

STANDARD OPERATING PROCEDURES

for

Laboratory Analysis: Zooplankton Indicator

Prepared by



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July 2017

A1. TITLE AND APPROVAL SHEET

Document Title:

Quality Assurance Project Plan for Laboratory Analysis: Zooplankton Indicator

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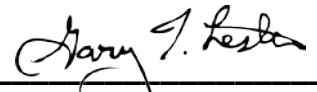
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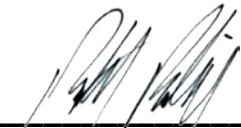
15/July/2017

EcoAnalysts, Inc. President/CEO, Project Manager:



Gary T. Lester / 15 July 2017

EcoAnalysts, Inc. Quality Assurance Manager:



Robert Bobier / 15 July 2017

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Table 1. Acronyms and Abbreviations

CEO	Chief Executive Officer
EPA	United States Environmental Protection Agency
DQO	Data Quality Objective
EcoAnalysts	EcoAnalysts, Inc.
QA	Quality Assurance
QAM	Quality Assurance Manager
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
QC	Quality Control
SOP	Standard Operating Procedure
US EPA	United States Environmental Protection Agency

DOCUMENT CONTROL

This document has been prepared according to the United States Environmental Protection Agency publication, *EPA Requirements for Quality Assurance Project Plans* (EPA QA/R5, March 2001). This QAPP will be reviewed annually and updated as needed. Updated versions of this QAPP will bear a new (x + 1) revision number.

GROUP A: PROJECT MANAGEMENT**A3. DISTRIBUTION LIST**

Each person listed on the Approval Signature Page and each person listed in Table 2 or his/her successor will receive a copy of the final approved version of this Quality Assurance Project Plan. A copy will also be made available to other persons taking part in the project and to other interested parties.

Table 2. QAPP for Laboratory Analysis: BMI Distribution List

Name	Title/Affiliation	Address	Phone/email
Gary Lester	CEO, Project Manager EcoAnalysts, Inc.	1420 South Blaine Street, Suite 14 Moscow, ID 83843	208-882-2588 ext 21 glester@ecoanalysts.com
Robert Bobier	QA Manager EcoAnalysts, Inc.	1420 South Blaine Street, Suite 14 Moscow, ID 83843	208-882-2588 ext 34 rbobier@ecoanalysts.com
William LaVoie	Taxonomy Coordinator EcoAnalysts, Inc.	1420 South Blaine Street, Suite 14 Moscow, ID 83843	208-882-2588 ext 80 blavoie@ecoanalysts.com
Megan Payne	Sorting Lab Manager EcoAnalysts, Inc.	1420 South Blaine Street, Suite 14 Moscow, ID 83843	208-882-2588 ext 59 mpayne@ecoanalysts.com

A4. PROJECT/TASK ORGANIZATION

The primary responsibilities of the principals are as follows:

EcoAnalysts Project Manager – Gary Lester, CEO

- Provides overall coordination of the project and makes decisions regarding the proper functioning of all aspects of the project; and
- Makes assignments and delegates authority as needed, to other parts of the project organization.

EcoAnalysts QA Manager – Robert Bobier

- Oversees transfer of samples and related records for the zooplankton indicator;
- Ensures the validity of data for the zooplankton indicator;
- Interacts with EcoAnalysts Project Manager on issues related to sample processing and schedules for conduct of activities;
- Collects copies of all official forms, evaluation checklists and reports;
- Oversees and maintains records of laboratory operations, but is not part of laboratory operations; and
- Directs laboratory audits.

