

***Alexandrium* Rapid Response Study
Survey Plan. Revision 1**

Massachusetts Water Resources Authority
Environmental Quality Department
Report 2013-06



Citation

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1. General

Project title	Massachusetts Water Resources Authority (MWRA) Harbor and Outfall Monitoring Program (HOM8)
Survey title	MWRA <i>Alexandrium</i> Rapid Response Study 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) 788-4951 or David Taylor (617) 788-4952
WHOI Contact Persons	Don Anderson (508) 289-2351 or Bruce Keafer (508) 289-2509
MA DMF Contact Persons	Michael Hickey (508) 990-2860 x122 or Terry O'Neil (508) 990-2860 x127
ME DMR Contacts Persons	Alison Sorois (207) 633-9401 or Kohl Kanwit (207) 633-9535
NH DES Contact Person	Chris Nash (603) 559-1509

2. Schedule of operations

Mobilization Date	On Call/Standby Status
Location	TBD
Departure/Sampling Date	Within 48 hours of notification by MWRA.
Planned Survey Duration	1 day (multiple surveys possible)
Maximum Duration	1 day (per individual survey)
Demobilization Date	Immediately following completion of the survey.
Location	TBD
Comments	As needed

3. Background Information

This document, Revision 1, updates the original 2006 and 2008 plans (Libby 2006) for the surveys to be conducted under MWRA's *Alexandrium* Rapid Response Study (ARRS). It updates the station locations to better match the new station locations of the regular water column monitoring, which was revised for 2011 as described in MWRA's Ambient Monitoring plan (MWRA 2010). This revision also updates and simplifies the description of how MWRA decides whether to conduct a survey.

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

4. Objectives

The goal is to characterize and understand an *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 distribution and 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 ARRS surveys supplement the nine regular MWRA bays water column monitoring (BWQM) surveys which are conducted each month from February to October. Because the BWQM surveys follow a set schedule and are about a month apart, they are not designed to adequately describe the development of a short-lived, seasonal bloom of a single species such as *Alexandrium*. The ARRS surveys supplement the BWQM surveys by providing information between the BWQM surveys. The ARRS surveys cover the ten BWQM stations where *Alexandrium* are regularly sampled plus nine additional stations. The additional station coverage is feasible within one day because the ARRS surveys measure a subset of the BWQM parameters shortening time on station, the principal measurement being *Alexandrium* cell abundance.

5. Decide whether to conduct an ARRS Survey

MWRA will begin conducting weekly surveys for *Alexandrium* when information suggests a bloom is present in Massachusetts Bay or imminent, and discontinue the ARRS surveys when the bloom has ended.

5.1. Basis for making the decision to carry out a survey:

1) Seasonal and weekly forecasts from researchers.

The first warning of conditions for the year is provided in April when WHOI researchers forecast the potential for a New England "Red Tide"² based on abundance of *Alexandrium* cysts in sediments in the Gulf of Maine. Modelers then use the cyst abundance data and current weather data to provide weekly forecasts of the growth and movement of the bloom.³ In 2013, researchers will deploy an experimental device to measure and report *Alexandrium* abundance near York ME.⁴

2) *Alexandrium* >100 cells/liter in samples collected during MWRA's regular BWQM surveys

The April or May surveys are the surveys most likely to detect elevated levels of *Alexandrium*, and MWRA expedites the counting of those samples.

ACTION: If the *Alexandrium* abundance in any BWQM sample exceeds 100 cells/L, MWRA will begin conducting the weekly ARRS survey.

3) High levels of PSP toxicity in blue mussels

² <http://www.whoi.edu/page.do?pid=117456&tid=3622&cid=133929>

³ <http://omglnx3.meas.ncsu.edu/GOMTOX/2012forecast/>

⁴ <http://www.whoi.edu/oceanus/viewArticle.do?id=78826>

The presence of paralytic shellfish poisoning (PSP) toxicity that can result from *Alexandrium* blooms is monitored in Maine, New Hampshire, and Massachusetts as part of their shellfish monitoring programs under the auspices of Maine Department of Marine Resources (ME-DMR)⁵, New Hampshire Department of Environmental Services (NH-DES)⁶ and Massachusetts Division of Marine Fisheries (MA-DMF)⁷.

