THE MEADOWS CENTER
FOR WATER AND THE ENVIRONMENT

TEXAS STATE UNIVERSITY

TEXAS STREAM TEAM

TEXAS STREAM TEAM
ESCHERICHIA COLI (*E. COLI*) BACTERIA
WATER QUALITY
COMMUNITY SCIENTIST MANUAL

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The Texas Stream Team encourages life-long learning about the environment and people's relationship to the environment through its multidisciplinary community science programs. We also provide hands-on opportunities for Texas State University students and inspire future careers and studies in natural resource related fields. Preparation of the Texas Stream Team *E. coli* Bacteria Water Quality Community Scientist Manual has provided Texas Stream Team with the chance to extend additional outreach and educational opportunities to Texans. Texas Stream Team values the staff contributions and recognizes each individual for their role.

The following staff and student workers assisted in the preparation of this report and are acknowledged for their contributions:

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1.0 INTRODUCTION

The Texas Stream Team *E. coli* Bacteria Water Quality Community Scientist Manual (*E. coli* Bacteria Manual) presents methods and procedures to become a certified Texas Stream Team *E. coli* Bacteria Water Quality Community Scientist. Certification enables community scientists to collect water quality data that meet the requirements of the Texas Commission on Environmental Quality approved [Texas Stream Team Quality Assurance Project Plan.]

Texas Stream Team has developed this community science program with input from the Environmental Protection Agency and the Texas Commission on Environmental Quality to address the following goals and benefits:

- Standardized training and quality assurance procedures help community scientists collect accurate, consistent information and improves data quality and integrity which can be used to make environmentally sound decisions.
- As recognized by the Environmental Protection Agency and Texas Commission on Environmental Quality, community scientists collect quality assured data that serve to supplement professionally collected data.
- Collection of quality assured data helps improve understanding of environmental issues and promotes communication and positive cooperation between Texans, professional monitors, and the regulated community.

Please note that to receive certification in the Texas Stream Team *E. coli* Bacteria Water Quality Community Scientist training, participants must be at least 14 years old (9^{th} grade), as well as Standard Core and/or Probe Core certified with at least six months of experience as an active core monitor.

The *E. coli* Bacteria Manual was developed to provide community scientists with clear instructions on how to collect *E. coli* bacteria water quality data and to educate community scientists about the importance of the monitoring they conduct.

This manual features information on procedures for collecting and/or analyzing *E. coli* bacteria. Texas Stream Team encourages new and veteran community scientists to develop a solid understanding of key concepts such as watersheds, stream order, and eutrophication. By raising awareness of nonpoint source pollution, Texas Stream Team educates community scientists on more effective strategies for protecting water resources and for resolving water quality problems that may originate at the community level.

1.1 WHAT IS TEXAS STREAM TEAM?

Texas Stream Team (formerly known as Texas Watch) is an environmental education and volunteer-based community scientist water quality monitoring program. Community scientists collect environmental and water quality information that may be used to promote and protect a healthy and safe environment for people and aquatic inhabitants. Texas Stream Team emphasizes communication about the environment, which is based on the premises that water issues are inextricably linked with air, biological, land, and human resource issues, and that the protection of the environment requires the active, positive, collaborative participation of all Texans.

Through Texas Stream Team, community members, students, educators, academic researchers, environmental professionals, and both public and private sector partners are brought together to conduct scientific research and to promote environmental stewardship.

Texas Stream Team encourages everyone to ask:

- What questions do we want to answer about the environment?
- What part of the environment are we most concerned with?
- What can I do to help preserve and protect the environment?

For those whose concerns are centered on water quality, Texas Stream Team helps design water quality monitoring programs to address specific concerns.

Recognizing the size and complexity of the water environment, the time and expense of monitoring water quality, and the significant role each one of us has in protecting Texas waters, the Texas Commission on Environmental Quality, the U.S. Environmental Protection Agency, and Texas State University have formed a cooperative partnership to support Texas Stream Team. Texas Stream Team is partially funded through an Environmental Protection Agency Nonpoint Source Pollution grant under Section 319 of the Federal Clean Water Act.

1.2 NONPOINT SOURCE POLLUTION

Getting to the Point

To a large extent, water quality within a watershed is linked to the actions of the people who live, work, and play within its boundaries. Water quality issues caused by human activities can be a result of either point source or nonpoint source pollution.

A point source is a single, identifiable source of pollution such as an end-of-pipe discharge from a municipal or industrial wastewater treatment plant. Point sources are regulated under the Federal Clean Water Act and Texas state law and are subject to permit requirements. These permits specify effluent limits, monitoring requirements, and enforcement mechanisms. Even though effluent discharges are permitted and regulated, point sources can still contribute to water quality degradation.

Nonpoint source pollution is pollution from sources which are diffuse and do not often have a single point of origin or are not introduced into a stream from a specific source. The pollutants are generally transported from the land by runoff. Nonpoint sources of pollution are largely unregulated and have not historically been evaluated in the same rigorous manner as point source pollution. Nonpoint source pollution originates from many different locations and sources. We have all seen trash in waterways following a rainfall event. Other contaminants, not so easily seen, enter our waters in much the same way.

Nonpoint source pollution occurs when rainfall runoff transports contaminants on the surface of the land into adjacent water bodies. Contaminated stormwater can cause impairment to the beneficial uses of streams, reservoirs, estuaries, and oceans. Pollutants carried by water percolating through the soil and entering aquifer recharge features can contaminate groundwater. Land management activities associated with agriculture, forestry, and residential and urban development can increase nonpoint source pollutants.

Nonpoint Source Pollution's Effects on Aquatic Ecosystems

Dissolved oxygen is a basic requirement for a healthy aquatic ecosystem. Most fish and insects breathe oxygen dissolved in the water. Some fish and aquatic organisms, such as gar and sludge worms, are adapted to low dissolved oxygen concentrations. However, most desirable fish species, such as largemouth bass and darters, become stressed if dissolved oxygen concentrations are below 4 milligrams per liter (mg/L). Insect larvae and juvenile fish are more sensitive and require even higher concentrations of dissolved oxygen to grow and reproduce.

Oxygen concentrations in the water column fluctuate under natural conditions, but depletion beyond normal fluctuations may be the result of human activities that introduce large quantities of biodegradable organic materials into surface waters. Biodegradable organic materials which include lawn clippings, raw and treated sewage, food processing wastes, rice field drainage, and pulp paper wastes, are some examples of oxygen depleting organic materials that enter surface waters. As these wastes decompose and break down into essential nutrient-enriched building blocks, many chemical and biological processes are directly affected.

Nutrients are fundamental building blocks for healthy aquatic communities, but excess nutrients (especially nitrogen and phosphorus compounds) may over stimulate the growth of aquatic plants and algae. Excessive growth of these plants, in turn, can clog waterways and interfere with boating and swimming. In addition, these plants will out-compete native submerged aquatic vegetation, and with excessive decomposition, lead to oxygen depletion or a condition called eutrophication. Oxygen concentrations often fluctuate widely, increasing during the day as aquatic plants conduct photosynthesis producing oxygen and falling at night as plants and animals respire, consuming oxygen.

Common Nonpoint Source Pollutants

Sediment from croplands, forestry activities, construction sites, and streambank erosion.

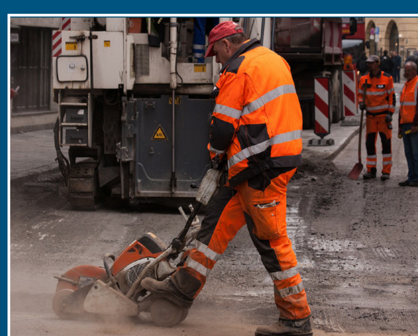
Nutrients from croplands, lawns and gardens, livestock operations, septic systems, and land waste application; sediments from erosion can reduce clarity and sun penetration in bodies of water, harming aquatic plant life and fish. Nutrients can also be carried by runoff from over-fertilized areas or decaying leaves and lawn clippings. Excessive nutrients in waterways can cause excess plant and algal growth, resulting in eutrophication (oxygen depletion) and fish kills.

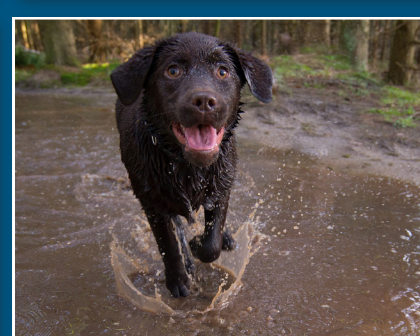
Bacteria from livestock, seepage from improperly maintained septic systems, leaking sewer lines, wildlife, and urban runoff.

Man-made chemicals, including pesticides from roadways, croplands, lawns, gardens, and forestry operations. Toxic materials, such as improperly applied pesticides or automotive products such as motor oil, engine degreasers, and antifreeze. These toxins can wash off from city streets and other areas or can result from illegal dumping.

Surface trash, such as plastic containers or cigarette butts; this trash is not only aesthetically unappealing, but residue from discarded containers can be washed into water bodies.

Fertilizers, malfunctioning septic systems, detergents and organic materials in treated sewage, and manure in agricultural runoff are examples of nutrient sources often responsible for water quality degradation. Rural areas are susceptible to groundwater contamination from nitrates found in fertilizer and manure. Nutrients are difficult to control because they typically recycle among the water column, algae, and bottom sediments. For example, algae may temporarily but significantly reduce phosphorus from the water column, but the nutrients will return to the water column and bottom sediments when the algae die and are decomposed by bacteria. Gradual inputs of nutrients tend to accumulate over time rather than leave the system.





Detecting and Tracking Nonpoint Source Pollution

Nonpoint source pollution is episodic. This means it typically enters our rivers and lakes during episodes of rainfall resulting in runoff, during isolated events such as incidences of illegal dumping, or in a random fashion, as when a sewer line overflows or breaks. It is difficult and expensive to monitor nonpoint source pollution using a fixed monitoring schedule and employing tests for only a few chemical variables. Analyzing data for trends and correlations over space and time provides an effective strategy to investigate nonpoint source pollution.

Conducting chemical and biological tests on water quality is like taking a snapshot of the river or lake at that moment in time. Trend analysis on water parameters measured provides clues to assess nonpoint source pollution. Analysis of chemical and biological test results over an extended period of time provides a foundation of background levels of dissolved oxygen values (oxygen concentrations will correspond to plant production and decomposition), rainfall contributions (streamflow values will change with runoff), and nutrient fluctuations (Secchi measurements can be used to determine the productivity status of a system, which is influenced by nutrient loading).

