WDFW Intertidal Forage Fish Spawning Habitat Survey Protocols

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

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Laboratory materials needed:

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

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

Procedure:

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

2. If preservative is present, pour off as much liquid as possible into the appropriate waste container and fill the Petri dish ~½-⅔ full with sediment. Use a pipette to remove any residual preservative or other liquid then use a paper towel to blot the subsample dry. Record the weight.

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

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

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

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

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

   then,

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

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

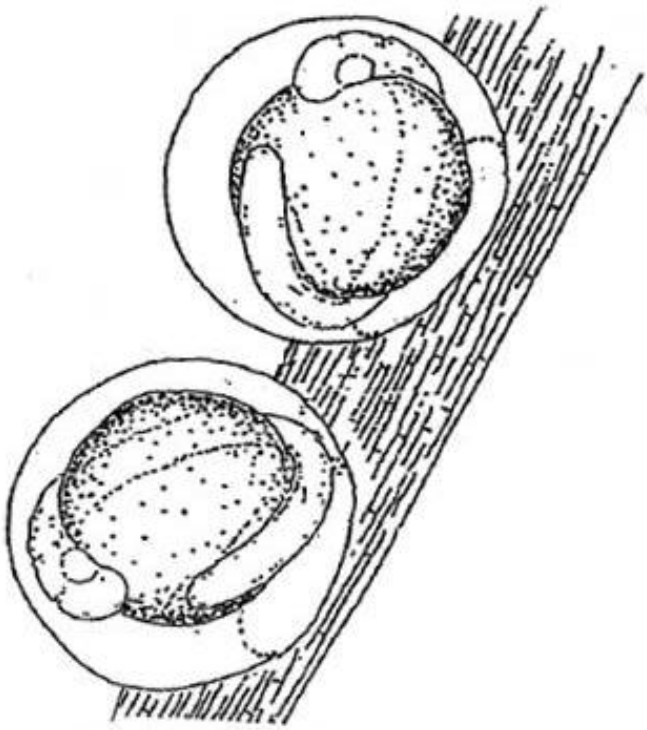
   (10+10+10) / (10+10+10+270) = 30/300 = 0.10

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

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

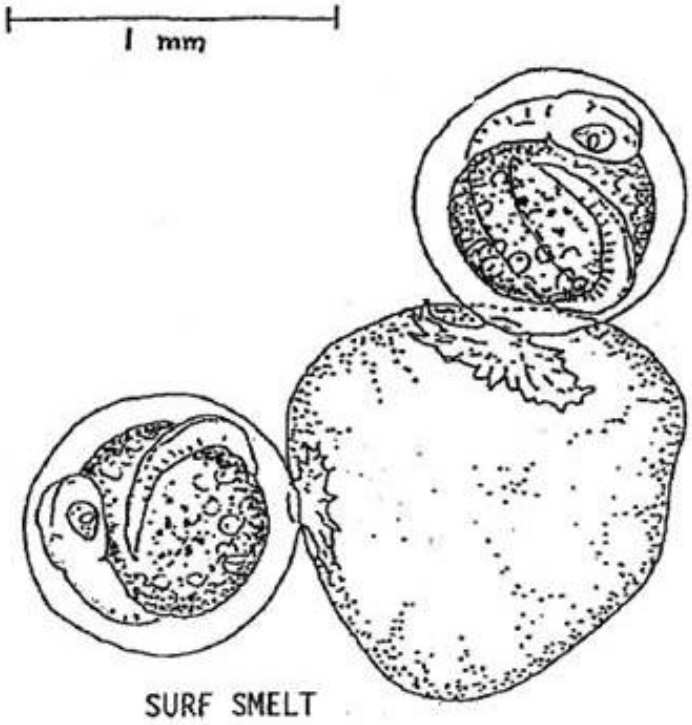
Original protocol by Doris Small, WDFW. Reformatted by Dayv Lowry, WDFW.

