Standing Survey Plan:
Rapid Response *Alexandrium* Survey

Massachusetts Water Resources Authority

Environmental Quality Department
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Standing Survey Plan: Rapid Response *Alexandrium* Survey
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1. General

Project title	Massachusetts Water Resources Authority (MWRA) Harbor and Outfall Monitoring Program (HOM4)
Survey title	MWRA Rapid Response *Alexandrium* Survey
Survey vessel	TBD[1]
Vessel requested by	TBD
Organization	TBD
Project Manager	Michael Mickelson
Organization	MWRA
Survey Chief Scientist	TBD
Organization	TBD
Address	TBD
Telephone	TBD
Fax	TBD
Cellular Phone (Field)	TBD
MWRA Contact Persons:	Michael Mickelson (617) 788-4746 or David Taylor (617) 788-4748
WHOI Contact Persons	Don Anderson (508) 289-2351 or Bruce Keafer (508) 289-2509
MA DMF Contact Persons	Michael Hickey (508) 563-1779 ext. 122 or David Whittaker (508) 563-1779 ext. 126
ME DMR Contacts Persons	Laurie Bean (207) 633-9555 Darcie Couture (207) 633-9570
NH DES Contact Person	Chris Nash (603) 559-1509

2. Schedule of operations

Mobilization Date	On Call/Standby Status
Location	Varies:
Transit	Basic surveys: small boat out of Gloucester. Full surveys: TBD.
Departure/Sampling Date	Within 48 hours of notification by MWRA.
Planned Survey Duration (Days)	1 (multiple surveys possible)
Maximum Duration (Days)	1 (per individual survey)
Demobilization Date	Immediately following completion of the survey.
Location	TBD
Comments	

3. Background Information

This document contains background information and details on surveys that are designed to respond to a large or unusual red tide bloom of *Alexandrium* in Massachusetts Bay. Although such blooms have historically been rare, an event may develop rapidly, therefore advance

[1] Entries coded "TBD" will be updated on an annual basis with MWRA's contractor for water quality monitoring

planning is warranted. This document describes the decision-making, communications, and logistics that may be appropriate in the event of an *Alexandrium* bloom.

The response supplements regular MWRA outfall monitoring, which is not designed to describe such a bloom or provide all the desired information relevant to possible outfall effects. The response may require targeted sampling using specific rapid methods for counting *Alexandrium*, multiple surveys, and additional stations.

4. Objectives

The goal is to characterize and understand a major *Alexandrium* bloom in Massachusetts Bay and determine whether the MWRA outfall influences the bloom. Data are collected during the surveys to obtain insight on the bloom dynamics and to evaluate the potential influence (impact) of outfall discharge on the bloom (e.g., localized and downstream, change in magnitude of bloom, etc.).

This plan outlines a stepwise series of activities that will be taken in the event of an *Alexandrium* bloom, ranging from an early warning step of any incidence of paralytic shellfish poisoning (PSP) in Maine to conducting a comprehensive monitoring effort in Massachusetts Bay ("full surveys"). The rationale behind the approach proposed, a presentation of the stepwise methodology for tracking and ultimately characterizing an *Alexandrium* bloom, and a summary of the field and laboratory procedures that would be used in the event that a rapid assessment is necessary are described below.

5. Survey justification and rationale

Alexandrium cell data are collected by State programs in Maine, New Hampshire and Massachusetts. The presence of PSP toxicity that can result from *Alexandrium* blooms is monitored in each of these states as part of their shellfish monitoring programs under the auspices of ME Department of Marine Resources (DMR), NH Department of Environmental Services (DES) and MA Division of Marine Fisheries (DMF). The MWRA HOM water quality surveys have detected *Alexandrium* in the bay on occasion, but the surveys have not been designed to specifically focus on this species or to fully define effects on this species by the discharged effluent. Previous major *Alexandrium* research efforts have studied the western Gulf of Maine including stations within Massachusetts Bay (Franks and Anderson 1992, Anderson *et al*. 2002, 2005a and others). Although these efforts are intermittent, they serve as the main source of data and understanding of *Alexandrium* bloom dynamics in the Gulf of Maine and Massachusetts Bay and form the basis for the development of this rapid-response monitoring plan.

The current understanding is that the *Alexandrium* blooms that affect Massachusetts Bay tend to originate off the mid-coast of Maine in April to May and are usually transported southward with the western segment of the Maine coastal current (Figure 1; Franks and Anderson 1992). The bloom may enter Massachusetts Bay under appropriate conditions - sustained winds from the northeast at the time the offshore bloom is passing the northern boundary of Massachusetts Bay

(i.e. downwelling favorable; Anderson *et al*. 2005a). There is no evidence that *Alexandrium* blooms in Massachusetts Bay are initiated internally.

The north to south transport of the bloom within Massachusetts Bay is also evident in data from the MA DMF PSP monitoring program. This program includes 14 primary sites along the Massachusetts shoreline (Figure 2). An examination of MA DMF data from 1980-1999 indicates that there are some clear patterns that are relevant to this project (D.M. Anderson *et al*., unpublished). Anderson focused on data from stations in Gloucester, along the south shore of Massachusetts, and in Cape Cod Bay. During each of the years that PSP toxicity was observed, it was first seen at the Gloucester station and then subsequently at stations to the south. The toxicity always progressed from the north to the south (see Figure 3 for 1993 data). Additionally, the peak level of toxicity was always higher to the north. Moreover, the data indicate the higher the toxicity in Gloucester, the higher the likelihood that toxicity will be observed to the south. The historic PSP toxicity data not only support the plume advection hypothesis, but also allow the transport time through the system to be estimated. Based on the 1980-1999 data, there is a delay of about 2 weeks between when toxicity is first observed at the MA DMF Gloucester station and when it is measured at the south shore sites and another week or two before it is seen in Plymouth or Sandwich (Figure 4).

The combination of N-S transport of the bloom in the Gulf of Maine, influence of favorable currents/winds for the bloom to enter Massachusetts Bay, and the historic trends in PSP toxicity form the basis of events that trigger the monitoring approach described in this document. The plume advection hypothesis and MA DMF PSP toxicity data will also serve as a backdrop for understanding if there is potentially a gross impact due to the MWRA bay outfall. If an *Alexandrium* bloom is more intense (abundance and higher toxicity) along the south shore than to the north or if toxicity is observed earlier at stations along the south shore than at Gloucester, this pattern would be different than that historically observed and consequently suggest an outfall effect.

MWRA has developed the response approach described in this survey plan to ensure data are obtained to appropriately describe the *Alexandrium* bloom and understand whether and how the outfall in Massachusetts Bay affects the bloom.

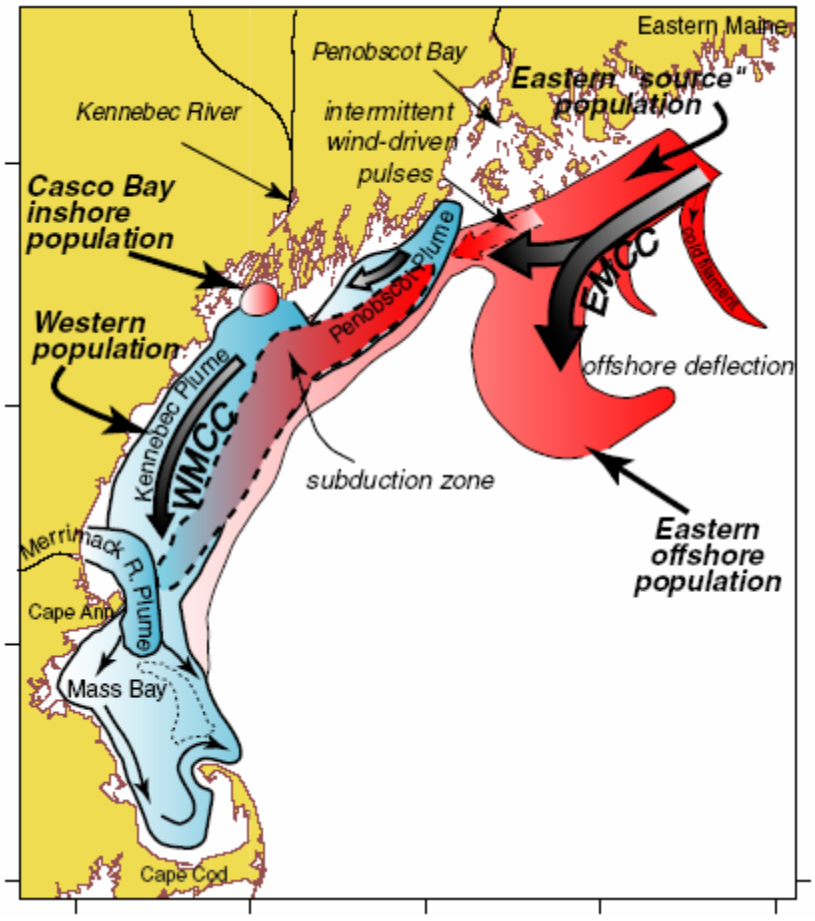

Fig 1. Conceptual model of *Alexandrium fundyense* dynamics in the Gulf of Maine. Eastern and western populations and associated transport via Eastern and Western Maine Coastal Currents. (This is figure 11 from Anderson et al. 2002)

