Environmental Resource Management Standard Operating Procedures Manual 2019

City of Austin
Watershed Protection Department
Environmental Resource Management Division

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1.0 Purpose

This Standard Operating Procedures Manual describes the procedures for both special study and routine activities of the Environmental Resource Management Division of the City of Austin Watershed Protection Department (WPD).

ERM staff collect data from multiple matrices throughout the greater Austin area in fulfillment of our stated mission:

> ...to protect and improve water quality in Austin's creeks, lakes and aquifers for our community and aquatic life by preventing, detecting, evaluating and reducing water pollution.

Program-specific information may either be found in the current version of the ERM Quality Management Plan or the Quality Assurance Project Plan (QAPP) for an individual project. Unless otherwise approved by the Quality Assurance Officer and/or data management team all data collected by ERM staff should be stored in the Field Sampling Database (FSDB). Procedures for appropriate data input to and export from the FSDB may be found in the most current version of the User Guide. In the near future, the FSDB shall be replaced with a new version (in development at the time of this document) called the Water Resources Monitoring (WRM) database.

The standard operating procedures presented in this manual are derived from reference materials and regionally specific professional experience developed in Austin from the past 30+ years of data collection. Many of the relevant procedures in this manual are similar or consistent with the most current version of the Texas Commission on Environmental Quality (TCEQ) Surface Water Quality Procedures manuals and or EPA Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers, for which the most current versions are available online.

2.0 Field measurements

Collection of in situ standard physical and chemical field data at the time of sample collection may reveal environmental perturbations and may be helpful in the evaluation of laboratory analysis results. This section details the calibration, maintenance, and use of multi-probe instrumentation as well as other field instruments (e.g. Secchi disk, flow meters, etc) used by ERM staff.

Handle and transport all sondes properly to prevent physical damage**:**

- Fasten the guard cautiously so that probes do not touch the guard. Grasp the top of the sonde (below the probes) so that fingers can guide the guard safely past the probes.
- Transport the sonde such that top of the cable is never bent.
- Be cautious when deploying to avoid any sudden impact. For example: Walk into the creek and set the sonde in the water instead of tossing the sonde out into the water.
- When placed in water, prevent bubble entrapment by lowering at an angle and rolling sonde. Limit static electricity build-up by securing sonde during travel and keeping it off any carpet in the vehicle.

All instruments should be properly calibrated prior to field deployment in accordance with the calibration guidance for each instrument described below. Calibration data must be recorded (in ink) in the calibration log which is stored in the ERM laboratory with all archived logs. Data to be recorded includes the initial readings of the probes submerged in laboratory standards (typically pH7, pH10, conductivity 1000 for standard field work) in addition to the initial reading of DO compared to the theoretical saturation value based on the water temperature and barometric pressure adjusted for elevation of the 11th floor. In addition to recording the initial readings, the calibration log must also include the supporting data including water temperature barometric pressure, battery level, purpose for calibration, and date/time/staff.

For any parameter, if the instrument does not read within the designated accuracy range during pre-calibration: 1) record parameter failure in the calibration Log, 2) attach a note to the instrument indicating the specific malfunction, the date and your name, 3) notify the designated team member responsible for routine maintenance for the instrument, and 4) select a different instrument for use. The designated team member responsible for routine maintenance shall investigate the error, perform maintenance, additional corrective action(s) and send the instrument in for repair (if necessary). Do not record or enter into the database measurements for parameters which do not properly calibrate prior to instrument deployment. Calibration results should be recorded in ink in the calibration Log, located in the ERM laboratory.

When instruments are returned from the field, the probes must be checked for accuracy. This process is termed "post-calibration" even though probes are not actually calibrated a second time. Results of post-calibration must be entered into the database to properly qualify field data accuracy. Data recorded in the database from instruments that fail post-calibration should be "J-flagged" (see database SOP/User Guide) with explanation in the comments field.

2.1 Hydrolab/Quanta Multi-probes

The Hydrolab and Quanta multi-probes ("sondes") are typically used for instantaneous field measurements of routine physical parameters. They are also deployed for extended periods to collect semi-continuous data (at 5- to 30-minute intervals). For example, these sondes can be used to collect the 24-hr average DO measurements used by TCEQ in the Clean Rivers Program.

General Maintenance

- For routine storage of sondes, use tap water in the sonde cups because the chlorine in tap water restricts algal growth on sensors. Add only a small amount of tap water for which to maintain 100% humidity but not enough to submerge the probes. Never store in distilled, deionized (DI), or reverse osmosis (RO) water.
- If a problem occurs during calibration with the pH probe, store sonde overnight with the probes submerged in a full-strength pH storage solution. The pH storage solution is a special solution purchased from the sonde manufacturer.
- Store so that the probes are not submerged in the water.
- Batteries on Surveyor (handheld display unit) should be charged when not in use.
- All sondes are on a bi-monthly (six times per year) maintenance schedule.

Maintenance Procedures

- **Clark Cell DO Sensor**
  - Remove and discard old membrane, shake out old electrolyte
  - Refill with DO electrolyte until the meniscus rises above the surface of the sensor
  - Tap the side of the probe to make sure there are no bubbles
  - Replace membrane and secure O-ring, ensure no bubbles or wrinkles in the membrane
  - Trim away excess membrane
  - Keep the sensor moist for 4-24 hours before using

- **LDO Sensor** This sensor requires very little maintenance, but they are expensive to replace and last about as long as the Clark Cell (approximately 3 years)
  - Wipe off lens with a clean non-abrasive cloth

    - Replace if cap is scratched or light can be seen through the top coating
    - (If trouble shooting, remove cap and look for moisture underneath)

- **Conductivity Sensor**
    - Clean inside the oval measurement cell with a non-abrasive cloth or cotton swab. Use soap if coated with dirt, grease, or biological growth. Then rinse with tap water.

- **pH Electrode**
    - If the sensor is coated with pollutant, clean glass with a very clean non-abrasive cloth or cotton swab with very mild soap. Never use alcohol on this probe.
    - Rinse with tap water.

- **pH Standard Reference Electrode**
    - Pull entire reference electrode away from the transmitter. Discard old solution. Fill with new solution and slide electrode back over the transmitter with cap facing upward to allow extra solution and air to flow out through the cap.
    - Rinse with tap water.
    - If reference electrode cap is one year old, looks fouled, or does not allow some reference solution through when sliding electrode back over transmitter, replace cap.

- **pH Integrated Sensor**
    - Cover the glass probe with finger, use screwdriver to remove pH electrode cap.
    - Use pipette to remove old solution, then flush the reservoir twice with new solution and finally fill reservoir to the brim with new solution.
    - Re-install electrode cap using the screwdriver.
    - If reference electrode cap is on year old, or looks fouled, replace cap.

- **Temperature Sensor**
    - Use soap or alcohol to remove any pollutants and rinse with tap water.

- **Circulator**
    - Turn on circulator to make sure it works.

- **Data Logging Batteries**
    - Replace batteries in data logging sondes when they get below 60% or 10.1 volts.

Following maintenance, complete the Hydrolab maintenance log sheet (Figure 2.1-1).

Hydrolab Monthly Maintenance Schedule
Instrument: **Year:**
Serial Number:

Monthly Maintenance	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Initial & Date												
Check circulator												
Check temperature probe												
Clean probes w/ alcohol												
Rinse probes with DI water												
Conductivity												
pH reference												
pH junction replaced: Y/N												
D.O. Membrane												
Store in 1:1 KCl/tap water												
Post maintenance Calibration												
Change logging batteries: Y/N												

Date/Initials	Notes

Figure 2.1-1. Hydrolab monthly maintenance log.

Calibration

Record the results of all calibration procedures in ink in the calibration logbook located in the ERM laboratory. Calibration results shall also be entered in the QA/QC Data Entry Form of the database. Consult database staff for guidance in entering calibration data into the database. Ensure that standards have been stored at room temperature. Do not use standards if temperature in storage area has exceeded recommended range listed on containers.

To begin, place the calibration cup (open at both ends) on the instrument; invert and place instrument in the designated sonde stand. Parameters should always be calibrated in the following order: conductivity, pH 7, pH 10, turbidity (optional), then DO. It is important to thoroughly rinse the probes with a triple wash of reagent grade DI water prior to calibrating each parameter. Thorough rinsing is critical because pH solution has a high conductivity and conductivity is used to compensate percent DO saturation. DO calibration should only be performed using reagent-grade or DI water.

Conductivity probe:

1. Rinse probe twice with reagent-grade or DI water, then place the probe into ambient air with no liquid (dry) in the calibration cup.
2. Record the stable reading in the "**COND 0 initial"** column in the logbook.
3. Press CALIBRATE, then select C and press ENTER.
4. Type in the correct value (0 in open air), press ENTER, and select Y (for Yes, save the calibration.).
5. Record the final reading in the "**COND 0 final"** column of the calibration logbook.

6. Rinse and then fill the calibration cup with the 1000 µS/cm standard. Allow time for reading to stabilize.
7. Record the stable reading in the "**COND 1000 initial"** column of the logbook.
8. Press CALIBRATE, then select C and press ENTER.
9. Type in the correct value (1000), press ENTER, and select Y (for Yes, save the calibration.).
10. Record the final reading in the **COND 1000 final** column of the calibration logbook.

<u>pH probe:</u>

1. Pour a small amount of reagent-grade or DI water into the calibration cup, then place the cap on top and shake well. Repeat three times.
2. Following the same procedure, rinse the probe with the 7.0 pH standard.
3. Fill the cup above the pH sensor with the pH 7.0 standard and allow time to stabilize.
4. Record the reading in the "**pH initial"** column in the logbook.
5. Press CALIBRATE, select pH, and press ENTER.
6. Type in the correct pH, and press ENTER, and select Y (for Yes, Save the Calibration).
7. Record the final reading in the "**pH final"** column in the logbook.
8. Rinse the probe well with reagent-grade or DI water, and then repeat from step 2 above using the 10.0 pH standard.
9. If probe fails to calibrate follow directions in maintenance procedures and try again.

<u>DO probes:</u>

Clark Cell Membrane Sensor

1. Check the DO membrane for wrinkles, tears or air bubbles and replace if necessary. See Maintenance Section in Hydrolab manual for replacement instructions.
2. Rinse thrice with reagent-grade or DI water.
3. Fill the cup with reagent-grade or DI water to just below the O-ring securing the DO membrane.
4. Remove any water droplets off the membrane by wiping with a Kimwipe.
5. Place the plastic storage lid on the cup leaving a small gap and allow five minutes to stabilize or until the temperature stabilizes.
6. Once the instrument has stabilized record the water temperature (from the display) and the barometric pressure in the logbook.
7. Use temperature and barometric pressure to determine the theoretical DO value (https://www.atxwatersheds.com/open_utils/theopostcal/).
8. Record this in the "**DO theo"** (theoretical) column in the logbook.
9. Record the actual DO reading in the **"DO initial"** column in the logbook.
10. Press CALIBRATE, type in the correct barometric pressure and DO value, and press ENTER.
11. Record the final reading in the "**DO final"** column in the logbook.

LDO Sensor

1. Check DO cap for scratches. Replace if damaged. See Maintenance Section in Hydrolab manual for replacement instructions.
2. Rinse thrice with reagent-grade or DI water.
3. Fill a 1L plastic bottle with 500 ml reagent-grade or DI water and agitate vigorously for 40 seconds.
4. Fill the cup within a ¼" of the top, submerging the LDO probe.
5. Place the plastic storage lid on the cup leaving a small gap and allow five minutes to stabilize or until the temperature stabilizes.
6. Once the instrument has stabilized record the water temperature (from the display) and the barometric pressure in the logbook.

7. Use temperature and barometric pressure to determine the theoretical DO value (https://www.atxwatersheds.com/open_utils/theopostcal/).
8. Record this in the "**DO theo"** (theoretical) column in the logbook.
9. Record the actual DO reading in the **"DO initial"** column in the logbook.
10. Press CALIBRATE, type in the correct barometric pressure and DO value, and press ENTER.
11. Record the final reading in the "**DO final"** column in the logbook.

Depth probe:

1. Pour out any solution and hold the Hydrolab downward (probes pointing down), resting the calibration cup on the floor.
2. Record the reading in the "**Depth"** column.
3. Press CALIBRATE, select D (for Depth), and press ENTER.
4. Type in 0.00 (change negative to positive if necessary), press Enter, and select Y (for Yes, Save the calibration).
5. Check the depth reading to make sure it is within range.

Turbidity sensor (self-cleaning):

1. Perform a 2 point calibration – see below for preparation of high end standard solution (32 NTU)
2. Clean all sensors and inside of storage cup using soft brush and mild soap, rinse thoroughly
3. Rinse the probe 3 times with reagent-grade or DI water
4. Fill storage cup 75% full of reagent-grade or DI water, screw on storage cap, invert sonde so that sensors point down
5. Find the option to operate the self-cleaning wiper (either "Clean" or "Turbidity[Rev]"), set it to 1, and select calibrate
6. Watch for one full rotation of the self-cleaning wiper
7. Find the option to calibrate the turbidity sensor, set 1 for the turbidity point, set 0.3 as the turbidity standard (it will not be 0 because the sensor can never be perfectly clean), and select calibrate
8. Dump the reagent-grade or DI water
9. Rinse the probe 3 times with the 32 NTU solution
10. Fill storage cup 75% full of 32 NTU solution, screw on storage cap, invert sonde so that sensors point down
11. Again, find the option to operate the self-cleaning wiper (either "Clean" or "Turbidity[Rev]"), set it to 1, and select calibrate
12. Watch for one full rotation of the self-cleaning wiper
13. Again, find the option to calibrate the turbidity sensor, set 2 for the turbidity point, set 32 as the turbidity standard, and select calibrate

Temperature probe:

- The temperature probe is factory calibrated and does not need further calibration, however, periodic verification with another thermometer is useful.

Turbidity probe for the Quanta:

1. Soap or rubbing alcohol may be used to remove grease, oil, or biological material.
2. Use a non-abrasive, lint-free cloth to clean the quartz glass tube. Scratched glass reduces the sensor's accuracy.
3. Rinse with water.

Mix 4 ml (4000 NTU) + 496 ml DI in a 500 ml Erlenmeyer flask to get 32 NTU solution

$C_1V_1 = C_2V_2$ or $V_1 = (C_2V_2) / C_1$

Where,

C_1 = Concentration of Stock Solution – 4000 NTUs
C_2 = Concentration of the Standard Needed – 32.0 NTU's
V_1 = Volume of Stock Solution Needed – 4 mg/l or 4000 ug/l
V_2 = Total Volume of Mixture – 500 ml (Stock Volume + Water)

Turbidity is a two-point calibration. A turbidity standard of 0.0 is treated as the "zero" and all other values are treated as the "slope". First calibrate "zero", then calibrate "slope". Calibrate turbidity with primary standards ('turbid-free' water, Formazin, and/or polystyrene beads). A check with a secondary standard (Quick-Cal Cube) is optional but should be done during routine maintain by mixing a check standard at concentration between the "zero" and "slope" standard. The 'turbid-free' water used by the City of Austin is from Milipore filter system (or DIUF water purchased from Fisher Scientific) and slope calibration standard 32.0 NTUs mixed from a 4000 NTU Formazin stock solution. Please use the following formula to prepare the standard:

$C_1V_1 = C_2V_2$ or $V_1 = (C_2V_2) / C_1$

Where,

C_1 = Concentration of Stock Solution – 4000 NTUs
C_2 = Concentration of the Standard Needed – 32.0 NTU's
V_1 = Volume of Stock Solution Needed – 4 mg/l or 4000 ug/l
V_2 = Total Volume of Mixture – 500 ml (Stock Volume + Water)

Step 1
Use 'turbid-free' water to calibrate the "zero" (Standard 1). Record calibrate in Calibrate Log for that instrument.

Step 2
Use Formazin and/or polystyrene beads to calibrate the "slope" (Standard 2). Record calibrate in Calibrate Log for that instrument. Primary standards must completely fill the optical area of the turbidity sensor plus ¼" (6 mm) of standard on both sides of the PVC body by filling the calibration cup to the top. Alternately, pour ≈1-¼" (32 mm) of standard into the storage cup and place the inverted sensors into the standard with bayonets disengaged.

Optional
After calibration with primary standards, the value of the optional Quick-Cal Cube secondary standard, if used, must be determined and recorded for each individual instrument. The Quick-Cal Cube value is determined by removing the storage/calibration cups, wiping the optical areas, both sensor and cube, clean and dry with a non-abrasive, lint 26 free cloth, and placing the ceramic glass cube into the turbidity sensor's optical area. Align the Quick-Cal Cube's pin with the turbidity sensor's recessed hole and, for optimum repeatability, rotate the Quick-Cal Cube clockwise to remove mechanical play in the pin/hole. To test for drift between primary calibrations, reinstall the Quick-Cal Cube

Notes:
'Turbid-free' water is available for purchase from chemical supply houses. However, it is far less expensive to make by passing reagent-grade water through a 0.1 µm or smaller filter.

Formazin and polystyrene beads are primary standards as defined by the EPA. Quick-Cal Cubes are secondary standards, which must be rechecked, and value recorded, after each primary standard calibration with each instrument. However, Quick-Cal Cubes save resources, both time and money, by allowing inexpensive and frequent calibration checks between permit and/or standard operating procedure required primary calibrations. Formazin requires daily preparation.

Polystyrene beads are instrumentation specific and beads formulated for one instrument design often read differently on a different instrument design. Hydrolab has polystyrene beads formulated for the Quanta Turbidity sensor. Please contact Customer Service or www.hydrolab.com for ordering information.

When using liquid standards, insure no bubbles in the optical area. The optical properties of bubbles affect the turbidity calibration. Gentle agitation easily dislodges bubbles.

When using Quick-Cal Cube standards, insure no water droplets in the optical area. The optical properties of water droplets affect the calibration check. Remove droplets with a non-abrasive, lint-free cloth. Turbidity is a two-point calibration. A turbidity standard of 0.0 is treated as the "zero" and all other values are treated as the "slope". First calibrate "zero", then calibrate "slope".

Hydrolabs Quanta Turbidity option is compliant with GLI Method 2, an EPA approved method, and ISO 7027:1999(E). GLI Method 2 is recognized by EPA as an approved method in Section 141.74 of the Federal Register Vol. 59 No. 232 (December 5, 1994). Reprints of both the GLI Method 2 documentation and the Federal Register reference are available on request. The Quanta's turbidity sensor, circuitry, software, and Quick-Cal Cubes were developed as a joint venture between Hydrolab Corporation and GLI International, Inc. and are protected by U.S. Patents #5,059,811 and #5,140,168. Other patents are pending.

Sonde Measurement

Remove the plastic screw cap from the sonde and attach the guard/weight. Place the instrument into the water so that all instruments are submerged. Turn on the sonde, and record measurements once readings have equilibrated to ambient conditions at that site (usually once temperature and conductivity stabilize). Prior to recording field measurements, be certain that a stirrer is attached and functioning or there is enough velocity (1 foot per second) at the site to insure proper water circulation around sensors.

For specifics on programming and downloading sondes in any situation, refer to Hydrolab manual "Datasonde 4 and MiniSonde User's Manual" or "Datasonde 3 Operating Manual". Download and use Hydras3LT for programming and downloading data from sondes.

Turn off the instrument and replace plastic screw cap. Be sure that enough liquid (1/4" to 1/2") remains in screw cap to keep sensors moist between readings. During use in the field, ambient site water can be used in the screw cap if tap water is discarded and/or not available. Tap water should replace site water following lab post-calibration (chlorine in tap water restricts algal growth on sensors).

Post-Calibration

Post-calibration values are compared to expected ranges to determine if field measurements may be reported with confidence. If post-calibration values fall outside error limits, all data for that parameter since the last calibration is unreliable. If the instrument fails to calibrate and properly post-calibrate again, maintenance is necessary.

Post-calibration must be performed after each use of the instrument. It is preferable that calibration and post-calibration are no more than 24-hours apart.

To post-calibrate, follow the initial calibration rinse procedures but DO NOT perform the calibration. Record the instrument reading for each standard in the calibration log (with notations indicating post-calibration). Record the instrument reading for each standard in the **initial** column, and the standard or theoretical value in the **final** column. If field sheets allot space to record post-calibration, populate those fields with the appropriate information as well.

If the instrument is to be used on the next consecutive day, the next morning's calibration should not be used as post-calibration for the prior day's records. The reason that this should not be done is because calibration of conductivity will affect (readjust) the dissolved oxygen result and will therefore no longer reflect a true post-calibration from the day before.

If measurements do not fall within the satisfactory error limitations (Table 2.1-1), appropriate routine maintenance (changing DO membrane, cleaning sensors, etc.) should be performed. If post calibration values are consistently out of limits, the instrument should be submitted for repair.

Table 2.1-1. Post Calibration Guidelines for Field Instruments

Field Parameter	**TCEQ error limits for multi-probes**	*Accuracy Value*		
		Hydrolab	Horriba	[a] Corning M90 [b] Hach pH tester
Dissolved Oxygen	± 0.5 mg/L	± 0.2 mg/L	± 0.1 mg/L	----
Temperature	± 1 C	± 0.15 (± 0.10) C	± 0.3 C	----
pH	± 0.5 su	± 0.2 su	± 0.05 su	0.02 (at room T)[b]
Sp. Conductance	± 5 %	± 1 % of range	± 1 % range	± 1.5 % range [a]
TDS	--	----	----	± 1.5 % range [a]
Depth	± 0.2 at 1 m	± 0.45 m	---	---
Turbidity	---	± 5 % of range	± 3 % range	----

2.2 Cole-Parmer pHCON10

The Cole-Parmer pHCON10 (19825-00) can collect pH, conductivity and water temperature.

Calibration

Calibrate the Cole-Parmer pHCON10 for pH using a two-point calibration curve.

1. Press MODE to select pH mode. It will appear in the upper right corner of the display.
2. Rinse the probe thoroughly with reagent-grade or DI water, then with the 7.0 pH solution.
3. Immerse the probe in a small amount of calibration buffer 7.0 in a clean beaker.
4. Check the upper left corner of the display for MEAS (measuring indicator); If it is not in this mode (it would show CAL), then press CAL/MEAS to switch the display.
5. Wait until the reading is stabilized (READY in upper left of display); write this value in the pH initial column in the logbook.
6. Press CAL/MEAS to begin calibration. The primary (first line) display will show the measured reading while the smaller secondary display will show the pH standard value. If the pH standard value showing is different from the standard you are using, press the up or down arrow key to scroll to the correct value.
7. When the READY indicator shows, the reading is stable, and you should confirm the calibration by pressing ENTER.
8. Press CAL/MEAS key and allow the reading to stabilize, then record the value in the pH final column of the logbook.
9. The secondary display automatically scrolls to the next buffer calibration option. If not, press the up or down arrow keys to select the next buffer.
10. Follow steps 2-9 for the second buffer 10.0 solution.

To calibrate for conductivity measurement:

1. Press MODE to select Conductivity. Make sure 'MEAS' is displaying.

2. Rinse probe 3 times with reagent-grade or DI water, then 3 times with the calibration standard solution.
3. Immerse the probe in the standard, then tap the probe to remove air bubbles.
4. When the READY indicator lights, record the value in the COND initial column in the logbook. Record the temperature reading (second line in display) also.
5. Press the CAL/MEAS key. The CAL indicator will show in the upper left of the display, and the first line of display will show the factory default value.
6. Press the up or down arrow keys to scroll to the value of the conductivity standard being used.
7. Press ENTER to confirm the calibration.
8. Upon calibration, the CON indicator appears and the meter switches back into measurement mode. The display shows the calibrated value. Record this in COND final column of the logbook.

Measurement

1. Turn the probe on and make sure 'MEAS' (not 'CAL') is displayed. Press MODE button until display reads MEAS.
2. Immerse the probe into the sample, stirring gently. Tap the probe to remove air bubbles.
3. When READY shows on the display, the reading is stable and may be recorded.
4. To switch from pH to Conductivity (display will read "μS"), press the MODE key

2.3 Hach 2100p Turbidimeter

The Hach 2100p turbidimeter may be used to measure turbidity (in NTU) in the field or the laboratory.

Measurement Tips

- Bring refrigerated samples to room temperature (place in a warm water bath if necessary) to prevent condensation that can interfere with measurement.
- Take a reading soon after filling sample vial to minimize settling
- Use only vials that appear extremely clean (no mineral deposits) and scratch-free. Wear cotton gloves and handle the vials only by the caps where possible.
- To ensure accuracy at low turbidity levels, use a single vial for all samples.
- Do NOT leave a vial in the cell compartment after taking a reading; this can compress the spring in the cell holder.
- Remove batteries from the instrument if it will not be used for more than one month

Checking the Turbidimeter Calibration

Prior to running samples, check the calibration date on instrument or in calibration log to determine if a formazin recalibrate is needed. If the calibration date is past two-months or if calibration checks with the Gelex Standards are not within 5 % of the standard values listed on vial, the instrument should be recalibrated according to the HACH 2100P Turbidimeter Instrument and Procedure Manual (Cat No 46500-88).

Calibration of the 2100P meter is based on formazin standards. Hach Company recommends using <0.1, 20, 100, and 800 NTU formazin standard for calibrating the instrument. Pre-mixed standards can be purchased from Hach or dilutions using 4000 NTU standard can be mixed to calibrate the instrument and assigning values to Gelex Standards. Gelex Secondary Standards are used for calibration checks and value most be assigned to the three check standards ranges: 0-10 - NTU, 0-100 NTU, and 0-1000 NTU. **A formazin recalibration should be performed at least once every three months.**

<u>DO NOT calibrate with Gelex Secondary Standards. Gelex standards are designed for instrument verification, not calibration.</u>

The calibration procedure

1. Rinse a clean sample cell with dilution water 3 times. Then fill the cell to the line (about 15 ml) with dilution water or use StabCal <0.1 NTU standard (Hach Cat. No. 26597-49).
2. Insert the sample cell into the cell compartment with orientation mark on cell aligned with cell mark on the cell compartment. Close the lid.
3. Press I/O to turn on instrument.
4. Press CAL.
5. Press Read. The instrument will count down from 60.
6. Remove the NTU standard and repeat step 5 and 6 with 20, 100, and 800 NTU standards.
7. After the 800 NTU standard, Press CAL to accept the calibration.
8. 8. Assign values to Gelex Secondary Standards by measuring each of three check standards and record the value on lid of Gelex vial.
9. Please record the date of calibration in calibration log and label the instrument and Gelex Secondary Standard with the calibration date.

The calibration is good for at least 3-months. The Gelex Secondary Standards can be used to validate the stored calibration during this 3-month period.

Measuring Turbidity

1. Press the I/O button to turn on the instrument. If the display does not show AUTO RNG, press the RANGE key once to select auto-range. For other range options, see the manual referenced above.
2. Check the display for SIG AVG; if present, use the Signal Average button to turn this function off. It is only needed if the sample causes a ‘noisy’ signal (constantly changing value).
3. Rinse the vial three times with reagent-grade or DI water, then three times with sample water.
4. Shake the container with the sample water well, then fill vial to the line (about 15 ml) with sample water. Cap the vial.
5. Quickly wipe the vial with a soft, lint-free cloth after applying a small bead of silicon oil from top to bottom, enough to coat the vial with a thin layer of oil. Avoid excess oil!! After a few samples, the cloth will contain enough oil and can be used without adding oil. Do not take long with this step, to minimize sample settling.
6. Flip open the instrument top and insert the sample vial, orienting it with the diamond facing front. Close the lid.
7. Press READ. The display will show ----NTU until it displays the final turbidity reading in NTUs. Record the value after the lamp symbol turns off.

2.4 Secchi Disk Transparency

A weighted, 20 cm diameter black-and-white Secchi Disk is used to assess transparency of standing water (TCEQ 2003). If collecting data on a sunny, or partly sunny day, avoid glare from the water surface affecting results by lowering the Secchi Disk on the shady side of the boat or use polarized sunglasses. Lower the disk into the water slowly, watching for it to disappear. Note the point on the rope where the disk disappears, then slowly raise the disk until it reappears and note that point. Record the mean of these two values. Repeat observation to corroborate results. If the water velocity or boat motion prevents the Secchi Disk from dropping straight down, additional weight should be added.

2.5 Manual Flow Measurements

ERM uses two different types of flow meters to manually measure flow in waterways; the Marsh-McBirney or the Sontek Flowtracker. The Marsh-McBirney uses an electrical signal to measure water velocity across a sensor while the Flowtracker uses an acoustic signal to measure water velocity. In addition, there are several methods to yield a semi-quantitative estimate of flow.

Marsh-McBirney Flow Meter

Instream flow measurement with the Marsh-McBirney Flow Meter is ideally performed in a straight, flat laminar channel free of large rocks, weeds and protruding obstructions. When these qualities are not present, it may be necessary to modify the channel cross-section to create more acceptable conditions. Building dikes to reduce dead water and shallow flows or removing rocks and debris from two meters upstream of the measurement transect is acceptable.

To begin, stretch a measuring tape across the stream at a right angle to the direction of flow. Measure and record the wetted stream width and determine the number of flow measurements to be made. The preferable number of flow measurements is 20-30. If the stream is greater than 5 feet (1.5 meters) wide, at least 10 measurements must be taken. If the stream is less than 5 feet, use flow sections of 0.5 feet (0.15 meters). Although the flow measurements do not have to be equal width, <u>no flow measurement section should have greater than 10% of the total flow</u>. Where flow is deeper or faster, it is good to measure smaller and more numerous sections

Record all pertinent information on the appropriate field sheet (Figure 2.5-1), including:

- Date and time
- Site name
- Staff measuring flow
- Current and antecedent weather conditions, including an estimate of flow severity (flow type)
- Total transect width
- All specifics (width, depth, velocity) for each measurement

Several factors should be considered when choosing a transect to measure flow. Try to find a narrow spot with constricted flow to minimize the chance of backwater spots. Immediately upstream of the transect should be free of large rocks or other obstructions that cause unusual water turbulence. The bottom of the stream should also be as uniform as possible. If measurements are taken at a manmade dam or culvert, make sure there are no leaks or diversions around the structure.

To measure flow, record the starting and ending point (bank to bank) of the measuring tape. With the intention of creating equal intervals record the tape measurement at each flow measurement. Adjust the height of the sensor to the appropriate depth. If the total depth in that section is greater than 2.5 feet, measure velocity at two different depths within that section (at depths of 0.2 times the total depth and 0.8 times the total depth).

