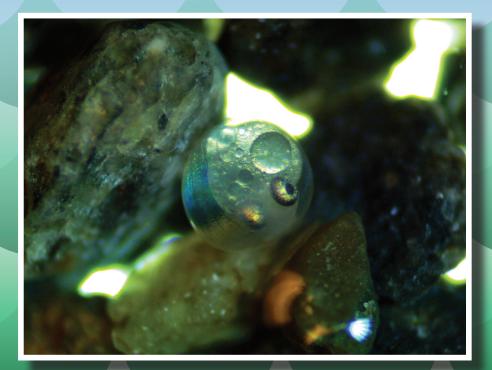
January 2015

FPA 15-01

Summary of Coastal Intertidal Forage Fish Spawning Surveys: October 2012 – October 2014

by Mariko Langness, Phillip Dionne, Daniel Masello, and Dayy Lowry





Washington Department of FISH AND WILDLIFE Fish Program Fish Management Division

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Washington Department of Natural Resources 1111 Washington St. SE, Olympia, WA, 98501 Agreement No. IAA 13-147

Fish Program Report Number FPA 15-01

Acknowledgments

Data collection for this project was a collaborative effort overseen by WDFW personnel and biologists from four tribal governments: Joe Gilbertson (Hoh Tribe), Jennifer Hagen (Quileute Tribe), Alan Sarich (Quinault Nation), and Joe Petersen (Makah Tribe). In addition to these individuals, several other staff contributed to data collection and sample analysis, including Bernard Afterbuffalo, Matt Bowen, Erin Dilworth, Ivan Eastman, Zac Espinoza, Rio Foster, Joe Garrick, Ben Gilles, Joe Gonce, Ruben Hernandez, Kristen Hinton, Colin Jones, Tyler Jurasin, Kira Kranzler, Joe LaShier, Russell Markishtum, Scott Mazzone, Graywolf Nattinger, Mario Reyes, Greg Urata, Brian Walker, Nick Ward, Alex Wise. Project oversight and supervision was provided by Lorna Wargo and Brad Speidel, mapping support was provided by Andy Weiss and Dale Gombert, and the statistical sampling design was developed by Kirk Krueger. Brian Benson and Marc McCalmon designed and managed the statewide intertidal forage fish database and were integral in implementing the integration of data from this study.



Abstract

Marine spatial planning (MSP) involves the identification and mapping of marine resources and human interactions with these resources, the weighing of costs and benefits to diverse stakeholders, and the development of long-term utilization plans. As part of a coast-wide MSP process funded by the Washington State Legislature, the Washington Department of Fish and Wildlife (WDFW), in collaboration with the Hoh, Makah, Quileute Indian Tribes, and Quinault Indian Nation, conducted a 24-month survey in an effort to document the presence of eggs deposited by forage fishes spawning in the intertidal. From October 2012 through October 2014, beaches along the Washington outer coast were surveyed for Surf Smelt Hypomesus pretiosus, Night Smelt Spirinchus starksi, and Pacific Sand Lance Ammodytes hexapterus spawn. The specific goals of the study were to: 1) subsample the breadth of intertidal reaches along Washington's outer coast monthly; 2) identify forage fish eggs found to the lowest taxonomic level possible; and 3) geo-reference all survey data to provide an easily accessible overview of sampling effort and egg detections to date. The results for year-one of this study are reported elsewhere (Langness et al. 2014) and the results for year-two are presented in detail here. Yeartwo results were integrated with year-one survey data to provide a comprehensive, two-year evaluation. Over the two-year survey period, we sampled 89% of possible spawning habitat on the outer coast, and documented 40 spawning sites. Over the 13-month survey from October 2013 to October 2014, 761 sites were allocated, and 654 (86%) were sampled. Smelt eggs were present at 32 of these sites, while eggs of any species were not detected at the remaining 622 sites. Of the sites where smelt spawn was present, samples collected from 20 of the sites met the WDFW standard of containing a minimum of 2 eggs. Ten of these 20 sites are newly documented spawning sites. Spawn was documented in each month from January through October, one month earlier and one month later than suggested by our prior survey year (Langness et al. 2014). The number of documented spawning sites peaked in May and the number of eggs peaked in September. The geographical range of spawning sites remained clustered within the Quinault and Kalaloch-Hoh-Quileute beach zones; ranging as far south as site 365 (south of the Quinault River) and as far north as site 555 (south of Goodman Creek). The recurring presence of eggs at different sites and the presence of multiple egg stages at a single site indicate that several spawning events occurred during the season. We expect that further sampling would identify a broader spatial and temporal range of smelt spawning along the outer coast. Sampling over multiple seasons would likely increase egg detections as some sites may have only limited use on a seasonal or annual basis. Continued sampling could also provide the opportunity to improve methods that would enable a higher detection probability and greater efficiency in sampling. As our comprehensive study allowed us to determine the areas of spawning on the outer coast, focused sampling on spawning beaches in the Quinault and Kalaloch-Hoh-Quil beach zones is suggested for any future research efforts.

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The process of Marine Spatial Planning (MSP) has developed over the past ten years to bring together stakeholders from diverse sectors that make use of the ocean, including governments, fishing and energy industries, conservationists, landowners, and recreationists, in order to identify, map, and allow for effective long-term utilization of the marine environment (Douvere 2008). Ultimately, this process is intended to minimize conflicts among sectors by spatiotemporally parsing both consumptive and nonconsumptive exploitation of the environment in such a way that the needs of all parties are met. Where contentious issues centering on incompatible activities arise, the MSP process acts as a mechanism by which competing uses can be weighed, the impact of trade-offs identified, and a data-driven compromise made (Douvere 2008; Lester et al. 2013; Samhouri and Levin 2012). In some cases, this optimized planning process has been shown to benefit numerous sectors in complex ways, such as increasing fishery profits by excluding fishing in target regions (e.g., Marine Protected Areas) while at the same time increasing ecotourism opportunities (Rassweiler et al. 2012).

As part of the first phase of a coast-wide MSP process funded by the Washington State Legislature and administered by the Washington State Department of Natural Resources (WDNR), the Washington Department of Fish and Wildlife (WDFW) was contracted to conduct surveys for eggs deposited by forage fishes that spawn in the intertidal (Surf Smelt *Hypomesus pretiosus*, Night Smelt *Spirinchus starksi*, and Pacific Sand Lance *Ammodytes hexapterus*) along the Washington coast from the mouth of the Columbia River north to Cape Flattery. Knowledge of these species is critical because of the role they play as mid-level prey in the marine food web (Penttila 2007; Simenstad et al. 1979) and because they are harvested for subsistence, as well as exploited recreationally and commercially (smelt only) by fishers in Washington. Due to the local knowledge of smelt fisheries possessed by coastal Indian Tribes, and their role as comanagers of the natural resources of Washington State, surveys were collaboratively conducted with members and employees of the Hoh, Makah, Quileute Indian Tribes, and Quinault Indian Nation.

WDFW and its collaborators have collected extensive data on the location and timing of smelt and Sand Lance spawning in Puget Sound over the past 35 years (Penttila 1995, 2000, 2007; Quinn et al. 2012), including the strait of Juan de Fuca (Shaffer et al. 2003), however a comparative paucity of effort has been expended along the outer coast. Sampling in Puget Sound has also identified seasonal and tide height-specific patterns in spawning distribution and a variety of targeted studies have further identified key environmental parameters associated with use of beaches for spawning, and egg survival (de Graaf 2008; Penttila 2001a, 2001b; Quinn et al. 2012; Rice 2006). As a result of these surveys and associated conservation efforts, the Hydraulic Code Rules of the Washington Administrative Code (WAC220-110) recognize intertidal forage fish habitat as a Saltwater Habitat of Special Concern and provide for a "no net loss" provision to protect these habitats. Additionally in order to protect both spawning adults and the eggs on the beach, certain seasonal windows have been designated "prohibited work times" (WAC220-110-271). A lack of knowledge about spawn timing and distribution along the outer coast has prevented the setting of prohibited work times relevant to intertidally spawning forage fish outside of Puget Sound.

The intertidal habitats in Puget Sound are generally less exposed to high-energy wave regimes, especially during winter storms, and typically vary substantially from those along the outer Washington coast. In accordance with traditional tribal knowledge of smelt occurrence along the outer coast, a handful of beach surveys conducted from 1994-1998 identified five spawning areas utilized by forage fish, one of which is inside Grays Harbor (WDFW, unpublished data). In addition to the sites identified by WDFW, Surf Smelt spawning is well known from Rialto Beach at the mouth of the Quillayute River, which has resulted in additional study of this locality because of the U.S. Army Corps of Engineers' potential use of the site to dump dredge spoils (ICF International 2010). Additional surveys have been conducted along the shoreline of the Olympic National Park by Park staff (Steve Fradkin, pers. comm.), but only data through the year 2000 have been made widely available (Fradkin 2001). Because so few locations have been sampled for forage fish spawning activity on the outer coast, the temporal, spatial, and environmental spawning preferences of these species is not well understood, and not accounted for in management planning activities.

Our first year survey (October 2012 – September 2013) provided essential data as to where and when smelt spawn on the outer coast (Langness et al. 2014). Following the same comprehensive sampling strategy, surveys continued monthly through October 2014. The second year survey remained largely exploratory in nature, and the specific goals of the study were to: 1) subsample the breadth of the outer coast monthly from October 2013 through October 2014; 2) identify forage fish eggs found to the lowest taxonomic level possible; and 3) geo-reference all survey data to provide an easily accessible overview of sampling effort and egg detections to date for use in MSP activities, and to guide future survey efforts. The sampling design was constructed to allow use of an occupancy model to predict the likelihood of finding eggs. The number of sites with documented spawn was low relative to the total number of sites sampled, and so model development has been delayed until a more robust data set is available.

Study Area and Design

Sampling sites were established along the Washington outer coast shoreline, from the Columbia River North Jetty to Cape Flattery, using a stratified random design. The shoreline (158 miles) was separated into 35 sampling "beaches" identified as "semi-exposed cobble-mixed coarse" and "exposed sandy" beach types based on WDNR ShoreZone line feature GIS data, and defined by breaks due to large estuaries (Willapa Bay or Grays Harbor), smaller estuaries and river mouths, or rocky headlands (Fig. 1). Extensive forage fish spawning surveys in Puget Sound (Penttila 1995, 2000, 2007), suggest that the chosen beach types have the potential to support spawning of Surf Smelt, Night Smelt, and Sand Lance. Each sampling "beach" was then subdivided into equal 1000-ft. long beach segments/sites (831 total), which is the current and historic mapping and sampling convention used by WDFW in Puget Sound, and assigned sequential beach segment ID/site numbers (Fig. 2). This site length allows sampling protocols to account for pocket beaches and heterogeneity in spawning environment without requiring sampling on a logistically unmanageable scale. "Beach zones" or "sampling regions" were created by an arbitrary grouping of beach segments into logistical sampling strata that roughly followed ownership or management of the land. Beach zones were named as follows: Long Beach, Twin Harbors, Copalis-Moclips, Quinault, Kalaloch-Hoh-Quil, and NW Coast.

