Suquamish Tribe

East Kitsap Intertidal Forage Fish Spawning Study

Quality Assurance Project Plan

EPA Grant No. PA-00J912

Suquamish Tribe, PO Box 498, Suquamish, WA 98392

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Abstract: This project develops and implements a monitoring plan to survey and document the location and extent of forage fish spawning in East Kitsap County. This project will utilize the methods and the systematic and comprehensive sampling strategy currently in use by the Washington Department of Fish and Wildlife (WDFW) on the outer Washington Coast and elsewhere in Puget Sound. This effort is intended to comprehensively update data collected by WDFW since 1972 and improve the accuracy of distribution (both spatial and temporal) of East Kitsap County beach spawning forage fish.
A. Project Management: Suquamish Tribe

A1. Approval Sheet:

Paul Dorn  
Suquamish Tribe  
Senior Research Scientist

Tom Ostrom  
Suquamish Tribe  
Salmon Recovery Coordinator

Donald Brown  
USEPA Region 10  
Quality Assurance Manager

Lisa Chang  
USEPA Project Officer

Dani Madrone  
Northwest Indian Fisheries Commission  
Puget Sound Recovery Projects Coordinator
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### A3. Distribution List

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<th>Name</th>
<th>Organization</th>
<th>Address</th>
<th>Phone #</th>
<th>Fax#</th>
<th>Email Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tom Ostrom</td>
<td>Suquamish Tribe</td>
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<td>360-598-4666</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mobile: 360-981-5388</td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Mobile: 360-981-7658</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phill Dionne</td>
<td>Washington Department of</td>
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<td>360 902-2946</td>
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</tr>
<tr>
<td></td>
<td>Fish and Wildlife</td>
<td></td>
<td>Mobile:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lisa Chang</td>
<td>USEPA</td>
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<td>206-553-2955</td>
<td><a href="mailto:chang.lisa@epamail.epa.gov">chang.lisa@epamail.epa.gov</a></td>
</tr>
<tr>
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<td>360-753-8659</td>
<td><a href="mailto:dmadrone@nwifc.org">dmadrone@nwifc.org</a></td>
</tr>
<tr>
<td>Lucy Yanez</td>
<td>NWIFC</td>
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<td>360-528-4328</td>
<td>360-753-8659</td>
<td><a href="mailto:lyanez@nwifc.org">lyanez@nwifc.org</a></td>
</tr>
</tbody>
</table>

Each person listed on the approval sheet and each person listed under Project/Task Organization will receive a copy of this Quality Assurance Project Plan (QAPP). Individuals taking part in the project may request additional copies of the QAPP from personnel listed under Section A4.

This document has been prepared according to the United States Environmental Protection Agency publication *EPA Requirements for Quality Assurance Project Plans* dated March 2001 (QA/R-5).

### A4. Project Management and Organization

#### A4.1 Roles and Responsibilities:

<table>
<thead>
<tr>
<th>Individual</th>
<th>Project Title</th>
<th>Responsibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tom Ostrom</td>
<td>Suquamish Tribe Project Manager</td>
<td>Overall project manager. Responsible for ensuring proper implementation of funding agreement with Northwest Indian Fisheries Commission Contract - East Kitsap Forage Fish Monitoring Project and FY-14 EPA Puget Sound Partnership Implementation Agreement (#14EPA PSP436)</td>
</tr>
<tr>
<td>Paul Dorn</td>
<td>Suquamish Tribe Project Supervisor</td>
<td>Responsible for implementation of the QAPP, field sampling, coordination with</td>
</tr>
</tbody>
</table>
Phill Dionne | WDFW Research Scientist/Forage Fish Project Manager/Project Quality Assurance Officer | Responsible for directing WDFW forage fish beach surveys, access, location, sampling, processing, and QA/QC.

Karin Feddersen-Lethe | USEPA | Quality assurance review and approval of this QAPP

Lisa Chang | USEPA | USEPA project officer

Dani Madrone | Northwest Indian Fisheries Commission | NWIFC Puget Sound Recovery Projects Coordinator; responsible for managing sub-award and ensuring overall project success

Lucy Yanez | Northwest Indian Fisheries Commission | Responsible for ensuring contract compliance

**USEPA Quality Assurance Manager:** The USEPA Quality Assurance Manager is Donald Brown. Karen Feddersen-Lethe has signatory authority on QAPPs for Donald and will review and approve this Quality Assurance Project Plan and any subsequent addenda or amendments to the Quality Assurance Project Plan submitted to the USEPA.

**USEPA Project Officer:** The USEPA Project Officer is Lisa Chang. The USEPA Project Officer is responsible for communicating and working with the Northwest Indian Fisheries Commission to ensure that all grant conditions are met.

Dani Madrone, NWIFC Puget Sound Recovery Project Coordinator and Lucy Yanez, NWIFC Contract Coordinator. The NWIFC Puget Sound Recovery Project Coordinator and the NWIFC Contract Coordinator shall be responsible for administration of the EPA sub-award contract with the Suquamish Tribe. They will ensure goals and objectives of the project are achieved and that project deliverables are complete and of required quality and that project completion dates are met. They will communicate the status of the project with EPA.

Tom Ostrom, Suquamish Project Manager. The Project Manager is responsible for the grant, QAPP, coordinating data management and analysis, and report completion. The Project Manager will report all data collection activities associated with this QAPP in regular progress reports (FEATS). The Project Manager is responsible for ensuring proper implementation of the underlying funding agreement with Northwest Indian Fisheries Commission (FY-12 EPA Puget Sound Partnership Implementation Agreement #12EPA PSP436).

Paul Dorn, Suquamish Tribe Project Supervisor. The Project Supervisor will assist in development of the QAPP, data management and analysis, and report drafting. The Project Supervisor will be responsible for coordination with WDFW’s Forage Fish Program and Lab of all field work, including sample scheduling and collection. The Project Supervisor will assign appropriate personnel to assist in the completion of project tasks. The Project Supervisor will train field personnel in proper operation of the forage fish egg sampling, collection, processing, and storage if needed. The Project Supervisor will communicate with the Suquamish Project Manager on work accomplished in this plan and any problems or deviations that need to be resolved.
Phillip Dionne, Project Quality Assurance Officer, manages WDFW’s Nearshore/Forage Fish Ecology Habitat Science Team. Responsible for directing Washington State’s forage fish program design, sampling protocol and collection methodology, data analysis, quality assurance/quality control of all processed samples, processed sample storage, and reporting results. Ensures methods, analyses, and outputs are consistent with historic and current forage fish monitoring projects in Washington State. Obtains landowner permission to access private property for this study.

A5. Problem Definition and Background

Forage fish are an essential ecosystem component of the Puget Sound food web and to the recovery of Puget Sound listed Chinook and Orca, and many other non-listed fish, marine mammal and avian species. The four major Puget Sound forage fish groups are surf smelt (Hypomesus pretiosus), Pacific sand lance (Ammodytes hexapterus), Pacific herring (Clupea harengus pallasi), and Northern anchovy (Engraulis mordax). Surf smelt and Pacific sand lance spawn intertidally and are the focus of this investigation. On line fact sheet references are available at:


As they have since time immemorial, Suquamish Tribal Members harvest forage fish for food and for trade. Forage fish populations are important to the economic and cultural wellbeing of the Suquamish Tribe. The Tribe therefore has a strong interest in protecting the habitat and nearshore processes that support these important treaty-protected resources. Suquamish Tribal Elders report current surf smelt abundance and spawning areas diminished from historic levels. Alteration of shoreline habitat by human activity and development is a contributing factor to this decline.

The purpose of this study is to document current distribution and timing of forage fish spawning in East Kitsap County, an important Near Term Action identified by the Puget Sound Partnership (Puget Sound Partnership, 2012). The Puget Sound Partnership Action Agenda recognizes the role the food-web plays in recovering and sustaining healthy populations of native species and specifically called out forage fish and protecting their spawning habitats as critical to the overall recovery of Puget Sound. The Action Agenda calls specifically for the use of complete, accurate, and recent information in making shoreline planning and land use decisions. This project contributes to this strategy by updating the surf smelt and sand lance spawning maps in East Kitsap County. This data will be used by federal, state, and local jurisdictions to identify protection and restoration strategies and actions that will maintain or improve nearshore habitat used by spawning forage fish. This project will better inform the potential impact of intertidal aquaculture projects in this area to forage fish populations.

The study will support the West Sound Watersheds Council’s Puget Sound Monitoring and Adaptive Management Program by providing timely status and trends data for identified species and food web Key Ecological Attributes. The study will document intra- and inter-annual variation in spawn timing and distribution within budget limitations if continued for multiple years.

The Suquamish Tribe is a co-manager of fin fish populations within their usual and accustomed fishing and hunting areas (U & A), which encompass East Kitsap County, Washington, the geographic area of this study. The Tribe will coordinate closely with WDFW's Marine Beach Spawning Fish Ecology Program to maximize the number of samples collected and the beach elevations that are sampled.
The Washington Department of Fish and Wildlife protects known, documented forage fish spawning habitat as a Saltwater Habitat of Special Concern from the impact of shoreline development under "no net loss" regulations codified in WAC 220-110. In addition, "prohibited work times" are established to protect spawning adults and eggs on beach substrate. WDFW has 40+ years of forage fish spawning data to support these efforts but some beaches have been surveyed infrequently and other beaches have never been surveyed.

To address forage fish data gaps, the Washington State Legislature passed Substitute Senate Bill 5166 on February 25, 2015 authorizing WDFW has undertaken a two year, 2015 - 2017, systematic forage fish survey to sample all beach habitat types in Puget Sound and the Strait of Juan de Fuca to better understand spawning habitat characteristics and forage fish population dynamics. While WDFW's field study approach is comprehensive, the large geographic scope limits most beaches to being sampled infrequently.

This study will allow the Tribe to support a more intensive WDFW forage fish beach spawning monitoring effort in East Kitsap County, an area of Central Puget Sound that is projected to grow significantly in human population, potentially impacting Suquamish Treaty Rights in their terminal U & A. The Tribe's research boat is better equipped to sample beaches year round than the smaller WDFW skiffs that are more impacted by weather events. Preliminary forage fish sampling using the Tribe's research boat achieved 100 % beach access, and can sample a larger area in a given time frame.

### A6. Project/Task Description and Schedule

#### Schedule and Summary of Major Project Tasks

<table>
<thead>
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<th>Task Name</th>
<th>Task Description</th>
<th>Start Date</th>
<th>End Date</th>
<th>Outputs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Quality Assurance Project Plan</td>
<td>Complete QAPP and acquire all approvals.</td>
<td>11/1/2016</td>
<td>3/31/2017</td>
<td>Approved QAPP</td>
</tr>
<tr>
<td>2. Acquire equipment</td>
<td>All supplies and equipment necessary for sampling</td>
<td>3/31/2017</td>
<td>3/31/2017</td>
<td>All equipment and supplies will be ready for sampling</td>
</tr>
<tr>
<td>3. Conduct sampling</td>
<td>Sample beaches from Foulweather Bluff to Yukon Harbor and Blake Island per schedule</td>
<td>4/1/2017</td>
<td>12/3/2017</td>
<td>Completed data sheets and physical samples. Early field work funded by other sources.</td>
</tr>
<tr>
<td>5. Summarize data monthly and produce final report</td>
<td>Summarize field sampling data, noting any deviations from QAPP criteria or procedures, using tables and maps.</td>
<td>4/1/2017</td>
<td>12/31/2017</td>
<td>Monthly field sampling reports summarizing observed data and final report summarizing all data.</td>
</tr>
</tbody>
</table>

The Suquamish Tribe will coordinate with WDFW to expand upon their 2015-2017 Region 3 study sampling design in East Kitsap (Figure 1). The Tribe will assist WDFW sampling two 24.4 km beach segments (from twelve total beach segments) randomly each month using the Tribes larger research
vessel to minimize weather schedule impacts. The Tribe will sample one additional 24.4 km beach segment monthly and will sample one index beach site twice monthly using the semi-lunar tidal cycle schedule.

The Tribe will provide a research boat, staff to run the boat, collect field samples, and process samples that aren’t processed by WDFW. Each sample site will be documented, described in detail in the following sections, using standardized WDFW methodology and forms, site photographed, samples labeled, samples stored in a cool location, and all equipment cleaned prior to leaving the site (Penttila, 2011). Methods and standard operating procedures are described in the appendices to this QAPP. The Suquamish Tribe and WDFW will share responsibility for sample processing (Dionne, 2015). The Tribe maintains a qualified laboratory and staff to process any samples that exceed WDFW’s laboratory processing capacity. The Tribe will follow all WDFW forage fish process and handling protocols (Appendix 3).

