PORTLAND HARBOR PACIFIC LAMPREY AMMOCOETE STUDY: LABORATORY TESTING PLAN

PREPARED BY STRATUS CONSULTING

FOR THE

PORTLAND HARBOR

NATURAL RESOURCE TRUSTEE COUNCIL

January 28, 2011

Portland Harbor Pacific Lamprey Ammocoete Study: Laboratory Testing Plan

Prepared for:

Portland Harbor Natural Resource Trustee Council

Confederated Tribes of the Warm Springs Reservation of Oregon
Nez Perce Tribe
Confederated Tribes of Siletz Indians
Confederated Tribes of the Umatilla Indian Reservation
Confederated Tribes of the Grand Ronde Community of Oregon
National Oceanic and Atmospheric Administration
Oregon Department of Fish and Wildlife
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January 28, 2011
SC12213

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1. Introduction

Contaminants such as chlorinated hydrocarbons, petroleum-related compounds, metals, and other hazardous substances have been released from various sources and have come to be located in Portland Harbor (hereafter, the Harbor) sediments. Many of these compounds are at elevated levels in the Harbor compared to upstream locations. Sediments from specific areas in the Harbor have demonstrated toxicity to benthic invertebrates, and sediment-associated biota and fish collected from the area have accumulated contaminants in their tissue.

Habitat in the Harbor may be an important resting and foraging area for Pacific lamprey ammocoetes (*Lampetra tridentata*; hereafter designated as ammocoetes) as they transition to the lower Columbia River and prepare for their marine life stage. Ammocoetes collected from the Harbor have accumulated higher concentrations in their tissue of some organochlorine compounds than ammocoetes collected upstream.

The Portland Harbor Trustee Council is evaluating potential natural resource injuries to ammocoetes. Insufficient information is available to determine if the levels of contaminants at which ammocoetes are being exposed exceed concentrations that could cause injuries or prevent colonization of the Harbor by ammocoetes. In addition, restoration efforts for ammocoetes could be more successful if sediment toxicity to the species were better understood.

1.1 Overview of Sampling and Testing Efforts

The toxicity of contaminated sediments collected from within the Harbor to ammocoetes will be evaluated using 45-day sediment bioassays in the laboratory. Ammocoetes collected from the Siletz River will be used to test ammocoete sensitivity to contaminated Harbor sediments by exposing ammocoetes to sediment collected from Harbor and to reference and control sediments. The sensitivity of ammocoetes to Harbor sediments will be assessed based on standard toxicological endpoints (e.g., mortality, growth, behavior, changes in detoxification enzymes). Phase 1 of this project included a series of tasks focused on methods development. Phase 2 is a pilot-scale experiment that has been designed based on the preliminary results of Phase 1 testing.

1.1.1 Phase 1

Phase 1 of this project focused on bioassay methods development, including exposure design, duration, and endpoint measurements. The results of these methods development experiments were considered when Phase 2 was developed.

1.1.2 Phase 2

Phase 2 will consist of a bioassay performed with sediments collected from multiple locations in the Harbor (Stratus Consulting, 2011c). In general, ammocoetes collected from the Siletz River will be exposed to sediments collected from multiple locations (e.g., nine different sites) in the Harbor, and the toxicity of these sediments will be assessed based on endpoints selected during Phase 1. This testing plan describes objectives, methods, and procedures for Phase 2 testing.

1.2 Problem Definition

Problem description: Lamprey ammocoetes are the only detritivorous fish present in the Lower Willamette River (Windward Environmental, 2007a). Survival, growth, and behavior of ammocoetes could be impacted from exposure to contaminants in sediment in the Harbor, and lamprey could avoid Harbor sediments because of the presence of contamination.

Conceptual model of potential hazard: Industrial and municipal sources have released contaminants into the Harbor. A wide variety of contaminants have been released from these sources and have come to be located in bed sediments. While some of these contaminants remain near their release points, others have been transported away from their sources into downstream areas (Integral Consulting et al., 2009). Some of these contaminated sediments are within depositional areas or other areas where ammocoetes would settle as they move downriver. Ammocoetes readily burrow into sediment at settling areas and filter feed within the sediment or at the sediment surface. They are potentially exposed to contaminants in pore water, transition zone water, surface water, and suspended sediment at the interface between surface water and sediment. Exposure also occurs when ammocoetes consume contaminated sediment and detritus. Contaminants could enter ammocoetes through dermal, ingestion, and gill pathways. Water toxicity tests conducted on ammocoetes suggest lamprey are moderately sensitive to contaminants (Windward Environmental, 2007b), but their response to contaminants from exposure in sediments has not been evaluated. Responses in ammocoetes exposed to sample sediments obtained from the Harbor in sediment toxicity tests will be used to determine whether sediment toxicity tests provide a suitable tool to identify and quantify injury in ammocoetes.

Primary study question: Do contaminant concentrations in Portland Harbor sediment cause identifiable and quantifiable injuries to ammocoetes?

Under this primary study question, the specific objective of this study is to:

- Determine whether measurable adverse effects can be observed in controlled laboratory exposures of ammocoetes to Harbor sediments.

1.3 Document Organization

The remainder of this document contains sections describing data management and quality assurance procedures, experimental methodology, and an appendix containing experimental standard operating procedures (SOPs; appendix).

2. Data Management and Quality Assurance

2.1 Project Organization

The overall project organization and the individuals responsible for tasks required for this toxicity test are presented in Figure 1.

