

Normandeau Associates, Inc.

**Marine Benthic Infauna Sorting and Taxonomic
Identification**

Quality Assurance Project Plan (QAPP)

Falmouth Office:
141 Falmouth Height Rd.
Falmouth, MA 02540
Telephone: 508-548-0700

Website: www.normandeau.com



May 2020

A. PROJECT MANAGEMENT

A1. TITLE AND APPROVALS

QUALITY ASSURANCE PROJECT PLAN


for

MARINE BENTHIC INFAUNA SORTING AND TAXONOMIC IDENTIFICATION

Normandeau Associates, Inc.

May 2020

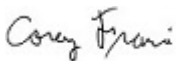
Review and Approvals



Ms. Deborah Rutecki
Normandeau Project Manager – Falmouth

5/15/2020

Date



Mr. Corey Francis
Normandeau QA Director

5/15/2020

Date

A2. TABLE OF CONTENTS

A.	PROJECT MANAGEMENT	1
A1.	TITLE AND APPROVALS	1
A2.	TABLE OF CONTENTS.....	2
A3.	DISTRIBUTION LIST	5
A4.	PROJECT AND TASK ORGANIZATION	5
A4.1	QAPP Introduction	5
A4.2	Project Organization	5
A5.	PROJECT/TASK DESCRIPTION	8
A5.1	Analysis of Benthic Infauna Samples	8
A5.2	Schedule of Deliverables	8
A6.	QUALITY OBJECTIVES AND CRITERIA	8
A7.	SPECIAL TRAINING/CERTIFICATIONS	9
A8.	DOCUMENTS AND RECORDS	10
A8.1	Documentation	10
A8.2	Laboratory Records and Deliverables	10
A8.3	Reports and Data Submissions	10
A8.3.1	Reference Collection Status Letter	11
A8.3.2	Sample Analysis Data Submissions	11
A8.3.2.1	Data Submissions to the Project Manager	11
A8.3.2.2	Infaunal Data Analyses	11
A8.3.2.3	Data Submissions by the Project Manager	11
A8.4	Project files	12
B.	DATA GENERATION AND ACQUISITION	13
B1.	SAMPLE HANDLING AND CUSTODY	13
B1.1	Sample Handling	13
B1.2	Sample Custody	13
B1.2.1	Sample Tracking	13
B1.2.2	Sample Custody	13
B1.2.3	Sample Archival Policies	15
B2.	Benthic Infaunal Analysis	16
B3.	QUALITY CONTROL	17
B4.	INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE ...	19
B5.	INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES	19
B6.	DATA MANAGEMENT	19
B6.1	Data Custody	19
B6.2	Laboratory Data and Data Reduction	20
B6.2.1	Statistical Analysis	20
B6.3	Data Set Structure	21
B6.4	Project Database Codes	21
B6.5	Data Submittal to Client and Selected Database	24
C.	ASSESSMENT AND OVERSIGHT	25

C1.	Assessment and Response Actions	25
C1.1	Assessments.....	25
C1.1.1	Laboratory Technical System Audits	25
C1.1.2	Performance Evaluation Sample Assessment	25
C1.1.3	Data Technical System Audits	25
C1.2	Assessment Findings and Corrective Action Responses.....	26
C1.2.1	Laboratory Corrective Action.....	28
C1.2.2	Corrective Action during Data Validation and Data Assessment	28
C2.	REPORTS TO MANAGEMENT	28
D.	DATA VALIDATION AND USABILITY	29
D1.	DATA REVIEW, VERIFICATION, AND VALIDATION.....	29
D1.1	Laboratory Data	29
D1.2	Data Management.....	29
D2.	VALIDATION AND VERIFICATION METHODS.....	29
D2.1	Laboratory Data	29
D2.2	Data Management.....	30
D2.3	Project Deliverables	30
D3.	RECONCILIATION WITH USER REQUIREMENTS.....	30
D3.1	Comparison to Measurement Criteria	30
D3.1.1	Accuracy and Precision Assessment	30
D3.1.2	Completeness Assessment.....	31
D3.1.3	Representativeness.....	31
E.	REFERENCES.....	32

LIST OF FIGURES

Figure 1. Outline of Normandeau Associates' quality assurance/ quality control (QA/QC) program.	7
Figure 2. Example of a Chain-of-Custody Form.	14

LIST OF TABLES

Table 1. Supplies, Acceptance Criteria, and Responsibility for Critical Laboratory Supplies.	19
Table 2. Benthic infaunal sample data codes. Data codes are from NCCA 2015 (US EPA 2016).....	22
Table 3. Benthic infaunal taxonomic identification data codes. Data codes are from NCCA 2015 (US EPA 2016).	23
Table 4. Example of Technical Systems Audit Checklist	27

LIST OF APPENDICES

Appendix A: Normandeau Associates, Inc. Laboratory Standard Operating Procedures:
Marine Benthic Infauna Sorting and Identification

A3. DISTRIBUTION LIST

Copies of this QAPP, and any subsequent revisions, will be distributed to Normandeau employees once approvals have been obtained. Normandeau personnel that participate in sorting or taxonomic identification of marine benthic infauna will receive either a PDF or hard copy of the QAPP.

A4. PROJECT AND TASK ORGANIZATION

A4.1 QAPP Introduction

This Quality Assurance Project Plan (QAPP) presents the organization, objectives, functional activities, and specific quality assurance (QA) and quality control (QC) activities associated with marine benthic infauna sorting and taxonomic identification that will be conducted for benthic projects for clients that include private and public businesses, non-governmental organizations (NGO), and federal, state and municipal agencies. This document also describes the specific protocols that will be followed for sample handling and storage, chain of custody, laboratory analyses, data review and validation, document management, data management, and data usability assessment. All applicable federal, state, and local laws and regulations are to be followed when conducting activities described in this Quality Assurance Project Plan.

This QAPP was prepared in accordance with US EPA guidance documents U.S. EPA QA/R-5 (2001, reissued 2006) and U.S. EPA QA/G-5 (2002). A laboratory standard operating procedure (SOP) will supplement this QAPP (Appendix A). The SOP will provide the additional operational details required to conduct benthic infauna sorting and taxonomic identification, and describes staff responsibilities and specific equipment.

In instances where the client or project has a project specific QAPP or other quality assurance (QA) materials, the project specific documents will supersede this QAPP.

A4.2 Project Organization

Normandeau Associates, Inc. (Normandeau) is an employee-owned environmental and regulatory consulting firm that was founded in 1970 and has been providing high quality scientific data collection services for 50 years. Today, we are considered one of the nation's foremost companies in the field. Normandeau's deep and broad background as both environmental and public involvement consultants provides a wealth of knowledge and experience in disciplines ranging from environmental assessment and permitting to stakeholder interviews and facilitation.

Normandeau works closely with clients, regulators and the public to seek solutions that enhance economic development, meet regulatory requirements, protect and restore our natural resources, and help improve our local communities. Normandeau performs a wide range of

monitoring studies for a variety of clients including utilities, renewable energy projects, dredging operations, the hydropower industry, and outfalls. Normandeau recognizes that the foundation of a monitoring program is scientifically-defensible data collection and analysis.

It is our policy to supply quality services, information, data, and products with timely delivery. Figure 1 illustrates our quality assurance/ quality control (QA/QC) program to achieve high-quality, cost-effective deliverables. This program is designed to meet or exceed the guidance criteria of the US EPA and to be consistent with the intent of federal regulations (10 CFR 50), which require that QA for data collection and laboratory services be separated from operational and budgetary concerns. Normandeau has a full time QA Director who supervises the implementation and documentation of QA Programs and reports directly to the President of the Company.

The Normandeau QA Program for data collection and laboratory services is comprised of two systems: a QC system and a QA system. The principal strengths of the QA Program are the functional independence of the systems and the common collection and interpretation point for quality related information—the QA Director. The QC system will be managed by the Program Manager and conducted by operational personnel, including our subcontractors. The QA system is managed by Normandeau's QA Director and uses project-independent technical personnel during performance and system audits.

Tasks in this QAPP fall under the QC system and will be performed by operational personnel including the Project Manager, QA Director, Scientists (I to Senior), and Technicians (I-V).

- The Project Manager is the primary person responsible for overseeing project activities including benthic infauna processing and the project work described in this QAPP. The Project Manager serves as an interface with the client.
- The QA Director the primary person responsible overseeing project QA/QC procedures. The QA Director is responsible for interacting and communicating certification and safety requirements, implementing the quality assurance programs, and reporting to the Project Manager the status of the project quality program.
- Scientists and Technicians, under the direction of the Project Manager, follow the procedures described in the Laboratory SOP to process benthic infauna samples and the criteria established in this QAPP. Sample processing includes preservation, sorting, taxonomic identification to lowest practical taxon and enumeration.

Laboratory personnel assignments may include cross-functional training and work performance in multiple areas of the operations. Multiple function training ensures laboratory back up personnel during high workloads.