For the second year survey, a new random draw of sites (831 sites) was allocated over a 12 month period, November 2013 through October 2014 (21-70 sites/month). October 2013 had 83 sites allocated from the previous 6 month period, May 2013 through October 2013. Due to safety concerns, sampling effort was reduced to 25% per beach zone for winter surveys, November 2013 to January 2014 (21 sites/month). With reduced winter sampling effort, 761 sites in total were allocated over the report period, October 2013 through October 2014. As sites could potentially be resampled, sampling coordinates for November 2013 through October 2014 were shifted 250 ft. north of the beach segment center. Beach segment south point coordinates were used for October 2013 sites. Sites were sampled by WDFW, Quinault, Hoh, Quileute, and Makah staff, based on ownership, management, or ease of access to the land where sites were located.

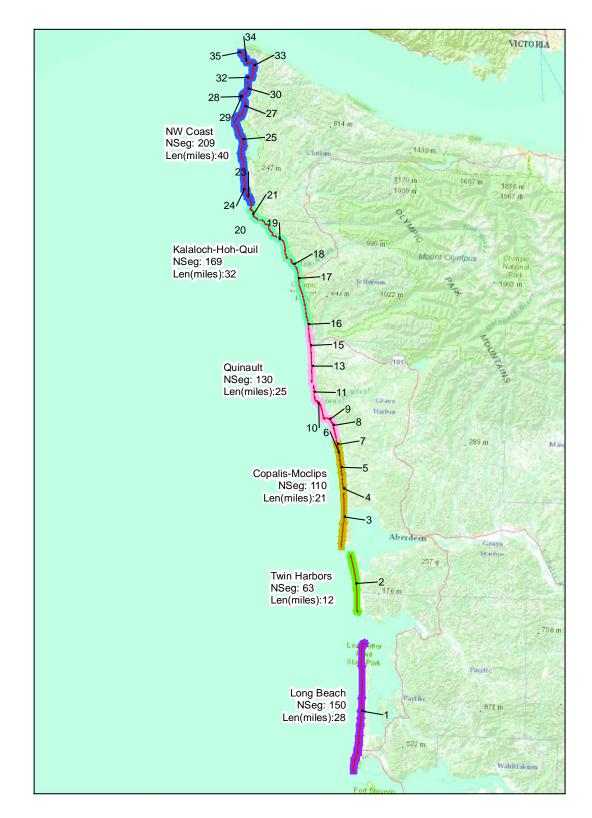


Figure 1. Study area along Washington outer coast, showing 6 defined "beach zones" (Long Beach, Twin Harbors, Copalis-Moclips, Quinault, Kalaloch-Hoh-Quil, and NW Coast) and 35 sampling "beaches".

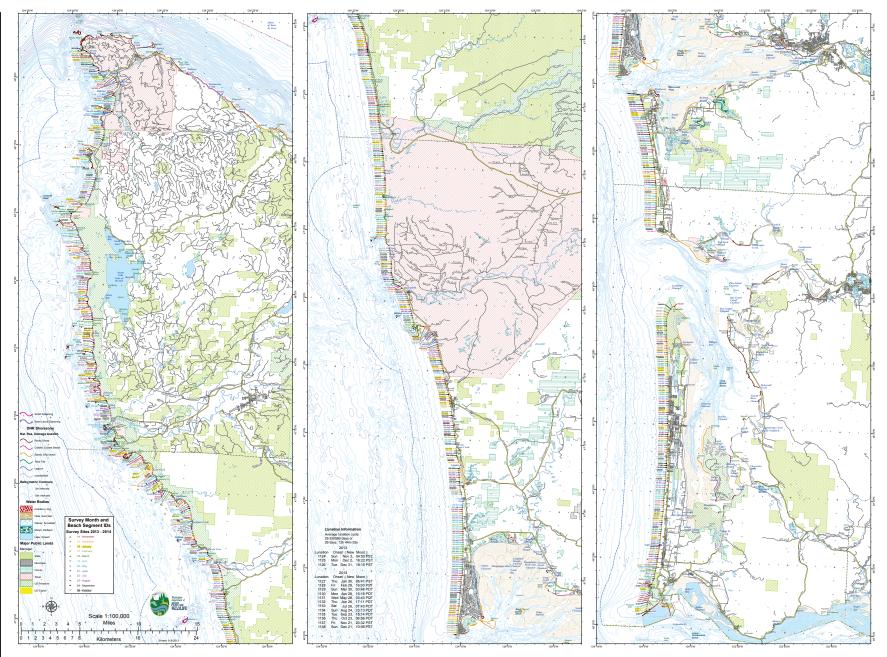


Figure 2. Planned sampling sites from November 2013 to October 2014. October 2013 sites are mapped on the prior survey year map. Visit wdfw.wa.gov to down-load report FPA 14-01.

Sampling Approach

Sample Collection

Sampling occurred monthly and represented a continuation of sampling that began 16 October 2012. Samples collected beginning the week of 1 October 2013 and ending 31 October 2014 (13 sampling months) are reported in detail here. Within a month, days during or after the highest tides and with the broadest temporal sampling windows were chosen. There is evidence from Puget Sound surveys that Surf Smelt and Pacific Sand Lance spawn during high tide events; the highest densities of deposited eggs for both species has been found to overlap in the upper third of the intertidal range (local equivalent of +7 MLLW to MHHW) (Moulton and Penttila 2006; Penttila 1978, 1995). Therefore, we aimed to sample on days that would allow for access near the upper tidal limit for an extended period of time, maximizing collection capacity for a given date.

Estimation of the upper third of the daily high tide range was determined using NOAA tide prediction charts (Fig. 3). Using these charts we were able to determine the approximate time at which only the upper third of the beach (~+6 MLLW to daily high tide) was exposed. If possible, we arrived at the site at this time, sampling from the high tide mark down to the water's edge. This allowed us to take a linear measurement of the beach face as an index of tidal height, and for use as an estimate of the upper third of the beach for that particular sampling day and location. This method was particularly effective for estimating the upper third of broad, flat, sandy beach sites at Long Beach, Twin Harbors, and Copalis-Moclips (Fig. 4A). At steep, cobble-course beaches (Fig. 4B), the linear distance of the upper third was shorter, and often sampling occurred from the upland toe or log line (if high tide mark unidentifiable) down to the estimated lower edge of the upper third of the exposed intertidal area.

This study used a variant of the bulk beach substrate sampling protocol used for spawning beach surveys in Puget Sound, standardized in the late 1990s by Dan Penttila and later codified into a manual (Moulton and Penttila 2006; Penttila 1995). The only major deviation from this standard protocol was that sediment samples were taken perpendicular to the beach face rather than parallel to the high tide line (Appendix; Protocol FF-01-C). This allowed us to survey the entire upper third of the recent tidal range in a single sample, circumventing a lack of knowledge about the specific tidal height at which eggs are deposited on beaches along the outer coast.

The modified protocol has since been further augmented to accommodate specific circumstances encountered only on the outer coast. Specific changes include: 1) addressing that a range of beach sediment particle sizes may be encountered within the upper third of the tidal range (unlike Puget Sound where sampling occurs at a known tidal elevation and band of similar sediment character); and 2) rewording the meaning of the "width" and "sample zone" data fields, with width representing the width from the "upper most" to "lower most" scoop on a transect, and sample zone representing the distance to the lowest sample scoop of a transect taken perpendicular to a landmark (Appendix; Field Data Sheet). For most sampling sites, the width and sample zone are the same distance unless extra samples are taken in the lower 2/3 of the tidal range (procedures for extra samples of the lower 2/3 of the beach are further detailed below). In addition, many of the landmark codes have been eliminated since they did not apply well to coastal sampling. Only two landmark codes are used: 1 – down beach from high tide mark, and 2 – down beach from upland toe.

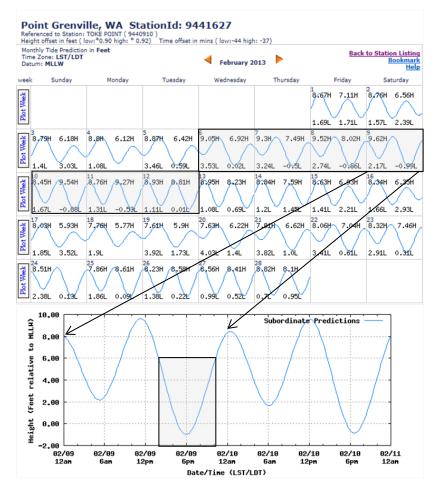


Figure 3. February 2013 tide chart of NOAA central coast site Point Grenville, WA. Highlighted days are preferable sampling days, allowing for access to the upper third of the beach for an extended period of time. On February 9, the time range is highlighted showing a potential 8 hour window for sampling between 2pm and 10pm.

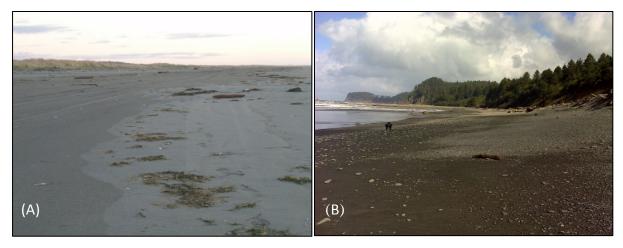


Figure 4. (A) Copalis Beach, a flat, broad, exposed, sandy beach type, showing high tide mark/wrack line; (B) Rialto Beach, a steep, semi-exposed, cobble-mixed course beach type.

The sampling sites were located using provided beach segment coordinates from WDFW GIS data. Upon arrival at the provided geographic latitude and longitude, the last high tide mark or wrack line was identified, and recorded as the actual longitude coordinate (due to the north to south orientation of the outer coast, there was no need to adjust the latitude coordinates). Pertinent habitat data were recorded, including the sediment character (particle size range), character of the uplands, and shading of the spawning substrate zone. Additionally, a subjective field assessment of spawn intensity apparent to the naked eye was conducted. When possible, photos were taken of the survey area at the site center facing each cardinal direction. The time of collection for each subsample was recorded and allowed us to determine tidal height with NOAA verified historic tide data (parameters: 6 minute water level intervals, MLLW, feet, LST/LDT) from the nearest harmonic tide sites on the outer coast (sites: Toke Point, Westport, and LaPush).

At each sampling site, three bulk sediment samples were collected; at the site center (monthly sampling coordinates) of the beach segment, 100 ft. north of the center, and 100 ft. south of the center. For each bulk sediment sample, four evenly spaced scoops of sediment were collected within the estimated upper third of the tidal range. The first scoop was collected at the high tide mark and the fourth at the lower edge (water side) of the upper third (Fig. 4A; Fig. 5). Each scoop was collected using a 16 oz. sample jar or large scoop to remove the top 2-4 in (5-10 cm) of sediment and placed in a plastic bag for later wet sieving and winnowing. Collecting three samples in this manner allows for evaluation of the intrasample egg detection rate at each beach where eggs are found in at least one sample. If sample sizes eventually become sufficient to allow formulation of an occupancy model, this ability to determine the occurrence rate of false negatives will become significant.

