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Hydrographic Study of Peirce Island Wastewater Treatment Plant Effluent in the Piscataqua River of Portsmouth, New Hampshire: Report of Findings from the December 10 – 14, 2012 Study Period

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Report of Findings from the December 10 – 14, 2012 Study Period

FDA Technical Assistance and Training Project

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TABLE OF CONTENTS

LIST OF FIGURES (APPENDIX I)

LIST OF TABLES (APPENDIX II)

1.0 INTRODUCTION

1.1 Executive Summary

In order to assist the New Hampshire Department of Environmental Services (NHDES) evaluate the impact of treated wastewater effluent from Peirce Island Wastewater Treatment Plant (WWTP) to the Lower Piscataqua River and Portsmouth Harbor a hydrographic dye study was conducted in December 2012 in Portsmouth, NH. Eight (8) shellfish cages with American oysters (*Crassostrea virginica)* and blue mussels (*Mytilus edulis*) were deployed both upstream and downstream of the Peirce Island WWTP in the Piscataqua River, Little Harbor, and the entrance of Little Bay. Eight (8) mini CTDs that monitor conductivity/salinity, temperature, and depth, and six (6) moored fluorometers, which measure dye tagged effluent from the Peirce Island WWTP were attached to the subsurface cages. A fifty (50) gallon mixture of Rhodamine WT dye and distilled water was injected into WWTP on December 11, 2012 for a half tidal cycle (approximately 12.4 hours). Additionally, boat tracking fluorometers connected with a mobile geographic information system (GIS) were used to measure dye levels on the surface in situ and in real time. Microbiological analyses of fecal coliform (FC), male-specific coliphage (MSC), Norovirus (NoV) genogroup I (GI) and genogroup II (GII), and Adenovirus (AdV) were conducted on WWTP influent and effluent composite samples collected with automated samplers to determine the WWTP efficiency in reducing indicator bacteria and viruses. Microbiological sampling and testing of oysters and mussels from the eight (8) sentinel cages was conducted to assess the impact of WWTP effluent on shellfish growing areas and growing area classifications. Prior to conducting the study, the assumption was that the FDA's recommended minimum dilution of 1000:1was not applicable in this situation because the recommended dilution is based on a WWTP having at least secondary treatment. The microbiological findings in shellfish samples, wastewater samples from the Peirce Island WWTP, and the results of the dye study, confirm that a minimum of 1,000:1 dilution with respect to Peirce Island WWTP is currently not applicable for this WWTP. The FDA and NHDES recommend continued MSC testing of wastewater samples from the WWTP before and after the WWTP upgrade. The FDA and NHDES recommend a future field study after the WWTP upgrade in order to delineate the 1,000:1 dilution zone.

1.2 Study Objectives

A hydrographic dye study of effluent from the Peirce Island WWTP was conducted by the FDA, the NHDES, and the City of Portsmouth on December 10 – 14, 2012 to assess the dilution, time of travel, and dispersion of effluent in the Piscataqua River. The hydrographic dye study objectives were to: (1) Determine the bacterial and viral conditions that could arise under a short term lapse in treatment and disinfection at the WWTP; (2) Determine the steady state bacterial and viral conditions in the shellfish growing waters during normal WWTP operation; and (3) Provide overall recommendations to the NHDES regarding the FDA's recommended minimum sized prohibited zone (1000:1 minimum dilution of effluent) for a Conditionally Approved classification of growing waters in consideration of the WWTP performance. A separate dye study was conducted at the Peirce Island WWTP by the Environmental Protection Agency (EPA) in 1999, but that study was limited to examining transport of effluent into Back Channel and

Little Harbor, but not the Piscataqua River. Furthermore, the study utilized older field methods that have since been replaced by methods and procedures in FDA's 1000:1 dilution guidance, as discussed below.

In conjunction with the hydrographic dye study, a microbiological assessment of the Peirce Island WWTP wastewater treatment efficiency and the microbiological quality of shellfish in the receiving waters was conducted. The objectives of the microbiological analyses were to: (1) Correlate the dye concentrations found at the cage locations with the bacterial and viral indicator findings in the shellfish; and (2) Research the dilution level needed to achieve a sufficient reduction in viruses to ensure the safety of shellfish harvested in proximity to the WWTPs as part of FDA's dilution guidance. On December 7, 2012, influent and effluent grab samples were collected from the Peirce Island WWTP. To quantify differences between effluent from the primary treatment facility on Peirce Island and effluent from secondary treatment facilities, additional influent and effluent samples of the Pease, Durham, Kittery, Dover, and Newington WWTPs were collected to compare the relative microbiological loadings between WWTPs. Composite samples of the Peirce Island WWTP influent and effluent were collected on December 10-12, 2013. Eight (8) cages filled with American oysters and blue mussels were deployed at various locations (stations) along the anticipated path of the dye tagged effluent. Wastewater and shellfish samples were analyzed for fecal coliform (FC), male-specific coliphage (MSC), Norovirus (NoV) genogroup I (GI) and genogroup II (GII), and Adenovirus (AdV). Due to laboratory difficulties with the MSC testing of the shellfish tested in 2012, additional shellfish samples were redeployed in late 2013. The microbiological study results from 2012 and 2013 are reported within this document.

1.3 Establishing Closure Zones for WWTP Discharges

In consideration of the 2014 National Shellfish Sanitation Program Model Ordinance, Section II, Chapter IV @.03 (E.)(5.) (Prohibited Classification – Wastewater Discharges), which states that determination of the size of a prohibited zone around a WWTP 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), the FDA has provided guidance to state shellfish control authorities to size prohibited zones around WWTP outfalls according to the following scenarios:

Scenario 1: In consideration of effluent discharged from a WWTP under **failure conditions** (such as a loss of disinfection or bypass), 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 MPN/100 ml within the harvest area

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 from a secondary treatment facility operating within its design parameters 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 WWTP 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).

Over the years, wastewater treatment technologies have improved. However, the FDA has maintained a conservative position recognizing that a WWTP may still be subject to failure regardless of the type of treatment system used, and experience at WWTPs all along the U.S. coastlines have verified that such failures do periodically occur. The FDA recognizes that with the advancement of technologies such as improved monitoring and alarm systems, it may be possible to operate a conditional area as outlined in Scenario 2 above, which allows additional shellfish growing areas to be harvested under certain conditions.

When a WWTP 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. 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. The FDA and Environment Canada conducted joint research involving a meta-analysis of the reduction of NoV and MSC concentrations by different methods of wastewater treatment reported in Joint United States-Canada Risk Assessment on Norovirus in Bivalve Molluscan Shellfish (Pouillot, 2015). The meta-analysis demonstrates that chlorine disinfection had little effect on the mean reductions of the NoV and MSC. WWTPs with ultra-violet (UV) disinfection demonstrated slightly higher mean reductions. In addition, this research demonstrated that a strong correlation 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.

To reasonably mitigate the risk of contaminating shellfish with viruses, the FDA recommends a 1000:1 dilution of sewage effluent as described in Scenario 2 as the minimum zone of dilution needed when the (secondary treatment) WWTP is operating under normal conditions. Included in this report is an estimation of the 1000:1 dilution area for the Peirce Island WWTP based on the steady state dilution levels found at the cage locations along the path of the WWTP effluent and the first day ebb tide excursion dye results. As noted, 1000:1 is the minimum dilution the FDA recommends in sizing a prohibited zone, but other factors are also considered in this report that may increase the amount of dilution needed within the prohibited zone to mitigate the risk of viruses reaching the shellfish growing areas.

1.4 Description of Peirce Island WWTP

The Peirce Island WWTP is located in the southeast portion of the island at 80 Peirce Island Road in Portsmouth, NH. The WWTP was constructed in 1964 and last underwent a major renovation in 2001. The WWTP is currently slated for another upgrade from primary treatment

only to primary and secondary treatment. The flows from the WWTP are in the range of 4.3 – 6.8 million gallons a day (MGD) (based on 2008 – 2010 data). The design flow is 4.8 MGD and the hydraulic capacity and peak hourly wet weather flows are 22 MGD. Wastewater is disinfected year round with sodium hypochlorite and dechlorinated using sodium bisulfite.

The Peirce Island WWTP outfall is located east of Peirce Island and southwest of Seavey Island. The outfall is designated with a pink marker in Figure 1. The outfall is a straight pipe that is estimated to achieve a 27:1 dilution (based on information from the plant's operators). Figure 1 also shows the proximity of the eight (8) sentinel shellfish cage locations with attached fluorometers and CTDs to the Peirce Island WWTP's outfall location.

1.5 General Description of Study Design

The sentinel shellfish cage locations were selected based on the results of EPA's previous dye study in 1999 (Bridges. T., EPA, 2001) and locations of concern identified by NHDES, such as Dover Point and the mouth of Sagamore Creek. Each of the eight (8) shellfish cages (custom-made by Brooks Trap Mill, Thomaston, ME) were filled with 33 American oysters in the range of 3 to 4 inches and 37 native blue mussels, approximately 2.5 inches in size. The American oysters were provided by the University of New Hampshire, the blue mussels were collected by NHDES near the mouth of Sagamore Creek, and all shellfish were depurated by Spinney Creek Shellfish, Inc. (Eliot, ME) prior to deployment. Six of the cages were equipped with WET Labs submersible fluorometers (WET Labs, Inc., Philomath, OR) to measure dye concentrations and all eight (8) cages were equipped with Star-Oddi miniature DST CTDs (Star-Oddi, Iceland) to monitor conductivity/salinity, temperature, and depth/pressure during the course of the dye study. The NHDES and the EPA deployed the sentinel shellfish cages on November 26, 2012 at various distances along the estimated path of the effluent plume. The cages were deployed in order to allow sufficient time for the shellfish to acclimate to their new environment and accumulate viruses and bacteria from the WWTP effluent prior to the FDA arrival on December 6, 2012. The FDA and the EPA attached the programmed WET Labs fluorometers and DST CTDs to the cages on December 9, 2012. Background tracking of the study area took place at this time to determine background levels of fluorescence in the Piscataqua River. The dye injection and dye tracking study occurred from December 11 – 13, 2012. The cages were collected after the dye study was completed to remove the shellfish and the instruments for analysis. The EPA provided a boat rigged to assist with the deployment and the lifting of cages and assisted with surface dye tracking work during the hydrographic dye study.

In addition to deploying the sentinel shellfish cages and conducting background tracking, the FDA and the NHDES conducted a drogue study on December 9, 2012 to determine the current velocity, direction, and dispersion in the Piscataqua River and to ascertain if the sentinel shellfish cages placed along the path of the effluent plume from the WWTP were positioned properly to maximize shellfish exposures to viruses in the effluent. The drogue study provided information about tidal cycles, including the current velocities and time of travel within the Piscataqua River, and assisted with planning the timing of the dye injection.

The dye for the comprehensive study was injected over half a tidal cycle and remained in the Piscataqua River system for greater than three (3) days. Boat tracking with towed WET Labs fluorometers was conducted to find the edges of the dye plume during daylight hours, in addition to the 24/7 dye readings recorded by the cage-attached submersible fluorometers.

2.0 METHODS

2.1 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 Piscataqua River were determined, using a combined total of nine (9) fluorometers. Six (6) were WET Labs FLRHB submersible fluorometers (WET Labs, Inc., Philomath, OR) attached to the sentinel shellfish cages deployed at stations along the anticipated path of the effluent throughout the course of the study. Two (2) were WET Labs FLRHRT fluorometers that were towed behind a boat and used for tracking the dye on each day of the study. The last fluorometer was a WET Labs FLRHRT fluorometer attached to a SeaBird CTD (conductivity/temperature/depth) profiling instrument to collect profiles of the dye levels at different depths. Unfortunately, one of the sentinel shellfish cages (Station 4) and the attached WET Labs FLRHB submersible fluorometer were lost due to the swift current at the location.

The FDA used the serially diluted dye standards to develop calibration curves for the FDA's WET Labs FLRHRT-586 and 2040 tracking fluorometers and the six submersible fluorometers – WET Labs FLRHB units 913, 915, 1730, 1731, 2032, and 2146. Prior to the study, the background fluorescence levels collected on December 9, 2012 in the Piscataqua River were subtracted, creating the curves used to calculate 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 and 100 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 the 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.

2.2 Drogue Study

Approximately fifteen (15) oranges and grapefruits and two NHDES "winged drogues" constructed from PVC pipe and aluminum sheet metal were used on December 9, 2012 to assess

the timing of tidal cycles, e.g. slack high/start of ebb tide, tidal velocity, and the influence of wind to estimate the velocity and direction of the effluent leaving the WWTP diffuser. The fruit drogues were released on the surface of the water, while two winged drogues were suspended to a depth of approximately 1 – 2 feet and 10 feet respectively in order to capture the tidal flow and be less influenced by surface winds. The winged drogues were constructed by fitting two sheets of aluminum forming an "x" shape help together by a PVC pipe with slits down the length of the pipe in which the sheets of aluminum slide into. The aluminum wings were held in place within the PVC pipe with hose clamps. Fish weights were suspended to bottom of the PVC pipe to provide extra weight and line attached to a toggle buoy was tied to the top of the PVC pipe to make the drogue visible while tracking via boat.

A portion of the fruit drogues and both of the winged drogues were released near the outfall prior to the turning of the tide from flood to ebb tide. The Global Positioning System (GPS) coordinates and time of release of the drogues was recorded in the FDA's RAFT-MAP application (Real-Time Application for Tracking and Mapping) using the GPS marker feature. Additional fruit drogues were dispatched in the afternoon north of Station 2, and prior to the switching of the tide from ebb to flood, to assess how the shifting tide would impact the movement of the drogues. These drogues were then tracked along the anticipated path of the effluent plume, and the approximate direction, distance, and time the drogues traveled were recorded in RAFT-MAP. The velocities of the drogues were later determined in ArcGIS Desktop 10.1 using this information.

2.3 Dye Injection

A total of 25 gallons of dye was injected at a constant rate into the Peirce Island WWTP effluent over a 12.4 hour period beginning at slack low tide (2:12 AM on 12/11/2012) continuing through a ½ tidal cycle and ending at 2:51 PM on the same day to demonstrate how pollutants disperse, transport, and build-up in the estuary. Based on the concentrations of dye in the estuary, a worst-case pollution condition can be simulated. To facilitate the pumping of dye, 25 gallons of distilled water was added creating a 1:1 dye dilution mixture (50 gallons in total). A Masterflex model 7553-20 variable speed peristaltic pump (Cole-Palmer Instrument Co.) with Masterflex Tygon L/S-15 tubing was used to withdraw the tracer dye solution from a large plastic container. A pump head size 7015 was used with a constant pumping rate of 255 ml/min which was maintained at about 145 revolutions/minute (rpm) head speed. The pump head speed was adjusted to 100 rpm from 2:25 PM on 12/11/2012 until the end of injection since the dye mixture was delivered slightly faster than had been calculated. The dye mixture was fed continuously into the final effluent following the chlorine treatment for approximately a 12 hours and 39 minutes injection period. The initial concentration of the dye in the effluent was determined using the WWTP's flow average over the course of the dye injection period.

2.4 Dye Tracking

The dye plume was followed during the last stages of flood tide and into the beginning of the ebb tide on December 11, 2012, and was tracked as it moved through the Piscataqua River on an ebb tide using the FDA's WET Labs FLRHRT tracking fluorometers and the SeaBird CTD profiling unit with an attached WET Labs FLRHRT fluorometer. The fluorometers were linked to

Panasonic Toughbook C-19 computers running the FDA's mobile GIS RAFT-MAP program, and the GPS coordinates for the outer edges of the dye were recorded.

Two boats were used, each with tracking fluorometers towed to determine dye levels in the estuary. One of the boats provided by the EPA was rigged for dropping and lifting the SeaBird CTD profiling unit to conduct profiles of the dye at depth in addition to towing the tracking fluorometer. Dye readings were taken on successive days (December 12 - 13) for high and low tides. Traverses in the estuary were performed on all the days of the study from north to south and east to west and vice versa, and dye readings also were recorded at the surface of each of the sentinel shellfish cage station locations to allow a comparison of surface readings with the bottom readings from the submersible fluorometers affixed to the cages. On December 11th, two boats recorded data a total of 32 traverses. On December 12th, one boat recorded data from 15 traverses and again from 4 traverses on December 13th. 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 WWTP by the final (five-point moving average) concentrations detected in the estuary. A separate outlier analysis was conducted using desktop ArcGIS to ensure that outliers were detected and removed from the final dataset.

2.5 Dilution Analysis - Dye Readings from Submersible Fluorometers

The fluorescence readings recorded by the submersible fluorometers at each of the five fixed sentinel shellfish cage stations (as previously noted, Station 4 was not recovered and thus no results are reported) were downloaded, converted to ppb using each fluorometer's linear calibration regression curve, and plotted alongside the CTD tidal depth curves for the period of the study.

Traditionally, determination of pollutant buildup of wastewater effluent discharged into receiving waters has been accomplished through dye tracing by injecting Rhodamine dye for several tidal days until an equilibrium in dye concentration values was achieved for the prevailing tidal and freshwater inflow conditions studied (Hetling et al. 1966; EPA 1992; FDA 2010). However, the superposition method (Kirkpatrick, 1993, Goblick et. al, 2011) was chosen to overcome the challenges and cost of injecting and monitoring continuously dye for several days. Since the FDA has successfully employed the superposition method to save time and resources, only a half tidal day (12.4 hour) dye injection was conducted. The superposition method was used to estimate the steady state condition for dye over multiple tidal days at each of the cage stations using data collected from December 11, 2012 – December 13, 2012. Superposition determinations are achieved by superimposing, in cumulative fashion, the measurements taken on each tidal day after the dye injection with the measurements recorded on the first tidal day. The process continues until a stable (peak) concentration dye value is obtained. The peak concentration value represents the buildup of pollutants to a steady state maximum concentration, and the timeframe to reach steady state represents the overall residence time of pollutants within the estuary.

For the steady state analysis, three categories of steady state dilution values were determined based on half tidal day average concentrations, half tidal day peak 1 hour concentrations and half tidal day maximum concentrations by plotting continuous dye concentrations recorded by submersible fluorometers at five sentinel shellfish cage locations (Stations 1-3, 5, and 7). For the day of the injection, December 11, 2012, peak 1 hour concentrations and average concentrations of dye were plotted. For the additional days that the instruments were in the water the remaining dye level for each half tidal day was added to the levels detected on day 1 and plotted. The half tidal day average concentration is based on determining the average dye concentration that occurred during each half tidal (12.4 hours) day – these levels are superimposed to determine a steady state peak. The half tidal day peak 1 hour concentration is based on finding peak concentrations that occur over an hour within each half tidal day - these levels are superimposed to determine a steady state peak. The peak 1 hour is an average of these peak readings over an hour. The half tidal day max concentration is based on maximum dye concentration that occurred during each half tidal day - these levels are superimposed to determine a steady state peak.

Following the superposition principle, remaining dye levels found in the system on successive tides were used to determine the steady state condition at each cage station. The steady state dilution was calculated by dividing the initial concentration of dye injected at the WWTP by the final (five-point moving average) concentrations detected at each station expressed as below:

$$D = \frac{C_{ww}}{C_{ss}}$$

Where:

D = dilution;
C_{ww} = initial concentration from the WWTP;
C_{ss} = steady state concentration at the fixed shellfish station;

2.6 Microbiological Analysis of Wastewater

Indicator Microorganisms

FC densities in the WWTP influent and effluent were determined using a conventional five-tube, three-dilution MPN 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.

Virus concentration and RNA extraction

Viral analysis for the sewage utilizes elution with an alkaline buffer followed by ultracentrifugation (Williams-Woods, et al., 2011). Concentrates were extracted for RNA with RNeasy Mini Kit (Qiagen, Valencia, CA) utilizing 6M guanidium isothiocyanate as a lysis

solution. Extracted RNA and DNA were tested by real-time reverse transcription (RT)-qPCR and qPCR respectively.

RT-qPCR

Positive controls used for NoV GI and GII were *in vitro* RNA transcripts of sequences cloned from positive clinical samples previously identified as NoV (Burkhardt, et al., 2006). Primers and probes for NoV GI and GII targeted the most conserved region of the open reading frame 1 (ORF1)-ORF2 junction. Real-time RT-qPCR for detection of NoV GI and NoV GII with an RNA IAC was performed in a 25-µl reaction volume by using a one-step RT-PCR kit (Qiagen). The primer concentrations for the NoV targets were 300 nM each, and the concentrations for the IAC primers (46F and 194R) were 75 nM each. The 5' nuclease probe concentrations for NoV and the IAC target were 100 and 150 nM each, respectively. The final concentration of $MgCl_2$ in the real-time RT-qPCR was 4 mM. Thermal cycling was run using the SmartCycler II system with the following conditions: 50°C for 3,000 s and 95°C for 900 s followed by 50 cycles of 95°C for 10 s, 53°C for 25 s, and 62°C for 70 s. Fluorescence was read at the end of the 62°C elongation step. Default analysis parameters were used, except that the manual threshold fluorescence units were set to 10. Samples positive with the initial primer and probe sets for NoV GI and/or NoV GII were subjected to a secondary detection assay. Amplification of the original RNA extract was performed with primers from the B region by conventional RT-PCR (DePaola, et al., 2010). Amplification of a second region of the genome is non-contiguous to the first and serves as an indication that the RNA was not degraded.

Adenovirus

The positive control used for Adenovirus (AdV) was serotype 41 isolated from a clinical stool sample, propagated in-house by utilizing the A-549 cell line. Real-time PCR for the detection of AdV was performed in a 25-mL reaction volume by using Platinum TAQ DNA Polymerase (Life Technologies, Grand Island, NY) as previously described with slight modifications (Williams-Woods, et al., 2011). A DNA IAC utilizing the 46F and 194R primers and the TxRed-labeled probe as previously described was added with final primer and probe concentrations of 0.75 mM and 1.5 mM, respectively (DePaola et al., 2010). Cycle parameters were slightly adjusted as follows: 95°C for 120 s followed by 50 cycles of 95°C for 3 s, 53°C for 10 s, and 65°C for 70 s. AdV primers and probe were previously described with slight modifications to the probe (Heim, 2003) whereby probe was FAM-ZEN labeled as a fluorescent dye on the 5' end and an Iowa Black quencher dye labeled on the 3'end. Fluorescence was read at the end of the 72°C elongation step. Default analysis parameters were used except that the manual threshold fluorescence units were set to 10.

Murine norovirus

The extraction control used for murine norovirus was purchased from ATCC PTA-5935 and propagated using the RAW264.7 cell line. Real-time RT-qPCR was utilized for the detection of murine norovirus (the extraction control virus) with an RNA IAC in a 25-µl reaction volume by using a one-step RT-PCR kit (Qiagen). Primers and probes were utilized as described in Hewitt, et al., 2009. Thermal cycling was run using the SmartCycler II system. Fluorescence was read at the end of the elongation step and the default analysis parameters were used except that the manual threshold fluorescence units were set to 10.