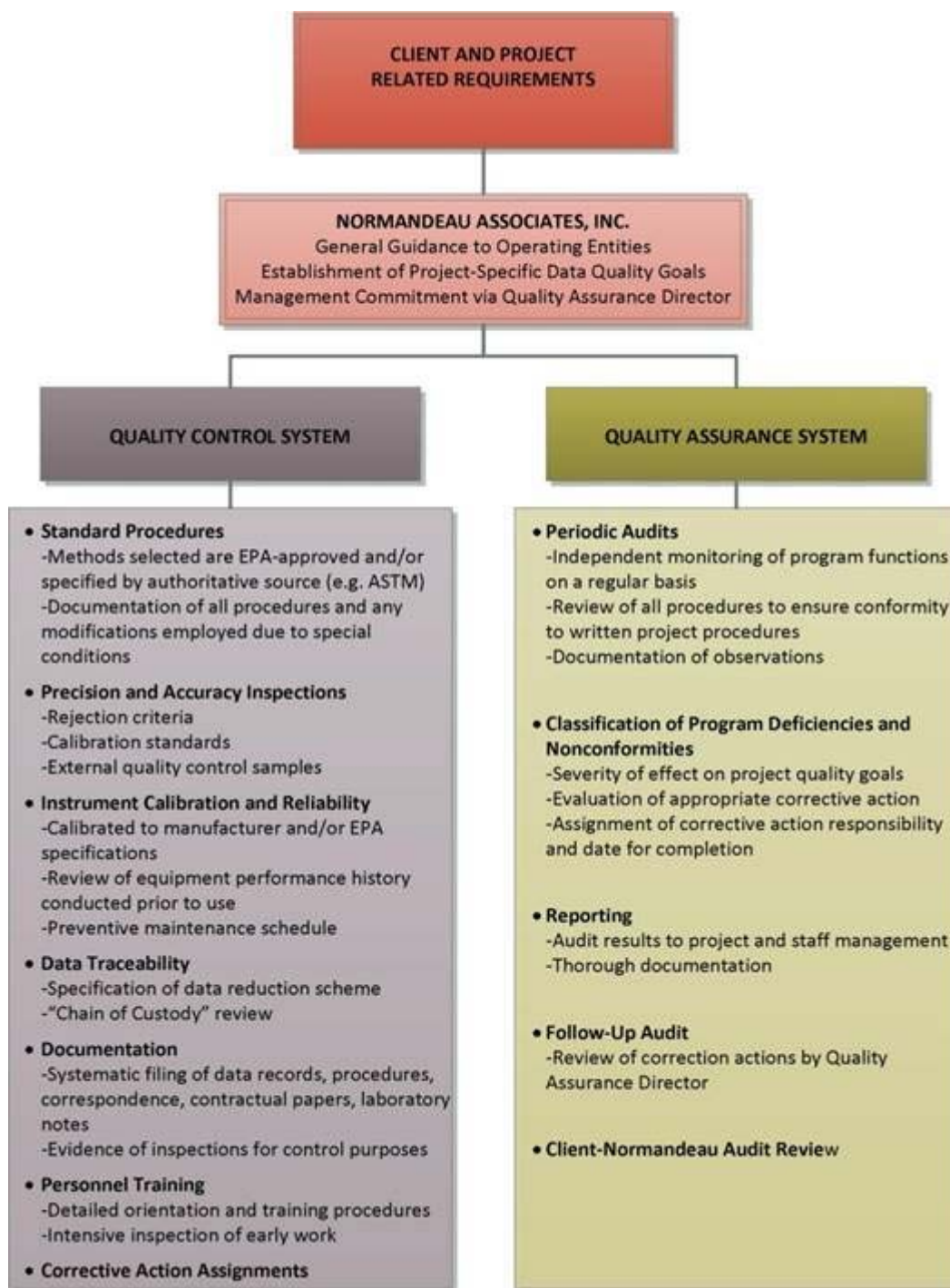


Figure 1. Outline of Normandeau Associates’ quality assurance/ quality control (QA/QC) program.

A5. PROJECT/TASK DESCRIPTION

Benthic infaunal communities are used to assess and establish benthic habitat types; assess the forage base for commercially and ecologically important species; and are used to assess the level of habitat health in embayments from healthy (low organic matter, high D.O.) to highly stressed (high organic matter, low D.O.). Communities in benthic assemblages respond to a variety of factors (e.g. sediment grain size, salinity, and temperature) and stressors in different ways allowing the type of habitat or stress that has influenced the assemblage to be identified (Pearson and Rosenberg 1978, Rhoads and Germano 1986, and Weisberg et al. 1997).

A5.1 Analysis of Benthic Infauna Samples

This project/task includes the identification and enumeration of the benthic fauna recovered from sediment grab samples. If requested by the client, results from the project sampling locations will be evaluated statistically to characterize benthic community structure and/or to assess embayment ecological health using benthic macrofaunal community parameters and multivariate analyses. Community parameters may include: total abundance, number of species (per grab), log-series alpha (Fisher's alpha), Shannon-Wiener diversity index (H'), Pielou's evenness (J'), Margalef's diversity index (D_{mg}), Simpson, and Average Taxonomic Distinctiveness (ATD). Multivariate analyses may include Bray-Curtis similarity hierarchical agglomerative clustering (cluster analysis), non-metric multidimensional scaling (MDS), and US M-AMBI (multivariate AZTI Marine Biotic Index in United States coastal waters). Analyses will be performed using Microsoft Excel, SAS, and/or PRIMER (Plymouth Routines in Multivariate Ecological Research) software programs.

If requested by the client, a reference collection of all infaunal taxa (identified and unidentified specimens) will be stored, maintained, and compiled throughout the project.

Details of sample handling and laboratory methods to be used in the benthic infaunal processing are provided in Sections B-1 and B-2, respectively, and can also be found in the Laboratory SOP in Appendices A.

A5.2 Schedule of Deliverables

The schedule of deliverables will be determine on a per projects basis. The schedule will be determine by client deliverable dates and requested milestones.

A6. QUALITY OBJECTIVES AND CRITERIA

Requirements for ensuring that the data are usable for their intended purpose (that is, are of suitable quality) include accuracy, precision, representativeness, comparability, and completeness. When these requirements are met, the final data product is technically defensible.

Data elements for this project are discussed in terms of the appropriate characteristics, defined as:

Accuracy: The extent of agreement between a measured value and the true value of interest.

Precision: The extent of mutual agreement among independent, similar, or related measurements.

Representativeness: The extent to which measurements represent true systems.

Comparability: The extent to which data from one study can be compared directly to similar studies.

Completeness: The measure of the amount of data acquired versus the amount of data required to fulfill the statistical criteria for the intended use of the data.

Quality objectives are given below. Details of how these criteria are met are presented in Section B3.

The data quality goals (DQGs) for the analysis of benthic infauna are: (1) all samples be processed, (2) all animals be removed for identification and enumeration, (3) all infaunal animals be counted accurately, (4) the taxonomic identifications be accurate (correct) as discussed in Section B3, and (5) the identifications correspond to those used throughout the project/program or the current consensus of the scientific community as documented by the World Register of Marine Species (WoRMS) or an equivalent source. At least 95 percent of all animals must be removed from a sample to pass the quality control (QC) evaluation as discussed in Section B3.

A7. SPECIAL TRAINING/CERTIFICATIONS

Laboratory personnel will be experienced in the laboratory techniques documented in this QAPP. Prior to starting work, any new personnel will be given instructions specific to the project and task being performed, covering the following areas:

- Organization and lines of communication and authority
- Overview of the QAPP
- QA/QC requirements
- Documentation requirements
- Health and safety requirements

Instructions will be provided and documented by the Program Manager and the QA Director. All Normandeau laboratory employees complete an annual hazard communication program that includes a formaldehyde standard.

Personnel responsible for shipping samples will also be trained in the appropriate regulations, i.e., Department of Transportation (DOT), International Civil Aviation Organization (ICAO), and International Air Transport Association (IATA).

A8. DOCUMENTS AND RECORDS

A8.1 Documentation

Initially, all data will be recorded either (1) electronically onto computer storage media or (2) manually into bound laboratory notebooks or onto established data forms. All data collection notes will be made in permanent ink, initialed, and dated, and no erasures or obliterations will be made. If an incorrect entry is made, the information will be crossed out with a single strike mark and the correct entry will be made, initialed, and dated by the person making the correction. Corrections to electronically captured data will be documented on a hard-copy of the data. Completed data forms or other types of hand-entered data will be signed and dated by the individual entering the data. Direct-entry and electronic data entries will indicate the person collecting or entering the data. It will be the responsibility of the Project Manager or designee to ensure that all data entries and hand calculations are verified according to the procedures described in Section D of this QAPP.

A8.2 Laboratory Records and Deliverables

Laboratory data reduction procedures will be performed according to the following protocol. All information related to analysis will be documented in controlled laboratory logbooks, instrument printouts, or other approved forms. All entries that are not generated by an automated data system will be made neatly and legibly in permanent, waterproof ink. Information will not be erased or obliterated. Corrections will be made by drawing a single line through the error and entering the correct information adjacent to the cross-out. All changes will be initialed, dated, and, if appropriate, accompanied by a brief explanation. Unused pages or portions of pages will be crossed out to prevent future data entry. Laboratory records will be reviewed by supervisory personnel on a regular basis, and by the QA Director periodically, to verify adherence to documentation requirements.

Data will be submitted as electronic data deliverables (EDD). The EDDs for benthic infauna including oligochaetes will be provided in Excel spreadsheets. Data deliverables will be provided to the client on the schedule described in the project scope of work and/or contract. Details of data management are discussed in Section B.

A8.3 Reports and Data Submissions

Documents and the data submission that could be generated under this QAPP are listed below. The due dates for these reports and data submissions are described in the project scope of work and/or contract.

