Evaluating the Dilution of Wastewater Treatment Plant Effluent, Treatment Efficiency, and Potential Microbial Impacts on Shellfish Growing Areas in Groton & Stonington, CT
Report of Findings from the May 18 – 22, 2015 Study Period
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FDA Technical Assistance and Training Project

Reported by:
David Lamoureux, Jr.
Kristin DeRosia-Banick
Connecticut Department of Agriculture
Bureau of Aquaculture
Milford, CT 06460

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Safety
Shellfish and Aquaculture Policy Branch
Field Engineering and Data Analysis Team
College Park, MD 20740-3835

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1.0 INTRODUCTION

1.1 Executive Summary

The U.S. Food and Drug Administration (FDA), Environmental Protection Agency (EPA) and the Connecticut Department of Agriculture Bureau of Aquaculture (DABA) conducted a study from May 18 – 22, 2015 in the Mystic River to assess the relative impacts from the Stonington (Mystic) water pollution control facility (WPCF) discharge on the downstream shellfish growing area located in the Towns of Groton and Stonington, CT (Mystic River/Mystic Harbor) and the existing Approved Area at the mouth of the Mystic River-Long Island Sound. Moored buoy stations equipped with WET Labs fluorometers (WET Labs, Inc., Philomath, OR) to measure dye concentrations were deployed at various locations along the anticipated path of effluent to measure dye concentrations at these stationary locations. Boat-towed tracking fluorometers were used to measure the surface level of dye-tagged effluent near each cage and in other parts of the Mystic River and Long Island Sound. Microbiological analyses of fecal coliforms (FC), male-specific coliphage (MSC), were conducted for samples from the WPCF. The results of the microbiological analyses and the dye study are presented in this report.

The results of the dye study indicated that surface dye levels differed from bottom dye levels near the cages and around the outfalls. This is likely due to the difference in water level between the surface and bottom depth, which in some cases was greater than 20 feet. This would result in higher concentrations at the surface during the first few days of tracking the dye, as it usually takes some time for the dye to reach lower depths. When the dye actually reaches the lower depths, it has been diluted throughout the water column and therefore dye concentrations would be lower in most cases at depth than at the surface. A full steady state analysis could not be done with all of the cage fluorometers due to variability in the dye data which made predicting the steady state build-up in the environment too difficult, consequently the overall findings of this report emphasize the surface dye concentrations and corresponding dilutions detected via boat tracking.

The minimum level of dilution recommended by FDA to mitigate the risk of viruses in treated effluent in a general classification scenario is 1000:1 dilution achieved within the Prohibited classification area.

In the case of the Mystic WPCF, the facility has advanced biological treatments with nitrogen removal, UV disinfection, Biomag settling and a number of back-up systems.

From the Proceedings of the Water Environment Federation, WEFTEC 2010: Session 81 through Session 90, pp. 6164-6177(14)[i]

> BioMag is an enhanced biological wastewater treatment process that uses magnetite, an inert iron ore ($Fe3O4$), to increase the specific gravity of biological floc. With a specific gravity of 5.2 and a strong affinity for biological solids, the ballast substantially increases the settling rate of the biomass. Increasing the specific gravity and settling rate of the biological floc provides the opportunity to increase the mixed liquor concentration, while still maintaining adequate settling and thickening in the secondary clarifiers. In the BioMag process, magnetite ballast is blended with a sidesteam of mixed liquor or return activated sludge (RAS), and the ballasted blend then flows back to the aeration basin. The solids then settle and thicken in the secondary clarifier. The majority of the settled solids (with ballast) are returned to the aeration basins as RAS, similar to a conventional activated sludge process. The magnetite recovery process is based on pumping the waste activated sludge (WAS) through a shear mill to separate the ballast from the floc. This stream then flows to a rotating drum lined with fixed magnets that enable

separation and recovery of magnetite from the biological floc. The recovered ballast is re-used. The WAS (minus the magnetite) is wasted to the plant's solids handling processes.

The Mystic plant is the first WPCF in Connecticut using the BioMag enhanced flocculation process to be assessed by the Bureau for effectiveness of microbial disinfection. Microbial analysis of influent, pre- and post-disinfected effluent conducted during the study period indicated that this facility was effective at reducing levels of bacterial and viral indicators. The study period coincided with a rainfall event of 0.50" on 5/19/15, which was on-going prior to and during the dye injection.

It is recommended that DABA staff periodically visit the WPCFs under adverse conditions to determine that operation remains consistent under a variety of rainfall and seasonal conditions. These evaluations should include confirmatory testing for fecal coliform and Male-Specific Coliphage (MSC), a viral surrogate, levels in the influent, pre- and post-disinfection effluent. In the event of a WPCF malfunction (including a bypass, loss of disinfection, or disruption of treatment leading to partially treated effluent), the conditional area should close to harvesting. The time of travel for effluent to reach the new conditionally approved area was calculated during the dye study as approximately 4 hours. Notification by WPCF personnel within the time of travel period is essential to ensure that the areas can be closed prior to contamination in the growing area from the event.

The results of this hydrographic dye dilution study indicate that a new Conditionally Approved area may be established in the Lower Mystic Harbor at a distance of approximately 1.6 miles from the outfall and outside of a minimum WPCF dilution zone of 1000:1 (Figures 8 and 9). This new Conditionally Approved area will be open for harvest on a seasonal basis during the months when marinas and mooring fields are not in operation. The operation of this newly classified Conditionally Approved area will require the implementation of a Conditionally Approved management plan and Memorandum of Understanding between all agencies responsible for managing the area.

There is also an opportunity for an additional Conditionally Approved area, in the portion of the Mystic River within which dilutions were found to range between 400:1 and 1000:1. If the quality of effluent remains consistent in the future during periods in which the proposed conditional area is in the open status, a 400:1 dilution zone could be justified, and would be consistent with FDA dilution recommendations for plants with UV disinfection that demonstrate exceptional treatment capabilities. The performance of the WPCF and associated impact to microbial quality of shellfish during higher risk periods, which include periods following significant rainfall events (>0.50"), shall be evaluated prior to any future upgrading in classification within the 400:1 to 1000:1 dilution zone.

It is recommended that the Prohibited area be expanded as a result of this study due to the high effluent concentrations identified outside of the existing Prohibited area (Figure 7). A minimum dilution value of 116:1 was recorded in the Northern Conditionally Restricted outside of the existing Prohibited classification area (Table 1).

Table 1. Minimum 5-point Moving Average Dilution Value Associated with Classification Polygons. Note 1000:1 Dilution FDA Recommendation for Conditionally Approved classification. Note that a lower dilution indicates a higher concentration of dye tagged effluent.

Classification	Minimum 5pt Average Dilution	Date and Time of Minimum Dilution
Prohibited	20.49	5/19/15 10:53
CR North	116.39	5/19/15 11:49
Restricted	635.23	5/20/15 15:27
CR South	585.36	5/19/15 17:53
CAPrior	461.41	5/20/15 15:42
CANew	1749.13	5/20/15 10:28
Approved	1586.59	5/19/15 12:00

Additional studies are needed to evaluate the effectiveness of sewage treatment under adverse environmental conditions and periods of high flow through the plant. These studies should also include testing of shellfish at three key sentinel locations in the Mystic River at the newly established classification lines and within the 400:1 to 1000:1 dilution zone concurrent with influent, pre- and post-disinfection effluent samples. Analysis shall include both fecal coliform and MSC levels in order to evaluate both bacterial and viral impacts to the growing area.

The resources needed to conduct such studies are above and beyond that required for routine management of shellfish growing areas, and would require the commitment of additional staff time and laboratory resources.

1.1 Study Objectives

The study objectives were to:
(1) Assess the dilution, time of travel, and dispersion of effluent in the Mystic River and Long Island Sound;
(2) Determine the bacterial and viral loads in raw, untreated wastewater and in pre-disinfected effluent and the efficiency of the WPCFs, which use a nitrate removal treatment process, BioMag flocculation process and UV disinfection, to reduce microbial loads before discharge to the receiving waters of the Mystic River;
(3) Provide FDA guidance to the CT DABA regarding the sizing of the prohibited area around the WPCF outfall based on dilution of effluent.

1.2 Study Area and Program Background

The study was performed in the Mystic River and Long Island Sound in Groton and Stonington Connecticut. The Mystic River/Mystic Harbor growing areas are jointed managed by the municipalities of Groton and Stonington. The Mystic River extends north to its primary tributary, Whitford Brook and flows south to southeast, prior to discharging into Long Island Sound. A comprehensive sanitary survey of the area was conducted in 2008 (Stonington) and 2010 (Groton) prior to this study. A prohibited zone is currently established around the WPCF outfall in the upper Mystic River which extends south to Murphy Point, Stonington. At the time of the study, there was no Conditionally Approved growing area available in the Mystic River/Mystic Harbor growing area. The written description of existing Groton and Stonington water quality classifications may be found in the Appendix.

The Approved classification area is located at a distance of 2.19 miles from the outfall. Approved classification areas in Groton and Stonington are available for shellfish harvest on a year-round basis. The results of this study indicate that the Approved classification line is appropriate based on the dilution of dye observed during the study and when the plant is operating under “normal” conditions, with the understanding that any bypass or treatment failure condition may require closure of Approved waters in Groton and Stonington.

Temporary closures for Approved and Conditionally Approved harvest areas are based on “triggers” or condition thresholds which are representative of conditions that exist when shellstock are likely unsafe for human consumption. As currently managed, a significant loss of treatment or sewage collection system failure would result in the closure of all Approved growing area waters in Groton and in Stonington east of Ram Island. In addition to a bypass situation, all approved shellfish growing areas in the CT waters of Long Island Sound are closed by the authority as a precautionary measure after 3.0” or more of rain. At the time of the study, there were no emergency closures in place and the Approved area was open and actively being harvested.

According to the NPDES permits for all coastal WPCFs in Connecticut, any loss of disinfection or sewage collection system bypass event must be reported to DABA within 2 hours of the failure. This 2 hour notification is a requirement of all coastal WPCF National Pollutant Discharge Elimination System NPDES permits in addition to being a requirement of the individual management plans for conditionally approved areas. Impacted areas are able to be closed to harvest immediately following this notification.

A shellfish harvesting area may close as a result of any of the following conditions: 1) when there is discharge or spillage of any substance that is considered hazardous to public health, 2) when there is the presence of biotoxins in concentration levels deemed to be detrimental to the public health, or 3) any other event such as a tropical storm, hurricane, tidal surge, etc. that could pose any significant potential public health threat. Closures resulting from discharges or spillage of hazardous substances and unusual weather events (tropical storms, hurricanes, heavy rains greater than 3 inches) are defined as Emergency Closures.

1.3 Description of the Stonington (Mystic) WPCF

The most significant pollution source with the potential to impact the growing waters of Groton and Stonington is the Mystic (Stonington) WPCF. The facility directly discharges to Prohibited classification receiving waters of the Mystic River.

The Mystic Water Pollution Control Facility located at 22 Edgemont Street, Mystic, CT has a current National Pollutant Discharge Elimination System (NPDES) Permit (CT0100544) which was issued by the Connecticut Department of Energy and Environmental Protection (DEEP) on May 21, 2012 and which will expire on May 20, 2017. The design flow of the facility is 0.80 MGD with an average total daily flow of approximately 0.50 MGD. There were no significant differences in flow rate during summer and winter months and dry (August) and wet (April) months. The method of disinfection utilized by the plant is ultra-violet (UV) light. The facility was in the design phase in 2011-2012 and was under construction in 2012-2015 to convert over to the UV disinfection method with a BioMag enhanced flocculation system installed. The construction portion of the project is completed. At the time of the study, the remaining portion of the project was to stress-test the BioMag system.

This facility is a significant direct point pollution source to the Mystic River, and as such must be carefully evaluated in order to ensure that shellfish harvested from waters adjacent to the facility are safe for consumption and not likely to be contaminated by pathogens. The DABA routinely samples at the outfall in the Mystic River and final effluent after significant rainfall events. The facility and collection system are evaluated by DABA interviews of WPCF staff and via written and electronic surveys on an annual basis. In addition, DEEP monthly monitoring reports and notices of violations are reviewed and evaluated for concerns on an annual basis.

The NPDES permit allows for up 260 CFU / 100ml for a single sample and 88 CFU/100ml for a 30 consecutive day geometric mean for fecal coliform bacteria. The facility experienced some difficulty maintaining treatment levels within permit parameters during the construction phase of the UV disinfection and Bio-Mag system. The DABA expects the quality of the effluent to improve once the plant operators have adapted to the new disinfection system equipment and required maintenance per the manufacturer's standards, which should allow for achieving sufficient treatment to meet NPDES permits parameters.

The facility was last inspected by DEEP on 5/2/2012. There were no NOV's issued. The DABA WPCF survey completed by the Mystic plant operator Gerry Minor states that no NOVs were issued 2011-2014. The upgrade at the facility included replacing the chlorine disinfection system with an UV light disinfection system, BioMag enhanced flocculation system, improved nitrogen removal, odor control, sludge processing and a complete equipment upgrade. The DEEP did not expect any impact to treatment during the construction and switch over to UV light disinfection. The DABA is monitoring the improvement to the facility and is evaluating the quality of the effluent to determine the impact to the growing area post-construction. The plant was diverting influent to the Stonington Borough plant during the construction phase of the project, with an average daily flow of 0.375mgd in 2013. The plant should be capable of treating approximately 0.5 mgd once the construction is completed the diversion is eliminated.

Influent at the Mystic WPCF consists of mainly domestic sewage and some pre-treated industrial wastewater. No septic sewage comes into the facility.

The effluent discharges into the river from an outfall pipe located at 41 20.924, 71 58.209. Treated effluent travels a short distance to the river and has a limited residence time in the outfall pipe.

1.4 General Description of Study Design

Prior to the dye study at the Mystic WPCF from May 19 – 21, 2015, a preliminary study was conducted on May 18, 2015 to gather background data in the Mystic River and Long Island Sound. The background fluorescence data was collected with two boat tracking fluorometers at the surface.

Four buoys equipped with WET Labs fluorometers (WET Labs, Inc., Philomath, OR) to continuously measure dye concentrations were deployed at strategic locations to assess the adequacy of existing classification lines. The buoys remained in the water until May 26, 2015, so that the equipment could collect data on remaining dye in the water column following the study.

Figure 1 shows a map of the study area with the four stationary fluorometer locations, the WPCF outfall overlaid with existing shellfish growing area classifications.

The dye for the comprehensive study for the Mystic WPCF was injected over a half tidal cycle (12.4 hours) from 5:10 AM to 5:34 PM on May 19, 2015 and was detected in the surface waters for two days following the injection. Boat tracking with towed WET Labs fluorometers was conducted to find the edges of the dye plume during daylight hours and the surface dye concentrations.

In addition to the dye study, DABA staff collected samples of influent, pre-UV treated effluent, and post-UV treated effluent from the WPCF, which were processed at the DABA laboratory in Milford. Samples were analyzed for fecal coliform and MSC. These analyses were performed to assess the efficiency of the WPCF secondary treatment process and UV disinfection at removing viruses and to better inform DABA's recommendations for sizing a prohibited area around the WPCF outfall.

2.0 METHODS -

See **Appendix 1** for detailed methods used in the study.

3.0 RESULTS

3.1 Weather Conditions

According to the NOAA's National Weather Service rain gauge station at the Groton Water Filtration Plant and the rain gauge located at the Stonington Shellfish Commission Chairman's house located at Lord's Point, Stonington, CT, during the Mystic comprehensive study period, the Groton weather station recorded a total of 0.32 inches of rain and the Stonington weather station recorded 0.85 inches on the morning of May 19, 2015. There were no significant rainfall events in the week prior to the study period. The wind conditions were not a significant factor during this study as the upper Mystic River is enclosed and is not as impacted by high wind as compared to the open waters of Long Island Sound. Dispersion of dye was not influenced by the wind, but was more significantly influenced by the high tidal range (approximately 7 – 8 feet) in the area and strong currents.

3.2 Dye Injection

Records from the Mystic WPCF showed that the 24 hour average flow during the dye injection period was between 0.27 and 0.69 MGD. This flow rate is less than what the plant is designed to process (0.80MGD). Using a mass balance approach based on these flows and the volume of dye injected, that the dye concentration leaving the Mystic WPCF (Cout) was calculated at 2153 ppb.

Equation 1. Dye Concentration Calculations

Cjug = Concentration of dye in jug (mixture of dye + DI water)

Dye = Amount of dye to be injected

Duration = Duration of injection

Qjug = Flow rate of jug

Qout = Flow rate of WPCF
Cout = Dye concentration in WPCF effluent
*Cjug * Qjug = Cout * Q out = Cest * Qest*

Dilution Calculation:
D = Cout/Cest

Equation 2. Mystic, CT. May 19, 2015 Dye Concentration Calculations.

Injection Time	Cjug	RPM	Qjug	Qjug	Qout	Cout	35to1	100to1	1000to1	10000to1
05/19/15	ppb		ml/min	gal/day	gal/day	ppb	ppb	ppb	ppb	ppb
6:00-18:00	100,000,000	123	30.56452	11.6270	540000	2153	62	22	2.15	0.22

3.3 Travel Time

This study determined the travel time of dye from the Mystic WPCF to the Approved growing area and to a newly proposed Conditionally Approved growing area. This was determined by tracking the leading edge of the dye on the ebbing tide during the initial dye tracking on 5/19/15 as seen in Figure 3 and Table 1. Based on an average velocity of 0.44 mph and the distance to the Approved growing areas, the time of travel the dye-tagged effluent to the Approved growing area outside of river was determined to be 6.4 hours. The time of travel to the proposed Conditionally Approved growing area was determined to be 4.17 hours.

As required by current NPDES permits for all coastal WPCFs in Connecticut, any loss of disinfection or sewage collection system bypass events must be reported to CT DABA within 2 hours of the failure. This 2 hour notification is a requirement of all coastal WPCF NPDES permits in addition to being a requirement of the individual management plans for conditionally approved areas. Impacted areas are able to be closed to harvest immediately following this notification. Based on these times of travel to the Approved or proposed Conditionally Approved growing areas, a 2 hour notification window is adequate for closure.

Table 2. Time of Travel Data from GPS of Leading Edge of Dye Tracking.

GPS Marker Note	Distance (miles)	Time	Time after Start Ebb	Time in Hours	Velocity
leading edge (LE) 1 14:12	1.55	14:12	3:31	3.52	0.44
LE 3 14:44	1.81	14:45	4:04	4.07	0.45
LE 5 14:51	1.88	14:51	4:10	4.17	0.45
LE 9 15:31	2.17	15:31	4:50	4.83	0.45
LE 15:51	2.36	15:51	5:10	5.17	0.46
LE 17:05	2.6	17:05	6:24	6.40	0.41
				Mean	0.44

High tide occurred at 10:41 AM (start of ebb)
Low tide occurred at 5:06 PM (end of ebb)

10:41

3.4 Dye Readings by Tracking Fluorometers

The accumulated 5-point moving average concentration values and the corresponding dilution levels for the three days of tracking during the Mystic study period (May 19 – 21, 2015) as determined by both WET Labs boat tracking fluorometers is presented in Figure 7.

The minimum concentration found via the boat tracking fluorometers within the existing Prohibited area on May 19th was 105 ppb, equivalent to a dilution of 20.5:1 (Figure 4). Concentrations as high as 18.49 ppb were measured outside of the existing Prohibited area, corresponding to dilution of 116:1. Dye was tracked upriver as far as the Route 1/East Main Street bridge (approximately 0.5 miles upriver from the outfall) because the entire river north is classified as prohibited and gathering data further north was deemed unnecessary. On the first day of tracking, dye was detected throughout the upper and lower Mystic River to approximately 2.42 miles south of the Mystic outfall as far as east of Ram Island in the Approved growing area. The highest concentration recorded on Day 1 of tracking in the Approved area was 1.357 ppb corresponding to a dilution of 1586:1. The minimum measureable concentration during day 1 tracking was 0.0102 ppb, corresponding to a dilution of 211,078:1, which is less than the 100,000:1 dilution level of 215,300.

The 100,000:1 dilution is significant because in the case of a raw sewage bypass, where a level of 1.4 x 106 FC/100ml is assumed for a raw sewage release, a dilution of at least 100:000:1 would be required to dilute the sewage sufficiently to meet the NSSP approved area standard of 14 FC/100ml. It is only when a dilution analysis determines that the location of the discharge is such that the dilution of effluent would be greater than 100,000:1 that the WPCF may be considered as located outside the zone of influence to the shellfish growing area.[iii] Therefore based on the results of this study, there are no waters within the study area identified as being outside of the zone of influence of the WPCF in the case of a raw sewage release. Based on the dilution information gathered during this study, if the fecal coliform level for a given loss of treatment situation can be estimated based on sampling results, the corresponding fecal coliform level in the growing area may be estimated allowing appropriate management decisions to be made in terms of closure to impacted growing areas.

