SAN JUAN COUNTY FORAGE FISH ASSESSMENT PROJECT

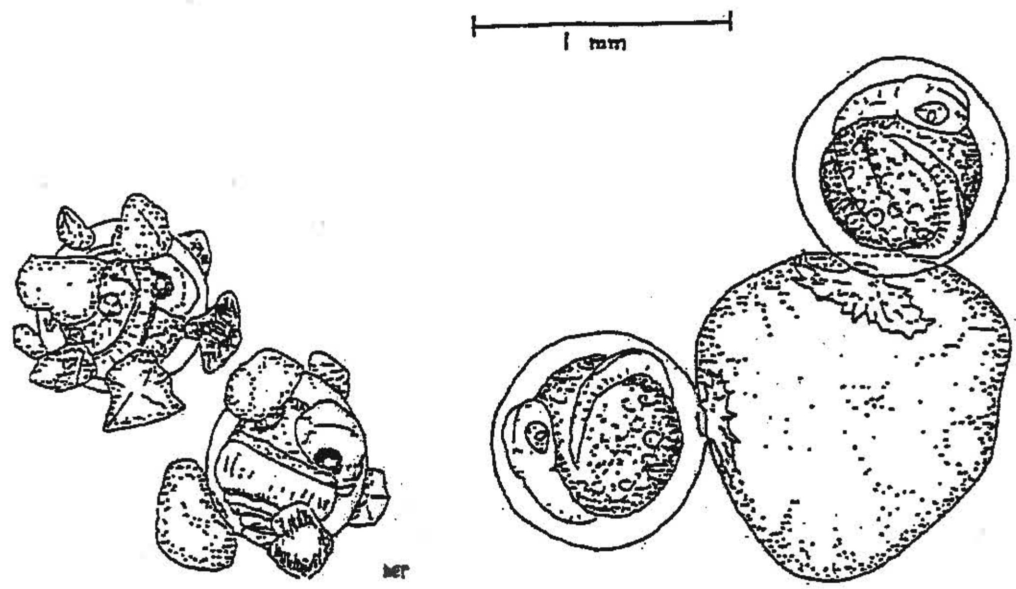
FIELD MANUAL

FOR SAMPLING FORAGE FISH SPAWN IN INTERTIDAL SHORE REGIONS

FIRST EDITION

MARCH 2001

(REVISED 2006)



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INTRODUCTION

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

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

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

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

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

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

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

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

STUDY DESIGN CONSIDERATIONS

Project Objectives

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

Sampling Schedule

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

SURF SMELT AND PACIFIC SAND LANCE SPAWN ASSESSMENT

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

Site Selection

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

Field Equipment

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

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

- Electrical tape

Equipment needed for condensing samples:

- Rack of sediment screens, size 4, 2, and 0.5 mm, preferably Nalgene instead of the more traditional brass screens,
- 2 - 5 gallon buckets modified to act as drain for screen rack,
- 2 Wash buckets,
- Plastic dishpan,
- 16 ounce plastic sample jar
- Stockard's Solution:
  50 ml formalin (37% formaldehyde)
  40 ml glacial acetic acid
  60 ml glycerin
  850 ml fresh water

Equipment needed to establish sample location:

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

Field Records

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

The data fields should be filled in as follows:

Last High Tide: time and elevation of the last high tide – can be obtained from a current tide chart.
Island: Island Sampled
Date of Sampling
Beach Number: Assigned Number for Beach being sampled.
Sample Number: Sample number from Sample Label.
Time: time sample label is removed from the beach (0000-2400 hr)
Latitude/Longitude: latitude and longitude in degrees, minutes, seconds
Beach: Character of the upper beach:
0 = mud,
1 = pure sand,
2 = pea gravel (fine gravel) with sand base,

3 = medium gravel with sand base,
4 = coarse gravel with sand base,
5 = cobble with sand base,
7 = boulder with sand base,
8 = gravel to boulders without sand base,
9 – rock, no habitat

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

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

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

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

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

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

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

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

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

Samplers: Names of personnel participating in the sample collection

Photo Taken: indicate number and direction of photographs

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

Relevant nautical charts are:

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

Relevant USGS topographic sheets are:

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

General Guidelines for Collecting Bulk Beach Samples

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

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

- Identify an approximately 100 ft stretch of beach to be sampled.
- Obtain location information for the transect by reading position information from a GPS or marking the location carefully on a large scale (1:24,000) USGS topographical sheet.
- Prepare a Sample Label to allow identifying the location (Beach Number) and collection time of the sample, deposit the label in the plastic bag (Figure 11).
- Start at one end of the transect, scoop a jar full of sand from the top 1-2 inch of beach and dump the sand into the plastic bag. The scooped area will likely be 3-4 ft long – the idea is to skim the eggs developing in the surface one-inch of substrate.

- Move 10 paces along the transect, obtain another scoop sample and place in the bag with the previous scoop.
- Repeat pacing and scooping until the four scoops have been obtained – this constitutes the bulk sample for the chosen transect.
- Seal the bag securely and place in a cool location. This is particularly important in warmer weather because high temperatures can cause mortality and decomposition in the eggs.
- Store in a secure location to ensure that the bags are not damaged during transit from the field.
- Take one or more photographs of the sampled beach. The photograph should be taken from one end of the sampled transect, looking towards the other end, so that the view is parallel to the beach. The photograph should show the sample relative to the last high tide line, if possible, and any other land marks that will help to establish the sample location. The direction of view (looking north, south, etc.) should be recorded on the field data sheet.

Condensing Bulk Samples

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

- Assemble the Nalgene screens on top of the drain bucket, with the largest mesh on top, grading to the smallest mesh on the bottom.
- Remove the sample label and place it in a 16 ounce sample jar.
- Mark the Beach Number and Sample number on the outside of the jar with the fine-tip marker pen.
- Add a portion of the sample to the top screen, thoroughly wash the sediment through the screen set with either salt or fresh water, which ever is readily available.
- Discard the sediment in the top screens, retain only the material in the bottom (0.5 mm) screen.
- Dump the material retained in the 0.5 mm screen into the dishpan.
- Add water until the material is covered by 1-2 inches of water.
- Swirl the water around the pan, adding rocking and bouncing motions to allow the eggs to migrate to the top of the sediment. The idea is similar to gold panning, try to winnow the eggs to the surface of the material.
- After swirling for 1-2 minutes, work the lighter fraction of material to one corner of the pan. Carefully dry up the lighter fraction by tipping the pan so that the water drains away, and skim the lighter fraction from the surface of the sand with the sample jar.
- Repeat the winnowing process two more times.
- Process the remainder of the sample in a similar fashion, each time adding the retained lighter fraction to the sample jar.
- Fill the sample jar with Stockard's Solution to preserve the eggs. Seal the jar securely, invert carefully several times to ensure that the preservative reaches all the eggs.

- Tape the jar shut with electrician's tape so that the preservative does not evaporate during storage.

