Sampling and Analysis Plan
Bitterroot Mainstem
Long-term Nutrient Trends Monitoring

2024

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ACRONYM LIST

Acronym	Definition
AFDW	ash free dry weight
BRPA	Bitterroot River Protection Association
cc	cubic centimeter
CFC	Clark Fork Coalition
COC	chain of custody
DEQ	Montana Department of Environmental Quality
DO	dissolved oxygen
FB	field blank
FD	field duplicate
H_2SO_4	sulfuric acid
g/m^2	gram per square meter
HDPE	high-density polyethylene
NH_3+NH_4-N	total ammonia as nitrogen
MAS	Monitoring and Assessment Program
µg/L	microgram per liter
µm	micrometer
mg/m^2	milligram per square meter
mL	milliliter
mm	millimeter
NO_2+NO_3-N	nitrate-nitrite as nitrogen
NPS	Nonpoint Source Program
QAPP	quality assurance project plan
QA/QC	quality assurance/quality control
SAP	sampling and analysis plan
SOP	standard operating procedure
SRP	soluble reactive phosphorus
TMDL	total maximum daily load
TP	total phosphorus
TPN	total persulfate nitrogen
TSS	total suspended solids

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1.0 INTRODUCTION

Water quality data collected from the Bitterroot River under this sampling and analysis plan (SAP) supports the Bitterroot Mainstem Long-term Nutrient Trends Monitoring Project (Riedl 2022). Nutrient data collection on the Bitterroot River has occurred periodically beginning in the early 2000s, when the river was monitored by the Tristate Water Quality Committee as part of the Clark Fork River Monitoring Program during total maximum daily load (TMDL) development. Monitoring also occurred in support of TMDL development in 2012, and most recently under the Bitterroot River Protection Association's (BRPA) volunteer monitoring program. The 2024 monitoring effort represents the sixth of at least 10 years of concerted data collection, with the opportunity to renew for another 10 years later. Refer to the quality assurance project plan (QAPP; Riedl 2022) for more background information.

The impetus behind establishing a long-term nutrient monitoring program on the Bitterroot River is supported by Montana Department of Environmental Quality's (DEQ's) Nonpoint Source (NPS) Program's and Monitoring and Assessment (MAS) Program's strategic long- term plans (DEQ 2019a, 2019b, respectively). One of MAS's stated strategic plan objectives is to track water quality change over time, particularly in watersheds where multiple DEQ program priorities align. 2019 marked the first year of implementing the NPS Program's focus watersheds strategy, and the Bitterroot River watershed (hydrologic unit code 17010205) is designated as the first focus area (DEQ 2019a). Through 2022, a majority of the NPS Program's financial and technical resources were prioritized in the watershed, thereby increasing momentum for education and outreach and voluntary restoration implementation. A major goal of the Bitterroot Mainstem Long-term Nutrient Trends Monitoring Project is to help demonstrate the success of this approach by tracking water quality within the Bitterroot River. Refer to the Bitterroot Watershed Focus Area Project Plan (DEQ 2019c) for more information about project activities in the Bitterroot Watershed during the focus area effort.

In the past, monitoring has been coordinated by the Clark Fork Coalition (CFC) with support from DEQ, and conducted by BRPA. However, CFC is no longer coordinating this effort. From 2023, monitoring will be conducted by BRPA in coordination with DEQ. BRPA will continue to collect water chemistry samples and Missoula County Water Quality District (MCWQD) will assist BRPA volunteers with collecting benthic algae samples, similar to past efforts. Equipment and supplies, such as bottles, coolers, filters, and syringes, for water chemistry monitoring will be dropped off in Missoula, MT, by DEQ personnel for BRPA efforts. Once sample efforts are completed, BRPA will drop samples off in Missoula, MT, with MCWQD to be picked up by DEQ personnel and dropped off to Energy Labs in Helena, MT.

2.0 OBJECTIVES AND DESIGN

This SAP covers water quality monitoring conducted at six stations located on the Bitterroot River, from Darby, MT, to Missoula, MT (**Table 2.1**; **Figure 2.1**). Rationale and historical data availability are in *Table 1.2* of the QAPP (Riedl 2022). Note that **Table 2.1** and site visit forms contain site COMBITR04, the Bitterroot River at Veterans Bridge. This site is included in the table and on the site visit forms for ease of data collection by the BRPA. Monitoring of this site is funded separately by BRPA. Also note that the 2024 SAP remains consistent with the 2023 SAP with respect to water chemistry monitoring. For algae monitoring, volunteers and MCWQD staff will collect algae chlorophyll samples. As in 2023, 9 composite benthic algae chlorophyll samples are collected from each site instead of 18 replicate samples.

Table 2.1 *Bitterroot mainstem long-term nutrient trends monitoring stations*

Site Name	Description	Latitude	Longitude
COMBITR02	Bitterroot River at Buckhouse Bridge	46.83194	-114.05306
COMBITR03	Bitterroot River at Florence Bridge	46.63309	-114.05096
BITR-C05BITRR24	Bitterroot River at Bell Crossing	46.4436	-114.12630
COMBITR04	Bitterroot River at Veterans Bridge	46.2792	-114.1606
BITR-C05BITTR03	Bitterroot River at Main Street Hamilton	46.2475	-114.17722
BITR-C05BITTR06	Bitterroot River at Darby Bridge	45.9725	-114.1411