- Microsoft Excel spreadsheets containing benthic infauna results
- Reference collection reports
- Technical reports
- Data submission to databases

A8.3.1 Reference Collection Status Letter

A reference collection letter will be prepared after benthic infaunal data analysis is complete if the client has asked for a reference collection in the scope of work. The letter will include:

- A hierarchical taxonomic list of all taxa comprising the collection, including the station ID from which the specimen came
- A project specific taxa code (if provided by the client) or Taxonomic Serial Number (TSN) for each taxa
- Identification of the storage location and the staff with custody of the collection

A8.3.2 Sample Analysis Data Submissions

A8.3.2.1 Data Submissions to the Project Manager

The benthic infaunal data submissions will include tables showing the station, replicate number, taxon name, and the number of individuals counted for each taxon. If the client provides a specific table format or list of parameters, the client provided material will supersede Normandeau formats.

A8.3.2.2 Infaunal Data Analyses

Prior to analysis of the infaunal data, some modifications to the dataset may be needed. For example, incidental pelagic organisms will be eliminated from calculations. Counts for higher taxa (i.e., specimens that cannot be identified to species level) will be included in calculations of abundance but not diversity. Only those individuals identified to species level will be included in all remaining calculations (e.g. number of species, diversity, evenness, multivariate analyses).

A variety of community parameter and indices, on project specific basis, may be calculated including: (1) species richness indices, (2) indices based on the proportional abundances of species (i.e., Shannon-Weiner index (H'), Pielou evenness index (J'), Margalef's index, and Total Taxonomic Distinctness), and (3) US M-AMBI. US M-AMBI will follow Pelletier et al. (2018) and will be used to determine embayment soft bottom habitat health. US-M-AMBI health condition categories include: bad (<0.20), poor (0.20 to 0.39), moderate (0.39 to 0.53), good (0.53 to 0.77), and high (>0.77). Cluster and non-metric multidimensional scaling analyses will be conducted to assess spatial trends in community composition between sites. Statistical routine packages (e.g. Primer, R) will be used to calculate these parameters and indices.

A8.3.2.3 Data Submissions by the Project Manager

All sample data will be processed into the appropriate application format as defined in the project's scope of work. If data analysis is conducted, processing of data is recommended to be

done using an advanced analytics, multivariate analyses, and data management software (e.g. R, SAS). Data processing will include error checking and checks to ensure that data sets meet scope of work requirements including database format specifications, allowable value requirements, and constraints. Data will be exported from the software (if used) in or entered into Microsoft Excel spreadsheets. The Project Manager or designee will then submit the data by email or upload it to a specified platform or database. If data are to be uploaded onto a third party database, the client will approve the data before it is upload to the database. The Project Manager will notify the client that the data was successfully uploaded to the database.

A8.4 Project files

The project files will be the central repository for all documents relevant to sample processing and analysis activities as described in this QAPP. The Project Manager is the custodian of the project files and will maintain the contents of the project files, including all relevant records, reports, datasheets, pictures, subcontractor reports, and data reviews in a limited access area and under custody of the Project Manager.

The project files may contain:

- Electronic copies laboratory datasheets
- Laboratory data deliverables
- Reference collection letter
- Technical reports
- Verification of data submission
- All custody documentation (chain of custody forms, etc.)

Electronic versions of reports and statistical analyses will be stored in a project-specific folder on a server or network. The original EDDs received from any subcontracted laboratory and project data will also be stored in a folder on the server. All the project records will be maintained for at least three years unless the scope of work specifics a longer period.

B. DATA GENERATION AND ACQUISITION

B1. SAMPLE HANDLING AND CUSTODY

B1.1 Sample Handling

Benthic infaunal samples (stored in sturdy coolers) will be delivered by a Normandeau field crew member or shipped to the laboratory. The laboratory will be contacted to arrange a time for sample drop off or notified that samples are to be shipped. This will allow laboratory staff to be prepared for sample receipt. The samples, while still in buffered 10% formalin, can be shipped by ground or 2-day express delivery if necessary for delivery to the laboratory. The lids on the sample jars will be taped and the jars inserted individually into large zip-locked or tied plastic bags lined with absorbent padding. On arrival at the laboratory, samples will be checked to ensure they arrived undamaged and that sample information on the sample labels matches the information on the COC forms. Damaged samples will be noted immediately, and appropriate corrective actions taken (see Section B1.2.2). If the scope of work requests samples are to be archived, one sample from each infaunal station will be randomly selected to archive (see Section B1.2.3) and the other(s) will be processed. The samples will be fixed in buffered 10% formalin (added at time of collection) for at least 48 hours, and then be transferred to reagent alcohol within 7 days of collection. The organisms will be picked from the samples and sorted into major taxonomic groups, and then identified to lowest practical taxon.

B1.2 Sample Custody

B1.2.1 Sample Tracking

Sample custody will be tracked through external and internal sample labels, and chain of custody (COC) forms (Figure 2).

B1.2.2 Sample Custody

Benthic infauna samples will be in the custody of the survey chief scientist or a field crew member from collection until they are transferred or shipped to the laboratory. COC forms (Figure 2) will accompany the samples. One complete (copied) set of the COC forms will be included in each shipping container and the original COC forms will be returned to Project Manager after the samples have been logged in at the laboratory. The signed original custody forms will be retained in the project files. Sample processing will occur in the laboratory. After the samples are processed, the laboratory will store the appropriate samples and specimens for the specific length of time for re-identification QC, voucher, or unforeseen circumstances based on project specific requirements.

www.normandeau.com Bedford: (603) 472-5191 Falmouth: (508) 548-0700

Relinquished by: (signature)	Received by: (signature)	Relinquished by: (signature)	Received by: (signature)	Relinquished by: (signature)	Received by: (signature)
Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:
Date:	Date:	Date:	Date:	Date:	Date:

Figure 2. Example of a Chain-of-Custody Form.

Transfer of benthic infaunal samples will be documented on the custody forms. All samples will be distributed to the appropriate laboratory personnel by hand or by a shipping service. A copy of the COC form will be retained by the field sample custodian in the field log. The original will accompany the samples to the laboratory for subsequent sample transfer. When samples arrive at the laboratory, custody will be relinquished to the laboratory staff. The laboratory staff will verify that the custody seals on the cooler are intact. The laboratory staff will then examine the samples, verify that sample-specific information recorded on the COC is accurate and that the sample integrity is uncompromised, and complete and sign the COC form so that transfer of custody of the samples is complete. Any discrepancies between sample labels and transmittal forms, the condition of the samples upon receipt, and any unusual events or deviations from the QAPP will be documented in detail on the COC, and the Project Manager notified. Copies of completed custody forms will be delivered (scanned and emailed or faxed) to the Project Manager within 24 hours of receipt.

B1.2.3 *Sample Archival Policies*

The types of materials that may be archived under this QAPP include samples, sample residues, a reference collection, and other infaunal specimens.

If archival samples are requested in the scope of work, one randomly selected sample from each station will be archived, and the other(s) will be processed. Archived benthic infaunal samples will be rinsed with fresh water over 500-µm-mesh (unless otherwise specified in the scope of work) screens and transferred to reagent alcohol for storage at the laboratory.

Benthic infaunal samples (both archived and processed samples) will be held until acceptance of final data or technical report by the client or for the period of time specified in the scope of work. These samples can then be disposed of after approval from the Program Manager. Processed samples will be maintained at the laboratory. Benthic infauna sample residues will be held until QC procedures are completed and the data is accepted by the Project Manager, then the residues may be discarded.

Reference collection specimens will be retained by the laboratory for the specified amount of time and then provided to the client or the next designated laboratory. Reference collection specimens will be clearly identified, labeled with the project name and unique identification number, and stored under appropriate conditions for the length of the storage period. Normandeau laboratory staff will maintain the collection and regularly check to ensure that it is stored properly to reduce the risk of alcohol evaporation and damage, and to ensure that labels are intact and legible. Vials in which the reagent alcohol level is low will be filled with clean alcohol. Any labels showing signs of deterioration will be replaced. Normandeau may retain other benthic infauna specimens to develop in-house reference collections unless otherwise prohibited by contractual agreement or scope of work requirements.

B2. BENTHIC INFAUNAL ANALYSIS

Samples will be rinsed with fresh water over 500-µm-mesh screens (unless otherwise specified in the scope of work) to remove any broken-up mud casts and transferred to reagent alcohol for storage prior to sorting. To facilitate the sorting process, all samples will be stained in a saturated alcoholic solution of Rose Bengal at least overnight, but no longer than 48 hours to avoid over-staining. After rinsing with clean fresh water, small aliquots of the sample will be placed into white plastic or enamel pans, and all organisms, including anterior fragments of polychaetes, will be removed and sorted to major taxonomic categories such as polychaetes, arthropods, and mollusks. Sorting will be done under a dissecting microscope, and organisms will be placed into vials of denatured 100% reagent alcohol (Appendix A).

After samples have been completely sorted, the organisms will be delivered to taxonomists for identification and enumeration. Identifications will be made to the lowest practical taxonomic level, usually species (Appendix A). For oligochaetes, only whole organisms or fragments that include the head will be counted and identified to the taxonomic level of Class. Nematodes will not be counted for infaunal analysis under this QAPP. Immature or damaged specimens that are missing the necessary diagnostic features for identification to the species taxonomic level will be identified to the lowest practical taxon. To ensure consistency for assessment of the benthic macrofaunal community, any incidental pelagic organisms or fauna attached to hard-substrates will not be identified.