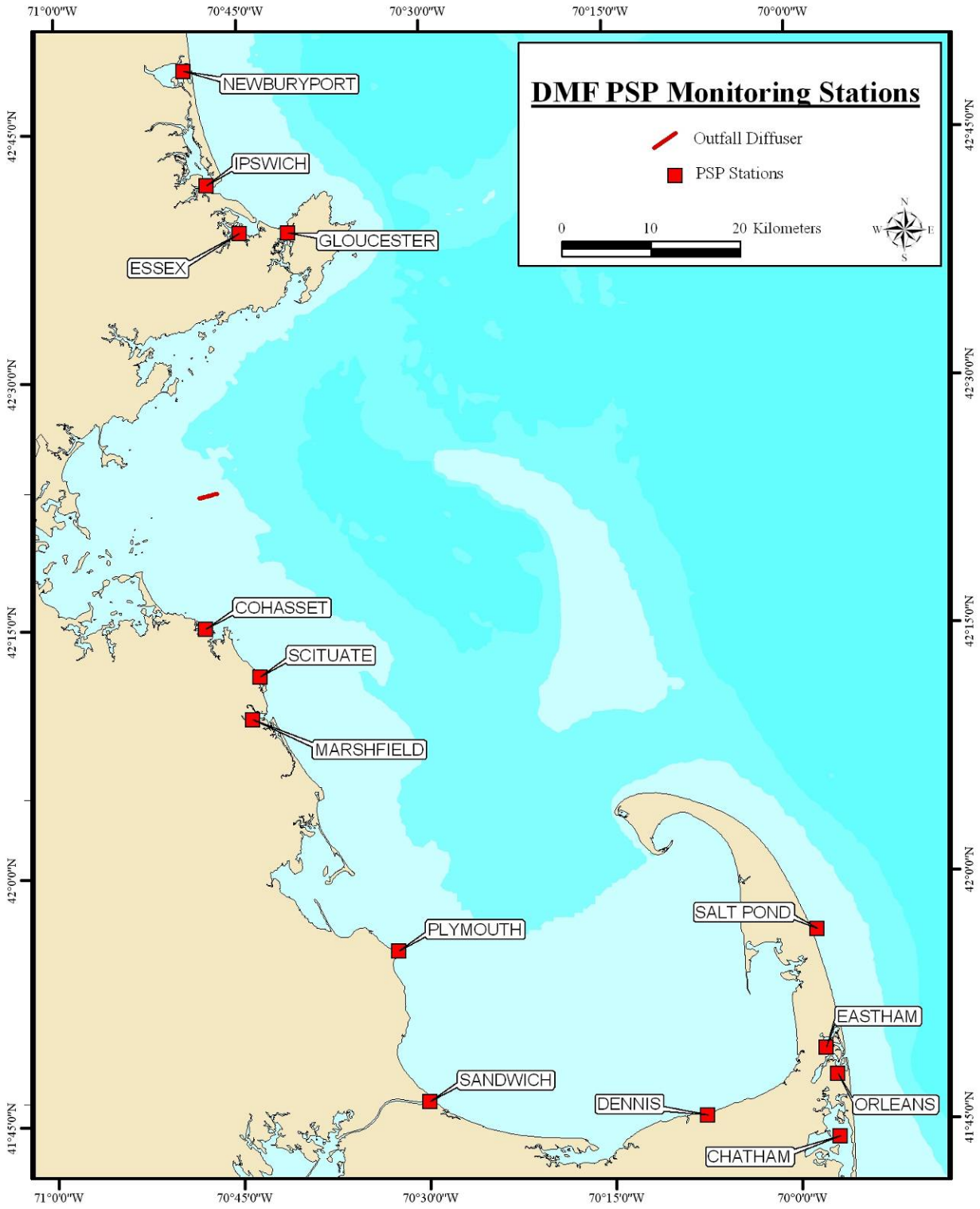

Fig 2. Map of primary Massachusetts DMF PSP toxicity monitoring stations

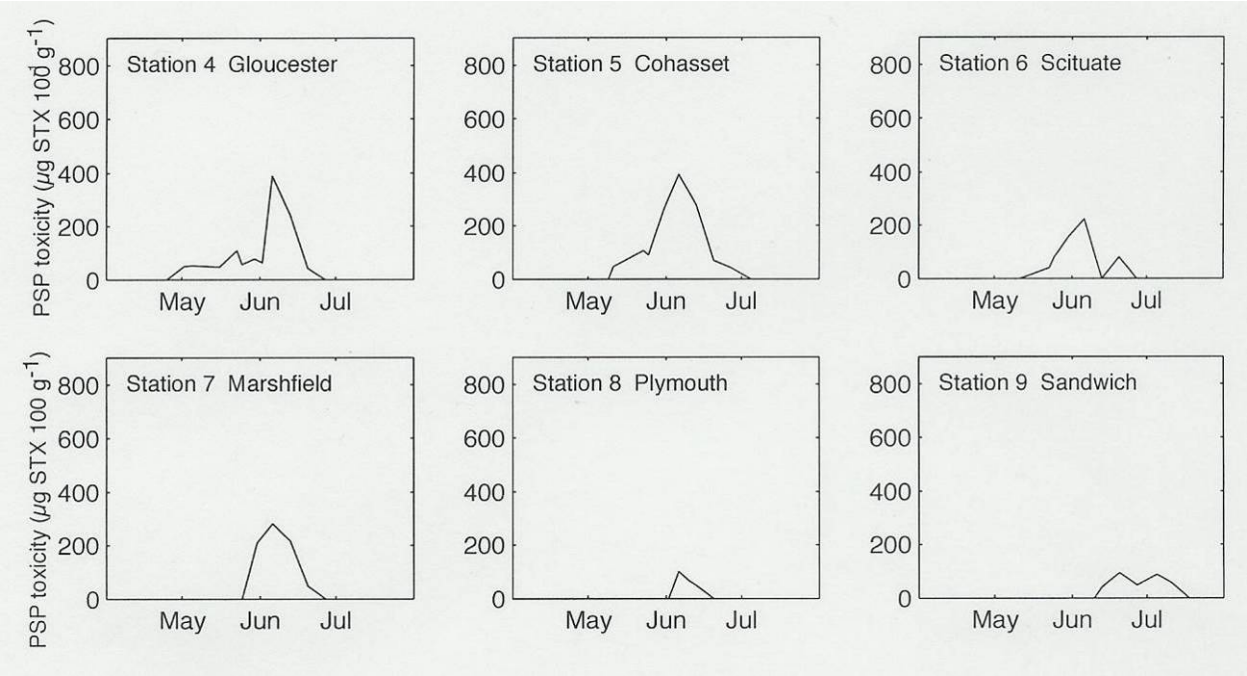

Fig 3. Time series of PSP toxicity in 1993 from Gloucester, south shore and Cape Cod Bay MA DMF stations. (D.M. Anderson et al., unpublished data)

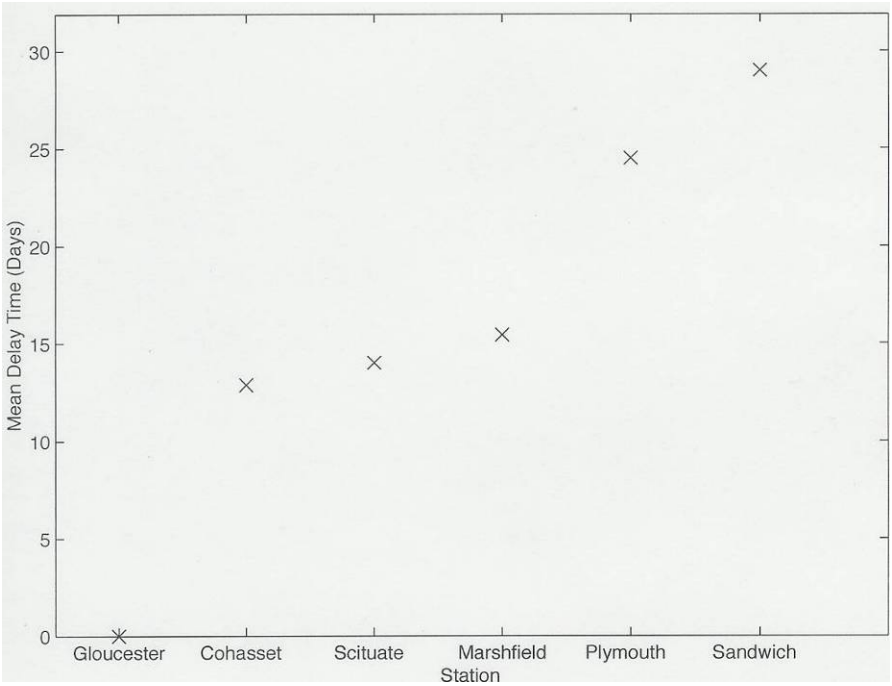

Fig 4. Average delay between PSP toxicity in Gloucester and stations along the south shore and in Cape Cod Bay based on 1980-1999 MA DMF data with 1996 omitted.

(D.M. Anderson et al., unpublished data)

6. Rapid Response Stepwise Methodology

A stepwise approach is proposed for the response monitoring to balance the need to keep the monitoring operationally predictable yet obtain defensible and useful data in the event of an *Alexandrium* bloom. The steps are triggered though a series of observations and findings. MWRA determines whether to activate each step.

Since MWRA began the outfall monitoring program in 1992, only two *Alexandrium* blooms (1993 and 2005) have caused substantial PSP toxicity in Massachusetts Bay. Prior to 1993, blooms were nearly annual events. The relative infrequency of recent events in Massachusetts Bay compared to those along the Maine coast suggests that most of the blooms are either weakening before entering the bay or not entering the bay. Thus this reponse plan includes a tiered response with upstream early warning triggers, a subsequent rapid monitoring effort ("basic survey"), and a more comprehensive "full" survey. The full survey is triggered by high cell counts observed in the basic survey. It is also triggered by high PSP toxicity at MA DMF stations within the bay. The individual steps and triggers for activation are highlighted in Figure 5 and discussed below.

Step 1: Early Warning

MWRA is alert to biotoxin data from ME DMR (south of Cape Elizabeth) and NH DES through a formal line of communication with relevant personnel. If any level of toxicity is detected, Step 2 will be triggered.

Website: http://www.state.me.us/dmr/rm/public_health/closures/pspclosures.htm
Red Tide Hotline: 207-633-9571
Maine DMR Marine Biotoxin Monitoring Program contact: Laurie Bean at 207-633-9555

Website: http://www.des.state.nh.us/wmb/shellfish/
Red Tide Hotline: 800-43-CLAMS (800-432-5267)
New Hampshire DES Shellfish Program contact: Chris Nash at 603-559-1509

Step 2: Initial Trigger

This step examines data from the Gloucester MA DMF site at the Annisquam Yacht Club and the three sites to the north (Figure 2). MWRA will look for MA DMF email alerts for toxicity measured by the MA DMF biotoxin monitoring program at these stations. The Gloucester (Annisquam) site is the most important to monitor based on historic data. If saxitoxin is detected (MDL of ~40 μg STX/100 g shellfish meat) by MA DMF, Step 3 will be triggered.

Website: http://www.mass.gov/dfwele/dmf/programsandprojects/psp_notice.htm#shelsani
Massachusetts DMF PSP Monitoring contact: Michael Hickey at 508-563-1779 ext. 122

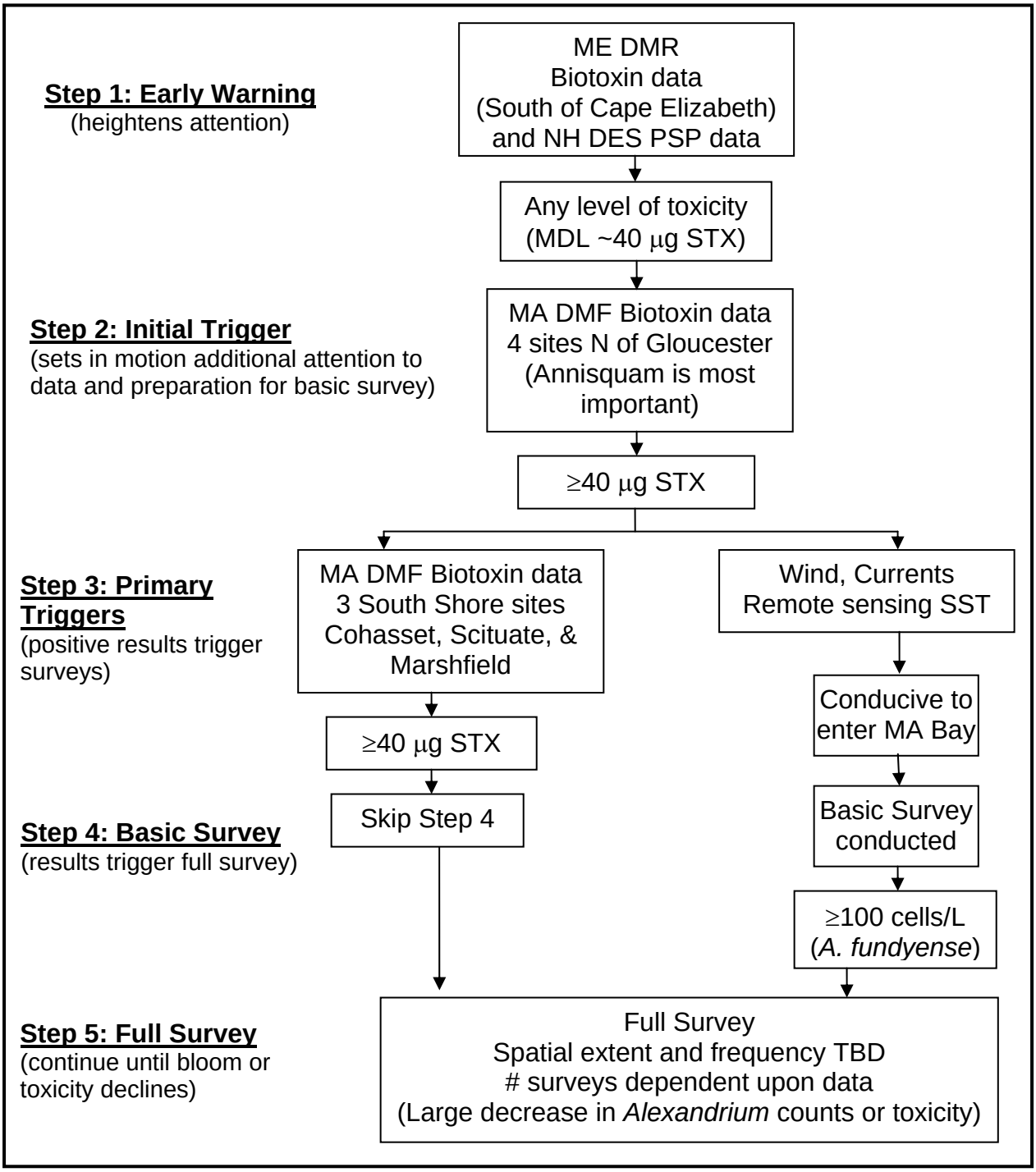

Fig 5. Flow chart of steps, trigger levels, and monitoring actions. Toxin units are μg STX/100 g shellfish meat.

