QUALITY ASSURANCE PROJECT PLAN

MACROINVERTEBRATE INVESTIGATION: CHELAN RIVER, WA

Revised August 2017
Prepared by Terraqua, Inc. for Chelan County Public Utility District

Author Information

Keith van den Broek
Terraqua, Inc.
2989 Entiat River Rd.
Entiat, WA 98822
www.terraqua.biz

Publication Information

This QA Project Plan was prepared by Terraqua for the Chelan County Public Utility District (Chelan PUD), Project No. 15-73. For more information on the plan or to request data/reports for this project, please contact Steve Hays, steve.hays@chelanpud.org

Project Location

Chelan River, Chelan County, Washington
T27N R22E 13; R23E 18-19, 29-30

Approved by:

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Keith van den Broek, Author / Project Manager (Terraqua) Date

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Steve Hays, Project Manager (Chelan PUD) Date

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Abstract

This Quality Assurance Project Plan describes the intended methodology to be used in determining the baseline condition of the macroinvertebrate community assemblage in the Chelan River, Washington. The results of this study are intended to provide a baseline for measuring success in meeting the Biological Objectives outlined in the Lake Chelan Settlement Agreement. Data collection will occur in the spring and summer/fall of 2016, and be targeted to assess the biomass, taxonomic classification, resource class and/or size distribution of the drift and benthic macroinvertebrate communities of the Chelan River. Work will be completed by Terraqua, Inc., with laboratory support from Rhithron Associates, Inc. (Rhithron), for the Chelan County Public Utility District (Chelan PUD).

Background

Study Area and Surroundings

Drift and/or benthic macroinvertebrate community composition will be assessed within four sections of the Chelan River, WA (T27N R22E 13; R23E 18-19, 29-30; Fig. 1): 1) above the Lake Chelan Dam (0.75 rkm); 2) “Reach 1” between the Dam and the top of the Chelan River Gorge (3.45 rkm); 3) the engineered Habitat Channel located within “Reach 4” (0.55 rkm); and 4) the powerhouse tailrace near the Columbia River confluence (0.2 rkm).

Water in Reach 1 (Fig. 2) flows from Lake Chelan either through a low level outlet structure or from the spillway. The bed of this relatively low gradient (1%) section is primarily composed of large cobbles and small boulders, with smaller cobbles and gravels generally limited to the margins of the river channel. This reach is moderately confined by hill slopes composed of glacial moraine deposits. Most fine bed materials are flushed out of the river during annual spill events, but pockets of medium sized cobble and small gravels exist in some areas. Channel width through Reach 1 averages 28 m, and is primarily confined to a single channel except for a short (~640 m) braided section near the lower end of the reach. Riparian vegetation is scarce throughout Reach 1, with the most significant stands of riparian cover existing along the braided section.

The Habitat Channel is an engineered sinuous stream channel parallel to and upstream of the main tailrace. It is watered by the mainstem Chelan River, but has supplemental flow pumped from the tailrace during peak salmonid spawning periods in the spring and fall. Substrate varies from large cobbles to small gravel and some areas of sands. Riparian vegetation is thick, and primarily dominated by willows.

The section of the Chelan River above the Dam is backwatered and typically slow water velocity and depths >2 m. Substrate is composed of small and large cobbles, gravels, sand and some fines. The section of river below the tailrace and Habitat Channel contains similar substrate and depths, but is also influenced by eddy flows as it joins the Columbia River. It is primarily watered by the tailrace but also includes flows from the Habitat Channel and an ephemeral floodplain channel, primarily hugging the north shore of this section.

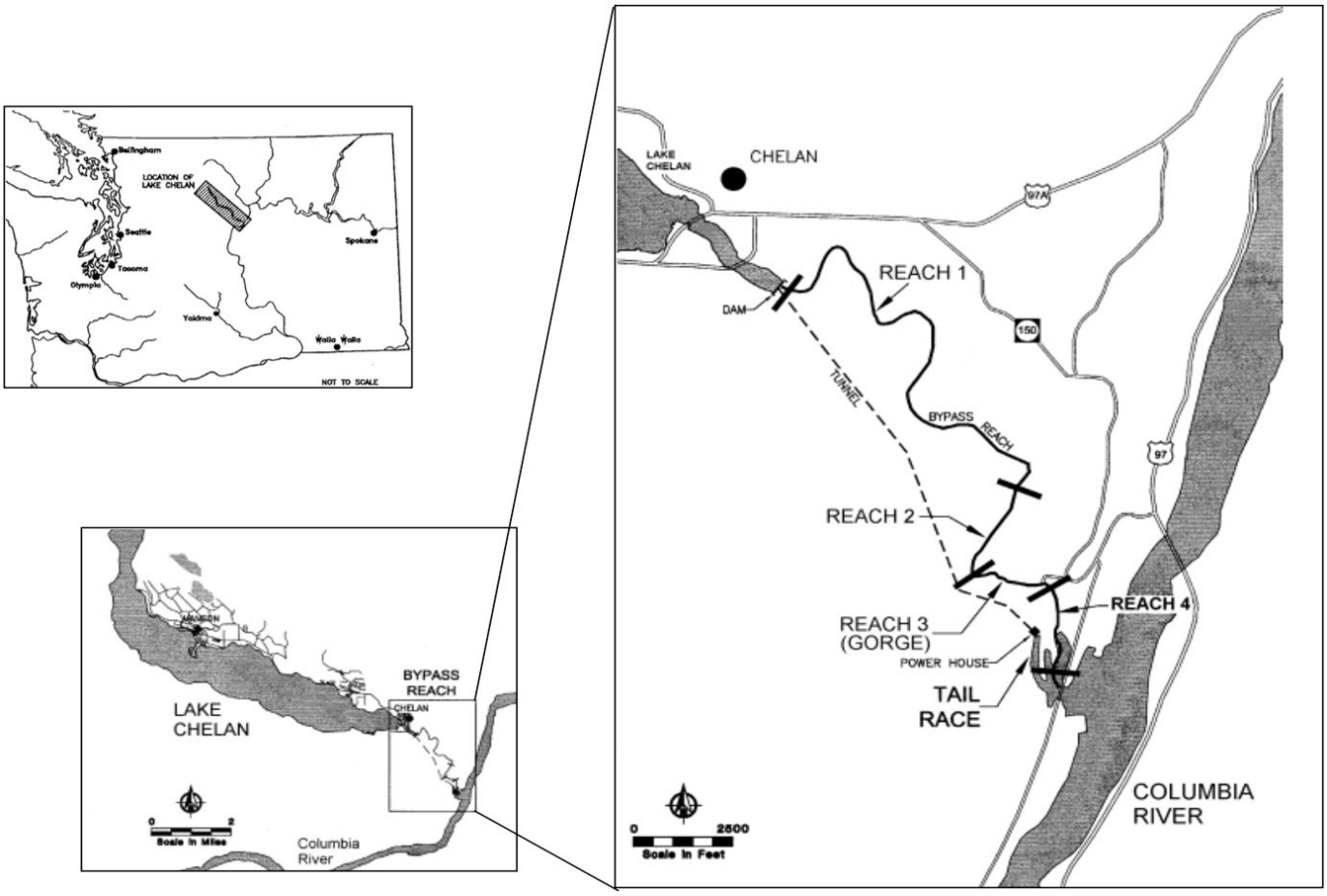


Figure 1. Lake Chelan Hydroelectric Project Area (from Lake Chelan Comprehensive Fishery Management Plan, 2003).