Infaunal data will be recorded on project-specific data sheets (Appendix A) and will then be entered into an electronic format. Data entered into an electronic database will either be manually verified for accuracy or will be entered in duplicate, and a comparison program run to identify any discrepancies.

If a project-specific reference collection is to be established, the first identifiable specimen of each taxa and life stage will be saved to ensure representation in the collection. The specimen will be placed into a vial with reagent alcohol and properly labeled. Other representative specimens may be saved if they are of excellent quality or illustrate a distinguishing feature. The reference collection will be used by project taxonomists to ensure comparability of the taxonomic identifications performed between surveys and laboratories. As taxa not previously identified during a program are encountered, they will be added to the collection. As part of the maintenance of the reference collection, taxonomists will review any possible inconsistencies between previous identifications and those made during the current survey. The taxonomic status of species in the collection will be evaluated as relevant systematic revisions appear in the scientific literature. If necessary, recommendations for changes in taxonomic usages will be made to the client. The reference collection will be maintained for the specified amount of time in the contractual agreement or scope of work. The collection will be sent to the Project Manager of the next survey or another designated laboratory.

Additional details on infaunal sample analysis methods that are not specified elsewhere in this QAPP are provided in the Laboratory Standard Operating Procedures (Appendix A). For any case in which the Laboratory Standard Operating Procedures are different from those described in this QAPP, the procedures described in this QAPP will be followed.

B3. QUALITY CONTROL

Accuracy

Benthic infauna will be identified by experienced taxonomists. In cases where different taxonomists identify replicates from the same station, discrepancies in species identifications will be recognized during data entry and reviewed. Taxonomic discrepancies will be addressed by communication among the taxonomists. In the case of questions about organisms in specific taxonomic groups, specimens may be sent to recognized experts for a second opinion on the identification. Standard taxonomic references will be used. Selected specimens of newly found species may be retained as part of reference collections or the laboratory in-house collection.

Precision

Sorting: Sorting technicians will remove all organisms from the samples and separate them into major taxonomic groups. All residual material will be labeled and stored for QC analysis. For the QC analysis, samples will be divided into batches of approximately 10 samples.

Approximately 10% of the samples from each batch will then be randomly chosen for an independent QC check. Only senior technicians will perform the QC evaluations (A senior technician is defined as having three or more years of sorting experience). Under no circumstances will the same individual who sorted the sample perform the QC evaluation. In most cases, a batch of samples is defined as ten consecutively sorted samples. By definition, at least 95% of all animals must be removed from a sample to pass the QC evaluation (i.e., the percent sorting error must be $\leq 5\%$). The following formula will be used to calculate the percent sorting error for each QC sample:

Number of animals found in QC inspection

----- $\times 100 =$ percent sorting error

Total number of animals present in sample

If more than 5% of the total organisms in the QC sample have been missed, the sample fails QC evaluation, and the all remaining samples from that batch will be re-sorted. Technicians will be informed of any necessary corrective measures. This procedure will be repeated until the batch of samples passes the QC evaluation. An exception will be made for low abundance samples (a sample with fewer than 60 organisms) that are chosen for the QC evaluation. Any low abundance sample in which three or fewer organisms were missed is considered to pass the sorting QC evaluation even if the percent sorting error is $>5\%$. Samples in which no organisms are present will be excluded from the sorting QC selection process. A record of all sorting QC evaluations will be maintained for each batch (Appendix A).

Identification and Enumeration: The same basic QC principles described in the Sorting section will in general apply to species identifications. At least 10% of the samples will be checked to detect any unacceptable identification and enumeration errors. Only senior taxonomists will perform the QC check. QC samples will be selected in the same manner as described in the Sorting section above. Additionally, the same percent accuracy level will be used to determine if a sample passes the QC evaluation and the same corrective measures will be implemented if a sample fails the QC evaluation. The following formula will be used to calculate the percent taxonomy error for each QC sample:

$$\frac{\text{Total number of taxonomy errors}}{\text{Total number of animals present in sample}} \times 100 = \text{percent taxonomy error}$$

In certain cases it may not be necessary to reprocess the entire batch of samples if only minor corrections are needed (e.g., name changes). When any misidentification is discovered, all previously identified samples containing that taxon will be rechecked. A record of all identification QC evaluations will be maintained (Appendix A).

Representativeness

Because all of the sample will be analyzed, representativeness will be determined by sampling factors.

Completeness

Since one sample from each station will be archived, the loss of one sample will still permit data to be obtained from the archived sample for that station. One hundred percent completeness is expected.

Comparability

Methods of analysis will be comparable to those used in other investigations found in the peer-reviewed literature and technical reports for the study area. Comparability of the identifications will be ensured through the use of standard taxonomic references and by comparison of specimens in the in-house reference collection or project specific reference collection (if requested). Taxonomists will be familiar with fauna from study area waters and those of the surrounding regions. The reference collection, if requested, will be maintained and, if new species are identified, expanded. Any new species that have not been reported in previous surveys or other studies conducted in the study area will be checked against similar taxa in the reference collection and carefully verified with recognized experts.

B4. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

Maintenance of and repairs to instruments will be in accordance with manufacturers' manuals.

Microscopes used for sorting of infaunal samples and taxonomic identification of specimens are cleaned and maintained as needed.

B5. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Critical supplies for laboratory activities will be the responsibility of the Project Manager or designee (Table 1).

Table 1. Supplies, Acceptance Criteria, and Responsibility for Critical Laboratory Supplies.

Critical Supplies and Consumables	Inspection Requirements and Acceptance Criteria	Responsible Individual
Jars for infauna samples	Visually inspected for cracks, breakage, and cleanliness. May be reused.	Laboratory Staff
Chemicals and reagents	Visually inspected for proper labeling, expiration dates, appropriate grade.	Laboratory Staff

If unacceptable supplies or consumables are found, the Project Manager will initiate corrective action. Corrective measures may include repair or replacement of measurement equipment, and/or notification to vendor and subsequent replacement of defective or inappropriate materials. All actions will be documented in the project files.

B6. DATA MANAGEMENT

B6.1 Data Custody

Each team member involved in this project is responsible for the internal custody of their electronic and hard-copy data until they are submitted to the Project Manager. All hand-entered data that is submitted electronically will receive 100% verification prior to submission, will be entered and checked using double data entry, or will be done using a software application (e.g. KeyesPunch™) which employs automated controls and data verification. Formats designed to comply with rules of the selected database (if upload requested) will be used in the application to constrain data entry. These features will ensure that any entry errors are caught and corrected as the operator keys the data.

B6.2 Laboratory Data and Data Reduction

All data generated by manually from optical field of a microscope and entered directly into an electronic format (e.g., Excel spreadsheet), or entered into laboratory data sheets and then manually entered into an electronic format will be reviewed. All manually entered data will receive 100% verification or will be entered and checked using double data entry.

Data reduction is the process of converting raw numbers (e.g., numbers of organisms per replicate) into data that can be displayed graphically, summarized in tables, or compared statistically for differences between mean values for sampling stations or times. Infauna data analysis discussed below require that some data be derived from the raw numbers for the technical reports. All data reduction will be performed electronically, either by the instrument software or in a spreadsheet, and will be validated according to procedures described in Section D2.

The format for final data submission is described below.

B6.2.1 Statistical Analysis

If the scope of work includes data analysis, benthic infaunal data may be analyzed using some or all (per project basis) of the following community parameters: abundance, Shannon-Wiener diversity index (H'), Pielou's evenness (J'), Margalef's diversity index, Simpson, and Total Taxonomic Distinctiveness (TTD). Other analyses may be performed if requested by the client.

Shannon-Wiener diversity index (H') characterizes the species diversity in a community and is calculated following

$$H' = -\sum_{i=1}^R p_i \ln p_i \quad (1)$$

where p_i is the proportion of individuals belonging to the i th species in the sample.

Pielou's evenness is calculated by

$$J' = H'/H'_{\max} \quad (2)$$

where H' is derived from the Shannon-Wiener diversity index and H'_{\max} is the maximum possible value of H' (if every species was equally likely), calculated by $H' = \ln S$. S is species richness, the total number of species in the sample. J' ranges between 0 and 1, the lower J' is the less evenness is a community between the species.

Margalef's diversity index (D_{Mg}) is calculated by

$$D_{Mg} = (S - 1)/\ln N$$

(3)

where S is species richness and N is the total number of individuals in the sample.

Multivariate analyses will be performed using a statistical software package (e.g., R, Primer) to determine the spatial patterns in the overall similarity of benthic assemblages in the survey area. Multivariate analyses will include hierarchical agglomerative clustering (cluster analysis) and non-metric multidimensional scaling (MDS; Clarke 1993, Warwick 1993, Clarke and Green 1988). Both of these analyses will be based on Bray-Curtis similarity. Prior to these analyses, the infaunal abundance data will be square-root or fourth-root transformed to ensure that all taxa, not just the numerical dominants, will contribute to similarity measures. The “similarity profile test” (SIMPROF) will be used to provide statistical support for the identification of faunal assemblages (i.e., selection of cluster groups). SIMPROF is a permutation test of the null hypothesis that the groups identified by cluster analysis do not differ from each other in multivariate structure.

US M-AMBI, a multivariate AZTI Marine Biotic Index (M-AMBI) adapted for US coastal waters, will be used to determine the health condition of sub-embayments and embayment. US M-AMBI will be calculated following Pelletier et al. (2018). US-M-AMBI health condition categories will include: bad (<0.20), poor (0.20 to 0.39), moderate (0.39 to 0.53), good (0.53 to 0.77), and high (>0.77). A statistical routines package (e.g. R, Primer) will be used to calculate this index.