3.0 RESULTS

3.1 Drogue Study

Figures 2 - 3 show the results from the drogue study from slack high tide to ebb from different water depths on December 9, 2012. Figure 2 shows orange and grapefruit drogues deployed at the surface near the Peirce Island WWTP outfall when the tide was at slack high before ebb tide. Because wind drag and surface waves can influence the speed and direction of the drogues, another drogue study using winged drogues made from aluminum sheet metal held by PVC pipes was conducted approximately 10 feet below surface water at slack high before ebb tide (Fig. 3). For calculating drogues travel velocities, ArcGIS Desktop was applied by dividing the distance the drogues traveled by the time required to travel that distance.

As shown in Figure 2, the first set of orange drogues was released near the Peirce Island WWTP outfall at first slack high around 6:41 AM on December 9, 2012. Oranges traveled 1.85 miles in 42 minutes (0.7 hours) along the lower Piscataqua River towards Station 2, which shows the average travel speed at slack high tide moving towards ebb tide was 2.64 mph (4.25 km/h). The oranges stayed in the conditionally approved area on New Hampshire side until 7:20 AM, and subsequently traveled to the prohibited area on Maine's side of the river. For comparison, a winged drogue was released around Station 4 at 6:56 AM approximately 10 feet beneath the water (Fig. 3). The winged drogue traveled 1.92 miles at a rate of 2.87 mph (4.62 km/h). Based on these drogue studies, no significant difference was found between the travel velocity on the surface or beneath the surface which indicates that the tidal currents were most dominant.

Moreover, tidal charts had predicted the turning of the tide from ebb to flood around 1:30 PM on December 9, 2012. However, using the results of the drogue study the FDA and the NHDES determined that the tide did not turn from ebb to flood until about 3:15 PM – 1.75 hours later than the NOAA chart had predicted.

3.2 Background Readings

Background fluorometer readings of the Portsmouth study area (including Little Bay, Piscataqua River, Peirce Island WWTP area, and Back Channel) were taken by two (2) tracking units and one (1) Seabird CTD. Background levels of FUs for the WET Labs FLRHRT-586 and FLRHRT-2040 tracking fluorometers were measured ranging from 83 - 92 FUs and 49-51 FUs, respectively. Based on background analysis for each unit, 86 and 51 were determined to be the background reading for the units 586 and 2040, respectively, for the study estuary. These are normal background levels for an estuarine system evaluated with the FLRHRT-586 fluorometer and are not considered indicative of excessive background levels. A background level of 86 FUs was subtracted from the fluorescence readings during the dye study. Similarly, background level of 51 was subtracted from the unit 2040 fluorescence readings during the dye study period. Five- point moving averages above these levels were considered dye readings. However, single or random data points above background levels that appear among a distribution of points below background were identified in ArcGIS and further analysis was conducted to identify and remove potential outliers.

Six (6) moored fluorometers (FLRBH 913, 915, 1730, 1731, 2032, and 2416) were programmed and deployed to fixed stations 1-5 and 7 between 1:30 PM and 2:30 PM on December 9, 2012. Background readings at the area were taken with the moored Wet Labs units before the dye injection. Based on the approximately 36 hours background collected before injection, 102, 79.5, 47, 53, and 48 FUs were chosen as background levels for stations 1, 2, 3, 5, and 7 (Station 4 was lost). Since different units have different characteristics based on individual calibrations, various background levels from different units are expected.

3.3 Dye Injection

Dye injection began at 2:12 AM on December 11, 2012 and continued for approximately 12 hours. Records from the Peirce Island WWTP showed that the average hourly flow for the period of injection was 5.408 MGD, which was close to the average daily flow rate range based on historical records, although slightly above the design flow. According to the state's records, from December 1, 2012 to December 10, 2012, about 10 days prior to the injection, approximately 0.68 inches of rain fell in the Portsmouth area, while on the day of injection, 0.01 inches of rainfall was recorded and observed. Based on the flow rate out of the WWTP during the injection period, the measured concentration of dye mixture in the jug, and the flow out of the jug, the concentration of dye flowing out of the WWTP's outfall was 1,370 ppb, which was calculated using a mass balance approach.

3.4 Travel Time

Since the dye injection started at slack low tide, this study determined the extent of dye travel on the following flood tide and successive ebb tide on the first day of the study (December 11, 2012). On December 11, the first flood tide was from 2:09 AM to 8:18 AM based on the tidal chart at Portsmouth Harbor from NOAA, although for safety considerations, the FDA could not track dye on the surface via boat during this tide (tracking was conducted only during daylight hours). However, submersible fluorometers at each station were able to detect dye in deeper waters during this timeframe. Station 7, which had an attached fluorometer was located 5.48 miles upstream of the Peirce Island WWTP in the Piscataqua River (Dover Point). Dye was detected at Station 7 at 6:45 AM, with a travel time of 4.55 hours. Thus, the calculated average travel velocity was 1.2 miles/hr. This velocity during flood tide should be considered to assess impact when a WWTP failure or a loss of disinfection has occurred. It should also be noted that the first detected dye level at Station 7 was 0.03 ppb and dye was as high as 0.2 ppb in less than half an hour. Thus, since Station 8 is in the Little Bay within 6.42 miles distance from the WWTP outfall, if the velocity of 1.2 miles/hr were applied during flood tide, it would take about 5.35 hours for sewage to reach shellfish at Station 8 (near the border of conditional approved and prohibited area) in the event of a WWTP failure or a loss of disinfection.

Since effluent travel time is a critical factor to manage conditionally approved shellfish growing areas, Figure 8 shows a dilution versus distance and travel time based on the first day's tracking of surface dye. According to the tracking data, nine (9) maximum concentrations (note: one (1) outlier data point is shown on the map but was not taken into account for analysis) within various upstream distances from the outfall were extracted and are shown on Figure 7. The tracking

began around 8:00AM on the first day and the first highest concentration (3.93 ppb) on the surface was detected at 8:08 AM within 300 meters radius distance (0.18 miles) from the outfall. Only one minute later the second highest dye level (0.82 ppb) was measured at 522 meters (0.32 miles). This patch of dye collected by boat tracking units moved into the Little Bay quickly. The highest concentration of 3.93 ppb was detected at the north of the Adams Point after only 3 hours 34 minutes of travel time based on the time detecting first highest dye level (8:08 AM) which means raw sewage or partially-treated sewage takes approximately 8 hours to reach into Great Bay on a flood tide if the WWTP malfunctioned. This timeframe is vital for proper management actions.

A more detailed travel time discussion is addressed in Section 3.7 based on station data for each fixed sentinel shellfish cage station. The information is also very significant for projecting fecal coliform (FC) levels with various scenarios of FC concentrations from the WWTP effluent (see Section 3.8).

3.5 Outlier Analysis for Dye Readings via Boat Tracking Fluorometers

In order to verify dye readings in critical areas downstream of the outfall particularly around Stations 1-4, data points were selected spatially, encompassing the entire time period of data collection, as shown in Figures 17 and 18. Figure 17 shows collected data around Stations 1 and 2 containing all date points with low dilution levels (<1,000:1) on December 11-12, 2012. At Station 1, all data was collected between 2:39 PM and 2:46 PM from the same track on December 12. It is clear that only 1 point (6.5 ppb) can be considered an outlier, however, the other less than 1,000:1 dilution data points (3-4 ppb) distributed between 43.055^0 and 43.056^0 (latitude coordinates) are considered valid data. For Station 2, two (2) tracks were conducted on December 11, 2012 and December 12, 2012, respectively. Higher concentrations (1.01 – 2.59 ppb) measured at GPS locations from 43.058^0 to 43.075^0 (latitude coordinates) on the first day (12/11/2012) between 3:26 PM and 3: 47 PM are verified as valid data. Similarly, outlier analysis for Stations 3 and 4 is illustrated in Figure 18. At Station 3, three (3) tracks of data were collected on the first day (12/11/2012) and second day (12/12/2012), a good quantity of low dilution data (<1,000:1) occurred on both days as expected. None of the data points at Station 3 were considered to be outliers. For Station 4, two points collected on December 11, 2012 were considered outliers located at 43.0738^0, -70.7295^0, however all data collected on December 12, 2012 were determined to be valid.

3.6 Dye Readings via Three-day Boat Tracking

The calibration curve data for the each fluorometer was programmed into RAFT-MAP. This information was used by RAFT-MAP to convert raw fluorescent dye readings from the fluorometer into ppb concentration units. A five-point moving average was applied to the dye concentration data in RAFT-MAP to smooth out any false high or low readings. Dilution was calculated by dividing the initial concentration of dye injected at the WWTP by the final (five-point moving average) concentrations detected in the estuary. The associated GPS coordinates for each dye concentration data point were recorded in RAFT-MAP. All of the data was

displayed on a GIS map in real-time as it was being collected. Dye concentration data points were color-coded representing different dye level ranges. The data could also be viewed in a tabular format in the "Data View" feature of RAFT-MAP and after the study the tabular data was exported and saved as .csv files.

All of the data and GIS layers stored in RAFT-MAP were downloaded as a geodatabase into ArcGIS Desktop after the study. Using ArcGIS, the FDA created refined GIS maps of the dye study data, including the 5-point moving average concentration points in the study area and the associated dilution levels, as well as other data of interest, such as travel times and modeling.

Figures 4-6 show the movement of dye in the growing areas, surface dye concentration levels (5-point moving average, hereafter all concentration levels in this report are 5-point moving averages) detected by two(2) boat tracking units and corresponding dilution levels during the study period. Dye injection began at the slack low tide and lasted actual 12.65 hours covering approximately a half tidal cycle. The average flow rate during injection was 5.408 MGD. Three days of dye tracking reveal dye movement through several tidal cycles for the purpose of determining where the dye ultimately dispersed and in what concentrations and dilutions. Usually, the first day tracking of dye is the most critical because it can provide essential information in terms of times of travel to the shellfish growing areas and what concentration levels corresponding to the tides. On the following two days, continued dye tracking can determine the flushing time (residence time) of potential contaminant residuals in the estuary.

On the first study day (Fig. 4), dye tracking began at approximately 8:00AM and ended around 4:30 PM which covered the entire ebb tide cycle and part of flood tide cycle. The highest concentration of 3.93 ppb was found near Peirce Island WWTP at the beginning of the ebb tide representing the lowest dilution (348:1) on the first day of the study and, in fact, for the whole study period as well. Since dye was injected from 2:12 AM on December 11, 2012 and there was a 1.75hour delay than the predicted tidal chart at Portsmouth Harbor based on the drogue study, it already experienced most of the flood tide (from 3:54 AM to 10:03 AM) at Portsmouth Harbor before tracking was conducted. However, the time of high tide at Dover Point and Adams Point were approximately 1.5 hours and 2 hours later relative to Portland, ME (Short, F.T. 1992), respectively. From the initial drogue study, a 1.75 hour delay time was also considered comparing with the tidal information from NOAA chart. Thus, flood tide did not change to ebb until approximately 11:33 AM at Dover Point and 12:03 PM at Adams Point/Little Bay area. During the first flood tide, dye was quickly flushed into Piscataqua River, Bellamy River, Oyster River, and Little Bay and was actively detected in the range of 0.01 – 3.95 ppb. The leading edge was found near the edge of Little Bay and Great Bay at approximately 11:30 AM. Based on the distance traveled to the leading edge (9.83 miles) during the first flood tide and about 1 hour into ebb tide, the average travel speed during the flood tide was 1.34 mph (9.83 miles / 7.3 hrs). As expected, the dye tagged effluent primarily stayed within the channel and concentration levels decreased with increase of distance. The concentration levels along the lower Piscataqua River were 0.21-0.3 ppb while the levels on the upper Piscataqua River were 0.04- 0.15 ppb during the first flood tide. However, the relatively quick 5 hours 19 minute travel time to reach Dover Point potentially raises a concern. The first ebb tide occurred at around 4:30 PM at Portsmouth Harbor and during the ebb tide, significant high concentrations took place at Stations 2, 4, and the southeast of Station 3 (east side of Pest Island). The detected peak concentration around Station 3

was 0.17 ppb, however around Station 2, and the south of Station 2, the highest concentration was measured 1.1-2.75 ppb ; Station 4, and the east side of Pest Island, had dye levels of 0.5-1.0 ppb. Although Stations 1-4 are located in the prohibited area, it was noted that all locations with higher concentrations were measured between 3:00 PM and 4:00 PM. This demonstrates that higher levels of pollutants can reach the conditionally approved area in 1-1.5 hours at ebb tide.

Figures 5 and 6 represent consecutive tracking results for the second and third day respectively. Unlike the first day, significantly higher dye levels (0.1 – 2.65 ppb) were measured in Spruce Creek and from the south of Station 3 (Back Channel) to the Sagamore Creek for day two (2) (Fig. 5) which represents dilutions between 516:1 and13,700:1. This shows pollutant concentrations are higher than the first day at these locations after approximately 32 hours (the third ebb tide). Similarly, in the east side of Station 1 in Little Harbor, the peak dye concentration was 3.59 ppb on the second day. Since dye levels on the second day were higher than on the first day in Back Channel, this indicates that a potential build-up can occur at these locations and also extended to the mouth of the Piscataqua River at the confluence of Atlantic Ocean. For the Little Harbor area, boat tracking did not cover the entire area on the first day (only covered the outside of the mouth) and dilutions ranged from 6,000:1 – 9,000:1 dilution. The second day tracking data in most of the Little Harbor area showed that the dilution decreased into the range of 1,000:1 – 10,000:1. However, some measurements show dilutions less than 1,000:1 at the mouth of Little Harbor. This illustrates that Little Harbor probably is not impacted until the second tidal cycle. It's worth noting that the locations where dilutions were lower than, and close to, 1,000:1 in Little Harbor is adjacent to the border of conditional approved area and approved area. However, on the other hand, as the FDA expected, the pollutants were flushed into the middle of the Great Bay and were diluted to much lower concentrations (<0.1 ppb representing greater than the dilution of 13,700:1) compared to the first day tracking results. As shown in Figure 6, the third day tracking was only conducted in Bellamy River, Oyster River, Little Bay, and Great Bay. As the FDA anticipated, most dye was flushed out from Great Bay (a portion of which is classified as a conditionally approved area) where dye was found at the range of 0.01-0.05 ppb which is close to the limit of detection of the fluorometers.

Figure 1 illustrates accumulated three (3) day (December 11-13, 2012) dye concentration levels with corresponding dilutions collected by two (2) boat tracking units. Blue data points represent dilutions between 1,000:1 and 10,000:1 while pink data points show dilutions less than 1000:1. It can be seen that not all dilutions in prohibited areas downstream of the Peirce Island WWTP outfall (hereafter referred to as "outfall") achieved 1,000:1 which the FDA typically recommends for WWTPs that have at a minimum, secondary treatment, and are operating within normal operating conditions. The growing area in the portion of Little Harbor (approximately 2 miles from outfall) raised a concern because this area is conditionally approved and lower dilutions (<1,000:1) occurred even close to the border of the conditionally approved area and approved area under 5.408 MGD flow rate during the study period. In addition, although a large quantity of data points on the portion of upstream and downstream of the outfall fell in the range of 1,000:1 to10,000:1, numerous data points are concentrated on the lower end of the dilution range among these areas including Little Harbor, finfish aquaculture site (Station 2), Back Channel, and Stations 3 and 4. Therefore, those areas are still a concern. However, all dilution levels were greater than 1,000:1 in Little Bay and Great Bay based on tracking data.

3.7 Dye Readings at Stations

Continuous data from submersible fluorometers at each station over the whole study area for three days were collected in order to measure pollutant levels at depth and estimate build-up over multiple tidal cycles during critical times and difficult periods via boat tracking. However, for comparison of dye concentrations and dilution levels on the surface and at depth, surface tracking data within a 500 meter buffer area at each of the station locations are extracted spatially, as shown in Figures 9 – 16. Meanwhile, Figures 19-23 show dye readings and estimated build-up recorded by the submersible WET Labs units at Stations 1-5 and 7. Based on the modified super-position method developed by the FDA, the half tidal day peak 1 hour and the half tidal day average concentrations were calculated, reflecting the relative build-up of pollutants from the WWTP outfall over several tidal days to a steady state condition. Plume tracking data with a 500 or 200 meter buffer area was also plotted for comparison. Wet Labs 1731 at Station 4 was lost due to strong current, therefore, no steady state analysis results were conducted at Station 4.

Station 1 was located at Sagamore Creek approximately 1.02 miles downstream from the outfall. Dye levels recorded by Wet Labs FLRHB 913 submersible fluorometer and boat tracking units within a 200 meter buffer zone around Station 1 over the period of study are shown in Figure 19. In addition to recorded dye concentrations, corresponding tidal depth (ft), which represent tidal cycles in the specific area, were recorded by CTD (unit 5487). In general, the tidal range near Stations 1 and 2 were 8-20 ft. Dye was detected by submersible fluorometer at Station 1 about 11 hours later (1:00 PM on December 11, 2012) after injection and reached a peak concentration (0.2 ppb) at the second low tide, representing 6,850:1 dilution. However, due to the close distance to the outfall, the higher concentrations were still on the surface, as the FDA expected. The maximum concentration of 1.17 ppb dye level was found within a 200 meter buffer zone on the surface which is about six (6) times than the maximum concentration of 0.2 ppb detected by submersible fluorometers at Station 1. At this station, dye was not evenly distributed in the first half tidal cycle since much higher dye levels were concentrated on the surface. Based on the super-position analysis, the half tidal day peak 1 hour steady state dilution was 3,355:1 after 3.5 tidal days. However, because the instruments were retrieved prior to reaching a steady state condition, dye levels were shown to be building with a steep gradient. Therefore, it illustrates that the pollutants residence time in Sagamore Creek area is likely greater than 4 tidal days. All build-up dilution levels around Station 1 were greater than 1,000:1; however, the minimum dilution from surface tracking by boat (1,171:1) was close to this FDA recommended dilution number (1,000:1).

On the east side of New Castle Island, Station 2 (finfish aquaculture site) began to pick up dye three hours earlier than Station 1, which is located at the west side of the New Castle Island. The travel time to Station 2 is faster than Station 1 although Station 2 is almost 1 mile farther from the outfall than Station 1. The submersible fluorometer recorded the maximum concentration of 0.21 ppb, yielding a dilution of approximately 6,571:1. It was shown that most dye concentrations fell into the range of 0.1-0.5 ppb within a 500 meter buffer zone of boat tracking data, representing the dilution of 2,740 – 13,700:1. Unlike Station 1, the maximum concentrations on the surface measured by boating tracking were 5 hours later than the maximum concentration recorded by the submersible fluorometer. Based on the drogue study, which

measured current velocities at different depths, the stratification causes different travel speeds at the surface and in deeper water. This was the likely reason that dye was detected earlier at the deeper submersible fluorometer than the surface tracking fluorometer. The interesting finding was the first mass of dye detected by the submersible fluorometer lasted 4 hours during the first ebb tide followed by a gap for over 3 half tidal days before being detected again on the fifth half tidal day for about 2 hours . However, these findings match the findings at Station 1, where there was a dye detection gap between the first day and the third day and on the third day (December 13. 2012) dye was still consistently detected at depth for several hours. Moreover, dye was measured by surface boat tracking around Station 2 on both December 11 2012and December 12, 2012 as well shown on Figure 17. The data from both boat tracking and submersible fluorometer proves that pollution once discharged can be still detected for at least three consecutive days.

Station 3 is adjacent to the Peirce Island WWTP which is approximately 0.15 miles downstream from the outfall. Figure 11 represents surface tracking via boat within a 500 meter buffer zone while Figure 21 represents continuous 5-point moving average concentration values and the corresponding dilution levels for the 3-day study as determined by the submersible WET Labs 1730. According to Figure 21, dye was captured by the submersible fluorometer 25 minutes after injection, which indicates that it was still at the end of ebb tide at the beginning of dye injection. The first peak concentration occurred at approximately 3:50 AM on 12/11/2012 which is shown on Figure 21 as well and indicates that tide began to switch from ebb to flood around 3:50 AM. It matches the result from the drogue study, which confirms that the tide began to change from ebb tide to flood tide approximately 1 hour and 45 minutes later than NOAA predicted tides at Portsmouth Harbor. During the whole study period, the minimum dilution found at depth was 681:1 (2.01 ppb) on the first day of injection when the ebb tide was near its lowest. Meanwhile, maximum dye detected on the surface (500 meter buffer around Station 3 on Figure 11) was approximately 2 times the levels detected at depth representing 349:1 dilution equivalent to a 5-point moving average concentration of 3.93 ppb. It is worth noting that although dye at depth reached the steady state with a dilution of 1,370:1 on the second day, it was still measured on the surface with a significant level of dye (2.86 ppb) on the second day (December 12, 2012), yielding a dilution of 479:1. Moreover, both the Station 3 outlier analysis (Fig.18) and the second day tracking (Fig. 5) show a significant amount of dye-tagged effluent was detected at less than 1,000:1 dilution around Station 3 on both the first and second day. Since the FDA did not track dye on the surface on the third day in the Little Harbor area, it is difficult to conclude if dye was completely flushed out within three (3) consecutive days. Based on all findings and analysis, the FDA estimates at least three (3) days are needed for pollutants discharged to be removed from Station 3 and the adjacent area.

At the east side of Goat Island near the outfall (0.28 mile downstream), Station 4 was located with an attached submersible WET Labs 1731. However, as described in section 2.1, the cage was lost due to strong current. Therefore, only plume tracking data is available for analysis. Figure 12 shows boat tracking results around Station 4 for a 500 meter radius buffer. Most 5-point moving average concentrations adjacent to Station 4 were in the range of 0.1- 0.5 ppb, however, dye levels of 0.5-1.0 ppb were also observed at Station 4 along the channel. As can be seen on Figure 18, significantly higher dye levels were measured in Spruce Creek on the second day about 3:40 PM (December 12, 2012) and dye levels of 0.1- 0.5 ppb were constantly detected

as far as the mid-way into Spruce Creek (Maine side). Although the dilution levels at most places were higher than 1,000:1, a significant amount of data close to the WWTP outfall shows dilutions still less than 1,000:1 or at the lower end of the range of 1,000:1 – 10,000:1. Since the growing area around Station 4 is prohibited, based on this dye study with 5.408 MGD flow rate, this area is not a concern.