In Step 2, MWRA also alerts its field teams that a bloom is occurring to the north and that preliminary preparations for survey activities should be initiated (not mobilization but initial coordination of boats and staff).

Note that toxicity data from MA DMF's south shore sites (Cohasset, Scituate, and Marshfield in particular) are monitored concurrently, but as toxicity at these sites would trigger a full survey, they are included as a primary trigger under Step 3 below.

Step 3: Primary Triggers

Under Step 3, MWRA will track emails from MA DMF regarding shellfish toxicity and will also access and evaluate meteorological and oceanographic data. These data sources may trigger survey activities and thus are the primary triggers for conducting field activities. Two types of results may trigger field surveys: (1) movement of *Alexandrium* bearing Gulf of Maine water into Massachusetts Bay or (2) high levels of STX shellfish meat from within Massachusetts Bay.

For Step 3 Trigger 1, MWRA will examine data on wind, ocean currents, and remote sensing sea surface temperature (SST) to determine the likelihood of the western Maine coastal current entering Massachusetts Bay. Sustained winds out of the northeast are conducive to flow into the bay and should become manifested in the surface water current data. Real time wind speed and direction and surface water currents are available from GoMOOS buoy A (www.gomoos.org) located to the south of Cape Ann in Massachusetts Bay. Remote sensing images for SST also provide information on water movement into the bay from the Gulf of Maine and will be inspected under this step. SST images are posted in near real time by the Satellite Oceanography Data Lab at University of Maine (http://wavy.umeoce.maine.edu/). MWRA will initiate a basic-survey under Step 4 if meteorological or oceanographic conditions are conducive for or indicate that Gulf of Maine water is entering Massachusetts Bay near Cape Ann[2].

For Step 3 Trigger 2, MWRA will track emails from MA DMF biotoxin monitoring program and focus on data from within Massachusetts Bay – specifically the south shore sites in Cohasset, Scituate, and Marshfield. If more than 40 µg STX/100 g shellfish meat is reported at any of these sites, MWRA will skip Step 4 and initiate mobilization for conducting a full survey under Step 5. The data from the south shore MA DMF stations will be monitored without Step 2 having triggered Step 3. Based on historic data it is very unlikely that toxicity will be seen along the south shore without first having been measured to the north, but in the unusual event that it is, Step 3 will trigger the full survey under Step 5.

Step 4: Basic Survey

A basic survey consists of two (2) transects run perpendicular to the coast to the south of Gloucester, MA. This survey's goal is to quickly obtain data to confirm the presence and quantify the abundance of *Alexandrium*. Basic survey details are provided in Section 7. The basic survey protocol includes collection of CTD profiles and water samples, for identification

[2] Professional judgment will be used in implanting this Plan. For example, in 2005, extremely high PSP levels measured at the Isle of Shoals(documenting a large population of cells North of Cape Ann) and the intense Northeast storm of May 7-9 led MWRA to bypass step 4 and initiate full surveys without waiting for the results of a basic survey or for toxicity to be observed on the South Shore.

and enumeration of the *Alexandrium* cells. The *Alexandrium* counts must be reported to MWRA within 72 hours of the basic survey. If the abundance is > 100 cells/L (*A. fundyense*) for any individual sample at any of the stations, MWRA will initiate the full-survey-scenario of Step 5.

Step 5: Full (comprehensive) Survey

A full survey will be conducted along an array of transects to the north, within and to the south of the nearfield area of the outfall. *In situ* hydrographic measurements and water samples for dissolved inorganic nutrients, chlorophyll, dissolved oxygen, plus *Alexandrium* and other phytoplankton taxa analysis will be obtained. *A. fundyense* counts will be relayed to MWRA within 72 hours of the survey. The full surveys will be conducted on a weekly basis until such time as *Alexandrium* abundance decreases below 100 cells/L or toxicity data from MA DMF is no longer elevated.

7. Survey locations and descriptions

Basic Survey:

Survey Name(s)	Rapid Response *Alexandrium* Basic Survey
Survey Area Locations	Massachusetts Bay, Table 1 and Figure 6
Survey Station Types	Vertical profiles with discrete water sample collection at the surface (1-2 m) and at ~10 m.
Number of Stations	6 stations

A complete hydrographic profile, including temperature, salinity, density, depth, time and position will be recorded at each station. Water samples will be collected at two depths [surface and mid-depth of the surface mixed layer (above the pycnocline); approximately 1-2 and 10 m] for *Alexandrium* identification and enumeration. Samples will be transferred directly to WHOI (Don Anderson/Bruce Keafer) within 24 hours of collection.

Full Survey:

Survey Name(s)	Rapid Response *Alexandrium* Full Survey (surveys will be numbered sequentially following the basic or first full survey)
Survey Area Locations	Massachusetts Bay, Table 1 and Figure 6
Survey Station Types	Vertical profiles with discrete water sample collection at three depths from the surface, mid-surface, and below the pycnocline. Phytoplankton samples to be collected at the surface (1-2m) and mid-surface (~10 m) depths.
Number of Stations	21 stations

A complete hydrographic profile, including temperature, salinity, density, depth, dissolved oxygen, fluorescence, time and position will be recorded at each station. In addition, water

samples will be collected at the surface, mid-surface, and below the pycnocline at each station and processed for analysis of dissolved inorganic nutrients according to procedures outlined in Libby *et al*. 2006. Chlorophyll and dissolved oxygen samples will be collected at the three depths at five of the stations for calibration of the *in situ* sensors – stations AF1, AF3, F13, N18, and F22. Triplicate DO samples will be collected from the surface and bottom waters at the first and last stations of the day. Duplicate samples for DIN and chlorophyll will be collected from the mid-depth sample at stations AF1, F13, and F22. The 20-µm screened water and whole water phytoplankton samples will be collected at two depths (surface and mid-surface) at all 21 stations. The screened samples will be analyzed using the fluorescent probe technique for *Alexandrium* identification and enumeration. The whole water phytoplankton samples will be stored and analyzed as needed to provide additional information on the phytoplankton community assemblage and abundances. The nutrient and chlorophyll samples will be transferred to MWRA upon completion of the survey. The DO samples will be analyzed on board the vessel. The screened phytoplankton samples will be transferred directly to WHOI (Don Anderson/Bruce Keafer) within 24 hours of collection.

Table 1. Planned Station Locations for survey AF06X.

Station	Latitude	Longitude
AF1	42.02788	-70.55467
AF2	42.09879	-70.52724
AF3A	42.22398	-70.43118
AF4	42.21997	-70.69553
AF5	42.30970	-70.65347
AF6*	42.40335	-70.50163
AF7*	42.47384	-70.45407
AF8*	42.54019	-70.54553
AF9*	42.58754	-70.60224
AF10*	42.54425	-70.70468
N04	42.44383	-70.73650
N10	42.33150	-70.83400
N16	42.39400	-70.75333
N18	42.36583	-70.77767
F05	42.13867	-70.65000
F06	42.17067	-70.57667
F07	42.19683	-70.51583
F13	42.26833	-70.73500
F17	42.34583	-70.57050
F22*	42.47983	-70.61767
F31	42.30633	-70.94000

*Stations sampled on both Basic and Full Surveys

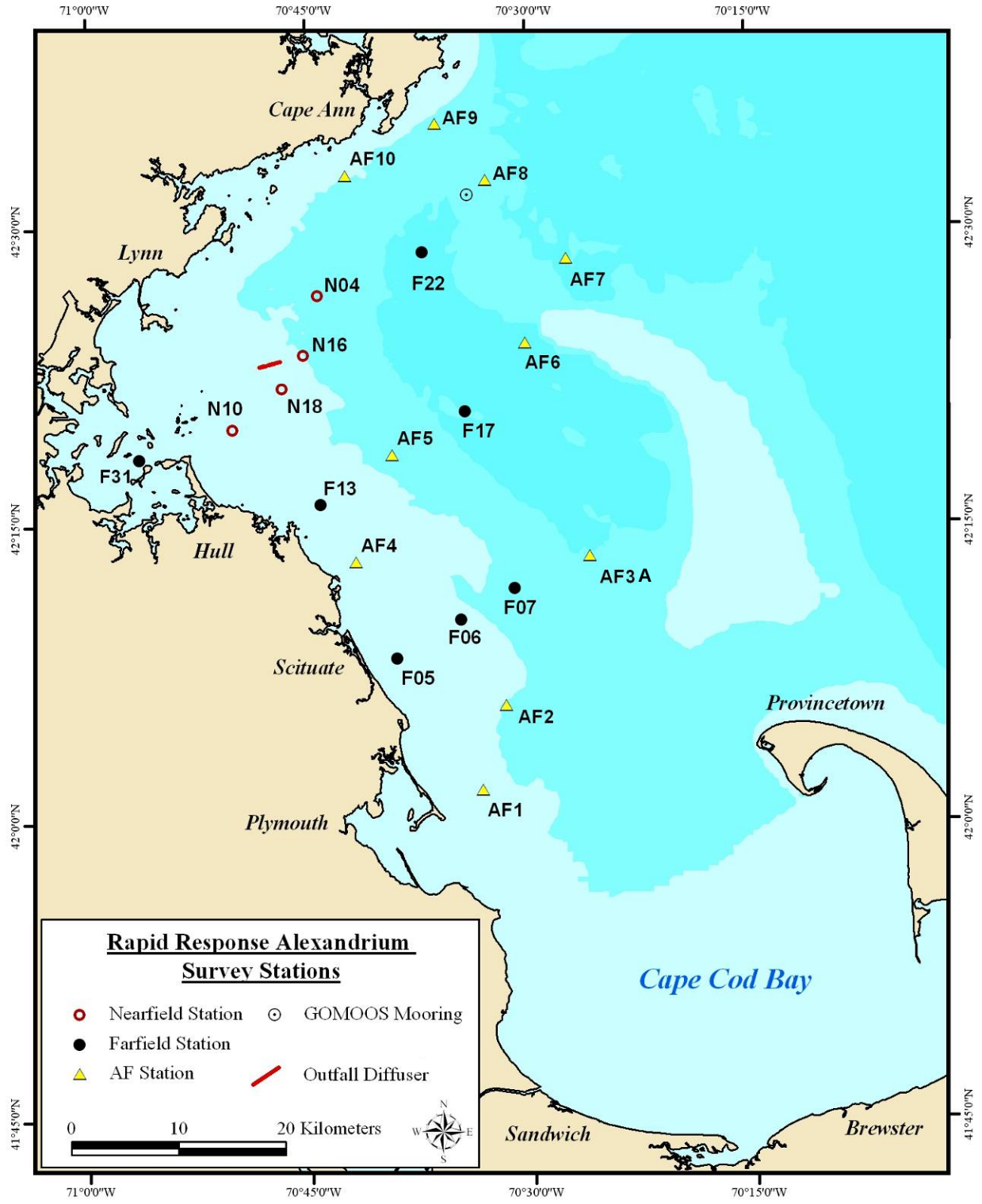

Fig 6. Planned station locations for basic and full surveys.

8. Survey/Sampling Methodologies

Method Descriptions:

The water quality monitoring QAPP of the current Harbor and Outfall Monitoring water quality monitoring consultant (*e.g.* Libby *et al.*, 2006) contains additional details on survey/sampling methods.

In Situ Measurements:

Continuous profiles for hydrographic and water quality measurements will be taken from near–surface to within 5 m of bottom at each station. *In situ* hydrography measurements for conductivity, temperature, sensor depth, fluorescence and dissolved oxygen will be taken at each station. Salinity and water density will be calculated from conductivity, temperature, and depth data. Water depth, navigational position, and time also will be recorded by the NavSam© system.

The basic survey may warrant a smaller vessel than the R/V used for the nearfield water column surveys. To support sample collections from such vessels, a hand-deployed CTD compatible with the contractor's navigation and sampling software and water collection system will be used.