When measuring velocity, the rod must be held vertical and straight up (no lean) with the bulb facing upstream. The bulb and stem should always be parallel with the flow, therefore angle the bulb into the flow as necessary and record the estimated angle (in degrees) that the bulb was adjusted away from being perpendicular to the transect. A protractor is the most accurate way to estimate angle, but best professional judgment is acceptable. Measure velocity for a minimum of 20 seconds, while standing in a position that least affects the velocity of the water passing the meter (at least 1.5 feet downstream). Ensure that unnatural turbulence is prohibited while taking flow (upstream staff, Marsh McBirney cord, etc). Repeat this procedure for as many flow measurements sections as necessary.

Discharge values less than 10 ft^3/s should be reported to two significant figures. Discharge values greater than 10 ft^3/s should be reported to the nearest whole number, but not more than three significant figures.

The flow meters should be calibrated quarterly. Whenever the batteries of the units are changed, the zero-adjustment is lost, and the units must be re-zeroed.

To zero the unit, place the probe in a plastic 5-gallon bucket (or pool of still water if in the field), three inches below the water surface, wait 10 to 15 minutes and press the STO and RCL buttons simultaneously. A "3" should appear. Press the down arrow until the meter reads zero. A "32" should then appear on the display and the meter will turn off.

WPDRD FLOW DATA FIELD SHEET

Date: **Time:** **Team:**

Site:

Meter: ____________ **Instrument No.** ________

Substrate: smooth/irregular/rough - bedrock, sand/silt, gravel, cobble, boulder

Total Transect (ft): ________ **Number of Intervals:** ________ **Section Intervals (ft):** ________

Sec.	Width (ft)	Depth (ft)	V_1 (fps)* 0.2 or 0.6	V_2 (fps)* 0.8	V_{avg}** (fps)	Discharge (cfs)	Notes
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
					Total:		

* Depth > 2.5', velocity measured at 0.2 and 0.8.
Depth < 2.5', velocity measured at 0.6.
** $V_{avg}=1/2(V_1+V_2)$

Form: ERM10-2003

Figure 2.5-1 Field sheet for flow data collection if electronic data collection is not available

Flowtracker

Downloading steps of Flowtracker training:

1. Download Flowtracker software from here: V:\SUPPORT_AV\Sontek_flow_tracker\Flowtracker
2. Plug Flowtracker in using grey cable (I'll leave this in Scotts care for GW Flowtracker, the SW Flowtracker has a cable in its case).
3. Turn on Flowtracker
4. In software on computer, click "Connect to a Flowtracker"
5. The connection settings should be to COM1 – then press "Connect"
6. Press "OK" when it tells you that it found the Flowtracker
7. On left hand side of Flowtracker program (below where the Connect to a Flowtracker button is) click "Recorder"
8. Select the files that you want to download (you can use ctl to select multiple)
9. Click "browse" and save in whatever folder you want to keep these in. Right now, we have "G:\ERM\WRE\EQRR_PM\Flowtracker_Downloads" setup to keep Flowtracker files in (you just need to select/create a folder for your project)
10. Press "download"
11. You can export files to pdf's by printing to a pdf and saving in the same folder as the Flowtracker files. The files then need to be combined into a single pdf for the day and sent to Rob, after manually entering the flow data into the database.

Caution – it is crucial that the "Site name" that you enter during setup in the field is what you want to be displayed in the database (aka fit as much site info – number and name – as you can). "File name" is more a double check for yourself.

Other Quantitative and Semi-quantitative Methods

If site conditions are inappropriate, or if a Marsh-McBirney or Flowtracker is unavailable, other quantitative and semi-quantitative methods can be used.

For some sites with relatively small discharges or where conditions dictate, a graduated container and timer can be used to make accurate discharge measurements. This is particularly true for springs. Locate a point or modify (with as little damage to vegetation or mineral deposits) a point where a bottle/flask/plastic bag can capture all the discharge water. A bag (such as a Whirl-Pak®) is sometimes the best option since it is flexible enough to conform to the shape of the flow path. The water collected in the bag can then be poured into a graduated container to measure the volume. Use the following procedure and modifying the site:

1. Test the site to see if the container is capturing all the flow
2. If all the flow is not captured, estimate the volume bypassing the container (in percent or volume)
3. Insert the container in the flow and start the timer.
4. Record the time and volume measured
5. Repeat three times
6. Average the result

The equation is: $Q = V/T$

Q= discharge
V = volume (either gallons, liters etc.)
T= time

Another method to estimate discharge is visually estimating the water velocity and measuring water depth and width. This is similar to using a flow meter and can yield a result in cubic feet per second. This technique is more appropriate for small tributaries/drainage ways with relatively low discharge and slow water velocities. Accuracy increases with lower discharge and smaller cross section. A leaf, blade of grass or some other floating object can be used to estimate water velocity and either counting seconds or using a timer. This technique can be done by measuring either the length of time for the object to move a known or estimated distance travelled over a known period of time. An average of at least three discharge estimates is recommended.

The equation is:
$Q = L \times D \times V$

Q = discharge
L = length of section in feet
D = average depth of section in feet
V = feet per second

An estimate of discharge can also be made visually. As a point of reference, use a known discharge to visually compare with. For example, a typical garden hose on full discharges about 5gpm. Experiment with the hose since it appears as if more water is discharge if the water is spread over a surface rather than in a confined stream.

2.6 Diurnal (24-hour) Field Measurements

Average measurements of dissolved oxygen may be submitted to TCEQ for inclusion in the 303(d)/305(b) review process by ERM staff in conjunction with the Clean Rivers Program. All procedures for 24-hour average field measurements should comply with TCEQ (2003) requirements.

All diurnal measurements for the Clean Rivers Program must occur within the index period (March 15-October 15), with at least $^1/_2$ to $^2/_3$ of measurements occurring within the critical period (July 1-September 30). Only the first 24-hours of measurements from the time of deployment may be used to calculate average values.

For any site, diurnal sampling events must be at least one month apart. Flow must be measured either during deployment or retrieval of the logging datasonde for diurnal sampling. Flow must be greater than the published 7Q2 value (TSWQS 1997).

For shallow streams, diurnal measurements should be collected from the surface, with the main goal to best represent the conditions of that water body. Diurnal sampling within reservoirs should be conducted within the mixed surface layer.

The minimum, maximum and average parameter concentration should be entered into the database along with the number of measurements used to generate those summary statistics. Datasondes must properly calibrate prior to deployment, and all parameters must meet post-calibration requirements (see table 2.1). Downloaded data files should be stored in the following folder subdirectory: Project Folder\Field Data\Datasonde. Data is turned into the designated DA/DS officer for entry into the database after which it is transferred into the subdirectory Loaded to the database.

2.7 Quantum Photometer Field Measurements

The photometer may be used to measure light extinction, an important parameter in algae modeling. To use the photometer:

1. Press power button to start.
2. Choose Calibration by pushing MODE once, then use UP/DOWN to choose Sun (not Electric) Calibration, since the meter will be used outdoors.
3. Choose Mode by pushing MODE twice, then use UP/DOWN to make choice:
   SMPL for manual measurements (field use) LOG for automatic measurements
   Once desired mode is blinking, press MODE two more times to begin.
4. To use SMPL Mode, Press SAMPLE when you want to take a measurement. It can store up to 99 readings; to review data, press UP/DOWN. To exit and return to sampling mode, press SAMPLE. You will have to press SAMPLE again to take another measurement.
5. When in LOG mode, meter will turn on to record data every thirty seconds. For more information on using this feature, go to www.apogeeinstruments.com
6. If you take 99 readings, and still need to record more data, you will need to re-set the meter, erasing all measurements. Press MODE twice (RUN should be blinking), then press DOWN and press MODE once.
7. For reservoir use, follow steps 1-3 above (choose SMPL mode)
8. To take the first reading, hold the meter just above the water surface, press SAMPLE and record the value in field notebook or datasheet.
9. Then lower the meter 10 cm (4 inches) into the water column and take another reading and record. Continue taking readings every 10 cm until you note a sharp decline in light levels.

2.8 Datasonde Programming

Hydrolab Programming for Continuous Sampling Using Hydras3 LT

A. Create file
   a. Open Hydras3 LT (available on disc)
   b. Operate datasonde > System (tab) > Set clock to PC time
   c. Online Monitoring (tab) > Start > verify there is enough internal battery voltage 11 volts (±2 volts)(this is an estimate)
   d. Log Files (tab) > Create > type name for file (site#_month_day_year_datasonde#) > select parameters (save parameters in a template by right clicking in parameter box)
   e. Set Start Logging and Stop Logging (allow extra time to elapse past the 24 hour cycle) > confirm other options are correctly set > Save Settings > ****Enable** (this is very important and easily forgotten)**

f. Close Hydras3 LT
g. Attach a sticker or tape to the datasonde indicating where it is to be deployed and what time and date the logging begins
h. You are now ready to deploy

B. Download file

Option 1:

a) Open Hydras3 LT
b) Select datasonde > select file to download > select “Save file to:” location > check “Delete files in datasonde after reading > Download Selected Files

Option 2

a) Open Hydras3 LT
b) Operate datasonde > Log Files (tab) > select file from “Log File” drop down list > download > File > EXPORT Excel
c) Open Excel > verify presence of all data
d) Hydras3 LT window > Delete > yes > ok

C. Delete file

a. Open Hydras3 LT
b. Select datasonde > Operate Datasonde > Log Files (tab) > Disable (if logging) > select file > Delete > yes > ok
c. Close Hydras3 LT

Hydrolab Programming For Continuous Sampling Using Hyperterminal

A. Connect to Hyperterminal
   a. Open Hyperterminal software (Start > Programs > Accessories > Communitactions > HyperTerminal)
   b. Connect cable to computer port Com1 and then connect to hydrolab
   c. Name new connection > connect using “COM1” > ok > Bits per second “19200 or 9600” (verify on Hydrolab) > Data bits “8” > Parity “none” > Stop bits “1” > Flow control “hardware” > ok

B. Begin Programming
   a. “Files” e> “Create” e> name file (site#_month_day_year_datasonde#) e> input start date e> input start time e> input finish date e> input finish time e> input interval (15 minutes) e> input sensor warm-up time (2min for DS3 or MS4, 30 seconds for MS5) e> input circulator warm-up (2min) e> enable audio (no) e> “file created press any key” e> “files” e> “status” e>

C. Download Files
   a. Connect to Hyperterminal
   b. “Files” e> “Transfer” e> “Sensors Off” e> “Spreadsheet” e> “XMODEM-1K” e> select appropriate log file “*log file name to be d/l*” e> “Transfer” (at top of page) > “receive file” > (address of file placement) > ok > “Transfer complete press any key” e> verify the file transferred using Excel and importing the .CSV file using the comma separation choice

D. Delete Files
   a. “Files” e> “Delete” e> “Select Log File:” # e> y e> press any key

3.0 Water Sample Collection

Water sample collection should be performed before any other activity is done at the site, unless holding time restrictions require field parameter collection first.

3.1 Site Selection & Access

Sampling locations should be chosen to best characterize water quality for a given stream reach or segment. Program specific sampling locations are listed in each QAPP.

When choosing sites, consider historical water quality data availability, location of flow gage stations, coordination with the EII and access. Sampling should be conducted at sites which can be accessed safely under most expected flow conditions. Sampling should be conducted on public property unless specific permission is received from private landowners to access sites on private property.

3.2 Surface Water Sample Collection

Prior to sample water collection, sample bottles should be labeled with (at a minimum) date, time, site name and database number, collecting staff, and preservation method. Use waterproof pens (such as Sharpies) for sample bottle labeling.

Unless specified in a Quality Assurance Project Plan, sample containers should be new, unused, clean polyethylene containers or glass jars. **DO NOT PRERINSE NEW BOTTLES WITH SITE WATER.** If a new bottle is not required (e.g. benthic macroinvertebrate collection), a re-used bottle should be rinsed three times with DI water prior to sample collection.

Surface water should be collected from the top one-third of the water column for shallow streams and from no greater than 0.3m deep for water bodies greater than 0.5m of depth. To the extent practicable, collect surface water facing upstream and from within the centroid of flow. Take precaution to ensure that no upstream disturbance has occurred (walking, sonde placement, sampling, etc. and avoid collecting and surface debris (scum, pollen, detritus, etc.) or bottom particulate into the container, especially for nutrient and chlorophyll-a samples that could be influenced by surface pollen. Sample bottles should be held upright with the mouth of the bottle facing the water surface, the bottle mouth should be submerged below the surface of the water, and then inverted to avoid direct sampling of the water surface.

When required for preservation, acid should already be present in the designated bottles (inserted by the bottle supplier). Special handling should be employed for sample bottles with preservatives such as sulfuric or nitric acid and for other additives such as sodium thiosulfate. Bottles with preservatives should not be submerged (to avoid washing out preservative), but rather sample water should be poured in from collection with a clean/unused bottle. If adding preservative while in the field, the appropriate amount of acid (see Section 3.10) should be added with a pipette, then samples should be capped and agitated to ensure adequate mixing and placed on ice for transport to the lab.

When collecting samples for bacteria analysis, leave a head space of at least ½ inch in bacteria bottles (often referred to as Bac-T bottles) to allow for agitation prior to filtering. Be sure to fill at least to the 100 ml line on the bottle. If several dilutions are anticipated, collect water in a 250 ml Whirl-Pak®, otherwise use the sample bottle specified in the QAPP for that project. It is preferable to decant sample bottles in the field to avoid problems with decanting in the lab that may result in a yield of less than 100 ml total volume.

Whole water samples for phytoplankton identification should be collected as surface samples in new or a triple rinsed 1 L plastic sample bottle, placed on ice and transported to the lab where they should be preserved with 32 ml of 10% formalin. Be sure to note volume of formalin added and notify the algal taxonomist as the preservative volume is subtracted from the final total sample volume prior to determination of cell abundances.

All samples should be immersed in an ice bath with the intent to promptly reach $\leq$ 4°C for transport to the lab and kept out of sunlight, making sure to meet holding times for each requested analysis.

Sample Collection with Kemmerer

Sampling water at depths below 0.3 m in a reservoir is conducted using a Kemmerer sampler, designed to collect approximately three liters of water at a range of depths. Additionally, the Kemmerer may also be used to generate split samples for quality control purposes.

Before deployment, the Kemmerer must be opened and pre-rinsed with ambient site water. Pushing the metal rod flush with the top seal will open the bottom seal of the container. The top seal can then be pulled up. Ensure that the seals stay open by holding the sampler by the rope and moving it gently. Make sure the metal messenger is not attached to the rope, and then lower the sampler to the desired depth. If collecting a sample near the lake bottom, allow the sampler to gently touch bottom and then raise it slightly to keep bottom substrate out of the sampler. When at the desired sampling depth, turn the top of the messenger to open it, attach it to the rope and then let it snap shut on the rope. Holding the rope straight up above the sampler, drop the messenger down the rope with some force to trigger the sampler to close. For best results, tug upward on the rope when you feel the messenger make contact with the Kemmerer to increase the force exerted on the trigger mechanism. Following retrieval of the sampler from depth, check the chamber to ensure that sediment was not inadvertently collected. If sediment is present in the chamber, release the water, rinse with ambient water and re-sample. If no sediment is observed in chamber, use the spout on the bottom of the sampler to fill the sample bottles. Once all sample bottles are filled, the remaining water can be released by opening the sampler over the edge of the boat.

3.3 Groundwater Sample Collection

In most springs, the near surface discharge is representative of the local groundwater. As a result, groundwater grab samples are collected at springs by directly filling the sample container as close to the spring's discharge point as possible. DO NOT PRERINSE CONTAINERS. If the spring's discharge is very low, a sterile Whirl-Pak® (or sterile intermediate container) is used to grab and transfer aliquots of sample water into the sample container. An alternative method to using a Whirl-Pak® is to use a portable peristaltic pump with sterile tubing to fill the sample container. In both cases, extreme care is used to avoid contaminating the sample with debris. In addition, the spring's discharge is noted on the field sheet by ether direct measurement or field observation estimation.

To collect a dissolved metals sample, it must be filtered in the field with peristaltic pump and in-line filter set. To minimize contamination unpowdered latex gloves are always worn during sample collection and when possible, an intermediate collection bottle is not used. When the peristaltic pump is used, field equipment blanks are collected before a ambient sample is collected for 10% of sample set at a site(s) with the same tube and filter that will be used the collect the sample. This involves pumping De-Ionized Ultra Filtered (DIUF) water though the in-line filter set into sample container for analysis. All quality control and sampling labeling protocols follow those in the most current TCEQ SWQM guidance available online.

Immediately after collection the appropriate preservation method is followed for each sample container. Samples are stored on ice in an ice chest and if required acidified with the appropriate acid for transport to the lab. Sufficient ice is used to lower the sample temperature to less than 4°C. Samples should be kept out of sunlight.

3.4 QA/QC Sample Requirements

Each sampling program shall identify the required quality assurance/quality control (QA/QC) measures as specified in the program specific QAPP. However, each surface and groundwater sampling program should collect a minimum of one field split or duplicate sample per program per sampling event and one field blank per program per year.

Field split, field duplicate, and blank samples should be labeled according to pre-assigned "dummy" names and database site numbers, as listed in each QAPP or as assigned by data team representative as necessary. "Dup", "Blank" or "Split" should not appear on the sample label or chain of custody form for any QA/QC sample submitted to a contract laboratory.

The pre-assigned site name, number, and time of sample should be recorded on the field sheet or electronic form for the site associated with the QC sample. The sample time for the blank, split, or duplicate should not be the same as the sample time of the parent site sample. Times for QC samples should be offset by a reasonable amount of time (typically 15 minutes). In general, blanks are associated with the first site in a sampling run, while splits and duplicates can be collected at any site. Record the site associated with the Blank/Split/Dupe through comment field in digital format, or hard copy field sheet.

Sample collection procedures are as follows:

Field split-A new, large container (typically a 1 L plastic) should be used to collect the water for use as a field split. At the sample site designated for field split collection, fill the large container with surface water. Prior to dispensing sample water, ensure that parent and split bottles are both labeled with their respective site name/number and different sample times (typically 15 min later). Fill sample bottles in like pairs by evenly distributing water from the large container back and forth incrementally until full (do not fill one wholly and then the other). Re-fill the large container to complete filling the sample bottle pairs as needed.

NOTE: For reservoirs, the Kemmerer sampler is the large container (see Section 3.2 for Kemmerer use)

Field duplicate- Label each bottle as described for the field split (one with regular site I.D. and one with field dupe information, different times for each). To fill, hold each pair of bottles (both 250 ml plastic bottles, etc) side by side and fill simultaneously from the water body.

Blank- Use Type III, distilled or clinical laboratory reagent-grade (not R.O.) water from the ERM 11th floor laboratory. Label each new bottle with the pre-assigned 'dummy' site name and DUDB number, including the time of actual sample collection. Reused bottles (e.g., for EII sampling analyzed in-house at ERM lab) should continue to be pre-rinsed three times with the Type III water prior to being filled. Fill the blank bottle set with Type III water. Place the sample bottles on ice in the cooler that is to be carried in the field for regular sample storage. Continue with regular sample collection.

3.5 Antecedent Flow Requirements

Baseflow conditions are those in which the waterbody is not significantly influenced by either stormwater runoff or abnormal conditions (e.g. turbid water from nearby dewatering or unauthorized discharge). Most sample program QAPPs require samples to be collected under baseflow conditions. All samples shall be collected under baseflow conditions unless otherwise specified in the program QAPP. Baseflow conditions must exist at all sites at the time of water quality sample collection unless specified in special studies fully documented in the program

QAPP. Baseflow conditions should be determined using antecedent rainfall information, but consideration may also include field observation and best professional judgment based on data.

Rainfall totals for the sampled watershed may be acquired using FEWS data, or other credible weather station information (i.e. RainVieux).

Rainfall totals will be calculated from the 24-hour, 48-hour and 72-hour periods preceding the estimated time of first sample collection and compared to the criteria in Table 3.5-1 to determine if baseflow conditions exist

Table 3.5-1 Antecedent rainfall totals upon which to determine baseflow conditions

Rainfall total in 24-hour period prior to sampling (Inches)	Time to wait until baseflow conditions return (Hours)
≥0.10	24
≥0.25	48
>1.00	72

3.6 Core Parameters

Lists of routine lab (Table 3.6-1) and field (Table 3.6-2) typically evaluated in water samples are listed in Table 3.6-1 and 3.6-2 The contract laboratory should be consulted for current costs. Additional parameters may be collected as specified in the sample program QAPP.

Table 3.6-1 Routine core laboratory parameters for water samples. Consult the current lab contract for parameter cost.

Parameter	*Bottle*	*Analytical Method*	*MDL*	*Reason for Monitoring*
Ammonia-Nitrogen (mg/L)	250 ml Plastic, H_2SO_4, Ice	EPA 350.1	0.02	NH3 is used by TCEQ for identifying secondary concerns; high levels of NH3 may be toxic to aquatic organisms; NH3 has multiple anthropogenic sources
Nitrate+Nitrite-Nitrogen (mg/L)		EPA 353.1	0.002	NO3+NO2 is also used by TCEQ for identifying secondary concerns in Texas waters; high levels of NO3 may be toxic to humans and aquatic life; NO3 has multiple anthropogenic sources
Total Kjeldahl Nitrogen		EPA	0.04	TIKN is the sum of organic nitrogen (ammonia and ammonium) and is informative to quantify the amount of nitrogen in organic form
Total Orthophosphate (mg/L)	250 ml Plastic, Ice	EPA 300.0	0.02	OP is the bioavailable portion of phosphorus in water; the state standard is for dissolved OP, and additional sampling techniques (field filtering of samples) would have to be employed for this data to be considered valid for inclusion in 303(d)/305(b) assessments. Total OP can still be used as a screening tool in non-official comparisons to dissolved OP standards.
Turbidity	WhirlPak (>15ml)	Trilogy Fluorometer		Turbidity informs water quality information that may not be reflected in TSS data due to cloudiness of fines or colloids that reduce light penetration and photosynthetic productivity
Total Suspended Solids (mg/L)	1L Plastic, Ice	EAP 160.2	0.5	TSS is useful in quantifying suspended sediment loads and is required by the masterplan. BMP design criteria in TCEQ Edwards Rules (and others) are based on TSS removal
Escherichia coli (cfu/100 mL)	125 ml BacTNa$_2$ S$_2$O$_3$, Ice	SM 9223 B	1	State standards for bacteria have shifted to *E. coli*.

Table 3.6-2 Routine core field parameters for water samples.

Field Parameter	***Recommended Methods***	***Reason for Monitoring***
Dissolved Oxygen, mg/L	Multi-probe (YSI, Hydrolab or Quanta)	Water quality standards exist for DO; despite high variability, DO is a useful screening tool in assessing water quality impairments
Water Temperature, °C	Multi-probe (YSI, Hydrolab or Quanta)	Water quality standards exist for temperature; temperature is useful in normalizing the concentrations of DO
Conductivity, μS/cm	Multi-probe (YSI, Hydrolab or Quanta)	Water quality standards exist for conductivity; conductivity does change with increasing urbanization; extreme concentrations of conductivity may be detrimental to humans and livestock
pH, standard units	Multi-probe (YSI, Hydrolab or Quanta)	Water quality standards exist for pH; extreme pH values may be harmful to aquatic life
Instream Flow, ft^3/s	Marsh-McBirney meter	Flow should be collected with field samples not only to enable pollutant loading calculations but also to document changing patterns in flow regimes over time

3.7 Field Data Collection Forms

Field data may be collected on a project-specific field sheet, such as the standardized field sheet shown in Figure 3.1-1 or a standardized project-specific field sheets such as those for depth profiles in reservoirs (Figure 3.1-2). Most modern data is collected in a customized Fulcrum application on iPads similar to the electronic format shown in Figure 3.1-3.

WPDRD FIELD DATA SHEET

Database Entry		
Locked:	P ☐	F ☐
Date:	________	________
Entered by:	________	________
Ref. No:	________	

Database No.: ________ Project: ________

Site Name: ________ Geologic Formation: ________

Date: ________ Watershed: ________

Time: ________ QA/QC Name: ________

Personnel: ________ Blank, Split, Duplicate

Current Weather: ________ Antecedent Weather: ________

________ Day Since Sign. Rain: (> 0.1") ________

________ FEWS Gauge/Other: ________

Flow Type: Baseflow (B) - Stormflow (S) - Special Events (E) - No Flow or Dry (N)

Flow Severity: Dry - **No** Flow (pools only) - **Low** Flow - **Normal** Flow - **High** Flow - **Flood**

Sample Type: Grab, Grab Composite **Medium:** Surface Water, Groundwater, Sediment, Soil, Other:______

Field Parameters:

Parameter	Value	Units	Passed Post-Calibration Yes	No	Parameter	Value	Units	Passed Post-Calibration Yes	No
Dissolved Oxygen	________	mg/l	☐	☐	Total Dissolved Solids	________	mg/l	☐	☐
Water Temp.	________	°C or °F	*		Flow	________	gpm or cfs	*	
pH	________	Std.Units	☐	☐	Conductivity	________	µS/cm	☐	☐
Turbidity	________	NTUs	☐	☐	Dissolved Oxygen %	________	% Sat.	☐	☐
Depth	________	ft or m	*						

***** Annual Calibration Check***

Method: Hydrolab-MiniSonde, Hydrolab-DataSonde, Hydrolab-Quanta, Cole Parmer, Other SN# or Instrument # ________

Flow : Estimate, Marsh McBirney, Measured w/ bottle, USGS gauge ________

Lab Analysis: **Laboratory Analyzing Samples:** COA-WWW, LCRA, COA-ERM, Other:________

Nutrients	Ions	Semivolatiles	Chlorophyll-α
TSS	Pesticides	PCBs	Isotopes
Bacteria	Herbicides	TOC	Others: ________
Metals	VOAs	VSS	Others: ________

Notes: (Sampling Location, Site Description - Algae Type, Algae % Cover, Substrate Description, Flow, Water Color and Clarity, General Vegetation, Benthics, Fish, Trash, Sample Collection and Field Measurement Location, Number of Grab Samples and etc.)

Calibration Notes: Instrument Passed Post Calibration (Yes or No) : ________

(Dissolved Oxygen **±0.5 mg/l**; pH **±0.5 Std.Units**; Conductivity and TDS **±5%**; Temp. **±1°C**, annual check; Depth **±0.2 m**, annual check; Turbidity **±0.5 NTUs**)

Form : ERM10-2003

Figure 3.7-1. Standard field data collection form.

LADY BIRD LAKE SAMPLING- FIELD SHEET

SITE__________________________ DATE___________

WEATHER_________________________________ TIME____________

DAYS SINCE LAST RAIN__________ STAFF___________

TOTAL DEPTH_______(Enter in DB at deepest depth)

DEPTH	TEMP	PH	DO	COND	SECCHI (record at surface)

FIELD NOTES:
Water color, clarity, surface

POST CAL
PH
______7.00_______10.00

COND
_______500

DO
______ ______ ______
actual theor temp

Instrument Number __________________

Figure 3.7-2 Standard reservoir field data collection form.

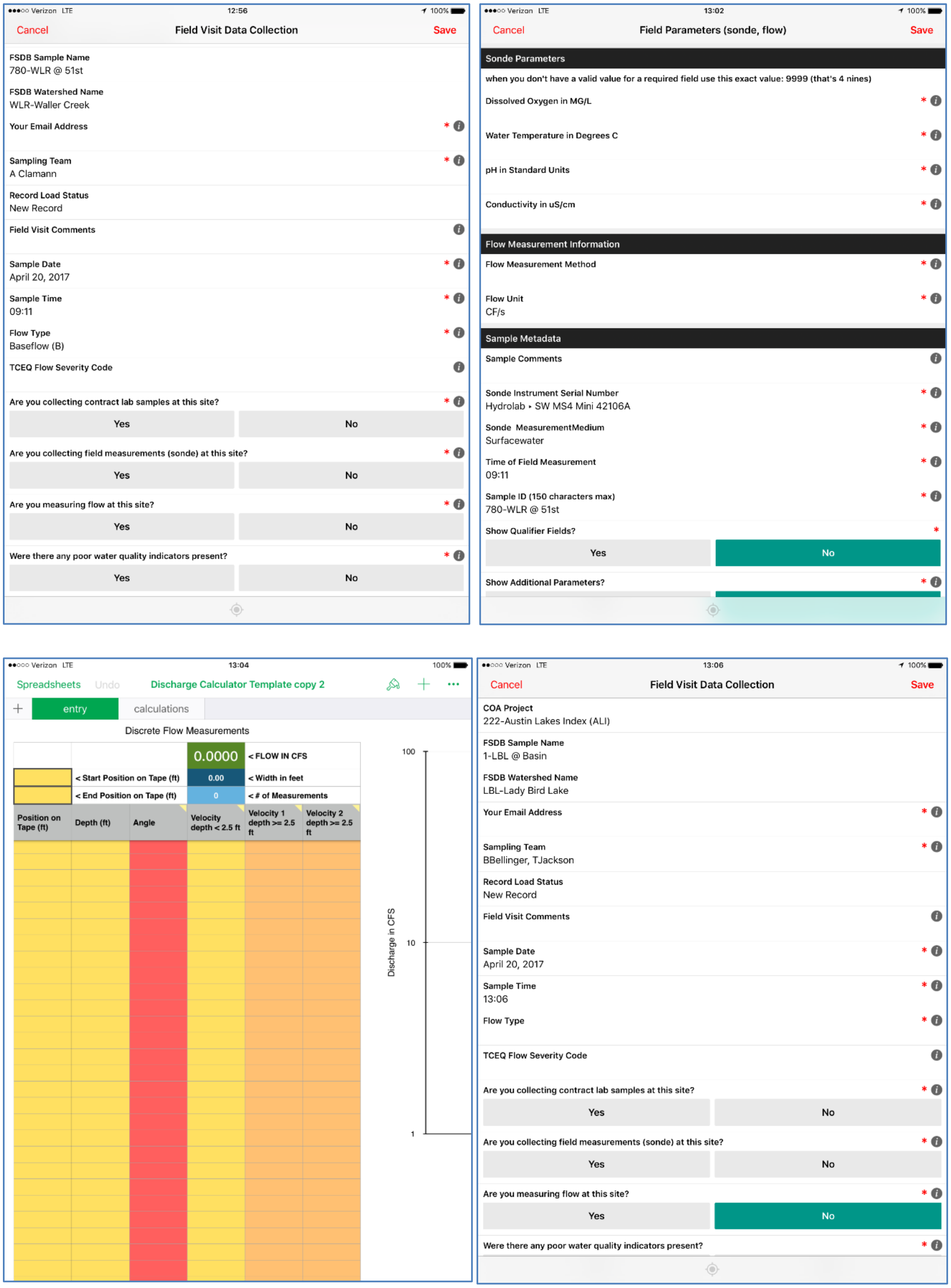

Figure 3.7-3 Examples of electronic format (Fulcrum app on iPad) for field data collection

3.8 Field Book Notation Guidelines

The preferred method for recording all quantitative and qualitative data is either on standardized hard copy or electronic field sheets. However, if neither are available/appropriate to collect all desired information, a field notebook can be used to record primary or ancillary information. Following entry of information in the database, pages of the field book should be scanned, and the electronic PDF linked to the Field Sample Reference Number for the purposes of archive and QA/QC.