On May 20, 2015 during the second day of tracking for the Mystic study, although overall dye levels dropped off in the river, concentrations as high as 6.8245 ppb were still detected outside of the existing Prohibited area as seen in Figure 5. The minimum dilution detected outside of the existing Prohibited area was 315.5:1, equivalent to a 5-point moving average concentration of 6.8245 ppb, at a distance of 1374 feet south of the of the outfall. A significant number of points with dilution values between 400:1 and 1000:1 were identified in the existing Conditionally Approved classification area. In addition, concentrations as high as 0.1821 ppb were recorded in the Approved area east of Ram Island, corresponding to a dilution of 11,823:1.

On May 21, 2015 relatively low concentrations were found throughout the river with approximately half of the recorded points being below the limit of detection (0.01 ppb). The highest concentration recorded on day 3 of tracking was 1.2461 ppb, corresponding to a dilution of 1727:1, with a significant number of points in the 0.2 to 0.3 ppb range, corresponding to dilutions of 10765:1 to 7177:1. Concentrations as high as 0.1213 were recorded east of Six Penny Island on day 3, corresponding to a dilution level of 17,749:1.

Accumulated dye values from the three study days show that no dilution values less than 400:1 were found in a newly proposed conditionally approved and existing approved growing areas as seen in Figure 7. However, dilution levels within the existing conditionally approved area were only slightly greater than 400:1, even at a distance of 1.7 miles from the outfall.

FDA normally recommends a 1000:1 dilution value as described in Section 2.1 of Appendix 1, but with UV disinfection and exceptional treatment capabilities at this plant, a 400:1 value *may* be adequate for future upgrades if additional studies are performed to investigate and verify the microbial treatment capabilities of the pant under a variety of environmental, meteorological, seasonal and facility conditions. With additional confirmatory testing of the effectiveness of advanced treatment and confirmatory testing of the microbial quality of shellfish in the growing area, the potential exists for future upgrades with in this area. These upgrades are not a given, as the upgrade potential will depend on the documented findings associated with such studies.

Based on the results of this study, a new Lower Mystic Harbor Conditionally Approved area is being proposed with a northern boundary line drawn south of a greater than 1000:1 dilution zone as seen in Figure 8. The proposed classification line could extend from the end of Main St. just north of the Noank Coop, and extending east to Mason's Island near Seal Rocks. This new area may be established without additional WPCF and shellfish studies, however a Conditional Area Management Plan and Memorandum of Understanding between all responsible parties will need to be established prior to opening the area for harvest. It should be noted that the operation of this proposed Conditionally Approved area hinges on operations at the plant, as this 1000:1 dilution appears to be adequate for mitigating the impacts of viruses on shellfish only when WPCFs are operating under normal conditions. Bypass and loss of disinfection operating under normal conditions. "Normal" means that the WPCF is operating fully within the plant's design specifications, including design flows; treatment stages; disinfection; as well as compliance with all permit conditions that relate to the WPCFs effectiveness in reducing enteric viruses in sewage.[iv]

In addition, based on the high concentration of dye tagged effluent identified outside of the existing Prohibited area, the Prohibited area will be expanded south to a new line drawn from Spence Point to Pine Point, which would encompass dilutions less than 400:1, and provide a more adequate buffer around the outfall. To our knowledge, there are no commercial or recreational shellfish activities within this newly expanded Prohibited area and we do not anticipate a negative impact to management of resources in the area.

At this time, no classification changes are being recommended in the Conditionally Restricted and Restricted waters west of Mason's Island. Relay from these areas should be limited to conditions when the water temperatures are above 50°F and should rely on a minimum 21-day relay period with confirmatory MSC and fecal coliform testing of relayed shellfish prior to market harvest. These recommendations are made in order to more effectively reduce concerns associated with viral pathogens, which take longer to depurate than bacteria. Due to the risk of human illness related to contamination of shellfish by bacterial and viral pathogens, it is imperative that no direct harvest of shellfish shall occur from the Restricted and Conditionally Restricted areas in the Mystic River complex.

3.5 Dye Readings at Buoys-Submersible Fluorometers

Dye readings recorded by the station 2 stationary WET Labs fluorometer 1730 and boat tracking fluorometers within a 200 meter radius of station is shown in Figure 16. The maximum dye concentration values are also plotted for both the submersible readings and the boat tracking readings. Along with the dye concentrations in each figure are the associated dilution levels for the study. The WET Labs fluorometer at stations 1, 3, and 4 malfunctioned and data was not consistent enough to be used in the analysis, however the Station 2 data was most critical in terms of this study. Tidal depth in feet is also plotted based on the Noank Tide Gauge (#8461NOA) located at Noank, Mystic River entrance, Connecticut 41.3167° N, 71.9833° W.

Continuous dye readings from the submersible fluorometer are plotted in Figure 16. Because the build-up of dye at other stations was unpredictable, accurate steady state dilution levels could not be determined using the superposition method. Based on the data collected at station 2, the highest recorded tracking reading within a 200 m buffer of station 2 was 0.8205 corresponding to a 2624:1 dilution. The high tracking reading was recorded on May 19, 2015 at 17:50 hours, at approximately low tide. The highest recorded station reading was 0.2861, corresponding to a dilution of 7525:1 was recorded on May 20, 2015 at 07:11 hours, at low tide. The high reading was recorded the day following the injection because a large amount of dye was still remaining in the upper reaches of the river due to the flooding tide, and dye would not have been able to reach this station until the tide turned and dye was moved south on the current. Remaining dye continued to be flushed out of the estuary on the second low tide on May 20th and during the first low tide on May 21st. No dye readings were observed at Station 2 after the first low tide on 5/21/15. It is not surprising that station readings are lower than the tracking readings; dye is held in the fresh water in the surface of the water column and becomes more well-mixed and diluted over time.

3.6 Profiles of Dye at Depth

Many profiles were conducted during the Mystic study using the SeaBird CTD interfaced with a WET Labs tracking fluorometer (WET Labs FLRHRT 586). Figure 21 shows the locations of profiles performed during the study. Figures 17 – 20 show dye data from profile fluorometer for the Mystic study where profiles were recorded in the plume of dye along with the salinity, temperature, and depth for that given profile. The depths and concentration recorded at each profile location varied significantly based on the distance from the outfall and amount mixing of dye within the water column. Profile data is presented in Figures 17 through 20, moving from north to south and are numbered 1 through 8. All profiles with the exception of Profile 8 were recorded on May 19, 2015. Profile 8 was recorded on May 21, 2015.

Profile 1 was taken 16 m north of the outfall and recorded data to a depth of 3.0 m. The highest concentration recorded was approximately 125 ppb at a depth of 1.5 m. Profile 2 was taken 5 m south of the outfall and recorded a high concentration of 12 ppb at the surface then dropped off to a concentration of between 4 and 6 ppb at a depth of 2.5 m. Temperature and salinity were consistent throughout the water column at the Profile 2 location. Profile 3 was taken 31 m east of the outfall and recorded a high concentration at the surface of 7 ppb and dropped off to a concentration of 1.3 ppb at a depth of 3.0 m. Temperature and salinity were consistent throughout the water column at the Profile 3 location. Profile 4 was taken at the existing Prohibited line and recorded a high concentration of 8 ppb and dropped off to non-detectable levels at a depth of 3.5 m. At the Profile 4 location, the salinity increases from 29 ppt at the surface to 30.5 ppt at a depth of 5 m, while temperatures decreased from 15°C to 13.5°C at depth.

Profile 5 was taken at a distance of 397 m from the outfall in the Conditionally Restricted portion of the river. In contrast to Profiles 1 through 4, Profile 5 recorded the highest dye concentration (0.6ppb) at a depth of 3 m, indicating that dye was starting to mix within the water column by the time it reached this point in the system. Temperature and salinity were consistent throughout the water column at the Profile 5 location. Profile 6 was taken at 1447 m from the outfall in the Restricted area of the river. Dye concentration continued to decrease however is now well-mixed throughout the water column at approximately 0.2 ppb. Temperature and salinity were fairly consistent throughout the water column at the Profile 6 location. Profile 7 was taken at a distance of 3107 m from the outfall in the existing Conditionally Approved area. Dye concentration at this location was less than the detection limit throughout most of the water column to a depth of 2.5 m, with a high reading of approximately 0.08 ppb. Detectable dye was concentrated at the surface at this location. Temperature and salinity were consistent throughout the water column at the Profile 7 location. Profile 8 was taken at a distance of 3656 from the outfall in the Approved area on May 21st. Dye readings were consistent throughout the water column at approximately 0.06 to 0.08 ppb to a depth of 4 m. Temperature and salinity were consistent throughout the water column at the Profile 8 location.

3.7 Short Term Failure - Dilution and Anticipated Fecal Coliform (FC) Concentrations

A short-term raw sewage failure at the Mystic WPCF is unlikely given the advanced SCADA systems, new technologies, and multiple back-up systems, but is possible due to bulb malfunctions and other potential issues with the UV system. However, both failure events are assessed below and should be considered as part of the conditional management plan for the newly proposed Lower Mystic Harbor Conditionally Approved area.

This study shows that the entire Mystic River estuary including approved, conditionally approved, and restricted areas would be impacted under a WPCF malfunction event as shown in Figure 22. Figures 22 symbolizes the existing classification overlaid with dye study data according these various dilution schemes: >=100,000:1, 10:000:1, 1000:1, and 400:1.

In the unlikely event of a raw sewage failure, FDA typically recommends a dilution of 100,000:1 for any approved areas within an accessible distance of the diffuser, assuming that fecal coliform levels would be in the range of 1.4×10^6 cfu/100 ml (100,000:1 dilution is needed to reduce failure load to 14 cfu/100 ml). As seen in Figure 22, there are no waters within the Mystic River estuary that are available at the >100:000:1 dilution (symbolized in blue), thus all waters in the study area could be impacted in the case of a raw sewage event or complete treatment failure.

If one were to assume that the treatment within the WPCFs could achieve a log reduction prior to disinfection then at a minimum 10,000:1 dilution may be adequate for a loss of disinfection failure, however most of the growing area is still impacted at less than the 10,000:1 dilution level (symbolized in yellow). As seen in Figure 22, the waters along the western shore of the proposed Lower Mystic Harbor Conditionally Approved area are the only waters which would potentially meet this 10,000:1 dilution level, however the majority of the area remains impacted at a less than 10,000:1 dilution level and should be closed to harvest in the event of a loss of treatment or raw sewage discharge.

3.8 Determination of 1000:1/400:1 Dilution – Mystic WPCF

Under Scenario 2 for sizing prohibited areas (see Section 1.2), the size of the prohibited zone can be reduced and a conditional area can be established if a 1000:1 dilution zone is achieved and other conditions are met. If a plant such as the Mystic WPCF can demonstrate effective UV disinfection and efficient virus removal, the potential exists for a 400:1 dilution zone to be established around the outfalls if studies document the effectiveness of disinfection in terms of bacterial and viral reduction.

Figure 22 shows the dilutions and existing shellfish classification areas for the combined 3 days of boat tracking of dye tagged effluent discharged from the Mystic WPCF. The dilution levels representing less than or equal to 400:1 are shown in red. It can be observed that dilution levels of 400:1 or less occurred approximately 0.3 miles downstream from the discharge location and beyond the existing Prohibited area extending into the upper Conditionally Restricted area. Dilutions greater than 400:1 were achieved throughout the managed Restricted, lower Conditionally Restricted, Conditionally Approved and Approved shellfish growing area.

Figure 23 shows the classifications proposed for this area based on the results of this study, which include establishing a new Lower Mystic Harbor Conditionally Approved area with a northern boundary line drawn from the end of Main St. just north of the Noank Coop, and extending east to Mason's Island near Seal Rocks. This new area is completely within the >=1000:1 dilution area.

It may be possible to establish an additional Conditionally Approved area within the 400:1 to 1000:1 dilution zone in this figure, provided that viruses and viral indicators in the WPCF effluent and growing area are determined to be consistently very low under a variety of scenarios.

3.9 Microbiological Analysis of WPCF Influent and Effluent

The results of viral and bacterial samples from the Mystic WPCF are used to document the effectiveness of the disinfection process. During the week of the dye study, male-specific coliphage (MSC) and fecal coliform bacteria samples were collected and analyzed at the Bureau of Aquaculture Laboratory. Results of these samples may be found in Table 3 below.

Table 3. DA/BA fecal coliform and MSC microbiological results for samples collected from the Mystic WPCF 05/19/15 through 05/21/15. Results are provided in either colony forming units (CFU) for fecal coliform bacteria or plaque forming units (PFU) for male specific coliphage.

Collection Date	Sample Type	Influent Fecal Coliforms (CFU/100 ml)	Influent MSC (PFU/100 ml)	Pre-UV Disinfection Fecal (CFU/100 ml)	Pre-UV Disinfection MSC (PFU/100 ml)	Final Effluent Fecal (CFU/100ml)	Final Effluent MSC (PFU/100 ml-LOD 9 PFU/100mL)**
5/19/2015	Composite Grab	210000	68000	2100	10	22	ND
5/19/2015	Composite Grab	220000	107200			60	ND
5/20/2015	Composite Grab	140000	56000	100	10	62	ND
5/21/2015	Composite Grab			7400	120	171	ND
5/21/2015	Composite Grab	400000	128000	2900	420	96	ND
5/21/2015	Sample Grab			700	52	50	ND
	CompositeMean	242500	89800	3125	140	82.2	ND
	LogMean*	5.38	4.95	3.49	2.15	1.91	-
* MSC results show a 5 log reduction from influent to final effluent							
** all post-treatment MSC results were non-detectable (level of detection 9 PFU/100mL)							

The influent (raw sewage), pre-UV disinfection (partially treated sewage) and post-UV disinfection (final effluent, treated sewage) samples collected during the dye study indicate that the WPCF was effective in reducing viral and bacteria levels prior to discharging to the Mystic River. The final effluent (post-UV disinfection) fecal coliform results were within the NPDES permit requirements per the USEPA and CTDEEP. There are no regulatory standards for MSC levels in sewage effluent, however all final effluent MSC results during the study were below the level of detection limits of the analysis, which is 9 PFU/100mL for the MSC procedure.

Another factor to consider when evaluating the effectiveness of WPCF disinfection and the associated impact to the growing area is to evaluate viral and bacterial indicators in shellfish tissues by placing shellfish cages at key monitoring locations in the Mystic River and Mystic Harbor growing area. Viral indicators survive for extended periods of time in cooler water temperatures, and seasonal impacts from the WPCF are most effectively evaluated when water temperatures drop below 50°F.

The resources needed to conduct such studies are above and beyond that required for routine management of shellfish growing areas, and would require the commitment of additional staff time and laboratory resources.

4.0 Conclusions and Recommendations

When considered collectively, the data from the hydrographic dye study and microbiological assessment of the Mystic WPCF support the following conclusions and recommendations:

- During the time of the study, the Mystic WPCF was efficient at removing fecal coliform indicator bacteria and in meeting its permitted requirements for fecal coliform;
- During the time of the study, the Mystic WPCF was efficient at removing MSC, which provides an indication that this WPCF is efficient in reducing the enteric viral load (such as NoV);
- The average flow during the dye injection period for the Mystic WPCF was 0.54 MGD, less than the design flow (0.80 MGD);
- Dilution levels of >=1000:1 are met relative to the Mystic WPCF dilution study approximately 1.6 miles downriver of the outfall within the currently classified Conditionally Approved area, however the existing classification line should be moved southward to avoid the higher 400:1 dilution area;
- During the Mystic WPCF study, dye-tagged effluent remained detectable near the growing area for at least 3 days based on tracking and also detected for longer periods at lower depths captured by the submersible fluorometers;
- The station data suggests that if a WPCF malfunction occurs, the residence time of pollutants discharged to the Conditionally Approved area could be at least 3 days. Based on environmental factors such as temperature and sunlight, viruses sequestered by shellfish could remain viable for a considerably longer time;
- The time of travel from the Mystic WPCF outfall to the northern line of the newly proposed lower Mystic Harbor Conditionally Approved area is 4.17 hours;
- In the unlikely event of a failure at the WPCF, these quick travel times necessitate the need for immediate notification from the WPCF and response on behalf of the CT DABA to ensure that harvesters are notified to stop harvesting in an adequate time frame based on the location of the harvest sites;
- The existing Prohibited area should be expanded to include areas of dilutions <=400:1;
- Based on the results of the study conducted for the Mystic WPCF, the 1000:1 dilution will be met within the newly proposed Prohibited, Conditionally Restricted and Restricted areas for average daily dry weather and wet weather flows and flows up to 0.75 MGD. Flows over 0.75 MGD would potentially result in dilutions less than 1000:1 in the proposed conditionally approved and existing approved areas and should be considered adverse conditions until additional data can be gathered that establishes otherwise;
- A new Lower Mystic Harbor Conditionally Approved area is being proposed with a northern boundary line drawn south of a greater than 1000:1 dilution zone as seen in Figure 8 and Figure 23. The proposed classification line could extend from the end of Main St. just north of the Noank Coop, and extending east to Mason's Island near Seal Rocks;
- Additional staff and resources will need to be leveraged in order to conduct MSC sampling of the Mystic WPCF and shellfish in the area, especially when WPCF flows exceed 0.75 MGD to assess the efficiency of the WPCF;

- Overall, the results of the study and modeling indicate that the growing area does not meet its classification – as currently managed. However, the classification changes hereby proposed would allow the growing areas to meet the appropriate classification based on current ISSC and FDA guidance;
- Although the authority has noted that the approved area is managed consistent with a conditionally approved area, it is important to note that the operation of the existing "approved" area needs to continue to have conditions established to close the area in the event of a WPCF malfunction as described in this report.

The authority recommends that a documented communication system be set up between CT DABA, the Mystic WPCF, and the licensed harvesters of the growing area, to ensure that harvesters are notified to stop harvesting in a sufficient period based on the location of the harvest sites in the unlikely event of a failure at the WPCF. If no harvesting takes place immediately after a failure event, this will allow the CT DABA, Ledge Light Health District and/or Stonington or Groton Shellfish Commissions additional time to close the growing area.

Based on the microbial findings in samples from the WPCF and the results of the dye study, the authority recommends a minimum dilution level of 1000:1 for Conditionally Approved areas operated with respect to the Mystic WPCF.

According to the 2015 ISSC Dilution Guidance for Prohibited Areas Associated with Wastewater Discharges:

> *Alternative options for calculating the size of the prohibited zone to mitigate the virological effects of WPCF discharges at the shellfish growing area may be used provided that they are based on sound scientific principles that can be verified. For example, it is reasonable to expect a potentially higher reduction in viral load from a properly maintained wastewater treatment system employing ultraviolet (UV) disinfection, tertiary treatment and operating under optimum design flow conditions. Regardless of the technology employed any proposed alternative minimum level of dilution for conditional management other than 1000:1 would need validation. However, when there is insufficient information available for a growing area to support the use of a lower level of dilution, the 1000:1 dilution should be employed.*

The minimum level of dilution recommended by FDA to mitigate the risk of viruses in treated effluent in a general scenario is 1000:1 dilution. FDA and DABA testing of effluent from other CT WPCF's utilizing UV disinfection has indicated that these facilities were efficient in reducing levels of enteric viruses and viral indicators. Based on the performance of the Mystic WPCF during the study period, if the quality of effluent remains consistent in the future during periods in which the conditional area is in the open status under higher flows and more challenging environmental and meteorological conditions, a 400:1 dilution zone may potentially be justified, and is consistent with FDA dilution recommendations for plants with UV disinfection that demonstrate exceptional treatment capabilities.

It is recommended that DABA staff periodically visit the WPCF to determine that operations remain consistent. Future studies should include confirmatory testing for MSC as a viral surrogate and fecal coliform, for both the treatment plant effluent as well as shellfish collected from the growing area at key sentinel locations. In the event of a WPCF malfunction or high flows (including a bypass, flows greater than 0.75 MGD, loss of UV disinfection, or disruption in treatment leading to partially treated effluent), the newly proposed Lower Mystic Harbor Conditionally Approved area should close to harvesting. Whenever possible, sampling during disruptions for MSC and fecal coliform will provide information for future management decisions. Continued timely notification by WPCF personnel is essential to ensure that the areas can be closed prior to contamination from the event.

APPENDIX 1 – METHODS (Section 2.0)

2.1 FDA Guidance on Establishing Closure Zones for WPCF Discharges

In consideration of Section II, Chapter IV @.03 E(5) (Prohibited Classification – Wastewater Discharges) of the National Shellfish Sanitation Program Model Ordinance, which notes that the determination of the size of a prohibited zone around a WPCF outfall shall include "the wastewater's dispersion and dilution, and the time of waste transport to the growing area where shellstock may be harvested" (iii), FDA has provided guidance to state shellfish control authorities to size prohibited zones around WPCF outfalls according to the following scenarios:

Scenario 1: In consideration of effluent discharged from a WPCF under **failure conditions** (such as a loss of disinfection), the prohibited zone should provide a sufficient amount of dilution to dilute the effluent discharged under failure conditions to the fecal coliform standard of 14 FC/100 ml within the boundaries of the prohibited zone.

OR

Scenario 2: In order to reduce the size of the prohibited zone, a conditionally approved zone may be operated if a factor of at least a 1000:1 dilution of effluent is achieved within the prohibited area to mitigate the impact of viruses, and there is a sufficient amount of time to close the conditional area to the harvesting of shellfish before the effluent discharged at the onset of a failure can travel to the boundaries of the prohibited zone

Note: the additional area beyond the prohibited zone to be closed under WPCF failure conditions should provide a sufficient amount of dilution to dilute the effluent discharged under failure conditions to the fecal coliform standard of 14 MPN/100 ml within the closed (due to failure) zone (consistent with Scenario 1).

