed some of these toxic organisms identified in estuarine and marine waters.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Type</th>
<th>Toxin or Effect</th>
<th>Illness or Event</th>
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</thead>
<tbody>
<tr>
<td><em>Alexandrium monilatum</em></td>
<td>Dinoflagellate</td>
<td>Ichthyotoxins</td>
<td>Fish Kills</td>
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<tr>
<td><em>Dinophysis acuminata</em></td>
<td>Dinoflagellate</td>
<td>Okadaic Acid</td>
<td>Diarrhetic Shellfish Poisoning</td>
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<tr>
<td><em>Karenia brevis</em></td>
<td>Dinoflagellate</td>
<td>Brevetoxins</td>
<td>Neurotoxic Shellfish Poisoning</td>
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<tr>
<td><em>Karenia spp.</em></td>
<td>Dinoflagellate</td>
<td>Ichthyotoxins</td>
<td>Fish kills</td>
</tr>
<tr>
<td><em>Karldininium veneficum</em></td>
<td>Dinoflagellate</td>
<td>Karlotoxin</td>
<td>Fish kills</td>
</tr>
<tr>
<td><em>Prorocentrum minimum</em></td>
<td>Dinoflagellate</td>
<td>Venerupin (?); low dissolved oxygen</td>
<td>“Mahogany Tide” Fish kill</td>
</tr>
<tr>
<td><em>Pyrodinium bahamense</em></td>
<td>Dinoflagellate</td>
<td>Saxitoxins</td>
<td>Paralytic Shellfish Poisoning</td>
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<tr>
<td><em>Pseudo-nitzchia spp.</em></td>
<td>Diatom</td>
<td>Domoic Acid</td>
<td>Amnesiac Shellfish Poisoning</td>
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<tr>
<td><em>Lyngbya spp.</em></td>
<td>Cyanobacterium</td>
<td>Suite of toxins including dermatotoxins</td>
<td>Swimmer’s itch Gastrointestinal inflammation</td>
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<tr>
<td><em>Oscillatoria spp.</em></td>
<td>Cyanobacterium</td>
<td>Suite of toxins including anatoxins and hepatotoxins (microcystins)</td>
<td>Neurotoxic and liver effects</td>
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<tr>
<td><em>Chattonella subsalsa</em></td>
<td>Rhaphidophyte</td>
<td>Brevetoxins: low dissolved oxygen</td>
<td>Fish kills</td>
</tr>
</tbody>
</table>
Participating Agencies

- **Alabama Department of Public Health**
  - The Mobile Division Laboratory in the Bureau of Clinical Laboratories identifies and enumerates HAB organisms. The microscopic analysis is based on the Utermohl method. Cell counts of *Karenia brevis* are in accordance with the state biotoxin contingency plan required by the National Shellfish Sanitation Program (NSSP). Water samples are submitted for a census of dinoflagellates and raphidophytes, some diatoms, some flagellates and some cyanobacteria. The laboratory reports results to collectors and conducts an informal email notification to other agencies such as ADEM, DISL, and ADCNR. Collection materials and sampling instructions are available through the laboratory. The lab maintains an Access database of sampling results. Metadata are available.
  - Seafood Branch- This branch of Environmental Health has responsibility for the shellfish growing areas and issues public health advisories in the event of a *Karenia brevis* red tide. The ADPH issues harvest-area closures in a red tide event based on *Karenia* cell counts with reopening of areas based on shellfish meat toxin levels. Samples are collected 8-10 time per year from specific shellfish areas to reflect the potential HAB in harvest waters. Additional water and shellfish sampling are conducted during a HAB event. Seafood Branch maintains a database of sample results.
  - Baldwin County Health Department environmentalists collect samples from specific BEACH sites as part of surveillance efforts on Alabama’s swimming beaches. On occasion environmentalists respond to complaints by collecting samples of discolored water or floating mats that may be indicative of HAB.