Living organisms in a stream or lake can provide information about what happened there over time. For example, monitoring a stream with healthy habitat and chemical water quality that meets local water quality standards, but no living organisms, indicates something may have happened there prior to sampling to account for the lack of living organisms. Perhaps a heavy rainstorm scoured the site and displaced the organisms. Perhaps some nonpoint source pollution lowered the dissolved oxygen concentration, causing the organisms to die or move downstream. There are many possible explanations, but by assessing the biological community of the stream over time alongside the water chemistry, the community scientist can learn more about the long-term conditions of the stream than if they performed only water chemical tests or field observations.

Water pollution from nonpoint sources is less obvious and more difficult to identify than from point sources and is not as easy to control through traditional management strategies. The variability of rainfall events and the complexity of the landscapes and geologic features lead to nonpoint source pollution phenomena which are highly variable and intricate. The lack of a single identifiable source of pollution makes it difficult to establish specific cause-and-effect relationships but reinforces the importance of analyzing trends and correlations drawn from consistent, long-term monitoring efforts.

Fecal Contamination and Water Quality

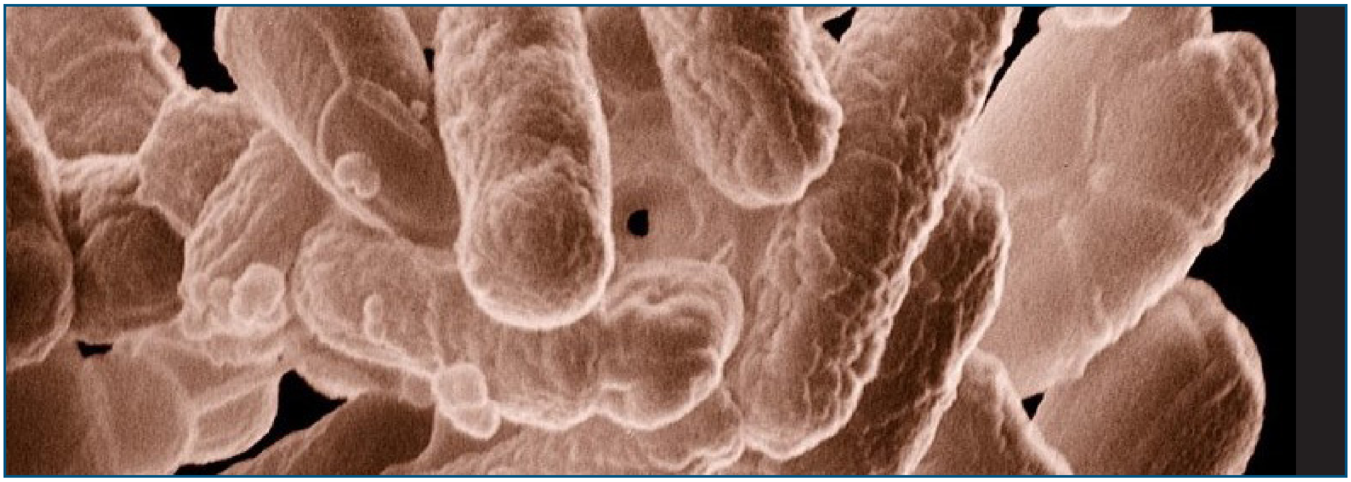
Fecal contamination in a water body is primarily measured to assess the potential health risks via primary or secondary contact recreation. Fecal bacteria enter the environment when they are expelled from the gut as fecal matter. Once in the environment, fecal bacteria enter a body of water either as nonpoint source pollution or point source pollution.

Fecal bacteria enter a water body in nonpoint source pollution when the source of the contamination comes from diffuse sources of runoff. For example, common nonpoint sources of fecal bacteria can include runoff from agricultural products (such as improperly managed manure), livestock and wildlife waste, and/or urban runoff containing pet waste or sewage. Fecal bacteria can also enter a waterbody in point source pollution, such as broken or malfunctioning sanitary sewer overflows (see Sanitary Sewer Overflows (SSOs) | National Pollutant Elimination System (NPDES) | US EPA) an illicit discharge from a specific source, or seepage from a broken or malfunctioning septic tank.

1.3 TEXAS STREAM TEAM *E. COLI* BACTERIA MONITORING

E. coli bacteria monitoring involves performing tests to determine their concentrations and to record core water quality parameters. These parameters are documented in a Group Monitoring Plan prepared by groups or organizations engaged in Texas Stream Team monitoring. A monitoring plan identifies the monitoring objectives and specifies the sites and parameters monitored and procedures to be implemented. A monitoring plan is unique to the conditions and needs of a site.

It is important to routinely collect *E. coli* bacteria water quality data because this information helps establish baseline conditions and identify abnormal environmental events when they occur. Baseline conditions represent background or normal environmental conditions for a waterbody, including an expected range of values for *E. coli* bacteria. An understanding of baseline conditions is established over time through substantial, routine observations. Once the background or normal environmental conditions of a water body are established, trend analyses can be performed. These analyses provide essential clues in assessing and managing nonpoint source pollution and protecting the health of Texans.



1.4 INTRODUCTION TO *E. COLI* BACTERIA

To determine the water quality of a body of water, water professionals perform tests for specific types of indicator organisms, whose presence signals that the water may have fecal contamination. Scientists test two general groups of fecal bacteria, coliform and Fecal streptococci bacteria (Environmental Protection Agency, 2012). Fecal streptococci are a group of bacteria found in the guts and feces of warm-blooded animals. Fecal streptococci have a comparatively strong resistance and can withstand adverse life conditions (Byappanahalli, 2012). Coliform bacteria are another type of bacteria found in the guts and feces of warm-blooded animals. Coliform bacteria can also be found naturally in the environment, such as in soil or wood (Environmental Protection Agency, 2012).

Not all fecal bacteria are harmful, however, certain strands such as *Escherichia coli* (*E. coli*) bacteria, can cause illness (most commonly gastroenteritis). While fecal bacteria in a water body might not be inherently harmful, their presence can indicate that pathogenic (disease-causing) microorganisms may be present (Environmental Protection Agency, 2012).

The fecal bacteria indicators that are most tested are total coliforms, fecal coliforms, *E. coli*, Fecal streptococci, and Enterococci. *E. coli* consists of a single species within the group of fecal coliforms, while all other fecal indicator bacteria consist of a variety of species that share common characteristics, such as behavior or habitat. For more information on the different types of bacteria commonly tested, visit the Environmental Protection Agency Water Monitoring & Assessment webpage (Environmental Protection Agency, 2012).

The Texas Commission on Environmental Quality sets statewide standards on acceptable levels of *E. coli* in freshwater, and Enterococci in saltwater bodies that are used for contact recreation activities. The Texas Commission on Environmental Quality categorizes contact recreation into two categories: primary and secondary. Primary contact recreation applies to situations where there is a higher risk of ingesting water (swimming, tubing, or surfing), while secondary contact recreation targets activities with a lower risk of ingesting water (fishing, kayaking, or wading in shallow waters) (Texas Commission on Environmental Quality, 2022).

The Texas Commission on Environmental Quality established a 10,000 uS/cm conductivity threshold for use of monitoring *E. coli* versus Enterococcus. If the conductivity measurement is <10,000 uS/cm, *E. coli* testing should be used as an indicator of contamination, but if >10,000 uS/cm Enterococcus testing should be used as an indicator of contamination for assessment of the contact recreation use. Information as to how the Texas Commission on Environmental Quality sets their standards and specifics regarding *E. coli* and Enterococci can be viewed at the Texas Administrative Code's directory (Office of the Secretary of State, 2022).

Padre Island National Seashore, Texas. Image acquired from the U.S. National Parks System.

Escherichia coli (E. coli)

Escherichia coli is a type of coliform bacteria. *E. coli* constitutes a diverse group of bacteria that live in the guts of people and warm-blooded animals. Most of the time, *E. coli* bacteria is harmless, and even provides essential functions to our digestive system and gut microbiota (Centers for Disease Control and Prevention, 2014).

Specific types of *E. coli* bacteria are considered pathogenic, meaning that they cause disease. Symptoms of illness caused by *E. coli* bacteria include vomiting, bloody diarrhea, and abdominal cramping (Byappanahalli, 2012). Typically, these infections will resolve on their own, however, in severe cases, they may require hospitalization. Pathogenic *E. coli* bacteria are usually introduced to the body by ingesting contaminated food or water, or by coming into contact with feces (Environmental Protection Agency, 2012).

Because specific strains of *E. coli* bacteria can cause illness, it is important to evaluate the *E. coli* bacteria content of a waterbody to establish the relative safety of contact recreation. The presence of *E. coli* bacteria indicates pathogens from waste may be reaching a body of water. Sources of *E. coli* bacteria include inadequately treated sewage, improperly managed animal waste from livestock, pets, aquatic birds and mammals, and/or failing septic systems.

Enterococci

Enterococcus is a genus of bacteria within the group of Fecal streptococci (Environmental Protection Agency, 2012). Enterococci live in the gut of humans and warm-blooded animals; however, these bacteria are more often associated with human fecal matter. For this reason, Enterococci are a relatively reliable indicator of potential human fecal contamination in a water body.

While Enterococci can also be found and monitored in freshwater, it is more commonly used as an indicator of water quality in coastal saltwater environments. This is because Enterococci can be differentiated from Fecal streptococci by its wide range of tolerable conditions. Enterococci can survive in higher salt concentrations, making it an ideal indicator bacterium in saltwater systems (Pepper et al., 2004).

Coliscan Easygel tests for *E. coli* in freshwater but can still indicate a level of contamination in saltwater, however there is currently no reliable and cost-effective method for monitoring Enterococcus that can be used by community scientists in saltwater.

The Environmental Protection Agency recommends collecting a combination of *E. coli* bacteria and Enterococci for water quality testing in recreational waters. In marine waters, only Enterococci is recommended. Currently, the Texas Stream Team only monitors *E. coli* bacteria in freshwater. Other methods are being explored to monitor enterococcus in saltwater.

1.5 GETTING STARTED WITH TEXAS STREAM TEAM

Please follow these steps to begin a monitoring project:

1. Schedule a training session(s) with a Texas Stream Team trainer in your area . All three training phases can be completed in one training session. If training phases are split up into multiple sessions, Training Phases I and II are generally scheduled with a group. After completing Phases I and II, Phase III can be scheduled at a later time to complete the *E. coli* bacteria training. Phase III will typically take place at the community scientist's monitoring site.
2. Select a monitoring site and request a site identification number based on the guidelines included in this manual under Section 2.1 - Choosing a Monitoring Location.