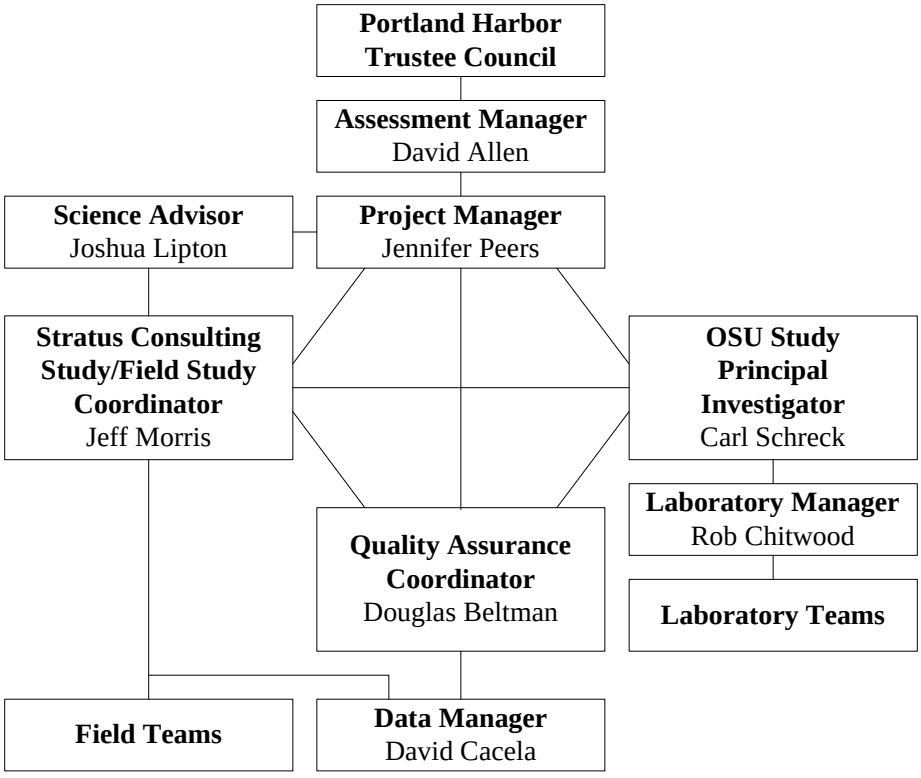

Figure 1. Organizational chart for Portland Harbor lamprey ammocoete study.

David Allen will serve as Stratus Consulting's Assessment Manager for the Portland Harbor natural resource damage assessment. Mr. Allen will have the ultimate responsibility for project oversight and communicating with the Trustee Council. Jennifer Peers (Stratus Consulting) will be the Project Manager (PM) responsible for study oversight, planning, budgeting, and day-to-day coordination with the trustees. Dr. Carl Schreck [Oregon State University (OSU)] will be the Study Principal Investigator responsible for all aspects of study management at OSU. Rob Chitwood (OSU) will serve as the Laboratory Manager at the OSU Fish Performance and Genetics Laboratory (FPGL). Dr. Jeff Morris (Stratus Consulting) will be the Stratus Consulting Study/Field Study Coordinator responsible for study design, planning, and implementation, as well as oversight of field teams. Dr. Joshua Lipton will serve as Stratus Consulting's Scientific Advisor on the project. David Cacela (Stratus Consulting) will be the project's Data Manager responsible for data archiving and analysis. Douglas Beltman (Stratus Consulting) will be the Quality Assurance Coordinator (QAC) responsible for quality assurance/quality control (QA/QC) oversight and communication between the Trustee Council, OSU, and Stratus Consulting.

2.2 Analytical Chemistry Samples Will Be Analyzed by CAS Laboratory Data Management

2.2.1 Toxicity testing records

During testing, file folders and laboratory notebooks from this study will be stored at the Fish Performance and Genetics Laboratory, 34349 Electric Road North, Oregon State University, Corvallis, OR 97331. At the conclusion of testing, copies of all files will be stored at Stratus Consulting Inc., 1881 9th Street, Suite 201, Boulder, CO 80302.

All sampling activities conducted at the FPGL will be documented on dedicated, preformatted data sheets or in laboratory notebooks. Entries will be made in waterproof ink, and corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Data sheets require a sampler's or analyst's initials. All data sheets will be filed in the project's dedicated file cabinet or three-ring binder. Raw data will be recorded on the appropriate data sheets. Data sheets and entries in laboratory notebooks will be copied and sent to Stratus Consulting. If the data are to be used in the project reports, they will be reduced for summarization, and the method of reduction will be documented in the report.

Preformatted data sheets may include the following:

- General forms
  - ***Daily Equipment Calibration.*** Form indicating that all monitoring equipment was calibrated and the standards used to calibrate these instruments.
  - ***Daily Water Quality Monitoring, Acclimation and Holding Tanks.*** Form designating the parameters to be monitored in acclimation and holding tanks each day, including water temperature, pH, dissolved oxygen, and conductivity.
  - ***Daily Mortality, Acclimation and Holding Tanks.*** Form for recording daily mortality from acclimation and holding tanks.
  - ***Photo Log.*** A table recording photo descriptions.
- Toxicity testing forms
  - ***Daily Water Quality Monitoring, Acclimation and Holding Tanks.*** Form designating the parameters to be monitored in exposure tanks each day, including water temperature, pH, dissolved oxygen, and conductivity.
  - ***Length, Weight, Ash Data.*** Form for recording lengths, weights, and ash content (if applicable) of fish before and after the testing period.
  - ***Ammocoete Loading.*** Form for recording the number of ammocoetes loaded into various containers at the beginning of each trial.
  - ***Daily Mortality and Sublethal Effects, Test Aquaria.*** Form for recording daily mortality and sublethal effects from test aquaria and containers during the test (if visible) and at the end of the test.

2.2.2 Chemical analysis records

All records of chemical analysis from CAS will be delivered electronically to Stratus Consulting. These records will include a project narrative documenting the receipt of the samples, copies of the completed chain-of-custody (COC) forms, and any problems encountered during receipt, storage, or analysis of samples. These deliverables will also include copies of the printouts generated during calibration of the analytical equipment and during sample analysis. Finally, these deliverables will also include an electronic database reporting analytical values for all samples, blanks, and QA/QC samples.

2.3 Assessment and Oversight

The appendix contains SOPs, which provide guidelines to ensure the reliability and validity of work conducted at the laboratory. Performance and system audits of laboratory activities may be conducted to verify that sampling and analysis are performed in accordance with the procedures established in this document and in the SOPs. Internal audits of activities (sampling and measurements) may be conducted by independent auditors. The audits may include examination of sampling records, instrument operating records, sample collection, handling and packaging in compliance with the established procedures, maintenance of QA procedures, and COC.

To ensure that all duties are being performed, all personnel will be trained to perform daily monitoring procedures by the Study Coordinator or Laboratory Manager and assigned a given set of duties. Daily checklist and daily water monitoring forms will be used to determine that routine jobs are being performed and that the water system is functioning properly. The person conducting the work will initial and date the appropriate form. This will provide the PM with the opportunity to verify that all work is being completed and that the water system is functioning properly. This process will ensure that the PM reviews all data collected.

