

**Casco Bay Red Tide 2006:
Intensified Paralytic Shellfish
Poisoning (PSP) monitoring
program**

**CASCO BAY ESTUARY PARTNERSHIP
AND
EPA OCEAN AND COASTAL PROTECTION DIVISION**

**Casco Bay Red Tide 2006:
Intensified Paralytic Shellfish Poisoning (PSP) monitoring program**

submitted to

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1.0 INTRODUCTION

Blooms of the toxic dinoflagellate, *Alexandrium fundyense*, have become common in Casco Bay over the last several decades (Keafer *et al.* 2005), often resulting in large-area closures of shellfish harvesting. In 2005, a red tide of *A. fundyense* was unusually intense and prolonged along the Maine coast (Anderson *et al.* 2005a), particularly in Casco Bay (pers. comm. Darcie Couture, DMR). As a result, two 2005 Casco Bay Estuary Partnership (CBEP) projects focused on clam beds were interrupted and indefinitely postponed. Based on Maine Department of Marine Resources (DMR) bloom projections, the historical existence of a large cyst bed offshore of Casco bay (Anderson *et al.* 2005b, Stock *et al.* 2005), and indications that high numbers of cysts were present there in fall 2005 (Anderson unpublished data), it was anticipated that the red tide in 2006 would again be more intense and prolonged than normal.

Maine DMR and the CBEP Casco Bay Clam Team were interested in enhancing monitoring efforts in Casco Bay to improve the ability to make more localized decisions on closing and reopening shellfish growing/harvesting areas based on paralytic shellfish poisoning (PSP) toxicity during the 2006 bloom season. A secondary goal was to develop a better understanding of *A. fundyense* bloom dynamics in Casco Bay. In 2005, the sampling procedure required low-tide sampling (a restrictive and time-consuming process) that limited the number of samples taken. Consequently, the closing/opening of large areas of Casco Bay were often based on data from a single, sometimes distant, point. The 2006 project increased the number stations, improved proximity of stations to specific harvest areas, and increased sampling frequency. The goals of the Intensified Paralytic Shellfish Poisoning (IPSP) monitoring program were to: 1) allow more refined management of specific resource areas for opening/closure; and 2) test a more efficient method for conducting routine monitoring for red tide by DMR along the Maine coast. In addition, CBEP and DMR wanted to examine bloom origin and development (outside Casco Bay, within Casco Bay or both) and correlations between water quality data, location, and bloom intensity.

A total of 43 stations were sampled by MER Assessment Corporation (MER) across Casco Bay on a series of weekly 2-day surveys from April to July. Mussels were collected for PSP toxin analysis from a large number of sites over a short period of time providing DMR with a "snapshot" of PSP across the bay. In addition to the mussel samples, *in situ* parameters were measured during CTD downcasts and coincident nutrient and phytoplankton samples were also collected. The data on *in situ* temperature and salinity, dissolved inorganic nutrients and phytoplankton abundance and community composition provide a comprehensive picture with which to understand the incidence and levels of PSP toxin measured. The 2006 dataset has already proved its worth by allowing DMR to restrict closures to specific areas, leaving nearby waters open for harvesting. The closures were also of shorter duration than during previous PSP events. Furthermore, the sampling method employed is more protective of human health than the previously used methodology due to the increased frequency and spatial scale of the testing. A closer examination of the data may provide added insight on the development, intensification, and termination of these red tide events and could also be useful in modifying monitoring approaches used in Casco Bay during future bloom events. These data are also useful in evaluating potential bloom mitigation efforts.

The objective of this report is to detail the monitoring approach taken during the 2006 Casco Bay PSP monitoring study, compile and evaluate the available data, characterize the 2006 bloom (spatially and temporally), and examine correlations between water quality, toxicity and phytoplankton data. Recommendations for future red tide monitoring efforts are also included.

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2.0 METHODS

2.1 Sampling Locations

A total of 43 stations were established within Casco Bay between the Fore River, Portland (at the western end of the bay) and Small Point in Phippsburg (at the eastern end; Figure 1). All 43 stations were located by GPS coordinates and recorded on MER's onboard Garmin Model 182 GPS chartplotter. Sampling was conducted on a series of 2-day surveys with 28 stations sampled on Day 1 in western Casco Bay and 15 stations sampled on Day 2 in eastern Casco Bay, shown in Figure 1. Three of the stations sampled on Day 2 represented existing stations (red dots) routinely sampled by DMR. These stations were included to allow comparison of boat-based and land-based sampling results. All other stations represented new stations that intensified the spatial proximity of sampling; most stations represented specific, individual harvesting areas (yellow or green stars). Six of the stations were located "offshore" adjacent to islands (blue triangles) to allow comparison of the chronology of inshore and offshore blooms.

Initial establishment and recording of sampling locations, along with collection of the first water column profiles, water samples for nutrient analysis, and vertical phytoplankton tow sampling was done on April 11 and 12, 2006, prior to bloom development. No mussel samples for toxicity testing were collected during the initial setup. Ten surveys for the collection of mussels and additional data and water samples were completed between May 9 and July 27, 2006. Full sampling was completed on all but two occasions, June 8 and July 11, when inclement weather rendered sampling unsafe.

2.2 Field Sampling procedures

The primary objective of the intensified sampling effort was to provide DMR with additional mussel tissue for biotoxin analysis from a larger number of sites, all taken within a short period of time, thereby providing "picture in time" sampling. The presence of the sampling crew at a variety of locations around the bay also offered the opportunity to collect additional data to be correlated with the incidence and levels of biotoxin found. This additional sampling included water samples for nutrient analyses, vertical-tow phytoplankton samples, and multi-parameter water column profile collection using a YSI 6600 profiler.

2.2.1 Mussel collection for biotoxin determination

The DMR Paralytic Shellfish Poisoning (PSP) toxicity testing protocol requires a minimum of 100g of mussel tissue per station for analysis. Initially, mussels were to be collected just subtidally, *i.e.* -1 to -2 feet MLW, either using a 9"x9" Ponar benthic grab sampler or a modified sea moss harvesting rake equipped with a catch basket and pole of sufficient length to allow sampling of -1 to -2 feet MLW irrespective of tide stage. However, an attempt to collect mussels using these methods during the initial station establishment runs of April 11 and 12 proved excessively time-consuming and unreliable; at certain sites, particularly at exposed, rocky island stations, no mussels were available for sampling.

An alternative buoy-based mussel collection method was developed to insure a reliable and adequate amount of mussel tissue at each collection (Figure 2). This was a modification of the method of Keafer et al., (2004). Accordingly, a buoyed 9-thread pot warp line (of sufficient length to allow retrieval at high water) moored either to a common cinder block or 25-pound mushroom anchor (offshore stations) was deployed at each station. PSP-free mussels (as determined by DMR routine sampling) were collected by DMR representatives. A sufficient number to provide an adequate amount of tissue for PSP testing were placed in meshed lobster bait bags on ice in a cooler prior to attachment to the buoyed lines. Bagged mussels were periodically delivered to MER either the night before or on the morning of a sampling date for attachment the same day or the following day.

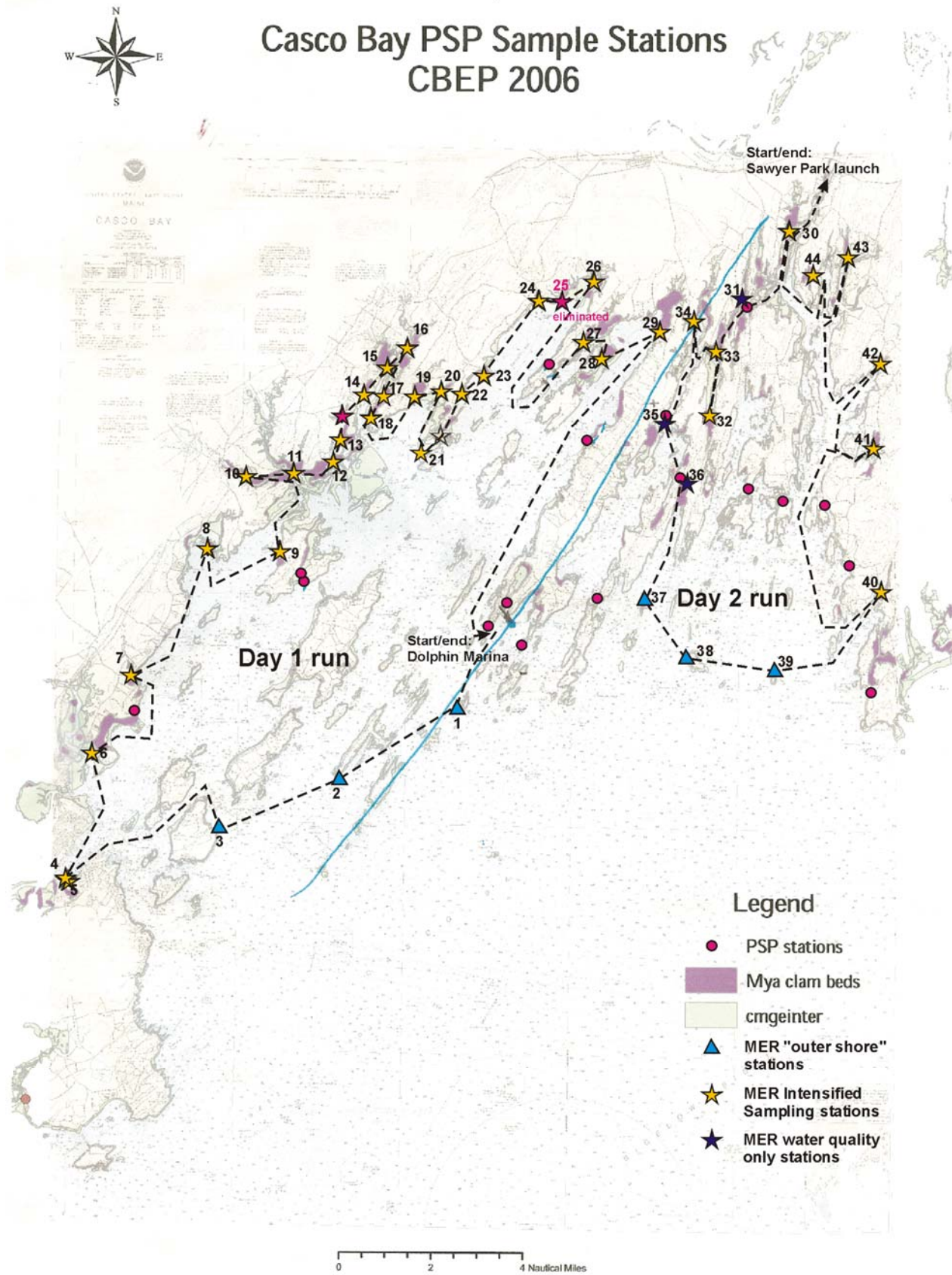


Figure 1. Intensified Sampling Station Location and Day-run Map

The first set of 4 bags of mussels per line was set out on April 20 and 21. The mussel-filled bait bags were attached to the buoyed lines by passing the bait bag drawstring through the lay of the rope, followed by two half-hitches to insure secure attachment of the bags to the lines even under rough sea conditions (Figure 2). The first collection of mussels for testing was done on May 9 and 10. At each station a bag of mussels was removed, the bag opened, and a plastic tag bearing the station number inserted into the bag to insure proper station identification by the testing laboratory. After insertion of the tag, the bag was closed and placed on ice in a cooler; at the end of each run day custody of the mussels was transferred to DMR for delivery to the PSP testing laboratory in Boothbay Harbor the following day.

