North Carolina Beach Monitoring Program Quality Assurance Project Plan



June 16, 2003 Revised April, 2024

North Carolina Department of Environmental Quality Division of Marine Fisheries

Shellfish Sanitation & Recreational Water Quality Section

Mission

To protect the public health by monitoring the quality of North Carolina's coastal recreational waters and notifying the public when bacteriological standards for safe bodily contact are exceeded.

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SECTION A: PROJECT MANAGEMENT

A3 - Distribution List

Joel Hansel, EPA Kathy B. Rawls, Director, DMF Patricia Smith, PIO, DMF NC Shellfish Sanitation & Recreational Water Quality (Wilmington) NC Shellfish Sanitation & Recreational Water Quality (Manteo) Cathy Akroyd, PIO, NC Division of Water Resources Rachel T. Noble, Ph. D. Institute of Marine Sciences, UNC Zack Moore, Division of Epidemiology Coastal County Health Directors

A4 - Project / Task Organization

Shannon Jenkins, Section Chief, Shellfish Sanitation and Recreational Water Quality Section Morehead City Office

Andy Haines, Assistant Section Chief, Shellfish Sanitation and Recreational Water Quality Section Morehead City Office

Erin Bryan-Millush, Recreational Water Quality Program Manager, Public Notification, Laboratory Quality Assurance Officer

Patricia Smith, Public Information Officer, DMF (Morehead City) Media Notification

The following staff members have responsibilities in both Shellfish and Recreational Programs:

Morehead City Office

Melissa Squibb, Laboratory Manager Michele Barger, RWQ Data Management and Lab Methods Zach Fasking, Sample Collection Phil Piner, Sample Collection Timmy Moore, Sample Collection Jason Hill, Sample Collection, Purchasing & Inventory

Wilmington Office

Holly White, Laboratory Manager Matthew Stokely, Sample Collection Lucas Edmondson, Sample Collection Kelly Brannigan, Shoreline Survey Phil D'Angelis, Shoreline Survey

<u>Manteo</u>

Candace Burrus, Laboratory Manager James Pace, Sample Collection Ann Daisey, Shoreline Survey

A5 - Problem Definition / Background

Coastal North Carolina has an abundance of surface water resources: 320 miles of Atlantic Ocean shoreline, 4,000 miles of estuarine shoreline and 2 million acres of shellfish growing waters. The beaches, coastal rivers and sounds play a large part in North Carolina's prosperous tourism industry, attracting more than 15 million vacationers each year. The resident population is growing rapidly, as more people find the North Carolina coast a desirable place to live and retire. As the population continues to grow, water quality is expected to decline as a result of increased run-off from land-disturbing activities.

In the mid-1990s, *pfiesteria* emerged in the national media as a possible public health threat, and North Carolina's Neuse River was in the spotlight for harboring the dinoflagellate organism. At that time, *pfiesteria* was found only in the brackish waters of the Neuse River, but the negative publicity carried the underlying possibility that all of North Carolina's coastal waters were unsafe for the consumption of seafood or for swimming. Water quality issues were rekindled in 1996 when a report from the Natural Resources Defense Council (NRDC) labeled North Carolina as a Beach Bum state for its lack of beach monitoring and public notification programs. When the legislature assembled the next year, it took very little lobbying to convince lawmakers to fund a recreational water quality program for coastal waters. In June 1997 the North Carolina Shellfish Sanitation Section was charged with monitoring coastal waters for two years as a pilot project. Approximately 300 sites were monitored weekly during the swimming season using fecal coliform as the indicator organism. As a result of the two years of monitoring, the reputation of North Carolina s coastal resources was being restored. In 1999 the program was funded permanently.

This document will report new program requirements and standards necessary for compliance with the Environmental Protection Agency s (EPA) guidance and will be a guide or protocol for operating the program. One aspect of the program that will not change is the

protocol for posting swimming advisories and methods for notifying the public. Advisories will still be based on the exceedance of the bacteriological standard; however, the standard will be enterococci instead of fecal coliform. The posting of advisories for single-sample maximum exceedances is another significant change to the program.

A6 - Project/Task Description and Schedule

The coastal counties are divided into three regions: Northern, Central and Southern (see Figures 1, 2, 3). The three regions combined have approximately 221 sites that are monitored either weekly or twice monthly during the swimming season. Monitoring sites that are adjacent to resort areas and public accesses, that are used daily, are Tier I beaches and are sampled weekly. Monitoring sites located along the ICW, tidal creeks & summer camps, that are not used daily, are Tier 2 beaches and are sampled twice monthly.

The Shellfish Sanitation and Recreational Water Quality Section (SSRWQ) has three State and Food and Drug Administration (FDA)-accredited laboratories in Morehead City, Wilmington and Manteo. Sample collection, laboratory analysis, and beach monitoring activities are conducted entirely by the SSRWQ staff; however, the public notification process is completed in conjunction with the Department of Environmental Quality and the Division of Marine Fisheries public information officers.

The State will continue to fund the Tier II monitoring sites and Tier 1 monitoring sites along federally protected reserves, approximately 50% of all the swimming areas, while the EPA grant will be used to fund the monitoring of the remaining Tier I beaches. Appendices 1, 2 and 3 list the monitoring sites that are supported by the state of North Carolina and the EPA grant. Appendix 4 contains the coordinates for each monitoring site or swimming area.

The EPA grant is also used to fund public education and outreach. Appendix 8 contains educational materials used at the public meetings; they are required by the EPA to be grant-eligible.



NC Recreation Water Quality Program Northern Region



Tier Identification and Regional Locations

Monitoring Stations

- 1 Daily Use
- 2 Non-daily Use

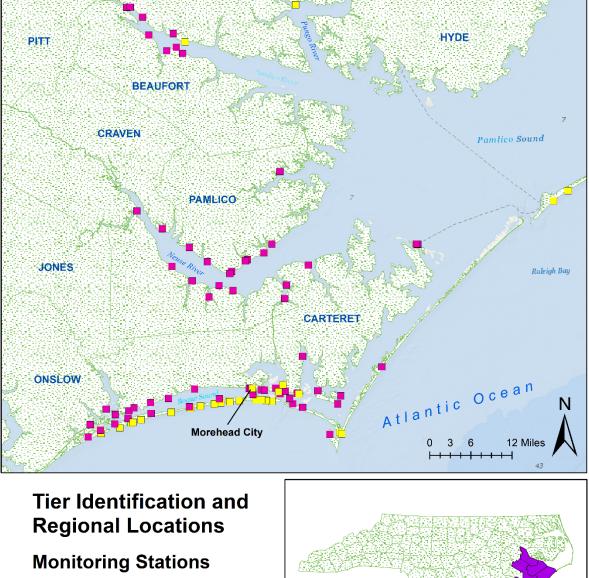




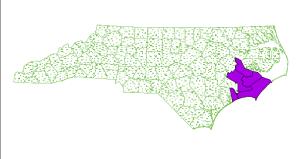
Shellfish Sanitation and Recreational Water Quality June 2021 Not to be used for navigational purposes.



NC Recreation Water Quality Program Central Region



- 1 Daily Use
- 2 Non-daily Use

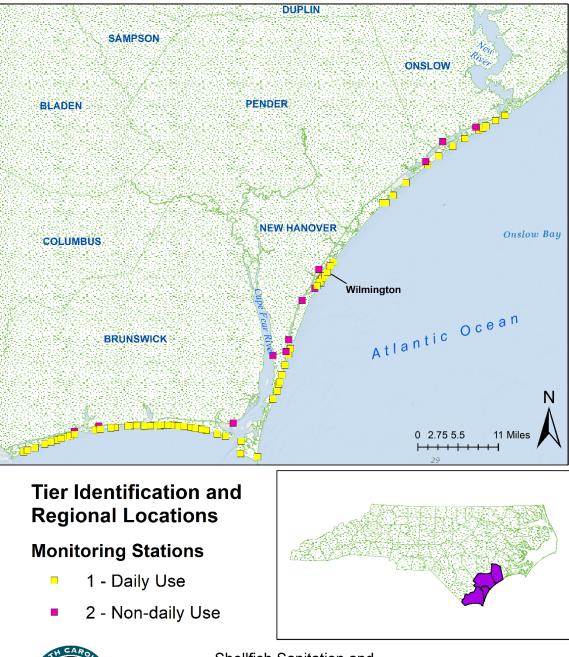




Shellfish Sanitation and **Recreational Water Quality** June 2021 Not to be used for navigational purposes.



NC Recreation Water Quality Program Southern Region





Shellfish Sanitation and Recreational Water Quality June 2021 Not to be used for navigational purposes.

A7 – Quality Objectives and Criteria

Primary Objective:

To protect the public health by monitoring the quality of North Carolina's coastal recreational waters and notifying the public when bacteriological standards for safe bodily contact are exceeded.

Quality Objectives:

- To identify swimming areas/beaches and classify them based on human recreational usage.
- To identify monitoring sites that exceed the enterococci geometric mean and singlesample maximum criteria using the Enterolert MPN method and EPA method 1600 forenumeration.
- To evaluate the public health significance of approximately twenty (20) ocean storm drains.
- To document trends in coastal bacteriological water quality.

Measurement Performance Criteria:

Swimming advisory signs are posted and press releases issued for Tier I swimming areas/beaches when a minimum of five (5) samples are collected, equally spaced over 30 days, has a geometric mean of **35 or greater enterococci per 100 ml** or, when both the initial water sample and resample collected exceeds **104 enterococci per 100 ml**. The public is notified via social media release, without an advisory sign when a single sample exceeds **104 enterococci per 100 ml**. The public **is notified via per 100 ml**. for a Tier I site. This is called a pending swimming advisory. A second sample will be collected immediately. If the second sample exceeds **104 enterococci per 100 ml**, the pending swimming advisory is converted into an advisory and the public will be notified by press release, and a sign will be posted.

Rescinding Tier I Advisories

Once the geometric mean exceeds the standard, the swimming advisory is not lifted until two consecutive weekly samples meet the EPA standard of **35 enterococci per 100 ml**. For a

geometric mean advisory to be rescinded, the site must have two consecutive good samples, with good being defined as 35 enterococci per 100 ml or fewer. However, if a situation occurs where two consecutive good samples are recorded, but the geometric mean still exceeds, the advisory will remain in place until the geometric mean falls below 35 enterococci per 100 ml and when two consecutive good samples are recorded concurrently within the same two week period.

Beaches that violate the single-sample maximum criteria for two consecutive tests are re-sampled at the time the sign is posted or the following week, dependent upon possible pollution source observation and at the program mangers discretion. If the re- sample is satisfactory, the advisory may be lifted. If the re-sample is unsatisfactory and the geometric mean is not exceeded, the sign remains posted. If the re-sampling causes the exceedance of the geometric mean, then the geometric mean criteria apply.

The timeframe for posting swimming advisory signs at Tier I beaches, based on the enterococci geometric mean, runs from the beginning of May through the end of September. Weekly sampling of Tier I beaches begins in April of each year so that a running geometric mean is established by May. April and October are considered the shoulder seasons. Seasonal low temperatures during April and October may prevent people from swimming. The project manager determines when advisory signs and public notification are initiated during the shoulder seasons.

Tier II beaches/swimming areas are sampled twice monthly from April to October, with the advisories based entirely on the single sample maximum criteria for two consecutive tests.

For **Tier II sites**, public notification and a swimming advisory sign are posted when both the initial water sample and resample collected exceeds **104 enterococci per 100 ml**. **Tier II** Beaches that violate the criteria are re-sampled at the time of the public notification and sign posting. If this sample exceeds **104 enterococci per 100 ml**, the site is not sampled until

the following week. Weekly sampling of the site continues until the enterococci counts are **104** enterococci per **100 ml or fewer**.

A pending swimming advisory, that is public notification via social media release without a sign, takes place when a single sample exceeds **104 enterococci per 100 ml**. If a second sample exceeds **104 enterococci per 100 ml**, the pending swimming advisory, is converted to an advisory and the public is notified, and an advisory sign is posted. Weekly sampling of the site continues until the enterococci counts are **104 enterococci per 100 ml or fewer.**

Other swimming advisories will be posted as precautionary measures when the following activities occur:

- Pumping of floodwaters between the primary dune and the ocean beaches.
- Disposal of dredge material from closed shellfishing waters on ocean beaches.
- Storm drains with discharges into ocean beaches.
- Storm drains that have flow reaching Tier I recreational waters only during wet weather events are posted with permanent advisory signs. The signs read as follows:

WARNING

STORM WATER DISCHARGE AREA

WATERS MAY BE CONTAMINATED BY DISCHARGE FROM PIPE. SWIMMING IS NOT RECOMMENDED WITHIN 200 YARDS OF THIS SIGN DURING ACTIVE DISCHARGE.

FOR MORE INFORMATION CALL 252 726 6827

OFFICE OF THE STATE HEALTH DIRECTOR

These pipes are also regular monitoring sites. When the bacteriological sampling results for these pipes exceed the standards during active or non-active discharge, bacteriological

advisory signs will be placed in addition to the permanent sign. The permanent warning signs only indicate a risk when storm drains are actively discharging. Adding the bacteriological advisory sign helps to notify the public that testing has indicated bacteria levels that exceed the standards for recreational use, a press release will be issued.

- Storm drains that have continuous active flow that reach ocean recreational waters are posted with permanent advisory signs. The signs read as follows:

WARNING

STORM WATER DISCHARGE AREA

SWIMMING WITHIN 200 YARDS OF THIS SIGN MAY INCREASE THE RISK OF WATERBORNE ILLNESS.

OFFICE OF THE STATE HEALTH DIRECTOR

Located within 200 yards of these pipes are also regular monitoring sites. When the bacteriological sampling results for these pipes exceed the standards, a bacteriological swimming advisory sign will be posted in addition to the permanent sign.