Forage Fish Eggs of Puget Sound



PACIFIC HERRING

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

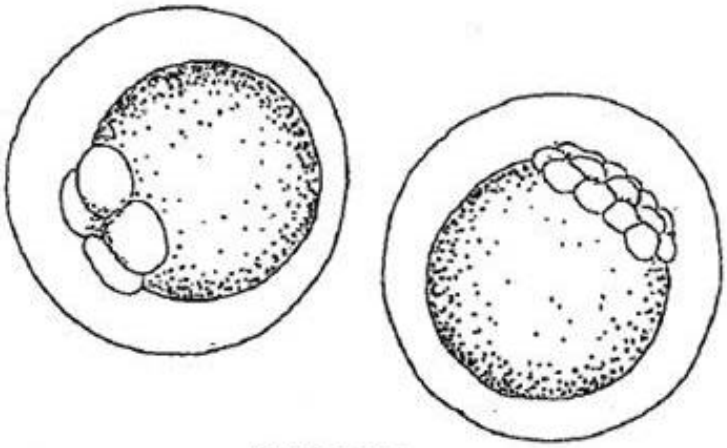


SURF SMELT

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

PACIFIC SAND LANCE

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

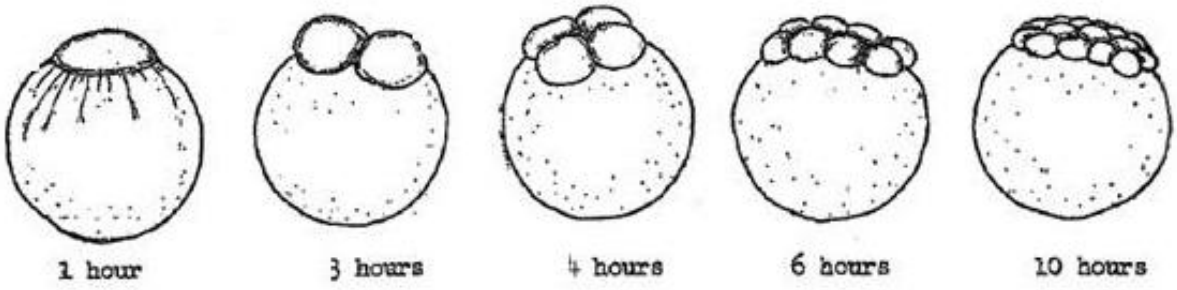


ROCK SOLE

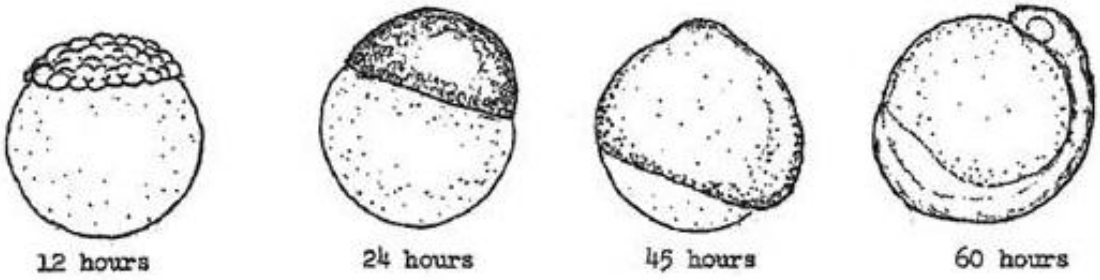
egg perfectly spherical; very clear; no visible attachment sites; non-self-adhesive.

D.E.P.

