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PROCEDURE FOR THE COLLECTION OF SEAWATER SAMPLES FOR BACTERIOLOGICAL EXAMINATION TO BE USED IN THE CLASSIFICATION AND MONITORING OF SHELLFISH GROWING WATERS

Introduction

The Connecticut Department of Agriculture, Bureau of Aquaculture (DOAG) is the lead agency on shellfish in Connecticut with the authority to classify shellfish growing areas and enact closures. The DOAG uses the guidelines and standards set forth by the National Shellfish Sanitation Program Model Ordinance (NSSP-MO) to classify and manage Connecticut's shellfish growing areas. *Growing area classifications are based on evidence of contamination found through sanitary surveys, which are performed every 12 years.* The survey identifies all actual and potential sources of pollution that may adversely impact the growing area, evaluates meteorological and hydrographic factors, and assesses water quality data. Properly classified shellfish growing areas ensure that shellfish are not harvested from areas contaminated with bacteria, viruses, and/or poisonous or deleterious substances.

In addition to an up-to-date sanitary survey, growing area classifications are supported by routine water sampling from established locations throughout Long Island Sound. Due to the extent of required sampling, the DOAG trains volunteers from municipal shellfish commissions to collect the required water quality monitoring samples for their programs. Certified volunteers coordinate sample collections with DOAG staff and deliver pre-approved samples to the Bureau of Aquaculture Laboratory in Milford or the Department of Public Health Laboratory in Rocky Hill.

Volunteer Eligibility

Members of municipal Shellfish Commissions, or other municipal Commission or Department responsible for shellfish, can be eligible to become a volunteer sampler. DOAG requires that all requests for volunteer training come from the Shellfish, or other municipal, Commissioner. No individual who has not been certified, or otherwise pre-approved, may collect seawater samples.

Please note that sampling for the majority of Connecticut growing areas is conducted year-round, with at least one collection per month. If you cannot commit to winter or year-round sampling, you may still become a volunteer, but you cannot be the sole volunteer for a Commission.

Volunteer Training Requirements

Volunteers are required to complete a two-part training consisting of an online component and a field site certification with a DOAG Analyst. The online training consists of a self-guided presentation outlining sampling, equipment, and laboratory requirements and includes an associated quiz. The in-field training will be available to Commissions annually at their request. Individuals who have not successfully completed the online training component are not eligible for field training. Analysts will evaluate each volunteer, who will be issued a certificate, if eligible. Certification is only required once per volunteer.

Volunteers should be prepared for several hours on the water. The in-field certification will consist of a full sample collection of the required seawater monitoring stations. Analysts will discuss the classifications of each area being sampled, the rationale behind the station locations, and identify potential sources of

pollution to the growing area. Training includes a review of the accurate completion of all required documentation.

In-field Certification Policy and Procedures

- Field certifications will be coordinated with DOAG Analysts and the Municipal Commission.
- Notify Analyst staff if you are missing any required supplies and they will be provided at the time of the training.
- All volunteers being trained in-field must first successfully complete the online training component.
- A DOAG Analyst will conduct the field certification to review sampling procedures and observe sample collection technique.
- The DOAG Analyst conducting the field training will transport the samples to the laboratory in Milford upon completion of the training. Commissions will be responsible for sample delivery under all other circumstances.
- Once both the online and in-field training are successfully completed, the DOAG will issue a certificate to the volunteer(s).