3. When establishing a monitoring group, complete a Texas Stream Team Group Monitoring Plan. The monitoring plan identifies the objectives of monitoring and specifies the sites and monitoring procedures. A copy of the Group Monitoring Plan and instructions can be obtained on the Texas Stream Team website.
4. Acquire monitoring supplies. Community scientists acquire supplies in a variety of ways. They may purchase supplies at their own expense or raise money from other sources such as a civic organization. Some Texas Stream Team partners provide supplies, and the Texas Stream Team office in San Marcos periodically has supplies to loan community scientists.
5. Begin monthly monitoring for at least one year. Record data on the *E. coli* Bacteria Environmental Monitoring Form, and send the data to your Texas Stream Team group Data Coordinator or to Texas Stream Team directly.
6. Contact Texas Stream Team for information on scheduling a training, completing a Group Monitoring Plan, acquiring monitoring supplies, or for any other questions.

Phone: (512) 245-1346
Email: TxStreamTeam@txstate.edu
Web: TexasStreamTeam.org

1.6 TRAININGS

Information describing the various levels of certifications and trainings offered by Texas Stream Team is provided here.

The longevity of the program is dependent upon the participation of our dedicated community scientists, and we encourage you to continue increasing your level of involvement by completing the required training to become a certified Texas Stream Team E.coli Bacteria Trainer/ Quality Assurance Officer, or a Texas Stream Team Advanced Water Quality Community Scientist.

Texas Stream Team Community Scientist Trainings

Texas Stream Team offers additional water quality and environmental monitoring trainings, including:

- Standard Core Water Quality Community Scientist Training
- Probe Core Water Quality Community Scientist Training
- *E. coli* Bacteria Water Quality Community Scientist Training
- Advanced Water Quality Community Scientist Training
- Macroinvertebrate Bioassessment Community Scientist Training
- Riparian Evaluation Community Scientist Training

This manual only includes information for the *E. coli* Bacteria Water Quality Community Scientist Training. Visit the Texas Stream Team Trainings and Programs page to learn more about other trainings offered by Texas Stream Team.

E. coli Bacteria Water Quality Monitoring Community Scientist Training

To receive certification as a Texas Stream Team *E. coli* Bacteria community scientist, the three-phase training program described on the following pages must be completed.

Each trainee is required to fill out the online Training Enrollment Form to become a certified Texas Stream Team *E. coli* Bacteria community scientist and begin monitoring activities.

PREREQUISITE

Prior to receiving certification for *E. coli* bacteria monitoring, participants must:

- Be at least 14 years-of age (9^{th} grade),
- Have completed and received certification in the Standard Core Water Quality Community Scientist Training or the Probe Core Water Quality Community Scientist Training, and
- Have completed at least six months of Core monitoring at an established monitoring site and submitted the corresponding data to Texas Stream Team.

PHASE I TRAINING

Phase I begins with an instructional classroom session, either in-person or virtually online, covering an introduction to Texas Stream Team, *E. coli* bacteria monitoring, and covering topics such as bacteria sources, standards, screening levels, and relevant natural processes.

Phase I training transitions to an interactive demonstration of monitoring procedures. A Texas Stream Team certified trainer explains how the monitoring equipment is used. The trainer demonstrates the *E. coli* bacteria monitoring procedures while trainees follow along with the demonstration. The trainees perform the *E. coli* bacteria water quality tests simultaneously and under close supervision of the trainer. Trainees record their results in the *E. coli* Bacteria Training Participant Packet on the Phase I Monitoring Form.

After all parameters are evaluated and the trainees are comfortable with the *E. coli* bacteria procedures, the trainee and trainer review the Phase I Monitoring Form. This form signifies the trainee's successful completion of Phase I for the parameters specified and indicates their understanding of the monitoring procedures and commitment to following all procedures.

PHASE II TRAINING

During Phase II, trainees demonstrate the monitoring procedures they learned during Phase I in the field with the assistance of the trainer. Whenever possible, the water body used for Phase II should be similar to the sites the trainees will eventually monitor as community scientists.

Trainees conduct *E. coli* bacteria water quality monitoring procedures on their own, with assistance from a trainer whenever necessary. The trainer observes the trainees' procedures, answering questions the trainees may have and assisting with data quality assurance.

After all questions have been answered and the trainee completes the Phase II Monitoring Form, the trainee and the trainer discuss the trainee's strong and weak points with respect to the monitoring procedures. The Phase II Monitoring Form is retained by the trainee for reference during Phase III.

PHASE III TRAINING

Phase III training can take place at the same time and location as Phases I and II, or it can take place at the trainee's approved monitoring site within three months of completing Phase II. The trainer observes the trainee as they conduct the monitoring at the site. The trainee should be able to work through the monitoring procedures and complete the Phase III Monitoring Form without the assistance of the trainer. After the trainee completes the training, the trainer discusses next steps for how to set up a monitoring site. The trainee then completes the online Measures of Success Survey.

Once Phase III has been completed, community scientists are required to attend one quality control field audit session every two years to comply with the Texas Stream Team Quality Assurance Project Plan. During Phase III, the trainer will conduct the trainees' first field audit session. The field audit session will include a detailed observation of the trainee's techniques to ensure monitoring is conducted following the Texas Stream Team protocol as described in this manual without the assistance of the trainer. A field audit session checklist is available on the monitoring form and will be used to document the session and to ensure monitoring protocols are adhered to by all trainees.

Texas Stream Team Trainer

E. coli bacteria community scientists may receive additional certification as a Texas Stream Team *E. coli* Bacteria Water Quality Trainer after completing the requirements described below.

- **Phase I -** Trainer trainee must be a certified Texas Stream Team *E. coli* Bacteria community scientist who has been actively monitoring for at least one year.
- **Phase II -** Trainee assists a certified trainer in planning, coordinating, and presenting at one community scientist training session.

- **Phase III –** Trainer trainee plans, coordinates, and presents all phases of one community scientist training assisted by a certified Texas Stream Team trainer.
- **Phase IV –** Trainer trainee submits the completed Trainer Enrollment Form to Texas Stream Team. If approved, the newly certified trainer will receive a certificate as a certified Texas Stream Team trainer.

A certified trainer may ask the trainer trainee to repeat any of the above phases if the trainer feels the trainer trainee is not ready to become a certified trainer. Certified trainers have authority to override prerequisites if the trainer trainee successfully completes all phases up to the certified trainer(s) standards.

The following are requirements to maintain active trainer status through the Texas Stream Team program:

- The trainer must participate in and/or lead at least one community scientist training session per year.
- The Trainer must attend the Annual Texas Stream Team Trainer meeting or have an alternate attend in their place.
- The Trainer should submit scheduled trainings to the Texas Steam Team online calendar.
- A Training Sign-In Sheet should be submitted to TxStreamTeam@txstate.edu after each training.
- The Trainer must complete a field audit session every two years. The field audit is designed to detect and correct discrepancies in monitoring techniques. This requirement is necessary to ensure data quality and comparability among community scientists statewide.

Note that if the requirements described above are not met, the trainer may have to repeat Phase III.

Texas Stream Team Quality Assurance Officer Training

TO PERFORM FIELD AUDIT SESSIONS:

Certified trainers concurrently become certified Quality Assurance Officers upon completion of the trainer certification. Community scientists can be certified to perform field audit sessions as a Texas Stream Team Quality Assurance Officer. Community scientists must observe a field audit session performed by a certified Quality Assurance Officer, then lead a field audit session with a Quality Assurance Officer present. Quality Assurance Officers must undergo field audit sessions every two years to maintain certification.

Certification

Upon completion of training phases I, II, and III, the trainee must complete the online Training Enrollment Form before a certificate of completion can be issued. The trainer will submit completed forms to the Texas Stream Team to create and distribute the certificates. The certification process serves as the record to document completion of the training and the first field audit session; therefore, it is critical that a legible form is submitted. If the trainer does not receive the completed form, a certificate of completion will not be generated and sent to the trainee. Texas Stream Team distributes certificates at the beginning of each month.

1.7 QUALITY ASSURANCE

Texas Stream Team data is collected under a Texas Commission on Environmental Quality approved Quality Assurance Project Plan. Quality assurance consists of community scientist activities that involve planning, implementation, documentation, assessment, reporting, and quality improvement to ensure that Texas Stream Team community scientist data are of the type and quality needed and expected by the agencies that provide financial support for the program, the Texas Commission on Environmental Quality and Environmental Protection Agency.

The approved Quality Assurance Project Plan documents the procedures Texas Stream Team community scientists implement to ensure that the resulting data are of high quality and meet project data quality objectives. The Quality Assurance Project Plan also ensures community scientists statewide use the same methods for all parameters measured including *E. coli* bacteria to ensure comparable results. For these reasons, it is critical all community scientists are aware of the Quality Assurance Project Plan requirements and implement the procedures as stated in the approved document.

Quality Control

Quality Control consists of the overall system of community scientist activities and compares the Texas Stream Team *E. coli* bacteria water quality data against defined standards to verify that they meet the stated requirements approved by the Texas Commission on Environmental Quality and Environmental Protection Agency.

Quality control measures implemented by Texas Stream Team *E. coli* bacteria community scientists include:

- **Buddy system:** We strongly recommend community scientists always monitor with another person for safety purposes, but also to confirm observations by conducting duplicate visual evaluations of measurements collected.
- **Field Audit Sessions:** Once trained, community scientists must attend one field audit session every two years. A field audit session includes observation of a community scientist conducting a monitoring event by either a Texas Stream Team Trainer, Quality Assurance Officer, or Texas Stream Team staff member. Observations are documented on a Field Audit Session Checklist and all discrepancies are discussed with the community scientist upon completion of the session. A training video describing the field audit procedure can be found here.
- **Cross Contamination:** Community scientists are trained to avoid contaminating sample containers, hands, tabletops, or any other surface or object. Always wear gloves or use hand sanitizer throughout procedure to prevent cross contamination from hands. Sterile petri dishes and pipettes are used for each sample volume to avoid cross contamination.

- **Sample Duplicate**: One water sample is collected but two duplicates are plated on separate petri dishes. The resulting bacteria counts from the duplicates are reported separately on the monitoring form then averaged. Duplicate samples are used to evaluate analytical or measurement precision, or how close the sample results are to each other.

- **Field Blank:** Field blanks are used to assess potential contamination from sample handling, airborne materials, equipment, media, and other sources. A field blank consists of a sterile diluent sample of 1 mL that is transported to the site and poured into a properly labeled Whirl-Pak® bag. If monitoring at more than one site, the field blank should be analyzed at a different site each sampling event to prevent bias. The blank sample is collected in the same type of container, labeled as a field blank, and handled and analyzed in the same manner as the bacteria sample(s) collected that day. It is used to detect errors or contamination in sample collection and analysis. The frequency of a bacteria field blank is one with every 10 samples.

