Section 5
Water Chemistry and Microbiology

Alan T. Herlihy[1] and Charles W. Hendricks[2]

There are two components to collecting water chemistry information: Collecting samples of stream or river water to ship to the analytical laboratory, and obtaining in situ measurements of specific conductance, dissolved oxygen, and temperature. At each river, teams fill one 4-L container and two 60 mL syringes with river water. These samples are stored in a cooler packed with plastic bags filled with ice and are shipped or driven to the analytical laboratory within 24 hours of collection (see Section 3). The primary purposes of the water samples and the field chemical measurements are to determine:

- Acid-base status (Acid Neutralizing Capacity, ANC)
- Trophic condition (nutrient enrichment)
- Chemical Stressors
- Classification of water chemistry type.

[1]Department of Fisheries and Wildlife, Oregon State University, c/o U.S. EPA, 200 SW 35th St., Corvallis, OR 97333.
[2]U.S. EPA, National Health and Environmental Effects Research Laboratory, Western Ecology Division, 200 SW 35th St., Corvallis, OR 97333.

Water from the 4-L bulk sample is used to measure the major cations and anions, nutrients, total iron and manganese, turbidity and color. The syringe samples are analyzed for pH and dissolved inorganic carbon. Syringes are used to seal off the samples from the atmosphere because the pH and dissolved inorganic carbon (DIC) will all change if the streamwater equilibrates with atmospheric CO2. Overnight express mail for these samples is required because the syringe samples need to be analyzed, and the 4-L bulk sample needs to be stabilized (by filtration and/or acidification) within a short period of time (72 hours) after collection.

In situ measurements are made using field meters and recorded on standard data forms. Specific conductance (or conductivity) is a measure of the ability of the water to pass an electrical current which is related to the ionic strength of a solution. Dissolved oxygen (DO) is a measure of the amount of oxygen dissolved in solution. In natural waters, minimal concentrations of oxygen are essential for survival of most aquatic organisms. Measures of DO and temperature are used to

assess water quality and the potential for healthy aerobic organism populations. Most of the procedures outlined in this section are similar to the ones utilized by the EPA in streams for the National Surface Water Survey (Kaufmann et al., 1988) and have been adapted from the Survey's field operations handbook (U.S. EPA, 1989).

5.1 Microbial Sampling

Separate samples are collected for the culture of bacteria that are present in water. The organisms are living entities and respond to fluctuations and nutrient stress just like higher forms. They may be native to the water or introduced to the stream or river by the addition of human or animal contamination. We are currently using culturing procedures that can differentiate between the two events based on relative numbers of bacteria of cultured bacteria.

Samples of stream and river water are to be taken in laboratory prepared sterile, glass bottles. These square bottles are designed for sample collection, transit and storage, and no other container should be used for bacteriological samples. Generally one (1) sample is collected per site, but, duplicate (or triplicate) samples from the same site may be requested for statistical purposes. Occasionally, two (2) or more samples at specific sites along a stream or river reach may be requested. The number of samples to be taken will be noted on the field activity sheets.

Ideally, microbiological examination of a water sample should begin promptly after collection to avoid unpredictable changes in the numbers of organisms present in the sample. If samples cannot be processed within 1 hour after collection, an iced cooler for storage during transport (overnight express mail) to the laboratory must be used. All microbiology samples are to be iced during transport and refrigerated in the laboratory, while awaiting analysis within 24 hours of collection.

5.2 Sample Collection

Before leaving the base location, package the sample containers (one 4-L cubitainer, two 60 mL syringes, and two 200 mL sterile glass bottles) and the stream sample beaker to prevent contamination (see Section 3). Fill out a set of water chemistry and microbial sample labels as shown in Figure 5-1. Attach a completed label to the cubitainer, each syringe, and each glass bottle and cover with clear tape strips as described in Section 3. Make sure the syringe labels do not cover the volume gradations on the syringe. In the field, make sure that the water chemistry labels all have the same sample ID number (barcode), and that the labels are securely attached. Also, make sure the microbial labels all have the same sample ID number (barcode), and that the labels are securely attached.