Laboratory Examination

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

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

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

QUALITY ASSURANCE/QUALITY CONTROL

Primary concerns for quality control include:

- sampling appropriate habitat,
- accurate identification of sample location,
- careful screening and winnowing of the bulk sample to retain the maximum number of eggs, and
- accurate identification of sampled eggs.

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

DATA REPORTING

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

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

REFERENCES

Penttila, D.E. 1990. Summary report, San Juan Co., surf smelt spawning beaches, February 1989 through May 1990. WDF Memorandum to Greg Hueckel, July 3, 1990. Seattle, WA. 7p.

Penttila, D.E. 1999. Documented spawning areas of the Pacific herring (*Clupea*), surf smelt (*Hypomesus*), and Pacific sand lance (*Ammodytes*) in San Juan County, Washington. Washington Dept. of Fish and Wildlife, Marine Resources Division. Manuscript Report. LaConner, WA. 27p.

Penttila, D.E. 2000. Previous surf smelt/sand lance beach sampling sites in San Juan County. Washington Dept. of Fish and Wildlife, Marine Resources Division. WDFW Memorandum to L. Moulton, February 14, 2000. LaConner, WA. 1p.

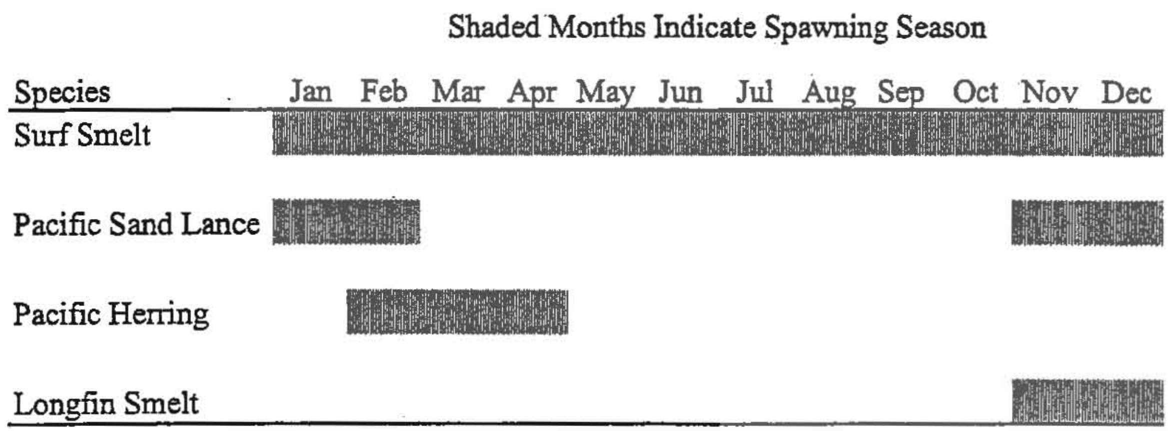
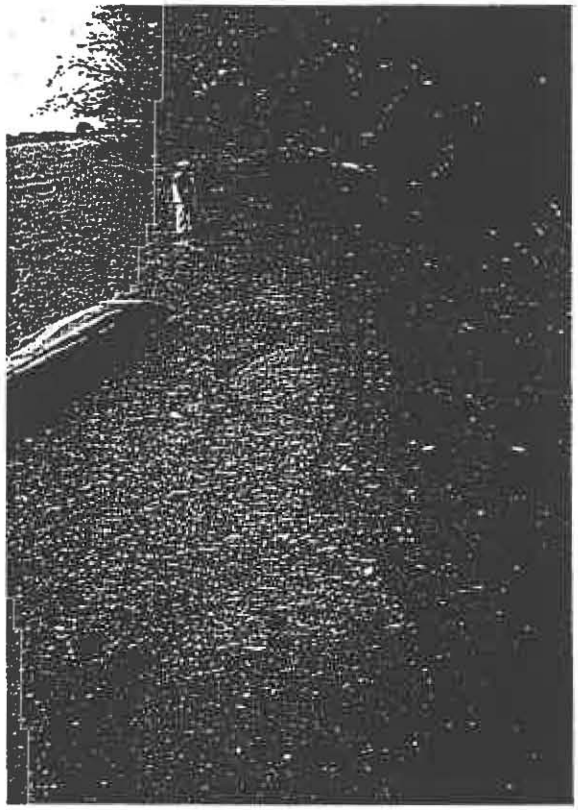


Figure 1. Spawning time of forage fish species in San Juan Islands (data from WDFW).



a. Surf smelt spawn deposit outlined to show extent of spawning activity – note proximity of spawn deposit to the high tide mark.

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

Figure 2. Fresh surf smelt and Pacific sand lance spawn deposits (photos by D. Penttila, WDFW).