Logistical Problems

All sites are easily accessed by vehicle or short hike along maintained trails. The surface elevation of Lake Chelan is strictly regulated through spillway releases at the dam, which typically results in variable high flows in Chelan River during spring runoff or fall drawdown. There may, therefore, be a tight time window in which sampling can occur at wadeable conditions in Reach 1 and the Habitat Channel to meet the objective for samples representative of spring and fall biota. Researchers must work closely with Chelan PUD dam operators to ensure crew safety and successful data collection around these logistical constraints.

One small section (<100 m) of the upper portion of Reach 1 was subjected to aerial fire retardant during the 2015 Chelan Complex fires, and there is still visible retardant coating much of the substrate in this section. As this may have effected a short-term impact on the localized benthic biota, this section of river will be excluded from any sampling.

History of Study Area and Settlement Agreement Biological Objectives

The Lake Chelan Hydroelectric Project (Project; FERC No. 637) serves a dual purpose of generating power and regulating the level of Lake Chelan. The Lake Chelan Settlement Agreement (SA; October 2003) was developed during the FERC relicensing process for the Project. The SA established a minimum flow of 80 cfs for the Chelan River, which had previously been dry from August-May in most years since the Project began operation in 1926, and called for habitat improvement features in an engineered "habitat channel" and the dam's tailrace to provide spawning habitat in the lower river.

Mandatory monitoring and evaluation activities have been implemented through the SA. These efforts track and document progress towards achievement of established Biological Objectives and provide information to inform adaptive management strategies. The Biological Objective in Reaches 1-3 of the Chelan River is to create habitat to support a viable cutthroat trout population of 200 fish. The Biological Objectives for the Habitat Channel and tailrace spawning areas are to provide spawning and rearing habitat for Chinook salmon and steelhead, to document that these fish are using the new habitat, and to show evidence that adult fish production (returning adults) originated from fish spawning in this habitat.

A number of criteria were established to measure components leading to success in achieving the Biological Objectives, including water quality requirements and standards for egg-fry survival. Other monitoring and evaluation activities specified in the SA include fish surveys and monitoring of benthic macroinvertebrate populations, which is the subject of this research project.

Parameters of Concern

The macroinvertebrate population structure in the Chelan River is previously unstudied. Macroinvertebrate colonization of the upper river is probably limited to aerial colonization or downstream drift of invertebrates or material via spillway input from Lake Chelan, which may be dominated by taxa not suited to residence in riverine habitat. The tailrace and Habitat Channel may be populated through all three possible routes: aerial colonization, downstream drift, and upstream dispersal. Productivity in all reaches of the Chelan River may be limited by high summer stream temperatures, poor nutrient input from the highly oligotrophic Lake Chelan, and subject to periods of intense scouring during regulated spill. Any macroinvertebrate population prior to development of the SA in 2003 was likely eradicated seasonally when the river went dry.

Project Description

Goals and Objectives

The goal of this project is to determine baseline condition of the benthic and drift macroinvertebrate population assemblage in the Chelan River in order to provide a metric for measuring success in meeting the Biological Objectives outlined in the Lake Chelan SA. Data collection will occur in the spring and fall, and be targeted to meet the following objectives:

- Assess biomass and taxonomic classification of the benthic macroinvertebrate community of the Chelan River;
- Assess biomass, taxonomic classification, resource class and size distribution of the drift macroinvertebrate community of the Chelan River;
- Assess quantity of organic debris in the Chelan River;
- Identify taxonomic classification of the benthic macroinvertebrate community immediately upstream of the Lake Chelan Hydroelectric Dam and the benthic and drift macroinvertebrate communities immediately downstream of the tailrace in order to determine the contribution of these habitats via upstream dispersal or downstream drift to the macroinvertebrate communities in the Chelan River; and
- Compare Chelan River macroinvertebrate community structure with that of comparable stream systems, with an emphasis, if possible, on other lake-fed, warm water salmonid bearing streams in the Pacific Northwest.

Information Needed and Sources

The project will collect drift and benthic macroinvertebrate samples. Concurrent metrics will include stream flow velocity and water temperature at each drift net transect, water temperature at each benthic sample site, and alkalinity within each stratum at the time of sampling. The study will leverage existing Chelan PUD data sources for dissolved oxygen, pH, conductivity and total stream discharge.

Target Population

The project targets the macroinvertebrate communities existing in the water column and benthos within four unique reaches of the Chelan River during the spring and fall, 2016.

Study Boundaries

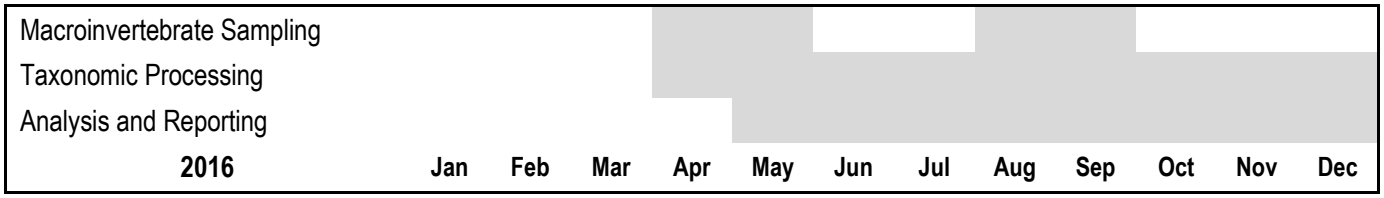
The study area encompasses the entire Chelan River excluding the gorge, which is considered poor habitat for macroinvertebrates and unsafe for researcher access. The river is stratified into four primary areas of interest, and Reach 1 is further stratified into three sections from which random sampling transects are chosen.