- **Quality Control Sessions:** Once trained, community scientists must attend one quality control session every two years. A Texas Stream Team Trainer, Quality Assurance Officer, or Texas Stream Team staff conducts Quality Control Sessions by observing community scientists as they conduct a monitoring event. Community scientists document observations on the Monitoring Form making sure results meet the data quality objectives.

- **Data Management:** Community scientists implement quality assurance procedures and checklists before entering data to the Dataviewer. See Section 3.1 - Data Management for additional information.

2.0 SAFETY CONSIDERATIONS

General Precautions

1. Read all instructions to familiarize yourself with the monitoring procedures before you begin. Note any precautions in the instructions.
2. Always wear gloves or use hand sanitizer prior to collecting bacteriological water sample(s).
3. Never monitor in unsafe, hazardous weather conditions. If you suspect hazardous weather conditions, do not attempt to travel to your monitoring location. Reschedule for a later time.
4. Follow the advice of all local, regional, state, and national weather agencies when it comes to your safety.
5. Follow all local, regional, state, and national laws while conducting your *E. coli* bacteria monitoring.
6. If you need to access private property to conduct *E.coli* bacteria monitoring, the Private Property Access Form must be submitted to Texas Stream Team PRIOR to accessing private property.
7. Keep all equipment and supplies out of the reach of young children.
8. In the event of an accident or suspected poisoning, immediately call the Poison Control Center at (800) 222-1222. Be prepared to provide information about the supplies ingested or exposed to.
9. Texas Stream Team strongly suggests that you always implement the buddy system and monitor with another person for safety purposes.
10. Always wash hands and clean all surfaces before and after each sampling event.

Protecting Yourself and Your Equipment

1. Avoid contact with skin, eyes, nose, mouth, and clothes while sampling.
2. Always wear safety goggles or glasses and rubber gloves.
3. Use the bottle caps, not your fingers, to cover bottles when mixing.
4. Wipe up any spills, liquid, or powder as soon as they occur. Clean area with a wet sponge, then dry.
5. Store supplies and equipment indoors at room temperature or as directed by the manufacturer. Do not expose supplies and equipment to direct sunlight for long periods of time and protect them from extremely high or low temperatures. Avoid storing equipment in an automobile.
6. Place empty bottles of media in the household recycling bin.
7. Dispose of used Petri dishes by lifting the lid and pouring 5 mL (about 1 teaspoon) of bleach, vinegar, or isopropyl alcohol into each dish. Place Petri dishes in a plastic bag and dispose in household trash.

Site Safety

1. Park your vehicle safely away from roads and out of the way of traffic. Be cautious of traffic when unloading or loading monitoring equipment and accessing your site.
2. If necessary, sample your site from bridges with pedestrian walkways, from docks, or from stream banks. If you must enter the water, always have a buddy or partner on the shore nearby and always wear a life jacket or U.S. Coast Guard approved personal floatation device if wading is necessary.
3. Approach your site carefully! Look out for traffic on bridges and when crossing roads. Be on the lookout for snakes, fire ants, wasps, poison ivy, Africanized honeybees, wild animals, broken bottles, or debris.
4. If using a boat or kayak to sample your site, learn and observe all U.S. Coast Guard and State of Texas regulations.

2.1 CHOOSING A MONITORING LOCATION

Historical water quality data is useful in assessing water quality. Therefore, it is preferable for community scientists conducting the *E. coli* bacteria monitoring to use existing monitoring sites with historical water quality data when possible. The Datamap can assist in determining if an established site is available in the community scientist's area of interest.

Activating an Inactive Site

Rather than establishing a new monitoring site, community scientists have the option of reactivating an inactive site. Inactive sites with historical data are useful for analyzing water quality and data trends. This information can then be used by water and resource professionals to make informed decisions about the management of a water body.

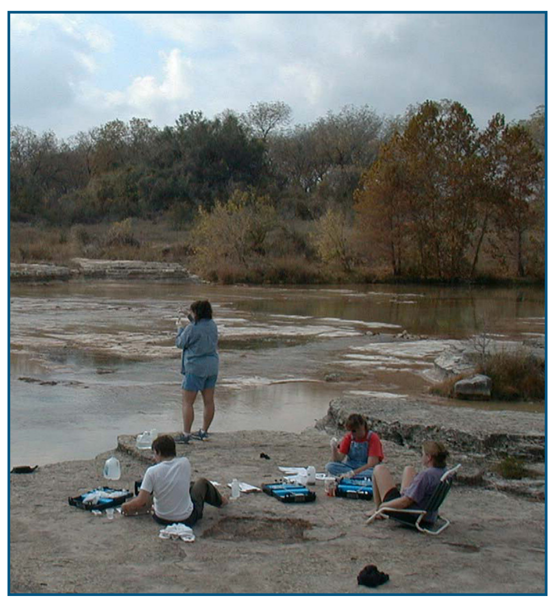
Due to these advantages, Texas Stream Team encourages community scientists to reactivate historic sites, prior to the creating a new site. Community scientists can view current and historic sites by accessing the Datamap, which can be used to identify an inactive site that appeals to you.

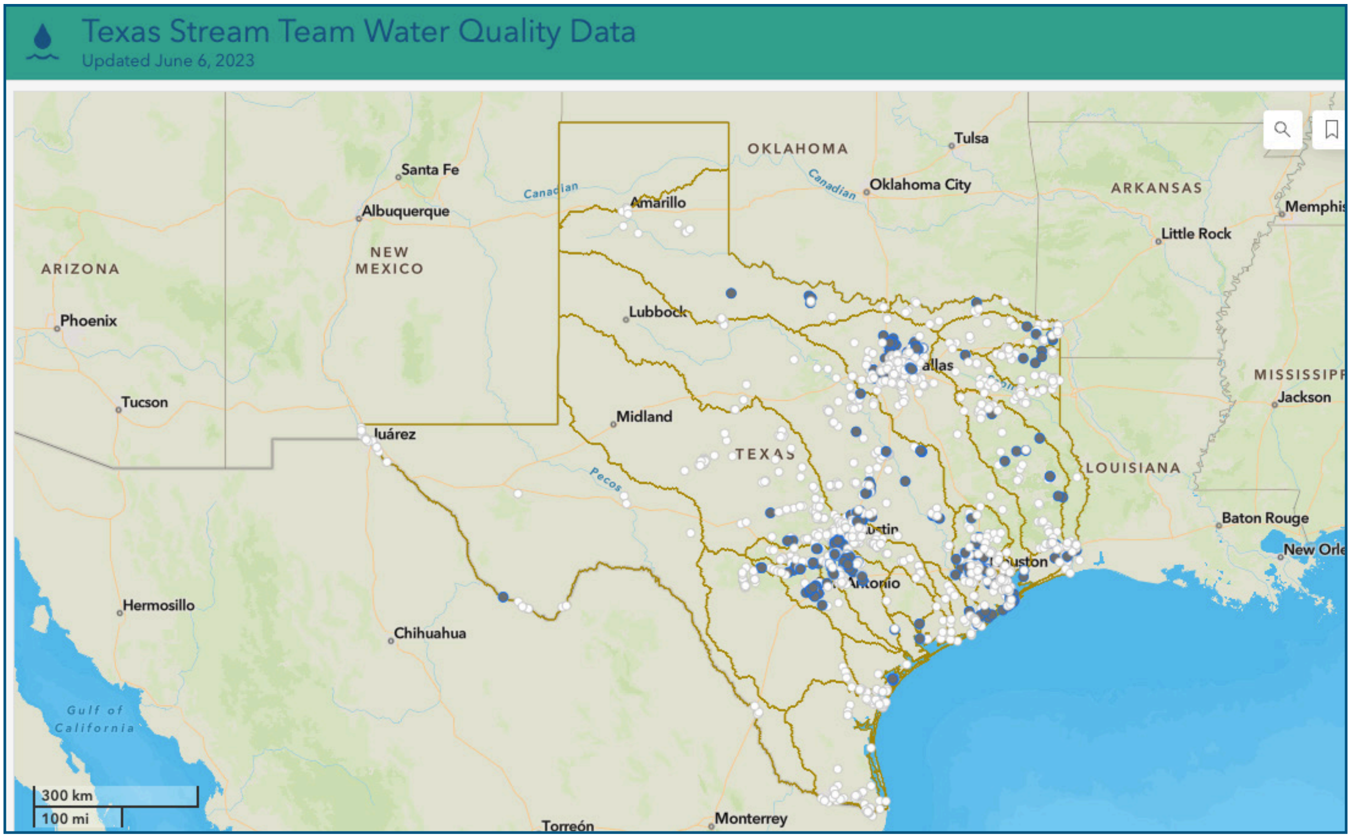
Creating a New Site

To create a new monitoring site, community scientists should submit an online New Monitoring Site Request Form to Texas Stream Team. Prior to completing the form, community scientists should review the Site Selection Guide. The Site Selection Guide lists the necessary qualifications a site must meet to be approved as a Texas Stream Team monitoring site. These qualifications must be met at every monitoring site to increase the comparability of the data collected and ensure the safety of the community scientist.

If the site is on private property, obtain the landowner's written permission granting access prior to monitoring the site. A Private Property Access form must be obtained and submitted to the Texas Stream Team for review before any Texas Stream Team monitoring can take place.

Once the Site Selection Guide has been reviewed and the new site has been confirmed, the New Monitoring Site Request Form is submitted with the new site information. Please note that data cannot be entered in the Waterways





Dataviewer without a site identification number that includes latitude and longitude coordinates and a short description. To determine the exact location of your site, use Google Maps, a U.S. Geological Survey topographic map (scale of 1:24000), a National Oceanic and Atmospheric Administration nautical chart, one of the several Street Atlas software systems that provide coordinates or street address, or a calibrated global positioning system (GPS) unit.

Following submission of the New Monitoring Site Request Form, Texas Stream Team staff will review the form and send an email with the site identification number once the site has been created. The site identification number will link the data collected and submitted to that specific site.

Something to consider when selecting a monitoring site: If your sample site is downstream of a wastewater treatment plant outfall, the effluent might contain chlorine disinfectant that could debilitate bacteria. At these sites, Texas Stream Team recommends community scientists use the Whirl-Pak® bag that contains 10 mg tablets of sodium thiosulphate to neutralize free chlorine in the sample.