2.4 Data Validation and Usability

2.4.1 Data review and validation

All data entry will be checked at the time of entry. At the completion of data entry, every entry will be verified. An additional data entry review will be performed by checking the ranges of given sets of data. Any data that appear to be suspect will be verified.

Laboratory data will be reviewed under the direction of the QAC as follows:

- Data will be screened for inclusion and frequency of specific QC information (e.g., detection limit verification, initial calibration, continuing calibration, duplicates, spikes, reagent blanks, field blanks). Requests for reanalysis or for additional QC supporting information will be made at this point if required.
- Measurement data will be reviewed in accordance with the following objectives:
    - ***Precision.*** A measure of mutual agreement among individual repeat measurements of the same analyte, usually under prescribed similar conditions. Precision is a measure of the reproducibility of analytical measurements.

- ***Accuracy.*** The degree of agreement of a measurement (or an average of measurements of the same parameter) with an accepted reference or true value. Accuracy is a measure of the bias in a system.

- ***Completeness.*** A measure of the total number of samples or data points obtained compared to the total number proposed. A measure of completeness is the fraction of measurement data that remains valid after discarding any invalid data due to laboratory QC rejection.

- ***Representativeness.*** The degree to which data accurately and precisely represent a characteristic of a population or an environmental condition. This includes comparing actual sampling procedures to those described in defined protocols, examining the results of QC blanks for external sample contamination, and identifying nonrepresentative data or data to be classified as questionable.

- ***Comparability.*** The confidence with which one dataset can be compared to another.

Data that do not meet QC targets will be identified and subjected to an analysis of outliers. They will be reviewed further, and a decision will be made as to their usability for meeting the data quality objectives of this project.

2.4.2 Reconciliation with data quality objectives

Identification of outliers involves a combination of objective mathematical criteria and professional judgment based on all information pertinent to a particular study. Outliers are identified by comparing replicate samples or by comparing sample results from the same and nearby locations.

This process will be followed using the conservative approach of assuming that each data point is valid (i.e., not an outlier); data points will be reclassified as outliers only if both objective and professional judgment evaluations lead to the conclusion that the data point is invalid.

3. Methods

3.1 Ammocoete Collection

The Aquatic Program Leader of the Confederated Tribes of Siletz Indians collected approximately 200 ammocoetes from various locations in the Siletz River during August and

September 2010 using electro-fishing gear. Ammocoetes were transported in aerated buckets or coolers with 1–2 inches of sediment to holding tanks at either the Lhuuke Illahee Fish Hatchery on the Siletz River or the FPGL. Ammocoetes were delivered to the FPGL on September 21, 2010. See the *Sampling Plan: Field Collection of Ammocoetes for Pacific Lamprey Toxicity Study* (Stratus Consulting, 2011a) for a detailed description of the lamprey collection and handling procedures.

3.2 Sediment Collection

Approximately 10 gallons of sediment were collected from 12 locations on the Willamette River during the week of July 26, 2010. Nine of these locations were contaminated areas in Portland Harbor and three locations were upstream reference areas. All sediment was maintained under COC by U.S. Fish and Wildlife Service (USFWS) personnel who were present during sediment collection. USFWS personnel stored the sediment at a secure USFWS facility until it was delivered to FPGL on July 30, 2010 by the same USFWS personnel. See the *Sampling Plan: Field Collection of Sediments for Pacific Lamprey Toxicity Study* (Stratus Consulting, 2011b) and the *Sampling Report: Field Collection of Sediments for Pacific Lamprey Toxicity Study* (Stratus Consulting, 2011c) for a detailed description of the sediment collection and handling activities.

3.3 Toxicity Testing

The testing facility for this study will be the FPGL, 34349 Electric Road North, Corvallis, OR 97331, operated by the OSU Department of Fisheries and Wildlife – U.S. Geological Survey Cooperative Fish and Wildlife Research Unit.

3.3.1 Laboratory holding and acclimation of ammocoetes

Ammocoetes will be held in large, round fiberglass tanks containing an 80:20 mixture of masonry sand and wood chips with well water flowing through the tanks. Water temperature and pH will be ambient and fluctuate seasonally from 12.8 to 14°C and from 6.83 to 7.04, respectively. The ammocoetes will be fed a 1:1 mixture of baker's yeast (1% body weight) and Encapsulon (larval fish diet; 1% body weight) three days a week. Ammocoetes will be acclimated to the laboratory well water for at least two weeks prior to being used in any test (PLA SOP P.2; see the appendix for SOP referenced).

3.3.2 Experiments

The purpose of these experiments is to observe the performance, growth, and behavior of ammocoetes in contaminated sediment from Portland Harbor and reference sediments from the Willamette River upstream from the Harbor.

Sediment: Twelve sediment types will be used in this experiment, including sediment collected from nine contaminated locations in Portland Harbor and two reference locations upstream from Portland Harbor [see *Sampling Report: Field Collection of Sediments for Pacific Lamprey Toxicity Study* (Stratus Consulting, 2011c]). Additionally, clean, sterilized masonry sand will also be used as a positive control for weight loss.

Exposure chambers: Mesh corrals will be used to house single ammocoetes in each exposure tank. These corrals will be constructed using 1/16-inch mesh nylon netting sewn into 3-inch-diameter × 15-inch-long cylinders that are open on the top (PLA SOP P.8). For each sediment type, five corrals will be placed upright in each of three 1-foot-diameter round fiberglass tanks. The open end of each corral will be attached at the top of the tank to a supporting bracket so that sediment and ammocoetes can be placed into the corrals. Approximately 4 inches of sediment will be placed in and around each corral in all three tanks. The methods for adding sediment to these exposures and collecting representative samples for analyses are described in PLA SOP P.5.

Lamprey ammocoetes: Ammocoetes will be collected from acclimation tanks the day before they are loaded into exposure chambers and sorted according to length. Ammocoetes within the 80–95 mm length range will be retained for testing and depurated for at least 24 hours prior to testing. Depuration shall occur in tanks with flowing well water and cotton gauze with no sediment. One depurated ammocoete will be placed into each exposure corral according to PLA SOP P.9. All exposure tanks will have water flowing through them for at least 24 hours prior to loading any ammocoetes for testing.