The Tribe's 2017 Final Forage Fish Report will describe the observed forage fish spawning distribution on East Kitsap nearshore beaches. The report will provide an annual summary of all field results followed by a monthly accounting of distribution of observed forage fish spawning by surveyed beaches. The Tribal East Kitsap Forage Fish Spawning Data will be available both on the Tribal web site and WDFW Forage Fish web site.
Figure 1. The location of the twelve East Kitsap 24.4 km forage fish beach segments sampled in this study.

See Figure 2 for more detail on how beach segment 10 is further divided into twenty 1.22 km segments.
Figure 2. Within each 24.4 km beach segment (Bainbridge Island segment #10 shown here) there are twenty 1.22 km sub-segments (numbered in large map above). Within each sub-segment there are twenty points spaced 200 feet apart and numbered 1 through 20 (numbered in the inserted map above). For each monthly survey a number (1-20) is randomly selected without replacement, and all the points corresponding to that number are surveyed in each of the sub-segments before a new number is randomly selected. Two beach segments are randomly sampled each month, and the order in which they are sampled is randomly assigned without replacement until all segments have been selected before a new order is assigned. This results in a random starting point and order for surveys and a consistent spacing at 1.22 km apart within each round of surveys. This grant supports beach segment #3, Point Bolin, being sampled every month and Battle Point index site being sampled twice monthly. More detail provided in sections B1, Sampling Design, and B2, Sampling Methods.
A7. Quality Objectives, Criteria, and Representativeness

The Tribe will document the current distribution and timing of East Kitsap forage fish beach spawning by following the qualitative approach established by WDFW. WDFW's approach documents observed spatial and temporal forage fish beach spawning. Quantitative methods for assessing trends in egg abundance require extensive randomized sampling that are beyond the scope of this project. Further, given the unknown East Kitsap forage fish abundance status, the Tribe desires not to cause excessive egg mortality resulting from extensive randomized beach sediment sampling. Consequently there is no hypothesis testing proposed for this study as the goal is descriptive. This study will accurately identify forage fish eggs, when present in beach sediments, their relative abundance by species, live/dead egg counts, and larval development stage. Larval development documents that multiple spawning events have occurred at that beach location. The study period is calendar year 2017. The study will sample all East Kitsap beaches from Foulweather Bluff to Yukon Harbor, including Bainbridge and Blake Islands.

Surf smelt and sand lance eggs are tiny, almost microscopic and attached to sand grains. Each spawning event may occur in relatively limited areas along a narrow band at a specific intertidal elevation. Surf smelt spawn higher on the beach (+7.5 to +10) than sand lance (+4 to +6). Storm events and nearshore currents may distribute their eggs up to 200 feet from their initial spawning area based on past research (Penttila, 2011; Quinn, 2012, Kranzler, 2014). It's possible to identify recent, active spawning sites visually, but many times the spawning events are not obvious to the human eye. No visually active spawning area is sampled unless the site is specifically identified in the random sample collection site protocol for that time period.

The Tribe will utilize two WDFW survey methodologies for this study. The first methodology will follow the 24.4 km Beach Reach sampling methodology. The standard operating procedures for this approach are documented in WDFW's Intertidal Forage Fish Spawning Habitat Survey Protocols, Procedures for Obtaining Bulk Beach Substrate Samples Guidelines (Penttila, 2011; Appendix 3). Sample bias is avoided by assigning randomized beach collection locations (Figures 1 & 2). The sampling locations and tidal elevations are generally representative of where forage fish may spawn. If the selected sample site is bedrock, bulkhead-filled or otherwise unsuitable forage fish spawning habitat, the sample may be moved 100' in either direction. The new site will be documented in the field records. The number and location of the beach sampling sites within each 24.4 km reach was determined by a Camano Island study (Quinn, 2012) and South Puget Sound Survey (Kranzler, 2015) to provide a positive correlation to forage fish spawning events, i.e., if forage fish spawning occurred nearby, it would be detected. The Suquamish Beach Reach #3 will be sampled monthly to provide a detailed survey of one reach over the course of this study. Two of the other beach reaches, from 1 to 12, will be randomly selected to be sampled each month.

Given that tidal elevations vary during the 24.4 km beach reach sampling times, it is not always possible to sample the lower tidal elevations that may contain suitable sand lance spawning habitat. The second sampling methodology used in this study is an Index Beach. The Tribe worked with WDFW to identify an index site, Battle Point (Bainbridge Island), that has well documented surf smelt and sand lance utilization based on historic WDFW forage fish use records and Suquamish Tribal beach seine records. Battle Point's natural processes remain intact, i.e., no shoreline armoring, active LWD recruitment, native vegetation present, and minimal human presence on the beach. Index beach sampling follows the WDFW index protocols: beach elevations are surveyed and staked at one foot elevations with six 16
One ounce samples collected at +4, +5, +6, +7, +7.5, and +8 elevations and two bulk samples (described above in standard operating procedures) at +5 and +8 elevations (Dionne, 2015, Figure 3). The 16 ounce samples provide for a quick survey at each one foot tidal elevation and are correlated to the two bulk samples collected at the same elevation, +5 and +7.5. The index site will be sampled twice monthly. All of the Beach Reach samples provide an opportunity to assess the presence of surf smelt, but may miss opportunities to assess sand lance. The Index Beach insures an equal opportunity to sample for the presence of both species at one location during every sampling event. In addition, the Index Beach offers an opportunity to evaluate spatial forage fish beach spawning changes that may occur seasonally.

Figure 3. Battle Point Index Sites sample collection sites consist of two staked transect lines, one on the north facing beach and one facing west. Each transect is fully sampled every two weeks.

A8. Special Training Requirements/Certification

WDFW scientists will provide forage fish protocol training to all staff working on this project including the Project Manager and the Project Supervisor. WDFW’s survey guidelines will be followed at all times during the project. At least one trained WDFW forage fish survey technician will be present at most sample sites.

Laboratory processing of the field samples requires careful adherence to the Vortex methodology to extract forage eggs (if present) from the sample. The volume retained after Vortex processing typically requires no further winnowing or reduction and can be inspected for eggs in a standard 10 cm petri dish.
in one or two batches. The laboratory procedure for preserving processed samples will be followed and is described in the "San Juan County Forage Fish Assessment Project: Field Manual for Sampling Forage Fish Spawn in Intertidal Shore Regions (Moulton and Penttila, 2001, rev 2006; Appendix 3). A Material Safety Data Sheet for Stockard's Solution is posted at the Olympia and Suquamish processing sites. All processed samples are disposed of following State hazardous materials protocols.

A9. Documents and Records

A9.1 Report format/information

Field and laboratory data will be recorded on standardized forms included in the field manuals and laboratory procedures (Moulton 2001, Dionne 2015; Appendices 2 & 3). The field data will be filled out completely for each sample site, complete with six digital photographs of each site, and site gps coordinates. The gps equipment accuracy is well within the 200 foot wide beach sample site location and the most recent high tide rack line accurately delineates the beach elevation for sample selection. Hand recorded data will be taken in indelible ink and any changes to the data will be made drawing a single line through the error, initialed by the trained staff person.

A9.2 Document/record control

WDFW retains the original field data and the Suquamish Tribe will keep a copy of East Kitsap field data and field photographs. The WDFW Forage Fish database is viewable on SalmonScape at the following link: http://wdfw.wa.gov/mapping/salmonscape/index.html. The database will be used to generate regional maps of the 24.4 km beach segments with color coded results of the beach sampling. The data will include the presence/absence of forage fish eggs at each sample site, the species observed, the live/dead egg count, and the embryonic development from unfertilized egg thru blastula to gastrula to notochord coil development to hatched larva observed.

A9.3 Other records/documents

Using the processed laboratory data, East Kitsap maps will be generated to summarize monthly forage fish observations and included in the final report along with detailed spreadsheets. Other records and documents that may be produced in conjunction with this Tribal project include:

- Amended QAPP
- Semi-annual FEATS progress reports to NWIFC
- Project final report
- PowerPoint presentations to local governments and organizations

A9.4 Storage of project information

The original field and laboratory forms, digital photographs, and processed samples will be stored by WDFW in Olympia at 1111 Washington Street SE, Olympia, Washington. The Suquamish Tribal Project Manager will retain all updated versions on the QAPP, be responsible for distribution of the current QAPP version, and insure that copies of all project information is stored at the Suquamish Tribe.
A9.5 Backup of electronic files

All electronic documents, data, spreadsheets, and databases will be backed up on the WDFW computer network and the East Kitsap Forage Fish study will be backed up on the Suquamish Tribe’s computer network.

B. Data Generation and Acquisition

B1. Sampling Design

The Tribe will utilize two WDFW sampling designs for this study, the beach reach and the beach index. The first methodology will follow the 24.4 km beach reach sampling methodology. The standard operating procedures for this approach are documented in WDFW's Intertidal Forage Fish Spawning Habitat Survey Protocols, Procedures for Obtaining Bulk Beach Substrate Samples Guidelines (Penttila, 2011; Appendix 3). Sample bias is avoided by assigning randomized beach collection locations (Figures 1 & 2). The sampling locations and tidal elevations are generally representative of where forage fish may spawn. If the selected sample site is bedrock, bulkhead-filled or otherwise unsuitable forage fish spawning habitat, the sample may be moved 100’ in either direction. The new site will be documented in the field records. The number and location of the beach sampling sites within each 24.4 km reach was determined by a Camano Island study (Quinn, 2012) and South Puget Sound Survey (Kranzler, 2015) to provide a positive correlation to forage fish spawning events, i.e., if forage fish spawning occurred nearby, it would be detected. The Suquamish beach reach #3 will be sampled monthly to provide a detailed survey of one reach over the course of this study. Two of the other beach reaches, from 1 to 12, will be randomly selected to be sampled each month.

The beach reach sampling design divides each shoreline into 24.4 km beach reach segments that are systematically sampled by boat. Past studies have determined that the minimum distance for autocorrelation between sampling sites was 1.22 km (Quinn, 2012; Kranzler, 2015). The 24.4 km beach segment length was chosen as the distance a small skiff and crew could sample within a day. There are twelve 24.4 km beach segments within East Kitsap (Figure 1). Each 24.4 km beach segment is partitioned into twenty 1.22 km sections. Within each 1.22 km section there are 20 beach sample points located 200’ apart (Figure 2).

There are two levels of randomization applied to the beach reach sampling strategy. First, within each 1.22 km beach length, each beach sample point is numbered 1 to 20. A number is randomly selected as a starting point, and all those numbered sampling points within each adjacent 1.22 km beach section are sampled. Second, the order of the 24.4 km beach segments is sampled is randomly selected each month, without replacement. Actual site conditions may preclude a beach being sampled due to bedrock conditions, being on a military base, or access denied, leading to some beach segments having fewer points than 20 sample points. Most 24.4 km beach samples will be sampled twice during the project, with one beach segment - the Suquamish reach # 3 - being sampled monthly.

It is not always possible to sample the lower tidal elevations when collecting beach reach sediment samples given tidal elevations may cover suitable sand lance spawning habitat. Using the index beach approach insures that both surf smelt and sand lance habitat is sampled consistently during every sample date. The Tribe worked with WDFW to identify an index site, Battle Point (Bainbridge Island), that has
well documented surf smelt and sand lance utilization based on historic WDFW forage fish use records and Suquamish Tribal beach seine records. Battle Point's natural processes remain intact, i.e., no shoreline armoring, active LWD recruitment, native vegetation present, and minimal human presence on the beach. Index beach sampling follows the WDFW index protocols: beach elevations are surveyed and staked at one foot elevations with six 16 ounce samples collected at +4, +5, +6, +7, +7.5, and +8 elevations and two bulk samples (described above in standard operating procedures) at +5 and +8 elevations (Dionne, 2015, Figure 3). The 16 ounce samples provide a quick survey at each one foot tidal elevation and are correlated to the two bulk samples collected at the same elevation, +5 and +7.5. The index site will be sampled twice monthly. The Index Beach insures an equal opportunity to sample for the presence of both species at one location during every sampling event. In addition, the Index Beach offers an opportunity to evaluate spatial forage fish beach spawning changes that may occur seasonally. WDFW's forage fish map (Figure 4) and the url: http://wdfw.wa.gov/conservation/research/projects/marine_beach_spawning/ documents the historic use of forage fish at both the beach reach and beach index sites.