Swimming advisories are not posted from November through March; however, all monitoring sites are sampled once per month during the non-swimming season.

Public notification and risk communication plan:

The health director or the environmental health supervisor of the local health department is the first to be informed of a swimming advisory. Discussion with the health department determines who the next contact should be, such as a town or county manager. The media are not contacted concerning a swimming advisory until all local and state officials are aware of the situation. The North Carolina Division of Marine Fisheries (NCDMF) then sends out the press release to the Associated Press and local community newspapers at the same time the advisory sign is posted. The press release advisories are aired on TV, radio stations and web sites (see Appendix 5 for press release templates and links to web sites).

Rescinding an advisory follows the same procedures in reverse. The first communication

involves the local health department, then the sign is removed and another press release is issued, declaring the waters are within the swimming standard.

If an advisory sign is needed on the ocean beaches, the sign will be placed on a post or posts at the interface of the wet and dry sand area of the beach. For estuarine waters, the sign may be posted by boat just offshore of the beach or on the shoreline. There may be instances where permission will have to be obtained to post signs on private property, such as on bulkheads or at entrances to marinas. Local Environmental Health Specialists or other local officials may be present when the signs are erected (see Appendix 6 for sign descriptions).

A8 - Special Training Requirements/Certification

SSRWQ laboratory personnel have been instructed in specific health and safety needs as required for employment. The laboratory maintains the required U.S. FDA and State certifications.

Field personnel are trained in small boat handling and navigation in coastal waters. Users of GPS equipment must be trained and certified before collecting field data for boundaries and monitoring sites. Personnel are trained in sample collection, transporting samples, recording field data, keying data into the database and following QA/QC protocols.

A9 - Documentation and Records

SSRWQ performs all sample collections and carries responsibility for handling all data collected in the field (see Appendix 7 for an example of the field sampling sheet). The SSRWQ laboratory is responsible for recording the bacteriological data on the field sheet (see Appendix 7). After laboratory staff record the bacteriological data on the field sheet, this information is immediately scanned and sent to data management personnel in Morehead City to be entered into an Oracle (IBEAM) database. Data entry for all data collected is managed and maintained in Morehead City. In the Oracle (IBEAM) database, exceedance limits are set to highlight bacteriological values that exceed the state and federal recreational water quality standards, those values are reported to the project manager for issuing public notifications immediately. A

back up of this data is recorded in Excel and is maintained at the main office. The Oracle database has a data exchange node that meets data reporting requirements to the EPA.

Hard copies of the laboratory data, laboratory quality assurance forms, and field sampling data sheets are archived indefinitely at the Morehead City office. Electronic copies of the bacteriological data are backed up and stored on the server in Morehead City, the state's Western Data Center. Sanitary survey reports of recreational waters and any other reports or audits are kept on file in the same manner.

Documentation of public notification, original press releases, are filed for one year; however, a spread sheet documenting advisories and rescinds will be filed electronically indefinitely.

SECTION B: MEASUREMENT AND DATA ACQUISITION

B1 - Sampling Process Design

The method for monitoring the 221 sites throughout coastal North Carolina began by grouping the sites in each region to create sampling runs or routes that the staff would travel for a particular day of sample collection. The northern region has four sampling runs consisting of 58 monitoring sites, the central region has eight sampling runs consisting of 100 monitoring sites and the southern region has four sampling runs with 63 monitoring sites. It is common for a sampling run to have a combination of Tier I and II swimming areas. Approximately half of the sampling runs are accessed by boat and half are reached by car and then wading into water from the beach. Weather conditions and tides have a strong influence on choosing a sampling run; therefore, the person collecting the water samples must plan his/her day accordingly. The sampling runs are close enough to one of the three regional laboratories to have water samples in the testing media before 2:00 p.m. each day (see holding time for samples in laboratory QAP Appendix 9).

Tier I beaches that require a minimum of five (5) samples in 30 days are tested using the following schedule:

Northern Region

Sampling Run	Day
Nags Head to Buxton	Monday
Corolla to KDH	Monday
Buxton to Ocracoke	Tuesday
KDH to Nags Head	Tuesday

Central Region

Sampling Run	Day
Topsail Island	Monday
Bogue Banks	Monday
Ragged Point to Belhaven	Tuesday
Pamlico River	Tuesday
Core Sound (Boat)	Tuesday
Core Sound (Truck)	Wednesday
East-side Bogue Sound	Wednesday
Upper Neuse	Wednesday
Lower Neuse	Thursday
West -side Bogue Sound	Thursday

Southern Region

Sampling Run	Day
South Carolina to Holden Beach	Monday
Oak Island to Southport	Tuesday
New Hanover County	Wednesday
Masonboro Sound	Thursday

If the above schedule cannot be met for a particular week, field staff will be responsible for ensuring that five (5) samples in 30 days are collected. This could involve going back to the same monitoring site twice in one week. Tier II swimming areas are sampled primarily by boat twice per month and do not require five (5) samples in 30 days, allowing more flexibility in scheduling the sampling runs.

There are several parameters of interest that are measured at each monitoring site that influence the transport and survival of microorganisms. Parameters collected include 24 hour rainfall total, air and water temperature, water depth/sample depth, wind speed and direction, current direction, tidal stage, time of sample collection and salinity. Time of sample collection is

critical for determining holding times. The other parameters can help in determining point and/or non-point sources of pollution that could possibly be impacting the monitoring location. Rainfall data are collected from rain gages scattered throughout the watersheds. Tidal stage and wind speed is determined by personal observation and verified by NOAA weather service. The presence of waterfowl and wildlife in proximity of the monitoring site is also recorded on the field data sheet.

B2 - Sampling Methods

Water samples are collected in 200 mL autoclaved wide mouth glass bottles with the site identification on the lid. Once the water sample is collected, the bottle is tipped to give one (1) inch of air space in the bottle. The water samples are stored immediately on ice in a cooler until all the samples are returned to the laboratory. The six-hour holding time for enterococci samples is not an issue because of the relative proximity of the sampling runs to one of the three laboratories. Quanti-trays with positive wells and plates with colonies are disposed of by placing them in orange biohazard bags and then autoclaving. The autoclaved bags are then taken to the landfill.

Approximately half of the beach monitoring will be accomplished by wading into the surf to collect the sample. When wading, the sampler will use a telescopic golf ball retriever, modified to hold the sample bottle, to reach out approximately 6 to 8 feet from the body in knee- deep water to collect the sample, being sure to collect behind wave break to avoid adding suspended sand and sediments to the sample. Many of the public accesses on the coastal rivers and sounds have long piers that extend out over the water. The sample should betaken 6 to 12 inches below the surface of the water at a location along the pier that receives the most use, e.g., ladders, etc. Sampling by boat takes place in an area the receives the most use with the sample collected 6 to 12 inches below the surface. A stainless steel rod with a sample holder will be used to collect the sample from the boat. Sampling personnel should avoid disturbing bottom sediment in either approach, to collecting the sample.

Dare County has eight ocean storm drains that extend to the water's edge at low tide. The mouths of these storm drains are partially or completely submerged at high tide. Samples are to be collected within 200 yards to either side of the Dare County drains. The water sample collected at the Hanby Beach storm drain in New Hanover County will be sampled in the same manner. The remaining storm drains in New Hanover, Brunswick and Carteret counties do not extend to the water's edge. These drains are sampled where the swash enters the surf. The water depth for sample collection at all storm drains is the same as the other monitor sites in the surf, just below the surface in approximately knee- deep water.

It may be necessary at some sites that exceed the geometric mean protocol to conduct additional sampling to define the extent of the pollution. Once the advisory sign is posted, sampling may be initiated at additional points on either side of the sign. The program manager determines when, if any, additional sampling will be conducted.

Personal watercraft rental sites in most cases are classified as Tier II sites. The samples are collected in the sound where the renters are allowed to ride. This is usually a sectioned-off area marked by buoys. If an advisory is needed, the sign will be posted near shore where watercraft users can see the sign before entering the riding area.

B3 – Sample Handling and Custody

The sample collectors are responsible for ensuring the samples are stored and handled properly while in the field. The samples are stored immediately on ice in a cooler to chill the sample and to limit the exposure to UV light. The time is recorded on the field-sampling sheet for each sample collected. The six-hour holding limit for enterococci is not a factor because each sampling run can be completed with samples back to one of the three laboratories within 5 hours. Laboratory personnel are responsible for recording the time on data sheets when samples are planted into the media and the times samples can be analyzed after incubation (see Appendix 9 for laboratory QA). Both laboratory and field personnel are responsible for signing off on the chain of custody checklist on the backside of the field-sampling sheet (see

Appendix 7, page 1).

B4 - Analytical Methods

Using the Idexx Enterolert method and EPA method 1600 are discussed in the laboratory quality assurance plan. A failure in any part of the laboratory procedure results in the collection of another sample. Laboratory staff members are responsible for ensuring that the project manager receives the bacteriological results immediately upon completion of the analysis, and correcting any laboratory procedures that may occur. The laboratory staff members are: Candace Burrus in Manteo, Melissa Squibb and Michele Barger in Morehead City, and Holly White in Wilmington.

B5 - Quality Control

A sample is collected in the field and labeled temperature control that is measured upon arrival to the laboratory. The laboratory refuses samples that are above the temperature at which they were collected and samples that exceed holding time. Split samples are also taken at different frequencies and shared among the three labs to compare results. During laboratory analysis, a pure culture of *Enterococcus faecium* is used as a positive control; *Serratia marcescens* is used as the negative control for the IDEXX method; and *Escherichis coli* is used as the negative control for the EPA method 1600.

The acceptance criteria for enterococci are based upon the MPN table provided by Idexx Laboratories. The smallest number of enterococci that can be analyzed is 9 organisms per 100 ml. The highest density of enterococci that can be analyzed is 2005 organisms per 100 ml. Higher densities can be analyzed by further diluting the sample. The critical value of enterococci is 104 organisms per 100 ml for single samples exceedances, are well within the capabilities of the Enterolert method. Idexx reports that Enterolert has a false negative rate 0.4% and a false positive rate of 5.1%.

Other analytical controls are detailed in the laboratory QAP in Appendix 9. Technicians at each regional office review each other's data entry for mistakes and random data entry

reviews are conducted in the main office for quality control purposes.

B6 – Instrument /Equipment Testing, Inspection, and Maintenance

The two major equipment items needed to do the recreational water quality method are an autoclave and an air incubator. A maintenance contract with the autoclave manufacturer requires that the autoclave have preventive maintenance once every two months by authorized technicians.

Laboratory personnel check the autoclave monthly for sterility. Air incubators are checked twice daily for proper temperatures using a certified thermometer.

B7 - Instrumentation Calibration and Frequency

Laboratory equipment is routinely inspected and calibrated at different times of the year to meet FDA and State certification requirements. The only field instruments that need calibrating are thermometers.

B8 - Inspection/acceptance of Supplies and Consumables

Lab supplies are ordered through major scientific supply companies and inspected upon receipt by the laboratory personal. All of the growth mediums and reagents are sterile upon arrival and received in sealed packaging. Each package has a certificate of sterility by the manufacturer.

The supplies will be returned to manufacturer if a seal is broken on any of the packaging. Field personnel inspect field supplies before leaving the office each day.

B9 – Non-direct Measurements

Tide tables are the only non-direct measurements that will be used in the project. Tide tables are often used when planning sampling runs by boat. Storms and wind can cause delays or early arrival in the actual tidal stage but are not a critical issue to the project.

B10 – Data Management

The data is currently being entered into an Integrated Build Environment & Application Management (IBEAM) database, which is accessed for public use through our program

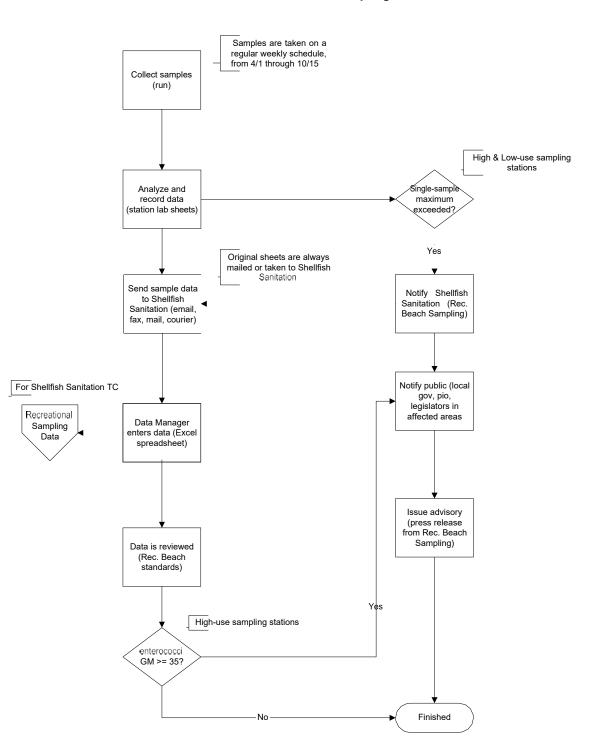
website:

https://xapps.ncdenr.org/mf/beaches/smplData.do;jsessionid=223BDF3CF7A1B5F0F1BD936 ADA6C7002

The IBEAM database is web-enabled and has both numerical and **Pr**ogram tracking, beach **A**dvisories, **W**ater quality standards, and **N**utrients (PRAWN) data.

Data is entered into the IBEAM database in Morehead City by the data manager and is stored in an ORACLE database housed in Raleigh, NC which is backed up nightly. All of the data from each region is entered into the Excel spread sheet from the Morehead City data manager for redundancy. Entries from the spreadsheet and the IBEAM database are compared to one another as a check (see Appendix 7). When data entered into IBEAM does not match with the spread sheet, the data manager is required to find and correct the mistake. Advisories are not posted until both databases can verify the geometric mean. The project manager reviews the database routinely to look at the geometric mean data and orders the posting of advisory signs and the issue of press releases accordingly.