MWRA focuses on data on PSP toxicity in blue mussels because the toxin concentration closely follows ambient *Alexandrium* abundance with a lag of a few days. Other species such as surf clams acquire and lose the toxin too slowly to be useful as bloom indicators.

MWRA receives the PSP toxicity results promptly from the state laboratories either directly or through the NortheastPSP Listserv⁸

ACTION: If the PSP toxicity in blue mussels at Cohasset, Scituate, or Marshfield (MA DMF stations) exceeds 40ug toxin per 100g shellfish meat, MWRA will begin conducting the weekly ARRS survey.

ACTION: If PSP toxicity in blue mussels exceeds 40ug at stations between Gloucester MA and Cape Elizabeth ME, assume that there is a bloom in the Gulf of Maine. MWRA will evaluate the likelihood that wind and currents will bring the bloom into Massachusetts Bay and staff will use professional judgment to decide whether to begin the weekly ARRS surveys.

Once MWRA begins the ARRS surveys, it will continue weekly sampling for *Alexandrium* until the measured *Alexandrium* abundance decreases below 100 cells/L and the toxicity data are no longer above closure levels (80 µg STX equiv. /100 g).

5.2. Carrying out an ARRS survey

A 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, and *Alexandrium* abundance will be obtained. *Alexandrium* counts (obtained using a species-specific ribosomal RNA probe) will be relayed to MWRA within 5 days of the survey. Surveys will be conducted on a weekly basis until *Alexandrium* abundance decreases below 100 cells/L and toxicity data from MA-DMF is no longer above action levels (80 µg STX equiv. /100 g). In addition to the rapid response surveys described in detail in the following sections, MWRA's BWQM surveys may be augmented to include additional stations as needed to characterize the bloom. Additional samples may also be collected from MWRA's Boston Harbor monitoring stations, MWRA's stations in Cape Cod Bay, or other surveys of opportunity.

⁵ http://www.state.me.us/dmr/rm/public_health/closures/pspclosures.htm

⁶ <http://des.nh.gov/organization/divisions/water/wmb/shellfish/index.htm>

⁷ http://www.mass.gov/dfwele/dmf/programsandprojects/psp_notice.htm#shelsani

⁸ <http://mailman.who.edu/mailman/listinfo/northeastpsp>

6. Survey locations and descriptions

Survey Name(s)	ARRS Survey AFYYX – concatenation of AF (<i>Alexandrium fundyense</i>), YY=year, and X=surveys will be numbered sequentially.
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 (if water depth is less than 25 m the 20 m sample will be marked as near bottom). A list of planned subsamples is listed in Table 2. Additional samples may be added if conditions warrant.

Table 1 Planned Station Locations for ARRS Surveys.

Station	Latitude	Longitude
AF1	42.02788	-70.55467
AF2	42.09879	-70.52724
AF4	42.21997	-70.69553
AF6	42.40335	-70.50163
AF8	42.54019	-70.54553
AF9	42.58754	-70.60224
N01	42.41933	-70.86450
N04	42.44383	-70.73650
N07	42.35633	-70.70617
N10	42.33150	-70.83400
N18	42.36583	-70.77767
F05	42.13867	-70.65000
F06	42.17067	-70.57667
F10	42.24233	-70.63733
F13	42.26833	-70.73500
F15	42.31550	-70.72767
F17	42.34583	-70.57050
F22	42.47983	-70.61767
F23	42.33917	-70.94200

Table 2. Planned Subsamples for ARRS Surveys.