2.2 CHOOSING A MONITORING TIME

E. coli bacteria monitoring should be conducted the same day as your Core monitoring. *E. coli* bacteria samples should be collected once a month and at regular intervals.

For example, if sampling is conducted monthly, try to sample every 30 days. If necessary, sampling can take place as early as 26 days after the last sampling event or as late as 34 days after the last sampling event.

Water quality and environmental conditions can change throughout the day, therefore monitoring at the same time and location helps to ensure the data collected on different sampling days using the same protocols are comparable. If you have questions about whether to cancel, postpone, sample early, or change your sampling location, call, or email your Training Coordinator or Texas Stream Team staff.

SAFETY CHECK: If conditions are not safe, do not sample.

2.3 EQUIPMENT AND SUPPLY LIST

E. coli Bacteria Water Quality Monitoring Equipment

E. coli bacteria monitoring involves performing tests for *E. coli* bacteria in a water body using Texas Stream Team approved *E. coli* bacteria monitoring supplies and equipment.

E. COLI BACTERIA MONITORING SUPPLIES

The supplies listed below are necessary for starting an *E. coli* bacteria monitoring program.

- Goggles
- Gloves – S/M/L
- Whirl-Pak® Bags
- 100 mL DI Water Bottle
- Petri dishes & Coliscan Easygel
- 5mL Graduated Sterile Pipettes
- Incubator
- MacroLens*
- Extension Pole**
- Ziploc Bags, Gallon
- Vinegar

*Community scientists can choose to use the MacroLens or a standard magnifying glass depending on their personal preference

**Not required but useful

E. COLI BACTERIA MONITORING REPLACEMENT REAGENTS

- 100 mL DI Water Bottle
- Petri Dishes & Coliscan Easygel

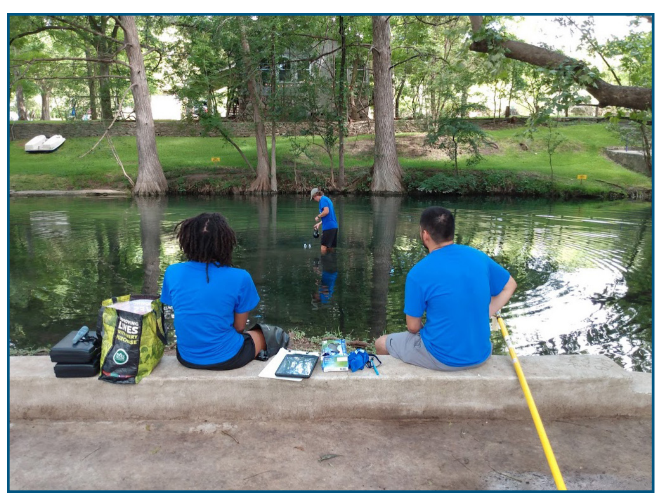
E. COLI BACTERIA MONITORING REPLACEMENT EQUIPMENT AND SUPPLIES

- Whirl-Pak® Bags
- 5mL graduated sterile pipettes
- Petri dishes
- Incubator
- MacroLens
- Magnifying Glass

Vendor and pricing information for ordering supplies and equipment can be found on the Texas Stream Team website. All items must be inspected upon receipt from the manufacturer and prior to each sampling event to ensure items have not exceeded expiration dates. Items should be checked for completeness, breakage, and to ensure they are operating properly.

Monitoring Reagents

Texas Stream Team requires all expiration dates be inspected before each monitoring event. When Coliscan Easygel are received, the production date (if known) or arrival date and the expiration date is provided on the box of media and Petri dishes. Media bottles should be kept frozen until they are ready to use and have a shelf life of up to one year. Thawed media is usable for up to two weeks when stored in a refrigerator. Medium can be refrozen, but repeated freezing and thawing should be avoided and is not recommended. Coliscan media should be at room temperature prior to use. Pre-treated Petri dishes should be stored at room temperature, out of direct light and in a closed bag or container to protect them from drying out and any contamination. Petri dishes also have a shelf life of up to one year. Divergence from any of these directions will compromise the quality and stability of reagents and shorten their shelf lives.



2.4 SAMPLING SEQUENCE

A typical monitoring sequence for a certified community scientist includes the following steps:

1. Print the Monitoring Form or enter your data directly into the Dataviewer using your account credentials.
2. Before sampling, review the field audit checklist on the second page of the Monitoring Form. Use this list as a guideline throughout the sampling event to ensure all protocols are adhered to.
3. Check reagent expiration dates.
4. Collect the *E. coli* bacteria water sample after completing Core monitoring.
5. At the monitoring site, document field observations on the Monitoring Form about the condition of the water, weather, and other pertinent facts. The following can be including in the Comments section:
    - Number of recreational swimmers, fishers, boaters, etc.
    - Any unusual water conditions, such as color or smell
    - The presence or evidence of wildlife
    - The presence of litter or trash
6. Collect the water sample.
7. If transporting the water sample to another location, immediately place the sample on ice in a portable cooler. If transportation is not necessary, be sure to store your water sample in the shade out of direct sunlight.
8. Use the sterilized pipette to draw the sample volume used to inoculate the media.
9. Once the media has been inoculated with sample water, plate the sample-media solution on petri dish on a level surface and allow to set.
10. Place the plated sample in the incubator for a minimum of 28 hours but not to exceed 31 hours.
11. After the sample has been incubated, remove the Petri dish lid, and count the number of *E. coli* bacteria colonies. Record the colony counts on the Monitoring Form.
12. Clean and store equipment. Dispose of plated samples using procedures discussed in Section 3.0 – Follow-Up and Clean-Up.
13. Legibly record all applicable data on the Monitoring Form. Remember to double-check for accuracy and readability.
14. Review and check-off the field quality control checklist on the second page of the Monitoring Form.
15. Make sure the Monitoring Form is completed, then sign and date.
16. Submit the form to your local Texas Stream Team Data Coordinator, or to Texas Stream Team by emailing a pdf or high-quality image to TxStreamTeam@txstate.edu.

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2.5 MONITORING PROCEDURES

Texas Stream Team community scientists perform *E. coli* bacteria monitoring on lakes, rivers, streams, and estuaries. The primary reason for *E. coli* bacteria testing is the determination of baseline conditions.

Coliscan Easygel is a method used by Texas Stream Team to test for *E. coli* bacteria and general coliform bacteria. Easygel is a pectin-gel that comes in a sterilized, two-piece unit, including a bottle of liquid medium and a Petri dish treated with a special formulation. In December 1999, the Environmental Protection Agency Region 4 approved Coliscan Easygel for use in bacteriological monitoring of surface waters as part of the program developed by the Alabama Water Watch under the direction of Dr. William G. Deutsch of the Department of Fisheries of Auburn University. As a result of this program and other studies, Coliscan Easygel has become the preferred method for bacteriological monitoring in community scientist water quality programs throughout the United States.

Accuracy of Coliscan Easygel is based on the reasonable performance of properly stored, pre-treated sterile plates, media, and pipettes. Extensive evaluation of the Coliscan Easygel method was conducted by Alabama Water Watch, Alabama Department of Fisheries, and Auburn University from February to September 1998 to confirm the accuracy of the method. The results indicated the Coliscan Easygel is a reliable and valid tool for the detection of fecal contamination through a variety of concentrations.

Field Observations

Conducting field observations adds important background information to your *E. coli* bacteria water quality monitoring results. This background information allows watershed managers and users of the data to better understand and predict trends in *E. coli* bacteria water quality data. For this reason, it is important to adhere to the procedures described in the Core Water Quality Community Scientist Manual to collect and report field observation data on the Monitoring Form.

If you have any questions regarding field observation procedures, please contact a Texas Stream Team Trainer and/or Data Coordinator, or reference Section 2.6 – Monitoring Procedures of the Core Water Quality Community Scientist Manual.

Comments Measurement

Record any explanatory information about the *E. coli* bacteria measurements in the Comments section. For example, if you needed to transfer the sample you can document it here. This is also the best place to describe:

- The biological conditions such as a plankton bloom, fish kill, presence and abundance of fish, aquatic insects, aquatic plants, and wildlife.
- The lake and stream use(s) like swimming, wading, boating, fishing, irrigation pumps, or navigation.
- In stream or drainage basin activities and events that are impacting water quality – bridge construction, soil washouts, herbicide or pesticide use, livestock watering, dredging, or changes in stream bottom.
- Type of floating debris found at the site.

Sample Collection

Always wear gloves or use hand sanitizer prior to collecting bacteriological water samples to prevent contamination.

> **Note:** If the sample site is downstream of a wastewater treatment plant outfall, the effluent might contain chlorine disinfectant that could debilitate bacteria. At these sites, Texas Stream Team recommends community scientists use the Whirl-Pak® bag that contains 10 mg tablets of sodium thiosulphate to neutralize free chlorine in the sample.

STEP 1: Before collecting the sample, label each Whirl-Pak® bag with the site ID, site description, date, and time collected. If it is appropriate to process a field blank sample, the Whirl-Pak® bag will have the previously mentioned information plus a "field blank" label. Refer to the Field Blank section to see if a field blank is necessary.

STEP 2: If conducting a field blank, prepare the field blank Whirl-Pak® bag first before collecting the water sample. Transfer roughly 1 mL of the sterile diluent from its original container to the field blank Whirl-Pak® bag while at the monitoring site. This will serve as the "field blank."

STEP 3: Collect bacteriological samples in sterile Whirl-Pak® bags. *Never pre-rinse the bacteriological sample container because it is sterilized.*

When collecting samples from a water body, drop the Whirl-Pak® bag to a depth of 0.3 m (1 ft), or roughly half the depth, in very shallow streams. Avoid contact with sediment to prevent contamination. With the open end facing upstream, push the opened mouth of the Whirl-Pak® bag upstream at this depth until full.

When collecting samples from a bucket of water, collect the bacteria sample before other monitoring activities occur. Before collecting the sample, rinse the bucket twice and discard the water downstream of the sample site. Pour water from the bucket into the Whirl-Pak® bag. Never dip the Whirl-Pak® bag into the bucket. This could introduce contamination.

STEP 4: Squeeze out the top 1 inch of water from the Whirl-Pak® bag and whirl the bag away from you to seal. The sealed bag must retain at least 50 mL of sample water but leave 1 inch of airspace to help mix the sample when it is inverted just before making dilutions.

STEP 5: Place sample(s) on ice immediately after collection. Bacteriological samples must be transported, processed (diluted and plated), and placed in an incubator within **8 hours** of sample collection. Do not report sample results that are not processed within the time limit or holding time.