When time and tides permitted, extra samples were taken in the lower two-thirds of the daily tidal range. During low tide, four additional evenly spaced scoops were taken below the lower edge of the upper third down to the edge of the water (Fig. 5). These extra samples were collected to determine if eggs could be detected in the lower elevations of the beach and because the gentle slope of southern beaches often made determining the exact extent of the upper third of the intertidal zone difficult.

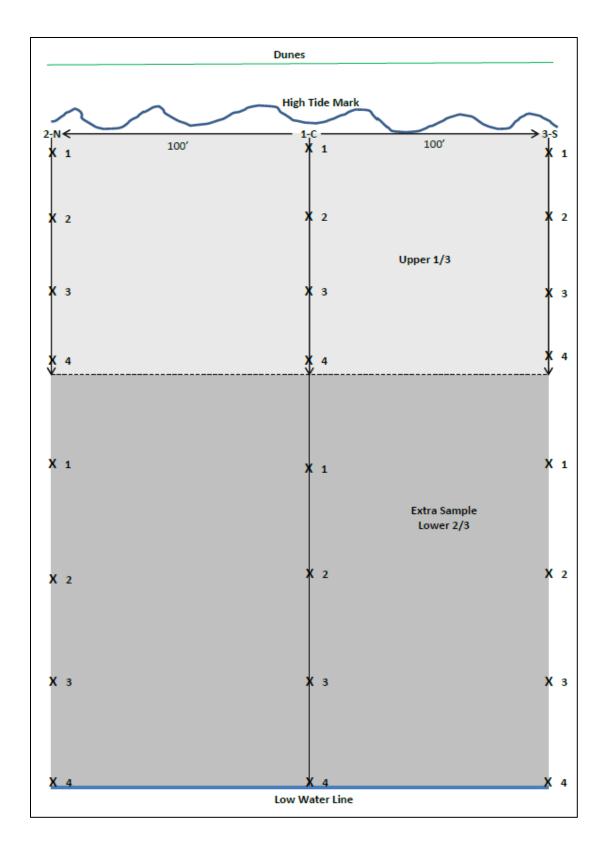


Figure 5. Sampling diagram. 1 - C = Sample 1 taken at monthly site coordinates, 2 - N = Sample 2 taken 100 ft. north of center, 3 - S = Sample 3 taken 100 ft. south of center. 1, 2, 3, 4 = scooped sediment.

Sample Processing

Bulk beach substrate samples were condensed in the field or lab to remove most of the sand and reduce the volume of sediment following Moulton and Penttila (2006) (Appendix: Protocol FF-02). The bulk sediment sample was run through a set of nested 4-mm, 2-mm, and 0.5-mm sieves, using buckets of shore water in the field or freshwater from a sink/hose setup in a lab. Materials from the 4-mm and 2-mm sieves were discarded and material from the 0.5-mm sieve (egg-sized material) was placed into a rectangular dishpan and covered with 1-2 in. of water. Eggs were then winnowed to the surface by swirling, rocking, and bouncing the dishpan for 1-2 minutes. Light material accumulated toward the center of the pan and was then worked to one corner. Tipping the pan, water was slowly drained away, drying and exposing the lighter fraction, which was skimmed from the surface using a spoon and placed into an 8 oz. jar. This winnowing process was repeated twice, or until the sample jar was roughly two-thirds full, completing a "winnowed light fraction sample" (Fig. 6). Samples were stored in a refrigerator for up to two weeks and, if left unexamined for eggs, preserved in 200 proof (90.48%) denatured ethanol. For sites within the Long Beach, Twin Harbors, and Copalis-Moclips beach zones, we maximized field collection on a given day by collecting bulk sediment samples and bringing them back to the lab for storage in a refrigerator or outside in a cool, shaded environment. These samples were condensed, and examined or preserved, within two weeks.



Figure 6. Sieving and winnowing process. Numbers to the lower left of each frame indicate the sequential process of sieving and washing (1-4), agitating (5), and winnowing the light fraction (6-8).

Winnowed light fraction samples were examined for forage fish egg presence/absence using the adapted Puget Sound forage fish egg presence/absence laboratory protocol, with the WDFW standard for documenting a spawning site for a given species at 2+ eggs (live or dead) per single "winnowed light fraction" sample (Appendix; Protocol FF-03). However, the standard for documenting a spawning site was altered so that for a given species 2+ eggs (live or dead) could be found in any of the three "winnowed light fraction" subsamples at a single site. This alteration was made in response to the large difference in the scale of the beaches between the outer coast and Puget Sound, and because we sampled at randomly selected sites and predetermined elevations, rather than selecting choice sites and elevations as has been the norm in Puget Sound. Winnowed light fraction samples were analyzed by scooping the evenly mixed sediment into a glass petri dish and thoroughly examined for eggs using a dissecting microscope with 10-20x power.

The abundance of forage fish eggs in all the collected samples was low enough so that complete analysis of the entire winnowed light fraction occurred. However, there was the option to subsample in cases of high spawn density. Up to half of the sample could be subsampled. All eggs found were removed and, if time permitted, the development stage of smelt eggs was determined using embryological stage categories created by Dan Penttila (Appendix; Protocol FF-04). The determined egg stages may not be an accurate reflection of the stages that were on the beach at the time of collection, as it is likely that eggs continued to develop or perish in the time between collection and preservation. All eggs were archived for potential future genetic testing.

Over the two-year survey period (October 2012 to October 2014), we directly sampled 89% of possible spawning habitat on the outer coast. Of the 831 segments/sites that make up the sampling universe, we sampled 743 at least once.

Of the 761 total planned beach sites from October 2013 to October 2014, 654 (86%) were sampled. Monthly sampling percentages ranged from 72-97% (Table 1). Further detail on the total number of sites sampled per month by collaborating entity, and overall sampling percentages, are provided in Table 1.

Of the 654 sites sampled, 30 involved collections outside the boundaries of the planned sites. Sampling outside of an allocated site occurred due to limited time to reach the site (i.e., tide was coming in and sampled nearest location) or inaccuracy in locating sites via GPS.

The loss of planned sampling was primarily due to difficult site access in parts of the Quinault, Kalaloch-Hoh-Quil, and NW Coast beach zones. The NW Coast beach zone was the least sampled, with 75% of planned sites sampled for the year (Table 2). Additional sampling percentages per year and month by beach zone are provided in Table 2. Sites located north of Johnson Creek up to Yellow Banks–Ozette were especially challenging to reach, particularly north of Cape Johnson and the area south of Yellow Banks to Norwegian Memorial. Poor weather conditions also reduced overall sampling efforts due to safety concerns, especially in remote locations. Sites that fell directly on a rocky headland (North Head or Taylor Point) were not sampled due to unsuitable habitat not identified by the GIS data layers. Additionally, stream outflows would sometimes be impassible and access to sites prevented or limited by these barriers.

Month	WDFW	Quinault	Hoh	Quileute	Makah	Total Sampled	Percent Sampled
October 2013	33	11	2	18	6	70	84%
November	10	3	2	2	3	20	95%
December	9	3	0	2	3	17	81%
January	9	3	3	2	3	20	95%
February	25	7	10	4	5	51	73%
March	28	9	11	6	10	64	93%
April	29	8	12	10	4	63	91%
May	26	10	8	10	7	61	90%
June	27	10	11	8	9	65	96%
July	26	10	10	13	7	66	97%
August	25	10	11	11	ND	57	84%
September	23	8	10	11	ND	52	76%
October 2014	26	9	4	9	ND	48	72%
Year 2 Total	296	101	94	106	57	654	86%

Table 1. Total sites sampled per month by collaborating entity, and overall sampling percentages. ND = No Data (data was collected but not received).

Table 2. Sampling percentages per month by beach zones; Long Beach, Twin Harbors, Copalis-Moclips, Quinault, Kalaloch-Hoh-Quil, and NW Coast.

Month	Long Beach	Twin Harbors	Copalis-Moclips	Quinault	Kalaloch-Hoh-Quil	NW Coast
October 2013	100%	100%	100%	85%	47%	90%
November	100%	100%	100%	100%	75%	100%
December	100%	100%	100%	100%	0%	100%
January	100%	100%	100%	100%	75%	100%
February	92%	100%	89%	64%	86%	39%
March	100%	100%	100%	82%	100%	82%
April	100%	100%	100%	73%	100%	82%
May	100%	100%	100%	91%	100%	65%
June	100%	100%	100%	91%	92%	94%
July	100%	100%	100%	91%	100%	94%
August	100%	100%	100%	91%	86%	59%
September	92%	100%	78%	73%	86%	53%
October 2014	100%	100%	100%	90%	71%	18%
Year 2 Total	99%	100%	97%	87%	78%	75%

Over the two year survey period (October 2012 to October 2014), 1489 planned sites were sampled and 40 spawning sites were documented (i.e., met the WDFW 2+egg standard) (Fig. 7). Of the 654 sites sampled in the second year survey, smelt eggs were detected at 32 sites, and were absent from the remaining 622 sites. Twenty of the 32 "smelt positive" sites met the WDFW 2+egg standard to document as a spawning site (Fig. 8). The 12 remaining "single egg" sites were detected in January, February, March, May, July, and August (Fig. 8, Table 4). Because Surf Smelt and Night Smelt eggs cannot be distinguished morphologically, the species of smelt spawning at these beaches cannot be definitively stated. Eggs were retained for potential future species identification using genetic tools.

Forage fish spawning was detected monthly starting in January and ending in October (Fig. 8). In January, one site was documented as a spawning site in the Kalaloch region south of Steamboat Creek. In February, three sites were documented, two in the Kalaloch region and one on the Hoh Shoreline. In March, two sites were documented, one in the Kalaloch region, and one on the Hoh Shoreline. In April, two sites were documented, one at Ruby Beach, and one north of Whale Creek. May had the greatest number of documented spawning sites for the survey year. Six sites were documented, two south of the Quinault River, one between the Queets River and Whale Creek, one at Browns Point Kalaloch, one near the mouth of the Hoh River, and one between Goodman Creek and Mosquito Creek. In June, three sites were documented, one near the Queets River documented; one in the Kalaloch region between Browns Point and Steamboat Creek and one near the mouth of the Hoh River. In August, one site was documented between the Quinault River and Point Grenville. In September, four sites were documented, two in the Kalaloch region near Browns Point, and two on the Hoh Shoreline. In October, one site was documented near Raft River (Fig. 8).

In addition to determining egg presence, several of the eggs were further examined to determine the development stage of the embryo using standardized stage categories (Moulton and Penttila 2006, and see Appendices). Table 3 further details the documented (2+ egg) spawning sites, number of samples with smelt eggs, total number of smelt eggs at each site, and smelt egg stage/condition. Table 4 details single egg sites, general location, and stage/condition if determined. September had the highest total egg count of any spawning month, with 142 eggs found. June and July also had higher total egg counts, with 137 eggs in June and 138 in July. Live egg counts were also greatest in September with 88 live eggs. June and July had lower live egg counts, with 7 live eggs in June and 55 in July.