Dye in deep water first reached Station 5 (Kittery WWTP) at 5:37 AM on December 11, 2012, approximately 3.42 hours after the injection (Fig. 22). Most dye levels were less than 1 ppb over the course of study. Due to the distance from the outfall (1.59 miles upstream of the Peirce Island WWTP outfall), the high concentrations did not occur at the first low tide following the injection but occurred at the second low tide between 00:21 – 1:11 AM on December 12, 2012. In less than 1 hour, sufficient data collected by the attached submersible fluorometer shows that the high concentrations were not outliers. The maximum concentration detected by submersible fluorometer was 6.65 ppb, yielding a dilution of 206:1, which is much lower than the minimum dilution of 3,342:1 detected by boat tracking (500 meter buffer), as shown on Figure 13. It is very interesting to have approximately 50 minutes of high dye levels at the end of first tidal cycle. However, it is very difficult to explain the reason for a significant amount of high concentrations of dye occurring in a short time other than attributing it to the complexity of mechanism of water flow, bathymetry and impact of locations because Station 5 was placed between the I-95 Bridge and Route 1 Bypass as well as other potential environmental factors. Nevertheless, neither the steady state peak 1 hour concentration, nor the steady state average concentrations, were impacted by the unusual spikes. Meanwhile, significant levels of dye at Station 5 were not detected both via surface tracking and submersible measurement on the third day, but low levels of dye (0.01 – 0.5 ppb) were periodically detected at depth increasing with decreasing tidal depths. Since FDA pulled out the submersible fluorometer a little early, additional data analysis was conducted to understand when dye can reach steady state status. Based on the projection calculation, the flushing time at Station 5 is four (4) days with a half tidal day peak 1 hour dilution of 1,539:1.

Station 6 was located 2.18 miles upstream of the Peirce Island WWTP outfall and the mouth of Spinney Creek which is located on the north side of I-95 Bridge. Figure 14 represents boat tracking concentration values and the corresponding dilution range levels within a 500 meter radius around Station 6 for the study period. The dye was relatively well distributed at Stations 6 with similar levels of dye found near the bottom of the water column as at the surface for many measurements. A few measurements with relatively lower dilution (above 0.1 ppb dye concentrations) occurred adjacent to the north side of the I-95 Bridge and at the Maine and New Hampshire border due to lower energy and Piscataqua River channelization comparing to more opened area downstream. Dye levels of 0.01 – 0.1 ppb were found on both the New Hampshire and Maine sides of the river.

Stations 7 and 8 were located further upstream from the WWTP outfall at Dover Point and Little Bay, respectively. As shown on Figure 23, dye was detected by Station 7 submersible fluorometer Wet Labs 2146 at 6:45 AM (12/11/2012), which is only 4.5 hours from the time of injection. Considering the factors of tidal change delay compared to the NOAA tidal chart at Portsmouth Harbor (around 1.75 hours late according to the drogue study), dye released from the WWTP at the beginning of flood tide and reached to the Dover Point area in less than 3 hours

representing the average travel velocity (during flood tide injection) of 1.94 mph (5.43 miles/2.8 hours). Therefore, assuming dye was released at the beginning of flood tide it will reach Station 7 much quicker, although the distance to the outfall is 5.43 miles. The peak concentration measured by the submersible fluorometer was 0.21 ppb, equivalent to a dilution of 6,523:1, whereas the minimum dilution on the surface detected by boat tracking was 8,059:1 (Fig. 15), which is close to the peak concentrations measured deeper in the water column. It shows dye mixed and distributed along the upper Piscataqua River. Similar to Station 5, dye reached Station 7 quickly, according to steady-state analysis, the half tidal day peak 1 hour steady state dilution of 4,567:1 based on additional projection analysis. Moreover, dye levels were in the range of 0.02 – 0.08 ppb for the whole course of study according to the submersible fluorometer data; the first day surface tracking data shows dye concentrations were relatively stable along the whole Piscataqua River, both observational results show the relatively channelized Piscataqua River has lower energy to circulate and mix dye compared to the high-energy estuaries which has more complex flow features and topography and strong tidal currents bringing in quick travel speed from the WWTP outfall during flood tide. Thus, quick travel time to Little Bay, and Bellamy River is still a concern.

At each of the stations, there is a clear relationship between dye levels and tidal depths, with higher concentrations of dye seen at low tide (with less water to dilute the dye) and lower concentrations of dye seen at high tide (with more water to dilute the dye). Nevertheless, based on the results stated above, it is difficult to draw a 1000:1 steady state dilution line or radius for the Peirce Island WWTP effluent. Moreover, large amounts of data were close to 1,000:1 dilution although they are greater than 1,000:1, especially the downstream of outfall in the estuary at Stations 1-4.

3.8 Projection of WWTP Performance Impact Based on Various Fecal Coliform Assumption

Due to the potential design flow changes for the new updated WWTP, the FDA applied additional analysis for the potential 6.13 MGD flow rate and assessed the impact of the Portsmouth growing area based on the assumption of non-disinfected effluent discharges with 100,000 FC/100ml, 500,000 FC/100ml, and 1,000,000 FC/100ml for disinfection failure scenarios (Fig. 24-26). The first step to projecting fecal coliform (FC) levels is to calculate the difference factor between projected flow rate (6.13 MGD) and the actual study flow rate (5.408 MGD) which is 1.13. Spatial analysis was used by ArcGIS to calculate new dilution levels for assumed 6.13 MGD based on the 1.13 difference factor and actual dilution levels for 5.408 MGD. The last step for calculating FC levels is using different FC effluent assumptions divided by the projected dilution levels.

In the NSSP Model Ordinance (MO), fecal coliform median or geometric mean MPN of water sample results shall not exceed 14 FC/100ml for approved and conditionally approved growing areas. Based on the spatial analysis, when assuming 100,000 FC/100 ml non-disinfected effluent discharging from the WWTP, most areas at downstream of outfall would have very high FC numbers, that is, 39, 21, and 87 per 100 ml for Station 1, 2, and 3, respectively. As can be seen in Figure 24, at the upstream of WWTP outfall, there were still plenty of data more than 14

FC/100ml along the Piscataqua River. A level of 29 FC/100 ml, which is more than two (2) times the criteria in the MO was still anticipated to be measured at Station 7 and adjacent waters. Meanwhile, Dover Point, Little Bay, the mouth of Oyster River, and the lower part of the Bellamy River also would not meet the criteria. However, the areas in the Great Bay, upstream of Bellamy River, Oyster River, some areas in the upstream of Piscataqua River, and upstream of Spruce Creek shows levels less than 14 FC/100ml. Figures 25-26 show the estimated FC levels in the study area assuming the two (2) non-disinfected effluent scenarios of 500,000 FC/100ml and failed disinfection of 1,000,000 FC/100ml. Basically, these two (2) scenarios do not have a large difference indicating that when the effluent concentration is greater than 500,000 FC/100ml most areas do not meet the FC standard, even in deep Great Bay. This shows if the WWTP disinfection failure occurs with a 6.13 MGD potential design flow rate, all areas including Piscataqua River and Portsmouth Harbor, Little Harbor, nearly the whole Bellamy River, Little Bay, and Great Bay will be largely impacted. However, if the effluent is less than 100,000 FC/100ml, it possible to classify a portion of Bellamy River other than the prohibited area, but other management might apply depending on other factors.

3.9 Projections for Different Wastewater Treatment Plant Flows

In order to assess WWTP performance and assist the NHDES for the conditional area management plans, simulated 3, 4, and 6.13 MGD flow rates were used to project corresponding dye concentrations and dilutions based on the current dye study. During the hydrographic dye study period, the flow rate at the WWTP was 5.408 MGD which is the baseline for comparing different scenario flow rate projections.

Figures 27-29 represent the 5-point moving average concentrations and dilutions for 3, 4, and 6.13 MGD flow rate scenarios, respectively, while Figure 1 shows the concentrations and dilutions for 5.408 MGD. Less than 1,000:1 dilution occurs around Stations 1 – 4 and partially Spruce Creek as well, when the flow rate was 5.408 MGD. It should be noted that quite a good amount of data fell within the lower end of 1,000 – 10,000:1 range. Downstream of Sagamore Creek and the mouth of Little Harbor should also be of concern. There is no significant improvement of those areas when the simulated flow rate is decreased to 3 or 4 MGD (Fig. 27 and 28). The less than 1000:1 dilution points upstream of Station 2, downstream of Station 3 and the east side of Station 4 were removed when the flow rate changed to 3 MGD, however upstream of Station 2, downstream of Spruce Creek, the mouth of Little Harbor, and adjacent to the WWTP outfall are still a concern. Therefore, the WWTP effluent does not significantly improve, even with a flow rate down to 3 MGD, considering this treatment plant only has primary treatment process. Based on the conversation with the NHDES, shellfish on the finfish aquaculture site (Station 2) probably does not need to be harvested year round, however, more information is needed for determining the harvest months and duration in a year (e.g. MSC). Since the WWTP will be upgraded and add a secondary treatment process, the treatment performance might be improved and a conditional management plan, if appropriate, could possibly be considered to guide future management decisions is this area, which is currently prohibited.

3.10 Microbiological Analysis of WWTP Influent and Effluent

Table 3 shows the fecal coliform (FC) and Male-Specific Coliphage (MSC) levels in the influent and final effluent at the Peirce Island WWTP over the course of the study period. Both grab samples and samples from ISCO automatic water samplers that were set up for collecting wastewater samples every six (6) hours were used for both influent and effluent sewage. Levels of FC in the influent ranged from 5.4×10^5 cfu/100ml to 9.25×10^7 cfu/100ml and MSC levels were in the range between 1.2×10^5 pfu/100ml and 1.586×10^6 pfu/100ml. The geometric mean of FC was approximately 4.9×10^6 cfu/100ml in the influent, while the geometric mean of MSC level was about 8.11×10^5 pfu/100ml. The final effluent of geometric mean FC level was reduced to only 3 cfu/100ml representing 6.17 of log reduction after treatment by WWTP, although one sample showed a high FC level of 54 cfu/100ml. However, the MSC remained at high levels in all collected effluent samples. The geometric mean of MSC level was as much as 1.29×10^5 pfu/100ml after being treated comparing 8.11×10^5 pfu/100ml of influent level resulting in less than a 1 log reduction of MSC. The results demonstrate that the Peirce Island WWTP, which only has primary treatment process, cannot provide efficient treatment for virus although FC removal is efficient and meets the EPA standards with a good log reduction. Sewage MSC testing data supports the fact that by adding secondary treatment a possible reduction of 2-3 logs of virus could be realized. This would reduce virus in the final effluent and potentially reduce the impacts of virus bioaccumulation in shellfish downstream.

3.11 Microbiological Analysis of Shellfish Samples

During study period, eight (8) sentinel shellfish cages were deployed at fixed locations. Due to the loss of Cage 4, no shellfish samples were retrieved and tested at the Goat Island west location. Moreover, all shellfish samples during the 2012 study period were only tested for Norovirus GI, GII, and Adenovirus (Table 4) without conducting FC, EC, and MSC testing as adequate sample holding times could not be achieved and stored samples were not in a desirable condition to test. As a result, additional shellfish (American oysters and blue mussels) were tested in FC, EC and MSC in 2013 as shown in Table 5.

As can be seen in Table 4(note: all results in Table 4 are reported in RT-qPCR Units/ gram of digestive diverticulum), Norovirus GI and GII were not detected in all shellfish cages for the 2012 study. However, Adenovirus results in shellfish samples from all cages were in the range of 1.9 and 94.1 RT-qPCR units/ grams. Although all cages were placed in the prohibited zones, Cage 1 is close to Little Harbor which is classified as Conditionally Approved Area. The high Adenovirus level (26 RT-qPCR units/ g) at Cage 1 raises a significant concern of Little Harbor classification. It is noteworthy that, results of blue mussel samples in 2013 (Table 5) show MSC levels greater than the 50 pfu/100g level established in the NSSP in Little Harbor (128 pfu/100g) at the sample location of LHOP2. Meanwhile, high number of MSC levels tends to occur during colder months with lower levels occurring in warmer months. Similarly, five (5) shellfish samples (including both American oysters and blue mussels) were collected and tested from the finfish aquaculture site in Portsmouth Harbor (Station 2) in 2013. MSC levels were found in two (2) out of eight (5) shellfish samples greater than 95 pfu/100g, the highest being 717 pfu/100g from blue mussels collected in December 2013. These numbers possibly indicate a WWTP

contamination issue at the finfish aquaculture site. Thus, in some circumstances (period of high viruses load in the effluent, colder water temperature periods) the poor WWTP efficiency in removing viruses is a potential public health risk with respect to shellfish harvesting in the Little Harbor area and nearby finfish aquaculture site.

Repeated shellfish testing at Stations 2, 3, 7, and 8 were conducted on 12/10/2013 using American oysters and blue mussels (Table 5). FC and EC were not detected in most samples except American oysters at Station 3 (20 cfu/100g for FC and 20 cfu/100g for EC) and blue mussels at Station 7 with 40 cfu/100g FC and 20 cfu/100g EC. However, seven (7) out of eight (8) samples had significantly high MSC levels relative to the 50 pfu/100g level established in the NSSP. The highest MSC level was 6,154 pfu/100g located at Station 3 which is very close to the Peirce Island WWTP outfall. The 2014 NSSP Model Ordinance layouts out criteria used for re-opening harvest areas after an emergency closure due to raw untreated sewage discharge (Section II, Chapter IV, @.03 (A.)(5.) (c.)(ii))) which states the analytical sample results shall not exceed background levels or a level of fifty (50) male-specific coliphage per 100 grams. During the 2015 ISSC conference, proposal 15-102 was adopted revising the language for emergency closures and states that the analytical sample results shall not exceed background levels or a level of fifty (50) male-specific coliphage per 100 grams or pre-determined levels established by the Authority based on studies conducted on regional species under regional conditions from shellfish samples collected no sooner than seven (7) days after contamination has ceased and from representative locations in each growing area potentially impacted; or until the event is over and 21 day have passed. Proposal 15-102 further defined a use for MSC for Conditional Management Plans based on the waste water performance (http://issc.org/client_resources/2015%20biennial%20meeting/tf%20i%20master.pdf).

The current and on-going studies take into consideration different shellfish species under regional conditions and can be used to establish a background level that may be potentially higher than 50 pfu/100g. Work to establish a background MSC level in Little Bay shellfish has not been conducted. A level of 1,212 pfu/100g and 412 pfu/100g MSC, were detected in American oysters and mussels, respectively within the Conditionally Approved Area at Station 8 in Little Bay, which is a magnitude or higher than the 50 pfu/100 grams level noted in the NSSP. These numbers suggest that the levels found may not be entirely attributed to background, but more information on Peirce Island WWTP performance and MSC removal efficiencies would be helpful in refining the analysis of the extent to which MSC levels in Little Bay shellfish can be attributed to MSC levels in Portsmouth/Peirce Island WWTP effluent. Further analysis of the data may be conducted to determine a correlation between factors including seasonality but especially a potential correlation between WWTP efficiency to remove MSC with rainfall and WWTP flow rate which may be useful in refining the Conditional Area Management Plan (CAMP) closure triggers. Thus, future studies on these topics should be pursued to generate data needed to incorporate the WWTP efficiency of removing viruses into Conditional Area Management Plans.

4.0 CONCLUSIONS AND RECOMMENDATIONS

When considered collectively, the data from the hydrographic dye study at the Peirce Island WWTP and microbiological assessment of WWTP influent and effluent, as well as

microbiological results of shellfish sampling, support the following conclusions and recommendations:

- The Peirce Island WWTP is very efficient (more than 6 log reduction) at removing FC indicator bacteria and meeting its permitted requirements for FC; whereas it is poorly efficient (less than 1 log reduction) at removing MSC, which provides an indication of efficiency in reducing the enteric viral load as the Peirce Island WWTP only employs primary treatment process;
- Adenovirus levels in the oysters cages were detected in the 2012 study which indicates shellfish were exposed to sewage and can potentially bioaccumulate unacceptably high levels of virus from the Peirce Island WWTP discharge;
- The average hourly flow rate at the Portsmouth WWTP during the dye injection was 5.408 MGD which is higher than the design flow rate (4.8 MGD);
- Based on the records, the Peirce Island WWTP regularly operates at flows above the 4.8 MGD design flow, which is considered as representative of a higher risk period closer to worst case rather than normal conditions;
- Due to the performance of the Peirce Island WWTP with regularly exceeding design flow and microbiological results of viruses levels in the WWTP effluent and in shellfish, the FDA 1,000:1 dilution guidance may not be sufficiently protective of public health;
- Based on the drogue study, the travel velocity on the surface and 10 feet underneath the water shows no significant difference. The speed at ebb tide can reach as high as 2.87 mph towards to the growing areas downstream of the Peirce Island WWTP;
- Tidal current influences play the most dominant role on the effluent travel direction and speed among other environmental factors. During high tide, dye moved to Little Bay along the Piscataqua River at a relatively fast velocity as high as 1.2 miles/hr. Less than 5.5 hours is needed for dye traveling from the Peirce Island WWTP outfall to the border of conditional approved and prohibited area in Little Bay and less than 8 hours reaching into Great Bay at flood tide condition;
- During the Peirce Island WWTP hydrographic dye study, dye-tagged effluent with high levels can reach to the approved growing area in the estuary in 1-1.5 hours at ebb tide and can be still detected for at least 3 consecutive days;
- Based on the surface tracking data, Little Harbor, finfish aquaculture site, Back Channel areas are a great concern due to numerous high dye levels that were detected;
- The station data suggests that if a WWTP failure occurs, the residence time of pollutants discharged could be approximately 4 days at the areas of Little Harbor and finfish aquaculture site;
- Based on the projection results for assessing impact of the Peirce Island WWTP performance, if the flow rate increased to a potential new design flow (6.13 MGD), and assumed 100,000 FC/100ml in non-disinfected effluent was applied, most areas at Little Harbor, finfish aquaculture site, Sagamore Creek, and Back Channel could be impacted heavily. If increasing FC discharge to 500,000 FC/100ml and of 1,000,000 FC/100ml (failed disinfection), most of the areas would be impacted ;
- The different flow rate projection results shows that decreasing flow rate to either 3 or 4 MGD had limited impact to the growing areas compared to the 5.408 MGD flow rate that occurred during the study since this treatment plant only has primary treatment

process. However, at higher flow rates the Peirce Island WWTP could impact the growing area heavily; and

- The WWTP efficiency of removing viruses should be addressed either through the CAMP and/or significant changes/upgrades to the WWTP.

Currently, the Peirce Island WWTP is operating over design flow frequently without secondary treatment process. Therefore, upgrading the WWTP (adding secondary treatment) is strongly recommended by the FDA. It is unclear what an upgrade might do to the viral output of the plant. However, limited sampling from nearby WWTPs that currently employ secondary treatment suggests that Peirce Island WWTP effluent may show significantly lower MSC levels after the upgrade to secondary treatment. If the upgraded WWTP effluent has significantly lower viral indicators, some growing areas (e.g. Little Harbor) may have a significantly reduced risk of chronic viral loading and accumulation in shellfish. Moreover, an upgraded WWTP should operate under normal conditions (not exceeding design flow). The FDA's 1,000:1 dilution guidance is not sufficient to protect public health around this particular WWTP, given that the facility only employs primary treatment and exhibits levels of MSC in final effluent that are much higher than nearby secondary WWTPs.

It is noted that although the Peirce Island WWTP has a SCADA system, NHDES staff are no longer on call after 9 pm. Therefore, if problems occur (e.g. bypasses, overflows) overnight, the effects of the pollution event would have been widely distributed throughout the estuary considering fast travel time. Due to the fast travel time to the growing areas showing approximately 1-1.5 hours to Little Harbor and the finfish aquaculture site during ebb tide, and 5.5 hours to the growing area in Little Bay at flood tide, the FDA recommends increasing communication between the WWTP operators and the NHDES in the event of a raw sewage failure or disinfection failure at the WWTP at any time is necessary. Therefore, immediate notification and response to the NHDES in the event of a bypass and/ or overflow could allow for prompt closure of the growing areas.

The growing area in Little Harbor is currently classified largely as Conditionally Approved. NHDES has determined that given the Peirce Island WWTP performance and the uncertainties in levels of viral loading and the accumulation of viruses in shellfish, the current classification and/or management of Little Harbor should be re-classified to Prohibited/Safety Zone until the Peirce Island WWTP is upgraded to secondary treatment. While that is occurring, periodic sampling of shellfish and effluent should be conducted on a seasonal basis to establish background levels of MSC. These background data could be compared to data generated after the WWTP upgrade in order to help determine if Little Harbor can again be classified as Conditionally Approved. FDA concurs with the NHDES determination.

The growing area of Portsmouth Harbor is classified as Prohibited/Unclassified area. The shoreline in the Atlantic Ocean from Little Harbor to Odiorne Point is classified as Approved Area with a small Restricted Area at the north of Odiorne Point. Based on the dye study results and the WWTP effluent microbiological results, NHDES has determined that these areas be included in an enlarged Prohibted/Safety Zone around the Peirce Island outfall. Portsmouth

Harbor is recommended as Prohibited/Safety Zone until a better understanding of virus levels in shellfish and completion of a comprehensive sanitary survey. FDA concurs with these classification changes. Additionally, the northern portion of nearshore and offshore Atlantic (from mouth of Little Harbor southward towards Odiorne Point) should be reclassified to Prohibited/Safety Zone as well. Since the WWTP will be upgraded and add a secondary treatment process, the treatment performance might be improved and a conditional management plan can also be adjusted from the current management decisions. Other management options can also be applied for the NHDES to manage depending on the continuous microbial testing results, the WWTP performance, and seasonal factors.

The Back Channel and Sagamore Creek are classified as Prohibited/Safety Zone and no commercial or recreational harvesting is currently occurring in either Back Channel or Sagamore Creek. However, there was once interest in using Sagamore Creek for washing commercially harvested Atlantic blue mussels. The 2012 study results show that both these areas are heavily impacted with rapid travel speed, and insufficient dilution, therefore both growing areas should be maintained the same Prohibited/Safety Zone classification.

The Upper Piscataqua River is Prohibited/Safety Zone. Due to the close distance from the Dover WWTP outfall, water quality issues, and study results, this area should be maintained the same. For the Lower Piscataqua River, the Peirce Island WWTP outfall lies within this growing area, thus this growing area is currently classified as Prohibited/Unclassified. Based on the dye study results, no management options to allow for shellfish harvesting should be pursued in the Lower Piscataqua River area. Current classification can be changed from Prohibited/Unclassified to Prohibited/Safety Zone.

For the Bellamy River, the area is classified as Conditionally Approved at the southern end (Clements Point to the mouth of the River at the Route 4/Scammel Bridge) while northern Clements Point areas are Prohibited. However, due to the concerns of rapid travel time and insufficient dilution of effluent during the dye study, NHDES recommends a change to the CAMP addressing recreational harvest management in the Bellamy River. Harvest in the Bellamy River will only be allowed on Saturdays, 9am-sunset. This tighter control of harvest, combined with the delayed 9am start time, will enable NHDES to implement emergency closures if the Peirce Island WWTP has any overnight issues with disinfection while NHDES staffs are not on call.