Organization and Schedule

Key Individuals

Project Manager (Chelan PUD) Oversight of project operations, deliverables and data quality assessment	*Steve Hays*
Contract Manager (Terraqua) Prime responsibility and authority over contractual obligations	*Michael B. Ward* President
Project Manager (Terraqua) Primary responsibility for project operations, analysis and reporting	*Keith van den Broek* Senior Fisheries Ecologist
Project Team (Terraqua)	
Contract technical representative	*Pamela Nelle*, Vice President
Study design & data analysis support	*Shubha Pandit*, Biostatistician
Assist with literature review, reporting	*Sara Smith*, Fisheries Ecologist
Assist with field sampling	*Various*, Ecological Technicians
Taxonomic Services Rhithron Associates, Inc.	Wease Bollman, Chief Biologist Jennifer Bowman, Vice President

Project Schedule



Quality Objectives

Measurement Quality Objectives (MQOs)

The goal of this study is to collect macroinvertebrate samples that are representative of community and ecological conditions present at the time of sampling. Samples will be collected using commonly adopted protocols similar to Adams (2010) and further developed and used by numerous regional monitoring programs, including the Pacific Northwest Aquatic Monitoring Partnership (PNAMP) (Hayslip 2007), Columbia Habitat Monitoring Program (CHaMP) (CHaMP 2015) and Large River Bioassessment Protocol (Flotemersch *et al* 2006). Taxonomic services will be provided by Rhithron following standard and widely accepted subsampling and measurement protocols (see Appendix A). This ensures data quality, and improves data comparability between reference streams.

Water temperature and flow velocity will be measured in situ. Accuracy is ensured by calibrating the instruments before and after use. Alkalinity will be measured in the field using direct reading titration. The titrator is calibrated in terms of total alkalinity expressed as parts per million (ppm) Calcium Carbonate ($CaCO_3$), +/- 4 ppm $CaCO_3$.

Targets for Precision, Bias and Sensitivity

Benthic sampling precision is affected by the extent of spatial variability between discrete sampling sites within a continuous reach, and drift sampling precision is affected by the extent of temporal variability in downstream drift past a discrete transect. Benthic sampling precision is improved by compositing eight replicate samples per reach. Drift sampling precision is improved by allowing collection nets to continuously sample over a long period of time (min. three hrs) determined to account for most natural variability in downstream drift.
Sampling bias is not quantifiable, but the study has been designed in a way that prevents introduction of systematic error. Habitat is relatively homogenous within each sample reach, and sites are distributed randomly across targeted channel unit types. Replication of composited sites follows widely adopted protocols and is assumed to be sufficient for an unbiased and representative sample. Sampling bias is further mitigated by strict adherence to sampling protocols and extensive training of experienced field personnel.

Targets for Comparability, Representativeness, and Completeness

The adopted field protocols for this project are widely used by several regional monitoring programs, allowing the collected data to be compared across programs and subbasins. Taxonomic analysis will be completed by a laboratory that is widely utilized for similar projects, using standard and comparable methodology. The Benthic Index of Biotic Integrity (B-IBI) will be used as a standard quantitative method for determining and comparing the biological condition of the macroinvertebrate community.

All sites except one are randomly selected and therefore representative of the complete range of physical conditions in all sample reaches. The single targeted drift sample site is representative of tailrace conditions in the only spatial area accessible to wadeable drift sampling protocols, and is considered sufficiently representative given the inherent constraints.

The target for data completeness is 100%. Sample contamination or dessication will be avoided by using appropriate sealed containers and shipping samples to the laboratory within 24 hours following completion of the sampling event. Sample loss is unacceptable given the small overall sample size, and therefore any samples lost will be replaced immediately with additional field effort if possible.

Sampling Process Design

Study Design

Sample events will represent the spring and fall macroinvertebrate communities, with two sample events included in the one-year study. Spring sampling will occur in April/May and fall sampling in August/September, contingent upon river conditions and logistical constraints.

Drift Sampling

We will use a probabilistic design for drift macroinvertebrate site selection within Reach 1 and the Habitat Channel, and a targeted design in the tailrace and above the dam, with a total of six drift sample sites and 11 drift samples collected during each sample event. Reach 1 is divided into three spatially balanced strata (upper, middle, lower), and the Habitat Channel, tailrace and above dam each comprise a single stratum. A master sample list defines transect coordinates at 50 m intervals within each stratum. One transect is randomly selected from each stratum for drift sampling. Drift nets will be set in suitable habitat, per protocol, within a maximum of 50 m upstream of the selected transect. If suitable habitat cannot be located within 50 m upstream of the chosen transect, this transect will be rejected and an alternate transect randomly chosen as a replacement. A targeted site will be chosen for drift sampling near the tailrace, as far downstream of the Habitat Channel as possible before water depth and/or flows exceed protocol parameters. The same transects will be sampled for drift in each season for comparability. Two drift nets will be set as replicates at each sampled transect, as far apart as possible for the conditions at that location. Each replicate drift net will represent a single unique sample, resulting in two samples per site, except that upstream of the dam, a single drift sample will be collected using a trawled net, and all trawls will be composited into a single sample for this stratum.

Benthic Sampling

Benthic macroinvertebrate sampling sites will be selected randomly by field crews to represent up to eight different riffle or fast-water habitats within each stratum, as defined above with the addition of strata in the reaches above the dam and below the tailrace, with a total of 16 replicate sites sampled in each stratum. If insufficient riffle or fast-water habitat units are present within a stratum, such as will be expected above the dam and below the tailrace, sites will be randomly selected based on accessibility and substrate. The 16 sites will be broken into two separate samples per stratum, with eight sites composited into each unique sample, for a total of two benthic samples per stratum and 12 benthic samples total collected during each sample event: Reach 1 Upper, Reach 1 Middle, Reach 1 Lower, Habitat Channel, Tailrace, and Above Dam.

Additional Data

Stream flow and temperature will be measured at the inlet of the drift sampling nets at each transect at the beginning and end of each set. Stream temperature will also be measured concurrent to each benthic sample collected. Alkalinity will be measured near the mid-point of each stratum, once per sample event.

Sampling Procedures

Field measurement and field sampling SOPs

Benthic sampling within wadeable areas will follow PNAMP protocols (Hayslip 2007). Within each of the six sampling sections, a total of 16 ft^2 of stream bottom will be sampled and composited into two samples for taxonomic processing. These will be representative of 16 randomly selected 1 ft^2 sites within each section as previously discussed. All benthic samples will be collected using a 1 ft x 1 ft D-frame kick net with 500 µm mesh.