All of the data on the server have timed backups that are stored on network drives. Redundancy in data entry prevents incorrect data from being stored on network. The following flow chart shows the process by which the data is generated and how the data is used.



Data Generation Routine Beach Sampling

SECTION C: ASSESSMENT AND OVERSIGHT

C1 – Assessments and Response Actions

As Section Chief, Shannon Jenkins has oversight for the shellfish and beach monitoring programs. Erin Bryan-Millush is the Beach Monitoring Project Manager and is responsible for regularly reviewing the progress of the project, compiling data and supervising employees.

The project manager is responsible for posting swimming advisory signs, directing public notification activities, and visiting the field offices regularly to assure adherence to the quality assurance project plan. Performance reviews are scheduled every six months for individual employee assessments. The project manager is responsible for any corrective action needed to ensure that the staff in the recreational water quality program is adhering to the QAPP and program objectives. The program manager is responsible for notification of health departments, local, state and federal governments as well as interest groups and the public; analyzing and preparing data for submittal to the Environmental Protection Agency and public outreach and education about the program.

The SSRWQ staff monitors the documentation of laboratory/field procedures and data analysis for their specific region throughout the beach-monitoring project.

C2 – Reports to Management

The recreational water quality data manager is responsible for submitting data summaries to the project manager routinely. Field and lab staff members are responsible for reporting quality assurance issues as they occur to the project manager. Individual reports for daily objectives and accomplishments are available for management at any time. The project manager reports to the EPA all swimming advisories as they are issued. An in-depth survey of the beach monitoring is reported to the EPA in January of each year.

SECTION D: DATA VALIDATION AND USABILITY

D1 – Data Review, Verification, and Validation

Each region has standardized field and laboratory procedures. Sample collection, handling of samples and lab analysis are all conducted in the same manner. Splitting samples and sharing them with each of the three labs to analyze helps verify that protocols are followed properly. Bacteriological data that is derived from samples where quality assurance is questionable will be rejected. The regional laboratories are state- and FDA-certified.

D2 – Verification and Validation Methods

Verification and validation are conducted by the staff members who record and enter the data. Laboratory staff are to ensure that samples are read and recorded correctly on to the field/lab form. The laboratory staff responsible for resolving laboratory issues are: Candace Burrus in Manteo, Melissa Squibb and Michele Barger in Morehead City, Holly White in Wilmington. Other staff members can be trained as backups when laboratory staff are on leave. Training records are kept in the laboratory quality assurance binders and signed off by the Quality Assurance Officer. The Project Manager and Quality Assurance Officer, Erin Bryan-Millush, will assist in possible issue resolution.

Controls are setup to verify that samples are being read properly (see Sect. B-5 Quality Control). The laboratory results are entered into the database by data manager or the project manager. The project manager performs random spot checks on both IBEAM and excel backup databases. The database is set up so that data summaries are verified by having the program to automatically calculate geometric means.

D3 – Reconciliation with User Requirements

The geometric means and single sample maximums are compared to the standards in the EPA s Beach Guidance manual for posting swimming advisories.

				SS			SEASON	SHOULDER SEASON	OFF SEASON
SITE	COUNTY	Tier	LOCATION	Max	EPA_ID	SEASON	FREQUENCY	FREQUENCY	FREQUENCY
C115						4/1-	5 in 30		
A	BEAUFORT	1	sound	104	NC442154	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30		
N87	BEAUFORT	1	sound	104	NC483929	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
C1	BRUNSWIC			104	NG 400745	4/1-	5 in 30	10/1.10/21 T	11/1 2/2 0 1
S1	K	1	ocean	104	NC499745	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S2	BRUNSWIC K	1	ocean	104	NC758778	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
32	BRUNSWIC	1	ocean	104	INC/30//0	4/1-	5 in 30	10/1-10/31 - 1 wice per month	11/1-3/3 - Once per monut
S3	K	1	ocean	104	NC848108	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
00	BRUNSWIC	1	occum	101	110010100	4/1-	5 in 30		
S5A	K	1	ocean	104	NC379310	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC					4/1-	5 in 30		
S5B	Κ	1	ocean	104	NC442815	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC					4/1-	5 in 30		
S5	K	1	ocean	104	NC175823	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC					4/1-	5 in 30		
S6A	K	1	ocean	104	NC971811	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30		
S6B	Brunswick	1	ocean	104	NC427139	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
0.00	BRUNSWIC			104	2101(4272	4/1-	5 in 30	10/1.10/21 T	11/1 2/2 0 1
S6C	K BRUNSWIC	1	ocean	104	NC164372	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S6	KUNSWIC	1		104	NC463026	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1 2/2 On as non-month
50	BRUNSWIC	1	ocean	104	NC403020	4/1-	5 in 30	10/1-10/31 - 1 wice per month	11/1-3/3 - Once per month
S9A	K	1	ocean	104	NC975034	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
57A	BRUNSWIC	1	occan	104	110775054	4/1-	5 in 30	10/1-10/51 - 1 wice per month	
S9B	K	1	ocean	104	NC345154	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
575	BRUNSWIC	-	- COOMI	101		4/1-	5 in 30		
S9C	Κ	1	ocean	104	NC140790	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC					4/1-	5 in 30		
S9D	Κ	1	ocean	104	NC861543	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC					4/1-	5 in 30		
S9	K	1	ocean	104	NC576773	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC					4/1-	5 in 30		
S10A	K	1	ocean	104	NC998441	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
610 D	BRUNSWIC			104	NGCIDDAC	4/1-	5 in 30		
S10B	K	1	ocean	104	NC642326	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S10C	BRUNSWIC K	1	ocean	104	NC521331	4/1- 9/30	5 in 30 Days	10/1 10/31 Twice not month	11/1 2/2 Once nor month
5100	K BRUNSWIC	1	ocean	104	110321331	4/1-	5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S10D	K	1	ocean	104	NC892936	4/1- 9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
5100	BRUNSWIC	1		101	110072750	4/1-	5 in 30	Tori Toro T Twice per month	The per month
S10E	K	1	ocean	104	NC654550	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC	_				4/1-	5 in 30		
S10F	K	1	ocean	104	NC457651	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC		1		1	4/1-	5 in 30		· · · · ·
S10G	Κ	1	ocean	104	NC517459	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC					4/1-	5 in 30		
S10	K	1	ocean	104	NC698990	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	BRUNSWIC					4/1-	5 in 30		
S11A	K	1	ocean	104	NC187482	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
012 1	BRUNSWIC			104	NGOOALLO	4/1-	5 in 30	10/1 10/21	
S13A	K	1	ocean	104	NC894449	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
\$12D	BRUNSWIC	1	00000	104	NC384247	4/1-	5 in 30	10/1 10/21 Turing non-manth	11/1-3/3 - Once per month
S13B	K	1	ocean	104	INC304247	9/30	Days	10/1-10/31 - Twice per month	11/1-5/5 - Once per month

<u>APPENDIX 1</u>: List of Tier One Monitoring Sites with sampling frequency and the single sample maximum.

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BRUNSWIC 1 ocean 104 NC873506 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month S14A K 1 ocean 104 NC873506 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month S14B K 1 ocean 104 NC742373 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month S14B K 1 ocean 104 NC143620 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month S15A K 1 ocean 104 NC497594 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month S15A K 1 ocean 104 NC497594 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month S16A K 1 sound 104 NC191050 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month S16B K 1 oce	S13C		1	ocean	104	NC404663			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
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BRUNSWIC i										
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	S15A		1	ocean	104	NC497594			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
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$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	S15		1	ocean	104	NC449749			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
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$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	510A		1	sound	104	NC309433			10/1-10/31 - 1 wice per month	11/1-3/3 - Once per month
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C3 CARTERET 1 ocean 104 NC895537 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C4A CARTERET 1 ocean 104 NC895537 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C4A CARTERET 1 ocean 104 NC343007 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C4B CARTERET 1 ocean 104 NC737019 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C4 CARTERET 1 ocean 104 NC714613 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C5 CARTERET 1 ocean 104 NC152475 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C5 CARTERET 1 ocean 104 NC152475 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C6							4/1-		•	· · · · ·
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C6 CARTERET 1 ocean 104 NC120547 $9/30$ Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C7 CARTERET 1 ocean 104 NC244236 $9/30$ Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C8 CARTERET 1 ocean 104 NC147416 $9/30$ Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month	C6A	CARTERET	1	ocean	104	NC102958			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
C7 CARTERET 1 ocean 104 NC244236 4/1- 9/30 5 in 30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C8 CARTERET 1 ocean 104 NC147416 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month										1
C7 CARTERET 1 ocean 104 NC244236 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month C8 CARTERET 1 ocean 104 NC147416 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month	C6	CARTERET	1	ocean	104	NC120547	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
C8 CARTERET 1 ocean 104 NC147416 4/1- 9/30 5 in 30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month							4/1-	5 in 30		
C8 CARTERET 1 ocean 104 NC147416 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month	C7	CARTERET	1	ocean	104	NC244236	9/30		10/1-10/31 - Twice per month	11/1-3/3 - Once per month
								5 in 30		
4/1- 5 in 30	C8	CARTERET	1	ocean	104	NC147416			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	~ ~									
	C9	CARTERET	1	ocean	104	NC475791			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
4/1-5 in 30 0/20 Days $10/1 10/21$ Twiss non-month $11/1 2/2$ Once non-month	C10.4	CADTEDET	1	0.0007	104	NC052661			10/1 10/21 T	11/1 2/2 On
	CIUA	CARTERET	1	ocean	104	INC952001			10/1-10/51 - 1 wice per month	11/1-3/3 - Once per month
C10B CARTERET 1 ocean 104 NC511988 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month	CIAP	CARTERET	1	00000	104	NC511099			10/1 10/31 Twiss nor month	11/1-3/3 - Once per month
C10B CARTERET 1 ocean 104 NC511988 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month 4/1- 5 in 30 5 5 5 10/1-10/31 - Twice per month 11/1-3/3 - Once per month	CIUD	CARTERET	1	occan	104	INCJ11900			10/1-10/31 - 1 wice per month	11/1-5/5 - Once per month
	C55A	CARTERET	1	sound	104	NC189579			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
COSA CARTERET 1 Sound 104 NC109379 9350 Days 10/1-10/31 - 1 wice per month 11/1-3/3 - Once per month 4/1- 5 in 30 4/1- 5 in 30 5000	<i>555</i> A		1	Sound	107	11010/3/7				The permonul
	C57	CARTERET	1	sound	104	NC101248			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
C69A CARTERET 1 sound 104 NC888920 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month	C69A	CARTERET	1	sound	104	NC888920		Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
4/1- 5 in 30									· ·	
C69C CARTERET 1 ocean 104 NC106127 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month	C69C	CARTERET	1	ocean	104	NC106127		Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
4/1- 5 in 30										
	N1A	CURRITUCK	1	ocean	104	NC109355			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
4/1- 5 in 30										
	N1	CURRITUCK	1	ocean	104	NC790915			10/1-10/31 - Twice per month	11/1-3/3 - Once per month
		GUDDUTUGU			1.0.4	MORECTOR			10/1 10/21 5	11/1 2/2 0
N2 CURRITUCK 1 ocean 104 NC856780 9/30 Days 10/1-10/31 - Twice per month 11/1-3/3 - Once per month									I III/I III//I Truess man magnetic	LICE IC Chase man mounth

N3	CURRITUCK	1	ocean	104	NC542433	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N4	CURRITUCK	1	ocean	104	NC846710	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N5A	DARE	1	ocean	104	NC995692	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N7A	DARE	1	ocean	104	NC658738	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N7	DARE	1	ocean	104	NC653898	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N12A	DARE	1	ocean	104	NC992884	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N12C	DARE	1	ocean	104	NC332962	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N12	DARE	1	ocean	104	NC933106	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N14A	DARE	1	ocean	104	NC148512	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N14	DARE	1	ocean	104	NC196750	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N15	DARE	1	ocean	104	NC701853	4/1- 9/30	5 in 30 Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N16A	DARE	1	ocean	104	NC297658	4/1- 9/30 4/1-	5 in 30 Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N16B	DARE	1	ocean	104	NC669365	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30		
N16D	DARE	1	ocean	104	NC706960	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N16E	DARE	1	ocean	104	TBD	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N16F	DARE	1	ocean	104	TBD	4/1- 9/30	5 in 30 Days	10/1-10/31 – Twice per month	11/1-3/3 – Once per month
N17A	DARE	1	ocean	104	NC703962	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N17B	DARE	1	ocean	104	NC194415	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N17	DARE	1	ocean	104	NC675298	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N18	DARE	1	ocean	104	NC888506	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N18A	DARE	1	ocean	104	TDB	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N18B	DARE	1	ocean	104	TDB	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N19A	DARE	1	ocean	104	NC325364	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N19B	DARE	1	ocean	104	TDB	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N22	DARE	1	ocean	104	NC239137	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N23	DARE	1	ocean	104	NC570729	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N25	DARE	1	ocean	104	NC944159	4/1- 9/30 4/1-	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N26B	DARE	1	ocean	104	NC144418	9/30	5 in 30 Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N26	DARE	1	ocean	104	NC289380	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month