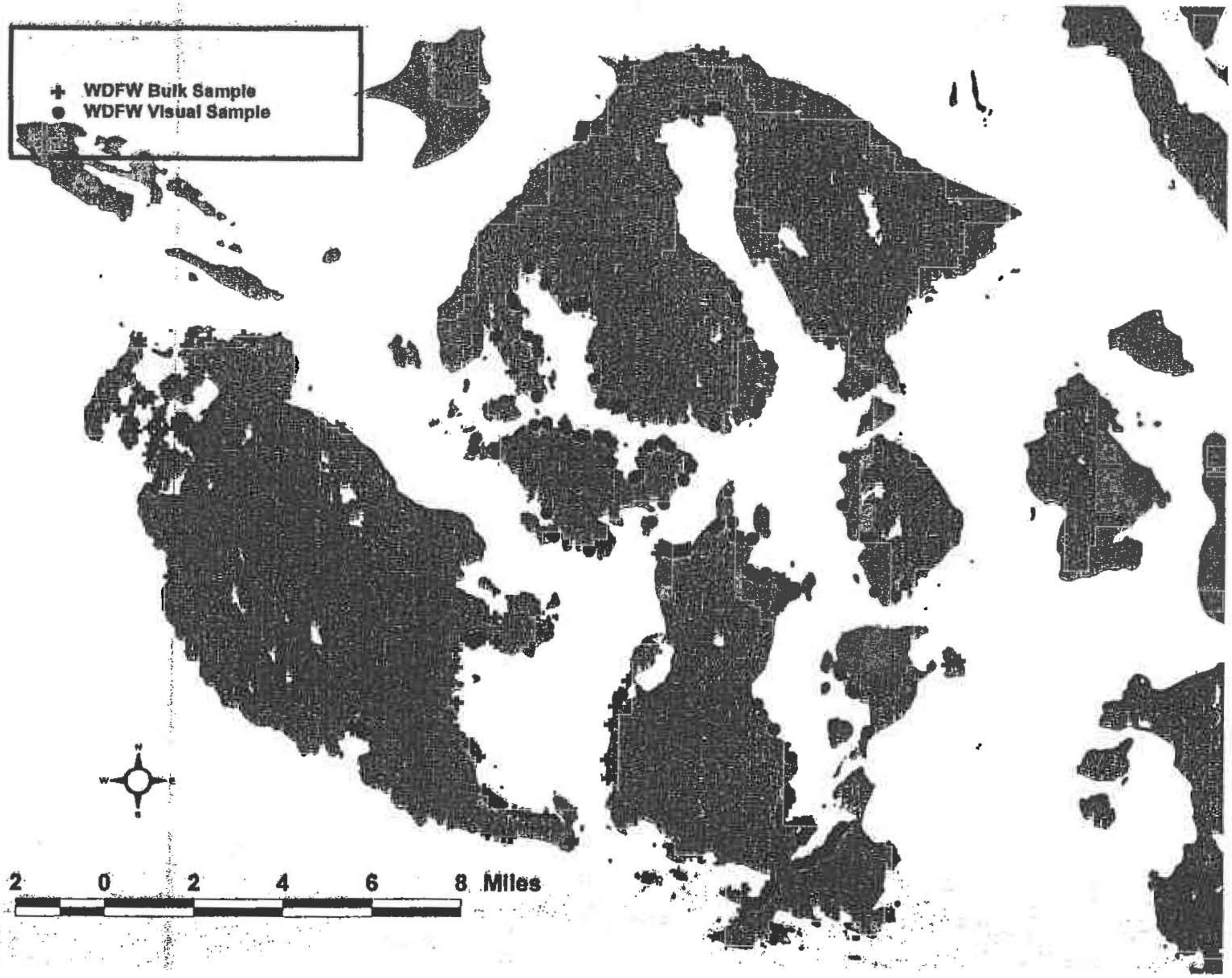


Figure 3. Distribution of intertidal sampling by WDFW to identify surf smelt and sand lance spawning beaches, 1989-2000.

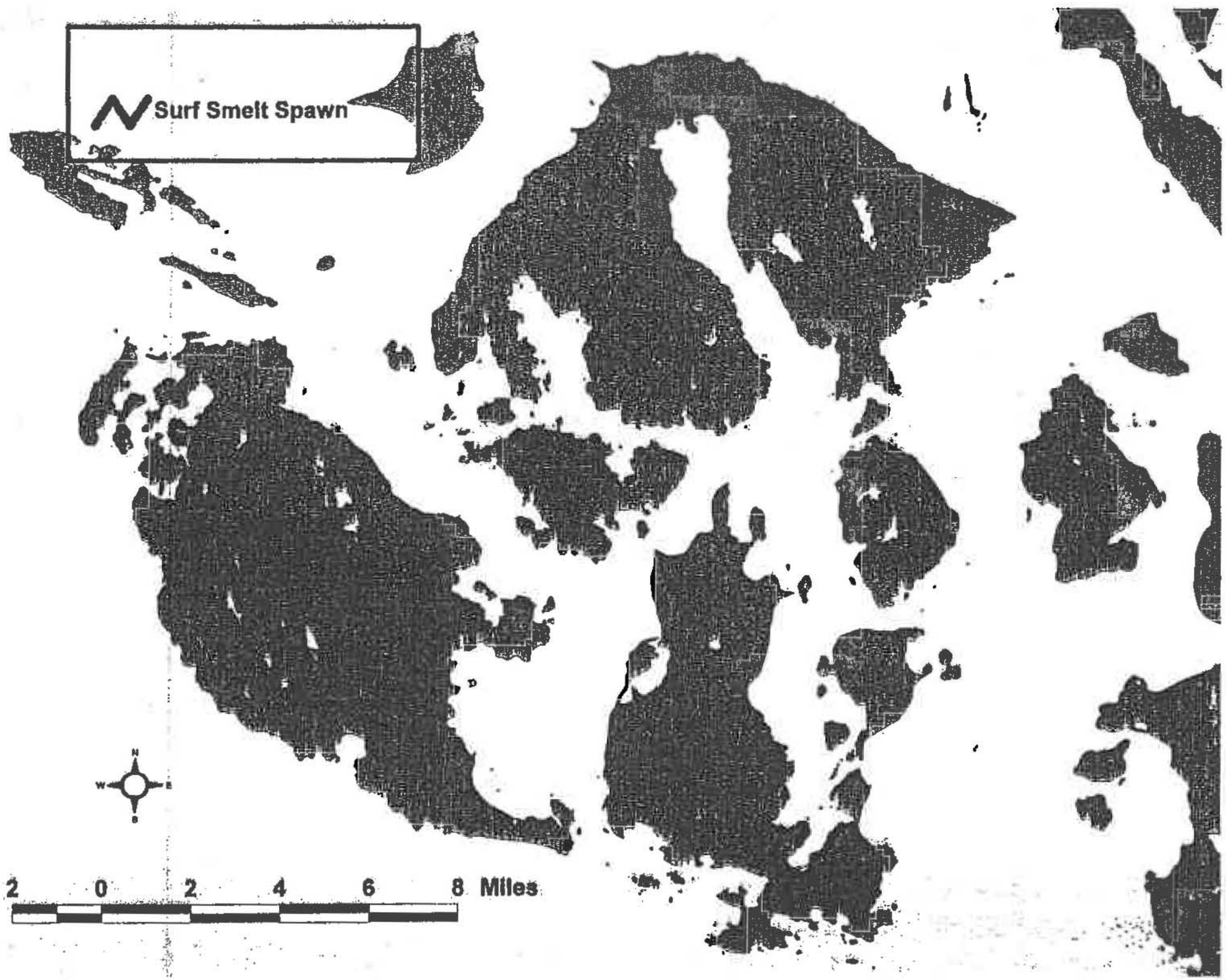


Figure 4. Results of WDFW sampling for evidence of intertidal spawning by surf smelt, 1989-2000.