FIELD BLANK

The frequency of a bacteria field blank is 1 with every 10 samples. If less than 10 bacteria samples are collected in a month, include at least 1 field blank per month. Follow routine handling, plating, and analysis procedures, and report the results on your Monitoring Form. There should be no *E. coli* bacteria colony growth on the field blank samples. If *E. coli* bacteria growth occurs, discard all data collected that day. Document the results on the Monitoring Form and consult your trainer.

Analyze *E. coli* Bacteria with Coliscan Easygel

DETERMINING SAMPLE SIZE

One water sample from each monitoring site is collected, however two replicates are analyzed, and a mean value is reported. To check for potential contamination issues, community scientists are required to conduct a field blank for quality control analysis for every ten samples tested, or at least once per month during routine *E. coli* bacteria monitoring procedures.

Community scientists typically use a 1 mL sample to inoculate the Coliscan Easygel media, but sample volumes may range in size from .5 mL to 5 mL. The Coliscan Easygel test can detect as little as one bacterial colony per sample and can be used to identify up to 200 colonies/sample. Concentrations exceeding 200 colonies/sample are recorded as too numerous to count. A black and white grid, which is the same size as the Petri dish, is provided to assist community scientists in counting *E. coli* bacteria colonies.

The ideal number of colonies resulting from a single prepared plate is 20 to 60, and not over 200. Since the number of resulting colonies is dependent on the sample size, it may be necessary to experiment with different sample volumes to determine the best sample size to use to achieve 20 to 60 colonies.

STEP 1: To establish a baseline for typical conditions, collect a 1 mL and a 5 mL sample volume during the first sampling event.

STEP 2: If the 1mL sample volume results in *E. coli* bacteria colony counts of 0 or only a few colonies, the sample volume should be increased to 3 mL or 5 mL. Conversely, if the 5 mL sample results in >60 colonies, the sample size should be decreased to 1 mL or 3 mL during the next sampling event.

> **Note:** Environmental and precipitation variables will influence levels of bacteria. We suggest you experiment with the sample volume used to inoculate the Coliscan Easygel media to capture the optimal colony count. Begin with a 1 mL and 3 mL sample volume then adjust accordingly. Keep in mind that pristine waters may require a 5 mL sample to achieve the preferred range of colonies.

PREPARATION

> **Note:** Coliscan Easygel should be removed from the freezer in time to ensure it has reached room temperature (typically 2-3 hours) before use. You can also place it in water to help thaw.

STEP 1: Prepare a minimum of 2 Petri dishes per sample/site, in addition to the field blank, if necessary.

STEP 2: Label the top of each Petri dish (the side without the treated film) with the station ID, site description, date, and the sample volume (1, 3, or 5 mL). If conducting a field blank, write field blank on the Petri dish as well.

PREPARING THE SAMPLE

STEP 1: Invert the Whirl-Pak® bag with the sample water a few times, avoid touching the lip of the bag when opening the bag.

STEP 2: Unwrap the pipette from the bulb end when ready to draw the sample. Avoid contact of the tip with anything except the sample water.

STEP 4: Submerge the bottom half of the pipette into the Whirl-Pak® bag and squeeze the bulb to expel the air. Draw the appropriate sample water volume (1, 3, or 5 mL) into the pipette by releasing the bulb slowly. Squeeze out any excess of the desired volume from the pipette.

STEP 5: Place the designated sample water volume from the pipette into the Easygel media bottle, cap, and swirl gently. Repeat steps 1-5 for second replicate.

STEP 6: If a field blank is necessary, withdraw 1 mL of sample water from the Whirl-Pak® bag labeled field blank and place in the Easygel media bottle, cap, and swirl gently.

STEP 7: Your samples are now prepared. Record the sample sizes on the Monitoring Form.

> **Note:** Once mixed with Easygel media, the prepared samples should either be plated within 10 minutes, kept on ice, or placed in a refrigerator and plated as soon as possible.

PLATING THE SAMPLE

STEP 1: Pour the prepared sample slowly into the bottom of the Petri dish (the side with the treated film).

STEP 2: Gently swirl until there is a smooth coating of the prepared sample across the bottom of the Petri dish (be careful not to splash over the side or on the lid).

STEP 3: Set on a level surface and allow between 5-45 minutes for the media to gel. This will help ensure that the sample will spread uniformly across the Petri dish and help prevent shifting or pooling of the media after placing in the incubator.

INCUBATION

STEP 1: Turn on the incubator far enough in advance to ensure a steady incubation temperature of 33° C +/- 3°C is reached before placing Petri dishes inside the incubator.

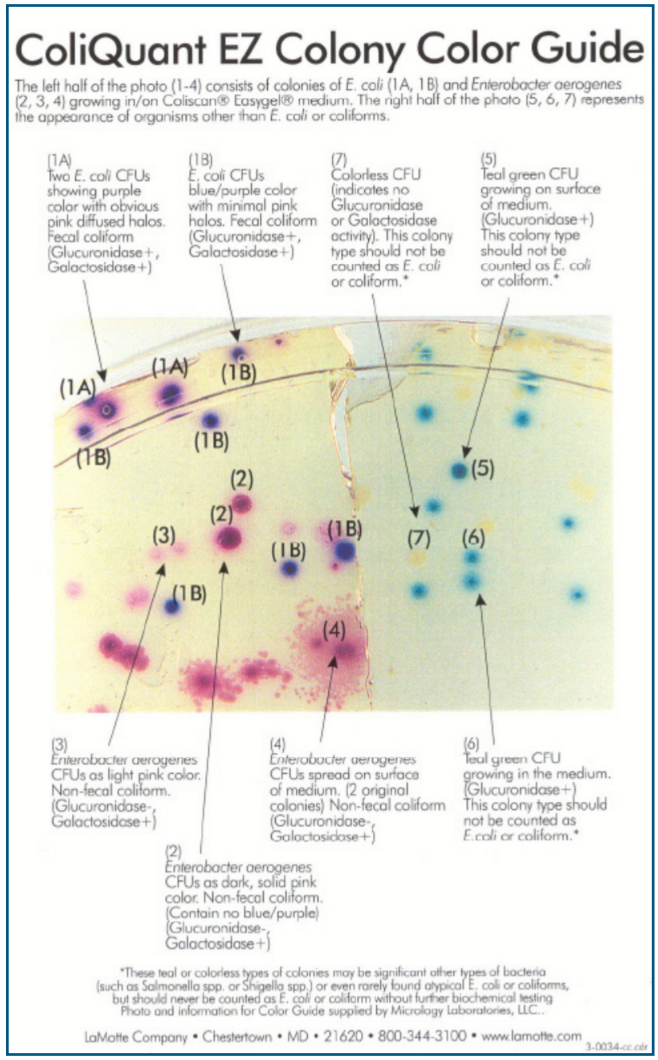
STEP 2: Place Petri dishes right-side up in the incubator. Colonies should be counted within **28-31** hours.

STEP 3: Record the incubation start and end times and temperature on the Monitoring Form.

Quality Control Check: Colonies should not be counted for a minimum of 28 hours and no counts should be made after 31 hours. For optimum results, count and record colonies at 28 hours of incubation.

COUNTING COLONIES

Upon incubation, the general coliforms and *E. coli* bacteria produce enzymes that react with color reagents in the media to produce pink/red colonies (general coliforms) or dark purple and dark blue colonies with and without pink diffused halos (*E. coli* bacteria). Do not count the pink, white, light blue, or teal/turquoise-colored colonies.



STEP 1: Count the number of individual and distinct dark purple and dark blue colonies with and without pink diffused halos. **Do not** count the pink, white, light blue, or teal/turquoise-colored colonies.

STEP 2: Record the number of *E. coli* bacteria colonies on the Monitoring Form for each sample and/or field blank.

DATA REPORTING

Results of the analysis for the 2 sample replicates per site plus the blank are reported on the Monitoring Form as "colonies per 100 mL" of sample water. To arrive at that number, first determine the dilution factor.

Dilution factor = 100 / sample size

For example, if you collected a sample size of 1 mL in the pipette and added to the Easygel Coliscan media, your dilution factor is: 100 mL/ 1 mL, or 100.

To determine the number of colonies per 100 mL, multiply the number of colonies counted x dilution factor.

#colonies/100mL = #colonies X dilution factor

For example, if you counted 25 colonies and had a dilution factor of 100 (1 mL sample size), the result is 25 x 100 = 2500 *E. coli* colonies/100 mL. If you counted 25 colonies and had a dilution factor of 33.3 (3 mL sample size), your result is 25 x 33.3 = 833 *E. coli* colonies/100 mL. And, if you counted 25 colonies and had a dilution factor of 20 (5 mL sample size) your result is 500 *E. coli* colonies/100 mL.

This information should be entered on the Monitoring Form to document the final results of each set of samples analyzed. Verify the dilution factor calculation is correct and marked accordingly on the Field Quality Control Checklist.

Sample Size	Dilution Factor
0.5 mL	100/0.5 mL = 200
1 mL	100/1 mL = 100
3 mL	100/3 mL = 33.3
5 mL	100/5 mL = 20

Note: Once the appropriate sample size is determined for a particular site (1, 3, or 5 mL), the sample size for both samples should remain the same for the next sampling event. For example, if after the first time you sample a site, you determine that a 3 mL sample size yields the ideal number of E. coli colonies (between 20-60 and not over 200), then the sample size for both samples for that site should be 3 mL during the next sampling event.

3.0 FOLLOW-UP AND CLEAN-UP

Clean-Up

WASTE DISPOSAL

STEP 1: To dispose of the used Petri dishes, lift the lid and pour 5 mL (about 1 teaspoon) of bleach or isopropyl alcohol into each dish.

STEP 2: Make sure the bleach has covered the entire dish and allow it to sit for a minimum of 15 minutes.

STEP 3: Place the dish in a sealed plastic bag and place in normal household trash.

STEP 4: Clean the inside of the incubator with diluted bleach solution and allow to air dry before next use.

STEP 5: Dispose of used gloves, Whirl-Pak® bags, sterile pipets, and Ziploc bags by placing them in normal household trash.

STEP 6: Dispose of expired reagents or waste chemicals by running cold tap water and slowly pouring the material down a sanitary sewage system drain with water. While pouring the waste into the drain, turn on the tap and keep it running throughout the process. This will dilute the waste chemicals.

> **Note:** Do not dispose of chemicals into a septic waste system, water body, or onto the ground.

STEP 7: After all supplies have been properly disposed, disinfectant should be used to clean tabletops or other work areas, and community scientists should wash hands thoroughly.