Entries in field notebooks should contain at a minimum the date, site name and number, initials of staff, and time of visit. Additional notes can include unusual circumstances, particularly those related to data collection (or lack of it). For example, if two sites in a sampling ‘run’ are dry or have no flow, this information should be recorded in the field notebook. Problems with sample collection or field equipment should be noted, especially if equipment fails and data is not collected.

In addition, if the individual does field work that does not involve sampling, such as Capital Improvement Project (CIP) reviews, notes from these site visits should also be kept in field notebooks to provide a permanent record of the visit.

3.9 Chain of Custody

Chain of custody forms provided by the contract laboratory, or generated using the ERM Event Log database form, will be completed for all samples submitted for analysis.

3.10 Preservation of Samples

All samples except benthic macroinvertebrates should be preserved on ice to a temperature of 4˚C unless otherwise specified in the QAPP for that project.

Nitrogenous nutrient samples require preservation with sulfuric acid (H_2SO_4) (or freezing) if the sample is not to be analyzed within 24-hours of sample collection. The preferred method is to collect sample in a bottle that has been “pre-acidified”. If not collected in a sample bottle with preservative already added, acidification of samples to a pH of less than 2 can be conducted in the field by ERM staff using H_2SO_4 solution at a dilution of 50% by volume of 36-37% H_2SO_4 with 50% by volume reagent-grade or DI water in accordance with the amounts shown in Table 3.10-1

Samples for total metals require preservation with nitric acid (HNO_3). The preferred method is to collect sample in a bottle that has been “pre-acidified”. If not collected in a sample bottle with preservative already added, then acid can be added by ERM staff in the field.

Table 3.10-1. The required acid addition are presented below by sample bottle type.

Bottle Type	Amount of Acid	# of Drops with Pipette
1 L	1 ml	10 drops
500 ml	0.5 ml	5 drops
250 ml	0.25 ml	3 drops

4.0 Sediment Sample Collection

Sediment collected in creeks and lakes should be representative of the sampling reach. Samples should be obtained from recently deposited fine bed sediments. Every effort should be made to collect sediment in depositional areas of lower hydrologic energy to insure a maximum amount of new, fine materials. Avoid hard-packed bank sediments and disturbed (scoured) areas, as these are most-likely not freshly deposited materials.

In shallow creeks, visual assessment of the entire area is possible before collecting the sample. In deeper areas such as reservoirs, visual inspection is impossible and several attempts to collect appropriate sediment may be necessary.

4.1 Equipment

Sampling equipment consists of a stainless steel or Teflon coated metal scoop, disposable pre-sterilized HDPE plastic scoops or the stainless-steel petite ponar dredge or Eckman dredge for collection in deeper water. The selection of sampling equipment is determined by the substrate (rocky areas and small pockets of deposited materials preclude the use of the dredge), the depth of the water and the parameters for analysis (to avoid contamination). Polystyrene scoops may not be used.

Only clean, washed sampling equipment should used. The sampling equipment is pre-rinsed at each site with native water. The equipment is rinsed between samples, removing all sediment from previous samples. For sampling in lakes or other bodies of water that are known to be infested with zebra mussels, it is imperative that equipment be bleached, otherwise sterilized or TPWD Clean-Drain-Dry protocols be followed.

4.2 Sample Collection

A minimum of three (preferably more, depending on the amount of sediment needed) sub-samples are collected and deposited into a clean glass bowl, which is pre-rinsed at each site with native water. All material is well mixed before subsampling into the proper container.

For lake sampling, only the top layer, or "recent deposits" are removed from the dredge sample as described in the most recent version of the SWQM manual (TCEQ 2003). Reservoir samples should be collected from aerobic zones, generally the uppermost 5cm of sediment. Blackened or otherwise obviously anoxic sediment in the grab should not be included in the composite.

Samples are then composited directly into properly labeled and certified clean glass containers or unused sterilized Whirl-Paks®. Care should be taken to minimize water content of sediment samples to avoid inflation of detection limits. If necessary, slowly decant overlying water from glass bowl after fine sediments have generally settled prior to emptying sample into final container.

If sediment and water samples are to be collected during the same sampling event, water samples should be collected prior to sediment samples.

Field notation should include at a minimum:

- Type of composite (both time and space-composited?)
- Number of grabs
- Duration of sampling
- General description of the sample including color, texture and odor.

4.3 Chain of Custody and Preservation

Chain of custody for sediment samples must be maintained and documented on the appropriate chain of custody forms provided by each contract laboratory. See FSDB guidance for more details.

Place samples on ice as soon as collected to cool to 4ºC for transport to the appropriate laboratory. Avoid exposure of sample containers to sunlight.

4.4 Routine Sediment Parameters

Routine sediment investigation parameters have been determined by committee and may be found in the ERM core parameter documentation (WRE 2003). For convenience, the list of core sediment parameters is presented (Table 4.4-1). Except for specific investigations with pre-determined objectives, this list may be considered the standard analytes for all sediment samples.

A need for periodic screening of chlorophenoxy herbicides and organophosphorus pesticides has been recognized and should occur on a project-by-project basis as suggested by data analysis.

Table 4.4-1. List of routine ERM sediment parameters

Category	Parameter	Lab*	Method
Nutrients	Ammonia	sub-contract	EPA 350.1
General	Oil and Grease	DHL	EPA 431.2
	Percent Moisture	DHL	ASTM D2216
	Texture	sub-contract	Hydrometer
	TOC (%)	DHL	EPA 9060
	TPH (by TX1005)	DHL	TX 1005
Metals	ICPMS Metals (As, Cd, Cr, Cu, Fe, Pb, Ni, Zn)	DHL	EPA 6020
	Mercury	DHL	EPA 7471A
Organics	PAHs	DHL	EPA 8270C
Pesticides	Organochlorine Pesticides	DHL	EPA 8081
Other	Polychlorinated Biphenyls	DHL	EPA 8080

* DHL and/or LCRA.

5.0 Biological Sample Collection

Routine biological sampling by ERM staff includes assessment of benthic macroinvertebrates, diatoms and algae.

5.1 Algae Transects

Monitoring of the relative coverage and composition of aquatic macrophytes and algae is used as another indicator of change in water quality over time. Coverage is estimated along linear transects by standard plant ecology techniques (Barbour et al 1980, Blum 1957, Hynes 1970). The distance that all plants or unvegetated substrates project through the plane of the line is tallied and expressed as a percentage of the total length of the line.

Pool Method
Surveys are typically conducted in pools, with each pool divided by three to six equally-spaced transects depending on the length of the pool. Measurement is typically performed with two staff members. As one person records the data the other attaches the tape measure across the stream, observes the benthic cover and calls out the number of feet observed in the various categories. If waters are deep, a diving mask may be used to observe the cover.

All aquatic vascular macrophytes are identified to the genus level using Correll and Correll (1972) as a reference. Non-filamentous algae with macrophyte morphology such as Nitella and Chara species are identified by genus. Commonly encountered spongy composites of blue-green algae, diatoms, and sediment are identified as "carpet algae" if the cover has enough integrity to bind together when disturbed. Substrate will be described as silt if it breaks into fine suspended solids when disturbed.

Filamentous algae are categorized as *Cladophora* species or *Spirogyra* species types depending on their texture and branching habit, as described below. All blue-green algae are lumped together, and un-vegetated substrates are identified as one of seven categories. Five categories of un-vegetated substrate are characterized by particle size (Compton 1985), and the other two un-vegetated substrates were characterized as bedrock and leaf litter (included any dead or decomposing organic matter). Altogether, 26 commonly encountered categories of cover are listed on the field data sheet, with several blank columns available for the addition of rarely encountered plants or substrates. These categories may be lumped into four super categories for analysis purposes: un-vegetated substrate, filamentous algae, non-filamentous algae, and aquatic macrophytes.

Cladophora species algae is coarse in texture and is multi-branched in morphology, making field identification of this genus rather easy. Other non-branching, slimy textured algae are identified as a *Spirogyra* type on the data sheet.

Riffle Transect Method
Transects may be conducted in riffles to estimate periphytic algae coverage, substrate composition and macrophyte coverage, using sample field sheet in Figure 5.1-1.

Site #
Site Name:
Date:
Observer:

Record % by Type

Type	Transect 1	Transect 2	Transect 3	Comments
Wetted Width (ft)				
Vascular macrophytes (*Justicia*, *Eleocharis*)				
Vascular algae (*Chara*, *Nitella*)				
Filamentous algae (*Cladophora*, *Spirogyra*)				
Abiotic stable substrate (bedrock, cobble)				
Abiotic unstable substrate (silt, sand)				
Organic material (leaf litter, carpet algae)				
Scenescent Algae/Periphyton				
	0	0	0	

General Comments:

Figure 5.1-1. Sample data sheet for algae transects in riffles.

Zig-Zag Method
Stream reaches may be characterized by a 50 point zig-zag transect moving from downstream to upstream using the sample field sheet shown in Figure 5.1-2. At each survey point a 1 ft-sq area quadrat will be placed with the survey point at the center. The composite cover is the percent of macroalgae (filamentous only) within the quadrat and will be noted categorically (0 = none, 1 less than 5%, 2 = 5-24%, 3 = 25-49%, 4 = 50-74%, 5 > 75%). If there is loose substrate within the quadrat, the closest rock (>32 mm but small enough to pick up with one hand) to the center of the survey area will be examined for percent cover of macroalgae (filamentous only) using the same categorical scale as the composite cover. In addition, this rock will be examined for thickness of microalgae (ie Diatoms). Thickness is to be measured using the following scale: 0 = Not Collected, 1 = Rough/Slimy, 2 = Visible - <0.5mm, 3 = 0.5-1.0 mm, 4 greater than 1.0 mm. If there are no sizeable rocks within the quadrat, larger rocks or bedrock within the quadrat should be examined for microalgae thickness. Microalgae thickness will not be collected if all substrate

within the quadrat is < 32 mm. If microalgae thickness is not recorded, then the reason for not collecting data must be noted in the comments field.

Site ______________________ Site # ______
Project ______________________ Project # ______
Date ______________________
Staff ______________________

Point	Composite Cover[1]	Rock Cover[1]	Thickness[2]	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
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38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				
50				

Cover (filamentous only)[1]	
0	None
1	<5%
2	5-24%
3	25-49%
4	50-75%
5	>75%

Microalgae Thickness[2]	
0	not collected
1	rough/slimy
2	visible - <0.5mm
3	0.5-1 mm thick
4	> 1mm

If thickness not collected note why
Transect: diagonal zig-zag from bank to bank, downstream to upstream
50 equally spaced points
At each survey point a 1 ft-sq quadrat will be used to estimate composite cover.
The rock closest to the center of the quadrat is to be examined for rock cover.
If there are no rocks >32mm and small enough to easily handle with one hand in the quadrat, then put a dash line under rock cover.
Using the same rock used for rock cover, examine the microalgae thickness. If no sizeable rocks are present within the quadrat, estimate the thickness on bedrock or large rocks within the quadrat. If no suitable substrate exists (ie rocks <32mm) then ma

Figure 5.1-2. Sample field sheet for ZigZag method for algae coverage.

5.2 Flow Requirements & Timing of sampling events

Historically (prior to 2006) biological samples were not collected when baseflow was absent. However, beginning in 2006init was determined that due to perennial pools serving as refugia and due to the frequency of cessation of flow of many streams, it was advantageous to sample streams even if they lacked flow in order to support the goal of characterizing and scoring of the biological community. Bioassessments since that time collected samples in perennial pools with kick nets in the absence of flow. Ideally, biological sampling should occur during baseflow conditions, however sampling may occur after, or during small rain events that do not scour substrate or otherwise significantly impact the biological community.

Biological sampling is prohibited within 2 weeks following a "scouring" event. Determination of a scour event can be based on best professional judgment based on the physical conditions at the site and using the best available remote sensing information for the given water body. Typically scour can be assumed to be daily mean flow that exceeds the 90th percentile. Daily mean flows at USGS gages (http://tx.waterdata.usgs.gov/nwis/current?type=flow) have been used to determine the 90th percentile mean daily flow values for representative EII gages (Table 5.2-1).

Table 5.2-1. Mean daily high flow values resulting in 2-week postponement of sampling.

USGS Gage	High Daily Mean Flow (cfs)
Bear Creek @ FM 1826 (08158810)	10
Bull Creek @ Loop 360 (08154700)	50
Walnut Creek @ Webberville Rd (08158600)	75

Screening level sampling and EII biological sampling should be conducted between the TCEQ SWQM index period (March 15 to October 15), and if possible within the TCEQ SWQM critical period (July 1 to September 30), but may be adjusted to optimize baseflow conditions and reporting schedule needs.

5.3 Benthic Macroinvertebrate Sampling

Benthic Macroinvertebrate Sampling from Creeks

Flow, collected by standard ERM field procedures as defined in the Standard Operating Procedures Manual, will be collected at each site when biological samples are collected and as directed by individual project QAPP. Field data will be entered in electronic data collection device as available, and when unavailable a field data sheet (see example Figure 5.3-1) shall be used.

Site Name: ______________ **Site #** ________ **Date:** ________ **Time:** ________

Benthic Macros

Conditions (check one)
- ☐ **Sampled** during Baseflow (B)
- ☐ **Sampled** pools No Flow (N)
- ☐ **Not Sampled** No Flow (N)

Equipment
- ☐ Surber ________ (surbers)
- ☐ Subsampler ________ (grids)
- ☐ Kicknet ________ (minutes)

QA Detritus? YES NO
Personel:__________

Notes

Diatoms

Conditions (check one)
- ☐ **Sampled** during Baseflow (B)
- ☐ **Sampled** pools No Flow (N)
- ☐ **Not Sampled** No Flow (N)

Substrate
Rock #1:
Rock #2:
Rock #3:

Notes
QA Detritus? YES NO
Personel:

Figure 5.3-1. Example biological sample field data sheet.

For all surveys, count, note and remove all Corbicula from samples (mussels have a tendency to foul a small sample vial). Permanent paper labels describing the location and time of sample collection will be placed in all field vials.

Field teams will need the following equipment: one surber sampler (1 ft^2, 500 μm mesh-size), one Caton sub-sampler, sample vials, mechanical pencils, picking pan(s) and extra forceps for each person who will be sorting. Three surber samples, including all detritus, will be collected from the bottom, middle and top parts of the riffle at each site.

Screening level programs and the EII are treated differently from intensive study programs like the Bull, Barton, or Walnut creek studies. For screening level programs and the EII, the three surber samples will be composited in the sub-sampler, distributing material evenly through the grid. For intensive study programs such as the Bull, Barton, or Walnut Creek studies, the three surbers will be preserved as three discrete samples and labeled appropriately so that they can be spatially located (a, b, c, etc., with notations on field sheets). Intensive study surbers should be picked in their entirety except as noted below for high abundance (>1000 organisms) surber samples.

Once the sample(s) have been deposited in the pan or sub-sampler, abundance is noted visually and used to determine if sub-sampling is necessary:

- For high abundance samples (>1000 organisms), one grid (out of 30) will be randomly selected. That grid will be removed and transferred to the picking pan to be completely picked. For screening level investigations and the EII program, sequential grids will be picked until the target number of 200 organisms (+/- 20%) is reached. For intensive study programs, each surber may be sub-sampled until 100 organisms per surber (for a total of 300 organisms) is reached. Additionally, intensive study surbers which are sub-sampled should be reviewed for large and rare organisms, which are stored in a fourth vial and used in calculation of richness/diversity metrics.
- (SCREENING LEVEL ONLY) Lower abundances will require more than one grid per person picking (2-9). As a guide, if each grid has < 7 organisms, you will have to pick the whole pan to get your target. Think rapid and pull enough grids to give each picker approximately 25–50 organisms.
- (SCREENING LEVEL ONLY) Extremely low abundance samples can be picked in their entirety and supplemented with sequential surbers until the target number of organisms is reached (200 +/- 20%). However, the original 3 surber composited sample will be maintained as a discrete sample, and each subsequent surber after that initial discrete sample will be maintained separately. Thus, the 3-surber composite will be one sample in the database and each subsequent surber will be one sample in the database.
- (FOR INTENSIVE STUDIES ONLY) In case of an excessively depauperate site, after the first three surbers have been sorted (3 samples in DB), successive surbers (one sample each) may be collected and sorted if the field staff desires to increase total number of organisms to 100 (+/-20%) in order to calculate. However, this is not required and the three-surber sample with uniform area corresponding to other sites should always be the base data source for analysis purposes.

For all cases, the number of surbers, number of grids picked if sub-sampled, and the estimated total number of organisms should be recorded in the lower portion of the field sheet. The number of grids/surbers sub-sampled is noted along with the estimated number of organism in each grid/surber to document the level of effort (area) to reach the target number of organisms.

If Intensive and EII surveys have crossover and the data will need to be used for both purposes, the Intensive survey method should be used.

Each sorted pan of benthic macroinvertebrate samples will be checked for missed organisms by a different member of the field staff. Quality control (QC) detritus will be retained for 10% of field collected benthic macro-invertebrate samples. QC detritus will be placed in a white sorting pan and thoroughly scanned with the naked eye at the ERM lab (i.e. without microscope or other visual aids) to ensure the same level of effort encountered in the field sorting is used in the processing of the QC detritus. Percent missed will be noted to monitor effectiveness of each field team.

Benthic Macroinvertebrate Sampling from Lakes

Littoral Zone Sampling

At each transect, three discrete kick net (500um net) samples will be collected on each side of the lake. Collection of each sample will begin in a maximum of 0.5 m of water no farther than 10 ft from shore, moving straight toward the shore along the transect line for a period of 30 seconds. Each kick net will be kept separate. Samples will be well rinsed, then dumped into pans for field picking, with all organisms but scuds picked and preserved in 89 % ethanol. Then the sample will be returned to the Caton subsampler, with 4 grids picked for scuds, to estimate total scud numbers.

Sublittoral Zone Sampling
Along each transect, 6 dredges (3/side) will be collected from the sublittoral area (> 1m, < 6 m) using the Petite Ponar dredge. All dredge material will be placed in the 500um kick net and rinsed well, then evaluated for abundance and if needed, subsampled to 200 +/- 20%. All sublittoral dredges will be composited into one sample.

Elutriation of Benthic Macroinvertebrate Sediment Samples
There are several conventional methods to extract benthic macroinvertebrates from sediment samples. Some methods re-suspend benthic macroinvertebrates through the use of solutions with higher specific gravities than the organisms. Commonly used solutions include: sugar, sodium chloride, calcium chlorides, D-mannitol, magnesium sulfate, and kerosene (Rosenberg *et al.* 2007, Hynes 1970). However, potential problems with this method are that heavier taxa (i.e. gastropods, trichoptera, and mollusks) will not be suspended within the solution (Rosenberg *et al.* 2007, Merritt *et al.* 1996), and taxa flotation time varies with type of solution used (Merritt *et al*. 1996). A method which resolves this problem is elutriation. Elutriation is the process of extracting benthic macroinvertebrates from inorganic materials (i.e. sand, pebbles, and rocks) by re-suspending them in water or other solutions through agitation (i.e. swirling) or flotation (Feldman *et al.* 2006). The City of Austin uses an elutriation method (adapted from Rosenberg *et al.* 2007, Feldman *et al.*2006) to process sediment samples for the purpose of identification and enumeration of benthic macroinvertebrates in streambed substrate.

Sediment Sample Elutriation Equipment

- 1 Bucket (dimension)
- 1 U.S. #35 sieve (500µm)
- 2 white sorting pans

Sediment Sample Elutriation Protocol

- Pour preservative off sample and through sieve
- Pour sediment into bucket
- Rinse sample bottle with water and pour into bucket
- Fill bucket with water to approximately 6” above sample
- Agitate/swirl sediment in bucket for approximately 1.5 minutes
- Pour water, floating material, and organisms off sample and into sieve
- Add more water and repeat process 10 times*

After elutriation, two samples will be produced for sorting; a sieved sample and an elutriated sample. Organisms transferred to sieve will be rinsed into a white sorting pan for processing, and any organisms stuck to sieve will be removed and placed into sample as well. The elutriated sample will also be placed into a separate white sorting pan.

The sieved sample should theoretically contain the majority of benthic macroinvertebrates, while the sediment left after elutriation could contain heavier organisms that did not separate out during the agitation process. If 90% of the organisms are not contained within the sieved sample place both samples back in the bucket and continue to wash until the samples are sorted as such (Feldman *et al.* 2006). After separation of the samples by elutriation, the sieved sample will be hand picked (without the aid of a microscope) in its entirety (or sub-sampled if needed) to obtain 200 organisms (± 20%). The elutriated substrate sample will be scanned for large organisms, and will be used as the Quality Analysis (QA) sample.

* If sediment sample is larger than ½ liter, sample can be divided into equal portions and elutriated according to the above process (Peterson 2007).

Benthic Macroinvertebrate Sampling Using Hester Dendy Samplers

Hester Dendy artificial substrate samplers (H-D) are used to sample benthic macroinvertebrates by first installing H-D and then allowing colonization over time. H-D are beneficial for direct comparisons between different sampling locations when there may be a detection bias among sampling localities using other equipment (Serber, Hess sampler) or use of other equipment is impractical due to site conditions. H-D are biased toward some taxa that are more likely to colonize the artificial substrate, so they may be less useful than other samplers at determining total biodiversity.

Field teams will need the following equipment: H-D samplers, rope, bricks or rocks to anchor the H-D, sample vials containing 70% ethanol, mesh bags, soft brushes, and collection pans.

H-D should be installed at least 6 weeks prior to sampling per EPA recommendations. Note that H-D may not be suitable for busy public areas, as they may be disturbed or stolen. H-D should be attached to bricks or large rocks with rope to avoid loss downstream and to suspend them above the substrate, so that they do not get buried in sediment. Placing H-D in a range of orientations relative to water flow may allow a more diverse group of organisms to colonize (Hill & Matter 1991). Placement in areas of high or low flow may also impact invertebrate colonization, so the ideal conditions should be determined based on the purpose of the project. For consistency, high flow could be standardized. Alternatively, a variety of flow conditions could be sampled.

When sampling macroinvertebrates from H-D, a fine mesh bag should be placed around the H-D prior to lifting it out of the water to avoid loss of macroinvertebrates. Two methods can be used for collect macroinvertebrates. The entire H-D can be placed in 70% ethanol to preserve macroinvertebrates. Alternatively (and necessary if the H-D will be redeployed immediately), the H-D can be scrubbed gently in a pan with a soft brush to remove invertebrates that will be preserved in 70% ethanol.

Note that previously deployed H-D versus new H-D can differ in colonization rates (Valenty & Fisher 2011), so the use of each should be standardized for projects.

Benthic Macroinvertebrate Sampling Using Hess Samplers

The Hess sampler is used to sample lotic habitats composed of sand, gravel, and small to medium-sized cobble, and can prevent the loss of organisms that may occur in areas that are too deep to use a Surber sampler. Care should be taken to place the Hess sampler down quickly and evenly on the substrate being sampled, in order to prevent the loss of motile organisms, however; too much turbulence should also be avoided to prevent flushing benthic macroinvertebrates out of the sample area. The net should be located downstream to capture invertebrates that are dislodged from the substrate during sampling. The handles on the sampler should be used to gently rotate and press the sampler into the substrate until the netted portion of the sampler (on the interior side of the sampler) is just above the level of the substrate. Use a swift stirring motion to loosen the sand-gravel-cobble, and lift macroinvertebrates into the water column, and gently sweep them into the open, netted portion if flow is low. Care should be taken to prevent specimens from washing out of the top portion of the sampler. Larger cobble or small boulders should be lifted out of the way to deploy the sampler, then placed in through the open top portion and gently brushed to remove adherent taxa (this will probably require a sampling partner, especially in deep water habitats). The substrate will be disturbed within the sample location for 45 seconds to dislodge invertebrates. If sampling is conducted in open or scoured areas, among boulders, heavily vegetated areas, or in hyporheic or lentic habitats, then another sampling device should be used.

Field teams will need the following equipment: one Hess sampler (0.086 m^2, 500 μm mesh-size), one Caton sub-sampler, sample vials containing 70% Ethanol, soft-touch forceps, and a picking pan for each person who will be sorting. Three Hess samples will be collected from each site, and benthic macroinvertebrates will be picked from the sample. SCUBA gear may be required in deep water habitats, such as Barton Springs Pool.

Hess samples may be preserved as discrete samples, or composited, and the choice to do so must be indicated in each project QAPP using this device. Discrete samples also need to be labeled appropriately so that they can be spatially located (a, b, c, etc., with notations on field sheets and on vial labels). Once the sample(s) have been deposited in the pan or sub-sampler, abundance is noted visually and used to determine if additional sampling, or sub-sampling, is necessary. The threshold for choosing to subsample or collect additional samples is QAPP specific, and will depend on the objectives of the study, but some thresholds used in previous COA studies are listed below as guidance.

1) Collecting Additional Samples:
   a) For screening level investigations, such as the EII program, three Surber samples have been collected and composited in most cases. Extremely low abundance samples were picked in their entirety and supplemented with sequential Surber samples until the target number of organisms was reached (200 +/- 20%). However, the original 3 Surber composited sample was maintained as a discrete sample, and each subsequent Surber after that initial composite sample was maintained separately. Thus, the 3-surber composite is one sample in the FSDB and each subsequent Surber is another sample in the FSDB. A similar method can be employed for screening programs using the Hess Sampler.
   b) For other special studies in low-abundance streams, and after the first three discrete samples have been sorted, successive samples (one sample each) have been collected and sorted to increase total number of organisms to 100 (+/-20%); however, this is not always required and the DADS Team should be consulted to determine whether or not additional sampling would be desired in this case.

2) Sub-sampling:
   a) A 30-grid, Caton sub-sampler is used for subsampling high abundance samples, or samples with a large amount of detritus. Grids (1 through 30) are randomly selected, and the contents of that grid are removed and transferred to a picking pan to be completed picked through.
   b) A general rule thus far for using the Caton Sub-sampler has been to pick out a minimum of at least 4 grids from the subsampler, then adding additional grids to reach the desired target number of organisms.

c) Samples which are sub-sampled should be reviewed for large and rare organisms, which are picked out of the entire sample, stored in a fourth vial, and used in calculation of richness/diversity metrics.
d) For several special studies of streams, subsampling has been used for samples containing >1000 organisms, and additional grids have been picked until the target level of 100 organisms (+/-20%) per sample unit has been reached.
e) For screening level investigations, such as the EII program, sequential grids have been picked until the target number of 200 organisms (+/- 20%) has been reached.

For all surveys, count, note and remove all Corbicula from samples (these clams can foul the sample when abundant). Permanent paper labels describing the location and time of sample collection will be placed in all field vials.

U.S.A., TX, Travis Co., Austin Eliza Stream Monitoring: Eliza Stream **Project # 591, Site # XX** (Hess Sampler) In 70% EtOH 04-MAY-2017 @ 15:00 Coll. T. Jackson	U.S.A., TX, Travis Co., Austin Eliza Stream Monitoring: Eliza Stream **Project # 591, Site # XX** (Hess Sampler) In 70% EtOH 04-MAY-2017 @ 15:00 Coll. T. Jackson	U.S.A., TX, Travis Co., Austin Eliza Stream Monitoring: Eliza Stream **Project # 591, Site # XX** (Hess Sampler) In 70% EtOH 04-MAY-2017 @ 15:00 Coll. T. Jackson
U.S.A., TX, Travis Co., Austin Eliza Stream Monitoring: Eliza Stream **Project # 591, Site # XX** (Hess Sampler) In 70% EtOH 04-MAY-2017 @ 15:00 Coll. T. Jackson	U.S.A., TX, Travis Co., Austin Eliza Stream Monitoring: Eliza Stream **Project # 591, Site # XX** (Hess Sampler) In 70% EtOH 04-MAY-2017 @ 15:00 Coll. T. Jackson	U.S.A., TX, Travis Co., Austin Eliza Stream Monitoring: Eliza Stream **Project # 591, Site # XX** (Hess Sampler) In 70% EtOH 04-MAY-2017 @ 15:00 Coll. T. Jackson

For all cases, the number of Hess samples, number of grids picked if sub-sampled, and the estimated total number of organisms should be recorded in the field notes. The number of grids/Hess samples sub-sampled is noted along with the estimated number of organism in each grid/Surber to document the level of effort (area) to reach the target number of organisms. Each sorted pan of benthic macroinvertebrate samples will be checked for missed organisms by a different member of the field staff.

5.4. Diatom Sampling

Routine diatom collections are sampled from periphyton growing on the surface of rocks (epilithon) in the same study riffle that benthic macroinvertebrates are collected. The objective is to collect a composite sample that is representative of the diatom community from each monitoring site's riffle. The Benthic/Diatom Survey Field Sheets (Figure 5.1) should be used to note flow conditions, size of rocks scraped and QA samples at each site. Additional field notes should be taken from each site to describe the amount and color of periphyton and any other details pertaining to the health of the diatom community (i.e., observable creek sedimentation, etc.). Sample bottles (250 ml amber plastic or otherwise opaque) should be labeled appropriately with site number, site name, collection date & time, and collector's initials.

The following procedure relates to the collection of periphyton for diatom community identification. Prior to collection, clean all equipment, including tray, rinse bottle, wire brush, and Petri dish. Take care to rinse all equipment several times with ambient stream water making sure everything is free from debris or material remaining from previous sampling and avoid rinsing in murky water if possible.