Ten of the 20 sites where eggs were found are newly documented spawning sites, i.e., previously undocumented in the first year of this survey or by any prior work. Six of the new sites fall near (within 5 beach segments) previously documented sites, expanding these general spawning

areas/clusters further north or south. However, four sites fall outside of these areas, including site 442 near the Queets River, site 508 near Ruby Beach, site 537 north of Hoh Head, and site 555 between Goodman Creek and Mosquito Creek (Fig. 8). The temporal range of spawning broadened for the ten previously documented sites in the second year survey; and all sites were re-documented in new months (Table 5). Site 527 was sampled frequently due to ease of access and to complete elevation sampling (Appendix; Beach Profiling and Elevation Sampling). The site was sampled each month, February through September, with eggs detected every month except for August.

In addition to the randomly selected sites surveyed each month, sixteen extra sites were sampled from December to August and were targeted based on likelihood of encountering forage fish eggs (i.e., adult smelt observed spawning at location or suitable substrate present). Ten of these extra sites fell within the Twin Harbors beach zone, two in Copalis-Moclips, and four in the Kalaloch-Hoh-Quil zone. Site 527 had smelt eggs present in February and the remaining sites did not have any forage fish eggs.

Six sites had additional samples collected in the lower 2/3 of the intertidal zone, and forage fish eggs were absent in all of these samples. Sampling effort in the lower 2/3 was completed at 3 sites in the Twin Harbors zone in March and April, 2 sites in the Kalaloch-Hoh-Quil zone in November and March, and 1 site in the NW coast zone in March. These sites were targeted based on the likelihood of encountering forage fish eggs.

Since no eggs were detected at the six lower 2/3 sites, focus shifted to documented spawning sites where eggs were likely to be detected again. In an effort to obtain precise spawning elevation and complete beach profiles; we targeted sites with a geodetic marker nearby. We chose site 527 near the mouth of the Hoh River, as multiple spawning events had been documented at this site and a geodetic marker (ECY) is located approximately 200 ft. inland. Further, the ease of access to the site allowed for sample collection on a regular basis and easier equipment transportation. As the results of this additional research are outside the project goals, further details are provided in the Appendix; Beach Profiling and Elevation Sampling.

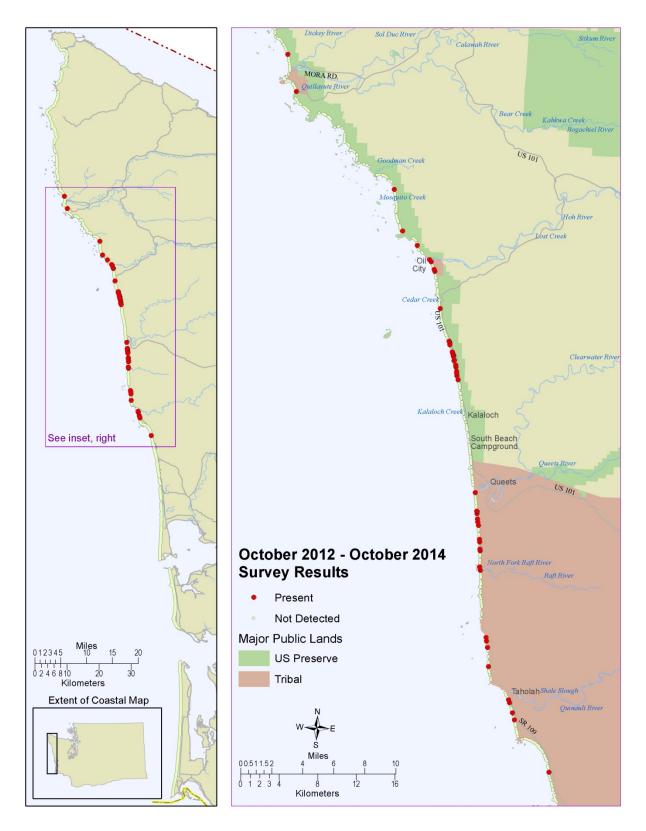


Figure 7. Locations of all sites sampled from October 2012 – October 2014. Documented smelt spawning sites (Present = 2+ eggs) and sampled sites where eggs were not detected are indicated.

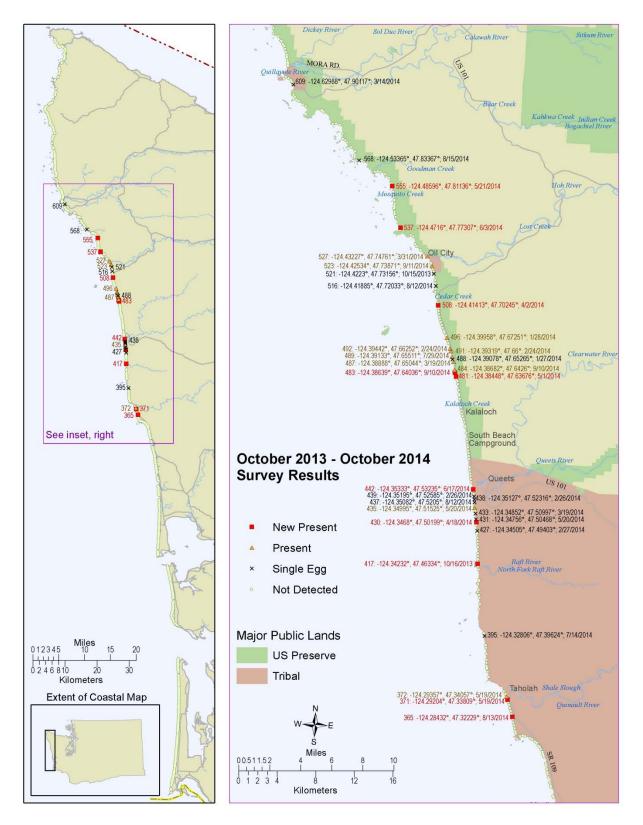


Figure 8. Locations of all sites sampled from October 2013 – October 2014. Documented smelt spawning sites (2+ eggs), both previously (present) and newly documented (new present), as well as single egg sites are indicated; also showing site #, longitude, latitude, and survey date.

Table 3. Documented smelt spawning sites from October 2013 – October 2014. Sites by month of sample collection, general location, number of samples with smelt eggs (of 3 samples collected per site; for site 527: of 24 in May, of 20 in June, of 18 in July, of 12 in September), total number of smelt eggs at site, and egg stage/condition.

Marida	Documented	Documented Spawning Site # General Location	Samples with	Number of	Smelt Egg Stage / Condition									
_				n –	Smelt Eggs at Site	Dead	1-Cell- Morula	Blastula	Gastrula	0.5 coil	1 coil	1.5 coil	>1.5 coil	Late-Eyed
October 2013	417	Raft River	2	2	1							1		
January 2014	496	Kalaloch	2	8	2			3	2	1				
	527	Hoh Shoreline	1	3	1						2			
February	492	Kalaloch	2	4	2				2					
	491	Kalaloch	2	4	1						2		1	
Manah	487	Kalaloch	2	6							1	3	2	
March	527	Hoh Shoreline	3	6	1			3	1					1
انب ۸	508	Ruby Beach	2	2				1			1			
April	430	N. Whale Creek	2	2	1									1
	371	S. Quinualt R.	1	2	1									1
	372	S. Quinualt R.	1	2	1									1
More	435	S. Queets R.	2	11	5								2	4
May	481	Kalaloch	2	9	5							4		
	527	Hoh Shoreline	1	2	1				1					
	555	Goodman Creek	2	2	2									
	442	Queets R.	3	6	6									
June	527	Hoh Shoreline	19	129	124					1		2		2
	537	N. Hoh Head	1	2							2			
July	489	Kalaloch	3	122	71			1		4	5	15	24	2
July	527	Hoh Shoreline	9	16	12					2	1	1		
August	365	S. Quinualt R.	1	2	1									1
	483	Kalaloch	2	8	8									
September	484	Kalaloch	3	111	28								83	
September	523	Hoh Shoreline	2	16	12								4	
	527	Hoh Shoreline	4	7	6									1

Month	Single Egg Site #	General Location	Egg Stage
October 2013	521	Hoh Shoreline	Dead
January 2014	488	Kalaloch	Gastrula
	427	Whale Creek	Not Determined
February	438	S. Queets River	Dead
	439	S. Queets River	Not Determined
March	433	S. Queets River	Dead
March	609	First Beach	Not Determined
May	431	S. Queets River	Not Determined
July	395	Camp Creek	Dead
	437	S. Queets River	Dead
August	516	Ruby Beach	1 coil
	568	Toleak Point	1.5 coil

Table 4. Single egg sites by month of sample collection from October 2013 – October 2014. Single egg sites do not meet the WDFW 2+ egg standard to document as a new spawning site.

Table 5. Multiple documented spawning sites by survey year and month.

Spawning Site #	General Location	1998 Survey	2013 Survey	2014 Survey
372	Quinault River		June	May
425	Whale Creek	July	July	
435	S. Queets River		July	May
484	Kalaloch	July		September
485	Kalaloch	July	May	
487	Kalaloch	July	April, September	March
489	Kalaloch		April	July
491	Kalaloch		March, June	February
492	Kalaloch		March	February
496	Kalaloch		March, June	January
523	Hoh Shoreline		August	September
				February, March,
				May, June, July,
527	Hoh Shoreline		April	September

This study was designed to inform the Washington State Marine Spatial Planning (MSP) process with regard to the presence and timing of forage fish spawning on coastal beaches. The year-two goals of our study were to: 1) subsample the breadth of the outer coast monthly from October 2013 through October 2014; 2) identify any forage fish eggs found to the lowest taxonomic level possible; and 3) geo-reference all survey data to provide an easily accessible overview of sampling effort and egg detections to date for use in MSP activities, and to guide future survey efforts. Results were then integrated with year-one survey data to provide a comprehensive, two-year evaluation of the spatiotemporal distribution of forage fish spawning within intertidal areas along the outer coast. Despite limited site access that, in some cases, reduced sample size, we were able to achieve our goals and documented 20 (10 new in year two) smelt spawning locations. Over the two-year survey period we documented 40 spawning sites. All survey data have been compiled into an ArcGIS geodatabase for easy integration with other resource distribution and exploitation data when proceeding with MSP activities on the outer coast.

Earlier survey efforts to document intertidal spawning forage fish on the outer coast of Washington State have been sparse relative to the efforts in the Puget Sound region. Previous sampling efforts on the outer coast have preferentially not sampled during winter months, presumably due to the logistical challenges of sampling during periods of high storm and wave activity, and because previous winter sampling efforts on the outer coast had detected no spawn between the months of November and February (Fradkin 2001; Penttila 2007). Despite the results of previous efforts, we continued to conduct surveys from November through February because: 1) previous sampling was not geographically comprehensive; and 2) we were using a modified sampling technique that covered a broader portion of the intertidal than has been previously sampled. By coordinating with tribal collaborators and having dedicated staff available to conduct surveys during the "off" season we had a substantial chance of documenting spawning in previously unconsidered locations and at novel times of the year.