Figure 4. Documented East Kitsap WDFW forage fish spawning locations.
B2. Sampling Methods

The study will follow WDFW's Intertidal Forage Fish Spawning Habitat Survey Protocols, Procedures for Obtaining Bulk Beach Substrate Samples guidelines (Penttila, 2011; Appendix 3). The sampling locations and tidal elevations are generally representative of where forage fish may spawn. Sampling will occur at low tide, using predicted tide tables to ensure access to the +7 to +10 surf smelt tidal elevation and +5 to +8 sand lance tidal elevation when possible. Sampling will take place on public beaches and where landowner permission has been obtained by WDFW.

Randomly selected beach coordinates for day will be loaded into the Tribal research boat. Upon disembarking from the boat, hand-held Garmin GPS equipment will be used to locate each beach survey site. To insure consistency among units, the make and model of each GPS unit will be recorded as well as the specifications of the unit's accuracy. The datum for each unit will be set to NAD 83 and positions recorded in decimal degrees. A GPS-equipped, waterproof/shockproof Nikon Coolpix AW130 GPS-equipped camera, or equivalent, will be used to take the six photos of each site with the photo numbers recorded on each sites WDFW Forage Fish Spawning Beach Survey form. This standardized form documents sample date, time, GPS coordinates, and codes used to record detailed beach characterization observations taken from WDFW’s Field Observation Sampling Code chart. A site label with date, location, beach station #, and sample # is placed in each sample bag before sealing the bag. The field technician signs each data form certifying compliance with WDFW-approved protocols are used to enter beach characterization data on each sites.

The sample collection procedure is described in WDFW Protocol FF-01 on-line at: http://wdfw.wa.gov/conservation/research/projects/marine_beach_spawning/training/protocol-field_bulk_sample_collection.pdf (Appendix 3). Substrate samples are collected along a 100 foot horizontal distance located below the most recent high tide rack line with the center point being the sites GPS coordinates. Predicted tide charts are corrected by actual Seattle NOAA tidal observations. A 16-ounce plastic sample jar is used to scoop bulk sediment from four 1 inch to 4 inches deep small three foot long trenches dug perpendicular to the 100 foot line at the zero foot, 33 foot, 66 foot, and 100 foot intervals. The sediment samples are placed into 8” by 24’ heavy duty polyethylene bags, label added, sealed and stored in a cool location for transport to the laboratory.

All sampling equipment will be inspected for any substrate, fauna, or flora. Potentially contaminated equipment will be rinsed in seawater and wiped down until all potential contaminants are removed. Paper towels will be bagged and disposed of in the marina garbage receptacle upon returning to moorage. No felt-soled boots or shoes will be allowed on the beach as they can't be effectively decontaminated. The decontamination procedures that will be followed are on-line at: http://www.ecy.wa.gov/programs/eap/InvasiveSpecies/AIS-PublicVersion.html.

The samples will be kept cool and processed as soon as possible following WDFW’s Vortex Method for Separation of Forage Fish Eggs From Beach Sediments Addendum to the 2006 revision of Field Manual For Sampling Forage Fish Spawn In Intertidal Shore Regions (Dionne, 2015; Appendix 3) which supplements WDFW's Intertidal Forage Fish Spawning Habitat Survey Protocols FF-02 available on-line at: http://wdfw.wa.gov/conservation/research/projects/marine_beach_spawning/training/protocol-field_substrate_winnowing.pdf. WDFW's Intertidal Forage Fish Spawning Habitat Survey Protocols "Laboratory procedure for counting and staging forage fish eggs obtained from processed "winnowed
light fraction" field samples (Protocol FF-04, Version 1.0, July 2011, Appendix 3). Online procedures for this protocol FF-04 are at: http://wdfw.wa.gov/conservation/research/projects/marine_beach_spawning/training/protocol-lab_spawn Enumeration.pdf. The Tribal laboratory will use an Omno binocular microscope to process samples. All processed samples are disposed of following State hazardous materials protocols.

B3. Sample Handling and Custody

Chain of custody is described in the WDFW forage fish sampling field and laboratory procedures (Penttila, 2011; Dionne, 2015; Appendix 3). The bulk sample bags will be transferred from the Tribal research boat into a WDFW for transport to the Olympia laboratory for processing. The processed samples will be preserved with Stockard's solution and stored in a well-ventilated area until disposal following State protocols (Appendix 3).

B4. Analytical Methods

No physical tests or chemical analyses will be used for this project. The processing of the bulk field samples begins with sieving through progressively finer sieves (4mm, 2mm, and 0.5mm mesh). The material remaining in the 0.5mm mesh is added to a hydrocyclone device to which a water vortex is applied to separate the forage fish eggs from the substrate. The sample fraction with the eggs is filled with Stockard's Solution, gently rotated to submerge all eggs in the preservative and stored in a cool location, not frozen, until placed in a petri dish for processing under a dissecting microscope. Eggs are removed by forceps and placed in a watchglass for species identification, development, and counting.

B5. Quality Control Requirements

Field staff will be trained in, and follow all WDFW forage fish spawning data protocols. Project supervisory staff will confirm field data entry, proper bulk sample processing, laboratory microscopy identification, and accurate project database entry is performed. 100% of the positive forage fish egg samples will be re-checked by the Project Quality Assurance Officer to verify correct species id and that all eggs were accurately counted. 10% of the negative forage fish sediment samples will be re-checked by the Project Quality Assurance Officer to confirm that no forage fish eggs were present. Any discrepancy between observations will be noted on the spreadsheet and the Project Quality Assurance Officer count will be the data used in all reports.

B6. Instrument/Equipment Testing, Inspection, and Maintenance

This section is not relevant to this project.

B7. Instrument/Equipment Calibration and Frequency

This section is not relevant to this project.

B8. Inspection/Acceptance for Supplies and Consumables

This section is not relevant to this project.
B9. Non-Direct Measurements (i.e. Secondary Data)

This project will not rely on any secondary data.

B10. Data Management

Data management is described in Section A.9 of this QAPP and the attached WDFW Forage Fish Beach Sampling and Laboratory Processing Procedures in Appendix 3.

C. Assessment and Oversight

C1. Assessment/Oversight and Response Actions
This QAPP describes a timeline of intensive scheduled field beach sample collection that will be processed and analyzed on a monthly basis noting the beach location and tidal elevation sampled, presence/absence of forage fish egg(s), live/dead egg counts, embryonic development stage, and any sample site deviations required (within protocol limits).

The limited project budget anticipates maximum personnel sampling effectiveness sampling, processing, and generating the final report. At deliverable deadline the Project Manager and the NWIFC Puget Sound Recovery Project Coordinator will review the outputs, assess project progress, and decide on any remedial actions.

C2. Reports to Management

In addition to the field sampling results maps, all activities and progress will be documented in regular biannual FEATS reports.

D. Data Review and Evaluation

D1. Data Review, Verification, and Validation Requirements

This QAPP shall govern the operation of the project at all times. Each responsible party listed in Section A.4 shall adhere to the procedural requirements of the QAPP and ensure that subordinate personnel do likewise.

This QAPP shall be reviewed during the project timeline to ensure that the project will achieve all intended goals. All the responsible persons listed in Section A.4 shall participate in the review of the QAPP. The Project Manager and the Project Quality Assurance Officer are responsible for determining that the data confirm to project standards. The Project will be modified, if necessary, as directed by the Project Manager. The Project Manager shall be responsible for the implementation of minor changes to the project and shall document the effective date of all changes made.
It is expected that ongoing and unexpected changes may need to be made to the project. The Project Manager shall consult with and receive approval from the EPA for any significant changes or deviations in the operation of the project. These significant changes must be formally submitted as an amendment to the QAPP. All verification and validation methods will be noted in the analysis provided in the final project report.

D2. Verification and Validation Methods

Data will be reviewed throughout the field beach sampling season by the Project Manager. Any anomalies will be corrected or identified and flagged in the final project report.

D3. Reconciliation with User Requirements

The final project report will provide a spreadsheet of all sampled beaches by date and location, presence absence of forage fish egg(s), species identification, a live/dead egg count, number of eggs observed, and egg development stage. This data will update East Kitsap beach forage fish spawning data maps and improve the regulatory protection for these beaches.

E. References


Lagness, M. P. Dionne, E. Dilworth, and D. Lowry. 2014 Summary of Coastal Intertidal Forage Fish Spawning Surveys: October 2012-September 2013. Washington Department of Natural Resources. Fish Program Report Number FPA 14-01


Pentilla, D. 2011. WDFW Intertidal Forage Fish Spawning Habitat Survey Protocols; Procedures for Obtaining Bulk Beach Substrate Samples. WDFW Protocol FF-01 Version 1.0, July 2011. Reformatted by Dayv Lowry


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Forage Fish Spawning Surveys
Puget Sound, WA
October 2015 - May 2016

1,201 Survey Sites
238 Sites had Surf Smelt Only
20 Sites had Sand Lance Only
4 Sites had both Surf Smelt & Sand Lance
Forage Fish
Spawning Surveys
Region 3
October 2016

74 Survey Sites
16 Sites had Surf Smelt only
0 Sites had Sand Lance only

- Surf Smelt Only
- Sand Lance Only
- Surf Smelt & Sand Lance
- No Eggs
Forage Fish Spawning Surveys Region 3 August 2016

60 Survey Sites

- 0 Sites had Surf Smelt only
- 0 Sites had Sand Lance only

- Surf Smelt Only
- Sand Lance Only
- Surf Smelt & Sand Lance
- No Eggs

Washington Department of FISH and WILDLIFE
Forage Fish Spawning Surveys
Region 3
July 2016

76 Sample Sites

7 Sites had Surf Smelt only
0 Sites had Sand Lance only

- Surf Smelt
- Sand Lance
- Surf Smelt & Sand Lance
- No Eggs

Washington Department of FISH and WILDLIFE

Washington Conservation Corps

Department of Natural Resources
Forage Fish Spawning Surveys Region 3 June 2016

77 Sample Sites

1 site had Surf Smelt only
0 sites had Sand Lance only

- Surf Smelt
- Sand Lance
- Surf Smelt & Sand Lance
- No Eggs
Forage Fish Spawning Surveys
Region 3
May 2016

70 Sample Sites
1 site had Surf Smelt Only
0 sites had Sand Lance

- Surf Smelt Only
- Sand Lance Only
- Surf Smelt & Sand Lance
- No Eggs
Forage Fish Spawning Surveys
Region 3
April 2016
34 Sample Sites
2 sites had Surf Smelt Only
0 sites had Sand Lance
Surf Smelt Only
Sand Lance Only
Surf Smelt & Sand Lance
No Eggs
Forage Fish Spawning Surveys Region 3 March 2016

52 Sample Sites

- 2 sites had Surf Smelt Only
- 0 sites had Sand Lance
- surf smelt only
- sand lance only
- surf smelt & sand lance
- no eggs
Forage Fish Spawning Surveys
Region 3
February 2016

65 Sample Sites

5 sites had Surf Smelt Only
0 sites had Sand Lance

- Surf Smelt Only
- Sand Lance Only
- Surf Smelt & Sand Lance
- No Eggs
Forage Fish
Spawning Surveys
Region 3
January 2016

58 Sample Sites

13 sites had Surf Smelt Only
0 sites had Sand Lance Only
1 site had both Surf Smelt & Sand Lance

- Surf Smelt
- Sand Lance
- Surf Smelt & Sand Lance
- No Eggs
Appendix 2. WDFW Forage Fish Spawning Beach Survey Form with Codes
Field Observation Sampling Code

Beach: Sediment character of the upper beach (particle size range in inches)
0 = mud (<0.0025)
1 = pure sand (0.0025-0.079)
2 = pea gravel (0.079-0.31, “fine gravel”) with sand base
3 = medium gravel (0.31-0.63) with sand base
4 = coarse gravel (0.63-2.5) with sand base
5 = cobble (2.5-10.1) with sand base
7 = boulder (>10.1) with sand base
8 = gravel to boulders without sand base
9 = rock, no habitat

Tidal Elevation: Determined in the office using location and time data provided.