Three rocks are taken from randomly selected areas in the study riffle; these areas should correspond to the general area where benthic macroinvertebrates are sampled (i.e., bottom, middle and top of riffle). The rocks should be collected in undisturbed areas, before sampling for macroinvertebrates. Rocks should be flat to facilitate a consistent sample area. To avoid sediment contamination in the sample, rinse off the bottom of each rock to ensure they are reasonably clean of debris and sediments before scraping. A small Petri dish ($19.6cm^2$) and a sharp scoring object are used to mark the area of the rock to be sampled. This area is then scraped with a wire brush, and the particulate matter deposited into a shallow collecting pan. A sufficient quantity of ambient creek water is used to flush epilithon from each rock. After each scraping, make sure that the wire brush is also thoroughly rinsed for finer plant material into the collection pan. After all three delineated rock areas have been thoroughly scraped and rinsed, the contents of the collection pan should be carefully poured into the labeled bottles; more water should be used to flush all remaining particulate matter from the pan.

During rinsing, care should be taken to keep the total volume of rinse water used to flush the three sample rocks, wire brush, and pan to a total of 250 mL for the site. To accomplish this, fill the rinse bottle with only 250 ml of creek water and take care that there is sufficient water remaining in the rinse bottle to ensure that the pan and brush can be rinsed at the end. The samples should be kept on ice until transported to the ERM lab. Upon arrival at the ERM lab the samples should be refrigerated and preserved with 10% buffered formalin (8 ml per 250 ml sample).

Duplicate samples have been collected from 10% of the total number of sites for historical sampling through FY 2013; however, duplicate sampling has been suspended until a review of the efficacy of this form of QC has been completed.

The Diatom Sample Log should be completed as each sample is placed in the refrigerator. When all samples from a project are logged in and preserved, the contract diatomist should be contacted for sample delivery/pick up. The date that the samples are delivered to the diatomist should be logged in as well.

Densiometer readings will be recorded to estimate percent canopy cover at each riffle where diatom samples are collected. Descriptions of densiometer use appear in the habitat section of this manual (Section 6.5).

10% Buffered Formalin is purchased and does not require mixing before use.

Diatom Identification and Processing

The diatomist shall follow the following protocol:
Samples are allowed to settle for 24 hours and then the supernatant is decanted to concentrate the material to about 20 ml. Approximately half of this concentrate is cleaned and half is reserved until the samples are completely processed. Samples are cleaned of organics and carbonates in preparation for microscopic analysis by boiling first in hydrogen peroxide and then in nitric acid. The oxidized, decalcified material is rinsed repeatedly until a pH of about 6-7 is obtained. A few drops of the cleaned, thoroughly mixed material is air-dried onto glass coverslips and mounted on glass slides using HYRAX ©, a resin with a high index of refraction. Slides are scanned at 1500x in a random pattern and all diatoms are recorded until 500 valves are counted. The counts are transferred from the original bench sheets into an Excel spreadsheet, converted to # cells/ml, proofed against the original data sheets, and submitted to the City of Austin for entry into the database.

Biochemistry of Periphyton Scrapings
Periphyton scrapings from rocks can also be analyzed in a laboratory for Volatile Suspended Solids (VSS), pheophytin, chlorophyll *a*, total nitrogen, and total phosphorus to characterize periphyton productivity at a site. Collection is identical to periphyton collection for diatom community identification. Three additional rocks are collected and scraped (if the original three are too small to accommodate additional scrapings), samples are composited into a separate 250 ml amber plastic bottle, and the sample is not preserved with formalin. Sample bottle labeling should ensure distinction between samples for diatom species identification and biochemical characterization. Immediately after collection, sample should be kept in the dark and placed on ice for transport to ERM's Contract Laboratory for analysis. It should be noted on the laboratory

COC that the total volume of the sample should be measured by the lab prior to sample processing.

5.5 Benthic Macroinvertebrate Reference Collection

The following steps are to be guidelines for the addition of specimens to the COA benthic macroinvertebrate reference collection. Through this protocol, we hope to achieve a uniform method to be followed so that maintenance of the collection can be passed on to different people while retaining the same basic organization and structure. Any changes to the protocol should be considered and agreed upon by all those in the Surface Team and should be uniformly applied to the collection. Please direct questions or concerns about the physical collection to the Section Manager (Mateo Scoggins). For Oracle database problems or questions, the FSDB manager (Rob Clayton).

City of Austin's benthic macroinvertebrate reference collection will be maintained by ERM taxonomists. The collection will be housed in the ERM lab.

Materials

All samples will be stored in 4-dram glass vials with a polyseal cap. Samples will be stored in 70% ethanol. Labels for the reference collection should be made following the labeling guidelines in section D.

a. Reference collection ethanol will be mixed from a standard of ethyl alcohol 200 proof (100%) to obtain a 70% mixture.
b. Archiving benthic macroinvertebrates for sampling projects will be stored in 70% that is mixed from a 95% denatured alcohol base stock solution.
c. Formula for mixing ethanol from base alcohol stock solutions
   (total quantity needed) X (% ethanol needed) / actual ethanol %
   For example if you wanted 20 L of EtOH at a 70% mixture and you had 95% base alcohol stock solution
   (20 L) X (70%) / 95% = 14.74 L of 95% alcohol needed and added to that is 5.26 L of deionized water.

Specimen Acquisition

Specimens should be pulled from all studies as new taxa are collected. Additional specimens of already referenced taxa should be stored if newer specimens exhibit better morphological traits, are in better condition than current specimens, or are a different life form of referenced specimen (i.e. larvae, pupae or adult). If a specimen is removed from a sample labels for both location and identification should be placed within the vial. The vial from which the specimen was removed should have a label stating that specimens were removed for addition to the reference collection (refer to label section below).

Specimen Identification

Specimens should be identified to the lowest practical taxonomic level. Immature benthic macroinvertebrates should also be left to the lowest taxonomic level (i.e. family) if further identification is made impossible due to lack of mature morphological characteristics. If a new taxon is collected and identified by a CoA taxonomist, two additional CoA taxonomists need to verify the identification before the new parameter is added into the database. If there is a dispute between identifications the specimen is left at the lowest agreed upon taxonomic level and it sent off to be verified by a 3rd party specialist.

Taxonomic Literature

For all general taxonomic identifications, the CoA taxonomists will primarily use the 4th edition of *An Introduction to the Aquatic Insects of North America* by R.W. Merritt, K.W. Cummings and M.B. Berg. Additional references include:

<u>Non-Insect Taxa</u>
Ecology and Classification of North American Freshwater Invertebrates by Thorp and Covich, 2nd ed, 2001.
<u>Ephemeroptera</u>
The Mayflies of Florida: revised edition by Berner and Pescador, 1988.
Odonata:
Dragonflies of North America by Needham, Westfall and May, 2000.
Damselflies of North America by Westfall and May, 2007.
Dragonflies and Damselflies of Texas and the South-Central United States: Texas, Louisiana, Arkansas, Oklahoma, and New Mexico by John C. Abbott, 2005.
<u>Plecoptera</u>
Nymphs of North American Stonefly Genera (Plecoptera), 2nd ed, by Stewart and Stark, 1993.
<u>Trichoptera</u>
Larvae of the North American Caddisfly Genera (Trichoptera), 2nd ed by G.B. Wiggins, 2000.
<u>Diptera</u>
Chironomidae – Identification manual for the larval Chironomidae (Diptera) for North and South Carolina by Epler, 2007.
\\coacd.org\dfs\wpdrd\EnvironmentalResourceManagement\WRE\Surface team\Literature\Chironomid_Epler_2007

This is not an exhaustive list of keys present in the COA taxonomic library. Regional keys pertinent to taxa present in our region are also used for certain groups. It is always prudent to refer to primary literature when taxonomic identification issues arise. Keys to species are located in the Surface water team's literature folder with each Order having its own file G:\EnvironmentalResourceManagement\WRE\Surfaceteam\Literature

Oracle Database Entry

Once a specimen has been verified it is ready to add to the reference collection. To enter a specimen in the Oracle database

- Main Menu selection: Supplemental Information
- Benthic Taxonomy
- Under the Order tab select the correct order and then select the "Run Query" selection in the lower left corner of the screen. All available genera and species will be listed. Highlight the correct taxon and then select the "Reference Collection" tab at the top of the screen.
- Fill in the correct information in the lines provided
- Once the information is entered and is checked for accuracy, select the "Save Changes" tab at the bottom of the screen. NOTICE that a new "Ref #" was generated for the line of information entered for this specimen. This number is posted on top of the vial that the NEW specimen is located in (refer to #8 for
- Now the specimen is ready to be placed within the reference collection

Reference Collection Labeling

- Two labels are needed for each specimen located within the reference

collection: location label and a determination label
- Labels should be typed using Times New Roman or Arial Font in 6 pt
- Labels should be typed and printed or copied onto 100% cotton bond paper. Print on an ink jet printer such as the ERM Lab Hewlett

Packard. The ink found in these printers can withstand submersion in 70% ethanol.

Locality Label

The locality label contains information pertinent to the *collection* of an insect only.

STATE: County, Project #, Site # Body of Water Abbreviated Location Lat/Long Date, Collectors Name

The abbreviated location will be the site name that is in the CoA database. The date should be in one of the following formats: 12-IX-2008 or 12-Sept-2008. This label with be affixed around the top of the vial facing outwards for easy reading of the label.

Determination Labels

Order Family *Genus species* Author, Date Det by, Date

This label will be placed within the vial and should not obscure the locality label.

Note:
If taxonomy changes DO NOT remove a previous determination label. Remove the label from the vial, fold the INCORRECT label length wise in half and return to the vial. Create a new determination label with the correct identification and other relevant information. This allows for tracking the history of the ID with dates and persons involved in the identification process.

Vial Number Labels

Each vial that has been added to the reference collection has a unique vial number (Ref #) that is generated and tracked in the Oracle Database. There are two colors of external labels used to track what level of verification the reference specimen is at. The bright green labels are used only for specimens that have been verified/identified by a third party professional. The bright orange labels are used for those specimens that have been identified by a COA taxonomist only. Specimens that bear the orange label should be treated with some caution since they may or may not have been identified correctly.

-Verified by a 3rd party professional

-Identified by a COA taxonomist only

Filing Specimens

After proper identification, labeling and entering into the database the specimen is ready to be placed within the reference collection cabinet. Storage of specimens is by Order, Family (alphabetical within each Order), and then by *Genus* (alphabetical within each family). Third party verified vials should be placed before CoA identified specimens within the same *Genus*.

Verification Contacts

Below are some examples of professionals/consulting services used for verification/identification of the Reference Collection. This is by no means a list of people that should only be used or even necessarily used again.

John C. Abbott, Ph.D.
email: *jabbott1@ua.edu*
Specializes in: Odonata and Plecoptera but will look at most other groups, excluding Diptera.

Chris Rogers
EcoAnalysts 1307 “L” Street, Davis, CA 95695, Phone: (530)756-4481,
email: *crogers@ecoanalysts.com*
Specializes in: Camaeridae, other arthropods

Gary Lester
EcoAnalysts, Inc., 105 E. 2nd St., Suite 1, Moscow, ID 83843
Phone: 208-882-2588 Fax: 208-883-4288 Email: *eco@ecoanalysts.com*
Specializes in: Insect and non-insect benthic invertebrates

James H. Kennedy
Professor of Biology, University of North Texas, Institute of Applied Sciences
P.O. Box 310559, Denton, TX 76203, Phone/FAX:940-565-2993, Email: *kennedy@unt.edu*
Specializes in: Diptera, specialty Chironomidae

Clint Robertson
Texas Mussel Watch, Texas Parks and Wildlife Department
4200 Smith School Road, Austin, Texas 78744
Email: Clint.Robertson@tpwd.texas.gov
Specializes in: Unionidae (Freshwater mussels)

Lori Tolle
5723 Blanford Street, Irondale, AL 35487 Email: *tolle004@bama.ua.edu*
Specializes in: Gastropoda & Fingernail Clams

Kathryn Perez
Department of Biology, University of Texas Rio Grande Valley
Edinburg, TX 78539 phone: 956.665.7145 Email: k*athryn.perez@utrgv.edu*
Specializes in: Gastropoda & Fingernail Clams

Barbara Winsborough
23606 Round Mountain Circle, Leander, Texas 78641 Email: *bwinsbor@io.com*
Specializes in: Diatoms

5.6 Larval Chironomidae (Midge) Mounting

Procedure for mounting midges for microscope identification:

1. Sort larval Chironomidae to subfamilies under dissecting microscope
2. Heat 10% KOH (potassium hydroxide) in 20 ml beakers (approximately on 55ºC on hot plate). Note: 10 grams of KOH is dissolved in 1 L H_2O to make a 10% solution

3. Place sorted larval Chironomidae in 10% KOH for undetermined length of time (this is done to separate muscle tissue from exoskeleton). Duration of this is based on size of larvae. Be careful in this step: small specimens will disappear quickly if KOH is too hot or left too long.
4. After KOH soaking, place larvae into acetic acid (vinegar, CH_3COOH) to neutralize the KOH. Allow specimens to sit in acetic acid for 30 seconds.
5. Place larvae into 100% EtOH to neutralize the acetic acid.
6. Remove larvae from EtOH and dab off excess alcohol.

   NOTE: Steps 2-6 can be eliminated based on taxonomist discretion if mounting is not for reference collection, or if specimens are not particularly large/dense.

7. Place larvae laterally on a glass slide
8. Drop 1-2 drops of CMC-10 (mounting medium) on top of larvae
9. Place glass cover slip on top of larvae and then "roll" the larvae ventral side up
10. Lightly press cover slip over the larvae to spread the head capsule apart so mouth parts can be exposed
11. Let slides dry for 24 hours and then proceed with larval Chironomidae identification using a compound light microscope.

Materials required include:

- Partitioned disposable petri dishes (Quad-plate, 100 x 15mm)
- 24-well culture dish
- 20 ml beakers
- Glass rods
- Minutia Pins
- Plastic or glass pipettes
- 70% Ethanol
- 100% Ethanol
- Acetic Acid
- 10% KOH solution
- Forceps (Dumont #5)
- Hotplate
- Cotton paper
- Pigma Pen
- Cover slips:(Round # 1 coverslips from 12 to 22 mm in diameter allow more rotation and better position of your specimens)
- Glass slides: (Rectangular/plain glass slides, 3''x 1', thickness: 0.96 to 1.06 mm)
- Stereoscope for sorting larvae
- Compound microscope 4X, 40X, 100X preferably with phase contrast (useful when observing small structures such as setae or Laterbourn organs)

5.7 Salamander Drive Survey Standard Procedure

A drive survey (Figure 5.7-1) involves searching the entire wetted surface of each mapped survey section, including in and under available cover (rocks, woody debris, leaf litter, plants, etc.) and in the top layer of sediment or detritus. Surveys should be performed in the same area for consistency. This applies for count or capture-recapture data when estimates of abundance and/or density are of interest and when effort should be consistent across time.

A drive survey may be performed for visual enumeration (i.e., counts) or for capturing salamanders. Starting at the downstream end of a section, all loose cover is removed, with the exception of heavy leaf litter, and placed slightly downstream as surveyors move upstream. A four to six-inch gap, perpendicular to the direction of flow, is created in the stream where all cover has been removed. This creates a reference point for all surveyors (who can be adjacent to each other moving upstream as they survey) where all salamanders behind the line have been counted/captured and all those in front have not; this also creates a cover-free zone that salamanders are less likely to cross, or more easily observed if they do cross. If counting only and not capturing, salamanders are chased in one direction, behind the observer (downstream), to ensure they are not double counted. For example, this can be accomplished by gently prodding the animal's tail with your finger and directing it downstream or coaxing the animal into your hand and gently placing it behind you.

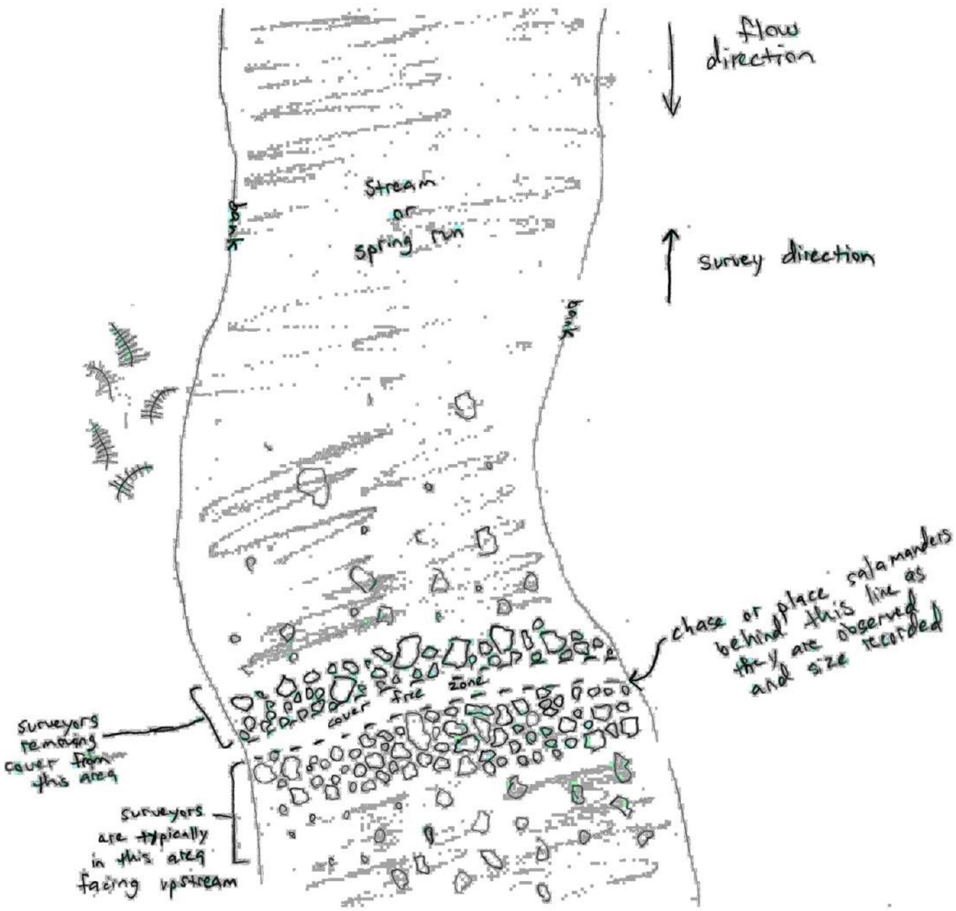

Figure 5.7-1 Drive survey

As salamanders are observed, they are categorized by their total length: < 1", 1–2", ≥ 2" or approximately < 25 mm, 25–50 mm", ≥ 50 mm". This is most easily done by having a length reference with you as you are searching- this can be something like a small ruler or other object of known length with which to compare the salamander. Dead, injured or otherwise odd salamanders are made note of. These are also included in the count tally. Notes should be added to the comment field when entering into the database. Surveys should not be done during storm flow conditions or when high turbidity may negatively affect capture probability

Special considerations

Salamander Capture- For capture-recapture studies, salamanders are captured by hand-nets, pool-nets, or other methods (e.g. scooped up by hand), rather than being chased downstream. Salamanders that are observed, but are not captured, are recorded as “missed.” Photographs of salamanders can be used for individual identification. Please refer to the section *Photographic Method for Individual Salamander Identification*.

Capturing salamanders relies on anticipating their behavior, having patience and a bit of luck. Lift each rock or cover object slowly. Sometimes salamanders will remain still or will only walk away after their cover is removed. This is ideal, and the salamander can be followed with a net. If possible, position a small net (a 6” aquarium net is a good size, but smaller sizes work well for tight spaces) so that the salamander is facing the inside of the net. Do not place the net too close to the salamander (give at least an inch or two distance), so as not to startle the animal. Gently touch the salamander’s tail to coax them into the net. The net must be flush against the substrate or they may crawl or swim under the net. You may place small stones to anchor the back of the net against weak flow. If the net flows inside out while in the water, or bunches up, it does not create an inviting space for the salamander to crawl into. If flow is high, you can pull the back of the net taut and rely on a second observer to goose the salamander into your net. If these methods do not work, then improvise. Sometimes salamanders will crawl into your hand rather than a net. Sometimes you can swipe a swimming salamander out of the water column. With the swipe of your hand can you can force water and create a current into your net to “sweep” a salamander. The downside of this technique is that if you miss, the water is cloudy, and you will not see where the salamander swam to. It can work well, particularly if the substrate is very flat and you have a large net, but the best method is to always try to gently coax the salamanders into swimming or crawling into your net.

Leaf Litter**-** Searching through leaf litter poses some challenges because it typically causes a greater disturbance of sediment than overturning rocks. This undoubtedly affects the probability of observing a salamander. In situations where leaf litter is very thick and can produce significant clouding of the water, the leaf litter mats can be prodded with your fingers instead of entirely disturbed by turning over. Gently prodding the mats with fingers and gently moving them by hand (as opposed to removal from search area) will disturb salamanders enough to swim from their cover so they can be observed. While this method is less thorough in terms of removing all cover, the probability of observation is much improved over creating a cloud of sediment through which nothing can be seen. Areas that accumulate lots of leaf litter will do so on a predictable and seasonal basis, so surveying these areas in a consistent manner is more important that surveying with the goal to remove all cover (in spite of sediment disturbance). Also note that this method does not work well in shallow water (e.g., less than 2 inches)—leaf litter must still be removed in the standard fashion. Alternatively, use of a large net to collect and sift through leaf litter can be very effective at capturing salamanders. This causes a lot of distrubance and will mobilize fine sediments, thus increasing turbidity in the area.

Turbulent Water**-** Clear plastic pans are used as viewing devices in turbulent water. When surveying areas with riffles that have moderate to high flow, visibility is reduced. Placing a clear bottomed pan on top of the water while removing cover objects underneath will greatly improve visibility. When capturing salamanders in turbulent water, positioning a net downstream of all cover objects will help catch them if they are flushed downstream.

III. What accompanying data is collected?

In addition to the number of salamanders in a given size class per section, we also record other types of data. These can be found in electronic data collection apps, or on standardized data sheets, and include the following: observers, start and stop times, discharge, pH, temperature, specific conductivity, dissolved oxygen, presence/absence of crayfish, fish and tadpoles, and percent leaf litter cover (a visual estimate of percent area in survey section that is covered by leaves). Furthermore, notes should be taken on any findings that may affect the interpretation of the data or be interesting from a natural history perspective. We record noteworthy (or uncommon) species of invertebrates observed, but since this will vary greatly by the skill and knowledge of observer (and is qualitative), there is no formal section for this data.

IV. Field Preparation and Materials

Prepare to get wet from the knees down. Although you won't be knee deep in water, you will probably be kneeling in the creek bed as you survey. Some springs are small enough that it is not necessary to get wet. Knee protection is advised, but not necessary. This can be in the form of strap on knee pads, or alternatively, a raised knee pad/stool.

Dive slates for waterproof record keeping are useful in addition to standardized record sheets where the data is eventually transferred to.

Shallow glass baking dishes or plastic pans can also be used for searching in turbulent water, as described above.

Photographic Method for Individual Salamander Identification

Computer-assisted individual identification from photographs is a reliable technique for tracking salamanders for capture-recapture (Bendik et al. 2013). To obtain photographs, place salamander in a container with shallow water and a grid in the background, to serve as a scale reference. The container we commonly use is referred to as a "salamander cartridge," a 22.5 x 8.5 x 4.7 cm acrylic box with a 5 mm grid printed on the bottom of each of four compartments, with internal dimensions of 5.3 x 7.9 cm. This is filled with approximately 1 cm of water to hold one salamander in each compartment. The container is placed (preferably) on a white background and each compartment is photographed using a flash. Alternatively, one may use another container with a flat surface and a standardized grid for scale.

The camera may be handheld or mounted on a stand. The goal is to achieve parallelism between the focal plane and the salamander dorsum. The greater deviation from parallelism, the greater potential for the scale reference to be skewed across the image plane. This can, in part, be overcome by measuring the scale within ImageJ along the distal to proximal axis of the salamander (hence the importance of using a grid scale rather than a rule). It is therefore best to achieve parallelism, and the use of a camera stand helps achieve this.

To efficiently photograph salamanders in the cartridge with the camera stand, load each cartridge, one salamander per compartment, and place underneath a DSLR camera with mounted flash(es), macro lens, and a wired trigger. Adjust focus and position of camera to achieve complete coverage of the compartment in the camera frame. Then, finely tune focus to the salamander dorsum (not the grid background). This will result in the sharpest image. Using base ISO of a camera (e.g., ISO 100 on a Nikon D7100) with a high pixel density (e.g. 24 mpix, APS-C), and a small f-stop (e.g., f/11 or f/13) should result in sharp pictures with adequate depth of field and plenty of leeway for head-cropping (the "mark" that is used for image matching).

To correctly position the salamander, use a plastic mechanical pencil with the lead retracted to gently prod or manipulate it into position. Sometimes they can be moved from their head (but not the gills), as they may stay rigid. Most other times it is a matter of prodding them until they are in the optimal position. The goal is to meet the following conditions, in order of importance, with regard to their position:

1. The entire salamander, from the head to the tip of the tail should be within the frame to allow for total length measurements.
2. The salamander's head should not be touching the edge, but its snout and the rest of its torso should be approximately 3 mm from the edge to avoid distortion created by the meniscus of the water.
3. The salamander should not be pushing up on its front legs, or in a position that otherwise makes achieving parallelism of the dorsum impossible.
4. The salamander torso should be as straight as possible.

The first two conditions are absolutely critical, while the second two are ideal, but not necessary, provided good focus is achieved on the head. Having a straight body helps keep consistency among measurements (and is easier to do), but sometimes you have to take what you can get. It is *not* a problem if the salamander's tail lies against the edge of the compartment. See Figure 5.7-2 for an example of an ideally positioned salamander.

Occasionally a salamander will be so active (particularly just after they have been captured) that they will not stay still, or they will not keep their head from the edge. In these cases it may be necessary to either let the salamander become accustomed to the cartridge space (though never unsupervised or without a lid, as they can crawl out), or given time to "calm down" in a mesh box within the stream, until they will stay still in the correct position. Alternatively, they can be photographed "on the move," which requires a deft hand to manipulate the salamander and accurate timing to capture the salamander in motion away from the edge. This takes some practice.

The camera should be set to auto-number each photograph taken, such that there is no overlap between previously taken images. The goal is to have a unique number for each photograph. Because the current file naming capability is limited to 99999, and additional prefix in the file name can be used to augment this number (as seen below) an additional 3 digits (in the D7100 and other Nikon DSLRs).

It is important to both lock photos (using the key button in playback mode) that are good, and to delete *ALL* unnecessary duplicates while in the field. This reduces the data entry burden in the office. Duplicate photos (same individual, same day) can cause problems if they creep into the analysis. Data including each photo number, the salamander location (e.g., section, box number) whether the salamander is gravid or not, are recorded on a Rite-in-the-Rain data sheet, along with any other interesting observations (e.g., injury, trematode metacircariae, gas bubbles). Later body length, total length, and other measurements are recorded on the same data sheet.

Figure 5.7-2. Example photograph showing ideal body position of salamander.

In the office, photos are stored locally with copies on G. RAW format is used, and measurements are taken on converted TIF files (because ImageJ can save the scale information). Photos are renamed on import to include the date the photo was taken, in the format YYYYMMDD, followed by an underscore and the photo name, e.g., 20160301_SA53994.NEF. Photos are cropped to include the entire head to just behind the gills (Figure 5.7-3). Wild-ID is then used for photo-matching (Bolger et al. 2012).

Figure 5.7-3. Example head crop.

The following settings are recommended for the Nikon D7100:

Nikon D7100 camera settings for salamander photography. If anything is confusing, please refer to the manual.

1. Attach flashes to front of macro lens using adapters (usually already on the lens).
2. Set manual focus (set on either lens or camera body) for camera stand use. Set to autofocus and AF-C if hand-holding.
3. Press button next to flash to pop up while camera is on.

4. Load two SD cards. One serves as a backup. Copy photos to local external hard drive and to the G: drive. Then format both memory cards.

The following settings should be saved to the user (U1 or U2) dial settings.

The first six settings for exposure can be changed from time to time, particularly if we decide to photograph a bug or something else when we are out in the field, or if they get accidentally changed.

- Mode **M** on top left dial
- Set matrix metering (near shutter release button, hold down and use thumb command dial). Matrix metering setting looks just like the image on the button.
- Aperture F/11–F/16 (to achieve acceptable depth of field without too much sharpness loss from diffraction).
- Shutter Speed 1/250s (this is the fastest flash sync speed).
- ISO 100 (base ISO).
- While Balance **Auto** (you may also pre-set this if you know how- see manual).

These settings usually will not get changed.

- Custom Settings Menu: **e** Bracketing/flash: **e3** flash cntrl for built-in flash: **CMD**: Built-in flash should have **--** for Mode. Set Group B to **TTL** and Channel to **1**. Make sure settings on external flashes match these settings.
- Shooting Menu: Role played by card in Slot 2: **Backup**
- Shooting Menu: Image Quality: **RAW**

Settings that should **never** be messed with because they impact our data storage and indexing of photos:

- File Naming: **SA5**
- **d7** File number sequence **ON**

5.8 Benthic Macroinvertebrate Sampling in Lentic Habitats

The sampling protocols described in this section are to be used only for bioassessments of pool, wetland or other lentic habitat for special studies which do not require the standard methods used for screening level and intensive study programs. Flow and standard physicochemical parameters will be collected by standard ERM field procedures as defined in the Standard Operating Procedures Manual, at each site when biological samples are collected and documented on field sheets. Field teams will need the following equipment: a standard D-frame kicknet (500µm mesh-size), a large tub or bucket, one Caton sub-sampler, picking pans, sample vials, and forceps.

Prior to disturbing the habitat, an investigation of the pool or wetland study area should be conducted in order to identify the various types and relative abundance of microhabitats including hard substrate, snags and woody debris, vegetated banks, submerged macrophytes, undercut banks, depositional areas, leaf packs etc. Although the field staff should concentrate the collection effort on the most productive portions of the study area, the sample should represent the variety of available microhabitats to the maximum extent practical. It is important for the sampler(s) to determine the approximate path for sweeping prior to beginning the collection, so that sweep time can be budgeted accordingly, and sample area will represent the longitudinal and cross-sectional heterogeneity of the aquatic habitat.