Drift sampling within wadeable areas will follow CHaMP protocols (CHaMP 2015). In all strata except Above Dam, two replicate samples will be collected at the probabilistically selected or targeted transect within suitable riffle or fast-water habitat. Drift nets (40 cm x 20 cm, 1000µm mesh) will be set for a minimum of six hours at each transect. They will be deployed at least two hours after sunrise, and retrieved at least two hours before sunset. Replicate samples are considered as a single sample per net, resulting in two samples per transect for taxonomic processing. Flow velocity entering the net mouth will be recorded at the start and end of sampling in order to calculate sample volume.

Benthic and drift samples will be collected by boat at the non-wadeable sites found in the Above Dam stratum. Average depth within this reach is 7 m at average spring and summer lake surface elevations, and sampled depth will not typically exceed 10 m. Sixteen replicate benthic samples will be collected in this stratum using a 6”x6”x6” AMS Ekman dredge sampler. The composited samples will be filtered through a 500 µm mesh net prior to preservation and holding. Drift samples will be collected using a single drift net as previously described, modified with a rigid metal frame and trawled from a downrigger by a motorized vessel. A minimum of two trawls will be conducted at depths of 3 m and 9 m along 2 random transect lines of approximately 500 m each, extending from the marker buoys above the dam to the Woodin Ave. bridge. The total covered trawl distance will be recorded as a GPS track log for measurement of sample volume.

Containers, Preservation, Holding Times

All benthic and drift macroinvertebrate samples including invertebrates and organic matter will be retained in sample jars containing 95% EtOH (decanted prior to shipping). All samples will be refrigerated within eight hours of collection, and shipped to Rhithron for taxonomic processing within 24 hours of completion of the sample event.

Invasive Species Evaluation

Evaluation of invasive species is not an explicit objective of this study; however, any invasive species captured will be identified during taxonomic processing.

Equipment Decontamination

Our equipment decontamination protocols follow the guidelines in the Invasive Species Management Protocols (WDFW 2012). All non-watershed specific field gear including waders, wading boots, sampling nets, etc. will be decontaminated following Level 1 and/or Level 2 decontamination protocols before being used in any other watershed. Our preferred method for Level 2 decontamination is freezing equipment at -10°C for a minimum of 8 hrs. Any gear which is not able to be decontaminated in this manner will be cleaned with Vikron detergent.

Sample ID

Sample jars will be labeled with project name, site ID, date, time, replicate and sample type.

Chain-of-Custody

A chain-of-custody (COC) document will be completed, and emailed to Rhithron upon shipment of samples. COC hard copies will be included in the shipment, and retained for our records. Rhithron will email confirmation upon receipt of the samples.

Measurement Methods

The Laboratory Quality Assurance Plan is included as Appendix A. Per standard protocols, Rhithron will randomly subsample each sample using a Caton grid subsampling device, and select a total of at least 500 (benthic) or 600 (drift) individual organisms for taxonomic identification to the lowest practical level and body length measurement consistent with WDOE and CHaMP protocols. A single dry mass measurement of all organic drift detritus will be recorded for each drift sample. Drift organisms will be sorted by resource class (aquatic organisms, emergent adults, terrestrial organisms), life stage (pupa, larva, adult, unknown) and size bin (3mm) for reporting of dry weight. Taxa and counts for each sample will be entered into Rhithron's customized laboratory information management system with standard metric calculations for aquatic invertebrate assemblages made using Rhithron's customized database application. Final invertebrate data will include sample identifiers, taxon names, counts, life stages, uniqueness designations, qualifiers and proportion of sample sorted. Summer samples will be processed and data returned within 90 days. Fall samples will be processed and data returned within 120 days.

Data Management Procedures

Field metadata are recorded electronically using a custom data collection app on either an Apple iPad Mini 2 or iPhone 6. Waterproof field journals are used as a backup in the case of technical issues. Recorded metadata associated with each drift sample include site ID, date, start/end time, start/end stream temperature, start/end flow velocity, physical habitat description and GPS coordinates. Recorded metadata associated with each benthic sample include reach number, replicate number, date, time, stream temperature, physical habitat

description and GPS coordinates. Additionally, alkalinity measurements are recorded for each reach on the date of sampling, and field notes each day include weather and environmental conditions, potential concerns or sources of bias, and notable observations.
Data collected electronically in the field are automatically backed up to a cloud server when the data collection device is within range of a cellular data or Wi-Fi network. Data are then manually downloaded and backed up to a local computer as .csv formatted spreadsheets, allowing compatibility with a range of software packages used for analysis.
Laboratory analysis results are stored on a local server hard drive, and disseminated electronically to the project managers upon completion of analysis. Physical sample materials are archived by Rhithron for up to one year (see Appendix A).

Audits and Reports

Rhithron will submit taxonomic analysis results to the project lead within 120 days of receipt of samples. Any problems and associated corrective actions will be reported to the project manager immediately. Unresolved problems may result in replacement samples if feasible, or dropping replicates from the analysis as a last resort.
The project manager will provide task summary reports to the project team within 30 days of completion of each field sampling event, and a final report within 90 days of completion of all field sampling and subsequent analyses.

Data Verification (Quality Control)

Data verification will ensure data are free of errors and omissions, and comply with MQO standards as discussed. Field staff are to validate all samples prior to leaving the site. This includes verification that all data specified in the Sampling Process Design were obtained, confirming correct labeling of each sample jar, and verification that all metadata fields have been populated completely and accurately. Any anomalous observations will be corrected or annotated as appropriate. The project manager at the taxonomic laboratory will provide data quality control (QC) per Appendix A.

Data Quality (Usability) Assessment

Data completeness will be assessed by examining the number of samples collected and analyzed compared to the sampling plan. Data usability is based on the laboratory's ability to yield taxonomic analysis results for each sample. The project manager will use professional judgment to determine that MQO standards were met for precision, bias and sensitivity. Samples must be representative of the population at large, and any extreme values (e.g., unexpected or dissimilar to comparison rivers) will be logically explained, or identified as potential indicators of bias.

Data from external efforts will be accepted for comparative analysis as long as they are collected using similar protocols and represent geographically close and/or geomorphically similar watersheds.

References

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Hayslip, Gretchen, editor (2007). Methods for the collection and analysis of benthic macroinvertebrate assemblages in wadeable streams of the Pacific Northwest. Pacific Northwest Aquatic Monitoring Partnership, Cook WA

Washington Department of Fish and Wildlife (WDFW; 2012). Invasive Species Management Protocols Version 2. WDFW Invasive Species Management Committee.