Wastewater treatment technologies have drastically improved throughout the past few decades. During this time FDA has maintained a conservative position recognizing that any WPCF may remain subject to failure. FDA recognizes that with advancements in technologies, including improved monitoring and alarm systems for a treatment bypass or loss of disinfection, it may be possible to operate a conditional area as outlined in Scenario 2 above. This allows additional shellfish growing areas to be harvested under certain conditions.

When a WPCF is operating normally, disinfection has been shown to be effective in reducing the coliform bacteria group (fecal coliform and total coliform) to levels below shellfish harvesting standards as can be seen in WPCF permit records kept in accordance with the Environmental Protection Agency (EPA) National Pollutant Discharge Elimination System (NPDES) Program. However, human enteric viruses such as noroviruses and hepatitis A virus are more resistant to disinfection and thus are not reduced to the same degree as the coliform bacteria group. In an effort to mitigate the risk of contaminating shellfish with viruses, FDA has recommended a 1000:1 dilution as described in Scenario 2 as the minimum zone of dilution needed when the WPCF is operating under normal conditions, unless an alternative approach is well supported by data. One of the alternative approaches recommended by FDA is to use 400:1 dilution for plants with UV disinfection that demonstrate exceptional treatment capabilities and the absence of significant levels of viruses and viral indicators in treated effluent. In the case of the Mystic WPCF, the use of a 1000:1 dilution zone is supported by the data presented in this report.

2.2 Dye Standard Preparation and Fluorometer Calibration

The dye tracer used in this study was Rhodamine WT, purchased from the Keystone Aniline Corporation, with a specific gravity of approximately 1.12 (20% as dry dye). Ten (10) standards were prepared from the stock solution of Rhodamine WT dye and distilled water by serial dilution, ranging from 100,000 parts per million (ppm) to 0.1 parts per billion (ppb).

The Rhodamine WT dye was detected and its concentrations in the Housatonic River and Long Island Sound were obtained using a combined total of seven fluorometers. Four of these were WET Labs FLRHB submersible fluorometers (WET Labs, Inc., Philomath, OR) that were attached to the four monitoring stations. Two were WET Labs FLRHRT fluorometers that were pulled behind a boat and used for tracking the dye on each day of the Mystic study. The final was a WET Labs FLRHRT fluorometer interfaced with a SeaBird SBE19-plusV2 CTD used for conducting profiles of the dye at depth while at the same time capturing conductivity, temperature, and depth data within the water column.

The dye standards were used to develop calibration curves for FDA's WET Labs FLRHRT 2040, 2487, and 586 tracking and profiling fluorometers and the six station fluorometers – WET Labs FLRHB units 915, 1730, 2032, and 2416. With the subtraction of background fluorescence levels in the bay, these curves were used to calculate part per billion (ppb) levels of dye based on the WET Labs' measured fluorescence units (FUs).

The y-intercept of the calibration curve was adjusted so that a "0.1 ppb" result read as a perfect "0.1" on the curve. The slope and x-axis values for the curve remained the same, but this adjustment caused a slight addition of error (5-10% error) to the higher concentrations on the curve, such as 10 ppb. However, higher accuracy at the lower end of the curve, 0.1 ppb, is more vital in order to optimize sensitivity in detecting the dye at low concentrations, as important data tends to fall within the 0.1-1 ppb range during FDA dye studies. Using a calibration curve adjusted in this manner is necessary when converting raw FU readings to ppb values if sensitivity in the 0.1-1 ppb range is critical for the study. The WET Labs limit of detection in distilled water is 0.01 ppb, with a limit of detection in estuary water of approximately 0.01 – 0.03 ppb dependent on the specific fluorometer.

Background readings were captured prior to the study. For the interfaced SeaBird CTD and WET Labs FLRHRT 586 fluorometer (a.k.a., the "profiler"), background levels were recorded in terms of voltage readings and were converted to ppb units by applying a conversion factor and calibration curve data. However, the average of the raw voltage readings was used to program the background level for the profiler in RAFT-MAP. Background levels for the station fluorometers were determined by plotting all of the data collected by the fluorometers and finding the baseline FU level for readings taken prior to the dye injection in comparison with those recorded after the dye injection at each station. Background levels for the tracking fluorometers were based on maximum FU readings in the growing area, excluding outliers, detected prior to the dye injection. These background levels were programmed into RAFT-MAP and automatically subtracted from the fluorescence readings recorded in the bay after the dye injection.

2.4 Dye Injection

For the Mystic dye injection, a total of 6 gallons of dye mixture was injected at a constant rate into the WPCF effluent over a 12.4 hour period from 05:34 5/19/15 – 17:58 5/19/15.
To facilitate the pumping of dye, 3 gallons of deionized water was added to 3 gallons of dye creating a 50:50 water/dye dilution mixture. A Masterflex model 7553-20 variable speed peristaltic pump (Cole-Palmer Instrument Co.) was used to withdraw the tracer dye solution from a large plastic holding bin, using Masterflex Tygon L/S-14 tubing. A pump head size 7014 was used with a constant pumping rate of 31 ml/min which was maintained at about 123 revolutions/minute (rpm) head speed. The tracer dye mixture was fed continuously into the final effluent following the UV treatment over the 12.4 hour injection period. The dye was injected just after the last set of UV lamps, which then traveled a short distance to the outfall in the river. The initial concentration of the dye in the effluent was determined using the WPCF's flow average over the period of the dye injection (0.54 MGD). Flow rates out of the WPCF were based on SCADA readings.

2.5 Dye Tracking

Boat tracking was conducted on each day of the study with two boat-towed fluorometers, the WET Labs FLRHRT-2040 and WET Labs FLRHRT-2487, to track the dye past the cages; to determine the shape and edges of the dye plume; and to assess the dye concentrations and dilutions in the surface waters. The fluorometers were linked to Panasonic Toughbook C-19 field computers operating FDA's custom-made mobile GIS software, RAFT-MAP (Real-Time Application for Tracking and Mapping).

Two boats were used on each study for dye tracking, with each instrument on a different boat. Dye readings were taken on successive days May 19 – 21, 2015 for high and low tides. Traverses were done on all the days of the study from north to south and east to west and vice versa, and dye readings were also recorded at each of the fixed station locations to show changes in dye concentration and build-up with time.

While traverses of the dye were being done with two of the WET Labs FLRHRT fluorometers, the other FLRHRT fluorometer (586) was interfaced with a SeaBird SBE19-plusV2 CTD used for conducting profiles of the dye at depth at various locations along the path of the dye plume, particularly near the WPCF outfalls and each of the station locations. Fluorescence data from the SeaBird interfaced with the WET Labs was transmitted in voltage readings, but these were later converted to ppb readings using the dye calibration data.

A five-point moving average was applied to the dye concentration data to smooth out any false high or low readings. Dilution was calculated by dividing the initial concentration of dye injected at the WPCF by the final (five-point moving average) concentrations in the river.

Using RAFT-MAP, the fluorometer dye concentration readings (in FUs) with the associated GPS readings were converted into ppb units and automatically plotted on a field GIS map in real-time on the boat. The GIS caches were later synchronized into ArcGIS Desktop to post-process the data (e.g., remove false positive readings); add scales, legends, station locations, growing area classification lines, and other map features; and provide additional information, such as the accumulated dye concentrations and locations of dye readings with ≤ 1000:1 dilution.

The Geostatistical Tool in ArcGIS Desktop was used to interpolate the data and estimate dye concentrations in areas where no dye tracking was conducted based on the surrounding areas where dye tracking was conducted. The results were mapped in ArcGIS as the total dye mass for an 0.54 MGD flow (the flow rate during the Mystic study) over the May 19 – 21, 2015 study periods. Typically, dye dilutes to very low or non-detectable levels in less than 6 days, as this was the case for the Mystic studies. The Mystic study dye diluted to very low levels in less than three days.

2.6 Dilution Analysis - Dye Readings from Station Fluorometers

One of the advantages of the station fluorometers over the boat-towed fluorometers is that they can detect dye every ten minutes for thirty second intervals over the entire study period and can therefore pick up dye readings at depth during hours in which boat tracking was not possible. The fluorescence readings recorded by the submersible fluorometers at each of the six stations were downloaded, converted to ppb using each fluorometer's calibration curve chart, and plotted in SigmaPlot alongside tidal depth curves for the study period. The recorded boat-towed fluorometer readings at the surface within a 200 meter radius of each station were included on the charts as well.

A five-point moving average was applied to the dye concentration data to normalize high or low readings in the data. Dilution was calculated by dividing the initial concentration of dye injected at the WPCF by the final (five-point moving average) concentrations detected in the river.

Since only a 12.4 hour dye injection was conducted, FDA and DABA attempted to use the superposition method (Kirkpatrick, 1993) to estimate the steady state condition for dye at each of the stations using data collected from May 19 – 21, 2015 Mystic study and allow an adequate amount of time for the dye to be flushed out of the system. FDA has successfully employed the superposition method in a number of recent studies and uses this method to save time and resources. By adding the dye levels for each 6 hour period of the study together, the accumulated dye concentration value and associated dilution value provides a good reference point for how much dye was reaching the station over the entire study period. Unfortunately for this study the superposition method could not be employed because the data did not look predicable in the environment. Meaning it was too hard to predict a build-up from the data collected because high concentration values did not coincide with low tides and low concentrations did not coincide with high tides for example. Therefore trying to quantitatively predict the steady state concentrations was not recommended in this situation.

For each station, the minimum dilution was based on either the maximum concentration from the station fluorometer or the maximum concentration detected by the boat-tracking fluorometer within a 200 meter radius of that station (excluding outliers). Analyses and conclusions were based upon the lower of these dilution values in a conservative approach.

2.7 Microbiological Analysis of Wastewater

Indicator Microorganisms

FC densities in the WPCF influent and effluent were determined at the DABA laboratory using the mTEC procedure.

MSC densities were determined by using a modified double-agar-overlay method initially described by Cabelli (1988); the *E. coli* strain HS(pFamp)R (ATCC 700891) was utilized as the bacterial host strain.

APPENDIX 2 – FIGURES

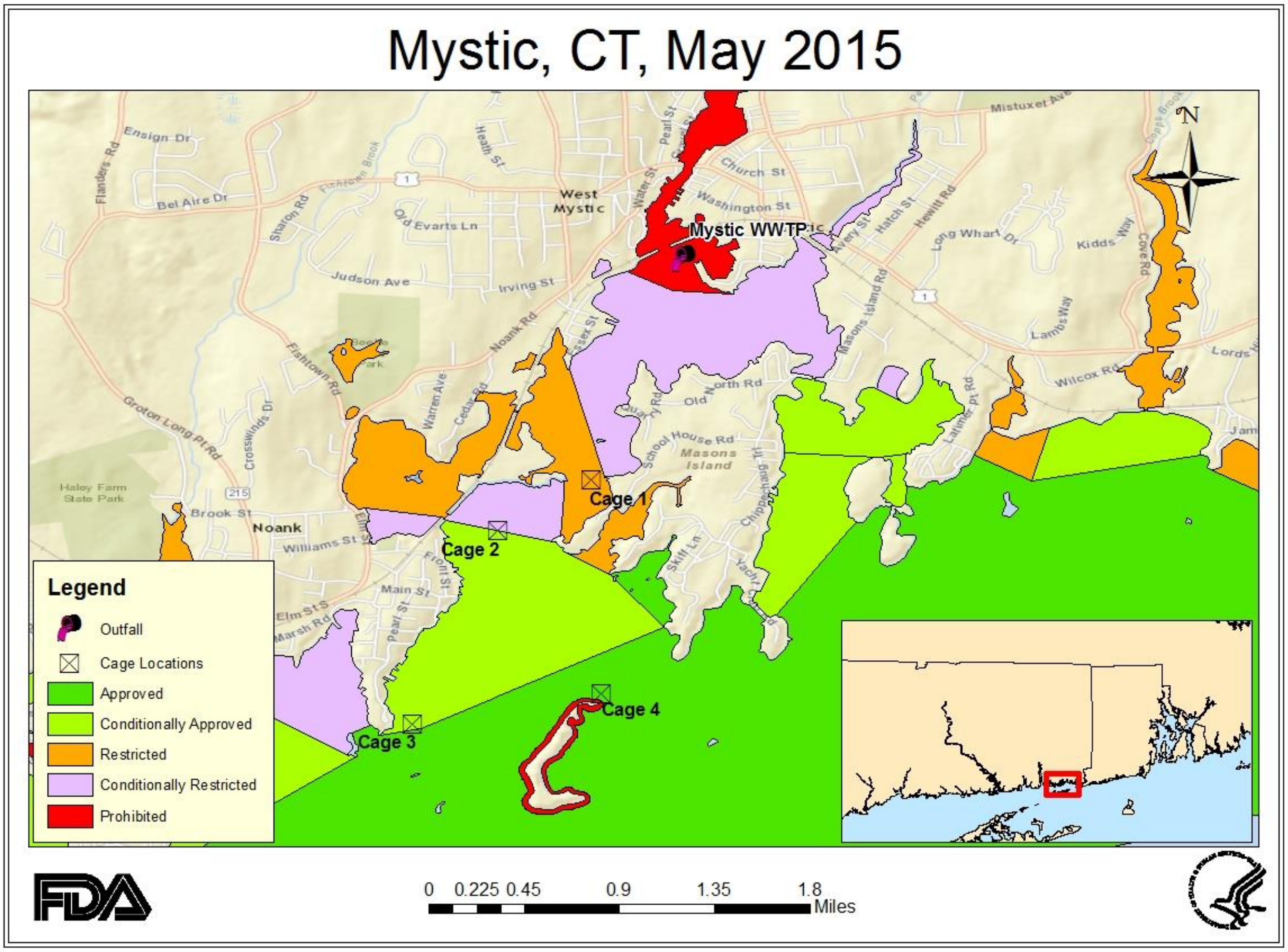


Figure 1. ***Map of Station Locations, Outfalls, and Classified Growing Areas Mystic, CT 2015***

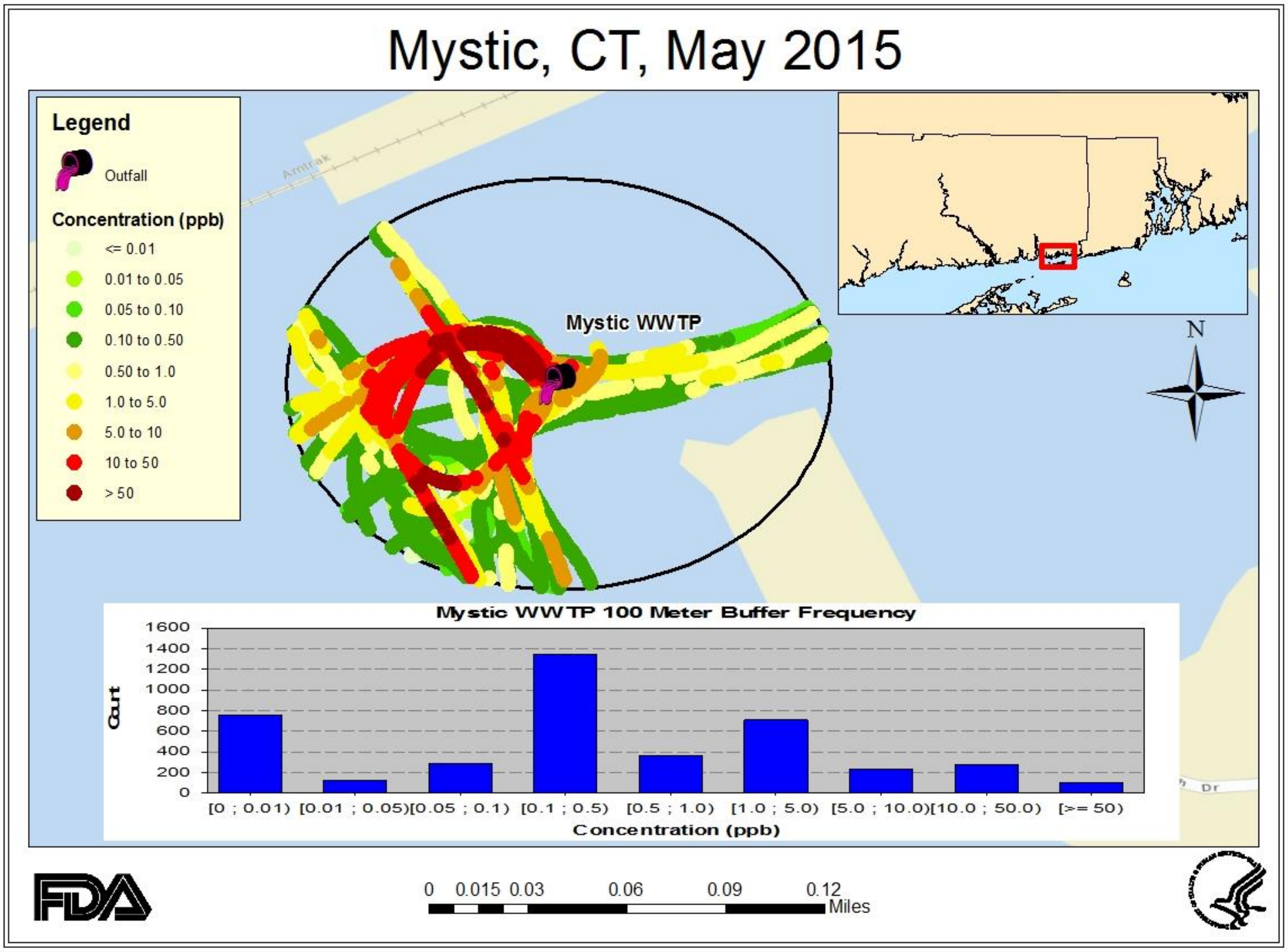


Figure 2. Mystic WPCF 100 Meter Buffer Frequency

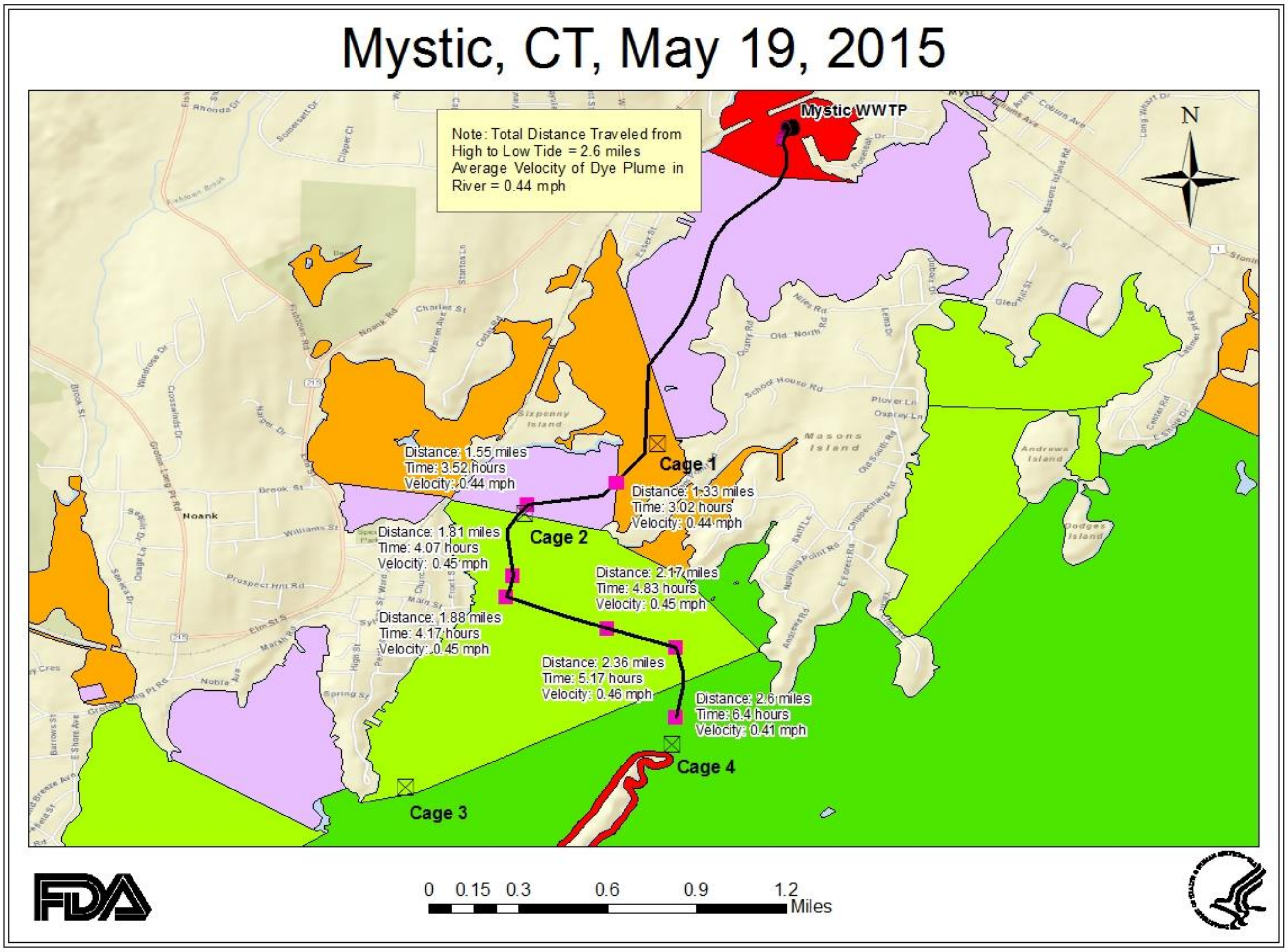


Figure 3. Velocity and Travel Time Estimates Based on Tracking Data.