Exposure parameters: Well water will be delivered to each exposure tank (12-inch diameter × 8-inch tall) at a rate of 200 mL/min. The exposure tanks will be exposed to a combination of ambient and artificial lighting on an ambient photocycle. Each exposure corral will receive 3 mL of a nutrient slurry three times per week. This dose will deliver approximately 60 mg of baker's yeast and 60 mg of Hatchery Encapsulon III to each corral three times per week. Ammocoetes will be exposed to their respective sediment types for 45 days. In each exposure tank, the dissolved oxygen shall be > 5 mg/L, the pH between 6 and 8, and temperature between 12 and 15°C.

Endpoints: Survival and behavior will be assessed during the test. Survival, behavior (during the 24-hour depuration period immediately after testing), growth (length and weight; PLA SOP P.7; P.14), total lipid content, total ash content (PLA SOP P.14), gill ATPase, and various detoxification enzymes may be assessed at the end of the test. Ammocoetes will also be depurated in individual containers for 24 hours after testing. Procedures for processing dead or live ammocoetes for further analysis are explained in PLA SOP P.13.

3.3.3 Handling of sediments

Prior to using any test sediments, the procedure for using and sampling sediment described in PLA SOP P.5 will be followed.

3.3.4 Bioassay testing

Water monitoring

Basic water chemistry, including pH, temperature, dissolved oxygen, electrical conductivity, and ammonia, will be measured in all holding, acclimation, and test tanks on test-specific schedules (PLA SOP P.1). Additionally, flow rates to all holding, acclimation, and test tanks will be verified at least every other day.

Ammocoete monitoring

Exposure tanks and corrals will be monitored for dead ammocoetes daily during the test. The procedures for determining if an ammocoete is dead and removing the ammocoete are explained in PLA SOP P.10. The procedure for processing dead ammocoetes for further analysis is described in PLA SOP P.13.

3.3.5 Quality assurance/quality control procedures

Documentation

All measurements and observations will be recorded on pre-formatted data sheets or in dedicated laboratory logbooks. All data sheet and logbook entries will be scanned electronically to PDF. Electronic copies will be sent to Stratus Consulting on a regular basis, and hard copies will be kept at FPGL until the conclusion of testing.

3.4 Sediment Analysis

3.4.1 Sample collection

Each sediment sample will be clearly labeled for easy identification. All labels will be printed using a laser printer or written with permanent, waterproof markers. All labels will be sealed to the bottles with clear packing tape to ensure that the labels will not fall off and to avoid contamination from printing ink. Each label will contain information on sediment source and treatment ID as described in PLA SOP P.12.

Sediment samples will be collected for contaminant analysis at the same time sediment is loaded into exposure tanks and corrals at the beginning of the test according to PLA SOP P.5.

During all laboratory studies and chemical analyses, disposable equipment will be used whenever possible. However, some equipment may need to be reused. In particular, large mixing containers used for sediment loading and sediment sampling equipment may require decontamination. This equipment will be decontaminated to minimize the introduction of cross-contamination errors.

Sediment loading and sampling equipment will be decontaminated according to PLA SOP P.4. Glassware used for the project must be cleaned and acid washed prior to use according to PLA SOP P.4. Analytical probes used for measuring dissolved oxygen, pH, temperature, and conductivity must be thoroughly rinsed with well water before and after measurements in exposure aquaria.

3.5 Sample Schedule

Water samples will be collected from acclimation/holding tanks and from a subset of exposure aquaria daily for basic water quality analysis (PLA SOP P.1) and weekly for unionized ammonia analysis (PLA SOP P.1). Sediment samples will be collected at the beginning of an exposure from each type of sediment being tested (PLA SOP P.5).

3.5.1 Sample shipping

All chemical analyses of samples will be completed at CAS in Kelso, WA. All samples will be shipped to CAS overnight on ice. Before sealing coolers for shipment, samples will be individually verified against completed COC forms (PLA SOP P.6). The cooler will be sealed with a COC seal to ensure against sample tampering.

3.5.2 Analytes and methods

All samples collected for this project will maintain strict COC procedures (PLA SOP P.6). Sample containers and any necessary preservatives will be provided by the analytical laboratory. Sample containers will be capped for storage after sample collection, filtering, and necessary preservation have been completed. Samples will be stored on ice in coolers for shipping to the laboratory.

Chemical analysis for specific analytes will follow QA/QC procedures outlined in PLA SOP P.11. Sample numbering, tracking, and reporting will follow procedures outlined in PLA SOP P.12.

Analytical methods will be consistent with, or equivalent to, U.S. Environmental Protection Agency (EPA) methods or some other commonly accepted or approved method, as approved by the QAC. All laboratory equipment and instruments will be operated, maintained, calibrated, and standardized in accordance with EPA-accepted or manufacturer's practices.

Analytical instruments and equipment should be calibrated before each use or on a scheduled, periodic basis.

Laboratory reagents will be reagent grade or higher quality. Calibration standards and laboratory control samples will be traceable to the National Institute for Standards and Technology, the U.S. Geological Survey, EPA, or other EPA-approved sources. Preparation and use of these samples will follow applicable EPA guidance. In addition to following standard analytical methods, CAS maintains SOPs for all aspects of sample handling, preparation, analysis, data reporting, and QA/QC procedures.

3.6 Corrective Actions

During the investigation, corrective actions may be required for analytical or equipment problems. Analytical and equipment problems may occur during sampling and sample handling, sample preparation, laboratory instrumental analysis, and data review.

During sample collection, technical staff and project personnel will be responsible for reporting all suspected deficiencies of any activity to the PM or designee. The PM will be responsible for assessing the suspected problem and making a decision based on the potential for the situation to affect the quality of the data. If corrective actions are deemed necessary, such actions will be fully documented in logbooks and in subsequent project reports.

Corrective action for measurements may include:

- Repeating the measurement to check the error
- Checking for all proper adjustments to ambient conditions such as temperature
- Checking batteries
- Recalibrating instruments
- Replacing the instrument or measurement devices
- Stopping work (if necessary).