If collecting in a pool habitat, the sweep effort should begin in the downstream end of the pool and progress to the upstream end. If collecting in a wetland or other shallow water habitat, the sweep effort should begin in the deepest part of the study area and progress toward the shoreline, or shallowest portion. Substrate, emergent vegetation and snags should be thoroughly disturbed with the foot (and sometimes hand) so that dislodged material will be swept into the net while progressing through the designated area. The total effort should be no more than 3 minutes of total sweep time. If more than one person is collecting, the total 3-minute time should be divided evenly between the collectors. If the kicknet becomes completely clogged prior to the completion of the predetermined area, and field staff determines that the area has been inadequately sampled, the contents of the kicknet should be discarded completely and started again in the same habitat type, but adjacent location.

After completion of a cumulative 3-minute sweep, the contents of the kicknet(s) should be inspected. Large debris can be removed from the sample after thoroughly rinsing over the net and inspecting thoroughly for adhering organisms. Silt and sand should be gently rinsed out of the net without losing any of the debris. The contents of the kicknet(s) should then be deposited into a single container (tub or bucket) to create a single composite sample. The net(s) should be inspected for clinging organisms which should be placed in the container with the remainder of the sample. The composite sample should be agitated thoroughly in order to homogenize the sample. Abundance is visually estimated and used to determine if the sample should be picked in its entirety, sub-sampled, or supplemented with additional kick/snag sampling according to the following protocol:

- Samples which appear to contain abundance near the target number of 200 organisms (+/- 20%) should be picked in their entirety.

- For high abundance samples (>500 organisms), the entire contents of the kicknet sample should be placed in a Caton subsampling tray and agitated to produce a homogeneous distribution of debris. One grid (out of 30) will be randomly selected. That grid will be removed and transferred to the picking pan to be completely picked. Sequential grids will be picked until the target number of 200 organisms (+/- 20%) is reached. Additionally, the remaining detritus of a kicknet sample which is sub-sampled should be reviewed for large and rare organisms. Any species which have not been identified as being collected at the site will be stored in a fourth vial and used in calculation of richness/diversity metrics.

- Extremely low abundance samples can be supplemented with additional kicks and snag samples if the field staff desires to increase total number of organisms to 100 (+/-20%), however, this is not required.

Each sorted pan of benthic macroinvertebrate samples will be checked for missed organisms by a different member of the field staff. The detritus from 1 out of every 10 samples will be preserved and returned to the lab for verification. Percent missed will be noted to monitor effectiveness of each field team.

For all surveys, count, note and remove all *Corbicula* from samples. Permanent paper labels describing the location and time of sample collection will be placed in all field vials. Field sheets should include details of sampling procedures including minutes of kick time, types of macrophytes, dominant types of debris, depth of water, numbers of snags, substrate, number of grids picked if sub-sampled etc. Specimens will be preserved in vials of 70% ethanol, labeled and transported to the City of Austin lab for identification. Benthic Macroinvertebrates will be identified to the lowest practical taxa.

6.0 Physical Habitat

Protecting the physical integrity of waterways is a vital component of an urban watershed protection and management program. Physical habitat data is collected in conjunction with the EII as well as historically for intensive study programs. Although modern bioassessment data is not submitted to the Clean Rivers Program, most parameters are collected in accordance with TCEQ protocols (tceq.texas.gov/publications/rg/rg-415).

6.1 Non-Contact Recreation

The aesthetics of Austin waterways are assessed for the EII program using the Non-contact Recreation Use field assessment form (Figure 6.1). This visual/sensory assessment of flow volume and water appearance is conducted through field observation of the creek and adjacent stream banks.

The area surveyed encompasses approximately 100 meters upstream of each site, 100 meters downstream and the visible area of each stream bank. Field assessment forms include a description of the conditions for six parameters in four scoring categories: Excellent, Good, Fair and Poor. Parameters are ranked on a 20-point scale that represents the overall conditions of stream bank at that site. Notes and photographs are used to document the conditions of the creek and the overall appearance of the creek at the time of the survey.

The visual/sensory assessment parameters are:

- **Clarity**: a visual assessment of turbidity. (mark N/A if dry)
- **Litter**: a visual survey of the litter conditions at each site.
- **Flow Volume**: amount of flow occurring at each site. (mark zero if dry)
- **Odor**: a sensory assessment of objectionable odors existing at each site.
- **Percent Algae Cover**: a visual assessment of algae occurring on or below the water's surface at each site. (mark N/A if dry)
- **Surface Appearance**: a visual assessment of the percent of organic floatables, plants and woody debris excluding algae at each site. (mark N/A if dry)

Figure 3: Non-Contact Recreation Field Assessment Form

DATE______________ TIME______________ OBSERVER____________

SITE________________________________

Indicator	Excellent (20-16)	Good (15-11)	Fair (10-6)	Poor (5-1)	Score
Clarity	Clear to slightly cloudy, visability mostly good	Slightly cloudy to cloudy, visability somewhat impaired	Cloudy to very cloudy, impaired visability	Very cloudy/murky, can't see below surface	Score:_____
Litter	Very little litter present in the creek and on the banks; no glass	Some litter present in the creeks and on the banks; mostly small items (paper, wrappers); no glass	Litter present in the creeks and on the banks; larger items (cups, containers, cans); a few pieces of glass	Large volume of litter in the creeks and on the banks; dumping; glass and/or sharp metal objects readily apparent	Score:_____
Flow volume	Steady, running flow; water reaches both channel banks	Moderately running; evidence of diminished flow; some areas of the creek channel are dry.	Slight, trickling flow, most of the creek bed is dry	Dry creek bed, Isolated stagnant pools	Score:_____
Odor	No odor to very faint offensive odor (e.g. fishy, sulphurous, petroleum, dead animal)	Slight offensive odor (e.g. fishy, sulphurous, petroleum, dead animal)	Noticeable offensive odor (e.g. fishy, sulphurous, petroleum, dead animal)	Strong offensive odor (e.g. fishy, sulphurous, petroleum, dead animal)	Score:_____
Percent Algae Cover	Surface less than 10% covered by algae	Surface 10-20% covered with algae	Surface 20-30% covered by algae	Surface more than 30% covered with algae	Score:_____
Surface appearance	Surface less than 10% covered by organic floatables, plants, woody debris, oil or foam, etc...	Surface 10-20% covered by organic floatables, algae, plants, woody debris, oil or foam, etc...	Surface 20-30% covered by organic floatables, algae, plants, woody debris, oil or foam, etc...	Surface more than 30% covered with organic floatables, algae, plant woody debris, oil or foam, etc...	Score:_____

Fish Presence / Absence (yes/no)

Total:

Greenbelt/ Buffer Rating: ________ Trail Access Rating:_________

Notes: ____

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Figure 6.1-1 Non-Contact Recreation assessment field sheet.

6.2 EPA Habitat Quality Protocol

The EPA Physical Habitat Quality evaluation is a visual assessment of existing erosion-related in-stream and riparian conditions conducted through field observation. The field assessment sheet is derived from Barbour et al (1999), and describes four categories of conditions (optimal, sub-optimal, marginal and poor) for 10 parameters.

Two scoring methods will be used for each site:
1.) “Potential”- describe each parameter as if there were normal base flow conditions. This is to be used if “current” conditions are dry.
2.) “Current”- describe each parameter exactly as it exists on the day of the survey. This would mean dry sites would score zero for epifaunal substrate, velocity depth regime, channel flow status and frequency of riffles.

- **Epifaunal substrate/available cover**: the complexity and quality of available cover for benthic macroinvertebrates and fish.
- **Embeddedness**: the degree that the stream bed substrate is covered by fine sediment, based on the USGS method (USGS 1993) in which five larger-sized particles are randomly selected along a stream cross-section and rated according to the amount of their surface area covered by fine sediment.
- **Velocity/Depth regimes**: the evaluation of a diverse stream morphology characterized by the presence of different velocity regimes.
- **Sediment deposition**: the changes in a stream’s morphology resulting from the erosive forces of water and the accumulation of sediments to form new surfaces. The amount and pattern of sediment deposition is indicative of the severity of stream bank erosion.
- **Channel flow status**: the volume of water present in the stream, visually estimated by comparing the width of water within the channel with the amount of exposed channel substrate.
- **Channel alteration**: the amount of human-induced modifications in the natural geometry of the stream channel including removal of meanders and reinforcing embankments of hard engineered materials.
- **Frequency of riffles**: a measure of the sinuosity of the stream based on the frequency of occurrence of riffles relative to the width of the stream
- **Bank Stability**: the current stability of the stream banks including consideration of the presence and magnitude of recent erosion or bank failure.
- **Vegetative Protection**: the amount and composition of vegetative cover present along stream banks.
- **Riparian vegetative zone width**: the width of the natural vegetative zone and amount of human influence within close proximity to the stream channel.

HABITAT ASSESSMENT FIELD DATA SHEET—HIGH GRADIENT STREAMS (FRONT)

STREAM NAME	SITE	
DUDB #________ RIVERMILE________	DAYS SINCE RAIN	
STAFF/YOU	DATE________ TIME________ AM PM	PROJECT

Parameters to be evaluated in sampling reach

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Velocity/Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Sow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Figure 6.2-1 Habitat assessment field data sheet (front)

Parameters to be evaluated broader than sampling reach

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank) Note: determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Total Score__________

Figure 6.2-2 Habitat assessment field data sheet (back)

6.3 Pfankuch Channel Stability Evaluation

A comprehensive evaluation of the stability and erosion occurring within stream reaches is conducted using the method developed for the U.S. Department of Agriculture by Pfankuch (1975) to answer three basic questions: (1) what are the magnitudes of the hydraulic forces at work, (2) how resistant are the various organic and inorganic bank and channel components to the recent stream flow exerted on them, and (3) what is the capacity to adjust and recover from potential changes in flow volume and increases in sediment production.

Although the Pfankuch channel stability evaluation is considered non-scoring, supplemental information for the EII, it does provide useful information about the capacity of streams to recover from change by systematic measurements of the resistive capacity of stream channels to the detachment of bed and bank materials.

The parameters evaluated are:

Landform Slope: the lateral extent and ease to which banks can be eroded and the potential slough which can enter the water. Bank slopes may be measured with a clinometer.
Mass Wasting: mass movement of banks by slumping or sliding introducing large volumes of soil and debris into the channel causing constriction and increasing sedimentation rates.
Debris Jam Potential: the potential for increasing woody impediments to the natural direction and force of flow of the channel.
Bank Protection from Vegetation: density, coverage and root mat continuity as a measure of protection of the bank from overland flows.
Degree of Entrenchment: ratio of bankfull width to valley width as a relative index of channel incision.
Bank Rock Content: the relative resistance of bank rocks to detachment by flow forces.
Obstructions, Deflectors and Sediment Traps: objects within the stream that change the direction and velocity of flow as indicators of increasing channel damage.
Cutting: the loss of aquatic vegetation by scouring or uprooting, sometimes characterized by vertical channel walls.
Deposition: a measure of the amount and size of material that has recently been moved in or around a channel.
Rock Angularity: resistance of rock movement is evaluated by visually estimation of the sharpness of rock angles and shapes.
Brightness: the brightness of rock surfaces is an indicator of rock movement.
Consolidation: a measure of the unstable substrates that form in loose attachments, as opposed to more stable materials that pack together with voids filled over time.
Bottom Size Distribution and Percent Stable Materials: the change from the natural variation of component size classes and the percentage of all components judged to be stable.
Scouring and Deposition: a rating of the percentage of channel bottom affected by scouring and deposition.
Clinging Aquatic Vegetation: an evaluation of the community and coverage of macro-aquatic biomass as an indicator of stream stability.

EII Reach Inventory and Channel Stability Evaluation

EII Site Number: | EII Site Name: | Stream: | Staff: | Date: | Time:

Days since last rain (+0.25"): | Avg. stream width: | Avg. stream depth: | Est. stream discharge (cfs):

Note flow condition (Flooding, high flow, moderate flow, low flow or dry): | Bankfull width: Riffle 1 = | Riffle 2= | Pool 1= | Pool 2=

Parameter	Excellent	Good	Fair	Poor	Score
Upper Banks (From normal high water mark to the next break in general slope. Relatively perennial with terr. plants and animals)					
Landform Slope	Bank slope gradient < 30% (2)	Bank slope gradient 30-40% (4)	Bank slope gradient 40-60% (6)	Bank slope gradient > 60% (8)	
Mass Wasting (existing or potential)	No evidence of past or potential for future mass wasting into channel. (3)	Infreq. and/or very small, mostly healed over, low future potential. (6)	Moderate freq. & size, with some raw spots eroded by high flows (9)	Freq. or large, causing sediment loss nearly yearlong (12)	
Debris Jam Potential (floatable objects)	Essentially absent from immed. channel (2)	Present, but mostly small twigs and limbs (4)	Present, volume and size are both increasing (6)	Moderate to heavy amounts, larger sizes (8)	
Bank Protection From Vegetation	90% + plant density, vigor and variety suggest deep, dense root mass (3)	70-90% density, fewer species or lower vigor suggest less dense or deep root mass (6)	50-70% density, lower vigor and still less species form a shallow/discont. root mass (9)	>50% density, fewer species, less vigor indicate poor, discont. or shallow root mass (12)	
Lower Banks (Intermittently submerged section of channel from waters edge to normal high water mark)					
Degree of Entrenchment (Entrenchment ratio*)	Little or no entrench., ratio >2.5 (1)	Minimal entrench., ratio of 2.0-2.5 (2)	Moderate entrench., ratio of 1.2-2.0 (3)	Highly entrenched, ratio <1.2 (4)	
Bank Rock Content	+65% w/ large, angular boulders (12" dia.) abund. (2)	40-65% small boulders to cobble (6-12" dia.) (4)	20-40% with most in the 3-6" dia. class. (6)	<20% rock fragments of gravel size (2-4" dia.) (8)	
Obstructions, Deflectors, Sediment Traps	Rocks, old logs firmly embedd., flow pattern of pool/riffle stable w/out cutting or deposition (2)	Some present, causing erosive cross currents and minor pool filling, obstructions and deflectors newer and less firm (4)	Moderately frequent, mod. unstable obstruct. & deflect. move with high water causing bank cuttin and filling of pools (6)	Freq. obstruct. and deflect. cause bank erosion yearlong, sed. traps full, channel migration occuring (8)	
Cutting	Little or none evident, infrequent raw banks less than 6" high (4)	Some, intermitt. at outcurves & constrictions, raw banks be up to 12" high (8)	Significant, cuts 12-24" high, root mat overhangs and sloughing evident (12)	Almost continuous cuts some over 24" high, failure of overhangs frequent (16)	
Deposition	Little or no enlargement of channel or point bars (4)	Some new increase in bar formation, most from course gravels (8)	Moderate deposition of new gravel & course sand on old/new bars (12)	Extensive dep. of predom. fine particles, accelerated bar development (16)	
Bottom (Generally submerged portion of channel, totally aquatic)					
Rock Angularity	Sharp edges and corners, plane surfaces roughened (1)	Rounded corners & edges, surfaces smooth & flat (2)	Corners & edges well rounded in two demensions (3)	Well rounded in all dimensions, surf. smooth (4)	
Brightness (Clean rocks)	Surfaces dull, darkened or stained, gen. not bright (1)	Mostly dull but may have up to 35% bright surfaces (2)	Mixture, 50-50% dull and bright, (+,- 15 %) (3)	Predom. bright, 65% exposed or scoured surf. (4)	
Consolidation or Particle Packing	Assorted sizes tightly packed and/or overlapping (2)	Moderately packed with some overlapping (4)	Mostly a loose assortment with no apparent overlap (6)	No packing evident, loose assortment, easily moved. (8)	
Bottom Size Dist. & % Stable Materials	No change in sizes evident, stable materials 80-100% (4)	Distribution shift slight, stable materials 50-80% (8)	Moderate change in sizes, stable material 20-50% (12)	Marked dist. change, stable materials 0-20% (16)	
Scouring and Deposition	Less than 5% of bottom affected by scouring and deposition (6)	5-30% affected, scour at constrict. and where grades steepen, some dep. is pools (12)	30-50% affected, dep. & scour at obstruct. constrict. and bends, pools filling (18)	More than 50% of bottom in a stat of flux or change nearly yearlong (24)	
Clinging Aquatic Vegetation (moss, algae)	Abundant, growth largely moss-like, dark green, perennial, in swift flow also. (1)	Common, algal forms in low velocity & pools, moss in pools and riffles (2)	Present but spotty, mostly in backwater areas, seasonal blooms make rocks slick (3)	Perennial veg. scarce or absent, yell.-gr., short term blooms may be present. (4)	

* *Entrenchment ratio = Width of the flood-prone area (width at 2X bankfull height) divided by width at bankfull.*

Reach Score of: <38 = Excellent, 39-76 = Good, 77-114 = Fair, > 115 = Poor

Total:

Notes: (Large erosion sites, niche-points, massive deposition, excessive debris, exceptional integrity,etc...)

Figure 3.2. EII Reach Inventory and Channel Stability Evaluation Form

Figure 6.3-1 Pfankuch Channel Stability Evaluation field sheet.

6.4 Densiometer

Densiometer readings will be made to estimate percent canopy cover at each riffle where diatom samples are collected. Three readings will be collected at each site, holding the densiometer out from the hip (arm open at a right angle) and level (Figure 6.4-1). The three readings will be averaged to yield average percent canopy cover.

How to Read the densitometer (Spherical densiometer Model-C)

1. Hold instrument level
   a. 12-18 inches in front of body
   b. Hold at elbow height – making sure that observers head is just outside of the grid area
2. Assume four equally spaced dots in each square of the grid
   a. Count dots equivalent to quarter-square canopy openings
   b. Enter the number of dots OCCUPIED by canopy or, if your data collection sheet allows it, the dots NOT OCCUPIED by canopy (verify you entered the number in the accurate category)
3. Electronic data-recording app will automatically translate count to percentage for the user, however, if not using an app, count can be manually calculated: Multiply the total count by 1.04 to obtain percent of overhead area **NOT OCCUPIED** by canopy
   a. The difference between this and 100 is an estimation of overstory density in percent.
4. Make three readings per location
   a. At upstream end of sample reach facing upstream, in middle of sampling reach facing downstream, in downstream end of reach facing downstream.
   b. Record and average

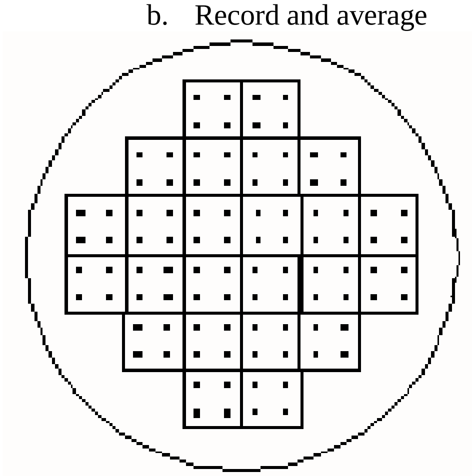


Figure 6.4-1. Spherical densiometer showing depicting the 96 points counted to estimate canopy coverage.

Historic densiometer readings on transects included three observations representing coverage at right bank, left bank, and center facing downstream.

6.5 Site Photograph Collection

Digital photographs will be taken to document the status of a sampling location and to informally track changes in the site over time. Good photographs are very useful to master-planning personnel and other office staff to understand the relationship of the data to creek reaches. A minimum of three photos should be taken for each site: one looking upstream from the sample location, one looking downstream from the sample location and one taken from the bank that encompasses the sampling location.

For intensive habitat transects, repeat this series of images for each station in the habitat survey.

ALL photos used for documentation purposes should be taken so that the image is wider than it is high; sometimes referred to as a "Landscape orientation." Additional images collected for educational or pedagogical purposes can be shot in any manner the shooter sees fit so long as they are in addition to the three landscape images taken at each sampling location. Check your photos on the viewing screen before leaving the site and if they are blurry, re-take them.

Take all photographs in the **LANDSCAPE** format. Photos should be named according to the site photo protocol listed below.

- *Site Photograph Naming Protocol*

Rename your photos using the following format:

SiteNum _ TransectNum { tX } - PhotoView { us, ds, ur, na } - Date {mm _ dd _ yyyy } .jpg

e.g. “502_t00-us-07_04_2007.jpg”

[use “na” for PhotoView if the photo was not taken as part of the riffle/reach characterization. “Na” stands for “not applicable”]

Check with the project manager regarding the storage of photos.

6.6 Lake Habitat

In each reservoir, sites have been *a priori* selected to represent the dominant habitats present and are approximately evenly spaced. Overall there are ten sites sampled at five locations; sites are paired to capture the habitats along the “upper” and “lower” shorelines of each reservoir. Habitat characteristics are categorized for three zones at each site and data are entered into field sheets (Figure 6.6-1).

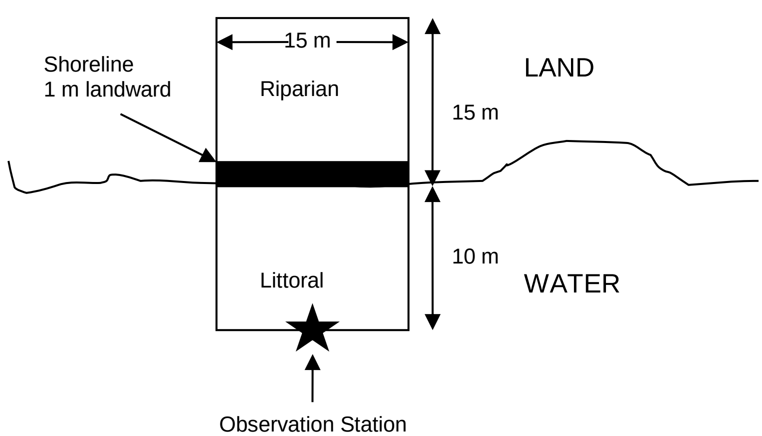


Figure 6.6-1 Zones for the characterization of habitat in lakes

Area assessed for riparian vegetation – 15m along shoreline, 15m back onto land
Area assessed for shoreline substrate and bank angle- 15m along shore and 1 m back
Area assessed for littoral zone- 15m along shoreline and 10m into lake

Aerial coverage within each habitat class is based on the "shadow" cast by a particular component within the layer. Total vegetation coverage in riparian and littoral zones can exceed 100%. Aerial coverage is scored using the following percentages:

- (0) none (i.e., component is 100% absent from the layer)
- (1) sparse <10%
- (2) moderate 10-40%
- (3) heavy 40-75%
- (4) dense >75%

RIPARIAN HABITAT

Shoreline Vegetation- characterize vegetation in riparian zone within height classes:

- Canopy (i.e., vegetation >5m in height) - Large (i.e., >0.3m diameter at chest height) and small (i.e., <0.3m diameter at chest height);
- Under story (0.5–5m height class) – woody shrubs/sapling; herbs, forbs, grasses; invasives;
- Ground cover (<0.5 meters height) – woody shrubs/sapling; herbs, forbs, grasses; invasives; barren/buildings

Canopy and understory also characterized as: deciduous; coniferous; mixed; none

SHORELINE HABITAT and BANK ANGLE

- Substrate size classes – bedrock; boulder; cobble/gravel; loose sand; silt/fines; vegetated (terrestrial); other. Aerial coverage is scored as above.

Bank Features- bank angle (Vertical >75°, Steep 30-75°, Gradual <30°)
Vertical and Horizontal distance from waterline to high-water mark
Human Influence can be "present" if visible within plot (boxed area above); "observed" if outside of the plot but visible, or absent.

LITTORAL HABITAT

Depth (m) at observation point
Within littoral 'plot'

- Pres/Abs surface film (algae, oil, scum, other)
- Bottom substrate if visible from boat (can also utilize an oar or other object to feel bottom texture) – bedrock; boulder; cobble; gravel; sand; silt/clay/mud; and woody debris. Aerial coverage is scored as above. Color and odor are also noted.
  Macrophytes – Submergent; emergent; whether macrophytes extend beyond 10m from shore. Aerial coverage is scored as above.
  Fish Cover- Large woody debris (LWD)/snags (> 0.3m diameter); small wood debris (SWD; < 0.3m diameter); overhanging vegetation <1m above surface; rock ledges/drop offs; boulders; human structures (i.e., docks, pilings, etc). Aerial coverage is scored as above.

6.7 Riparian Functional Assessment

Riparian Functional Assessments (RFA) will be performed for all established Riparian Zone Restoration (RZR) projects. Data collection occurs during leaf-on conditions from May to early November. Currently measured parameters are listed in Table 6.7-1.

Table 6.7-1 City of Austin metrics for evaluating the functional condition of riparian zones.

Metric	Rational
Soil Compaction	Soil compaction or bulk density is one of the most discerning variables separating forest cover from turf grass (Pouyat et al. 2007). Increasing soil compaction can reduce the soil's ability to function for structural support, water and solute movement, and restrict root growth (USDA 2008). Compaction can result in shallow rooted plants and poor plant growth, reduced vegetative cover, increased erosion, and reduction in water infiltration (USDA 2008). Improvements in soil compaction can be gained by reducing disturbance from vehicle and foot traffic and increasing soil organic matter content (USDA 2008).
Plant cover and structural diversity	High cover and structural diversity of vegetation (groundcover, shrub, middle and upper canopy) indicates a productive plant community, high species diversity, adequate food resources and habitat for wildlife, and reduced flood impacts along banks (Stacey et al. 2006). Structural diversity is often absent in riparian areas that have been heavily damaged by human activities and can result in native wildlife species being extirpated from the area (Stacey et al. 2006). Patches of dense vegetation, both native and exotic, also play a key role in trapping sediment during periods of over-bank flow (Stacey et al. 2006).
Native tree demography and succession	Size and age class distribution of all dominant tree species indicates recruitment success and disturbance intervals. Missing age classes are often a result of disruptions to natural ecosystem processes and can result in successional changes and species loss (Stacey et al. 2006). Dominant species exert the most influence, and thus the greatest functional changes will occur if the abundance of these species is altered (Richardson et al. 2007). The understory (sapling) community reflects a habitat's current ecological condition; while overstory (tree) communities are reminders of past environmental condition (Woosley et al. 2005). If the understory composition is different from the overstory, then a shift has occurred in the environmental conditions of a site either by anthropogenic or natural causes and may change the site's ecological function.
Riparian zone width	A wide riparian buffer has been shown to filter pollutants, control erosion, prevent flooding, and provide habitat and nutrient inputs into the stream (Barbour 1999; Fischer and Fischenich 2000). Increased riparian zone width in restored systems has been shown to positively impact macroinvertebrate diversity in Austin streams (Chin et al. 2010). Riparian zone width is also the best predictor of nitrogen loading to water bodies when buffers are "relatively leaky" (Baker et al 2006).
Canopy Cover	Temperature heterogeneity within the stream channel is associated with increased aquatic species richness and ecosystem function (Woosley et al. 2005). The amount of solar shading provided by adjacent and in stream riparian vegetation is critical for maintain temperature refugia. Decreased streambank vegetation cover, increased channel width, and reduced stream depth increases exposure, raises water temperatures and impacts aquatic life (Stacey et al. 2006).
Large woody debris (LWD)	The amount, composition, and distribution of LWD in the stream channel and along the banks provides important aquatic habitat (Stacey et al. 2006). As the diversity and abundance of LWD and other in-stream habitat decreases, habitat structure becomes monotonous, species diversity decreases, and potential for recovery following disturbance is reduced (Barbour 1999). Streams with adequate LWD generally have greater habitat diversity, a more natural stream shape, and greater resistance to flood events (Stacey et al. 2006).
Snags	Snags provide important ecological services to riparian forests (Groffman et al. 2003, Gurnell et al. 2005) and represent a potential source of large woody debris for the stream
Soil organic carbon	Continuous soil moisture monitoring is cost prohibitive for this study. Measuring total soil organic carbon from soil samples collected in each plot and each site as a surrogate to directly measuring soil moisture. The underlying assumption is that organic carbon retains water and thus organic carbon content and soil moisture are positively correlated (Rawls, Pachepsky et al. 2003).
Hydrophytic vegetation	
Instream Shade	Temperature heterogeneity within the streamis associated with increased aquatic species richness and ecosystem function (Woosley et al. 2005). The amount of solar shading provided by adjacent and in stream riparian vegetation is critical for maintain temperature refugia. Decreased streambank vegetation cover, increased channel width, and reduced stream depth increases exposure, raises water temps and impacts aquatic life (Stacey et al. 2006).

Establishing sampling transect
In each study site, a 100 m transect is defined parallel to the stream centerline. Three 10x10 m plots are marked on each bank at 0-10, 45-55, and 90-100 m along the transect, beginning at bankfull and perpendicular to the stream (Figure 2). Only plots that reflect the site designation are collected. That is, if a site is designated as reference and a plot had been disturbed (mowed, built, etc.), that plot data does not get collected. If a site is designated as degraded and there are plots that would be considered reference (vegetation undisturbed in the last ~40 years), there is no data collected on those plots. If a site is designated as degraded (undergoing restoration) there are plots where restoration is not taking place (mowed continued, etc.) there is no data collected on those plots.
RFA parameters

Soil Compaction
At the center of each plot, soil compaction is measured with a Humboldt Corps of Engineers Cone Penetrometer at three different points and each measurement is recorded. Soil compaction measurements are comparable when soil moisture is similar. For RFA, this period coincides between 6-11 days after a rain event ≥ 0.7 inches.

Plant Structural Distance
Within each plot, the percent plant cover is visually estimated and recorded for each vegetation layer: canopy (vegetation at ≥5 m), understory (0.5 m<x<5 m), and ground cover (<0.5 m). A vector of the form [x_1, x_2, x_3] is created for each plot where canopy represented x_1, understory represented x_2, and ground cover represented x_3. A weighted Euclidean distance is calculated for each plot from the zero vector [0, 0, 0], using the following equation:

$$Structural\ Distance = \sqrt{3{x_1}^2 + 2{x_2}^2 + {x_3}^2}$$

Weights are assigned to the canopy and understory layers as they are thought to provide higher functionality thn the ground cover and represent later stages in a forest succession.