The results of samples collected during November and December were consistent with the results of previous studies, with no spawn detected. However, spawn was documented in each month from January through October, one month earlier and one month later than documented during our prior survey year (Langness et al. 2014). Though the numbers of eggs collected in January and October was generally low, it indicates that the spawning season on some beaches of the outer coast is longer than previously thought. The number of documented spawning sites peaked in May and the number of eggs peaked in September. This pattern differs from our prior survey year in which both the number of documented sites and eggs peaked in June and July. Still, these seasonal trends in spawn abundance coincide with the results of a previous study in which peak egg densities occurred from May through September (Fradkin 2001).

Spawning sites are located in the northern central coast, ranging as far south as site 335 (south of Wreck Creek) and as far north as site 624 (near Ellen Creek) (Fig. 7). The geographical range of spawning sites didn't extend further north or south in the second year survey, and sites remained clustered within the Quinault and Kalaloch-Hoh-Quileute beach zones, ranging as far south as site 365 (south of the Quinault River) and as far north as site 555 (south of Goodman Creek) (Fig. 8). However, these general spawning areas/clusters broadened within this geographical range, adding 10 new sites. At this time, the results of this study do not allow us to definitively state the mechanisms influencing this spatial distribution. Surf Smelt and/or Night Smelt likely demonstrate some annual migration/movement along the coast and may simply spawn when they are physiologically ready. Additionally, spawn timing may be related to a suite of environmental conditions that would promote egg development. Also, given that our detected. Subsequent analysis of this false negative rate will occur coincident with the eventual development of an occupancy model, provided additional sampling occurs in subsequent years.

When comparing the first and second year survey results, similarities were observed for the percentage of documented sites, and mean number of eggs (per sample with eggs). The percentage of documented sites for the second year survey was 3.1% in comparison to 3.8% for the first year. The mean number of eggs (per sample with eggs) for the second year survey was 24 in comparison to 26 for the first year. These results reflect a slight decrease in documented site occurrence and detected egg abundance for the second year survey, but are considered marginal. The decrease is most notable in the Quinault beach zone, with a 5% decrease in the number of documented sites, and mean number of eggs (per sample with eggs) reduced from 42 to 4. The mechanisms influencing the observed decrease are difficult to definitively state without further investigation into our detection rates. Additional years of study are necessary to more thoroughly understand yearly temporal, spatial, and spawn abundance variations.

Many of the spawning locations noted in this study were identified within close proximity to freshwater outflows, small streams or large river mouths, as has been observed elsewhere. Freshwater outflows to the intertidal zone may provide eggs with the needed moisture to prevent egg desiccation, heat stress, and mortality. This could be particularly important on the exposed beaches of the outer coast where there is often little marine riparian cover to provide shade. In Puget Sound, the relative humidity of the spawning substrate, surface temperature of the sediment, and light intensity have been linked to fluctuations in habitat suitability that correlate with egg survival rate (Rice 2006). The interaction of freshwater outflows with nearshore waves resulting in the accumulation of sediment near the mouths of rivers and streams and the local attenuation of wave energy may also influence the ability of forage fish to utilize intertidal habitat, and influence the retention of spawn in that habitat. In Puget Sound, Surf Smelt are known to be highly tolerant of variable salinity regimes and immersion in freshwater outflows is not uncommon (Penttila 1978). In California, the most favored Surf Smelt spawning beaches are

coarse sand pea-gravel beaches, with some freshwater seepage (Leet et al. 2001). Perhaps, feeding adult smelt are attracted to these nutrient-rich sandflats, an area that would also provide desirable habitat for rearing juvenile smelt. Although an interesting observation, additional investigation is needed to assess this potential affinity to freshwater outflows. As several spawning sites were near large river mouths (Hoh and Queets), this could include further investigation into how proximal spawning is to a freshwater outflow based on volume (small outflow, stream, or river).

Coastal survey efforts to date have produced 12 multiple-documentation spawning sites. Spawning on these sites has been documented over several months during surveys conducted in 1998, 2013, and/or 2014. The recurring presence of eggs at these sites is indicative of a broader temporal spawning range. Further, analysis of the developmental stage of a subset of the eggs collected indicates the presence of multiple stages at the same site, suggesting overlapping broods and multiple spawning events. Surf Smelt eggs may hatch as soon as two weeks after being spawned; and spawning events in Puget Sound are commonly superimposed on each other. Thus, it is not uncommon for an area to contain two to five individual broods of eggs (Penttila 2007). The recurring presence of eggs at different sites and the presence of multiple egg stages at a single site indicate that several spawning events occurred during the season. However, multiple sites were sampled where only one egg was found, indicating that as comprehensive as our sampling was, bi-weekly as opposed to monthly sampling may be justified to document additional spawning sites.

Because Surf Smelt and Night Smelt eggs cannot be distinguished morphologically, the species of smelt spawning at these beaches cannot be definitively stated. Most documented spawning sites in Puget Sound have been documented as Surf Smelt spawning sites. However, Night Smelt have been recently documented in the Salish Sea and northern Puget Sound. An egg specimen collected near Discovery Bay, WA (Salish Sea) was misidentified as a Longfin Smelt but based on a study using molecular markers to distinguish smelts found in the gut contents of fishes, the specimen was identified as a Night Smelt (*Spirinchus starski*) (Paquin et al., in press). Additionally, Night Smelt have been observed spawning on coastal beaches during early spring by tribal fishermen. Although not officially documented, it provides some insight into the possibility that observed smelt spawn may be Night Smelt. In California, Night Smelt are known to spawn earlier (before June) in the season than the spawning of Surf Smelt, predominately in the summer (Leet et al. 2001). Genetic identification of the eggs collected in this study, and elsewhere in Puget Sound, would allow for positive identification of Surf and/or Night Smelt, and may be carried out in the future. Further, we would gain a better understanding of the spatiotemporal patterns of each species.

No Sand Lance eggs were discovered over the duration of the two survey years. Sand Lance generally spawn in the winter in Puget Sound and on beaches with grain sizes smaller than those

favored by Surf Smelt, and generally spawn lower on the beach than Surf Smelt (Penttila 1995; 2001b). Given this predilection, we anticipated that the detection probability for Sand Lance eggs in the Long Beach, Twin Harbors, and Copalis-Moclips beach zones might be higher than for Surf Smelt. In the few surveys that have historically occurred on the outer coast, Sand Lance have been documented to spawn in December inside Grays Harbor and in June in Grenville Bay just south of the mouth of the Quinault River. Our lack of Sand Lance egg detections could be a result of our sampling protocol, a lack of spawning occurrence altogether, or our focus on exposed beaches on the outer coast, as opposed to more protected beaches inside Grays Harbor, Willapa Bay, etc. Additionally, though we sampled hundreds of beaches, our sample size could have simply been insufficient.

Based on our success in documenting spawn in previously undocumented times and areas, we expect that further sampling would identify a broader spatial and temporal range of smelt spawning on the outer coast. Half of the sites documented in the second year survey were previously undocumented (10 of 20). Also, previous work on Rialto Beach and in Puget Sound has shown both seasonal and annual variability in egg density even during peak months of spawning activity (Fradkin 2001; Penttila 2007). This suggests that given the opportunity to continue sampling over multiple seasons, the potential to document spawning sites would increase, as some sites may have only limited use on a seasonal or annual basis. Continued sampling could also provide the opportunity to improve methods that would enable a higher detection probability and greater efficiency in sampling. Due to the comprehensive nature of our coast-wide survey, workloads did not provide ample opportunity to investigate and improve our methods. The beach profiling and elevation sampling pilot study (see appendix) provided insight into the distribution of spawn across tidal elevations on the outer coast. However, additional research is needed that would require an increase in sampling at known spawning sites. As our comprehensive study allowed us to determine the areas of spawning on the outer coast, focused sampling on spawning beaches in the Quinault and Kalaloch-Hoh-Quil beach zones is suggested for any future research efforts.

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Beach Profiling and Elevation Sampling

Introduction

The Washington Department of Fish and Wildlife (WDFW) and its collaborators have collected extensive data on the location and timing of smelt and Sand Lance spawning in Puget Sound. Additionally, tide height-specific patterns have been identified, indicating that Surf Smelt and Pacific Sand Lance spawn during high tides, depositing eggs along the upper third of the intertidal range (Surf Smelt +7 ft. to extreme high water; Sand Lance +5 ft. to MHHW) (Penttila 2007; Moulton and Penttila 2006; Penttila 1978, 1995). However, a comparative paucity of effort has been expended along the outer coast. Thus, this pilot study was designed to identify the specific tide heights at which smelt eggs are deposited on the outer coast.

Methods

In an effort to obtain precise spawning elevations on the outer coast, we selected a known spawning site with a geodetic marker nearby. We chose site 527 (from the two-year Marine Spatial Planning study) near the mouth of the Hoh River, as multiple spawning events had been documented at this site and a geodetic marker was located approximately 200 ft. inland. Further, the ease of access to the site allowed for sample collection on a more regular basis and easier equipment transportation. The Hoh geodetic control monument (ECY – Hoh) was installed by the Washington State Department of Ecology in February 2013. The monument is located next to a fire hydrant in the middle of a turnaround circle at the dead end of Lower Hoh Road (230255.6 Easting, 89499.93 Northing - WA State Plane North; 47.74825, -124.43196 NAD83) and has an elevation of 4.475 m NAVD88. The material is an epoxy on the cement base that surrounds a water line cover (Kaminsky et al. 2013).