Most area in Little Bay is classified as Conditionally Approved. Two Prohibited areas (near Station GB6A and GB17) were put in place to examine seasonal sewage risk concerns from mooring fields and marinas -- the “Prohibited” approach was adopted over a seasonal to enable more practical management of open/closed seasons. However, recreational harvest management in Little Bay must change owing to the rapid travel time and insufficient dilution of effluent from the dye study results. NHDES recommends two changes to the area’s classification. The first changes is to more tightly control the time when recreational harvesters have access to the growing area, and enhance communications among NHDES, NHF&G, the WWTP, and recreational harvesters so that public health risks related to a disinfection failure at the WWTP can be effectively managed. As with the Bellamy River, recreational harvest will be allowed on Saturdays, 9am-sunset. Commercial harvest will be allowed throughout the week because

NHDES must grant harvest approval to each commercial harvest before it occurs. The second change involves modifying the classification of the area directly adjacent to the General Sullivan Bridge and Dover Point from Conditionally Approved to Prohibited. The Dover Point area showed a steady-state dilution of approximately 4,500:1. FDA's guidance on 1,000:1 dilution is not applicable to the Peirce Island wastewater treatment facility because it does not employ secondary treatment. There is no formal guidance on an appropriate steady state dilution level for a primary plant, but given the observed MSC levels in effluent and the frequency with which the current plant exceeds its design flow, NHDES recommends closure of this area until the facility is upgraded. FDA concurs with this recommendation.

The elevated MSC levels in Little Bay shellfish observed in December 2013 warrant additional study to examine background levels of MSC in shellfish, as well as studies to more closely examine how MSC concentrations and removal efficiencies vary at Peirce Island under different operational conditions, particularly in the fall, winter, and early spring when MSC levels in the environment may be the highest. NHDES and FDA will work together to further examine these issues to determine if additional adjustments to the CAMP for Little Bay are needed to protect public health. These adjustments will be examined for Lower Little Bay (Fox Point to Dover Point) and possibly for the Bellamy River, where the dye study suggested dilution in the range of 1,000:1 to 10,000:1. Areas south of Fox Point (Upper Little Bay) seemed to show dilution of greater than 10,000:1 and therefore show potentially less risk from viral contamination. Future annual and triennial reports will highlight the ongoing analyses of these issues.

Much of Great Bay is Conditionally Approved classification. The southwestern section of Great Bay in proximity to the Newmarket and Exeter WWTPs is Prohibited Area and four other small areas are Restricted due to water quality issues. The classification is recommended to remain the same in Great Bay based on the dye study results. Additionally, the Conditional Area Management Plan is recommended to be amended to establish performance standards/closure criteria for a lapse in treatment occurring at the Peirce Island WWTP. In order to accurately reflect the real virus levels and background levels at growing areas, the FDA recommends collecting more data on mussels and/or oysters periodically before and after the Peirce Island WWTP upgrade to understand background better.

Based on the microbiological findings in shellfish samples, wastewater samples from the Peirce Island WWTP, and the results of the dye study, a minimum of 1,000:1 dilution with respect to Peirce Island WWTP is currently not applicable for this WWTP unless a future upgrade (secondary treatment) is under operation and a future field study similar in scope can demonstrate a minimum dilution of 1,000:1. The FDA recommends continued MSC testing of wastewater samples from the WWTP before and after the WWTP upgrade. When the Peirce Island WWTP is upgraded to secondary treatment many of the classification changes should be revisited because of the underlying assumptions about the microbiological quality of the WWTP effluent (assumed FC load in undisinfected effluent, assumed MSC load in final effluent) are expected to significantly improve. A future field study is also recommended after the WWTP upgrade in order to evaluate the 1,000:1 dilution zone.

5.0 ACKNOWLEDGEMENTS

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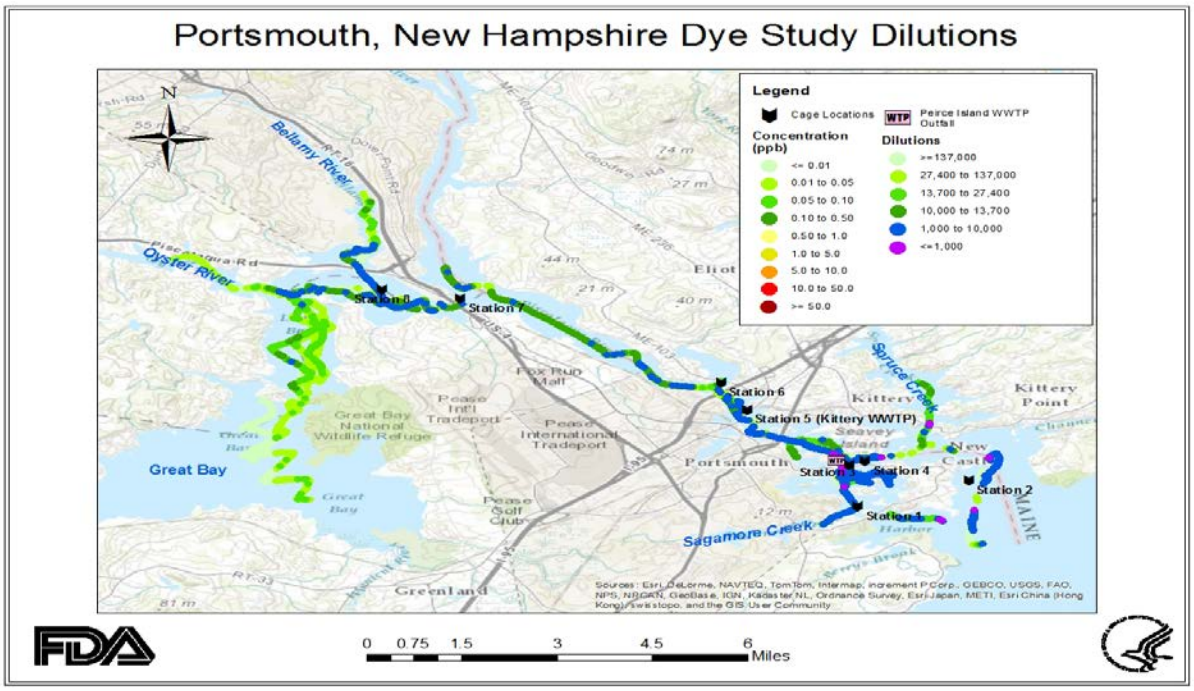
APPENDIX I



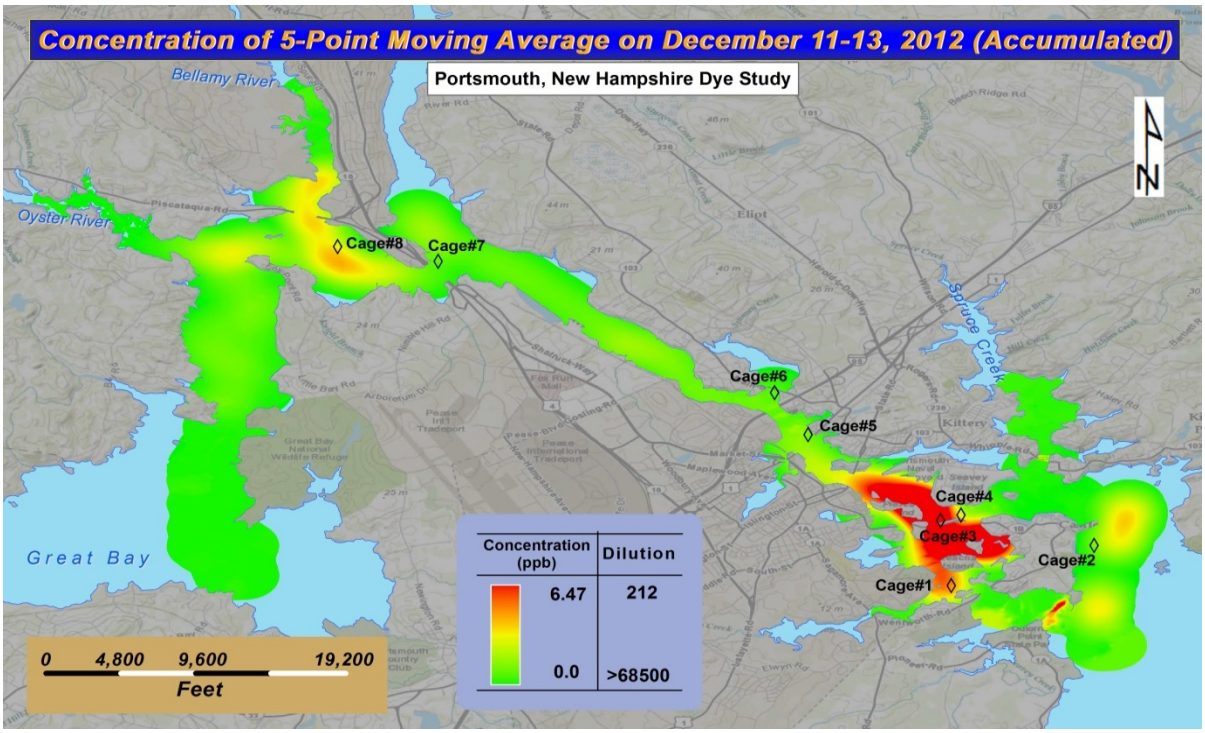


Fig. 1

Portsmouth, NH study area, station and submersible fluorometer locations, and dye concentration and dilutions from plume tracking during study period under 5.408 MGD (12/11/2012 – 12/13/2012)