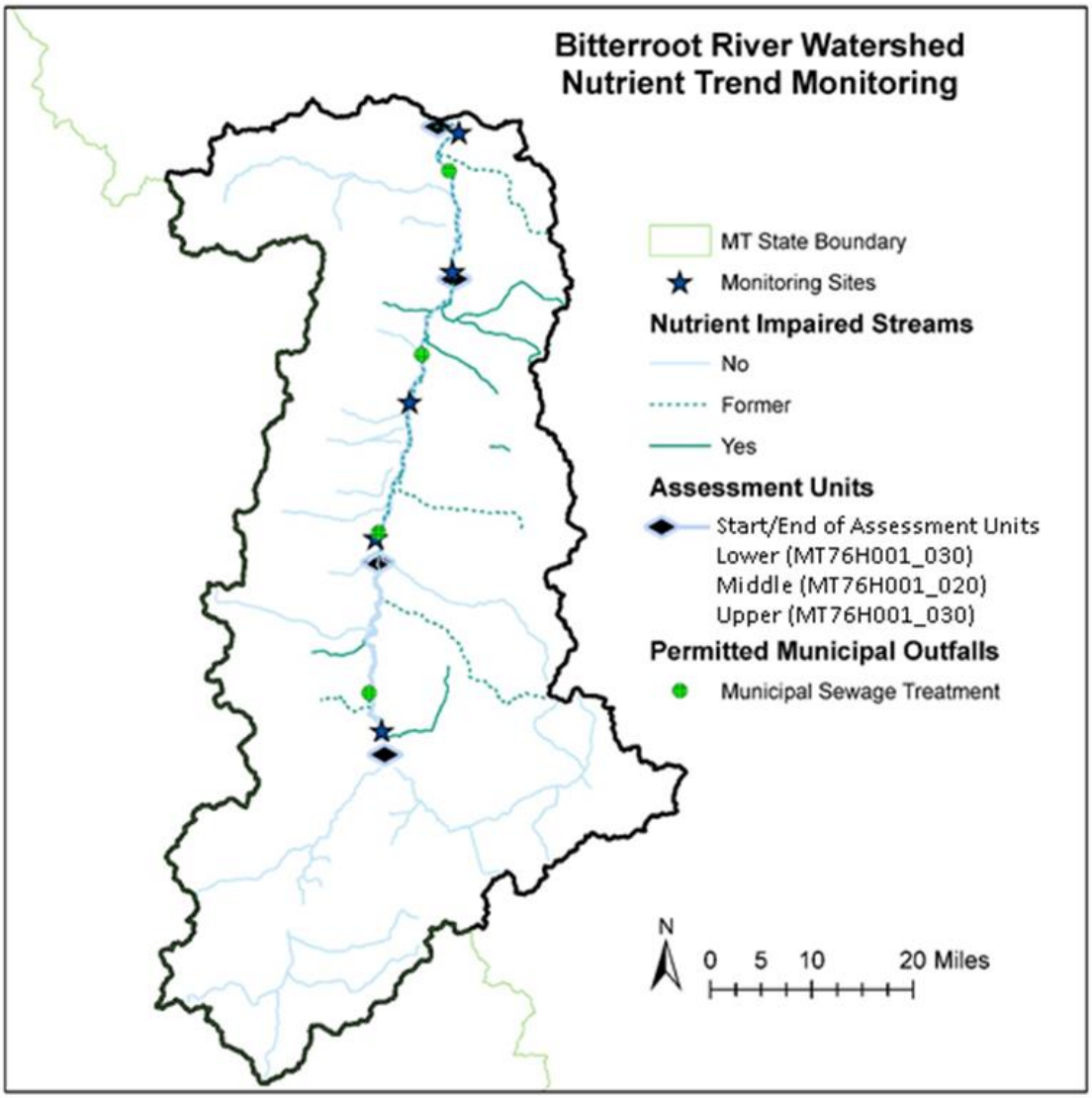


Figure 2.1. *Map of the Bitterroot Watershed showing assessment units, formerly and currently nutrient impaired assessment units, monitoring sites, and permitted municipal wastewater treatment facilities.*

The primary goal of this long-term monitoring project is to conduct nutrient trend analyses. The data collected will address these specific objectives:

- Determine if nutrient concentrations are being maintained (i.e., meeting water quality criteria).
- Identify where and when changes in nutrient and algal concentrations occur.
- Ensure data collection is sufficient to identify trends and an efficient use of resources.

Monitoring parameters for all sites included in **Table 2.1** include:

- Measure field parameters
    - Turbidity
    - Temperature
    - Dissolved oxygen (DO)
    - pH

  - Specific conductivity
- Collect and analyze nutrient/lab samples
  - Total persulfate nitrogen (TPN)
  - Total phosphorus (TP)
  - Nitrate + nitrite nitrogen (NO_2+NO_3-N)
  - Ammonia (NH_3+NH_4-N)
  - Soluble reactive phosphorus (SRP)
  - Total suspended solids (TSS)
- Collect and analyze benthic algae samples
  - Chlorophyll-*a*
  - Ash free dry weight (AFDW)
  - Visual assessment of benthic algae

Monitoring will be conducted by BRPA with support from MCWQD. Site visits will occur eight times (twice in July, August, September, and October) for nutrient monitoring and two times (August 1-2 and September 5-6) for benthic algae monitoring. A detailed schedule is provided in **Table 2.2**.

Table 2.2 *Water quality sampling schedule and sampler roles.*

Month	**Date***	**Parameters**	**QA/QC**	**Sampler**
July	8-9	Field, nutrients	FB & FD at Veterans	BRPA
July	22-23	Field, nutrients	FB & FD at Buckhouse	BRPA
August	1-2	Algae	N/A	MCWQD
August	5-6	Field, nutrients	FB & FD at Main Street	BRPA
August	19-20	Field, nutrients	FB & FD at Florence	BRPA
September	5-6	Algae	N/A	MCWQD
September	9-10	Field, nutrients	FB & FD at Darby	BRPA
September	23-24	Field, nutrients	FB & FD at Bell Crossing	BRPA
October	7-8	Field, nutrients	FB & FD at Veterans	BRPA
October	21-22	Field, nutrients	FB & FD at Buckhouse	BRPA

* Dates may vary slightly due to unforeseen circumstances. Sampling will occur within one week of this date.

3.0 FIELD SAMPLING METHODS

3.1 EQUIPMENT

Required equipment for each entity collecting samples includes:

- 250 ml sampling bottles with white caps for TPN samples (1 for each station per sampling event, field blank, and duplicate)
- 500 ml sampling bottles with yellow caps for total phosphorus, nitrate + nitrite, and ammonia samples (1 for each station per sampling event, field blank, and duplicate)
- Sulfuric acid (1 vial for each sampling event, field blank, and duplicate)
- 120 ml sampling bottles with white caps for soluble reactive phosphorus samples (1 for each station per sampling event, field blank, and duplicate)
- 1000 ml sampling bottle for TSS samples (1 for each station per sampling event, field blank, and duplicate)
- 1-60 cc syringe to collect water for filter (1 for each station per sampling event, field blank, and duplicate)
- 0.45 µm disposable or acid rinsed filters (at least 2 for each routine sample, field blank and duplicate, plus several extras; one used for triple rinsing 120 ml bottles plus at least one for collecting the sample itself)
- Field meter(s) and calibration solutions
- Algae sample containers (47 mm plastic petri dishes, zip lock bags), during algae collection only
- Sampling hoop and template; single edge razor blades, during algae collection only
- Plastic basin to hold samples, during algae collection only
- Chain of custody (COC) form(s): one for nutrient grab samples and one for algae samples (**Attachment A**)
- Site visit form(s): one for field parameters and nutrient grab samples and one for algae samples (**Attachment A**)
- Aquatic Plant Visual Assessment forms (during algae collection only; **Attachment A**)
- Pencils and waterproof pen, plus clear plastic tape to protect label
- Cooler and ice (wet and dry, as appropriate)
- De-ionized water (~1 gallon in liter bottles)
- Packaging tape and preprinted shipping labels

Energy Laboratories will supply labeled bottles, syringes, filters, preservatives, and coolers. BRPA and MCWQD will use their field meters. The University of Montana will provide supplies for algae sampling. DEQ will provide COCs and site visit forms, and examples are provided in **Attachment A**.

3.2 FIELD PARAMETERS

Prior to data collection, the BRPA or MCWQD will calibrate a YSI DSS or Pro Plus meter according to instructions in the user manual. During each sampling event at each sampling site, in situ measurements of field parameters and turbidity will be conducted using a YSI ProDSS or Pro Plus meter (**Section 2.0**). These measurements must be taken prior to collecting water quality samples or other physical disturbances to the water column and substrate. The results of the field measurements will be recorded on routine site visit forms for each monitoring station.

