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Assessing Land-Based Impacts to Spawning Habitat of Surf Smelt

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PROJECT TITLE: ASSESSING LAND-BASED IMPACTS TO SPAWNING HABITAT OF SURF SMELT

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Abstract

The three most abundant forage fish species in the Salish Sea, Pacific herring (*Clupea pallasi*), surf smelt (*Hypomesus pretiosus*), and sand lance (*Ammodytes hexapterus*), all spawn in nearshore shallow subtidal or intertidal habitat. These habitats are also the areas of the Salish Sea most likely to receive significant inputs of land-based pollution, such as stormwater runoff, combined sewer overflows, or surface oil spill "bathtub rings". Although methods exist to deploy caged herring embryos as tools to measure the biological effects of *in situ* exposure, these methods are restricted to investigations of the shallow subtidal zone. The potential impact of oil spills on surf smelt and sand lance was identified as a key data gap in Puget Sound. Surf smelt are obligate intertidal spawners resulting in different exposure characteristics and life history-specific physiological requirements that could modify their sensitivity or responses to pollutant exposure. Here we describe *in situ* methods for deploying surf smelt embryos in Puget Sound beaches intended to improve contaminant monitoring in intertidal habitats.

1. Background

Forage fish are keystone species in Puget Sound – important planktivores and prey for salmon, seals, and seabirds. The three most abundant forage fish species in the Salish Sea, Pacific herring (*Clupea pallasii*), surf smelt (*Hypomesus pretiosus*), and sand lance (*Ammodytes hexapterus*), all spawn in the nearshore intertidal or shallow subtidal habitat. These habitats are also the areas of the Salish Sea most likely to receive significant inputs of chemical pollutants from land-based sources. These sources include those derived from urban development, such as stormwater runoff or combined sewer overflows, those from non-urban areas including rural or residential sources such as agricultural runoff and failing septic systems, but also those potentially affecting all shorelines, such as petroleum deposited as a "bathtub ring" following a maritime surface spill. Increased shipment of diluted bitumen products by rail from the Pacific Northwest interior to Salish Sea ports is another new land-based source of potential oil spills through train derailments or other accidents.

For largely historical reasons stemming from the 1989 Exxon Valdez oil spill (EVOS) in Prince William Sound (PWS), Alaska, Pacific herring have become widely recognized and studied as a forage fish species that is particularly sensitive to oil pollution in its spawning habitat. The collapse of the PWS herring population following EVOS prompted over two decades of studies characterizing the acute and long-term effects of low-level oil pollution on this species (Carls et al., 1999; Cypher et al., 2019; Incardona et al., 2009; 2021; Marty et al., 1997). The recognition of herring embryos' sensitivity to oil spills subsequently led to studies assessing potential injury to herring following the 2007 Cosco Busan bunker oil spill (CBOS) in San Francisco Bay. In parallel to EVOS, field studies in the wake of the CBOS involved sampling of herring embryos that were deposited naturally in nearshore areas by normal spawning activity. However, given the relatively unpredictable nature of spawning location by herring, we pioneered the use of caged herring embryos to assess exposure to and injury from oil in a controlled manner to

increase coverage beyond areas that received natural spawn (Incardona et al., 2012a). These methods for capturing ripe herring to produce laboratory-fertilized embryos for controlled deployment studies were later refined and applied to monitor the effects and effectiveness of creosote-treated piling removal in relatively pristine areas of the Salish Sea (Quilcene Bay on Hood Canal) (West et al., 2019), as well as assessing the success of remediating highly contaminated habitats in Port Gamble Bay, WA (West et al., in prep). These most recent studies have reinforced the sensitivity of developing herring to fossil fuel-derived and other pollutants and have informed management decisions regarding the timing and methods of creosote piling remediation and other toxics-reduction techniques. Importantly, recent laboratory studies demonstrated significant overlap between the adverse impacts on herring of both oil spills and urban highway runoff, likely due to common levels of polycyclic aromatic compounds (PACs) in both sources (Harding et al., 2020).

These studies with caged Pacific herring embryos provide the basis for a widely useful monitoring tool for the adverse biological effects of contaminants in nearshore areas. However, in association with the normal life history of herring, this means the utility of herring is restricted as a representative of the shallow subtidal/low intertidal zone. The two other major forage fish species of the Salish Sea, sand lance and surf smelt, are obligate intertidal spawners, with the latter requiring a daily cycle of water immersion and air-exposure for ideal development (Misitano, 1977). Thus, surf smelt represent a different habitat zone with different exposure characteristics and have life history-specific physiological requirements that could modify their sensitivity or responses to pollutant exposure. Additionally, the potential impact of oil spills on surf smelt was identified as a key data gap by the Puget Sound Nearshore Partnership (Penttila, 2007). Due to their relatively short life span, surf smelt populations may be particularly vulnerable to extended spawning habitat degradation resulting from oil spills or repeated stormwater runoff exposure.

Previous studies demonstrate the sensitivity of surf smelt to contaminants and the need for further research and monitoring. Surf smelt embryos exposed to crude oil exhibited forebrain and retina damage and reduced hatching success (Hawkes and Stehr, 1982). Additionally, Morgan and Levings (1989) found that surf smelt embryos and larvae were most sensitive to contaminated sediments compared to Pacific herring and lingcod. The association of surf smelt spawning with the upper intertidal zone also renders them potentially more susceptible to multi-stressor interactions including elevated temperature, desiccative stress and exposure to ultraviolet (UV) radiation. An extremely potent interaction between UV from sunlight and petroleum pollution was demonstrated for Pacific herring following the CBOS (Incardona et al., 2012a; 2012b). This interaction is likely to be significant for surf smelt exposed to either land-based or maritime sources of petroleum compounds.

Over the last two years we have been developing methods to collect ripe surf smelt from spawning aggregations and produce synchronously fertilized embryos for laboratory studies, parallel to what has been established for Pacific herring. The initial primary goals have been to

determine the effects of crude oil, urban stormwater runoff, and diluted bitumen on surf smelt in controlled laboratory studies. However, recognizing that the broadly distributed populations of surf smelt have a wider temporal range of spawning throughout the Salish Sea, this species also has the potential to form the basis of a more widely applicable monitoring tool relative to herring. In essence, herring embryos can be produced and deployed from roughly February through early May. Surf smelt populations that we readily access throughout greater Puget Sound provide embryos nearly year-round except for March and April (Penttila, 1978). Therefore, not only does out-planting of caged surf smelt embryos provide tools directly applicable to management of this species, but it may also provide a tool more broadly applicable to studying the overall health of the Salish Sea upper intertidal zone practically year-round.

2. Hypotheses and aims

The objective of this project was to establish methods for out-planting manually spawned surf smelt embryos into upper intertidal beach sediments to develop a monitoring tool to evaluate the impact of contaminants on the intertidal ecosystem and forage fish embryo health in Puget Sound. Using techniques for manual spawning already developed by the authors, we set out to 1) design smelt embryo cages that would support normal embryonic development, protect them from predation, and resist vandalism and 2) develop methods to deploy surf smelt embryo cages long enough for them to develop to near-hatching, and retrieve a sufficient mass of embryos samples to assess survival, developmental success, and contaminant profiles.

3. Methods and experimental design

This study comprises a combination of research activities conducted by the authors over approximately the past five years developing techniques to manually spawn smelt for toxicological studies, with the practical questions being addressed herein related to the feasibility of deploying embryos in natural habitats to evaluate status, trends, and impacts of chemical contaminants in Puget Sound. As such it presents both unpublished methodological information related to the embryo-creation steps with the cage development and deployment activities funded in this current study. Moreover, it combines this pre-existing knowledge (e.g., the adherent properties of embryos to certain substrates) with design features of the cages described below.

Embryo Deployment Cage Design

We designed embryo cages for deploying surf smelt embryos from approximately 18 hours post-fertilization until late embryonic development (just before hatching), a period lasting about 2 weeks in summer or as long as 4-8 weeks in winter (Penttila, 2007). The goal was to develop a deployment system with cages that are easily assembled, deployed, and retrieved, easily and effectively anchored to withstand several weeks of inclement weather and shifting

substrates, provide protection from desiccation, predators and vandalism, and support normal embryonic development.

To achieve these goals, we developed an apparatus comprising two primary elements – an outer cage with relatively large-diameter, rigid mesh, which houses a smaller, inner embryo chamber with smaller-diameter, flexible mesh (Figure 1). The outer protective cage, measuring 23 cm in length x 9 cm diameter is a commercially-available wire bait cage consisting of a cylinder of epoxy-coated wire mesh with plastic end caps (one cap is removable via screw-mount). Inner embryo chambers measuring approximately 5 cm diameter x 6 cm length were designed to fit inside the outer cage, which is anchored to the beach substrate. The inner embryo chambers are a slight modification of similar containers developed by the authors to incubate smelt embryos in the lab. These were constructed from short sections of 2-inch-diameter PVC pipe fitted with 40-micron Nitex mesh et both ends. After addition of embryos the Nitex mesh is held at both ends with PVC coupler rings (Figure 1). This cage-within-a-cage design was selected to satisfy the following key needs:

- Sand exclusion: the purpose of the 40-micron mesh at either end of the inner egg chamber was to exclude as much sand and silt as possible to prevent embryos from sticking to ambient particles. Smelt embryos adhere to sand and other silica-containing matter, which can interfere with microscopic observation of the embryos and also contaminate embryo samples for analytical chemistry.
- Water flow: it is important that water can move easily into and out of the inner embryo chambers – both for normal embryonic development and to ensure that embryos are exposed to the same sediment pore water (and contaminants) that naturally spawned embryos would experience. The 40-micron Nitex mesh at both ends of the inner embryo chamber was selected to balance the need for water flow with particle-exclusion.
- Predator protection: the cage protects developing embryos from egg predators, including small fish and invertebrates. The inner embryo chambers were placed inside commercially available bait cages for additional predator protection and to facilitate attachment of the inner embryo chambers to anchoring devices. We compared two commercially available bait cages and opted for rigid plastic-coated wire-mesh bait cylinders measuring 23 cm long x 9 cm diameter (Figure 1). These bait cages were a dark color that made them less noticeable to curious passersby and were easy to attach to anchors.
- Embryo mass: the size of inner embryo chambers was optimized to house sufficient mass of embryos (approximately 3 gm) for analytical chemistry
- Anchoring: Due to the dynamic nature of the intertidal habitat, sturdy anchors are required to keep the cages in place. To mimic naturally spawned embryo placement, we aimed to anchor the embryo cages in the top 10 cm of sediment. While multiple anchoring methods are possible, the best design featured a horizontal placement of the cage apparatus, anchored by means of nylon conduit ties to a length of metal rebar, bent into a U-shape, and driven into the substrate. Straight rebar lengths were also

tested with cages oriented vertically in the substrate (Figure 2), however the horizontal orientation seemed most likely to position all embryos at a consistent and environmentally realistic substrate depth.

Figure 1: Photo of embryo cage materials including commercially available bait cage, two-inch PVC pipe sections, coupling rings, and 40 micron Nitex mesh.

Figure 2: Embryo deployment cage anchor methods.

Study area

Surf smelt spawn throughout Puget Sound, providing various locations for collection of ripe smelt throughout the year. We utilized popular sites for recreational and commercial smelt fishing to opportunistically harvest smelt gametes from fishermen's catch (Table 1, Figure 3). Additionally, we conducted a reconnaissance survey to identify local beaches with habitat characteristics consistent with commonly used surf smelt spawning beaches that could be used for embryo cage deployments. Sites were evaluated based on the sediment grain size, accessibility, current and historical wild smelt spawning activity, and proximity to potential sources of contamination. For initial smelt deployments, we avoided known sources of pollution to remove contamination as a factor in our assessments of the effectiveness of the cage design and deployment methods. A list of ideal beaches that were identified is shown in Appendix A. Sites used for cage deployments are shown below in Table 1 and Figure 3. Utsalady Beach, on the northwest side of Camano Island, was used as the primary deployment beach throughout the study. Utsalady Beach is a well-known smelt spawning beach with no suspected or known sources of contamination, which supports surf smelt spawning activity year-round. Tulare Beach is a finer-grained beach that was selected as a second deployment location to test the embryo deployment methods across a variety of beach substrate types.

