

SEAN environmental contaminants SOP 4: field and laboratory guidelines for tissue analysis of bay mussels

Version 1.0

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1 Introduction

This standard operating procedure (SOP) covers all the necessary information for SEAN staff and partners to plan and execute the field work to collect, preserve, and ship bay mussels (*Mytilus trossulus*) to a laboratory contracted by the National Oceanic and Atmospheric Administration's Mussel Watch Program (NOAA MWP) for analysis of a broad suite of metals, polycyclic aromatic hydrocarbons (PAHs), and persistent organic pollutants (POPs). See SOP 6 for more details regarding specific analytes and data reporting procedures. Tasks related to this SOP include obtaining the permits required to perform the field sampling, gathering required supplies, observing collection standards, and methodically handling and preserving samples for shipping. It is important that all points of this SOP are read, clearly understood, and implemented as necessary starting six to nine months before field collection activities are planned. The project leader (currently, the SEAN ecologist) is responsible for ensuring this SOP is followed safely and accurately. Please refer to SOP 1 for operational safety considerations for each contaminants monitoring effort.

In 2007, a baseline assessment involving 65 samples of bay mussels was conducted in all three SEAN parks. Since that initial work, SEAN has continued collecting mussels every two years from five sites in GLBA and one site in SITK. The latest report is here:

Tallmon, D. A. 2012. Contaminants assessment of intertidal resources in southeast Alaska national parks—2007 to 2011. Natural Resource Technical Report NPS/SEAN/NRTR—2012/630. National Park Service, Fort Collins, Colorado.

The next sampling event is planned for late summer 2017. The current version of this SOP envisions that SEAN will maintain monitoring at seven sites: five in GLBA, one in KLGO, and one in SITK. During Fiscal Year 2018, SEAN will partner with NOAA MWP to synthesize all existing data to determine the implications of reducing sampling effort to every 5 years and whether trends in any analytes warrant more frequent analysis. To date, no such threats have been identified and results appear to demonstrate relatively pristine water quality in nearshore marine zones.

2 Field procedures

1 Permitting

Each SEAN park unit may require a permit for specimen collection activities. Contact the research permit coordinator for each park for current policy and application procedures. In some cases collections may be covered under an existing multi-year research permit. It is possible to obtain one permit that covers collection activities in all park units involved, but researchers should consult with permit coordinators for the preferred approach. Each year an Annual Investigator's Report is required. This report updates the details of the original study plan (if necessary), summarizes the study activities over the preceding year, and estimates funding expenditures. See these websites for more information on NPS Research Permit and Reporting System (RPRS) and GLBA-specific information on research in wilderness lands:

- <https://irma.nps.gov/rprs/Home>
- <http://www.nps.gov/glba/naturescience/research-permit-application-process.htm>

2 Supplies and personnel

Six to nine months before sampling is planned, the Project Leader should ensure that the field supplies required for collecting and shipping bay mussels in a clean manner are organized and in working order (Table SOP-4.1). At GLBA, a research vessel, usually the R/V Fog Lark, must be scheduled for sampling with a park-certified staff to captain. At SITK and KLGO, one person can accomplish field tasks, while at GLBA a minimum of two people are necessary for collection activities because a boat is required for every site except Bartlett Cove.