Station	Depth (m)	Level/ depth code	Protocols				Comments
			CH	DO	IN	AL	
AF1	28.5	AF1 E	1		1		CH Dup IN 20m Alex
		AF2 20m	1		1	1	
		AF4 10m	1		2	1	
		AF5 A	1		1	1	
AF2	41	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
AF4	25	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
AF6	92.5	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
AF8	59.5	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
AF9	46	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
F05	17.5	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
F06	34	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
F10	33	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
F13	25	AF1 E	1		1		CH Dup IN 20m Alex
		AF2 20m	1		1	1	
		AF4 10m	1		2	1	
		AF5 A	1		1	1	

Station	Depth (m)	Level/ depth code	Protocols				Comments
			CH	DO	IN	AL	
F15	38	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
F17	78	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
F22	81	AF1 E	1	3	1		DO CH w/ dup Dup IN 20m Alex
		AF2 20m	1		1	1	
		AF4 10m	2	3	2	1	
		AF5 A	1	3	1	1	
F23	25	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
N01	30	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
N04	52	AF1 E	1		1		CH 20m Alex
		AF2 20m	1		1	1	
		AF4 10m	1		1	1	
		AF5 A	1		1	1	
N07	52	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
N10	26.5	AF1 E			1		
		AF2 20m			1		
		AF4 10m			1	1	
		AF5 A			1	1	
N18	27	AF1 E	1	3	1		DO CH w/ dup Dup IN 20m Alex
		AF2 20m	1		1	1	
		AF4 10m	2	3	2	1	
		AF5 A	1	3	1	1	
Total Samples			22	18	80	43	
Bottle Blank					1		
Blanks			2		3		