For problems encountered in the laboratory, corrective action may be taken at several different levels, including:

- Reanalyzing samples, if holding time criteria permit
- Evaluating and amending analytical procedures
- Accepting data and acknowledging uncertainty levels.

References

Integral Consulting, Windward Environmental, Kennedy/Jenks Consultants, and Anchor QEA. 2009. Portland Harbor RI/FS Remedial Investigation Report. Draft. Prepared for the Lower Willamette Group by Integral Consulting, Windward Environmental LLC, Kennedy/Jenks Consultants, and Anchor QEA LLC. October 27.

Stratus Consulting. 2011a. *Sampling Plan: Field Collection of Ammocoetes for Pacific Lamprey Toxicity Study*. Prepared for the Portland Harbor Natural Resource Trustee Council. Stratus Consulting Inc., Boulder, CO. January 28.

Stratus Consulting. 2011b. *Sampling Plan: Field Collection of Sediments for Pacific Lamprey Toxicity Study.* Prepared for the Portland Harbor Natural Resource Trustee Council. Stratus Consulting Inc., Boulder, CO. January 28.

Stratus Consulting. 2011c. *Sampling Report: Field Collection of Sediments for Pacific Lamprey Toxicity Study*. Prepared for the Portland Harbor Natural Resource Trustee Council. Stratus Consulting Inc., Boulder, CO. January 28.

Windward Environmental. 2007a. Portland Harbor RI/FS Round 3 Lamprey Ammocoete (*Lampetra* sp.) Toxicity Testing Quality Assurance Project Plan Addendum: Phase 2 Lamprey Ammocoete Collection and Testing. Prepared for the Lower Willamette Group. July 6.

Windward Environmental. 2007b. Portland Harbor RI/FS Round 3 Lamprey (*Lampetra* sp.) Phase 1 Toxicity Testing Report. Prepared for the Lower Willamette Group. July 6.

A. Standard Operating Procedures: Pacific Lamprey Ammocoete Testing

Project-specific procedures list

PLA SOP P.1	Laboratory equipment calibration and use
PLA SOP P.2	Culture and handling of test ammocoetes
PLA SOP P.3	Humane procedures for anesthesia and euthanasia of ammocoetes
PLA SOP P.4	Procedure for equipment and glassware decontamination
PLA SOP P.5	Use and sampling of sediments in the laboratory
PLA SOP P.6	Chain-of-custody procedures
PLA SOP P.7	Determining lengths and weights of ammocoetes
PLA SOP P.8	Procedure for constructing ammocoete corrals
PLA SOP P.9	Adding ammocoetes to exposure aquaria
PLA SOP P.10	Procedure for observing and removing dead ammocoetes from exposure corrals
PLA SOP P.11	Analytical procedures for sediment characterization
PLA SOP P.12	Procedures for sample labeling
PLA SOP P.13	Procedures for processing dead or live ammocoetes for further analysis
PLA SOP P.14	Procedures for determining dry weight and ash content of ammocoetes

PLA SOP P.1

Laboratory equipment calibration and use

Routine water quality parameters, including pH, temperature, dissolved oxygen, conductivity, and ammocoete weights, will be measured using the following equipment (owned by the Fish Performance and Genetics Laboratory at OSU or Stratus Consulting) according to the manufacturer's instructions:

- American Marine Pinpoint pH meter
- Extech dissolved oxygen meter and thermometer
- Oakton Acorn Con 6 conductivity meter
- Ohaus Navigator analytical balance.

All equipment will be calibrated daily before use according to the manufacturer's instructions.

Other water quality parameters, including ammonia, hardness, and alkalinity, will be measured using aquaculture test kits from Lamotte Company, Chestertown, Maryland.

PLA SOP P.2

Culture and handling of test ammocoetes

Ammocoetes will be maintained at the Fish Performance and Genetics Laboratory in fiberglass, plastic, or glass aquaria. Water will be supplied through polyvinyl chloride (PVC) pipes at a flow necessary to maintain at least 5 mg/L dissolved oxygen in the aquarium. Water pH, temperature, conductivity, and dissolved oxygen (PLA SOP P.1) levels will be measured daily. Ammonia concentrations will be analyzed weekly (PLA SOP P.1). Cleaning, aeration, and water flow rates will be adjusted accordingly to maintain acceptable culture conditions.

Dead ammocoetes will be removed daily, and both live and dead ammocoetes will be visually inspected periodically for possible disease. If mortality rates increase substantially or if the ammocoetes show evidence of disease, live ammocoetes showing possible disease symptoms will be collected and transported to the State Fisheries Pathologist for inspection. Following diagnosis of disease, ammocoetes will be treated for disease per the State Fisheries Pathologist's recommendations. The ammocoetes will be allowed to recover for at least two weeks prior to testing in any toxicity experiments. If possible, ammocoetes will be replaced by certified disease-free ammocoetes of the same species.

Following removal of all ammocoetes from an aquarium, the aquarium will be disinfected with benzylklonium chloride or equivalent disinfectant solution followed by several rinses with well water. All culture equipment (nets, siphons, buckets) that comes in contact with any culture or exposure aquarium will be disinfected with benzylklonium chloride or equivalent disinfectant solution prior to subsequent use. All sampling containers and analytical equipment (probes, electrodes) will be rinsed thoroughly with well water prior to and after collecting measurements.

PLA SOP P.3

Humane procedures for anesthesia and euthanasia of ammocoetes

Ammocoetes that are to be used in toxicity tests will be anesthetized prior to or during testing using a 100-mg/L dose of buffered MS222. Following toxicity experiments, remaining live ammocoetes that were exposed to contaminated sediments and (or) ammocoetes that are required for physiological or histological samples (e.g., lipid, ash, enzyme analysis) will be euthanized with an overdose of MS222 (> 250 mg/L) followed by severing of the spinal cord by pithing. The Institutional Animal Care and Use Committee at OSU has accepted these procedures for the euthanasia of ammocoetes (ACUP ID 4022).

PLA SOP P.4

Procedure for equipment and glassware decontamination

Sediment mixing and sampling equipment, aquaria, and other non-disposable equipment that comes in contact with contaminated sediments will be decontaminated before re-use. All equipment will be scrubbed with Liqui-Nox® laboratory-grade detergent or equivalent detergent and rinsed generously with well water. All equipment will then be rinsed with a 10% solution of nitric acid to desorb metals. Following this acid wash, equipment will be rinsed with well water.