Appendix A

Rhithron Associates, Inc.

Laboratory Quality Assurance Plan

Quality Assurance/Quality Control
Policies and Procedures

Version 16.1.a
Revised January, 2016

Corporate Approval

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Wease Bollman — Date
President / Chief Biologist
Rhithron Associates, Inc.
33 Fort Missoula Road
Missoula, MT 59804

_____ _____

Jennifer Bowman — Date
Vice President
Rhithron Associates, Inc.
33 Fort Missoula Road
Missoula, MT 59804

Document Name: Laboratory Quality Assurance Plan	Document revised: January 22, 2014 Effective date: Date of last signature
Document number: Revision 14.1.d	Issued by: Rhithron Associates, Inc. Chief Biologist

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1. Macroinvertebrate project management

g. ***Scope and quality objectives:*** Rhithron processes and identifies macroinvertebrate samples from clients throughout North America. The data generated from these samples needs to be consistently and reliably generated to support the uses to which the data is put, typically, to assess water quality and habitat integrity in surface water systems. Various methods and protocols are applied to samples, depending on client-specifications and project goals. Thus, samples must be handled with the utmost attention and care, the client-specified protocol including required taxonomic resolution must be faithfully followed. All client-required deliverables must be quality-assured and delivered within specific timeframes. Quality control/quality assurance (QA/QC) systems must be implemented, and all procedures and protocols, including QA/QC procedures and results must be documented and delivered along with other project deliverables.
The potential for introducing uncertainty into macroinvertebrate sample analysis arises at multiple places in the process. Implementation of all provisions of the Standard Operating Procedures for Macroinvertebrate Sample Analysis (SOP, current version 13.2.c) will allow the qualified, trained staff to meet data quality objectives (DQO) for all projects. Rhithron's internal DQOs for macroinvertebrate sample analysis are summarized as follows:

   i. All chain-of-custody documentation is maintained
   ii. Sample sorting efficiency is maintained at greater than 90% for each sample
   iii. Taxonomic accuracy and precision is maintained at ≥95%(Bray Curtis similarity), ≤5 Percent Difference in Enumeration (PDE) and ≤10 Percent Taxonomic Disagreement (PTD).
   iv. Bias is minimized, representativeness, comparability, and completeness of data is maximized.
   v. All client project requirements and specifications are met or exceeded
   vi. Quality-assured, completed projects are delivered by the client's specified due date.

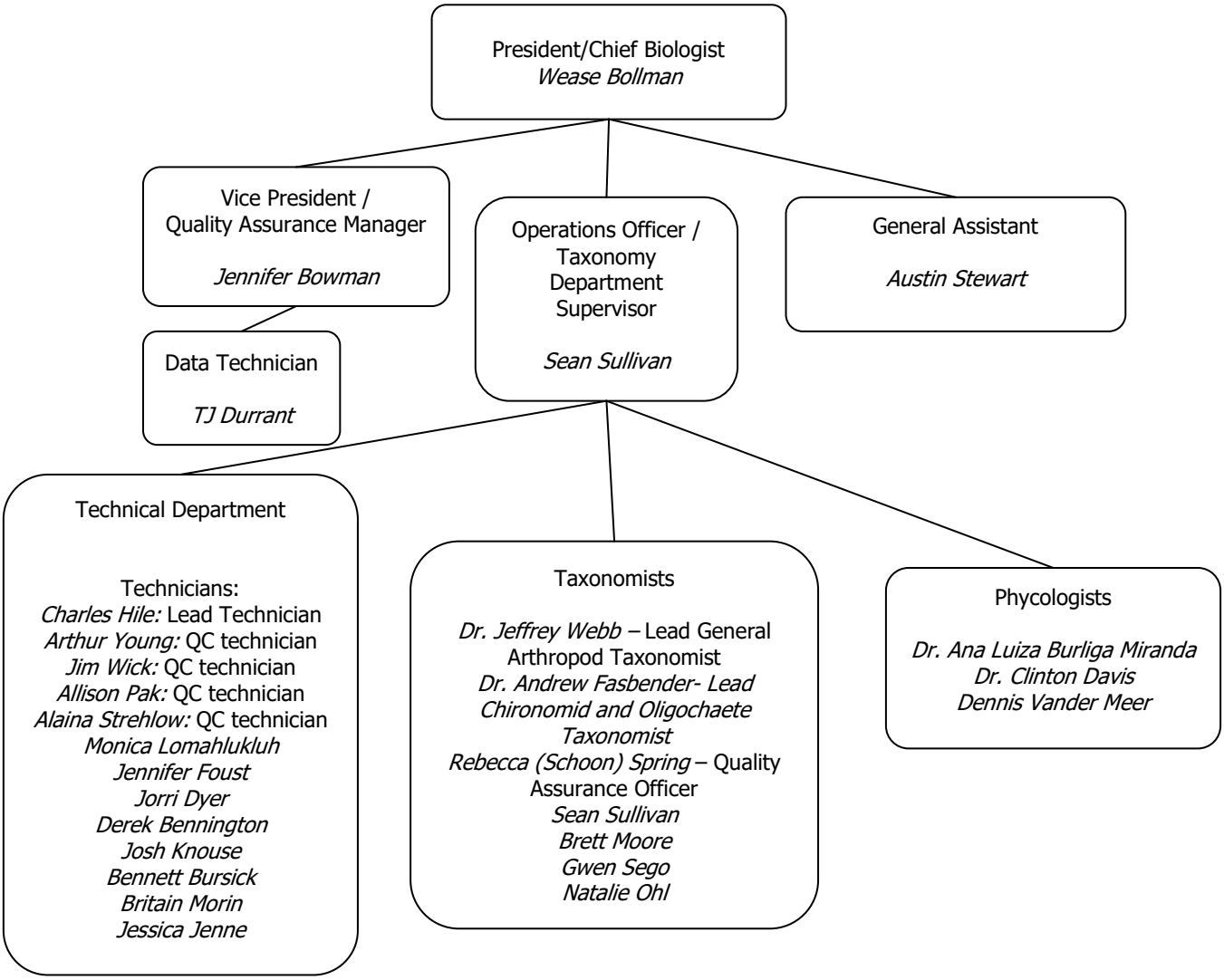
h. ***Laboratory organization:*** The organizational chart in Figure 1 shows the Rhithron personnel responsible for the various tasks associated with macroinvertebrate and periphyton sample analysis, and illustrates the pathways of communication that are used to assure the quality of Rhithron's work.

   i. ***Responsibilities related to the analytical protocols:*** Variations for individual projects are communicated to the Technical staff by the Lead Technican at weekly meetings, where projects scheduled for the upcoming week or on-going projects are discussed and reviewed. Specific project guidelines are printed on the inventory/sign-out sheet, which is available at all times to the technicians. QA/QC procedures are implemented in the Technical Department by trained QC technicians; a minimum of 10% of samples processed by technicians at Rhithron are randomly selected by the Operations Officer and subjected to QA/QC procedures that evaluate sorting efficiency. QC Technicians record sorting efficiency for each sample on sample benchsheets. QA/QC failures are addressed immediately by technicians. Periodic comparisons of subsample similarity are performed on randomly selected samples at least once per week. Random selection of samples for this QA/QC check is provided by the Lead Technician. Oversight of these functions is provided by the Lead Technician and the Operations

Officer. The Lead Technician enters sorting efficiency statistics for every sample into the Rhithron database.