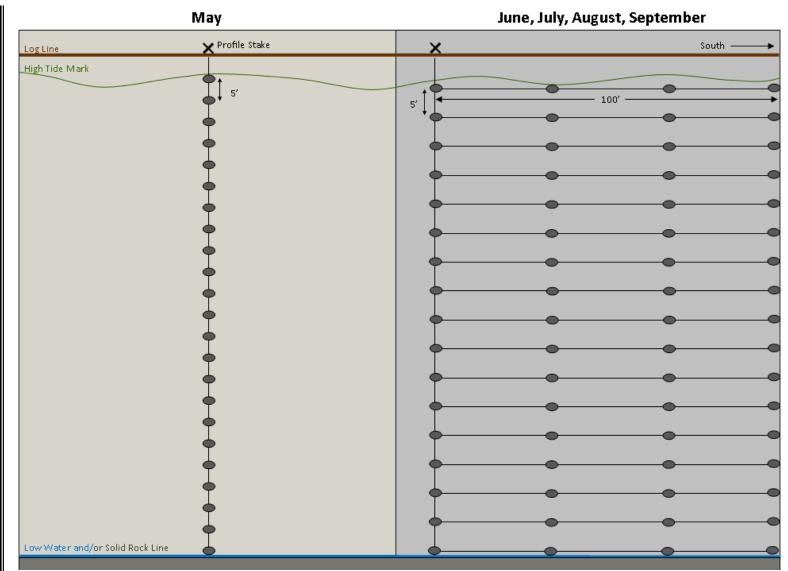
We used a simple, low-cost beach profiling method developed by Delgado and Lloyd (2004). Two main tools were used to obtain beach profile data. The first tool is a setsquare constructed of a horizontal aluminum pipe joined at one end with another vertical aluminum pipe, at a ninety degree angle. Our desired sample interval was 5 ft. and, thus, the length of the horizontal rod. The vertical rod was 3 ft., an adequate length given the estimated slope of the beach. Additionally, a circular level was fixed near the unattached end of the horizontal pipe (Fig. 1). The second tool was a stadia rod with a level affixed to ensure the rod was vertical while taking measurements (Fig. 1). Additional tools included a compass and measuring tape/rope to position the direction of the profile. For sediment collection, a 16 oz. sample jar was used to remove the top 5-10 cm (2-4 in) of sediment which was then placed in a plastic bag for later wet sieving and winnowing (Moulton and Penttila 2006).

Sampling methods were first tested in May 2014. Using the setsquare and vertical stadia rod we started the profile from the geodetic marker (47.74825, -124.43196 NAD83) towards the desired profile start, compass bearing S195W. After working over a large man-made boulder line, we set a profile stake (47.74770, -124.43219 NAD83) behind the log line and worked seaward at compass bearing S215W. Sediment sampling started at the high tide mark and occurred every 5 ft. (horizontal distance) from this reading, where 1 scoop of sediment was collected along the transect line (Fig. 2). Sampling stopped at the water line and/or where the sediment character changed from gravel/cobble to solid rock/boulder. A total of 23 samples were collected over a linear distance of 110 ft.

In June, we started the beach profile from the profile stake (47.74775, -124.43231 NAD83), compass bearing S210W. New profile stake coordinates were used as we did not have coordinates from the surveys conducted in May at the time of sampling. Starting from the profile stake broadened our sampling window and allowed us to take elevation readings/samples further than in May. Sediment sampling started at the high tide mark and continued every 5 ft. (horizontal distance) from this reading. However, instead of only 1 scoop of sediment collected every 5 ft. along the profile transect line, 4 scoops of sediment were collected at even intervals along a 100 ft. perpendicular transect set south from our profile transect every 5 ft. (Fig. 2). By increasing our sampling area and sample volume we hoped to increase egg detections. Sampling stopped at the low water/solid rock line and a total of 20 samples were collected over a linear distance of 95 ft. In order to complete the profile and obtain precise spawning elevations, we profiled from the geodetic marker to the profile stake, compass bearing S195W. Sampling methods in July, August, and September were performed the same, with the exception of using original profile stake coordinates from May (47.74770, -124.43219 NAD83). Eighteen samples were collected in July over a liner distance of 85 ft.; 17 in August over 80 ft.; and 12 in September over 55 ft.



Appendix Figure 1. Beach profiling sampling apparatus: A) Stadia rod with attached level, B) Aluminum setsquare consisting of a 3 ft. vertical rod and 5 ft. horizontal rod with attached circular level.



Appendix Figure 2. Beach profiling and elevation sample collection diagram. Grey ovals = sample scoops. From June to September, four scoops were collected for one elevation sample.

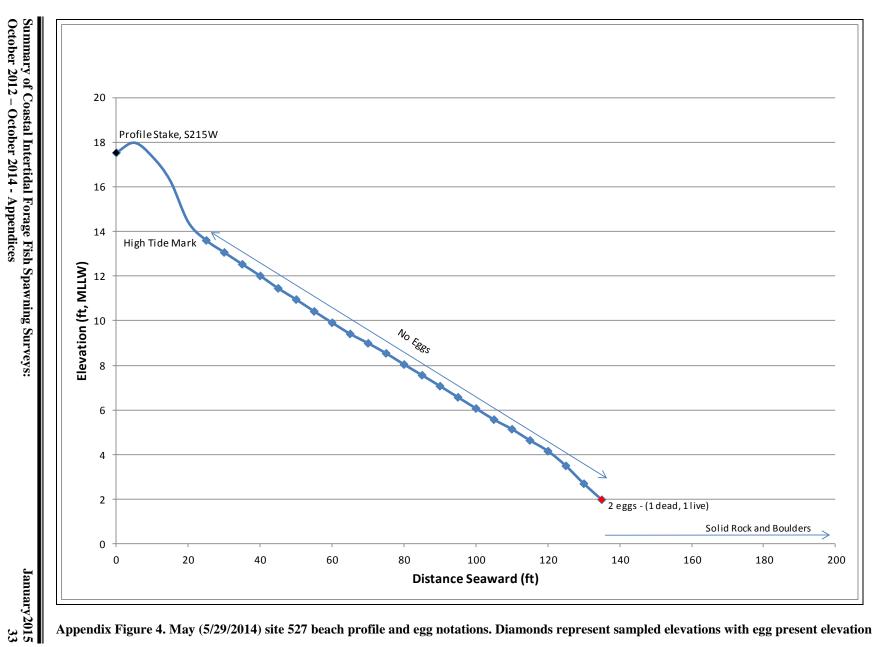
January2015 30 In order to reference all beach elevations to MLLW (US_ft) and compare results to Puget Sound historical spawning elevations, a vertical datum transformation was performed using NOAA's VDatum software (Figure 3). The geodetic elevation 4.475 m (NAVD88) converted to 15.5499 ft. (MLLW). From hereafter, all beach elevations are in reference to MLLW and measured from the elevation of the permanent monument.

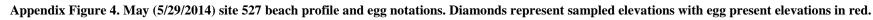
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Northing:	89499.93	to DMS	Re	set	Latitude:						
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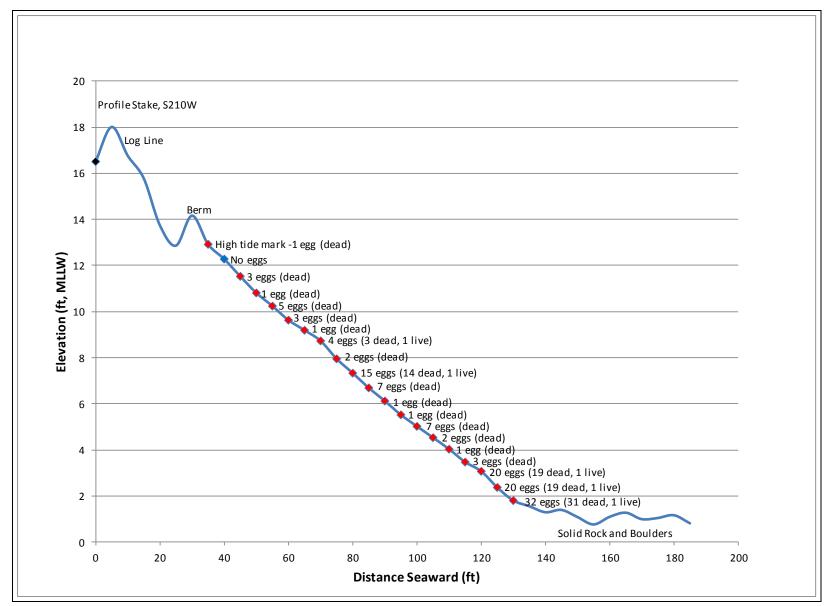
Appendix Figure 3. VDatum Fields. <u>http://vdatum.noaa.gov/</u>

Results

In May, 2 eggs (1 live and 1 dead) were found in total at +2.0 ft. No eggs were found at any other elevation (Figure 4). In June, eggs were present at each sample elevation (+1.8 ft. to +12.9 ft.); however, no eggs were found at +12.3 ft. (Figure 5). In total we found 129 eggs; 124 dead and 5 live. One live egg was found in each of the three lower elevation samples (+1.8 ft. to +3.1 ft.), as well as one each at +7.3 ft. and +8.7 ft. (Figure 5). In July, eggs were intermittently present between +3.0 ft. to +9.4 ft., with live eggs found between +6.6 ft. to +9.4 ft. (Figure 6). In total we found 16 eggs; 12 dead, 4 live. No eggs were found in August (Figure 7). In September, eggs were present between +9.2 ft. to +14.9 ft., with one live egg found at 13.9 ft. (Figure 8). In total we found 7 eggs; 6 dead, 1 live.



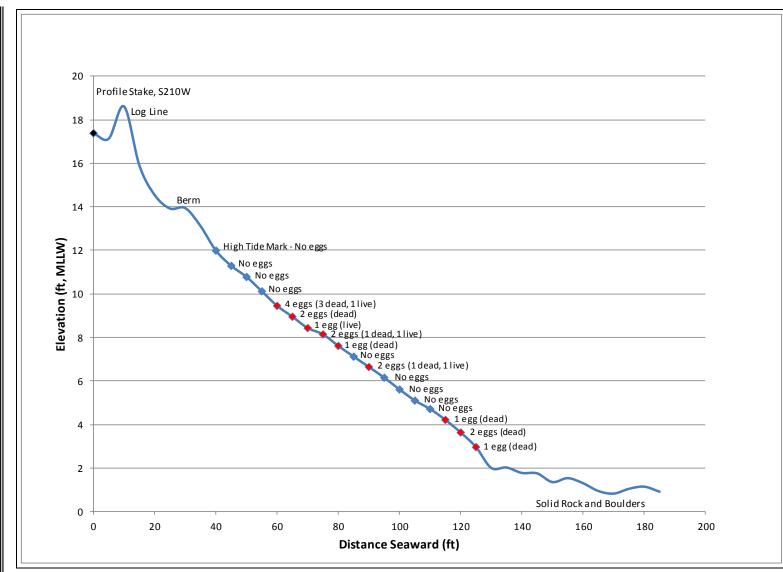


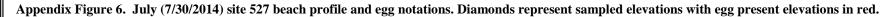




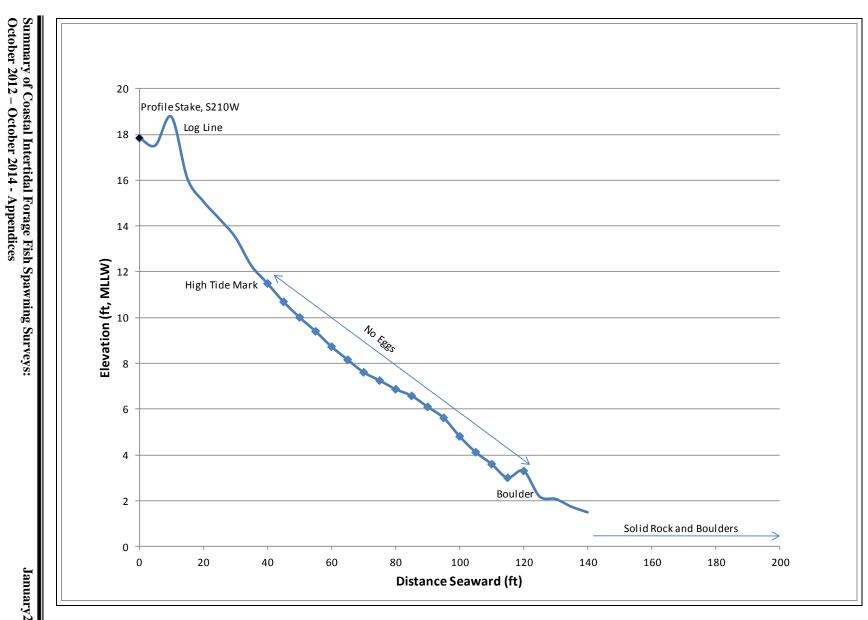
Appendix Figure 5. June (6/29/2014) site 527 beach profile and egg notations. Diamonds represent sampled elevations with egg present elevations in red.