Table 1: Study locations used for collection of ripe adult smelt and embryo cage deployments

Site	Site Name	Latitude	Longitude	Description
A	Ross Point	47.53980°	-122.66208°	Winter adult smelt collection site
B	Twin Rivers	48.16500°	-123.94956°	Summer adult smelt collection site
C	Maple Grove	48.25288°	-122.51766°	Summer adult smelt collection site
D	Utsalady Beach	48.25393°	-122.49807°	Summer adult smelt collection site and primary deployment location
E	Tulare Beach	48.10342°	-122.34423°	Deployment location

Figure 3: Map of adult surf smelt collection locations (orange pins) and embryo cage deployment locations (yellow pins).

Experimental design

Four deployment events were used to evaluate optimal methods for deploying manually spawned surf smelt embryos into the upper intertidal sediments of spawning beach habitats in Puget Sound (Table 2). The primary factors tested for optimization in the first deployment were tidal elevation and adherent vs non-adherent embryos. Subsequent deployments were conducted to determine whether our methods were repeatable across seasons and sites. For each deployment, all embryos were created from a single spawning action and deployed on the same day to minimize variation in gamete quality, deployment duration, and environmental variables.

Table 2: Overview of timeline and description of each deployment trial conducted

Deployment Trial	Dates	Location	Description	
1	1/14/21 – 2/8/21	Utsalady Beach	Comparison of adherent and non-adherent embryos at three tidal elevations on Utsalady Beach	9 cages (3 low, 3 mid, 3 high tidal elevations) 2 inner embryo chambers per cage (1 adherent, 1 non-adherent)
2	5/16/21 – 5/26/21	Utsalady Beach Tulare Beach	Comparison of Utsalady Beach (coarse substrata) and Tulare Beach (finer-grained substrata). Three inner embryo chambers were included in each embryo cage to allow for easier monitoring of developmental stage throughout the deployment	12 cages (3 low, 3 mid, 3 high tidal elevations) at two sites (9 cages at Utsalady Beach and 3 cages at Tulare Beach) with 3 inner embryo chambers of non-adherent embryos per cage
3	7/31/2021	NA	Poor fertilization. Field deployment cancelled.	
4	8/20/21 – 8/31/21	Utsalady Beach Tulare Beach	Replication of Deployment 2	Same as for Deployment Trial 2

Adherent vs non-adherent embryos

Surf smelt eggs are known to adhere to sand or gravel particles by a proteinaceous "suction cup" that forms during fertilization. The "suction cup" adheres to any silica-containing material, such as sand and glass, but not to other materials like plastic, including the nylon used in Nitex mesh. We have used this property to developed techniques for manually spawning surf smelt to produce embryos that are either attached to a substrate (adherent) or remain loose and unattached (non-adherent). Non-adherent embryos can be produced in controlled fertilizations by avoiding contact with silica-containing materials during early embryonic development. In our laboratory exposures, we found the latter were easier to sample for analytical chemistry and loose, demersal embryos are easily incubated in mesh-bottom PVC cups similar to what are typically used in salmonid culture. Adherent embryos, on the other hand, are useful for repeated developmental assessments of specific embryos attached to glass slides.

For the first deployment, two inner embryo chambers were included in each cage – one with embryos adhered to a glass slide inserted into the inner chamber and one with loose, non-adherent embryos in the inner chamber (Figure 4).

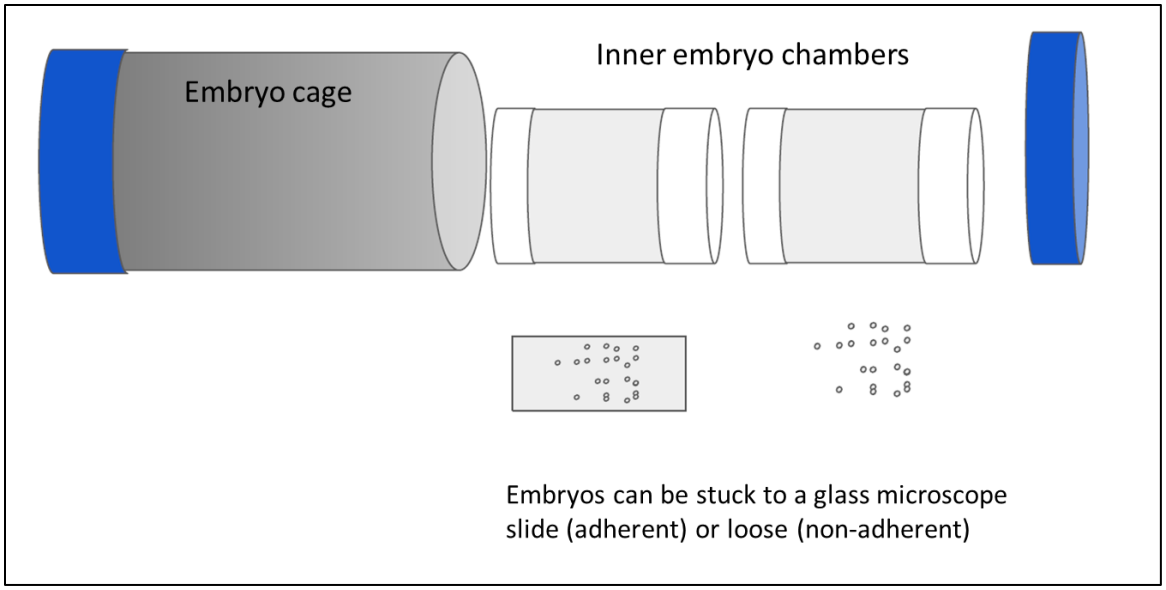

Figure 4: Schematic of cage design used in first deployment to compare adherent and non-adherent embryos. Two inner embryo chambers (one with adherent eggs and one with non-adherent) were included in each embryo cage.

Tidal elevation

Surf smelt spawn at high tide, depositing their embryos in the upper intertidal between +7 and +11 feet above mean lower low water (Penttila, 1987; 2007, Middaugh et al., 1987). We deployed our embryo cages at +6.0 ft or higher to mimic the natural tidal elevation of surf smelt embryos while trying to reduce risk of desiccation. Cages were deployed at 3 tidal elevations ranging between +6.0 ft above mean lower low water and the maximum high-water level for the subsequent tides following 2 weeks after deployment. Embryo cages were buried just

beneath the sediment surface (within 10 cm) to mimic, as much as possible, the depth of naturally spawned embryos.

Manual Surf Smelt Spawning and Fertilization

Collecting adult smelt for spawning

Because fishing for surf smelt is a common activity throughout Puget Sound, ripe fish can typically be obtained from recreational or commercial fishers near to potential deployment beached. Outreach efforts typically lead to enthusiastic involvement of fishers, who are willing to donate fish for these studies. Smelt are usually caught at high tide as the fish approach the beach for spawning. The method recreational fishers use to catch surf smelt varies from beach to beach, but in all cases, it involves capturing the fish with dip nets as the school of smelt nears the spawning beach during daytime or nighttime, depending on the tide and fish behavior (See Appendix B). Commercial fishers visually spot the school during daylight hours and surround it with a beach seine deployed from a small boat. Once fishers agree to donate, live fish are selected at random from buckets of their collected fish.

Table 3: Dates and locations of adult smelt capture for gamete collection.

Date	Location	Description
1/12/2021	Ross Point	High tide was +11.52 ft at 15:15. First smelt caught at 14:27. Total of 10 fish (4 females, 6 males) caught by recreational fishermen
5/14/2021	Twin Rivers	High tide was +5.43 ft at 16:51
7/30/2021	Utsalady Beach	High tide was + 11.38 ft at 22:11
8/18/2021	Maple Grove	High tide was + 10.53 ft at 21:01

Manually spawning smelt

Once in-hand, a slight squeeze to the abdomen of spawn-ready fish easily produces eggs or milt. Difficulty in obtaining eggs or milt using slight pressure indicates the fish are not spawn-ready, and so are not used. Once a ripe female is identified, an alcohol wipe is used to clean the genital pore and a paper towel is used to dry the fish. Pressure is again applied to the abdomen and eggs flowing freely out of the female are collected into a 50-ml capacity, conical bottomed, polypropylene sample tube (Figure 5). A single female can produce 10-20 ml (approximately 10-20,000) of eggs and typically the eggs of two females are combined into a single tube. Two to three ml of milt each is collected from ripe male fish, with milt combined from up to five fish. Eggs and milt are then combined, and sufficient clean seawater added to fill the tube, which is then capped tightly and inverted several times. This allows the sperm to access all the eggs and prevents eggs from clumping together. The sealed tube is then placed in a styrofoam block on gel ice packs in a cooler and transported to the lab and stored at 4°C. This method typically produces 85-95% fertilization rates. Alternatively, when male and female gametes have been transported separately from the beach and subsequently combined in the lab for fertilization, fertilization rates were typically much lower. Best results are obtained when fertilization is carried out immediately after gamete collection.

Figure 5: Mark Tagal stripping gametes from surf smelt at Twin Rivers, WA on May 14, 2021. Recreational smelt fisherman in background.

Fertilization rate

For smelt, we have found reliable fertilization rate and viability checks are challenging to conduct before 12 hours post fertilization (hpf). Therefore, the fertilization rate in this study was checked 18-20 hours after manual spawning. Tubes of fertilized eggs were decanted and rinsed with clean seawater. Approximately 100 eggs were

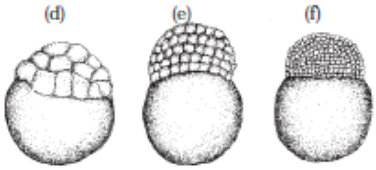

Figure 6: Blastodisc observable during the blastula period after 12 to 18 hours, indicating successful fertilization and early growth. Image taken from Hill and Johnson (1997).

removed from each tube of fertilized eggs and placed in a petri dish. The number of eggs that exhibited a well-formed blastodisc (Figure 6, adapted from Hill and Johnson, 1997) were counted and compared to the total number of eggs. A spawn batch was considered successful if the fertilization rate was greater than 70%.

Embryo Cage Deployments

Embryo preparation and assembly of the inner embryo chamber

Embryos were held at 4°C in their original fertilization tubes during the approximately 18-hour period between collection and fertilization check. After successful fertilization was confirmed, the tubes of embryos were transported in coolers with ice packs to the deployment location, and the cage units were assembled. At the deployment site all embryos were combined in a clean Nitex mesh strainer and rinsed with seawater obtained from the deployment beach. Embryos for the non-adherent embryo trials were weighed into a clean weigh boat and transferred into each inner embryo chamber, after which the Nitex mesh caps were secured with coupling rings. For the adherent embryo trials, approximately 100 embryos were placed on a glass slide until they adhered, after which the slide was placed inside an inner embryo chamber.

Embryo cage assembly and deployment

Two to three inner embryo chambers were placed inside each deployment cage (depending on the deployment) and cage lids were screwed on securely. For a subset of cages, HOBO electronic temperature and light data loggers were included inside the bait cages to record real-time temperature data for calculation of degree days (see below) and estimating developmental timeline. All cages were labeled with a "WDFW Contaminant Monitoring Study" placard and a unique identifier. At each beach deployment location, a small hole (approximately 15 cm deep) was dug and the cage was placed horizontally in the hole. Horseshoe-shaped rebar anchors were placed around the embryo cages and carefully pounded into place. Embryo cages were then secured to the rebar anchors using nylon conduit ties at either side (Figure 2). After the anchor was securely in place, the sand/rocks were spread back over the embryo cage, just covering it (Figure 7).