Specific Protocols and Procedures related to sample analysis and QA/QC for individual projects are communicated to the taxonomy staff by the Taxonomy Project Manager,

Figure 1. Rhithron Associates, Inc. organizational chart: January 2016



who assigns taxonomists to projects. The Protocol and Procedure (P&P) document specific for each project records project specifics, including QC protocols. P&P documents are kept in a manual in the taxonomy laboratory and are also available to taxonomists on the Rhithron network server. The Vice President randomly selects a minimum of 10% of completed samples, and re-identification of these samples is assigned to taxonomists by the Taxonomy Department Quality Assurance Officer. The Quality Assurance Officer calculates sample similarity statistics and provides these to the Taxonomy Department Supervisor, who institutes corrective action where needed. Corrective action may involve review of taxonomic determinations, additional QA/QC for the project, or sending specimens to systematic authorities for verification.

ii. ***Responsibilities related to the QC functions for sample analysis***:
The Lead Technician is responsible for the implementation of sample processing QA/QC procedures involving sorting efficiency. Standard operating procedure requires at least 10% of samples to be evaluated for sorting efficiency; these checks are performed immediately after a sample is processed. The QC Technician is selected by random rotation; thus the QA/QC process is shared by all trained QC Technicians. Sorting efficiency results are compiled by the Lead Technician, who institutes additional training for technicians with poor sorting efficiency statistics. Failure of the subsample similarity procedure results in review of sample handling procedures by all technical staff.

QA/QC procedures for taxonomy fall under the authority of the Taxonomy Department Supervisor and Taxonomy Department Quality Assurance Officer, who review all sample similarity statistics other QC parameters and identify areas in taxonomic determinations or enumeration that require corrective action. The Quality Assurance Officer generates similarity statistics and other QC parameters for comparison of identifications and enumerations, and implements corrective actions when needed. The Taxonomy Department Supervisor assures that corrective action is taken by taxonomy staff members. Corrective action may include additional QA/QC for a project, or submittal of specimens to systematists for verification of identification. The Taxonomy Department Supervisor indicates to the Quality Assurance Officer when additional QA/QC is needed for a project.

i. ***Training/Certification:*** Quality analysis of macroinvertebrate samples requires a laboratory staff with extensive training and experience.
    i. Laboratory technicians perform macroinvertebrate sample sorting: each technician completes an extensive step-by-step in-house training program. Each laboratory technician is required to maintain an average sorting efficiency of ≥90%.
    ii. Quality control technicians perform sample sorting, and are additionally responsible for the quality checks on at least 10% of samples in each project. QC technicians have at least one year of experience in the technical laboratory, and have passed written and practical examinations that

document their understanding and proficiency at providing sorting QC. They are also required to maintain sorting efficiency for their own samples at ≥90%.

iii. Staff taxonomists hold SFS (Society for Freshwater Science, formerly the North American Benthological Society or NABS) Level 2 certifications in taxonomic groups in which they work.

iv. The Taxonomy Department Quality Assurance Officer holds multiple SFS Level 2 certifications, and has at least 3 years of experience in the Taxonomy Laboratory.

j. ***Documentation and records***:

i. Samples are received and sample metadata is logged into the Rhithron database (RAILISv.1.2.1) by the Data Technician. Logging in samples involves comparing the information on a chain-of-custody document to the information on sample container labels. An internal inventory is produced, and each sample is assigned a unique Rhithron identifier (RAI number). The internal inventory is printed out and serves as an inventory/sign-out sheet when the project is in the custody of the technical department.

ii. The chain-of-custody (COC) document is signed by the Data Technician after sample log-in, and the Data Technician confers with the client about discrepancies, damage, or other problems with the samples as they have arrived.

iii. A copy of the COC is made, and the original is returned to the client. Internal COC records for transfer of samples between departments are kept on the COC copy made at this time.

iv. Transfer of sample custody within the Technical Department is recorded and tracked on the inventory/sign-out sheet.

v. Sample processing information is recorded by sorting technicians on paper benchsheets and these data are transferred to the Rhithron database by the Lead Technician.

vi. Transfer of sample custody within the Taxonomy Department is recorded on a sample COC sheet created by the Taxonomy Department Project Manager.

vii. Taxonomic and count data, and all associated data generated by taxonomists is entered into the EPIC (v.1.7) data entry program, from which it is uploaded to the Rhithron database. Data output in the form of taxa and metrics lists are generated for each sample. Both paper and electronic formats can be generated.

viii. Processed and unprocessed sample remnants are either retained and stored in a secure facility or returned to clients.

ix. The Rhithron database resides on the Rhithron network server, which is backed up daily both externally and to 3 internal drives.

k. ***Data generation and acquisition for macroinvertebrate analysis:*** Processing, analytical and archival methods for macroinvertebrates follows specified standard operating procedures and relies on standard resources and references. Detailed procedures are found in the Rhithron Standard Operating Procedures (SOP: current version 13.2.c).

i. Performance objectives: Technical laboratory (sample sorting): The goal of sample processing is to sort invertebrates from substrate in such a manner

that results in an unbiased, representative subsample containing the appropriate number of organisms. The number of organisms is typically determined by the project specifications.