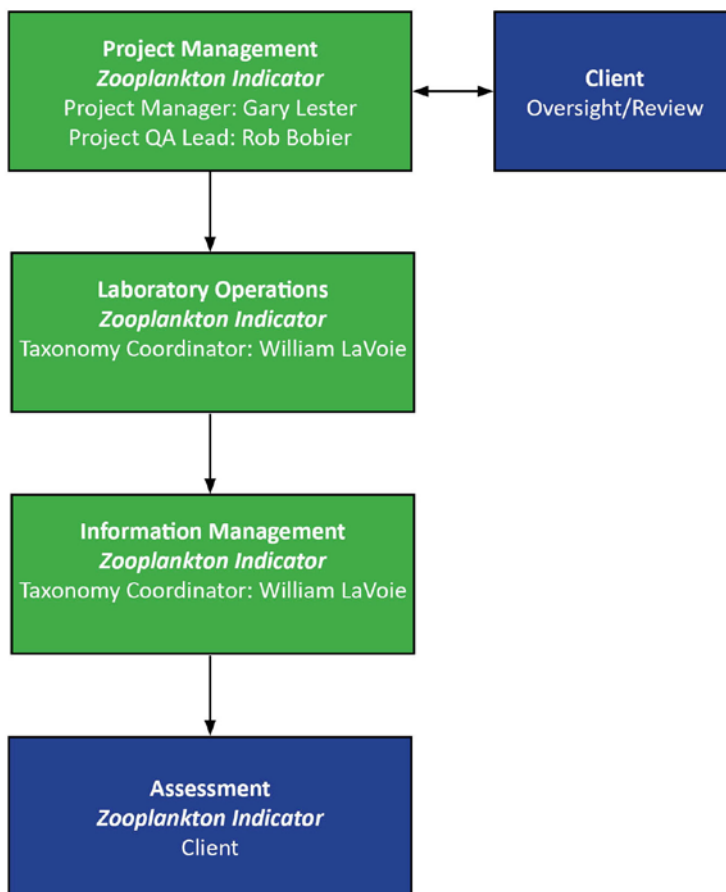
EcoAnalysts Taxonomy Coordinator – William LaVoie

- Oversees analysis of zooplankton samples; and
- Ensures the validity of data for the zooplankton indicator.

Table 3. Principal Contact List

Gary Lester CEO, Project Manager EcoAnalysts, Inc. 1420 South Blaine Street Suite 14 Moscow, ID 83843 208-882-2588 ext. 21 208-883-4288 glester@ecoanalysts.com	Robert Bobier QA Manager EcoAnalysts, Inc. 1420 South Blaine Street Suite 14 Moscow, ID 83843 208-882-2588 ext. 34 208-883-4288 rbobier@ecoanalysts.com	William LaVoie Taxonomy Coordinator EcoAnalysts, Inc. 1420 South Blaine Street Suite 14 Moscow, ID 83843 Phone: 208-882-2588 ext. 80 Fax: 208-883-4288 blavoie@ecoanalysts.com
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Figure 1. Project Organization



The QA Manager is independent from project staff that generates data. The QA Manager, Robert Bobier, is responsible for managing this QAPP and is available to address project QA/QC problems and concerns.

A5. PROBLEM DEFINITION/BACKGROUND

This QAPP addresses the laboratory analysis of zooplankton indicator samples. This Laboratory Operations Quality Assurance Project Plan is a tool to guide EcoAnalysts’ laboratory operations for processing zooplankton samples. This plan contains elements of project management, data quality objectives, measurement and data acquisition, and information management for processing zooplankton samples.

This QAPP covers in scope the processing of zooplankton samples collected from all water body types (coasts and estuaries, wetlands, lakes, and rivers).

A6. PROJECT/TASK DESCRIPTION

Ecoanalysts is adequately equipped and staffed to conduct highly specialized analyses related to the zooplankton indicator. EcoAnalysts complies with all methods, procedures, and QA/QC requirements as described in required laboratory methods manuals. Prior to initiation of task orders, EcoAnalysts' laboratory operations may be evaluated by EcoAnalysts' QAM.

Zooplankton samples collected and preserved at each site will be processed and identified at Ecoanalysts' laboratory to the lowest practicable level or level required. The taxonomy coordinator will oversee and periodically review the work performed by taxonomists.

A7. QUALITY OBJECTIVES AND CRITERIA

For analyzing zooplankton indicator samples, performance objectives (associated primarily with measurement error) are established (following USEPA Guidance for Quality Assurance Plans EPA240/R-02/009). The following sections describe approaches for evaluating zooplankton indicator sample analyses.

A7.1 Taxonomic Precision and Accuracy

Taxonomic precision will be quantified by comparing whole-sample identifications. To calculate taxonomic precision for zooplankton, 10 percent of the samples will be randomly-selected for re-identification and percent similarity will be calculated. Percent similarity is a measure of similarity between two communities or two samples (Washington 1984). Values range from 0% for samples with no species in common, to 100% for samples which are identical. It is calculated as follows:

Equation 1. Percent Similarity

$$PSC = 1 - 0.5 \sum_{i=1}^K |a - b|$$

where:

a and b = for a given species, the relative proportions of the total samples A and B, respectively, which that species represents.