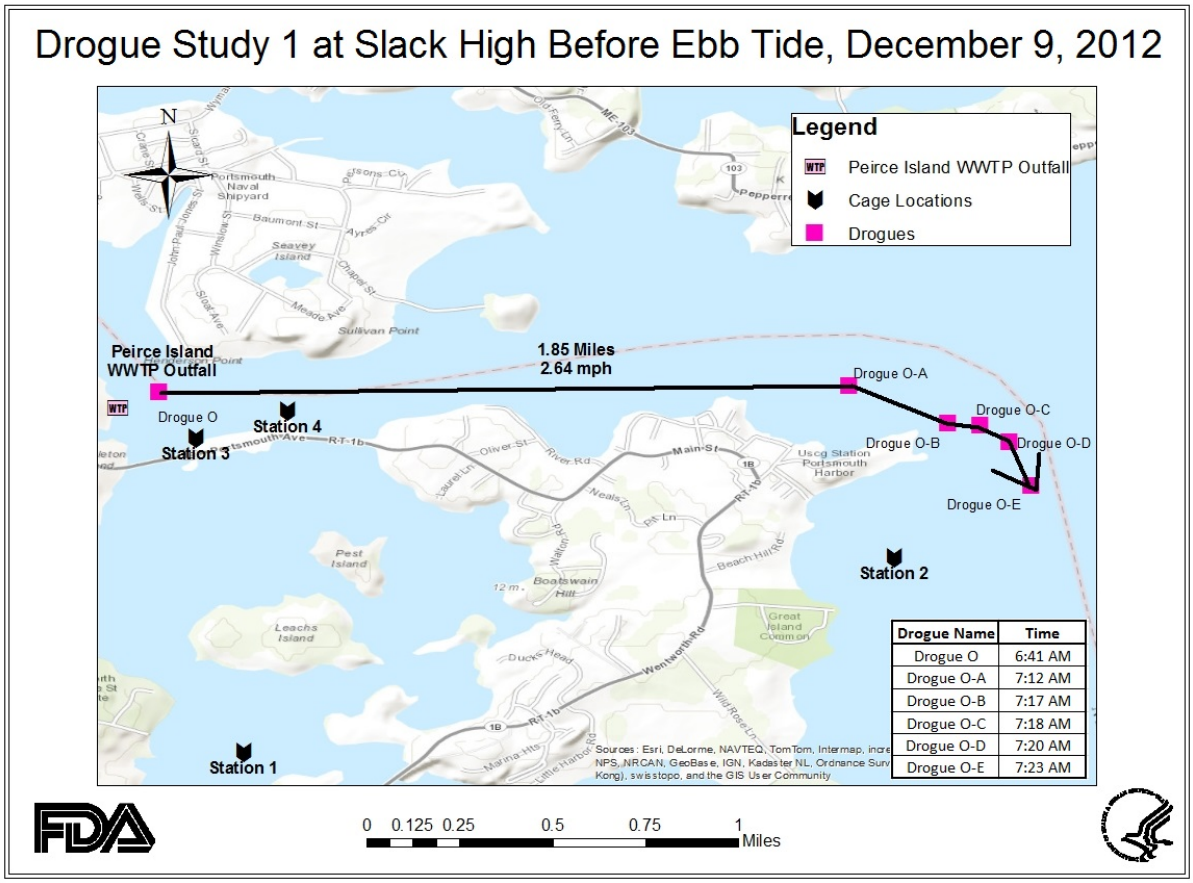


Fig. 2

Drogue study at slack high before ebb tide on the surface

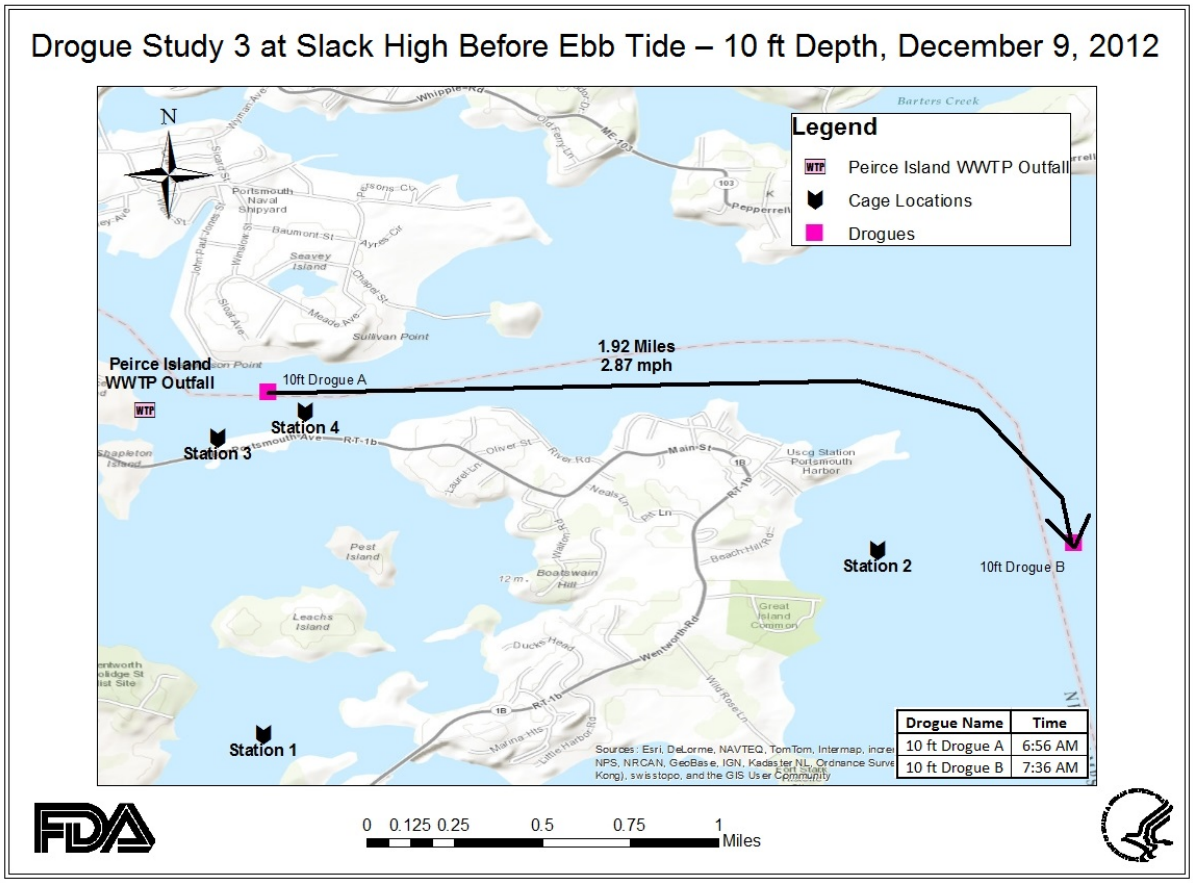


Fig. 3

Drogue study at slack high before ebb tide at 10 feet depth

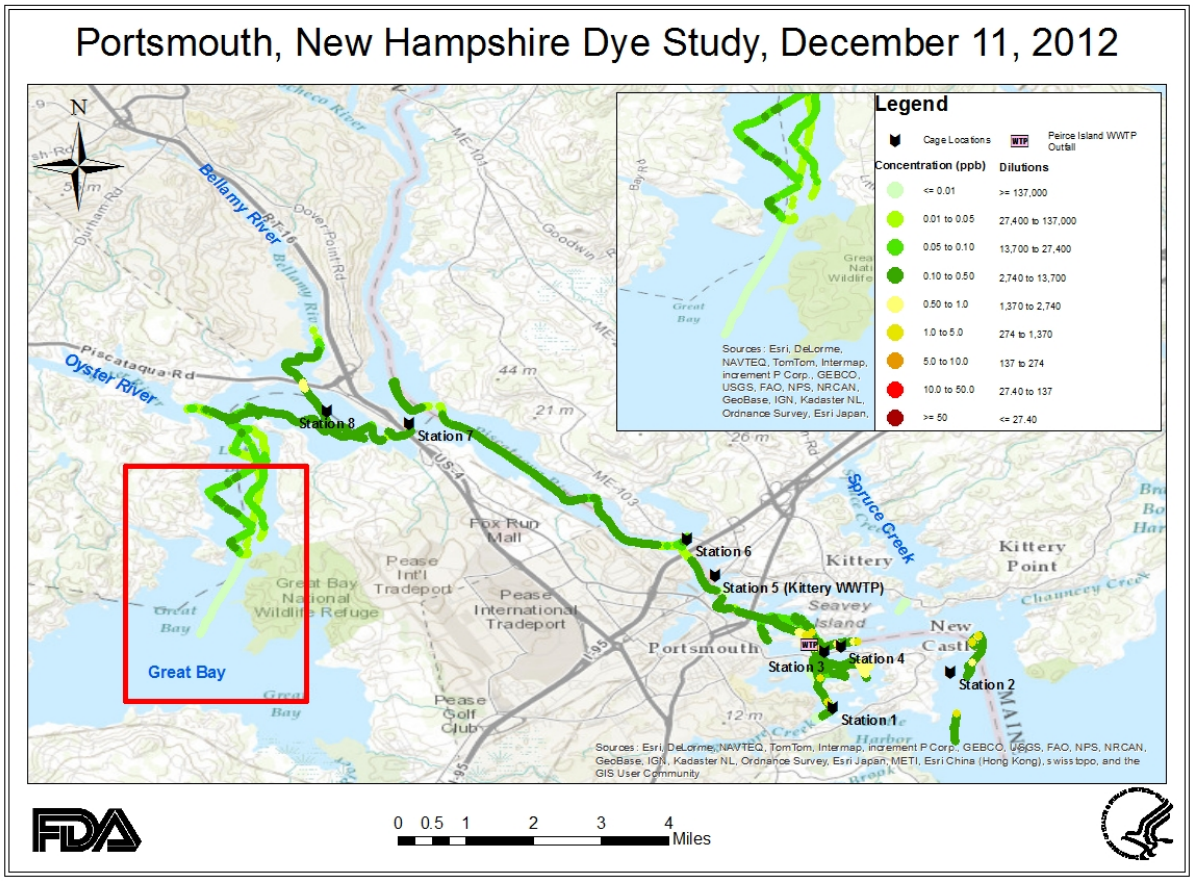


Fig. 4

The plume tracking on the first day (12/11/2012) under 5.408 MGD

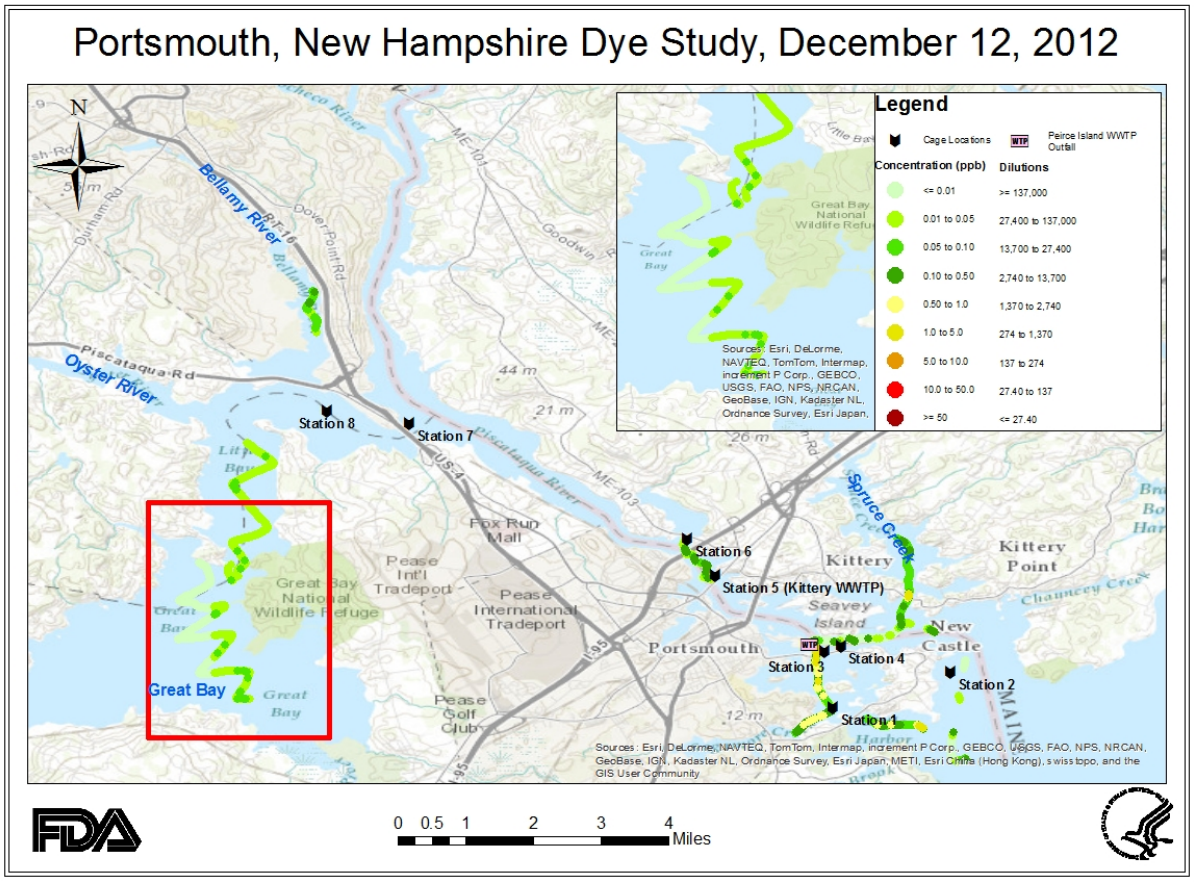


Fig. 5

The plume tracking on the second day (12/12/2012) under 5.408 MGD

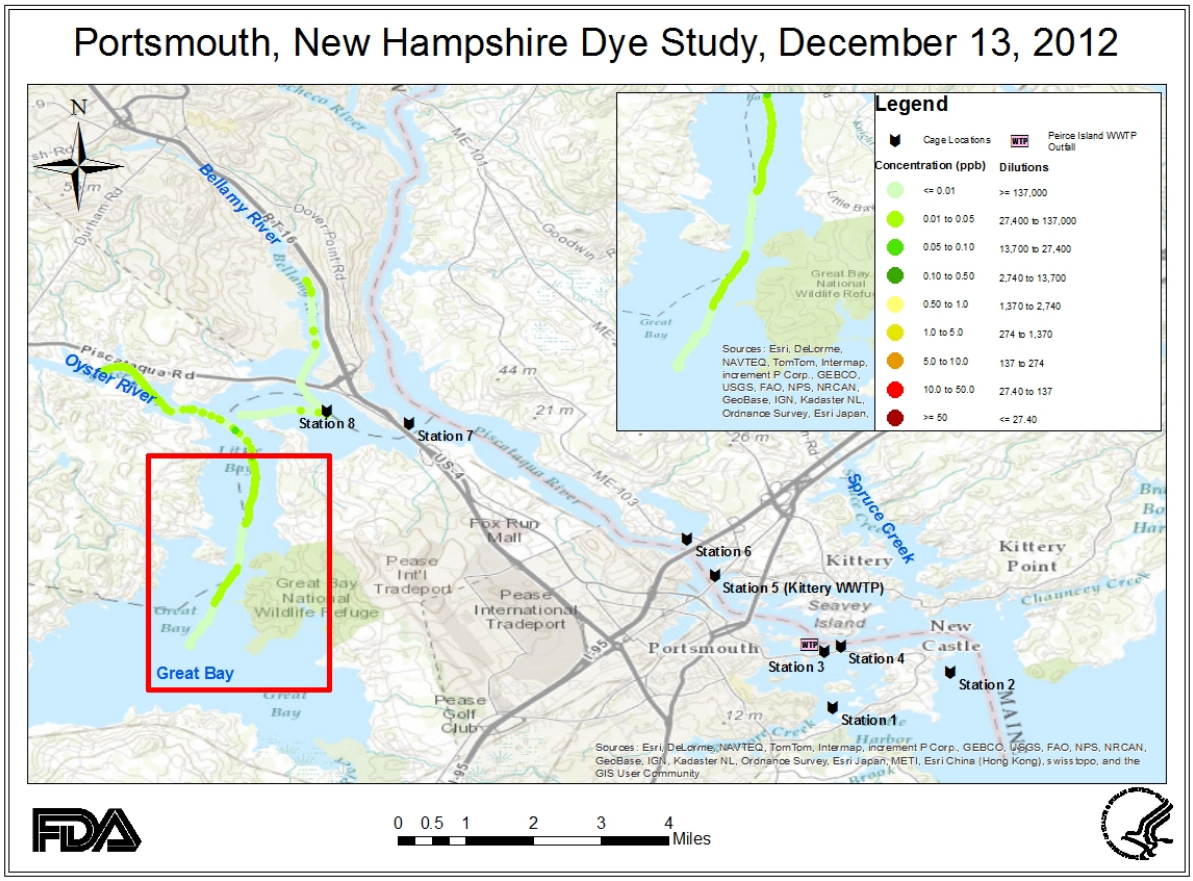


Fig. 6

The plume tracking on the third day (12/13/2012) under 5.408 MGD

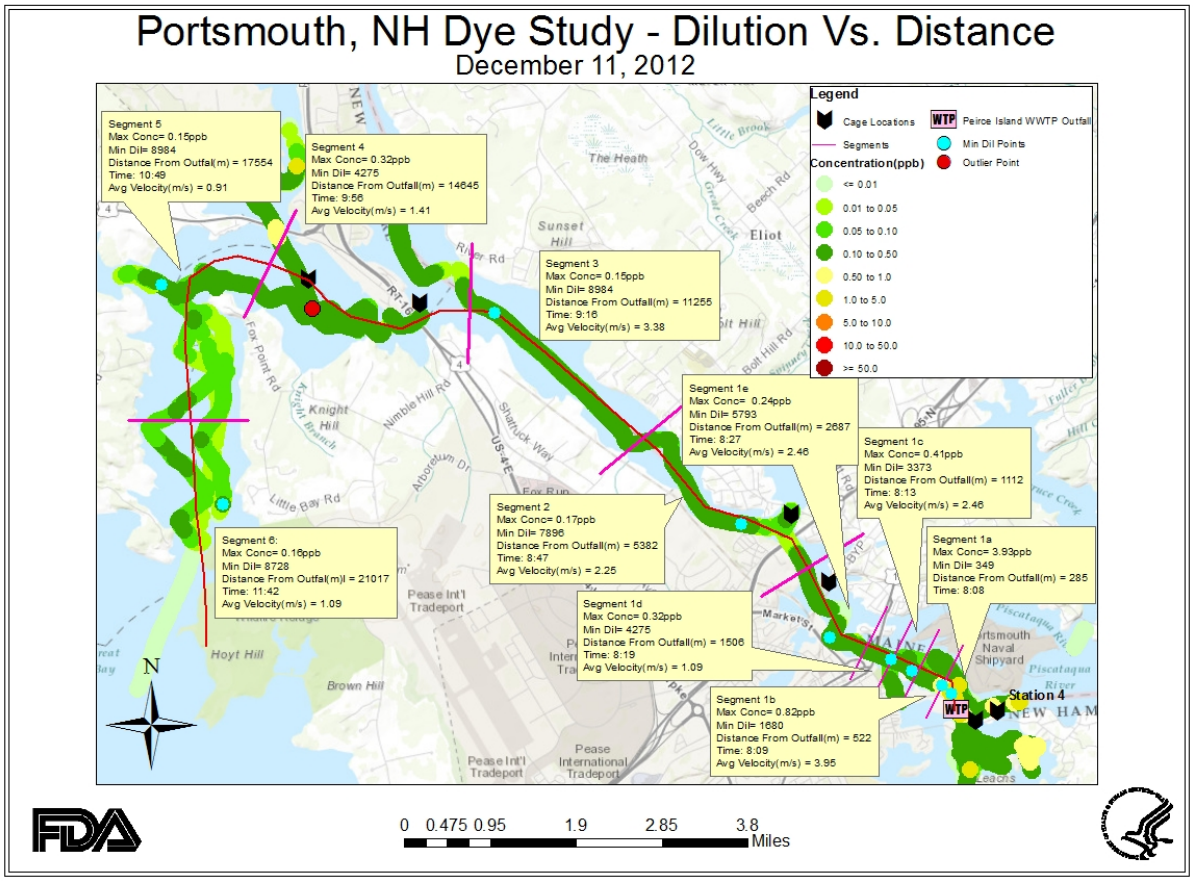


Fig. 7

Maximum concentrations at various distances from the outfall on the first day of plume tracking (12/11/2012)

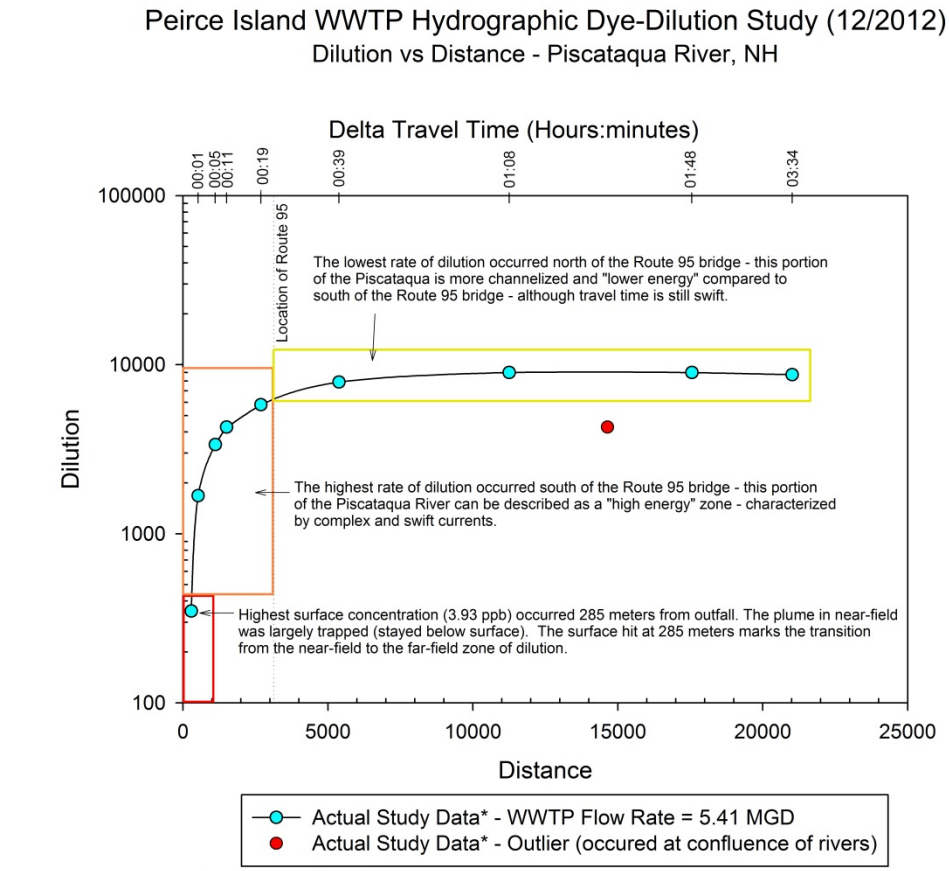


Fig. 8

Dilution vs. distance on the first day of plume tracking (12/11/2012)