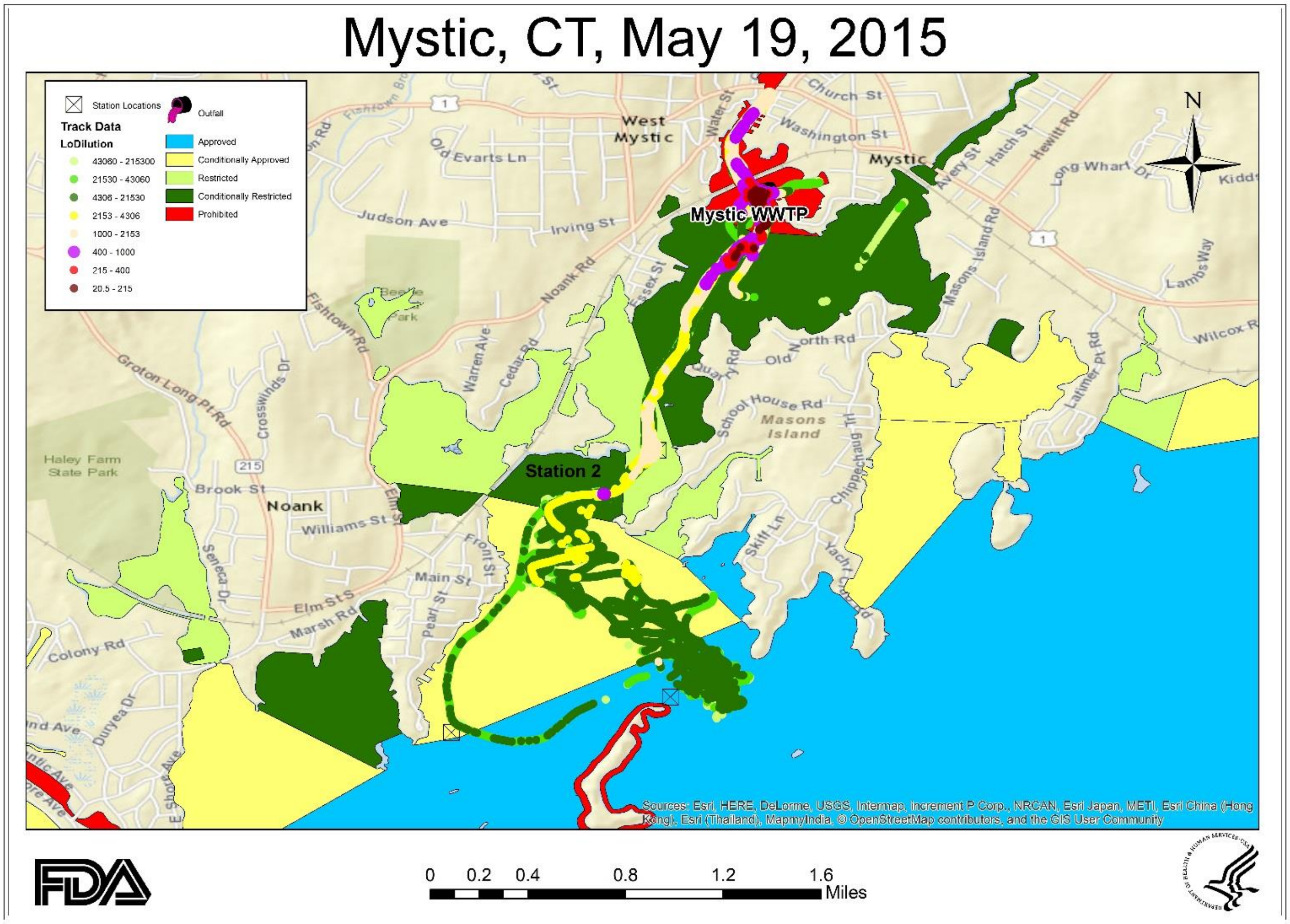


Figure 4. Dilution Levels for Day 1 Boat Tracking – Mystic Study

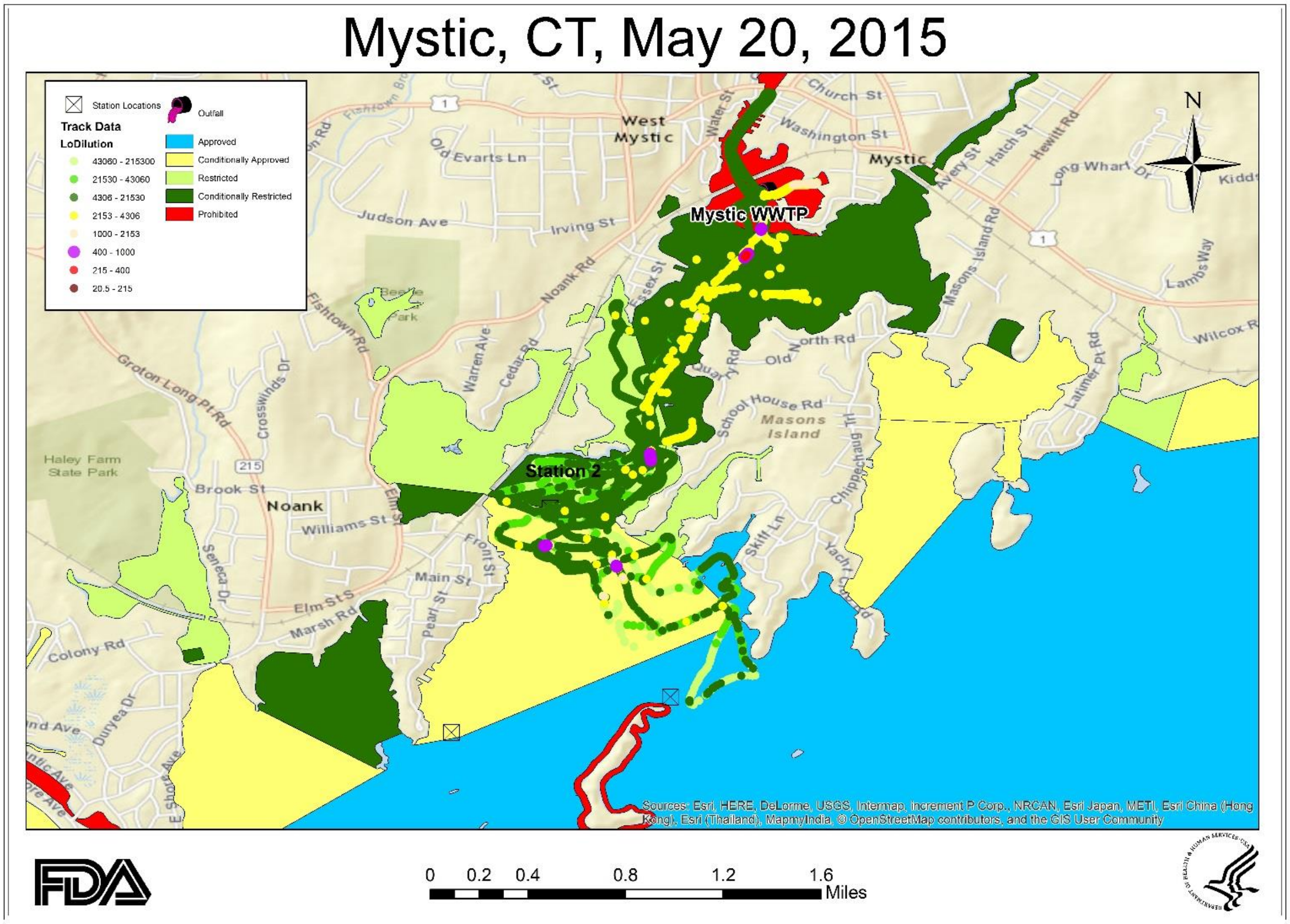


Figure 5. Dilution Levels for Day 2 Boat Tracking - Mystic Study

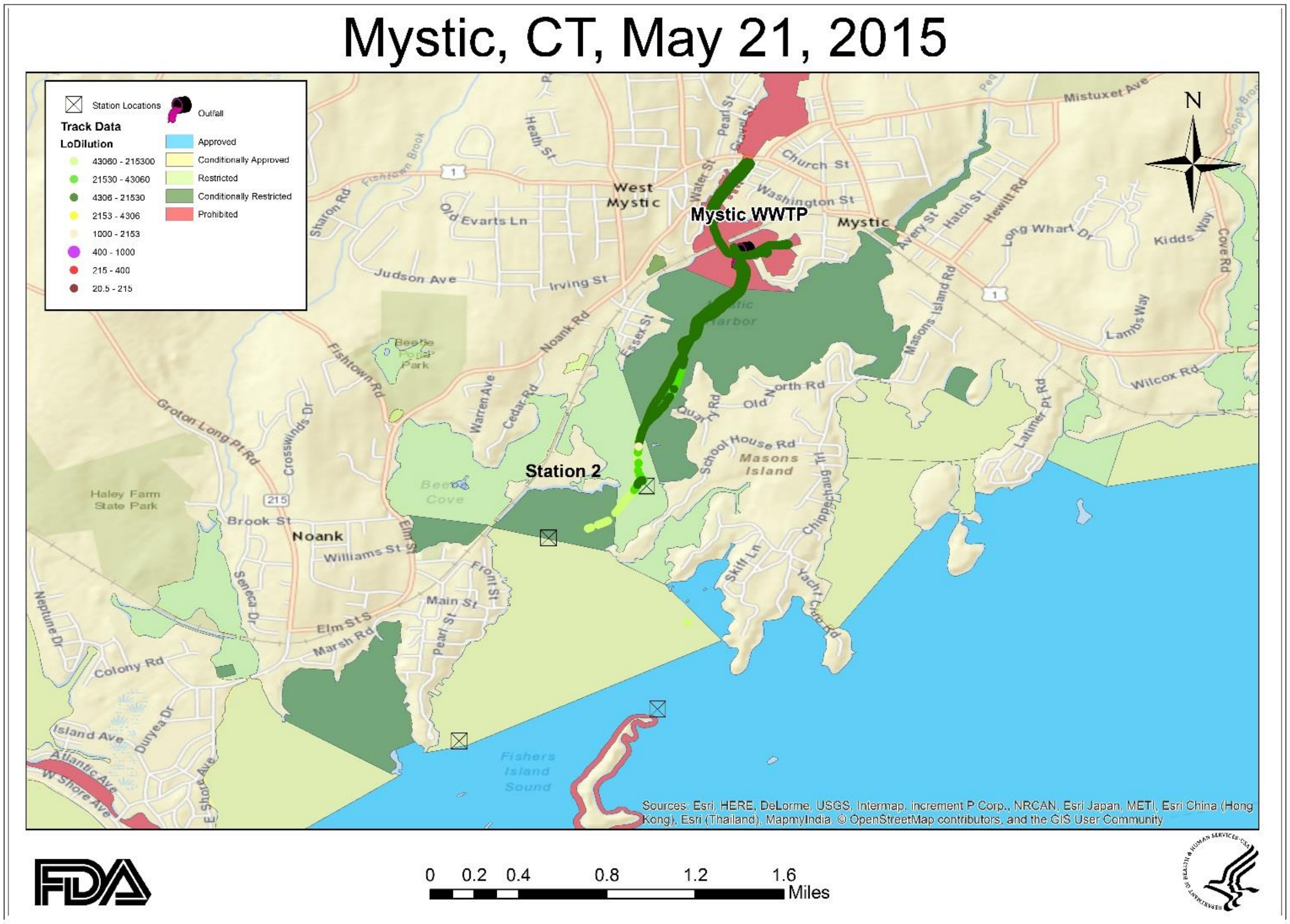


Figure 6. Dilution Levels for Day 3 Boat Tracking - Mystic Study

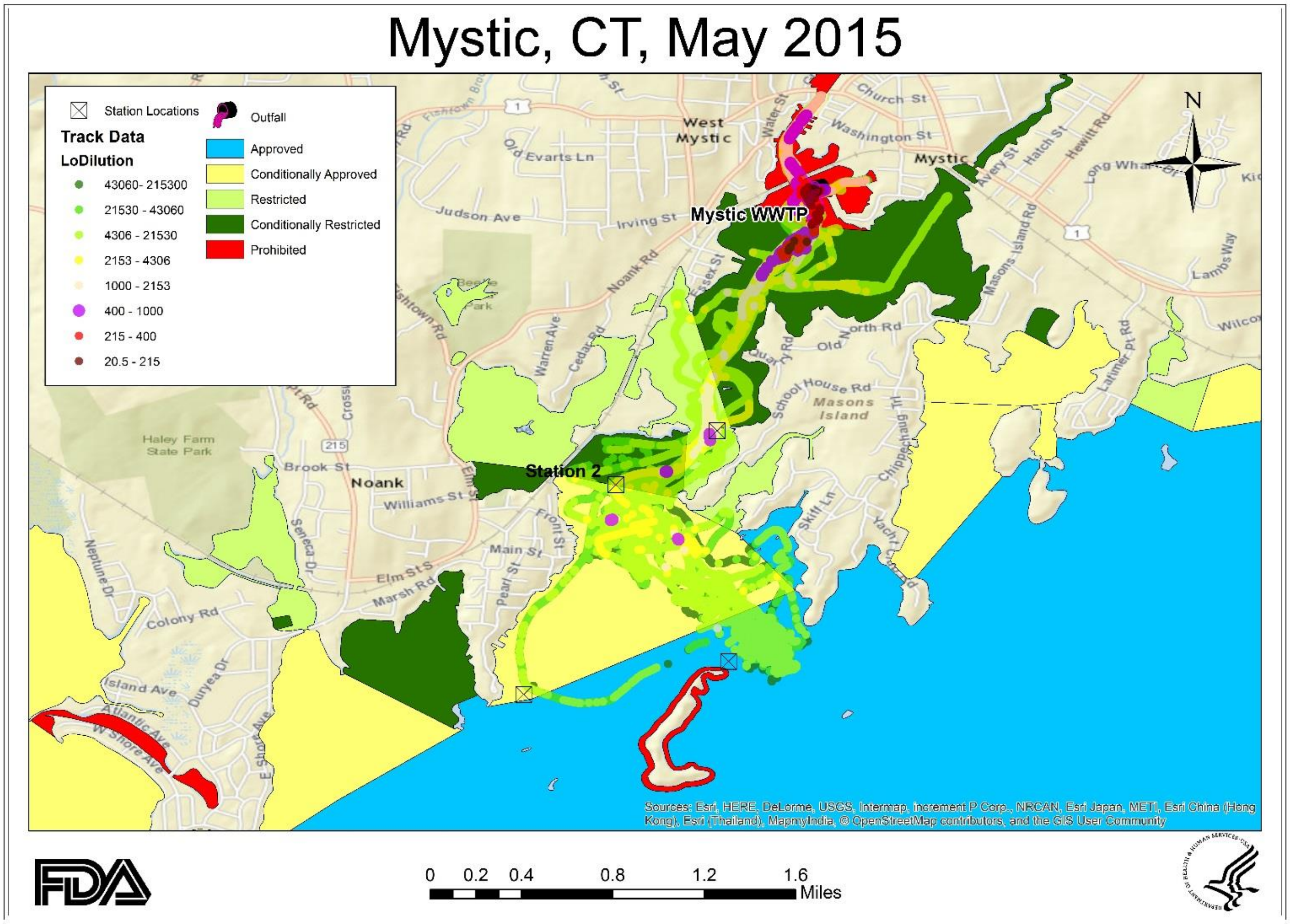


Figure 7. Combined 5-Point Moving Average Dilutions Values from 3 Days of Tracking with Existing Classification – Mystic Study

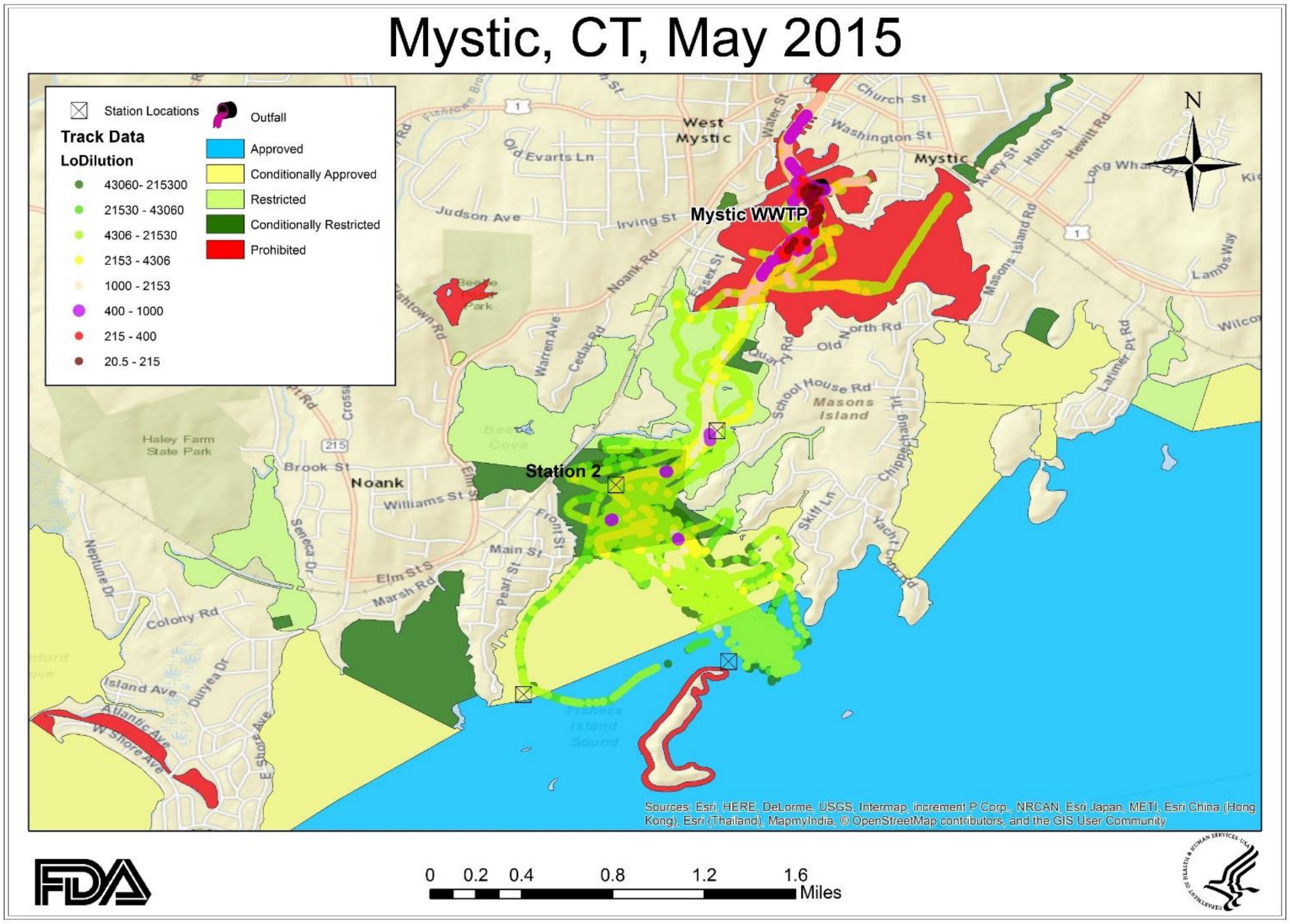


Figure 8. Combined 5-Point Moving Average Dilutions Values from 3 Days of Tracking with Proposed Classification Change – Mystic Study

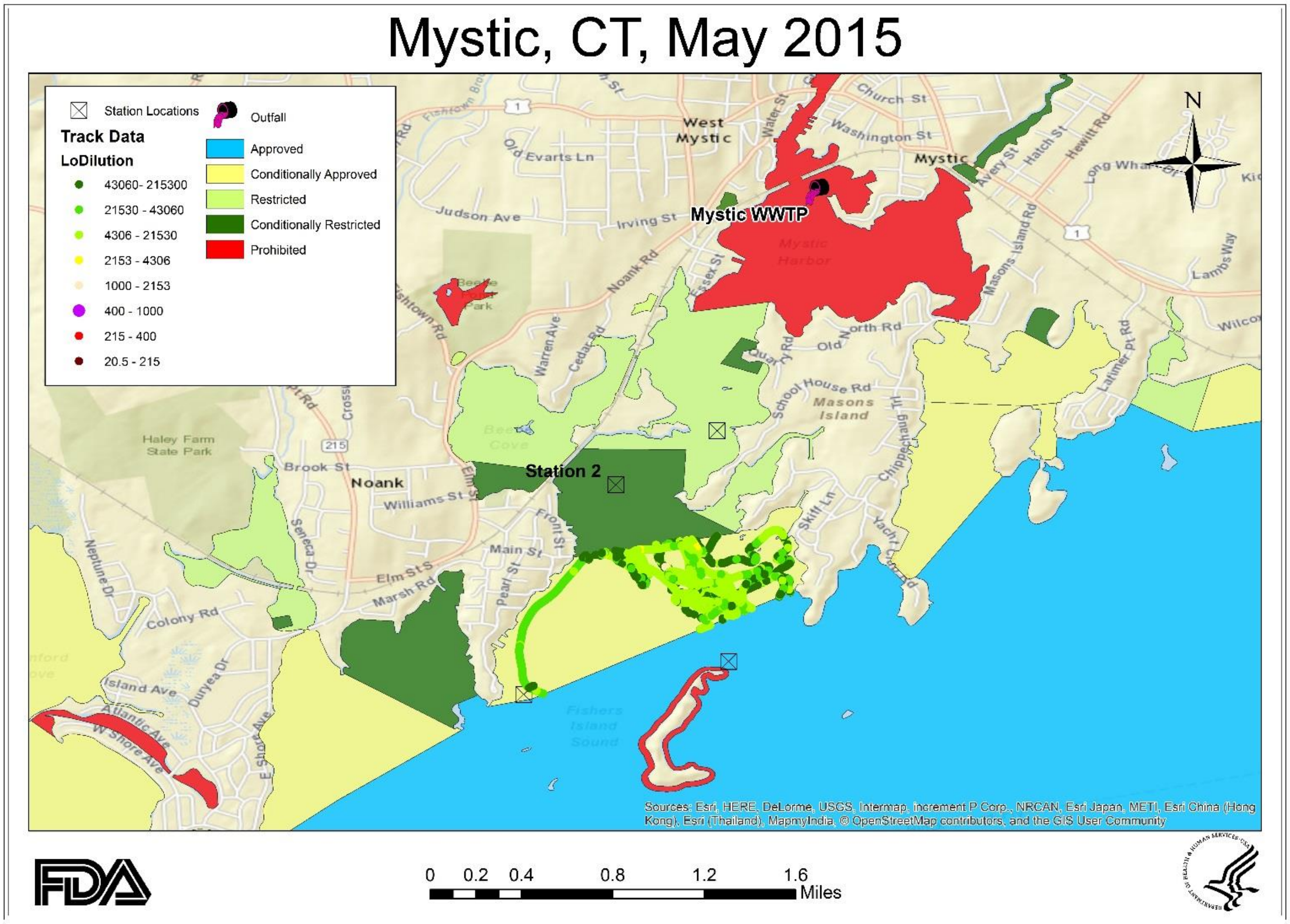


Figure 9. Combined 5-Point Moving Average Dilutions Associated with New Classification Polygon – Mystic Study. Note minimum dilution value of 1749:1 in proposed Conditionally Approved area.