- **Alabama Department of Environmental Management**
  - The ADEM responds to fish kill reports by assessing the site for parameters (temperature, salinity, and dissolved oxygen) that may indicate the source of the kill. Samples for microscopic analysis are collected and the Department of Conservation and Natural Resources (ADCNR) may be called for consultation. Reports are cataloged in Complaint/Incident Reports quarterly within the agency. Additionally, ADEM voluntarily collects surf water samples at EPA BEACH sites for microscopic phytoplankton exams. Microscopic identification and enumeration are done by ADPH. Samples are collected weekly during the active swimming season and monthly in the winter, giving an indication of phytoplankton from Florida Point at the Alabama state line to Dauphin Island Public Beach.

- **Alabama Department of Conservation and Natural Resources**
  - The ADCNR investigates fish kills and reports of discolored water and enforces shellfish water closure. Additionally, ADCNR personnel collect water samples in the Weeks Bay National Estuarine Research Reserve monthly for identification and enumeration of HABs by ADPH. The ADCNR currently funds monitoring efforts at DISL (see below).

- **Dauphin Island Sea Lab**
  - DISL personnel in the MicroAlgal Lab collect samples for HABs under revolving research grants of 2-3 year duration and during HAB event response. Microscopic identification and enumeration are done by ADPH. Collection sites are in the bay waters as well as the off-shore waters.
Extensive water quality data are collected with these samples. Databases of water-quality and HAB counts are maintained.
- DISL personnel in the MicroAlgal Lab provide logistical, analytical and advisory support for the AVMMN monitoring, including sample collection and identification, water-quality analyses and data management. A database of water-quality and HAB counts is maintained.
  - The US Food and Drug Administration- Division of Seafood Science and Technology, Chemical Hazards Branch
    - FDA provides toxin testing of shellfish in a *Karenia brevis* HAB event. Arrangements for analysis are made through the ADPH Seafood Branch to the Chemical Hazards Branch.

**Outreach: AVMMN**

The Microalgal Lab at DISL has initiated a bi-weekly volunteer monitoring effort with members of Little Lagoon Preservation Society, Wolf Bay Watershed Watch; Weeks Bay Reserve Foundation and others. The effort is part of NOAA’s Phytoplankton Monitoring Network. Taxonomic data are reported on the SEPM website. DISL provides logistical support for sampling and identification and analytical support for determination of additional water-quality parameters (T, S, pH, DO, Secchi depth, DIN, DIP, TN, TP, Chlorophyll *a*). A website for inquiry-driven reporting of these data as well as background and historical information on HABs in local waters is under development at DISL.
## Personnel and Contact Information

<table>
<thead>
<tr>
<th>Agency</th>
<th>Contact</th>
<th>Email</th>
<th>Telephone</th>
<th>Address</th>
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</tbody>
</table>
Sampling Scheme

- Routine Monitoring - Biotoxin Contingency Plan for NSSP
  - Event Response
  - Voluntary Collections associated with BEACH grab samples
    - Response to Event and Complaints
  - Research and Event Response
    - Response to Event and Complaints
      - Surveillance
      - Event Response
  - ADPH Seafood Branch
    - ADEM and Baldwin Co HD
      - Academia
    - Dept of Conservation Marine Resources
      - Volunteer
- Samples Received in the Lab
Lab Results Distribution

Fax, Phone, Email, Access Data Base and Hard Copy

Result

All results generated by the lab are faxed.

Event Response –
All Results by Fax, email and phone results in toxic event within 24 hours of receipt in the lab

Response to Event and Complaints
Reports are emailed, faxed and phoned if a toxic event

Results are faxed and email notification if a toxic event

Response to Event and Complaints
Results are faxed and email notification if a toxic event

Surveillance Results reported to ADPH Seafood Branch and others as necessary

Event Response
Public Health HAB Coordinator

Event Response or Alert
Email or Phone Call

Agencies

ADPH Seafood Branch - Public Health HAB Coordinator

ADEM and Baldwin Co HD

Academia

Dept of Conservation Marine Resources

Volunteer

Contacts in Adjacent States
Public Health Coordinator Role

- FDA Toxin Analysis Unit
  - Arrangements are made to conduct toxin studies

- State Health Officer
  - ADPH Environmental Health
  - Closure notices prepared when cell counts are >5000 K. brevis/l in shellfish growing areas
  - Public Health Advisories for Swimming Area

- Mobile Laboratory
  - Event Response Sample Analysis

- Dept of Conservation Marine Resources
  - Enforce Shellfish Growing Area Closure

- FDA Shellfish Specialist

- Local Press
  - Publish Public Health Advisory

- Local Health Depts.