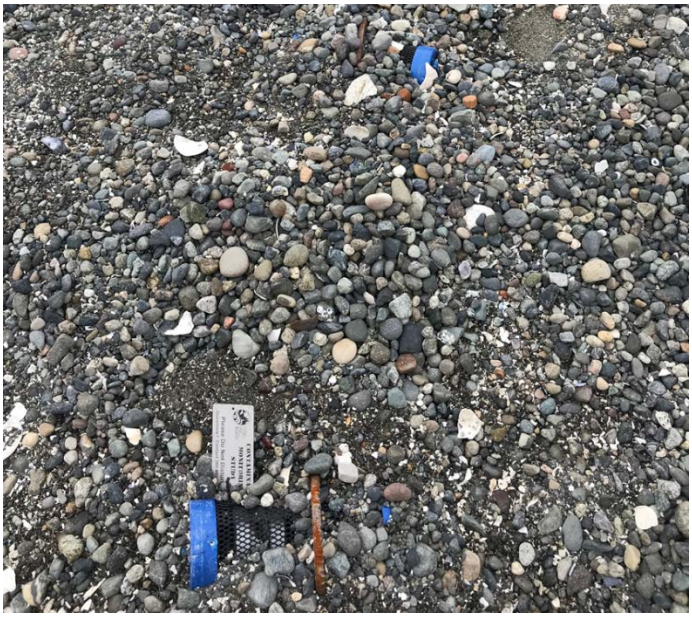

Figure 7: Embryo cage deployment

During deployment, cages were monitored on a regular basis to ensure cages remained securely anchored, maintained appropriate sediment depth, and embryonic development was

progressing as expected based on calculated degree days and past developmental studies. To monitor embryonic stage, cages were dug up and opened. If possible, electronic data loggers were awakened and temperature data was downloaded for calculation of degree days. One inner embryo chamber was opened, and a subset of embryos was placed in a 50 ml conical-bottomed polyethylene sample tube and placed in a cooler with ice packs. The inner embryo chamber was replaced, and the embryo cage was reassembled and re-buried. Embryos were transported back to the lab for microscopic evaluation of developmental stage.

Embryo cage retrieval and sample collection

Embryonic development of surf smelt proceeds quicker or slower depending on the ambient temperature (Penttila, 2007; Yap-Chiongco, 1941). As such, we sought a method for estimating the time for embryos to reach a given developmental stage across different seasons and temperature regimes. The degree-day (DD; °C·days) approach is a method of quantifying an organism's cumulative thermal experience that is increasingly being used to describe fish growth and development (Chezik, 2013). In an effort to retrieve embryo cages at a consistent developmental stage, we aimed to retrieve embryos at approximately 175 DD, calculated as the sum of daily mean temperatures (°C). If available, daily mean temperatures from electronic data loggers were used, otherwise, average daily air temperatures reported for Everett, WA or Seattle, WA were used depending on data availability.

Retrieved embryo cages were placed in a cooler and transported to the lab for processing and microscopic assessment of developmental stage and survival. In the lab, a clean plastic spoon was used to transfer subsets of loose/non-adherent embryos into seawater-filled petri dishes to assess the number of live embryos that reached various developmental stages. Observations of surf smelt embryonic development have been published (Yap-Chiongco, 1941) and are largely consistent with embryonic development of Pacific herring as described by Kawakami et al. (2011). Due to the improved visualizations and increased accessibility of more recent publications, we compared the development of surf smelt in this study to the Pacific herring embryonic stages according to Kawakami et al. (2011). Embryos with the appearance of an optic vesicle (Figure 8f) or appearance of lens and otic vesicle (Figure 8g), but prior to appearance of melanophores on the eye was considered an early-stage embryo. Embryos with melanophores on the eye (Figure 8i), were considered eyed embryos. Embryos with fully pigmented eyes and melanophores on the body (Figure 8k) were considered late-stage/pre-hatch embryos. Adherent embryo slides were placed in a seawater-filled petri dish and assessed similarly for developmental stage and survival. All embryos were euthanized by over-anaesthetization in MS-222 for greater than 10 minutes followed by submersion in dilute sodium-hypochlorite solution.

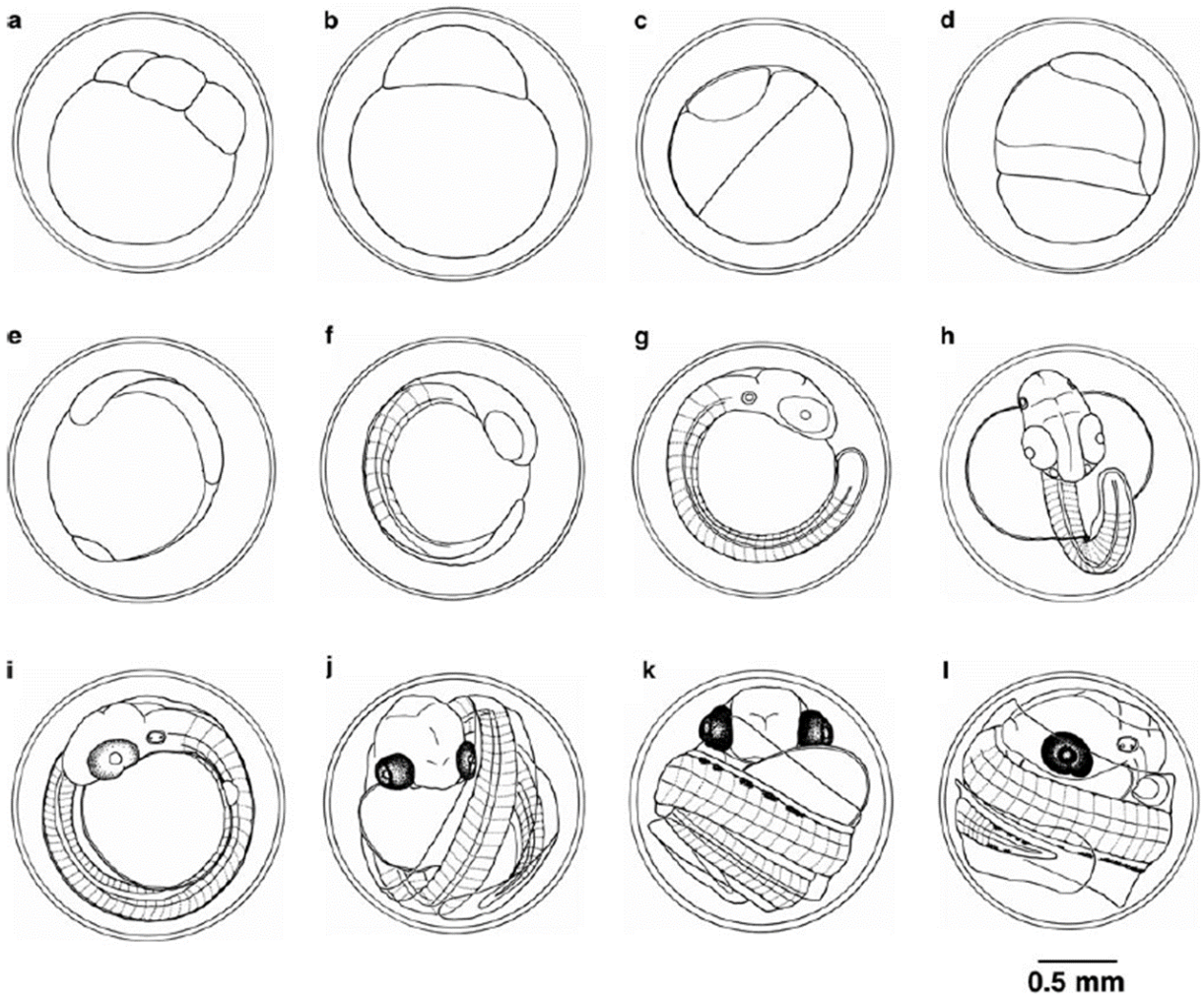

Figure 8: Stages of Pacific herring embryonic development through pre-hatch stage. Appearance of the lens and otic vesicle can be seen in g) and appearance of melanophores on the eye is shown in i). Figure modified from Kawakami et al., 2011.

Deployment Trial 1: January 2021

Mark Tagal collected gametes from live, ripe smelt caught by recreational fishermen at Ross Point, near Port Orchard, WA (Table 3, Figure 3) on Tuesday, January 12, 2021. Eggs and milt were obtained from four females and six males and combined immediately after collection resulting in ~ 50 g of fertilized embryos. Embryos were maintained in 50 ml conical-bottomed, polypropylene sample tubes in ambient seawater overnight at 4°C. Fertilization rate was calculated as 95% the next morning (19 hours post fertilization).

Embryo cages were deployed at Utsalady Beach on the north end of Camano Island (Figure 3). In this first deployment, each embryo cage included one inner embryo chamber with adherent embryos and one with non-adherent embryos to allow evaluation of whether both embryo types develop similarly and exhibit similar survival to late-stage embryonic development. This

comparison also helped determine which type of embryo is more practical for field deployment and sampling. Three cages were deployed at three tidal elevations ranging from +6 ft to +9 ft, for a total of nine cages. Six of the cages were deployed on a private beach to the west of the public boat ramp and the other three cages were deployed on a public beach to the east of the boat ramp (Figure 9). Electronic data loggers were deployed inside three of the deployment cages, one at each tidal elevation.

Figure 9: Embryo cage deployment locations at Utsalady Beach, Camano Island, WA

On February 8, 2021 (27 days post fertilization [dpf]; ~158°C·day) all cages were retrieved and transported from the deployment location to the lab in a cooler with wet ice. In the lab, three random subsets of embryos from each non-adherent embryo chamber were examined and survival assessment was conducted. Additionally, one random subset of embryos from each non-adherent embryo chamber was collected to assess developmental stage. All nine slides containing adherent-type embryos were examined and analyzed for survival. Electronic data loggers were recovered at the cage retrieval and light and temperature data for the entire deployment were successfully uploaded.

Deployment Trial 2: May 2021

Smelt gametes were collected from live, ripe adult smelt caught by recreational fishermen at Twin Rivers (Figure 3, Table 3) on May 14th, 2021. Eggs and milt were combined immediately after collection, resulting in approximately 90 g of fertilized eggs. Fertilized embryos were maintained in 50 ml conical-bottomed, polypropylene tubes in ambient seawater collected from the Twin Rivers Beach at 4°C. The fertilization rate was greater than 90% approximately 48 hours post fertilization.

On May 16, 2021, smelt embryos were transported to the deployment locations in a cooler with wet ice. Two sites were selected for deployment in this second trial to compare development success between a beach typified by coarse substrata, (the same Utsalady Beach site used in Deployment Trial 1) with a beach typified by finer-grained substrata. This second location, Tulare Beach, was located in Port Susan, in the Whidbey Basin, approximately 16 km north of Everett, WA (Figure 3).

All embryos used in Deployment Trial 2 were the non-adherent type. Embryos were weighed into each inner embryo chamber (2.5 g/chamber) and three inner embryo chambers were placed in each cage. A total of 12 embryo cages were deployed at three tidal elevations ranging between +7 ft and + 10 ft. Nine embryo cages were deployed at Utsalady Beach as in deployment 1 and three embryo cages were deployed at Tulare beach. The embryo cages were placed at slightly higher tidal elevations to be more consistent with natural surf smelt spawning habitat. Electronic data loggers were included in three of the deployment cages at each site. On May 20, 2021 (5 dpf) the 3 cages deployed at the public beach at Utsalady were retrieved due to a public complaint of a tripping hazard. Because of this short incubation time, no embryos from those three cages were used for development/survival measurements. Most remaining embryo cages were checked on May 24, 2021 (8 dpf); samples of embryos were removed from one inner embryo chamber from cages 1, 3, 5 at Utsalady Beach and cage 1 at Tulare Beach to evaluate developmental assessment. All cages were retrieved May 26, 2021 (10 dpf) and examined microscopically for developmental stage and survival. Data loggers were recovered, however due to battery issues or user error, no data was recorded. As a result, degree days for Deployment Trial 2 were estimated from reported average daily air temperatures from Everett, WA and/or Seattle, WA depending on availability.

Deployment Trial 3: July 2021

Gametes were collected from ripe male and female smelt caught by a commercial fisherman off of Utsalady Beach, WA on July 30, 2021 (Figure 3, Table 3). Gametes were collected when the fisherman came ashore, approximately one hour after the fish were caught. Approximately 100 grams of eggs were fertilized and maintained in 50 ml conical-bottomed, polypropylene tubes in ambient seawater overnight at 4°C. The next morning, cell masses had turned white, indicating the embryos were non-viable. Field deployment was cancelled due to low fertilization rate/viability.

Deployment Trial 4: August 2021

On August 18, 2021, gametes were collected from live, ripe smelt caught by a commercial fisherman during a spawning event near the Maple Grove boat launch on Camano Island, WA (Figure 3, Figure 10, Table 3). Eggs and milt were immediately combined after collection resulting in approximately 100 grams of fertilized eggs. At 8 hours post fertilization, fertilization rates were > 95% and early embryonic development was progressing normally (Figure 11). Embryos were maintained in 50 ml conical-bottomed, polypropylene tubes in ambient seawater at 4°C for approximately 48 hours before deployment.