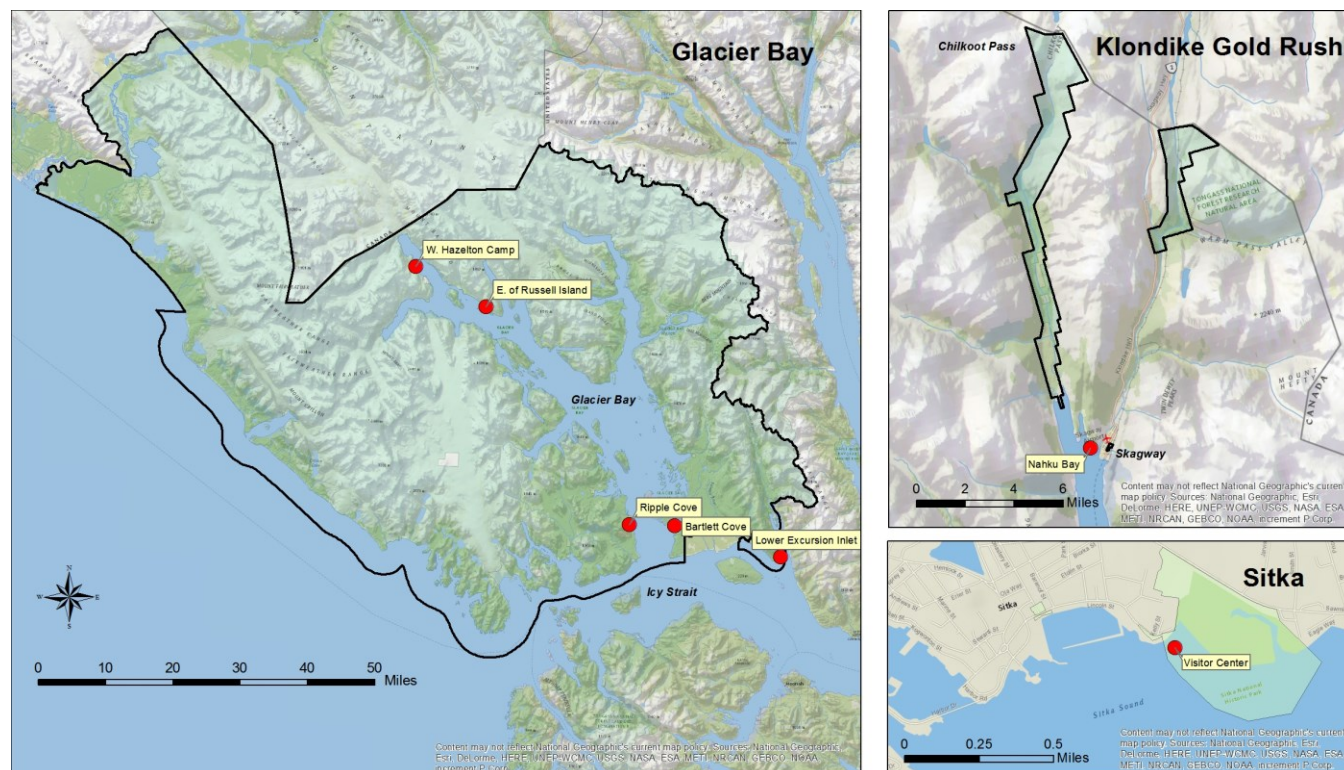
Table SOP-4.1. Checklist for field collection.

-
- ☐ GPS
 - ☐ Camera
 - ☐ 40 mL scintillation vial per site (or similarly sized vial; one per site)
 - ☐ Scotch or electrical tape for sealing vial lids
 - ☐ Gallon-size heavy-duty freezer bags (3-5 per site depending on mussel shell size)
 - ☐ Pencils
 - ☐ Rite in the Rain notebook
 - ☐ Sample labels
 - ☐ Field data sheets (one per site)
 - ☐ Sharpies
 - ☐ Thermometer (0.5°F or greater accuracy)
 - ☐ Transport cooler(s)
 - ☐ Store-bought bagged ice
 - ☐ Colander(s)
-

One cooler will be needed for shipping samples from each park. For GLBA, a 48 quart Coleman cooler is adequate and affordable, while the single site samples at SITK can be shipped using “lunch-sized” coolers with approximately 9 quart capacity. If equipped, cooler handles should be removed before shipping. At this time, due to budget and logistics constraints, histopathology samples, which require samples to stay cool but not frozen, are no longer being collected at SEAN monitoring sites. Therefore, samples should be frozen before shipping to ensure good condition upon arriving to the contracted laboratory. More details on shipping are found in the collection procedures section below.