The following steps will be followed to collect instantaneous measurements using the YSI meter:

1. Remove the YSI meters from its case, attach the cable to the handheld unit if not already connected, and turn the instrument on.
2. Place a thermometer in a shaded location and allow it to equilibrate. When stable, record the air temperature on the Routine Site Visit Form.
3. The ODO sensor must be calibrated at the start of each sampling day. Place a small amount of clean water (~5 ml) in the calibration cup. Make sure there are no water droplets on the ODO sensor cap or temperature sensor. Attach the probe guard and carefully slide the assembly into the calibration cup. Make sure a seal is not created around the probe. Atmospheric venting is required for accurate calibration.
4. Place the meter in a safe place in the shade with the unit turned on and with ODO calibration selected, then allow the meter to sit undisturbed for approximately 10- to 15-minutes. This allows the barometric pressure to stabilize and calibrates the meter in preparation for DO readings at each site. When the DO reading is stable, accept ODO calibration.
5. Wade with the meter into a safe location of the stream. Field measurements should be taken at a location that is well-mixed, has steady flow, is not excessively turbulent, stagnant or in an eddy, and is free of upstream obstructions and disturbances (for example, not directly downstream from a bridge, boulder, tree, people, dogs), and deep enough so the sensor can be entirely submerged.
6. Facing upstream into the direction of flow, fully submerge the probes. Take care not to kink the cable. If the river bottom is very fine silt or mud, hold the sensors above the river bottom so the sensors are suspended above and not buried in sediment. Gently swirl and shake the sensors to release any air bubbles that are trapped in the sensor guard.
7. Observe the display screen and wait a minute or more until the numbers stabilize. NOTE: It is permissible to collect the Nutrient Grab Samples (**Section 3.3**) while allowing the probes to equilibrate, but care must be taken to place the probes upstream from where the samples will be collected to avoid any disturbances.
8. Record the field parameter values on the Routine Site Visit Form, carefully noting the units. Finally, log the values on the YSI handheld, taking care to select the correct sampling site.

3.3 NUTRIENT GRAB SAMPLES AND PHOTOS

BRPA volunteers will collect water samples at respective sites (**Table 2.1**). A sample duplicate and field blank for each parameter will be taken at one site during each sampling event. **Table 2.1** provides the dates when quality assurance/quality control (QA/QC) samples will be taken and the monitoring station where duplicate samples will be collected. **Table 4.1** summarizes sampling volumes, containers, preservation and holding time requirements for nutrient samples collected from the Bitterroot River. Step-by-step processes for sample collection are found below, and corresponding SOP references are included in the QAPP (Riedl 2022).

Prior to collecting each sample, use a pencil or a permanent, fine-point marker to fill out the label on the sample bottles needed for each water sample at the site. Include the Sample ID (site ID), date collected, time of first sample collected at the site, preservative, and sampler's initials. Cover each label with clear plastic tape to protect the label from water damage. Sample bottles and labels that require preservatives will be color-coded and the label will indicate which acid is required (e.g., yellow cap for sulfuric acid).

To collect the samples:

1. Carry the bottles and wade into the stream to a sampling location that is well-mixed (water is flowing steadily, is not stagnant or an eddy) and upstream from any disturbances (e.g., areas where people or dogs have disturbed the streambed or water).
2. Submerge the bottle (for unfiltered samples) or syringe (for filtered samples) upstream from where you are standing and below the water surface to not collect surface scum, but so the mouth of the bottle is elevated away from the river bottom to not collect sediments from the stream bottom.
3. Follow either unfiltered or filtered grab sampling procedures described below, as applicable, for each sample.
4. Check the appropriate boxes on the Routine Site Visit Form. Ensure that all required data and information are recorded accurately on both the Site Visit Form and Energy Lab COC.

Unfiltered Grab Samples

The following samples will be collected in high-density polyethylene (HDPE) bottles provided by the lab using unfiltered grab sampling techniques:

- TPN: 250 ml square bottle with white lid
- TP, nitrite plus nitrate (NO_2+NO_3), and ammonia (NH_3+NH_4): 500 ml bottle with yellow lid
- TSS: 1000 ml square bottle

1. Triple-rinse the bottles and lids: facing upstream into the direction of the flow, collect a small amount of water in the bottle, replace the lid, and shake gently. Discard this rinse water behind you. Repeat this process three times to triple-rinse the bottle.
2. Collect the sample: Submerge the sample bottle deep enough so that the mouth of the bottle is below the water surface but not so deep that you scoop river bottom sediments. Fill the bottle up to the shoulder, leaving a small amount of "head space," and securely tighten the lid.
3. Add the vial of sulfuric acid preservative to the TP, NO_2+NO_3, NH_3+NH_4 sample: Carefully unscrew the lids of the TP, NO_2+NO_3, NH_3+NH_4 sample bottle and sulfuric acid preservative vial, dump the entire content of the acid vial into the TP, NO_2+NO_3, NH_3+NH_4 sample bottle, securely tighten the lid on the sample bottle, carefully reseal and discard the empty vial, and gently invert the sample bottle three times to mix the preservative into the sample. Nitrile gloves may be worn while handling the sulfuric acid vial but are not mandatory.

Filtered Grab Samples

SRP samples will be collected in 120 ml HDPE bottles with white lids provided by the lab using filtered grab sampling techniques:

1. Open the new 60 cc syringe package, remove the syringe, and discard the packaging. Triple-rinse the syringe by drawing stream water into the syringe, gently shaking, and compressing the syringe to force the water out three times.
2. Fill the syringe with stream water.
3. Open a new 0.45 µm filter package by gripping the blue ring and peeling the cover open. Screw the filter onto the syringe and discard the packaging. Pass a small amount of water through the filter to "prime" it.

Note: Avoid contaminating the filter before and during sample collection by not touching the filter tip anywhere besides the blue ring.

4. Triple-rinse the sample bottle with filtered water. Draw water into the syringe from below the water surface. Push a small amount of water (approximately 10-20ml) from the syringe through the filter into the sample bottle. Replace the lid and shake gently. Discard this rinse water behind you. Repeat

this process three times to triple-rinse the bottle with filtered water. When finished rinsing, unscrew and discard the filter used for rinsing.

5. Refill the syringe with ambient stream or lake water, open and attach a new filter, and pass a small amount of water through the filter to "prime" it.
6. Once the bottle has been rinsed, fill the bottle with filtered water. Since the bottle is 120 ml and the syringe holds only 60 ml, filling the bottle will require approximately two refills of the syringe. When the syringe is empty, grip the filter's blue ring, unscrew the filter and refill the syringe, taking care not to contaminate the filter. If the filter is not clogged, screw the filter back onto the syringe and continue filtering until the bottle is sufficiently full. If the filter clogs mid-way through filtering, unscrew and discard the clogged filter, refill the syringe, screw on a new filter, pass a small amount of water through the new filter, and continue filtering. Repeat this process until the sample bottle is full.

Note: Be sure to leave enough headspace in this sample bottle so it can expand when frozen without breaking, about ¼" below the shoulder of the bottle.