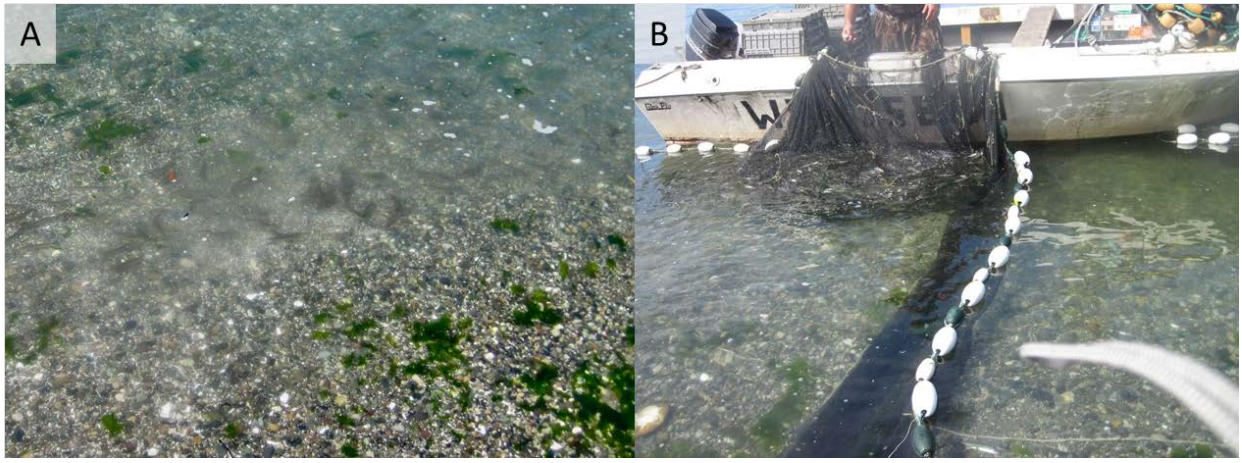

Figure 10: A) Active surf smelt spawning event at Maple Grove boat launch, Camano Island, WA on August 18, 2021. B) Commercial fisherman capturing smelt in a beach seine.

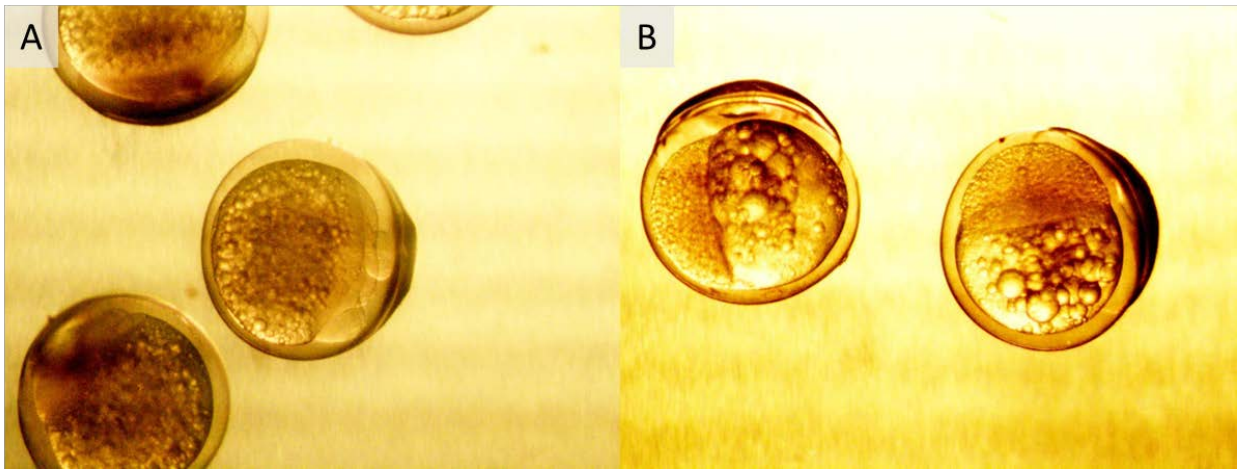

Figure 11: A) Surf smelt embryos at 4-cell stage (4 hpf) and B) at late blastula stage (~24 hpf)

On August 20, 2021, smelt embryos were transported in a cooler to the Utsalady Beach and Tulare Beach deployment sites to test effects of tidal elevation and beach substrate. At Utsalady, six cages were prepared, each containing 3 inner embryo chambers with non-adherent embryos. The six cages were deployed on the same private beach to the west of the Utsalady boat ramp used in the previous deployment trials. The embryo cages were placed at three tidal elevations, between +6.2 ft and +8 ft. Three electronic data loggers were included in deployed embryo cages. An additional three embryo cages each with 3 inner embryo chambers were deployed at the same Tulare Beach location as Deployment Trial 2. On August 24, the embryo cages were monitored. Cages with data loggers were dug up and opened and data was uploaded from the temperature loggers before the cages were reassembled and re-deployed. All 12 embryo cages from both Utsalady and Tulare were retrieved August 31 (13 dpf). Four random subsets of non-adherent embryos were collected from each embryo cage for a total of 48 samples that were assessed for survival. Electronic data loggers collected data for only the first four days of the deployment, likely due to user error during the re-deployment on August

24th. Measured average daily temperatures from the data loggers were compared to reported average daily air temperatures at Everett, WA for the same days. When temperature data for Everett were missing, daily temperatures for Seattle, WA were substituted. Measured cage and reported air average daily temperatures were similar, so degree days were estimated from daily average air temperatures.

4. Results

Manual spawning resulted in fertilization rates ≥ 90% for all attempts where gametes were stripped from live fish and eggs were fertilized immediately in the field. All cages were successfully retrieved across the three deployment trials. Deployed surf smelt embryo survival rates ranged from 41 - 86% in Deployment Trial 1 to 0% in Deployment Trial 2 (Table 4). Embryo survival was significantly lower in August (11%) compared to January (54%) (Table 4, ANOVA of the proportion of embryos surviving by deployment month, $F_{(1,13)}= 94.14$; $p<0.001$).

Table 4: Fertilization rate, estimated degree days, and mean percent embryo survival (± standard deviation) across deployment trials.

Trial	Dates	Fert. rate	Est. DD	Cage	Site	Tidal Elevation	Mean percent survival ± SD (Total N)		Notes
							Non-adherent	Adherent	
1	1/14/21-2/8/21	95%	158	1	Utsalady Private W	Low	41 ± 5% (178)	40% (112)	Rinsed, very hard to count slide
				2	Utsalady Private W	Mid	60 ± 13% (203)	35% (153)	Rinsed, very hard to count slide
				3	Utsalady Private W	High	56 ± 8% (172)	52% (84)	
				4	Utsalady Private E	Low	48 ± 7% (169)	-	Slide broke, very few embryos adhered
				5	Utsalady Private E	Mid	57 ± 6% (176)	74% (103)	
				6	Utsalady Private E	High	86 ± 2% (200)	-	Unable to count slide
				7	Utsalady Public	Low	46 ± 9% (145)	-	Unable to count slide
				8	Utsalady Public	Mid	44 ± 8% (167)	-	Rinsed, unable to count slide
				9	Utsalady Public	High	51 ± 9% (150)	11% (56)	
2	5/16/21-5/26/21	≥90%	148.9	1	Utsalady Private W	Low	0%	NA	Retrieved May 20, 2021 due to a public complaint
				2	Utsalady Private W	Mid	0%	NA	
				3	Utsalady Private W	High	0%	NA	
				4	Utsalady Private E	Low	0%	NA	
				5	Utsalady Private E	Mid	0%	NA	
				6	Utsalady Private E	High	0%	NA	
				7	Utsalady Public	Low	0%	NA	
				8	Utsalady Public	Mid	0%	NA	
				9	Utsalady Public	High	0%	NA	
				10	Tulare Beach	Low	0%	NA	
				11	Tulare Beach	Mid	0%	NA	
				12	Tulare Beach	High	0%	NA	
3	7/30/2021	<70%	NA	NA	NA	NA	NA	NA	Deployment cancelled due to low fertilization rate.
4	8/18/21-8/31/21	≥95%	247.8	1	Utsalady Private W	Low	89 ± 3% (422)	NA	
				2	Utsalady Private W	Mid	87 ± 8% (252)	NA	

	3	Utsalady Private W	High	88 ± 9% (230)	NA	
	4	Utsalady Private E	Low	91 ± 4% (326)	NA	
	5	Utsalady Private E	Mid	89 ± 2% (275)	NA	
	6	Utsalady Private E	High	90 ± 10% (275)	NA	
	7	Tulare Beach	Low	0%	NA	fungus present in inner embryo chamber
	8	Tulare Beach	Mid	0%	NA	
	9	Tulare Beach	High	0%	NA	

Deployment Trial 1: January 2021

Cage design

The inner embryo chamber/outer cage design allowed sand and shells to penetrate the outer cage, without getting into the inner embryo chambers. This allowed the embryos to be near the substrate and sediment pore water that they would normally be exposed to, without the sediment sticking to the eggs and interfering with microscopic examination and sampling for analytical chemistry (Figure 12).

Figure 12: Outer cages allowed movement of sand and shells (A, B, C), while inner embryo chambers remained clear of debris (D).

Embryonic development and embryo survival

Daily average temperatures ranged from 1°C to 7°C resulting in slow embryonic development. Embryo cages were deployed for 25 days before reaching the targeted ~175 DD. Actual measured degree days ranged between 153 and 162 based on the electronic data loggers (Figure 13), which we would expect to correspond to an eyed embryo developmental stage based on our previous laboratory studies (NOAA, unpublished data). However, embryonic development was highly variable. Within the same cup, there were groups of embryos that had barely visible eyes and some with well-defined and silvered eyes (Figure 14). Embryo survival ranged from 41 to 86% (Table 4). During microscopic evaluation, a few invertebrates were observed in the egg cups – however evidence of predation (e.g., large numbers of empty chorions) was not observed, suggesting the cage and egg cups were successful at protecting the smelt embryos from predators.

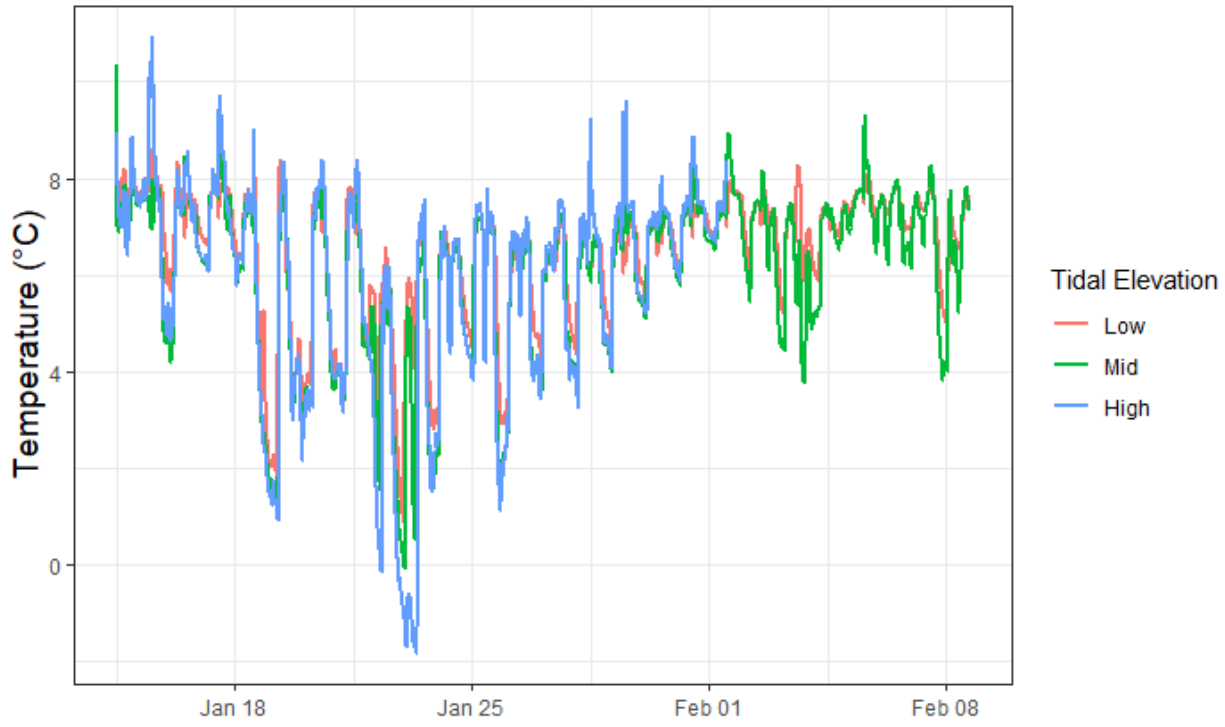

Figure 13: Temperature profiles for embryo cages at the low (red), middle (green) and high (blue) tidal elevations. The high tidal elevation data logger only captured data until February 2, 2021, when it was retrieved.