HAB Event
Public Health HAB Coordinator
Communicates with the Appropriate Representatives of These Agencies
References

1. Model Ordinance 2003
Appendix

1. Model Ordinance  Marine Biotoxin Control 2003
2. Laboratory Collection Procedure
3. Laboratory Procedure for the Enumeration of *Karenia brevis*
II.02 Guidance for Developing Marine Biotoxin Contingency Plans

@.04 Marine Biotoxin Control.
A. Contingency Plan.

(1) The Authority shall develop and adopt a marine biotoxin contingency plan for all marine and estuarine shellfish growing areas.

(2) The plan shall define the administrative procedures and resources necessary to accomplish the following:

(a) Initiate an emergency shellfish sampling and assay program;
(b) Close growing areas and embargo shellfish;
(c) Prevent harvesting of contaminated species;
(d) Provide for product recall;
(e) Disseminate information on the occurrences of toxic algal blooms and/or toxicity in shellfish meats to adjacent states, shellfish industry, and local health agencies; and
(f) Coordinate control actions taken by Authorities and federal agencies.

(3) Except that the Authority shall classify as prohibited any growing areas where shellfish are so highly or frequently affected by marine biotoxins that the situation cannot be safety managed, the presence of marine biotoxins shall not affect the classification of the shellfish growing area under §.03. The Authority may use the conditionally approved classification for areas affected by marine biotoxins.

(4) The plan may include agreements or memoranda of understanding, between the Authority and individual shellfish harvesters, to allow harvesting in designated parts of a growing area while other parts of the growing area are placed in the closed status. Such controlled harvesting shall be conducted with strict assurances of safety, such as by batch release of shellfish lots only after samples of each lot are tested and found to be below the action levels specified in §C.

B. Marine Biotoxin Monitoring. In those areas where marine biotoxins are likely to occur in shellfish, representative samples of shellfish shall be collected during all harvest periods. Samples shall be collected from indicator stations at intervals determined by the Authority, and assayed for the presence of toxins in accordance with §C.

C. Closed Status of Growing Areas.

(1) A growing area, or portion(s) thereof as provided in §A.(4), shall be placed in the closed status for the taking of shellstock when the Authority determines that the level of biotoxin present in shellfish
In the event of a suspected HAB, samples can be collected for identification as detailed below.

1. The ADPH Mobile Lab supplies collection protocols, collection bottles and preservatives, and standard report forms.

2. Contact the ADPH lab to arrange for a microbiologist to receive and examine the samples. If collection supplies are not on hand, they can be obtained from the lab.

3. If there is a fish kill, collect a sample of the dead fish. Identify the fish types if the kill is multi species.

4. Document the sampling location by site name and GPS if possible.

5. Record the physical data of the area with regard to the extent of the bloom or fish kill, water flow, activities in the area, the water depth (shallow or deep), air temperature. Is there respiratory or eye irritation from aerosols, etc?

6. Record water quality data such as temperature, salinity, color, turbidity, etc.

7. Wear gloves to collect samples.

8. Collect samples for microalgal identification, live and preserved. Glass containers are best for live and preserved. Live samples are important for determining color and motility in most phytoplankton and sheath formation in cyanos. Preserved samples are for biomass quantification and multi species identification.
   a. Plastic drinking water bottles may be used for live samples if lab sample bottles are not available.
   b. Lugol’s iodine is the preservative of choice for phytoplankton samples at the ADPH Mobile lab. The preserved sample with adequate Lugol’s iodine in it is the color of strong tea.

9. Store samples appropriately and transport to the lab as soon as possible
   a. Live samples should be cooled but not iced. Maintaining a temperature similar to the bloom condition is preferred. Wrap bottles in wet newspaper and transport in a dark box at ambient temperature.
   b. Preserved samples are protected from light and shipped at ambient temperature.