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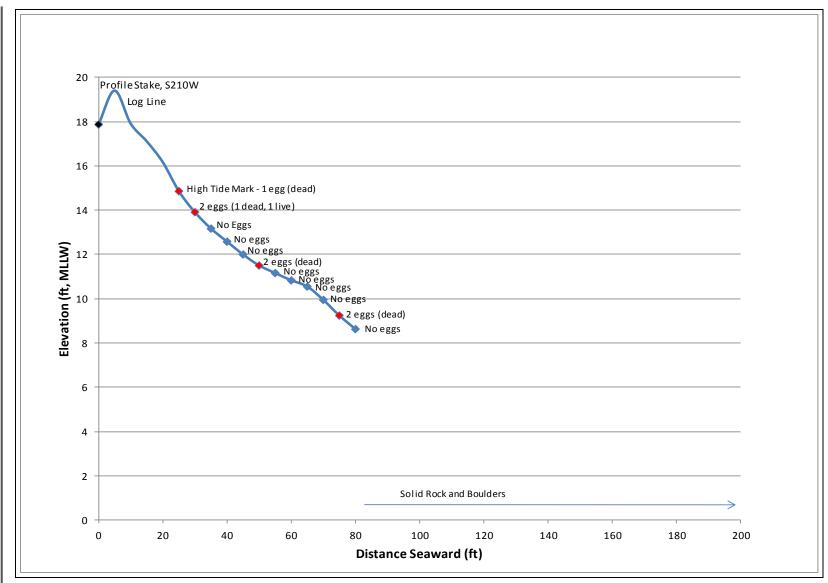


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Appendix Figure 7. August (8/28/2014) site 527 beach profile and egg notations. Diamonds represent sampled elevations.



Appendix Figure 8. September (9/26/2014) site 527 beach profile and egg notations. Diamonds represent sampled elevations with egg present elevations in red.

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Discussion

This pilot study was designed to identify the specific tide heights at which smelt eggs are deposited on the outer coast. The study was small in scope but we were able to produce data in an area of limited research. We found that smelt eggs are deposited and/or distributed on the outer coast across a broad tide range, unlike Puget Sound where eggs are deposited along a narrow substrate band near the high tide mark (Moulton and Penttila 2006; Penttila 1978, 1995). The range varied between sampling months; where in some months eggs were found only in low or high elevations, across all elevations (intermittently or continually), or not at all. Sampling conducted in May detected spawn at a low tide height, while June had the broadest spawning band of any sampled month, ranging from +1.8 ft. to +12.9 ft. (Figure 5). The highest June egg counts fell within the lower 3 elevation samples, however. July had a relatively broad spawning tide-height range; however, in comparison to June, spawning heights were more intermittent and the highest egg counts fell within the upper elevations. No eggs were found in August, and much like our comprehensive study, explanation for this kind of temporal pattern cannot be definitively provided. Eggs may have been absent, or eggs were present but not detected. September sampling detected spawn in the upper tide range, but the height of the beach had increased following a prior storm and the lowest elevation sample was collected at +8.6 ft.

Little effort has been expended on determining smelt spawn elevation on the outer coast; and the only known comparable study was performed at Rialto Beach by the Olympic National Park. Their study results determined that eggs were 3 times more abundant at low tidal elevations with 6% more spawning gravel (Fradkin 2001). Fradkin (2001) suggests this pattern to be "representative of open coast distributions caused by oceanic wave actions that disperse eggs deposited at the high tide line across a broad elevational band and more deeply into the beach substrate." The broad spawn elevation pattern observed at our study site can be attributed to the same open coast distributions. Further, this pattern isn't unlikely given outer coast conditions where wind and wave exposure is greater, there is a lack of vegetative shading, and sands are constantly shifting in the upper beach/dunes. In these conditions it may be beneficial for spawning smelt to place eggs lower on the beach in a well-drained, sandy substrate that is regularly inundated, thereby reducing egg desiccation stress. A Camano Island, Puget Sound habitat use study determined that smelt eggs at lower elevation transects generally had lower mortality rates than eggs at higher transects (Quinn et al. 2012). Based on these results, it is suggested that thermal and desiccation stress were both minimized lower on the beach.

A suite of beach physical characteristics have been correlated to egg abundance and survival. Characteristics such as sediment particle size, beach slope, sinuosity, concavity, aspect, solar radiation, and wood band width have been studied. By conducting monthly beach profiling at this known spawning site we were able to quantify the morphology and evolution of this beach and record changes in beach height, width, and slope. Though none of these characteristics have been directly correlated to spawning success, some interesting observations were made. Most notable was the change in beach slope, with a comparatively steeper beach in the summer (June – August) than spring (May) and fall (September). Interestingly, the highest egg count (total and live) occurred in June, suggesting a possible correlation between a steeper nearshore slope and spawning success.

Of further interest, we compared our estimated upper third distance methods to actual tidal height distance using our May beach profile. Using the estimation methods from our comprehensive study, we estimated a linear upper third distance of 125 ft. After completing our profile, we determined that sampling to the lower extent of the upper third of the intertidal (+6 ft.) would only require a linear distance of 75 ft. This suggests that our estimation methods covered a long enough distance to reach eggs found at +2 ft. or 110 ft. linear distance. However, sampling to only +6 ft. would have left the eggs found at +2 ft. undetected. Based on our monthly profiles, eggs would have been detected in the upper third (+6 ft. to high tide mark) in every month but May and August, but total egg counts would be reduced as eggs in the lower elevations would have gone undetected.

In summary, the results of this pilot study suggest that outer coast smelt spawn is deposited and/or distributed over a broad tide elevation range (+1.8 ft. to +14.9 ft.). However, additional research is needed; and sampling at additional spawning sites and further development of sampling methods are suggested. Sampling other spawning sites was attempted but referencing a geodetic monument proved to be difficult. Few geodetic monuments were within a reasonable distance to spawning sites. Further we were not able to use our apparatus as many of the existing monuments were located up high on a bluff or headland. In response to this challenge we attempted to set benchmarks using a Trimble GPS unit. However, we were not able to successfully create a stable benchmark as the unit couldn't obtain an accurate elevation (within 10 cm) at any desired upland location due to overhead vegetation. Future research would require additional investigation into setting up permanent monuments near spawning sites and/or using other electronic apparatuses such as Theodolite or Total Station.

WDFW Intertidal Forage Fish Spawning Habitat Survey Protocols

Procedures for obtaining bulk beach substrate samples -- Coastal

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)

<u>Note</u>: 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 upper third of the daily tidal range. It may also be necessary to obtain **permission to access the beach** from private or corporate landowners.

Procedure:

- 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)*.
- 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, the vegetated edge of the upland dune, and the edge of the water.
- Measure the distance from the landmark to the water side of the upper third of the daily tidal range. Note that linear measurements along the beach face serve as an index of tidal height but do not directly quantify *vertical* tidal height. The goal is to sample across the upper third of the daily tidal range.
- Standing at a randomly selected location at the water side of the proper tidal range, record a GPS fix on the data sheet.
- 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 4 above. Place the sediment in an 8 inch x 24 inch polyethylene bag or

Protocol FF-01-C

large, sturdy ziplock. You may need to take two scoops to get sufficient sediment, depending on the coarseness of the beach.

- 6. Walk several paces away from the water, repeat the sediment scooping action, and place the sediment in the bag. Move an additional several paces up the beach and repeat. Move an additional several paces, approximately to the high tide mark, and repeat. The bag should now have sediment from four locations in the upper third of the daily tidal range and be at least ³/₂ full.
- Using the measuring tape, move 100 ft along the beach, record a GPS fix, and repeat steps 5 and 6 using a new collection bag. Repeat this process again, filling a total of three bags at a given site.
- 8. Once three samples are collected at a site either: a) 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;" or b) continue on to the next sample site in order to maximize collection capacity for a given date.
- 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.

* Moulton, L.L., and Penttila, D.E. 2001. Field manual for sampling forage fish spawn in intertidal shore regions. Field Manual, MJM Research and Washington Department of Fish and Wildlife, Lopez Island, WA. PDF available on request from Dayv Lowry at WDFW (dayv.lowry@dfw.wa.gov).

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

WDFW Intertidal Forage Fish Spawning Habitat Survey Protocols

Procedures for recovering "winnowed 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

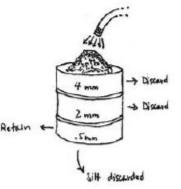
400-ml wide-mouthed sample jars

Freshwater hose work area with sufficient drainage (or extra buckets for saltwater rinsing) Area to discard waste gravel

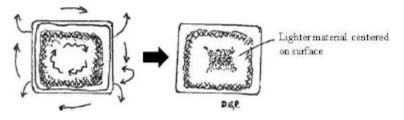
Ethyl alcohol or Stockard's solution[†] (only needed when samples will not be analyzed immediately) Pencil and Rite-in-the-Rain paper (cut into small squares for labeling samples)

Procedure:

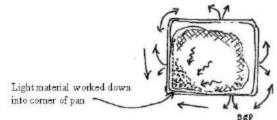
- Thoroughly wet-screen material through set of 4-mm, 2-mm, and 0.5-mm sieves/screens, using buckets of shore-side water at site or freshwater hose elsewhere. Screens should be carefully cleaned between samples.
- 2. Discard material retained in 4-mm and 2-mm sieves/screens.
- Place material from 0.5-mm sieve/screen ("egg-sized material") in rectangular dishpan and cover with ~1 inch of water.



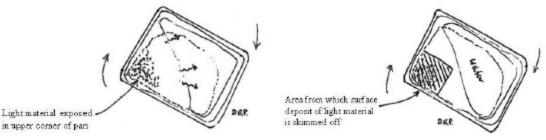
4. Rotate/tilt/yaw dishpan of material to impart rotation to water and cause lighter material to rise to the surface, where it should accumulate toward the center of the pan. Observe behavior of shell fragments and organic particles to get indication of behavior of forage fish eggs.



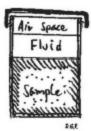
Tilt/swirl/agitate pan contents to move lighter material accumulated at center down to lower left corner of pan.