The results for abundance, H' diversity, J' evenness, Margalef’s diversity index, Simpson, TDD and US M-AMBI will be tabulated in an Excel spreadsheet for delivery to the Project Manager. Hierarchical agglomerative clustering (cluster analysis) and MDS multivariate analyses will be provided in graphic format to the Project Manager.

B6.3 Data Set Structure

Electronic Data Deliverables will be prepared by the laboratory in a structure and format that complies with Normandeau format, client specific format, or a selected database (if upload to a database is requested).

B6.4 Project Database Codes

Standardized codes and qualifiers help to ensure consistency over time in a laboratory and a benthic monitoring project/program. The data codes that may be used with the benthic infaunal samples are presented in Table 2. Benthic infaunal taxonomic identification data codes are shown in Table 3. These database codes are generally consistent with National Coastal Condition Assessment (NCCA) program.

Table 2. Benthic infaunal sample data codes. Data codes are from NCCA 2015 (US EPA 2016).

Field	Format	Description
LAB NAME	Character	Name of lab
DATE RECEIVED	MMDDYY	Date sample was received by lab
SITE ID	Character	Site identification code as used on sample label
SAMPLE_NUMBER	Numeric	Sample number as used on field sheet (on sample label)
SAMPLE_TYPE	Character	INFAUNAL, SEDIMENT, or DESTRUCTIVE
DATE COLLECTED	MMDDYY	Date sample was taken
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.
		Flag
		Definition
		OK
		Sample is in good condition
		C
		Sample container is cracked
		ML
		Sample label is missing
		NP
		Not enough preservative used
		Q
		Other quality concerns, not identified above (explain in COND_COMMENTS)
COND_COMMENTS	Character	Explanation for Q FLAG (if needed)

Table 3. Benthic infaunal taxonomic identification data codes. Data codes are from NCCA 2015 (US EPA 2016).

Field	Format	Description
LAB NAME	Character	Name of lab
DATE RECEIVED	MMDDYY	Date sample was received by lab
SITE ID	Character	Site identification code as used on sample label
SAMPLE_NUMBER	Numeric	Sample number as used on field sheet (on sample label)
DATE COLLECTED	MMDDYY	Date sample was taken
DATE TAXON	MMDDYY	Date that the taxonomist started identifying organisms in the sample
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.
FAMILY	Character	Taxonomic family
GENUS	Character	Taxonomic genus
SPECIES	Character	Taxonomic species
TSN	Numeric	Taxonomic Serial Number is a unique and persistent identifier for a scientific name in the Integrated Taxonomic Information System (ITIS). If taxon is not in this list, provide citation for reference used to identify organism in CITATION field
TAXA NAME	Character	Complete taxon name
ABUNDANCE TOTAL	Numeric	Total number of individuals
DISTINCT	Character	Distinct taxa in sample (y/n)
CITATION	Character	Citation for reference used to identify organism, if taxon not present in ITIS.
QA FLAG (if appropriate)	Character	QA/QC flag (lab may use its own flags, if defined in QA_COMMENTS field)
		FlagDefinition
		DDDamaged Organism, poor condition or fragments
		NPNot enough preservative used
		UNUnknown. Identification is tentative. Organism has been sent to expert taxonomist for definitive identification.
		NTNot able to meet target level for identification (may be used with other codes, or explain in QA_COMMENTS field)
		SSample shipping problem (explain in QA_COMMENTS field)
		QOther quality concerns, not identified above
COND_COMMENTS	Character	Explanation for Q FLAG (if needed)
LAB COMMENTS	Character	General laboratory analysis comments

B6.5 Data Submittal to Client and Selected Database

Prior to submittal to the client and/or selected database (if requested), all data will receive a quality assurance review by laboratory staff and the Project Manager during which a software application will be used for logical error checks and to check for violations of database constraints and business rules. Any issues will be corrected in the data files. Any irresolvable issues in the data files identified by quality control checks will be mentioned in the data deliverable to the client.

Electronic data submissions will be made by the Project Manager or a designee using email or a secure File Transfer Program (FTP) site. Submissions by the Project Manager or a designee to the selected database (if requested) will be made through the database interface.

C. ASSESSMENT AND OVERSIGHT

C1. ASSESSMENT AND RESPONSE ACTIONS

This section identifies the number, frequency, and type of planned assessment activities that will be performed to assure implementation of this QAPP. These activities will be overseen by the QA Director.

C1.1 Assessments

C1.1.1 *Laboratory Technical System Audits*

Laboratory audits may be conducted by the QC/QA Manager at the project start up and then periodically as part of its analytical monitoring program. The laboratory audit checklist (Table 4) will review the following:

- QA organization and procedures
- Personnel training and qualifications
- Sample log-in procedures
- Sample storage facilities
- Analyst technique
- Adherence to laboratory SOPs and this QAPP
- Compliance with QA/QC objectives
- Instrument calibration and maintenance
- Facility security
- Waste management
- Data recording, reduction, review, reports, and archival
- Cleanliness and housekeeping

Preliminary results of the systems audit will be discussed with the management staff. A written report that summarizes audit findings and recommends corrective actions will be prepared and submitted to the Program Manager. The results of the audit, including resolution of any deficiencies, will be included in the QA reports, as described in Section C2.

C1.1.2 *Performance Evaluation Sample Assessment*

Proficiency testing for infaunal taxonomic analyses is accomplished through regular communication and inter-calibration of infaunal samples among taxonomists.

C1.1.3 *Data Technical System Audits*

Data will be audited under the direction of the QA Director as part of the data validation process (Section D). Raw data will be reviewed for completeness and proper documentation. Errors noted in data audits will be communicated to analysts and laboratory management and corrected data will be verified. Audits of the data collection procedures at contracted laboratories will be the responsibility of the contracted laboratories. Each contracted laboratory

is fully responsible for the verification and validation of the data it submits. Data must be submitted in QAPP-prescribed formats; no other formats will be acceptable. During the time that work is in progress, the contracted laboratory's QA Director or his/her designee will conduct an inspection to evaluate the laboratory data-production process. All data must be reviewed by the contracted laboratories QA Director or designee prior to submission to the Project Manager.

C1.2 Assessment Findings and Corrective Action Responses

All technical personnel share responsibility for identifying and resolving problems encountered in the routine performance of their duties. Issues that affect the schedule, cost, or performance of project tasks will be reported to the Project Manager. The Project Manager will be accountable for overall conduct of a specific project, including the schedule, costs, and technical performance. The Project Manager will be responsible for identifying and resolving problems that (1) have not been addressed in a timely manner or successfully at a lower level, (2) influence multiple components of the project, or (3) require consultation with the client. He/she will be responsible for evaluating the overall impact of the problem on the project and for discussing corrective actions with the client. The Project Manager will also identify and resolve problems that necessitate changes to this QAPP. Problems identified by the QA Director will be reported to the Project Manager and corrected as described in Section C2.

Corrective actions may result from planned audits or from unanticipated events that occur during the course of the project. Significant events that result in deviations from this QAPP will be recorded through the "Extraordinary Event/Nonconformity" (EE/NC) reporting process. The appropriate corrective actions to address any such events will be assessed by the QA Director in consultation with the Project Manager and client. The QA Director will generate and/or review all corrective actions required during the project and monitor their effectiveness in meeting project quality objectives. The Project Manager will review these issues on a regular basis, but the QA Director will bring serious issues to the Project Manager's attention immediately. The Project Manager will report any corrective actions in project QA/QC Corrective Action Log.

Corrective action is the process of identifying, recommending, approving, and implementing measures to counter unacceptable procedures or out-of-limit QC performance that can affect data quality. Corrective action can occur laboratory analyses, data validation, and data assessment. All corrective action proposed and implemented should be documented in the QA reports (Section C2). Corrective action should only be implemented after approval by the Program Manager or a designee.

Table 4. Example of Technical Systems Audit Checklist

Project:	
Facility Location:	
Auditor:	
Are equipment adequate to perform the analyses of interest?	
Review procedures and engineering controls for minimizing cross contamination.	
Review techniques for conformance to approved SOPs.	
Are personnel qualified and trained? Is there a formal training program and are records of training and proficiency maintained?	
Is there a designated sample custodian? Is there a sample inspection checklist?	
Is the laboratory area secure?	
Review internal chain-of-custody procedures.	
Are samples being analyzed in conformance to the cited methods?	
Are QC samples and checks being performed at the frequencies stated in the cited methods?	
Are records complete, accurate, up-to-date, and in conformance to good recordkeeping procedures?	
How are project-specific requirements communicated to the bench level?	
Review data reduction, review, and reporting processes.	
Review data archival process (paper and electronic).	
Review audit and corrective action program.	
Additional Comments:	
Auditor:	Date:

C1.2.1 *Laboratory Corrective Action*

Corrective action in the laboratory is specified in laboratory SOPs and may occur prior to, during, and after initial analyses. Conditions, such as broken sample containers, may be identified during sample log-in or analysis. Following consultation with laboratory analysts and supervisory personnel, it may be necessary for the QA Director to approve the implementation of a corrective action. If the problem makes it impossible to achieve project objectives, the laboratory manager will be notified, who will in turn notify the Project Manager. The Project Manager will communicate with other members of the project team, as necessary.