According to the initial plan, which called for up to eight weeks of sampling, four bags were to be placed on each line at the start of the project and a single bag removed each week for three weeks, after which four more bags would be added to each line with which to complete the project. However, two to three weeks into the project, as the *Alexandrium* bloom spread more widely across the state, it became increasingly difficult for DMR to locate PSP-free mussel in any large quantity and the number of bags available for replacement was limited. Since there were 28 stations in the western bay and only 15 in the eastern bay area, available bags on any given sampling day had to be spread more thinly amongst the western stations than the eastern stations, consequently resulting in fewer bags per line at western stations. On June 7, there was only one bag left at the western stations (PSP-1 through PSP-18). This bag was collected on June 15 and new bags were added. Thus, the June 20 sampling at stations 1-18 collected bags that had only been deployed for one week. This problem was resolved toward the middle of June when sufficient PSP-free mussels once again became available to allow full stocking of nearly all stations in both the western and eastern sections of the bay. However, the different deployment times for each bag have the potential to add to the variability in the toxicity data.

2.2.2 Dissolved inorganic nitrogen (DIN)

Water samples were collected according to the Standard Operating Procedure developed by Friends of Casco Bay (FOCB), a brief step-by-step summary of which is included in Appendix A. Sampling was done by MER staff having received training from FOCB staff. Water samples for nutrient analysis were taken just below the surface and in a manner to reduce the possibility of sample contamination from surface scum or debris or sediment disturbances using a 50cc syringe. The syringe was rinsed with sample water three times prior to attaching a 35µm disposable filter. After rinsing the syringe, the filter was attached and water drawn in and a small amount of water dispensed into the sample vial, the vial capped, and the sample shaken to rinse the vial; this was repeated three times before securing a final sample. Once the final sample was secured, the sample was dispensed through the filter into the rinsed, pre-labeled sample vial (Figure 3). The sample was immediately preserved by the addition of two (2)



Figure 2. Buoy-line assembly and attached mussel-filled bags



Figure 3. Nutrient sample collection technique

drops of chloroform to the sample vial. The sample vial was then placed in a pre-labeled plastic sealable-type bag, and placed on ice in a cooler. Custody of the samples was transferred to DMR at the end of each run day, along with the mussels, to be placed in a freezer at DMR shortly after sample collection for storage pending delivery to the processing laboratory.

As of January 2007, the nutrient data were unavailable and have not been included in this report. It is anticipated that they will be available later this winter and would then add to characterization of the bloom and water quality from April to July 2006.

2.2.3 Vertical phytoplankton tows

Vertical phytoplankton tows were conducted by DMR staff at each station by lowering and raising a 20- μ m mesh phytoplankton net through the water column 10 times between the surface and a depth of approximately 15 ft; at certain stations at low water the depth of the drop had to be reduced to avoid collection of sediment in the sample. Once on the surface the net was held vertically and rinsed, the glass, cod-end sample jar removed from the net, and the sample placed on ice in a cooler (Figure 4). Phytoplankton samples were handled entirely by DMR personnel from collection through delivery to the DMR laboratory, according to DMR's Standard Operating Procedures.



Figure 4. Phytoplankton net tow

Although the method of collection was over a standardized depth and collection time, the volume of water sampled was not measured so these are considered qualitative samples. Presence/absence and %abundance are how DMR currently looks at the data (pers. comm. Allison Sirois, DMR). The total phytoplankton counts and *Alexandrium* counts have been used in this report to compare across stations, but the data should not be compared to other programs or areas.

2.2.4 Water column profiles

Water column profiles were taken by MER personnel using an YSI 6600 series sonde equipped with a pressure sensor to measure depth, a temperature-conductivity (salinity) sensor, a dissolved oxygen sensor, a pH sensor, and a self-wiping turbidity probe. Data collected included depth (m), temperature ($^{\circ}$ C), salinity (psu), dissolved oxygen (mg/L and % saturation), and turbidity (NTU). Due to problems with the

dissolved oxygen sensor those data are not evaluated in this report. The report focuses on the temperature and salinity *in situ* data. Data for each cast were stored on internal memory, but data collection was monitored using an YSI MD650 handheld digital display unit. The data collection interval was set at 0.5 seconds and the rate of descent maintained at approximately 0.1 to 0.2 m/sec. Data were collected only as the sonde descended through the water column (downcasts). Replicate casts were initially planned for each station on each sampling date, as per the MER's SOPs for use of the YSI 6600 profiler and data logging procedures (refer to Appendix B). Single rather than replicate casts were made during most of the surveys due to memory and time constraints. On those surveys where replicate casts were made there was essentially no difference between the casts. Sonde data were downloaded onto a PC at the end of each run day and exported into an Excel[®] spreadsheet to allow further analysis and graphing.

2.2.5 PSP Toxicity Testing

Shellfish samples must consist of at least 12 individuals large enough to yield a minimum of 100g of shucked meats. Samples were transported to the DMR PSP testing lab on ice accompanied by a chain of custody form indicating the sampler, the date and time of collection, location of sample collection, and the species. According to the PSP laboratory protocol, upon arrival at the lab, the receiving staff member signs for the samples, records the time of delivery and temperature of the samples, logs them into the Sample Log, and places them in the sample refrigerator. Samples are maintained at 0°C to 4°C and are processed within 24 hours of receipt.

The toxicity testing process involves several steps. First, one shellfish sample at a time is placed in a rinsing screen. The shellfish are separated and rinsed thoroughly with tap water. Following rinsing, each shell is opened with a shucking knife, and the adductor muscle and any connective tissue is cut as close to the shell as possible to allow removal of the whole animal from the shell without slicing into the main body tissue. Once all of the shellfish from the sample have been shucked, the meats are thoroughly rinsed with tap water to remove sand, shell, and foreign material. The rinsed meats are then transferred onto a #10 mesh sieve, arranged in a single layer, and allowed to drain for 5 minutes. Once drained, the shucked meats are placed in a clean blender jar and blended for 60 to 120 seconds to produce a uniform homogenate. The homogenate is then processed according to a boiling-acid AOAC PSP extraction method. An aliquot of the extracted material is centrifuged for 5 minutes, and the supernatant decanted into a clean tube. A Mouse Bioassay is performed following standard protocols, using the supernatant extraction, to determine µg of saxotoxin (STX) equivalents/100g of shellfish tissue.

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3.0 DATA RESULTS

The data fall into a number of different categories based on the sampling methods used (*in situ* profiles, near surface mussel deployments, and phytoplankton tows). The *in situ* downcast profiles have not been examined on an individual basis in this report. Instead, surface (<2 m) and average *in situ* values were calculated from the downcast data. A table of these surface and average *in situ* values and the associated toxicity and phytoplankton (total and *Alexandrium*) is presented in Appendix C. Since there was little difference between the surface (<2 m) and average *in situ* values and the station depths ranged from 1.7 m to 23.4 m, the surface values have been used for all analyses in this report.

3.1 Temporal Patterns

3.1.1 PSP Shellfish Closures

The 2006 *Alexandrium* bloom began with a relatively early closure of the Lumbo's Hole area of Harpswell Sound on April 25th, which was about a week earlier than the 2005 closures. This area is suspected of having a local, self-seeding population or seedbed of *Alexandrium* cells and is typically one of the first closures in western Maine. The first soft-shell clam closure was put in place on May 11th closing much of Casco Bay to shellfishing (Figure 5), but due to the efforts of the intensified PSP monitoring program many of the upper bays from the Harraseeket River west to the New Meadows River remained open to harvesting. On June 14th, all of the western Maine waters from Cousins Island in Casco Bay to the NH border were closed, but the upper reaches in eastern Casco Bay remained open. On June 22, there was a slight modification to the closures, shifting the line in Harpswell Sound from Ewing Narrows to the north to a line from Otter Brook Rd. to Prince Point (Figure 5). By June 28th, the bloom had begun to decline and PSP toxicity decreased below regulatory levels in western Casco Bay. DMR was able to open many areas of the bay to shellfish harvesting by late June (Figure 5). The soft-shell clam closure covering western Maine (NH border to Port Clyde including waters of Casco Bay in bottom panel of Figure 5) was lifted on July 13th.

3.1.2 IPSP Monitoring Program Data

The spring of 2006 was one of the wettest springs on record in New England causing major flooding in many areas. Major rain events occurred throughout the May-July sampling period with seven events totaling over one inch of precipitation at the Portland Jetport (Figure 6). The rains led to widespread bacterial closures of shellfishing areas that "lessened" the impact of PSP closures, but restricted the harvesting of shellfish nonetheless. The storms that brought these rains also pushed offshore waters toward shore on occasion. Wind and current data from the Gulf of Maine Ocean Observing System (GoMOOS) buoys was monitored closely by regional resource managers and scientists. Figure 7 shows the direction and strength of prevailing winds and surface water currents at GoMOOS buoy C off Casco Bay from March to August 2006. Two features of interest are the northeasterly winds in early and mid May and again in early June. These strong Nor'easters resulted in current flows that were alongshore brining coastal Gulf of Maine waters into Casco Bay from the northeast.

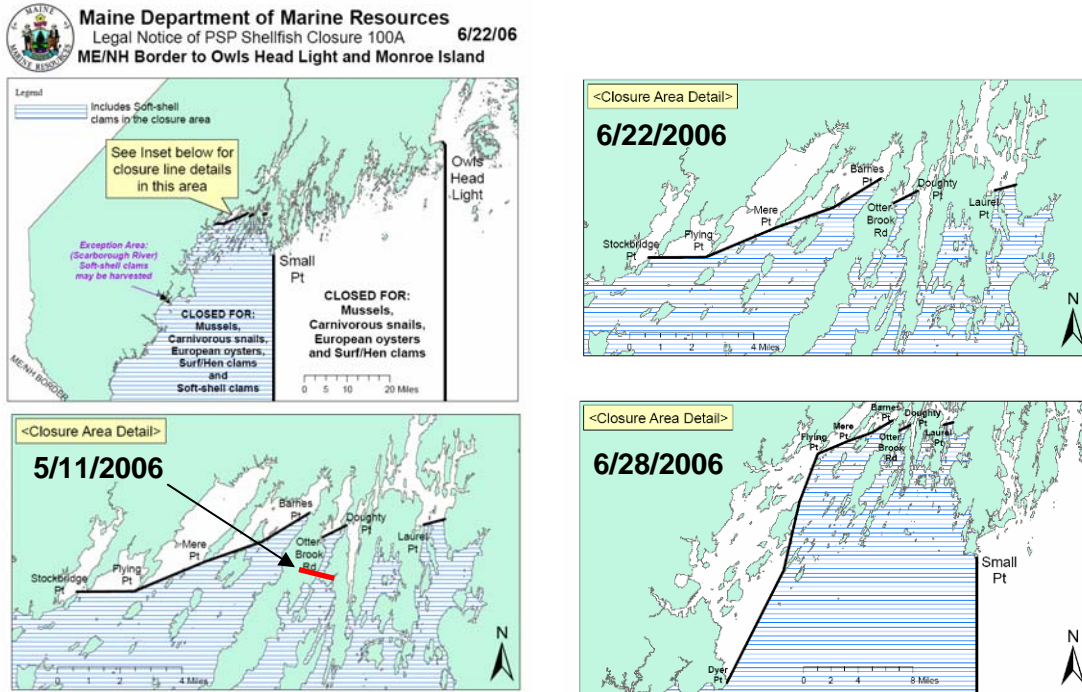


Figure 5. Maine DMR PSP shellfish closure maps for western Maine and Casco Bay from May 11th, June 22nd, and June 28th. Note that the original May 11, 2006 notice is not available. The only change from May 11 to June 22 is the expansion to the north in the Harpswell area above Ewing Narrows (red line represents May 11th closure boundary).