Embryonic Development Stages – Pacific herring

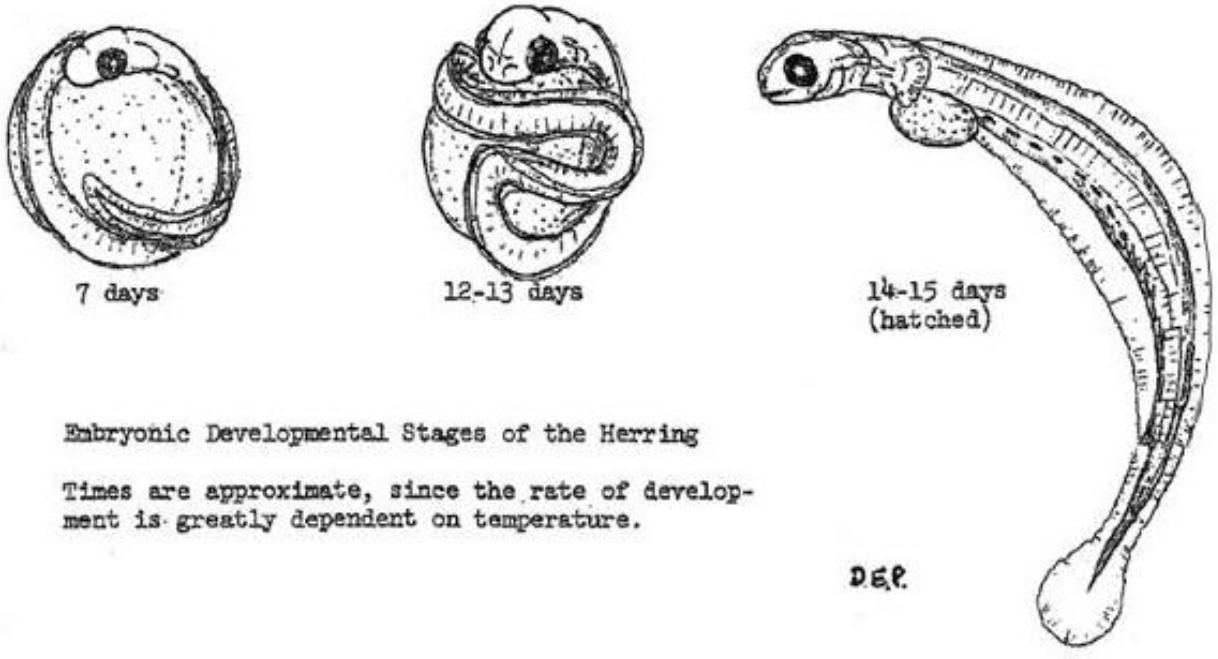


1 hour | 3 hours | 4 hours | 6 hours | 10 hours



12 hours | 24 hours | 45 hours | 60 hours

72 hours | 5 days | 6 days



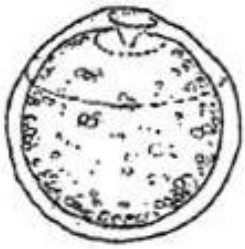
7 days | 12-13 days | 14-15 days (hatched)

Embryonic Developmental Stages of the Herring

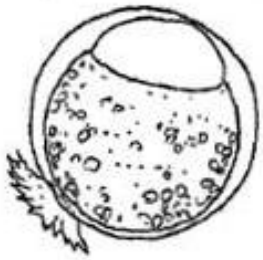
Times are approximate, since the rate of development is greatly dependent on temperature.

D.E.P.

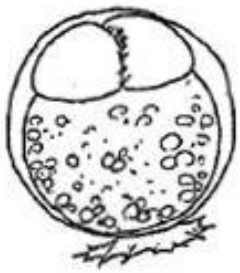
Embryonic Development Stages – Surf smelt



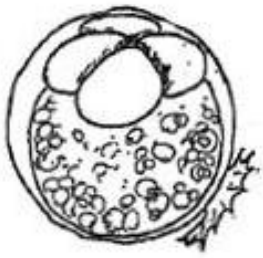
Unfertilized Egg



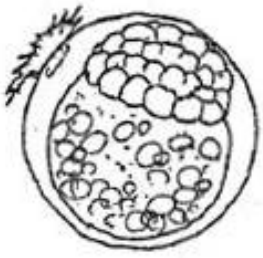
1 cell
½ hour



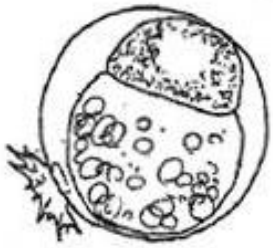
2 cells
1 hour



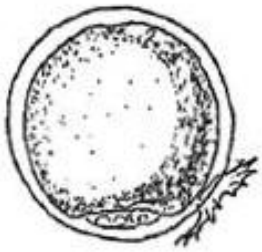
4 cells
3 hours



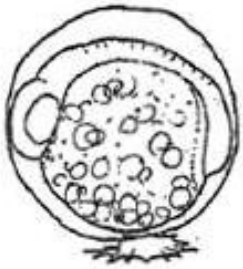
early blastula
5 hours



late blastula
8 hours



yolk plug (gastrula)
14 hours



1½ days

4 days

6 days
(1½ cells)