These corrective actions will be performed prior to release of the data from the laboratory. The corrective action will be documented in both the laboratory's corrective action files, and in the data report generated by the laboratory. If the corrective action does not rectify the situation, the laboratory will contact the Project Manager or a designee, who will determine the action to be taken and inform the appropriate personnel.

C1.2.2 *Corrective Action during Data Validation and Data Assessment*

The need for corrective action may be identified during either data validation or data assessment. Potential types of corrective action may include reanalysis of samples by the laboratory. These actions are dependent upon the ability to mobilize the survey crew and whether the data to be collected are necessary to meet the required QA objectives. If the data validator or data assessor identifies a corrective action situation that impacts the achievement of the project objectives, the Project Manager will be responsible for informing the appropriate personnel.

C2. REPORTS TO MANAGEMENT

QA reports will be prepared by the QA Director and submitted on an as-needed basis to the Project Manager. QA reports will document any problems identified during the analysis programs and the corrective measures taken in response. The QA reports will include:

- All results of laboratory audits
- Problems noted and actions taken during data validation and assessment
- Significant QA/QC problems, recommended corrective actions, and the outcome of corrective actions

A summary of QA issues, audit findings, and significant nonconformities will be included in the project notes or QA/QC Corrective Action Log.

D. DATA VALIDATION AND USABILITY

This section details the QA activities that will be performed to ensure that the collected data are scientifically defensible, properly documented, of known quality, and meet project objectives. Two steps are completed to ensure that project data quality needs are met:

- Data verification/validation
- Data usability assessment

D1. DATA REVIEW, VERIFICATION, AND VALIDATION

D1.1 Laboratory Data

Prior to the release of any data from the laboratory, the data will be reviewed and approved by laboratory personnel. The review will consist of a tiered approach (Section D2.2) that will include reviews by the person performing the work, by a qualified peer, and by supervisory and/or QA personnel.

D1.2 Data Management

The review process will include verification of manually entered data and QC checks run in a software application prior to submitting the data to the client. Detailed descriptions of these processes are included in Sections B6 and D2.

D2. VALIDATION AND VERIFICATION METHODS

D2.1 Laboratory Data

As a part of data validation, staff will ensure that:

- The QC checks specified in Section B3 were conducted and met the acceptance criteria
- All data that are hand-entered (*i.e.*, typed) will be 100% validated by qualified personnel prior to use in calculations or submission to the Project Manager
- All manual calculations will be performed by a second staff member to verify that calculations are accurate and appropriate
- Calculations performed by software will be independently verified at a frequency sufficient to ensure that the formulas are correct, appropriate, and consistent, and that calculations are accurately reported

Once data have been generated and compiled in the laboratory, senior scientists in the laboratory will review the data to identify and make professional judgments about any suspicious values. All suspect data will be reported, but flagged with a qualifier. These data may not be used in calculations or data summaries without the review and approval of the appropriate senior staff. No data measurements will be eliminated from the reported data or

database and data gaps will never be filled with other existing data. The loss of any samples during shipment or analysis will be noted.

D2.2 Data Management

Laboratory data will be reviewed by the Project Manager prior to the electronic submission to the client. Data review may include methods such as plots, logical checks, and range checks to identify suspect values. Routine system back-ups are performed daily. Data provided electronically to facilitate data handling will be verified against the hard copy data. Detailed description of data management and review is provided in section B6 of this QAPP.

D2.3 Project Deliverables

Upon completion of the verification/validation process, a dataset will be prepared for submittal to the client. This documentation will include the required elements outlined in the contractual agreement or scope of work

D3. RECONCILIATION WITH USER REQUIREMENTS

This element describes how the verified/validated project data will reconcile with the project DQOs, how data quality issues will be addressed, and how limitations on the use of the data will be reported and handled. The purpose of this section is to indicate the methods by which it will be ensured that the data collected for the client fall in line with the DQOs as described in Section A6 of this QAPP. To meet these DQOs, a combination of qualitative evaluations and statistical procedures will be used to check the quality of the data. These procedures will be used by the laboratory and by the Project Manager or a designee.

The data generated must meet client needs as defined in the project DQOs defined in Section A6 of this QAPP. The primary objectives for assessing the usability of the data are to ensure that (1) all data are complete and defensible, and (2) data are of the quality needed to meet the overall objectives of the client.

D3.1 Comparison to Measurement Criteria

D3.1.1 Accuracy and Precision Assessment

The accuracy and precision of the data generated during a project will be assessed by comparison to the DQOs specified in Section A6. Data that fail to meet the data quality criteria may necessitate sample reprocessing, analysis of archival material, or flagging of the data, depending on the magnitude of the nonconformance, logistical constraints, and schedule.

D3.1.2 *Completeness Assessment*

Completeness is the ratio of the number of valid sample results to the total number of results planned for collection. The goal is to generate valid, usable data. However, in environmental sampling and analysis, some data may be lost due to sampling location logistics, or field or laboratory errors. The overall completeness goal is 100% of samples to be analyzed. The Project Manager will assess the completeness of the overall data generation against the project goals. Following completion of the analysis and data review, the percent completeness will be calculated and compared to the project objectives stated in Section A6 using the following equation.

$$\% \text{ Completeness} = \frac{\text{Number of valid/usable results obtained}}{\text{Number of valid/usable results planned}} \times 100$$

If this goal is not met, data gaps may exist that will require evaluation to determine the effect on the intended use of the data. Sample re-analysis or analysis of archived material may be appropriate depending on criticalness of the missing data, logistical constraints, and schedule.

D3.1.3 *Representativeness*

Representativeness expresses the degree to which data accurately and precisely denote a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition within a defined spatial and/or temporal boundary.

The assessment of representativeness in the will consist of verifying that the proper analytical procedures and appropriate methods (SOPs) were used.

E. REFERENCES

- Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18:117-143.
- Clarke K.R., and R.H. Green. 1988. Statistical design and analysis for a 'biological effects' study. *Marine Ecology Progress Series* 46:213-226.
- Germano, J.D., D.C Rhodes, R.M. Valente, D.A. Carey, and M. Solan. 2011. The use of sediment profile imaging (SPI) for environmental impact assessments and monitoring studies: Lessons learned from the past four decades. *Oceanography and Marine Biology: An Annual Review* 49: 235–298.
- Pearson, T.H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology Annual Review* 16, 229–311.
- Pelletier, M.C., D.J. Gillett, A. Hamilton, T. Grayson, V. Hansen, E.W. Leppo, S.B. Weisberg, and A. Borja. 20018. Adaption and application of multivariate AMBI (M-AMBI) in US coastal waters. *Ecological Indicators* 89: 818-827.
- Rhoads, D.C. and J.D. Germano. 1986. Interpreting long-term changes in benthic community structure: a new protocol. *Hydrobiologia* 142: 291–308.
- US EPA. (U.S. Environmental Protection Agency). 2001(reissued 2006). EPA Requirements for Quality Assurance Project Plans, March 2001, (EPA QA/R-5), EPA/240/B-01/003, United States Environmental Protection Agency, Washington, D.C., <http://www.epa.gov/quality/qs-docs/r5-final.pdf>.
- US EPA. (U.S. Environmental Protection Agency). 2002. EPA Guidance for Quality Assurance Project Plans, December 2002, (EPA QA/G-5), EPA/240/R-02/009, United States Environmental Protection Agency, Washington, D.C.
- US EPA (U.S. Environmental Protection Agency). 2016. National Coastal Condition Assessment 2015: Laboratory Operations Manual. Version 2.1, May 2016. EPA-841-R-14-008. U.S. Environmental Protection Agency, Office of Water, Washington, DC. 214 pp.
- Warwick, R.M. 1993. Environmental impact studies on marine communities: pragmatical considerations. *Australian Journal of Ecology* 18:63-80.
- Weisberg, S.B., J.A. Ranasinghe, D.D. Dauer, L.C. Schnaffer, R.J. Diaz, and J.B. Frithsen. 1997. An estuarine benthic index of biotic integrity (B-IBI) for Chesapeake Bay. *Estuaries* 20(1): 149-158.

APPENDIX A

Laboratory Standard Operating Procedures

Benthic Infaunal Samples

Normandeau Associate, Inc.

Laboratory Standard Operating Procedures

Marine Benthic Infauna Sorting and Identification



May 2020

Laboratory Standard Operating Procedures for Benthic Infauna Samples

Samples are preserved in the field with formalin and delivered or shipped to the laboratory. The following section describes the laboratory procedure for sorting and taxonomic identification of macrobenthic organisms collected from soft-bottom infauna surveys using a Van Veen grab. These methods are consistent with the laboratory procedures outlined in the National Coastal Condition Assessment (NCCA) 2015 Laboratory Operations Manual (US EPA 2016).

1.0 Health and Safety

In addition to the laboratory's requirements, persons using this SOP must abide by the following health and safety procedures:

1. Wear proper personal protection clothing and equipment (e.g. lab coat, gloves, and protective eyewear/goggles).
2. When working with potentially hazardous chemicals (e.g. formalin, reagent alcohol, Rose Bengal) or biological agents (benthic organisms and sediments), laboratory personnel must follow all manufacture's Safety Data Sheet recommendations, and avoid inhalation, skin contact, eye contact, or ingestion. If skin contact occurs, remove clothing immediately and rinse thoroughly. Wash the affected skin areas thoroughly with large amounts of soap and water. When working with formalin, laboratory personnel must follow the OSHA Formaldehyde Standard (29 CFR 1910.1048).