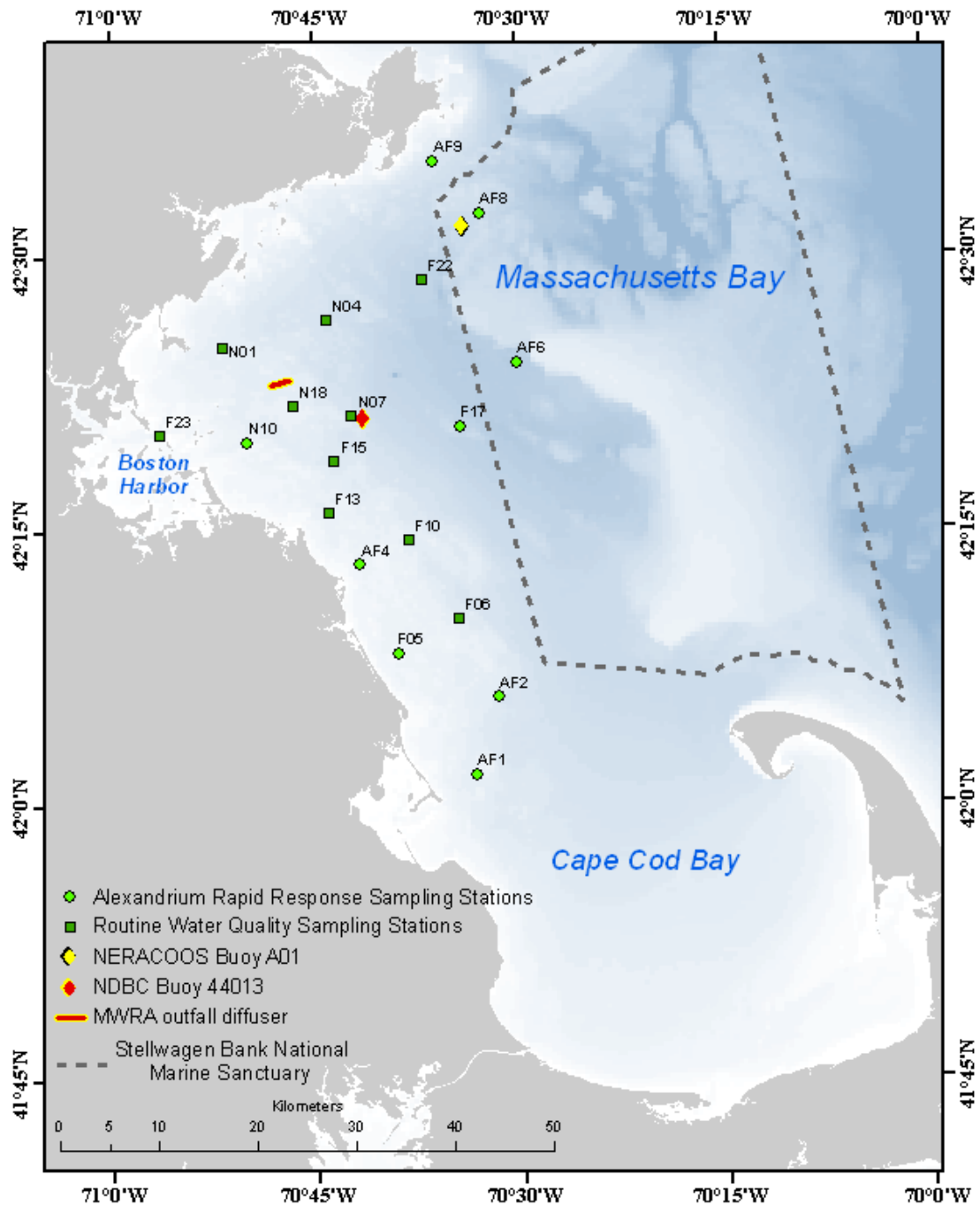


Figure 1. Planned station locations for ARRS surveys. Both the “*Alexandrium* rapid response sampling stations” and the “routine water quality sampling stations” are included in the ARRS surveys. When appropriate, additional stations in Cape Cod and Boston Harbor may be sampled.

7. 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.* 2011) 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.

Discrete Samples:

Water samples will be collected at 4 depths: surface, ~10 meters, ~20 meters and near bottom at each station and subsampled as described in Table 2. Once the Rosette system is onboard, subsamples will be obtained for each of the parameters being measured. All samples will be processed for analysis according to procedures outlined in Libby *et al.* 2011 and summarize in Table 3. Dissolved inorganic nutrient samples will be collected at each discrete water depth sampled. Chlorophyll samples will be collected at three depths at five of the stations for calibration of the *in situ* fluorescence sensor – stations AF1, F13, N04, N18, and F22. Triplicate DO samples will be collected from the surface, 10 m, and bottom waters at stations F22 and N10 for calibration of the *in situ* DO sensor. Duplicate samples for DIN and chlorophyll will be collected from the 10 m sample depth at stations AF1, F13, and F22. The 20- μ m screened water will be collected at two depths (surface and 10 m) at all 19 stations with additional samples collected at 20 m at stations AF1, F13, N04, N18, and F22. The screened samples will be analyzed using the fluorescent gene-probe technique for *Alexandrium* identification and enumeration (Anderson *et al.* 2005). 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 *Alexandrium* samples will be transferred directly to WHOI (Don Anderson/Bruce Keafer) within 24 hours of collection.

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.* 2011).

Table 3 Sample Handling and Preservation Requirements

Parameter	Container	Sample Size	No. of Containers	Sample Preservation	Holding Time
<i>Alexandrium</i>	15-ml centrifuge tube	4 L filtered thru 20- μ m mesh final volume after rinse 10-14 mL	43	5% formalin <4° C dark	Transfer to Methanol within 24 hours
Dissolved inorganic nutrients	125-mL polyethylene bottle	40 mL	84 including dups, 3 blanks, and 1 bottle blank	Freeze until analysis	28 days to analysis
Chlorophyll <i>a</i> and phaeopigments	Whatman GF/F in foil	25 – 400 mL filtered through GF/F	24 including dups and 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 <i>et al.</i> 2011 and titrate within 24 hours	24 hours to titration

8. Laboratory Analysis

The Water Quality Monitoring QAPP (*e.g.* Libby *et al.* 2011) contains details on the nutrient, chlorophyll, dissolved oxygen, and *Alexandrium* analytical methods. A summary of the *Alexandrium* methods is below.