Collecting Field Duplicate and Field Blank samples

1. To the extent possible, Field Duplicate samples should be collected in parallel with routine samples. For example, collect the TSS routine sample followed by the TSS duplicate sample.
2. De-ionized water is used to prepare the Field Blank samples. Samples should be prepared at the sample site after collecting the routine samples and duplicates. The same rinsing procedure that is used for routine samples should be used for all Field Blank sample containers, although the rinsing volumes may be reduced if necessary.

Photo documentation

During routine field parameter and nutrient grab sample site visits, take upstream, downstream, and across (river right or river left) photos. Record the photo number and brief description on the Routine Site Visit Form.

3.4 BENTHIC ALGAE SAMPLE COLLECTION

Algae samples

The University of Montana uses two methods of sampling benthic chlorophyll depending on the dominant algae type at the sampling site (DEQ 2021, Watson 2018). These methods include template samples (a rock scraping method) and hoop samples (a bulk sampling method). At river sites where microalgae films (diatom algae) dominate, the template method is used. At river sites dominated by heavy growths of filamentous green algae, samples are collected using the hoop method. It is likely that the template method will be the only method needed for the Bitterroot River since heavy growth of green algae (such as Cladophora, which occurs in the upper Clark Fork River) is not known to be present. **Table 4.1** summarizes sampling volumes, containers, preservation and holding time requirements for benthic algae samples.

To quantify biomass, sampling occurs at three subsites representing the river reach (which is 100-300 hundred meters long) at each site. The reach should be a minimum of 100 meters, but additional length may be necessary to capture subsites in appropriate indicator zones. The same reach should be sampled each year. Algae samples are only collected from an indicator zone and indicator time. The indicator zone should have a depth of approximately 30 cm and a flow velocity of about ½ meter per second (not so fast that sampling is difficult, but not so slow that there is no flow). Within this zone, rocks that are 10-20 cm in longest dimension (between the size of a ½ brick and a brick) are randomly collected. The

indicator time for collecting the algae samples is August and early to mid-September. This is the time and place where heavy growths are most likely to interfere with beneficial uses, and limiting sampling to this depth, substrate type, and timeframe reduces variability. Photos of the surrounding landscape and the latitude and longitude of each subsite is recorded using a camera and a GPS, respectively, to help navigate to the subsites during future sampling events.

Individual template samples are stored in a 47 mm petri dish; individual hoop samples are stored in Ziploc bags. If sampled rocks are bare, note the number of bare rocks on the Benthic Algae Site Visit Form. Within each reach, three sub-sampling locations are identified, and six rocks are randomly sampled within each sub-sampling location. Of these six rocks, algal material scraped from every two is composited, resulting in three samples per sub-sampling location, and nine samples for the reach.

Where microalgae films dominate the site, six rocks are sampled randomly from each sub-site within the indicator zone. The samples are collected working upstream, and selected by periodically stopping without looking, then taking the closest rock to the sampler's foot that meets the requirements for depth (30 cm +/-5) and size (10-20 cm in longest dimension). Rocks are collected in a plastic basin and carried to shore where they are kept in the shade until sampled within 5- to 10-minutes of removal from the water. Algal material is collected by scraping a 2inx2in area on the surface of the rock and transporting the material into a 47mm petri dish. Single edge razor blades and flexible templates with a 2x2 square hole are used for algae collection. Samples from every two rocks are composited as one sample, resulting in three samples per sub-sampling location, and nine samples for the reach. After each composite sample is collected, cover with tinfoil and place in a plastic Ziploc bag labeled with the site name. The samples are then placed immediately in a cooler and frozen as soon as practicable.

Where heavy filamentous growths cover the bottom, many rocks are actually bare even though the heavy filaments obscure the entire stream bottom. Under these conditions, random sampling of rocks would likely underestimate actual benthic biomass levels. At such sites, samples are collected by tossing a 30 cm diameter hoop (heavy gauge copper wire works well) at a random point in the 30 cm depth zone and collecting all the algae inside the hoop (any filaments extending beyond the hoop are cut off). The substrate found at the site should be noted since not all the rocks in the hoop may be 10-20 cm in size. Nine hoop samples are collected for a reach if all are hoop samples.

Use a Benthic Algae Site Visit Form for each site visited. Record each of the three composited samples collected from each of the three subsites on the Benthic Algae Site Visit Form. A total of nine samples will be collected at each site. Clearly document that each sample is a composite of two replicates.

Algae photos

At each sub-site, take at least one each of the following photo types:

(1) the surrounding landscape to document the sub-site's location,
(2) of the substrate within the indicator zone, and
(3) photos of substrate pieces used for sampling if water is too turbid for a benthic photo.

For each photo, record the photo number, photo type, and pertinent sub-site information on the Benthic Algae Site Visit Form.

Aquatic Plant Visual Assessment

During each site visit, field personnel will visually assess and record the general composition, amount, color, and condition of aquatic plants using the Aquatic Plant Visual Assessment Form (**Attachment A**). This information helps describe the health and productivity of the aquatic ecosystem, records nuisance aquatic plant problems, documents changes in the plant community over time, and can be used to help corroborate quantitative algae biomass results.

1. At each sub-site, evaluate the entire wetted stream bottom as it appears 5 meters above and 5 meters below the transect (i.e., 10 linear meters total).
2. Record Actual Cover in Channel: Refers to the aerial coverage of the stream bottom by the plant type in question, within the evaluation zone; circle the percent coverage category that most closely fits what you see.
3. Record Predominant Color: The colors of aquatic plants are clues to their identity, state of growth, and health of the aquatic ecosystem, record the predominant color that is observed of the plants or algae from the pick list, using the letter codes; be sure to lift your sunglasses to accurately view colors.
4. Record Condition: Aquatic plants go through seasonal cycles of growth, maturity, and decay. The condition of a plant or algae will indicate the approximate stage of this seasonal cycle. It can also help explain cases where, for example, AFDW to chlorophyll-*a* ratios are found to be unusually high. Growing plants and algae show new growth and bright colors. Mature plants and algae are larger but have more subdued colors because of age, epiphytes, and sediment deposits. Decaying plant and algae display a loss of both pigmentation and physical integrity. Record conditions as Growing, Mature, or Decaying on the form using the letter codes.
5. Record Thickness Category for Microalgae: Non-filamentous microalgae can be present on stones and fine sediment surfaces and can develop a fairly wide array of chlorophyll-*a* levels depending upon the mat thickness. The categories (Thin, Medium, Thick) will help corroborate chlorophyll-*a* and AFDW measurements collected and show the progression of algal growth at a site. Use a mm-scale ruler to measure the mat thickness.
6. Record Length Category for Filamentous Algae: Increasing length of filamentous algae has been associated with recreation impacts and highly enriched waters tend to grow long filaments, 1-2 meters or more in length at times. Record filamentous algae filament lengths as Short or Long on the form. When filaments are >2 cm in length, record their approximate lengths in the comments section.