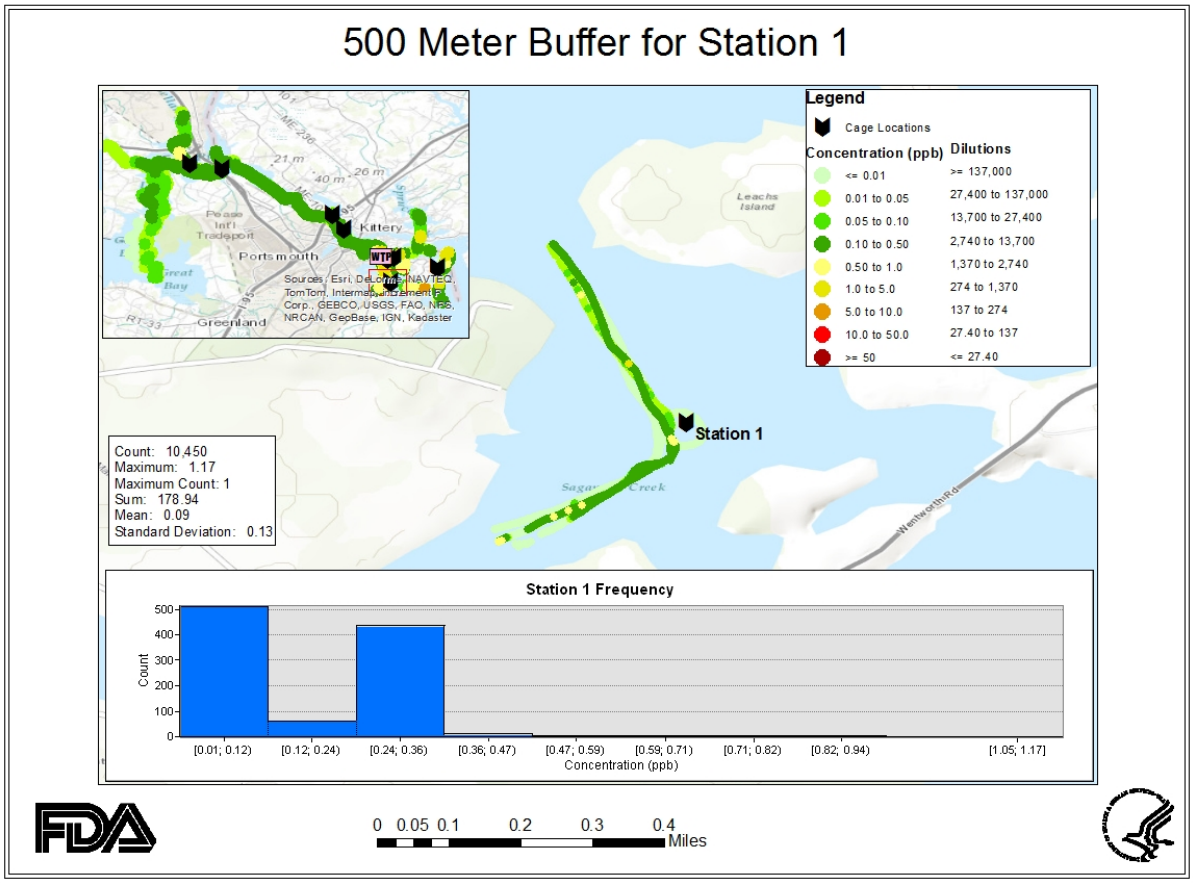


Fig. 9

500 meter buffer zone of plume tracking results around station 1

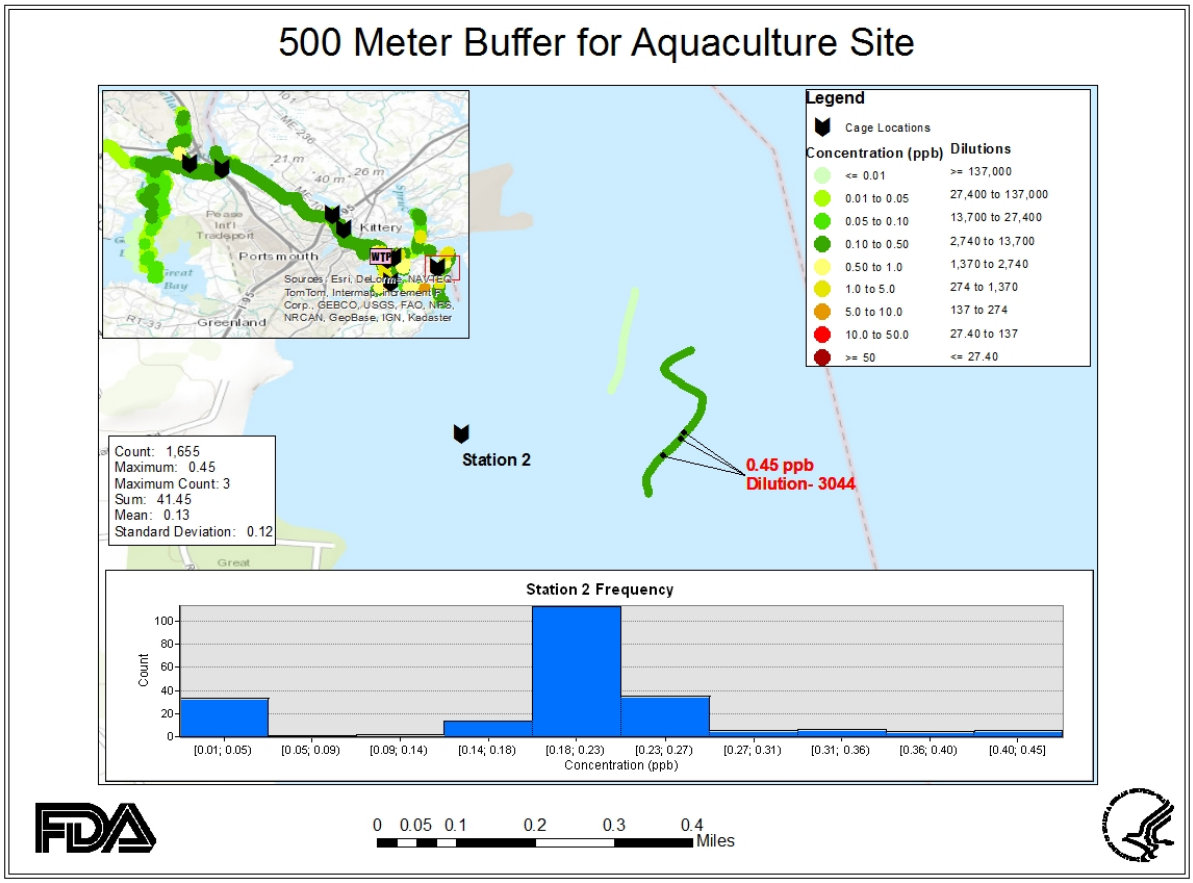


Fig. 10

500 meter buffer zone of plume tracking results around station 2

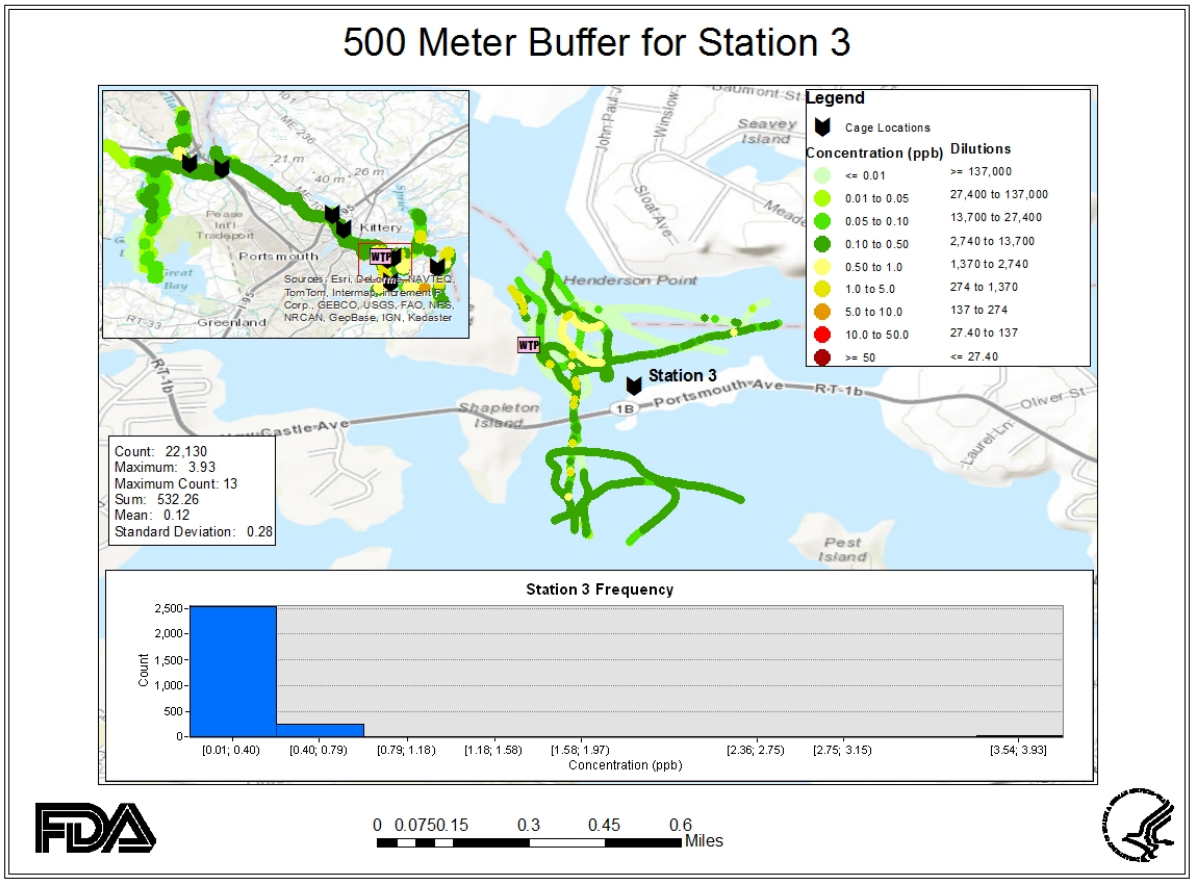


Fig. 11

500 meter buffer zone of plume tracking results around station 3

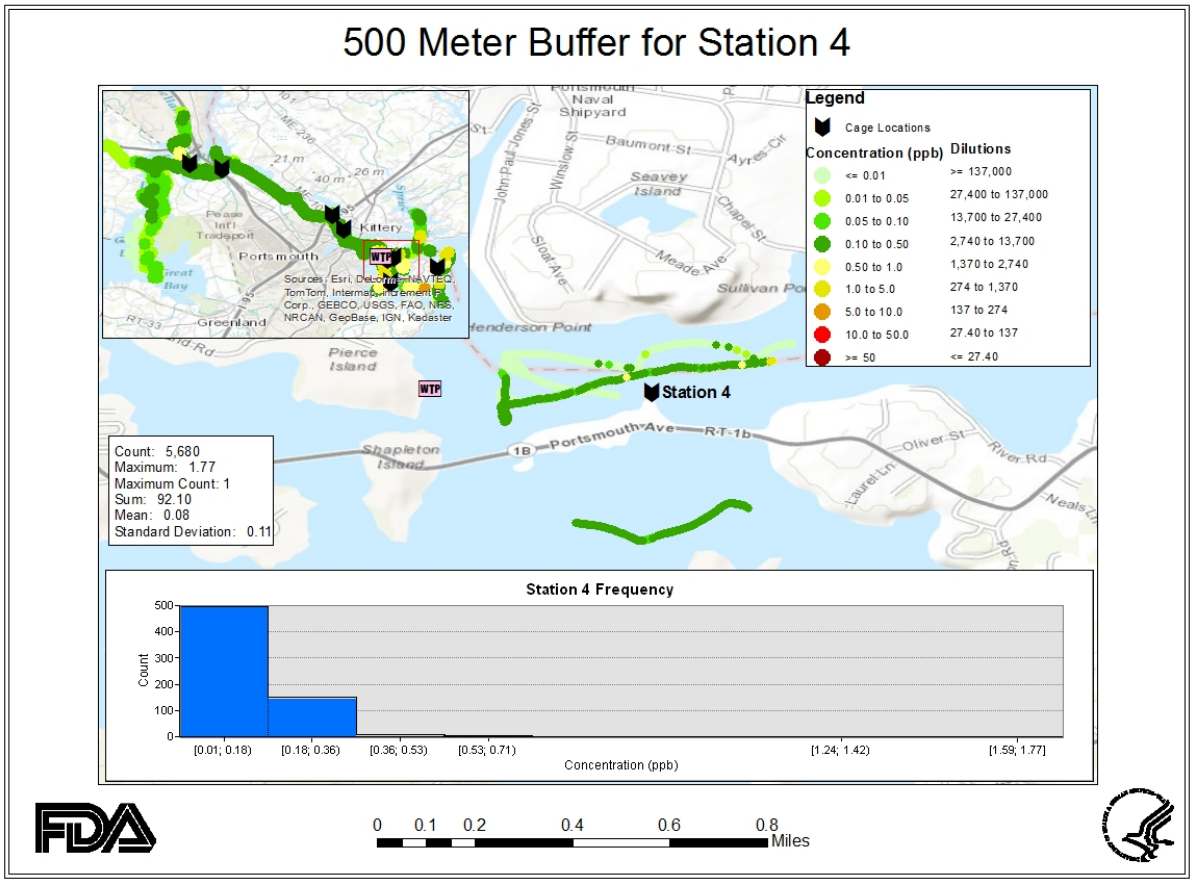


Fig. 12

500 meter buffer zone of plume tracking results around station 4

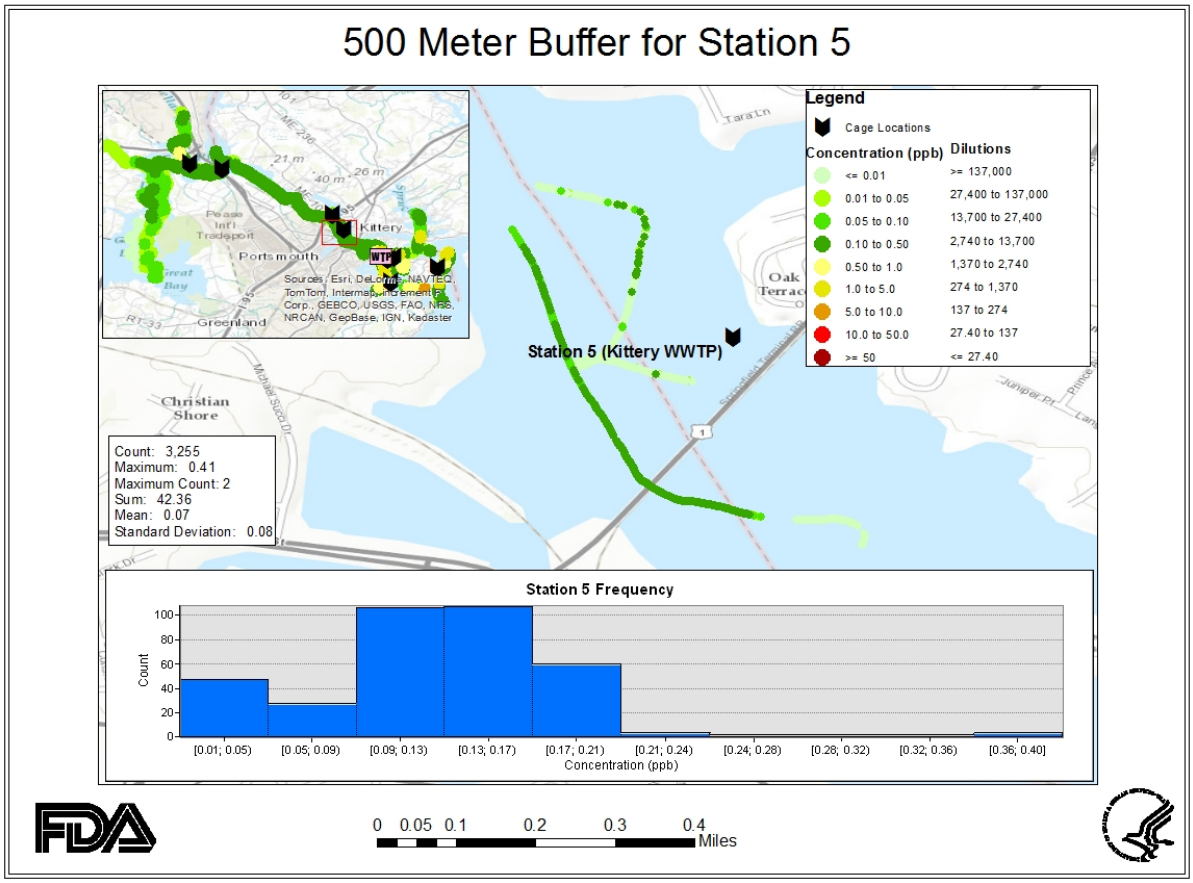


Fig. 13

500 meter buffer zone of plume tracking results around station 5

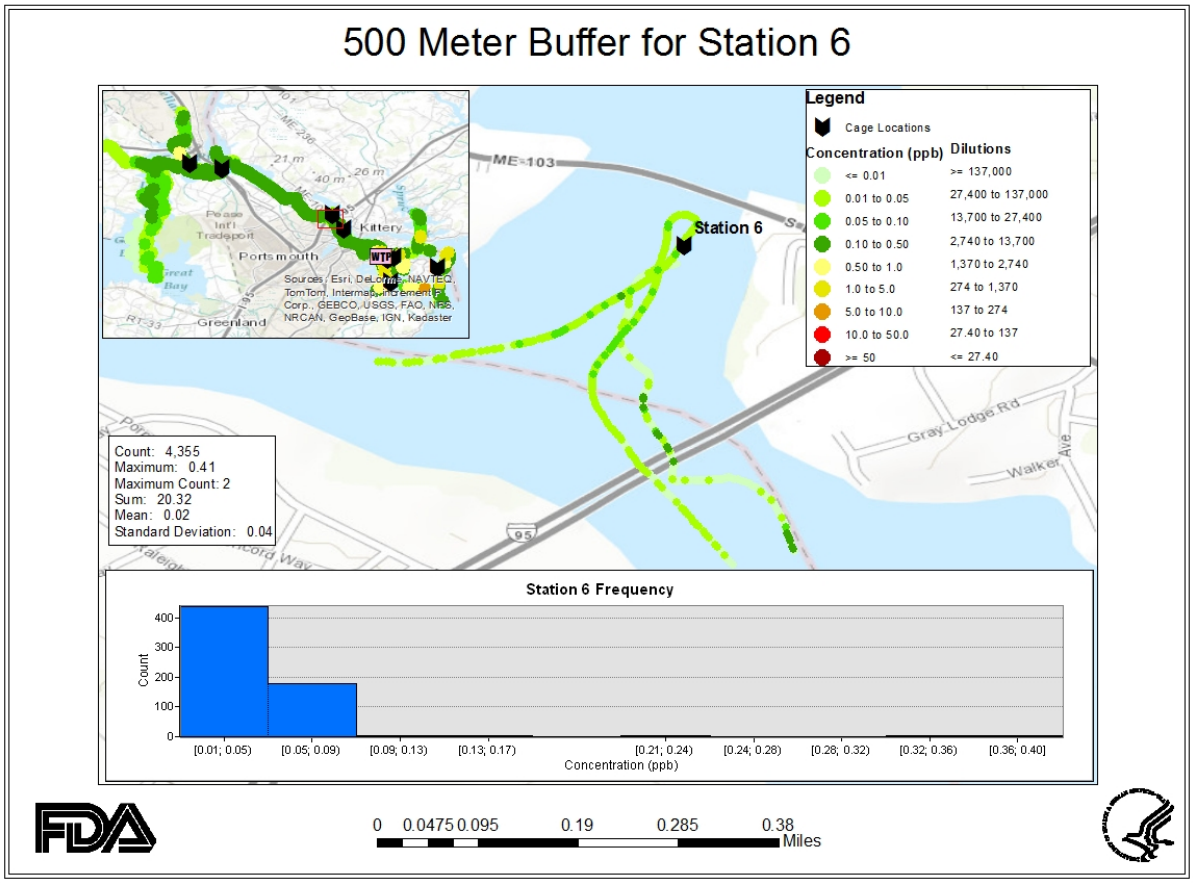


Fig. 14

500 meter buffer zone of plume tracking results around station 6

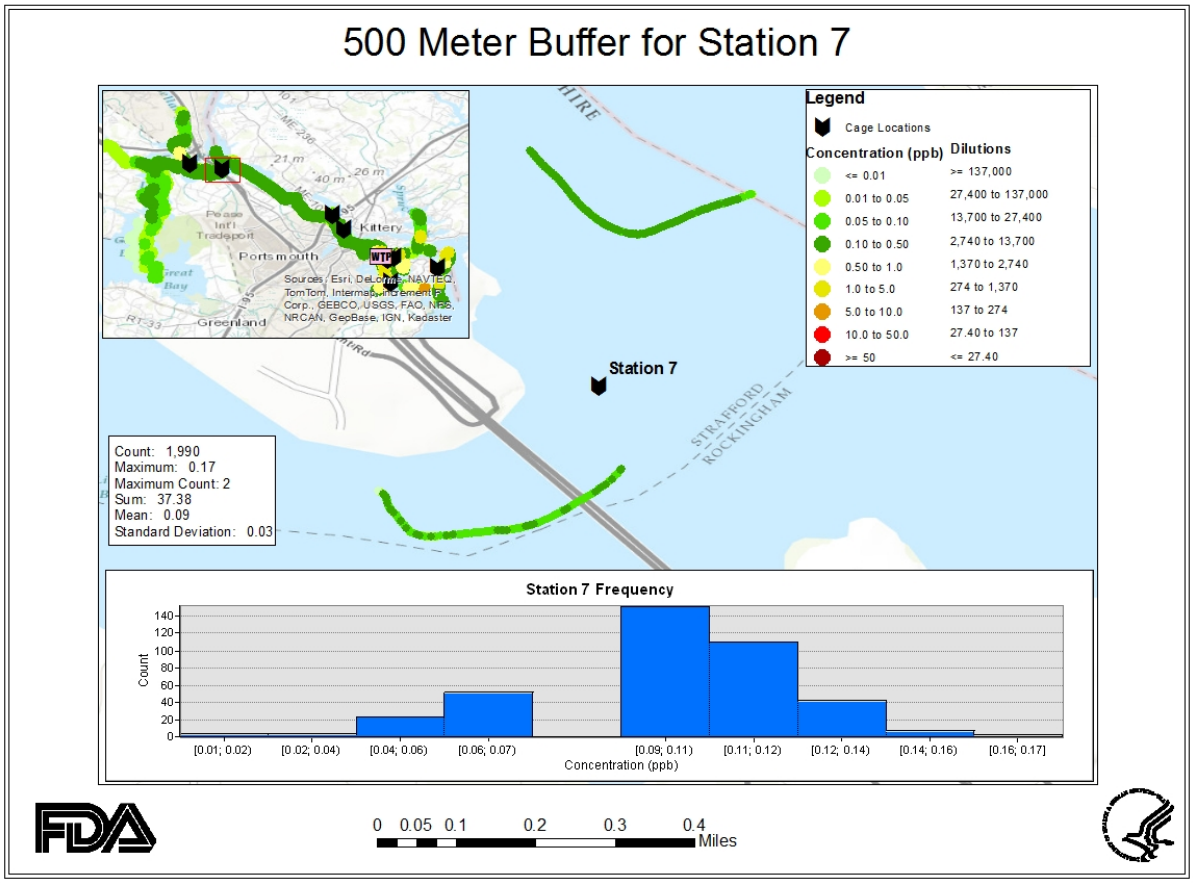


Fig. 15

500 meter buffer zone of plume tracking results around station 7

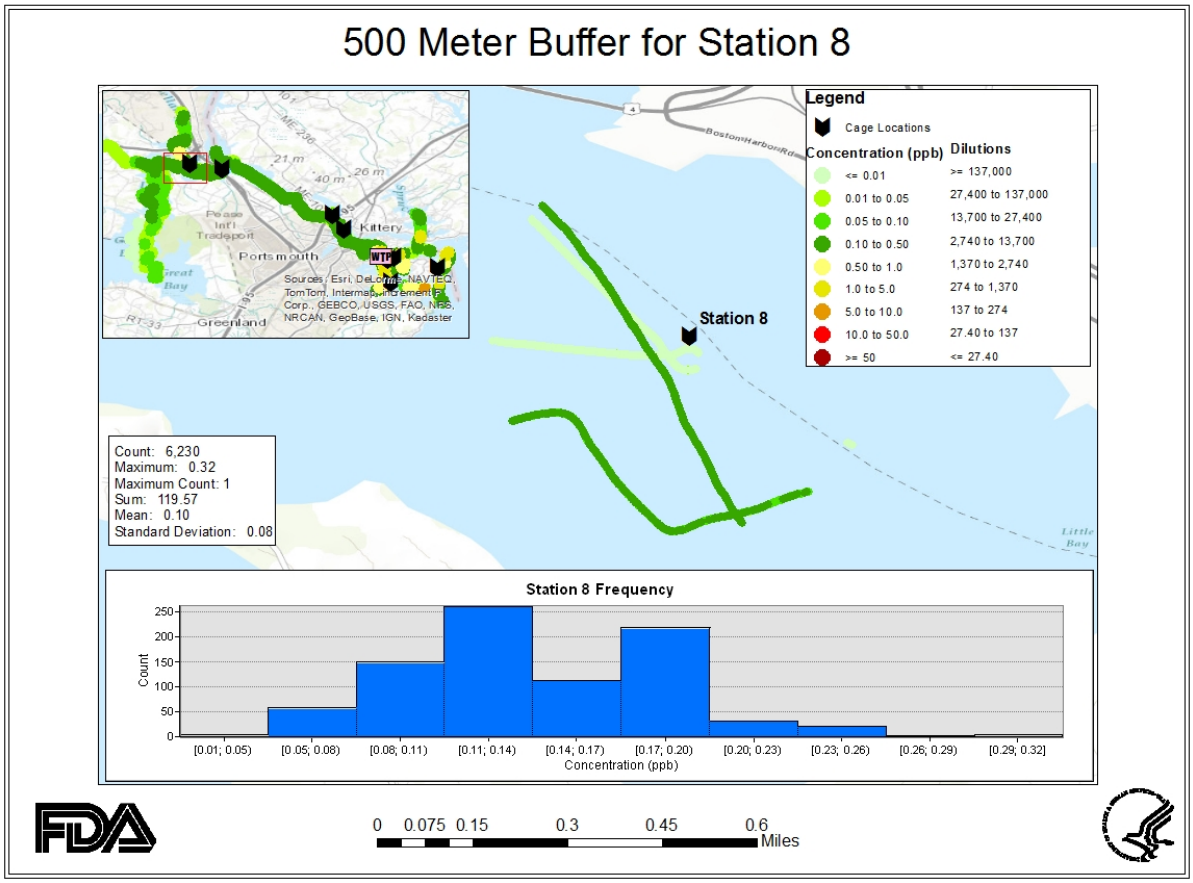


Fig. 16

500 meter buffer zone of plume tracking results around station 8

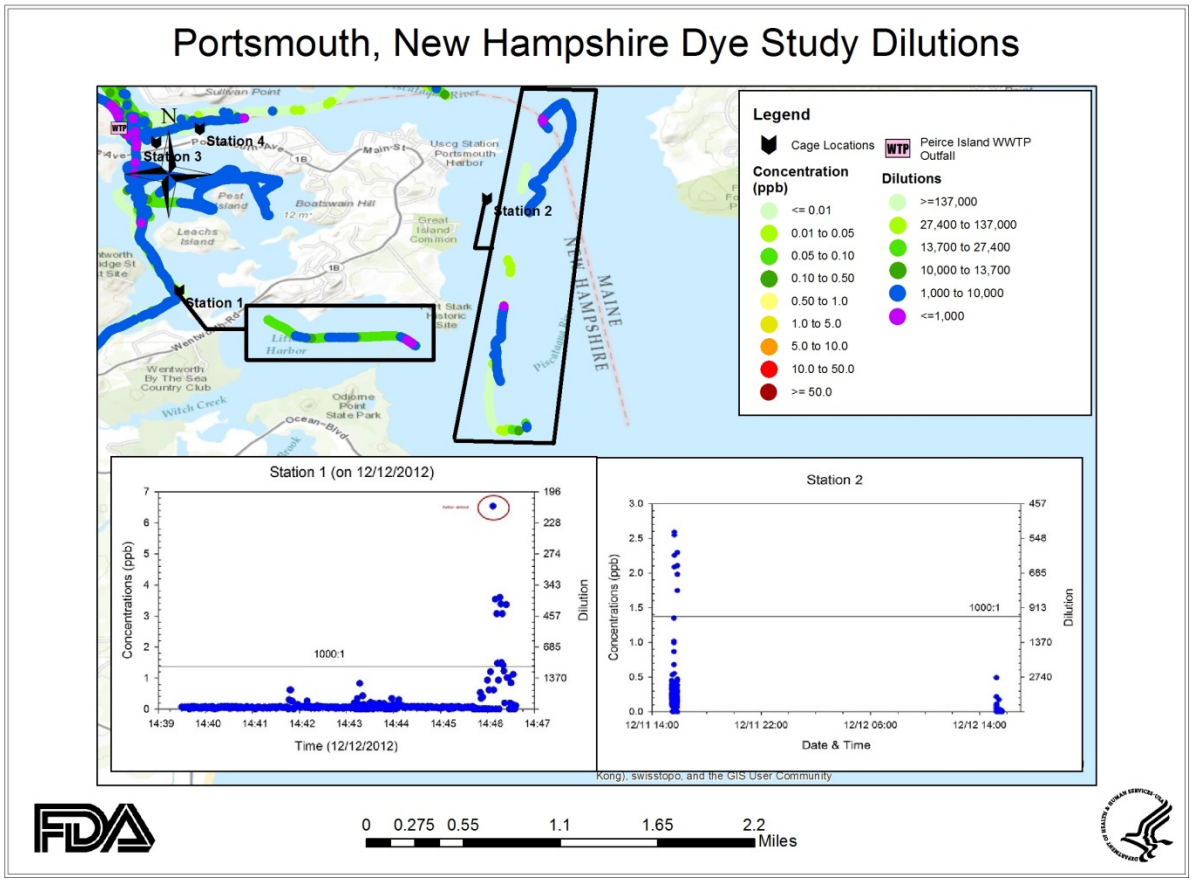


Fig. 17

Plume tracking outlier analysis at stations 1 and 2

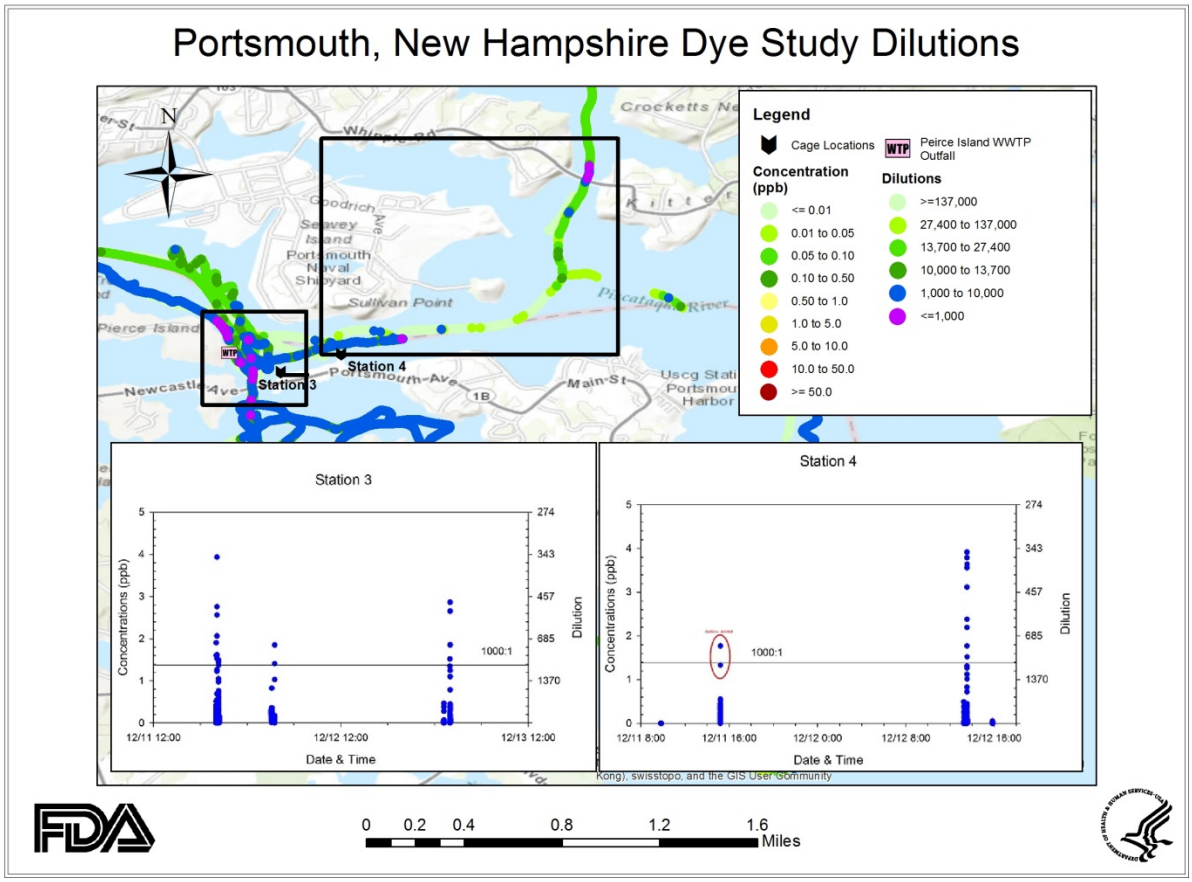


Fig. 18

Plume tracking outlier analysis at stations 3 and 4

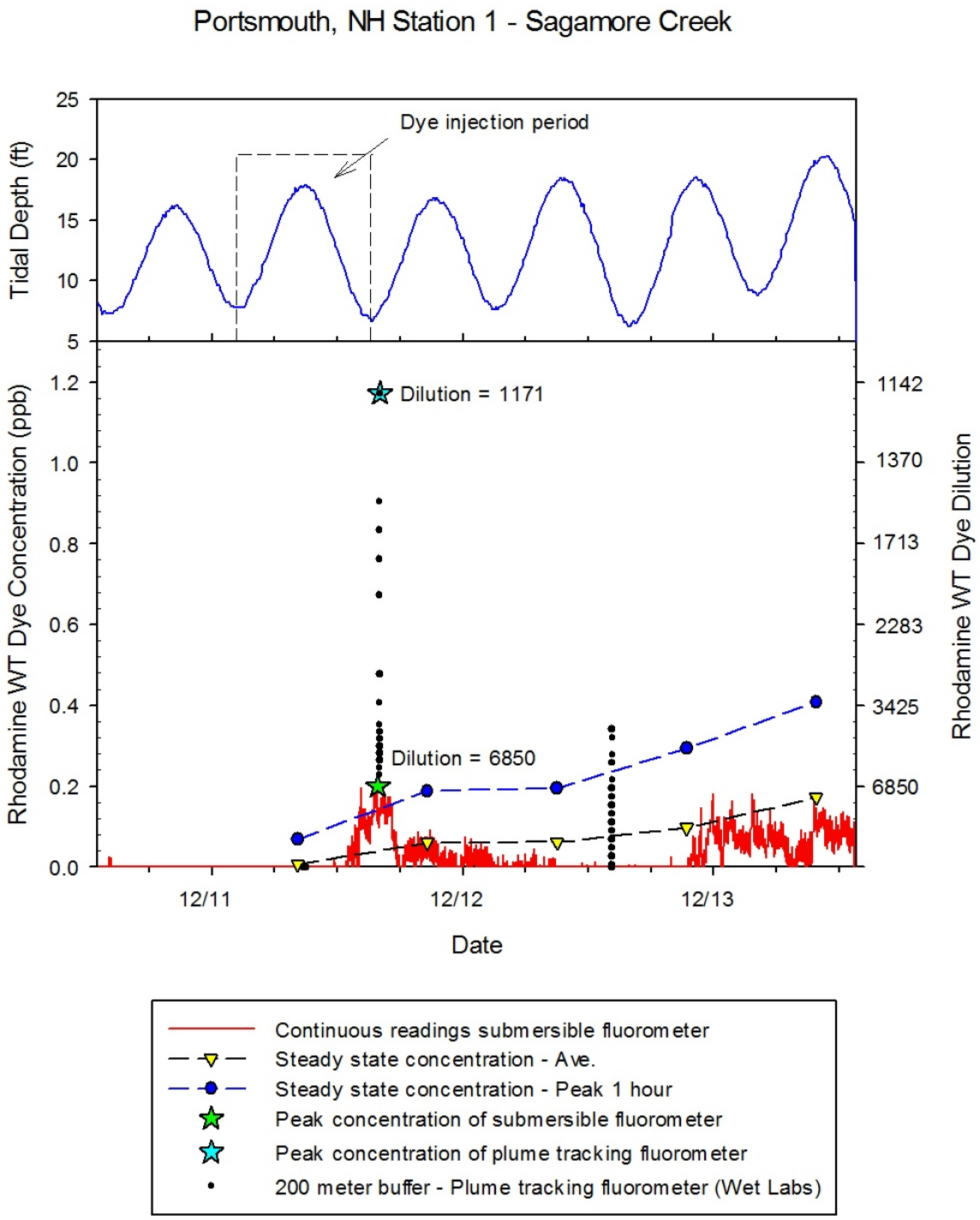
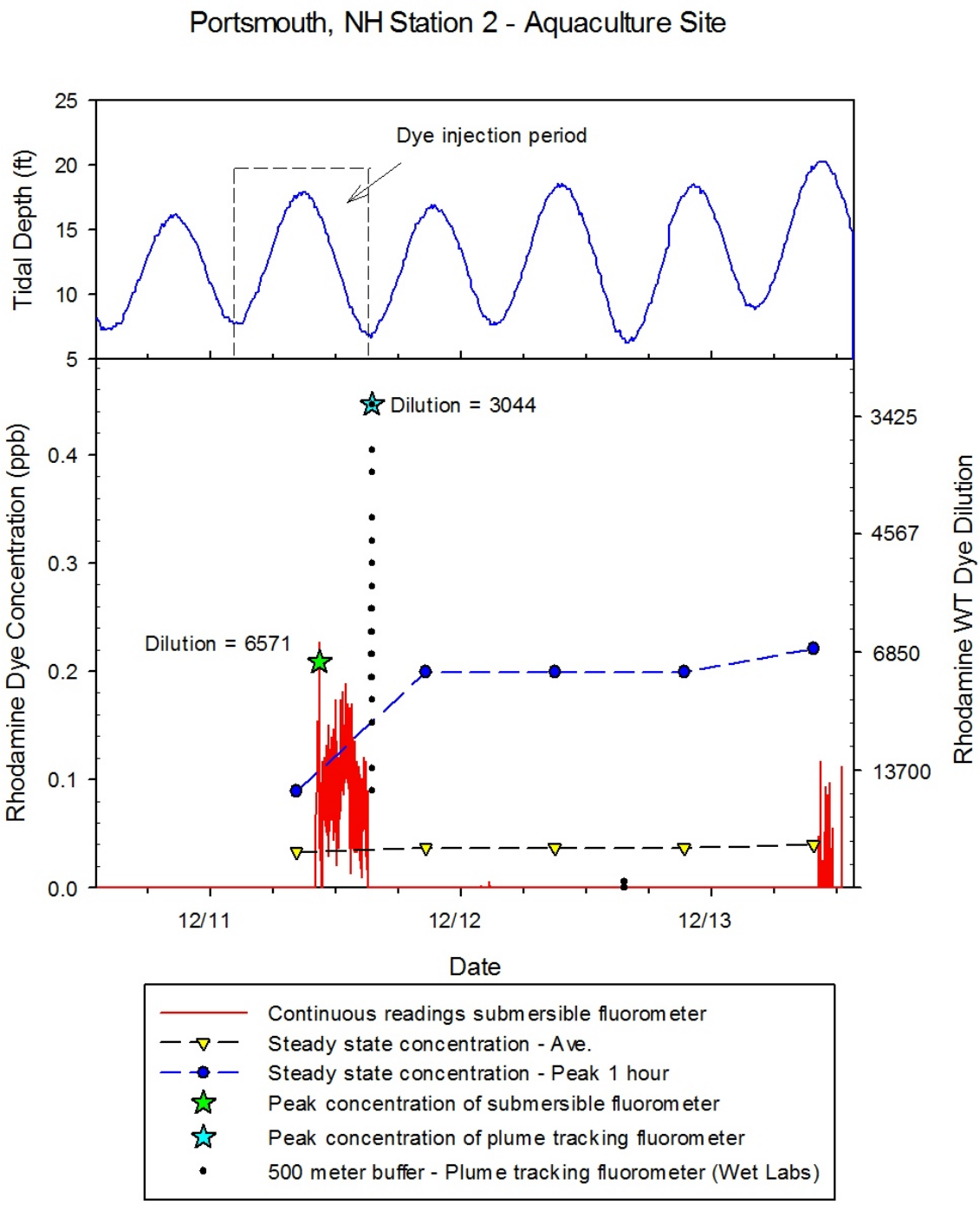


Fig. 19