PLA SOP P.5

Use and sampling of sediments in the laboratory

All reference, control, and test sediments must be combined and mixed prior to use. Because several gallons of sediment will be collected from each site, it may be necessary to use a mechanical mixing device such as a rotating cement or tile grout-type mixer attached to a power drill to homogenize the sediment. The mixing equipment will be decontaminated according to PLA SOP P.4. All sediment collected from a particular location will be placed in the mixer and rotated until the contents have a homogenous appearance in terms of color and texture.

Once homogenized, the sediment will be used to fill test aquaria, and a subsample will be collected for physical characterization and chemical analysis. Subsamples will be collected using a decontaminated stainless steel scoop and placed into pre-labeled glass jars according to the analytical laboratory's instructions. These subsamples will be shipped overnight on ice to CAS in Kelso, WA, following proper COC procedures (PLA SOP P.6). As needed, additional sediment samples may be collected from aquaria during or after an exposure test. These additional samples will be collected by inserting a decontaminated stainless steel tube into the sediment, capping the top of the tube to provide suction, and extracting the tube from the sediment. The entire contents of the tube will be homogenized in a decontaminated stainless steel bowl, loaded into glass jars, and shipped to CAS as described for samples collected at the beginning of an exposure.

To ensure that each exposure chamber receives a representative sample of the sediment, the following fractional loading and sampling procedure will be used.

Sediment will be scooped out of the mixing container in small (~ 10- to 20-oz) increments and placed in one corral, then in the exposure tank, then in the next corral, and then in the exposure tank in an alternating fashion so that the corrals and surrounding areas around the corrals are filled at about the same time. The corrals will be placed in supporting PVC pipes (3-inch inner diameter) while they are filled so that they hold their shape. The PVC pipes will be removed when the corrals and tank are filled. The depth of sediment should be the same inside and outside the corrals after all the tanks and corrals have been filled.

Sediment samples will be collected as part of the exposure tank and corral loading process. These samples will be shipped to CAS for analysis and archiving. In order to collect a representative sediment sample for analysis, additional mock corrals (e.g., glass beakers) will be filled in the same manner as the experimental containers (i.e., include the beakers in the sediment loading rotation among all corrals). Additionally, all available sediment for a particular treatment must be used when beginning an experiment. If more sediment is available than will be needed, discard containers (e.g., 5-gallon buckets) will be used as part of the filling rotation. The sediment in these discard containers will not be used for testing or analysis. For example,

approximately 10 gallons of contaminated sediment will be collected from each field site to complete Task 7 of the SOW. If three 1-foot-diameter tanks are used for each treatment and filled with 4 inches of sediment, the total volume of sediment that will be needed to begin the test and collect a sample for analysis will only be approximately 6 gallons. Therefore, additional discard containers will be used when loading each treatment. The purpose of the discard container is to ensure that all the sediment collected in the field has an equal chance of being placed into a test corral and that the sample sent to CAS is representative of the sediment in all of the corrals.

At the conclusion of testing, all contaminated and reference sediment will be sealed in 5-gallon plastic buckets or an equivalent holding container and turned over to OSU's Environmental Health and Safety facility for disposal.

PLA SOP P.6

Chain-of-custody procedures

The transportation of all sediment, water, and tissue samples will be documented using standard COC procedures. At a minimum, the sample ID, date, time, sample matrix, and analyses to be performed will be recorded as each sample container is loaded into a cooler or other appropriate container. The COC form will also include the contact information of the sample shipper or study coordinator and will be signed and dated by said shipper (see Figure A.1 for an example form). One copy of the COC form will be retained by the shipper and the other copy will be sealed in a Ziplock plastic bag and taped to the inside of the shipping container lid. The container will be sealed with signed and dated COC seals fixed across the lid of the container so that the container cannot be opened without tearing the seals. Sample containers will be hand-delivered or shipped overnight to the analytical laboratory. The samples must remain in the possession of the shipper or in a secure location until possession is remitted to the shipping company or analytical laboratory.