Shading: Shading of spawning substrate zone, averaged over the 1,000 foot station and best interpretation for the entire day and season
1 = fully exposed
2 = 25% shaded
3 = 50% shaded
4 = 75% shaded
5 = 100% shaded

Uplands: Character of the uplands (up to 1,000 ft from high water mark)
1 = natural, 0% impacted (no bulkhead, riprap, housing, etc.)
2 = 25% impacted
3 = 50% impacted
4 = 75% impacted
5 = 100% impacted

Smelt, Sand Lance, Rock Sole: subjective field assessment of spawn intensity apparent to the naked eye:
0 = no eggs visible
L = light, but apparent
M = medium, readily visible
H = heavy, broadly abundant
W = eggs observed in winnow

Photos: Take 6 site photos standing at the center of the site, and record the file number of the 1st photo in the 6 photo series.
*Photo 1: Completed sample tag
*Photo 2: Sediment w/ scale at transect
Photo 3: Beach backshore
Photo 4: Beach right
Photo 5: Beach foreshore (towards water)
Photo 6: Beach left
*If multiple samples are collected at a single station, then only photos 1 and 2 need be repeated for each sample.

**I certify that to the best of my abilities, the surveys recorded on this data sheet and the associated samples were collected and documented in accordance with WDFW approved protocols, and the information I am providing are the true and accurate results of these surveys.

Signature:

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FORAGE FISHES AND THEIR CRITICAL HABITAT IN THE NEARSHORE ZONE OF THE PUGET SOUND BASIN

KEY POINTS

1. Seasonal forage fish spawning activity is an important ecological feature of a significant portion of the shoreline of the Puget Sound basin (for detailed maps see http://fortress.wa.gov/dfw/gispublic/apps/salmonscape/default.htm).

2. Located in the intertidal/nearshore zone, forage fish spawning habitats are vulnerable to the effects of shoreline usage and development.

3. Preservation of spawning habitats is essential for forage fish preservation.

4. Substantial amounts of forage fish spawning habitat have been degraded or destroyed by the cumulative impact of shoreline usage and development in Puget Sound.

5. All known forage fish spawning habitat sites are currently protected from net loss by specific language in the WDFW Hydraulic Code (WAC 220-110), local shoreline master programs, and critical areas ordinances.

6. Our knowledge of the location and temporal usage patterns of forage fish spawning sites is incomplete. Additional sites continue to be identified, and/or the spawning timeframe more completely described, in on-going surveys.

7. Forage fish spawning habitat preservation cannot depend solely on public acquisition, restoration, or mitigation. Few restoration/mitigation efforts have been rigorously evaluated with regard to long term improvement or replacement of spawning habitat.

8. Given widespread privatization of tidelands in the Puget Sound basin, forage fish spawning habitat preservation will increasingly depend on the application of regulations to private property.

9. Adherence to private property rights must be balanced with effective stewardship and preservation of the public’s forage fish resources and associated critical habitat.

10. The need for public education about forage fish, their critical habitat, and their ecological role is constant to maintain a well-informed citizenry.

Original document by Dan Pentilla, WDFW Habitat Science Division, La Conner, WA, 2006. Adaptation by Dayv Lowry, WDFW Habitat Science Division, Olympia, WA, 2011.
Appendix 3. WDFW Forage Fish Spawn Sampling Field Manual and Forage Fish
Spawning Habitat Survey Protocols FF-01, FF-01, FF-03, & FF-04
SAN JUAN COUNTY FORAGE FISH ASSESSMENT PROJECT

FIELD MANUAL

FOR SAMPLING FORAGE FISH SPAWN IN INTERTIDAL SHORE REGIONS

FIRST EDITION

MARCH 2001

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SAN JUAN COUNTY FORAGE FISH ASSESSMENT PROJECT

FIELD MANUAL
FOR SAMPLING FORAGE FISH SPAWN IN
INTERTIDAL SHORE REGIONS

INTRODUCTION

With the listing of many Puget Sound salmon stocks as threatened or endangered, the issue of maintaining salmon forage fish stocks has been identified as a high priority by the San Juan County Marine Resources Committee (SJC MRC). All the important forage fishes, i.e. surf smelt, Pacific sand lance, and Pacific herring, depend on nearshore habitats for spawning and rearing. Protection of nearshore habitats utilized as spawning and rearing areas for forage fish will be needed if salmon recovery is to be successful. Recovery of bottomfish within SJC was also identified in 1996 as a key priority by the SJC MRC. These species have since become a high priority throughout Puget Sound because six stocks have been identified for potential listing as threatened or endangered species. The same forage fish species of interest in salmon recovery will be vital for the success of any program to restore bottomfish stocks.

Washington Department of Fish and Wildlife (WDFW) presently attempts to protect all known, documented Pacific herring, surf smelt, and Pacific sand lance spawning sites from impacts of shoreline development. “No net loss” regulations for the protection of known spawning sites of these species are included in the wording of the Washington Administrative Code “Hydraulic Code Rules” (WAC 220-110), which are applied by WDFW marine habitat managers during considerations for granting Hydraulic Permits for in-water shoreline development proposals. However, the forage fish habitat protection regulations only apply to shorelines where spawn has actually been detected by WDFW or other qualified surveyors. Thus it is critical for overall protection of these habitats that spawn deposition site inventories be complete and comprehensive. Not all outwardly suitable-appearing shorelines seem to be used by spawning forage fishes. On the other hand, large areas of formerly productive spawning habitat have been degraded or destroyed by shoreline practices in the absence of a database (or concern) regarding forage fish spawning activity.

Surveys to identify spawning areas were conducted by WDFW between 1989 and 1999, which documented 14 surf smelt spawning beaches, and 8 Pacific sand lance spawning beaches (Penttila 1999). WDFW was conducting a systematic survey of forage fish spawning beaches from 1991-1996 throughout Puget Sound, but lost funding for the effort in 1997, just as the San Juan County beaches were to be surveyed. “As a result of the diminished program, only a small portion of the potential beach spawning habitat has been surveyed” (Penttila 1999).

Surf smelt in the San Juan area spawn year-round, with no particular spawning season more dominant than another (Penttila 1990, 1999, Figure 1). Eggs, about 1 millimeter in diameter, are deposited in the upper intertidal zone on mixed sand and gravel beaches (Figure 2). After spawning, the eggs are dispersed across the beach by wave activity, so more of the beach is used for incubation than is used for actual spawning. Surf smelt can spawn on the same beach through the year, so eggs are likely to be present at any time. For example, at Hunter Bay and N. Shaw Island
index sites, smelt eggs were found during 13 of 16 visits from February 1989 to May 1990 (Penttila 1990).

WDFW conducted field surveys of spawn visible to the eye from 1989 to 1990 and “bulk sampling” (i.e., composited sediment samples from potential spawning beaches) from 1993 to 2000 to identify surf smelt spawning areas within San Juan County. The bulk sampling method consists of collecting beach samples and subjecting the sample to laboratory examination for egg presence. This method is considered a much more accurate measure of spawning activity than the visual method. A total of 208 visual samples and 286 bulk samples were taken during the survey periods. Most of the visual surveys were on Orcas, Lopez and Shaw islands, while bulk sampling was primarily on San Juan, Orcas and Lopez islands (Penttila 2000). The distribution of sampling is illustrated in Figure 3. As presented above, fourteen beaches within San Juan County have so far been identified as supporting spawning by surf smelt (Penttila 1999, Figure 4). The visual sampling method is considered relatively inefficient for identifying spawning locations, thus WDFW recommends that the locations surveyed in 1989-1990 that did not yield eggs should be resurveyed using the bulk method (Penttila 2000).

Results of bulk sampling indicate that not all beaches with appropriately-sized sand and gravel are used for spawning. Usage appears greatest on beaches with over-hanging vegetation. Over-hanging vegetation provides shade, which reduces egg mortality caused by desiccation. The shading is likely to be particularly important for the portion of the stock that spawns from late spring to early fall, when low tides are during the day and exposure to warm, dry air is greatest.

The intertidal nature of Pacific sand lance spawning was not known until 1989 (Penttila 1999). Pacific sand lance appear to use the same spawning substrate as surf smelt, as eggs from both species are often in the same sample. Pacific sand lance, however, will also use pure sand beaches that are not utilized by surf smelt. Fresh spawn appears as shallow, circular pits on the upper beach (Figure 2). The pits disappear rapidly after spawning as wave action re-works the beach sediment. Spawning by Pacific sand lance is during the winter, from early November through February (Figure 1). Development of the 0.6-0.8 mm eggs takes about 4 weeks, depending on temperature, thus incubating eggs could be present into late March.

The bulk sampling method described for assessing surf smelt spawning is also used to document Pacific sand lance spawning. The visual method is virtually useless for detecting Pacific sand lance eggs because these eggs are covered with sand grains and are essentially undetectable with the naked eye. Eight Pacific sand lance spawning areas were found during the bulk sampling conducted from 1993 to 2000, with the distribution as depicted in Figure 5.
STUDY DESIGN CONSIDERATIONS

Project Objectives

The primary objective of the SJC forage fish assessment is to identify county beaches that are utilized as spawning areas by surf smelt and Pacific sand lance. A secondary objective is to identify subtidal regions supporting Pacific herring spawning.

Sampling Schedule

Planning for surveys needs to consider spawning time when designing surveys intended to identify spawning locations (Figure 1). In the San Juan Islands, surf smelt spawn year-round (Penttila 1999). Pacific sand lance begin spawning in November, continuing through February.

SURF SMELT AND PACIFIC SAND LANCE SPAWN ASSESSMENT

Sampling for surf smelt and Pacific sand lance eggs consists of 1) obtaining a bulk sample of mixed sand and gravel from the upper intertidal region of an appropriate beach, 2) condensing the bulk sample to a manageable volume, and 3) examining the condensed sample under a dissecting microscope to determine the presence or absence of eggs.

Site Selection

Not all beaches represent potential surf smelt or Pacific sand lance spawning areas. Potential spawning areas are composed of a mixture of sand and small gravels, usually with fine shell fragments mixed in. Spawning and incubation areas are normally in the +7 to +9 foot MLLW tide zone. Areas that are shielded from direct sunlight by over-hanging vegetation are often more heavily used than areas where vegetation has been removed. Examples of spawning areas are shown in Figure 6. Note that in Blind Bay, only a portion of the potential habitat appears to be actually used for spawning and that the utilized area corresponds to the area with most over-hanging vegetation. Close-ups of areas containing appropriate substrate are in Figure 7. Eggs can sometimes be seen through a visual assessment (Figure 8).

Field Equipment

Equipment needed for collecting bulk beach samples to assess surf smelt and Pacific sand lance:

- 16 ounce plastic jar
- 8 inch x 24 inch polyethylene bags (to hold bulk sample)
- waterproof labels
- Pencil w/#2 lead
- Waterproof marker (fine tip)
• Electrical tape

Equipment needed for condensing samples:

• Rack of sediment screens, size 2 and 0.5 mm, preferably Nalgene instead of the more traditional brass screens,
• 2 - 5 gallon buckets modified to act as drain for screen rack,
• 2 Wash buckets,
• Plastic dishpan,
• 16 ounce plastic sample jar
• Stockard’s Solution:
  50 ml formalin (37% formaldehyde)
  40 ml glacial acetic acid
  60 ml glycerin
  850 ml fresh water

Equipment needed to establish sample location:

• Chart or map of beach to be sampled, 1:24,000 scale
• Integrated digital camera/GPS system
• 100 ft fiberglass tape for measuring distances

Field Records

Environmental characteristics of the sampled location are recorded to help analyze results of sampling. These records are entered on the field data sheet, which is completed at the time of sampling (Figure 14). Personnel involved in sampling need to be listed on the bottom of the sheet in case there are questions regarding the data. The data sheet will be reviewed after the crew has returned from the field. The reviewer will indicate that the sheet has been completed by signing the space labeled “Reviewed by”.

The data fields should be filled in as follows:

Last High Tide: time and elevation of the last high tide – can be obtained from a current tide chart.
Island: Island Sampled
Date of Sampling
Beach Number: Assigned Number for Beach being sampled.
Sample Number: Sample number from Sample Label.
Time: time sample label is removed from the beach (0000-2400 hr)
Latitude/Longitude: latitude and longitude in degrees, minutes, seconds
Beach: Character of the upper beach:
  0 = mud,
  1 = pure sand,
  2 = pea gravel (fine gravel) with sand base,
3 = medium gravel with sand base,
4 = coarse gravel with sand base,
5 = cobble with sand base,
7 = boulder with sand base,
8 = gravel to boulders without sand base,
9 = rock, no habitat

Uplands: Character of the uplands (up to 1,000 ft):
1 = natural, 0% impacted (bulkhead, rip-rap, housing, etc.);
2 = 25% impacted; 3 = 50% impacted; 4 = 75% impacted, 5 = 100% impacted

Sample Zone: Distance of collection parallel from a land mark in feet to the nearest ½ foot. Used
to determine the tidal elevation of the spawn deposit

Land Mark: Land mark for sample collection:
1 = down beach from last high tide mark
2 = up beach from last high tide mark
3 = down beach from second to last high tide
4 = down beach from upland toe
5 = up beach from waterline at the time noted

Tidal Elevation: This is determined in the office using the location and time data.