Steady state super-position Rhodamine WT dye concentrations and plume tracking dye concentrations at station 1



Note: CTD data at station 2 is not correct. So use the cloest station - station 1's depth data for station 2.

Fig. 20

Steady state super-position Rhodamine WT dye concentrations and plume tracking dye concentrations at station 2

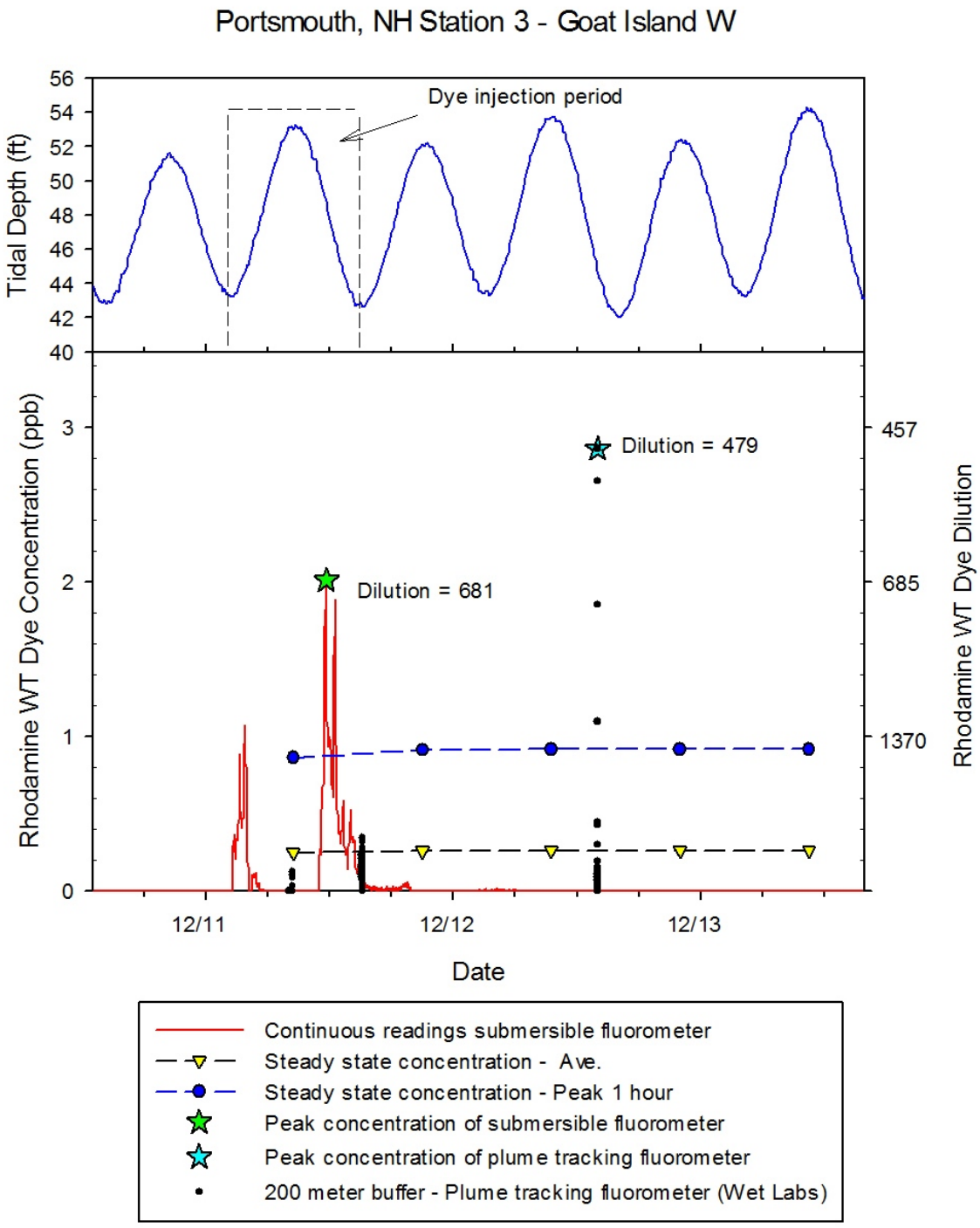
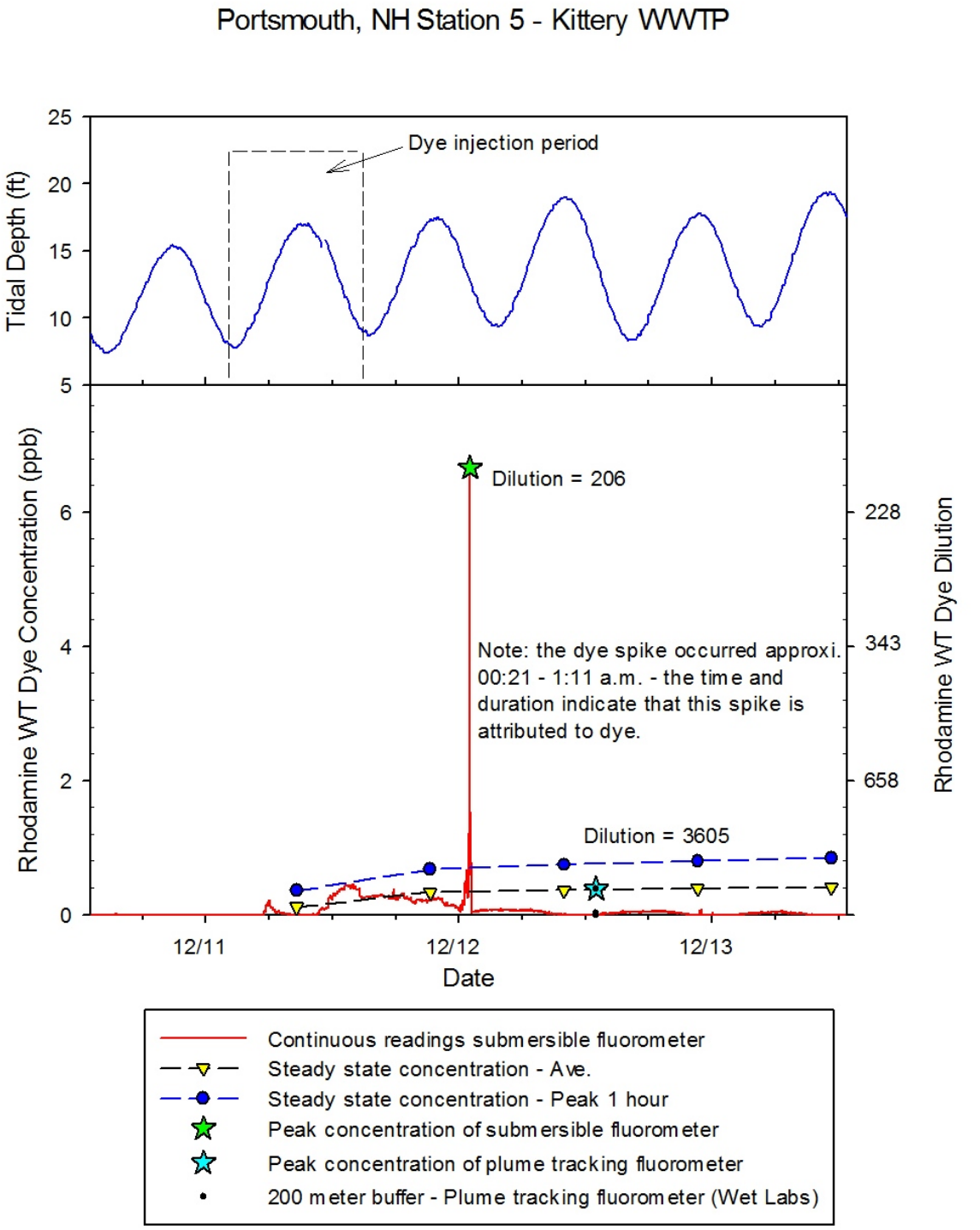


Fig. 21

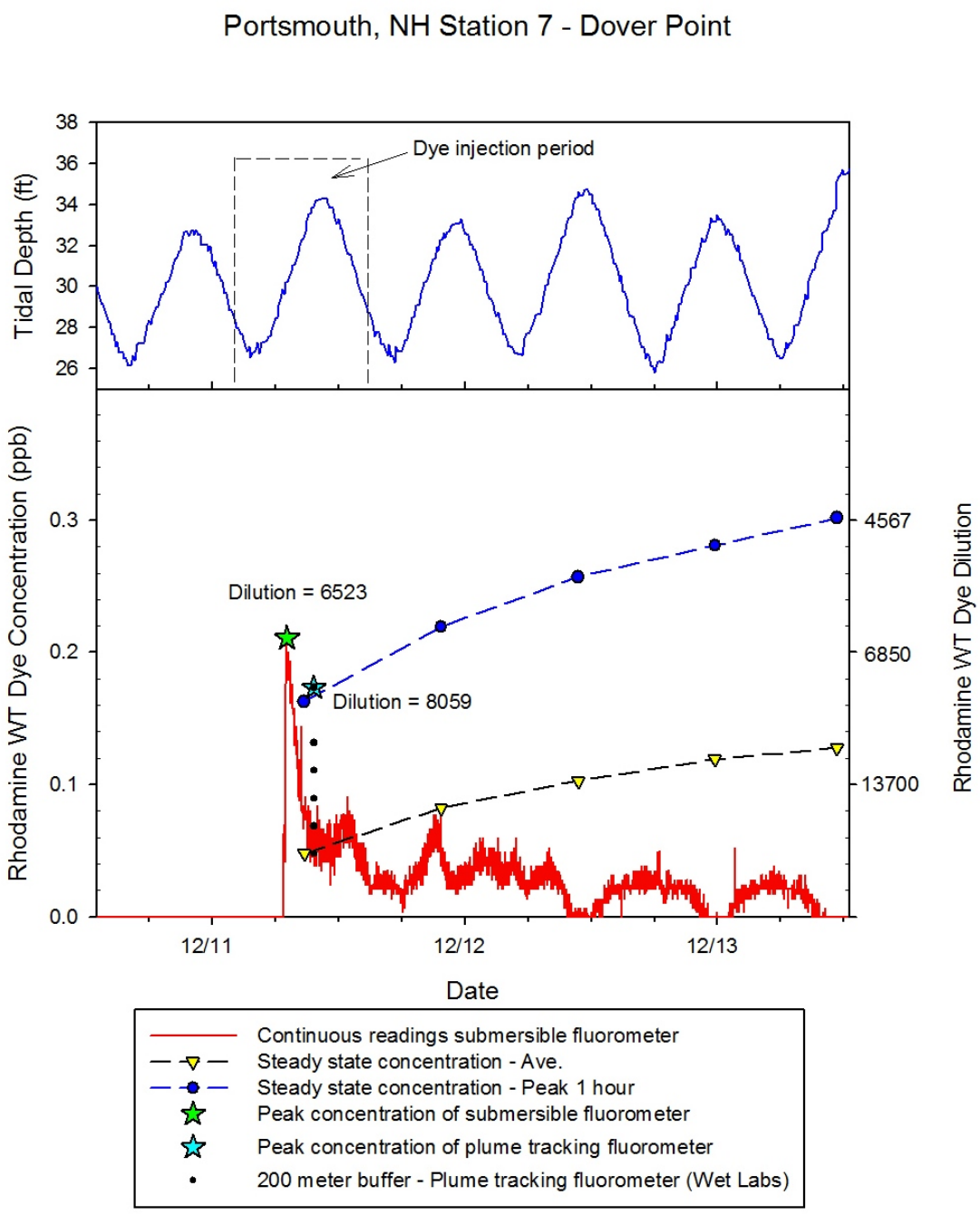
Steady state super-position Rhodamine WT dye concentrations and plume tracking dye concentrations at station 3



Note: CTD data at station 5 is not correct. Most likely it was outof water. So use the closest station - station 6's depth data for station 5.

Fig. 22

Steady state super-position Rhodamine WT dye concentrations and plume tracking dye concentrations at station 5



Note: CTD data at station 7 is not correct. Most likely it was outof water. So use the closest station - station 8's depth data for station 7.

Fig. 23

Steady state super-position Rhodamine WT dye concentrations and plume tracking dye concentrations at station 7

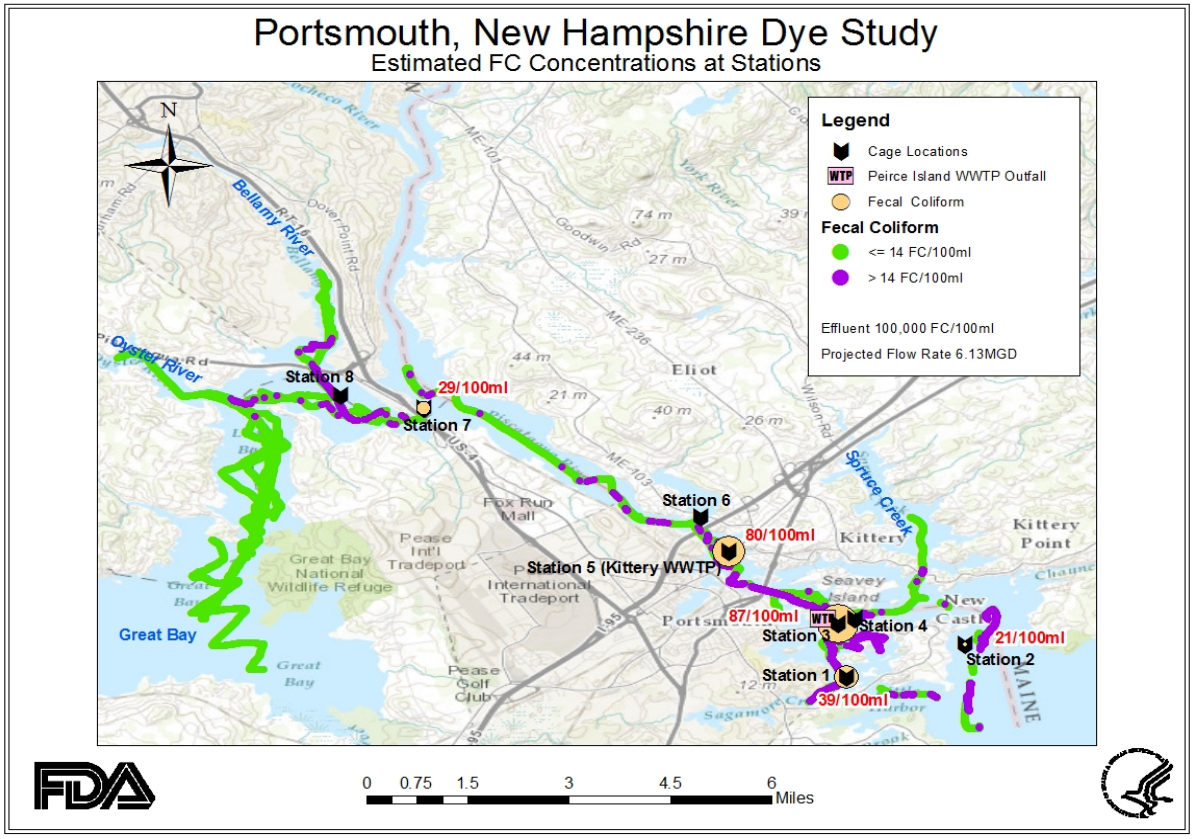


Fig. 24

Fecal coliform projection based on wastewater effluent of 100,000 FC/100 ml under 6.13 MGD flow rate