2.0 Laboratory Equipment

■ 2.1 Sample Preparation – Sorting

The following equipment and materials are required for sample preparation, subsampling, sorting, and taxonomic identifications:

- U.S. 35 sieve (500 μ m or 0.5 mm) for soft-bottom infaunal samples
- Round buckets
- Standardized, or gridded screen - 40 Mesh (380- μ m openings, T304 stainless steel wire, 34GA (0.010"))
- 6-cm scoop
- White or clear plastic or enamel pan (6" x 9") for sorting
- Teaspoon
- Permanent ink pen (e.g. Pigma Micron® pen, Sharpie)
- Pencil or alcohol resistant ink pen
- Dropper
- Fine-tipped forceps (watchmaker type, straight and curved)

- Vials with caps or stoppers
- Sample labels for vials
- Reagent alcohol (5% methanol, 5% isopropanol, 85% ethanol)
- Stereo zoom microscope (6-10X magnification or greater)

■ **2.2 Sample Preparation - Taxonomy Identification**

The following equipment is required for benthic macroinvertebrate taxonomic identification:

- Stereo dissecting microscope with fiber optics light source (50-60X magnification)
- Compound microscope (10, 40, and 100X objectives, with phase-contrast capability)
- Petri dishes
- Permanent ink pen (e.g Pigma Micron® pen, Sharpie)
- Pencil or alcohol resistant ink pen
- Dropper
- Fine-tipped forceps (watchmaker type, straight and curved)
- Vials with caps or stoppers
- Sample labels for vials
- Reagent alcohol in a plastic wash bottle
- Taxonomic Bench Sheet
- Hand tally counter
- Taxonomic keys (For example, Smith 1964, Gosner 1971, Bousfield 1973, Abbott 1974, Fauchald 1977, and Pollock 1998).

3.0 Sample Receipt

The laboratory procedure for benthic samples begins with the receipt of the samples at the subcontracted laboratory.

1. Record receipt of samples and sign the Chain of Custody (COC) form (Figure 1).
2. Inspect each jar THE SAME DAY THEY ARE RECEIVED:
 - a. Add 10% Formalin to the jar, if necessary (i.e., to cover the contents completely).
 - b. Verify that the date collected, site identification, and sample number on the label also appear on the Chain of Custody form in the shipment.
 - c. Notify the Project Manager if any jars were broken and/or there are discrepancies between the custody form and jars.
3. Store the sample containers at room temperature until sorting begins. Replace the 10% buffered formalin with reagent alcohol within 7 days of collection for better preservation of the organisms.

4. To facilitate the sorting process, all samples will be stained with Rose Bengal. Add Rose Bengal to the reagent alcohol to the point of saturation. Samples should be stained at least overnight but no longer than 48 hours before sorting the infaunal samples to avoid over-staining.
5. Maintain the Chain of Custody form with the samples; it will be needed if the samples are transported to any other location (e.g., for taxonomic identification, external quality control (QC) evaluation).

4.0 Sample Preparation - Sorting

This section describes the steps for the sorter in preparing the sample and picking organisms.

1. Remove the lid from the sample container and remove the internal sample label.
2. Carefully decant the reagent alcohol from the sample container by pouring the fluid through a 0.5 mm or 1.0 mm (selected based on the sample type being sorted) sieve into a separate container. Inspect the mesh of the sieve for any organisms and return any organisms found to the sample container so they can be included in the sample sort process.
3. Remove sieved organisms from the sample container and place into a sorting tray.
4. Sort all samples under a minimum of 6x dissecting microscope. Remove the macroinvertebrates from the detritus with forceps. In general, do not remove:
 - Empty snail or bivalve shells
 - Organisms of water surface-dwelling or strict water column arthropod taxa, and meiofauna.
 - Incidentally-collected terrestrial taxa.
 - Fragments such as legs, antennae, gills, wings, or tails.
 - For Oligochaeta, attempt to remove only whole organisms or fragments that include the head. Do not remove fragments without the head.
 - In case of uncertainties, place the organism in the sort vial for the taxonomist to make the final determination.
5. Place picked organisms of the same type into a single set of jars and vials containing reagent alcohol.
6. Remove the remaining material left on the sorting pan (i.e. material such as sticks, organic debris) and place it in a separate container with preservative (reagent alcohol). Label the container "Picked," on both internal and external labels.
7. Label the vials and jars of sorted organisms and material with an external label using a permanent ink pen. Internal sample labels should be made of cotton rag paper or an acceptable substitute and written with pencil or alcohol resistant ink pen.
8. Retain the vials and materials for the time period specified in Section 9.0.

9. Thoroughly clean all sample preparation and sorting equipment and make sure all equipment is free of organisms prior to sorting the next sample.

5.0 Taxonomic Identification

The taxonomist performs the following steps in identifying the benthic macroinvertebrates:

1. Upon receipt of a set of sample vials from the sorter:
 - a. Compare all site identification codes and sample numbers on the form with those entered on the labels of samples, and resolve any discrepancies with the sorter.
 - b. Determine if any vials are broken. For any broken vial, attempt to recover as much of the sample as possible. Describe the damage on the Taxonomic Bench Sheet.
 - c. Maintain the Chain of Custody form with the sample vials; it will be needed to return/store them.
2. Empty one sample vial at a time into a small Petri dish. Add reagent alcohol to keep the organisms covered. Remove the internal sample label and complete the top portion of a Taxonomic Bench Sheet, using the information from the label.
3. View the sample to ensure that all necessary diagnostic characters have been observed, according to the taxonomic key or other literature.
4. Identify organisms to the lowest practical taxonomic level (species is the target for all organisms with the exception of meiofauna, (due to being smaller than 0.5 mm). Additional exceptions include Oligochaeta (Class) and Chironomidae (Family) in samples from marine, polyhaline and mesohaline regions. If a laboratory or individual taxonomist is having trouble reaching the species level for a taxonomic group but not for an individual organism which might be damaged or otherwise difficult to identify, the lab must contact the project lead for guidance. Add any necessary data qualifiers.
5. Record the identifications. For example, using the Taxonomic Bench Sheet, record the identification in the Column labeled "taxon." Enter the number of larvae, pupae, and adults, or total count (e.g. mollusks), if appropriate life history column does not apply, of each taxon under the appropriate columns.
 - a. Refer to either website to check the scientific name to be sure that there have not been any name changes: 1) <https://www.itis.gov/> or 2) <http://www.marinespecies.org/aphia.php?p=search>
 - b. If the target taxonomic level cannot be achieved due to immature or damaged organisms this should be noted.
 - c. If damaged organisms can be identified, they are counted ONLY if the:
 - 1) Fragment includes the head, and, in the case of arthropods, the thorax;

- 2) Oligochaetes have a sufficient number of segments in the head;
 - 3) Mollusk shell (bivalve or gastropod) is occupied by an organism;
 - 4) Organism is the sole representative of a taxon in the sample.
- d. If a unique taxon is determined for which the appropriate taxonomic level is not available in the literature and there are other taxa in that taxonomic level:
- 1) Provide good quality digital photographs of the organism to outside experts for identification; and
 - 2) Include the tentative identification in the database with a data qualifier so that these organisms can be distinguished from other organisms in the data analysis.
 - 3) When the outside expert identifies the organism, update the database with the correct identification.
6. Compare taxa names from the taxa list provided by the project manager to the names used for the identifications. Check the non-matches and correct them.
 7. Complete the identification by entering the totals for each developmental stage and the total number of each taxon in the cells at the bottom of the sheet. Cross-check to be sure the totals were summed correctly.
 8. Return the identified organisms to the original sample vial, fill with reagent alcohol, and cap tightly.
 9. Return or store the samples according to laboratory protocols and requirements.
 10. Verify that all required data has been recorded by the taxonomist or QC personnel. If the results were recorded on paper, provide the Taxonomic Bench Sheet to data entry staff.

6.0 Data Entry

All data generated by taxonomic identification will be manually read from the instrument display (optical field of a microscope) and entered directly into an electronic format (e.g., Excel spreadsheet), or entered into laboratory data sheets, and then manually entered into an electronic format. All manually entered data will receive 100% verification or will be entered and checked using double data entry. Standardized codes and qualifiers help to ensure consistency over time in laboratories and benthic monitoring programs. Tables 1 and 2 identify the data codes to be used for benthic infauna samples.

Table 1. Benthic infaunal data codes. Data codes are from NCCA 2015 (US EPA 2016).

Field	Format	Description
LAB NAME	Character	Name of lab
DATE RECEIVED	MMDDYY	Date sample was received by lab
SITE ID	Character	Site identification code as used on sample label
SAMPLE_NUMBER	Numeric	Sample number as used on field sheet (on sample label)
DATE COLLECTED	MMDDYY	Date sample was taken
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.
		Flag
		Definition
		OK
		Sample is in good condition
		C
		Sample container is cracked
		ML
		Sample label is missing
		NP
		Not enough preservative used
		Q
		Other quality concerns, not identified above (explain in COND_COMMENTS)
COND_COMMENTS	Character	Explanation for Q FLAG (if needed)

Table 2. Benthic infaunal taxonomic identification data codes. Data codes are from NCCA 2015 (US EPA 2016).