3 Sampling sites

In SITK, the single sampling location is within short walking distance from the Visitor Center. In KLGO, Nahku Bay is accessed by foot from a trail departing from Skagway town center. In GLBA, all sites except for Bartlett Cove require access via vessels capable of shore landings (Figure SOP-4.1). Currently, the SEAN conducts the majority of GLBA field work from the R/V Fog Lark, an 8.5 m Munson landing craft stationed in Bartlett Cove. Four of the five long-term GLBA sites are distributed across the north-south gradient of the main bay. These sites track the main axis of vessel traffic, including the cruise ship route from the lower bay to upper Tarr Inlet. The Lower Excursion Inlet site was chosen to assess any potential impacts resulting from activities at the fish processing plant located just outside park boundaries on the east side of Excursion Inlet.



Site ID	Name	Park	Latitude	Longitude
GBEI	Lower Excursion Inlet	GLBA	58.38483	-135.46693
GBBC	Bartlett Cove	GLBA	58.44984	-135.89995
GBRC	Ripple Cove	GLBA	58.45086	-136.08684
GBHC	W. Hazelton Camp	GLBA	58.99271	-136.99306
GBRI	E. of Russell Island	GLBA	58.91107	-136.69620
NBES	Nahku Bay	KLGO	59.45330	-135.33650
SITK	Visitor Center	SITK	57.04780	-135.32060

Figure SOP-4.1. Long-term sampling locations in SEAN parks. Site IDs assigned by Mussel Watch data manager.

4 Collection procedures at each site

Samples should be collected within three weeks of the average sampling date from previous years. From 2007 to 2013, the average day of sampling at GLBA and SITK was August 19; therefore, samples each collection year should be gathered between July 29 and September 9. At each site, select a day and time within the date window that will allow collectors to arrive at low tide when mussels are easily accessible.

For each site, record the following on the paper field data sheet (Figure 2.2): Site ID, date, time of collection, water temperature near water line, approximate height of collection above the water level, height of the highest mussel distribution relative to water level, confirmation that salinity sample was collected, number of mussels collected.

These data should be kept on the field data sheet and also with each waterproof label that is eventually shipped with the samples to contaminants laboratories. Labels can be made using sections of waterproof notebook paper. All measures should be recorded with traditional units (i.e., feet and Fahrenheit) to be consistent with the NOAA MWP. Visually estimate the height of collection and height of highest mussel distribution in feet. Both measurements refer to the vertical height above the water level at the time of collection. For example, if samples are collected three feet above the water level sample height is indicated as 3 or if samples are collected at water level, the height of collection is 0. Mussel Watch does not use metric measurements. Time of collection and height measurements allow for future calculations of what tide stages mussels become accessible at each site.

Collect a vial of seawater for salinity from the water's edge. The SEAN ecologist should retain a box of glass 40 mL scintillation vials for this purpose. To flush foreign objects from the vial, rinse several times in sample water before retaining the sample. Tape the lid securely with scotch or electrical tape. Place the vial in an ice chest with the mussels. Measure the sea water temperature at the time of collection using any standard outdoor thermometer with 0.5°F or greater accuracy.

Collect mussels as close to the coordinates listed in Figure SOP-4.1 as possible. Pick from a variety of mussel beds. Don't collect all mussels from exactly the same area. If the average mussel shell is larger than ½ inch, collect 100 mussels per site. If mussels are small, collect 180. Only pick mussels with completely closed shells. Consolidate all mussels from one site into the fewest number of bags possible. Using a colander, rinse as much sediment as possible from the mussel shells before placing inside sample bag. Mussels should be immediately placed in a cooler with ice following collection. Ice should be kept in sealed bags and remain isolated from samples to avoid contamination with foreign water. Contact with standing water will also cause the mussels to open. Open shells introduce the possibility for contamination or depuration. If the water is fresh, mussels will die and be rendered useless for analysis. Since the samples will be analyzed for trace metal and organic contaminants. Avoid situations where these substances can be introduced (oil, fuel, pesticide, PCBs, exhaust fumes, flaking or rusty metal). Only collect samples from natural substrates such as large rocks.