1				1		4/1-	5 in 30		
N27	DARE	1	ocean	104	NC566464	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N29B	DARE	1	ocean	104	NC991015	4/1- 9/30	5 m 50 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N29	DARE	1	00000	104	NC995992	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
1129	DARE	1	ocean	104	NC993992	4/1-	5 in 30		11/1-5/5 - Once per monut
N30	DARE	1	ocean	104	NC440580	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N30A	DARE	1	ocean	104	TDB	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
2122	DADE	,		104	NG007125	4/1-	5 in 30	10/1 10/21 T	
N32	DARE	1	ocean	104	NC927135	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N33B	DARE	1	ocean	104	TDB	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N34	DARE	1	ocean	104	NC422633	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30		<u> </u>
N37	DARE	1	ocean	104	NC189209	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N39	DARE	1	ocean	104	NC380367	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N40	DARE	1	ocean	104	NC560974	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
1110			occun		110000711	4/1-	5 in 30		
N85A	DARE	1	ocean	104	NC635010	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N85	DARE	1	ocean	104	NC524248	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N88	DARE	1	sound	104	NC981058	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
1100	DARE	1	sound	104	NC981038	4/1-	5 in 30		11/1-5/5 - Once per monut
N91	DARE	1	sound	104	NC952532	9/30	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N91A	DARE	1	sound	104	TDB	4/1- 9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N141.4	IWDE	1		104	NC7009(9	4/1- 9/30	5 in 30	10/1 10/21 Traine man month	11/1.2/2 Once non-meth
N41A	HYDE	1	ocean	104	NC709868	4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N41	HYDE	1	ocean	104	NC368134	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
N42	HYDE	1	ocean	104	NC562881	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
2142	INADE	1		104	NG219225	4/1-	5 in 30	10/1 10/21 T	
N43	HYDE NEW	1	ocean	104	NC318235	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S18B	HANOVER	1	ocean	104	NC918299	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S18C	NEW HANOVER	1	ocean	104	NC274681	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	NEW					4/1-	5 in 30		
S18D	HANOVER NEW	1	ocean	104	NC301060	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S18	HANOVER	1	ocean	104	NC593669	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S19B	NEW HANOVER	1	ocean	104	NC765666	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	NEW					4/1-	5 in 30		
S19C	HANOVER NEW	1	ocean	104	NC796965	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S19	HANOVER	1	ocean	104	NC530102	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S20A	NEW HANOVER	1	ocean	104	NC616697	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	NEW		occuli			4/1-	5 in 30		
S21B	HANOVER NEW	1	ocean	104	NC939344	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S22A	HANOVER	1	ocean	104	NC375708	4/1- 9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
SOD	NEW	1	cound	104	NC748601	4/1-	5 in 30	10/1 10/21 Twice non-me-	11/1-3/3 - Once per month
S22B	HANOVER	1	sound	104	NC748601	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month

1	NEW	l	1	1		4/1-	5 in 30		
S22C	HANOVER	1	sound	104	NC252230	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	NEW					4/1-	5 in 30		
S22E	HANOVER	1	sound	104	NC230511	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
600F	NEW			104	210240062	4/1-	5 in 30		11/1.2/2
S22F	HANOVER	1	ocean	104	NC349062	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S22G	NEW HANOVER	1	sound	104	NC771581	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
3220	NEW	1	sound	104	NC//1501	4/1-	5 in 30	10/1-10/31 - 1 wice per month	11/1-3/3 - Once per monut
S23A	HANOVER	1	ocean	104	NC773758	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	NEW					4/1-	5 in 30		
S23B	HANOVER	1	ocean	104	NC632805	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
	NEW					4/1-	5 in 30		
S23	HANOVER	1	ocean	104	NC294779	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
G 40 4	NEW			104	210545001	4/1-	5 in 30		11/1.2/2
S48A	HANOVER	1	sound	104	NC547001	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
C13	ONSLOW	1		104	NC577316	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
015	UNSLOW	1	ocean	104	NC377310	4/1-	5 in 30	10/1-10/31 - 1 wice per month	11/1-3/3 - Once per monut
S29A	ONSLOW	1	ocean	104	NC266219	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
52711	0102011	-		101	11020021)	4/1-	5 in 30		
S29C	ONSLOW	1	ocean	104	NC440062	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30		
S30A	ONSLOW	1	ocean	104	NC741107	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30		
S30C	ONSLOW	1	ocean	104	NC645929	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
C20E	ONSI OW	1		104	NC900472	4/1-	5 in 30	10/1 10/21 Tarris a man are with	11/1 2/2 Once non-month
S30E	ONSLOW	1	ocean	104	NC899473	9/30 4/1-	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S30F	ONSLOW	1	ocean	104	NC471307	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
5501	OI (DEC II	1	occum	101	1101/150/	4/1-	5 in 30		
S30	ONSLOW	1	ocean	104	NC865785	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30	•	
S25A	PENDER	1	sound	104	NC467377	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30		
S28B	PENDER	1	ocean	104	NC403790	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
639 C	DEVIDED			104	NG04770/	4/1-	5 in 30	10/1 10/21 T	11/1.2/2
S28C	PENDER	1	ocean	104	NC947796	9/30	Days 5 in 30	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
S28	PENDER	1	ocean	104	NC098150	4/1- 9/30	5 in 30 Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
520	TENDER	1	occan	107	1100/0150	4/1-	5 in 30		11/1 5/5 - Once per monut
S29B	PENDER	1	ocean	104	NC594897	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
		l				4/1-	5 in 30		1
S29	PENDER	1	ocean	104	NC526485	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30		
C51C	CARTERET	1	sound	104		9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month
						4/1-	5 in 30		
C48C	CARTERET	1	sound	104	NC727802	9/30	Days	10/1-10/31 - Twice per month	11/1-3/3 - Once per month

]
SITE	COUNTY	TIER	LOCATION	SSMax	EPA_ID	SEASON	SEASON_FREQUENCY	SHOULDER SEASON FREQUENCY	OFF_SEASON_FREQUENCY
C7B	CARTERET	2	sound	104	NC429821	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C22A	ONSLOW	2	sound	104	NC930678	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C23B	ONSLOW	2	sound	104	NC611568	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C25	ONSLOW	2	sound	104	NC499511	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C27A	ONSLOW	2	sound	104	NC892318	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C30A	CARTERET	2	sound	104	NC377628	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C30	CARTERET	2	sound	104	NC254979	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C31A	CARTERET	2	sound	104	NC615139	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C33A	CARTERET	2	sound	104	NC927355	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C34A	CARTERET	2	sound	104	NC456163	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C35A	CARTERET	2	sound	104	NC722475	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C36	CARTERET	2	sound	104	NC623291	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C39A	CARTERET	2	sound	104	NC272309	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C40A	CARTERET	2	sound	104	NC637063	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C40	CARTERET	2	sound	104	NC320933	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C47A	CARTERET	2	sound	104	NC411851	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C47B	CARTERET	2	sound	104	NC160448	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C48B	CARTERET	2	sound	104	NC135340	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C51B	CARTERET	2	sound	104	NC958671	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C51	CARTERET	2	sound	104	NC659044	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C53A	CARTERET	2	sound	104	NC852484	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C54A	CARTERET	2	sound	104	NC907794	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C55B	CARTERET	2	sound	104	NC381539	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C56A	CARTERET	2	sound	104	NC730320	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C56	CARTERET	2	sound	104	NC179171	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C58	CARTERET	2	sound	104	NC997135	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C59A	CARTERET	2	sound	104	NC380630	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C60A	CARTERET	2	sound	104	NC935797	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C62	CARTERET	2	sound	104	NC951761	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C64	CARTERET	2	sound	104	NC341418	4/1-10/31	2x Monthly 2x Monthly	2x Monthly	11/1-3/3 - Once per month
C65A	CARTERET	2	sound	104	NC852895	4/1-10/31	2x Monthly 2x Monthly	2x Monthly	11/1-3/3 - Once per month
C66	CARTERET	2	sound	104	NC626501	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C69D	CARTERET	2		104	NC347773	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
			sound						
C75A	CARTERET	2	sound	104	NC861560	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month

<u>APPENDIX 2</u>: List of Tier II Monitoring Sites with sampling frequency and the single sample maximum.

C81A	CARTERET	2	sound	104	NC434812	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C81B	CARTERET	2	sound	104	NC134999	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C85	CARTERET	2	sound	104	NC586930	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C89A	PAMLICO	2	sound	104	NC624353	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C90A	PAMLICO	2	sound	104	NC990224	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C92A	PAMLICO	2	sound	104	NC155524	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C92	PAMLICO	2	sound	104	NC174861	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C93	PAMLICO	2	sound	104	NC854048	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C94	PAMLICO	2	sound	104	NC490467	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C95	PAMLICO	2	sound	104	NC902753	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C96A	PAMLICO	2	sound	104	NC589816	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C99	CRAVEN	2	sound	104	NC461235	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C100A	CRAVEN	2	sound	104	NC558811	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C104	CRAVEN	2	sound	104	NC193701	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C105B	CRAVEN	2	sound	104	NC821771	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C106A	CRAVEN	2	sound	104	NC905913	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C107	CRAVEN	2	sound	104	NC619539	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C110A	CRAVEN	2	sound	104	NC608977	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C111A	BEAUFORT	2	sound	104	NC105938	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C111	BEAUFORT	2	sound	104	NC556462	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C112A	BEAUFORT	2	sound	104	NC482470	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C114	PAMLICO	2	sound	104	NC659798	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C117A	BEAUFORT	2	sound	104	NC968346	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C118	BEAUFORT	2	sound	104	NC778708	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
C119A	BEAUFORT	2	sound	104	NC575571	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C120A	BEAUFORT	2	sound	104	NC808817	4/1-9/30	5 in 30 Days	2x Monthly	11/1-3/3 - Once per month
C126A	BEAUFORT	2	sound	104	NC635491	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
N29A	DARE	2	sound	104	NC347842	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
N33	DARE	2	sound	104	NC868201	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
N39A	DARE	2	sound	104	NC807801	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S30D	ONSLOW	2	sound	104	NC187979	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S35A	BRUNSWICK	2	sound	104	NC451008	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S37	BRUNSWICK	2	sound	104	NC803771	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S43A	BRUNSWICK	2	sound	104	NC642348	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S45A	NEW HANOVER	2	sound	104	NC853733	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S45	NEW HANOVER	2	sound	104	NC813450	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S48	NEW HANOVER	2	sound	104	NC519384	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S54A	ONSLOW	2	sound	104	NC431213	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month

S54	PENDER	2	sound	104	NC215656	4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S68	NEW HANOVER	2	sound	104		4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S69	NEW HANOVER	2	sound	104		4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month
S70	NEW HANOVER	2	sound	104		4/1-10/31	2x Monthly	2x Monthly	11/1-3/3 - Once per month

APPENDIX 4: Station Locations

STATION	LATITUDE	LONGITUDE	STATION	LATITUDE	LONGITUDE	STATION	LATITUDE	LONGITUDE
Central Region		Northern Region			Southern Region			
C2	34.69394638	-76.69724201	N1A	36.43585876	-75.83968249	S1	33.86356188	-78.51527162
C3A	34.69700737	-76.72403037	N1	36.39663574	-75.82791646	S2	33.86657985	-78.50650485
C3	34.69583086	-76.71178281	N2	36.37644043	-75.82329652	S3	33.87140195	-78.48822958
C4A	34.69664188	-76.7809633	N3	36.32844831	-75.8094266	S5A	33.87876782	-78.46523232
C4B	34.69494278	-76.80578492	N4	36.2787749	-75.79195055	S5B	33.88302916	-78.44991014
C4	34.69731061	-76.73911912	N5A	36.19613529	-75.75606546	S5	33.88626276	-78.43631643
C5A	34.69084475	-76.84535789	N7A	36.12166838	-75.72180795	S6A	33.89445128	-78.40493636
C5	34.69293202	-76.82888335	N7	36.14536226	-75.73410635	S6B	33.8897331	-78.42605463
C6A	34.68284924	-76.9069427	N12A	36.04417804	-75.67403845	S6C	33.89770606	-78.39411407
C6	34.68690932	-76.88116866	N12C	36.021895	-75.659347	S6	33.89277787	-78.41128612
C7B	34.68640917	-76.9098865	N12	36.06682161	-75.68917936	S9A	33.90618264	-78.34359023
C7	34.67524399	-76.95655585	N14A	36.01420467	-75.65463709	S9B	33.90753083	-78.33264435
C8	34.66073634	-77.03419145	N14	36.01566147	-75.65553878	S9C	33.91440851	-78.24638171
С9	34.65548359	-77.05547456	N15	35.99848263	-75.64574377	S9D	33.91007377	-78.30708153
C10A	34.65419598	-77.06031474	N16A	35.97131922	-75.63000341	S9	33.91058882	-78.29699199
C10B	34.64475863	-77.08942269	N16B	35.987269	-75.639445	S10A	33.91370788	-78.19015525
C13	34.63316855	-77.13784688	N16D	35.967732	-75.628239	S10B	33.91385405	-78.15849676
C22A	34.65263906	-77.16531317	N16E	35.9736341	-75.6310685	S10C	33.91422538	-78.17983866
C23B	34.62592827	-77.17088553	N16F	35.9801065	-75.6348122	S10D	33.91428501	-78.17192491
C25	34.63982813	-77.13963815	N17A	35.95880885	-75.62327886	S10E	33.91389885	-78.20595714
C27A	34.68483268	-77.12323518	N17B	35.962546	-75.625329	S10F	33.91360537	-78.2137974
C30A	34.653	-77.102	N17	35.96544073	-75.62690244	S10G	33.91284741	-78.22805586
C30	34.67275548	-77.10026206				S10	33.91393669	-78.2605221
C31A	34.67982058	-77.06532832	N18A	35.9468879	-75.6161109	S11A	33.9134154	-78.26723028
C33A	34.66383475	-77.06795354	N18B	35.941775	-75.6133612	S13A	33.90945097	-78.11682515
C34A	34.67360202	-77.00797809	N18	35.9315153	-75.60799394	S13B	33.9080251	-78.10693851
C35A	34.686	-77.052	N19A	35.91036276	-75.59617157	S13C	33.91156206	-78.13334012
C36	34.69660735	-77.00918628	N19B	35.8857165	-75.5819142	S13	33.91272275	-78.14684113
C39A	34.70469152	-76.96295099	N22	35.846914	-75.56304911	S14A	33.90324283	-78.08117223
C40A	34.702	-76.823	N23	35.7986653	-75.54036219	S14B	33.90563435	-78.09105227
C40	34.72365189	-76.89543785	N25	35.74029911	-75.50219232	S15A	33.89165045	-78.03533021
C47A	34.70909932	-76.74541518	N26B	35.6086426	-75.46491865	S15	33.89616568	-78.05453433