Total Woody Species Richness, Woody Species Richness Distance, and Forest Wetland Affinity
All species of trees present within each demographic category; mature, sapling, or seedling, are recorded. Total woody species richness is calculated for each plot and a species richness vector of the form [y_1, y_2, y_3] is created for each plot where mature trees represented y_1, saplings represented y_2, and seedlings represented y_3. The Euclidean distance is calculated for each plot from the zero vector [0, 0, 0], using the following equation:

$$Richness\ Distance = \sqrt{{y_1}^2 + {y_2}^2 + {y_3}^2}$$

The wetland status indicator of each of the woody species is recorded from the USDA plant database. Obligates are assigned a value of five, FACW species a four, FAC species a three, FACU species a two, and upland species are assigned a value of one. The Forest Wetland Affinity is calculated as the average wetland status indicator and is calculated for each plot.

Riparian Zone Width

On both banks at 5, 50, and 50 m on the transect, the width of the riparian zone, defined in this study as the zone with vegetation left undisturbed for at least a full growing season, is measured beginning at bankfull and perpendicular to the stream centerline (Figure 6.7-1)

In-stream Cover

At the center of the channel, canopy cover projected over the stream is measured at 5, 50, and 95 meters with two methods: a concave densiometer facing downstream (percent) and a visual estimate (currently used in the plant structure diversity and shown to be robust to distinguish undisturbed and degraded sites).

Snags and Large Woody Debris within the plots

All snags, dead standing and fallen trunks and large branches with a minimum of 20 cm in diameter and 3 ft in length, present within each plot are counted.

Soil organic matter

On degraded sites only. Soil samples are collected within the sampling plots, within 1 m of the center of the plot. All non-decomposed organic matter, Horizon O, from the soil should be removed. Using a soil spade, an unstratified composite soil sample of ~0.5 Kg of soil are collected within the first 6" of soil from at least three points within 1 m of the sampling plot center. Samples collected will be stored in 1-gallon sealable plastic bags. Samples must be stored in refrigerator until sent to the lab for testing. In addition, soil texture will be included in the requested laboratory analyses as a covariate that may help account for variation in soil organic matter.

Field data collection

Field data is collected during the end of April and the month of May. A Fulcrum app in an iPad is used to record the data unless a technical error takes place and data is then recorded on paper data sheets.

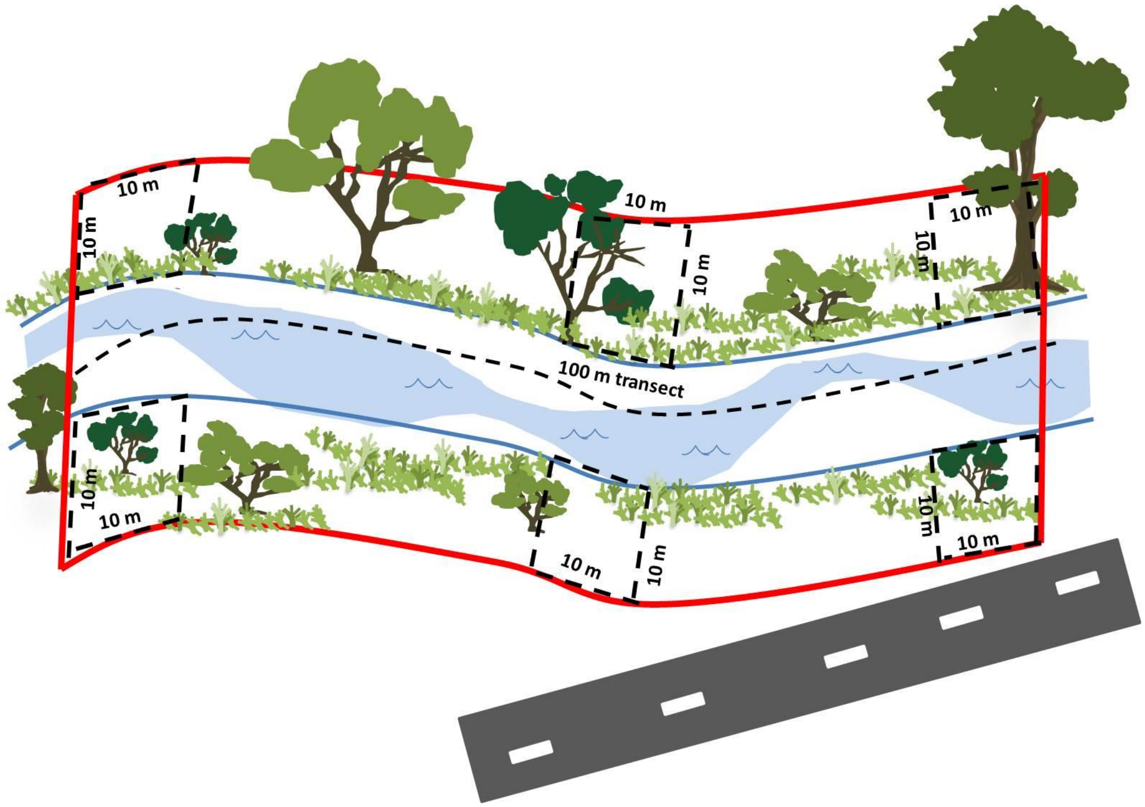


Figure 6.7-1 100m transect with $100m^2$ sampling plots for riparian assessments

7.0 ERM Laboratory Protocols and Analytical Techniques

Some quantitative environmental analyses may be performed in the ERM water quality laboratory (Table 7.1-1). However, data analyzed at the ERM laboratory or any other non-NELAP-accredited facility at the present time may not be submitted to TCEQ for inclusion in the 303(d)/305(b) assessment process under the Clean Rivers Program.

Table 7.1-1 Water quality analyses that can be conducted at the ERM laboratory.

Parameter	Method	Method Equivalencies	Preservative	Holding Time	Detection Limits	Suggested QA/QC
Orthophosphorus	Hach 8048	USEPA 365.1 SM 4500-P-E	ice and/or refrigeration to 4°C	24 hrs. with refrigeration	0.02 ppm	1 split for every 10 samples
Nitrate	Hach 8192 (LR)/ 8171(MR) Cadmium reduction method	InQuest nitrate screen zinc reduction method	ice and/or refrigeration to 4°C	Store at 4degrees c. or lower, run sample within 48 hrs	0.02 ppm	N/A
Turbidity*	Hach 8237 (FTU)	Hach 2100P	ice and/or refrigeration to 4°C	Store at 4°C or lower, run sample within 48 hrs	0.01 NTU	1 split for every 10 samples
	Hach 2100 (NTU)	SM 2130 B	ice and/or refrigeration to 4°C	Store at 4degrees c. or lower, run sample within 48 hrs	0.01 NTU	1 split for every 10 samples
	Trilogy fluorometer	ISO 7027	ice and/or refrigeration to 4°C	Store at 4degrees c. or lower, run sample within 48 hrs		1 split for every 10 samples
Total Suspended Solids	SM 2540D	n/a	ice and/or refrigeration to 4°C	24 hrs.- 7 days	0.5 mg/L	1 split per TSS run (2L collected)
E coli*	Colilert		ice and/or refrigeration to 4°C	8 hours from collection to incubation	<1 MPN	N/A

* Parameters that are still routinely evaluated in the ERM lab as of Jan 2020, all others are outsourced

7.1 Ohmicron Nitrate Analysis

The Ohmicron spectrophotometer may be used to measure nitrate-nitrogen (NO_3 as N) and polycyclic aromatic hydrocarbons (PAH) in soils. Note that nitrate analysis at ERM lab is not currently a preferred method due to cost and availability of reagents (as of 02/27/2009).

When analyzing nitrate on the Ohmicron spectrophotometer, always wear gloves and goggles.

Supplies

- Water samples
- Nitrate test kit
- Reagent-grade or DI water (~50 ml)
- Eppendorf Micro-pipetter
- Eppendorf micro-pipette tips
- Spectorophotometer DR/2000
- Test tube rack
- Sample list data sheet

1. Set out and label Ohmicron tubes with the name of each sample and each QA/QC check (blank, low standard, high standard and the designated split). Place the tubes in the Ohmicron rack. Note that although the meter will print out the results, the printout will not contain site names. Thus, it is CRITICAL to keep the tubes in order throughout the testing process. Samples should be allowed to come to room temperature.
2. When analyzing samples on the HACH DR2000 spectrophotometer, set method number to 0, and dial in wavelength to 500 nm. Use adapter for test tubes and close lid to prevent ambient light during reading.
3. Create standards listed below to make calibration curve. Place Nitrate Developer in tubes last using a micropipette and run all of the test tubes at the same time. Change pipette tips after each solution is added to each tube. Once developer has been added, start timer for 3 minutes and mix until color changes to pink. Record absorbance, and use regression to generate calibration curve.
   0 – 1000 µl reagent-grade or DI water + 100 µl Nitrate Developer
   2.3 – 1000 µl Nitrate Standard + 100 µl Nitrate Developer
   0.23 – 900 µl reagent-grade or DI water + 100 µl Nitrate Standard + 100 µl Nitrate Developer (make 2 test tubes of this dilution)
   1.15 – 500 µl reagent-grade or DI water + 500 µl Nitrate Standard + 100 µl Nitrate Developer (make 2 test tubes of this dilution)
4. Read samples. Run a blank (same as 0 dilution) and lab split for every 10 samples. Write sample name on each tube. Add 1000 µL sample water and 100 µL nitrate developer to each tube. Change pipette tip after each solution is added to the test tube. Add developer after each water sample has been put into the corresponding test tube to maintain consistent developing times. Start timer for 3 minutes and mix until color change occurs. Read absorbance and calculate nitrate concentration using calibration curve. Readings must occur between 3 and 5 minutes after developer addition to sample. Wipe tubes with a chem-wipe prior to reading to remove fingerprints.
5. Dispose of completed samples in the sink.

7.2 Ohmicron PAH analysis

PAH may be analyzed in soil and sediment samples, although an extraction must be performed on the samples prior to analysis with the Ohmicron spectrophotometer.

Rapid Prep PAH Soil Extraction
Always wear gloves and goggles when performing soil extraction protocol.

1. Collect soil by weight (> 10 grams)
2. Weigh 10-25 grams separately on a large filter paper and put in oven to dry.
3. Add 20 ml of PAH Extraction solution to collection device containing 10 grams of sample soil.
4. Replace cap and shake for one minute. Let stand for at least five minutes, until liquid appears clear and soil has settled.
5. Use filter apparatus to remove small amount of liquid:
   a. Disassemble filtration plunger from filtration barrel.
   b. Insert bulb pipet into top (liquid) layer in extraction jar and draw up sample. Transfer at least ½ bulb capacity into the filtration barrel. *Do NOT use more than one full bulb.*
   c. Press plunger firmly into barrel until adequate filtered sample is available or unit snaps together.
   d. Repeat for each sample to be tested.

Dilute the filtered extract into the appropriate extract diluent as described by analyte directions. For PAH analyses, use 250 µl filtered extracted to 12.24 ml diluent volume.

PAH Analysis

Always wear gloves and goggles when using the Ohmicron spectrophotometer for PAH analyses. Be sure that the 450 nm filter block is in the spectrophotometer when analyzing PAH.

1. Turn on the Rapid Assay Spectrophotometer to allow for warm up.
2. Prepare PAH Enzyme conjugate for use- reconstitute as directed.
3. Remove upper rack from magnetic base. Label test tubes for use (Table 7.2-1).

Table 7.2-1 Test tube lab instructions for PAH analysis.

Tube #	Content/Label
0	Diluent/Zero
1,2	Standard 0, 0 ppb
3,4	Standard 1, 2.0 ppb
5,6	Standard 2, 10.0 ppb
7,8	Standard 3, 50.0 ppb
9	Control
10 thru # of samples	Sample 10 … # of samples

4. Add 250 µL of either standard, control or diluted soil extract to the bottom of each test tube by inserting the pipet tip all the way into the tube without touching the sides or the bottom of the tube.
5. Add 250 µL of enzyme conjugate down the inside wall of each tube by aiming the pipet tip ¼” to ½” below the tube rim without touching the rim or tube wall with the pipet tip; deliver liquid gently.
6. Add 500 µL of thoroughly mixed PAH Antibody Coupled Magnetic Particles down the inside wall of each tube by using the technique described in #4. Vortex for 1 to 2 seconds at low speed to minimize foaming.
7. Incubate for 30 minutes at room temperature.
8. Combine the upper rack with the magnetic base; press all tubes into base; allow 2 minutes for the particles to separate.
9. Do not separate upper rack from lower base; using a smooth motion, invert the combined rack assembly over a sink and pour out the tube contents; keep inverted and gently blot the test tube rims on several layers of paper towels.
10. Add 1 ml of PAH washing solution down the inside wall of each tube and vortex each tube. Wait 2 minutes. Repeat step #8.
11. Lift the upper rack (with the tubes) off the magnetic base; add 500 µL of color reagent down the inside wall of each tube by using the technique previously described. Vortex for 1 to 2 seconds at low speed to minimize foaming.
12. Incubate for 20 minutes at room temperature (15-30ºC.) During this period add 1 ml washing solution to a clean test tube for use as an instrument blank in Step 12.
13. Add 500 µL of stopping solution down the inside wall of each tube using the technique previously described. Read results at 450 nm within 15 minutes after adding the stopping solution. Multiply test results by appropriate dilution factor then divide by dry weight calculation for final concentrations.

7.3 NH_3 by Hach Spectrophotometer

This method for ammonia as nitrogen analysis by Hach spectrophotometer is adapted from FWPCA (1969) Methods for Chemical Analysis of Water and Wastes. Always wear gloves, goggles and dust mask when performing this analysis.

1. Set out and label small post-its with the name of each QA/QC check (blank and standards), each site and the designated split sample(s).
2. Rinse each cuvet 3 times with the sample (or standard) it will contain during test.
3. Select HACH method 385, then press enter.
4. Rotate wavelength dial to 655 nm.
5. Press read/enter (display will show *mg/L N* NH_3 *Salic*).
6. Add one *Ammonia Salicylate* powder pillow to each bottle, INCLUDING the blank.
7. Stopper and shake each bottle until the reagent has dissolved.
8. Press Shift/Timer (a 3-minute countdown will begin).
9. When the timer beeps, remove the stoppers from each bottle, setting them behind the bottle they were removed from with the wide end down. This prevents cross contamination between samples.
10. Add one *Ammonia Cyanurate* powder pillow to each bottle.
11. Stopper and shake until dissolved.
12. Press Shift/Timer (a 15-minute countdown will begin).
13. When timer beeps, clean the outside of the blank with a Kimwipe or lint-free cloth and place into the meter. Close the cover.
14. Press ZERO to zero the machine.
15. Clean the outside of all sample and standard cuvettes with a Kimwipe or lint-free cloth and place them into the meter one a time. Press READ/ENTER.
16. Record the results on the lab bench sheet.
17. Dispose of bottle contents down the sink with ample tap water.

7.4 Orthophosphorus by Hach Spectrophotometer.

This method for orthophosphorus (reactive) as phosphorus analysis by Hach spectrophotometer is adapted from FWPCA (1969) Methods for Chemical Analysis of Water and Wastes. Always wear gloves and goggles when performing this analysis.

1. Materials:
   a. spectrophotometer
   b. post-it notes
   c. samples
   d. glass vials
   e. stoppers
   f. paper towels
   g. lint free cloth or Kimwipes
   h. Phosphate Standard
   i. 100 ml volumetric flask (labeled A)
   j. distilled water
   k. Phosver3 pillows
   l. Gloves & goggles
   m. scissors
   n. lab bench sheet
   o. basin for fluids
2. Prep: Set out and label small post-its labeled "High Standard," "Low Standard," "Blank" and one for each of the site numbers. **Do not exceed 20 samples at a time**.
3. Standards Prep:
   a. Rinse bottle 3 times with Phosphate standard, then fill bottle with Phosphate standard to 25 ml line. **This is the "High" standard for QA/QC**.
   b. Using the "A" 100 ml volumetric flask (round bottom) dilute 20 ml of Phosphate standard with 80 ml of distilled water. Rinse bottle 3 times with the solution,

then fill bottle with dilute solution to 25 ml line. **This is the "Low" standard for QA/QC.**

c. Rinse a bottle 3 times with distilled water, then fill bottle to 25 ml line. **This is the "Blank" or zero standard for QA/QC.**

4. Prep: Rinse each bottle 3 times with the each sample. Fill bottle to 25 ml line. For every 10 samples do a sample split.
5. Prep: Tap Phosver3 pillows to settle reagent at bottom and cut off top of pillow. Squeeze pillow sides to open pillow. Prepare one pillow for each sample, split, and standard bottle.
6. Analysis: Turn on Spectrophotometer, allow 15 seconds for self-test.
7. Analysis: Enter method: 491 for spec #1, or 496 for spec #2 and #3. Press READ/ENTER.
8. Analysis: If requested, turn dial to 890nm. Press READ/ENTER.
9. Analysis: Clean "Blank" bottle with lint-free cloth or Kimwipe.
10. Analysis: Place "Blank" bottle in spectrophotometer, press "ZERO".
11. Analysis: Wait until screen reads "0.00"
12. Analysis: Hold down "SHIFT" and press "Timer" and quickly proceed to next step.
13. Analysis: Add contents of Phosver3 pillows to the High, Low and sample bottles – **DO NOT add Phosver 3 pillow to Blank sample.**
14. Analysis: Put stopper in each bottle, shake bottle vigorously, remove stopper.
15. Analysis: Clean off each bottle with lint-free cloth or Kimwipe.
16. Analysis: After 2 minute timer alarm, place each bottle in spectrophotometer, press READ/ENTER and record readings.
17. Dispose of bottle contents in sink with plenty of water.

7.5 Total Suspended Solids

Total suspended solids (TSS), also known as total non-filterable residue, is analyzed by Standard Methods 2540-D. Always wear latex gloves to ensure that human oil and dirt from skin does not impact this test.

Pre-weighing Filters

1. Wear rubber gloves at all times.
2. Count and sort metal pans so that numbers are laid out incrementally.
3. Using forceps place one glass fiber filter on each numbered metal pan.
4. Place the metal pans in order on a drying rack.
5. Place drying rack in oven for at least 24 hrs.
6. Calibrate and zero scale before weighing pans and filters.
7. Remove drying rack from oven and weigh each metal pan three times. Be sure to pick up metal pan between weighing.
8. Zero scale after ten pans or sooner if drift is occurring.
9. Place each weighed pan/filter in desiccator after weighing. Verify desiccant is active.

Filtering/Sample Analysis

1. Wear rubber gloves at all times.
2. Set up filtering apparatus with suction hose connected.
3. Place one filter in each filtering apparatus and prepare to filter sample water. Pay careful attention to keep the corresponding pan and filter together.
4. Mark the location of the sample next to the number of the pan/filter.
5. Turn on suction.
6. Shake sample bottle thoroughly before pouring sample.
7. Rinse 500 ml graduated cylinder 3 times with sample water.

8. Measure and pour 500 ml of sample water into filtering apparatus.
9. Measure and pour another 500 ml of sample water into filtering apparatus (1L total)
10. Rinse graduated cylinder 3 times with DO water, pouring each rinse into filtering apparatus.
11. When sample has completed filtering, rinse apparatus 3 times with DO water.
12. Turn off suction.
13. Remove filter from filtering apparatus and place on corresponding pan.
14. Place pan/filter in oven for 24 hrs
15. After 24 hours remove pan/filter and weigh each pan/filter three times.
16. Discard filter and wash metal pan.

7.6 Fecal Coliform

Fecal coliform are part of the total coliform group that ferment in lactose in 24 hours ($^{+}/_{-}$ 2 hours) at 44.5°C. These gram-negative non-spore-forming rod bacteria will produce acidity appearing as a blue colony in the membrane filter procedure. The major species is *Escherichia coli* and is indicative of fecal pollution and possible presence of enteric pathogens. Fecal coliform is no longer the preferred indicator bacteria in Texas, and for TCEQ assessments *E. coli* will be used instead of fecal coliform if sufficient data exist. If fecal coliform assessment is desired, the IDEXX method can be used in the same manner that *E.coli* is assessed (see Section 7.10) with the rubric provided by the IDEXX protocol.

7.7 Mettler Balance Operation and Calibration

Avoid unplugging or moving the balance! The balance is designed to be continuously plugged into the power supply at all times. If the balance has been unplugged or has otherwise lost power, it must be recalibrated (see below).

Always wear latex gloves when using the scale to prevent oil and dirt from human skin from affecting measurements.

Calibration
Calibration should only be performed by trained personnel wearing latex or lint-free gloves.

1. Wait to calibrate at least 30 minutes after power has been restored.
2. Confirm that balance is level using the bubble level located on the back of the balance.
3. Remove all items from the pan and clean any dust or dirt from pan using camel hair brush.
4. Press the ZERO (“TARE”) key. The balance will display: 0.0000 grams.
5. Locate the 30 g and 50 g calibration weights – always handle weights using a latex or lint-free glove and keep weights in designated container when not in use.
6. Place the 30 g weight on the scale, close the door and record the reading once the “U” has disappeared. The balance should display 30.0000 grams.
7. Replace the 30 g weight with the 50 g weight and repeat the procedure. –The balance should display 50.0000 grams.
8. Remove the 50 g weight from the scale.
9. Press “TARE” and hold it down until “CAL” appears in the readout.
10. Place the 30 g weight on the scale, close the door and wait until the readout settles to “30.000”
11. Remove the 30 g weight, press “TARE” and hold it down until “CAL” appears.
12. Place the 50 g weight on the scale, close the door and wait until the readout settles to “50.000”.
13. Remove the 50 g weight, replace in the appropriate container.

Measurement

1. Put on gloves to prevent oils, etc from affecting measurements
2. Press “TARE” to zero the scale.
3. Wait until the “U” on the left hand side of the readout disappears.
4. Place container (vial, paper, etc) on the scale and close the door.
5. Place the material to be weighed on the scale, and record the readout once the “U” disappears.

7.8 Glassware washing procedure

1. Wash all glassware using the liquinox low-phosphorus (brown) soap.
2. After scrubbing glassware, rinse thoroughly with tap water at least six times until all evidence of soap is gone.
3. Rinse three times with reagent-grade or DI water, then hang glassware on rack or place on drying rack to air dry.

7.9 Shelf Life Tracking of Laboratory Chemicals

- When unpacking shipment, inspect the shipping invoice and compare to the PRF to ensure that all supplies ordered have been received. Give the invoice to appropriate Admin staff after verification.
- Apply HazMat label, inserting Health, Fire, Reactivity and Hazard numbers obtained from the MSDS book. Include the date received on the edge of the Label.
- Ensure the expiration date is prominently shown and add “date opened MM/DD/YYYY” for reagent grade water and pH/conductivity standards
- When ordering a new chemical or standard, request a MSDS sheet, insert in the MSDS book and mail or fax a copy to the safety office.

7.10 *E. coli* by IDEXX Colilert method

Sample Collection
Collect samplings using a 125 ml “bacT” bottle with sodium thiosulfate (Na2S2O3). Sodium thiosulfate is a dechlorinating agent that neutralizes any residual halogen and prevents continuation of bactericidal action during sample transit. Make sure the bottle is filled exactly to 100 ml (bottom of meniscus at top of the line). If collecting a sample for a dilution, a 250ml flip bag may be used, rather than a 125 ml bacT bottle, or fill the 125 ml sample bottle in the field and pour off until 100 ml remains. Chlorine in samples must be neutralized with sodium thiosulfate, typically previously added to purchased or lab-provided sample bottles. Check the incubator temperature before leaving for the field; if it does not read 35º ± 0.5º C, <u>carefully</u> change the dial- use caution, as small turns (half clicks) can change the temperature by a degree.

Lab Procedures
1. Plug in the IDEXX Quanti-Tray Sealer and turn the power switch on. The amber power light should illuminate. Allow about 10 min for the sealer to warm up; when ready, the green light will come on.
2. Place an empty rubber insert of the Quanti-Tray Sealer with the large cutout facing away from the Sealer. While waiting, prepare the samples.
3. If the sample was not collected in a labeled bacT bottle, label a sterile bacT bottle for each sample with site name and db number, and dilution amount if appropriate. If not running a dilution, shake the flip bag well and fill the bacT bottle up to the 100 ml mark**. Make sure to fill exactly (not more or less) to the 100 ml mark with sample, as sample results are reported in MPN per 100 ml.**

4. If running a dilution, use the smallest graduated cylinder to measure out the appropriate amount of sterile water, pour into the bacT bottle, then fill the bottle up to the 100 ml mark with the sample water. (If more accurate measurement is needed, use a volumetric pipette, making sure to use a different pipette for each sample).
5. Record the sample volume, sterile water volume and dilution factor in the IDEXX sample log binder specific to the type of sample collected (i.e. surface water, groundwater, spill), along with site name, db number, date and time of collection.
6. Hold the nutrient indicator snap pack with its neck inside the bacT bottle mouth; snap the pack open and make sure all the powdered contents go into the sample.
7. Cap the bottle and shake gently to dissolve the powder; vigorous shaking will produce undesirable foam.
8. Once the powder is dissolved, use one hand to hold a Quanti-tray upright with the well side facing your palm, and squeeze the upper part of the tray so it bends toward your palm. Being careful not to touch the inside of the tray, gently pull the foil tab to separate the foil from the tray.
9. Pour the sample/reagent mix directly into the tray, avoiding contact with the foil tab. Tap the small wells 2-3 times to release bubbles, then allow the foam to settle.
10. Place the sample-filled tray in the rubber insert with the plastic wells facing down, making sure the tray is well seated in the insert. Push gently on the tray until the sealer motor pulls the tray in.
11. Remove the (now) sealed tray and insert from the rear of the sealer as it is pushed through.

Incubator Use
1. The incubator temperature should be at 35 º $\pm$ 0.5º C.
2. Place the sealed trays in the incubator; stacking up to seven per shelf is allowable.
3. Samples should be read at 24 hours; results are valid at 24-28 hours with peak accuracy occurring closer to the 28 hour mark.
4. Record the date and time of placement in the incubator in the IDEXX sample log binder.

Counting Techniques
1. The sample tray is made up of 48 small wells and 49 large ones. Wells that are positive for *E. coli* will be yellow and will fluoresce under a 6 watt, 365 nm UV light.
2. Use the standard comparator tray for verification- if a sample is less yellow and has less fluorescence than the comparator, it is negative for *E. coli*.
3. Count all the wells, both large and small, that are yellow and fluoresce. Use best professional judgement to determine if it is equal to or greater than the comparator.
4. If any wells present indeterminate reads then place tray back in the incubator for the full 28hrs and re-read.
5. If wells still display indeterminate reads after 28hrs (or timing does not allow incubation beyond 24 hrs), then read tray with best professional judgement, request a team member to read the tray, and average the two counts.
6. Record #large and small, yellow and fluoresce, separately in the IDEXX sample log binder. Samples that appear positive before 24 hours and negatives after 28 hours are also valid.

Determining Results
1. Use the Quanti Tray/2000 MPN table on the IDEXX sample log binder to find the Most Probable Number (MPN) for *E. coli*. Record the number from the table in the IDEXX sample log binder, multiply by the dilution factor where appropriate, and record the final result.
2. Results should be recorded as they are found in the table (or after factoring in dilutions) and may include decimal places.
3. If multiple dilutions are run, record the *E. coli* values in the data base from the dilution using the largest volume of sample water (i.e., with a 10% and a 50% dilution, record the value from the 50% dilution).

Dispose of used sample trays in the regular trash. Caps to bacT bottles are to be discarded in the trash while empty bottles may be recycled. For web-based information about Colilert methods, equipment, see https://www.idexx.com/water/products/colilert.html

7.11 Chlorophyll-a in-vivo fluorescence

Sample Collection
Collect ambient water samples preferably in a amber plastic bottle, although a 250 ml whirlpack bag may be used if kept in the dark. Place all samples on ice immediately after collection. Note cuvettes must be handled wearing gloves to prevent skin oils from fouling surface. All samples should be completely agitated prior to sub-sampling.

Lab Procedures
1. Insert the chlorophyll-a in-vivo module completely into the Trilogy fluorometer. Note that the green exposed circuit board of the module should face the back of the instrument. Turn on the instrument and select module "Blue." You will be using RAW Mode to measure influorescence.
2. Measure background fluorescence for the waterbody: Rinse syringe with ambient (unfiltered) sample water three times. Draw 4 ml of sample into the disposable plastic syringe. Screw 0.45 µm filter disk onto syringe. Inject syringe thru filter into 13x13x100 mm disposable, methylacrylate plastic cuvette to rinse cuvette. Rinse cuvette three times with filtered water. Unscrew filter to refill syringe as necessary, then fill cuvette with at least 3 ml of sample water. Clean outside of cuvette with Kimwipes, then quickly insert cuvette into module to limit sample settling. If necessary, gently invert cuvette a few times with finger over opening to prevent settling.
3. Hit Measure. Do NOT open the lid during measurement. Record raw fluorescence of filtered sample.
4. Measure total sample fluorescence: You may reuse the cuvette from the filtered sample for the same site only if rinsed again. Rinse 13x13x100 mm disposable, methacrylate plastic cuvette three times with unfiltered ambient site water. Fill cuvette with at least 3 ml of sample, wipe outside with Kimwipes.
5. Quickly insert cuvette into module, hit Measure as before, and record raw fluorescence of unfiltered sample. Dispose of cuvette, syringe and filters.

7.12 Turbidity using Trilogy fluorometer

Make sure the Trilogy fluorometer is OFF and unplugged. Insert the Turbidity Optical Application Module into the fluorometer; align the kit with the sample compartment, locking firmly into place. (Don't touch sides of Module – use handle)

Plug in and turn on the fluorometer, touch module key "Turbidity". If screen is blank, touch the screen where the turbidity button should be based on the Figure 7.12-1. Confirm.

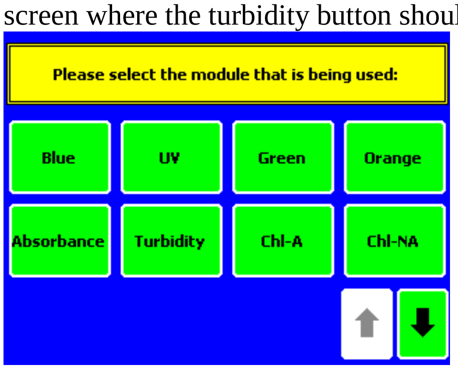


Figure 7.12-1 Trilogy Fluorometer turbidity module screen

Check the upper right portion of the Home Screen (Figure 7.12-2) to determine which mode is 'on': Raw Mode or Direct Concentration Mode. Turbidity should be measured in the Direct Concentration Mode. To change from Raw Mode, press the Mode button. If not, set to Direct Concentration, touch MODE key (lower right) to switch to Direct Concentration.