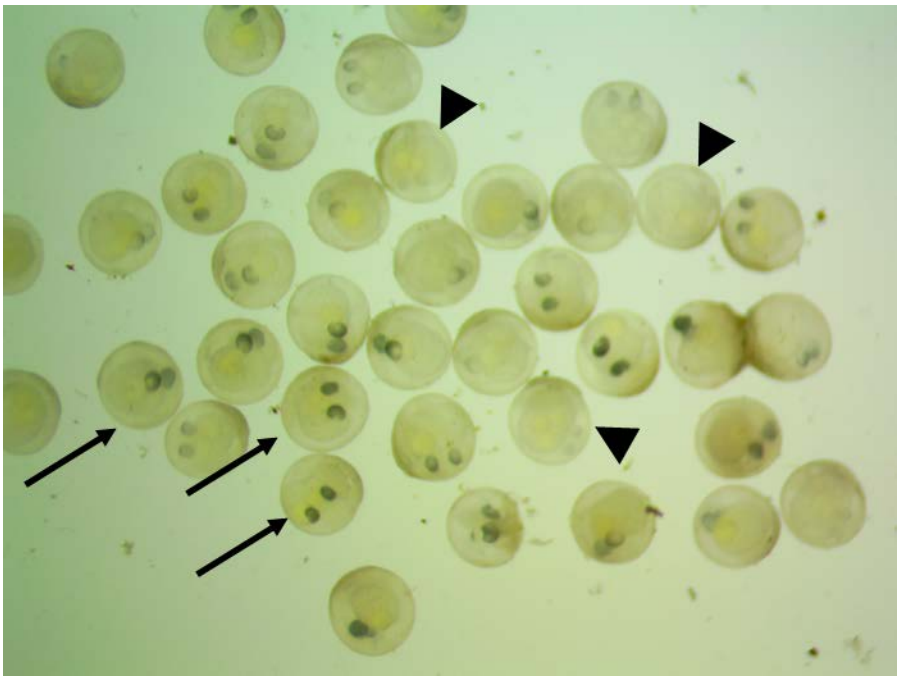

Figure 14: Variability in developmental stage within a deployment cage for non-adherent embryos. Arrowheads indicate embryos with minimal eye pigmentation. Arrows indicate embryos at more advanced stages of development with darkly pigmented eyes.

Table 5: Percentage of smelt embryos that reached early embryonic development with appearance of optic vesicle vs eyed-embryo stage with eye pigmentation

Cage	Site	Tidal Elevation	Early-stage embryo	Eyed-embryo	Dead	Total n
1	Private W	Low	24%	18%	58%	170
2	Private W	Mid	40%	27%	32%	245
3	Private W	High	1%	21%	78%	94
4	Private E	Low	31%	41%	28%	95
5	Private E	Mid	41%	35%	25%	133
6	Private E	High	12%	59%	29%	97
7	Public	Low	0%	58%	42%	105
8	Public	Mid	12%	27%	61%	164
9	Public	High	9%	38%	52%	107

Adherent vs non-adherent embryos

Adherent embryos appeared in excellent health and seemed to develop more quickly and synchronously than non-adherent embryos (Figure 15). Adherent embryos consistently had darkly pigmented eyes and melanophores along the egg yolk and tail, whereas non-adherent embryos had more variable eye pigmentation and no visible melanophores. However, the slides often acted as a substrate for biofilm growth which made microscopic examination difficult or impossible (Table 4, Figure 16). For three of the nine cages, biofilm growth prevented

microscopic evaluation of developmental stage or survival and precluded any statistical analysis.

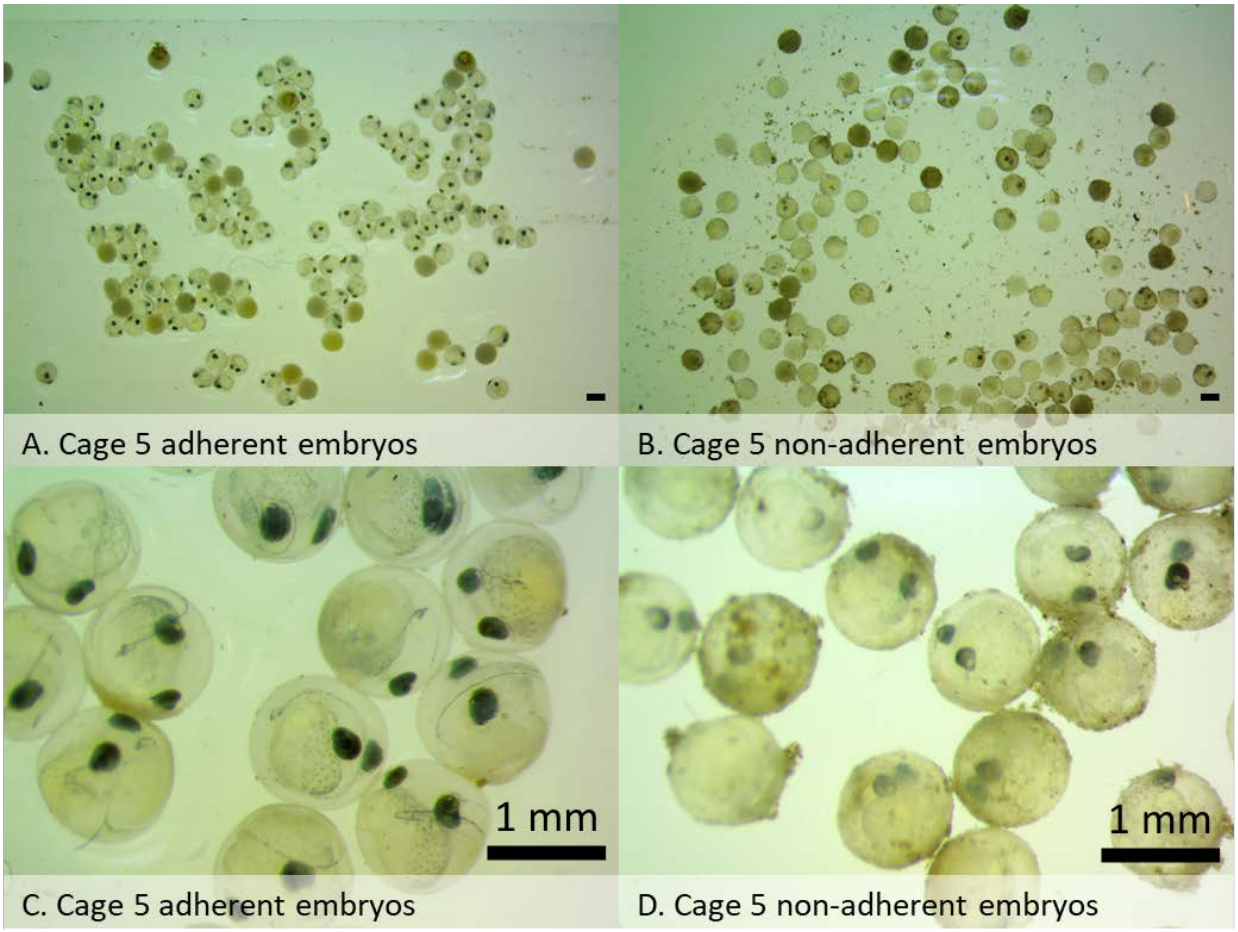

Figure 15: Adherent and non-adherent embryos from the same deployment cage.

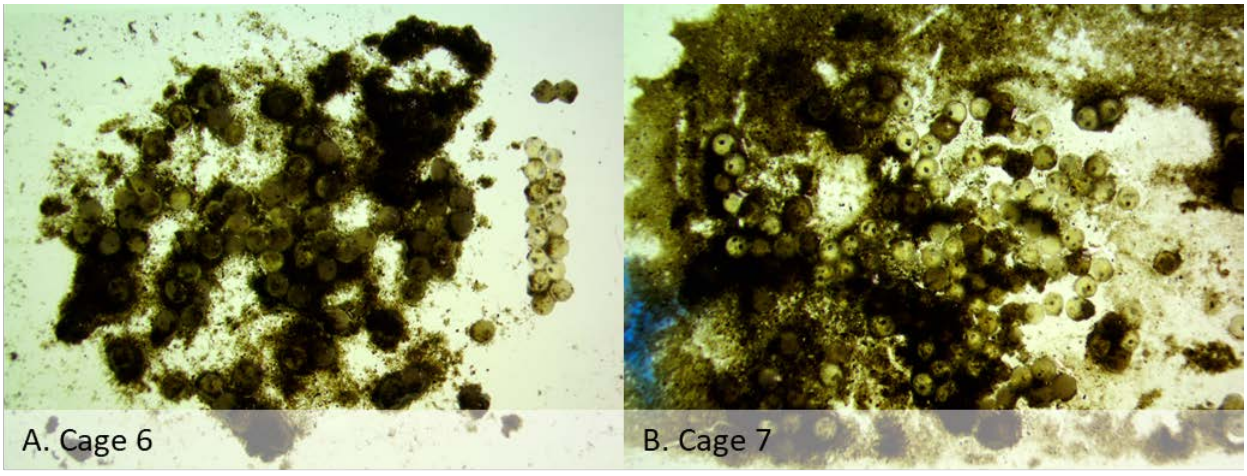

Figure 16: Deployed adherent embryo slides with biofilm fouling.

Tidal Elevation

There were no apparent differences in embryo development, fouling of the adherent embryos, or embryo survival based on tidal elevation. The mean percentage of embryos that reached the eyed stage was lower for the mid tidal elevation stratum (30%) compared to the low and high elevations (both 39%), but there was not a significant difference in the developmental stage based on tidal elevation (Table 5, ANOVA of the proportion of embryos reaching eyed stage by tidal elevation, $F_{(2,6)}$= 0.3666; p=0.7076). Extreme fouling that prevented accurate survival counts was observed in one embryo cage at each tidal elevation suggesting this occurred independent of tidal elevation, however we did not have sufficient power to test this statistically (Table 4). Although mean survival of embryos was greatest in the high elevation stratum (64%), followed by mid (54%) and low elevation (45%) strata, these differences were not statistically significant (Table 4, ANOVA of the proportion of surviving embryos by tidal elevation, $F_{(2,8)}$ =1.90; p=0.23, with both normality (Shapiro-Wilk test, p=0.51) and homoscedasticity (Brown-Forsythe test, p=0.52) assumptions met)).

Deployment Trial 2: May 2021

During the May deployment, daily air temperatures were warmer than Deployment Trial 1 (January) with average temperatures ranging from 11.7°C to 15.8°C and maximum temperatures ranged from 15.5°C to 23.9°C. This resulted in a shorter development time for Deployment Trial 2 (10 days, 148.9°C·day) versus Deployment Trial 1 (25 days, 158°C·day). Embryos were retrieved after 10 days (148.9°C·day) which we would expect to correspond to an eyed embryo stage.

On the fifth day of the May deployment, a citizen contacted WDFW to report that the embryo cages were a tripping hazard. This was not entirely unexpected, but it highlighted the benefit of working with landowners to deploy the cages on private beaches or in secluded areas where they are less likely to attract attention. As quickly as possible following the complaint, the cages from the public beach were retrieved. It looked as though the cages had been dug up and upon inspection, there was 100% mortality in those cages. On day five of the deployment, the embryos at the adjacent private beach at Utsalady appeared healthy and had developed to an early-stage embryo where the tail is freed from the yolk (Figure 17). The sand had moved around during the previous tides, but the embryo cage was packed with sand and shells, allowing the embryos to stay moist even at low tide (Figure 18). However, surf smelt embryos at Tulare beach on day five showed 100% mortality. The sand and sediment moved considerably with the tides at this location and the embryo cages were found on the surface. One anchor had come completely loose (or had been pulled up) and was also lying on the surface (Figure 19).