Discrete Samples:

Discrete water samples will be collected from surface and mid-surface (basic) or from three depths from surface, mid-surface and below the pycnocline (full) depending on the survey. These water samples will be collected with Niskin bottles deployed by hand (basic) or with a Rosette system (basic/full). Niskin bottles will not be closed until the *in situ* sensors have reached equilibrium to ensure sample data represent the collected water.

Once the Rosette system (or hand-deployed Niskin bottle) is onboard, subsamples will be obtained for each of the parameters being measured. The processing steps for the nutrient, dissolved oxygen, chlorophyll and whole water phytoplankton that will be collected during the full survey can be found in the current Water Quality Monitoring QAPP (*e.g.* Libby *et al*. 2006). The *Alexandrium* samples will be collected as 4-liter 20-µm screened samples from the surface and mid-surface (~10 m) waters (Table 1). These samples will be rinsed into 15 ml centrifuge tubes with filtered seawater (a funnel may be used), then the appropriate volume of formalin added. For example, if there are 10 ml of sample add 0.5 ml concentrated formalin (37% formaldehyde). *Alexandrium* samples are stored upright on ice and in the dark. To ensure that the fluorescent probe method works, the formalin fixed samples must be centrifuged and transferred into methanol within 24 hours.

Table 2. Sample Handling and Preservation Requirements

Parameter	Container	Sample Size	No. of Containers (basic/full)	Sample Preservation	Holding Time
Alexandrium	15-ml centrifuge tube	4 L filtered thru 20-µm mesh final volume after rinse 10-15 mL	12/42	5% formalin <4° C dark	Transfer to Methanol within 24 hours
Dissolved inorganic nutrients	125-mL polyethelyne bottle	40 mL	0/66	Freeze until analysis	28 days to analysis
Chlorophyll *a* and phaeopigments	Whatman GF/F in foil	25 – 400 mL filtered through GF\F	0/18	Freeze filter until analysis	4 weeks to analysis
Dissolved oxygen	300 mL glass BOD bottle	300 mL	0/23	Fix as described in Libby 2006 and titrate within 24 hours	24 hours to titration
Phytoplankton (whole water)	1000 mL HDPE bottle	850 mL	0/42	Preserve with Utermöhl's solution.	6 months to analysis

All sample bottles will be labeled with sample labels and stored appropriately.

QA/QC –Data quality requirements for navigational and hydrographic data collection, hydrographic profiling, and water sampling are detailed in the current Water Column Monitoring QAPP (*e.g.* Libby *et al.*, 2006).

9. Laboratory Analysis

The Water Quality Monitoring QAPP (*e.g.* Libby *et al.*, 2006) contains details on the nutrient, chlorophyll, dissolved oxygen, and whole water phytoplankton analytical methods.

The *Alexandrium* samples will be identified, counted, and quantified using a fluorescent probe technique. The samples will be delivered to WHOI where the sample will be centrifuged and the formalin removed by aspiration leaving the pellet intact. The pellet will then be resuspended with 100% cold methanol for analysis and storage. For optimal results, this process should occur within 24 hours after fixation in formalin. The sample cannot tolerate long time periods in formalin because the rRNA signal in the cell is lost due to excessive cross-linking of the nucleic acids by the formalin. Although 24 hours is the optimal time frame, it is expected that the fluorescent probes will provide acceptable results on samples stored up to one week in formalin (Anderson pers. comm.).

Fluorescent probes will be used to confirm and enumerate the *Alexandrium fundyense* that are present. This requires the use of a molecular probe that has been developed for this species. With the appropriate filters on an epifluorescent microscope, a sample can be labeled with the probe and the *A. fundyense* distinguished (Anderson *et al*. 2005b; Keafer *et al*. 2005; Gribble *et al*. 2005). The NA-1 probe conjugated to Texas Red will be used to identify and enumerate *A. fundyense* (North American ribotype). The samples will be examined for the presence of *A. fundyense* cells using a Zeiss epifluorescence microscope at 100X magnification. The microscope will be fitted with filter sets complementary to the probe/fluorochrome combination used. Control samples containing cells of *A. fundyense* will be processed simultaneously to confirm the reliability of the staining procedure.

It is also possible, that in the future, sample throughput could be increased even further utilizing sandwich hybridization assay technology.

10. Sample Handling and Custody

Sample containers and preservation are defined in Table 2.

The nutrient and chlorophyll samples will be delivered to MWRA for analysis upon completion of the survey directly or via FedEx. The *Alexandrium* samples will be delivered by hand directly from the vessel to Bruce Keafer or other designated staff (WHOI) within 24 hours of collection. Samples will be packed in a cooler with ice; no additional packing protection or preservation is required. The whole water phytoplankton samples will be delivered to the analyst following completion of the survey.

All custody forms will be completed during field collection. Labels will be affixed to the samples as well as the custody forms. The Chief Scientist will retain custody of samples during the survey. He is responsible for verifying each sample ID vs. the custody forms prior to delivering the samples to respective laboratories. Upon delivery, the subcontractor laboratory personnel will examine the samples versus the custody forms, verify that sample-specific information has been recorded on the custody forms and that the sample integrity is uncompromised. The laboratory sample custodian will send the original custody forms back to the Consultant's sample custodian, who will maintain the original custody forms in the custody log. The sample custodian will maintain the custody forms in the sample custody log and copies will be provided to the Consultant's Laboratory Manager.

11. Sequence of Survey Task/Events

Table 3. Schedule for Mobilization and Departure

Day 1	MWRA identifies possible need for basic or full survey, and notifies consultant. Consultant mobilizes for survey at most convenient location.
Day X	MWRA decides to proceed with basic or full survey, and notifies consultant.
Day X plus 12 - 36 hours	Consultant conducts survey.
Day X plus 36 - 60 hours	Consultant delivers *Alexandrium* samples to WHOI.
Day X plus 4 - 5 days	WHOI notifies MWRA and consultant of *Alexandrium* count results. MWRA evaluates data based on process outlined in Fig 5.
Day X plus ~7 days	Consultant conducts a follow-up survey each week until notified to discontinue by MWRA.

If weather delays are experienced, the survey schedule (Table 3) may be postponed for up to 24 hours. Delays will be determined as a joint decision between the Consultant Chief Scientist and the MWRA HOM project manager or Area Leader. The vessel captain has final authority over all vessel operations.

A 12-hour survey day has been planned. Every effort will be made to ensure that the collected samples are delivered to WHOI within the 24 hours of collection.

12. Communication

Open lines of communication between MWRA and the Consultant's team staff and clear chain-of-command within both organizations are critical to the timely conduct of any rapid response survey requested by the MWRA. Any of the following MWRA staff are responsible for requesting a rapid response survey:

- Dr. Michael Mickelson 617 788-4746
- Mr. Kenneth Keay 617 788-4742
- Dr. Andrea Rex 617 788-4708

These MWRA staff will notify at least two of the Consultant's Management Staff of an event in order of calling priority. At least four Consultant contact staff will be identified prior to the field year:

Communications with the subcontractor laboratories are **critical** to schedule sample delivery and analytical schedules (staff availability). The primary contacts for the phytoplankton analytical subconsultant will be identified annually; contacts at WHOI are:

- Mr. Bruce Keafer 508 289-2509
- Dr. Don Anderson 508 289-2351

13. Navigation and Positioning Control

The onboard sampling and navigation computer and software will use a differential GPS system. This system will be used to determine vessel position during sampling operations. At the start of each station, the vessel will be positioned upwind or upcurrent of the station position so that the vessel remains within 300 m of the targeted station location during sampling operations (*e.g.* Libby *et al.*, 2006).

14. Vessel, Equipment, and Supplies

A boat capable of sampling all 21 stations within an approximately twelve-hour field day will be used to conduct the full surveys. This will normally be the vessel used for nearfield water quality monitoring surveys. The vessel will need to be equipped with a motorized A-frame to support CTD operations, have adequate lab and deck space, and a top speed of approximately 14 knots to accomplish the surveys.

Equipment from vessel

A-frame, electrical and hydraulic power, lab space, backup navigation equipment, and safety equipment will be provided by the vessel.

Equipment from Consultant

CTD, Rosette system, surface and underwater irradiance sensors, DO probe, chlorophyll fluorescence sensor, barcode printer, color printer, navigation equipment, computer equipment, data acquisition software, and safety equipment will be provided by the Consultant. To support sample collections from an alternative vessel, a hand-deployed CTD and water collection system will suffice but must be compatible with the Consultant's navigation and data acquisition software. The use of this portable equipment will allow the basic or full survey be conducted from an alternative vessel on short notice.

Expendable supplies from vessel

The Consultant will maintain a supply of nutrient, phytoplankton, and *Alexandrium* sample bottles (a total of 100 of each). Sufficient supplies to complete two full surveys will be maintained at all times. One set of sample bottles will stored aboard the R/V, ready for immediate deployment. The Consultant will store a second set of bottles on-site at their local field facility.

These pre-positioned supplies will be checked every three months for integrity and availability. Supplies will be replenished as required after any survey activities.

Expendable supplies from Consultant

Computer discs, printer paper, and barcode labels.

Supplies from MWRA

Cleaned and rinsed nutrient sample bottles will be provided by MWRA.

15. QA/QC Procedures

Prior to data collection, the Chief Scientist is responsible for verifying that all equipment is calibrated and functioning properly. Refer to the current QAPP for Water Column Monitoring (e.g. Libby *et al.*, 2006) for sample collection quality assurance procedures.

16. Documentation Procedures

The Chief Scientist is responsible for ensuring that all events occurring during the survey are adequately documented in the Survey Log. It is anticipated that all data for this survey will be recorded on Station Log forms, the Survey Sensors and Equipment list, and the Survey Personnel Log-in sheet. The vessel captain will maintain a log of events in the Captains log.

17. Scientific party

Two survey teams will be on standby to ensure that adequate staff is available to conduct surveys within the required time frame following notification of an *Alexandrium* event. The basic surveys can be accomplished with one Chief Scientist and one Technician. The full surveys will require an additional technician.

Table 4. Survey Personal and Responsibility

	Participants	Survey Responsibility	Organization
1	TBD	Vessel Captain	TBD
2	TBD	Vessel Captain	TBD
4	TBD	Chief Scientist	TBD
5	TBD	Chief Scientist	TBD
6	TBD	Chief Scientist	TBD
7	TBD	Technician	TBD
8	TBD	Technician	TBD
9	TBD	Technician	TBD

18. Reporting requirements

Debriefing Telephone Call	Yes
No. of Days after demobilization	One day after sample collection
Survey report due date	Four weeks after completion of the final survey.
Final Report/Other Document Description	Data report and interpretive reports TBD.
Final Report Due Date	TBD

19. References

Anderson DM, Keafer BA, Churchill JC, Loder T. 2002. Post-outfall Surveys of Toxic *Alexandrium fundyense* Populations in Massachusetts Bay, 2001. Final Report to the Massachusetts Water Resources Authority 2002-08. 36 p.

Anderson DM, Keafer BA, Geyer WR, Signell RP, Loder T. 2005a. Toxic *Alexandrium* blooms in the western Gulf of Maine: The plume advections hypothesis revisited. Limnol. Oceanogr. 50: 328-345.

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Gribble KE, Keafer BA, Quilliam M, Cembella AD, Kulis DM, Anderson DM. 2005. Distribution and toxicity of *Alexandrium ostenfeldii* (dinoflagellata) in the Gulf of Maine, USA. Deep-Sea Research II 52:2745-2763.

Keafer BA, Churchill JH, McGillicuddy DJ, Anderson DM. 2005. Transport of toxic *Alexandrium fundyense* populations within a nearshore coastal plume in the Gulf of Maine. Deep-Sea Research II 52: 2674-2697.

Libby PS, Mansfield A , Buhl R, Lescarbeau G, Leo W, Keller A, Borkman D, Turner J, Oviatt C. 2006. Combined Work/Quality Assurance Plan For Water Quality Monitoring: 2006-2007. Boston: Massachusetts Water Resources Authority. Report Enquad 2006-03. 75pp + apps.