[illegible]

Figure SOP-4.2. The SEAN marine contaminants field data sheet, deliverable EC_F. Printable versions of this sheet can be found on the SEAN Environmental Contaminants original source data tab, toolbox link at https://science.nature.nps.gov/im/units/sean/EC_main.aspx.

Collect all paper field data sheets in a file over the course of field work. They will be turned into a deliverable in section 3, below.

5 Preserving and shipping samples to lab

Samples must be collected, kept cool, and frozen as soon as possible upon returning from the field. Distant sites at GLBA will most likely take two days to sample, so it is important to keep samples cool the entire time before freezing. As of spring 2017, FedEx in Juneau ships next day for items mailed before 9:45AM. SEAN staff should have access to a federal FedEx account that is considerably cheaper than other delivery options such as USPS or UPS. Check with delivery provider for shipping details before sampling to ensure samples will be delivered on time. Remember to minimize standing water to the extent possible. Time samples to arrive at labs on a weekday so they do not sit unopened for long periods of time.

To prepare samples for shipping (Figure SOP-4.3), divide the mussels from each site into one or two sealed bags for a larger contaminants lab ice chest. Include salinity vials with site labels in the contaminants ice chest. Store vials on top of the mussels to avoid crushing the vial during shipping. Ensure each bag has a

waterproof label with pertinent data described above. Again, be sure ice is in sealed bags and of sufficient quantity to keep samples frozen or nearly frozen until lab arrival. Add as much ice as can fit in the cooler, and consider adding insulation such as cardboard under the lid if there is room. Always ship at the fastest speed possible and hand samples directly to a shipping agent. Do not leave samples unattended at a drop location. Use at least 3 separate bands of fiber tape to seal coolers. Determine current addresses to send samples to and prepare labels. These will be provided by the Mussel Watch program representative. If necessary, put three address labels on each ice chest. Cover address labels with wide clear tape. Typically, FedEx creates their own shipping labels, so this step can be skipped if FedEx is used.

1) Put ice in sealed bag on bottom of cooler:



2) Add frozen mussel samples and seawater vial



3) Place as much ice in sealed bags as possible on top of samples without crushing samples



4) Seal with fiber tape



5) Place address labels (may not be necessary if using FedEx)



Figure SOP-4.3. Packing and shipping process for bay mussel samples.

6 Project oversight and main points of contact

Before shipping, contact the Mussel Watch Program for the most recent analytical lab contacts. The following people were the main contacts for Mussel Watch in 2017:

Mussel Watch Program

Dennis Apeti
 dennis.apeti@noaa.gov
 Office: (240) 533-0337

Contaminants lab contacts

Amanda Brewster
 amandabrewster@tdi-bi.com
 B&B Laboratories
 14391B South Dowling Road
 College Station, TX 77845
 Office: (979) 693-3446

Juan Ramirez, Environmental Lab Manager
 juanramirez@tdi-bi.com
 B&B Laboratories
 14391B South Dowling Rd.
 College Station, TX 77845
 Office: (979) 693-3446
 Cell: (979) 777-0793

3 Submitting, validating, and accepting bay mussel field collection sheets (EC_F)

For this deliverable, the complete sets of paper field collection sheets that have accumulated over the survey year for all sites are scanned into a single PDF. The PDF is submitted through the validation / quality control iterations until accepted. It is stored in the repository and disseminated over the web.

