

APPENDIX 4

EC MARINE WATER QUALITY SAMPLING PROTOCOL

1. Introduction:

All water samples for bacteriological analyses are collected in sterile 250 mL wide-mouthed bottles (polypropylene or glass) at a depth approximately 20cm below the water surface. All water samples collected are held in an insulated cooler on ice or ice packs.

Prior to sampling, the receiving laboratory has been contacted at least 24 hours in advance to confirm sample delivery time and analysis within prescribed limits. All equipment and supplies are prepared prior to leaving. These include sample bottles, coolers, sampling rod, GPS, chart, sampling station maps with descriptions, field thermometer, pencils, markers, field book, and watch.

Sampling is carried out under various environmental conditions including adverse weather such as heavy periods of precipitation, dry conditions, and different tidal stages. Sampling stations are located through GPS or triangulation and/or using sampling station maps and descriptions. Hydrological conditions including surface water temperature and tidal cycle are recorded as well as meteorological conditions. During sample collection, all relevant information is recorded in a field book. This would include all the items listed in under 7. Sample Collection Information Recorded in Field Book below.

A temperature blank is used to determine the temperature at time of collection (measured in field) and at the time of delivery (measured by laboratory). All sample bottles are cleaned, rinsed with distilled water, sterilized and kept closed until utilized. All samples are identified with location and a sampling station number. After collection, sample is immediately placed in a clean cooler which is maintained between 0°C and 10°C. Samples are delivered to the approved laboratory within 6 hours of collection of the first sample. Samplers ensure that, prior to delivery to the laboratory, all sample bottles and field books are checked for complete and accurate information.

2. Safety Precautions:

Field staff must undergo appropriate boating and vehicle safety training.

- 2.1 All microorganisms must be treated with caution and are to be considered hazardous. Aseptic technique is required. All samples/ sample containers not identified as “sterile” should be treated as potential hazards and may contain pathogenic microorganisms.
- 2.2 Eating and Drinking are PROHIBITED while sampling.
- 2.3 Technicians must handle samples with caution following the assumption that all samples are potential hazards.
- 2.4 Coolers must be disinfected before and after each use.
- 2.5 Technicians must wash hands effectively, as soon as possible, after handling the samples.

- 2.6 Open cuts/ sores must be bandaged to prevent accidental infection; these bandages should be changed frequently.

3. Apparatus:

- 3.1 Insulated cooler with ice/ice packs
- 3.2 250ml wide-mouth sterile sample bottle (polypropylene or glass)
- 3.3 Sampling rod with thermometer or with stand alone armor-cased thermometer
- 3.4 Field log book
- 3.5 Sampling map
- 3.6 Watch with 24 hour format
- 3.7 Label Tape for Bottles
- 3.8 Pencils, Indelible waterproof felt pens
- 3.9 GPS, Compass
- 3.10 Applicable Personal Protective Equipment (PPE)

4. Interferences:

- 4.1 Sample bottles must remain closed at all times (opened only just prior to use) to decrease probability of contamination. Occurrences of the cap becoming loosened or removed unintentionally will increase the chance of cross contamination of the sample and it will not be truly representative of natural environment. Caps should be held with open end facing down to reduce the risk of air contamination. All accidentally opened bottles should not be used, but returned to the lab for re-sterilisation. If lots of bottles are found to have numerous loose caps, the supervisor should be notified immediately and corrective action taken as soon as possible.
- 4.2 Sample bottles must be kept in an insulated cooler between 0⁰C and 10⁰C. If samples do not arrive at the laboratory between 0 and 10⁰C, the laboratory supervisor should be notified immediately. Corrective action such as continued holding on ice / ice packs or refrigeration may be employed if time permits, however explanations of non-conformances are to be recorded on the data sheets
- 4.3 Samples must be delivered to laboratory within 6 hours of collection of first sample to allow for 2 hours of processing. Samples outside this parameter are recorded on sample data sheets. No samples will be processed if beyond 24 hours after collection.
- 4.4 If sampling location is deemed inaccurate, another sample will be taken in appropriate location.

5. Procedure:

- 5.1 Water Quality Sampling Protocol
 - 5.1.1 Prepare a clean, insulated cooler with an appropriate number of sterile, labelled, sample collection bottles and enough ice or ice packs suitable to maintain samples in the range of 0°C – 10°C.

- 5.1.2 In the field log book, record growing area identification, date of survey, sampler's names, current weather conditions (wind, air temperature, sun, % cloud cover), tidal state, and sample station numbers.
- 5.1.3 Navigate to sample station locations using the station map, station descriptions, and GPS / compass as required to ensure accuracy and consistency.
- 5.1.4 Upon reaching sample station, select a pre-labelled, sterile sample bottle. Insert bottle into the sampling rod and dip into seawater to rinse. Remove and hold bottle cap with open side facing down, ensuring that its interior does not contact any surface.
- 5.1.5 Plunge the sampling rod into undisturbed water up to the 20 cm mark. Allow several seconds to fill and withdraw sample. Pour off excess water to allow for an approximate 1 inch air space for subsequent agitation in the laboratory (Maintain a minimum of 200 ml sample size).
- 5.1.6 Replace the bottle cap aseptically ensuring that neither its interior, nor the bottle neck contacts any surface. Place bottle in cooler ensuring ice or ice packs do not contact bottle cap.
- 5.1.7 Observe temperature from integrated thermometer or, in the case of separate tank thermometer, plunge to a 20 cm depth, then observe.
- 5.1.8 In the field log book, record sample time, water temperature, and any other relevant observations such as presence of birds, boat / cabin activity, high flow rates, spills, or other possible pollution sources (See Appendix A).
- 5.1.9 Repeat steps 5.1.3 through 5.1.8 for all sample stations.
- 5.1.10 An extra sample is to be taken at the first sample station to serve as a temperature control for later laboratory use. Label as 'temperature control' or TC.
- 5.1.11 After completion of sampling run, transport all samples to the laboratory within a 6 hour time frame beginning with time of first sample. Ensure that adequate ice / ice packs are in place to keep temperatures between 0°C and 10°C.
- 5.1.12 Upon return to laboratory, transfer custody to laboratory staff and ensure that information from field log book is copied to the laboratory data collection sheet and on the Sample Log In Sheet. It is very important to remember to use aseptic technique to collect and process samples and to show accountability.

6. Acceptance Criteria and Corrective Actions:

- 6.1 Temperature control must be within 0°C and 10°C. Discarding of samples depends on the temperature trend of the sample and if transport time was insufficient to cool samples to the accepted parameter (i.e. did temperature of sample decrease during transport). If samples are deemed out of compliance by the laboratory supervisor, they are discarded.
- 6.2 All non conformances must be reported to the lab supervisor immediately.

7. **Sample Collection Information Recorded in Field Book:**

- date of each survey
- growing area identification
- name of location or site
- state of tide
- station number
- time of each sample
- water temperature (at each station)
- water temperature blank
- precipitation in last 24 – 48 hours
- sun (% cloud cover)
- wind: direction and speed
- air temperature (optional)
- height of waves(optional)
- turbidity (optional)
- other potential pollution sources (birds, anchored vessels, marine mammals, etc.)
- sampler's names

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