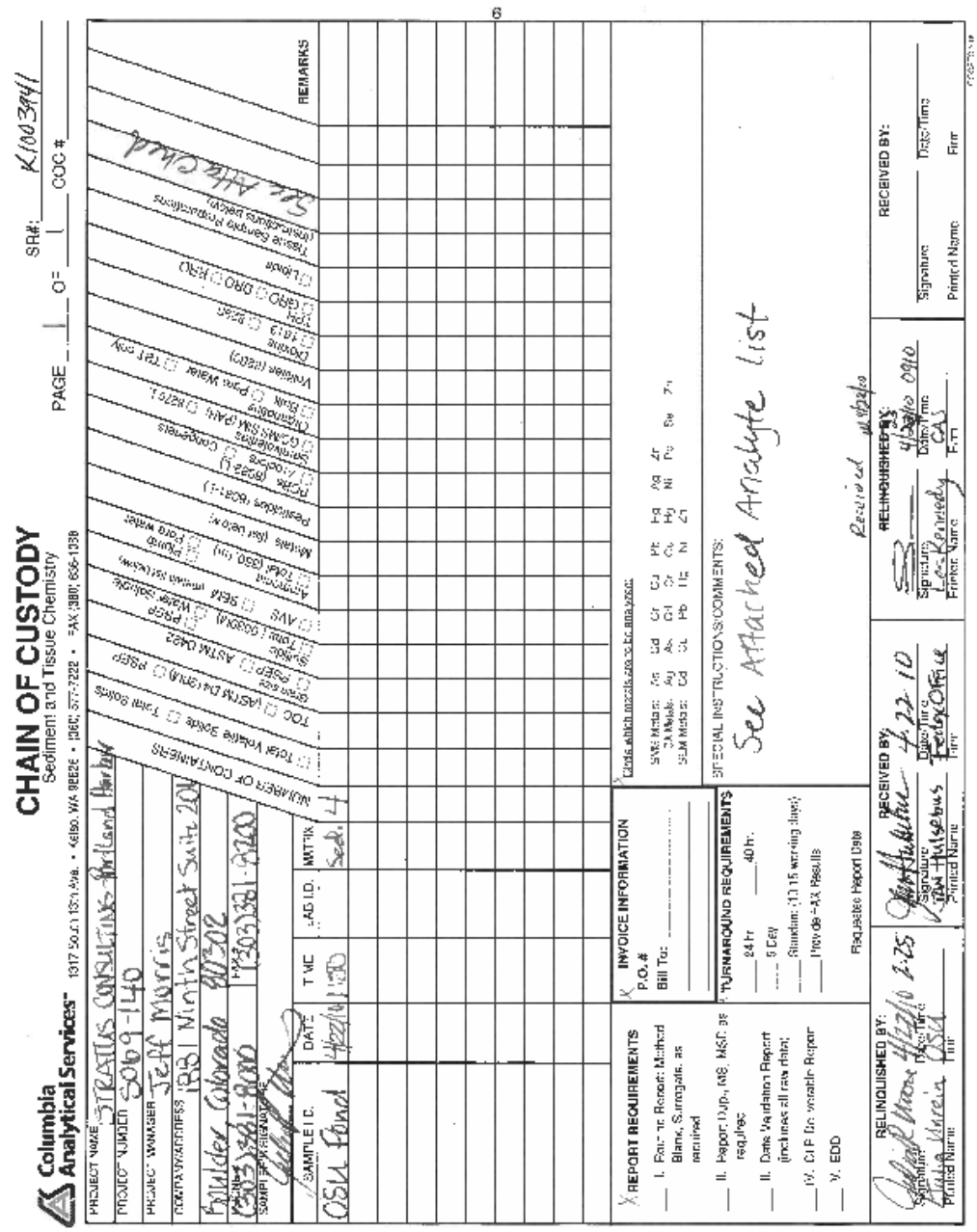

Figure A.1. Example of a completed COC form.

PLA SOP P.7

Determining lengths and weights of ammocoetes

Lengths and weights of ammocoetes may be determined before, during, and after testing. All ammocoetes used during testing will be weighed and measured individually. Head-to-tail lengths will be determined to the nearest millimeter using a wet measuring board. Weights of ammocoetes being loaded into testing corrals or other containers will be determined to at least the nearest 10 mg in a small beaker containing well water. Beakers containing water will be used to tare the analytical balance (Ohaus Navigator or equivalent) prior to weighing each ammocoete. At the conclusion of each test, ammocoetes will be anesthetized (PLA SOP P.3) and weighed on the analytical balance. Weights and lengths of all ammocoetes will be recorded on data sheets.

PLA SOP P.8

Procedure for constructing ammocoete corrals

Cloth with sufficiently small mesh to prevent escape or entrapment of ammocoetes will be sewed into sock-shaped corrals using nylon thread. The dimensions of the corrals will vary with each test design. The tops of the corrals will be open and extend out of the water in each exposure tank to allow for observations.

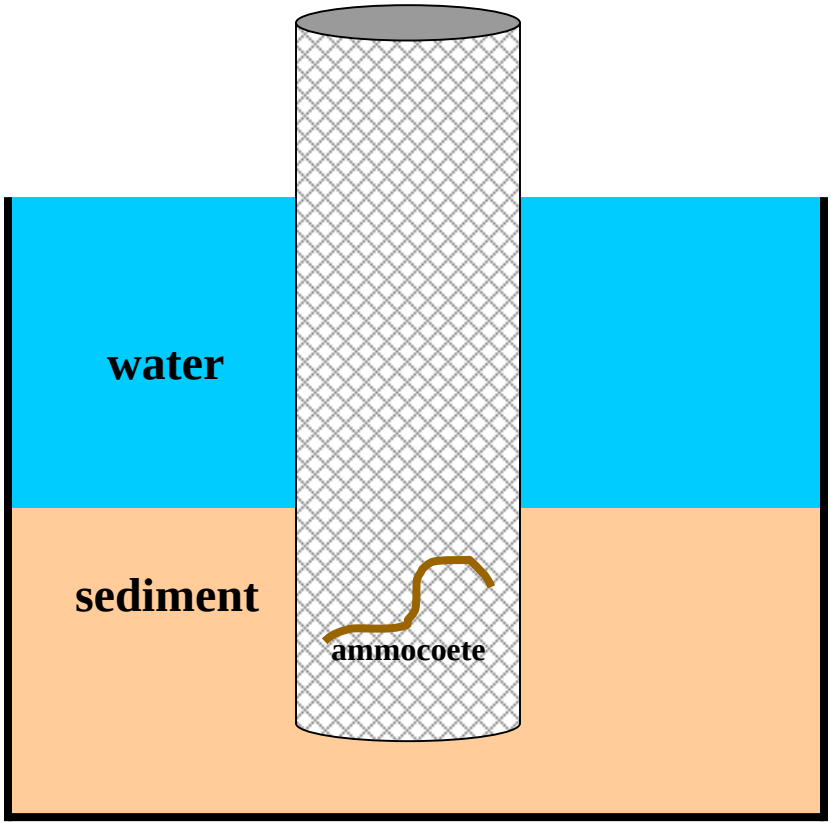

Figure A.2. Aquarium containing sediment, water, and a lamprey corral with an ammocoete.

PLA SOP P.9

Adding ammocoetes to exposure aquaria

The following procedure will be used to add ammocoetes to exposure aquaria during all testing:

1. Disinfect all ammocoete handling and holding equipment with benzylklonium chloride or other approved disinfectant.

2. Remove ammocoetes from holding tanks by scooping sediment from the holding tanks into OSU's lamprey separator (water sprayed onto sediment that washes into large stainless steel table where ammocoetes are visible in 1–2 inches of standing water and gently netted) or using an equivalent separation technique.

3. Depurate these ammocoetes by collecting a sufficient number of appropriately sized ammocoetes to conduct a specific experiment and holding these ammocoetes in a sufficiently sized tank or tanks containing cotton gauze and flowing well water. Allow the ammocoetes to depurate for 24 hours.

4. Weigh and measure each ammocoete loading into each corral (PLA SOP P.7).

5. Add one ammocoete to each corral in each tank, rotating between all tanks so that all corrals in all tanks are loaded sequentially and all corrals in a single tank are not loaded first.

6. Load ammocoetes according to the pre-designated random order on the loading data sheets and note on the data sheet after each ammocoete has been loaded.