The procedure to collect a water chemistry sample is described in Table 5-1. The sample is collected from the middle of the flowing stream whenever feasible or if the X Site is not accessible take the sample from the river channel at the last sample transect. Throughout the sampling process, it is important to take precautions to avoid contaminating the sample. Rinse all sample containers three times with portions of stream water before filling them with the sample. Many sites have a very low ionic strength and can be contaminated quite easily by perspiration from hands, sneezing, smoking, insect repellent, or other chemicals used when collecting other types of samples. Thus, make sure that none of the water sample contacts your hands before going into the cubitainer. All of the chemi-

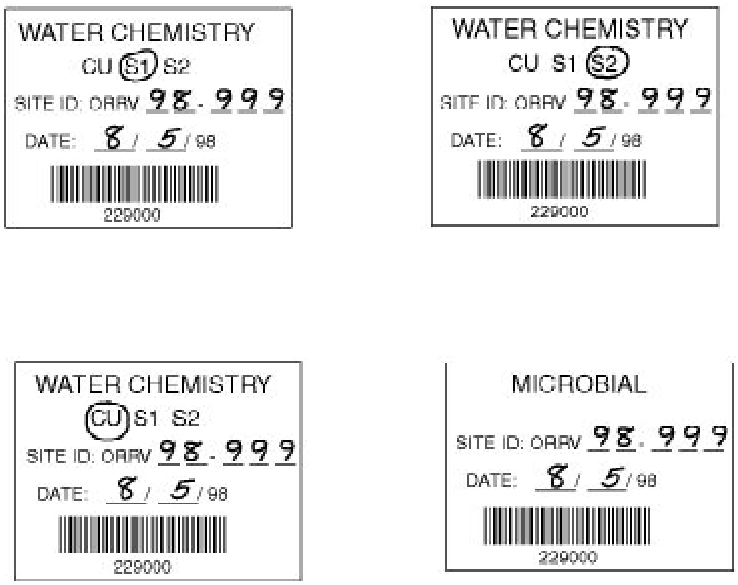

Figure 5-1. Completed sample labels for water chemistry and microbiology.

cal analyses conducted using the syringe samples are affected by equilibration with atmospheric carbon dioxide; thus, it is essential that no outside air contact the syringe samples during or after collection.

The procedure to collect a microbial sample is described in Table 5-2. The sample is collected from the middle of the flowing stream or river channel at the last sample transect. Collect samples that are representative of the water being tested and use aseptic techniques to avoid sample contamination. Take care to avoid contact with the bank or stream bed; otherwise, contamination of the sample may occur making it useless for analysis.

Record the information from the sample label on the Sample Collection Form as shown in Figure 5-2. Note any problems related to possible contamination in the comments section of the form.

5.3 Field Measurements

Table 5-3 presents the procedures for obtaining field measurement data for the water chemistry indicator. The conductivity and dissolved oxygen meters are checked in the field using the same procedures as those used at a base location (Section 3). The quality control check sample solution (QCCS) is prepared according to directions presented in Section 3. The results of field checks of these meters, as well as the measured values for specific conductance, dissolved oxygen, and stream temperature, are recorded on the Field Measurement Form as shown in Figure 5-3.

5.4 Equipment and Supplies

A list of equipment and supplies required to collect samples and field data for the water chemistry and microbiology indicator is presented in Figure 5-4. This checklist is similar

Table 5-1. Sample Collection Procedures for Water Chemistry.

Collect the water samples from either the X site or the last transect in a flowing portion near the middle of the river.

1. Rinse the 500 mL sample beaker three times with river water. Discard the rinse downriver.

2. Remove the cubitainer lid and expand the cubitainer by pulling out the sides. NOTE: DO NOT BLOW into the cubitainers to expand them, this will cause contamination.

3. Fill the beaker with river water and slowly pour 30-50 mL into the cubitainer. Cap the cubitainer and rotate it so that the water contacts all the surfaces. Discard the water downstream. Repeat the above rinsing procedure two more times.

4. Collect additional portions of river water with the beaker and pour them into the cubitainer. Let the weight of the water expand the cubitainer. The first two portions will have to be poured slowly as the cubitainer expands. Fill the cubitainer to its maximum volume. Rinse the cubitainer lid with streamwater. Eliminate any air space from the cubitainer, and cap it tightly. Make sure the cap is tightly sealed and not on at an angle.

5. Place the cubitainer in a cooler on ice and shut the lid.

6. Submerge a 60-mL syringe halfway into the river and withdraw a 15-20 mL aliquot. Pull the plunger to its maximum extension and shake the syringe so the water contacts all surfaces. Point the syringe downstream and discard the water by depressing the plunger. Repeat the rinsing procedure two more times.