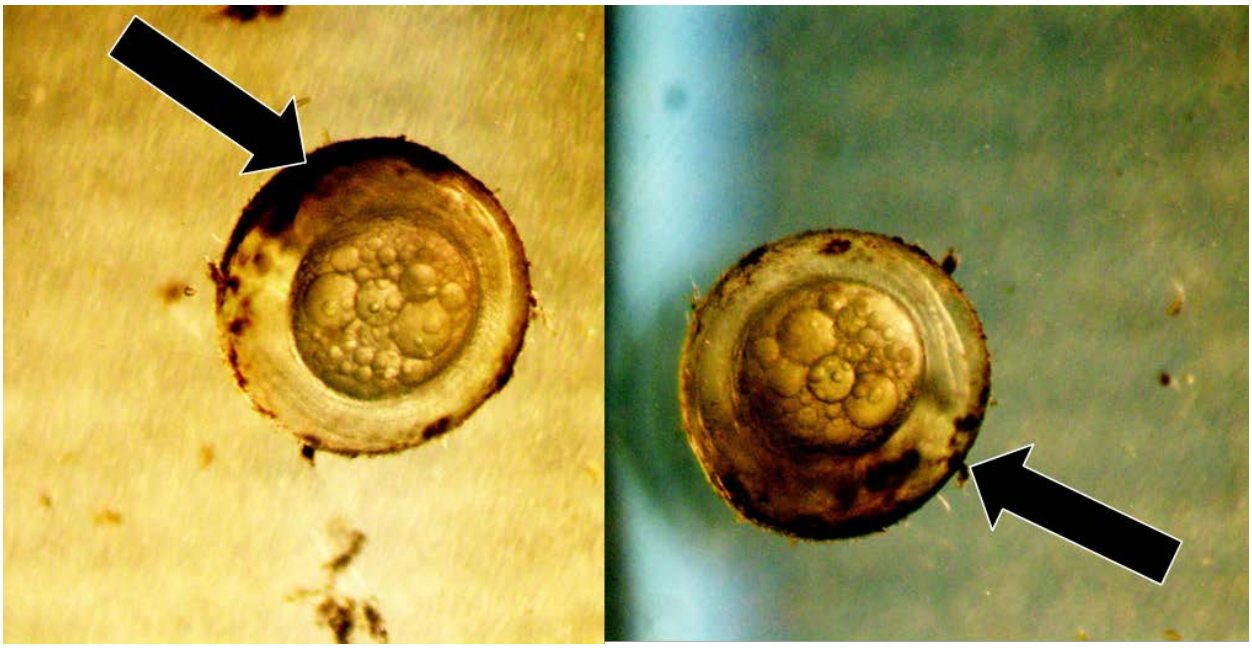

Figure 17: Surf smelt embryos deployed at the private beach west of Utsalady boat ramp at 6 dpf. Arrows point toward the embryo head.

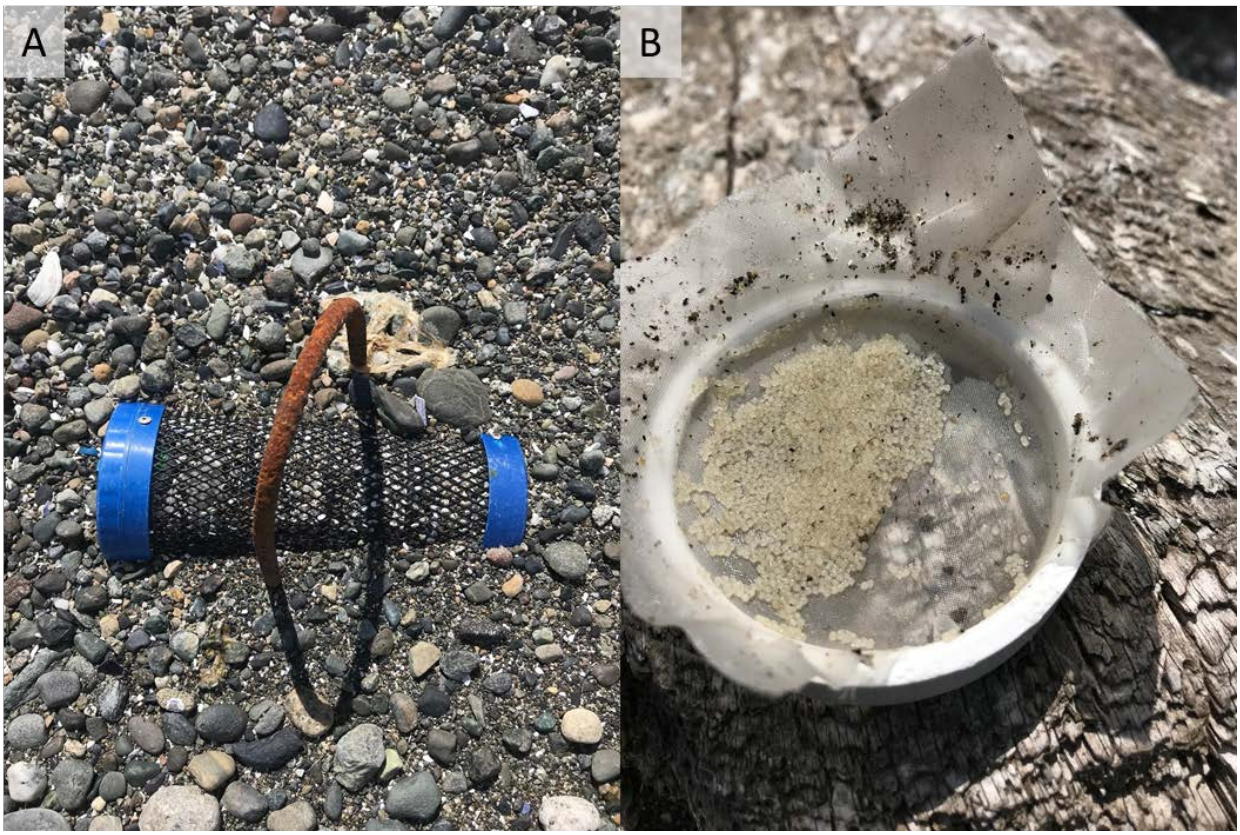

Figure 18: Surf smelt embryo cages monitored at Utsalady Beach on May 20, 2021 were near the surface, but packed with sand and shells so embryos were still moist at low tide.

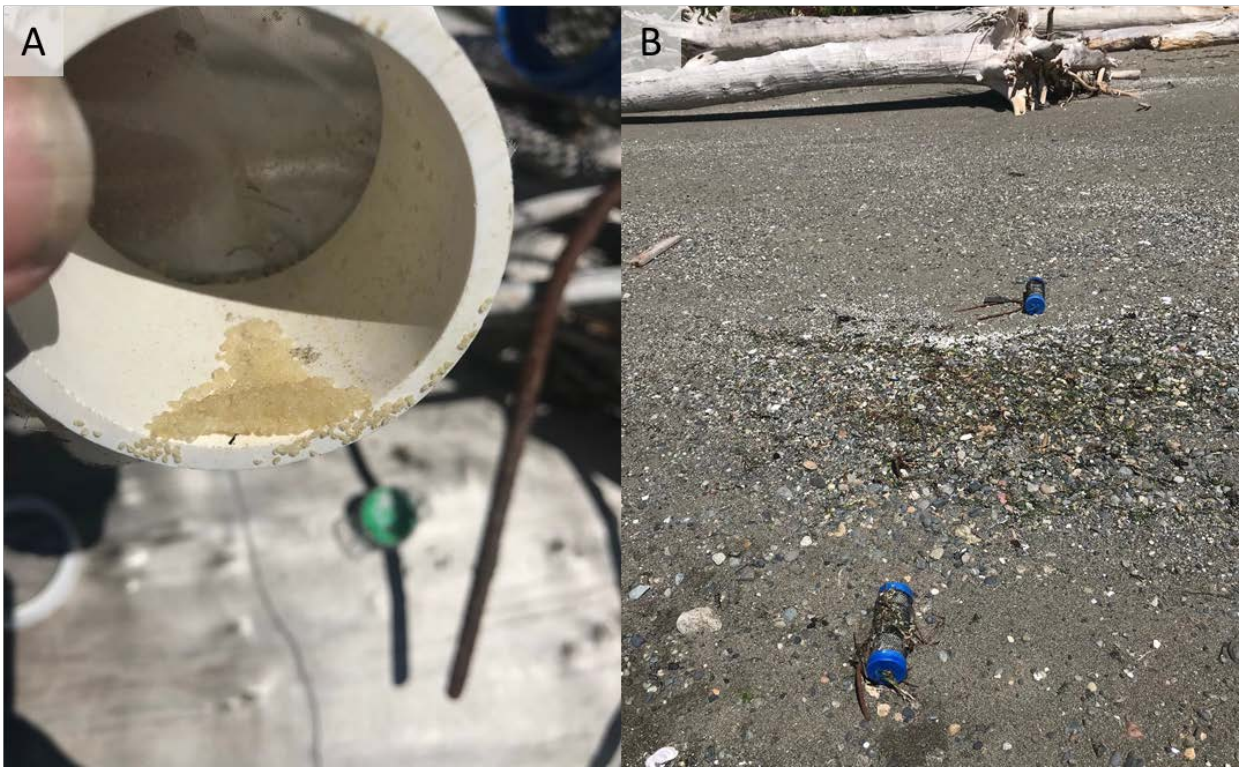

Figure 19: Surf smelt embryo cages monitored at Tulare Beach on May 20, 2021 were laying on the beach surface.

On the ninth day of deployment (May 24, 2021), the remaining cages at both locations were monitored and 4 cages were subsampled for microscopic examination. At this time, there was 100% mortality in all monitored samples, and the embryos appeared dried out and rubbery. All embryo cages were then retrieved, and 100% mortality was confirmed in all cages from both locations. On the day of retrieval, cages at Tulare beach were submerged even at low tide.

Deployment Trial 4: August 2021

During the August deployment, average daily temperatures ranged from 16.4°C to 18.9°C and maximum daily air temperatures ranged from 19.4°C to 27.2°C resulting in an estimated 194°C·day by 13 dpf. At 10 dpf (~143°C·day), the eyes had formed, and the embryos were mobile. As with the February deployment, there were no significant differences in survival based on tidal elevation (ANOVA of the proportion of surviving embryos by tidal elevation, $F_{(2,9)}$= 0.1283; p= 0.88). However, embryo survival was much lower in August compared to February. Mean smelt embryo survival ranged from 9% to 13% at Utsalady Beach and was 0% at Tulare Beach (Table 4). As with the May deployment, wave action moved the rocks and sand around considerably at Tulare Beach and the embryo cages were resting on top of the beach at low tide, although embryo cages remained damp even at low tide (Figure 20).