Smelt, Sand Lance, Rock Sole, Herring: subjective field assessment of spawn intensity:
0 = no eggs in field,
1 = very light, observed in field,
2 = light, observed in field
3 = light medium, observed in field
4 = medium, observed in field
5 = medium heavy, observed in field
6 = heavy, observed in field
7 = very heavy, observed in field
8 = eggs observed in the winnow

Width: Width of the potential spawning substrate to the nearest foot

Length: Length of the beach up to 1,000 feet (500 feet on either side of the station) or "C" if
continuous.

Shading: Shading of spawning substrate zone, averaging over the 1,000 foot station and best
interpretation for the entire day:
1 = fully exposed,
2 = 25% shaded,
3 = 50% shaded,
4 = 75% shaded,
5 = 100% shaded

Comments: additional information to be entered into the computer, evaluated on a station by
station basis.

Samplers: Names of personnel participating in the sample collection

Photo Taken: indicate number and direction of photographs

Prepare a map of each location sampled using a 1:25,000 scale NOAA nautical chart or 1:24,000
scale USGS topographic sheet. Mark each sample location on the map with appropriate sample
number so that the exact site can be re-visited, if needed. Use a GPS to obtain latitude and longitude of each sampled location, but priority should be placed on an accurate map.

Relevant nautical charts are:

18429 - Rosario Strait – Southern Part
18430 - Rosario Strait – Northern Part
18432 - Boundary Pass
18433 - Haro Strait – Middle Bank to Stuart Island
18434 - San Juan Channel

Relevant USGS topographic sheets are:

Blakely Island, Wa. 48122-E7-TF-024
Eastsound, Wa. 48122-F8-TF-024
False Bay, Wash. N4822.5-W12300/7.5
Friday Harbor, Wa. 48123-E1-TF-024
Lopez Pass, Wash. N4822.5-W12245/7.5
Mt. Constitution, Wa. 48122-F7-TF-024
Richardson, Wash. N4822.5-W12252.5/7.5
Roche Harbor, Wa. 48123-E2-TF-024
Shaw Island, Wa. 48122-E8-TF-024
Stuart Island, Wa. 48123-F2-TF-024
Waldron Island, Wa. 48123-F1-TF-024

General Guidelines for Collecting Bulk Beach Samples

Examine the beach to evaluate the most likely zone to contain eggs (+7 to +9 feet MLLW). This zone will be in the upper third of the beach, near the upper tidal limit. Typically, this zone is 1 or 2 vertical feet below the low line. For surf smelt eggs, the zone is characterized by mixed sand and small gravel. For Pacific sand lance eggs, the zone is similar, but can extend into pure sand. Mud or muddy sand are not acceptable substrates, nor are larger gravels, cobbles or solid rock and talus shores.

The sample is composed of four (4) scoops of gravel evenly spaced along a 100 ft stretch of beach (see Figure 10).

- Identify an approximately 100 ft stretch of beach to be sampled.
- Obtain location information for the transect by reading position information from a GPS or marking the location carefully on a large scale (1:24,000) USGS topographical sheet.
- Prepare a Sample Label to allow identifying the location (Beach Number) and collection time of the sample, deposit the label in the plastic bag (Figure 11).
- Start at one end of the transect, scoop a jar full of sand from the top 1-2 inch of beach and dump the sand into the plastic bag. The scooped area will likely be 3-4 ft long - the idea is to skim the eggs developing in the surface one-inch of substrate.
• Move 10 paces along the transect, obtain another scoop sample and place in the bag with the previous scoop.
• Repeat pacing and scooping until the four scoops have been obtained – this constitutes the bulk sample for the chosen transect.
• Seal the bag securely and place in a cool location. This is particularly important in warmer weather because high temperatures can cause mortality and decomposition in the eggs.
• Store in a secure location to ensure that the bags are not damaged during transit from the field.
• Take one or more photographs of the sampled beach. The photograph should be taken from one end of the sampled transect, looking towards the other end, so that the view is parallel to the beach. The photograph should show the sample relative to the last high tide line, if possible, and any other land marks that will help to establish the sample location. The direction of view (looking north, south, etc.) should be recorded on the field data sheet.

Condensing Bulk Samples

The bulk egg samples can be processed in the field to remove most of the sand and reduce the volume of the sample. This is done by washing the eggs from the sand and discarding the barren sediment (Figure 12). The eggs are lighter than the sand and gravel and will move upward during the washing process, allowing them to be skimmed from the surface of the material (Figure 13). The washing is conducted as follows:

• Assemble the Nalgene screens on top of the drain bucket, with the largest mesh on top, grading to the smallest mesh on the bottom.
• Remove the sample label and place it in a 16 ounce sample jar.
• Mark the Beach Number and Sample number on the outside of the jar with the fine-tip marker pen.
• Add a portion of the sample to the top screen, thoroughly wash the sediment through the screen set with either salt or fresh water, which ever is readily available.
• Discard the sediment in the top screens, retain only the material in the bottom (0.5 mm) screen.
• Dump the material retained in the 0.5 mm screen into the dishpan.
• Add water until the material is covered by 1-2 inches of water.
• Swirl the water around the pan, adding rocking and bouncing motions to allow the eggs to migrate to the top of the sediment. The idea is similar to gold panning, try to winnow the eggs to the surface of the material.
• After swirling for 1-2 minutes, work the lighter fraction of material to one corner of the pan. Carefully dry up the lighter fraction by tipping the pan so that the water drains away, and skim the lighter fraction from the surface of the sand with the sample jar.
• Repeat the winnowing process two more times.
• Process the remainder of the sample in a similar fashion, each time adding the retained lighter fraction to the sample jar.
• Fill the sample jar with Stockard's Solution to preserve the eggs. Seal the jar securely, invert carefully several times to ensure that the preservative reaches all the eggs.
• Tape the jar shut with electrician's tape so that the preservative does not evaporate during storage.

Laboratory Examination

Laboratory examination begins with a further condensing of the sample. The winnowing process conducted in the field is repeated using a shallow tray to separate eggs from sand. Final separation is performed under a dissecting microscope at 10-20x, where surf smelt eggs become quite visible. Pacific sand lance eggs are surrounded by sand grains, thus it is necessary to search for clumps of sand grains, then tease off the sand with fine-tipped forceps or dissecting needles to reveal the egg.

Eggs will be counted by species and the counts entered on the lab data form (Figure 13). The lab data form will only be used by those individuals specially trained in lab processing of samples and identifying eggs.

Eggs found during the smelt/Pacific sand lance spawn assessment will be archived for confirmation of species and spawn age analyses. Up to 100 random eggs of each species present will be labeled and preserved in Stockard's Solution in a vial, to be forwarded to WDFW staff, or other knowledgeable experts, for inspection. A number of non-egg objects may be encountered in preserved upper intertidal substrate samples that may be misidentified as forage fish eggs or empty egg shells, including invertebrate eggs, algal fruiting bodies, flatworms and their egg cases, certain thecate or arenaceous foraminifera, decalcified gastropods, and fragments of annelid worm tubes. Relative abundance and ages of forage fish eggs in the samples will be recorded, as these provide information of the relative frequency and density of spawning.

QUALITY ASSURANCE/QUALITY CONTROL

Primary concerns for quality control include:
• sampling appropriate habitat,
• accurate identification of sample location,
• careful screening and winnowing of the bulk sample to retain the maximum number of eggs, and
• accurate identification of sampled eggs.

The best way to ensure quality of the data is to make sure samplers are appropriately trained and understand the importance of careful sample processing and complete recording of sample-related information. Accuracy of screening and winnowing procedure can be measured by seeding a sand-gravel sample barren of eggs with a known number of eggs, then processing the sample to see how many eggs are actually detected.
DATA REPORTING

Data reporting should include all information collected during sampling. Usually, this reporting is in the form of summary tables that present information recorded on field and lab data sheets. The format of the tables can be similar to that of the data sheets to simplify reporting. Reporting should include:

1. a listing of all sites sampled, whether eggs were found or not,
2. detailed location information so that any site can be re-sampled, if necessary,
3. a summary of sampling at each site, including environmental conditions and number of samples taken,
4. a summary of findings for each site, including number of eggs by species found in each sample.

REFERENCES


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Shaded Months Indicate Spawning Season

Figure 1. Spawning time of forage fish species in San Juan Islands (data from WDFW).
a. Surf smelt spawn deposit outlined to show extent of spawning activity – note proximity of spawn deposit to the high tide mark.

b. Pacific sand lance spawn deposit with characteristic pitting (pits are circled to highlight).

Figure 2. Fresh surf smelt and Pacific sand lance spawn deposits (photos by D. Penttila, WDFW).
Figure 3: Distribution of intertidal sampling by WDFW to identify surf smelt and sand lance spawning beaches, 1999-2000.
Figure 4. Results of WDFW sampling for evidence of intertidal spawning by surf smelt, 1989-2000.
a. Surf smelt spawning area (patterned area) at Hunter Bay, Lopez Island (note that spawning area has been reduced by dock and launch ramp construction).

b. Surf smelt spawning area (arrow to patterned area) in Blind Bay, Shaw Island (note relationship of spawning area to over-hanging vegetation).

Figure 6. Representative surf smelt spawning beaches in San Juan County (aerial photos from WDOT, 1986).
a. Pocket beach west of Blind Bay on Shaw Island, surf smelt spawning area.

b. Mud Bay, Lopez Island, surf smelt spawning area.

Figure 7. Examples of surf smelt spawning beaches in San Juan County (photos by D. Peattila, WDFW).
a. Surf smelt eggs - 2 eggs are on the large black stone at the tip of the forceps. Eggs are approximately 1 mm in diameter (photo by L. Moulton).

b. Heavy deposition of surf smelt eggs in situ (photo by D. Peartlie, WDFW).

Figure 8. Examples of surf smelt eggs in field conditions.
Figure 9. Field data form used to record data associated with surf smelt and Pacific sand lance bulk sampling.
a. Obtaining beach subsample to examine for eggs.

b. Adding subsample to composited sample in bag.

Figure 10. Sampling mixed sand/gravel beach for surf smelt and Pacific sand lance eggs (photos by L. Moulton).
San Juan County MRC
FORAGE FISH PROJECT
SAMPLE NO:
FFP-000 1

DATE: _________  TIME: _________
BEACH NO: _______________________
SAMPLER: _______________________

Figure 11. Label used to identify each bulk sample
a. Standardized screens (4 mm, 2 mm, and 0.5 mm) are used to remove excess large material from the sample.

b. Sample is washed carefully to ensure eggs are removed from the large gravels and are deposited in the smallest material.

Figure 12. Screening bulk sediment sample to separate egg-bearing sediments from larger material (photos by L. Moulton).
a. Pan is swirled to separate eggs from sediment.

b. Lighter fraction of egg-bearing sediment is collected in a sample jar.

Figure 13. Winnowing bulk sediment sample to separate egg-bearing sediment from barren sand (photos by L. Moulton).
WDFW Forage Fish Spawning Habitat Survey Protocols:

Procedures for recovering "winned light fraction" subsamples of forage fish egg-sized material from bulk samples of beach surface substrate.

1. Wet-screen material through set of nested 4 mm/2 mm/.5 mm screens, using buckets of shore-side water at site or fresh-water hose elsewhere. Screens should be carefully cleaned between samples.

2. Discard material retained in 4 mm and 2 mm screens.

3. Place material from .5 mm screen ("egg-sized material") in rectangular dish-pan and cover with 1 inch of water.

4. Rotate/tilt/yaw dish-pan of material to impart rotation to water, and cause lighter material to rise to surface and accumulate toward center of deposit in pan. Observe behavior of shell fragments and organic particles to get indication of behavior of forage fish eggs.

5. Tilt/swirl/agitate pan contents to move lighter material accumulated at center down to lower left corner of pan deposit.

6. Carefully tilt pan to decant water to opposite corner of pan, slowly exposing lower left corner material above water's surface.
7. Holding pan in this tilted position, carefully scoop surface 1" of material from lower left corner into wide-mouth sample jar.