7. Submerge the syringe into the river again and slowly fill the syringe with a fresh sample. Try not to get any air bubbles in the syringe. If more than 1-2 tiny bubbles are present, discard the sample and draw another one.

8. Invert the syringe (tip pointing up), and cap it with a syringe valve. Tap the syringe lightly to detach any trapped air bubbles. With the valve open, expel the air bubbles and a small volume of water, leaving between 50 and 60 mL of sample in the syringe. Close the syringe valve. If any air bubbles were drawn into the syringe during this process, discard the sample and fill the syringe again (step 8).

9. Repeat Steps 6 through 8 with a second syringe. Place both syringes in a small plastic tote and store with the cubitainer in an ice filled cooler. Keep samples on ice until they reach the laboratory.

10. Record the site number (Sample ID) on the Sample Collection Form along with the pertinent river information (river name, ID, date, etc.). Note anything that could influence sample chemistry (heavy rain, potential contaminants) in the Comments section. If you had to move to another part of the reach to collect the sample, place the letter of the nearest transect in the "Station Collected" field. Record more detailed reasons and/or information in the Comments section.

Table 5-2. Sample Collection Procedures for Microbiology.

Collect the water samples from the last transect in a flowing portion near the middle of the stream. The following procedures describe sampling of Non-Wadeable streams and rivers for bacterial analysis. These techniques have been proven useful in evaluating indicators of functional diversity and sanitary significance, including the quality of all types of waters.

1. Keep the sampling bottle closed until it is to be filled.

2. Remove stopper and cap as a unit; do not contaminate inner surface of stopper or cap and neck of bottle.

3. Fill container without rinsing.

4. Water samples are taken from the upstream side of a boat by holding the bottle near its base in the hand and plunging it, neck downward, below the water's surface. Turn bottle until neck points slightly upward and mouth is directed toward the current.

5. If there is no current, as in the case of a reservoir, create a current artificially by pushing bottle forward horizontally in a direction away from the hand.

6. If it is not possible to collect samples from these situations in this way, attach a weight to base of bottle and lower it into the water.

7. When the sample is collected, leave ample air space in the bottle (at least 2.5 cm) to facilitate mixing at later stages in the examination of the water.

8. Replace stopper or cap immediately and apply electricians tape around the neck to send the bottle. If used, secure the hood around outside of the neck of the bottle and cap.

9. Place the microbial sample with the chemistry syringes in a small plastic tote and surround with ice in a cooler.

10. Repeat steps 1 through 9 if duplicate or triplicate samples are desired.

11. Record the barcode number (Sample ID) on the Sample Collection Form along with the pertinent river information (river name, ID, date, etc.). Note anything that could influence sample chemistry or microbiology (heavy rain, potential contaminants) in the Comments section. If you had to move to another part of the reach to collect the sample, place the letter of the nearest transect in the "Station Collected" field. Record more detailed reasons and/or information in the Comments section.

Reviewed by (initial) DKA

SAMPLE COLLECTION FORM - RIVERS (continued)

SITE NAME: BEAVER RIVER DATE: 8 / 5 / 98 VISIT ☐1 ☒2 ☐3 ☐

SITE ID: ORRV 98-999 TEAM ID (X): ☒1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8

CHEMISTRY AND MICROBIAL WATER SAMPLE (Chem: 4-L Cubitainer and 2 Syringes, Micro: Glass Bottle)

	SAMPLE ID (BARCODE)	TRANSECT	FLAG	COMMENTS
CHEMISTRY	243012	K		
MICROBIAL	243113	K		

SEDIMENT TOXICITY SAMPLES

SAMPLE ID (BARCODE)	FLAG	COMMENTS

FISH TISSUE SAMPLES - PRIMARY SAMPLE (min. 50g total wgt)

SAMPLE ID (BARCODE) → 243223

LINE	SPECIES CODE	COMMON NAME	NUMBER OF INDIVIDUALS	FLAG
P1	COTTZZZZ	UNKNOWN COTTIA	11	

IS COMPOSITE SAMPLE COMPOSED OF INDIVIDUALS COLLECTED FROM THROUGHOUT REACH? (X) → ☐YES ☒NO

IF NO, EXPLAIN: COLLECTED AT ONE PROFILE

FISH TISSUE SAMPLES - SECONDARY SAMPLE (where available; 5 individuals)