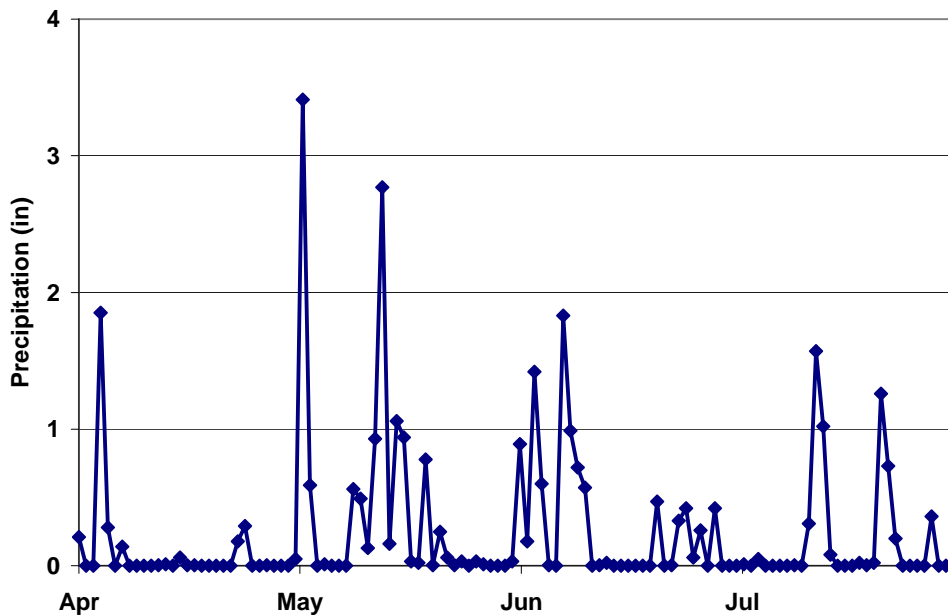


Figure 6. Precipitation (in) at Portland International Jetport (April-July 2006)

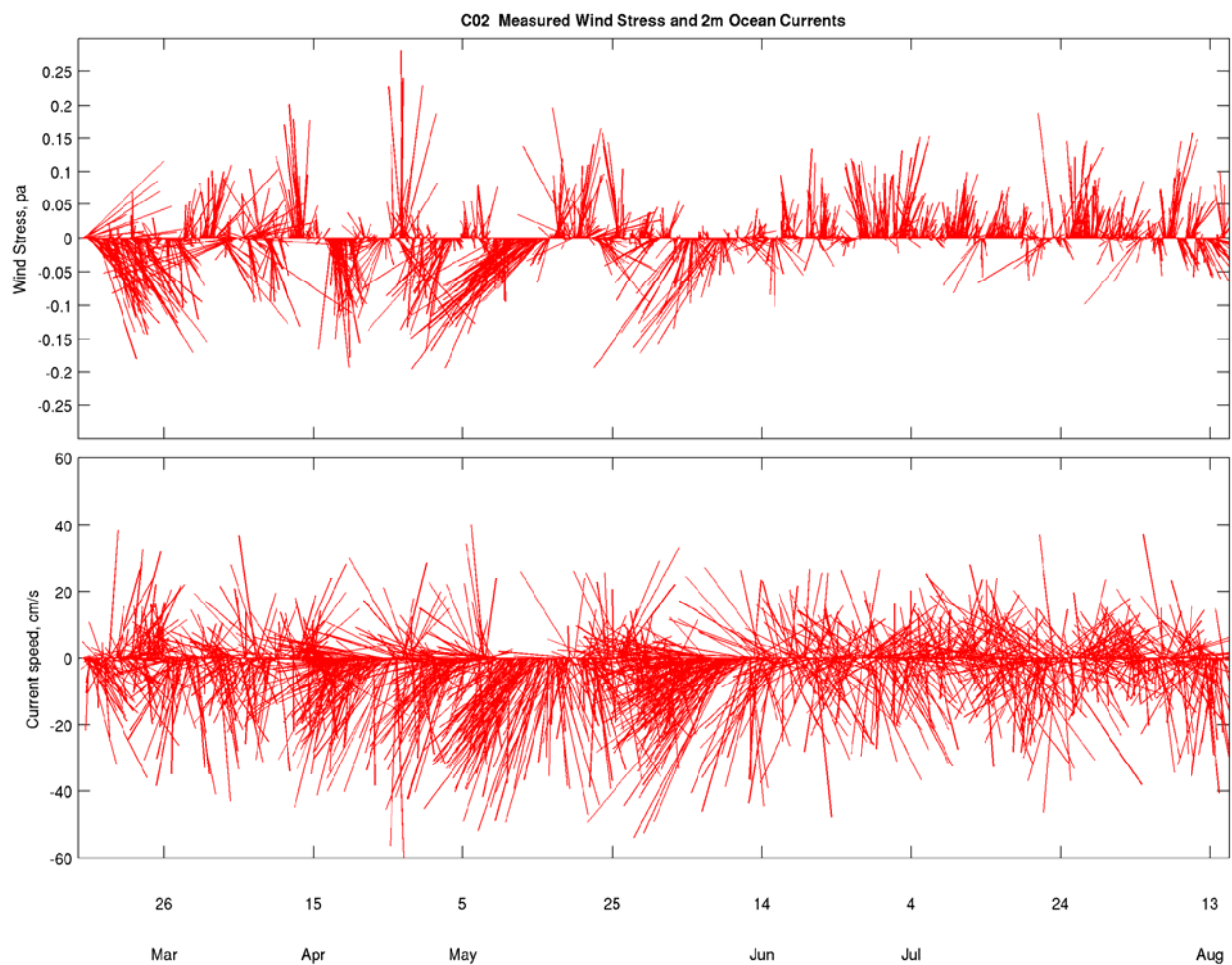


Figure 7. Wind and current data from GoMOOS buoy C in Casco Bay (April-July 2006). Plot provided by Physical Oceanography Group, University of Maine (<http://gyre.umeoce.maine.edu/gomoos.php>)

Surface water temperatures ranged from $\sim 5^{\circ}\text{C}$ in April at the offshore and Portland Harbor stations (1-5) to $>22^{\circ}\text{C}$ in the upper reaches of the New Meadows River in late July. Time series plots of temperature suggest more temporal than spatial variability across the bay. Although there is a general pattern of increasing temperatures by date, the May to July surveys appear to break out into three separate temperature regimes. Surface water temperatures were around $10\pm 2^{\circ}\text{C}$ during the first three May surveys, $15\pm 2^{\circ}\text{C}$ for the 5/31 to 6/16 surveys, and $20\pm 2^{\circ}\text{C}$ for the remaining surveys. The only exceptions are the offshore and Portland Harbor stations that remained cooler than the other areas during late June and July.

In contrast, surface salinity values showed a number of clear temporal and spatial patterns based on station data (Figure 8). Lower salinity values were consistently observed in Portland Harbor (stations 4 and 5 are influenced by the Fore River and station 5 is in very close proximity to the South Portland Sewer District's Fore River outfall) and in association with the Presumpscot (station 6) and Royal (stations 10 & 11) rivers. The lowest values at these stations (<15 PSU) were observed on the May 16-18 survey which was conducted after a five day period when ~ 6 inches of rain had fallen in the area (see Figure 6). Other spikes in salinity observed at these stations were also associated with rain events. The surveys in mid and late June had consistently lower salinities across all stations (except those directly influenced by riverine runoff). The four stations in eastern Casco Bay most exposed to offshore waters (stations 37-40) exhibited much lower salinities than non-riverine stations. This is suggestive of an offshore influence via the Kennebec River plume and will be looked at further in the next section focused on spatial characterization.

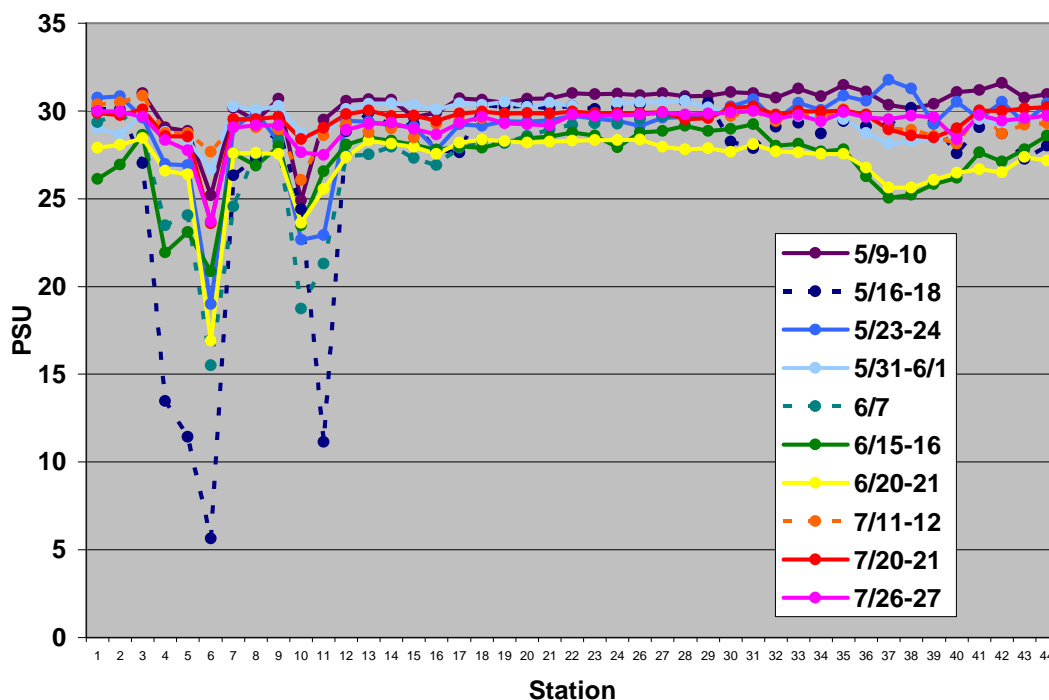


Figure 8. Surface (<2 m) water salinity by station during the ten May to July surveys

As with salinity, there are some clear spatial and temporal patterns in the shellfish toxicity data (Figure 9) though the two are not correlated. The lower salinity water observed in eastern Casco Bay during the June 15-16 and June 20-21 surveys are coincident with elevated toxicity. However, the highest toxicity values are well up in Harpswell Sound and New Meadows River and apparently are not directly associated with any Kennebec River/offshore water influence. A statistical comparison of the salinity and toxicity data using the non-parametric Spearman's rank correlation analysis indicated that there was a significant inverse correlation between the two parameters ($P=0.02$), but the correlation coefficient is very low (-0.12) suggesting that it is a very weak relationship. A subsequent analysis was done after removing

the river influenced stations 4, 5, 6, 10 and 11, and showed a similar finding. The non-river correlation was significant ($P=0.0015$) and suggested a slightly stronger relationship between the parameters (correlation coefficient of -0.18), but certainly is not indicative of a conclusive finding.