4.0 SAMPLE HANDLING AND LABORATORY ANALYTICAL PROCEDURES

Appropriate storage times for water quality samples are shown in **Table 4.1**. All water quality samples will be immediately placed into a cooler with ice following collection. SRP samples will be frozen on dry ice in the field. Once all water chemistry samples are collected, BRPA will drop samples off in Missoula, MT, with MCWQD. Algae samples will be dropped off with Dr. Vicki Watson (University of Montana). All samples will be stored according to the preservation requirements in **Table 4.1** below

If samples are to be shipped the day following data collection activities, freeze applicable samples (**Table 4.1**) in a freezer overnight upon completion of field work. If samples are to be shipped immediately after data collection activities (on the same day), ship on ice; ensure that adequate ice is used to maintain samples at proper storage temperatures and use expedited shipping options to ensure that all samples arrive at the laboratory within required holding times (note short holding times for soluble reactive phosphorus of 2 days if not frozen, and 7 days for TSS). However, shipping should not be necessary for 2024 sampling.

Table 4.1 *Sample analytical methods, volumes, containers, preservation, and holding time for nutrient samples. Lower reporting limits than typically required by DEQ's monitoring suite are required for this type of trend monitoring project.*

Analyte	Method	Project Required Quantitation Limit	Container	Sample Volume	Preservation	Holding Time*
Total persulfate nitrogen	A4500-N-C	40 µg/l	Acid-washed HDPE	250 ml	Cool on ice in field (freeze if need be)	28 days (45 days if frozen)
Total phosphorus	EPA 365.1	2 µg/l		500 ml	H_2SO_4, cool on ice in field	28 days
Low level nitrate + nitrite-nitrogen (NO_2+NO_3-N)	EPA 353.2	2 µg/l				
Total ammonia-nitrogen (NH_3+NH_4-N)	EPA 350.1	10 µg/l				
Soluble reactive phosphorus	EPA 365.1	1 µg/l		120 ml	Field filter 0.45 µm, freeze with dry ice in field	45 days, if frozen 2 days if thawed
Total suspended solids	A 2540 D	4,000 µg/l		1000 ml	Cool on ice in field	7 days
Chlorophyll-*a*	A 10200H	Template: 2 mg/m^2	47 mm petri dish			90 days

		Hoop: 0.1 mg/m^2	OR zip lock bag		Prevent light exposure; cool on ice in field, freeze in lab	
Ash free dry weight	A 10300 C (5)	Template: 2 g/m^2 Hoop: 0.1 g/m^2	47 mm petri dish OR zip lock bag		Cool on ice in field; freeze in lab	120 days

* For samples stored on ice or refrigerated, unless otherwise noted.

Samples will be packaged appropriately to avoid damage and transported on ice (or dry ice if samples need to be frozen). COC/site visit forms will be completed in the field as each sample is collected and will be kept with the samples at all times. Field duplicates and field blanks will be entered on the same chain of custody form as the routine samples collected at a site. Whenever samples are transferred, the "relinquished by" portion of the COC/site visit form will be completed. BRPA volunteers will drop off water chemistry samples and COCs to the Missoula County Water Quality District office and will be stored according to **Table 4.1** until DEQ staff can transport the samples to Energy Labs in Helena, MT. Water chemistry samples and the completed Energy Labs COC will be delivered to Energy Laboratories by DEQ staff for nutrient analysis; the Routine Site Visit Forms will be retained by BRPA. Benthic algae samples and algae COC/site visit forms will be delivered to the University of Montana Watershed Health Lab.

Samples are kept in the dark on ice until they reach the lab. SRP samples must be frozen in the field and before transporting to the lab. If additional holding time is needed (**Table 4.1**), labs may freeze any sample other than TSS until thawed for analysis; otherwise, samples will remain cooled on ice. If more details on sample handling are needed, see *Attachment A* of the QAPP (Riedl 2022).

Algae template samples will be composited in the field; algae hoop samples will be stored individually in Ziploc bags. In the lab, hoop samples should be subsampled, then the subsamples will be composited to obtain chlorophyll-*a*. The remaining hoop samples will be analyzed for AFDW individually. Algae results should be reported as weighted average, referencing the Benthic Algae Site Visit form for total surface area sampled, in mg/m^2.

5.0 QUALITY ASSURANCE AND QUALITY CONTROL REQUIREMENTS

More detail about the quality assurance process for this project can be found in *Sections 3.0* and *5.0* of the QAPP (Riedl 2022).

5.1 Field Quality Control

Field personnel will collect field duplicates and field blanks (not trip blanks) for at least 10% of all water quality grab samples or at least once per sampling event (one station per event; **Table 2.1**).

> **FIELD BLANK** - Field blanks are used to determine the integrity of the handling of the samples, preservative and/or containers, and monitors whether site conditions and reagents are sources of contamination. Field blanks will consist of laboratory-grade deionized water transported to the field and poured into a prepared sample container and treated the same (i.e., same rinsing,

collection, preservation and storage protocols) as grab samples taken from the site. For filtered samples, de-ionized water is run through a filter and treated as a sample.

DUPLICATE SAMPLES - Field duplicates are used to determine the precision of the sampling and analytical process and monitor the homogeneity of the collected samples. Duplicate samples should be taken simultaneously and handled with the same procedures as the original.

Data quality indicators described in **Section 5.0** are the quantitative and qualitative criteria established for a sampling design in order to meet the project's objectives. The data quality indicators are used during the data review process to validate data.

5.2 Laboratory Quality Control

Immediately upon receipt of samples from the field, the laboratory personnel will:

1. Review the chain of custody form for completeness and for clarity of instruction;
2. Inspect the cooler to make sure the samples have been kept at the proper temperature; and
3. Inspect the samples for leakage or breakage and to confirm that sample labels are consistent with the chain of custody forms.

The laboratory personnel will log in and store samples in accordance with the laboratory's procedures and will immediately notify the field personnel if any deficiencies are observed upon sample receipt. The laboratory personnel will notify the quality assurance program if the deficiencies have compromised the analysis and results or if resolution of a sampling/analysis issue is needed. The laboratory will note any deficiencies in a case narrative.

The project labs must follow procedures consistent with their own QA plans and laboratory certification requirements, including sample tracking and chain of custody procedures. For laboratories performing water chemistry analyses, a quality assurance package must be provided with each analytical report. The laboratory quality assurance package will include a duplicate split sample, and spike, blank, and reference sample analyses. Lab splits are run on a minimum of every 20 samples for a given analyte to determine error due to lab procedures. During analysis, verification standards are run regularly to check instrument calibration. Matrix interference is assessed by spiking samples with a range of known quantities of the analyte (at a minimum of every 20 samples). The standards and spikes should be within the same concentration range as the samples being analyzed.

5.3 Instrument Calibration, Testing, and Maintenance

Field personnel will maintain all field instruments and sampling equipment in proper working order, with regular maintenance being performed as required by the manufacturer. Prior to mobilization to the field, personnel will inspect the equipment to make sure it is in proper working order. Field personnel will enter maintenance notes into the field logbook.

BRPA personnel will perform field instrument calibration for pH, conductivity, turbidity, and barometric pressure prior to the first sampling event each month, and calibration for pH and conductivity will be verified with YSI Confidence Solution® before the second sampling event each month. The ODO sensor will be calibrated at the start of each sampling day as described in **Section 3.2**.