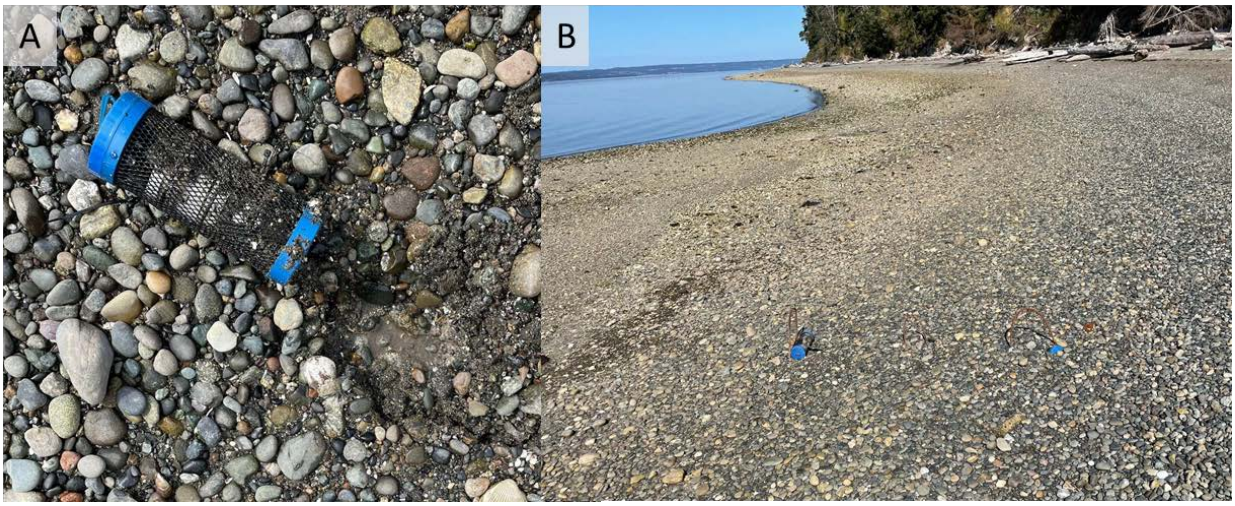

Figure 20: A) Embryo cages deployed at Tulare beach were submerged in water at low tide. B) Wave action moved the rocks and sand around considerably at Tulare beach, leaving the embryo cages on the surface of the beach.

5. Discussion

This study successfully demonstrated a method for deploying surf smelt embryos into the upper intertidal zone of Puget Sound beaches to use as a monitoring tool to assess the health of the intertidal habitat and forage fish embryos. The intertidal habitat lies at the interface between land and sea, making it susceptible to land-based sources of pollution including stormwater runoff, combined sewer overflows, or surface oil spill "bathtub rings". Surf smelt and other forage fish species spawn in the intertidal and shallow subtidal habitats, placing vulnerable life stages (e.g., embryos and newly hatched larvae) in harm's way. This method to deploy manually-spawned smelt embryos provides an *in situ* monitoring tool to assess the impacts of pollution to the upper intertidal habitat or fish embryos therein.

Cage design

Overall, the combination of outer cage with inner embryo chamber met most of the criteria that we were hoping to achieve in this study. We modified existing methods for rearing surf smelt in a laboratory setting and optimized them for use in the field. From our deployments, we determined that the optimal smelt embryo deployment cage consisted of two or more inner embryo chambers inside a larger bait cage, anchored in place with U-shaped rebar. The deployment cages were easy to assemble, deploy and retrieve and were able to support normal embryonic development through the pre-hatching stage. These methods are similar to methods previously used to deploy herring embryos in the shallow subtidal for contaminant monitoring (Incardona et al., 2012a; West et al., 2019). The outer bait cage permitted movement of sand and shells, allowing the embryos to be close to the sediment, sediment pore water, and potential associated contaminants. The Nitex mesh of the inner embryo chambers appeared to exclude beach substrate and allowed sufficient water flow to support development of both adherent and non-adherent embryos. The outer bait cage and inner embryo chambers together

excluded most organisms and there was no evidence of smelt embryo predation. The anchoring method was sufficient to withstand wave action and keep cages in place, however, it was not able to maintain a constant depth within the beach substrate. Under natural circumstances, the shifting of the sand and rocks by wave action helps to bury newly fertilized eggs in the top few cm of substrate, slowly shifting the embryos deeper into the substrate and lower in tidal elevation as the embryos develop (Penttila, 1978). However, the anchors held the cages and embryos at a constant depth regardless of how the gravel moved around with the tides, resulting in some cages becoming buried and others becoming exposed, the latter potentially exposing the embryos to thermal and desiccative stress. Therefore, embryo cages should be monitored every other day throughout the deployment period and repositioned to account for shifting substrate elevation, particularly during periods of warm weather.

Manual surf smelt spawning and fertilization

This study corroborated previous lab studies (NOAA, unpublished data) showing that fertilization rates and embryo viability are highest when gametes are stripped from live fish and when fertilization occurs as soon as possible after strip-spawning. Ripe smelt were spawned immediately after capture in three of the four deployment trials, resulting in > 90% fertilization rates. These methods are consistent with previously published surf smelt fertilization methods, in which surf smelt eggs were stripped and fertilized at the site of collection (Hawkes and Stehr, 1982). One fertilization attempt resulted in poor fertilization, likely because gametes were stripped from dead or dying fish that had been caught by a commercial fisherman approximately one hour prior to the fertilization attempt.

Embryo survival and development

Survival of smelt in the deployed cages that had mostly remained buried, with substrate surrounding the inner embryo chambers throughout the incubation period (Utsalady; 54% in February, Trial 1 and 11% in August, Trial 4) was roughly consistent with reports of surf smelt embryo survival in the field. Quinn et al. (2012) reported embryo survival rates in wild surf smelt of 25% in summer and 80% in September. Penttila (1978) reported survival of naturally spawned surf smelt embryos within the first 2-4 days after fertilization as 20-40%. This author concluded that while 70-80% survival may be achieved in ideal conditions, 10% survival is likely under normal field conditions. Such field assessments likely underestimate embryo mortality, particularly in later stages of development, due to dead embryos detaching from the beach substrate and washing away, whereas our study accounted for all embryos over the entire incubation period.

Both field and laboratory studies have indicated that thermal stress and desiccation are among the primary causes of mortality in wild surf smelt embryos (Penttila, 1978; Rice, 2006; Lee and Levings, 2007). This could explain the higher survival of embryos during our winter deployment compared to our May and August deployments. The upper lethal temperature for surf smelt embryos has been reported to be around 30°C (Rossel and Dinnel, 2006). Maximum air temperatures reached 23.9 and 27.2°C in our May and August deployments, respectively.

However, it is possible that the gravel surface could have exceeded air temperatures, approaching the upper lethal temperature for surf smelt.

Perhaps more likely is that as wave action shifted the sand and gravel around, embryo cages spent too much time at the surface of the substrate where they were vulnerable to desiccation. It appears that the combination of cages remaining buried, with substrate entering the cage and packing around the inner embryo chamber, most successfully mimicked the natural condition of wild spawned embryos and resulted in the greatest survival. To ensure this for future studies we recommend checking cages periodically to ensure they remain buried properly in the substrate to achieve this condition throughout the incubation period.

We observed more variation in developmental timing of field-deployed embryos than in previous laboratory experiments, particularly in the non-adherent embryos. In the lab, smelt embryos typically reach specific developmental milestones within a relatively narrow time frame (NOAA, unpublished data). We suspect that in field deployed embryos, development in some embryos progresses to a given stage, arrests, and the embryo dies. Because it is difficult to determine using visual examination whether an embryo is alive (unless it has begun to deteriorate), embryos having died at differing developmental stages over a short time period present the appearance of a wide range of developmental stages within a sample. Lee and Levings (2007) found that embryos exposed to a low relative humidity treatment (79.8%) reached the eyed stage before development arrested and eggs decayed.

Field deployed embryos may also experience different micro-scale environmental conditions within the inner embryo chamber, perhaps related to temperature or oxygen. In particular, it appeared that non-adherent embryos that had clumped together within the chamber exhibited a broader range of developmental stages. Such clumping may reduce the oxygen supply for individual embryos if it reduces the embryo surface area exposed to water (or air). This problem could be exacerbated if larger masses of eggs are needed for contaminant analyses. Adhering eggs to a glass plate in a monolayer could mitigate the clumping issue, but may result in biofilm growth, which is problematic both for observing embryo development as well as analytical chemistry for contaminants.

Loosanoff (1938) found that embryos that had been emersed for 36 hours had a dry, shriveled appearance but remained viable and could develop to hatching when placed back in seawater, suggesting they are somewhat resistant to desiccation. Indeed, an immersion/emersion cycle appears to be necessary for optimal smelt development. The embryos in our cage deployments would have been out of the water for no more than 12 hours at a time, suggesting that dessicative stress alone should not have been a significant source of mortality. Later in the season, at the beginning of the May deployment, embryos were emersed for ~9 hours a day from mid-day to late afternoon. This is the hottest part of the day and could have allowed for sediment temperatures to increase and dry out on consecutive days. Moreover, the cages deployed in May were observed to protrude from the substrate at the day-five check, and there appeared to be little substrate inside the cage surrounding the inner embryo chamber, possibly

increasing their exposure to excessive heat. In August, the longest low tides occurred earlier in the day, resulting in embryos being submerged during the hottest part of the day.

Adherent vs non-adherent embryos

Embryos retrieved from Deployment Trial 1 indicated that overall, adherent embryos appeared healthier, developed more synchronously and reached a more advanced stage of development than the non-adherent embryos. Adherent eggs were typically configured on the glass slides in a monolayer of embryos rather than in clumps, as were the non-adherent embryos, which may have promoted better embryonic development. Using a lower embryo density in the non-adherent inner embryo chamber could possibly promote less embryo clumping and better development. This would preclude the ability to perform analytical chemistry on the embryos, however, use of a passive sampler such as low density polyethylene strips (LDPSs) could be paired with the embryo cages to overcome this challenge. Polycyclic aromatic hydrocarbon (PAH) concentrations in LDPSs have been shown to be well correlated with PAH levels in herring embryos although the pattern of accumulated PAHs differed, so data from LDPSs should be used with caution (West et al., 2019).

Tidal Elevation

No significant differences in smelt survival or development were observed related to tidal elevation within a deployment. However, across deployments, we observed higher mortality (100%) and embryos appeared dried out and rubbery when cages were planted at slightly higher tidal elevations in May. This observation is confounded by differences in the degree to which cages remained buried in the substrate between the deployments. Published research on optimal tidal elevation is contradictory. Loosanoff (1938) deployed smelt embryos at various tidal elevations and substrate depths and found highest embryo survival at +10 and +11 tidal elevations within the top 2.5” of sediment. On the other hand, lower mortality of smelt embryos was observed at lower tidal elevations (+8.5 ft) compared to higher elevation (+10.5 ft) in field surveys of wild smelt embryos (Quinn et al., 2012). It’s possible that in field comparisons of mortality, dead embryos are more likely to detach and be washed away at lower elevations (Penttila, 1978). Additionally, these studies do not provide sufficient detail about the season, air or sediment temperatures, or other site characteristics (e.g., shading) that could impact temperature or relative humidity of the tidal elevations examined and subsequent embryo survival. There is likely a complex interplay between tidal elevation, depth, temperature, and relative humidity that defines an ideal spawning habitat. As such, these methods (e.g., appropriate tidal elevation) may need to be optimized for different seasons and sites.

Given the ability of wave action to move the beach substrate around and away from the embryo cages, deployment elevations in the lower range of natural spawning habitat (+6 to +8 ft) may help reduce the risk of desiccation, particularly during summer deployments. Alternatively, deploying cages in higher tidal deployments may reduce the degree to which wave action moves the substrate around. This also likely depends on the substrate size and

prevailing wave action at a particular site. In any case, to ensure cages remain sufficiently buried throughout the deployment period, it seems clear they should be checked periodically, and re-buried if needed.

Site selection

Embryos deployed at Utsalady Beach exhibited better survival than Tulare (which showed 100% mortality). Utsalady Beach is a common surf smelt spawning site and popular location for recreational smelt fishing. The beach is a gently sloping shoreline, with substrate consisting of sand, shell fragments, and small cobble. There are few overhanging trees that shade the beach, however the beach is north-facing, which limits solar exposure. Tulare Beach is an open shoreline with limited shading from trees, and areas of similar or finer substrate, however it is west facing, such that beach surface temperatures could become quite elevated due to more direct solar exposure. The embryo cages should have provided some shading to the embryos; however, it may not have been sufficient to reduce thermal and desiccative stress resulting from high solar exposure. A side-by-side deployment with contaminant analyses at Tulare and Utsalady Beaches in the winter, when thermal and desiccative stress are less extreme, would help determine whether temperature is a factor that contributed to the high embryo mortality at Tulare beach. A potentially more likely and simpler explanation for high mortality at Tulare is that cages at that site more consistently became exposed, whereas cages at Utsalady more consistently remained buried (except for Trial 2 in May).