APPENDICES

Appendix A

Standing Survey Plan **Date: May, 2008**

Rapid Response *Alexandrium* Survey

Survey AF08X

Survey Date: At the request of MWRA

1. General

Project title	Massachusetts Water Resources Authority (MWRA) Harbor and Outfall Monitoring Program (HOM6)
Survey title	MWRA Rapid Response *Alexandrium* Survey AF08X
Survey vessel	*R/V Aquamonitor or other as required*
Vessel requested by	Matt Fitzpatrick
Organization	Battelle
Project Manager	Michael Mickelson
Organization	MWRA
Survey Chief Scientist	Matt Fitzpatrick or Jessica Fahey
Organization	Battelle
Address	397 Washington Street, Duxbury, MA 02332
Telephone	(781) 934-0571
Fax	(781) 934-2124
Cellular Phone (Field)	(617) 968-1812
MWRA Contact Persons:	Michael Mickelson (617) 788-4746 or Ken Keay (617) 788-4947
MWRA Contract Number	S366
WHOI Contact Persons	Don Anderson (508) 289-2351 or Dave Kulis (508) 289-2859
MA DMF Contact Person	David Whittaker (508) 563-1779 ext. 126 or Michael Hickey (508) 563-1779 ext. 122
ME DMR Contact Person	Darcie Couture (207) 633-9570

2. Schedule of operations

Mobilization Date	On Call/Standby Status
Location	Varies: Duxbury or Hewitts Cove Marina in Hingham
Port	Hingham.
Departure/Sampling Date	Within 48 hours of notification by MWRA.
Planned Survey Duration	1 day
Maximum Duration	1 day
Demobilization Date	Immediately following completion of the survey.
Location	Hingham/Duxbury, Massachusetts.
Comments	

3. Background Information

This document contains background information and details on surveys that are designed to respond to a large or unusual red tide bloom of *Alexandrium* in Massachusetts Bay. Although such blooms have been infrequent in the past, an event may develop rapidly so advance planning is warranted. This document describes the decision-making, communications, and logistics that may be appropriate in the event of an *Alexandrium* bloom.

The response supplements regular MWRA outfall monitoring, which is not designed to describe such a bloom, nor can it provide information relevant to possible outfall effects. The response may require targeted sampling using specific rapid methods for counting *Alexandrium*.

4. Objectives

The goal of this monitoring effort is to characterize and understand a major *Alexandrium* bloom in Massachusetts Bay and determine if the MWRA outfall influences the bloom. Data collected during the surveys are intended to obtain insight on the bloom dynamics and to evaluate the potential influence (impact) of outfall discharge on the bloom (e.g., localized and downstream, change in magnitude of bloom, etc.).

The plan outlines the steps that will be taken in the event of an *Alexandrium* bloom. These steps range from an early warning step of notification of any incidence of paralytic shellfish poisoning (PSP) in Maine to the ultimate step of conducting a comprehensive monitoring effort in Massachusetts Bay. The following sections provide information on the rationale behind the approach proposed, a presentation of the stepwise methodology to tracking and ultimately characterizing an *Alexandrium* bloom, and summarizes the field and laboratory procedures that would be used in the event that a rapid assessment is necessary.

5. Survey justification and rationale

The presence of PSP toxicity that can result from *Alexandrium* blooms is monitored in Maine, New Hampshire, and Massachusetts as part of shellfish monitoring programs under the auspices of ME Department of Marine Resources (DMR), NH Department of Environmental Services and MA Division of Marine Fisheries (DMF). The MWRA HOM water quality surveys have measured *Alexandrium* in the bay on occasion (1992-2004) and surveys such as these were conducted in 2005 and 2006 during large regional Alexandrium blooms. The rapid response survey was designed based on past *Alexandrium* research efforts focused along the western Gulf of Maine (Franks and Anderson 1992, Anderson *et al*. 2002, 2005 and others). The survey design was modified from the initial rapid-response monitoring plan (Libby 2006) based on the lessons learned during the 2005 and 2006 blooms. The modified design is presented in this document.

MWRA has developed the response approach described in this survey plan to ensure data are obtained to appropriately describe and understand whether and how the outfall in Massachusetts Bay contributes to such observations.

6. Rapid Response Methodology

In the event that PSP has triggered shellfish closures in Massachusetts Bay, a rapid response *Alexandrium* Survey will be initiated. Other factors such as closures in New Hampshire and Southern Maine combined with recent north and northeast wind may also initiate a survey (Libby 2006).

7. Survey locations and descriptions

Survey Name(s)	Rapid Response *Alexandrium* Survey AF08X
Survey Area Locations	Massachusetts Bay, Table 1 and Figure 1
Survey Station Types	Vertical profiles with discrete water sample collection at the surface (1-2 m), ~10 m, ~20 m and near bottom (within 5 meters).
Number of Stations	19 stations

A complete hydrographic profile, including temperature, salinity, density, fluorescence, dissolved oxygen, depth, time and position will be recorded at each station. Water samples will be collected at 4 depths - surface, ~10 meters, ~20 meters and near bottom. A list of planned subsamples is listed in Table 2. Additional samples may be added if conditions warrant. Samples for *Alexandrium* will be transferred directly to WHOI (Don Anderson/Dave Kulis) within 24 hours of survey completion.

Table 1. Planned Station Locations for survey AF08X.

Station Id	Latitude	Longitude
AF1	42.02788	-70.55467
AF10	42.54425	-70.70468
AF2	42.09879	-70.52724
AF4	42.21997	-70.69553
AF5	42.3097	-70.65347
AF6	42.40335	-70.50163
AF8	42.54019	-70.54553
AF9	42.58754	-70.60224
F05	42.1386667	-70.65
F06	42.1706667	-70.5766667
F07	42.1968333	-70.5158333
F13	42.2683333	-70.735
F17	42.3458333	-70.5705
F22	42.4798333	-70.6176667
F31	42.3063333	-70.94
N04	42.4438333	-70.7365
N10	42.3315	-70.834
N16	42.394	-70.7533333
N18	42.3658333	-70.7776667

Table 2. Planned Subsamples for survey AF08X.

Station	Depth (m)	Level/depth code	Protocols				
			CH	DO	IN	AL	WW
AF1	28.5	AF1 E	1		1		
		AF1 20m	1		1	1	1
		AF1 10m	2		2	1	1
		AF1 A	1		1	1	1
AF2		AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF4		AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF5	50	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF6	92.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF8	59.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF9	46	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF10	28.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
F05	17.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
F06	34	AF1 E			1		
		AF1 20m			1		

Rapid Response *Alexandrium* Survey

Survey AF08X

Survey Date: At the request of MWRA

Station	Depth (m)	Level/depth code	Protocols				
			CH	DO	IN	AL	WW
		AF1 10m			1	1	
		AF1 A			1	1	
F07	53.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			2	1	
		AF1 A			1	1	
F13	25	AF1 E	1		1		
		AF1 20m	1		1	1	1
		AF1 10m	2		2	1	1
		AF1 A	1		1	1	1
F17	78	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
F22	81	AF1 E	1	3	1		
		AF1 20m	1		1	1	1
		AF1 10m	2	3	2	1	1
		AF1 A	1	3	1	1	1
F31	17	AF1 E			1		
		AF1 10m			1	1	
		AF1 A			1	1	
N04	52	AF1 E	1		1		
		AF1 20m	1		1	1	1
		AF1 10m	1		1	1	1
		AF1 A	1		1	1	1
N10	26.5	AF1 E		3	1		
		AF1 20m			1		
		AF1 10m		3	1	1	
		AF1 A		3	1	1	
N16	44.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
N18	27	AF1 E	1		1		
		AF1 20m	1		1	1	1
		AF1 10m	1		1	1	1
		AF1 A	1		1	1	1
Blanks			2		3		

Note: Triplicate dissolved oxygen samples will be collected at the first 3 depth station of the day and F22 at surface, 10m and near bottom depths.

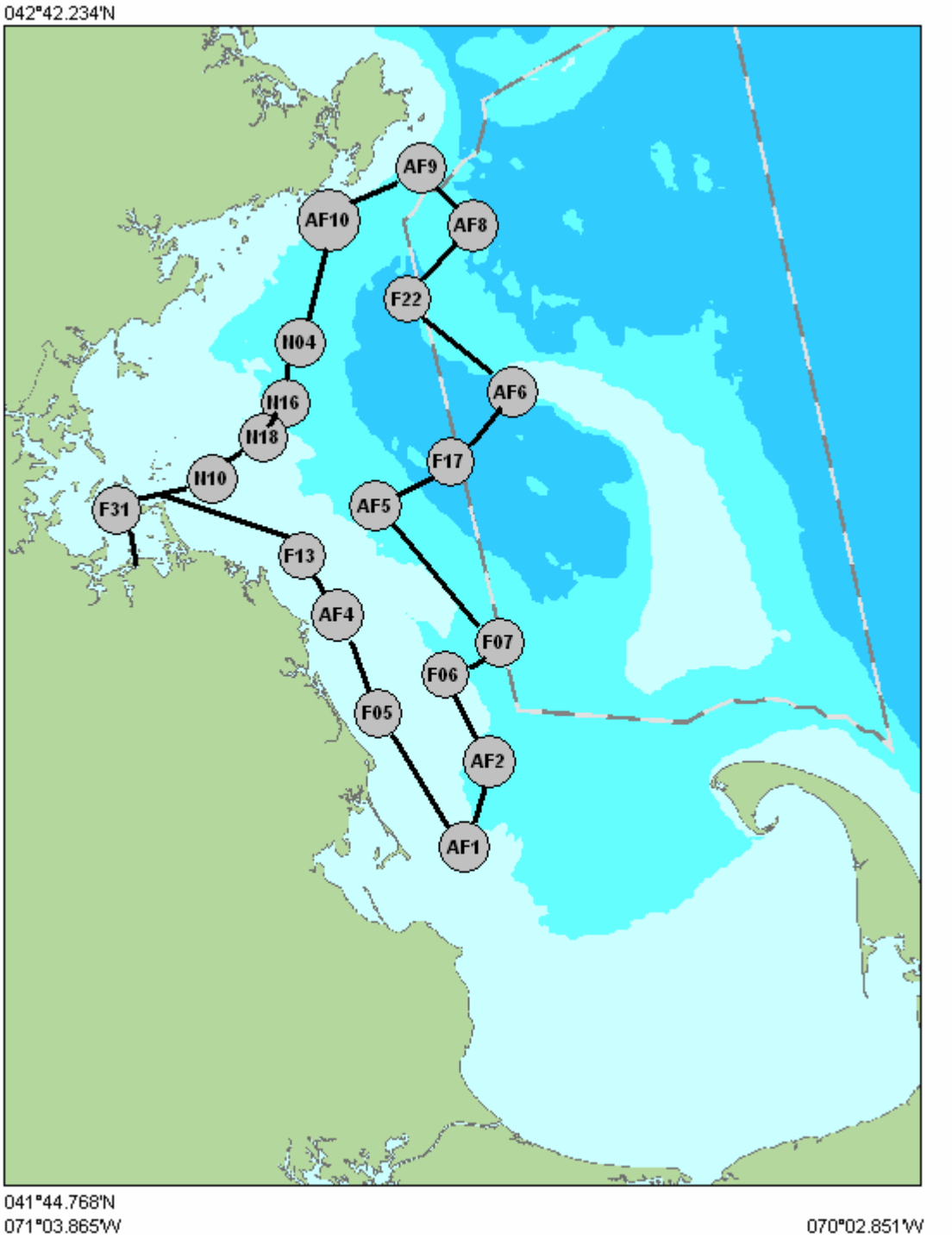

Figure 1. Planned station locations- the track line can be done in the order that is most conducive to weather.

8. Survey/Sampling Methodologies

Method Descriptions:

The water quality monitoring CWQAPP (Libby *et al.*, 2008) contains additional details on survey/sampling methods.