More frequent calibration may be performed at the discretion of field personnel and if warranted by weather conditions or if problems with the instruments are suspected. Post-sampling calibration will be

used when warranted to verify accuracy. Calibration procedures will conform to manufacturer specifications. Any deviations from manufacturer specifications will be identified in the SAP or final project report.

All calibration data and notes will be entered into the field logbooks, instrument calibration sheets, and/or the field meter's internal log of calibration.

Laboratory instrument calibration and maintenance will be the responsibility of the analytical laboratory and is described in their respective laboratory quality assurance plans (DEQ 2022). The laboratory will retain records of maintenance and calibration for future reference.

5.4 Inspection of Lab and Field Supplies and Materials

Prior to mobilization to the field, all field monitoring supplies and materials will be inspected by the sampler to ensure they are in proper condition and working order. Any problems as well as application of maintenance requirements will be documented in the field and instrument logbooks. Extra monitoring supplies and containers will be brought into the field in the event that contamination or damage occurs.

6.0 Data Analysis, Record Keeping, and Reporting Requirements

Refer to *Section 4.0* of the QAPP (Riedl 2022) for more information about data management, reporting, and records management.

7.0 Training

Field samplers will either be experienced environmental scientists or will be trained and supervised by experienced personnel. All field samplers and data managers assigned to this project will adhere to sampling method protocols and standard operating procedures (SOPs) described in this SAP to ensure compliance with data quality objectives. The University of Montana (Vicki Watson) will train DEQ staff and BRPA volunteers on collecting algae samples. If required, DEQ will assist with training field personnel for sampling nutrients following DEQ's SOPs. The project manager will review and approve the selection of field personnel annually.

Nutrient samples are analyzed at Energy Laboratories and benthic algae samples are analyzed at the University of Montana following EPA-approved SOPs.

Prior to sampling, a briefing is held to review the requirements of the project in conjunction with the requirements of the QAPP. DEQ QA/QC personnel review the sampling procedures, and the project manager routinely provides onsite inspections during selected monitoring events. Field personnel are routinely involved with the development and/or modification of the QAPP, SOP, and SAP documents. The quality assurance officer or designee will discuss any QA/QC issues with the project manager but will not be involved in the data collection/analysis/interpretation/reporting process except in a review or oversight capacity.

8.0 CHANGES TO THE FIELD SAMPLING PLAN

Modifications to the approved plan will be documented in a post-season addendum to this SAP and reflected in subsequent year SAPs (as appropriate) and in the final report.

9.0 PROJECT TEAM AND RESPONSIBILITIES

Refer to *Section 1.6* of the QAPP (Riedl 2022) for more information about the project team.

10.0 REFERENCES

Montana DEQ. 2019a. Montana DEQ Nonpoint Source Program 20-Year Vision and Strategic Plan. Helena, MT: Montana Dept. of Environmental Quality.

Montana DEQ. 2019b. Montana's Water Quality Monitoring and Assessment Strategic Plan. Helena, MT: Montana Dept. of Environmental Quality.

Montana DEQ. 2019c. Bitterroot Watershed Focus Area Project Plan. Helena, MT: Montana Dept. of Environmental Quality.

Montana DEQ. 2021. Sample Collection and Laboratory Analysis of Chlorophyll-a Standard Operating Procedure. WQPBWQM-011, Version 8.0. Helena, MT: Montana Department of Environmental Quality, Water Quality Planning Bureau.

Montana DEQ. 2022. Quality Assurance Project Plan for Water Quality Planning Bureau Environmental Data Operations. WQDWQPBQAP-01, Version 1.0. Helena, MT: Montana Department of Environmental Quality, Water Quality Planning Bureau.

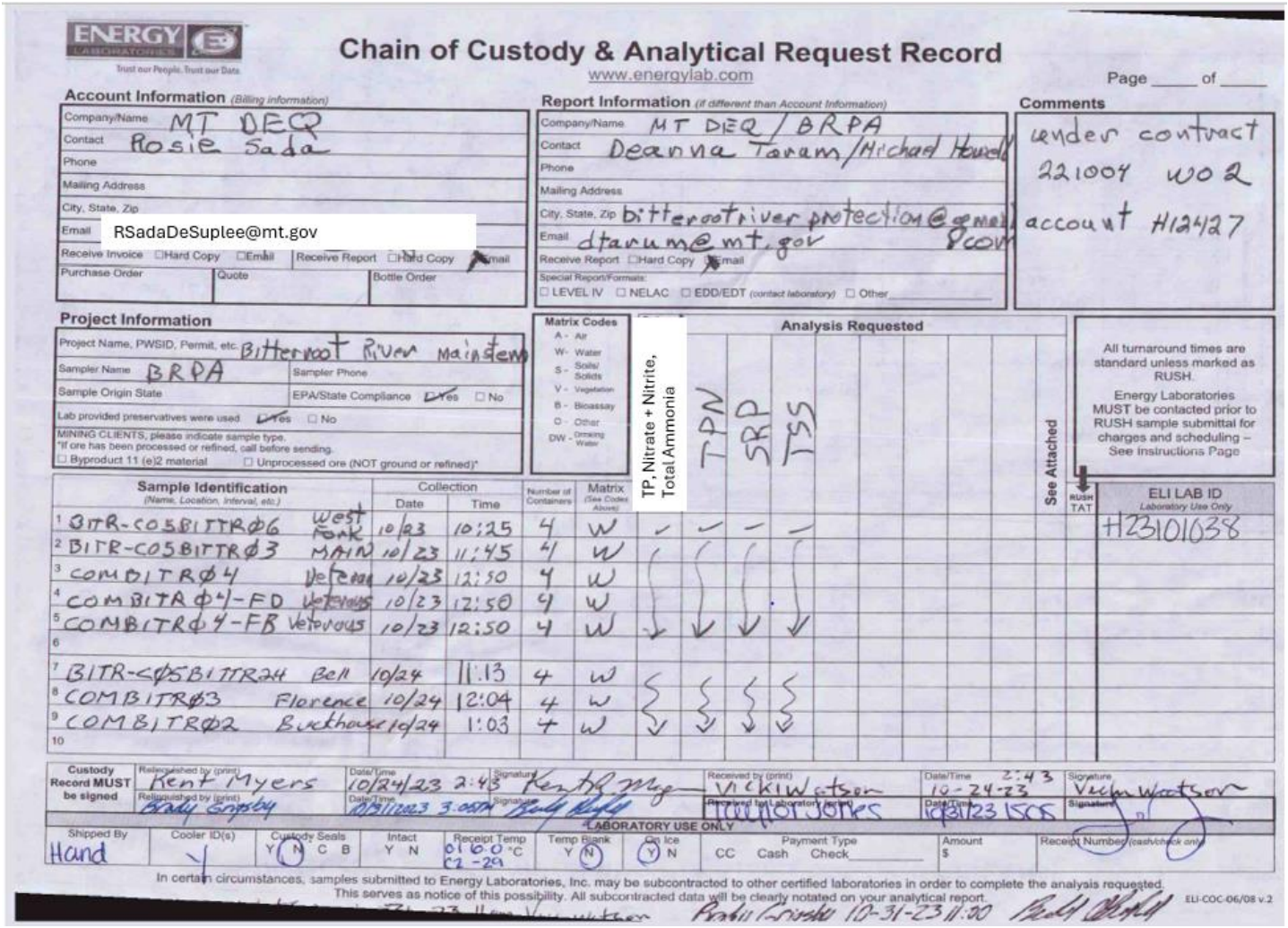
Riedl, Hannah. 2022. Quality Assurance Project Plan 2019-2039: Bitterroot Mainstem Long-term Nutrient Trends Monitoring. Document ID: BRMMASQAPP-19. Version 3.1. Helena, MT: Montana Department of Environmental Quality, Water Quality Planning Bureau.