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



- 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.
- Repeat steps 4-7 approximately three more times, or until the sample jar is ~²/₃ 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% nondenatured ethyl alcohol should be used.



- 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).
- 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.

⁷ Stockard's solution contains formaldehyde, which is carcinogenic. 11 Stockard's solution = 50 ml formalin (37% aqueous formaldehyde), 40 ml glacial acetic acid, 60 ml glycerin, 850 ml fresh water (11 = 0.2642 gal; 1 gal = 3.785 l).

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

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:

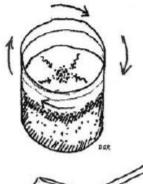
Fume hood (alternatively, winnowed light fraction samples can be carefully washed before analysis)* Latex or nitrile gloves* Spoon Oval microscope dish Dissecting microscope with 10-20x power Watchglasses/small Petri dishes Fine-point (watchmakers) forceps Data/tally sheets Paper towels Buckets/pans/sample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

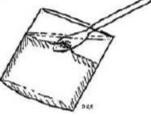
*Depending on the preservative used, samples may be toxic or carcinogenic. Take proper precautions.

<u>Note</u>: 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."

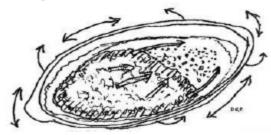
Procedure:

- 1. Stir "winnowed light fraction" sample jar contents with spoon.
- Swirl jar in clockwise manner to impart rotation to fluid and surface layer of contents, causing light material to move to center of jar.
- Carefully tilt jar. Slowly scoop center mound of light material with spoon into oval microscope dish.
- Repeat steps 1-3 four times, accumulating about 400 grams of light material in microscope dish.

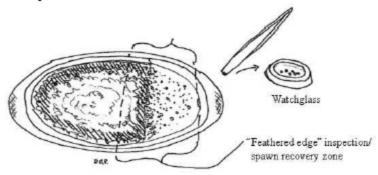




 Add water to microscope dish. Swirl/tilt/yaw dish to suspend lightest material and concentrate it along feathered edge of the deposit in the dish.



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



- 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 "winnowed light fraction." The WDFW standard for documenting a spawning site for a given species is 2 eggs in a single "winnowed light fraction" sample.
- Either preserve eggs for future counting and staging, or identify eggs in watchglass (see attached egg identification guide) to determine the species present.
- Complete survey findings, as well as preserved egg samples if taken, should be sent to Dayv Lowry at <u>Dayv.Lowry@dfw.wa.gov</u> and/or WDFW, Habitat Program, 1111 Washington St SE, Olympia, WA 98501.

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

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

<u>Note</u>: 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:

- 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.
- If preservative is present, pour off as much liquid as possible into the appropriate waste container and fill the Petri dish ~½-⅔ 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.
- 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.

- 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:

(Weight of combined subsamples) / (Weight of total sample) = (decimal conversion factor)

then,

(# eggs in combined subsamples) / (decimal conversion factor) = (# 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:

(10+10+10) / (10+10+10+270) = 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.

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

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

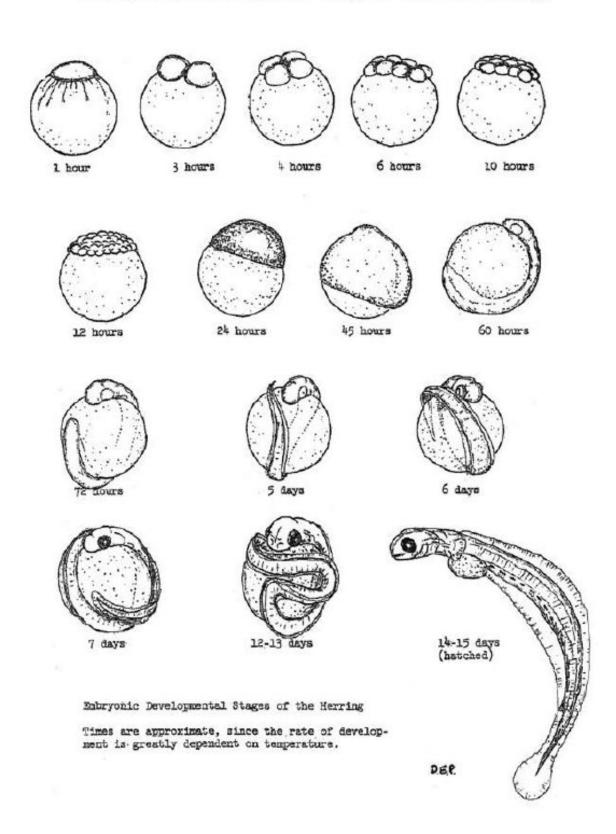
1 mm PACIFIC HERRING SURF SNELT almost entirely deposited on marine vegetation; distinct shell attach-ment sites; self-adhesive in layers or clumps. single pedestal-like attachment site; son-self-adhesire; entirely in beach sediment particlas. ROCK SOLE PACIFIC SAND LANCE

Forage Fish Eggs of Puget Sound

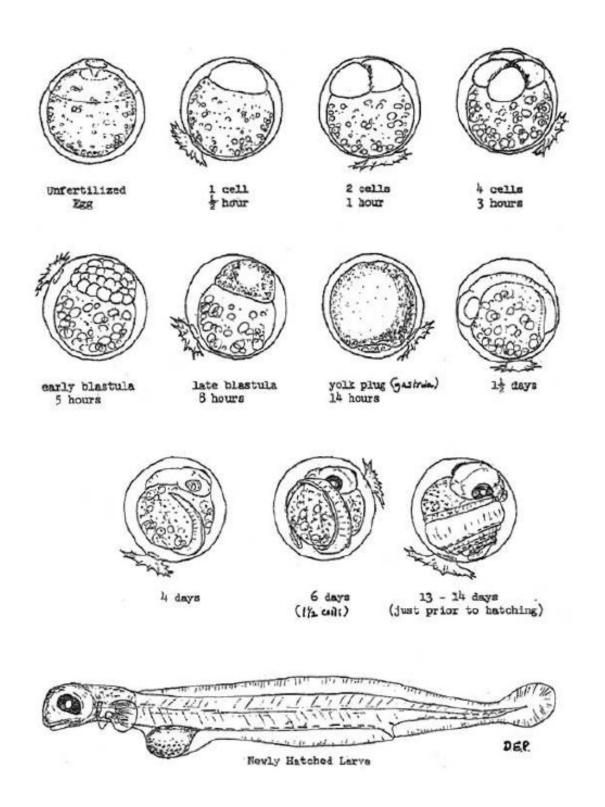
egg perfectly spherical; very clear; no visible attachment sites; non-self-adhesive.

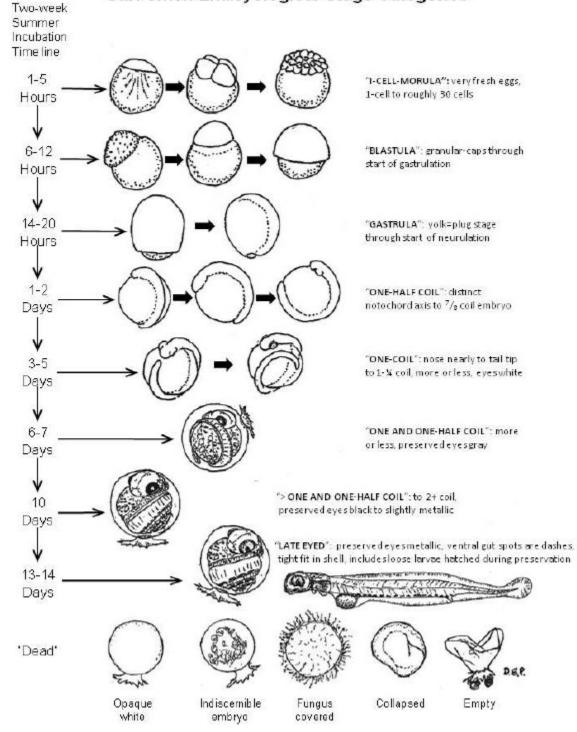
DER

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



Embryonic Development Stages – Pacific herring





Surf Smelt Embryological Stage Categories

	Last High Tide Time (24-hr): Day Elevation:				Year	Forage Fish Spawning Beach Survey							Reviewed by:						Pageof	
	Beach Station #	Latitude (decimal degrees)		25)	Longitude (decimal degrees)		Beach	Uplands	Width	Length	Sample #	Time (24-hr)	Landmark	Sample zone	Tidal elevation	Shading	Smelt	Sand lance	Rock sole	Comments
1																				
z																				
з				_																
4																				
5				_																
6																				
7				_																
8																				
9																				
10																				
11																				
12																				
13																				
14																				
15																				

Summary of Coastal Intertidal Forage Fish Spawning Surveys: October 2012 – October 2014 - Appendices

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Field Observation Sampling Code

Beach: Sediment character of the upper third of 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

Note: Record code that depicts the dominant substrate for the station. If there is no dominant substrate, record all substrate codes observed in the comments.

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

Width: Width from upper most to lower most sample scoop on a transect; in feet to the nearest ½ foot.

Length: Length of beach segment up to 1,000 feet (500 feet on either side of the station center).

Sample #: Unless otherwise noted, it is assumed that for a given station with three samples:

- 1 = Center sample (Recorded coordinate)
- 2 = North sample (100 ft. north of center)
- 3 = South sample (100 ft. south of center)

Landmark: landmark for determining sample zone where collection occurs 1 = down beach from last high tide mark4 = down beach from upland toe

Sample Zone: Distance to lowest sample scoop of a transect taken perpendicular to the landmark; in feet to the nearest ½ foot.

Tidal Elevation: Determined in the office using NOAA verified historic tide data and location/ time data provided.

Shading: Shading of spawning substrate zone, averaged over the 1,000 ft. 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

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

Forage Fish Spawning Beach Survey Sample	Anah	vsis
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Page___of___

	order	Beach Station #	Sample #	Collection Date	Analysis Date	Species	Total Eggs counted		Denominator of portion sampled*	Comments			
						Surf smelt							
1			1			Sand lance							
						Rock sole							
						Surf smelt							
2			2			Sand lance							
						Rock sole							
						Surf smelt							
з			3			Sand lance							
						Rock sole							
						Surf smelt							
4		1			Sand lance								
						Rock sole							
			2	2			Surf smelt						
5					2	2			Sand lance				
								Rock sole					
						Surf smelt							
6			3			Sand lance							
						Rock sole							
						Surf smelt							
7		1			Sand lance								
						Rock sole							
					Surf smelt								
8			2			Sand lance							
						Rock sole							
						Surf smelt							
9			3			Sand lance							
						Rock sole							

*The "Denominator of portion sampled" is the value to multiple by to expand to the whole sample. For example, if you analyze 1/4 of the whole sample, this value would be 4. This value must be an integer, therefore if more than 1/2 of the sample is processed, then the whole sample must be processed and reported as 1.

Reviewed by:



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