1 Project leader (or delegate) tasks

1. Gather all of the field collection sheets that have been recorded during the field surveys for the year
2. Review sheets and fill in any missing information, if necessary.
3. Arrange observer forms in chronological order (earliest sampling first).
4. Verify the set is complete. There should be at least one sheet for every sampled site. If a sheet is missing and cannot be found after diligent search, insert a sheet of paper in the stack stating the site name and “No Field Observer Form.”
5. Scan the entire packet into a single PDF file using a color scanner. Be sure the scanner is set for two-sided scanning.
6. Save the PDF on the SEAN network at
 \\sean.glba.nps.gov\Data\SEAN_Data\Work_Zone\EC\EC_F\yyyy\EC_F_yyyy.PDF, where yyyy is the single year the samples were collected.
7. Request validation by sending a notification email to the Data Manager, specifying in the message body that deliverable EC_F, for year yyyy is ready; the Data Manager will know how to locate and work with the file named “EC_F_yyyy.PDF”.

2 Data manager tasks

1. On receipt of the submission, assign the next formal Submission Number to this file, as found in the master Submission_Log table.
 1. Use the “Update Submission Log” web tool at
http://sean-dm.glba.nps.gov/0_submission_update.aspx.
 2. Edit in the Submission Log details up through the Submission_Date column.

2. Copy the file into the staging area for validation at a new subdirectory named with the submission number in \\sean.glba.nps.gov\Data\SEAN_Data\Staging\EC\EC_F.
 1. Validate the submission according to current criteria.
 2. Record validation summary data in the Submission_Log using the web tool.
 3. If submission fails mandatory criteria, reply with a “failure email” that includes:
 1. The submission number assigned
 2. The deliverable ID
 3. The protocol ID
 4. Documentation listing all the specific mandatory criteria failed
 4. If submission passes mandatory criteria, reply with a “success email” that includes:
 1. The submission number assigned
 2. The deliverable ID
 3. The protocol ID
 4. Documentation listing any specific optional criteria failed
 5. Request to certify deliverable as complete

3 Project Leader Tasks

1. On receipt of a failure email:
 1. Review the paper stack is in chronological order.
 2. Rescan or edit the PDF to ensure it is in the proper form.
 3. Resubmit for validation.
 4. If failure cannot be resolved, email the Data Manager that the submission is withdrawn.
2. On receipt of a success email, review any failed optional criteria:
 1. The following tasks are the responsibility of the Project Leader and may not be delegated.
 2. If the review of failed optional criteria indicates the validated data are acceptable reply with a “certification email” stating the deliverable is accepted and may be disseminated.
 3. Otherwise, when the validated data are unacceptable:
 1. Reply with a “withdrawal email”, stating the deliverable is withdrawn.
 2. Take remedial action to obtain a corrected deliverable.
 3. Restart the process from the beginning.

4 Data Manager Tasks

1. On receipt of a withdrawal email:
 1. Mark the withdrawal in the Submission_Log’s Status column using the web tool.
 2. Terminate the process.
2. On receipt of a certification email:
 1. Install this file in the NPS Data Store under Project Environmental Contaminants using current practices. Typically this is done by cloning an existing EC_F reference, updating the metadata, and uploading a new file.
 2. Sensitive information may not be disseminated. (Sensitivity is highly unlikely for this deliverable.) If sensitive:
 1. Mark the Data Store reference as “internal” rather than “public” and indicate sensitivity in the Notes attribute of the reference.
 2. Mark the sensitive designation in the Submission_Log status column using the web tool.
 3. Notify the submitter of the situation.

4. Complete the deliverable tracking spreadsheet cells referring to dissemination with the word SENSITIVE.
5. Do not attempt to install the sensitive item in the web repository

4 Laboratory analysis procedures

The SEAN does not have facilities for preparing bay mussel tissue or analyzing contaminant concentrations, so lab work will be conducted by approved labs that can meet the necessary standards for analysis that are comparable with previous sampling efforts. Thus far, B&B Laboratories in College Station, Texas has been the contracted NOAA MWP lab for all contaminants analyses. All laboratory method citations can be found in Table 3.1 of the Environmental Contaminants protocol narrative (Sergeant et al. 2017).

SOP 6 details the final reporting structure from the lab analysis, including spreadsheet structure for results served on the SEAN website.