C47B	34.71695021	-76.74687376	N26	35.68026788	-75.47987822	S16A	33.88092603	-77.9978067
C48B	34.7223024	-76.75529379	N27	35.58499122	-75.46100908	S16B	33.85655697	-77.99993404
C48C	34.719	-76.724	N29A	35.53366568	-75.47583433	S16C	33.84994935	-77.95970035
C51B	34.71779444	-76.71602039	N29B	35.43844176	-75.48355779	S18B	33.99032437	-77.90864444
C51C	34.722	-76.747	N29	35.53145704	-75.4680156	S18C	33.99347899	-77.90761908
C51	34.72189453	-76.75116269	N30A	35.3469802	-75.5005235	S18D	33.979636	-77.912031
C53A	34.72161621	-76.68715455	N30	35.37705603	-75.49214639	S18	33.96407925	-77.92111895
C54A	34.70926372	-76.67636051	N32	35.32189827	-75.50682384	S19B	34.0119747	-77.89960854
C55A	34.728	-76.668	N33B	35.2985092	-75.5113233	S19C	34.03131962	-77.89204232
C55B	34.72273139	-76.66801969	N33	35.2987193	-75.5145622	S190	33.99806545	-77.90506805
C56A	34.70972225	-76.63204321	N34	35.25404181	-75.52084915	S20A	34.05247005	-77.88310913
C56	34.71488538	-76.6626949	N37	35.23213845	-75.60439615	S21B	34.06373015	-77.87869288
C57	34.71368384	-76.68021494	N39A	35.221141	-75.657925	S21B	34.19308497	-77.80444986
C58	34.68723685	-76.64406304	N39	35.22352804	-75.64332968	S22B	34.19639699	-77.80541608
C59A	34.67938603	-76.61910806	N40	35.20439656	-75.70266471	\$22C	34.20391228	-77.79942986
C60A	34.69922408	-76.65151301	N41A	35.1578687	-75.84206461	S22E	34.20169548	-77.80127128
C62	34.78922414	-76.60785984	N41	35.18077289	-75.77833293	S22F	34.20732533	-77.79409262
C64	34.71390843	-76.5788927	N42	35.12323539	-75.92108097	S22G	34.1942071	-77.8074981
C65A	34.70213019	-76.52039585	N43	35.1023641	-75.95907669	S23A	34.23375944	-77.77398669
C66	34.684697	-76.528839	N85A	36.00594398	-75.64988082	S23B	34.22623357	-77.78119418
C69A	34.62393278	-76.52515449	N85	36.01151804	-75.6530312	S23	34.21386241	-77.78829205
C69C	34.62126555	-76.52076971	N87	35.53305101	-76.61257371	S25A	34.35025327	-77.65349171
C69D	34.620872	-76.551299	N88	35.95228739	-75.63296435	S28B	34.3893186	-77.59791736
C75A	34.76136856	-76.41306228	N91	36.01837068	-75.72686409	S28C	34.35018617	-77.64663866
C81A	35.0183845	-76.31227876	N91A	36.0183936	-75.7261233	S28	34.36481114	-77.62854856
C81B	35.01925089	-76.31549635				S29A	34.46037931	-77.48462902
C85	34.98	-76.596				S29B	34.44117942	-77.51882273
C89A	35.02698691	-76.68830774				S29C	34.47525938	-77.45583558
C90A	35.00897073	-76.70949719				S29	34.42419862	-77.54583075
C92A	34.99274117	-76.75734228				S30A	34.49577289	-77.41161113
C92	34.99526013	-76.7524396				S30C	34.50980464	-77.38126226
C93	34.97097118	-76.79361553				S30D	34.49718715	-77.42823821
C94	34.96662672	-76.79839251				S30E	34.51987886	-77.35868008
C95	34.99261474	-76.85546497				S30F	34.49934837	-77.40462231
C96A	35.02378442	-76.90118418				S30	34.49169917	-77.42103306
C99	35.0646135	-76.97017789				S35A	33.91338647	-78.33582797
C100A	35.10307527	-77.03494406				S37	33.90336872	-78.39430835

C104	34.98458069	-76.94728472		S43A	33.91731846	-78.01610671
C105B	34.95276437	-76.89564438		S45A	34.05787965	-77.88872179
C106A	34.91765438	-76.85336357		S45	34.05201506	-77.91788558
C107	34.94159687	-76.82645276		S48A	34.18760111	-77.81363808
C110A	34.930135	-76.791425		S48	34.18308545	-77.81912255
C111A	35.53559212	-77.04134147		S54A	34.46965981	-77.50848406
C111	35.53510588	-77.05028196		S54	34.43076997	-77.55032884
C112A	35.51387771	-77.00994522		S68	34.081	-77.882
C114	35.18037399	-76.66325786		S69	34.159	-77.849
C115A	35.45970011	-76.90123053		S70	34.22	-77.809
C117A	35.43516185	-76.90837622				
C118	35.44854314	-76.92409325				
C119A	35.44135996	-76.9490002				
C120A	35.4754323	-76.99484537				
C126A	35.47781425	-76.93127503				

APPENDIX 5: Press release templates

Roy Cooper *Governor*

Elizabeth S. Biser Secretary, DEQ



Kathy B. Rawls, Division Director

Release: Immediate	Contact: Erin Bryan-Millush
Date:	Phone: 252-808-8153

Water quality swimming advisory issued for (sound-side/ocean-side) site in X County

MOREHEAD CITY – An advisory against swimming was posted today at a(n) (sound-side/ocean-side) site in X County, where state officials found bacteria levels in the water that exceed the state's and Environmental Protection Agency's recreational water quality standards.

The advisory is for **PUBLIC DESCRIPTION** in **TOWN**. Test results of water samples taken on **DATE** and **DATE** indicate bacteria levels that exceed the state and federal action levels of 104 enterococci per 100 milliliters for Tier (1 or 2) (daily or non-daily) use sites. Swimming areas are classified based on recreational use and are referred to as tiers.

The N.C. Recreational Water Quality Program tests water quality at ocean and sound beaches in accordance with federal and state laws. Enterococci, the bacteria group used for testing, is found in the intestines of warm-blooded animals. While it does not cause illness, scientific studies show that enterococci may indicate the presence of other disease-causing organisms. People swimming or playing in waters with bacteria levels higher than the action level have an increased risk of developing gastrointestinal illness or skin infections.

This advisory is not a beach closing, nor does the advisory affect the entire **X** area. Swimming advisories are for waters within 200 feet of the sign. The sign posted reads as follows:

ATTENTION SWIMMING IN THIS AREA IS NOT RECOMMENDED. BACTERIA TESTING INDICATES LEVELS OF CONTAMINATION THAT MAY BE HAZARDOUS TO YOUR HEALTH. THIS ADVISORY AFFECTS WATERS WITHIN 200' OF THIS SIGN. OFFICE OF THE STATE HEALTH DIRECTOR

State officials will continue testing the site, and they will remove the sign and notify the public again when the bacteria levels decrease to levels below the standards.

State recreational water quality officials sample 215 sites throughout the coastal region, most of them on a weekly basis, from April to October. Testing continues on a reduced schedule during the rest of the year, when the waters are colder.

For more information on the N.C. Recreational Water Quality Program or to a view a map of testing sites, visit the <u>program's website</u>, and follow the <u>program's Twitter feed</u>.

###

Website: http://www.ncmarinefisheries.net Facebook: https://www.facebook.com/NCMarineFisheries Instagram: https://www.instagram.com/NC_DMF Twitter: https://twitter.com/NC_DMF P.O. Box 769, 3441 Arendell St., Morehead City N.C. 28577 APPENDIX 6: Advisories Signs



APPENDIX 6: Advisories Signs Continued



APPENDIX 6: Advisories Signs Continued



OFFICE OF THE STATE HEALTH DIRECTOR

RUN: COR	LIO	RUN: COROLLA/KDH_1@F4_COUNTY: DARE STATE: NO	ñ		D/	DATE COLLECTED:	CTED:	41124	14				BY WHOM:	A
AIR TEMPERATURE	ERA	TURE 60 OF TEMP. CONTROL 40	0	् म	D	DATE/TIME RECEIVED IN LAB: 4 1/24 -	RECEIVE	DINL/	18:4	1/24	õ	SIAM	BY WHOM:	18
RECIPIT/	ATIO	PRECIPITATION, LAST 24 HOURS 011 IN	INCHES		D/	DATE/TIME PLANTED: 4 1/24	LANTEI	Ē	124	- 110	113AM		BY WHOM:	K
Ef +		S.m Media Lot # らいっこ	20	ľ	D,	DATE/TIME REPORTED: 4/2/24	REPORT	۹ با	2/24	L.	1/13 AM		BY WHOM:	C.
-1	41		-				WIW	-	WA	WATER	DEPTH	Ŧ	RESULTS	LIS
fi A	- 9	LOCATION OF STATION	x m -	TIME	Div.	TIDE	Dir.	VeL	Temp	% Sait	Water	Samp	Pos. Wells	MPN Enters.
AL VIN		Convila. 2.0 miles north of N. 1	-	931	Z	Y4FLD	32	5	S	26	2	~	a	<10
IN 1	-	Corolla, ramp at end of paved road	-	926	z	ÿ4£~D	ŝ	Ñ	Š	26	2	-	0	<10
N2 2.	-	Corolla, ramp across from Corolla Lighthouse		016	Ζ.	YUELD !	ş	2	5	26	2	-	0	<10
S S		Corolla, Albacore St. beach access	-	859	z	VUELD	ş	ŝ	15	26	0	-	0	<10
N4 4	-	Pine Island., Curr. Co. So. Beach Access	-	848	Ζ	YHELD	N N	3	N.	26	2	-	0	510
NSA 5A		Duck -Beach Acc. #1 Sprigtail Drive		828	2	YHELD	52	Ñ	S	26	2	-	0	<10
L 41		Southern Shores, Hillcrest Dr beach access	-	814		VYFLD	ž	ñ	5	24	Ν	-	0	<10
NTA TA		Southern Shores-1.5mile N. K.H Pier Beach Ace	-	800	z	145-D	52	Ň	5	28	Ν	-	0	<10
NI2 12	-	Kitty Hawk Bath House near S.R. 1206	-	346	Z	MELD	SE	Š	SI	28	2	~	0	<10
NIZA 17	12A	KDH. Avalon Pior	-	136	Z	ISTELD	212	õ	Ñ	28	2	-	0	<10
NI2C 12	122	KDH, Prospect Aye.	-	727	г	LAST E8B	N Z	Ñ	5	22	2	-	0	<10
16N	-	ROANOKE SD Colington Swim Beach	-	108		3/4 680	SN	Ñ	56	e	2	1	0	<10
NOIN GIA		Swim dock at the end of Colington De.	-	707	Z	34600	ŝ	Ñ	56	v	N	-	-	01
N14 14		KDH Bathhouse at Occan Bay Dr.	-	653	Z	3/4880	ŝ	Ā	ō	28	ы	-	0	< 10
NI4A 14A		KDH RO Pipe at Oregon Street	-	655	z	3/1600	5 Z	Ñ	Ś	28	P3			10
N85 85	-	KDH, D/P Cavalier by the Sea	-	644	Ζ	34683	Σ	Ñ	5n	28	2	-	-	10
N85A 82	12	KDH S/D at Martin Street	-	636	2	Huess>	SW	51	5	28	r	-	-	10
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APPENDIX 7: Example of Field Sampling Sheet with Lab results