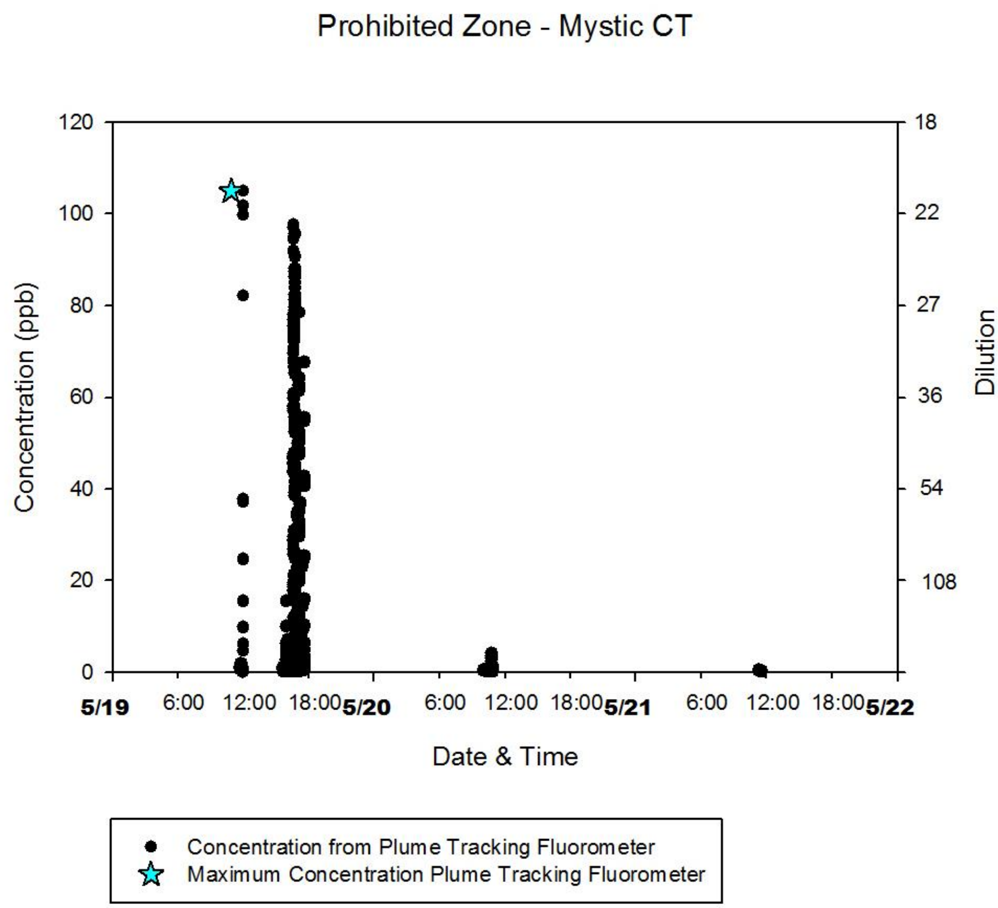


Figure 10. Combined 5-Point Moving Average Concentration Values and Associated Dilution readings within Prohibited zone. Mystic, CT.

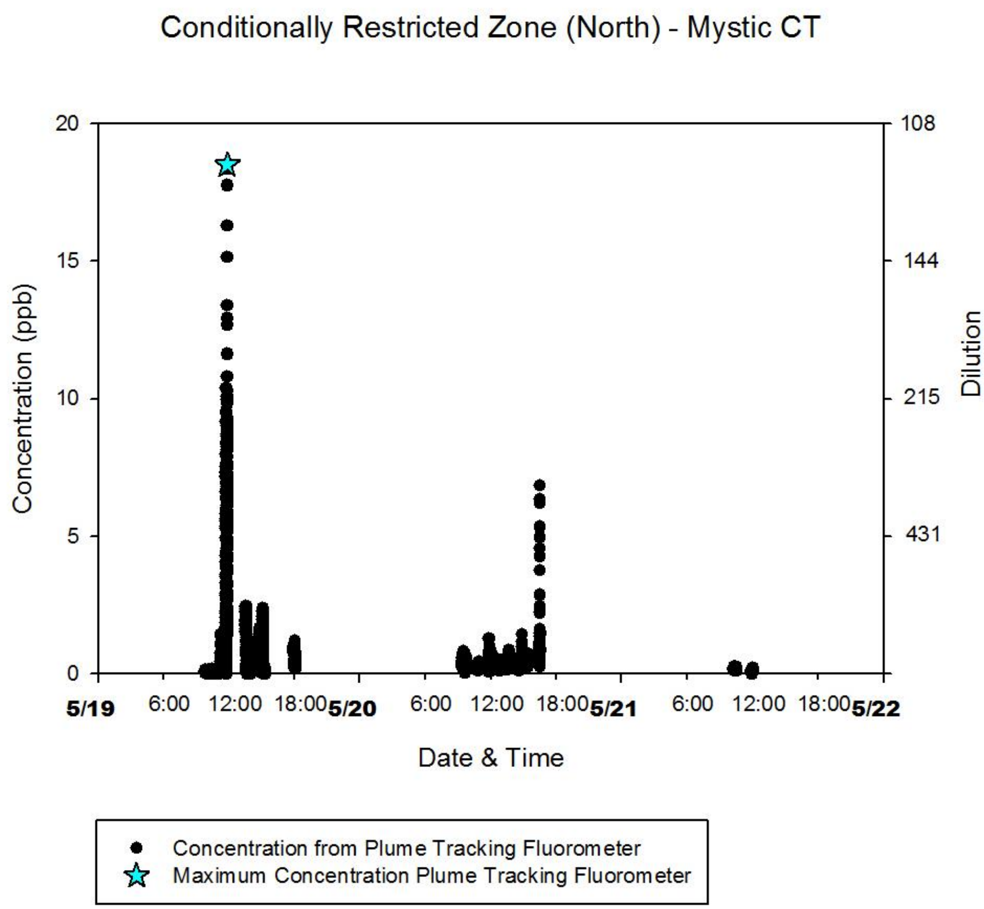


Figure 11. Combined 5-Point Moving Average Concentration Values and Associated Dilution readings within Conditionally Restricted Zone (North) Mystic, CT.

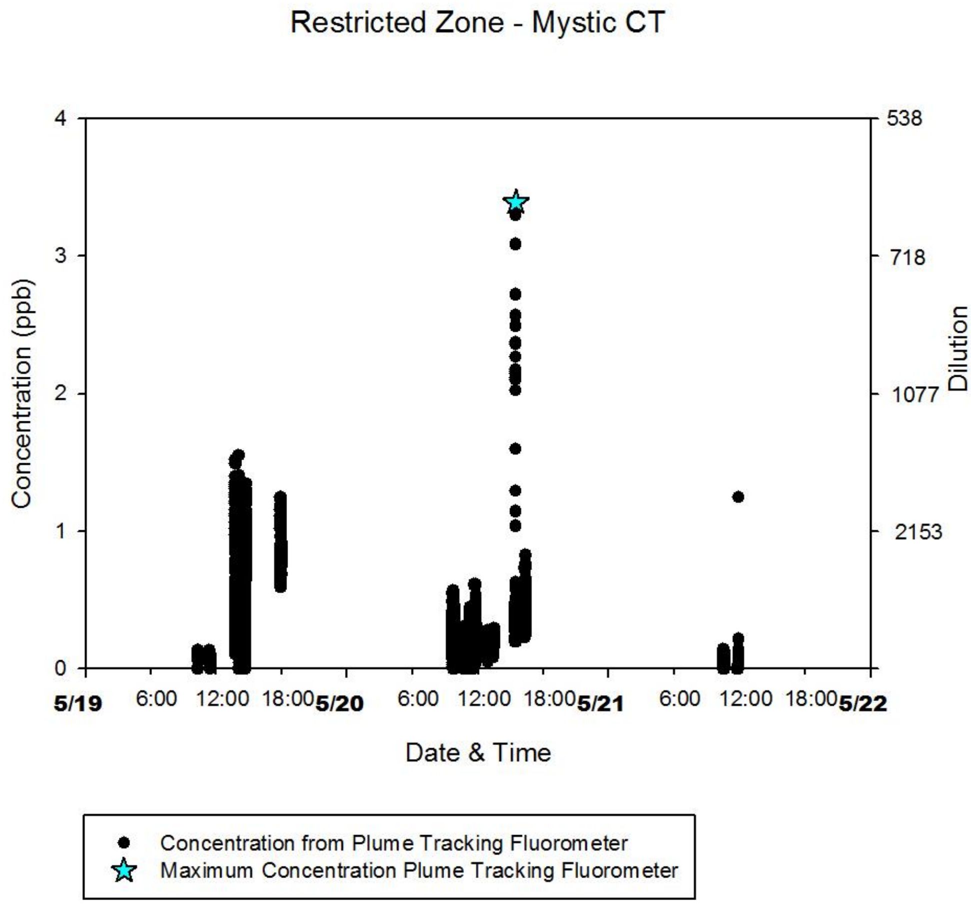


Figure 12. Combined 5-Point Moving Average Concentration Values and Associated Dilution readings within Restricted zone. Mystic, CT.

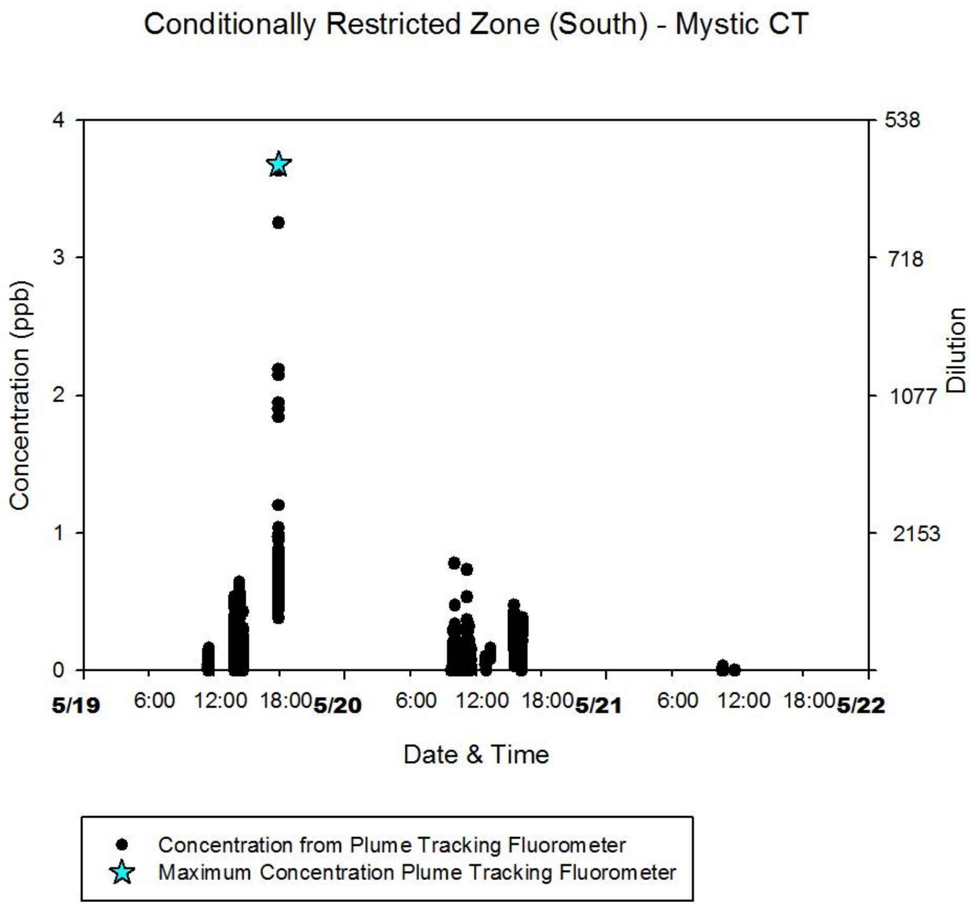


Figure 13. Combined 5-Point Moving Average Concentration Values and Associated Dilution readings within Conditionally Restricted zone (South). Mystic, CT.

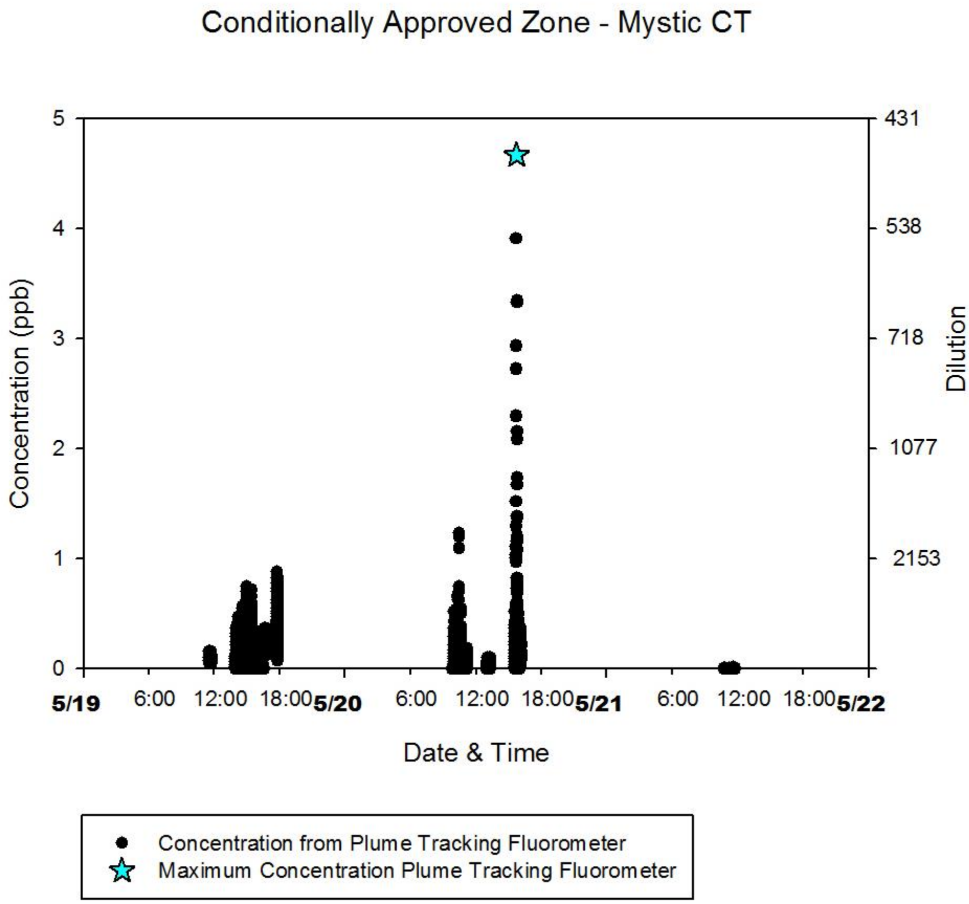


Figure 14. Combined 5-Point Moving Average Concentration Values and Associated Dilution readings within Conditionally Approved zone. Mystic, CT.

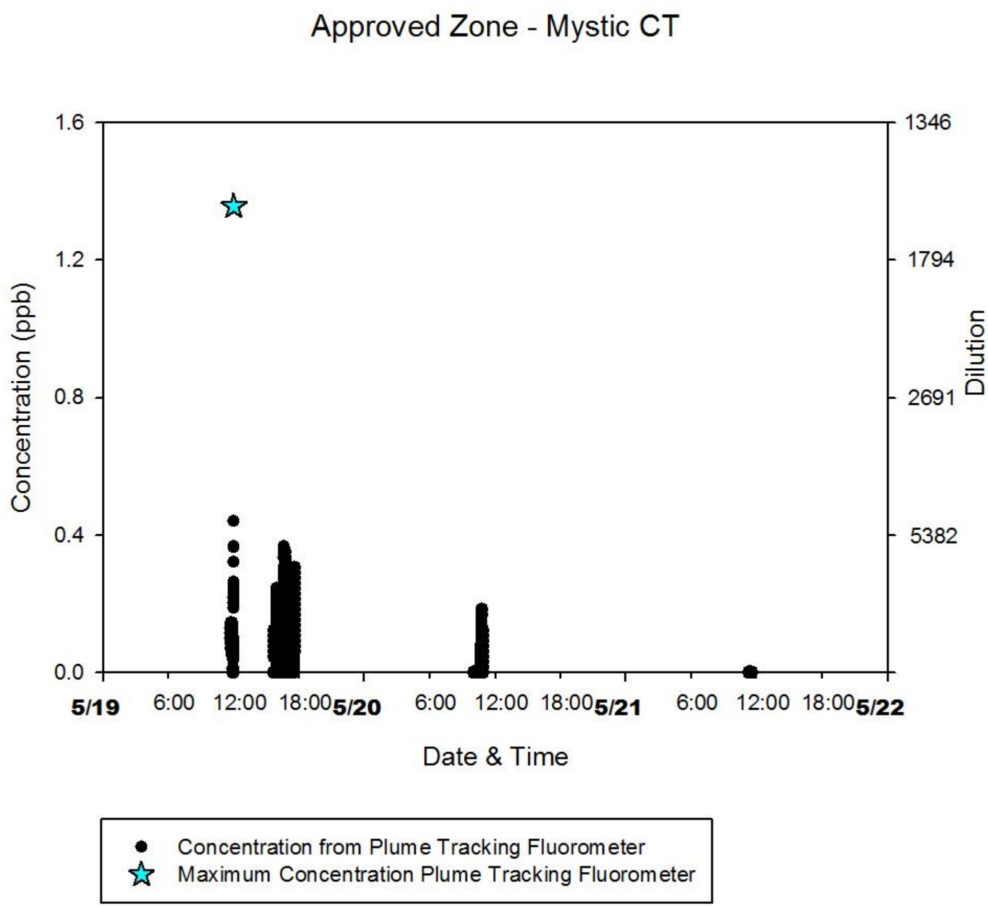


Figure 15. Combined 5-Point Moving Average Concentration Values and Associated Dilution readings within Approved zone. Mystic, CT.