STORING OF CHEMICALS

Supplies should always be stored in a cool, dark place away from children and pets.

Follow-Up

SUBMITTING A TRAINING ENROLLMENT FORM

The Training Enrollment Form must be submitted to participate in the Texas Stream Team program. Note that the Texas Stream Team cannot certify individuals who do not submit the Training Enrollment Form.

The trainer will send training documentation to Texas Stream Team to be processed by staff. Your certificate will be emailed to community scientists the month following the training.

Upon completion of the training, Texas Stream

Team *E. coli* Bacteria community scientists can begin monitoring. To get started, certified community scientists need to obtain monitoring supplies, select a site, and create a monitoring schedule.

MONITORING EQUIPMENT

To obtain equipment and supplies for the *E.coli* Bacteria training, go to the Texas Stream Team Monitoring Equipment Form. **Please note**, Texas Stream Team is a federally recognized statewide monitoring program with an approved Quality Assurance Project Plan, therefore all monitoring must be conducted using approved methods and with the equipment listed, unless prior approval has been granted by Texas Stream Team staff. The Texas Stream Team is entirely grant funded, and, therefore, unable to provide extensive funding assistance to all community scientists and partners across the state. Texas Stream Team highly encourages community scientists and partners to seek alternate funding sources. Please refer to the Funding Sources Document for assistance.

MONITORING SITE

To select a site, community scientists can begin by referencing the Datamap. The Datamap includes all historic and current water quality monitoring sites. Community scientists can choose to reactivate an inactive site, or they can create a new site using the New Monitoring Site Request Form. For more information on site selection, go to Section 2.1- Choosing A Monitoring Location.

MONITORING SCHEDULE

Community scientists must create a monitoring schedule that allows time to travel to the monitoring site and conduct the sampling following their Core monitoring. To ensure data quality, the Texas Stream Team requests community scientists conduct *E. coli* bacteria water quality monitoring at least once a month, at the same time. For more information on scheduling your sampling time, see Section 2.2 - Choosing a Monitoring Time.

3.1 DATA MANAGEMENT

Community scientists are required to use the *E. coli* Bacteria Monitoring Form to record measurements at their monitoring site(s). Test results are always recorded on the form as they are completed in the field. All applicable sections of the Monitoring Form should be completed. For example, if information is not collected for a parameter, the space on the form remains blank.

Recording Data

To ensure compliance with the approved Quality Assurance Project Plan, community scientists should observe the following rules when completing the Monitoring Form:

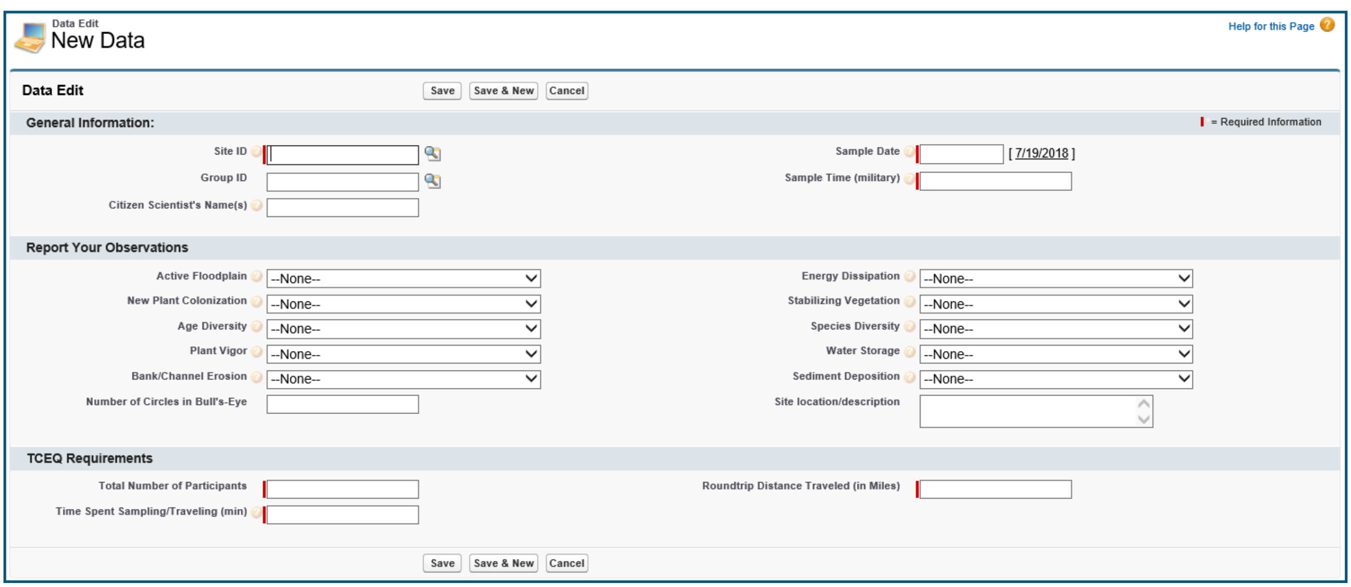
1. Write legibly in ink or pencil if using the hard copy version of the Monitoring Form.
2. Correct errors with a single line strike-through followed by initials of the individual making the correction and date the correction was made.
3. Complete the Field Quality Control Checklist on the form to confirm protocols were followed.
4. Sign and date the form once complete for validation.

Before monitoring data can be entered into the Dataviewer, it must undergo a quality control check to ensure the data are of the highest quality and meet the following conditions:

1. Data is collected by a certified community scientist that has met all training requirements as described in this manual.
2. Data is collected using the protocols, equipment, and the Field Quality Control Checklist provided on the form described in this manual.
3. All data entries are legible if using the hard copy version of the Monitoring Form.
4. The Monitoring Form is complete and includes a signature by the community scientist that conducted the monitoring event.
5. All quality assurance and quality control protocols described in this manual have been implemented and met to the best of the community scientist's ability. See Section 3.2 - Data Entry Checklist.

Entering Data into Waterways Dataviewer

Once the Monitoring Form is complete and meets the quality control checks, the next step is to enter the data into the Dataviewer. There are two ways to enter the data:


Data Edit
New Data
Help for this Page
Data Edit Save Save & New Cancel
General Information:
= Required Information
Site ID
Sample Date [7/19/2018]
Group ID
Sample Time (military)
Citizen Scientist's Name(s)
Report Your Observations
Active Floodplain --None--
Energy Dissipation --None--
New Plant Colonization --None--
Stabilizing Vegetation --None--
Age Diversity --None--
Species Diversity --None--
Plant Vigor --None--
Water Storage --None--
Bank/Channel Erosion --None--
Sediment Deposition --None--
Number of Circles in Bull's-Eye
Site location/description
TCEQ Requirements
Total Number of Participants
Roundtrip Distance Traveled (in Miles)
Time Spent Sampling/Traveling (min)
Save Save & New Cancel

1. Monitoring Forms get forwarded to the group Data Coordinator. The group Data Coordinator conducts a quality control check and enters the data into the Dataviewer. If your group does not have a Data Coordinator, skip to the second option.

2. Monitoring Forms are submitted to Texas Stream Team by emailing scanned or high-quality photocopies to TxStreamTeam@txstate.edu for entry by Texas Stream Team staff. Texas Stream Team staff conduct a quality control check and enters the data into the Dataviewer.

Before group Data Coordinators can access the Dataviewer to enter data, they must first request an account and receive assigned login credentials. A request for an account can be made by filling out the Dataviewer Account Request Form.

Once the data from the Monitoring Forms are entered into the Dataviewer, the group Data Coordinator, Texas Stream Team staff, and/or designee verifies the data entry, and the data becomes publicly available.

If the data do not meet the checks described above, the data are flagged upon entry to the Dataviewer for further review by Texas Stream Team staff or designee. The purpose of flagged data is to note inconsistencies or identify data that may have excessive variability. The Dataviewer is designed to recognize and flag data that do not meet requirements in the checklist. Therefore, it is critical for community scientists to comply with the protocols described in this manual to ensure data measurements are of the highest quality and can be used to promote and protect a healthy and safe environment for people and the aquatic life.

3.2 DATA ENTRY CHECKLIST

The data entry checklist is used by the Texas Stream Team community scientist and Data Coordinator to verify that data are collected using approved protocols prior to entering data into the Waterways Dataviewer.

General Procedures

- Samples were transported on ice if testing did not occur at monitoring site.
- Gloves were worn or hand sanitizer was applied throughout.
- None of the reagents used for testing were expired.
- All reagents were stored at room temperature or in an environment protected from extreme weather prior to use.
- Sampling was conducted at approximately the same time/day as previous sampling events at this site, preferably before noon or after 4pm (16:00).
- Monitoring sample was collected from the centroid of flow with minimal streambed disturbance.
- All equipment was rinsed twice with sample water before test was conducted.
- All equipment was rinsed twice with deionized water after testing was completed.
- All relevant measurements are recorded in appropriate fields on monitoring form.

Field Observations

Suggested rainfall resources: Community Rain, Hail & Snow Network , Weather.com and Lower Colorado River Authority's Hydromet Page.

- **Days since last significant precipitation**: Report whole numbers. If it is raining when the sample is collected, or has rained within the past 24 hours, report a value of <1. Otherwise, report the actual number, if known, or a 'greater than' value.
- **Rainfall accumulation**: Report inches of rain within the last 3 days.
- **Algae:** Recorded algae observed on the water surface and below the water surface.
- **Water Color:** Observed water color in a plastic cup or bucket with a white background.
- **Water Clarity:** Observed the relative cloudiness of the water from bridge or banks.
- **Water Odor:** Tested by wafting from plastic cup or bucket.
- **Present Weather:** Marked cloudy if there is at least one cloud in the sky.

E. coli Bacteria Tests and Measurements

- The sample depth is either 0.3 m or half of the total depth.
- A field blank is analyzed at least every 10th sample.
- Hold time between sample collection time and incubation time does not exceed 8 hours.
- Incubation time is between 28 to 31 hours.
- Incubation temperature is 33˚C ± 3˚C. Optimal colony number is achieved.

3.3 EQUIPMENT MAINTENANCE AND STORAGE

The importance of proper maintenance and storage of all monitoring equipment cannot be overstated. The accuracy of the measurements depends on proper maintenance and storage. The time, effort, and expense that goes into conducting Texas Stream Team water quality monitoring is highly valued, therefore do not dismiss this very important step that will add value to the quality of data produced and increase the longevity of the equipment.

Quality Control Check: Do not use soap when cleaning your equipment. This can leave a residue, which can alter your results.