SAMPLE ID (BARCODE) → 243224

LINE	SPECIES CODE	COMMON NAME	TOTAL LENGTH (MM)	FLAG
S1	PTYCOREG	NORTHERN SQUAWFISH	220	
S2	PTYCOREG	NORTHERN SQUAWFISH	220	
S3	PTYCOREG	NORTHERN SQUAWFISH	265	
S4	PTYCOREG	NORTHERN SQUAWFISH	290	
S5	PTYCOREG	NORTHERN SQUAWFISH	215	

IS COMPOSITE SAMPLE COMPOSED OF INDIVIDUALS COLLECTED FROM THROUGHOUT REACH? (X) → ☒YES ☐NO

IF NO, EXPLAIN:

LINE	COMMENT OR FLAG EXPLANATION FOR FISH TISSUE

Flag codes: K = Sample not collected; U = Suspect sample; F1, F2, etc. = misc. flag assigned by field crew. Explain all flags in Comments sections.

Rev. 05/29/98 (_rvscmb.98)

SAMPLE COLLECTION FORM - RIVERS - 2

Figure 5-2. Sample Collection Form (page 2), showing data recorded for water chemistry and microbial samples.

Table 5-3. Procedures for Streamside and In Situ Chemistry Measurements.

Specific Conductance

1. Check the batteries and electronic functions (e.g., zero, "red line") of the conductivity meter as instructed by the operating manual.

2. Insert the probe into the "RINSE" container of the quality control check sample (QCCS) and swirl for 3 to 5 seconds. Shake off the probe and transfer to the "TEST" container of QCCS; let stabilize for 20 seconds. Record the conductivity of the QCCS on the Field Measurement Form.

   If the measured conductivity is not within 10% or 10 S/cm of theoretical value, repeat the measurement process. If the value is still unacceptable, flag the conductivity data on the Field Measurement Form.

3. Submerge the probe in an area of flowing water near the middle of the channel at the same location where the water chemistry sample is collected. Record the measured conductivity on the Field Measurement Form.

Dissolved Oxygen and Temperature

1. Inspect the probe for outward signs of fouling and for an intact membrane. Do not touch the electrodes inside the probe with any object. Always keep the probe moist by keeping it inside its calibration chamber.

2. Check the batteries and electronic functions of the meter as described in the operating manual. Record the results of these checks on the Field Measurement Form.

3. Calibrate the oxygen probe in water-saturated air as described in the operating manual. Allow at least 15 minutes for the probe to equilibrate before attempting to calibrate. Try to perform the calibration as close to stream temperature as possible (not air temperature) by using stream water to fill the calibration chamber prior to equilibration. For doing the elevation correction, the elevation of the sample site is given on the site Information sheet in the dossier for the site. Record the pertinent calibration information on the Field Measurement Form.

4. After the calibration, submerge the probe in midstream at mid-depth at the same location where the water chemistry sample is collected. Face the membrane of the probe upstream, and allow the probe to equilibrate. Record the measured DO and stream temperature on the Field Measurement Form. If the DO meter is not functioning, measure the stream temperature with a field thermometer and record the reading on the Field Measurement Form along with pertinent data flags and comments.

Reviewed by (initial) DKA

FIELD MEASUREMENT FORM - STREAMS/RIVERS

SITE NAME: BEAVER RIVER DATE: 8 / 5 / 98 VISIT ☐0 ☒1 ☐2 ☐3

SITE ID: ORRV 98-999 TEAM ID (X): ☒1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8

WEATHER CONDITIONS (X)

CLOUD COVER	☒ < 5%	☐ 5-25%	☐ 25-50%	☐ 50-75%	☐ >75%
PRECIPITATION	☒ NONE	☐ LIGHT	☐ MODERATE	☐ HEAVY	
PREVIOUS PRECIPITATION (24 H)	☒ NONE	☐ LIGHT	☐ MODERATE	☐ HEAVY	
AIR TEMPERATURE XX	28 °C				

IN SITU MEASUREMENTS

STATION ID: K Assume X-site unless marked

		FLAG	COMMENTS
QCCS COND µS/CM XXXX	75		AT TRANSECT "K"
STREAM/RIVER COND µS/CM XXXX	110		AT TRANSECT "K"
STREAM/RIVER DO MG/L XX.X	8.8		AT TRANSECT "K"
STREAM/RIVER TEMP °C XX.X	17.6		AT TRANSECT "K"