A MQO of ≥85% is recommended for percent similarity of taxonomic identification. If the MQO is not met, the reasons for the discrepancies between analysts should be discussed. If a major discrepancy is found in how the two analysts have been identifying organisms, the last batch of samples that have been counted by the analyst under review may have to be recounted.

Additionally, percent similarity should be calculated for re-processed subsamples. This provides a quantifiable measure of the precision of subsampling procedures. A MQO of ≥70% is recommended for percent similarity of subsamples. If a sample does not meet this threshold, additional subsamples should be processed from that sample until the MQO is achieved.

Corrective actions for samples exceeding these MQOs can include defining the taxa for which re-identification may be necessary (potentially even by a third party), for which samples (even outside of the

10% lot of QC samples) it is necessary, and where there may be issues of nomenclatural or enumeration problems.

Accuracy of taxonomy will be qualitatively evaluated through specification of target hierarchical levels (e.g., family, genus, or species); and the specification of appropriate technical taxonomic literature or other references (e.g., identification keys, voucher specimens). Samples will be identified using the most appropriate technical literature that is accepted by the taxonomic discipline and reflects the accepted nomenclature. Where necessary, the Integrated Taxonomic Information System (ITIS, <http://www.itis.usda.gov/>) will be used to verify nomenclatural validity and spelling.

A8. SPECIAL TRAINING/CERTIFICATION

Training of EcoAnalysts' project staff, when needed, is done internally through assistance from senior project staff. All identifications are completed by taxonomists certified in the appropriate area.

A9. DOCUMENTATION AND RECORDS

All versions of the QAPP are retained by EcoAnalysts. EcoAnalysts retains sorting bench sheets indefinitely. Taxonomic data are entered into EcoAnalysts' custom LIMS program by taxonomists during the identification process. Sample data are retained by ecoanalysts indefinitely following completion of the project.

GROUP B: DATA GENERATION AND ACQUISITION

B1. SAMPLING DESIGN

The specific details for the collection of samples associated with different indicators are described in the zooplankton indicator-specific sections of the field QAPP or client field manual.

B2. SAMPLING METHODS

The specific details for the collection of samples associated with different indicators are described in the zooplankton indicator-specific sections of the field QAPP or client field manual.

B3. SAMPLE HANDLING AND CUSTODY

Immediately upon receipt of zooplankton samples, all containers are inspected for damage or leakage. Sample labels are checked against chain of custody forms and/or packing slips and any discrepancies are noted. Receipt records are reported to the client within one business day of sample receipt. Chain of custody logs are reported throughout the project according to timelines and methods requested by the client.

Samples are logged into the EcoAnalysts, Inc. custom Laboratory Information Management System, LIMS, database and assigned a unique sample tracking number.

B4. ANALYTICAL METHODS

B4.1 Preparing Zooplankton Samples

Samples are checked out via LIMS. A sheet is printed out containing all of the sample information and sorting protocols designated for it. Samples are rinsed with 70% ethanol into a 500 ml jar, and the sample is weighed.

B4.1 Taxonomic Identification and Enumeration of Zooplanktons

After weighing the sample, a 1 mL Hensen-Stempel pipette is inserted into the sample and is used to homogenize the sample, mixing it in a random fashion (not swirling). The sub-sample is captured during the mixing process to avoid bias due to sinking of heavier planktonic organisms.

The subsample taken from the homogenized sample is rinsed into a watch glass with 70% ethanol. Based on the organism density of the first 1 mL of the subsample, more 1-mL aliquots are added until the target count of 200-400 non-rotifer zooplankters is present in the watch glass. If the target count is exceeded in one mL of sample, a secondary dilution is made by transferring aliquots from the sample into a second beaker and diluting this subsample. After weighing the secondary dilution, aliquots are then taken from this secondary dilution for analysis. Identifications are taken to the lowest practical level (Genus and species for Cladocera, Cyclopoida, Calanoida, and Anostraca, family level for Diptera, Hydracarina, and order level for Harpacticoida). The length measurements of 15 individuals of the top five dominant taxa are taken.

The entire contents of the watch glass are counted to allow proper abundance calculations. After identification, enumeration, and measurements, the sample (and the secondary dilution, if used) is weighed to calculate the total volume analyzed.

After initial analyses are complete, 10% of the samples will be randomly selected for re-identification. See Section A7.1 for taxonomic precision and accuracy measurement quality objectives. The final data will be adjusted according to the recommendations of both taxonomists.