The toxicity data show an early toxicity event during the May 9-10 survey at the “outer shore” stations in eastern Casco Bay (37-39) and in the lower reaches of the New Meadows River (stations 40-42). These stations remained above $100 \mu\text{g STX equiv./100g}$ on May 16-18 and elevated toxicity was also measured at “outer shore” stations 1 and 2 that are located off of some of the outer islands of the bay further to the west. These two stations were not sampled on May 9-10 so the westward extent of the bloom at the offshore stations is not known for that survey. Station 4 in Portland Harbor also had toxicity $>80 \mu\text{g STX equiv./100g}$ on May 16. By the time of the next survey (May 23-24), toxicity levels in the New Meadows River had increased sharply and remained elevated at the offshore stations. Toxicity $>80 \mu\text{g STX equiv./100g}$ was also showing up at the three stations in Middle Bay (27-29). These four areas – offshore island stations in western Casco Bay, offshore stations in eastern Casco Bay, Middle Bay and upper Harpswell/New Meadows River – were the only areas where toxicity levels above the $80 \mu\text{g}$ regulatory threshold were observed for the rest of the surveys. Except for the one measurement of $103 \mu\text{g STX equiv./100g}$ at station 4 in Portland Harbor, all toxicity values at stations 4 to 26 (Portland Harbor east to Maquoit Bay) were $<80 \mu\text{g STX equiv./100g}$.

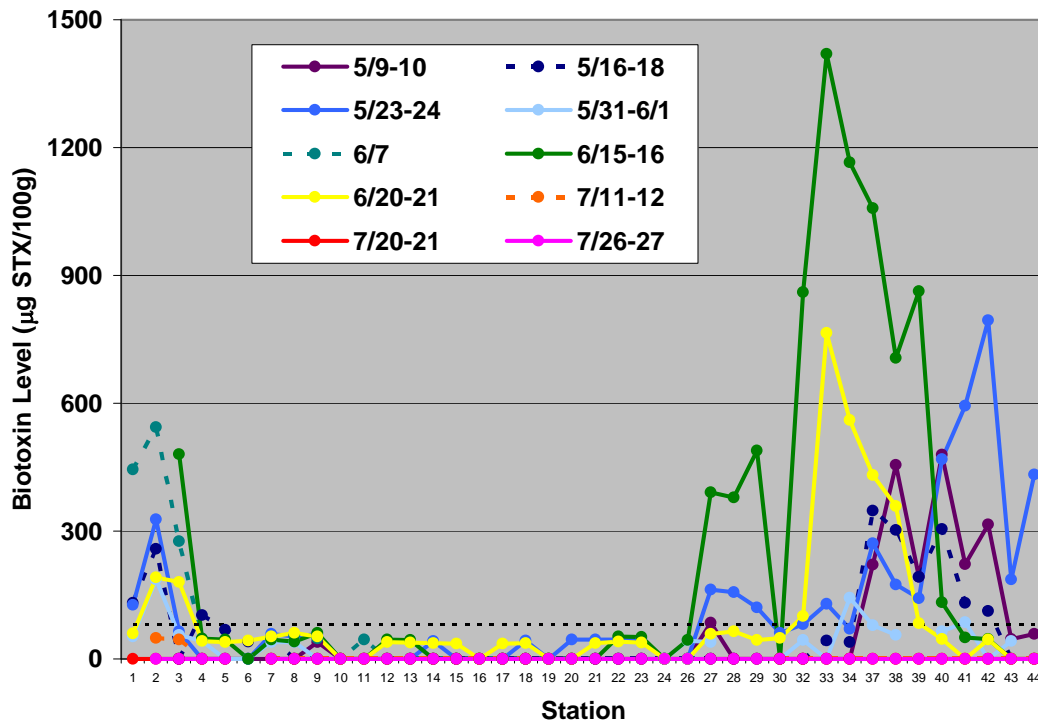


Figure 9. Biotoxin levels ($\mu\text{g STX equiv./100g}$ tissue) by station during the ten May to July surveys. The dotted black line denotes the $80 \mu\text{g STX}$ regulatory threshold. (Note stations 31, 35 & 36 are not shown as they are water quality only stations see Figure 1)

Toxicity levels decreased from mid to late May, but were still above the regulatory threshold at a few stations. On June 7 only the western Casco Bay stations were sampled. The offshore stations 1-3 exhibited very high toxicity suggesting that values were also likely high at the eastern bay offshore stations. Maximum toxicity measurements were made during the June 15-16 survey reaching $1,420 \mu\text{g STX equiv./100g}$ at station 33 and $>700 \mu\text{g STX equiv./100g}$ at offshore stations 37-39 and stations 32 and 34 (Figure 9). Middle Bay stations also peaked at levels of $380-490 \mu\text{g STX equiv./100g}$ and

comparable levels were also measured at station 3 off of Cushing Island (stations 1 and 2 were not sampled during this survey). By June 20-21 toxicity levels had decreased by ~50% in these waters, but still remained well above the regulatory threshold. The June 20-21 survey was hampered by having mussel bags deployed for only one week at stations 1-18. It is unclear how this shortened deployment time impacted the data, but the decrease in levels at stations in eastern Casco Bay (not impacted by the shortened deployment) and the comparable decrease in levels at station 3 suggests that the impact was minimal. Great effort will be taken to avoid this issue during future monitoring studies. By July 11-12, all toxicity levels were below the regulatory threshold and the only measurable toxicity was measured at stations 2 and 3 (<50 µg STX equiv./100g). No toxicity was detected during the last two surveys in July.

The phytoplankton data are semi-quantitative because they were taken using a plankton net, which can clog and thus prevent accurate flow measurements. As a result, the method focused on key species and the three most abundant species while counting a limited set of fields. However, given the consistent methods used and the lack of obvious clogging of the nets the data are internally comparable and are examined on a station-to-station basis over the May to July surveys. The phytoplankton data are presented and discussed in terms of relative abundance units (RAU) rather than the more quantitative cells per unit volume. The most obvious patterns that are shown in Figure 10 are the differences in western and eastern Casco Bay total phytoplankton and *Alexandrium* counts. Total phytoplankton counts are much higher in western Casco Bay with the highest relative abundance value (1,200 RAU) seen in Portland Harbor at station 4 in mid-July. Total phytoplankton counts were routinely above 200 RAU at stations 1-29 during many of the surveys, while counts ≥ 200 RAU were only measured for four instances in western Casco Bay with a maximum of 307 RAU at station 37 (outer shore) in mid-May. Also there was a relatively consistent level of elevated phytoplankton counts at stations from Yarmouth (station 12) to Middle Bay (station 29) observed during the June 20-21 survey (Figure 10a). These counts were dominated by the diatom *Skeletonema costatum* and suggest a nearshore diatom bloom of this species in western Casco Bay. Total phytoplankton and *Skeletonema* counts were dramatically lower at the eastern Casco Bay stations (30-44). This serves to highlight the different water masses and complex flow within Casco Bay.

An opposite pattern was observed for the *Alexandrium* with higher relative abundance generally seen in eastern Casco Bay and offshore (Figure 10b). There was an early spike in *Alexandrium* counts during the May 9-10 survey, which included the maximum relative abundance for the program of 33 RAU at station 41 in New Meadows River. Elevated counts were also seen at other stations in the offshore waters of eastern Casco Bay and New Meadows River and were concomitant with elevated toxicity levels (Figure 9). Elevated *Alexandrium* counts continued to be observed at stations in eastern Casco Bay on May 16-18. These first two May surveys were the only times that *Alexandrium* counts of >5 RAU were measured at western Casco Bay stations 4 through 29. From late May to mid June, *Alexandrium* counts were elevated at the offshore stations 1 and 2 in western Casco Bay. During the June 15-16 survey, a second major peak in *Alexandrium* was observed in eastern Casco Bay that was coincident with the peak in toxicity (Figure 9) though *Alexandrium* was nearly absent from the offshore stations 37-39 that showed high toxicity. By June 20-21, *Alexandrium* counts were ≤ 2 RAU at all stations except station 44 in upper New Meadows River. These low relative abundances and the decrease in toxicity measured during this survey compared to the June 15-16 survey suggest that the end of the *Alexandrium* bloom occurred in mid June. Although some of the patterns in toxicity and *Alexandrium* data appear to be related, statistical analyses (Spearman's rank correlations) indicated that there was no correlation between *Alexandrium* relative abundance and toxicity. This could be due to a number of factors including the non-quantitative nature of counts, possible clogging of the phytoplankton sampling net, rapid toxin uptake and depuration rates of mussels, and errors that may have been due to varied deployment times of mussels.

3.2 Spatial Characterization

Temperature patterns across the bay showed typical inshore to offshore gradients of decreasing temperatures (see Appendix D). The warmest temperatures were consistently observed in the upper reaches of the embayments from Maquoit Bay east to New Meadows River. Water temperatures decreased to the southern extent of the bay and offshore to the east. Salinity patterns across the bay were more complex and driven by a combination of the river and outfall inputs to Portland Harbor (Fore River) and at stations near the mouths of the Presumpscot and Royal Rivers and the influence of offshore waters (Figure 11). The offshore waters exhibited inputs of both more saline waters into the bay (e.g. 5/23-24 and 7/11-12) or fresher waters likely due to the influence of the Kennebec River plume (e.g. 6/15-16 and 6/20-21). It had been hypothesized that there might be a relationship between salinity and toxicity or *Alexandrium* abundance, but given the varied inputs of low salinity waters (river and offshore riverine plume) and the combination of both higher and lower salinity water inputs into the bay from offshore, only a weak inverse correlation was observed. This analysis is tenuous and collection of more fully resolved and quantitative datasets in the future may provide conclusive results.