In Situ Measurements:

In situ hydrographic measurements will be taken during the vertical downcast for conductivity, temperature, depth, dissolved oxygen, chlorophyll *a*, transmissometry, irradiance, and altitude. Salinity and water density will be calculated from conductivity, temperature, and depth in real time. Concurrently with the recording of the profile measurements, measurements of total incident photosynthetically active radiation (white light), (scalar hemispherical), water depth, navigational position, and time will be measured. Furthermore, light irradiance will only to be measured from ½ hour after sunrise to ½ hour before sunset. Weather and waves permitting, the vessel will be oriented to avoid shading light sensors during measurements; otherwise unsatisfactory light measurements will be annotated in the survey log book. Readings of the two light sensors will be compared together on deck (in air) at least once each day as a rough check of the consistency of sensor calibrations.

Discrete Samples:

Discrete water samples will be collected from surface, ~10 meters, ~20 meters, and near bottom using a Rosette system. Niskin bottles will not be closed until the *in situ* sensors have reached equilibrium to ensure sample data represent the collected water.

Once the Rosette system is onboard, subsamples will be obtained for each of the parameters being measured. The processing steps for the nutrient, dissolved oxygen, chlorophyll and whole water phytoplankton that will be collected during the full survey can be found in the CWQAPP (Libby *et al*. 2008). The *Alexandrium* samples will be collected as 4-liter 20-µm screened samples from the surface,10 meter, and 20 meter waters (Table 2). These samples will be rinsed into 15 ml centrifuge tubes with filtered seawater (a funnel may be used), then the appropriate volume of formalin added. For example, if there are 14 ml of sample add 1 ml concentrated formalin (37% formaldehyde). *Alexandrium* samples are stored upright on ice and in the dark. To ensure that the probe method works, the formalin fixed samples must be transferred into methanol within 24 hours.

Table 3. Sample Handling and Preservation Requirements

Parameter	Container	Sample Size	No. of Containers	Sample Preservation	Holding Time
Alexandrium	15-ml centrifuge tube	4 l filtered thru 20-μm mesh final volume after rinse 12-14 mL	43	5% formalin <4° C dark	Transfer to Methanol within 24 hours
Dissolved inorganic nutrients	125-mL polyethelyne bottle	40 mL	83 including 3 blanks	Freeze until analysis	28 days to analysis
Chlorophyll *a* and phaeopigments	Whatman GF/F in foil	25 – 400 mL filtered through GF\F	25 including 2 blanks	Freeze filter until analysis	4 weeks to analysis
Dissolved oxygen	300 mL glass BOD bottle	300 mL	18	Fix as described in Libby 2008 and titrate within 24 hours	24 hours to titration
Phytoplankton (whole water)	1000 mL HDPE bottle	850 mL	15	Preserve with Utermöhl's solution.	6 months to analysis

All sample bottles will be labeled with sample labels generated by NavSam© and stored appropriately.

QA/QC –Data quality requirements for navigational and hydrographic data collection, hydrographic profiling, and water sampling are detailed in the Water Column Monitoring CW/QAPP (Libby *et al.*, 2008).

9. Laboratory Analysis

The Water Quality Monitoring CW/QAPP (Libby *et al.*, 2008) contains details on the nutrient, chlorophyll, dissolved oxygen, and whole water phytoplankton analytical methods.

The *Alexandrium* samples will be identified counted and quantified using fluorescent probes (Don Anderson/Dave Kulis). The samples will be delivered to WHOI where the sample will be centrifuged and the formalin removed by aspiration leaving the pellet intact. The pellet will then be resuspended with 100% cold methanol for analysis and storage. For optimal results, this process should occur within 24 hrs after fixation in formalin. The sample cannot tolerate long time periods in formalin because the rRNA signal in the cell is lost due to excessive cross-linking of the nucleic acids by the formalin. Although 24 hr is the optimal time frame, it is expected that the probes will provide acceptable results on samples stored up to one week in formalin (Anderson pers. comm.). Fluorescent probe analysis will be used to confirm and enumerate

Alexandrium fundyense present (Anderson *et al*., submitted; Keafer *et al*., submitted; Gribble *et al*., submitted).

Data generated during the analysis will be submitted to Battelle in an excel document once it becomes available.

10. Sample Handling and Custody

Sample containers and preservation are defined in Table 3.

The nutrient and chlorophyll samples will be delivered to MWRA within 2 days of survey completion. The *Alexandrium* samples will be delivered by hand directly from the R/V *Aquamonitor* to Dave Kulis (WHOI) within 24 hours of collection. Samples will be packed in a cooler with ice; no additional packing protection or preservation is required. Whole water samples will be stored at Battelle until further notice is given by MWRA.

All Battelle custody forms will be completed during field collection. Labels generated by the NavSam© program will be affixed to the samples as well as the Battelle custody forms. The Chief Scientist will retain custody of samples during the survey. He is responsible for verifying each sample ID vs. the custody forms generated by NavSam© prior to delivering the samples to respective laboratories. Upon delivery, the subcontractor laboratory personnel will examine the samples versus the custody forms, verify that sample-specific information has been recorded on the custody forms and that the sample integrity is uncompromised. The laboratory sample custodian will send the original custody forms back to the Battelle sample custodian, who will maintain the original custody forms in the Battelle custody log. The Battelle sample custodian will maintain the custody forms in the sample custody log and copies will be provided to the Battelle laboratory Manager.

11. Sequence of Survey Task/Events

Table 4. Schedule for Mobilization and Departure

Day 1	Notification of possible need for survey by MWRA. Mobilize for survey at most convenient location.
Day 1 plus X days	Notification by MWRA to conduct survey.
Day X plus 12 - 36 hours	Conduct survey.
Day X plus 36 - 48 hours	Deliver *Alexandrium* samples to WHOI. Deliver nutrient samples to MWRA.
Day X plus 48 - 72 hours	Notify MWRA of *Alexandrium* count results
Day X plus ~7 days	Conduct follow-up survey as data warrant and until notified to discontinue by MWRA

If weather delays are experienced, the survey schedule may be postponed for up to 24 hours. Delays will be determined as a joint decision between the Battelle Chief Scientist and the

MWRA HOM6 project manager or Area Leader. The vessel captain has final authority over all vessel operations.

A twelve-hour day has been planned. Every effort will be made to ensure that the collected samples are delivered to URI and WHOI within 24- hours of collection.

12. Communication

Open lines of communication between MWRA and the Battelle team staff and clear chain-of-command within both organizations are critical to the timely conduct of any rapid response survey requested by the MWRA. Any of the following MWRA staff are responsible for requesting a rapid response survey by Battelle:

- Dr. Michael Mickelson (617) 788-4746
- Mr. Kenneth Keay (617) 788-4947
- Dr. Andrea Rex (617) 788-4708

These MWRA staff will notify any one of the following Battelle Management Staff of an event in order of calling priority (at least two Battelle staff will be notified):

- Mr. Scott Libby 207-725-9572 (W)
- Mr. Matt Fitzpatrick 781-952-5351 (W)
- Ms. Jeanine Boyle 781-952-5327 (W)
- Ms. Ellie Baptiste-Carpenter 781-952-5361 (W)

Communications with the subcontractor laboratories are **critical** to schedule sample delivery and analytical schedules (staff availability). The primary contacts at WHOI are:

- Mr. Dave Kulis 508-289-2859 (W)
- Dr. Don Anderson 508-289-2351 (W); 508-289-2745 (Lab)

13. Navigation and Positioning Control

The Battelle onboard sampling and navigation computer (NavSam©) will use a differential GPS system. This system will be used to determine vessel position during sampling operations. At the start of each station, the vessel will be positioned upwind or upcurrent of the station position so that the vessel remains within 300 m of the targeted station location during sampling operations (Libby *et al.*, 2008).

14. Vessel, Equipment, and Supplies

The 45–ft vessel *R/V Aquamonitor* will be used as the primary support vessel during both the mini and full survey operations. The vessel is equipped with a motorized A-frame to support CTD operations, has adequate lab and deck space, and a top speed of 14 knots.

Equipment from vessel

A-frame, electrical and hydraulic power, lab space, backup navigation equipment, and safety equipment will be provided by the vessel.

Equipment from Battelle

CTD, rosette system, surface and underwater irradiance sensors, DO probe, chlorophyll fluorescence sensor, optical beam transmittance sensor, altimeter, barcode printer, color printer, navigation equipment, computer equipment, NavSam© data acquisition software, DO titrator, BOD bottles, nutrient filtration systems, zooplankton nets, respiration incubation system, and safety equipment.

Expendable supplies from vessel

Battelle will maintain a supply of nutrient, phytoplankton, and *Alexandrium* sample bottles. Sufficient supplies to complete two full surveys will be maintained at all times.

These supplies will be checked every three months for integrity and availability. Supplies will be replenished as required after any survey activities.

Fuel and water.

Expendable supplies from Battelle

Nutrient sample bottles, plankton bottles, preservatives and other solutions, foil pouches, computer discs, printer paper, and barcode labels.

Supplies from MWRA

Nutrient sample bottles will be provided by MWRA along with LIMs Ids.

15. QA/QC Procedures

Prior to data collection, the Chief Scientist is responsible for verifying that all equipment is calibrated and functioning properly. Refer to the CWQAPP for Water Column Monitoring (Libby *et al.*, 2008) for sample collection quality assurance procedures.

16. Documentation Procedures

The Chief Scientist is responsible for ensuring that all events occurring during the survey are adequately documented in the Survey Log. It is anticipated that all data for this survey will be recorded on Station Log forms, the BOSS Survey Sensors and Equipment list, and the Survey Personnel Log-in sheet. The vessel captain will maintain a log of events in the Captains log.

17. Scientific party

A diverse survey team (Table 5) will be on standby to ensure that adequate staff is available to conduct surveys within the required time frame following notification of an *Alexandrium* event. A survey requires four individuals and a captain to be completed safely. Staff required to support these surveys include:

Table 5. Survey Personal and Responsibility

	Participants	**Survey Responsibility**	**Organization**
1	Bob Carr	Vessel Captain	Battelle/consultant
2	Matt Fitzpatrick	Chief Scientist/NavSam©	Battelle
3	Bob Mandeville	NavSam©	Battelle
4	Jessica Fahey	Chief Scientist/Technician	Battelle
5	Annie Murphy	Technician	Battelle
6	Mike Walsh	Captain/Technician	Battelle

18. Reporting requirements

Debriefing Telephone Call	No
No. of Days after demobilization	Day of sample collection
Survey report due date	Within 2 weeks of the final *Alexandrium* survey of the year.
***Alexandrium* probe counts**	As soon as available.
Collection, *in situ*, and *Alexandrium* database file	Within 1 week of the final *Alexandrium* survey of the year.

19. References

Anderson, D.M., D.M. Kulis, B.A. Keafer, K.E. Gribble, R. Marin, and C.A. Scholin. In press. Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using molecular probes. Deep Sea Research II.

Anderson, D. M. B.A. Keafer, J.C. Churchill, and T. Loder, 2002. Post-outfall Surveys of Toxic *Alexandrium fundyense* Populations in Massachusetts Bay, 2001. Final Report to the Massachusetts Water Resources Authority 2002-08. 36 p.

Anderson, D. M. B.A. Keafer, W.R. Geyer, R.P. Signell, and T. Loder, 2005. Toxic *Alexandrium* blooms in the western Gulf of Maine: The plume advections hypothesis revisited. Limnol. Oceanogr. 50: 328-345.

Anderson, D.M., Kulis, D.M., Keafer, B.A., Gribble, K.E., Marin, R., Scholin, C.A., 2004a. Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using molecular probes. Deep Sea Research II submitted.

Franks, P.J.S. and D.M. Anderson. 1992a. Alongshore transport of a toxic phytoplankton bloom in a buoyancy current: *Alexandrium tamarense* in the Gulf of Maine. Marine Biology 112:153-164.

Gribble, K.E., Keafer, B.A., Quilliam, M., Cembella, A.D., Kulis, D.M, Anderson, D.M., Distribution and toxicity of *Alexandrium ostenfeldii* (dinoflagellata) in the Gulf of Maine, USA. Deep Sea Research II submitted.