8. Repeat steps 4-7, about 3 more times, until sample jar is about 2/3 full of material.

9. Top-off sample jar with Stockard’s Solution preservative, and shake well to distribute preservative to all material.

10. Preserved samples will emit carbon dioxide as acidic preservative dissolves shell material in the samples. Lids should be loosely-fitted initially to allow escape of gas.

11. Escaping gas will also result in preservative escaping jars. Samples should be stored in leak-proof containers, and stored in well-ventilated areas to prevent accumulation of carbon dioxide in enclosed spaces.

12. Preserved samples may be archived for 10+ years without loss of data.

**Bulk substrate sample processing materials:**

Nested set of 4 mm/2 mm/.5 mm screens (Nalgene preferred over brass which bends/distorts over time)
buckets for discarded gravel
1-2 gallon plastic dish-pans
400 ml wide-mouth sample jars
Stockard’s Solution preservative (one gallon will preserve about 30 winnowed-light-fraction samples)
freshwater hose work-area with sufficient drainage
area to discard waste gravel
WDFW Forage Fish Spawning Habitat Survey Protocols:

Laboratory procedures for recovering forage fish eggs from preserved "winnowed light fractions" (screened beach substrate subsamples).

1. Stir winnowed light fraction sample-jar contents with spoon.

2. Swirl jar in clockwise manner to impart rotation to fluid and surface layer of contents, causing light material to move to center of material in jar.

3. Carefully tilt jar, slowly scoop center-mound of light material with spoon into oval microscope dish.

4. Repeat steps 1-3 four times, accumulating about [ ] of light material in microscope dish.

5. Add water to microscope dish, swirl/tilt/yaw dish to suspend lightest material and concentrate it along a feathered edge of the deposit in the dish.

6. Carefully place dish on microscope stage, inspecting zone around feathered edge of deposit of material in dish, removing eggs to watch glass with forceps.
7. Reverse dish, repeat steps 5-6 three times or until eggs cease to be detected around feathered-edge of deposit of material in dish.

8. If single egg is recovered in steps 1-7, repeat with second sample of material from jar of winnowed light fraction.

9. Identify eggs accumulated in watch-glass, count and/or record number of eggs in each embryological-stage category on data sheet

Lab materials:

Fume hood (alternatively, carefully rinse preservative from winnowed light fraction samples before processing).

Paper-towels
lab-gloves (keeps preservatives off skin)
Microscope with 10-20X
buckets/pan (to catch drips, accumulate completed samples, etc.)
Oval microscope dish
watch-glasses/small petri dishes
fine-point (watchmakers) forceps
table-spoon
 tally sheets/multi-place counter
CHARACTERISTICS OF THE EGGS OF FOUR SPECIES OF INTERTIDAL-SPAWNING MARINE FISHES FROM THE PUGET SOUND BASIN.
WDFW SURF SMELT EMBRYOLOGICAL-STAGE CATEGORIES

1-5 Hours → "1-CELL-MORULA": Very fresh eggs, 1-cell to roughly 30-cells

6-12 Hours → "BLASTULA": granular-caps through start of gastrulation

14-20 Hours → "GASTRULA": yolk-plug stage through start of neurulation

1-2 Days → "ONE-HALF COIL": distinct notochord axis to 7/8 coil embryo

3-5 Days → "ONE-COIL": nose nearly to tail tip to 1-1/4 coil, more or less, eyes white

6-7 Days → "ONE AND ONE-HALF COIL": more or less, preserved eyes gray

10 Days → "=>ONE AND ONE-HALF COILS": to 2+ coil, preserved eyes black to slightly metallic

13-14 Days → LATE-EYED": preserved eyes metallic, ventral gut spots are "dashes", "tight fit" in shell, includes loose larvae hatched during preservation

"DEAD": opaque-white/without discernable embryo/ fungus-covered eggs/ collapsed amnion/ eggs...
"NON-FORAGE FISH EGG-LIKE" OBJECTS ENCOUNTERED IN PUGET SOUND BEACH SUBSTRATE SAMPLES

These objects may be mis-identified as forage fish eggs with the naked eye, but can be easily distinguished from them under microscopic examination.

*Gromia* protozoan: benthic protozoan with 1-4 mm spherical, soft shell, contents granular and brown in color, no attachment sites.

Worm? egg cases: wrinkled-ovoid, 1 mm in length; purple/brown transparent in color, filled with round eggs or oval larvae, may have sand grains attached, could be mistaken for sand lance egg shells when empty.

Sand-covered beach worms: a 1-2 cm annelid, plain in form and white in color, is common in gravel beaches; when disturbed, they may coil-up tightly and secrete mucus, collecting coats of sand grains and thus resembling sand lance eggs to the naked eye.

Annelid sand-tube fragments: irregular fragments or sections of chitinous worm tubes with sand grains attached, could be confused with sand lance eggs.

Coiled-up flatworms: a 2-4 mm white flatworm may be common on Hood Canal beaches; when disturbed, it may coil-up into a globular shape resembling a loose, dead smelt egg to the naked eye.

Plant seeds/flower parts: a variety of shore-zone plant seeds and miscellaneous parts find their way onto the beach, none closely resemble forage fish eggs under a scope.

Conifer pitch droplets: often perfectly spherical, variable in size, clear to red-brown in color, no embryo-like internal structure, either deform un-elastically or shatter into fragments when forcepped.

Algal fruiting bodies and fragments: certain red algae shed fragments, ovoid-roundish in shape, variable in size, pink/green in color, no embryo-like internal structure under scope.

Coiled-up sphaeromtid isopods: can common on estuarine beaches, juveniles can be 1-2 mm in diameter when tightly coiled, gray in color, obviously a segmented arthropod under a scope.

Ostracods: 1-2 mm ovoid crustaceans with "bivalved" carapaces, light-brown in color, a central eye-spot and swimming legs are distinguishable under a scope.

Mites: 2-3 mm arachnids, light brown in color, body segmentation and walking legs obvious under a scope.

*Assiminia* snails: a globular gastropod, 1-3 mm in size, common in upper intertidal gravel. The
decalcified protein "ghost" of the shell with the coiled animal, can be distinguished from fish eggs under a scope.

Lacuna snail egg masses: 1-3 mm hemispherical jelly masses, white to yellow in color, commonly clustered at tips of eelgrass blades and other marine vegetation; distinguished from herring eggs by shape, texture, and presence of large numbers of tiny eggs imbedded in them under magnification.

Slag pellets/agate: Some eroding rock formations will yield tiny spherical translucent-quartz inclusions onto beaches; beaches in the area of old mills may have spherical slag-droplets formed when burning material was dumped into the water, obviously neither will deform when forcepped.

Carbonized spheres: spherical solid objects of unknown origin, flat-black in color, no internal structure, shatter to fragments when forcepped.

Invertebrate? fecal pellets: variety of ovoid/cylindrical brown objects, shatter to earthy fragments when forcepped.

"Non-forage" marine fish eggs: a few other marine fish species deposit benthic adhesive eggs on marine vegetation and other solid surfaces in the near-shore zone. While they may not be identifiable to species themselves, all are distinguishable from forage fish eggs by density or area of total deposit, size, color, embryo structure, or occurrence context.

Dan Penttila
WDFW Habitat Program
LaConner
Vortex method for separation of forage fish eggs from beach sediment

Addendum to the 2006 revision of
Field Manual for Sampling Forage Fish Spawn in Intertidal Shore Regions

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July 2015
Introduction

Washington Department of Fish and Wildlife (WDFW) biologists have assessed marine shorelines for evidence of forage fish spawning (presence of eggs) since the 1970’s. During this time, we have developed effective protocols for collecting and identifying forage fish eggs from beaches. These protocols are contained in the San Juan County forage fish assessment project: Field Manual for sampling forage fish spawn in intertidal shore regions (field manual; Moulton and Penttila 2001, revised 2006). The field manual describes the sampling process from beach site selection and sediment sample collection through condensing bulk sediment samples to laboratory analysis.

The current document, Vortex method for separation of forage fish eggs from beach sediments, describes an alternative method for condensing bulk samples to concentrate eggs to those described in the field manual. The vortex method generally results in a smaller volume of beach material retained for lab analysis and thus aids in egg identification by reducing the amount of material that must be sorted through. We intend the vortex method to be used in place of the “winnowing” method described in steps 3 through 8 on pages 24 and 25 of the field manual by Moulton and Penttila (2006).

As described in the on pages 24 and 25, the first step in treating the bulk sample is to sieve the sample through progressively finer sieves (4 mm, 2 mm, and 0.5 mm mesh). Only the material collected in the 0.5 mm sieve is retained for further processing. During the winnowing process, the condensed sample material is transferred to a square wash basin where it is covered with a thin layer of water and agitated to suspend and concentrate the lighter material, including eggs above the heavier material. This top layer of lighter material is collected and retained for laboratory analysis (examination of material by microscope) to identify and count the eggs.

The vortex method also follows sieving. The condensed material collected in the 0.5 mm sieve is added to a hydrocyclone device consisting of a circular bowl and a recirculating electric water pump to create a vortex that concentrates the light material. Thus, this method replaces the agitation process described above.

We compared the two condensing methods and found the vortex method has a higher egg recovery rate than the winnow method (average smelt egg recovery rate, winnow method: 59%, vortex method: 90%) and results in a smaller volume of material to process in the lab. In light of these improvements in efficiency, we recommend the vortex method for condensing bulk samples after sieving. However, before any modifications are made to your sampling program, be advised that careful consideration should be given to potential impacts to results and whether results from the two methods are directly comparable. Please consult with WDFW staff if you would like to discuss compatibility with WDFW data standards.

This document contains a description of the process and system that we have designed and tested. Modifications to the process or system we describe below may alter the efficiency of the system and consequently lead to results that are not comparable with our results. Those who intend to utilize the vortex method should obtain training prior to implementation. Biologists using these methods for regulatory surveys must complete the WDFW training. Additional information and resources for training are provided on page 11 of this document.
How it works

- The movement of the water through the bowl creates a vortex resulting in a pressure gradient.
- The material in the water moves from higher pressure at the edge to lower pressure in the middle of the bowl.
- Less dense materials, such as eggs, move towards the center faster than more dense materials.
- The raised cone in the middle of the bowl reduces the amount of sand and other dense material that leaves the bowl.
- The water leaving the blue bowl passes through a 0.5 mm sieve before being returned to the water reservoir.
- The sieve collects only the material that is egg size or greater.
Materials

A list of URLs for parts vendors is included on page 12 of this document.

One 18 gallon tote with lid
One blue bowl gold concentrator
One 750 to 1000 gph submersible electric water pump
One, two foot length of ¼” flex hose
One, ¾” hose clamp
One, ¾” male thread hose end kit
One adjustable hose valve
One quick connect hose fittings kit with female thread
One, 0.5 mm sieve (this can be the same sieve used to sieve the bulk sample)
Three shims
One, 250 to 1000 ml wash bottle
One rubber spatula
One plastic spoon
Sample jars

Tools for assembly:
Screw driver
Metric ruler
Permanent marker
Box cutter

Optional: The unit can be configured with a bilge pump and 12 volt battery to allow for use at locations where electricity is not available.
Assembly

1. First assemble the pump with the flex hose, hose clamp, male hose end, adjustable valve and one side of the quick connect hose fitting. Attach the other side of the quick connect hose fitting to the blue bowl.

2. Use a nylon stocking or pantyhose to stretch over the water intake of the pump to act as a filter and ensure that any eggs that may inadvertently fall into the water reservoir are not passed though the pump to other samples.

3. Use a ruler and a permanent marker to make a mark 2 cm below the inner edge of the blue bowl at several locations around the bowl.
Assembly

4. Next, modify the tote lid by cutting two holes; one for the pump and one for water to return after passing through the blue bowl and the sieve.

The pump hole should be large enough for the pump to pass through and should be located so that the flex hose can be easily connected to the blue bowl without kinking.

The water return hole should be smaller than the outer diameter of your sieve so that the sieve can rest on the lid without falling through the hole. Sieves are generally 8" to 12" in diameter.
Set up

1. Remove any equipment stored within the tote and place the tote on a relatively level surface.

2. Add enough water to the tote so that the pump will be covered by several inches of water when connected.

3. Attach the tote lid, place the 0.5 mm sieve over the water return hole, place the blue bowl on top of the sieve, and connect the pump to the bowl.

4. Add water to the bowl to aid in determining if it is near level. Use the shims to level the bowl if needed by placing them under the edge of the sieve.
Sample processing

Note: Before each sample is processed, the bowl and sieve should be rinsed and the pump should be run briefly with the valve open while disconnected from the bowl to avoid any possible cross contamination between samples.