Watson, V. 2018. Field Methods of the UM Watershed Health Clinic. Missoula, MT: University of Montana.

11.0 ATTACHMENT A

Site visit forms

Example Energy Labs Chain of Custody form.



ENERGY LABORATORIES
Trust our People. Trust our Data.

Chain of Custody & Analytical Request Record

www.energylab.com

Page _____ of _____

Account Information (Billing information)

Company/Name: MT DEQ
Contact: Rosie Sada
Phone:
Mailing Address:
City, State, Zip:
Email: RSadaDeSuplee@mt.gov
Receive Invoice: ☐ Hard Copy ☐ Email | Receive Report: ☐ Hard Copy ☒ Email
Purchase Order | Quote | Bottle Order

Report Information (if different than Account Information)

Company/Name: MT DEQ / BRPA
Contact: Deanna Taram / Michael Howell
Phone:
Mailing Address:
City, State, Zip: bitterootriver protection@gmail.com
Email: dtarum@mt.gov
Receive Report: ☐ Hard Copy ☒ Email
Special Report/Formats: ☐ LEVEL IV ☐ NELAC ☐ EDD/EDT (contact laboratory) ☐ Other

Comments

under contract
221004 WO 2
account H12427

Project Information

Project Name, PWSID, Permit, etc.: Bitterroot River mainstem
Sampler Name: BRPA | Sampler Phone:
Sample Origin State | EPA/State Compliance: ☒ Yes ☐ No
Lab provided preservatives were used: ☒ Yes ☐ No
MINING CLIENTS, please indicate sample type. "If ore has been processed or refined, call before sending.
☐ Byproduct 11 (e)2 material ☐ Unprocessed ore (NOT ground or refined)*

Matrix Codes: A - Air; W - Water; S - Soils/Solids; V - Vegetation; B - Bioassay; O - Other; DW - Drinking Water

All turnaround times are standard unless marked as RUSH.
Energy Laboratories MUST be contacted prior to RUSH sample submittal for charges and scheduling – See Instructions Page

Sample Identification (Name, Location, Interval, etc.)	Collection Date	Collection Time	Number of Containers	Matrix (See Codes Above)	TP, Nitrate + Nitrite, Total Ammonia	TPN	SRP	TSS	See Attached	RUSH TAT	ELI LAB ID (Laboratory Use Only)
1 BITR-CO5BITTRØ6 West Fork	10/23	10:25	4	W	✓	✓	✓	✓			H23101038
2 BITR-CO5BITTRØ3 MAIN	10/23	11:45	4	W	✓	✓	✓	✓			
3 COMBITRØ4 Veteran	10/23	12:50	4	W							
4 COMBITAØ4-FD Veterans	10/23	12:50	4	W							
5 COMBITRØ4-FB Veterans	10/23	12:50	4	W	✓	✓	✓	✓			
6											
7 BITR-CØ5BITTR24 Bell	10/24	11:13	4	W	✓	✓	✓	✓			
8 COMBITRØ3 Florence	10/24	12:04	4	W	✓	✓	✓	✓			
9 COMBITRØ2 Bicthouse	10/24	1:03	4	W	✓	✓	✓	✓			
10											

Custody Record MUST be signed	Relinquished by (print)	Date/Time	Signature	Received by (print)	Date/Time	Signature
	Kent Myers	10/24/23 2:43	Kent Myers	Vickie Watson	10-24-23 2:43	Vickie Watson
	Brady Snyder	10/31/2023 3:00PM	Brady Snyder	Heather Jones	10/31/23 1505	

LABORATORY USE ONLY

Shipped By	Cooler ID(s)	Custody Seals Y N C B	Intact Y N	Receipt Temp °C	Temp Blank Y N	On Ice Y N	Payment Type CC Cash Check	Amount $	Receipt Number (cash/check only)
Hand	✓	Y (N) C B		01.6.0 °C C2-29	Y (N)	(Y) N			

In certain circumstances, samples submitted to Energy Laboratories, Inc. may be subcontracted to other certified laboratories in order to complete the analysis requested. This serves as notice of this possibility. All subcontracted data will be clearly notated on your analytical report.

Brady Snyder 10-31-23 11:00

ELI-COC-06/08 v.2

Nutrient Site Visit Form:

BITTERROOT RIVER HEALTH CHECK
Bitterroot River Mainstem
ROUTINE SITE VISIT FORM

SITE NAME _____

SITE ID: _____ DATE: _____ ARRIVAL TIME: _____

SITE LOCATION: Latitude _____ Longitude _____ Elevation _____

SAMPLER(S) (Printed Names): _____

Signatures _____

OTHER PARTICIPANTS: _____

1. Place an air temperature thermometer in a shaded location and allow it to equilibrate. When stable, record the air temperature below.
2. Remove the YSI meter from its case, attach the cable to the handheld unit if not already connected, and turn the instrument on. Place the unit in the shade. Refer to the log book to confirm that the unit has been calibrated or verified (with YSI Confidence Solution) within last week.
   Calibration date: _____ Verification date (if appropriate): _____
3. Calibrate the ODO sensor at the first site of each sampling day (see **Section 3.2** of the SAP).
   ☐ Check box to confirm that the ODO sensor has been calibrated
4. Find a location to place the sensors that is well-mixed; is not excessively turbulent, stagnant or in an eddy; is free of upstream obstructions and disturbances (e.g., not directly downstream from swimming people or dogs); and is deep enough so that the sensors can be entirely submerged. Gently swirl and shake the sensor assembly to release any air bubbles that are trapped in the sensor guard, then position the sensors so that they are facing upstream and are not in silt or mud.
5. Observe the display screen and wait a minute or more until the numbers stabilize. NOTE: It is permissible to proceed with collection of the Nutrient Grab Samples while allowing the probes to equilibrate, but care must be taken to place the sensors upstream from where the samples will be collected to avoid any disturbances.
6. Record the field parameter values below, paying close attention to the units (e.g., °C vs. °F). Finally, log the values on the YSI handheld, taking care to select the correct sampling site.
7. Collect the Nutrient Grab Samples as described in **Section 3.3** of the SAP. Record the time of the first sample collected at the site. Use this time on all the bottle labels for the site.
   Time of first sample collected at site: _____
8. Document the condition of the site by taking photos looking upstream, downstream, and across stream. Note any unusual or notable circumstances in the space provided below.
   ☐ Upstream photo #_____ ☐ Downstream photo #_____ ☐ Across stream photo #_____

YSI Pro DSS screen parameters

(Air temperature)	_____°C	Spec. Conductance	_____µS/cm
Water temperature	_____°C	Total dissolved solids (TDS)	_____ mg/L
Barometric Pressure	_____ mmHg	pH	_____ (standard)
Dissolved Oxygen	_____ DO mg/l	NTU	_____NTU

YSI ProPlus parameters

(Air temperature)	_____°C	Spec. Conductance.	_____ µS/cm
Water temperature	_____°C	pH	_____ (standard)
Barometric Pressure	_____ mmHg	Turbidity: #1 _____	#2 _____ #3 _____
Dissolved Oxygen.	_____ DO mg/l	Average: _____cm.	_____NTU

Turn off YSI and place in shade or in carrying case once the field parameters have been logged.