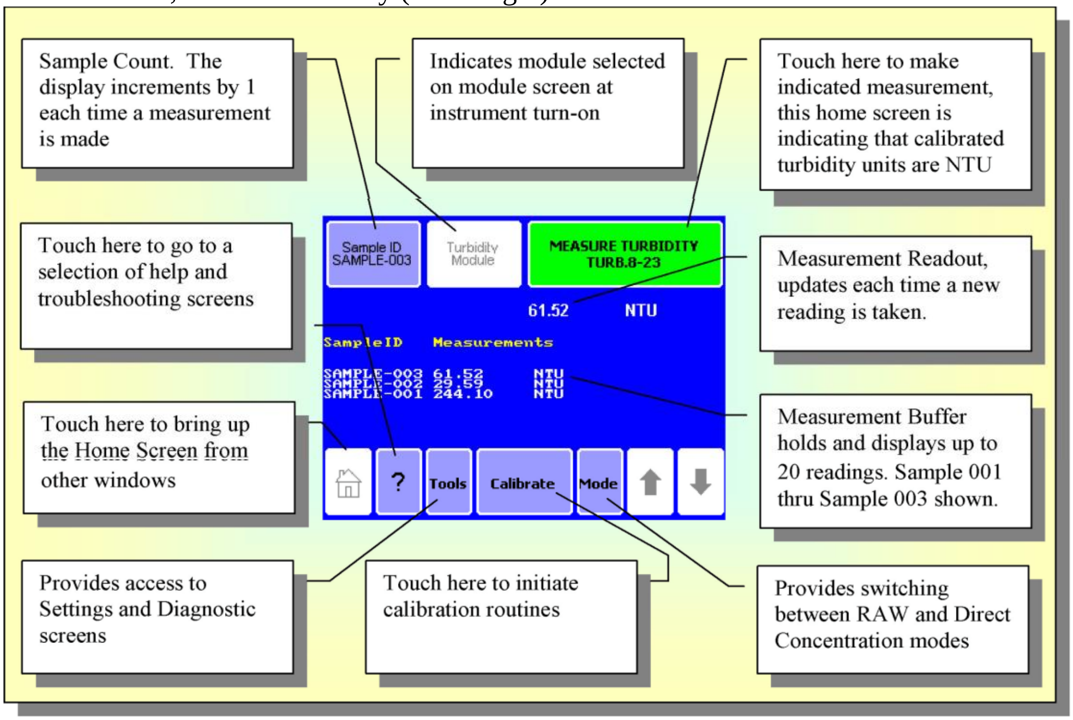


Figure 7.12-2 Trilogy Fluorometer turbidity home screen

- The message "You must calibrate..." appears briefly, then screen returns to Home Screen. Touch Calibrate and Use Stored Calibration. A choice of stored calibrations appears, touch the most recent (ex TURB 9-10) key, then Select.
- The Home Screen displays "MEASURE TURBIDITY *TURB-9-10"* in the upper right corner. The chosen calibration file is shown here in italics, it will vary depending on the name of the file.
- To measure turbidity, rinse the disposable methylacrylate cuvette three times with sample water (or standards, if checking calibration or calibrating), then fill with at least 3 ml sample water.
- Wipe the cuvette with chem wipe and insert into module. Close the lid.
- Touch MEASURE TURBIDITY. Do NOT open the lid while instrument is measuring.

Prior to measuring water samples, run a 'calibration check' by running Trilogy turbidity standards. Standards range from 0 to 10 NTUs. Use at least three (low, med and high) of the six standards provided. Record these as calibration check on the data sheet. If the standards are not within acceptable ranges (+/- 5%) (Table 7.12-1), perform the instrument calibration as described in the Trilogy Laboratory Fluorometer User's Manual. www.turnerdesigns.com

Table 7.12-1 Acceptable range for turbidity standards

NTU reading	+/- 5%
0.05	0.0025
1	0.05
2	0.1
4	0.2

6	0.3
8	0.4
10	0.5

To minimize measuring time, from the Home Screen, touch Settings, then Lid Start key to turn this feature ON. With this on, measurement begins as soon as the lid is closed, with no need to touch the MEASURE key.

Note: a blank can either refer to Deionized water or the 0 NTU standard, either can be used.

7.13 Lab Safety, Chemicals, Container and Waste Disposal

Emergency Numbers

The following signage for lab emergency numbers shall be unobstructed and prominently posted at all times near the phone and on the exterior of the front door of the laboratory.

Police & Fire Department	9-911
Poison Control	9-1-800-222-1222

Building/Lab Information: 974-6572
One Texas Center
505 Barton Springs Rd, 11th Floor

Sample containers stored in the ERM lab

All samples MUST contain a proper label and be LOGGED into the lab.

- Unlabeled and/or un-logged samples may be disposed
- Samples that have exceeded the holding times may be disposed
- Any mixed reagents or standards without a proper hazard warning label or content label with date mixed may be disposed

Labels: Name of Collector
Date
Site location
Preservation method (i.e. Formalin %, EtOH %, etc.)

Log: Name of Collector
Contact Information (your phone extension is fine)
Date of collection
Type of samples (i.e. Bacteria, TSS, etc.)
Number of Samples
Holding Time

Hazard Identification labeling

- All chemical containers must have a legible, firmly attached label describing contents.
- Labels on incoming containers of hazardous chemicals must not be removed or defaced. Any labels that are damaged must be immediately replaced with labels containing the same identification, warning and source information.
- Material safety data sheets (MSDS) received with incoming shipments of hazardous chemicals must be maintained and made readily accessible to laboratory employees.

- A hazard review of new materials not previously used in the laboratory must be completed before actual handling of the material begins.
- Chemical substances (or by-products) developed in the laboratory are assumed to be hazardous in the absence of other information.
- Standards mixed in the laboratory must be labeled with
    - Name of standard
    - Date mixed
    - Date no longer usable
    - Name of person who made the standard
    - How to dispose of contents (i.e. sink)
    - Cover container with lid or paraffin paper

Emergency Procedures

Each laboratory employee shall be familiar with the location, application, and correct ways to operate the following equipment.

1. Fire extinguishers
2. Fire alarms
3. Eye wash stations
4. First aid kits
5. Chemical response spill kits
6. Location of emergency telephone numbers and telephones

Eyewash

1. Eyewashes should be within the work area for immediate emergency use if required.
2. Inspections:
    a. Access to the eyewash should always be available. This means that no unwashed glassware or other items should be blocking the use of the sink in the laboratory. A clear path must always be maintained; always keep all field gear and ice chests pushed back from the sink area.
    b. Adequate eyewash flow should be observed by operating the device at least weekly for a one-minute period. This flow test also serves to clear sediment or rust that may accumulate in piping.
3. Eyewashes will be inspected annually by a representative of the Safety Office

Fire Extinguishers

1. Fire extinguishers are serviced annually through Pete Binion
2. Fire extinguisher should be the ABC model
3. Fire extinguisher should be within 30 feet of travel from the fire hazard(s) and located along a normal path of travel
4. Access to fire extinguisher must be maintained and free of equipment and the location should be conspicuously marked
5. The fire extinguisher type and size must be selected for the appropriate hazards.

Fire Alarms & Evacuation Route

a. Fire alarms should be conspicuously marked.
b. Evacuation Route should be posted in the laboratory and should mark nearest evacuation route.

First Aid Kits

1. First aid kits should be available and maintained for treatment of minor injuries or for short-term emergency treatment before getting medical assistance.
2. First aid kits are to be inspected monthly and refilled as necessary.

Broken Glass
Equipment (broken beakers, pipets, etc. that are waste) should be promptly swept up and disposed of in rigid cardboard containers or the small plastic Glass Refuse container on the lab counter. When the container is full tape it shut.

Chemicals
Each person working in the laboratory is responsible to ensure that all wastes are disposed of properly.

- Waste disposal procedures must be in compliance with State and Federal regulations. All waste containers must be labeled with the contents and the date of accumulation. Waste EtOH must be stored in flame cabinet in the laboratory.
- Hoods shall not be used for disposal (i.e., evaporation) of volatile chemicals.
- Drains shall not be used for disposal of chemicals unless it has specifically been approved by Pete Binion or MSDS.
- Chemical waste is removed from laboratories by persons who are responsible for regulatory compliance once the waste is removed from the generation site.

Container integrity is the responsibility of the generator. Containers must be compatible with the material inside and must be leak proof. Waste containers must be placed in secondary containment.

8.0 Salamander Count Mark & Recapture Database

8.1 Jollyville salamanders

Field Visit
Project: 118
Sample Site: ####
Date: MM/DD/YYYY format
Col Entity: 1 "WRE"
Personnel: Limited space in this field, so you may need to use initials
Comments: Any information not routinely collected, but important, goes here.

Field Sample
Time: enter 9999 if no time; otherwise simply enter time or approximate time survey started (military time is used for afternoon times: 1 o'clock = 1300)
Lab: 19 "Field"
Medium: 6 "Benthic Cover"
Sample Type: 6 "Visual Observation"
Depth: Leave blank.
Sample ID: Enter the section here, such as "section 3." Use numerals.
QC type: Leave blank for most purposes
Weather: e.g. "sunny and hot." Not a critical field.
Flow Type: B "base flow" S "storm flow" E "special event flow" N "no flow" U "unknown flow"
Comments: Here I generally indicate who surveyed the section, and sometimes a site description.

Notes: Sample ID field is where the section number for the survey is input. Do not update this field with a description, simply input the section number.

If the site was dry when you were scheduled for a survey it is important to create a field visit and field sample entry for that date and site, even if there is no data collection on the salamanders or water quality. Input "N" in the drop down menu for "flow type" and any other pertinent information in the comments field. Do not fill out the sample results tab in this situation

Sample Results
Enter the results from the survey for each field sample (i.e. each section number). You can load up the default parameters by clicking the "Proj Param" button; this will automatically fill in the appropriate data fields. Pay attention to the units and be sure to adjust if necessary. 1535, 1536, and 1537 are the parameter numbers for the three salamander size categories which should be input for every section that was surveyed. Be sure to enter zeroes for actual counts of no salamanders for each size class not observed. **Do not enter a zero if it is missing data- leave blank.**

8.2 Mark-Recapture Data

Project 528
Enter mark-recapture records under the **salamander mark** tab, after completing the previous three tabs, as noted above. Data structure is similar for project 118 entries, with the exception that salamander counts by size (parameter numbers 1535 1536 1537) are not tallied for any marked salamanders- these data are entered by individual in the **salamander mark** tab. Individuals are identified based on their unique marked pattern of elastomer. For each unique combination of marks, there is a numerical ID number- this number is what gets entered under the heading "salamander." These numbers can be acquired when generated, using the

Access database "salamander tracker" created by Rob Clayton (located on the "salamanders" laptop). They can also be referenced from this Access database for recaptured salamanders (already have a number) or by double clicking within the open space of the "salamander" column of the Oracle database. This will open the "salamander tracking form" (see below).

Once the salamander ID is entered, the Tab key will move your cursor to the next input fields, recapture, gravid and dead. The Space Bar will enter a check mark in these fields; only do so for a positive recaptured, gravid, or dead individual. Tab over to the three size categories. The first one is for snout-vent length, which used to be measured in the field. We no longer measure individuals in the field, but from photographs, thus SVL is no longer recorded. Enter the total length and the SVL surrogate, SPHLL, or Snout-to-Posterior-Hind-Limb-Length, a measurement from the snout to the middle of the back between the hind legs where the hind legs intersect the body closest to the tail (see "how to measure salamanders from photographs"). Enter any relevant comments, such as whether the tail looks to be regenerating or recently chopped- this is usually noted as "stub" or "stub tail." This will help to identify total lengths that don't necessarily reflect age. Finally, hit the photo button to link this record to a photo of the individual taken during that session. Simply browse to where the photo is stored (on a network drive, not a local one- photos are safer there). The photo is used for verification and, in the absence of a full set of marks, identification. Photos are identified by sequential number- this is easy to set up in a DSLR, such as the D80 we are currently using.

Salamander tracking form

This form can be used to query salamander ID numbers using the mark combo, or vice versa. Incomplete combos can also be used to query salamander IDs, and this form also displays any attached photograph of the animal. The numbers 1-6 indicate the location of the salamander's body of the elastomer, and the pull down menu gives the option of choosing a color, a dash "-" indicated no mark, or a blank space (null), indicating any of the above possible. The status column menu options are "in use," indicated that that combo being searched for has been assigned to a salamander, or blank (null), which would return marks that have not been assigned in addition to those that have (all possible results of your query). When using this form to ID a salamander for which not all the marks have been identified, e.g. only two marks observed in field, it is important to select the "in use" status so it only returns results of combos that have been used already (because you don't want to see unused combos). When there are a large number of results for a salamander query intended to ID an unknown or unconfirmed salamander, it is often more useful to execute a special query with the SQNAV tool, because more specific parameters can be selected to help narrow the search down (e.g. Lanier Spring, K or B is position 2, less than 50mm TL). The salamander tracking form is mostly useful for querying salamander ID numbers from marks noted on the field data sheets when entering data. Here's an example of a workflow to accomplish finding and selecting a salamander marked KnOW in the field (1st pos Black, 2nd pos no mark, 3rd pos Orange, 4th pos White):

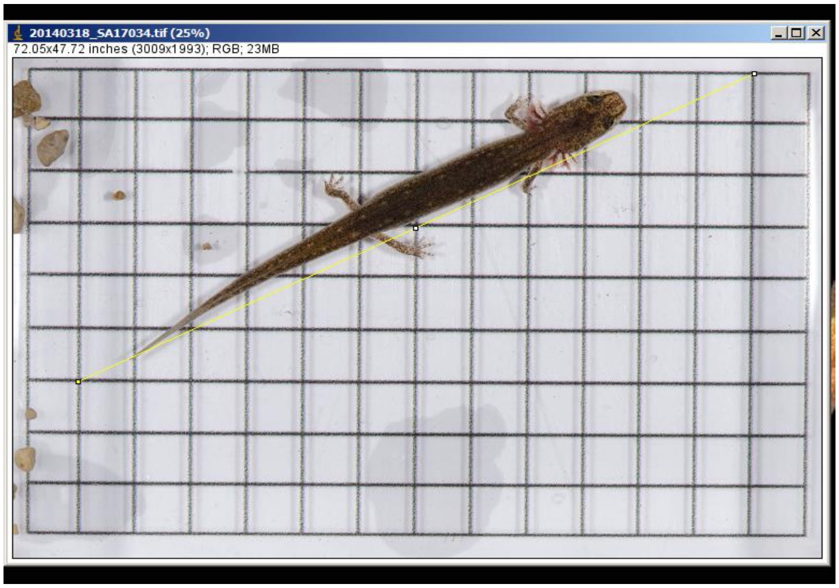
1. Double click in white space under "salamander" column where new record is to be entered.
2. Salamander tracking form opens.
3. Hit "enter query." This clears the form.
4. Tab, this selects column 1. Hit key "K"
5. Tab, "-", Tab, "O", Tab "W" Tab x4, "I". This will enter the correct combo to position 4, and then select the "in use" option under status. Optionally you can fill out all six combos and then there is only one possible answer so the status option is moot.
6. Select "run query" One result should appear- if more than one appears, select the correct one with the mouse or arrow keys so that it is highlighted yellow. A few have the same exact ID at the same site (due to mistakes) and they have duplicate numbers- using the linked photos will help ID which salamander to select. Simply double click the photo link and the photo

will open in the default photo program in Windows (this can be changed on your computer at your discretion).

7. Once you've selected the correct ID, hit "select for observation" at the bottom. Confirm your choice by hitting enter or with the mouse, and this will return you to your position in the salamander mark form.

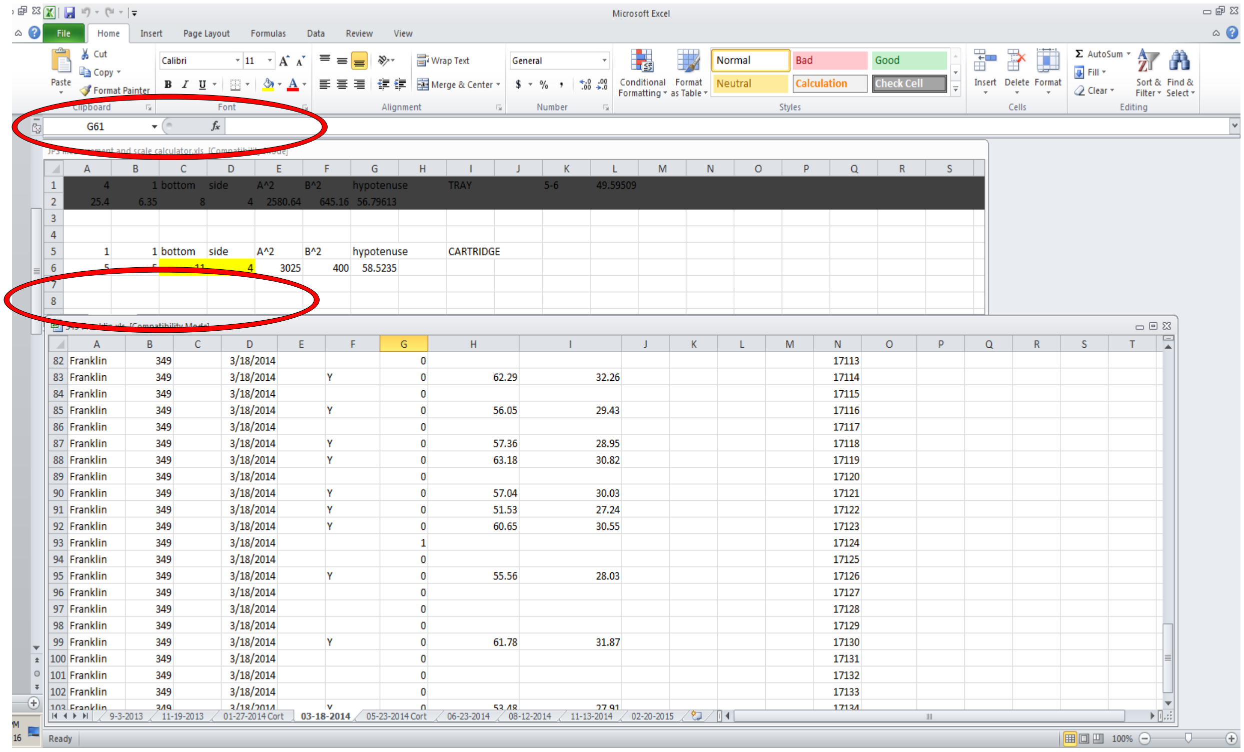
8.3 Measuring salamanders from photographs

- We use the program ImageJ, which is available as free public domain software from http://rsb.info.nih.gov/ij/.
  - Open salamander image from its location within ImageJ, or to make life easier, set ImageJ as the default program to open jpegs in Windows (right click-> open with-> ImageJ -> check make default program). The salamander image is usually converted to a tif imager. Double check the photo number.
  - The photograph should be in focus, the salamander flat (but not necessarily straight) in the frame, the entire salamander within the picture, and against a grid background of known dimensions. If salamander is not entirely in photo; please make a comment on the data sheet as well as the excel file.
  - We use a either laminated grid paper siliconed to the bottom of a shallow white plastic tray, where four grid squares equals one inch, or more typically, a plastic cartridge with 5 x 5 mm grid squares On the ImageJ toolbar, choose the straight line selections tool, 5th from the left. Left-click and drag a known distance with this tool, creating a straight yellow line. Release mouse button to finish line. Placing the mouse cursor over the white end points of the line, the cursor turns to a hand, which can be used to drag and resize the line you just created. Ideally the scale is drawn parallel to the salamander to accommodate any distortion or projection error along that axis. The length can be quickly calculated as the hypotenuse of a triangle using a pre-formulated excel sheet. Zoom in (+) and out (-) to make sure the line is in the center of the square intersection.

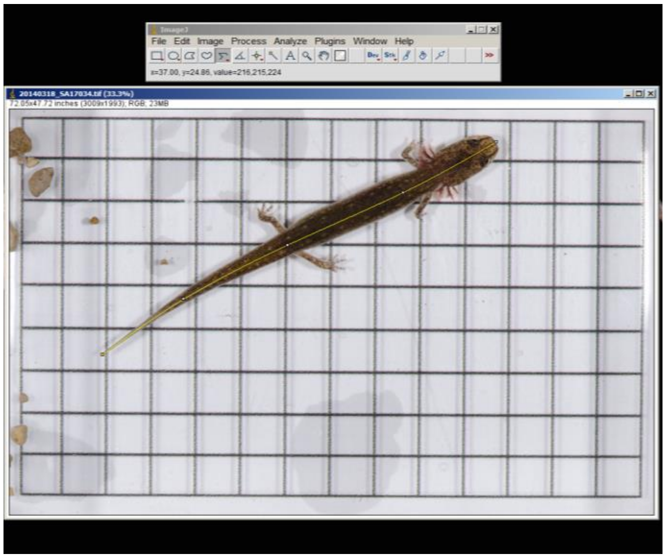


*The plus and minus sign on the keyboard is useful for enlarging and decreasing the image.

- Below is an example of an excel sheet open for calculating the scale length as well as a data entry sheet for the results.



- Once the scale line is drawn, select Analyze from the menu and scroll down to set scale. `Tab` to "known distance" and enter the length of the scale, then hit enter.
- Now you are ready to measure. Go back to the straight line tool button and right-click, then select segmented lines.
- A single left-click starts the line, so start on the tip of the tail or tip of the snout and begin tracing down the middle of the salamander, attempting to represent its true length as well as possible (again, it helps to zoom in). Additional clicks will create anchor points, which are useful with the salamander is in any curved position. Holding `space` will help navigate while zoomed in.
- Finish the line by double left-clicking. Then, *double click the segmented line button which will fit a smoothed line; you will need to ensure that the lines are still on the centerline of the salamander.



- Now press `Ctrl + M`, or select measure from the analyze menu (but the hot key is quicker). This should give you the total length. Then you can either make an additional line for the SPHLL measurement (body length) or trim your first line by hovering the mouse over any anchor point you wish to remove and holding `Alt` while left-clicking. You can also create new anchor points by using `Shift + left-click`. This measurement should look something like this,

bringing the endpoint of the line to the midsection of the tail just at the end of the legs, as if a line were drawn from between both legs where the back of the hind leg joins the body. This method is more consistent than guessing where the vent might be and is reliably reproducible.

    - `Ctrl + M`, and you have both measurements. In case you don't see or lose the results, they are displayed in a separate window by ImageJ called "results"; just look for it minimized somewhere on the taskbar.
- Record your measurements on the data sheet (making sure you are on the appropriate date and survey) and in the excel file.
- Save .tif files from ImageJ – these save the scale data so that it does not have to be re-scaled if additional measurements are required in the future.
- Add any comments from the data sheet to the excel file.
- Update salamander team on the SLACK channel.
- Check through your work and make sure the photo number (ID) all match up. *BE AWARE, that the photo numbers may not be in order, e.g. in the series 14931, 14932, 14935, the photo numbers 14933 and 14934 are omitted.
- Always compare your data sheet with the excel file before and after your measurements.

9.0 Data Analysis Protocols

The implementation of standardized analysis protocols will not only be useful to ensure that all analyses are statistically correct, but also should increase the efficiency of data analysis efforts.

Presented below are standardized methods for statistical analysis by type of test. For each type of test, there is a referenced method and a tool available to ERM staff. The statistical software tools available to staff at the present time are:

Statistica, release 6.1
SAS, version 9.1.3
Microsoft Excel, version 2000 and up

For each type of test, the user must consider the distribution of the data (e.g., is data normally distributed?) and the presence of censored observations (e.g., does the data contain non-detects?). Additionally, the quality of data to be used for analysis, determined by the automated qa/qc flagging procedure, must also be considered.

9.1 QC Flag Handling

Prior to data analysis, the data quality flags (Table 9.1-1) generated by the automated QA/QC Flagging Process (WRE 2004) should be reviewed.

Table 9.1-1. List of potential flags generated by QA/QC process

QC Flag	Description	Reason for Flag
U	Results are completely usable	All applicable tests OK
R	Results are completely unusable	Failure of any major test (logical test, lab standard, lab split, field split, post-cal, holding time)
J	Results are to be considered an estimated value	Failure of one or more minor tests (field blank, lab blank, field replicate, lab matrix spike) but no failure of any major test
S	Result outside of standard range but not rejectable	Result outside $5^{th}/95^{th}$ percentile but all other applicable tests OK
?	No sample result data exists	No result entered in FSDB; data entry error

Rejected data (R-flags) should be excluded for all analyses, but will remain in the database not only to track qc failures over time (e.g., are the number of major qc test failures increasing at a given lab over time), but also as placeholders for billing and audit purposes.

Use of estimated data (J-flags) and data outside of standard range (S-flag) is permissible if the use of these data are allowable within the data quality objectives of the QAPP for the specific program.

9.2 Distributional Testing

Parametric analyses should be used on data that is normally distributed, or by transformation can be approximated by the normal (or a known) distribution. Non-parametric analyses, though valid on normally-distributed data , will be of reduced power and thus should be limited to non-normally distributed datasets where practical.

In some situations, it may be valid to rank-transform (rank-order) the data (**PROC RANK** in SAS, **Rank** from the **Vars** menu on the Statistica toolbar) and use normal-theory methods for data analysis (Iman 1982). Parametric tests on ranked data approximate their equivalent nonparametric procedures (Conover and Iman 1981).

Histograms and stem-and-leaf plots will give a visual impression of the shape of the dataset, but are not adequate tools for discrimination (Gilbert 1987). Two acceptable goodness-of-fit tests are the Kolmogorov-Smirnov (Pr > D) (D'Agostino and Stephens 1986) and Shapiro-Wilk (Pr < W) (Shapiro and Wilk 1965) options. Both test the null hypothesis that the values of the analysis variable are a random sample from the specified distribution. Pr values less than the critical value α (generally taken as 0.05) reject the hypothesis (i.e., the data is not normally distributed). For smaller sample sizes, it is recommended that a larger α-value (0.1) be used to increase the power to detect non-normality (Helsel and Hirsch 1995). Sample sizes must be greater than or equal to 3 to perform tests for normality.

The Shapiro-Wilk test is the preferred test of normality because of its good power properties as compared to a wide range of alternative tests (Shapiro et al 1968, EPA 1998). However, the Shapiro-Wilk test (with Royston's extension as calculated in SAS and Statistica) is only valid for samples up to 2,000 observations (Royston 1982). Use the Kolmogorov-Smirnov test for samples with more than 2,000 observations.

Both options may be requested in SAS using the **PROC UNIVARIATE** procedure with the **NORMAL** option. Both tests are available in Statistica from the **Basic Statistics/Tables > Descriptive Statistics** menu under the **Normality** tab (choose histogram or frequency table from the normality tab).

Lilliefors probabilities (Lilliefors 1967) should be used to determine the significance of the K-S statistic in Statistica, as the K-S statistic is not valid if the parameters of the hypothesized distribution are estimated from the data set. **PROC UNIVARIATE** in SAS does not rely on Lilliefors adjustment, but rather computes the critical values (Pr > D) of the modified D statistic (the K-S statistic) by the more reliable method of using simulations of large numbers of samples (10,000) so that the critical values do not involve the actual sample size (Stephens 1974). Thus, no additional adjustment of critical values (Pr > D) in SAS output is necessary.

Datasets may be transformed by a variety of methods, then tested for goodness-of-fit of the assumed distribution (see "Ladder of Powers" in Helsel and Hirsch 1995, see "Chapter 13" in Sokal and Rohlf 1995), but be sure to correctly re-transform output data back to original units and be aware of any transformation bias (Helsel and Hirsch 1995). The Shapiro-Wilk test on the logarithms of the data is a valid test to determine if the distribution of the data is a lognormal distribution (Gilbert 1987), as is often encountered in environmental science.

Tests for distribution of data with censored observations are difficult. If the distribution is known for a dataset with censored observations, parametric analyses may be used. In general, datasets with censored observations will be analyzed with non-parametric methods.

9.3 Summary Statistics

Summary statistics, including descriptive statistics such as the mean, median, standard deviation, and percentiles, may be calculated on any dataset without censored observations using any commonly available software. For example, summary statistics may be calculated in SAS with the **PROC MEANS**, **PROC UNIVARIATE**, and **PROC SUMMARY** procedures. Summary statistics may be calculated in Statistica from the **Basic Statistics/Tables** > **Descriptive Statistics** menu.

The choice of using a median or mean value to describe a dataset should be dependent upon the intended use of the dataset. Median values are more resistant to outliers or extreme observations, while mean values are strongly influenced by outliers and extreme observations (Helsel and

Hirsch 1995). The use of median and interquartile range (IQR, the 75th percentile minus the 25th percentile) are encouraged for the estimation of the "typical" value (Helsel 1990). Note that the IQR equals 1.35 times the standard deviation for a normally distributed dataset, although for strongly skewed distributions the IQR will often be much smaller than the standard deviation and a better estimate of the variability of the bulk of the data. Other measures of location may be useful, depending on the distribution of the data. For positively skewed data (such as bacteria) the geometric mean, or mean of the logarithms of the data transformed back to the original units (Sokal and Rohlf 1995), is frequently used as a more unbiased estimate of the median (Helsel and Hirsch 1995).

For datasets with censored observations, there are acceptable statistical methods for the calculation of summary statistics. Substitution methods (e.g., using ½ the detection limit for censored observations) should always be avoided (Helsel and Hirsch 1995, Helsel 2005) unless replicating a published procedure used by another agency (see TCEQ 2004). Methods such as trimmed means and Winsorized means (Gilbert 1987, EPA 1998, Sokal and Rohlf 1995) may be used if the distribution of the data is known to be reasonably symmetric and the percentages of data "trimmed" are explicitly stated (Helsel and Hirsch 1995), but should be used with caution as these techniques involve the exclusion of both high and low values from the summation of the dataset.