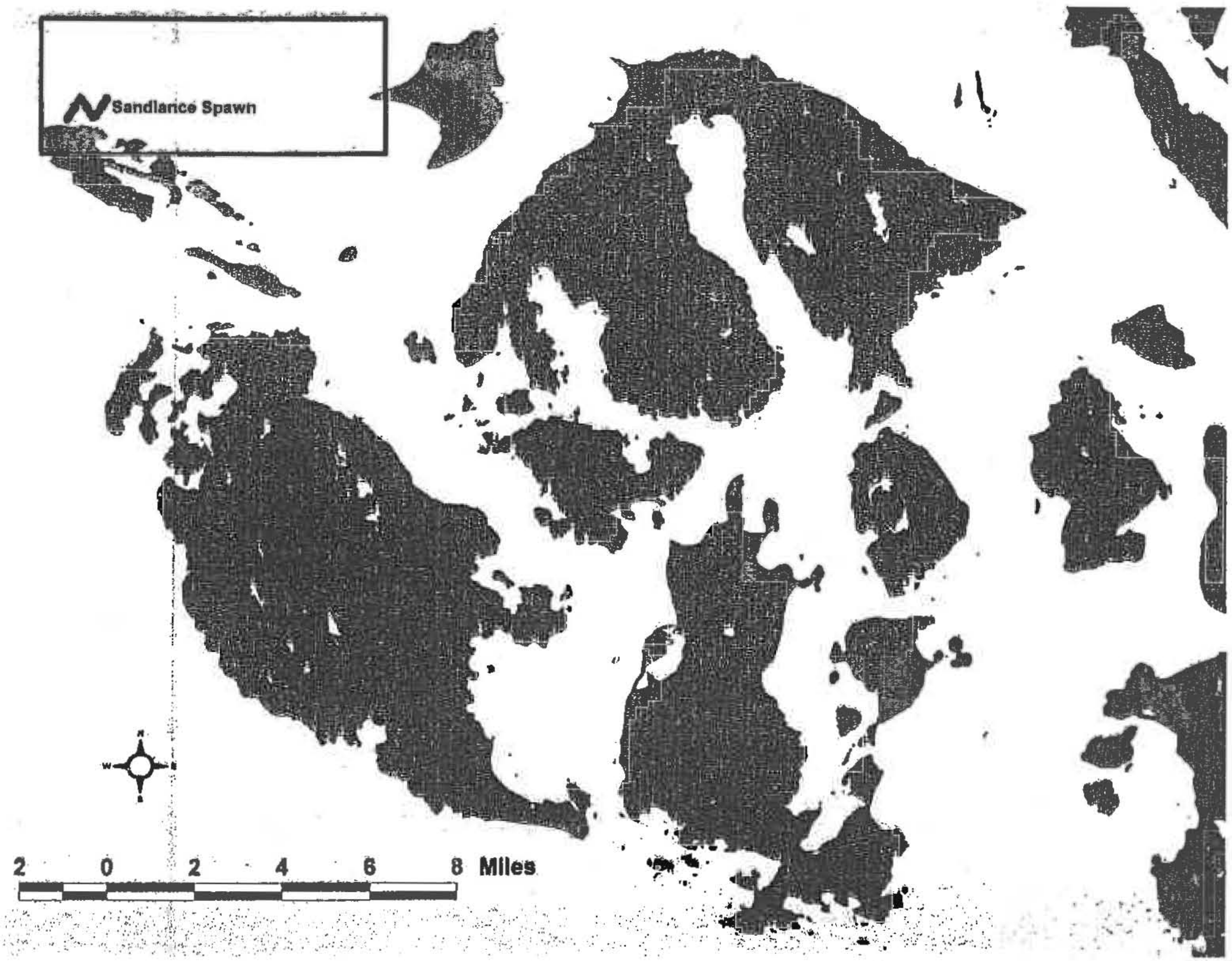
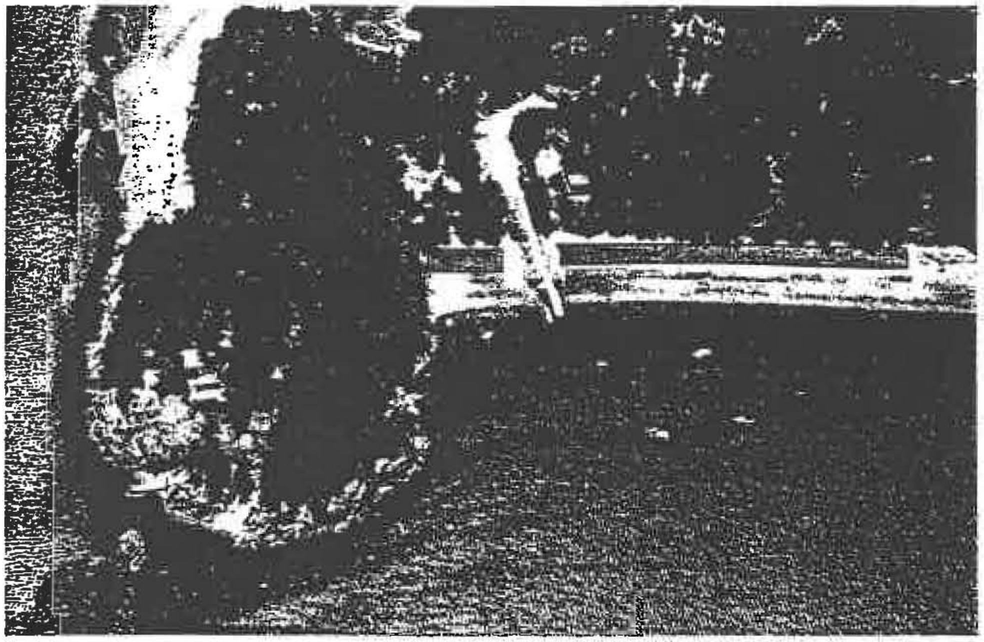


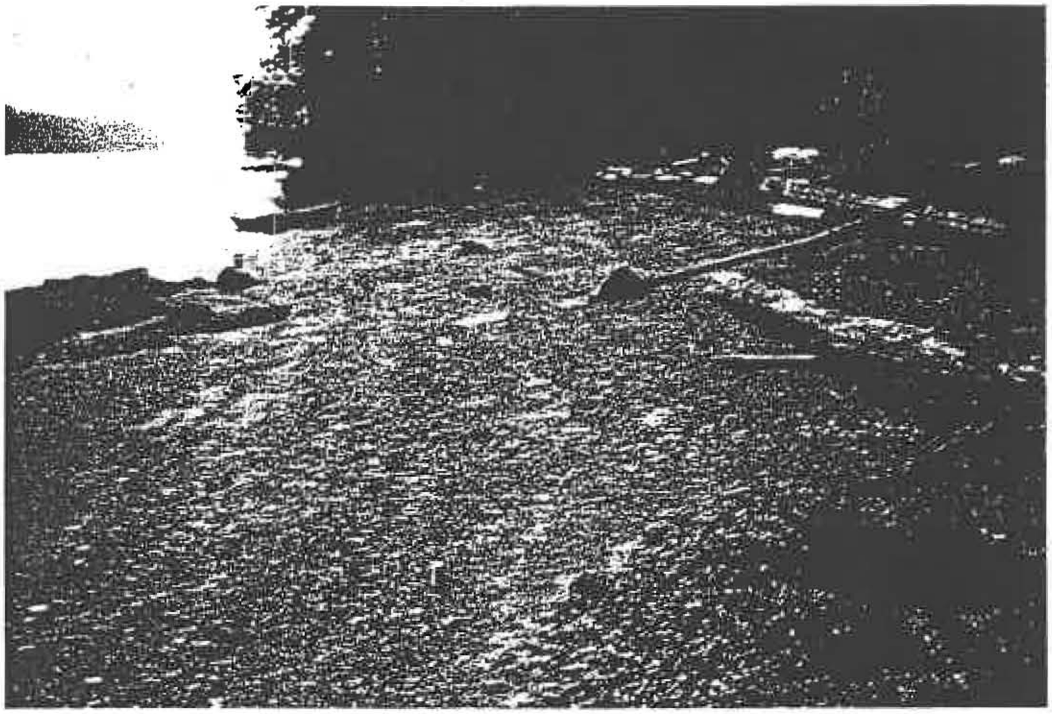
Figure 5. Results of WDFW sampling for evidence of intertidal spawning by Pacific sand lance, 1989-2000.



a. Surf smelt spawning area (patterned area) at Hunter Bay, Lopez Island (note that spawning area has been reduced by dock and launch ramp construction).