FISH TISSUE SAMPLES - SECONDARY SAMPLE (where available; 5 individuals)

INITIAL O_2 (MG/L) XX.X	INITIAL INCUBATION TEMP (°C) XX.X	INCUBATION TIME (24-HR TIME) START (HH:MM)	INCUBATION TIME (24-HR TIME) FINISH (HH:MM)	DURATION OF INCUBATION (HH:MM)	FLAG	COMMENTS
9.0	18.4	18:30	22:30	4 : 00		

SAMPLE ID (BARCODE)	FINAL O_2 (MG/L) XX.X	FLAG	COMMENTS
243663	7.5		INITIAL D.O. AT 1000' ELEVATION
243664	7.2		FINAL D.O. AT 2200' - METER CALIBRATED
243665	7.8		FOR EACH ELEVATION
243666	8.1		
243667	7.0		
CONTROL	8.8		

OXYGEN METER CALIBRATION INFORMATION

MEMBRANE CHECK ☒ ELECTRONIC ZERO ☐ RED LINE ☒

CALIBRATION CHAMBER TEMPERATURE: °C

SATURATED O_2 @ @ TEMP: MG/L

STATION ELEVATION (FROM TOPO. MAP OR ALTIMETER): 1000 FT

ELEVATION CORRECTION FACTOR: x

The calibration value is obtained by multiplying the saturated DO concentration times an elevation correction factor (obtained from the tables on the back of the YSI meter). Adjust the meter reading to the calibration value.

CALIBRATION VALUE: MG/L

COMMENTS:

Flag codes: K = no measurement or observation made; U = suspect measurement or observation; Q = unacceptable QC check associated with measurement; F1, F2, etc. = miscellaneous flags assigned by each field crew . Explain all flags in comments section.

Rev. 05/29/98 (strvfldm.98)

FIELD MEASUREMENT FORM - STREAMS/RIVERS - 1

Figure 5-3. Field Measurement Form (page 1), showing data recorded for water chemistry.

Equipment And Supplies For Water Chemistry		
Qty.	Item	
1	Dissolved oxygen/Temperature meter with probe	
1	DO repair kit containing additional membranes and probe filling solution	
1	Conductivity meter with probe	
1	500-mL plastic bottle of conductivity QCCS labeled "Rinse" (in plastic bag)	
1	500-mL plastic bottle of conductivity QCCS labeled "Test" (in plastic bag)	
1	500-mL plastic bottle of deionized water to store conductivity probe	
1	Field thermometer	
1	500 mL plastic beaker with handle (in clean plastic bag)	
1	4-L cubitainer with completed sample label attached (in clean plastic bag)	
2	60 mL plastic syringes (with Luer type tip) with completed sample labels attached	
1	200 mL sterile glass microbial bottles with completed sample label attached (in clean plastic bag)	
1	Plastic container with snap-on lid to hold filled syringes	
2	Syringe valves (Mininert® with Luer type adapter, or equivalent, available from a chromatography supply company)	
1	Cooler with 4 to 6 plastic bags (1-gal) of ice OR a medium or large opaque garbage bag to store the water sample at streamside	
1	Sample Collection From	
1	Field Measurement Form	
	Soft-lead pencils for filling out field data forms	
	Fine-tipped indelible markers for filling out labels	
1 roll	Electricians tape	
1 copy	Field operations and methods manual	
1 set	Laminated sheets of procedure tables and/or quick reference guides for water chemistry and microbiology	

Figure 5-4. Checklist of equipment and supplies for water chemistry.

to the checklist presented in Appendix A, which is used at the base location (Section 3) to ensure that all of the required equipment is brought to the Non-Wadeable stream or river. Use this checklist to ensure that equipment and supplies are organized and available at the stream or river site in order to conduct the activities efficiently.

5.5 Literature Cited

Kaufmann, P., A. Herlihy, J. Elwood, M. Mitch, S. Overton, M. Sale, J. Messer, K. Reckhow, K. Cougan, D. Peck, J. Coe, A. Kinney, S. Christie, D. Brown, C. Hagley, and Y. Jager. 1988. Chemical Characteristics of Streams in the Mid-Atlantic and Southeastern United States. Volume I: Population Descriptions and Physico-Chemical Relationships. EPA 600/3-88/021a. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1989. Handbook of Methods for Acid Deposition Studies: Field Operations for Surface Water Chemistry. EPA 600/4-89/020. U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C.