Reporting the percentiles and median of dataset as "less-than" (or censored) values when those values fall on non-detect data points is an acceptable (and non-parametric) method of calculating summary statistics for censored datasets (Gilbert 1987, Helsel 2005).

The Kaplan-Meier (K-M) survival analysis technique (Kaplan and Meier 1958) is the preferred non-parametric method for estimating summary statistics that is valid when less than 50% of the observations are censored (Allison 1995, Helsel 2005). If more than 50% of the data are censored, the median cannot be estimated with the K-M method (although mean values are still generated). Note that K-M operates only on right-censored data ("greater-thans"). Environmental data that is left-censored ("less-thans") must be transformed by subtracting all observations (censored and un-censored) from an arbitrary constant larger than the largest value in the dataset (Helsel 2005). Estimates of the median and mean must then be re-transformed back to original units after analysis by again subtracting from the same arbitrary constant. The estimates of variability (standard error and standard deviation) do not need to be re-transformed.

K-M is available in SAS (see Allison 1995) using the **PROC LIFETEST** (with the additional ODS statement to output the statistics to another dataset):

```
ODS OUTPUT Quartiles=q Means=m;
PROC LIFETEST data=<data-name>;
    TIME <analysis variable>*<censoring variable>(censored indicator);
```

Note that the standard error may be converted to a standard deviation using the approximation (Helsel 2005):

$$\text{Std Error} * (\sqrt{n}) \approx \text{Std Deviation}$$

If the smallest observation is a censored observation, the estimate of the mean will have a small positive bias (Allison 1995, Helsel 2005) . The estimate of the median would remain unbiased, and thus would be preferred over the mean (Allison 1995).

Maximum likelihood estimation methods are valid if the distribution of the data is known and the sample size is large (n>25,50), and may be useful for highly censored (<80%) datasets (Helsel 2005). Helsel and Cohn (1988) developed a MLE method for calculating summary statistics for lognormally-distributed datasets (standalone program and SAS macro are available at http://www.practicalstats.com), although the sample size is limited to 1,000 detected observations and 1,000 non-detect observations, and datasets must be input one-at-a-time. Estimates of percentiles (median and IQR) are reliable even if the dataset is not normally distributed (Helsel and Hirsch 1995), but locational estimates (mean and standard deviation) are very sensitive to the values of the largest observations and are not reliable if the distribution is not lognormal (Gilliom and Helsel 1986). The Helsel MLE program accepts left-censored data, and does adjust for transformation bias (Helsel and Cohn 1988), inherent in the computation of the mean of transformed data (Helsel and Hirsch 1995).

The **PROC RELIABILITY** procedure in SAS may be used to perform MLE of the mean and standard deviations for datasets of known distributions (normal, lognormal, weibull, etc). The RELIABILITY procedure will accept left-, right- or interval-censored data. An example of the SAS statements syntax for MLE with probability plot for a lognormal distribution is:

```
PROC RELIABILITY data=<data-name>;
    DISTRIBUTION lognormal;
    PPLOT <analysis variable>*<censoring variable>(censored indicator);
```

MLE methods are available in Statistica from the **Advanced Linear/Nonlinear Models** > **Nonlinear Estimation**, although the user must specify the regression equation.

For datasets with 50-80% censored observations, robust regression on order statistics (robust ROS), or regression on probability plots, may be used. Robust ROS assumes that only the non-detect values follow a specified distribution (Helsel and Cohn 1988, Helsel 2005). Values for non-detect observations are predicted based on their normal scores from a regression model of the detected observations on a probability plot. Predicted values for non-detects are used in combination with detected values to compute summary statistics (Helsel 2005). Note that retransforming individual values prior to calculation of summary statistics avoids all transformation bias.

Standalone programs for robust ROS by probability plotting (assuming a lognormal distribution for non-detect values), including SAS macro's, may be found at the website: http://www.practicalstats.com/Pages/software.html . Robust ROS is a useful alternative for small datasets (n<30), where MLE becomes inaccurate (Helsel and Cohn 1988). The robust log probability plotting regression method output from the program by Helsel listed above is one method for calculating a non-parametric robust ROS. Again, this program is limited to 1,000 detect and 1,000 non-detect datapoints, and datasets must be input one-at-a-time.

No method is valid if more than 80% of the data is censored (Helsel 2005).

Statistica does not calculate valid mean estimates for censored data sets from the **Survival Analysis** menu. However, valid percentile estimates for censored data are available from the **Survival Analysis** > **Product Limit (Kaplan-Meier)** menu.

In summary:

- For less than 50% censored observations, use non-parametric Kaplan-Meier.
- For large samples (n>50) and 50-80% censoring (with data transformed to approximate normal, lognormal distribution), use MLE (adjusted for bias).
- For small samples (n<50) and 50-80% censoring (or unknown distribution), use robust ROS.
- For >80% censored observations, report data as censored percentiles.

9.4 Comparison Testing

Statistical tests are the most quantitative methods to determine whether hypotheses of system functions can be substantiated or whether they must be modified or rejected outright (Helsel and Hirsch 1995).

Hypothesis tests should be conducted according to the following structure (taken from Helsel and Hirsch 1995):

1. Choose the appropriate test
2. Establish the null and alternate hypotheses
3. Decide on an acceptable error rate (α)
4. Compute the test statistic from the data
5. Compute the p-value
6. Reject the null hypothesis if $p \leq \alpha$

The default acceptable error rate is 0.05 for all tests used by ERM.

Consider the distribution of the data if using parametric hypothesis tests. The power of parametric tests to reject H_0 when H_0 is false can be very low when applied to non-normal data (Bradley 1968). Nonparametric tests are particularly appropriate for small datasets (unless experience supports the assumption of normality) and for strongly skewed data with outliers (Helsel and Hirsch 1995). Parametric tests on ranked data approximate their equivalent nonparametric procedures (Conover and Iman 1981).

The null hypothesis is assumed to be true about the system prior to data collection, usually stating the null situation (no difference between groups). The alternate hypothesis is the situation anticipated to be true if the evidence show the null hypothesis is unlikely.
Note that a one-sided test is a comparison of a "treatment" to a "control," where only one direction of difference is expected (e.g., current conditions are significantly higher than background historical conditions). A two-sided test is a comparison between two groups where either may be better or worse than the other (e.g., is site A different from site B).

Independent datasets are those for which there is no natural way to pair the data (Gilbert 1987, Helsel and Hirsch 1995), as opposed to datasets with paired observations (sometimes referred to as dependent samples).

Comparing means to a constant value
A one-sample test involves the comparison of a population parameter (the mean, variance, etc) to a fixed threshold value (Hays 1988, EPA 1998). An example of the use of a one-sample test is to compare the mean of one parameter from a single site to an effects level to determine if the mean concentration exceeds the effect levels.

The primary assumptions of a (parametric) one-sample t-test are (EPA 1998):
1. Random sampling (independence of the data values)
2. The sample mean must be normally distributed

A one-sample t-test may be performed in Statistica from the **Basic Statistics/Tables** > **t-tests, single sample** menu. A one-sample t-test may be performed in SAS using the **PROC TTEST** specifying the **H0** option:

```
PROC TTEST data=<data-name>  H0=<threshold-value>;
        VAR <response-variable>;
```

The t-test is not resistant to outliers because the sample mean and standard deviation are influenced greatly by outliers (EPA 1998). The Wilcoxon signed rank test is more resistant to outliers (as it is based on the ranks of the data, EPA 1998), but still requires the assumption that the observations are symmetrically distributed about the mean (SAS Help Files). The sign test is a nonparametric one-sample t-test that does not require an assumption about the distribution of the data (Gilbert 1987) and may be used to test whether the *median* of a population differs from a specified value (Helsel and Hirsch 1995, SAS Help Files). Both the Wilcoxon signed rank test and the sign test may be requested in the SAS **PROC UNIVARIATE** using the **Mu0** option:

```
PROC UNIVARIATE data =  =<data-name>  Mu0=<threshold-value>;
        VAR <response-variable>;
```

Comparing two independent groups

To compare the means of two different (independent) groups without censored observations from a normal distribution, a parametric two-sample t-test may be used (EPA 1998).

The primary assumptions of a (parametric) two-sample independent t-test are (Bowker & Lieberman 1972, EPA 1998):

1. Random sampling (independence of the data values)
2. Independent groups
3. The means of the groups must be normally distributed
4. The variances of the two groups must be equal (homogeneity of variance)

Two sample independent t-tests are available in Statistica from the **Basic Statistics/Tables** > **t-test, independent, by groups** or **t-test, independent, by variables** menu. Two-sample independent t-tests are available in SAS using the **PROC TTEST** procedure:

```
PROC TTEST  data=<data-name> COCHRAN;
        CLASS <grouping-variable>;
        VAR <response variable>;
```

To verify the assumption of the independent two-sample t-test that the variances of both groups are equal, use the Brown-Forysthe variation of Levene's (option **HOVTEST=BF** in SAS) test (1974), shown to yield better power to detect variance differences while protecting the Type I error probability than other homogeneity of variance tests (Conover et al 1981, Olejnik and Algina 1987). The Cochran and Cox (1950) or Satterthwaite (1946, Helsel and Hirsch 1995, EPA 1998) computational methods (specified as options in the PROC TTEST statement in SAS) should be used if the variances of the two groups are heterogeneous. If the Brown-Forysthe test rejects the assumption of homogeneity of variance, use Welch's variance-weighted ANOVA instead of the usual ANOVA to test for differences between group means.

```
PROC GLM data=<data-name>;
        CLASS <grouping-variable>;
        MODEL <dependent variable> = <grouping-variable>;
        MEANS <grouping-variable> / HOVTEST WELCH;
```

If the variances are different between the two groups, then the two groups are most likely from different populations. Tests for the homogeneity of variance have low power for small sample sizes and are of less importance for large sample sizes when the null hypothesis of equal variances is frequently rejected (Cody and Smith, 1997).

The strict assumptions of the parametric t-test are often difficult to satisfy with water resource datasets, and the use of non-parametric methods (rank sum tests) are encouraged (Helsel and Hirsch 1995).

To compare the means of two different (independent) groups without censored observations using a non-parametric test, use a Mann-Whitney (equivalent to the Wilcoxon rank-sum) test (Gilbert 1987, Helsel and Hirsch 1995, Cody and Smith 1997, EPA 1998, Helsel 2005). The Mann-Whitney U-test for two samples is available in Statistica from the **Nonparametrics** > **Comparing two independent sample (groups)** menu. One-sided and two-sided comparison tests (the Wilcoxon rank-sum) are available in SAS using the **PROC NPAR1WAY** specifying the **WILCOXON** option. Evaluate the results under the "Wilcoxon Two-Sample Tests" header of the SAS output.

```
PROC NPAR1WAY data=<data-name> WILCOXON;
        CLASS <grouping variable>;
        VAR <response variable>;
        EXACT WILCOXON;
```

For even moderate sample sizes, the Wilcoxon test is almost as powerful as the parametric equivalent (the t-test), and should be used if there is a question of the distribution of the data. Specifying EXACT causes SAS to compute exact p-values, and should be requested for small sample sizes less than 10 (Helsel and Hirsch 1995, Cody and Smith 1997). When interpreting the results of the Wilcoxon rank sum test output by PROC NPAR1WAY in SAS, use the 1-sided or 2-sided p-value statistics appropriately. If a directional alternative hypothesis can be justified (is A > B), then use the 1-sided test results. If simply testing the alternative hypothesis that the samples are different (A>B or B>A), use the 2-sided test values (Cody and Smith 1997).

The rank-sum test is a test for whether one group tends to produce larger observations than the second group. P-values less than the critical value lead to rejection of the null hypothesis, or small p-values indicate the samples are from different populations. No assumptions about how the data are distributed in either group are required (Helsel and Hirsch 1995).

Computations of the p-value using a parametric t-test on rank transformation of the data for moderate sample sizes (conservatively, larger than 25 observations in both groups) is valid (Helsel and Hirsch 1995).

To test if two groups are distributed identically, use the non-parametric Kolmogorov-Smirnov two-sample test (Sokal and Rohlf 1995), which is sensitive to differences in location, dispersion and skewness and is based on the unsigned differences between the cumulative frequency distributions of the samples. The Kolmogorov-Smirnov two-sample test may be requested in SAS using the **PROC NPAR1WAY** procedure requesting the **EDF** option.

```
PROC NPAR1WAY data=<data-name> EDF;
        CLASS <grouping variable>;
        VAR <response variable>;
```

Nonparametric survival analysis techniques adapted to water resource data are the preferred method of comparing two different (independent) groups with censored observations. The rank-sum test is a valid nonparametric method for independent comparison of censored datasets with only one detection limit, lower than the lowest detected value (Helsel 2005). Standard nonparametric tests can always be computed on censored data by censoring all values below the highest detection limit to a common (censored) value (Helsel 2005). **Left-censored environmental data should be "flipped" prior to employing survival analysis techniques.**

Score tests, in which the distribution functions are compared between groups, should be used for comparison testing of censored data with more than one detection limit (Helsel 2005). If uncensored data are input to these tests, the results are identical to the Wilcoxon rank-sum test (Helsel 2005). Like other nonparametric tests, score tests are invariant when applied to data transformed using power functions, and thus there is little reason to use transformations when performing score tests (Helsel 2005).

To compare the means of two different (independent) groups with censored observations using a non-parametric test in Statistica, use **Advanced Linear/Non-Linear Models** > **Survival Analysis** > **Comparing two samples** and choose **Peto and Peto generalized Wilcoxon** test from the Two Sample Test tab. Statistica calculates the Peto and Peto (1972) generalized Wilcoxon modification of the Gehan (1965) test.

To compare the means of two different (independent) groups with censored observations using a non-parametric test in SAS, use the **STRATA** option in **PROC LIFETEST**, and interpret the results for tests of equality over strata (Allison 1995). The log-rank output (aka Mantel-Haenszel test), is closely related to the proportional hazards model and is more to differences between groups that occur at later points in time whereas the Wilcoxon test is more powerful where event times have log-normal distributions with a common variance but with different means between the groups (Allison 1995). The Wilcoxon statistic is equivalent to the Gehan Wilcoxon test used in Statistica, and by specifying the option **TEST=MODPETO**, you can request the modified Peto and Peto test (Prentice 1978), generally found to be the score test yielding the best overall performance for datasets with unequal sample sizes between groups (Latta 1981).

```
PROC LIFETEST data=<data-name>;
    TIME <response-variable>*<censoring variable>(censored-indicator);
    STRATA <grouping-variable> / TEST = (MODPETO);
```

Additionally, **PROC LIFETEST** in SAS may be useful for screening for the effects of quantitative covariates using the **TEST** statement, but may not be adequate for examining the effects of variables controlling for other covariates (Allison 1995).

Comparing two dependent (paired) groups

A variation on the two-sample design occurs when observations in each group are purposely paired with one another to block out sources of background noise and focus on the effect being studied (Helsel 2005). Independent t-tests may not be used on this type of comparison as the samples are no longer independent (Cody and Smith 1997).

Use a parametric two-sample paired t-test for normally distributed paired differences. In Statistica, use the **Basic Statistics/Tables > t-test, dependent samples** menu to perform two-sample parametric paired t-test. To compute parametric two-sample paired t-tests in SAS, use **PROC MEANS** as SAS does not include a paired t-test as part of PROC TTEST (Cody and Smith 1997). This will compute the probability that the difference between the two groups is significantly different from zero:

```
DATA <data-name>;
    INPUT var1 var2;
    DIFF = var2-var1;
PROC MEANS data=<data-name> N MEAN STDERR T PRT;
    VAR DIFF;
```

Assumptions of the paired t-test (Helsel and Hirsch 1995) are:

1. The paired differences are normally distributed around their mean
2. The two groups of data have equal variance and shape.

Use the sign test or the Wilcoxon signed-rank test for two-sample nonparametric matched pair comparison tests. The sign test determines whether x is generally different than y for data pairs (Helsel and Hirsch 1995). The sign test does not require an assumption about the distribution of the data (Gilbert 1987, Helsel and Hirsch 1995). The Wilcoxon signed rank test is more resistant to outliers (as it is based on the ranks of the data, EPA 1998), but still requires the assumption that the observations are symmetrically distributed about the mean (Gilbert 1987, Helsel and Hirsch 1995, SAS Help Files). However, asymmetry must be severe to have a substantial effect on the resulting p-value of the Wilcoxon signed rank test (Helsel and Hirsch 1995).

The sign test and the Wilcoxon signed-rank test may be calculated in SAS using the **PROC UNIVARIATE** procedure on a variable representing the difference between the two groups in question. This will test if the difference between the two groups is equal to zero. P-values less than the critical value lead to rejection of the null hypothesis and indicates that the groups are different:

```
DATA <data-name>;
    INPUT var1 var2;
    DIFF = var2-var1;
PROC UNIVARIATE data=<data-name>;
    VAR DIFF;
```

The sign test may be calculated in Statistica using the **Nonparametrics** > **Comparing two dependent samples (variables)** menu. The sign test may be applied to censored datasets, and is valid for all sets of observations except when both pairs of a set are censored (Helsel 2005).

Comparing three or more groups

To compare more than two groups, use analysis of variance (ANOVA). The assumptions for ANOVA are basically equivalent to those of the parametric t-test: independence between groups (unless using a repeated measures design); normally distributed sample means; homogeneity of variance (Cody and Smith 1997, Bowker and Lieberman 1972, Spiegel 1992). ANOVA is "robust," and departures from the assumptions may not invalidate the analysis (Cody and Smith 1997, Lindman 1974). Balanced designs (equal N in all groups) are preferred if group variances are unequal (Cody and Smith 1997). The null hypothesis of ANOVA is that the means of all groups are equal.

For uncensored datasets that contain normally-distributed independent groups with equal variance, use ANOVA to compare the means of the groups. ANOVA may be performed in Statistica from the **ANOVA** menu.

In SAS, ANOVA may be specified using the **PROC ANOVA** (balanced designs or one-way ANOVA only) or **PROC GLM** (valid for both balanced and unbalanced designs) procedures.

```
PROC ANOVA data=<data-name>;
    CLASS <grouping-variable(s)>;
    MODEL <dependent-variable(s)>=<grouping-variable(s)>;

PROC GLM data=<data-name>;
    CLASS <grouping-variable(s)>;
    MODEL <dependent-variable(s)>=<grouping-variable(s)>;
```

Although the F-distribution is robust to deviations from normality (Lindman 1974), non-parametric methods for comparing the medians of two or more groups are available in SAS and Statistica. The Kruskal-Wallis test is an extension of the Wilcoxon rank sum test and may be used for non-normal data in SAS, and can even accommodate a moderate number of non-detect values (Gilbert 1987). The Kruskal-Wallis test, based on ranks, tests the null hypothesis that the populations being compared have the same median. Kruskal-Wallis may be requested in SAS using the PROC NPAR1WAY procedure:

```
ODS KruskalWallisTest=<output-data-name>;
PROC NPAR1WAY data=<data-name> WILCOXON;
    CLASS <grouping-variable>;
    VAR <dependent-variable>;
```

Request the Kruskal-Wallis test in Statistica using **Nonparametrics > Comparing Multiple Independent Samples (groups) > Kruskal-Wallis ANOVA & Median Test**.

To compare the means of two or more groups with censored observations using a non-parametric test in Statistica, use **Advanced Linear/Non-Linear Models** > **Survival Analysis** > **Comparing Multiple Groups**. The multiple-sample test implemented in Statistica is an extension (or generalization) of Gehan's generalized Wilcoxon test, Peto and Peto's generalized Wilcoxon test, and the log-rank test. To compare the means of two or more different groups with censored observations, use the **PROC LIFETEST** procedure with a **STRATA** statement, specifying **TEST=WILCOXON** for the Wilcoxon score test (two or more samples).

```
PROC LIFETEST data=<data-name>;
    TIME <response-variable>*<censoring variable>(censored-indicator);
    STRATA <grouping-variable> / TEST = (MODPETO);
```

Multiple Comparison Tests

Once the null hypothesis of an ANOVA for more than two groups has been rejected (or the means of three or more groups are unequal), multiple comparison tests may be used to determine which of the group means are different (Bowker and Lieberman 1972, Spiegel 1992).

The recommended MCT in SAS is the Ryan-Einot-Gabriel-Welsch multiple range test, specified in the **MEANS** statement as the **REGWQ** option.

```
PROC GLM data=<data-name>;
    CLASS <grouping-variable>;
    MODEL <dependent-variable>=<grouping-variable>;
    MEANS <grouping-variable> / REGWQ;
```

9.5 Correlation/Trend Analysis

A response variable (referred to as the “dependent” or “y-axis” variable) is the variable whose variation is being assessed. The explanatory variable (referred to as the “independent” or “x-axis” variable) is used to explain why and how the magnitude of the response variable changes (Helsel and Hirsch 1995).

Correlation

Correlation coefficients measure the strength of association between two continuous variables (Helsel and Hirsch 1995).

Kendall’s tau measures the strength of the monotonic relationship between x and y and is based on ranks making it not only resistant to outliers, but also useful when some of the data are censored (Helsel and Hirsch 1995). Kendall’s tau is related to the sign test, and the magnitude of differences between data pairs are not considered, only the (positive/negative) direction (Helsel and Hirsch 1995). For small sample sizes (n<10), exact test should be used to compute the significance of tau (Helsel and Hirsch 1995). Kendall's tau-b is an adjusted statistic useful when large numbers of ties occur. (Helsel 2005).

Spearman's rho is a non-parametric test based on ranks (Helsel and Hirsch 1995), similar to the signed-rank test in that the magnitude of the differences between data values are considered. Rho is essentially the linear correlation coefficient computed on the ranks of the data (Helsel and Hirsch 1995). Rho measures monotonic correlation (an example of positive monotonic correlation is that as X increases, Y consistently increases although the pattern may or may not be linear). Rho is valid for censored datasets when data are censored at a single detection limit (Helsel 2005). Whenever using Spearman's rho for small sample sizes (n<20), exact p-values should be used (Helsel and Hirsch 1995).

Pearson's r measures the strength of the linear association between two variables (Helsel and Hirsch 1995), and is a parametric test (Helsel 2005). Pearson's r is not resistant to outliers, as it involves the mean and standard deviation of both variables (Helsel and Hirsch 1995).

Neither Kendall's tau, Spearman's rho or Pearson's r will detect non-monotonic relationships.

Trend Analysis
For normally-distributed data with no censored observations in SAS, use the **PROC REG** procedure. In Statistica, use the **Multiple Regression** menu options.

For non-normally distributed data with no censored observations, rank the data and then input the ranks to a normal linear regression analysis.

PROC RANK data=<*data-name*> OUTPUT = <*output data-name*>;
PROC GLM data=<*ranked-data*>;
MODEL <*dependent-variable*>=<*independent-variable*>;

For non-parametric datasets with censored observations, use Cox's proportional hazards regression in SAS with the **PROC PHREG** procedure (Allison 1995). PHREG is designed for right censored data, so left-censored environmental data must be flipped by subtracting the observations from a number larger than the maximum observation. The sign of the output parameter estimate does not need to be adjusted. Although there is no direct measurement of the "slope" of the regression line, the hazard ratio can be used to estimate the percent change in the dependent variable per unit change in the independent variable (Allison 1995, see page 117). Additional explanatory variables, not of direct interest, that interact with time may be specified in the model statement or with the STRATA statement. The Efron (1977) method is the best approximation for tied values (Allison 1995).

PROC PHREG data = <*data-name*>;
MODEL <*dependent-variable*>*<*censoring-variable*>(*censoring-indicator*)=<*independent-variable*> / ties=efron;
STRATA <*additional-explanatory-variable*>;

9.7 Benthic Macroinvertebrate Metric Calculation

Standard benthic macroinvertebrates community indices are calculated programmatically by the FSDB staff using the WRE_Maint.BIO_METRICS PL/SQL package. Metric scores are stored in the FSDB under the associated field visit, with a lab of **None**, sample medium **Benthic Macroinvertebrate**, and sample type **Calculation**.

Unless specified in the QAPP for a special project, the metrics for a given field visit, excluding qa/qc samples like detritus checks, are calculated using all samples composited together to yield a single set of metrics. Metrics may be calculated at any time by request of field staff.

Table 9.7-1 Standard benthic macroinvertebrate community metrics calculated by WRE_Maint.BIO_METRICS package and stored in the FSDB

Metric Name	Calculation Method	Source Notes	Use*
# Organisms	Total number of benthic organisms in sample		
# Taxon	Number of unique benthic taxa	TCEQ 2005, Plafkin et al 1989[1]	Qual, Quant, EII
% Dominance (1 taxa)	Number of organisms of most numerous single taxon as percent of total number of organisms	Plafkin et al 1989, TCEQ 2005	Qual
% Dominance (3 taxa)	Number of organisms of top 3 most numerous taxa as percent of total number of organisms	TCEQ 2005	Quant, EII
% Dominant Guild	Number of organisms of most numerous feeding group as percent of total number of organisms	TCEQ 2005	Qual
HBI	Average PTI value of all organisms; organisms with no known PTI value in the FSDB are excluded from the calculation of the HBI	Hilsenhoff 1987	Qual, EII
# Diptera Taxa	Number of unique taxa in order Diptera	TCEQ 2005	Quant
# Ephemeroptera Taxa	Number of unique taxa in order Ephemeroptera	TCEQ 2005	Quant, EII
% EPT	Number of organisms in orders Ephemeroptera, Plecoptera and Trichoptera as percent of total organisms	TCEQ 2005	Quant, EII
# EPT Taxa	Number of unique taxa in orders Ephemeroptera, Plecoptera and Trichoptera	Plafkin et al 1989	Qual, EII
EPT/EPT+Chironomidae	Number of organisms in Ephemeroptera, Plecoptera and Trichoptera orders as percent of the sum of number of organisms in Ephemeroptera, Plecoptera Trichoptera orders and Family Chironomidae		
% Chironomidae	Number of organisms in Chironomidae family as percent of total organisms	TCEQ 2005	Qual, Quant, EII
% Elmidae	Number of organisms in Elmidae family as percent of total organisms	TCEQ 2005	Qual
# Non-Insect Taxa	Number of unique taxa not in Class Insecta	TCEQ 2005	Qual
% Collector-Gatherer	Number of organisms in CG feeding group as percent of total organisms	TCEQ 2005	Qual, Quant
% Predator	Number of organisms in Predator feeding group as percent of total organisms	TCEQ 2005	Qual, EII
% Filterer	Number of organisms in Filterer feeding group as percent of total organisms	TCEQ 2005	Quant
% Grazers	Number of organisms in Scraper feeding group as percent of total organisms	TCEQ 2005	Quant
% Trichoptera as Hydropsychidae	Number of organisms in Family Hydropsychidae as percent of total organisms in Order Trichoptera	TCEQ 2005	Qual
Ratio of Intolerant to Tolerant	Ratio of number of organisms with PTI value ≥ 6 to number of organisms with PTI < 6	TCEQ 2005	Qual
# Intolerant Taxa	Number of unique taxa with a PTI value ≤ 4	TCEQ 2005	Quant, EII
% Tolerant	Number of organisms with a PTI value ≥ 8.5 as percent of total number of organisms	TCEQ 2005	Quant
Quantitative ALU	TCEQ quantitative aquatic life use score	TCEQ 2005	
Qualitative ALU	TCEQ qualitative aquatic life use score	TCEQ 2005	

*Qual=Qualitative ALU (Harrison 1996), Quant=Quantitative ALU (Davis 1997), EII=Use in EII

1. Modified to include count of all taxa, not just at family level.

9.8 Diatom Metric Calculation

Standard diatom metrics (Table 9.8-1) are calculated programmatically by the FSDB staff using the WRE_Maint.BIO_METRICS PL/SQL package. Metric scores are stored in the FSDB under the associated field visit, with a lab of **None**, sample medium **Diatom**, and sample type **Calculation**.

Unless specified in the QAPP for a special project, the metrics for a given field sample are calculated individually (i.e., separately for samples and field replicates). Metrics may be calculated at any time by request of field staff.

Table 9.8-1 Standard diatom community metrics calculated by WRE_Maint.BIO_METRICS package and stored in the FSDB

Metric Name	Calculation	Use
# Organisms	Total number of diatom organisms in sample	
# Taxon	Total number of unique diatom taxa in sample	
PTI	Average pollution tolerance index value[1]	EII
Cymbella Richness	Number of taxa in the *Cymbella, Encyonema, Encyonemopsis*, and *Reimeria* genera	EII
% Motile Taxa	Number of organisms in *Navicula, Nitzschia, Surrirella, Craticula, Diadesmis, Luticola, Sellaphora, Hippodonta, Tryblionella, and Geisselaria* genera as percentage of the total number of organisms	EII
% Similarity to Reference	Community similarity index calculated using percentages of organisms in both reference and test samples. Reference conditions are generated from data collected at reference sites in biologically equivalent time periods.	EII
# Deformed Cells[2]	Number of deformed organisms	
# Deformed Taxa[2]	Number of taxa observed with deformed organisms	

1. See SR-02-02 for info on PTI value calculation
2. Metric calculated only if deformed cells tracked by diatomist.

9.9 Eutrophication Index Calculation

As part of the Austin Lake Index, an index of eutrophication is derived based on Chl *a* concentration and the relative abundances of Cyanobacteria, Green algae, Diatoms, and Chrysophytes. Percentages of Cyanobacteria and Green Algae typically increase with eutrophication whereas the percentage of Diatoms and Chrysophytes generally decreases with eutrophication. The eutrophication index is made of mean scores derived from Chl a, proportion of eutrophic algal indicators, and the proportion of "non-eutrophic" indicators.

Chl *a* concentration for a sampling year are converted to a quality (*q*) value (0–100) based on reservoir data collected between 2000–2010. The final Chl *a q*-score for each reservoir is defined as the mean of all *q*-values generated from samples collected over a single season.

The eutrophic phytoplankton score is derived from:

$$100 - \max(\text{percent of cyanobacteria, percent of green algae})$$

The sum proportion of cyanobacteria and green algae are subtracted from 100 to standardize with index score calculation in other systems. The final score is calculated from the mean of all scores generated from samples collected over the season.

The non-eutrophic phytoplankton score is derived from:

Max(percent of chrysophytes, percent of diatoms)

The final score is calculated from the mean of all scores generated from samples collected over the season.

A final grand mean is generated from the means of the q-score and phytoplankton indicator scores which represents the final reported Eutrophication Index score for a reservoir.

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