Once your vortex unit is setup and the bulk sample has been sieved to retain the sediment in the 0.5 mm sieve, you are ready to run the sample.

1. Open the valve about ½ way and turn on the power to the pump.
   
The pump should not be left on with the valve closed as the hose may rupture.

2. Use the valve to adjust the flow as needed to ensure that water is not overflowing the outer edge of the bowl.

3. Add up to about 60 oz. of the sieved sediment to the bowl. The rubber spatula and wash bottle may be used to help add the sediment to the bowl.
   
   If you have more sediment you may need to divide the sample and repeat the process.

4. Once the sediment has been added, open the valve all the way, or until the water is about 1 to 2 cm from the edge of the bowl.
   
   It is common for the water level to drop after you add sediment due to the decreased water velocity caused by the rough surface of the sediment.
Sample processing

5. Using a sturdy plastic spoon, stir the sediment from the middle to the edge of the bowl by sliding the spoon down the edge of the cone, across the bottom of the bowl, then up the side.

A plastics spoon is preferred because it will not scratch the surface of the bowl. Scratches may affect the flow of water and may create areas where sediment or eggs could be trapped.

Move around the perimeter of the bowl as you stir while paying special attention to areas where the sediment has piled up or accumulated around the cone. This will help suspend eggs and ensure that they aren’t being buried under the sand.

6. Stir for 1 to 3 minutes, and then allow the bowl to run undisturbed for about 10 seconds before turning off the pump and closing the valve.

It is important to close the valve quickly after turning off the pump to minimize the amount of material that may be sucked back into the hose.

7. Once the water has settled, examine the sediment in the area immediately around the cone for eggs. If eggs are observed, skim them off with a spoon and add them to the sample jar.
Sample processing

8. Remove the blue bowl from the sieve and with the aid of a wash bottle, rinse the material captured by the sieve into a sample jar.

9. Once the material from the sieve is in the sample jar, strain off as much water as possible (being careful not to lose eggs), cover the sample material with preservative, and insert the appropriate sample label before securing the lid to the sample jar.

The sample is now ready for lab processing.
Notes for lab processing

The laboratory procedures described in the field manual by Moulton and Penttila (2006) describe the process of further winnowing and reducing the sample prior to analysis with a dissecting microscope.

We have found that the volume of material retained after processing with the vortex method is typically so small that no additional winnowing or reduction is necessary. Instead, the entire preserved sample can generally be inspected for eggs in a standard 10 cm petri dish in just two or three batches.

For samples with a high volume of material in the condensed sample, it may be appropriate to apply the additional condensing process described in the field manual laboratory procedures.
Additional Resources

For training, consultation, or more information about WDFW forage fish studies, please contact Phillip Dionne at: Phillip.Dionne@dfw.wa.gov; 360-902-2641

Sampling protocols, identification guides, maps and other materials are available online at: wdfw.wa.gov/conservation/research/projects/marine_beach_spawning/

Field Manual:
Parts vendors

The use of product brand names, images, vendor names and web addresses for the sources or descriptions of materials are included for convenience to aid in the identification of the materials used by WDFW in the development of these methods and do not represent an endorsement of the vendor or the product by the WDFW or its staff.


Blue bowl (includes hose valve): http://www.blackcatmining.com/mining-equipment/blue-bowl.cfm

750 – 1000 gph water pump: http://www.ebay.com/itm/Active-Aqua-Submersible-Water-Pumps-Aquarium-Reservoir-Fountain-Pond-Hydroponics-/111476699981

¾" flex hose: http://www.blackcatmining.com/mining-equipment/flex-hose.cfm

¾" quick-connect hose connection (with or without valve): http://www.amazon.com/Gilmour-2939Q-Premium-Complete-Quick-Connect-dp-B000E1AHVW
¾" male thread hose repair kit: http://www.tacomascrew.com/Products/Couplers-Connectors/Gilmour-01M-Garden-Hose-Repair-Ends?CAWELAID=12016860000024660&CAGPSPN=pla&catargetid=12016860000026509&cadevice=c&gclid=CKD8kczP6sYCFZJgfgod9PMKiw

0.5 mm sieve: https://www.fishersci.com/shop/products/fisherbrand-u-s-standard-stainless-steel-sieves-8-in-dia-2-in-d/0488110g
A 1/50 inch fine mesh sieve is an alternative: http://www.goldfeverprospecting.com/keclsc.html

Shims: http://www.homedepot.com/p/Unbranded-8-in-Composite-Shim-Bundle-of-12-SHM1-12-TW/202807695

Rubber spatula: http://www.amazon.com/Farberware-Color-Silicone-Spoon-Spatula/dp/B005GT01KE
Protocol FF-01

WDFW Intertidal Forage Fish Spawning Habitat Survey Protocols

Procedures for obtaining bulk beach substrate samples

Field materials needed:

- Measuring tape (100+ feet)
- 16-ounce plastic jar or large scoop
- 8 inch x 24 inch polyethylene bag (or large, sturdy ziplock)
- Handheld GPS device
- Tide table
- Digital camera (optional)
- Hypsometer (if available)
- Data sheet (preprint on Write-in-the-Rain paper if possible)

Note: Sampling should occur on the lowest tide practicable. Prior to sampling any site consult tide tables to ensure you will be able to access the +7-9 (surf smelt) and +5-8 (sandlance) tidal height. It may also be necessary to obtain permission to access the beach from private or corporate landowners.

Procedure:

1. Upon arriving on the beach, fill out the header information on the attached data sheet. Do not fill in “Reviewed by.” Before conducting the first sample, describe the character of the upland and beach environment using the codes provided on the back of the data sheet. For additional details on sample codes see Moulton and Penttila (2001)*.

2. Identify a landmark from which you will measure the distance to the bulk substrate sample tidal elevation. Typical landmarks include the upland toe of the beach, the last high tide mark or wrack line, and the edge of the water.

3. Measure the distance from the landmark to the tidal elevation to be surveyed. Note that linear measurements along the beach face serve as an index of tidal height but do not directly quantify vertical tidal height. If available, a hypsometer can be used to measure vertical sampling height.

4. Stretch a measuring tape at least 100 feet along the selected tidal height. Note that beach contours may cause the landmark to be ‘wavy’ and that the tape should remain a consistent distance from the landmark.

5. Standing at one end of the measuring tape, record a GPS fix on the data sheet.

Version 1.0, July 2011
6. Using a 16-ounce sample jar or large scoop remove the top 5-10 cm (2-4 in) of sediment from the location recorded in Step 6 above. Place the sediment in an 8 inch x 24 inch polyethylene bag or large, sturdy ziplock. You may need to take two scoops to get sufficient sediment, depending on the coarseness of the beach.

7. Walk ten paces (single steps) along the measuring tape, repeat the sediment scooping action, and place the sediment in the bag. Move an additional ten paces and repeat. Move an additional ten paces, approximately to the end of the tape, and repeat. The bag should now have sediment from four locations along the tape and be at least ½ to ¾ full.

8. If additional transects, representing various tidal heights, along the beach are to be surveyed, place the sample bag in a cool, shady place and repeat the above procedures at these additional locations. If no additional samples will be taken, move on to wet sieving and winnowing the sample as described in the companion protocol “Procedures for recovering “winnowed light fractions” subsamples of forage fish egg-sized material from bulk beach substrate samples.”

9. If you have a camera, take several photos of the survey area showing sampling locations. Be sure to take photos from several perspectives (i.e., both up and down, as well as along, the beach). For each photo, record the cardinal direction you are facing on the data sheet in the comments field.

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Original protocol by Dan Penttila. WDFW. Reformatted by Dayv Lowry. WDFW.

Version 1.0, July 2011
Forage Fish Spawning Surveys

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<th>Longitude (decimal degrees)</th>
<th>Beach</th>
<th>Uplands</th>
<th>Landmark</th>
<th>Sample</th>
<th>Zone</th>
<th>Tidal elevation</th>
<th>Smelt</th>
<th>Sand base</th>
<th>Rock sole</th>
<th>Herring</th>
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Samplers: ____________________

Version 2.0, July 2011
Field Observation Sampling Codes

**Beach**: Sediment character of the upper beach (particle size range in inches)
0 = mud (<0.0025)
1 = pure sand (0.0025-0.079)
2 = pea gravel (0.079-0.31, “fine gravel”) with sand base
3 = medium gravel (0.31-0.63) with sand base
4 = coarse gravel (0.63-2.5) with sand base
5 = cobble (2.5-10.1) with sand base
7 = boulder (>10.1) with sand base
8 = gravel to boulders without sand base
9 = rock, no habitat

**Tidal Elevation**: Determined in the office using location and time data provided.

**Smelt, Sand Lance, Rock Sole, Herring**: subjective field assessment of spawn intensity apparent to the naked eye:
0 = no eggs visible
1 = very light, sparse
2 = light, but apparent
3 = light medium, visible
4 = medium, readily visible
5 = medium heavy, abundant
6 = heavy, broadly abundant
7 = very heavy, widespread
8 = eggs observed in the winnow

**Uplands**: Character of the uplands (up to 1,000 ft from high water mark)
1 = natural, 0% impacted (no bulkhead, rip-rap, housing, etc.)
2 = 25% impacted
3 = 50% impacted
4 = 75% impacted
5 = 100% impacted

**Width**: Width of the potential spawning substrate band to the nearest foot. Judged by character of sediment and presence of spawn, when possible.

**Landmark**: landmark for determining sample zone where collection occurs
1 = down beach from last high tide mark
2 = up beach from last high tide mark
3 = down beach from second to last high tide mark
4 = down beach from upland toe
5 = up beach from waterline at the time noted

**Length**: Length of the beach up to 1,000 feet (500 feet on either side of the station). The value “C” may be assigned if surveyed beach is continuous with other potential sample sites.

**Shading**: Shading of spawning substrate zone, averaged over the 1,000 foot station and best interpretation for the entire day and season
1 = fully exposed
2 = 25% shaded
3 = 50% shaded
4 = 75% shaded
5 = 100% shaded

**Sample Zone**: Distance of sample zone transect parallel to the landmark, in feet to the nearest ½ foot. Used to determine the tidal elevation of the spawn deposit.

Version 2.0. July 2011
5. Tilt/swirl/agitate pan contents to move lighter material accumulated at center down to lower left corner of pan.

6. Carefully tilt pan to decant water to opposite corner of pan, slowly exposing lower left corner material above water’s surface.

7. Holding pan in the tilted position, carefully use a wide-mouthed sample jar to skim the surface 1 inch of material from the lower left corner of the deposit.

8. Repeat steps 4-7 approximately three more times, or until the sample jar is ~3/4 full of material.

9. If sample will not be analyzed within a few days in the laboratory, top-off sample jar with ethyl alcohol or Stockard’s solution† and shake well to distribute fluid. Note that long-term storage is also possible with these preservatives. If genetic samples are desired 95% non-denatured ethyl alcohol should be used.

10. Fit lid loosely onto sample jar to allow gas to escape (preserved samples will emit carbon dioxide as the acidic preservative dissolves shell material in the sample).

11. Store sample jars in leak-proof containers in well-ventilated area to prevent accumulation of carbon dioxide in enclosed areas. Note: both gas and some preservative, if present, will escape.

† Stockard’s solution contains formaldehyde, which is carcinogenic. 1 l Stockard’s solution = 50 ml formalin (37% aqueous formaldehyde), 40 ml glacial acetic acid, 60 ml glycerin, 850 ml fresh water (1 l = 0.2642 gal; 1 gal = 3.785 l).

Original protocol by Dan Penttila, WDFW. Reformatted by Dayv Lowry, WDFW.

Version 1.0, July 2011
WDFW Intertidal Forage Fish Spawning Habitat Survey Protocols

Procedures for recovering “winned light fractions” subsamples of forage fish egg-sized material from bulk beach substrate samples

Field materials needed:

Nested set of 4-mm, 2-mm, and 0.5-mm sieves/screens (Nalgene or stainless steel preferred over brass, for durability)

Buckets for discarded material (2-4), may have several large holes drilled near lip as rinse water outlets

1-2 gallon plastic dishpans

400-ml wide-mouthed sample jars

Freshwater hose work area with sufficient drainage (or extra buckets for saltwater rinsing)

Area to discard waste gravel

Ethyl alcohol or Stockard’s solution† (only needed when samples will not be analyzed immediately)

Pencil and Rite-in-the-Rain paper (cut into small squares for labeling samples)

Procedure:

1. Thoroughly wet-screen material through set of 4-mm, 2-mm, and 0.5-mm sieves/screens, using buckets of shore-side water at site or freshwater hose elsewhere. Screens should be carefully cleaned between samples.