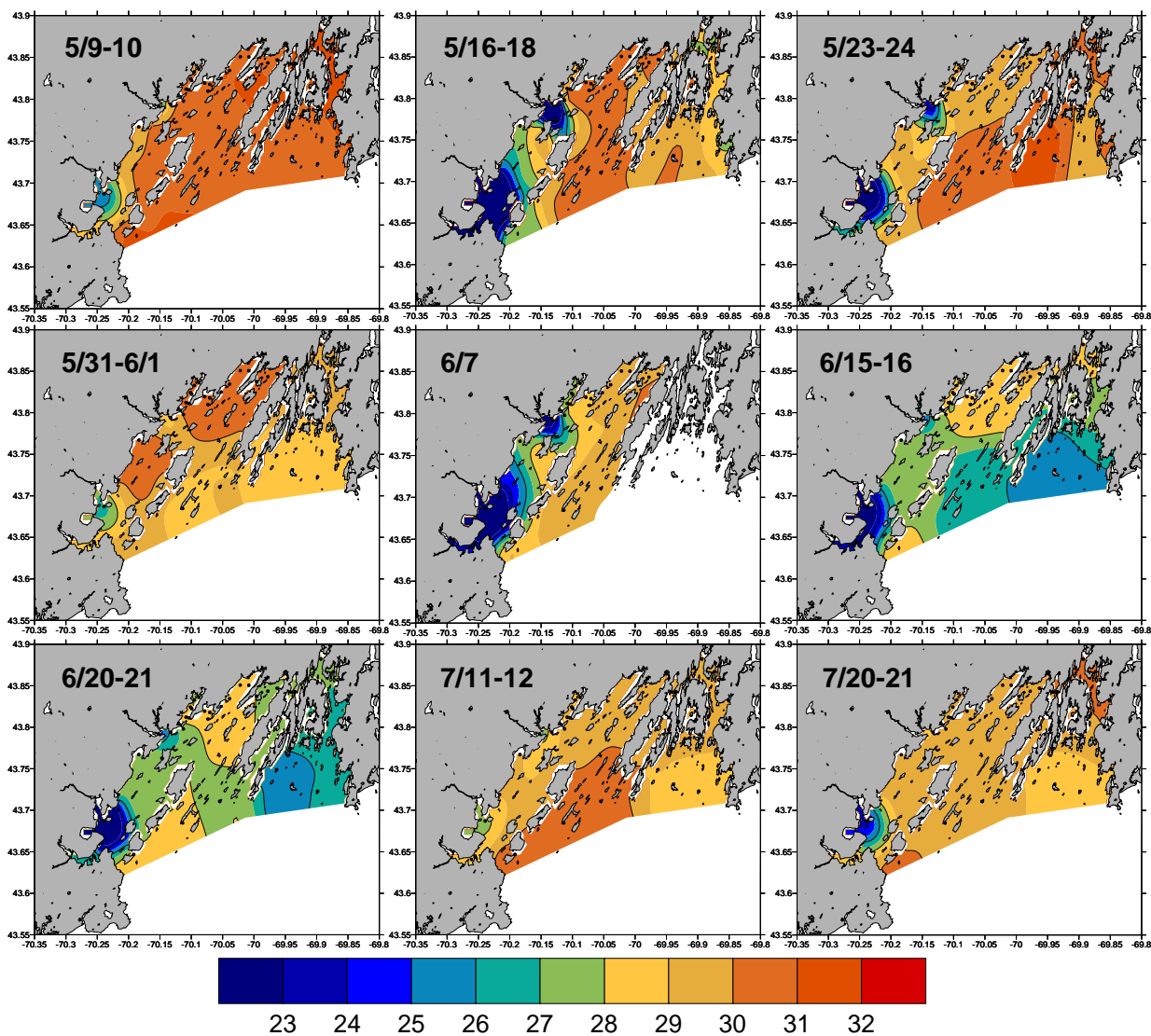


Figure 11. Contours of surface water salinity (PSU) during the nine surveys from May 9-10 to July 20-21.

The trends in toxicity discussed above are illustrated in Figure 12. Elevated toxicity levels were observed in eastern Casco Bay during the first survey in May 9-10 (no mussels were collected/analyzed for this study during the April 11-12 preliminary survey). Toxicity continued to be observed in eastern Casco Bay and New Meadows River during the remaining May surveys and was also seen at the offshore stations on islands in western Casco Bay. The toxicity peaked during the June 15-16 survey with levels >800µg STX equiv./100g at stations in Harpswell Sound and Cove and offshore stations in eastern Casco Bay. By late June, toxicity had decreased sharply and was well below 50 µg STX equiv./100g at all stations by July 11-12 (only detectable at stations 2 and 3 off of Cliff and Cushing Islands, respectively).

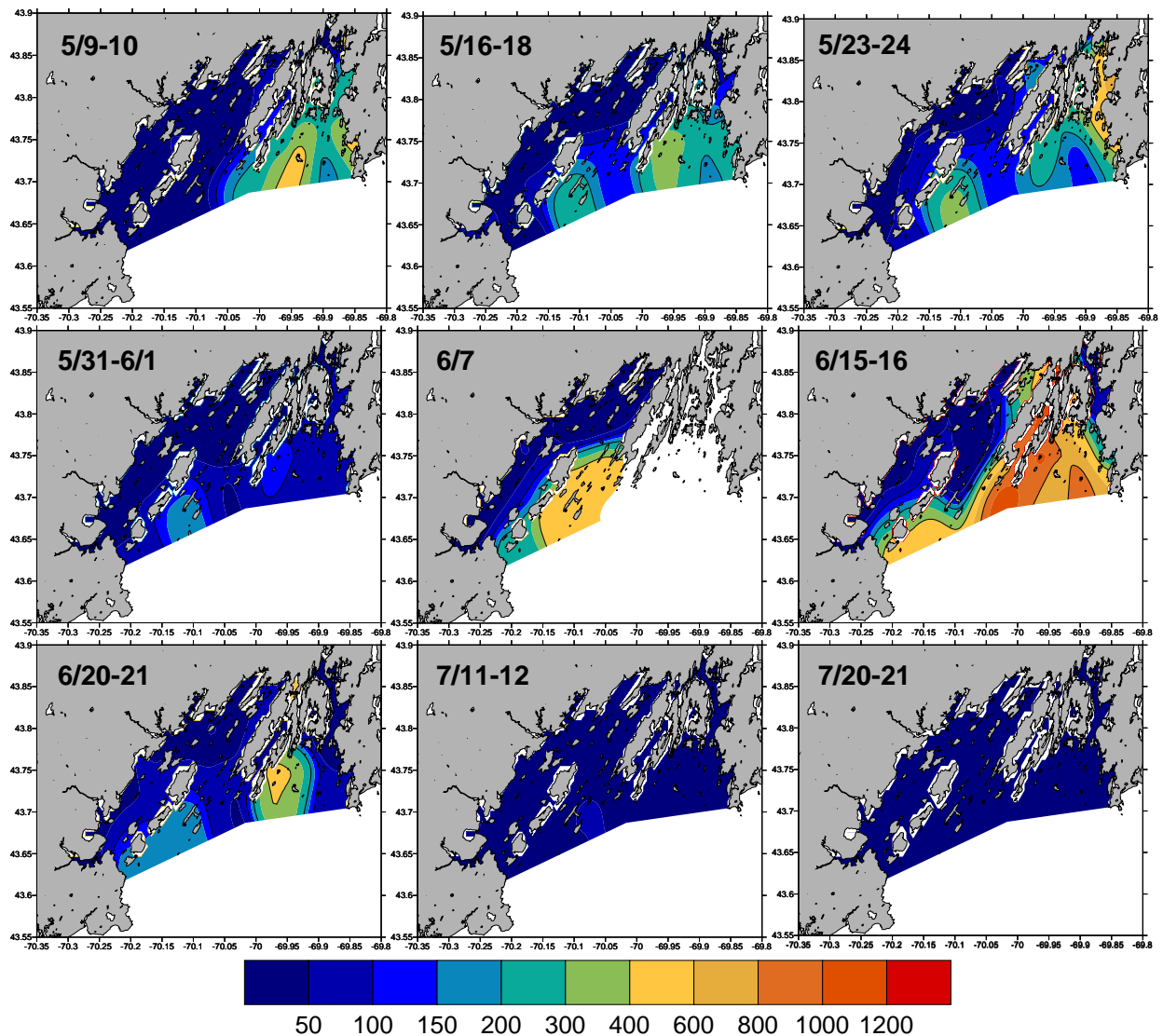


Figure 12. Contours of biotoxin levels (µg STX equiv./100g tissue) during the nine surveys from May 9-10 to July 20-21.

The eastern Casco Bay stations were not occupied during the June 7 survey due to inclement weather. This is unfortunate as toxicity peaked at offshore stations 1 and 2 on June 7th (445 and 544 µg STX equiv./100g, respectively) and may have been higher in eastern Casco Bay as well. *Alexandrium* net tow counts were low but highest offshore (11 RAU at station 1). Scientists from Woods Hole Oceanographic Institute (WHOI) were conducting a major survey effort in the Gulf of Maine in early to mid June 2006. Their quantitative data on surface *Alexandrium* counts are presented in Figure 13. The maximum *Alexandrium* abundance for the June 6 to 12 leg of the survey was 5,516 cells/l and it was collected just to

the west of Small Point in eastern Casco Bay on June 8th. High *Alexandrium* abundances (>1,000 cells/l) were observed from Cape Ann east to Port Clyde. The return leg of the WHOI survey was conducted a week later (June 13th), *Alexandrium* abundances had decreased off of Casco Bay to <400 cells/l with higher abundances observed to the south (Figure 13). As mentioned above, toxicity levels peaked during June 15-16 reaching levels of >800 µg STX equiv./100g in eastern Casco Bay although *Alexandrium* net tow counts were much lower only reaching 12 RAU at station 2 near Cliff Island. Qualitatively it is interesting to note that although *Alexandrium* counts were low in the Casco Bay dataset the relative contribution of *Alexandrium* (percent of total count) was very high throughout eastern Casco Bay (Figure 14). The WHOI data are presented to highlight a number of points. The first is to emphasize the magnitude of the regional bloom, which in combination with the toxicity and qualitative counts in Casco Bay data suggests a direct input of offshore cells on at least the outer waters of the bay. Additionally, in order to compare across coincident datasets similar methods should be used; at a minimum quantitative *Alexandrium* counts are needed.

A comparison of data from the June 15-16 and June 20-21 IPSP surveys shows some of the trends discussed earlier (Figures 14 and 15). The western and eastern Casco Bay areas show clear differences in all 4 parameters presented in these figures. Clearly, even though the *Alexandrium* relative abundance counts are low, the toxic dinoflagellate represents a more dominant portion of the total phytoplankton community in eastern Casco Bay than in western waters. Given the high toxicity (especially on June 15-16) and the counts from the WHOI cruise, it is likely that the net tow counts are off by a couple orders of magnitude. Quantitative counts would certainly have provided better insight on the toxicity vs. cell abundance relationship. The apparent bloom of *Skeletonema* (which dominated total phytoplankton counts during these surveys) started in Maquoit Bay on June 15-16 and covered much of the inshore waters of western Casco Bay by June 20-21.