7. Repeat steps 4–6 until all corrals/tanks are fully loaded (e.g., one per corral, five per tank).

8. Visually confirm that each ammocoete has been loaded into each corral before it burrows into the sediment. Injured or dead ammocoetes that are noted within 2 hours of initial loading can be replaced.

PLA SOP P.10

Procedure for observing and removing dead ammocoetes from exposure corrals

Exposure tanks will be observed for mortalities (dead ammocoetes are visible above the sediment surface) and abnormal behavior (i.e., not burrowing into the sediment) daily and at the end of each exposure.

1. During testing, when an apparent dead ammocoete is observed, closely observe the ammocoete for any movements. If movement is observed, the ammocoete is not dead. When observing ammocoetes that are not active, gently stimulate any ammocoete in question with a gentle burst of water from a pipette. If the ammocoete is dead, remove it using clean tongs. Weigh and measure the dead ammocoete and place it into a labeled plastic vial or equivalent container and place the container in a -80°C freezer for future possible analysis.

2. After dead ammocoetes are removed from an exposure corral, immediately record the corral ID on the designated data sheet.

3. After all exposure aquaria containing the same sediment have been observed and dead ammocoetes removed, thoroughly rinse tongs and glass rod pipette with well water before proceeding to the next sediment type.

4. At the end of each exposure, determine if ammocoetes buried in the sediment in each corral are dead or alive. To make this determination, remove a corral from the exposure aquaria by gently lifting straight up on the exposed sides of the corral and twisting, if necessary, to dislodge the corral from surrounding sediment. Once the corral is free, gently invert the corral into a large glass dish or equivalent container containing 1–2 inches of well water and spread out the sediment until the ammocoete is located. If the ammocoete appears dead, follow steps 2–3 above. If the ammocoete is alive, proceed to processing and anesthesia procedures described in PLA SOP P.3 and PLA SOP P.13.

PLA SOP P.11

Analytical procedures for sediment characterization

CAS in Kelso, WA, will analyze sediment samples. The analyses that may be conducted for all or some of the sediment samples are listed in Table A.1.

Table A.1. Sediment sample analytes and methods

Analysis	Analytical method	Container	Preservation temperature	Maximum holding time
Metals and metalloids	EPA 6020/7471A	Two 16-oz glass jars	4°C	6 months (28 days for mercury)
Pesticides	EPA 8081A	With metals	4°C	14 days
PCB aroclors	EPA 8082	With metals	4°C	14 days
Acid volatile sulfide	EPA 821/R-91-100	One 4-oz glass jar	4°C	14 days
Sediment particle size analysis	PSEP Grain Size	With metals	4°C	NA
Organotins	Krone	With metals	4°C	14 days
TPH	NWTPH-Dx	With metals	4°C	14 days
Total solids	TS-MET	With metals	4°C	28 days
TOC	PSEP TOC	With metals	4°C	7 days
Ammonia	EPA 350.1M	With metals	4°C	7 days

PCB: polychlorinated biphenyl.
TPH: total petroleum hydrocarbons – gas, diesel, and residual range hydrocarbons.
TOC: total organic carbon.

In addition to following standard analytical methods, CAS maintains SOPs for all aspects of sample handling, preparation, analysis, data reporting, and QA/QC procedures not described in standard analytical methods.

PLA SOP P.12

Procedures for sample labeling

Each water and sediment sample will be clearly labeled for easy identification. All labels will be printed using a laser printer or written with permanent, waterproof markers. All labels will be sealed to the bottles with clear packing tape to ensure the labels will not fall off and to avoid contamination from printing ink.

The label will convey the following information:

- Sediment source or aquarium ID
- Replicate number
- Sample date
- Analyses required [Portland Harbor Suite (PHS)].

The identification code will have the following information:

Source/ID – replicate information – sample date – analysis required

The form of the identification code will be:

XXX-0/0-00/00-YYY

Source/ID (XXX):

MSC = Masonry Sand Control
SRR = Siletz River Reference
OSP = OSU Pond
OST = Oregon Steel
SC1 = Schnitzer 1
ARM = Arco/Mobil
GAA = Gasco 1 (alternate)
GA2 = Gasco 2
AR1 = Arkema 1
AR2 = Arkema 2
SWI = Swan Island
RE1 = Reference Site 1
RE2 = Reference Site 2
RE3 = Reference Site 3
GPH = Gasco Portland Harbor

Replicate information (0/0) = 1/1 = only 1 sample
1/2 = 1 of 2 samples

Sample date (00/00/0000) = date (day/month/year); Example: 15/01/2010 = 15 January 2010

Analysis required (YYY) = PHS (or other pre-established analytical suite)

PLA SOP P.13

Procedures for processing dead or live ammocoetes for further analysis

If ammocoetes are alive, they will be anesthetized and (or) euthanized according to PLA SOP P.3 prior to the collection of blood or tissue samples. Ammocoetes should be weighed and measured according to PLA SOP P.7 and then frozen or preserved (e.g., Davidson's solution) as appropriate for the type of analyses to be conducted. These analyses may include measuring lipid or enzyme content, tissue contaminant concentration, histology, stage of metamorphosis, and others.

PLA SOP P.14

Procedures for determining dry weight and ash content of ammocoetes

Dead or euthanized ammocoetes should be dried to a constant weight in a standard drying oven at a relatively low temperature (e.g., 55–75°C). Samples should be periodically (e.g., every 24 hours) removed from the drying oven, allowed to cool to room temperature in a desiccator, and re-weighed. Each time a sample is re-weighed, record the weight and compare it to the last weight to determine if the sample is completely dry or is continuing to lose moisture. The sample is considered dry when the variation in sample weights between drying periods is within the usual variability in the analytical balance.

Dried ammocoetes or subsamples of dried, ground ammocoetes (if additional analyses are required on the sample) will be placed in pre-weighed aluminum boats with the sample ID scratched into the boat (any ink mark will be lost during combustion) and sealed by folding the boat in and pinching the sides shut to prevent loss of particles. Re-weigh this loaded, sealed boat to determine dry sample weight. Then combust the boat in a muffle furnace at 500°C for 5 hours. Combusted samples should remain sealed in their boats, cooled to room temperature in a desiccator, and re-weighed to determine the ash content of the sample.