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

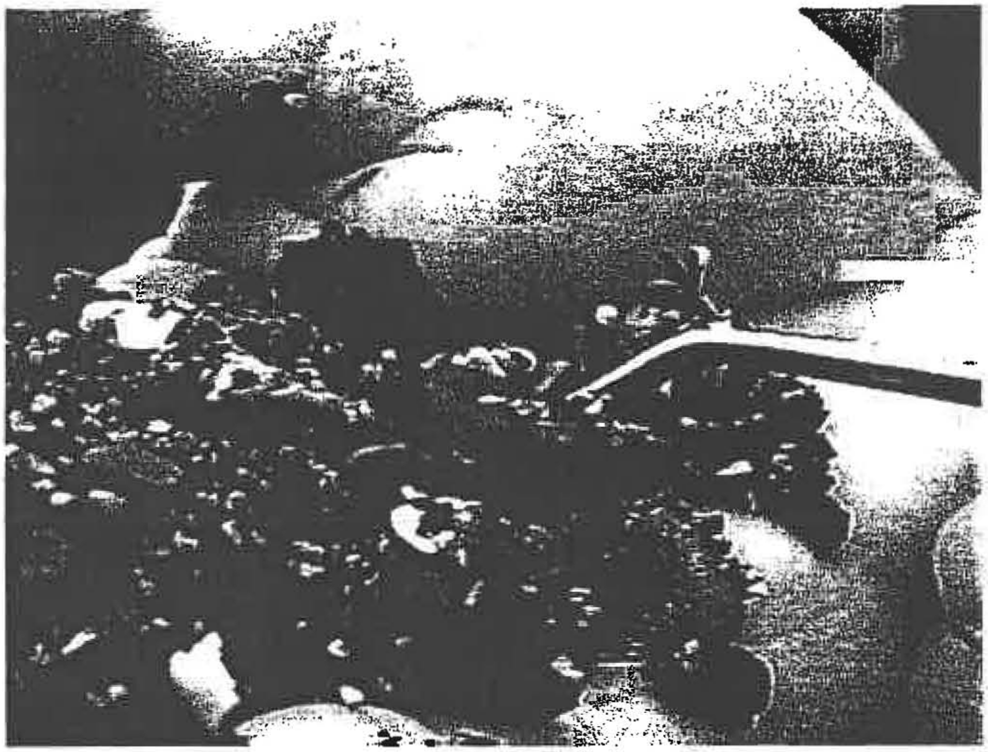
Figure 6. Representative surf smelt spawning beaches in San Juan County (aerial photos from WDOT, 1986).



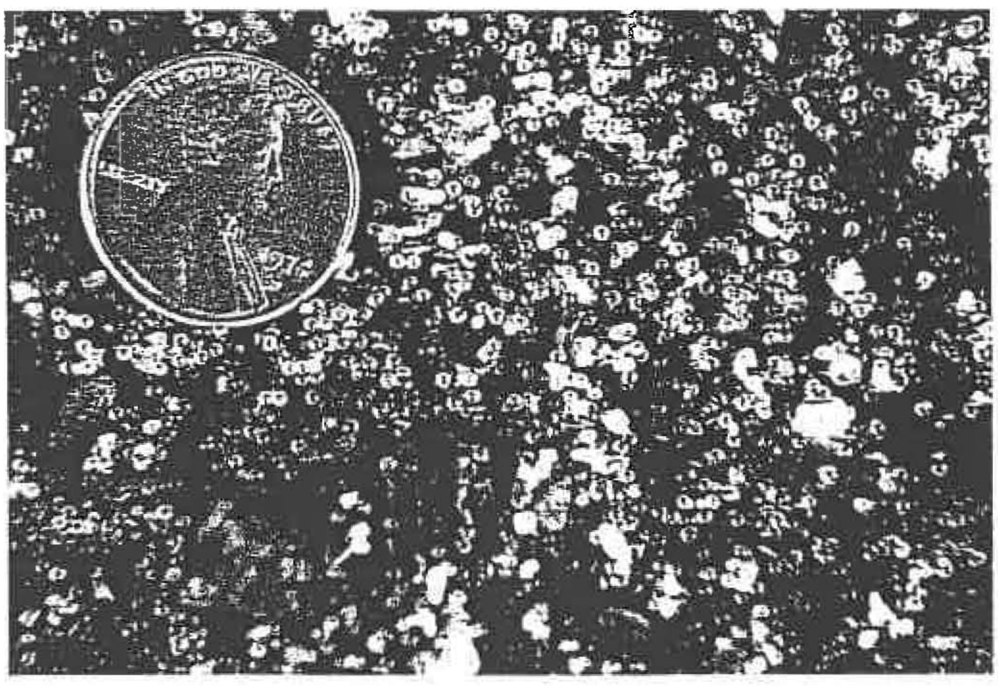
a. Pocket beach west of Blind Bay on Shaw Island, surf smelt spawning area.

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

Figure 7. Examples of surf smelt spawning beaches in San Juan County (photos by D. Penttila, WDFW).



a. Surf smelt eggs - 2 eggs are on the large black stone at the tip of the forceps. Eggs are approximately 1 mm in diameter (photo by L. Moulton).



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

Figure 8. Examples of surf smelt eggs in field conditions.

last high tide	
time	elevation

second effective high tide	
time	elevation

SJC Forage Fish Spawning Surveys

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Reviewed By: ______________________

Island

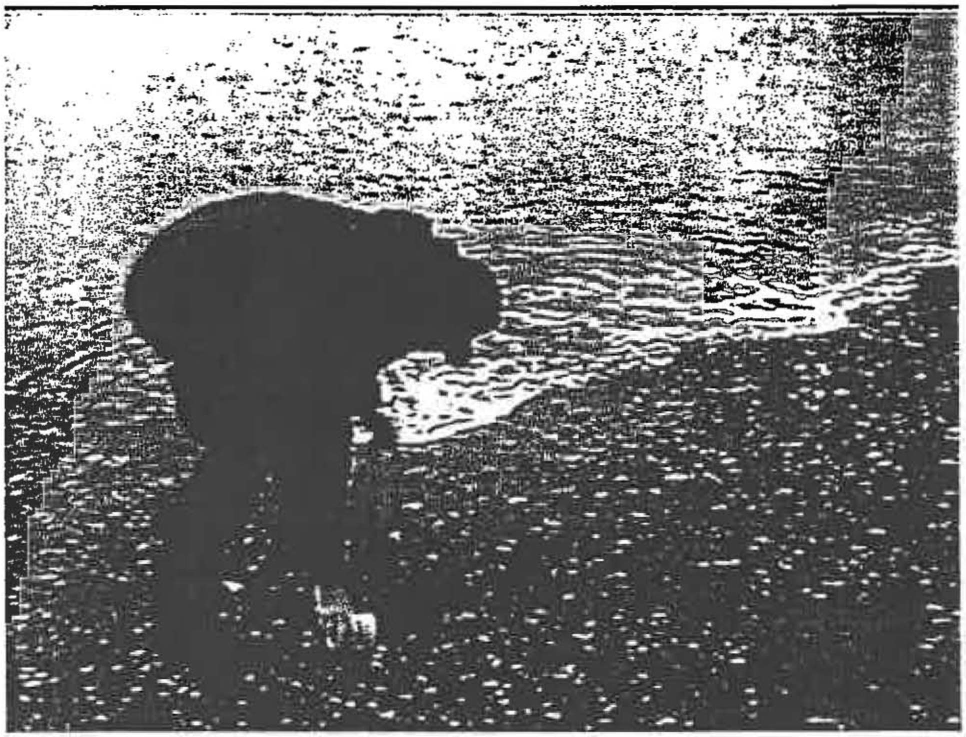
day	month	year

Beach Number	Sample Number	Time	latitude			longitude			beach	uplands	sample zone	land mark	tidal elevation	smelt	sand lance	rock sole	herring	width	length	shading	comments
			o	'	"	o	'	"													