General Overview of Sampling Requirements

- Samples must always be collected during low or ebbing tidal conditions. Sample collection may begin 1.5 hours after high tide and can continue through 1.5 hours after low tide. Samples collected during high tides will not be used in data analysis for shellfish growing waters.
- Volunteer samplers must be prepared to commit to doing a full sampling run for their area(s), including sample delivery to the lab. A Commission can elect to use a designated courier at their own expense, but the courier must accept the samples directly from the Commission in a cooler(s) on ice and meet the time constraints for sample delivery/processing.
- Direct shellfish harvesting only occurs in Approved and Conditionally Approved areas, as classified by DOAG, when in the open status. Harvesting does not occur in Restricted Relay, Conditionally Restricted Relay, or Prohibited areas.
- There are three types of sample runs. Open runs are only used for Conditionally Approved areas, demonstrate that water quality is acceptable while the area is in the open status year-round, and are described below. Adverse pollution condition (APC) runs are used for Conditionally Approved and Approved areas, demonstrate water quality trends when an area is adversely impacted by rainfall, and are described below. Reopening runs are required for Approved and Conditionally Approved areas following rainfall events, unacceptable sample results, sewage bypasses, or other emergency closures.
- The DOAG manages each Conditionally Approved area with a specific rainfall trigger that dictates when areas need to be closed. A town may have multiple Conditionally Approved areas with different rainfall triggers. All Approved areas are precautionarily closed following rainfall events $\geq$3.0". Please note that when an area is closed, a subsequent rain event over an area's rainfall trigger will restart the closure timeframe, such that the closure will be extended by an additional 14 days without reopening samples.
- Following a closure, the municipality must collect reopening water and/or shellfish tissue samples to reopen their Conditionally Approved or Approved areas prior to the NSSP-MO required 14 day closure period. Additional sampling will also be required to reopen an area that has been closed due to unacceptable sample results, sewage bypass, or other emergency closure conditions.

- Inadequate open and APC sampling, or failing water quality results, will result in an Administrative closure or classification downgrade by DOAG.

Conditional Area Open Sampling Run Criteria

Conditionally Approved areas are managed by DOAG and town officials under a Management Plan. *At a minimum*, a full sampling run is collected once per month year-round while the area is in the open status. If the Commission is unable to collect an open run, they must collect a make up open run the following month.

Please note that the DOAG manages some Conditionally Approved areas with a seasonal closure during the active boating season or when there is a known seasonal water quality decline.

Adverse Pollution Condition (APC) Sampling Run Criteria

The collection of APC samples is required from every Approved and Conditionally Approved area station. In Connecticut, APC are defined as samples collected on day 0-4 following a rainfall events ≥0.5" measured by the official rain gauge for the area, with day 0 being the day the rainfall event ends. APC sample runs can be collected when the area is in the open or closed status.

A minimum of 5 APC samples from every station is required annually. If 5 APC samples can not be collected in a calendar year, additional APC sampling to make up the difference will be required the following year so that the 3 year total APC count at each station equals 15.

Please note that the failure to collect the required number of samples from your growing areas may result in Administrative closure.

Sample Scheduling

Municipal Shellfish Commissions are responsible for tracking their sampling to ensure that requirements for open and APC sampling are met annually. DOAG Analysts will provide periodic updates to ensure that sampling goals are met.

Each Commission will have a standard number of stations for each collection that is provided by DOAG. Please note that you may be occasionally asked to modify your routine collection. Unless an area is being actively sampled for a potential upgrade via discussions with DOAG Analysts, no Restricted or Prohibited stations are to be collected by volunteers.

DOAG Laboratory, Milford, CT

- Commission designees shall schedule all sample collections **at least 24 hours** in advance during normal business hours with a DOAG Analyst or laboratory personnel. 24 hours in advance does not include weekends when staff are not routinely working.
- No samples may be collected on Fridays or Saturdays. Sample delivery to the DOAG is limited to Monday-Wednesday from 8:00 a.m. to 4:30 p.m. and Thursday from 8:00am to 1:00 pm. No samples will be accepted after 1:00 pm on Thursday.

DPH Laboratory, Rocky Hill, CT

- Commission designees shall schedule all sample collections **at least 24-36 hours** in advance with DPH lab staff. Again, 24 hours in advance does not include weekends.
- Please submit the DOAG Seawater Collection Form with the samples. The DPH laboratory will provide you with additional chain of custody paperwork to fill out.

- No samples may be collected on Fridays or Saturdays. Sample delivery to the DPH laboratory in Rocky Hill is limited to Monday-Thursday from 8:00 a.m. to 1:00 p.m. No samples will be accepted after 1:00 p.m. on Thursday.

All sample plans for the following week should be submitted to DOAG *by Fridays at noon*. Samples scheduled over a weekend for the following Monday may be rejected by the lab.