Keafer, B.A., Churchill, J.H., McGillicuddy, D.J., Anderson, D.M., Transport of toxic *Alexandrium fundyense* populations within a nearshore coastal plume in the Gulf of Maine. Deep Sea Research II submitted.

Libby PS. 2006. Standing survey plan: rapid response Alexandrium survey. Boston: Massachusetts Water Resources Authority. Report 2006-05. 19 p.

Libby PS, Fitzpatrick M, Buhl R, Lescarbeau G, Leo W, Keller AA, Borkman DG, Turner JT and Oviatt CA. 2008. Combined work/quality assurance project plan (QAPP) for water column monitoring 2008 - 2009, tasks 4, 5, 6, 7, 8, 11. Boston: Massachusetts Water Resources Authority. Report 2008-02. 98 p.

Appendix B

Standing Survey Plan **Date: May, 2009**

Rapid Response *Alexandrium* Survey
Survey AF09X

Survey Date: At the request of MWRA

1. General

Project title	Massachusetts Water Resources Authority (MWRA) Harbor and Outfall Monitoring Program (HOM6)
Survey title	MWRA Rapid Response *Alexandrium* Survey AF09X
Survey vessel	*R/V Aquamonitor or other as required*
Vessel requested by	Matt Fitzpatrick
Organization	Battelle
Project Manager	Michael Mickelson
Organization	MWRA
Survey Chief Scientist	Matt Fitzpatrick or Jessica Fahey
Organization	Battelle
Address	397 Washington Street, Duxbury, MA 02332
Telephone	(781) 934-0571
Fax	(781) 934-2124
Cellular Phone (Field)	(617) 968-1812
MWRA Contact Persons:	Michael Mickelson (617) 788-4951 or Ken Keay (617) 788-4947
MWRA Contract Number	S366
WHOI Contact Persons	Don Anderson (508) 289-2351 or Dave Kulis (508) 289-2859
MA DMF Contact Person	Terry O'Neil (508) 990-2860 ext. 127 or Michael Hickey (508) 990-2860 ext. 122
ME DMR Contact Person	Darcie Couture (207) 633-9570

2. Schedule of operations

Mobilization Date	On Call/Standby Status
Location	Varies: Duxbury or Hewitts Cove Marina in Hingham
Port	Hingham.
Departure/Sampling Date	Within 48 hours of notification by MWRA.
Planned Survey Duration	1 day
Maximum Duration	1 day
Demobilization Date	Immediately following completion of the survey.
Location	Hingham/Duxbury, Massachusetts.
Comments	

3. Background Information

This document contains background information and details on surveys that are designed to respond to a large or unusual red tide bloom of *Alexandrium* in Massachusetts Bay. Although such blooms have been infrequent in the past, an event may develop rapidly so advance planning is warranted. This document describes the decision-making, communications, and logistics that may be appropriate in the event of an *Alexandrium* bloom.

The response supplements regular MWRA outfall monitoring, which is not designed to describe such a bloom, nor can it provide information relevant to possible outfall effects. The response may require targeted sampling using specific rapid methods for counting *Alexandrium*.

4. Objectives

The goal of this monitoring effort is to characterize and understand a major *Alexandrium* bloom in Massachusetts Bay and determine if the MWRA outfall influences the bloom. Data collected during the surveys are intended to obtain insight on the bloom dynamics and to evaluate the potential influence (impact) of outfall discharge on the bloom (e.g., localized and downstream, change in magnitude of bloom, etc.).

The plan outlines the steps that will be taken in the event of an *Alexandrium* bloom. These steps range from an early warning step of notification of any incidence of paralytic shellfish poisoning (PSP) in Maine to the ultimate step of conducting a comprehensive monitoring effort in Massachusetts Bay. The following sections provide information on the rationale behind the approach proposed, a presentation of the stepwise methodology to tracking and ultimately characterizing an *Alexandrium* bloom, and summarizes the field and laboratory procedures that would be used in the event that a rapid assessment is necessary.

5. Survey justification and rationale

The presence of PSP toxicity that can result from *Alexandrium* blooms is monitored in Maine, New Hampshire, and Massachusetts as part of shellfish monitoring programs under the auspices of ME Department of Marine Resources (DMR), NH Department of Environmental Services and MA Division of Marine Fisheries (DMF). The MWRA HOM water quality surveys have measured *Alexandrium* in the bay on occasion (1992-2004) and surveys such as these were conducted in 2005, 2006 and 2008 during large regional *Alexandrium* blooms. The rapid response survey was designed based on past *Alexandrium* research efforts focused along the western Gulf of Maine (Franks and Anderson 1992, Anderson *et al*. 2002, 2005 and others). The survey design was modified from the initial rapid-response monitoring plan (Libby 2006) based on the lessons learned during the 2005 and 2006 blooms. The modified design was used in 2008 and is presented in this document.

MWRA has developed the response approach described in this survey plan to ensure data are obtained to appropriately describe and understand whether and how the outfall in Massachusetts Bay contributes to such observations.

6. Rapid Response Methodology

In the event that PSP has triggered shellfish closures in Massachusetts Bay, a rapid response *Alexandrium* Survey will be initiated. Other factors such as closures in New Hampshire and Southern Maine combined with recent north and northeast wind may also initiate a survey (Libby 2006).

7. Survey locations and descriptions

Survey Name(s)	Rapid Response *Alexandrium* Survey AF09X
Survey Area Locations	Massachusetts Bay, Table 1 and Figure 1
Survey Station Types	Vertical profiles with discrete water sample collection at the surface (1-2 m), ~10 m, ~20 m and near bottom (within 5 meters).
Number of Stations	19 stations

A complete hydrographic profile, including temperature, salinity, density, fluorescence, dissolved oxygen, depth, time and position will be recorded at each station. Water samples will be collected at 4 depths - surface, ~10 meters, ~20 meters and near bottom. A list of planned subsamples is listed in Table 2. Additional samples may be added if conditions warrant. Samples for *Alexandrium* will be transferred directly to WHOI (Don Anderson/Dave Kulis) within 24 hours of survey completion.

Table 1. Planned Station Locations for survey AF09X.

Station Id	Latitude	Longitude
AF1	42.02788	-70.55467
AF10	42.54425	-70.70468
AF2	42.09879	-70.52724
AF4	42.21997	-70.69553
AF5	42.3097	-70.65347
AF6	42.40335	-70.50163
AF8	42.54019	-70.54553
AF9	42.58754	-70.60224
F05	42.1386667	-70.65
F06	42.1706667	-70.5766667
F07	42.1968333	-70.5158333
F13	42.2683333	-70.735
F17	42.3458333	-70.5705
F22	42.4798333	-70.6176667
F31	42.3063333	-70.94
N04	42.4438333	-70.7365
N10	42.3315	-70.834
N16	42.394	-70.7533333
N18	42.3658333	-70.7776667

Table 2. Planned Subsamples for survey AF09X.

Station	Depth (m)	Level/depth code	Protocols				
			CH	DO	IN	AL	WW
AF1	28.5	AF1 E	1		1		
		AF1 20m	1		1	1	1
		AF1 10m	2		2	1	1
		AF1 A	1		1	1	1
AF2	41	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF4	25	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF5	50	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF6	92.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF8	59.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF9	46	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
AF10	28.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
F05	17.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
F06	34	AF1 E			1		
		AF1 20m			1		

Rapid Response *Alexandrium* Survey

Survey AF09X

Survey Date: At the request of MWRA

Station	Depth (m)	Level/depth code	Protocols				
			CH	DO	IN	AL	WW
		AF1 10m			1	1	
		AF1 A			1	1	
F07	53.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			2	1	
		AF1 A			1	1	
F13	25	AF1 E	1		1		
		AF1 20m	1		1	1	1
		AF1 10m	2		2	1	1
		AF1 A	1		1	1	1
F17	78	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
F22	81	AF1 E	1	3	1		
		AF1 20m	1		1	1	1
		AF1 10m	2	3	2	1	1
		AF1 A	1	3	1	1	1
F31	17	AF1 E			1		
		AF1 10m			1	1	
		AF1 A			1	1	
N04	52	AF1 E	1		1		
		AF1 20m	1		1	1	1
		AF1 10m	1		1	1	1
		AF1 A	1		1	1	1
N10	26.5	AF1 E		3	1		
		AF1 20m			1		
		AF1 10m		3	1	1	
		AF1 A		3	1	1	
N16	44.5	AF1 E			1		
		AF1 20m			1		
		AF1 10m			1	1	
		AF1 A			1	1	
N18	27	AF1 E	1		1		
		AF1 20m	1		1	1	1
		AF1 10m	1		1	1	1
		AF1 A	1		1	1	1
Bottle Blank					1		
Blanks			2		3		

Note: Triplicate dissolved oxygen samples will be collected at the first 3 depth station of the day and F22 at surface, 10m and near bottom depths.

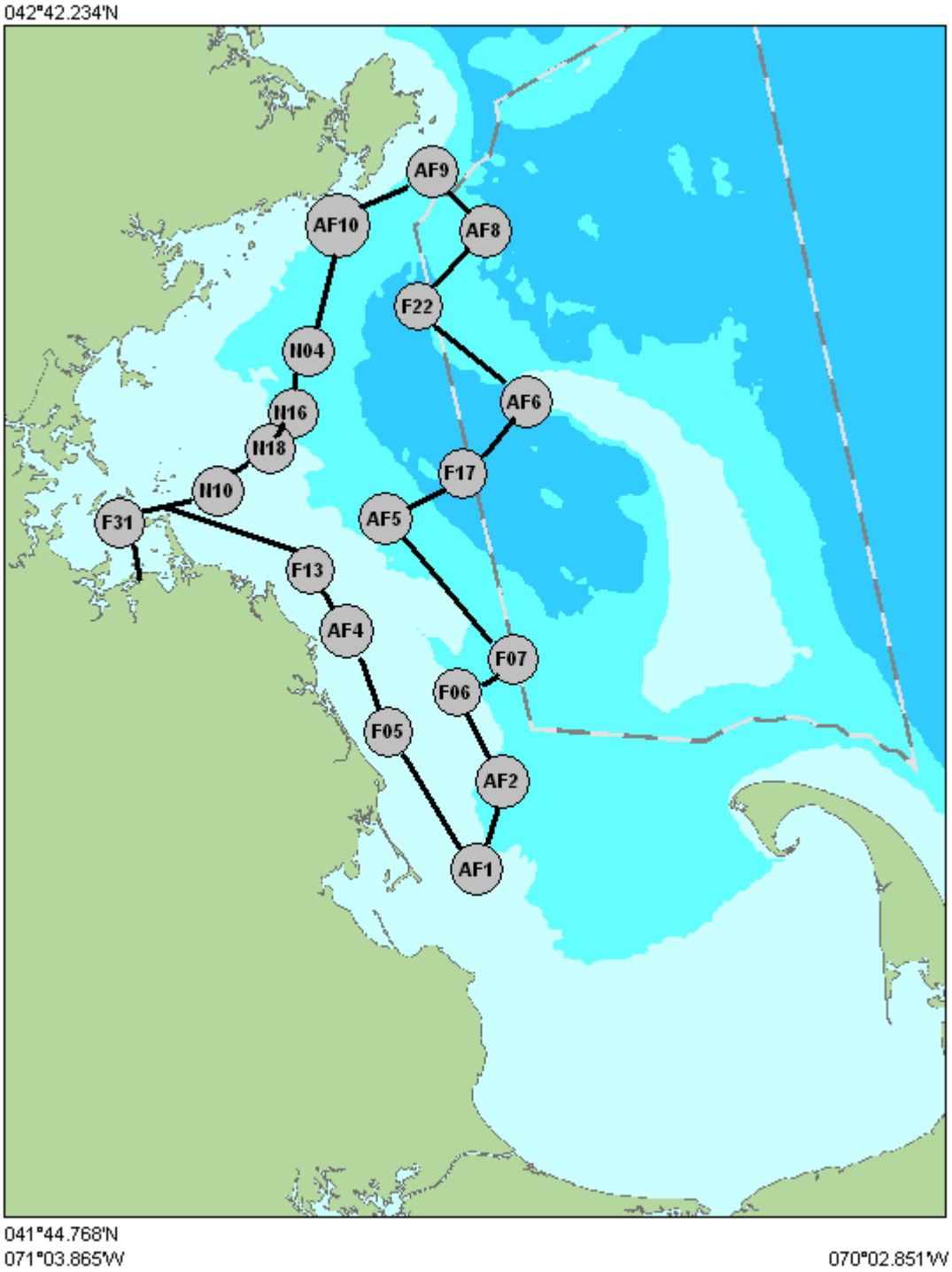

Figure 1. Planned station locations- the track line can be done in the order that is most conducive to weather.