10. Provide contact information so that results may be called, faxed, or emailed. Note chain of command contacts as necessary.

11. If toxicity studies are necessary, obtain collection bottles and follow protocol provided by the lab.
When possible please make prior arrangements with the lab before bringing in samples to be examined for dinoflagellates. Samples should be transported to the lab as soon as possible.

**Sampling Supplies**

The laboratory supplies the following items for sample collection:

- Glass sample bottles for phytoplankton monitoring or plastic bottles for toxicity studies
- Small test tube of Lugol’s iodine (approx. 7 mls.)
- Sample identification tags
- Laboratory report form

You may also need the following items:

- A box and or cooler for transporting samples to the lab.
- Preserved samples may be transported at ambient temperatures or cooled.
- Live (unpreserved) samples should be cooled (not iced) over ice packs in an insulated container.
- Thermometer for hydrographic data
- Salinometer or refractometer
- Bottom sampler (Lemott) for multiple depth sampling

**Sample Collection**

Phytoplankton samples may be collected from the surface during mid-day hours or from multiple depths if necessary or as appropriate for a particular organism. Samples may be collected in the sample bottle (surface) or a special sampler at prescribed depths for transfer to the glass sample bottle. Identify the samples with location (site name, GPS coordinates, etc.) and other pertinent data on the tag and the report form.

**Preserved samples for identification and enumeration**

Please fill bottles to the top allowing room for the addition of Lugol’s iodine. Samples are preserved with Lugol’s iodine immediately by pouring the contents of the small test tube directly into the sample bottle. Use care in handling to avoid contact with the iodine. Replace the cap and invert the bottle gently several times to mix the iodine into the sample.

**Live samples for identification only**

Live samples are important for cyano and flagellate identification. Fill live sample bottles about half to two-thirds full. Cool immediately. Insulate the samples from direct exposure to ice or cold packs using cardboard or newspaper. Try to maintain temperatures similar to bloom conditions. Transport to the lab within 24 hours of collection.

**Samples for Toxicity Studies**

Collect 500 to 1000 mls of water in a plastic bottle (brown is preferred). Allow room for expansion because the samples will be frozen for preservation. Label appropriately.

**Salinity**

Salinity is helpful in phytoplankton studies. If not measured on-site, you may collect a separate salinity sample (about 25 mls) so that the lab can perform the measurement. Please attach to the dino sample or label with the location.

Rev. 6/02
1. Background Information

1.1. *Karenia brevis* is a dinoflagellate responsible for Neurotoxic Shellfish Poisoning (NSP) respiratory irritation, and animal mortality. Red tide is a term used to describe the harmful bloom or increased concentration of microalgae. Cell concentrations may reach in the millions of cells per liter in these harmful algal blooms.

1.2. “The NSSP Model Ordinance mandates that growing areas be placed in the closed status when cell counts for members of the genus *Karenia* in the water column exceed 5,000 cells per liter of water.” (Guidance for the Developing Marine Biotoxin Contingency Plans)

1.3. In Chapter IV @.04 Marine Biotoxin Control C. Closed status of Growing area (1) (b) (ii) the cell counts for *Karenia brevis* organisms in the water column exceed 5,000 per liter.

2. Equipment

2.1. Light microscope, preferably phase contrast illumination, with objectives sufficient to identify distinguishing characteristics of the *Karenia* species.

2.1.1. 10X, 20X or 40X

2.1.2. 10X wide field oculars

3. Materials

3.1. Sample collection jars or bottles with sample identification tags and test request forms

3.2. Container, such as an ice chest, to store and ship samples to lab in the dark.

3.3. Acidified Lugol’s iodine sufficient to preserve cells in a water sample

3.3.1. Samples should be preserved immediately upon collection.

3.3.2. The staining of the water should resemble strong tea. Ex: 7 mls of Lugol’s in 1 liter of seawater.

3.4. Borosilicate tubes with nonreactive liner caps for storing Lugol’s iodine.

3.5. Chamber slide to contain 3 or 11 mls of water. Ex: Lab-Tek II by Nalge Nunc

3.6. Cover slips, 24X64 mm

3.7. Pipettes for filling the chamber slides

3.8. Timer for timing the sample settling period

3.9. Counting tally

3.10. Ocular micrometer (recommended)

3.11. Ocular reticule grid

3.12. Dinoflagellate reference materials such as manuals, on-line information, etc.

4. Formulations

Care should be taken to avoid contact with the ingredients of this Lugol’s iodine. Wear gloves and appropriate eye protection when handling. Follow standard lab safety practices.