Equipment

- 125 ml single-use sterilized plastic specimen bottles supplied by the DOAG in Milford, or 125 ml reusable plastic bottles provided by the CT Department of Public Health (DPH) laboratory in Hartford for collecting seawater samples.
  The use of alternate bottles is not allowed. Any samples submitted for bacteriological sampling using unauthorized containers will be rejected.
- Use a water-sampling tool consisting of a four (4) foot, or longer, handle with a clamp or holder at one end that securely holds a sample bottle in a vertical position, minimizing contact with mouth of the bottle and threads. A mark on the stick positioned at 18" above the mouth of the bottle indicates the depth of sample collection.
- Cooler to hold samples. Place ice and frozen ice packs in cooler to cool and maintain the temperature of the samples to 50^0 F (10^0 C), or less, until refrigerated or delivered to testing laboratory.
- Tide chart for the area being sampled.
- GPS unit or nautical chart of area showing locations of sampling stations established by DOAG.
- Shellfish seawater analysis laboratory collection forms supplied by DOAG, DPH Laboratory or private laboratory, respectively.
- Disposable gloves as needed (recommended when working in areas suspected of being contaminated by sewage).

Sample Collection

- Using a waterproof pen, record the seawater monitoring station number on the label located on the side of the bottle. The monitoring station number consists of the Town's state tax number followed by a dash (-) and DOAG's assigned station number.
- Collection bottles must be properly stored to protect them from contamination. Do not use bottles that have been submerged in water or are visibly soiled. Bottles with loose or missing caps, soap film deposits or any other extraneous particles found in the interior of the bottle, severely deformed (concave) or cracked bottles, or bottles lacking labels should be returned to the Laboratory and not used to collect samples. Contaminated bottles may be used for the temperature control.
- Samples should be collected from stations where the water depth is at least three feet. If there is less than three feet of water at the station location, note "shallow water" under "remarks" on the laboratory collection form.
- Do not break ice to collect a sample. If a location is iced over, note that on the laboratory collection form, do not collect the sample, and move to the next location. If there is ice floating in the water at a location, try and keep the ice from entering the sample bottle.
- If wading in to collect samples, avoid disturbing the sediment as much as possible and allow adequate time for it to settle before collection. Sediment in the sample can result in elevated, unrepresentative fecal coliform levels.
- At the first station take two samples, label one "TC" (temperature control) and label the other with

the station number. The "TC" sample will be handled the same as other samples, but will only be used to indicate the temperature of the water samples when they arrive at the laboratory.

- Place the capped bottle in the sampling tool with the bottle opening facing up, then remove the cap. Do not contaminate the interior of the bottle, cap or cap threads at any time.
- Using the sampling tool, quickly plunge the bottle below the surface of the water to a depth of at least 12 inches. When sampling by boat avoid collecting boat wash or bilge water. Always collect samples upstream, or water that is flowing toward you.
- Remove the sampling tool from the water. Make sure that there is at least a ½ inch air space at the top of DOAG single use sterile bottles. If necessary, pour water out of sample bottle. NOTE: A one-inch air space is needed for Public Health Department laboratory reusable bottles. **This air space is critical for proper mixing of the water sample in the laboratory.** At least 100 ml of water is needed for DOAG analysis. As a guide, fill DOAG bottles just below the bottom edge of the bottle cap and just below the "neck" of the Health Department reusable bottles
- Replace the cap <u>securely</u> on the bottle, *then remove* the bottle from the clamp. Promptly place bottle in the cooler with ice and ice packs.
- If a sample bottle is damaged during collection such that a crack is visible, collect another sample with a new bottle following the previous steps.
- Record the station number, time of collection (military time), and note any potential sources of bacteria, or anything else unusual (i.e. birds, water running out of pipes, suds in water, dredging operations, sewage-like odor, etc.)
- Keep bottles in upright position to prevent loss of sample and/or submersion in ice melt.

Samples will be rejected under the following circumstances:

- The lab may reject samples that were not planned in advance.
- Samples collected by untrained and uncertified individuals.
- Samples delivered without a temperature control OR with a temperature control $>50^0$ F (10^0 C).
- Samples delivered with inadequate processing time. Samples must be <u>processed</u> *within 30 hours of the first sample collected.*
- Bottles that are cracked or broken, have a leaking or mis-threaded cap, or have the seal in the bottle or threads.
- Frozen samples or samples with visible ice crystals in them.
- Samples without an adequate air gap.

The DOAG cannot use any sample data processed by a laboratory that is not NSSP standardized. Currently, only the DOAG and DPH labs are standardized for the shellfish program.

<u>Contact Information</u>

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