8. Survey/Sampling Methodologies

Method Descriptions:

The water quality monitoring CWQAPP (Libby *et al.* 2008) contains additional details on survey/sampling methods.

In Situ Measurements:

In situ hydrographic measurements will be taken during the vertical downcast for conductivity, temperature, depth, dissolved oxygen, chlorophyll *a*, transmissometry, irradiance, and altitude. Salinity and water density will be calculated from conductivity, temperature, and depth in real time. Concurrently with the recording of the profile measurements, measurements of total incident photosynthetically active radiation (white light), (scalar hemispherical), water depth, navigational position, and time will be measured. Furthermore, light irradiance will only to be measured from ½ hour after sunrise to ½ hour before sunset. Weather and waves permitting, the vessel will be oriented to avoid shading light sensors during measurements; otherwise unsatisfactory light measurements will be annotated in the survey log book. Readings of the two light sensors will be compared together on deck (in air) at least once each day as a rough check of the consistency of sensor calibrations.

Discrete Samples:

Discrete water samples will be collected from surface, ~10 meters, ~20 meters, and near bottom using a Rosette system. Niskin bottles will not be closed until the *in situ* sensors have reached equilibrium to ensure sample data represent the collected water.

Once the Rosette system is onboard, subsamples will be obtained for each of the parameters being measured. The processing steps for the nutrient, dissolved oxygen, chlorophyll and whole water phytoplankton that will be collected during the full survey can be found in the CWQAPP (Libby *et al*. 2008). The *Alexandrium* samples will be collected as 4-liter 20-µm screened samples from the surface,10 meter, and 20 meter waters (Table 2). These samples will be rinsed into 15 ml centrifuge tubes with filtered seawater (a funnel may be used), then the appropriate volume of formalin added. For example, if there are 14 ml of sample add 1 ml concentrated formalin (37% formaldehyde). *Alexandrium* samples are stored upright on ice and in the dark. To ensure that the probe method works, the formalin fixed samples must be transferred into methanol within 24 hours.

Table 3. Sample Handling and Preservation Requirements

Parameter	Container	Sample Size	No. of Containers	Sample Preservation	Holding Time
Alexandrium	15-ml centrifuge tube	4 l filtered thru 20-μm mesh final volume after rinse 12-14 mL	43	5% formalin <4° C dark	Transfer to Methanol within 24 hours
Dissolved inorganic nutrients	125-mL polyethelyne bottle	40 mL	83 including 3 blanks and 1 bottle blank	Freeze until analysis	28 days to analysis
Chlorophyll *a* and phaeopigments	Whatman GF/F in foil	25 – 400 mL filtered through GF\F	25 including 2 blanks	Freeze filter until analysis	4 weeks to analysis
Dissolved oxygen	300 mL glass BOD bottle	300 mL	18	Fix as described in Libby 2008 and titrate within 24 hours	24 hours to titration
Phytoplankton (whole water)	1000 mL HDPE bottle	850 mL	15	Preserve with Utermöhl's solution.	6 months to analysis

All sample bottles will be labeled with sample labels generated by NavSam© and stored appropriately.

QA/QC –Data quality requirements for navigational and hydrographic data collection, hydrographic profiling, and water sampling are detailed in the Water Column Monitoring CW/QAPP (Libby *et al.* 2008).

9. Laboratory Analysis

The Water Quality Monitoring CW/QAPP (Libby *et al.* 2008) contains details on the nutrient, chlorophyll, dissolved oxygen, and whole water phytoplankton analytical methods.

The *Alexandrium* samples will be identified counted and quantified using fluorescent probes (Don Anderson/Dave Kulis). The samples will be delivered to WHOI where the sample will be centrifuged and the formalin removed by aspiration leaving the pellet intact. The pellet will then be resuspended with 100% cold methanol for analysis and storage. For optimal results, this process should occur within 24 hrs after fixation in formalin. The sample cannot tolerate long time periods in formalin because the rRNA signal in the cell is lost due to excessive cross-linking of the nucleic acids by the formalin. Although 24 hr is the optimal time frame, it is expected that the probes will provide acceptable results on samples stored up to one week in formalin

(Anderson pers. comm.). Fluorescent probe analysis will be used to confirm and enumerate *Alexandrium fundyense* present (Anderson *et al*. 2005; Keafer *et al*. 2005; Gribble *et al*. 2005).

Data generated during the analysis will be submitted to Battelle in an excel document once it becomes available.

10. Sample Handling and Custody

Sample containers and preservation are defined in Table 3.

The nutrient and chlorophyll samples will be delivered to MWRA within 2 days of survey completion. The *Alexandrium* samples will be delivered by hand directly from the R/V *Aquamonitor* to Dave Kulis (WHOI) within 24 hours of collection. Samples will be packed in a cooler with ice; no additional packing protection or preservation is required. Whole water samples will be stored at Battelle until further notice is given by MWRA.

All Battelle custody forms will be completed during field collection. Labels generated by the NavSam© program will be affixed to the samples as well as the Battelle custody forms. The Chief Scientist will retain custody of samples during the survey. He is responsible for verifying each sample ID vs. the custody forms generated by NavSam© prior to delivering the samples to respective laboratories. Upon delivery, the subcontractor laboratory personnel will examine the samples versus the custody forms, verify that sample-specific information has been recorded on the custody forms and that the sample integrity is uncompromised. The laboratory sample custodian will send the original custody forms back to the Battelle sample custodian, who will maintain the original custody forms in the Battelle custody log. The Battelle sample custodian will maintain the custody forms in the sample custody log and copies will be provided to the Battelle laboratory Manager.

11. Sequence of Survey Task/Events

Table 4. Schedule for Mobilization and Departure

Day 1	Notification of possible need for survey by MWRA. Mobilize for survey at most convenient location.
Day 1 plus X days	Notification by MWRA to conduct survey.
Day X plus 12 - 36 hours	Conduct survey.
Day X plus 36 - 48 hours	Deliver *Alexandrium* samples to WHOI. Deliver nutrient samples to MWRA.
Day X plus 48 - 72 hours	Notify MWRA of *Alexandrium* count results
Day X plus ~7 days	Conduct follow-up survey as data warrant and until notified to discontinue by MWRA

If weather delays are experienced, the survey schedule may be postponed for up to 24 hours. Delays will be determined as a joint decision between the Battelle Chief Scientist and the

MWRA HOM6 project manager or Area Leader. The vessel captain has final authority over all vessel operations.

A twelve-hour day has been planned. Every effort will be made to ensure that the collected samples are delivered to URI and WHOI within 24- hours of collection.

12. Communication

Open lines of communication between MWRA and the Battelle team staff and clear chain-of-command within both organizations are critical to the timely conduct of any rapid response survey requested by the MWRA. Any of the following MWRA staff are responsible for requesting a rapid response survey by Battelle:

- Dr. Michael Mickelson (617) 788-4951
- Mr. Kenneth Keay (617) 788-4947
- Dr. Andrea Rex (617) 788-4940

These MWRA staff will notify any one of the following Battelle Management Staff of an event in order of calling priority (at least two Battelle staff will be notified):

- Mr. Scott Libby 207-725-9572 (W)
- Mr. Matt Fitzpatrick 781-952-5351 (W)
- Ms. Ellie Baptiste-Carpenter 781-952-5361 (W)

Communications with the subcontractor laboratories are **critical** to schedule sample delivery and analytical schedules (staff availability). The primary contacts at WHOI are:

- Mr. Dave Kulis 508-289-2859 (W)
- Dr. Don Anderson 508-289-2351 (W); 508-289-2745 (Lab)

13. Navigation and Positioning Control

The Battelle onboard sampling and navigation computer (NavSam©) will use a differential GPS system. This system will be used to determine vessel position during sampling operations. At the start of each station, the vessel will be positioned upwind or upcurrent of the station position so that the vessel remains within 300 m of the targeted station location during sampling operations (Libby *et al.,* 2008).

14. Vessel, Equipment, and Supplies

The 45–ft vessel *R/V Aquamonitor* will be used as the primary support vessel during both the mini and full survey operations. The vessel is equipped with a motorized A-frame to support CTD operations, has adequate lab and deck space, and a top speed of 14 knots.

Equipment from vessel

A-frame, electrical and hydraulic power, lab space, backup navigation equipment, and safety equipment will be provided by the vessel.

Equipment from Battelle

CTD, rosette system, surface and underwater irradiance sensors, DO probe, chlorophyll fluorescence sensor, optical beam transmittance sensor, altimeter, barcode printer, color printer, navigation equipment, computer equipment, NavSam© data acquisition software, DO titrator, BOD bottles, nutrient filtration systems, zooplankton nets, respiration incubation system, and safety equipment.

Expendable supplies from vessel

Battelle will maintain a supply of nutrient, phytoplankton, and *Alexandrium* sample bottles. Sufficient supplies to complete two full surveys will be maintained at all times.

These supplies will be checked every three months for integrity and availability. Supplies will be replenished as required after any survey activities.

Fuel and water.

Expendable supplies from Battelle

Nutrient sample bottles, plankton bottles, preservatives and other solutions, foil pouches, computer discs, printer paper, and barcode labels.

Supplies from MWRA

Nutrient sample bottles will be provided by MWRA along with LIMs Ids.

15. QA/QC Procedures

Prior to data collection, the Chief Scientist is responsible for verifying that all equipment is calibrated and functioning properly. Refer to the CWQAPP for Water Column Monitoring (Libby *et al.,* 2008) for sample collection quality assurance procedures.

16. Documentation Procedures

The Chief Scientist is responsible for ensuring that all events occurring during the survey are adequately documented in the Survey Log. It is anticipated that all data for this survey will be recorded on Station Log forms, the BOSS Survey Sensors and Equipment list, and the Survey Personnel Log-in sheet. The vessel captain will maintain a log of events in the Captains log.

17. Scientific party

A diverse survey team (Table 5) will be on standby to ensure that adequate staff is available to conduct surveys within the required time frame following notification of an *Alexandrium* event. A survey requires four individuals and a captain to be completed safely. Staff required to support these surveys include:

Table 5. Survey Personal and Responsibility

	Participants	**Survey Responsibility**	**Organization**
1	Bob Carr	Vessel Captain	Battelle
2	Matt Fitzpatrick	Chief Scientist/NavSam©	Battelle
3	Bob Mandeville	NavSam©	Battelle
4	Jessica Fahey	Chief Scientist/Technician	Battelle
5	Annie Murphy	Technician	Battelle
6	Patrick Curran	Technician	Battelle

18. Reporting requirements

Debriefing Telephone Call	No
No. of Days after demobilization	Day of sample collection
Survey report due date	Within 2 weeks of the final *Alexandrium* survey of the year.
***Alexandrium* probe counts**	As soon as available.
Collection, *in situ*, and *Alexandrium* database file	Within 1 week of the final *Alexandrium* survey of the year.

19. References

Anderson, D.M., D.M. Kulis, B.A. Keafer, K.E. Gribble, R. Marin, and C.A. Scholin. 2005. Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using molecular probes. Deep Sea Research II 52 (19-21), 2467-2490.

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Anderson, D. M. B.A. Keafer, W.R. Geyer, R.P. Signell, and T. Loder, 2005. Toxic *Alexandrium* blooms in the western Gulf of Maine: The plume advections hypothesis revisited. Limnol. Oceanogr. 50: 328-345.

Franks, P.J.S. and D.M. Anderson. 1992a. Alongshore transport of a toxic phytoplankton bloom in a buoyancy current: *Alexandrium tamarense* in the Gulf of Maine. Marine Biology 112:153-164.

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