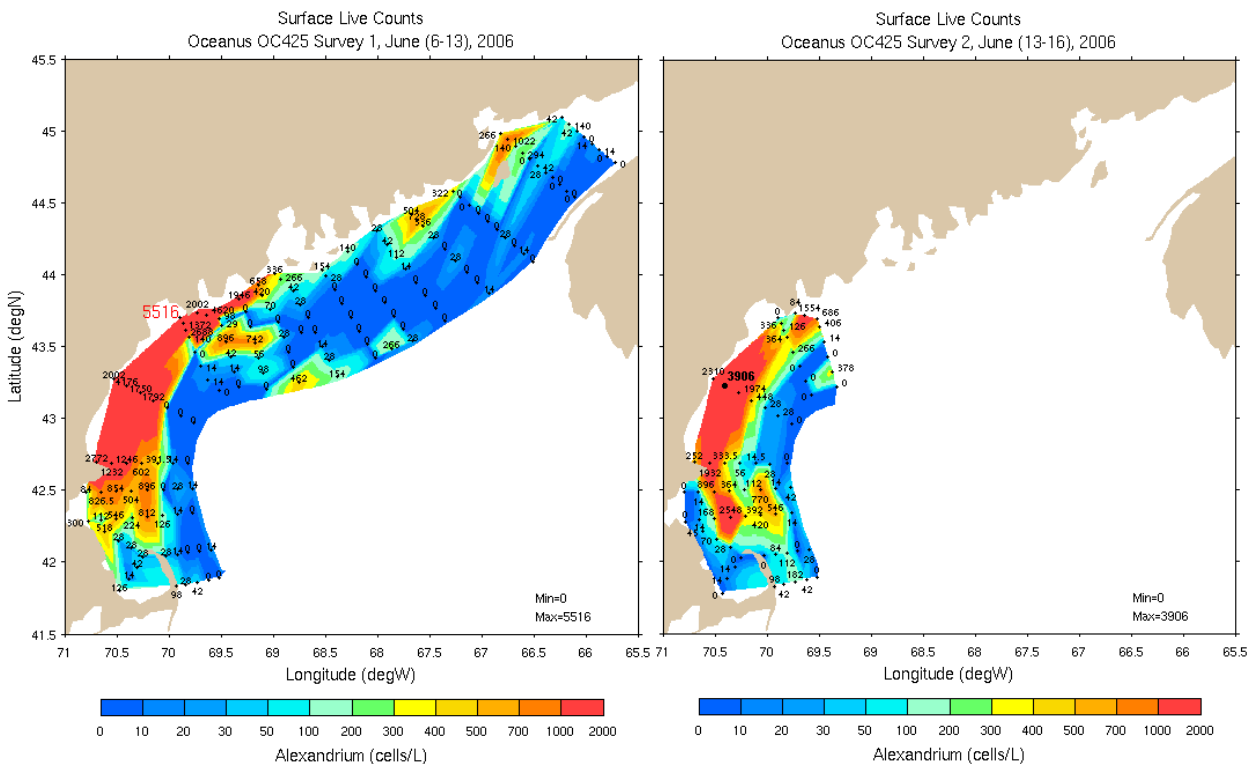


Figure 13. Surface water microscope counts of *Alexandrium* (cells/l) aboard the *R/V Oceanus* on June 6-13 and June 13-16, 2006 (Anderson, McGillicuddy, Keafer, unpublished data available at http://science.whoi.edu/users/olga/alex_surveys_2006/WHOI_Alexandrium_Surveys_2006.html)

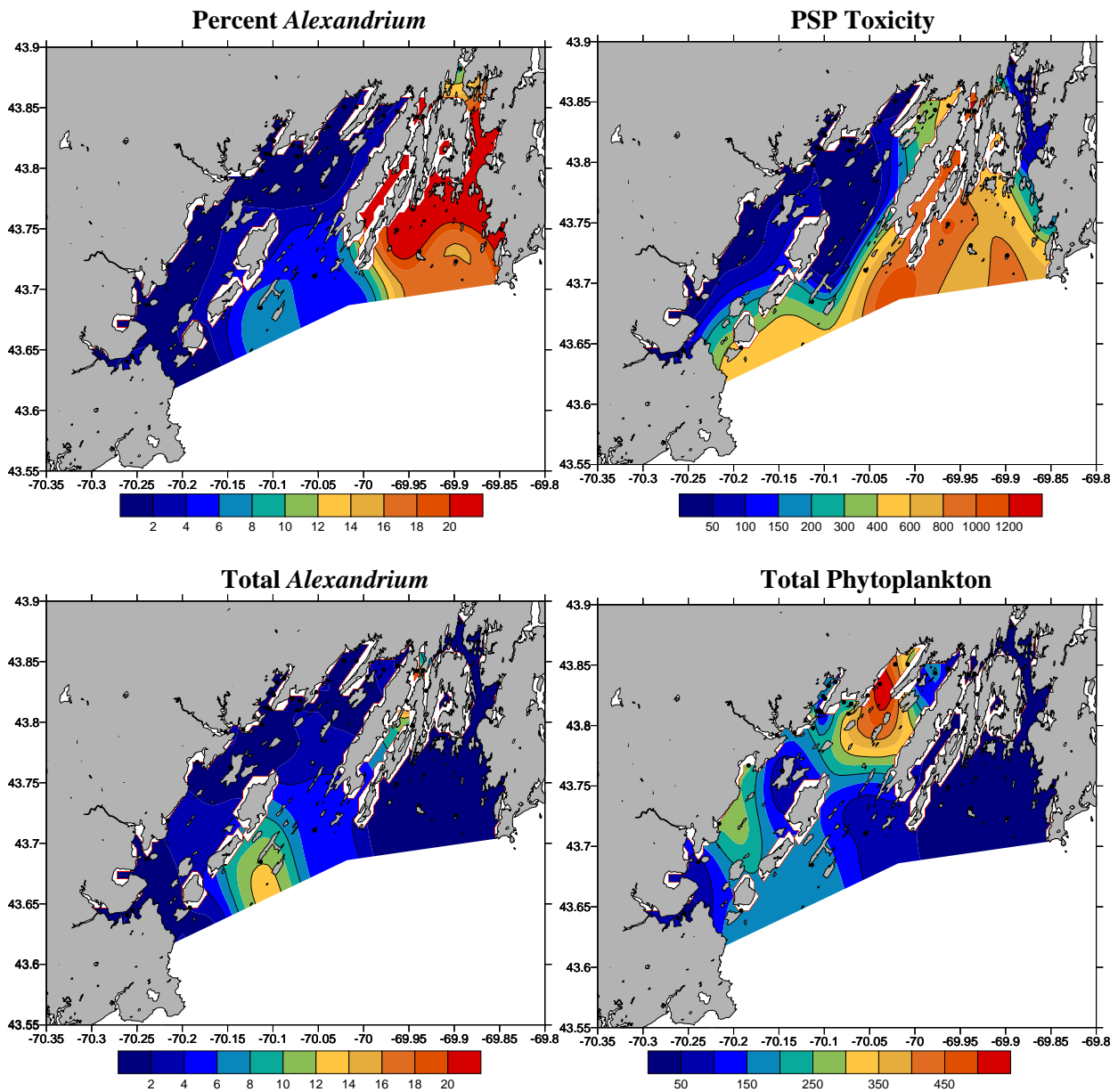


Figure 14. Contours of percent *Alexandrium*, toxicity (μg STX equiv./100g tissue), *Alexandrium* and total phytoplankton relative abundance net tow counts during the June 15-16 survey.

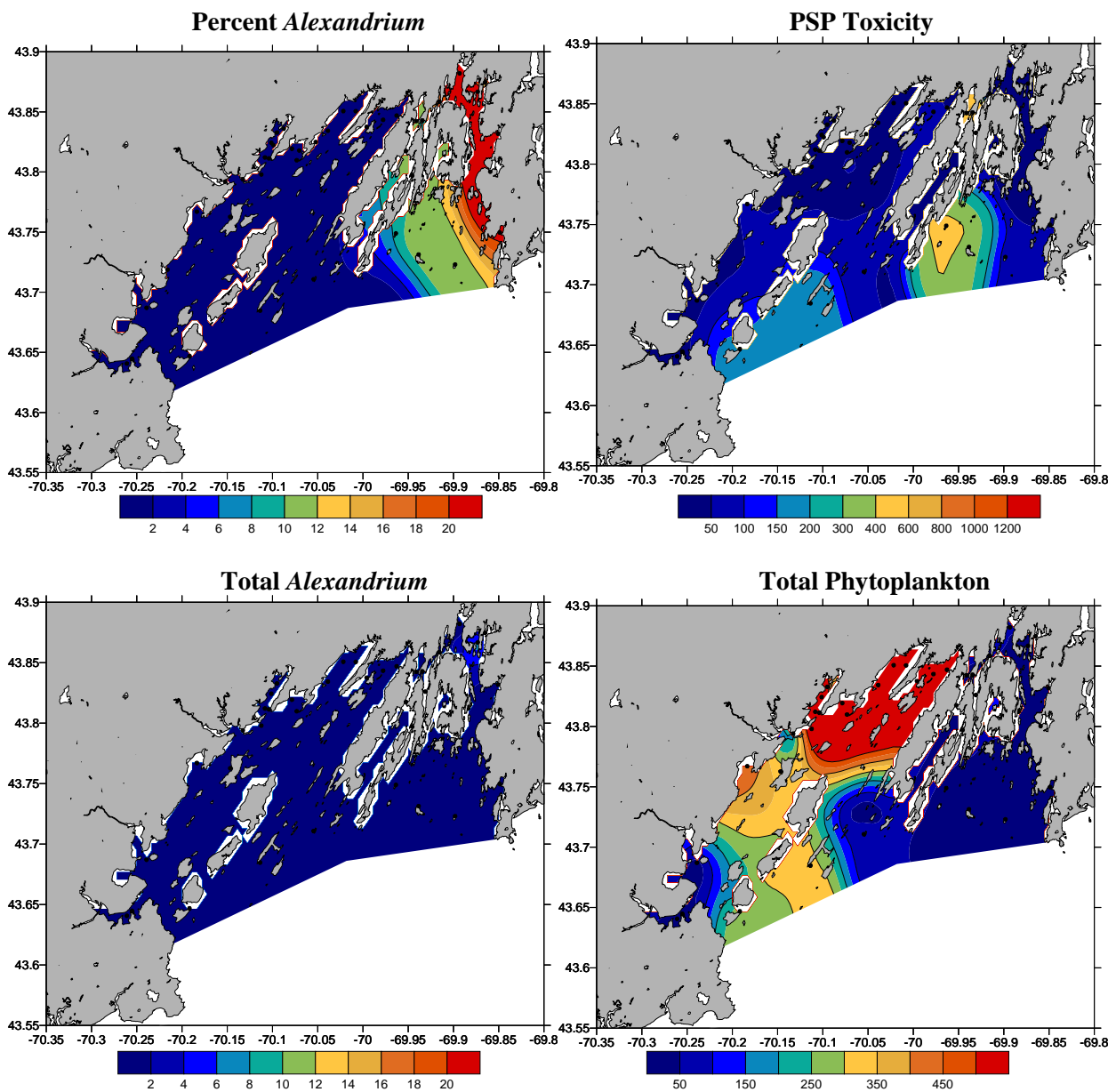


Figure 15. Contours of percent *Alexandrium*, toxicity (μg STX equiv./100g tissue), *Alexandrium* and total phytoplankton relative abundance net tow counts during the June 20-21 survey.

4.0 DISCUSSION

The goals of the Intensified Paralytic Shellfish Poisoning (IPSP) monitoring program were to 1) allow more refined management of specific resource areas for opening/closure and 2) test a more efficient method for conducting routine monitoring for red tide by DMR along the Maine coast. This would enable those responsible for protecting public health to provide adequate protection from the risks associated with the consumption of shellfish, while also minimizing the impact of such PSP closures on the shellfish harvesting industry. In addition, CBEP and DMR wanted to examine *A. fundyense* bloom dynamics in Casco Bay.

4.1 2006 IPSP Monitoring Program

A comparison of the 2005 PSP closures in Casco Bay and those of 2006 clearly illustrates the level of refinement this project allowed. The 2005 PSP event was exceptional both with respect to intensity and duration of the bloom. As a consequence, the entire bay had to be closed from May 4 to July 15, 2005. In 2006, soft-shell clamming areas of Casco Bay were closed for a similar duration from May 11 to July 13, 2006. By comparison, although PSP closures of shellfishing areas were required in response to the 2006 *Alexandrium* bloom, the intensified sampling program allowed approximately 11,000 acres of shellfish growing area that had been closed during the 2005 event to remain open to harvesting during the full duration of the bloom (Figure 16). It was noted that “all of the area in the upper New Meadows River, the upper bays and coves of Harpswell, and possibly Middle and Maquoit Bays in Brunswick would have been closed due to PSP for clams” on May 11th “if we didn't have that extra data” provided by the IPSP program (pers. comm. Darcie Couture, Director MDMR Biotoxin Program).