Samplers: ____

Figure 9. Field data form used to record data associated with surf smelt and Pacific sand lance bulk sampling.

2/26/2001



a. Obtaining beach subsample to examine for eggs.

b. Adding subsample to composited sample in bag.

Figure 10. Sampling mixed sand/gravel beach for surf smelt and Pacific sand lance eggs (photos by L. Moulton).

San Juan County MRC
FORAGE FISH PROJECT

SAMPLE NO:
FFP-000 1

DATE: ________ TIME: ________
BEACH NO: ________________
SAMPLER: ________________

Figure 11. Label used to identify each bulk sample

a. Standardized screens (4 mm, 2 mm, and 0.5 mm) are used to remove excess large material from the sample.

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

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

a. Pan is swirled to separate eggs from sediment.

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

Figure 13. Winnowing bulk sediment sample to separate egg-bearing sediment from barren sand (photos by L. Moulton).

SJC Forage Fish Spawn Sample Analysis

Page _____ of _____

Recorder ______________________

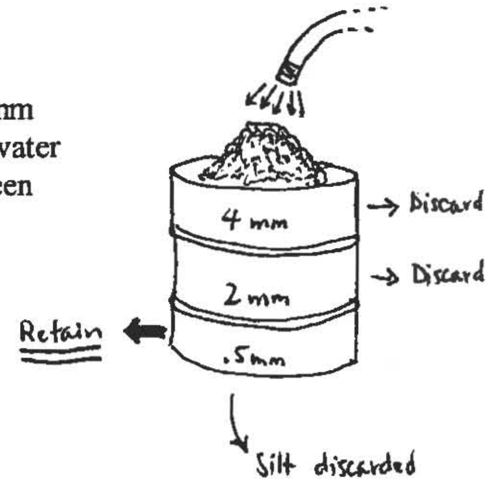
Island	month	day	year

Beach Number	Sample Number	species	1 cell to morula	blastula	gastrula	1/2-1 coil	1 coil	11/2 coil	>11/2 coil	late eyed	dead	# eggs	% dead	est. # broods	wt. substrate	eggs/ gram	remarks

Figure 14. Lab data form used to record data associated with surf smelt and Pacific sand lance bulk sampling.

WDFW Forage Fish Spawning Habitat Survey Protocols:

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

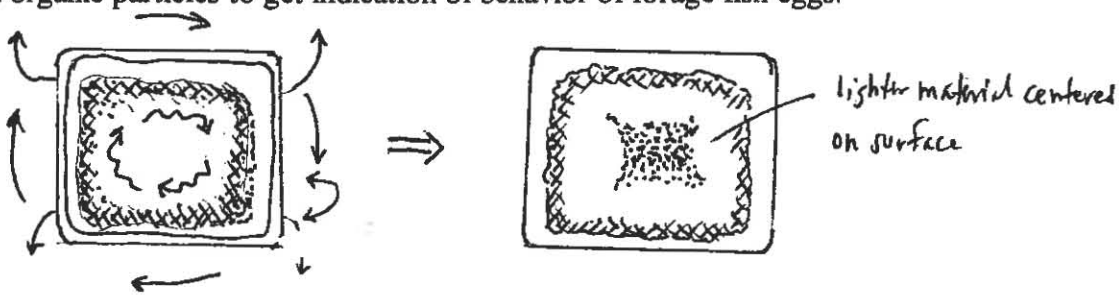


1. Wet-screen material through set of nested 4 mm/2 mm/.5 mm screens, using buckets of shore-side water at site or fresh-water hose elsewhere. Screens should be carefully cleaned between samples

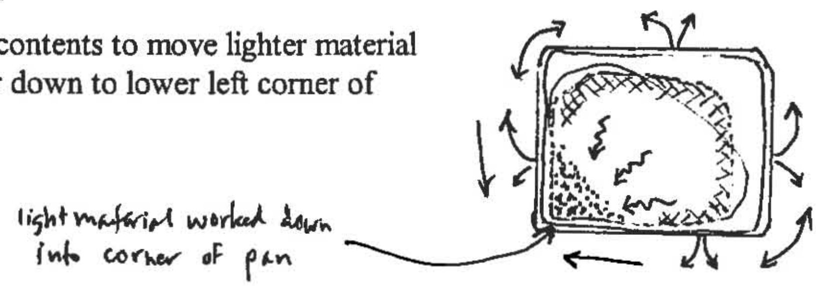
2. Discard material retained in 4 mm and 2 mm screens.

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

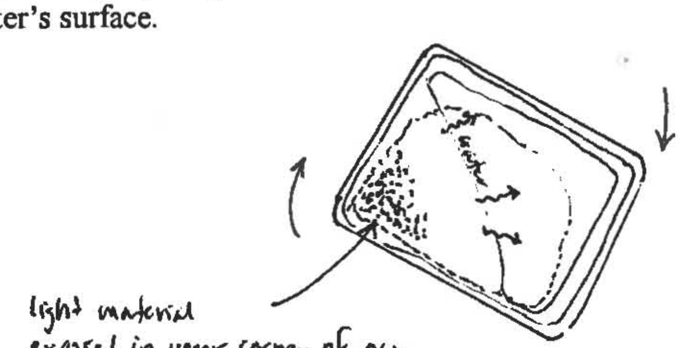
4. Rotate/tilt/yaw dish-pan of material to impart rotation to water, and cause lighter material to rise to surface and accumulate toward center of deposit in pan. Observe behavior of shell fragments and organic particles to get indication of behavior of forage fish eggs.