In addition to environmental variables, embryo cages can be disturbed by curious passersby. During our May deployment, a well-meaning citizen reported the embryo cages as a tripping hazard at Utsalady Beach. One can imagine that a U-shaped rebar anchor might be particularly hazardous to recreational fishermen fishing for smelt at high tide when the embryo cages would be under the water and hard to see. As such, it would be preferable to work with private landowners or find remote areas for use as deployment locations. For example, there is optimal spawning habitat on the northwest side of Camano Island, between Utsalady and Maple Grove boat ramps which may provide a more ideal location for cage deployment. The beach is only accessible by boat and has ample overhanging trees that provide shading to the beach. In situations where samples need to be deployed along public shorelines or in contaminated areas that are selected because of contamination (rather than optimal deployment conditions), more efforts may be needed to either completely bury the anchors and cages and monitor them during the deployment period to ensure they remain buried, or provide clear warning signage to beach users.

6. Management implications for marine conservation

Developing appropriate tools to measure the exposure of marine organisms to toxic contaminants and the impact of those contaminants on biota is a key component of the plan for recovering the Puget Sound ecosystem. Our work here describes a method to evaluate the exposure of a sensitive life stage of a keystone species, in a major spawning habitat potentially

impacted by toxic contaminants. We envision these techniques will be used by remediation and conservation practitioners to assess the degree to which smelt reproduction may be impaired by contaminants in their spawning habitat, and to evaluate baseline conditions of chemical contaminants in surf smelt embryos for comparison with conditions after an oil spill.

We plan to publish these methods as a WDFW technical report and conduct outreach to remediation, conservation, and oil spill prevention and cleanup practitioners. This includes Washington Department of Ecology's Spill Prevention, Preparedness and Response Program, Water Quality Program, and Toxics Cleanup Programs, WDFW's Oil Spill Prevention and Response program and NOAA's Northwest Fisheries Science Center Ecotoxicology Program.

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Harding, L. B., Incardona, J. P., & West, J. E. (December 15, 2020). Assessing land-based impacts to spawning habitat of surf smelt. Oral presentation to WDFW Habitat Program's Shorelines Quarterly Meeting.

Appendix A

Location	Tulare boat ramp
Ownership	Private
Contact	Bob
Access	24 hours
Freshwater input	No
Historical spawning	Yes
Recent observed spawning	No
Body of water	Port Susan

Very gradual sand and gravel beach on the shores of port susan. Two possible locations. Historical record of spawning, locals however do not report recent spawning. Marine mammals observed hunting forage fish in this area

https://www.arcgis.com/home/webmap/viewer.html?webmap=19b8f74e2d41470cbd80b1af8dedd6b3

Tulare beach

Location	Kayak Point beach park
Ownership	Public
Contact	None
Access	Limited
Freshwater input	yes
Historical spawning	Yes
Recent observedspawning	unknown
Body of water	Port Susan

Very gradual sand and gravel beach on the shores of port susan. Two possible locations. Historical record of spawning, locals however do not report recent spawning.

https://www.arcgis.com/home/webmap/viewer.html?webmap=19b8f74e2d41470cbd80b1af8dedd6b3

Kayak point

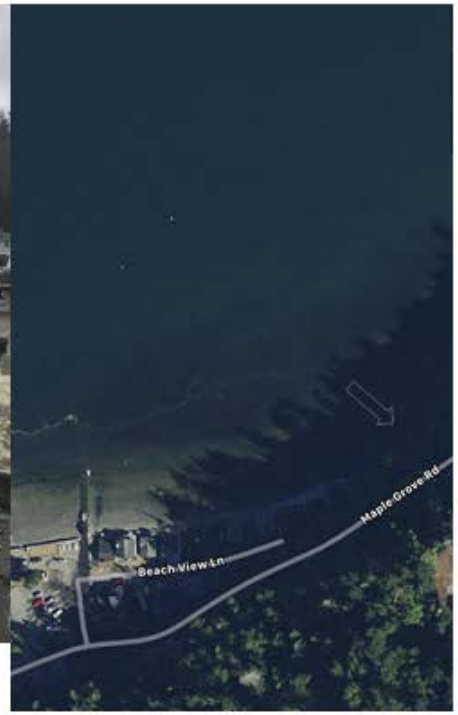
Location	Utsaladyboat ramp
Ownership	Public /Privalp
Contact	#278
Access	24 hours
Freshwater input	yes
Historical spawning	Yes
Recent observedspawning	yes
Body of water	Utsalady/ Skagit

gradual sand and shell beach on the shores of Utsalady bay, North Camano Island. Many possible locations, public and private. This areas supports both a commercial and recreational fishery for surf smelt. At times large schools are in the area

https://www.arcgis.com/home/webmap/viewer.html?webmap=19b8f74e2d41470cbd80b1af8dedd6b3

Utsalady boat ramp

Location	Island county boat ramp
Ownership	Public /Private
Contact	
Access	24 hours
Freshwater input	yes
Historical spawning	Yes
Recent observedspawning	yes
Body of water	Skagit

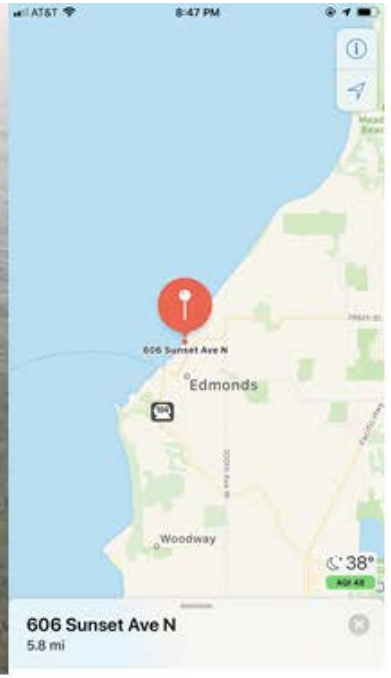


gradual sand and shell beach on the shores west Utsalady bay, North Camano Island. Many possible locations, public and private. This areas supports both a commercial and recreational fishery for surf smelt. At times large schools are in the area. Public beach accessible by small boat.

https://www.arcgis.com/home/webmap/viewer.html?webmap=19b8f74e2d41470cbd80b1af8dedd6b3

Island County boat ramp

Location	North of edmonds ferry
Ownership	Public
Contact	
Access	24 hours
Freshwater input	Storm drain
Historical spawning	Yes
Recent observedspawning	unknown
Body of water	Puget Sound



Armored beach in urban area with stormwater outfall. Historic spawning ground for surf smelt. Observed current spawning unknown. This area is accessible to the public.

https://www.arcgis.com/home/webmap/viewer.html?webmap=19b8f74e2d41470cbd80b1af8dedd6b3

606 sunset beach/Edmonds

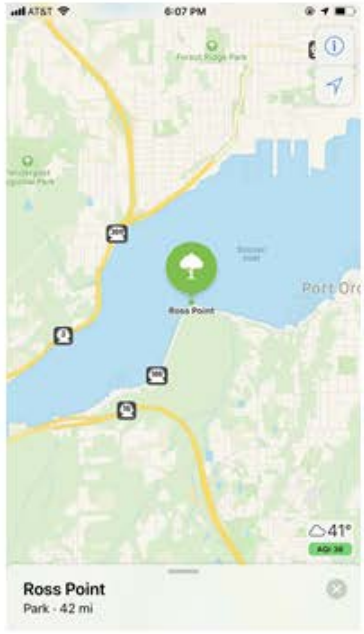
Location	Northcoast central Whidbey
Ownership	Private
Contact	Vikki
Access	24 hours
Freshwater input	unknown
Historical spawning	No
Recent observedspawning	unknown
Body of water	Saratoga Passage/ Holmes Harbor

Natural tree covered beach next to urban breadwater. Light gravel. No historic spawning, no recent observed spawning of smelt, but sand lance known to spawn there

https://www.arcgis.com/home/webmap/viewer.html?webmap=19b8f74e2d41470cbd80b1af8dedd6b3

Vikki Beach

Location	Ross Point
Ownership	Public
Contact	None
Access	24 hours
Freshwater input	Storm drain
Historical spawning	yes
Recent observedspawning	yes
Body of water	Sinclairinlet



Natural tree covered beach near to urban breadwater. Industrial waterway. Light gravel. Known Spawning ground. Supports sizable recreational fishery Would be nice to see how the eggs are doing on the beach that many are spawned at!!

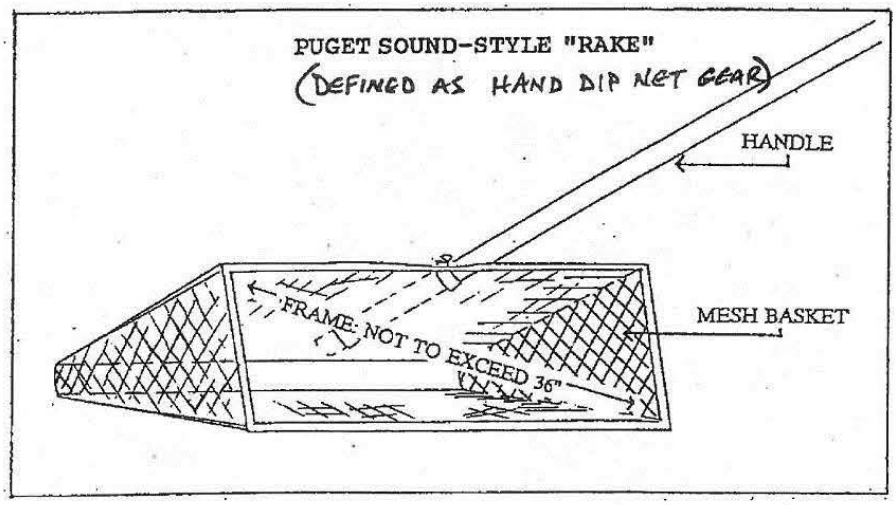
https://www.arcgis.com/home/webmap/viewer.html?webmap=19b8f74e2d41470cbd80b1af8dedd6b3

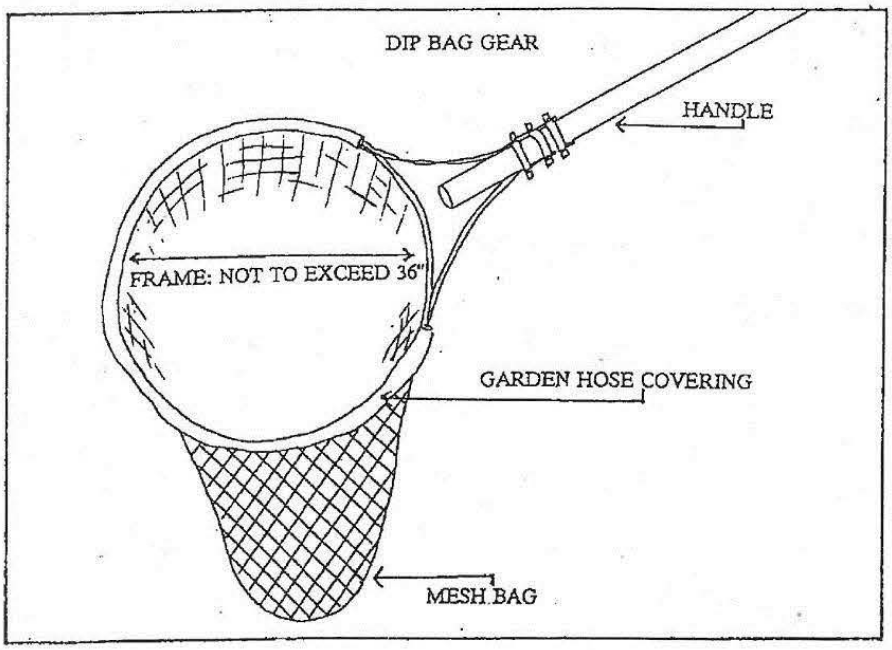
Ross Point

Additional potential deployment locations include Weaverling Spit, Fidalgo Island as described in Rossell and Dinnel (2006) and Cavelero's Beach, Camano Island; Penn Cove, Whidbey Island; south shore of Liberty Bay; southeast shore of central Eld Inlet; and the south shore of Hood Canal just east of Twanoh State Park as described by Penttila (1978).

Appendix B

Recreational surf smelt dip net gear. Image excerpted from the Washington State Surf Smelt Fact Sheet (https://wdfw.wa.gov/sites/default/files/publications/01219/wdfw01219.pdf).





Two of the most commonly used recreational surf smelt dip bag nets.