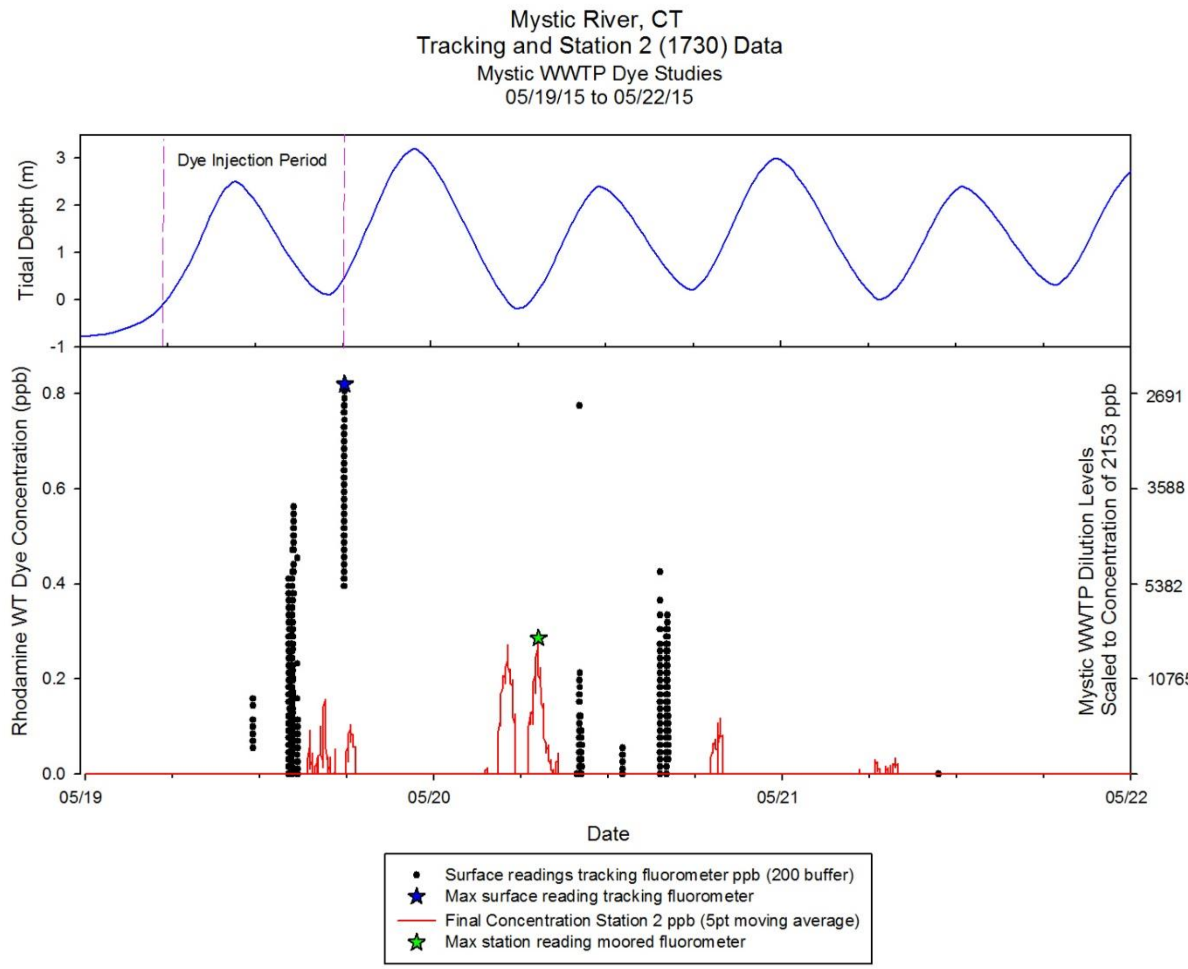


Figure 16. Station 2 Wet Labs 1730 Data – Mystic. Dilution scaled to concentration of 2153 ppb. The black dot plot indicates surface readings collected by tracking fluorometer within a 200 meter buffer around Station 2 moored fluorometer. The red line plot indicates the dye concentrations collected by the Station 2 (1730) fluoromoter, moored during study period. The top graph indicates the tidal height during the study period.

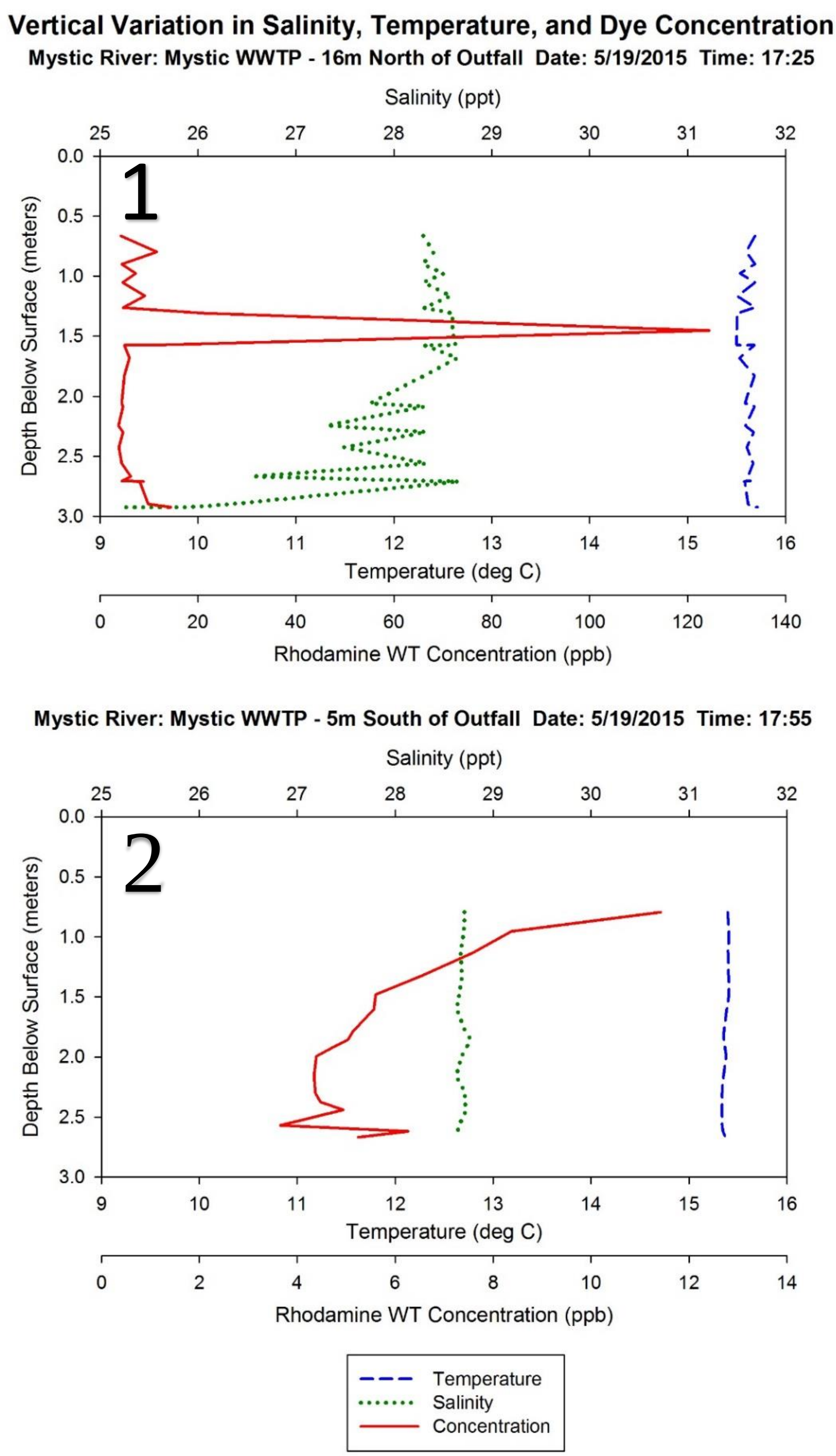


Figure 17. Seabird CTD with WET Labs Profile Fluorometer – Mystic Study

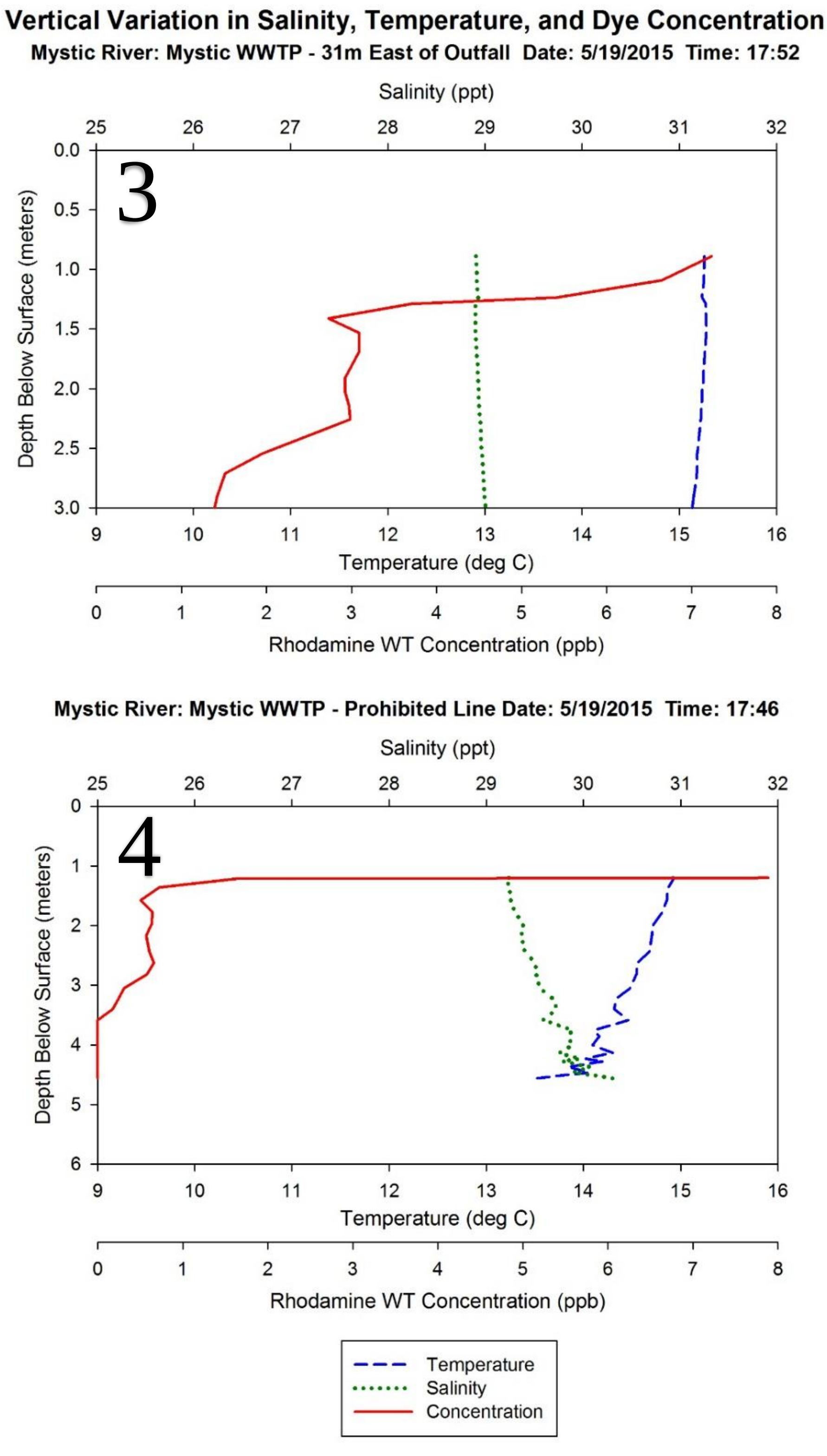


Figure 18. Seabird CTD with WET Labs Profile Fluorometer – Mystic Study

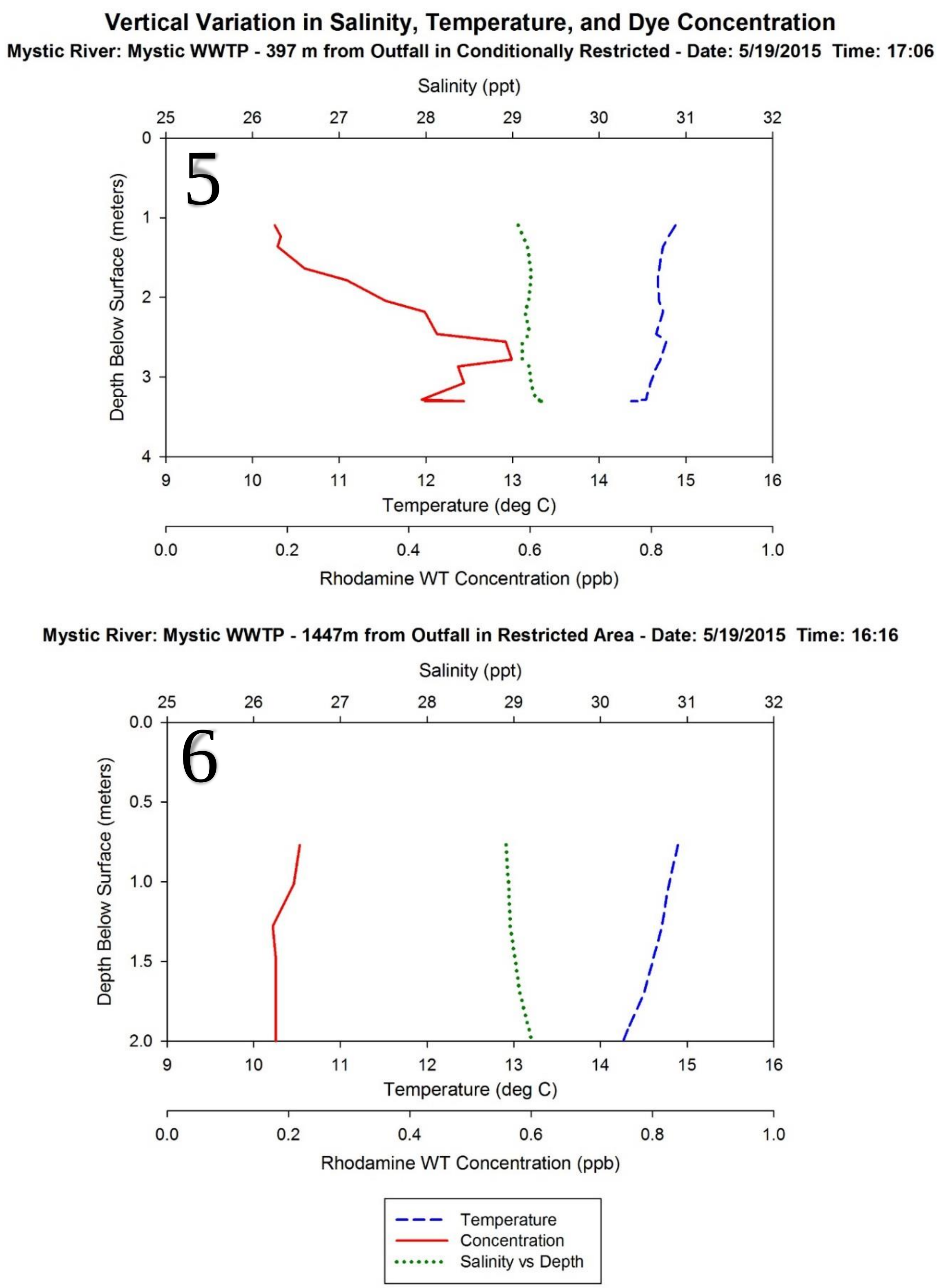
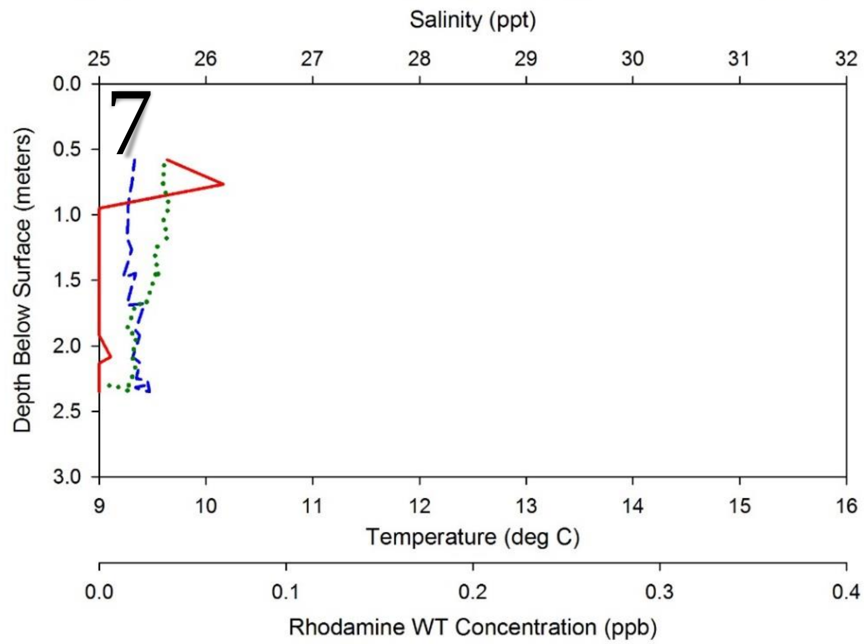


Figure 19. Seabird CTD with WET Labs Profile Fluorometer – Mystic Study

Vertical Variation in Salinity, Temperature, and Dye Concentration

Mystic River: Mystic WWTP - 3107m from Outfall in Cond. Approved Area - Date: 5/19/2015 Time: 14:22



Mystic River: Mystic WWTP - 3656m from Outfall in Approved Area - Date: 5/21/2015 Time: 11:29

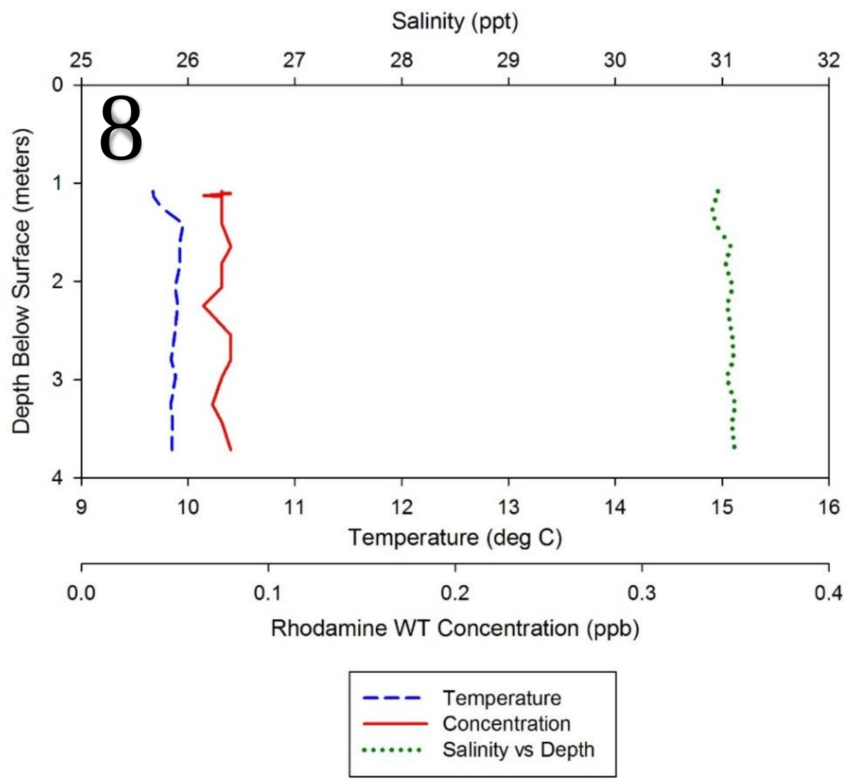


Figure 20. Seabird CTD with WET Labs 586 Profile Fluorometer – Mystic Study

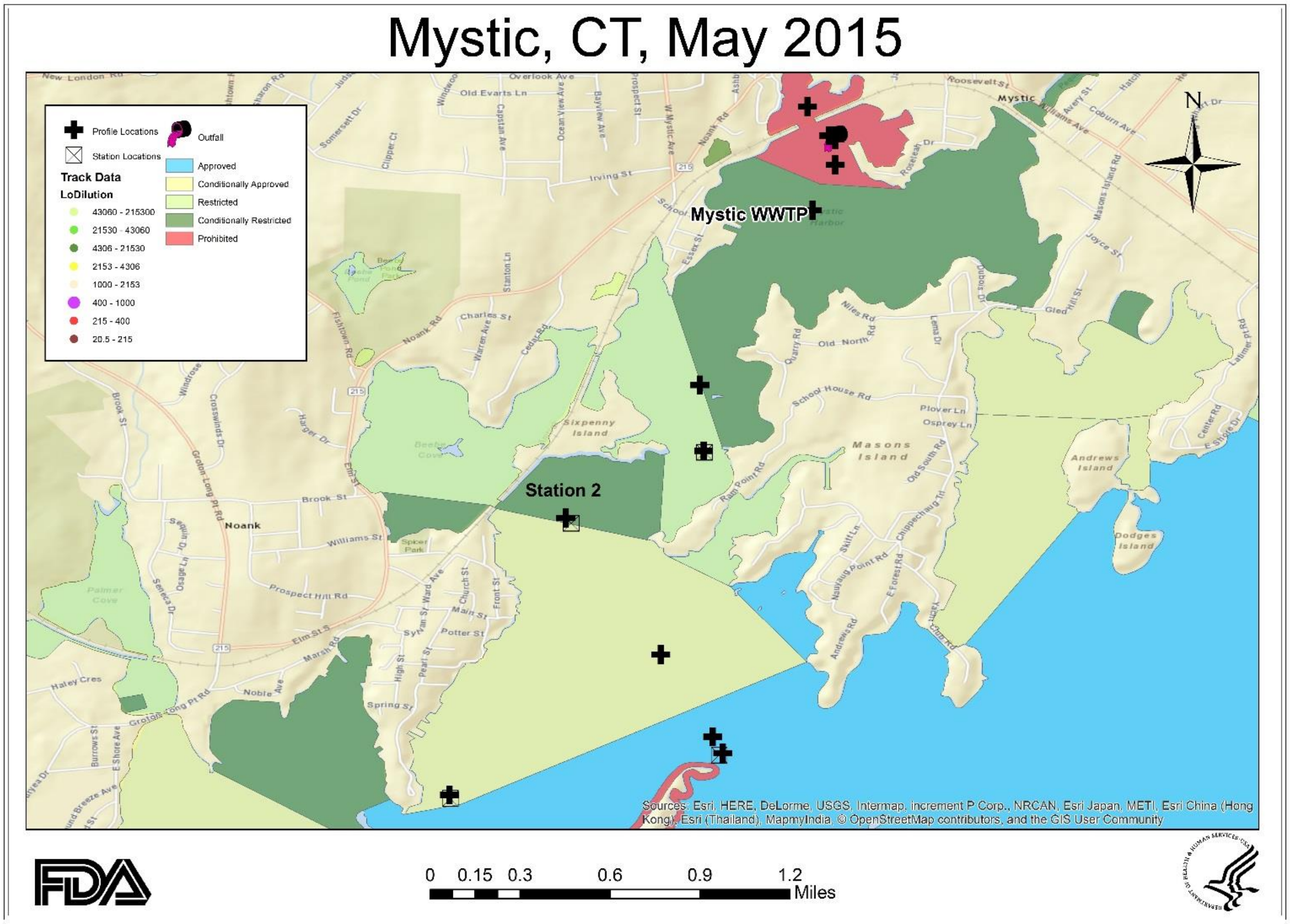


Figure 21. Locations of Seabird CTD with WET Labs 586 Profile Fluorometer – Mystic Study