APPENDIX 7 Continued: Example of IBEAM and Excel Databases

		ing Run: Cord	olla/KDH										MPNGM		FUGM Calculat	or Find Sam	pies
	Report Date: 04/02/2024 Plant Time: 11:13 (H 24hr Precip: None	HH24:mm)	"				Report Time Air Temp		(HH24:r	nm)		Ar Ar	a miners Idy Haines In Daisey andace Burru	▲ → 5 ▼ ←	Candace Burn	J5 ▲ ▼	
Tier	Location	Time (HH24:mm)	Current	Tide		V Dir.	Vind Vel.(mph)	V Temp(°F)	later Salt(%o)		epth Smpl(ft)	Lab Err		Bacteriol	ogical Samples		
) •	Atlantic Oc Corolla, 2.0 Miles North Of N1	09:31	N V	1/4 Flood	~	SW 🗸	11 - 15 🗸	51	28	2	1 🗸		MPN 9	MPNGM 9.2(117)	CFU	CFUGM ERROR	
)	Atlantic Oc Corolla, Ramp At End Of Paved Road	09:26	N V	1/4 Flood	~	sw 🗸	11 - 15 🗸	51	26	2	1 🗸		MPN 9	MPNGM 9(117)	CFU	CFUGM ERROR	
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	Corolla, Albacore St. Beach Access	08:59	N V	1/4 Flood	~	sw 🗸	11 - 15 🗸	51	26	2	1 🗸		MPN 9	MPNGM 9(117)	CFU	CFUGM ERROR	
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5 4/1/2024	S 1	9 C	NEV HAN	Carolina Beach- P	ublic Beach Acces	ss at Hamlet Ave.			CAROLINA BEACH	- HAMLET AVE.	VB	ocean	1 1	NC796965	9		32	59	1/2 FLD	N/A	SV	64	0
46 4/1/2024	S 4	5 A	NEV HAN	Public Boh on Mas	sonboro Sd - end o	of Florida Ave in Ca	rolina Beach		JUNCTION OF SNC	OV'S CUT AND M	VB	sound	2	NC853733	9		32	59	1/2 FLD	N/A	SV	64	0
7 4/1/2024	S 2			Vehicle Access, 60					VEHICLE ACCESS /	AT DUNE MARKE	VB			NC939344	20		4	59	1/2 FLD	N/A	SW	64	0
8 4/1/2024			NEV HAN	PUBLIC ACCESS,	PERIVINKLE LA	NE IN CAROLINA E	BEACH		PUBLIC ACCESS, F	PERIVINKLE LANI	VB			NC616697	9		32	59	1/2 FLD	N/A	SV	64	0
9 4/1/2024	S 4		NEV HAN	Cape Fear River, ju	unction of Snow's	Cut and Cape Fear I	River		CAROLINA BEACH	I STATE PARK	VВ			NC813450	20		6	59	1/2 FLD	N/A	SV	64	0
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4/1/2024			NEV HAN	Banks Channel - W	/aynick.blvd. Appro	ox. 150 yds N of Iula :	St.		BANKS CHANNEL	- HANOVER SEA:	VВ	sound		NC748601	9		30	59	1/4 FLD	N/A	SV	60	0
6 4/1/2024				Carna Access, cor					Cama Access, come		VВ			NC771581	42		30	59	1/4 FLD	N/A	SV	60	0
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6 4/1/2024			NEV HAN	Stone Street Public	c Access - Ocean	side Wrightsville Be	ach		STONE STREET (V	B)	VВ			NC349062	9		32	59	1/4 FLD	N/A	SV	60	0
7 4/1/2024			NEV HAN	Ocean pier at Salis	bury St. public acc	ess			JOHNNY MERCER	PIER	VB			NC294779	9		32	59	1/4 FLD	N/A	S∀	60	0
8 4/1/2024				L-Shaped Lot, Wrig					L-Shaped Lot, (WB)		VВ			NC632805	9		32	59	1/4 FLD	N/A	SV	60	0
9 4/1/2024				N. end of Wrightsvi		access #2 off Lum	ina Dr.		SHELL ISLAND		VВ			NC773758	9		32	59	1/4 FLD	N/A	SV	60	0
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1 4/1/2024	N			Corolla Ramp, end					ATLANTIC OC Co		CC			NC790915	9		26	51	1/4 FLD	N	SV	60	0
2 4/1/2024				Ocean-side of Cor			n Corolla		ATLANTIC OC Ra		CC	ocean		NC856780	9		26	51	1/4 FLD	N	SV	60	0
3 4/1/2024				Corolla, Albacore					COROLLA, ALBACO		CC			NC542433	9		26	51	1/4 FLD	N	SV	60	0
4 4/1/2024				Currituck S Beach		land			ATLANTIC OC Pir		CC			NC846710	9		26	51	1/4 FLD	N	SV	60	0
5 4/1/2024			DARE	Beach access at S					Ocean - Beach acc. 4		CC			NC995692	9		26	51	1/4 FLD	N	SV	60	0
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4/1/2024			DARE	Beach Access at 9					Ocean - KDH, AVAL		KH			NC992884	9		28	51	1ST FLD	N	SV	60	0
4/1/2024			DARE	Prospect Ave Bea					ATLANTIC OCKDF		KH			NC332962	9		28	51	LST EBB	N	SV	60	0
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2 4/1/2024 3 4/1/2024			DARE			rive in Colington Ha	arbor		Swim dock at the end		RI	Soana	1		10		5	56	3/4 EBB	N	SV	60	0
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4/1/2024	N 8		DARE	Drain Pipe at MP 8					KDH, D/P ACROSS		KH			NC524248	10		28	51	3/4 EBB	N	SV	60	0
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APPENDIX 8: Educational Materials

Recreational Water Quality Program—an overview of the new standard

Does anyone check the waters on the coast to see if it's safe to swim and play in them? Yes, the Recreational Water Quality Program operated by the Shellfish Sanitation Section of the Division of Marine Fisheries monitors coastal waters on a year-round basis.

Since its inception in 1997, the program has fulfilled its purpose: "To protect the public health by monitoring the quality of North Carolina's coastal recreational waters and notifying the public when bacteriological standards for safe bodily contact are exceeded".

The program has identified 221 specific locations where the public swims and plays in coastal waters and has sorted the areas into three tiers, determined by use patterns and the resultant risks of someone becoming ill from contaminated water. Approximately 65 percent of these sites are Tier I sites, those with the most use. Examples of these areas include oceanfront beaches, summer camp swimming areas and jet-ski rental facilities. The other 35 percent of the sites are Tier II areas. These areas have less usage and are generally accessible only via watercraft. Examples of these sites include public access areas, boat ramps, and other places where smaller numbers of people use the water.

The ocean beaches and other high usage areas are sampled once per week from the beginning of April to the end of September. All stations are sampled twice per month during October, and then once per month in the winter and spring, November through March.

The program uses enterococci as the indicator organism for contamination, based on current US EPA recommendations. Enterococci are the organisms most consistently present in the intestinal tracts of animals and are most closely associated with incidents of human illness. The program uses an EPA-mandated standard of 35 enterococci per 100ml of water, based on a geometric mean (logarithmic average) using the most recent five sample results collected over the previous 30 days. It also uses a single-sample maximum of 104 enterococci per 100 ml of water to indicate that the water in question exceeds that standard. Exceeding either of these standards will resultin public notification procedures being activated.

When the standards are exceeded, local officials are notified, a press release is issued, and a sign is posted at the affected area, warning the public of the risks associated with swimming or playing in the water at this site. The program will continue to sample the area repeatedly. When the area ceases to exceed the standard, the sign is removed, local officials are notified again, and another press release is issued. Point-source contamination sources are also the sites of sign postings. Sources such as sewage treatment plant outfall pipes are permanently posted to warn swimmers not to swim or play in such areas. More problematic are pipes with stormwater discharges. These discharges are heavily contaminated but discharge only intermittently, during and after rainfall events. Currently the program posts these pipes when they begin discharging during a storm and removes the posting 24 hours after the discharge stops.

In an effort to raise awareness of the program and to solicit input from the public, several public meetings have been scheduled in the past years and are currently scheduled on a needed basis. These meetings will be held primarily in beach communities along the coast and will involve local officials, travel/tourism representatives, property owners and members of the general public. Media packages will be developed to publicize the meetings.

Erin Bryan-Millush leads the program from the Morehead City office. The recreational water quality program consists of five water samplers stationed in Manteo (1), Morehead City (2), and Wilmington (1).

We hope to keep North Carolinian residents and visitors protected when swimming in theses coastal waters for generations to come!

APPENDIX 8 continued: Educational Materials.

THE FACTS: RECREATIONAL WATER QUALITY MONITORING IN NORTH CAROLINA



ATTENTION SWIMMING IN THIS AREA IS NOT RECOMMENDED. BACTERIA TESTING INDICATES LEVELS OF CONTAMINATION THAT MAY BE HAZARDOUS TO YOUR HEALTH. THIS ADVISORY AFFECTS WATERS WITHIN 200 FT. OF THIS SIGN.

OFFICE OF STATE HEALTH DIRECTOR

I saw this sign at the beach. Who put it there, and what does it mean?

It means testing shows that state and federal bacteria levels for swimming water quality were exceeded. Therefore, state officials recommend that you do not swim within 200 feet on either side of the sign. The main goal of the Shellfish Sanitation and Recreational Water Quality Section is to protect the public health by monitoring the quality of North Carolina s coastal recreational waters and notifying the public when bacteriological levels for safe bodily contact are exceeded.

The section started monitoring coastal recreational water quality in 1997. The coastal waters monitored include the ocean beaches, sounds, bays and estuarine rivers. Unfortunately, North Carolina does not have a statewide monitoring program for inland recreational waters. The public should avoid freshwater swimming after heavy rain, especially near storm drains.

Are North Carolina's beaches safe for swimming?

Yes. North Carolina has miles of beaches with excellent water quality, and the state has an extensive monitoring program to test the waters and identify any temporary problems that might arise. The data that has been collected since the program began show that our swimming beaches have been under advisory for an average of less than 1 percent of the swimming season for each year. While the waters of North Carolina are generally very clean, it is important to monitor them continually, so the public can be informed of any localized problems.

How many stations do you monitor and how often do you monitor them?

The Recreational Water Quality staff tests 221 sites throughout the coastal area at different frequencies, depending on the time of year and use patterns of the site. Ocean beaches and other high usage areas are sampled once per week between April 1 and Sept. 30; lower usage areas are sampled twice per month. All stations are sampled twice per month during October, and then once per month in the winter, November through March. Staff members collect approximately 6,000 samples per year.

What are the recreational water quality levels?

The sections staff tests for a type of bacteria called enterococci, which are found in the intestines of warm-blooded animals such as birds, dogs, raccoons and people. Enterococci will not make you sick; however, it is often associated with other bacteria and viruses that can cause water-borne illness. The U.S. Environmental Protection Agency found that enterococcus closely correlates with incidence of human illness.

To comply with the swimming water quality levels set by the EPA and the state, water test results have to fall below a set average as well as a single-sample level. The average is the geometric mean of five weekly samples taken within a 30- day period. The geometric mean cannot exceed 35 enterococci per 100 milliliters of water. In addition, swimming advisories may be posted if a single sample exceeds the level set for it based on usage. Advisories based on single sample results are retested at the time of the posting.

What happens if the swimming water quality levels are exceeded?

If the swimming water quality level is exceeded at a site, the staff sends out a press release to inform the public and an advisory sign is posted at the swimming site. Discharges of stormwater and floodwater into the swimming area also trigger swimming advisories that last for 24 hours after the discharge has ended.

Where are the disease-causing organisms coming from?

Disease-causing organisms, or pathogens, can come from both human and animals. Stormwater runoff from agricultural and urban areas delivers pathogens from humans, livestock, wildlife, and pets into recreational waters. Poorly treated wastewater from treatment plants, malfunctioning septic systems and boat discharges are sources of human fecal contamination. Bacteria can also be introduced directly into the bathing area from swimmers.

Will I get sick if I swim in waters under a swimming advisory?

Not necessarily, but you are at an increased risk.

What kind of illnesses can I contract from swimming in polluted waters?

The most common are diarrheal diseases that can be caused by bacteria, viruses, and parasitic protozoa. Ear, nose, throat, skin and respiratory infections are also commonly associated with swimming in contaminated water.

What should I do if I become ill after swimming?

If you develop diarrhea or an infection after swimming in North Carolina s coastal waters, seek medical treatment and then please contact the Shellfish Sanitation and Recreational Water Quality Section of the Division of Marine Fisheries at (252) 515-5613. The Recreational Water Quality Program s staff would like to know about any possible water-borne illness outbreaks as soon as possible to prevent more people from becoming ill.

How long does a swimming advisory stay posted?

If the advisory is issued due to the single sample maximum level, it will be re-sampled routinely. The sign will remain posted as long as the standard is exceeded. This means the result of an immediate resample may lift the advisory as quickly as 24 hours after posting. Once the geometric mean exceeds the

standard, the swimming advisory is not lifted until two consecutive weekly samples meet the EPA standard of 35 enterococci per 100 milliliters.

I have more questions - where can I go for answers?

If you have further questions about the Recreational Water Quality Program, you can call Erin Bryan-Millush with the Program at (252) 515-5613 or view the Programs Website at <u>https://www.deq.nc.gov/about/divisions/marine-fisheries/shellfish-sanitation-and-recreational-waterguality/recreational-water-quality</u>.

Printing of this document was funded entirely through a grant from the U.S. EPA.

STORMWATER DRAINPIPE SIGNS IN NORTH CAROLINA





What do these signs mean?

These signs are posted where stormwater pipes are discharging water into coastal swimming areas. They recommend that people do not swim within 200 yards on either side of the sign. You may see these signs at drainpipes in Carteret, Dare and New Hanover counties. The flow may not be visible if the mouth of the pipe is in the surf. If so, the sign on the top left will be posted.

People are advised not to swim in the area when the pipe has stormwater coming from it. The Recreational Water Quality Program's tests have shown that after rainfall, the runoff coming out of the pipe often exceeds state and federal standards for bacteria. Swimming in the waters near the pipe can cause an increased risk of illness.

What is stormwater runoff?

When rain falls, the water that isn't able to sink into the ground washes everything lying on hard surfaces (roads, driveways, roofs and parking lots) into pipes, some of which empty into coastal waters. The contaminants on the ground can include pet and wildlife waste, gas and petroleum products, pesticides and fertilizers. The state's Recreational Water Quality program tests for bacteria found in the intestines of warm-blooded animals, including people. If it is present in the water at high enough levels, people swimming or playing in the water run an increased risk of developing a gastrointestinal illness (diarrhea and/or vomiting) or a skin infection, particularly people with compromised immune systems. It is important to note that unlike some states, North Carolina does not have sanitary sewer outfalls discharging from pipes to our ocean beaches. Our stormwater collection systems are separate from the wastewater treatment systems and do not connect with those pipes.

Why are these signs displayed?

Past data show that stormwater tends to have high bacteria counts. Therefore, people are warned to swim away from the signs, so they don't expose themselves to an increased risk of illness. This way, if people see discharge coming from the pipe, they can play it safe and avoid swimming near it.

Is it okay for my children to play in the ponds and streams created by these pipes?

Some pipes discharge onto the beach sand, creating a pond or stream. Some parents like their children to play in these puddles or ponds because they think the children are safer away from the waves and current, but this is not a good idea. These ponds are different from natural tidal pools in that they contain all the pollutants of stormwater without the dilution effect of the ocean. Allowing children to play in them, particularly small children who may swallow water, exposes them to an increased risk of getting sick.

Will I get sick if I swim in waters under a swimming advisory? What kind of illnesses could I get from swimming in polluted waters?