B5. QUALITY CONTROL

Zooplankton samples are checked for quality control. See Section A7.1 of this QAPP for quality objectives and methods.

B6. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

All microscopes and laboratory equipment are inspected regularly according to manufacturer recommendations.

B7. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

All microscopes and laboratory equipment, including digital imaging equipment, are calibrated regularly according to manufacturer recommendations. Calibration will be checked throughout the project and equipment will be recalibrated if necessary.

B8. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Supplies and consumables include alcohol and sample jars. Supplies and consumables are purchased only from reputable and reliable suppliers and are inspected for usability upon receipt.

B9. NON-DIRECT MEASUREMENTS

EcoAnalysts maintains a library of current taxonomic references. These are used for taxonomic identification purposes when such need arises. Taxonomists are responsible for using current references and publications.

B10. DATA MANAGEMENT

As the zooplankton sample is being identified, the taxonomist enters data directly into the computer using a custom built LIMS database and user interface. The data entry program has several features built into it, including steps for taxonomic identification of a specimen, the number of specimens in each taxon, life stage information, taxonomic notes, etc. There is a visual confirmation at each step which prompts for a user confirmation. A running tally of invertebrates as well as the number and type of taxa in the sample are displayed on the screen; therefore, a taxonomist can quickly look for low or high counts as a flag for major discrepancies. Note: With this process, we have successfully eliminated the need for handwritten bench sheets, thereby doing away with a secondary step of data entry and the errors associated with it.

Throughout the project and sample analysis, data entry is double checked for accuracy. Using our networked computer systems, the appropriate data are combined for each sample to obtain the sorting statistics and comprehensive taxa lists and counts.

Data are delivered in an electronic format specified by the client and emailed to the technical contact(s). Hard copies and/or copies on compact disc can be mailed to the client upon request. The delivery schedule will be agreed upon by the client and EcoAnalysts in advance, specifying the sample lots, dates, and components. EcoAnalysts, Inc. retains all raw data files used and derived in our projects.

Quality assurance data sheet checks include scanning for apparent entry errors, measurement errors, omissions, and anomalies. Suspect data are flagged and/or excluded from use. Data may be presented in table, graph, and chart format. Unusual data are rechecked to verify their accuracy.

GROUP C: ASSESSMENT AND OVERSIGHT

C1. ASSESSMENT AND RESPONSE ACTIONS

The project manager, Gary Lester, is responsible for all reporting, tracking, and overall project management including field activities, reviewing the data, reporting, and forwarding all data to the client for inspection. Megan Payne and Pat Barrett are responsible for laboratory operations involving processing zooplankton indicator samples for projects. Robert Bobier, EcoAnalysts QAM, is authorized to oversee all activities as required for quality assurance.

C2. REPORTS TO MANAGEMENT

Draft reports of project findings will be prepared for the client on a regular basis, as requested. Problems that arise during the project are corrected and reported to client and EcoAnalysts staff via this report. The project manager will submit a final report prior to the conclusion of the task order. All data are tracked through use of EcoAnalysts' LIMS. The data compiled during this project are incorporated into spreadsheets and sent to the client and if requested, will be uploaded to the client's database.

GROUP D: DATA VALIDATION AND USABILITY

D1. DATA REVIEW, VERIFICATION, AND VALIDATION

All raw data are transcribed into EcoAnalysts' LIMS. Hard copies of raw data are organized and filed. Statistical analyses on replicate samples are recorded so that the degree of certainty can be estimated. All laboratory analytical results are cross checked to ensure data are complete and error free. Data are archived using EcoAnalysts' LIMS on EcoAnalysts' servers.

D2. VERIFICATION AND VALIDATION METHODS

Project staff follows the EPA *Guidance on Environmental Verification and Validation* (EPA QA/G-8) whereby the data are reviewed and accepted or qualified by project staff.

D3. RECONCILIATION WITH USER REQUIREMENTS

Upon receipt of results of each sample group, calculations and determinations of precision and accuracy are made and corrective action implement, if needed. If data quality does not meet project specifications, the deficient data are flagged and the cause of failure evaluated. Any limitations on data use are detailed in the project reports and other documentation. For the data to be considered valid, data collection procedures, the handling of samples, and data analysis must be monitored for compliance with all the requirements described in this QAPP. Data are flagged and qualified if there is evidence of habitual violations of the procedures described in this QAPP. Any limitations placed on the data are reported to the data end user in narrative form.