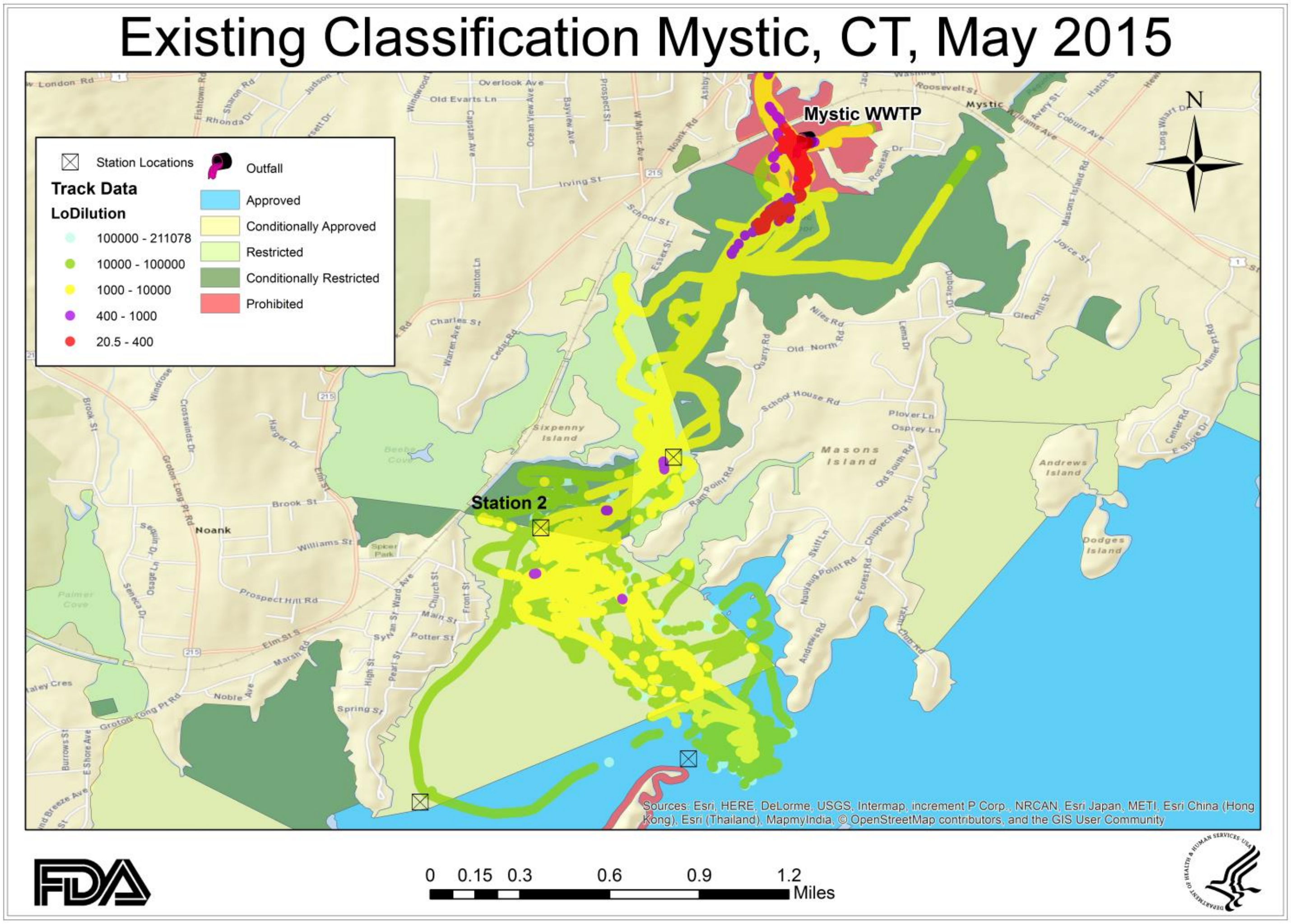


Figure 22. Combined 5-Point Moving Average Dilutions Values from 3 Days of Tracking with Existing Classification Under Recommended Dilution Schemes – Mystic Study

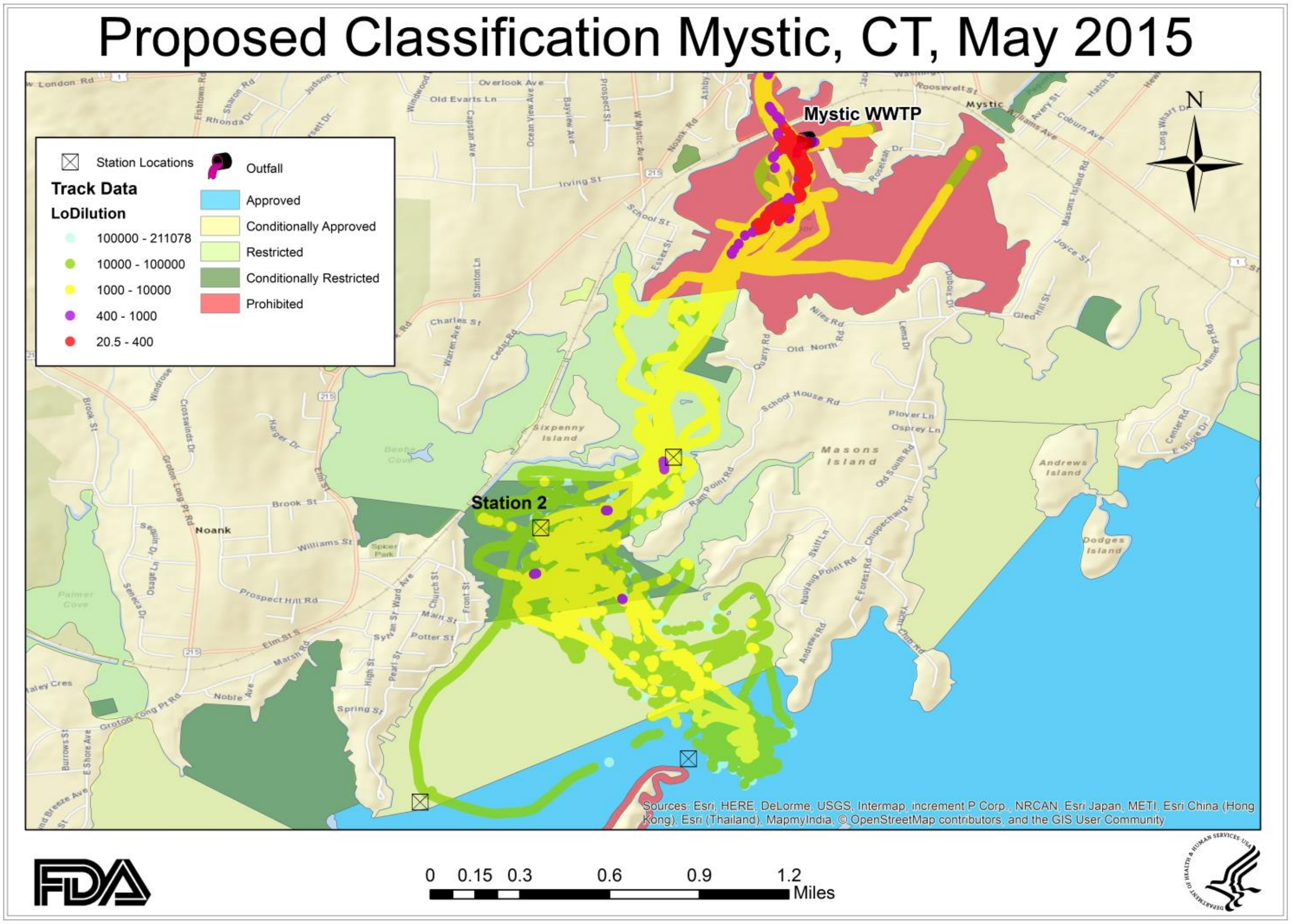


Figure 23. Combined 5-Point Moving Average Dilutions Values from 3 Days of Tracking with Proposed Classification Under Recommended Dilution Schemes – Mystic Study

References:

[i] Water Environment Federation (WEFTEC). 2010: WEFTEC 2010: Session 81 through Session 90 , 6164-6177(14). Full-scale BioMag Demonstration at the Mystic WPCF and Establishing the Basis-of-Design for a Permanent Installation. Downloaded on 11/30/15 at: http://www.ingentaconnect.com/content/wef/wefproc/2010/00002010/00000010/art00024

[iii] Interstate Shellfish Sanitation Conference. 2015. Dilution Guidance for Prohibited Zones Associated with Wastewater Discharges

[iv] Interstate Shellfish Sanitation Conference. 2015. Dilution Guidance for Prohibited Zones Associated with Wastewater Discharges.

Proposal Subject	Dilution Guidance for Prohibited Zones Associated with Wastewater Discharges
Specific NSSP Guide Reference	NSSP Guide Section IV. Guidance Documents Chapter II. Growing Areas
Text of Proposal/ Requested Action	Refer to 2015 Proposal Package
Public Health Significance	The public health purpose of this guidance is to provide the scientific basis and recommendations for determining appropriately sized Prohibited Areas (closure zones) around waste water treatment plants (WWTP). Section II, Chapter IV. @.03 (5) currently mandates that a prohibited zone be established, but there is no specific guidance information on how to calculate the size of the prohibited zone to ensure that microbiological pathogens (particularly viruses) from WWTP do not adversely impact the growing area at the time of harvest. It is expected that this guidance will provide all ISSC stakeholders with better information on which to make informed, scientifically based decisions
Cost Information	
Action by 2013 Task Force I	Recommended referral of Proposal 13-118 to an appropriate committee as determined by the Conference Chairman with additional instructions to the ISSC Executive Office to create a workgroup to meet quarterly and report back to the Conference at the next ISSC meeting.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-118.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-118.
Action by 2015 Growing Area Classification Committee	Recommended adoption of Proposal 13-118 with substitute language as follows: Determining Appropriately Sized Prohibited Areas Associated with Wastewater Treatment Plants <u>Introduction</u> The original National Shellfish Sanitation Program (NSSP) principles have proved effective in controlling bacterial illness associated with shellfish harvested from polluted waters. These principles, namely a robust sanitary survey, regular water and shellfish monitoring using bacterial indicators, controlled harvest times and labelling the origin of shell stock remain applicable as the primary preventative food safety control measures for growing areas. However, there is now ample scientific evidence to show that the current bacterial indicators are inadequate to predict the risk of viral illness for the following reasons: (1) Enteric viruses are resistant to treatment and disinfection processes in a wastewater treatment plant (WWTP) and are frequently detected in the WWTP's final effluent under normal operating conditions (Baggi et al. 2001; Burkhardt et al. 2005, Pouillot et al. 2015). (2) Shellfish can bioaccumulate enteric viruses up to 100-fold from surrounding water (Seraichekas et al. 1968; Maalouf et al. 2011). (3) Certain enteric viruses are retained by molluscan shellfish to a greater extent and for longer than the indicator bacteria currently used to classify shellfish growing areas (Sobsey et al. 1987; Dore & Lees 1995; Love et al. 2010). It has been well documented that enteric virus detection is not indexed by levels of conventional indicator bacteria.

	For several decades now viral illnesses, in particular norovirus (NoV) and Hepatitis A (HAV), have been the most common food safety problem associated with bivalve molluscan shellfish (Woods 2010; Iwamoto et al 2010; Scallan et al. 2011; Batz et al. 2012; Hall et al 2012). NoV genogroups I, II and IV and HAV are typically associated with ill-individuals and transferred by the fecal-oral route. Because WWTPs do not completely remove infectious enteric viruses emphasis should be placed on the importance of ensuring there is adequate dilution between a sewage source and a shellfish growing area. In addition to the risk of enteric viruses WWTP effluents may also contain other chemicals and deleterious substances including pharmaceuticals, nanoparticles, and other contaminants of emerging concern. Establishment of a prohibitive area in proximity to WWTP discharges is an effective strategy to reduce the risk posed by both enteric viruses and other contaminants found in WWTP effluents. This guide provides information on the recommended dilution rates with respect to enteric viruses to ensure WWTP effluent does not cause a significant viral food safety risk within shellfish growing areas. The guide also considers the factors that should be used to assess a WWTP. <u>Delineation of the Prohibited Zone around a Wastewater Treatment Plant</u> The NSSP Model Ordinance Section II, Chapter IV. @.03 (2) (b) and @.03 E(5) states that all growing areas which have a sewage treatment plant outfall or other point source outfall of public health significance within or adjacent to the shellfish growing area must have a prohibited classification established adjacent to the outfall taking account of the following factors: (1) The volume flow rate, location of discharge, performance of the wastewater treatment plant and the microbiological quality of the effluent; (2) The decay rate of the contaminants of public health significance in the wastewater discharged; (3) The wastewater's dispersion and dilution and the time of waste transport to the area where shellstock may be harvested; and (4) The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries. There are several important considerations for the shellfish authority to consider when establishing the size of each prohibited zone: (1) The area to ensure that there is adequate dilution when the WWTP is operating as normal. “Normal” means that the WWTP is operating fully within the plant’s design specifications, including design flows; treatment stages; disinfection; as well as compliance with all permit conditions that relate to the WWTPs effectiveness in reducing enteric viruses in sewage. Below is not an exhaustive list but serves as examples of situations that could occur and are critical for Shellfish Control Authorities (SCAs) on evaluating each WWTP when developing Conditional Area Management Plan (CAMP): <u>Bypassing stage of treatment</u> A plant may be considered operating outside of normal operation if a treatment stage such as primary or secondary treatment is bypassed which

may result in an increased load of solids in the disinfection step and reduce the effectiveness of disinfection. An additional example would be when a WWTP experiences a loss in disinfection and thus the ability to effectively treat the final effluent. SCAs should determine the significance of these types of events and make appropriate provisions in the CAMP.

Operating outside design specifications/other types of failures or events

It is not uncommon for a WWTP to periodically experience mechanical failures of equipment that could alter the treatment of sewage. Additionally, a WWTP may also need to periodically perform routine maintenance to the various stages of treatment and may need to temporarily take a portion of a treatment stage off-line for cleaning. Other unexpected maintenance may need to occur for example bio-fouling of filters or membranes used in treatment. SCAs should be informed by WWTP operators of these events to determine if any additional temporary action is needed if not addressed in the CAMP.

Operating above design flow

Some WWTPs may operate above its design flow and not necessarily bypass any particular stage of treatment. During these events it is typical for WWTP operators to adjust the operation of the WWTP which may include reducing the treatment time in the aeration stage and/or solids separation/settling stage of treatment. Under some circumstances this could lead to a significant reduction in the effectiveness of disinfection. SCAs may consider assessing the efficiency of WWTPs to determine the significance of these type of events and if additional provisions should be made in the CAMP.

WWTP permit violations

If a WWTP is exceeding the permitted bacterial indicator levels in the final effluent this indicates that effectiveness of the disinfection step has been reduced. Other measured parameters in the effluent (e.g. TSS, BOD) may also indicate a reduction in treatment efficiency as occurred. SCAs may consider assessing the efficiency of WWTPs to determine the significance of these type of events and if additional provisions should be made in the CAMP.

Situations where compliance with permit but risk to shellfish growing area

There could be situations in which a particular WWTP could be in compliance with a permit, and could still pose a risk to the shellfish harvest area. For example, a WWTP may have permit conditions to allow for flow blending during high flow periods where a portion of the sewage may receive full treatment but a portion of the sewage may only be partially treated and "blended" in the final disinfection step. Although this may be an acceptable practice under a permit it could result in conditions in which the efficiency of the WWTP to remove enteric viruses is considerably reduced. SCAs may consider assessing the efficiency of WWTPs to determine the significance of these type of events and if additional provisions should be made in the CAMP.

(2) That the collection system has no malfunctions, bypasses or other factors that would lead to significant leakages of untreated sewage to the marine environment.

(3) That there is adequate detection and response time when any malfunction occurs to ensure that all harvesting ceases and closures are enforced, so that contaminated product does not reach the market.

Additional considerations

It is critical for SCAs to communicate with WWTP operators and ensure that there is no confusion over how SCAs define "outside of normal operation" in a Conditional Area Management Plan (CAMP) which may differ from how "malfunctions" or "violations" are defined in a permit. The SCAs also need to ensure that the WWTP operators understand the CAMP and that shellfish growing areas may close based on conditions of the CAMP even though the WWTP is operating in compliance within permitted conditions. Thus, it is important to communicate with WWTP operators to ensure that when shellfish closures occur and are reported that SCAs are using terminology that is understood by both parties.

Guidelines for Dilution, Dispersion, and Time of Travel of Effluent

Dilution refers to the dilution of effluent that occurs when the effluent is subjected to a number of physical processes in the receiving waters including turbulent mixing of the effluent in the vicinity of the outfall and at further distances primarily through tidal action, wind, and density stratification. Dispersion refers to the spread, location, and shape of the effluent discharge plume with time as it leaves the WWTP outfall. Time of travel refers to the time it takes effluent to reach the shellfish harvest site starting from the point of discharge.

It is essential to recognize that water samples collected near discharge outfalls are not useful for determining the size of prohibited zones because normal operating conditions in WWTPs can effectively reduce or even eliminate the fecal and total coliforms which are the current indicator microorganisms used to assess treatment efficiency. In contrast, many human enteric viruses are not inactivated by functioning WWTP treatment and disinfection systems, hence the need for an adequate dilution zone between the outfall and the shellfish resource.

It is important to consider not only the WWTP discharge, but also overflow points on the collection system such as those from pumping stations. While a malfunctioning WWTP may provide partial treatment, the discharge from a collection system is untreated and may be a more common failure point in the overall system.

When determining if a WWTP or collection system discharge within the watershed or catchment area draining to a shellfish estuary potentially impacts a shellfish growing area, in the absence of a performance history of the treatment and collection system, and a database of influent and effluent quality, the NSSP recommends that a worst case raw sewage discharge be assumed. In this circumstance, if a level of 1.4×10^6 FC/100ml is assumed for a raw sewage release, a 100,000:1 dilution would be required to dilute the sewage sufficient to meet the approved area standard of 14 FC/100ml. If dilution analysis determines that the location of the discharge is such that the dilution of effluent would be greater than 100,000:1 then the WWTP could be considered located outside the zone of influence to the shellfish growing area. Different dilution ratios may be applied depending on the known concentration of sewage, provided that the water quality objective of the downstream harvest area is met.

In areas where the required WWTP discharge dilution is less than 100,000:1 and/or a raw sewage release results in FC levels in the growing area of >14 FC/100 ml a conditional management may be considered. However, conditional management is only recommended for, highly efficient WWTPs that are well monitored to detect malfunctions and changes in effluent quality and when the shellfish authority has the resources to effectively administrate and patrol the conditions of the growing area management plan.