Not necessarily, but you are at an increased risk. The most common illnesses are gastrointestinal diseases with symptoms such as diarrhea and vomiting. Ear, nose, throat, skin and respiratory infections are also commonly associated with swimming in contaminated water. If you become ill after swimming in North Carolina's coastal waters, seek medical treatment and then please contact us at the phone number below. We would like to know about any possible waterborne illnesses as soon as possible to prevent others from becoming ill. You may contact our office at

(252) 515-5613 or by e-mail at erin.bryan-millush@deq.nc.gov or visit us online at

https://www.deq.nc.gov/about/divisions/marine-fisheries/shellfish-sanitation-and-recreational-waterquality/recreational-water-quality

Printing of this document was funded entirely through a grant from the U.S. EPA.

APPENDIX 9: Laboratory Quality Assurance Plan NORTH CAROLINA

SHELLFISH SANITATION LABORATORIES

RECREATIONAL WATER QUALITY MONITORING QUALITY ASSURANCE PLAN

Effective June 2, 1997

Revised June 10, 2002 Revised May 15, 2003 Revised April 30, 2008 Revised May 5, 2009 Revised May 1, 2012 Revised Sept. 22, 2016 Revised March 15, 2017 Revised October 11, 2017 Revised April, 2021 Revised, May 2022 Revised, April 2024

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	Brain Heart Infusion Broth + 6.5% NaCl	
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1. INTRODUCTION

The **Quality Assurance Plan (QA plan)** is intended to add additional standardization to the Shellfish Sanitation and Recreational Water Quality laboratories and is designed to reduce errors caused by analysts, equipment, supplies and analytical methods. The full implementation of the plan will substantiate the validity of the data and produce results of high integrity.

All personnel with laboratory responsibilities will have an active role in the Quality Assurance Program with overall implementation the responsibility of the analyst-in-charge of individual laboratories.

The major components of the QA plan are quality control and

quality assessment. **Quality control** consists of a set of measures intended to identify and ensure proper functioning of materials, equipment and procedures which directly impact the quality of data. **Quality assessment** involves evaluation of both those established procedures and the implementation of those procedures.

Performance evaluations may be internal or external and are a means of asserting and validating the level of effectiveness of the **QA plan**. Results of evaluations will be maintained in each laboratory and in the Morehead City office files. The Food and Drug Administration and the State Laboratory of Public Health conduct laboratory evaluations of the Shellfish Sanitation and Recreational Water Quality Programs. The laboratories participate in NSSP Shellfish Split Sample Proficiency testing annually and Potable Water Proficiency testing for the State Laboratory of Public Health twice a year.

2. PERSONNEL CHART

Shellfish Sanitation and Recreational Water Quality Laboratory Management Structure

Erin Bryan-Millush

Laboratory Supervisor Recreational Water Quality Program Supervisor Quality Assurance Officer

Michele Barger

Environmental Tech. II

Melissa Squibb

Microbiological Laboratory Specialist Manager of Morehead City Laboratory

Holly White Microbiological Laboratory Technician Manager of Southern Region Laboratory

Candace Burrus

Microbiological Laboratory Technician Manager of Northern Region Laboratory

Assistants

Timmy Moore

Env. Specialist

Morehead City Lab

Zach Fasking Env. Tech II Morehead City Lab

Phil Piner Env. Tech II Morehead City Lab

Jason Hill Env. Specialist Morehead City Lab

> James Pace Env. Specialist N. Region Lab

Matthew Stokley Env. Tech II S. Region Lab

Lucas Edmonson Env. Specialist S. Region Lab

3. TRAINING REQUIREMENTS

The laboratory supervisor will be responsible for ensuring that all staff are adequately trained to perform the procedures assigned. Training will be provided by the laboratory Quality Assurance Officer. Trainees will be required to demonstrate proficiency prior to completion of training.

All personnel working in the laboratory will be trained in the following areas:

- preparation of media and reagents
- calibration of instrumentation
- sample preparation and inoculation
- laboratory safety
- quality control

4. GENERAL LABORATORY PRACTICES

The procedures and protocols of analyses are defined in <u>Laboratory Procedures for the</u> <u>Examination of Sea Water and Shellfish</u>, 4th Edition; <u>Bacteriological Analytical Manual</u>, 8th Edition, 1995; EnterolertTM-A Rapid Method for the Detection of Enterococcus spp., Idexx Laboratories, Inc., Westbrook, ME; <u>Method 1600</u>: <u>Enterococci in Water by Membrane</u> <u>Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar</u> (mEI) and <u>Standard</u> <u>Methods for the Examination of Water and Wastewater</u>, 20th Edition, 1998. General guidelines for laboratory practices are listed below.</u>

- a. Proper labeling, storage and/or disposal of supplies.
- b. Laboratory:
 - bench-tops will be disinfected before and after each use
 - work area adequate for workload and storage
 - work area clean and well lighted.
- c. Environmental contamination beyond laboratory control will be reported promptly to the Quality Assurance Officer and/or the Laboratory Supervisor.
- d. The laboratory staff is responsible for reporting equipment breakdowns to the Laboratory Supervisor.
- e. Laboratory staff will keep the laboratory prepared to handle all regularly schedule workloads at all times.
- f. A copy of the **Quality Assurance Plan** will be located in an accessible area for all personnel to obtain.
- g. Records are maintained in the Shellfish Sanitation and Recreational Water Quality Section quality assurance notebook.

5. <u>QA - MATERIALS</u>

5.1 Glassware

- a. All broken glassware will be discarded.
- b. Glassware will be of borosilicate non-fluorescent glass or other non-toxic material.
- c. Culture tubes and durham tubes will be non-fluorescent.
- d. Washing and sanitizing will be in accordance with procedures in <u>Laboratory</u> <u>Procedures for the Examination of Seawater and Shellfish</u>, 4th Edition.
- e. Inhibitory residue test will be performed after glassware is washed and dried using Bromothymol Blue solution, but only if detergents are used for washing glassware.

5.2 Pipets

- a. Will be borosilicate glass or other non-toxic disposable plastic.
- b. Pipets conform to the requirements cited in <u>Recommended</u> <u>Procedures for the</u> <u>Examination of Sea Water and Shellfish</u>.
- c. Sterility of reusable pipets will be determined after each sterilization.
- d. Efficiency of hot air drying oven will be tested with spore strips quarterly to ensure proper sterilization of pipettes.
- e. Sterility of disposable pipets will be determined for each batch.

5.3 Dilution Bottles

- a. Will be borosilicate glass or other non-toxic material.
- b. Graduation levels marked on side.
- c. Screw caps used with glass sample vessels. Use autoclave tape on the cap to show the sample vessels are sterile and also for labeling sample number.

5.4 Sample Bottles

- a. Will be borosilicate glass or suitable non-toxic material capable of being washed and sterilized.
- b. Will contain sufficient volume of sample for all required test plus adequate space to allow for effective shaking.

c. Will be checked for sterility after every sterilization.

5.5 Inoculating Equipment

- a. Wire loops may be used as recommended in <u>Recommended Procedures</u> for the <u>Examination of Seawater and Shellfish</u>.
- b. Single service disposable hardwood applicators may be used and will be 0.2 to 0.3 cm in diameter and at least 2.5 cm longer than fermentation tubes; sterilized in autoclave, dried in hot air oven and stored in stainless steel containers. Sterility of hardwood applicator sticks will be tested after each sterilization. See data sheet on page 27.
- c. Sterile disposable inoculating loops, 10 µl or 1µl may be used.

5.6 Dehydrated Media and Enterolerttm Reagent Snap Packs

- a. Stored as specified on the manufacturer's label.
- b. All media received is labeled with date received and date opened.
- c. Lot number and expiration date will be recorded for each batch of media.
- d. Dehydrated media which is discolored, caked, or expired is properly disposed (or sent back to central office for manufacture reimbursement).
- e. Enterolert reagent snap packs are discarded when expired or sent back to central office for manufacture reimbursement if the snap pack appears to be damaged and/or punctured and the contents appear to be discolored or caked.

5.7 Water

- a. Only deionized or distilled water is used for media and reagent preparation.
- Deionized water supply is monitored for conductivity using the Myron 750 Series II Digital Conductivity Controller to ensure that conductivity is less than 2 micro Siemens.
- c. Make up water will be tested monthly for pH (5.5 7.5), total residual chlorine (mg/L < 0.1) and bacterial level (<1000 CFU/mL) and annually for dissolved metals (Cd, Cr, Cu, Ni, Pb, Zn).
- d. Stored volumes of sterile, lab pure water are replaced frequently.

5.8 Media Preparation

a. Culture media preparation is according to the method of analysis and will be as

described in <u>Laboratory Procedures for the Examination of Seawater and Shellfish,</u> <u>5th Edition</u> and <u>Standard Methods for the Examination of Water and Wastewater</u>, 20th Edition.

- b. Membrane Filtration culture media preparation is according to <u>Method 1600</u>: <u>Enterococci in Water by Membrane Filtration</u> <u>Using membrane-Enterococcus</u> <u>IndoxyI-β-D-Glucoside Agar</u>, (mEI)
- c. SPC agar in flasks and Nutrient broth will be stored under refrigeration for a period not to exceed three months.
- d. All prepared media will be properly labeled with type and preparation date.

6. UNBUFFERED DILUTION WATER

- a. Deionized water is to be sterilized for 15 minutes at 121°C and stored at room temperature.
- b. Place deionized water in 500 mL flask with screw cap. Fill to below 450 mL graduation. Mark cap with sterilization tape and back off 1/4 turn.
- c. For storage of all deionized water, rotate flasks so that most recently prepared water will be used last. Write sterilization date on sterilization tape on cap.

7. MEDIA PREPARATION

7.1 ENTEROLERT[™] - used to enumerate Enterococci

One snap pack of reagent for 100 mL water sample. Order from IDEXX Laboratories, Inc., One

IDEXX Drive, Westbrook, Maine 04092 (1-800-321-0207).

7.2 mEl Agar – used to enumerate Enterococci

Combine ingredients and heat to boil for 1 minute in 4 L stainless steel beaker. Stir continuously to prevent burning and sticking of media. When completely dissolved and tempered in waterbath check pH, record and adjust by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Pour hot media equally into two 1 L flasks, to prevent boiling over in autoclave. Sterilize in autoclave at 121°C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.1 ± 0.2). Temper in waterbath. Once tempered add measured amount of Nalidixic Acid and Triphenyltetrazolium Chloride. Dispense 5 mL of warm agar into 9x50mm petri dishes and allow to solidify. Store upside down in a \leq 4°C refrigerator.

7.3 Nalidixic Acid – added to mEI agar to enumerate Enterococci

Ingredients: Nalidixic Acid – 0.48 g 10 N NaOH – 0.4 mL Deionizedwater – 10 mL

Combine ingredients and filter-sterilize. Add to tempered mEI agar at 5.2 mL per liter.

7.4 Triphenyltetrazolium Chloride (TTC) - added to mEI agar to enumerate Enterococci

Ingredients: Triphenyltetrazolium Chloride (TTC) – 0.1 g Deionizedwater – 10 mL

Combine ingredients and filter-sterilize. Add to tempered mEI agar at 2 mL per liter.

7.5 Brain Heart Infusion Broth (BHIB) - used in the colony verification process

Ingredients: Commercially prepared DIFCO[™] Brain Heart Infusion Broth (BHIB) – 37 g Deionized water – 1 L

Combine ingredients in 4 L stainless steel beaker. Stir and allow media to go into solution. When completely dissolved, check pH, record, and adjust if necessary by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense 9 - 10 mL of media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121°C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.4 ± 0.2). Cool at room temperature. Tighten caps and refrigerate.

7.6 Brain Heart Infusion Broth (BHIB) with 6.5% NaCI - used in the colony verification process

Combine ingredients in 4 L stainless steel beaker. Stir and allow media to go into solution. When completely dissolved, check pH, record, and adjust if necessary by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense 9 - 10 mL of media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121°C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.4 ± 0.2). Cool at room temperature. Tighten caps and refrigerate.

7.7 Brain Heart Infusion Agar (BHIA) - used in the colony verification process

Ingredients: Commercially prepared Brain Heart Infusion Agar (BHIA) – 52 g Deionized water – 1 L

Combine ingredients and heat to boil for 1 minute in 4 L stainless steel beaker. Stir continuously to prevent burning and sticking of media. When completely dissolved and tempered in waterbath check pH, record and adjust by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense 9 - 10 mL of hot media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121°C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.4 ± 0.2). Cool at room temperature with culture tubes at an angle, so agar solidifies as a slant. Tighten caps and refrigerate.

7.8 Bile Esculin Agar (BEA) - used in the colony verification process

Ingredients: Commercially prepared Bile Esculin Agar (BEA) – 64 g Deionized water – 1 L

Combine ingredients and heat to boil for 1 minute in 4 L stainless steel beaker. Stir continuously to prevent burning and sticking of media. When completely dissolved and tempered in waterbath check pH, record and adjust by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense 9 - 10 mL of hot media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121°C for 12 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 6.6 ± 0.2). Cool at room temperature with culture tubes at an angle, so agar solidifies as a slant. Tighten caps and refrigerate.

7.9 Nutrient Broth – used for routine QA check of sample collection bottles and pipettes

Ingredients: Commercially prepared DIFCO[™] Nutrient Broth – 8 g Deionized water – 1 L

Combine ingredients in 4 L stainless steel beaker. Stir and allow media to go into solution. When completely dissolved, check pH, record, and adjust if necessary. Dispense media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121°C for 12 to 15 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 6.8 \pm 0.2). Cool at room temperature. Tighten caps and refrigerate.