4.1. Acidified Lugol’s Iodine

4.1.1. 100 gms Potassium Iodide (KI)

4.1.2. 1 liter deionized water

4.1.3. 50 gms iodine (crystalline)

4.1.4. 100 ml glacial acetic acid

4.1.5. Dissolve KI in deionized water then dissolve iodine crystals. Add glacial acetic acid. As the solution nears saturation, decant so that any possible precipitate is
removed. Store in amber glass at room temperature. Expires two years from preparation.

4.1.6. Dispense sample preservation volumes to nonreactive tubes (glass) and use caps with nonreactive liners. Ex: 7 mls for 1 liter of seawater.

5. Sample Collection
5.1. Samples should be taken from collection sites representative of the shellfish growing area.
5.2. Surface grab samples are satisfactory. Fill bottles to the top with seawater.
5.3. Preserve samples with Lugol’s immediately after collection.
5.4. Store out of the sunlight for transportation back to the lab.
5.5. Record hydrographic data of the area such as temperature and salinity on an accompanying request form.

6. Chamber set-up
6.1. The identification of a sample is checked for agreement with the request form when samples are delivered to the lab.
6.2. The preserved sample is gently mixed by inversion about 10 times to distribute cells evenly throughout the bottle.
6.3. Prepare a chamber slide by removing the plastic cover and placing a glass cover slip diagonally across the chamber. When the chamber is filled with sample, the cover slip will slide into place on the top of the chamber due to surface tension.
6.4. Use a pipette of the appropriate dispensing volume fill the chamber.
6.5. Set a timer for 15-20 minutes to allow the contents of the sample to settle to the bottom.

7. Calculations
7.1. Chamber factors
7.1.1. Counting chambers should be checked for the number of reticle grids across and down the chamber for each microscope and analyst. This will be used in cases of high counts when representative fields are counted rather than a whole chamber Ex:
7.1.2. Calibration of the 11 ml Lab-Tek chamber cell by analyst A gave an area of 800 grids (10 down and 80 across) using the 10X objective and 10X ocular
7.1.3. 10 grids with an average of 60 cells each are counted
7.1.4. Multiply 60 cells X 800 to calculate the chamber count = 48,000 Karenia brevis cells per chamber.

7.2. Concentration Factor
7.2.1. The Concentration Factor will correct for the volume of sample counted as a portion of a 1000 ml sample of seawater.
7.2.2. \[
\frac{1000 \text{ mls}}{\text{mls of seawater counted}} = \text{Concentration Factor}
\]

8. Counting cells
8.1. View chambers with the 10 X objective and phase contrast illumination.
8.2. Karenia brevis cells are typically 18-45 µm and stained golden by the Lugol’s. Fat droplets in cytoplasm produce a characteristic refractive or “glowing”, and a lacy quality to the cell.
8.2.1. Other Karenia species have distinctive properties. Use reference materials to aid in the identification of all Karenia species.
8.3. Blooms may have multiple Karenia species. Enumerate each separately.

8.4. For samples with high cell counts use a reticle grid to count a representative number of fields.

8.4.1. Average the number of cells seen per grid

8.4.2. Apply the Chamber factor to calculate the Raw Cell Count

8.5. Record cell counts

8.6. Multiply by the concentration factor to calculate a raw cell count per liter

8.7. Round counts to two significant figures to avoid overstating the precision of the count. This is the reportable Cell Density per Liter.

8.8. If a sample yields between 3,000 and 7,000 cells per liter, count 3 representative chambers and average for the collection site Cell Density per Liter.

9. Reporting Results

9.1. Report cell densities per liter to the Shellfish Authority

10. References

10.1. Model Ordinance 2003