5 Submitting, validating, and accepting bay mussel laboratory technical report (EC_G)

For this deliverable, a laboratory technical report detailing all analytical results and the associated QA/QC procedures are provided to SEAN as a single PDF file. The PDF is submitted through the validation / quality control iterations until accepted. It is stored in the repository and disseminated over the web.

5 Project leader (or delegate) tasks

1. Lab reports will most likely be sent electronically as a PDF. If the report employs some other medium, make a PDF file from it. Save the PDF on the SEAN network at \\sean.glba.nps.gov\Data\SEAN_Data\Work_Zone\EC\EC_G\yyyy\EC_G_yyyy.PDF, where yyyy is the single year the samples were collected.
2. Request validation by sending a notification email to the Data Manager, specifying in the message body that deliverable EC_G, for year yyyy is ready; the Data Manager will know how to locate and work with the file “EC_G_yyyy.PDF”.

6 Data manager tasks

1. On receipt of the submission, assign the next formal Submission Number to this file, as found in the master Submission_Log table.
2. Use the “Update Submission Log” web tool at http://sean-dm.glba.nps.gov//0_submission_update.aspx.
 1. Edit in the Submission Log details up through the Submission_Date column.
 2. Copy the file into the staging area for validation at a new subdirectory named with the submission number in \\sean.glba.nps.gov\Data\SEAN_Data\Staging\EC\EC_G.
3. Validate the submission according to current criteria.
 1. Record validation summary data in the Submission_Log using the web tool.
 2. If submission fails mandatory criteria, reply with a “failure email” that includes:
 1. The submission number assigned
 2. The deliverable ID

3. The protocol ID
4. Documentation listing all the specific mandatory criteria failed
3. If submission passes mandatory criteria, reply with a “success email” that includes:
 1. The submission number assigned
 2. The deliverable ID
 3. The protocol ID
 4. Documentation listing any specific optional criteria failed
 5. Request to certify deliverable as complete

7 Project Leader Tasks

1. The following tasks are the responsibility of the Project Leader and may not be delegated.
2. On receipt of a failure email:
 1. Edit the PDF to ensure it is in the proper form.
 2. Resubmit for another validation test.
 3. If failure cannot be resolved, email the Data Manager that the submission is withdrawn.
3. On receipt of a success email, review any failed optional criteria:
 1. If the review of failed optional criteria indicates the validated data are acceptable then reply with a “certification email” stating the deliverable is accepted and may be disseminated.
 2. Otherwise, when the validated data are unacceptable:
 1. Reply with a “withdrawal email”, stating the deliverable is withdrawn.
 2. Take remedial action to obtain a corrected deliverable.
 3. Restart the process from the beginning.

8 Data Manager Tasks

1. On receipt of a withdrawal email:
 1. Mark the withdrawal in the Submission_Log’s Status column using the web tool.
 2. Terminate the process.
2. On receipt of a certification email:
 1. Install this file in the NPS Data Store under Project Environmental Contaminants using current practices. Typically this is done by cloning an existing EC_G reference, updating the metadata, and uploading a new file.
 2. Sensitive information may not be disseminated. (Sensitivity is unlikely for this deliverable.) If sensitive:
 1. Mark the Data Store reference as “internal” rather than “public” and indicate sensitivity in the Notes attribute of the reference.
 2. Mark the sensitive designation in the Submission_Log status column using the web tool.
 3. Notify the submitter of the situation.
 4. Complete the deliverable tracking spreadsheet cells referring to dissemination with the word SENSITIVE.
 5. Do not attempt to install the sensitive item in the web auxiliary repository.
3. Otherwise:
 1. Mark the certification in the Status column in Submission_Log using the web tool.
 2. Install this file on the NPS web sites in the ... \AUXREP\EC\EC_G folder, make a web link for it, test and deploy it using current practices.

3. Update the deliverable tracking spreadsheet milestones with the date of completion for the EC_G and propagate it through the web sites using current practices.