1. ***Objectives:*** Aspects of sample processing by Rhithron's technical staff that are important to subsequent data quality include the following:
    - ***a.*** The target count of organisms is achieved within the specified tolerance limits.
    - ***b.*** The client-specified protocol is faithfully followed.
    - ***c.*** Sorting efficiency is maintained at an average level no lower than 90% for each project, thus assuring sorting accuracy and precision.
    - ***d.*** The appropriate paperwork is associated with the correct sample.
    - ***e.*** All data pertinent to the sub-sampling procedure, including fraction of sample used to obtain the target number of organisms, condition of the sample, any problems associated with sorting, and quality assurance procedure outcomes and statistics, etc. are recorded on sample benchsheets.
2. ***QA/QC plan:*** Accomplishment of these performance objectives is evaluated by the following QA/QC plan.
    - ***a.*** Target count: Under-processed samples are detected at the time of taxonomic identifications by the taxonomists or at the time of data entry by the Lead Technician. If the sample was not fully picked in the processing stage, under-processed samples are revisited by the sorting technician, who distributes the unpicked sample portion into the appropriate number of Caton tray grids, and sorts the sample until the target count is reached.
    - ***b.*** Adherence to specified procedure: Daily oversight by the Lead Technician assures that client-specific protocols are followed in the technical department. Documentation for each project in progress is reviewed periodically.
    - ***c.*** Sorting efficiency: Quality control procedures for initial sample processing and subsampling involves checking sorting efficiency. These checks are conducted on at least 10% of the samples by independent observers who microscopically re-examine 100% of sorted substrate from each QC sample. All organisms that were missed are counted. Sorting efficiency is evaluated by applying the following calculation:

$$SE = \frac{n_1}{n_2} \times 100$$

where: SE is the sorting efficiency, expressed as a percentage, n_1 is the total number of specimens in the first sort, and n_2 is the total number of specimens in the second sort plus the first sort. Sorting efficiency is recorded on the benchsheet, and this data is entered into the Rhithron database.

d. Correspondence of sample and paperwork: Two technicians check the correspondence of sample and paperwork before each sample is processed. Technicians check the RAI number, the client's sample identifiers, and the number of jars associated with that sample. Both technicians sign the benchsheet, which is generated by Rhithron's database for each specific sample, when this step is completed. Using a "buddy" system insures that there are no mismatches between labels, spreadsheets, other data materials and the corresponding sample. Correct labeling of the sample fractions resulting from the processing procedures is assured by the provision of database-generated labels, which are attached to the benchsheet for each sample.

e. Complete recording of appropriate sub-sampling data: Benchsheets for samples that have been processed are collected daily by the Lead Technician, who checks for completeness of sub-sampling data, checks for missing data, and enters this data into the Rhithron database. Since these checks are performed daily, obtaining the data for each sample is assured.

f. Corrective actions: If 90% sorting efficiency is not achieved for a given sample, a failure is recorded on the benchsheet and in the database. A failure of any sample triggers assessment of an additional 10% of samples. For large projects, additional QC samples may be stratified by the technician whose sample failed the QA/QC check. Sorting efficiency statistics for each technician and for the entire laboratory are reviewed monthly. Sorting efficiency for each project is reported to the client in the technical summary document. Technicians who do not maintain the target sorting efficiency are given remedial training, and larger portions of the samples they process are examined for the sorting efficiency test until they are able to maintain the target sorting efficiency.

ii. Performance objectives: Taxonomy Department (macroinvertebrate identification and enumeration): The goal of the taxonomic portion of sample processing is to identify and enumerate organisms accurately and precisely, to the taxonomic resolution required by the project. Bias is minimized and data is reported completely. Materials related to the project, including labeled microscope slides, labeled vials with identified organisms, and laboratory benchsheets are handled carefully and are archived on the completion of identification and enumeration, and after all QA/QC procedures and data reviews have been completed. Deliverables such as voucher collections are assembled accurately and completely. Higher levels of taxonomy applied to organisms that cannot be identified to taxonomic targets are explained and qualified in all cases. Life stages are accurately recorded in the data.

1. ***Objectives:*** Aspects of invertebrate identification and enumeration by Rhithron's taxonomy staff that are important to subsequent data quality include the following:
    - ***a.*** The accuracy and precision of identifications and enumerations is maintained such that Bray-Curtis similarity between quality checked samples is 95% or greater, the Percent Difference in Enumeration (PDE) is 5% or less, and the Percent Taxonomic Disagreement (PTD) is 10% or less.
    - ***b.*** Bias is minimized, and data completeness is assured.
    - ***c.*** The client-specified protocol, including specified target number of organisms and the required taxonomic resolution, is faithfully followed.
    - ***d.*** All client-requested deliverables are provided, including reference collections.
    - ***e.*** A summary of QA/QC procedures and results, and sample processing procedures is documented and delivered along with client-requested deliverables.
2. ***QA/QC plan:*** Accomplishment of these performance objectives is evaluated by the following QA/QC plan.
    - ***a.*** Accuracy of taxonomy is evaluated by adherence to target taxonomic resolution requirements, and by the use of appropriate technical taxonomic literature or other references (e.g., identification keys, voucher specimens). Taxonomic precision is assessed by the re-identification of a randomly-selected 10% of samples in a blind procedure. The results of the QC process are evaluated by the calculation of the Bray-Curtis similarity, the PDE and the PTD.
        - ***i.*** The percent taxonomic disagreement (PTD) is calculated by the following equation:
        $PTD = (1 - \left(\frac{comp_{pos}}{N}\right)) \times 100$ where $comp_{pos}$ is the number of agreements and N is the total number of organisms in the larger of the 2 counts. The lower the PTD, the more similar are taxonomic results and the overall taxonomic precision is better. Rhithron's quality objective for PTD is 10% or less.
        - ***ii.*** The percent difference in enumeration (PDE) is calculated by the following equation:

        $PDE = \frac{|n1-n2|}{n1+n2} \times 100$ Where *n1* is the number organisms counted by the original taxonomist, and *n2* is the number of organisms counted by the QC taxonomist. The lower the PDE, the more precise the enumeration.

iii. Rhithron's quality objective for PDE is 5% or less. The Bray-Curtis similarity (aka Sorenson similarity index) is calculated by the following equation:

$$\boldsymbol{Similarity_{ij}} = \frac{2C_{ij}}{S_i + S_j}$$ Where C_{ij} is the sum of the lesser value of a taxon in common between both samples. S_i and S_j are the total number of organisms counted in each sample.