Field	Format	Description
LAB NAME	Character	Name of lab
DATE RECEIVED	MMDDYY	Date sample was received by lab
SITE ID	Character	Site identification code as used on sample label
SAMPLE_NUMBER	Numeric	Sample number as used on field sheet (on sample label)
DATE COLLECTED	MMDDYY	Date sample was taken
DATE TAXON	MMDDYY	Date that the taxonomist started identifying organisms in the sample
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.
FAMILY	Character	Taxonomic family
GENUS	Character	Taxonomic genus
SPECIES	Character	Taxonomic species
TSN	Numeric	Taxonomic Serial Number is a unique and persistent identifier for a scientific name in the Integrated Taxonomic Information System (ITIS). If taxon is not in this list, provide citation for reference used to identify organism in CITATION field
TAXA NAME	Character	Complete taxon name
ABUNDANCE TOTAL	Numeric	Total number of individuals
DISTINCT	Character	Distinct taxa in sample (y/n)
CITATION	Character	Citation for reference used to identify organism, if taxon not present in ITIS.
QA FLAG (if appropriate)	Character	QA/QC flag (lab may use its own flags, if defined in QA_COMMENTS field)
		FlagDefinition
		DDDamaged Organism, poor condition or fragments
		NPNot enough preservative used
		UNUnknown. Identification is tentative. Organism has been sent to expert taxonomist for definitive identification.
		NTNot able to meet target level for identification (may be used with other codes, or explain in QA_COMMENTS field)
		SSample shipping problem (explain in QA_COMMENTS field)
COND_COMMENTS	Character	QOther quality concerns, not identified above
		Explanation for Q FLAG (if needed)
LAB COMMENTS	Character	General laboratory analysis comments

7.0 Sample and Record Retention

1. Infauna samples (both archived [if requested] and processed samples) and sample materials, including vials, slides, and sorting residuals, will be held until acceptance of the data deliverable or technical report by the client. These samples can then be disposed of after approval from the Program Manager. Processed samples will be maintained at the laboratory that conducted the sorting and identification. Sample residues will be held until the data is accepted by the client, and then may be discarded. Reference collection specimens (if requested) will be retained by the laboratory for the length of time specified in the contractual agreement or scope of work, and then provided to the next designated laboratory. Reference collection specimens will be clearly identified, labeled with the project name and unique identification number. Materials shall be stored in a cool location away from sunlight. The laboratory shall periodically check the reference collection and sample materials for degradation and refill jars and vials with reagent alcohol if necessary.
2. All the project records, including laboratory notebooks and the reference library, will be maintained at least 3 years.

8.0 Sample QC

Benthic samples will be checked for QA/QC following the procedure detailed in the Normandeau Marine Benthic Infaunal QAPP and presented below.

The data quality goals for analysis of benthic infauna are (1) all samples be processed, (2) all animals be removed for identification and enumeration, (3) all infaunal animals be counted accurately, (4) the taxonomic identifications be accurate (correct), and (5) the identifications correspond to those used throughout the monitoring program. At least 95 percent of all animals must be removed from a sample to pass the quality control (QC) evaluation.

■ Sorting

Sorting technicians will remove all organisms from the samples and separate them into major taxonomic groups. All residual material will be labeled and stored for QC analysis. For the QC analysis, samples will be divided into batches of approximately 10 samples. Approximately 10% of the samples from each batch will then be randomly chosen for an independent QC check. Only senior technicians will perform the QC evaluations (A senior technician is defined as having three or more years of sorting experience). Under no circumstances will the same individual who sorted the sample perform the QC evaluation. In most cases, a batch of samples is defined as ten consecutively sorted samples. By definition, at least 95% of all animals must be removed from a sample to pass the QC evaluation (i.e., the percent sorting error must be $\leq 5\%$). The following formula will be used to calculate the percent sorting error for each QC sample:

$$\frac{\text{Number of animals found in QC inspection}}{\text{Total number of animals present in sample}} \times 100 = \text{percent sorting error}$$

If more than 5% of the total organisms in the QC sample have been missed, the sample fails QC evaluation, and the all remaining samples from that batch will be re-sorted. Technicians will be informed of any necessary corrective measures. This procedure will be repeated until the batch of samples passes the QC evaluation. An exception will be made for low abundance samples (a sample with fewer than 60 organisms) that are chosen for the QC evaluation. Any low abundance sample in which three or fewer organisms were missed is considered to pass the sorting QC evaluation even if the percent sorting error is >5%. Samples in which no organisms are present will be excluded from the sorting QC selection process. A record of all sorting QC evaluations will be maintained for each batch.

■ **Identification and Enumeration**

The same basic QC principles described in the Sorting section will in general apply to species identifications. At least 10% of the samples will be checked to detect any unacceptable identification and enumeration errors. Only senior taxonomists will perform the QC check. QC samples will be selected in the same manner as described in the Sorting section above. Additionally, the same percent accuracy level will be used to determine if a sample passes the QC evaluation and the same corrective measures will be implemented if a sample fails the QC evaluation. The following formula will be used to calculate the percent taxonomy error for each QC sample:

$$\frac{\text{Total number of taxonomy errors}}{\text{Total number of animals present in sample}} \times 100 = \text{percent taxonomy error}$$

In certain cases it may not be necessary to reprocess the entire batch of samples if only minor corrections are needed (e.g., name changes). When any misidentification is discovered, all previously identified samples containing that taxon will be rechecked. A record of all identification QC evaluations will be maintained.

9.0 Storage

1. Upon completion, the sorted material and the vials of identified animals are boxed for off-site storage.
2. A Storage Label (Figure 2) will be completed and attached to one end of the storage box. The following information is to be provided:
 - a. Project Name, Box Number, and Project Date
 - b. Brief description of the package contents
 - c. Storage Date
 - d. Preservative
 - e. Manager's Name

3. On the Sample Storage Sheet (Figure 3) an accurate listing of the project name and number, collection date, station/replicate, and description for each sample will be completed. While only one task should be entered on a Sample Storage Sheet, multiple boxes may be included on a Sample Storage Sheet.
4. The original Sample Storage Sheet is placed in the Laboratory Storage Book and a copy placed in the box.
5. Samples are stored in the laboratory's storage facility until the data deliverables or technical report is approved by the client. With permission from the Program Manager, following data deliverable or report approval, samples are removed from storage and prepared for sample disposal as described below in Section 10

10.0 Disposal

1. Upon authorization from the Project Manager, samples will be removed from the storage facility and prepared for sample disposal.
2. Sample disposal, the separation of liquids and solids from processed samples, is conducted at the laboratory's ventilated hood by trained individuals.
3. Liquids and solids (including sample residue, glass vials, animals and electrical tape) from sample residue are separated, containerized and labeled following appropriate hazardous waste requirements.
4. The laboratory will adhere to local and state Hazardous Waste Small Generators requirements and hold wastes on site until the subcontracted hazardous waste vendor arrives and removes the wastes. Appropriate documentation, for example copies of the Hazardous Waste Manifest and the Land Ban Form, are filed with the laboratory's manager.

11.0 References

- 29 CFR 1910.1048. Occupational Safety and Health Administration, Department of Labor. Occupational Safety and Health Standards, Toxic and Hazardous Substances. Formaldehyde. Title 29 Code of Federal Regulations. Part 1910.1048.
- Abbott, R.T. 1974. American Seashells. The Marine Mollusca of the Atlantic and Pacific Coasts of North America. Van Nostrand Reinhold Company, New York.
- Bousfield, E.L. 1973. Shallow-water Gammaridean Amphipoda of New England. Comstock Publishing Associates, Ithica, NY.
- Fauchald, K. 1977. The Polychaete Worms. Definitions and Keys to the Orders, Families and Genera. Natural History Museum of Los Angeles County. Los Angeles.
- Gosner, K.L. 1971. Guide to Identification of Marine and Estuarine Invertebrates. Wiley Interscience, New York.

- Pollock, L. 1998. A Practical Guide to the Marine Animals of Northeastern North America. Rutgers University Press. New Brunswick, New Jersey.
- Smith, R.I. 1964. Keys to Marine Invertebrates of the Woods Hole Region. Marine Biological Laboratory, Woods Hole, MA.
- US EPA (U.S. Environmental Protection Agency). 2016. National Coastal Condition Assessment 2015: Laboratory Operations Manual. Version 2.1, May 2016. EPA-841-R-14-008. U.S. Environmental Protection Agency, Office of Water, Washington, DC. 214 pp.

www.normandeau.com Bedford: (603) 472-5191 Falmouth: (508) 548-0700

Relinquished by: (signature)	Received by: (signature)	Relinquished by: (signature)	Received by: (signature)	Relinquished by: (signature)	Received by: (signature)
Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:
Date:	Date:	Date:	Date:	Date:	Date:

Normandeau Associates, Inc.
Benthic Infauna Laboratory Standard Operating Procedures

PROJECT NAME:			BOX NUMBER:	
PROJECT CODE:			MANAGER:	
PROJECT DATE:			STORAGE DATE:	
SAMPLE DESCRIPTION: 				
SAMPLE INFO:			PRESERVATIVE:	
<input type="checkbox"/> RESIDUE	<input type="checkbox"/> CONT.	<input type="checkbox"/> VIALS	<input type="checkbox"/> 6% FORM.	<input type="checkbox"/> 70% ETOH

BoxLabel.AI_2015

Figure 2. Example of a Storage Label.

Storage Sheet

Project Name: _____

Project Year: _____

Page _____

Project Code: _____

Project Manager: _____

Project Task: ICH(IP) ____, ICH(Ent) ____, Benthos ____, Other _____

[illegible]

Figure 3. Example of a Sample Storage Sheet.