The *Alexandrium* samples will be identified, counted, and quantified using a fluorescent probe technique that accurately identifies toxic *A. fundyense* (Anderson *et al.* 2005). 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 to prevent loss of rRNA due to excessive cross-linking of the nucleic acids by the formalin. However, the fluorescent probes can be used with acceptable results on samples stored up to one week in formalin (Anderson pers. comm.).

A fluorescent rRNA probe that has been developed for *A. fundyense* [North American ribotype (NA-1) probe conjugated to Texas Red] will be used to identify and enumerate the *A. fundyense* in the samples using a Zeiss epifluorescence microscope with appropriate filters, at 100X magnification. Control samples containing cells of *A. fundyense* will be processed simultaneously to confirm the reliability of the staining procedure (Anderson *et al.* 2005, Keafer *et al.* 2005, Gribble *et al.* 2005).

9. Sample Handling and Custody

Sample containers and preservation are defined in Table 3.

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.

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 confirm 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.

10. Sequence of Survey Task/Events

Table 4 Schedule for Mobilization and Departure

Day 1	MWRA identifies possible need for survey, and notifies consultant. Consultant mobilizes for survey at most convenient location.
Day X	MWRA decides to proceed with survey, and notifies consultant.
Day X plus 12 to 36 hours	Consultant conducts survey.
Day X plus 36 to 60 hours	Consultant delivers <i>Alexandrium</i> samples to WHOI.
Day X plus 5 to 6 days	WHOI notifies MWRA and consultant of <i>Alexandrium</i> count results. MWRA evaluates data based on process outlined in Figure 3.
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 4) 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.

11. 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 or their designates are responsible for requesting an ARRS survey:

- Dr. Michael Mickelson 617 788-4951
- Mr. Kenneth Keay 617 788-4947
- Dr. Andrea Rex 617 788-4940
- Dr. David Taylor 617 788-4952

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:

- TBD (Name) telephone
- TBD (Name) telephone
- TBD (Name) telephone
- TBD (Name) telephone

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

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

12. 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.* 2011).

13. Vessel, Equipment, and Supplies

A boat capable of sampling all 19 stations within an approximately twelve-hour field day will be used to conduct the 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 survey be conducted from an alternative vessel on short notice.

Expendable supplies from vessel

The Consultant will maintain a supply of nutrient and *Alexandrium* sample bottles (a total of 100 of each). Sufficient supplies to complete two ARRS surveys will be maintained at all times. The Consultant will store the 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

Clean nutrient sample bottles will be provided by MWRA.

14. 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.* 2011) for sample collection quality assurance procedures.

15. 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.

16. Scientific party

The survey team 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 (Table 5).

Table 5 Survey Personnel and Responsibility

	Participants	Survey Responsibility	Organization
1	TBD	Vessel Captain	TBD
2	TBD	Chief Scientist	TBD
3	TBD	Lead Technician	TBD
4	TBD	Technician	TBD
5	TBD	Technician	TBD

17. Reporting requirements

Debriefing Telephone Call	Yes
No. of Days after demobilization	~5 days; once <i>Alexandrium</i> data are available
Survey report due date	Four weeks after completion of the final survey.
Final Report/Other Document Description	Data report and interpretive reports at MWRA's discretion.
Final Report Due Date	As specified by MWRA.

18. References

Anderson DM, Kulis DM, Keafer BA, Gribble KE, Marin R, Scholin CA. 2005a. Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using molecular probes. Deep-Sea Research II 52:2467-2490.

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