b. Bias is minimized by the use of taxonomic literature and resources that are accepted by the industry and reflects the most current accepted nomenclature. A bibliography of Rhithron's taxonomic library is maintained in a literature database. Consultation with experts and systematists occurs frequently. High quality optical equipment is used and regularly maintained. Geographic distributions of identified animals are checked and experts consulted when uncertainties arise, to assure credible identifications.
c. Data completeness is addressed by indicating reasons why taxonomic targets are occasionally not met. These are essential data components that are required by the EPIC (v.1.7) data entry program. Reasons include: damage to specimens, poor preservation, early instar or immaturity, and life stage. When metric calculation is required by a project scope, these specimens are included in the calculation of compositional metrics or tolerance indices, but are not included in calculations of richness metrics unless their uniqueness from other specimens is confidently ascertained.
d. Corrective actions include:
    i. Taxonomic discrepancies are examined and discussed by the original taxonomist and the QC taxonomist. Discussions may include the Taxonomy Department Supervisor, Project Manager, Quality Assurance Officer as well as other staff taxonomists. Discrepancies and disagreements that cannot be resolved internally are submitted via vouchered specimens or digital photographs to experts or systematists for resolution. Taxa lists may be changed when disagreements are resolved.
    ii. When QC parameters exceed Rhithron's quality objectives, additional samples are randomly selected and reidentified.

l. Data management

i. Scope: The goal of data management is to consistently, reliably, and accurately generate valid data products in conformance with client-specified requirements. Data management includes tracking the status of data as they are collected, transmitted, and processed.

ii. ***Objectives:*** Aspects of data management that are important to subsequent data quality include the following:
    1. Data files are accurate and data entry is error free.
    2. Data is delivered to the client in the format specified by the scope of work.
    3. QA/QC protocols and results, and any corrective actions taken, are reported to the client, along with a detailed description of sample processing procedures.
    4. Client approval is obtained for any changes to the project protocols.
    5. Clients are informed of any problems that could affect the quality of the data.
    6. Data storage is appropriately protected.

Accomplishment of these performance objectives is evaluated by the following QA/QC plan.

iii. ***Sample intake and chain of custody documentation:*** Sample intake procedures insure that each project is complete and in appropriate condition for further processing, and that internal documentation is created that adequately tracks sample location at all times while a project is in Rhithron's custody.
    1. Sample intake is managed by the Data Technician, who checks the condition of each sample and compares sample container labeling against the client-provided chain-of-custody (COC) document. Any discrepancies, damage, or missing containers are reported by the Data Technician to the client immediately. After difficulties are rectified, the Data Technician signs the COC, makes a copy of the COC, and returns the original signed COC to the client.
    2. The Data Technician transfers sample shipment metadata to the Rhithron database; at this time, each sample is assigned an internal laboratory identifier (RAI number), which is used to track project and individual sample progress through the laboratory to project completion. Sample metadata may include site name, client sample identifiers, replicate numbers, sample collection dates, number of jars in each sample, and other distinguishing notations, or other information that the client may require in a subsequent data deliverable. The Data Technician is responsible for generation of internal laboratory COC documents, and for assuring that COC documents are filed at project completion with other project paperwork.
    3. Final decisions about alterations to sample processing or identification protocols are made by the client. Any circumstances or problems that may compromise the validity or usefulness of the data are reported to the client by the Chief Biologist and/or the Operations Officer.
    4. Before sending the project, the project specifications received from the client are reviewed to make sure that all deliverables are completed to the specifications of the client's scope of work. A technical summary of QA/QC statistics for each sample and the protocols employed in sample processing and identification is

prepared by the Chief Biologist and is sent to the client along with data deliverables.

5. Data is stored on a Dell PowerEdge 6000SC Server supported by Windows 2003 Small Business Server Operating System. The server is configured with RAID 5 hard drive and a remote server backup. A hard drive configuration setup with RAID 5 allows for fault tolerance in case of server failure and uses at least three hard drives with striping of data across two drives and parity on the third drive, thus ensuring data recovery. Rhithron employs automated off-site data backups.

iv. Technical department data:

1. Technicians use an internal COC document to sign-out samples and to sign samples back in on completion of the sorting and sub-sampling procedures.

2. Technicians record sample sorting and sub-sampling information on the technical department benchsheet. This information includes: the number of grids sorted, preliminary counts of organisms sorted, technician identification, time expended for sample sorting and sub-sampling, and notes related to the condition of the sample.

3. QC technicians record the outcome of QC procedures, which are carried out on at least 10% of sorted samples. The QC parameter is reported as sorting efficiency.

4. The Lead Technician is responsible for transfer of technicians' data from benchsheets directly to RAILIS. The Lead Technician performs evaluations to ensure that QC is maintained throughout the sorting/sub-sampling procedure, and that client-specified protocols are followed.

5. Technical data entry is reviewed and verified by the Data Technician, who compares benchsheet information to entered data.

v. Taxonomic data:

1. Taxonomic data is entered by taxonomists, using a proprietary data-entry software application (EPIC v.1.7). The EPIC software uses drop-down taxa lists and incorporates several required fields for each taxonomic data entry. Required fields include correctly-spelled taxonomic name, count, uniqueness code, life stage, qualifier, and comments. Direct data entry by taxonomists minimizes errors due to misspellings, data loss or corruption at transfer, and maximizes completeness and thoroughness of the data.

2. Data errors associated with misidentification of specimens are corrected after QC procedures are complete. Verification of specimens by outside authorities may also result in changes to entered data.

3. QC sample parameters are reviewed by the Taxonomy Department Quality Assurance Officer, who determines whether quality criteria for samples and projects are met.

4. Final data review is a line-by-line review and verification of all deliverable data: the Taxonomy Department Quality Assurance Officer performs this review. Each line of data is scanned for completeness, and each taxonomic entry is reviewed for reasonableness, which

includes considerations of geographic distributions as well as ecological information implied by other taxa reported in the sample (e.g. indicators of lotic vs. lentic environs).

vi. Post-analysis archiving

1. Sorted and unsorted sample fractions, all vials and slides are securely contained, clearly labeled with the RAI number, organized by project, and archived at the Rhithron laboratory for a period of time specified by the client or for one year, whichever period is longer. Archived sample materials are examined for integrity biannually.

vii. Assessment and oversight provisions

1. Oversight of each project, at every stage of its progress, is provided by the project management group, which consists of the Taxonomy Department Supervisor, Vice President, and Chief Biologist. A weekly meeting of this team is held at which progress is reviewed and deficiencies, protocols, QA/QC statistics, and other pertinent topics are reported and reviewed. A project progress log, in which daily issues pertinent to each project are recorded, is kept by the Vice President and updated daily. Corrective actions are determined, and surveillance for these actions provided for by this team.
2. When laboratory procedures for a project are completed, the oversight group performs a complete project audit, in which the client-provided scope of work and the project progress log are reviewed. Decisions made regarding the project as it progressed through the laboratory are reviewed, uncorrected mistakes, if any, are identified, and data deficiencies, subsequently reported to the client by means of the technical summary, are discussed.