Programmatically, boat-based sampling offers numerous advantages over the traditional sampling of *in situ* mussels on mudflat beds. The principal advantage is the independence from tide stage. Under the traditional approach, all sampling is restricted to low or near-low tide when naturally-occurring mussels can be collected. In contrast, mussels suspended from floating buoys can be accessed at all times, irrespective of tide stage. Furthermore, since mussels occur at or near the low water mark, traditional sampling requires the sampler to drive to the collection flat, don hip boots, walk across the flat (mud) to the low water mark, collect the necessary mussels, walk back across the flat, take off the hip boots, and proceed to the next sampling station. Depending on the distance between stations, this procedure can severely limit the number of stations sampled per tide, and therefore, per day, since none are sampled at night. Indeed, a traditional sampler usually can cover a maximum of 8 to 10 stations in a single day. That is, 2 to 3 stations per hour assuming a 3 to 4 hr window of opportunity around low tide. The DMR currently has about 6 personnel available for sampling, thus only about 48 samples can be collected statewide on any given day (pers. comm. Darcie Couture, DMR). By contrast, 30+ sampling stations can be covered during an 8- to 9-hour boat-based sampling day, or 3 to 4 stations per hour. If closely spaced, as many as 5 to 6 stations could be sampled per hour, including the collection of associated data such as water column profiling, nutrient sampling, etc. at each station.

The spatial distribution of traditional sampling stations can also be constrained by physical or legal obstacles, *e.g.* obstructions (ledges, cliffs) and distance to low water in the former case and trespassing restrictions in the latter. Consequently, sampling is often restricted to public rights of way, such as bridges, municipal or state parks and boat landings, or properties of cooperative private citizens. Boat-based sampling clearly circumvents such restrictions and additionally allows sampling at remote locations, including mainland areas not accessible by road, mid-water areas of channels, bays, and coves, and offshore islands. Boat-based sampling also allows, or at least facilitates, collection of additional data on water column parameters that would be difficult, if not impossible, using the traditional approach since such sampling requires carrying additional, sometimes heavy, equipment and access to deeper water, *i.e.* 10-15 ft. of depth.

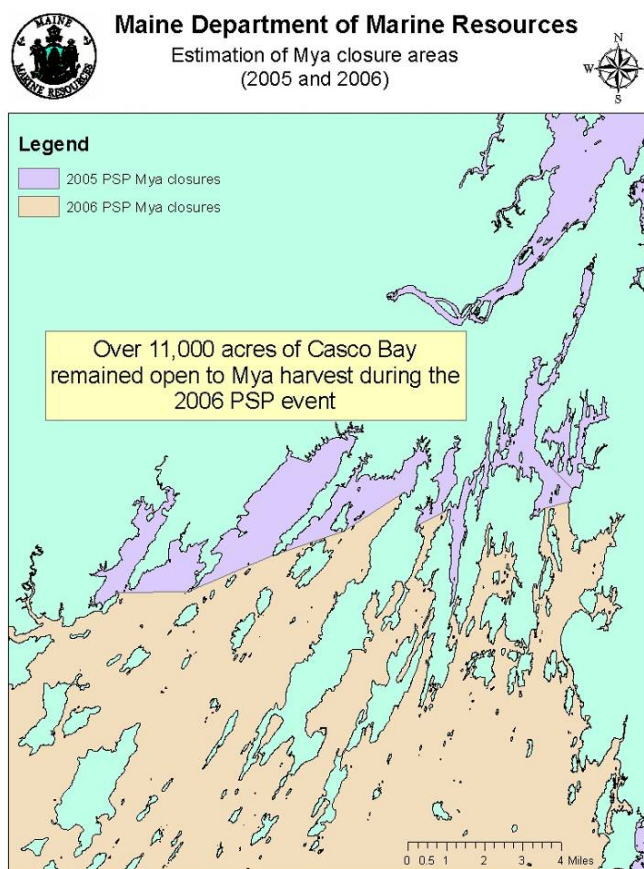


Figure 16. Comparison of 2005 and 2006 soft shell clam (*Mya*) closures in eastern Casco Bay. The tan area was closed in 2005 and 2006. The purple are remained open throughout the 2006 red tide.

The IPSP dataset for the 2006 *Alexandrium* bloom provided some insight into bloom dynamics in Casco Bay. As to the origin of the bloom, there were no definitive results. The first DMR shellfish closure of the 2006 season was the Lumbo's Hole area of Harpswell Sound, which is an area that consistently shows early toxicity on an annual basis and is suspected of having a local *Alexandrium* population. There are sophisticated genetic methods that could be used to ascribe local or regional origins of *Alexandrium* cells/bloom. These methods or perhaps sediment sampling to assess the number of *Alexandrium* cysts in the local area would be appropriate approaches for determining if the Lumbo's Hole *Alexandrium* are a self-seeding population.

Elevated *Alexandrium* net tow counts and toxicity observed at the start of the IPSP monitoring in early May were primarily influenced by offshore waters. High *Alexandrium* counts and toxicity were measured at the outer shore stations 37-39 and stations 40-42 off of the lower New Meadows River. Toxicity reached a maximum (1,420 μg STX equiv./100g) on June 16 at station 33 in Gurnet Strait located well inland off of the upper reaches of the New Meadows. Station 34 in Long Reach (off of the upper reaches of Harpswell Sound) and station 37 offshore of Ram Island also had toxicity measurements $>1,000$ μg STX equiv./100g. Relatively high *Alexandrium* net tow counts were also found at station 33 and 35 (but not station 37) during this survey. It is not possible to attribute the toxicity or *Alexandrium* in the locations to either an inshore or offshore source with the data available. However, given the very high abundances of *Alexandrium* observed in the surface waters further offshore in Casco Bay and the Gulf of Maine during the WHOI R/V *Oceanus* survey, the most likely source of the cells and toxicity is the offshore, regional bloom. This pattern of offshore delivery of cells to Casco Bay has been observed during the ECOHAB-Gulf of Maine program, and is well documented by Keafer *et al.* (2005).

4.2 Recommendations for Future Monitoring

The IPSP project was clearly very successful, but it did experience actual and potential problems that need to be addressed for future monitoring efforts. Recommendations to resolve these problems are detailed below. Many have already been discussed with CBEP and DMR and are being incorporated into monitoring plans for 2007. The recommendations focus on:

- Mussel bag deployment protocols
- Buoyed mussel toxicity vs. intertidal toxicity
- Quantitative phytoplankton and *Alexandrium* sampling
- Optimization of monitoring station number and location

4.2.1 Mussel Deployments

One of the most serious problems faced during the 2006 monitoring program was the unavailability of PSP-free mussels for timely deployment throughout the project period. As stated earlier, the progression of the *Alexandrium* bloom eastward along the coast made it increasingly difficult to secure adequate amounts of mussels to fully replace sampled mussels after the first few weeks of the project. This resulted in relatively rapid turnover of mussels in the western portion of the bay where on one occasion, specifically the run of June 20, mussels were only out for a week before collection and analysis.

Although DMR felt a full week was an adequate exposure time for toxicity detection (and the results appear to support this contention), a more consistent period of exposure across the entire bay would undoubtedly be preferable. To insure this, DMR is already considering mass collection of PSP-free mussels in the early spring of 2007 for placement in either recirculating or flow-through tanks at its Boothbay Harbor facility. Early collection of mussels would not only assure an adequate supply for the entire project period, but with the option to switch tanks to recirculation, would also assure PSP-free mussel tissue for deployment even if an *Alexandrium* bloom were to develop in the waters surrounding Boothbay Harbor.

Assuming an adequate supply of mussels is available, sufficient bags should be placed on the buoyed line at each station to allow collection of mussels with similar exposure times throughout the bay on each collection date. This can be accomplished by sequencing the bag collection off of each line on successive sampling dates. Accordingly, for example, on the first collection run the top bag (T_0+1), and only the top bag, would be collected from all lines and replaced; on the next run, the second bag down (T_0+2) would be collected and replaced from all lines, on the third run, the third bag down (T_0+3) would be collected and replaced, and so on. Experience has shown that a standard Spongex 6"x14" lobster buoy can support 4-6 bags of mussels without risk of sinking, even when heavily fouled.

According to this collection schedule, the maximum submersion time for test mussels would be approximately 4-5 weeks, *i.e.* (T_0+4) assuming the time between T_0 and the first collection is one week and sampling occurs weekly. As a result, T_1 would yield toxicity levels after one week exposure, T_2 after two weeks exposure, and so on, effectively yielding the *cumulative* toxicity level for the period of exposure. From a purely public health point of view, the *cumulative* toxicity level is probably all that is needed. However, the underlying question that cannot be answered using the cumulative procedure is the rate of incremental toxicity increase as affected by temperature, salinity, *Alexandrium* concentration, etc. If knowing the rate of incremental toxicity increase (ITI) were of value, this might be measured through the inclusion of an ITI-specific bag at each station. Fortunately, bait bags are available in a variety of colors, thus allowing color-coding of bags for specific purposes. The bags used to-date for the IPSP project have been orange, but by including a single green bait bag filled with PSP-free mussels on each station line that would be collected and replaced weekly (after only 1-week exposure) along with the routine orange bag, ITI data could easily be obtained. This would, however, double the number of samples generated weekly by the project and may strain the DMR analysis resources. This weekly

approach was taken by Keafer *et al.* (2004) in their mussel bag deployments, and very useful information was obtained.

The ITI information yielded through this additional sampling effort would probably be more of scientific rather than practical (public health) interest. However, if rapid increases in ITI could be correlated to specific environmental or weather conditions, such information might assist in the refinement and improvement of cost-effectiveness of monitoring efforts. For example, if routine monitoring indicates that toxicity levels are below quarantine levels and both environmental and weather conditions, as indicated by an ITI study, are not favorable to a rapid increase in toxicity, monitoring efforts might be temporarily relaxed at a cost-savings not only in the collection process, but in the laboratory as well. On the other hand, if environmental and weather conditions indicate a rapid increase in toxicity is likely, despite being below quarantine, monitoring efforts might be intensified, expanded, or initiated at other locations along the coast previously believed to be outside of the bloom effects. Another advantage of the weekly ITI approach would be that the transport of *Alexandrium* cells into the bay could be documented by the time-course of toxicity in offshore versus onshore mussel bags. This pattern would not be as evident in shellfish left for weeks between measurements.

4.2.2 Buoy vs. Intertidal Toxicity

On several occasions during this project, simultaneous samples were collected from both buoys and intertidal mussel beds when low tide permitted sampling of naturally-occurring mussels adjacent to sampling buoy sites. As Table 1 shows, with the exception of one site on June 1 and similar results on June 20, the buoyed mussels consistently resulted in higher toxicity levels than those recorded from the naturally-occurring intertidal mussels, with means of 65.3 and 47.6 μg STX equiv./100g, respectively. This is a 37% higher value for buoyed over natural mussels. A box-plot comparison of buoy-based and intertidal mussels shows the comparative ranges of the two mussel groups (Figure 17; note intertidal mussel outlier at 229 μg STX equiv./100g).