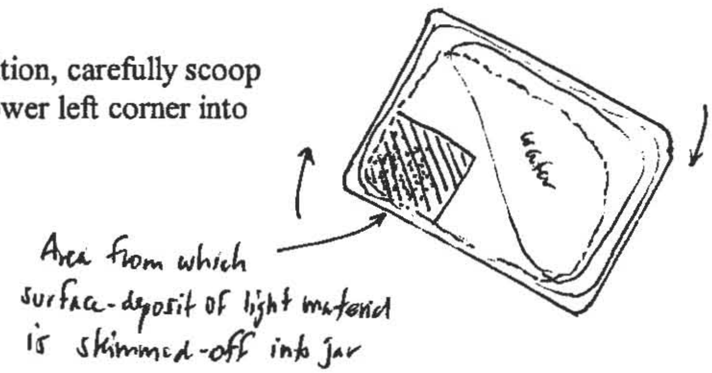
5. Tilt/swirl/agitate pan contents to move lighter material accumulated at center down to lower left corner of pan deposit.



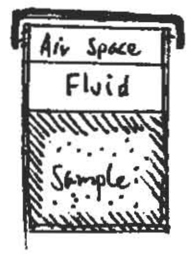
6. Carefully tilt pan to decant water to opposite corner of pan, slowly exposing lower left corner material above water's surface.



7. Holding pan in this tilted position, carefully scoop surface 1" of material from lower left corner into wide-mouth sample jar.



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



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

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

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

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

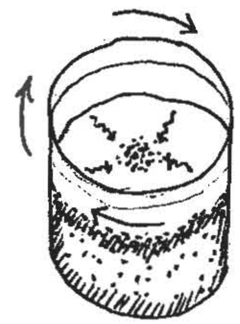
Bulk substrate sample processing materials:

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

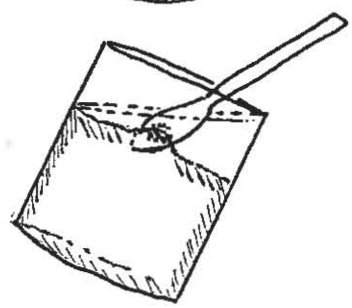
WDFW Forage Fish Spawning Habitat Survey Protocols:

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

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



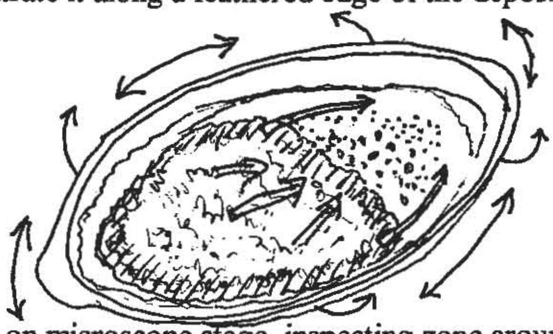
2. Swirl jar in clockwise manner to impart rotation to fluid and surface layer of contents, causing light material to move to center of material in jar.



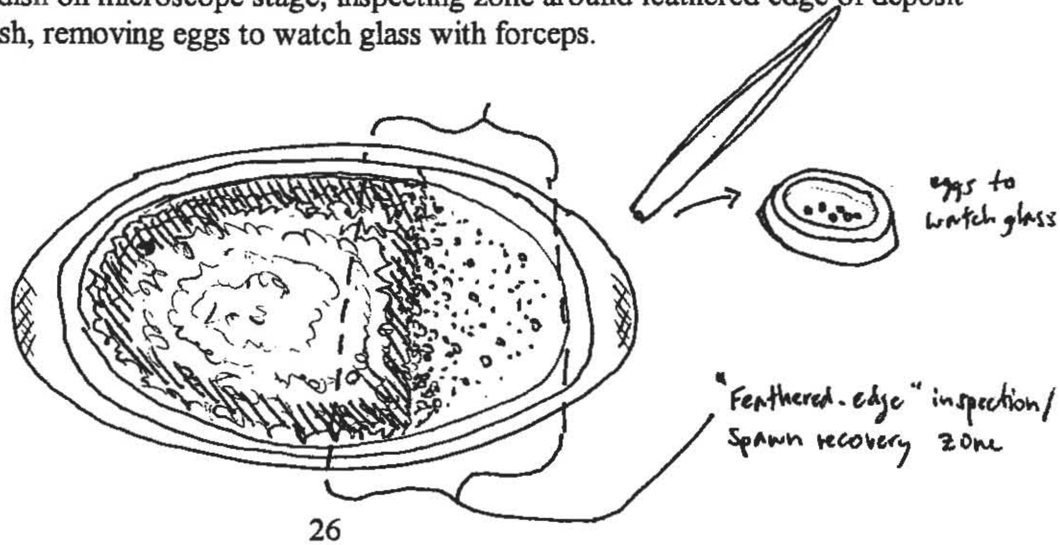
3. Carefully tilt jar, slowly scoop center-mound of light material with spoon into oval microscope dish.

4. Repeat steps 1-3 four times, accumulating about 400 grams of light material in microscope dish.

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



6. Carefully place dish on microscope stage, inspecting zone around feathered edge of deposit of material in dish, removing eggs to watch glass with forceps.



7. Reverse dish, repeat steps 5-6 three times or until eggs cease to be detected around feathered-edge of deposit of material in dish.

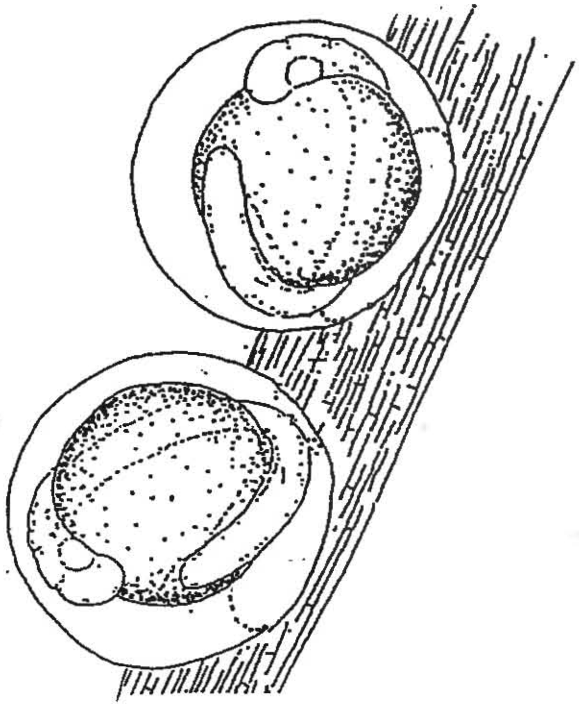
8. If single egg is recovered in steps 1-7, repeat with second sample of material from jar of winnowed light fraction.