In all cases the FDA recommends the minimum of a 1000:1 dilution around a WWTP outfall to mitigate the impact of viruses on shellfish growing areas.

A dye study can be used to measure the dilution and dispersion of the effluent during specific discharge conditions. Computer modeling programs can also be used to estimate the dispersion and dilution of the effluent plume from WWTPs and collection system overflows.

<u>Scientific Rationale for 1000:1 Dilution Guidance</u>

In 1995 the FDA determined the 1000:1 dilution was necessary using the most relevant the scientific literature available at that time (Kohn, et al. 1995; Havelaar et al. 1993; Kapikian et al. 1990; Liu et al. 1966). In 2008 FDA performed an investigation in the upper portion of Mobile Bay, Alabama, the results of which were published in the Journal of Shellfish Research (Goblick, et al., 2011). The article describes how FDA used technical advances to assess the 1995 1000:1 dilution recommendation. The Mobile Bay study confirmed that this level of dilution was appropriate to mitigate the risk of viruses discharged in treated wastewater effluent.

Since the 2008 Mobile Bay study there have been major advances in the detection and enumeration of NoV in wastewater and shellfish and fluorometer technologies have enabled more sophisticated hydrographic dye study methods. Using these advances, FDA has now conducted numerous dye studies supplemented with the testing of shellfish sentinels for enteric viruses and their surrogates. The findings from these studies demonstrate that achieving a steady-state 1000:1 dilution level in the requisite Prohibited area appears to be adequate for mitigating the impacts of viruses on shellfish when WWTPs have typical treatment and disinfection practices, such as secondary treatment and chlorination, and when operating under normal conditions.

While evaluating the 1000:1 dilution level Male Specific Coliphage (MSC) results in shellfish from the 2008-2015 studies were evaluated. These collaborative studies with State Shellfish Control Authorities and Industry were conducted in the Gulf, Mid-Alantic, East and West Coast, and under varying hydrographic and meteorological conditions. Various additional factors were considered such as type of wastewater treatment and disinfection technology, seasonal conditions, and shellfish species etc. and are represented in the data collected. In some cases, data was collected during a period of which the WWTP was considered to be operating outside of “normal” operating conditions. In other cases, the WWTP was considered not suitable for conditional area management due to design/poor performance even during routine/normal operation. Focus was given to the MSC threshold of 50 PFU/100 grams of shellfish tissue which is the level used for re-opening harvest areas after an emergency closure due to raw untreated sewage discharged from a large community sewage collection system or a WWTP (Model Ordinance (Section II, Chapter IV,

@.03 A(5)(C)(ii))). From the 2008-2015 studies, a total 216 samples were assessed including conditions when the WWTPs were considered operating normally as well as under a bypass or degraded operation conditions. In summary, 216 samples were analyzed for MSC of which 176 samples (81%) were positive for MSC; 118 samples (67%) contained MSC levels > than 50 PFU/100 grams; and 43 samples (20%) had MSC levels > 50 PFU/100 grams and wastewater effluent dilution was greater than 1000:1. These results are shown in Figure 1 and Table 1 below.

Figure 1: Comparison of dilution in receiving water and MSC levels in shellfish – all conditions

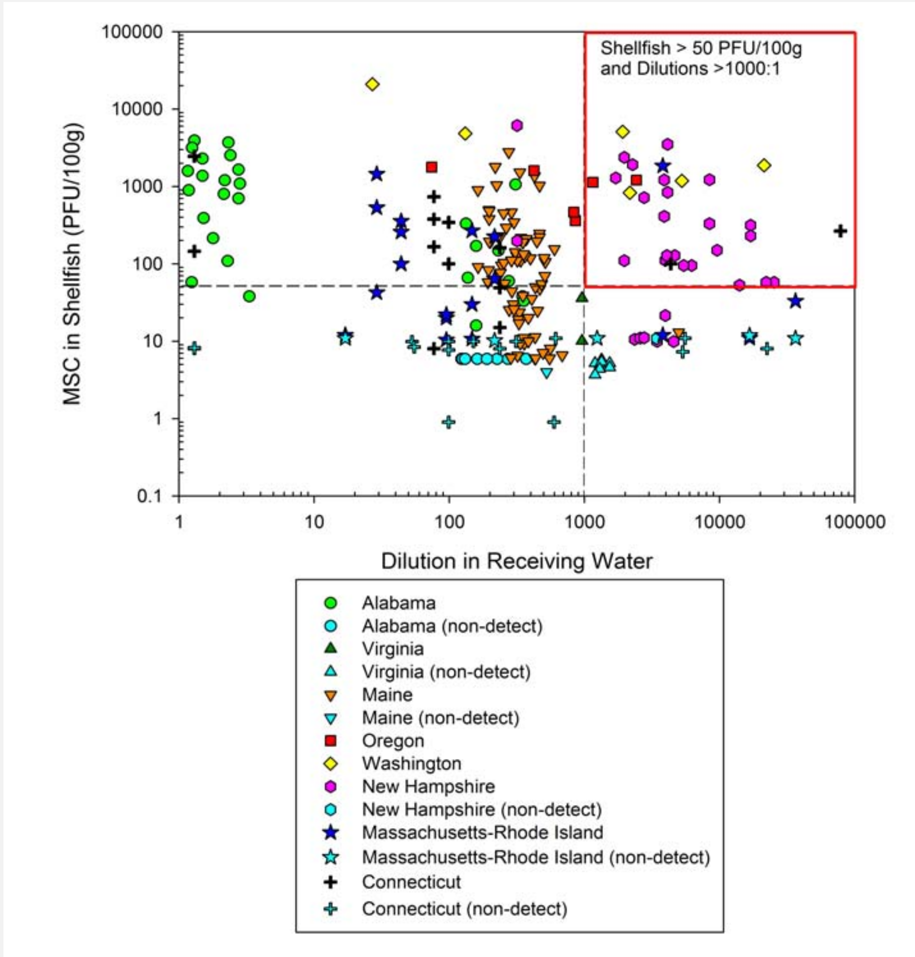


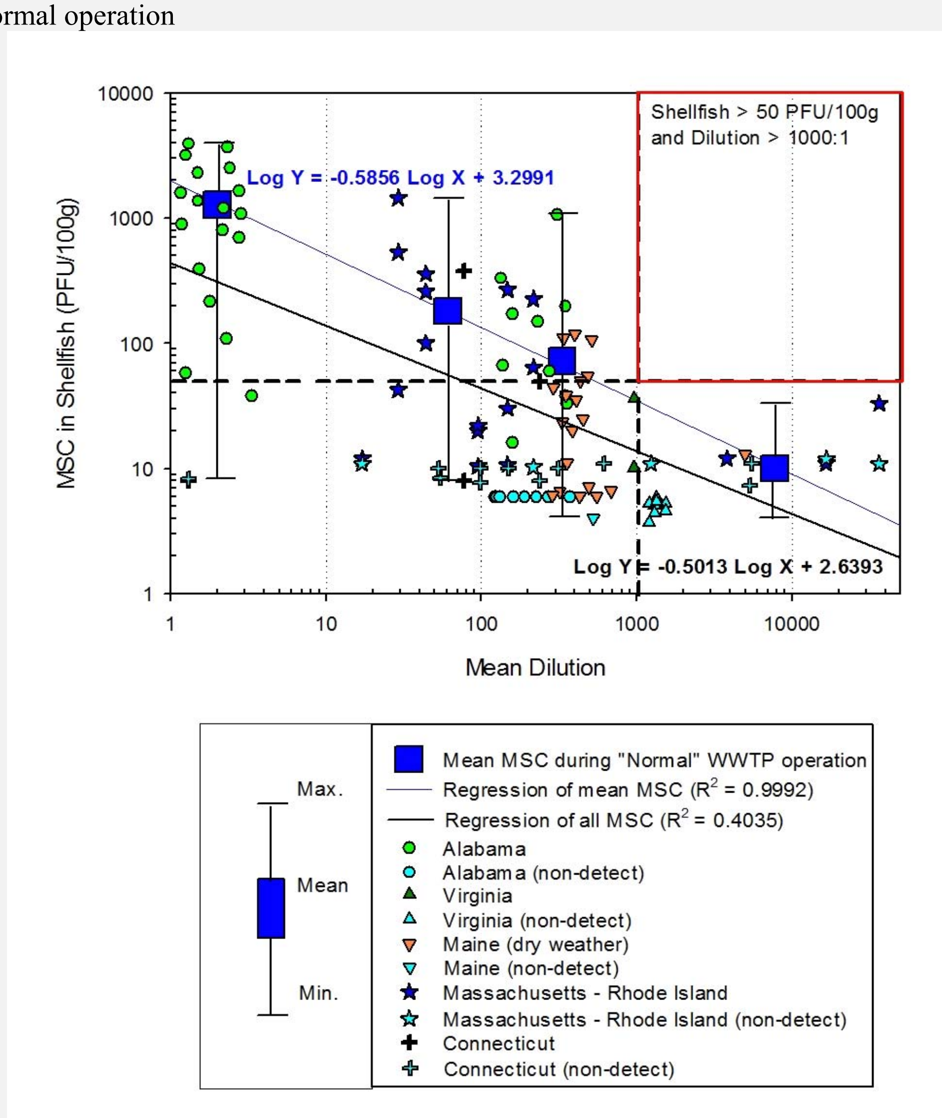
Table 1: MSC in shellfish operating under "normal" and outside of normal operation

MSC Results	All Conditions (n=216)	Normal Operating Conditions (n=129)
MSC detectable	81% (176)	62% (80)
MSC levels >50 pfu/100g	67% (118)	36% (46)
MSC levels >50 pfu/100g and Dilution in Growing Area >1000:1	20% (43)	0% (0)

In separating the data attributed to "normal" operation from other conditions, 129 of the 216 total samples were considered to be attributed to "normal" WWTP operation, also shown on Table 1. Eighty seven (87) samples were removed as they were attributed to conditions of WWTP malfunction or situations considered not suitable for conditional area management. From the 87 samples, 80 were associated with degraded WWTP performance or malfunction of which 6 were associated with a primary bypass, 13 were associated within a period of a WWTP upgrade during which the WWTP reportedly was operating an extended period (weeks) without disinfection, 31 were associated with degraded treatment quality because of rainfall/flows exceeding the WWTP design capacity, and 30 were attributed to a WWTP with no secondary treatment and operated frequently with flows exceeding the design capacity. Of the remaining 7 samples, 6 were associated with a WWTP utilizing unconventional disinfection technology (membrane filtration) and demonstrated poor performance in removing viruses compared to other conventional technologies during normal operating conditions, and 1 sample was attributed to a potential point source sewage discharge other than the WWTP.

When considering the remaining 129 samples attributed to "normal" WWTP operating conditions there were no samples that were above 50 PFU/100 grams when dilution was greater than 1000:1. In comparison, of the 87 samples attributed to malfunction or unsuitable conditions, 43 samples exceeded 50 PFU/100 grams when dilution was greater than 1000:1. These results are shown in Figure 2 below.

Figure 2: Comparison of dilution in receiving water and MSC levels in shellfish under normal operation



Comparing MSC with NoV sample results, out of the 216 samples analyzed for MSC, 161 samples were also analyzed for NoV. Of the 161 samples tested for NoV, 66 were positive (41% of total) were positive for NoV. Out of the 66 NoV positive samples, 62 (94% of total) were also positive for MSC and 53 (85% of total) had levels greater than 50 PFU/100 grams. There were only 4 cases where NoV was positive but MSC was not detected. However, in these cases, 3 of the sample results were near the Limit of Detection (LOD) for NoV enumeration. In one case it is suspected that both MSC and NoV may have been present but not likely viable as the WWTP utilized UV disinfection and was operating under normal conditions. These results are shown in Figure 3 and Table 2 below:

Figure 3: Comparison of MSC and NoV results

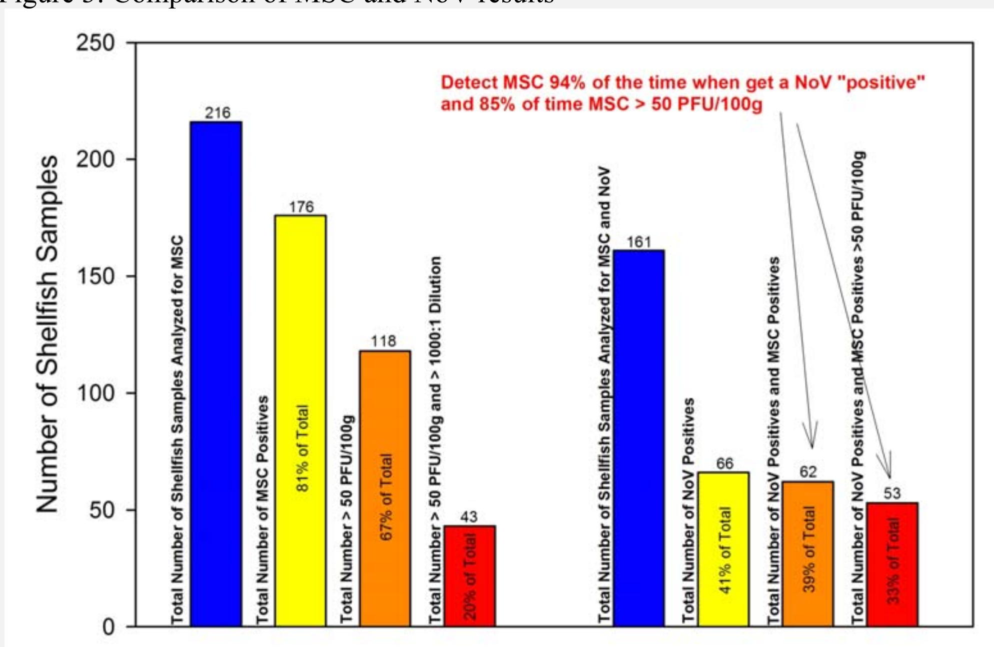


Table 2: Comparison of MSC and NoV Results in shellfish

MSC and NoV Results	
NoV detected in shellfish	41% (66 of 161)
MSC detectable	39% (62 of 161)
MSC negative when NoV detected (MSC<10 pfu/100g)	7% (4 of 66)*
MSC present when NoV detected (MSC>10 pfu/100g)	94% (62 of 66)
MSC present when NoV detected (MSC>50 pfu/100g)	85% (53 of 66)

*NoV detected at LOD of Assay

The overall results of FDA's field studies demonstrate a strong relationship between increased levels of enteric viruses and MSC and decreased levels of dilution. This trend was observed in all of the studies conducted by FDA at conventional WWTPs. These results also emphasize the critical need for sufficient notification time, meaning travel time from the WWTP discharge in the prohibited area is long enough to close the shellfish growing area in the event of a malfunction. This preventative measure may necessitate the Prohibited Area be larger than the zone necessary to achieve 1000:1 dilution. Furthermore, this analysis demonstrates the need to individually assess each WWTP, to assess their performance to remove enteric viruses.

In addition to the FDA field studies, as part of a Joint United States-Canada Norovirus in Bivalve Molluscan Shellfish Risk Assessment, a Meta-Analysis of the

Reduction of NoV and MSC Concentrations by Wastewater Treatment was conducted (Pouillot, 2015). The meta-analysis included previously unpublished surveillance data from the United States and Canada and relevant data reported in the literature (2,943 measurements in total).

For WWTPs with mechanical systems and chlorine disinfection, mean log10 reductions were 2.4 log10 gc/liter, for NoV GI, 2.7 log10 gc/liter, for NoV GII, and 2.9 log10 PFU per liter for MSCs. Comparable values for WWTPs with lagoon systems and chlorine disinfection were 1.4 log10 gc/liter for NoV GI, 1.7 log10 gc/liter for NoV GII, and 3.6 log10 PFU per liter for MSCs. WWTPs with ultra-violet (UV) disinfection demonstrated slightly higher mean log10 reductions with 3.0 log10 gc/liter, for NoV GI, 3.3 log10 gc/liter, for NoV GII, and 4.3 log10 PFU per liter for MSCs. The results of the reduction of NoV and MSC are shown in Table 3 below:

Table 3: Log reduction in NoV and MSC in treated wastewater with disinfection

Wastewater Treatment and Disinfection	Log_{10} NoV GI Reduction	Log_{10} NoV GII Reduction	Log_{10} MSC Reduction
Mechanical with Chlorine Disinfection	2.4	2.7	2.9
Lagoon with Chlorine Disinfection	1.4	1.7	3.6
Mechanical with UV Disinfection	3.0	3.3	4.3

This meta-analysis also demonstrated that Chlorine Disinfection had little effect on the mean reductions of the NoV and MSC. The mean log10 reduction that occur due to mechanical and biological treatment of the facility (prior to disinfection) were 2.2 log10 gc/liter, for NoV GI, 2.5 log10 gc/liter, for NoV GII, and 2.4 log10 PFU per liter for MSCs which varied little from mean log reduction after disinfection. In addition, a strong correlation, 0.8, existed between the reductions of NoV GII and MSC that occurred following treatment at the same WWTP indicating that MSCs could be useful in evaluating the efficiency of a WWTP.

Alternate Options

The FDA studies also suggested that certain factors, such as the quality of sewage treatment or the time of year, may exert influences on the levels of viruses discharged. However, at this time FDA does not have reliable data to justify specific dilution levels associated with environmental variables. It is recognized that such criteria could be determined by SCAs on a case by case basis, where factors of WWTP performance, disinfection method, tidal flushing, shellfish species and seasonal impacts may vary.

For example, in consideration of a raw sewage discharge, a lower dilution level than a 100,000:1 could be justified provided that specific data to that particular WWTP demonstrates that a lower bacteriological level associated with a potential raw sewage discharge is supported. Additional or other site specific information also can be used to justify alternative approaches that take into account other factors (such as no prior history of raw sewage discharges or containment structures sufficiently sized to accommodate a raw sewage event preventing a discharge).

Alternative options for calculating the size of the prohibited zone to mitigate the virological effects of WWTP discharges at the shellfish growing area may be used provided that they are based on sound scientific principles that can be verified. For example, it is reasonable to expect a potentially higher reduction in viral load from a properly maintained wastewater treatment system employing ultraviolet (UV)

	disinfection, tertiary treatment and operating under optimum design flow conditions. Regardless of the technology employed any proposed alternative minimum level of dilution for conditional management other than 1000:1 would need validation. MSC could potentially be used on a case-by-case basis as the validation process (for example to validate treatment efficiency) if demonstrated it is a successful/feasible strategy for the given location/situation. However, when there is insufficient information available for a growing area to support the use of a lower level of dilution, the 1000:1 dilution should be employed. If MSC is selected as an alternative option for calculating the size of the prohibited zone of a WWTP discharge, the authority should select an MSC criteria that adequately protects shellfish growing areas from virological effects and should be based on the most recent data and regional studies. References Baggi, F., A. Demarta, and R. Peduzzi. (2001) Persistence of viral pathogens and bacteriophages during sewage treatment: lack of correlation with indicator bacteria. Res. Microbiol. 152, 743–751 Batz, M. B., Hoffman, S., Morris, G.J. Ranking the Disease Burden of 14 Pathogens in Food Sources in the United States Using Attribution Data from Outbreak Investigations and Expert Elicitation. Journal of Food Protection, Vol 75 (7):1278-1291 Burkhardt, W. III, J.W. Woods, and K.R. Calci. 2005. Evaluation of Wastewater Treatment Plant Efficiency to Reduce Bacterial and Viral Loading Using Real-time RT-PCR. Poster Presentation, ASM, Atlanta, GA, Annual Educational Conference. Dore, W.J. and D.N. Lees. 1995. Behavior of *Escherichia coli* and male-specific bacteriophage in environmentally contaminated bivalve molluscs before and after depuration. Appl. Environ. Microbiol. 61:2830-2834. Goblick, G.N., Anbarchian J M,. Woods J.,, Burkhardt W. and Calci K. 2011. Evaluating the Dilution of Wastewater Treatment Plant Effluent and Viral Impacts on Shellfish Growing Areas in Mobile Bay, Alabama. Journal of Shellfish Research, Vol. 30 (3), 1-9. Hall AJ, Eisenbart VG, Etingue AL, Gould LH, Lopman BA, Parashar UD. 2012. Epidemiology of foodborne norovirus outbreaks, United States, 2001-2008. Emerg Infect Dis 18:1566-1573. Havelaar, AH, M. van Olphen, and Y.C. Drost. 1993. F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. Appl. Environ. Microbiol. 59(9):2956-2962. Iwamoto, M., Ayers, T., Mahon, B and Swerdlow, D.L 2010. Epidemiology of Seafood-Associated Infections in the USA. Clinical Microbiology Reveiws. April,2010 . p399-411. Kapikian, AZ and Chanock RM. 1990. Norwalk Group of Virus in Virology. New York, NY: Raven Press Ltd. pp. 671-693. Kohn, et al. 1995. An Outbreak of Norwalk Virus Gastroenteritis Associated with

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Action by 2015 Task Force I	Recommends adoption of Growing Area Classification Committee recommendation on Proposal 13-118.