WASTE BOTTLES

Dispose of all waste chemicals by slowly flushing them down the drain of a sanitary sewer with plenty of water. Allow the tap to run while flushing the waste. Do not dispose of chemicals into a septic waste system, water body, or onto the ground. Once emptied, rinse waste bottles twice with tap or deionized water. Allow them to dry before storing them in a secure area.

INCUBATOR

Prepare a dilute bleach solution by mixing 1/3rd cup of bleach with one gallon of water (New Hampshire Department of Health and Human Services, 2011). While wearing gloves, wipe the inside of the incubator with the diluted bleach solution and allow the incubator to air dry before the next use. After cleaning, always store cleaning supplies in a safe area not accessible to children and pets.

Images acquired from [the official government website of Knox County, Tennessee] (Knox County Tennessee Stormwater Compliance, n.d.).

3.4 REPORTING UNUSUAL ACTIVITY AND UNLAWFUL EVENTS

Illicit discharge

An illicit discharge can have different meanings across different regulatory agencies. For the purposes of Texas Stream Team monitoring activities, an illicit discharge is defined as any event wherein a storm drain has a measurable flow during dry weather conditions (Center for Watershed Protection, 2004).

Illicit discharges are usually produced from a singular source or operation and can be further broken down into categories based on their frequency, flow-type, and mode of entry (Center for Watershed Protection, 2004). Illicit discharges can be either direct or indirect. An illicit discharge has a direct mode of entry when the discharge is directly connected to a storm drain through a sewage pipe, shop drain, or other kind of pipe (Center for Watershed Protection, 2004). An indirect discharge occurs when flows generated outside of the storm drain enter the system, either through inlets, or by infiltrating the joints of a pipe (Center for Watershed Protection, 2004).

Illicit discharges include any instances wherein chemicals or waste are discarded into a sanitary sewer drain. Examples of illicit discharges include improperly discarded oil and grease, runoff from excessive fertilizers and pesticides, and illegal dumping of hazardous chemicals (Center for Watershed Protection, 2004). Other examples include septic tank seepage, laundry wastewater, or illegal sanitary sewer connections (Center for Watershed Protection, 2004). For information about illicit discharge, and additional examples of illicit discharges that might be expected corresponding to land use, visit the Illicit Discharge Detection and Elimination: A Guidance Manual for Program Development and Technical Assessments.

HOW TO REPORT AN ILLICIT DISCHARGE

To report an illicit discharge, please contact your city office. Many cities allow community members to anonymously report illicit discharges online. Check your city's Department of Water or Department of Public Works for an online form, or, alternatively, you can contact your city office at their main office phone line.

For assistance with reporting illicit discharges, you can also contact Texas Stream Team at TxStreamTeam@txstate.edu or by calling (512) 245-1346.

Wildlife Kills and Pollution Events

The Texas Parks and Wildlife Department's Kills and Spills Team (KAST) is comprised of a group of biologists who investigate fish and wildlife kills. KAST biologists evaluate both unnatural and natural events to assess the impacts to fish and wildlife resources and to determine the causes of the events. KAST biologists work to:

1. Determine the causes of wildlife kills and/or pollution events.
2. Attempt to minimize environmental damage resulting from wildlife kills and/or pollution events.

3. Obtain compensation for environmental damage and restore the affected environment resulting in Kill or Spill.

   (Kills and Spills Team, n.d.)

REPORTING A KILL OR SPILL

Prompt notification is essential to a successful investigation, and the sooner that KAST biologists are notified of a potential wildlife kill or pollution event, the better the chances are that useful evidence can be collected, and conclusive actions can be taken.

When reporting a Kill or Spill, make a note of the:

1. Location, date, and time
2. Water color, clarity, and odor
3. Number, size, and species of affected organisms
4. Recent weather
5. Condition and behavior of animals or organisms
6. Condition of plants/other organisms

   (Kills and Spills Team, n.d.)

To contact KAST, call (512) 389-4848 or contact your regional KAST biologist. You can find your regional KAST biologist at www.tpwd.gov/landcover/water/environconcers/kills_and_spills/regions.

Image acquired from the TPWD KAST webpage.

Texas Commission on Environmental Quality Compliance and Enforcement

The Texas Commission on Environmental Quality Office of Compliance and Enforcement is responsible for enforcing compliance with state environmental law, responding to emergencies and natural disasters, overseeing dam safety, and monitoring air quality (Office of Compliance and Enforcement, 2023).

The Texas Commission on Environmental Quality divides the state of Texas into four areas, with further regional divisions. Within their defined administrative region, each regional office is responsible for:

- Investigating compliance at permitted air, water and waste facilities
- Investigating complaints at facilities and operations- permitted or not- from community members, businesses, and other concerned parties
- Developing enforcement actions and referrals for violations
- Environmental education and technical assistance for communities
- Monitoring the quality of ambient air, surface water, and public drinking water (Office of Compliance and Enforcement, 2023)

Reporting an Environmental Problem

Concerned community members can file an Environmental Complaint with the Texas Commission on Environmental Quality. In general, the Texas Commission on Environmental Quality can assist with any complaint, provided that you have:

- Seen water that may be polluted.
- Seen or smelled something unpleasant in the air.
- Seen land that may be contaminated.
- Are having problems with your drinking water.
- Have information or evidence about an environmental problem.

- Are having problems with an individual or company licensed or registered by the Texas Commission on Environmental Quality.
- Need assistance or information regarding environmental laws, possible pollution sources, or other questions relating to Texas Commission on Environmental Quality Compliance and Enforcement.

  (Office of Compliance and Enforcement, 2023)

For more information on what Texas Commission on Environmental Quality can and cannot help you with, please visit the Texas Commission on Environmental Quality website.

To report an Environmental Problem, contact the Texas Commission on Environmental Quality Office of Compliance and Enforcement at their 24-hour line 888-777-3186 or fill out their online form.

If you would prefer to contact your regional Texas Commission on Environmental Quality Field Office, you can find applicable contact information on the Texas Commission on Environmental Quality website.

4.0 REFERENCES, ONLINE RESOURCES, AND GLOSSARY

References

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Online Resources

E. coli Bacteria Equipment Form

Dataviewer Account Request Form

Equipment Form

Funding Guidance

Group Monitoring Plan

Measures of Success Survey

New Monitoring Site Request Form

Private Property Access Form

Site Selection Guide

Supply Order Form

Texas Stream Team Calendar

Texas Stream Team Dataviewer and Datamap

Texas Stream Team Online Store

Texas Stream Team Partners List

Texas Stream Team Quality Assurance Project Plan (QAPP)

Texas Stream Team Trainers List

Texas Stream Team Trainings and Programs

YouTube Quality Control and Parameter Videos

For additional forms and resources please visit the Texas Stream Team Forms and Resources page.

Glossary

Algae - Plants that lack true roots, stems, and leaves. For the physical assessment described herein, algae consist of nonvascular plants that attach to rocks and debris or are free floating in the water. Such plants may be green, blue-green, or olive in color, slimy to the touch, and usually have a coarse filamentous structure.

Bank - The portion of the channel that tends to restrict lateral movement of water. It often has a slope less than 90° and exhibits a distinct break in slope from the stream bottom. Also, a distinct change in the substrate materials or vegetation may delineate the bank.

Channel - That portion of the landscape which contains the bank and the stream bottom. It is distinct from the surrounding area due to breaks in the general slope of the land, lack of terrestrial vegetation, and changes in the composition of substrate materials.

Colony – The amount of *E. coli* or *Enteroccocus* bacteria found in a water sample. Expressed in colonies/100 mL.

Contact Recreation - Recreational activities involving a significant risk of ingestion of contaminant water, including wading by children, swimming, water skiing, diving, and surfing.

Detritus - Decaying organic material.

Effluent - Wastewater (treated or untreated) that flows out of a treatment plant or industrial outfall (point source), prior to entering a water body.

***Enteroccocus* -** A bacteria that live in the gut of humans and warm-blooded animals, however, associated with human fecal matter. Enterococci are a relatively reliable indicator of potential human fecal matter contamination in a water body (mainly salt water).

Escherichia coli (*E. coli*) - A type of diverse coliform bacteria that live in the guts of people and animals. Most of the time, *E. coli* bacteria is harmless, but some can be pathogenic, meaning that they cause disease. Used as an indicator of fecal contamination typically in freshwater.

Estuary - Regions of interaction between rivers and near shore ocean waters, where tidal action and river flow create a mixing of fresh and salt water.

Eutrophic - Refers to shallow, murky bodies of water that have excessive concentrations of plant nutrients resulting in increased algal production.

Fecal Coliform Bacteria - Bacteria found in the intestinal tracts of warm-blooded animals. Organisms used as an indicator of pollution and possible presence of waterborne pathogens.

Field Blank – A sterile diluent sample used to assess for potential contamination from sample handling, airborne materials, equipment, media, and other sources.

Habitat - The area in which an organism lives.

Hold Time – The maximum time a water sample can be kept on ice before being processed.

Nonpoint Source - Pollution sources which are diffuse and do not have a single 38 point of origin or are not introduced into a receiving stream from a specific outfall. The pollutants are generally carried off the land by stormwater runoff. The commonly used categories for nonpoint sources are: agriculture, forestry, urban, mining, construction, dams and channels, land disposal and saltwater intrusion.

Nutrient - Any substance used by living things to promote growth. The term is generally applied to nitrogen and phosphorus in water and wastewater, but is also applied to other essential and trace elements.

Outfall - A designated point of effluent discharge.

Point Source - A specific location from which pollutants are discharged. It can also be defined as a single identifiable source of pollution (e.g., pipe or ship).

Pollution - the man-made or man-induced alteration of the chemical, physical, biological and radiological integrity of water (EPA CWA definition).

Primary Contact Recreation – Activities in waterbodies that pose a higher risk of ingesting water such as swimming, surfing, water-skiing, and other activities that are likely to result in immersion.

Reservoir - Any natural or artificial holding area used to store, regulate, or control water.

Runoff - The part of precipitation or irrigation water that runs-off land into streams and other surface water.

Secondary Contact Recreation – Aquatic activities with a lower risk of ingesting waters such as wading, fishing, and kayaking.

Sediment - Particles and/or clumps of particle of sand, clay, silt, and plant or animal matter carried in water and are deposited in reservoirs and slow-moving areas of streams and rivers.

Water Quality Standards - Established limits of certain chemical, physical, and biological parameters in a water body; water quality standards are established for the different designated uses of a water body (e.g., aquatic life use, contact recreation, public water supply).

Watershed -The area of land from which precipitation drains to a single point. Watersheds are sometimes referred to as drainage basins or drainage areas.