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

Lab materials:

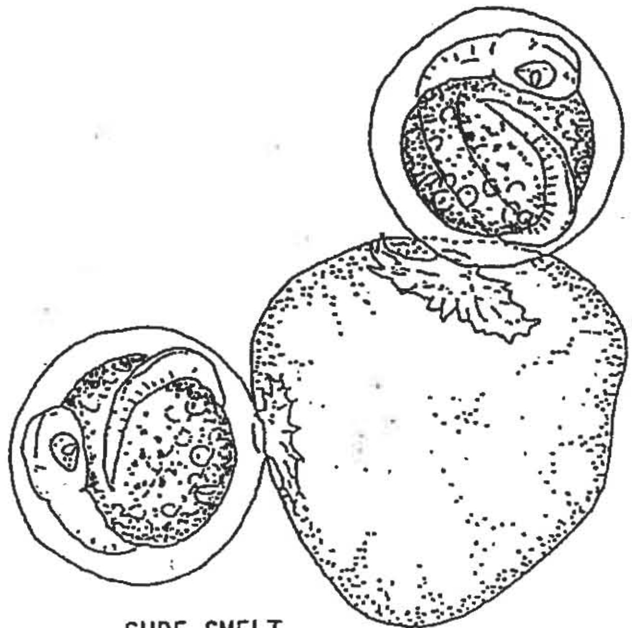
Fume hood (alternatively, carefully rinse preservative from winnowed light fraction samples before processing).
Paper-towels
lab-gloves (keeps preservatives off skin)
Microscope with 10-20X
buckets/pans (to catch drips, accumulate completed samples, etc.)
Oval microscope dish
watch-glasses/small petri dishes
fine-point (watchmakers) forceps
table-spoon
tally sheets/multi-place counter

1 mm



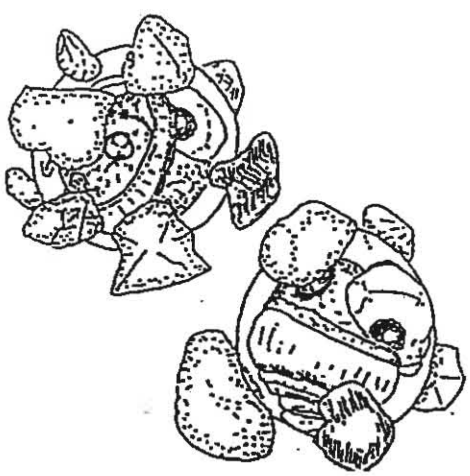
PACIFIC HERRING

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



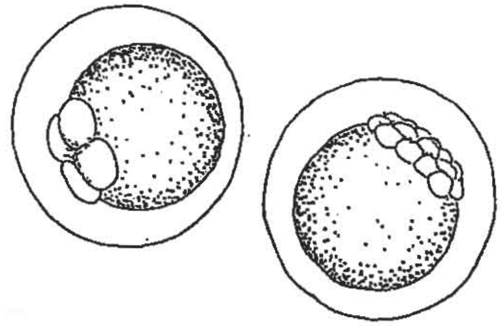
SURF SMELT

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



PACIFIC SAND LANCE

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



ROCK SOLE

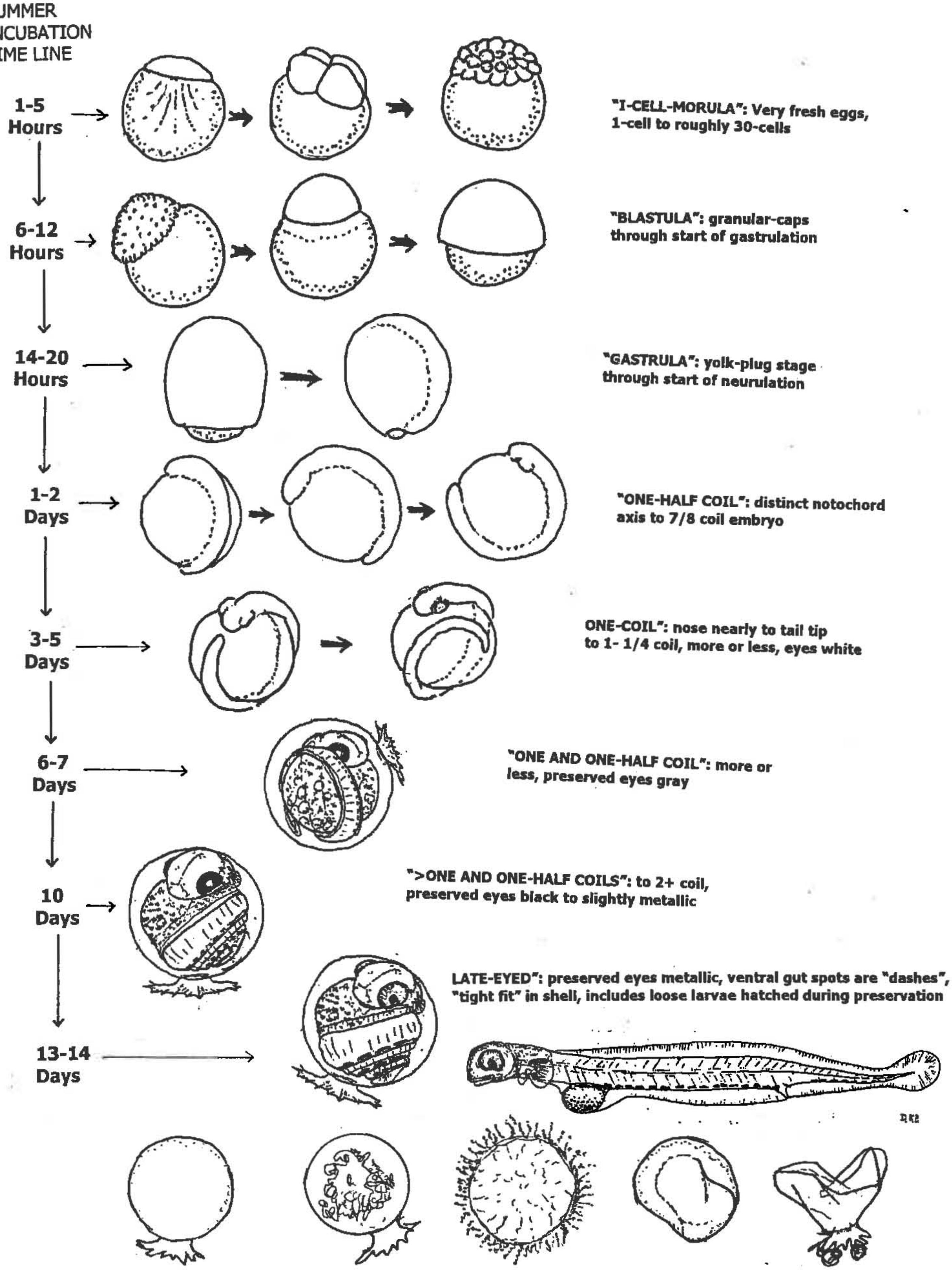
egg perfectly spherical; very clear; no visible attachment sites; non-self-adhesive.

D.E.P.

CHARACTERISTICS OF THE EGGS OF FOUR SPECIES OF INTERTIDAL-SPAWNING MARINE FISHES FROM THE PUGET SOUND BASIN.

TWO-WEEK
SUMMER
INCUBATION
TIME LINE

WDFW SURF SMELT EMBRYOLOGICAL-STAGE CATEGORIES



"DEAD": opaque-white/without discernable embryo/ fungus-covered eggs/ collapsed egg-shells/ empty egg-shells

"NON-FORAGE FISH EGG-LIKE" OBJECTS ENCOUNTERED IN PUGET SOUND BEACH SUBSTRATE SAMPLES

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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