2. Discard material retained in 4-mm and 2-mm sieves/screens.

3. Place material from 0.5-mm sieve/screen (“egg-sized material”) in rectangular dishpan and cover with ~1 inch of water.

4. Rotate/tilt/yaw dishpan of material to impart rotation to water and cause lighter material to rise to the surface, where it should accumulate toward the center of the pan. Observe behavior of shell fragments and organic particles to get indication of behavior of forage fish eggs.

Version 1.0, July 2011
WDFW Intertidal Forage Fish Spawning Habitat Survey Protocols

Laboratory procedure for determining forage fish egg presence/absence from preserved “winnowed light fraction” beach substrate samples

Laboratory materials needed:

Fume hood (alternatively, winnowed light fraction samples can be carefully washed before analysis)*
Latex or nitrile gloves*
Spoon
Oval microscope dish
Dissecting microscope with 10-20x power
Watchglasses/small Petri dishes
Fine-point (watchmakers) forceps
Data/tally sheets
Paper towels
Buckets/pans/sample jars (to collect waste, accumulated samples, etc.)

*Depending on the preservative used, samples may be toxic or carcinogenic. Take proper precautions.

Note: This procedure describes a second reduction of bulk substrate material collected during field sampling and is best used for determining spawn presence/absence. If detailed egg stage counts are needed, use the associated document “Laboratory procedure for counting and staging forage fish eggs.”

Procedure:

1. Stir “winnowed light fraction” sample jar contents with spoon.

2. Swirl jar in clockwise manner to impart rotation to fluid and surface layer of contents, causing light material to move to center of jar.

3. Carefully tilt jar. Slowly scoop center mound of light material with spoon into oval microscope dish.

4. Repeat steps 1-3 four times, accumulating about 400 grams of light material in microscope dish.
5. Add water to microscope dish. Swirl/tilt/yaw dish to suspend lightest material and concentrate it along feathered edge of the deposit in the dish.

6. Place dish on microscope stage. Inspect zone around feathered edge of deposit. Remove eggs to watchglass with forceps.

7. Reverse dish to redistribute sediment. Repeat steps 5-6 three more times, or until eggs cease to be detected around feathered edge of deposit. Species assignment may be made at this time or after completing processing (see attached egg identification guide).

8. If steps 1-7 produce zero eggs, or only a single egg, repeat the procedure with a second sample of material from the same jar of “winned light fraction.” The WDFW standard for documenting a spawning site for a given species is 2 eggs in a single “winned light fraction” sample.

9. Either preserve eggs for future counting and staging, or identify eggs in watchglass (see attached egg identification guide) to determine the species present.

10. Complete survey findings, as well as preserved egg samples if taken, should be sent to Dayv Lowry at Dayv.Lowry@dfw.wa.gov and/or WDFW, Habitat Program, 1111 Washington St SE, Olympia, WA 98501.

Original protocol by Dan Penttila, WDFW. Reformatted by Dayv Lowry, WDFW.
Forage Fish Eggs of Puget Sound

PACIFIC HERRING
almost entirely deposited on marine vegetation; distinct smell attachment sites; self-adhesive in layers or clumps.

SURF SMELT
single pedestal-like attachment site; non-self-adhesive; entirely in beach sediment particles.

PACIFIC SAND LANCE
relatively small; multiple sand grain attachment sites; egg off-round/milky; 1 large oil droplet in yolk.

ROCK SOLE
egg perfectly spherical; very clear; no visible attachment sites; non-self-adhesive.
WDFW Intertidal Forage Fish Spawning Habitat Survey Protocols

Laboratory procedure for counting and staging forage fish eggs obtained from processed “winnowed light fraction” field samples

Laboratory materials needed:

Petri dishes/measuring plates
Spoon
Balance or scale
Disposable pipette
Paper towels
Dissecting microscope with 10-20x power
Fine-point (watchmakers) forceps
Watchglasses
Data/Tally sheets

Note: This procedure describes the analysis of “winnowed light fraction” sediment samples and is best used for quantifying spawn abundance/intensity by species. If spawn presence/absence is needed, use the associated document “Laboratory procedure for determining forage fish egg presence/absence.”

Procedure:

1. Thoroughly mix the contents of the condensed “winnowed light fraction” sample obtained from field processing of bulk sediment samples. Place a Petri dish or measuring plate on a balance/scale and tare (i.e., zero) the device.

2. If preservative is present, pour off as much liquid as possible into the appropriate waste container and fill the Petri dish ~¼-½ full with sediment. Use a pipette to remove any residual preservative or other liquid then use a paper towel to blot the subsample dry. Record the weight.

3. Using a dissecting microscope and forceps, count and record the developmental stage of all eggs in the subsample, using the diagrams below. Eggs may be removed to a watchglass and separated by species (using diagrams below) prior to staging. Record counts on data sheet provided below.

4. Repeat steps 1-3 until all sediment in the sample jar has been examined. When counting and staging is complete, preserve the collected and separated eggs along with the entire sample, appropriately labeled with collection date, location, sampler, and other information.

Version 1.0, July 2011
5. Combine the weight of all sediment subsamples to obtain a total weight for the sample. Record this value in the comments field of the data sheet. This will be used to calculate egg density by species.

6. The abundance of sand lance, role sole, and other eggs is typically low enough that complete analysis of the “winnowed light fraction” can occur. For surf smelt subsampling may be required due to high spawn density. If this is the case, steps 1-3 should be repeated at least 3 times. The remaining “winnowed light fraction” sample must then have residual liquid poured off, be blotted dry, and be weighed. The total number of eggs in the original sample may then be estimated by dividing the combined weight of all subsamples by the total sample weight (remaining plus all subsamples), and then dividing the number of eggs in the combined subsamples by this value. Specifically:

\[
\frac{\text{Weight of combined subsamples}}{\text{Weight of total sample}} = \text{(decimal conversion factor)}
\]

then,

\[
\frac{\# \text{ eggs in combined subsamples}}{\text{(decimal conversion factor)}} = \# \text{ eggs in total sample}
\]

Example: From a wet “winnowed light fraction” sample you remove and dry three sediment subsamples weighing 10 g each. You count 200 eggs in the first subsample, 150 in the second, and 250 in the third. You then dry and weigh the remaining sediment in the sample jar and find it weighs 270 g. You have sampled 0.10 of the total sample:

\[
\frac{10+10+10}{10+10+10+270} = \frac{30}{300} = 0.10
\]

To get the number of eggs in the total sample, divide the number of eggs you counted (200+150+250 = 600) by 0.10 to get 6000 total eggs. The egg density is 20 eggs/g.

7. Complete survey findings, as well as preserved egg samples if retained, should be sent to Phillip Dionne at Phillip.Dionne@dfw.wa.gov and/or WDFW, Habitat Program, 1111 Washington St SE, Olympia, WA 98501.

Original protocol by Doris Small, WDFW. Reformatted by Dayv Lowry, WDFW.

Version 1.0, July 2011
Forage Fish Eggs of Puget Sound

PACIFIC HERRING
almost entirely deposited on marine vegetation; distinct shell attachment sites; self-adhesive in layers or clumps.

SURF SMELT
single pedestal-like attachment site; non-self-adhesive; entirely in beach sediment particles.

PACIFIC SAND LANCE
relatively small; multiple sand grain attachment sites; egg off-round/milky; 1 large oil droplet in yolk.

ROCK SOLE
egg perfectly spherical; very clear; no visible attachment sites; non-self-adhesive.
Embryonic Development Stages – Pacific herring

Embryonic Developmental Stages of the Herring

Times are approximate, since the rate of development is greatly dependent on temperature.
Embryonic Development Stages – Surf smelt

- Unfertilised egg
- 1 cell: 1/2 hour
- 2 cells: 1 hour
- 4 cells: 3 hours
- Early blastula: 5 hours
- Late blastula: 6 hours
- Yolk plug (gastrula): 1 1/2 hours
- 1 1/4 days
- 4 days
- 6 days (1 1/2 cell)
- 13 - 14 days (just prior to hatching)

Newly Hatched Larva
Surf Smelt Embryological Stage Categories

Two-week Summer Incubation Time line

1-5 Hours

6-12 Hours

14-20 Hours

1-2 Days

3-5 Days

6-7 Days

10 Days

13-14 Days

"Dead"

"1-CELL-MORULA": very fresh eggs, 1-cell to roughly 30 cells

"BLASTULA": granular-caps through start of gastrulation

"GASTRULA": yolk-plug stage through start of neurulation

"ONE-HALF COIL": distinct notochord axis to 3/4 coil embryo

"ONE-COIL": nose nearly to tail tip to 1-1/4 coil, more or less, eyes white

"ONE AND ONE-HALF COIL": more or less, preserved eyes gray

"> ONE AND ONE-HALF COIL": to 2+ coil, preserved eyes black to slightly metallic

"LATE EYED": preserved eyes metallic, ventral gut spots are dashes, tight fit in shell, includes loose larvae hatched during preservation

Opaque white
Indiscernible embryo
Fungus covered
Collapsed
Empty
Identification Guide to Larval Forage Fishes of Puget Sound

Pacific herring, Clupea pallasii
1. Head-vent distance about 90% of standard length
2. Ventral chromatophores in two parallel rows, with a distinct break to closer spacing, with no overlap, about the middle of the gut
3. About 40 myomeres between the pectoral fins and vent
4. Yolk sac immediately behind pectoral fins, unpigmented

Surf smelt, Hypomesus pretiosus
1. Head-vent distance about 80% of standard length, 17-20 ventral gut spots
2. Ventral chromatophores in a single row, with 2 parallel rows above it on the anterior ¼ of the gut
3. About 50 myomeres between the pectoral fins and vent
4. Yolk sac markedly behind pectoral fins, ventral surface with many tiny chromatophores

Pacific sand lance, Ammodytes hexapterus
1. Head-vent distance about 60% of standard length
2. Ventral chromatophores in two parallel continuous rows, becoming very closely spaced posterior of vent
3. About 35 myomeres between the pectoral fins and vent
4. Yolk sac immediately behind pectoral fins, ventral line with 3 chromatophores

All figures approximately 15 times natural size.
Larval Forage Fishes of Puget Sound

Pacific herring, *Clupea pallasii*
- Row of chromatophores on either side of gut, with distinct break to closer spacing along posterior half of gut; no overlapping

Surf smelt, *Hypomesus pretiosus*
- Row of chromatophores on either side of gut, overlapping with single row of chromatophores on ventral midline of gut
- Rayless adipose fin present

Pacific sand lance, *Ammodytes hexapterus*
- Widely spaced row of chromatophores on either side of gut
- Wide membranous fins continuous on dorsal and ventral midlines, fin rays appearing posterior to anus

All figures approximately 10-12 times natural size.
Identification of Post-Larval Forage Fishes of Puget Sound

Pacific herring, *Clupea pallasii*
- Insertion of dorsal fin posterior to pelvic fins
- Rows of ventral chromatophores distinct only anterior to pelvic fins

Surf smelt, *Hypomesus pretiosus*
- Insertion of dorsal fin at or slightly anterior to pelvic fins
- Rows of ventral chromatophores very distinct along entire gut, interrupted at pelvic fins
- Distinct, rayless adipose fin present

Northern Anchovy, *Engraulis mordax*
- Insertion of dorsal fin posterior to pelvic fins
- Rows of ventral chromatophores continuous along entire gut
- Mouth large, subterminal with overhanging upper jaw

Pacific sand lance, *Ammodytes hexapterus*
- Dorsal fin continuous from pectoral fins to caudal peduncle
- General body form long, slender, with snake-like swimming motions
- Head pointed with distinctly jutting lower jaw
- Pelvic fins absent

Fishes of this stage are 30-35 mm in standard length and semi-transparent. All figures are pictured 3-4 times their natural size.
# Forage Fish Spawn Sample Analysis

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<th>Sample Location</th>
<th>Day</th>
<th>Month</th>
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<th>Beach Number</th>
<th>Sample Number</th>
<th>Species</th>
<th>1 cell to morula</th>
<th>Blastula</th>
<th>Gastrula</th>
<th>1/2 - 1 coil</th>
<th>1 coil</th>
<th>1 1/2 coil</th>
<th>&gt;1 1/2 coil</th>
<th>Late eyed</th>
<th>Dead</th>
<th># Eggs</th>
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<th>Est. # broods</th>
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Version 1.0, July 2011