7.10 Standard Methods Plate Count Agar - used for routine QA check of DI system

Ingredients:

Commercially prepared DIFCO[™] Plate Count Agar – 23.5 g Deionized water – 1 L

Combine ingredients in 4 L stainless steel beaker. Stir to mix thoroughly. Heat to boil (using double boiler). Boil for 1 minute to completely dissolve media. When completely dissolved and tempered in waterbath check pH, record and adjust by adding 1 mL of 1M Sodium Hydroxide at a time until range is met. Dispense media into 150 mm X 20 mm screw cap culture tubes. Caps backed off 1/4 turn, label and date. Sterilize in autoclave at 121°C for 12 to 15 minutes. Remove from autoclave when chamber pressure reaches "0". Check pH, and record (range between 7.0 \pm 0.2). Cool at room temperature. Tighten caps and refrigerate.

8. QA - EQUIPMENT MAINTENANCE AND MONITORING

8.1 Thermometers

- a. Will be clearly marked and readable.
- b. Calibrated against NIST standards thermometer annually; labeled and dated at last calibration with ± temperature fluctuation.
- c. NIST standards thermometer calibrated annually by a certified laboratory.
- d. Ice point of NIST standards thermometer to be determined annually by certified laboratory.
- e. Will be properly immersed as required by manufacturer.
- f. Air incubator thermometers will be immersed in liquid.
- g. Graduated at 0.1°C intervals for incubators and water baths.

8.2 Air Incubators

- a. Shall maintain a uniform and constant temperature and must not vary more than \pm 0.5°C in the areas used.
- b. Will be of adequate capacity for work load.
- c. Twice daily temperature records maintained by analyst with at least 4 hours between measurements.

8.3 Water Bath

- a. Shall maintain temperature of $45.0 \pm 0.2^{\circ}$ C.
- b. Twice daily temperature records maintained by analyst with at least 4 hours between measurements.
- c. To be covered, and adequate water level maintained.
- d. Water mechanically circulated.
- e. Cleaned and disinfected quarterly (more often if needed).

8.4 Autoclave

- a. Will maintain a pressure range of 15 to 18 psi. during sterilization.
- b. Temperature maintained between 121-123°C for sterilization. Maximum registering

working thermometer to be used with every load and records maintained. Working thermometer to be checked against a NIST calibrated maximum registering thermometer yearly.

- c. Routine cleaning weekly to include drain trap.
- d. Spore suspensions (*Bacillus stearothermophilus*) will be used monthly to evaluate the effectiveness of the autoclave sterilization process and results recorded. Heat sensitive tape will be used with each autoclave use.
- e. NIST calibrated maximum registering autoclave thermometer to be re-calibrated every 5 years.
- f. Autoclaves will be serviced annually, or as needed, by a qualified technician and records maintained.
- g. Autoclave cycle to be timed quarterly.

8.5 Balances

- a. Provide a sensitivity of 0.1 g at a load of 150 g.
- b. Calibrated monthly using NIST class S weights.
- c. Calibration records maintained.

8.6 pH Meter

- a. Have standard accuracy of 0.1 pH unit.
- b. Calibrated with 2 buffers that include correct pH range of medium being prepared. Choosing between 3 standard buffers of 4.0, 7.0 and 10.0. Standard buffer solutions are used fresh daily.
- c. Electrode efficiency determined with each use (record slope after calibration).
- d. Calibration log to be maintained.

8.7 Hot Air Drying Oven

- a. Temperature for sterilization of glassware will be maintained at 170° C.
- b. Spore strips (*Bacillus subtilis*) will be used quarterly to evaluate efficiency of sterilization.

9. QUALITY CONTROL CHART

APPARATUS	TEST	FREQUENCY
Thermometers	NIST Calibration	Annually
Water System	Conductivity	Monthly
	Chlorine	Monthly
	рН	Monthly
	Standard Plate Count	Monthly
	Heavy Metals	Annually
Autoclave	Maximum registering temp.	Each use
	B. stearothermophilus	Monthly
	Time Cycle	Quarterly
Balance	Calibration –	Monthly
	NIST Class S weights	
pH Meter	Calibration – 2 out of 3 buffers	Each use
	Electrode efficiency	Each use
Incubators	Record temperature	Twice daily
Waterbaths		
Refrigerators		
Drying Oven	Record temperature	Each use
	Spore strips	Quarterly
Glassware	Pipette sterility	Each sterilization and Monthly
		in use
	Bottle sterility	Each sterilization and Monthly
		in use
Plastic ware	Disposable pipette sterility	Each batch
	IDEXX vessel sterility	Each lot number
Sterilized deionized water	Water sterility	Each sterilization

10.ANALYSES PERFORMED

PROCEDURES FOR THE ENUMERATION OF ENTEROCOCCUS SP. IN SEAWATER

- 10.1 <u>Enterococcus</u> <u>sp.</u> detection using Enterolert[™] reagent fluorescence method.
- a. Pour sterile deionized water into a sterile, transparent, non-fluorescent, borosilicate glass vessel to the 90 mL fill line. Repeat for the number of samples to be processed. Also fill three additional vessels to the 100 mL fill line for quality control.
- b. Label the autoclave tape on top of the sterilized sample vessels in consecutive numerical order and the quality control vessels as media, E.f. (*Enterococcus faecium* positive control), and S.m. (*Serratia marcescens* negative control). Inoculate the controls.
- c. Number and date the 51-well Quanti-Trays[™] on the paper side in the same consecutive order as the sample vessels. Label the control trays as above and include the date.
- d. Count number of Enterolerttm snap packs to be used. Carefully separate the snap packs.
- e. Up to 30 minutes prior to the receipt of samples in the lab, add the contents of one Enterolerttm snap pack to each glass sample vessel containing 90 mL sterile deionized water. Shake to completely dissolve reagent. Un-dissolved reagent particles may cause green fluorescence in Quanti-Tray wells after incubation. Hold at room temperature.
- f. Turn on the Quanti-Tray[™] Sealer at least 10 minutes prior to use.
- g. Seawater samples kept on ice or refrigerated at or below 10°C before examination. Temperature control collected in field by samplers and examined when received in laboratory. Samples will be processed within 6 hours of sampling.
- h. Samples are to be shaken 25 times within 7 seconds in a 1 foot arc.
- i. 10 mL from water sample bottle is pipetted into a glass sample vessel containing 90 mL sterile deionized water and Enterolert media, making the dilution for marine waters. Cap and mix well before transferring to the Quanti-Tray. Repeat procedure for all samples and controls.
- j. After reagent has dissolved, pour sample into Quanti-Tray[™] following Enumeration Test Procedure 1 through 4 from IDEXX brochure.
- k. Place sample filled Quanti-Tray[™] onto rubber tray carrier with plastic well side down. Insert carrier and tray into Quanti-Tray[™] Sealer and remove from Sealer once the tray is sealed. Repeat for all samples and controls.
- Incubate at 41°<u>+</u> 0.5° for 24 hours. Do not stack trays more than 5 high and leave space between stacks in the incubator. It is critical to maintain correct and stable temperature throughout the entire incubation time to prevent false positives from occurring in Enterolert.

- m. During incubation, if certain samples are suspected to yield high counts, samples can be removed briefly at the 20-hour mark for examination of positive fluorescent wells, at least 95% of growth will have taken place (Gil Dicter, IDEXX). Samples must be immediately returned into the incubator for the remaining incubation period. It is important to keep this practice to a minimum to reduce the risk for false positive or false negative results. "This indicator bacterium will exponentially double every 20 minutes (bacterial growth curve) and if present even at levels as low as 2/100ml will fluoresce under 365 nm UV light in less than 24 hours and fluorescence can be observed at 20 hours. Trays can be observed for the presence or absence of fluorescence prior to 24 hours and then placed back into the incubator for total time of 24 hours and should have no detrimental effect on the final results. If all the wells fluoresce at that time further incubation is not required." By Gil Dicter, IDEXX Labortories.
- n. Final examination of Quanti-Trays should be conducted at the 24-hour time period. Under incubation (read too early) would cause positive wells to be too weak to read. Over incubating (read too late) would increase the chances of false positives.
- o. Look for fluorescence with a 6-watt, 365-nm, UV light within 5 inches of the sample in a dark environment. Face the light away from your eyes and towards the sample.
- p. When reading results from marine water or brackish water samples, it is not recommended to leave the samples on the bench exposed to light or under the UV lamp too long before collecting results.
- q. Count positive (fluorescent) wells and refer to the 51-well Quanti-Tray[™] MPN table for results. The MPN value is multiplied by a factor of 10 due to the 1/10 dilution made for marine waters.

Result Interpretation

Lack of fluorescence = negative for entercocci Blue fluorescence = positive for enterococci

- 10.2 <u>Enterococcus sp.</u> detection using <u>Method 1600</u>: <u>Enterococci in Water by</u> <u>Membrane Filtration Using membrane</u>-<u>Enterococcus IndoxyI-β-D-Glucoside</u> <u>Agar</u>, (mEl)
 - a. Mark petri dishes containing mEI Agar with sample identification and sample volume on the back-side of the dish containing agar.
 - *b.* Place a sterile membrane filter on the filter base, grid-side up and attach the funnel to the base so that the membrane filter in now held between the funnel and the base. *NOTE: When using a new funnel, the same funnel may be used for multiple volumes for the same site starting with the smallest volume first.
 - c. Shake sample bottle 25 times to distribute the bacteria uniformly and measure the desired volume of sample into the funnel, starting with the smallest volume first. Leave pipette in sample bottle for distributing of

additional volumes. It is not necessary to shake sample between concurrent sample volumes, use pipette to stir sample by drawing up and down with pipette aid twice before measuring sample volume. Current volumes as of April 2021 are 50mL and 100 mL. **NOTE: When analyzing smaller volumes (e.g., <20mL), add 20-30 mL of PBS to the funnel. This will allow even distribution of the sample on the membrane.*

- d. Filter the sample, and rinse the sides of the funnel at least twice with 20-30 mL of sterile buffered rinse water. Turn off the vacuum and remove the funnel from the filter base.
- e. Use sterile forceps to aseptically remove the membrane filter from the filter base, and roll it onto the mEI agar to avoid the formations of bubbles between the membrane and the agar surface. Reset the membrane if bubbles occur. Run the forceps around the edge of the filter outside the area of filtration, close to the edge of the dish, to be sure that the filter is properly seated on the agar. Close the dish, invert, and place into a tight fitting plastic container.
- f. Process controls to accompany samples throughout incubation.
 - I. Media sterility: place one plate of sterile mEI agar into plastic container
 - *II.* Method blank: measure 50 mL of sterile PBS and follow steps d. and e. *NOTE: you can use the same filter funnel used for the Method blank for the Negative control.
 - III. Negative control: inoculate 100 mL of PBS with *E. coli* from dilute suspension. Shake to mix and follow steps d. and e., dispose funnel.
 - IV. Positive control: inoculate 100 mL of PBS with 1 BioBall[™] containing *E. faecalis*. Shake vigorously 25 times to dissolve, allow contents to settle for ~5 minutes then using a new filter funnel, pour contents in filter funnel **NOTE: you may need to rinse inside of dilution bottle and pour additional contents into filter funnel to ensure all cells are filtered*, then follow steps d. and e.
 - V. When using the UV sterilizer, be sure to process an extra method blank for every 15 samples processed.
- g. Place plastic container with inverted plates in air Incubator at 41°C ± 0.5°C for 24 ± 2 hours. Record time placed in air incubator on sampling sheet.
- h. Using refractometer, read salinity of each sample and record on sampling sheet.
- i. Record sterilization date of mEI agar used for analysis on sampling sheet.
- j. After incubation count and record colonies on those membrane filters containing the acceptable range of 20-60 colonies ≥0.5 mm in diameter with a blue halo regardless of colony color. Refer to Method 1600 EPA-821-R-09-016 appendix B: C.3.6
 - I. If all membrane filters are below the acceptable range of 20-60 colonies, select the most nearly acceptable count or counts (if two are more counts are equal but have different volumes than take the average of those numbers as the final CFU and record result on sampling sheet.
 - II. If counts from all membrane are zero, calculate using count from largest filtration volume. Calculate the number of colonies per 100 mL that would have been reported if there was one colony on the filter representing the largest filtration volume. Report this result as a less than value.
- k. Results are reported as CFU/100 mL of sample.

Result Interpretation

Colonies with a blue halo regardless of colony color = positive for enterococci

Verification Procedure

Colonies ≥0.5 mm in diameter of any color having a blue halo after incubation on mEI agar are considered to be "typical" enterococci colonies. Verification of colonies may be required in evidence gathering and it is also recommended as a means of quality control. The verification procedure follows.

- a. Using a sterile inoculating loop or needle, transfer growth from the centers of at least 10 well-isolated typical and at least 10 well-isolated atypical colonies into a BHIB tube and onto a BHIA slant. Incubate broth for 24 ± 2 hours and agar slants for 48 ± 3 hours at $35^{\circ}C \pm 0.5^{\circ}C$.
- b. After a 24-hour incubation, transfer a loopful of growth from each BHIB tube to BEA, BHIB, and BHIB with 6.5% NaCl.
 - I. Incubate BEA and BHIB with 6.5% NaCl at $35^{\circ}C \pm 0.5^{\circ}C$ for 48 ± 3 hours.
 - II. Incubate BHIB at $45^{\circ}C \pm 0.5^{\circ}C$ for 48 ± 3 hours.
- c. Observe all verification media for growth.
- d. After 48-hour incubation, perform a Gram stain using growth from each BHIA slant.
- e. Gram-positive cocci that grow and hydrolyze esculin on BEA (i.e., produce a black or brown precipitate), and grow in BHIB with 6.5% NaCl at 35°C ± 0.5°C and BHIB at 45°C ± 0.5°C are verified as enterococci.

11.LAB FORMS -

- (1) Reference Master Lab Sheets on shared drive for the current up-to-date documents used to verify quality control and quality assurance on items used to process recreational water quality samples.
 - a. LBS 1 Temperature Records ALL
 - b. LBS 2 Bromothymol Blue Test
 - c. LBS 5 Autoclave Sterilization Record
 - d. LBS 8 Oven Drying Pipettes