Table 1. Comparison of toxicity results from simultaneous samplings of buoyed and naturally-occurring mussels at selected sites on selected dates

Sampling date	Toxicity level (μg of STX equiv./100g)	
	Buoy	Intertidal
5/18/2006	132	60
6/1/2006	64	44
6/1/2006	0	48
6/15/2006	48	0
6/16/2006	50	0
6/16/2006	47	0
6/16/2006	0	0
6/20/2006	181	229
Mean	65.3	47.6

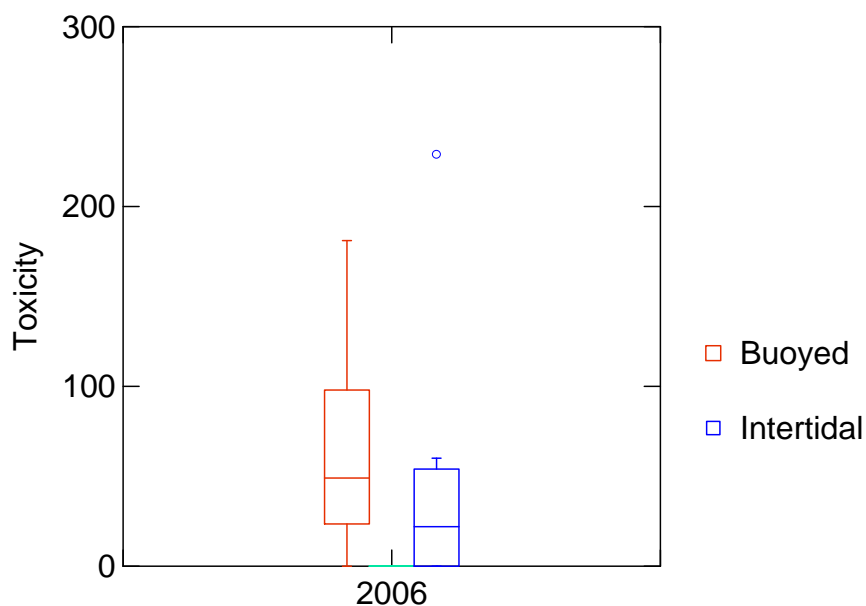


Figure 17. Box-plot graph comparison of the composite toxicity results from simultaneous samplings of buoyed and naturally-occurring mussels at selected sites.

This comparison demonstrates that the buoy-based mussels are at least equally protective, if not more protective, of public health. This is not surprising since the buoyed mussels are constantly submerged while most naturally-occurring mussels are out of the water, if only for a brief time in deeper intertidal areas. Additionally, naturally-occurring mussels undergo changes in the depth of the water column over the bottom, possibly reducing their exposure to the highest *Alexandrium* cell concentrations at high tide. In contrast, the buoyed mussels are always within the top meter of the water column, presumably continually exposed to the highest cell concentrations.

Although the higher level of toxicity found in buoyed mussels over natural mussels may be advantageous in protecting public health, concern has been expressed that buoy-based sampling, if it were to entirely replace natural mussel sampling, might ultimately result in larger, more frequent, and longer closures as a result of the sampling method. After all, the shellfish being harvested are typically those in the intertidal waters, so the toxin measurements should be reflective of the actual risk, not a hypothetical risk. Concern has thus been expressed over what appears to be an additional margin of safety offered by the buoy sampling method. Furthermore, the 80 μ g STX equiv./100g tissue quarantine level is already conservative and protective. A suggestion has been made that work be done to more accurately determine the difference between the toxicity levels found using the two methods and the reliability and repeatability of those differences. If the differences are found to be accurate, reliable, and repeatable, perhaps consideration should be given to the development of a new buoy method-specific quarantine standard. Clearly, much more work would need to be done comparing the toxicity levels of both methods before any such method-specific quarantine standard could be considered.

4.2.3 Quantitative *Alexandrium* Sampling

One major drawback of the 2006 monitoring program was the semi-quantitative phytoplankton and *Alexandrium* sampling. Not only does the plankton net tow method introduce uncertainties due to clogging, but routine microscope observations sometimes cannot distinguish *Alexandrium ostenfeldii* (which does not produce saxitoxins) from *A. fundyense*, which does (Anderson *et al.* 2005c). The consistency in the IPSP field and laboratory methods allowed us to make broad comparisons across stations and surveys within the program, but did not allow for comparisons to coincident or historical

monitoring data. For example, in 2006, data from the WHOI Gulf of Maine survey provided regional context for the Casco Bay bloom, but direct comparisons with the IPSP plankton data were not possible. The opportunity to compare data from site-to-site, and across coincident monitoring or research programs requires the use of similar, quantitative methods. Our understanding is that DMR will be using the *Alexandrium* whole-cell DNA probe-based method developed at WHOI (Anderson *et al.* 2005c) to measure abundance quantitatively in 2007 (pers. comm. Allison Sirois, DMR and Don Anderson, WHOI). This is a major step forward, and will do much to increase our understanding of the bloom dynamics of this organism in nearshore waters.

A major research initiative (GOMTOX¹) is slated to begin conducting surveys in the Gulf of Maine in 2007. The GOMTOX research team consists of scientists from WHOI, University of Maine and other research institutions as well as resource managers from Maine (DMR) and Massachusetts (DMF). One of the major objectives of the project is to understand the transport pathways of *Alexandrium* and associated nearshore shellfish toxicity. Pursuant to this objective is their stated goal to “assist managers, regulators, and industry to fully exploit nearshore and offshore shellfish resources threatened by PSP, with appropriate safeguards for human health.” This provides an excellent opportunity to place the Casco Bay intensive monitoring data in the context of the regional *Alexandrium* dynamics and hopefully address some of the remaining questions on delivery of offshore cells and the subsequent development of blooms within the bay.

4.2.4 Optimization of Monitoring Approach

One aspect of the monitoring program that we were asked to examine was station spacing and optimization of the program by reduction in the number of stations. Are there stations that are not statistically different from one another? The 2006 IPSP dataset, although useful, is relatively limited with regards to this type of statistical analysis especially since two of the primary parameters (total phytoplankton and *Alexandrium* abundance) were not sampled quantitatively and cannot be used. Nevertheless, a preliminary assessment was made using single factor ANOVAs and a multiple comparison procedure (Tukey’s test) based on the available surface water temperature, salinity, and mussel bag toxicity data.

The 15 stations in eastern Casco Bay are fairly well distributed across the bay and various embayments. The 28 stations in western Casco Bay fall into a number of geographical groupings that appear to have similar patterns in temperature, salinity and toxicity data. The groups are as follows: Portland Harbor (stations 4 & 5), Royal River (stations 10 & 11), Harraseeket River (stations 13 to 18), Wolfe’s Neck (stations 19 & 20), Maquoit Bay (stations 22 & 23), and Middle Bay (stations 27 to 29). None of the single factor ANOVAs were significant and the multiple comparison procedure indicated that the stations within any given group were not significantly different from each other. That being said, all parameters were run individually. A multiple comparison procedure where all parameters are evaluated together might yield slightly different results, particularly if there is interaction between the parameters. The current plan is to make quantitative *Alexandrium* abundance measurements this year and the resulting 2007 dataset would be amenable to this more rigorous statistical analysis to optimize the monitoring program. However, if budgetary and time constraints require the program be modified in 2007, the following changes are recommended based on the 2006 results/statistics and knowledge of the sampling area:

- Drop station 4 in Portland Harbor – station 5 is close to the South Portland Fore River WWTP and may provide important information on nutrients from that source and any interactions with phytoplankton/*Alexandrium* blooms.
- Drop station 10, which is upstream of station 11 on the Royal River.

¹ See <http://www.whoi.edu/sbl/liteSite.do?litesiteid=13193&articleId=20194> for more information

- Drop stations 13, 14, and 16 in Harraseeket River. Stations 12, 18, 17 and 15 should continue to provide high data density for this important shellfishing area.
- Drop station 19 or 20, and move the remaining station to an area between the two current locations.
- Drop station 22 or 23, and move the remaining station to an area between the two current locations.
- Drop station 28 in Middle Bay – stations 27 (to the west in Merepoint Bay) and 29 (in upper Middle Bay) should be adequate for this area.

4.3 Summary

Overall, the 2006 monitoring effort met the goals of the IPSP program. Using the IPSP data, DMR was able to refine their approach to closures and openings in Casco Bay. This resulted in keeping ~11,000 acres open to shellfishing in the upper reaches of eastern Casco Bay that had been closed during the 2005 *Alexandrium* bloom. The IPSP monitoring data also helped speed reopening of clam flats in Casco Bay. The June 28th modification of the western Maine closure notice (see Figure 5) was a direct result of the IPSP effort. Darcie Couture noted that the June 28th “opening is due in large part to the extensive buoy monitoring project that we have been doing with Casco Bay Estuary Partnership, as well as to the additional staff and overtime funds which were made possible by the legislature this year. These things allowed us to maintain more open area during the peak of the Western Maine bloom, as well as open more areas sooner. This is exactly what I had hoped would be the case when we asked for help, and so far it is proving to be a huge success story.” Thus, the second goal of the IPSP program clearly showed that this is a more efficient method for conducting routine monitoring for red tide along the Maine coast. The IPSP monitoring enabled DMR officials to adequately protect human health from the risks associated with the consumption of shellfish, while also minimizing the impact of such PSP closures on the shellfish harvesting industry.

An increased understanding of bloom dynamics of *A. fundyense* in Casco Bay was not fully realized, but a clear eastern vs. western Casco Bay difference was observed. The source of *Alexandrium* cells for the Lumbo’s Hole toxicity is still unknown, but recommendations on how to determine the source have been made. For the bay in general, it is not possible to attribute the toxicity or *Alexandrium* to either an inshore or offshore source with the data available. However, given the very high abundances of *Alexandrium* observed regionally in the Gulf of Maine during the WHOI *R/V Oceanus* survey, the most likely source of the cells and toxicity is the offshore, regional bloom.

The IPSP project was clearly very successful, but improvements could be made. We recommend that CBEP and DMR:

- 1) Continue the IPSP program, and expand it to new areas, as it has the potential to minimize the areas closed during red tides.
- 2) Examine the Maine coast to see if areas can be identified where IPSP approach would work (i.e., where there are convoluted peninsulas and sounds with complex hydrography that could lead to different levels of toxicity in relatively close areas.)
- 3) Conduct statistical analyses on a full complement of quantitative station data to see which are coherent, and therefore which might be redundant.
- 4) Consider mass collection of PSP-free mussels in the early spring of 2007 for placement in flow-through or recirculating tanks at DMR’s Boothbay Harbor facility.

- 5) Continue mussel bag experiments on buoys, looking at mussel toxicity levels for both short- and long-term deployments and comparison of buoy-suspended and intertidal mussel toxicity. These data should be correlated with *Alexandrium* cell abundance.
- 6) Conduct quantitative *A. fundyense* counts using probes that also allow the separation of *A. fundyense* from *A. ostenfeldii*.
- 7) Collect and examine water quality data in 2007 to see if there is a correlation between *A. fundyense* cell abundance and areas of Casco Bay with elevated nutrient levels from anthropogenic sources. This type of analysis can be done for the 2006 nutrient data once available, but only by relating water quality parameters to levels of shellfish toxicity.
- 8) Conduct an economic analysis of the actual cost of the IPSP program versus the value of the shellfish resource that is kept open due to the intensive sampling. It may be that this type of program can be justified on a sustained basis, without reliance on federal subsidies for the costs of the monitoring.

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