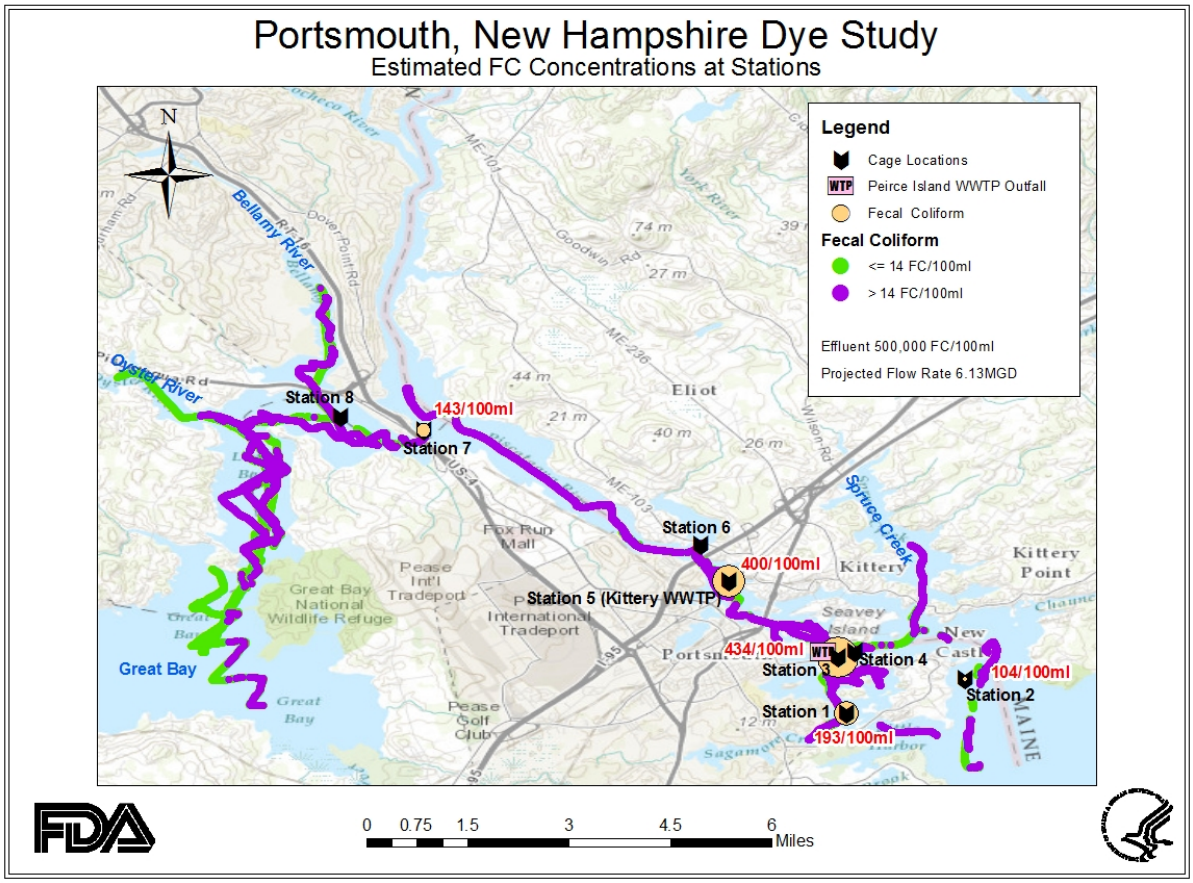


Fig. 25

Fecal coliform projection based on wastewater effluent of 500,000 FC/100 ml under 6.13 MGD flow rate

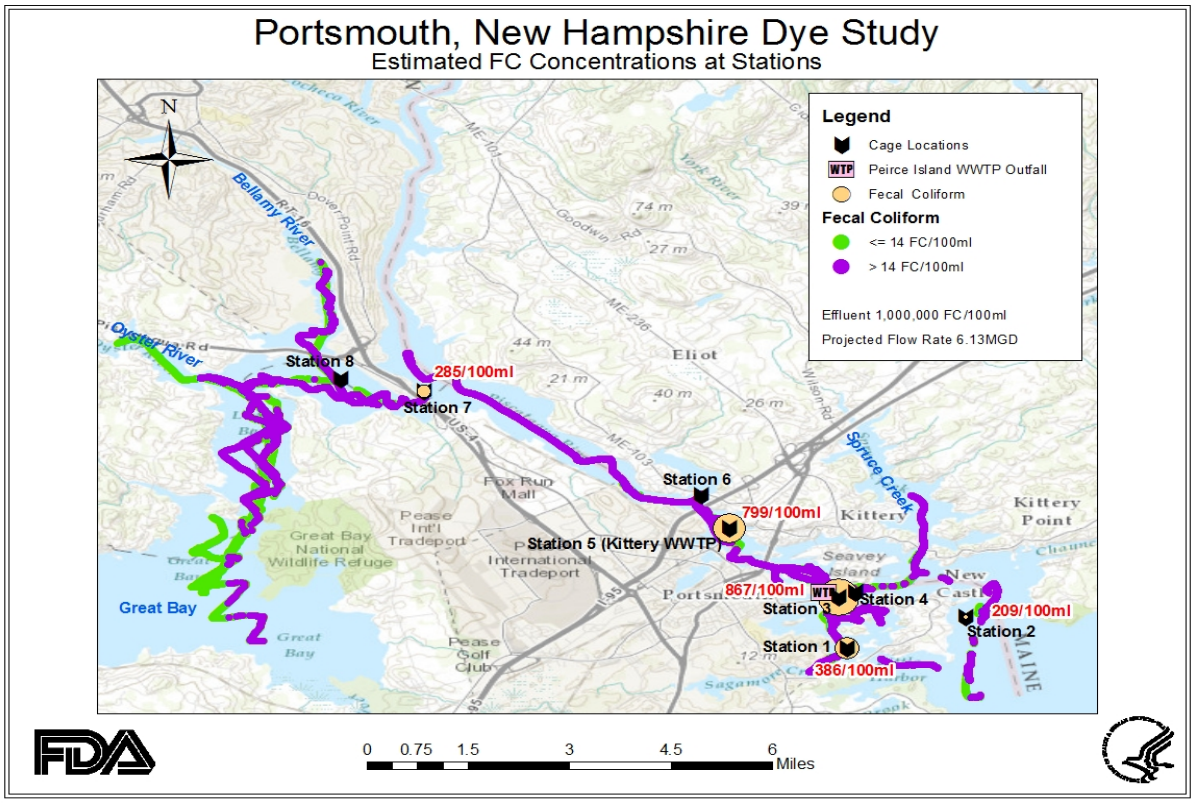


Fig. 26

Fecal coliform projection based on wastewater effluent of 1,000,000 FC/100 ml under 6.13 MGD flow rate

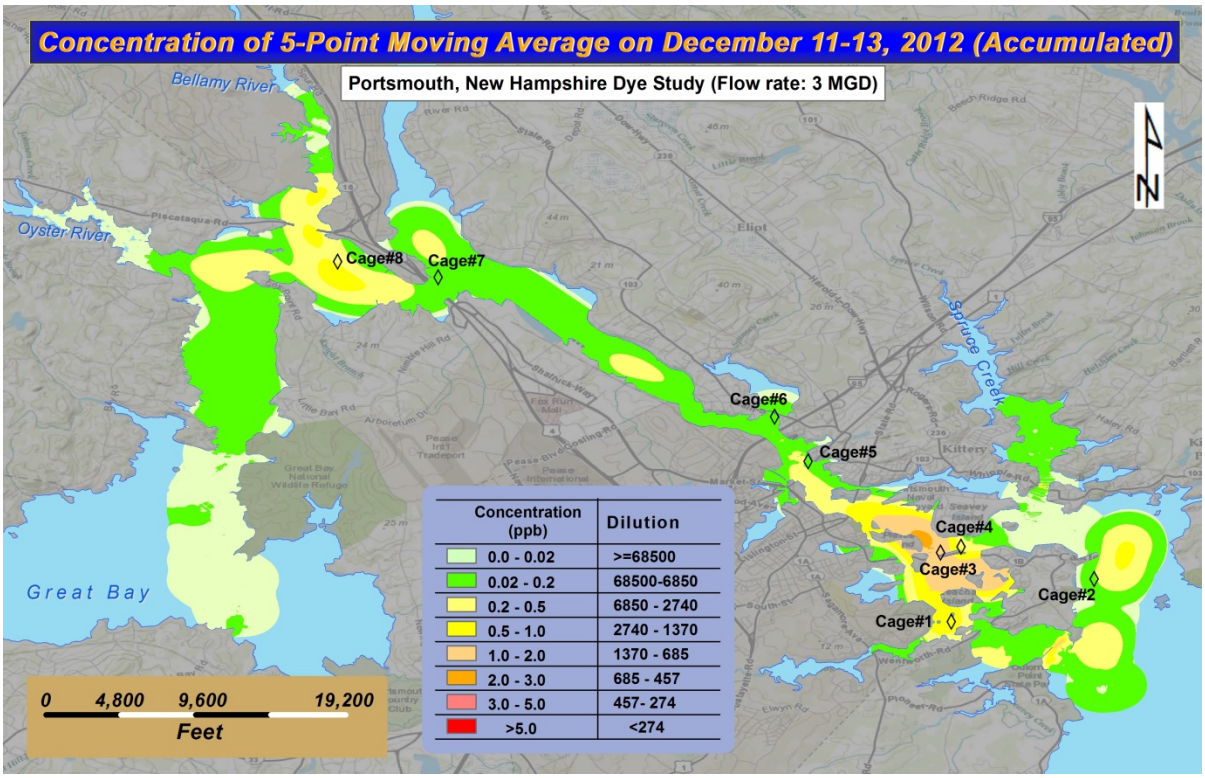


Fig. 27

Dye concentrations and dilutions for projected 3.0 MGD wastewater flow rate

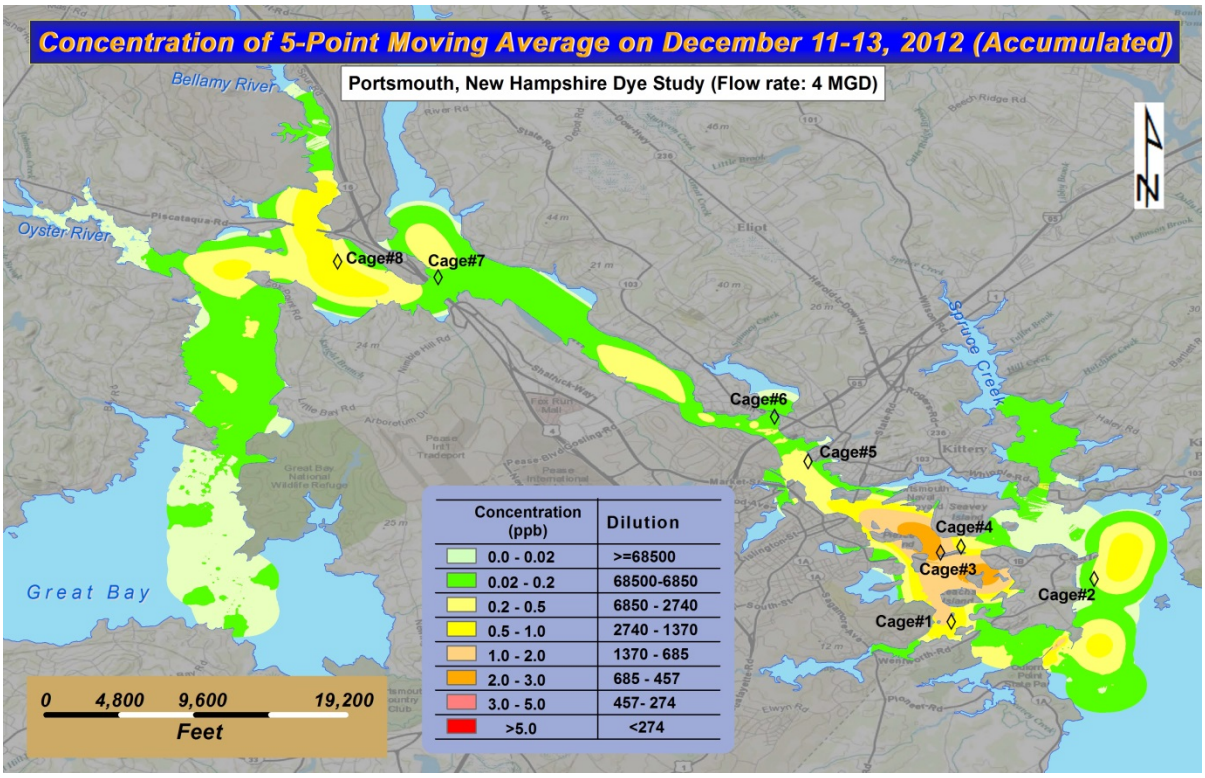


Fig. 28

Dye concentrations and dilutions for projected 4.0 MGD wastewater flow rate

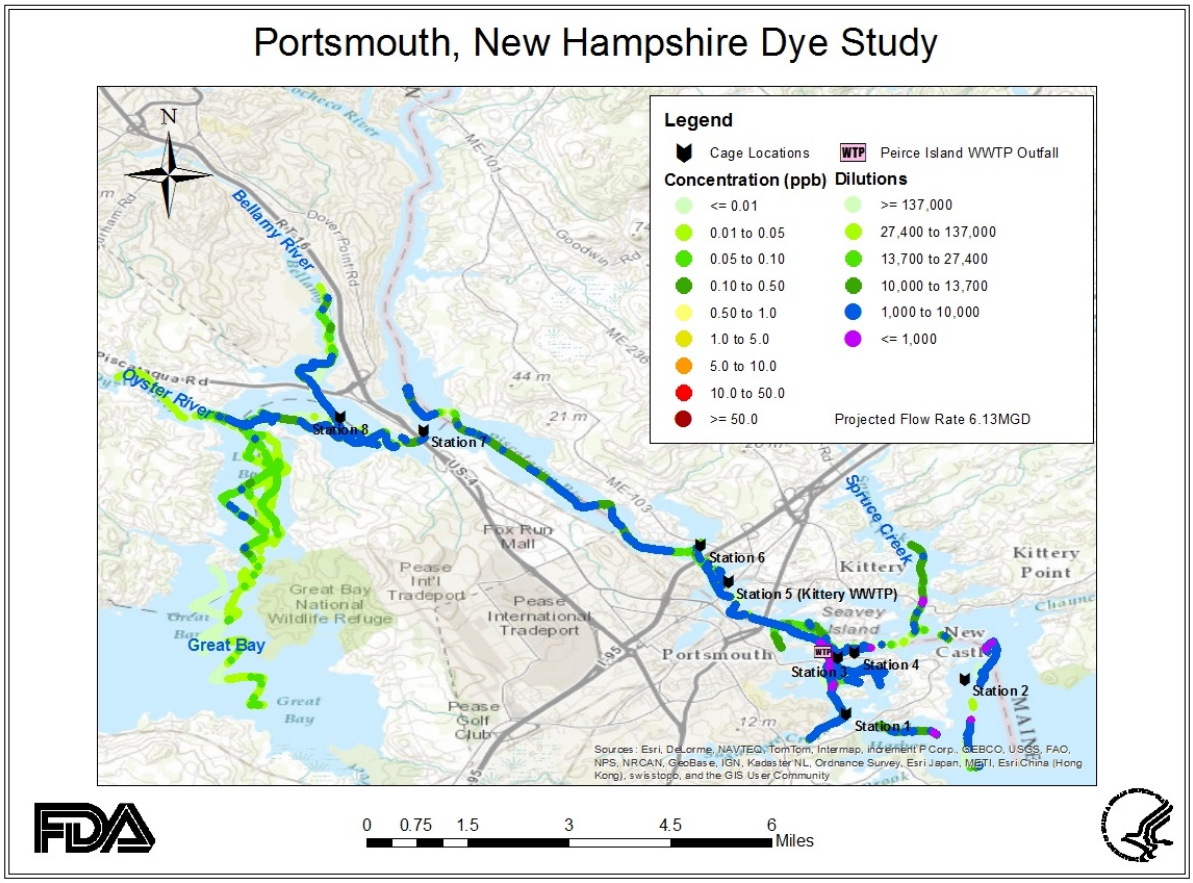


Fig. 29

Dye concentration and dilutions for projected 6.13 MGD wastewater flow rate

APPENDIX II

Table 1

Steady-state dilution analysis based on submersible (station) fluorometer data during study period (2012)

Station numbers	Dilution - SP peak 1 hour	Dilution - SP Ave.	Dilution - Peak conc.	Location
1	3355	7960	6849	Sagamore Creek
2	6201	34210	6571	Aquaculture Site
3	1494	5263	681	Goat Island west (near outfall)
5	1622	3380	206	Kittery WWTP
7	4542	10717	6523	Dover Point

Table 2

Comparison of dilution analysis based on surface tracking and submersible data during study period (2012)

Station numbers	Surface plume tracking	Submersible stations		Location
	Dilution – peak conc. (500 meter buffer)	Dilution - SP peak 1 hour	Dilution - peak conc.	
1	1171	3355	6849	Sagamore Creek
2	3044	6201	6571	Aquaculture Site
3	349	1494	681	Goat Island west (near outfall)
4	774	N/A	N/A	Goat Island east (near outfall)
5	3341	1622	206	Kittery WWTP
6	3341	N/A	N/A	Spinney Creek
7	8059	4542	6523	Dover Point
8	4281	N/A	N/A	Little Bay

Table 3

Peirce Island WWTP sewage sample data

Sewage samples					
Sample #	**Sample description**	**Date collected**	**Time**	**FC cfu/100ml**	**MSC cfu/100ml**
12	Influent	12/07/2013	9:57	< 49999	1260000
22	Influent	12/10/2013	11:10	540000	604000
23	Influent	12/10/2013	12:00	92500000	120000
30	Influent 6h comp	12/11/2013	6:00	8000000	1586000
31	Influent 6h comp	12/11/2013	0:00	4000000	988000
60	Influent 6h comp	12/12/2013	0:00	2650000	1190000
61	Influent 6h comp	12/12/2013	6:00	3250000	1354000
			Geometric Mean	*4895561*	*810912*
			Log	*6.69*	*5.91*
1	Final Effluent	12/07/2013	10:07	54	28400
18	Final Effluent	12/10/2013	11:00	<0.9	53200
19	Final Effluent	12/10/2013	12:10	<0.9	38200
24	Final Effluent 2h comp	12/10/2013	14:15	<0.9	127000
25	Final Effluent 2h comp	12/11/2013	4:15	<0.9	119800
26	Final Effluent 2h comp	12/11/2013	6:15	1	96600
27	Final Effluent 2h comp	12/11/2013	8:15	1	152600
28	Final Effluent 2h comp	12/11/2013	10:15	1	123800
29	Final Effluent 2h comp	12/11/2013	12:15	8	105800
32	Final Effluent 1h grab	12/11/2013	2:15	<0.9	128800
33	Final Effluent 1h grab	12/11/2013	3:15	<0.9	131200
34	Final Effluent 1h grab	12/11/2013	4:15	<0.9	136200
35	Final Effluent 1h grab	12/11/2013	5:15	<0.9	156200
36	Final Effluent 1h grab	12/11/2013	6:15	<0.9	Lab error
37	Final Effluent 1h grab	12/11/2013	7:15	<0.9	Lab error
38	Final Effluent 1h grab	12/11/2013	8:15	<0.9	237000
39	Final Effluent 1h grab	12/11/2013	9:15	<0.9	283800
40	Final Effluent 1h grab	12/11/2013	10:15	<0.9	205600
41	Final Effluent 1h grab	12/11/2013	11:15	<0.9	233600
42	Final Effluent 1h grab	12/11/2013	12:15	3	245200
43	Final Effluent 1h grab	12/11/2013	13:15	<0.9	230600
			Geometric Mean	*3*	*128150*
			Log	*0.52*	*5.11*
			Log Reduction	***6.17***	***0.80***

Table 4

Enteric virus levels in shellfish during study period (2012)

Sample Description	Sample Type	Collection date	Norovirus*				Adenovirus*		Extraction Efficiency**
			GI MPN	GI SC	GII MPN	GII SC	MPN	SC	
Cage 1	Oyster	12/17/2012	<1.5 CI(0.21-11)	<1	<1.5 CI(0.21-11)	<1	3.2 CI(0.46-23)	25.9	Not Done
Cage 2	Oyster	12/17/2012	<1.5 CI(0.21-11)	<1	<1.5 CI(0.21-11)	<1	6.9 CI(1.8-27)	13.6	Not Done
Cage 3	Oyster	12/17/2012	<1.5 CI(0.21-11)	<1	<1.5 CI(0.21-11)	<1	6.9 CI(1.8-27)	10.4	72%
Cage 4	Cage was lost. No samples were retrieved.								
Cage 5	Oyster	12/17/2012	<1.5 CI(0.21-11)	<1	<1.5 CI(0.21-11)	<1	6.9 CI(1.8-27)	29.1	Not Done
Cage 6	Oyster	12/17/2012	<1.5 CI(0.21-11)	<1	<1.5 CI(0.21-11)	<1	6.3 CI(1.5-26)	94.1	56%
Cage 7	Oyster	12/17/2012	<1.5 CI(0.21-11)	<1	<1.5 CI(0.21-11)	<1	6.9 CI(1.8-27)	11.4	Not Done
Cage 8	Oyster	12/17/2012	<1.5 CI(0.21-11)	<1	<1.5 CI(0.21-11)	<1	3.2 CI(0.46-23)	1.9	Not Done
Negative Controls	Oyster	N/A	<1.5 CI(0.21-11)	<1	<1.5 CI(0.21-11)	<1	<3.1 CI(0.42-23)	<1	63%

Note: * All results are reported in RT-qPCR Units/ 100 grams of digestive diverticulum

** Extraction Efficiency calculation: Log (Retrieved) / Log (Spike) x 100

Table 5

Male-Specific Coliphage, Fecal Coliform, *E coli* levels in shellfish (2013)

Sample ID	Cage	Latitude (N)	Longitude (W)	Sample Description	Sample	Sample Date	FC/100g	EC/100g	MSC/100g
ACAQ2		43.072000	-70.711000	ACAQ2 - UNH Pier	Mussels	6/25/2013	--	--	150
ACAQ1	Cage 2	43.067210	-70.709500	Off shore Mussel	Mussels	6/25/2013	--	--	10.6
ACAQ2		43.072000	-70.711000	ACAQ2 - UNH Pier	Mussels	8/26/2013	240	--	53
ACAQ1	Cage 2	43.067210	-70.709500	Off shore Mussel	Mussels	8/26/2013	6	--	10
LHOP2		43.051500	-70.727500	LHOP2 - Little Harbor	Mussels	8/26/2013	41	--	11
LHOP2		43.051500	-70.727500	LHOP2 - Little Harbor	Mussels	11/17/2013	40	<18	127.8
ACAQ1	Cage 2	43.067210	-70.709500	Site 2 Aquaculture Site	Mussels	11/17/2013	20	20	95.1
ACAQ2		43.072000	-70.711000	UNH Pier	Mussels	11/17/2013	40	<18	57.6
ACAQ1	Cage 2	43.067210	-70.709500	Site 2 Mussels	Mussels	12/10/13	<18	<18	717.0
ACAQ1	Cage 2	43.067210	-70.709500	Site 2 Oysters	Oysters	12/10/13	<18	<18	11.1
LPRGIW	Cage 3	43.071850	-70.736630	Site 3 Mussels	Mussels	12/10/13	<18	<18	6154.1
LPRGIW	Cage 3	43.071850	-70.736630	Site 3 Oysters	Oysters	12/10/13	20.0	20.0	198.5
UPRHP1	Cage 7	43.124900	-70.825440	Site 7 Mussels	Mussels	12/10/13	40.0	20.0	3515.0
UPRHP1	Cage 7	43.124900	-70.825440	Site 7 Oysters	Oysters	12/10/13	<18	<18	840.9
LBDP1A	Cage 8	43.123240	-70.843220	Site 8 Mussels	Mussels	12/10/13	<18	<18	1212.0
LBDP1A	Cage 8	43.123240	-70.843220	Site 8 Oysters	Oysters	12/10/13	<18	<18	412.1