SAMPLE CHECK LIST

	Sample	Duplicate	Blank
1. Total Suspended Solids (TSS)			
1 Liter bottle – White cap			
Rinsed three times in ambient river water and then filled?	_____	_____	_____
Firmly capped and placed in cooler?	_____	_____	_____
2. Total Persulfate Nitrogen (TPN)			
250 ml bottle – White cap			
Rinsed three times in ambient river water and then filled?	_____	_____	_____
Firmly capped and placed in cooler?	_____	_____	_____
3. Total Phosphorus (TP), Nitrite + Nitrate (NO_{2+3}), Ammonia (NH_{3+4})			
500 ml bottle – Yellow cap			
Rinsed three times in ambient river water and then filled?	_____	_____	_____
Preservatives (Sulfuric acid, H_2SO_4) added?	_____	_____	_____
Firmly capped and placed in cooler?	_____	_____	_____
4. Soluble Reactive Phosphorus (SRP)			
120 ml bottle – White cap - FILTERED			
Syringe rinsed 3 times in ambient river water and then filled?	_____	_____	_____
Bottle rinsed 3 times with FILTERED water and filled with			
FILTERED water (leaving room for expansion)?	_____	_____	_____
Firmly capped and placed in cooler with DRY ICE?	_____	_____	_____

DEPARTURE TIME: _____ AM PM

NOTES:

Algae COC and Site Visit Form (1 of 2)

Site Visit Form

PROJECT NAME: Bitterroot River Benthic Algae Volunteer Monitoring Program

Date:_____ Arrival Time:_____ am pm

Personnel Names:_____

Station Name ID (*circle one*):	COMBITR02 (Buckhouse)	COMBITR03 (Florence)	BITR-C05BITRR24 (Bell Crossing)	COMBITR04 (Veterans Bridge)	BITR-C05BITTR03 (Hamilton)	BITR-C05BITTR06 (Darby Bridge)
Latitude and Longitude:	46.83012, -114.05406	46.633056, -114.04917	46.4432, -114.123767	46.2792, 114.1606	46.2475, -114.17722	45.9725, -114.14111

SUBSITE 1: (indicator zone 30 cm deep; velocity ½ m/s; rocks 10-20 cm longest dimension)

Latitude_____ Longitude_____

Benthic algal samples collected (3 composites of 2 templates – 6 templates total):

# of templates:_____ # of hoops:_____ (only for heavy filamentous growth)

SUBSITE 2: (indicator zone 30 cm deep; velocity ½ m/s; rocks 10-20 cm longest dimension)

Latitude_____ Longitude_____

Benthic algal samples collected (3 composites of 2 templates – 6 templates total):

# of templates:_____ # of hoops:_____ (only for heavy filamentous growth)

SUBSITE 3: (indicator zone 30 cm deep; velocity ½ m/s; rocks 10-20 cm longest dimension)

Latitude_____ Longitude_____

Benthic algal samples collected (3 composites of 2 templates – 6 templates total):

# of templates:_____ # of hoops:_____ (only for heavy filamentous growth)

Date/ time samples were frozen: _____

Samples delivered to UM Watershed Health Clinic lab:

Relinquished by:_____ Date/Time:_____

Received by:_____ Date/Time:_____

Algae COC and Site Visit Form (2 of 2)

PHOTOGRAPH DOCUMENTATION

Subsite 1 – Photos of subsite location, substrate within indicator zone and algae on substrate	
Photo number	Photo Description
Was an Aquatic Plant Visual Assessment Conducted?	Yes No
Subsite 2 – Photos of subsite location, substrate within indicator zone and algae on substrate	
Photo number	Photo Description
Was an Aquatic Plant Visual Assessment Conducted?	Yes No
Subsite 3 – Photos of subsite location, substrate within indicator zone and algae on substrate	
Photo number	Photo Description
Was an Aquatic Plant Visual Assessment Conducted?	Yes No

COMMENTS

Example Aquatic Plant Visual Assessment Form

Print out DEQ of photos

Waterbody: Bitterroot Location: Veterans Bridge

Date: 9-14-19 Station ID:

Visit No.: 1

Subsite	1	Latitude 46° 16' 40" N	Longitude 114° 9' 40" W	
AQUATIC PLANT VISUAL ASSESSMENT FORM	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	G = Green GLB=Green/light brown LB= Light brown BR = Brown/reddish DBB =Dark brown/black	Gr = Growing M = Mature D = Decaying	Thin = < 0.5 mm thick Medium = 0.5-3 mm thick Thick = > 3 mm thick Short = < 2 cm long Long = > 2 cm long
	Actual Cover in channel (circle one)	Predominant Color	Condition	For microalgae & filamentous algae: Record thickness or length category
Microalgae	0 1 2 (3) 4	GLB	M	Medium / Short
Filamentous Algae	0 1 2 (3) 4	GLB	M	Thick / long
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			

COMMENTS

Subsite	2	Latitude 46° 16' 41" N	Longitude 114° 9' 40" W	
AQUATIC PLANT VISUAL ASSESSMENT FORM	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	G = Green GLB=Green/light brown LB= Light brown BR = Brown/reddish DBB =Dark brown/black	Gr = Growing M = Mature D = Decaying	Thin = < 0.5 mm thick Medium = 0.5-3 mm thick Thick = > 3 mm thick Short = < 2 cm long Long = >2 cm long
	Actual Cover in channel (circle one)	Predominant Color	Condition	For microalgae & filamentous algae: Record thickness or length category
Microalgae	0 1 2 (3) 4	GLB	M	Medium / short
Filamentous Algae	0 1 (2) 3 4	GLB	M	Medium / Short
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			

COMMENTS

Subsite	3	Latitude 46° 16' 43" N	Longitude 114° 9' 41" W	
AQUATIC PLANT VISUAL ASSESSMENT FORM	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	G = Green GLB=Green/light brown LB= Light brown BR = Brown/reddish DBB =Dark brown/black	Gr = Growing M = Mature D = Decaying	Thin = < 0.5 mm thick Medium = 0.5-3 mm thick Thick = > 3 mm thick Short = < 2 cm long Long = >2 cm long
	Actual Cover in channel (circle one)	Predominant Color	Condition	For microalgae & filamentous algae: Record thickness or length category
Microalgae	0 1 2 (3) 4	GLB	M	Medium / Short
Filamentous Algae	0 1 (2) 3 4	GLB	M	Medium / Short
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			

COMMENTS

There is a lot of loose microalgae here & lots of water bugs n crustaceans