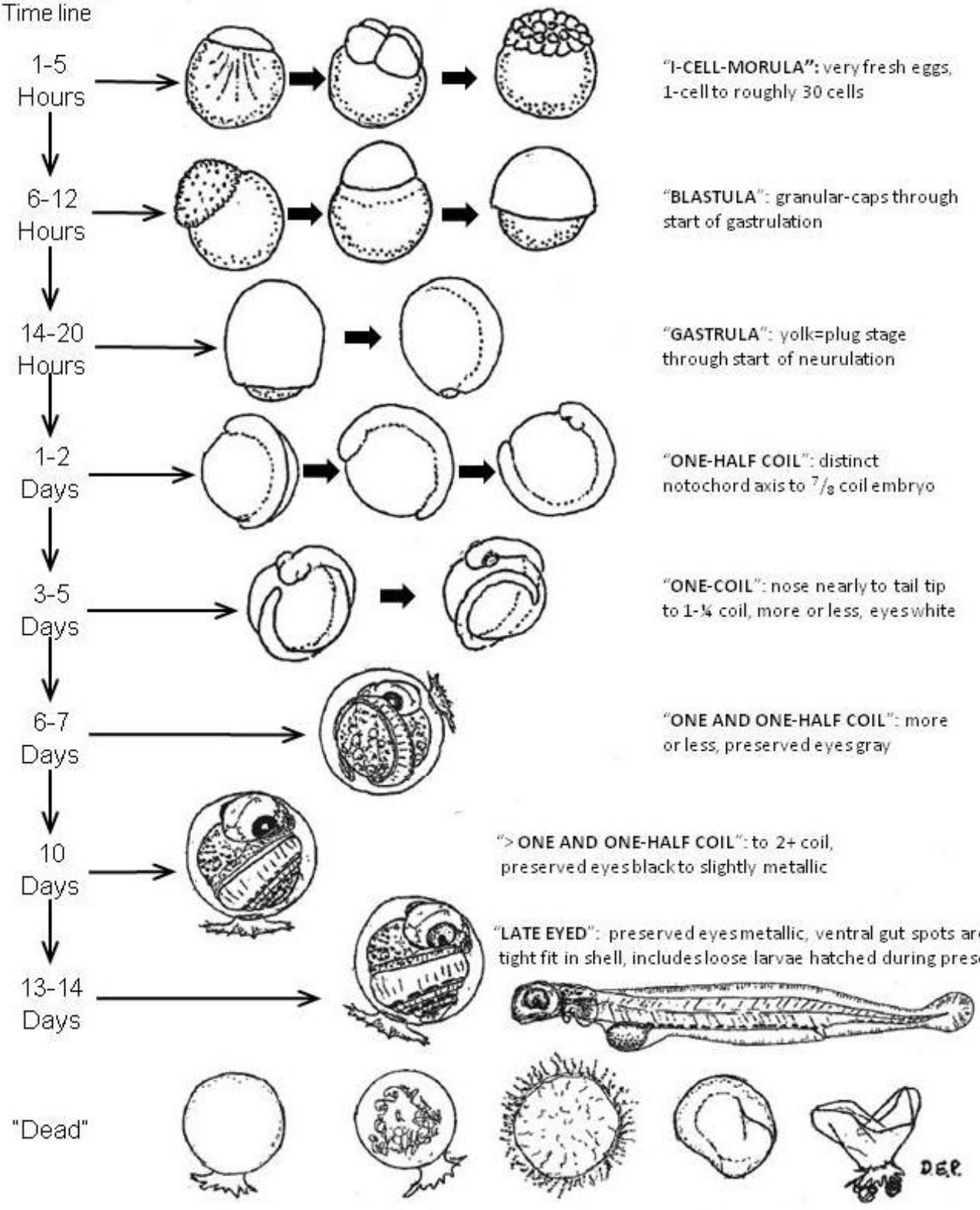
13 - 14 days
(just prior to hatching)

D.E.P.

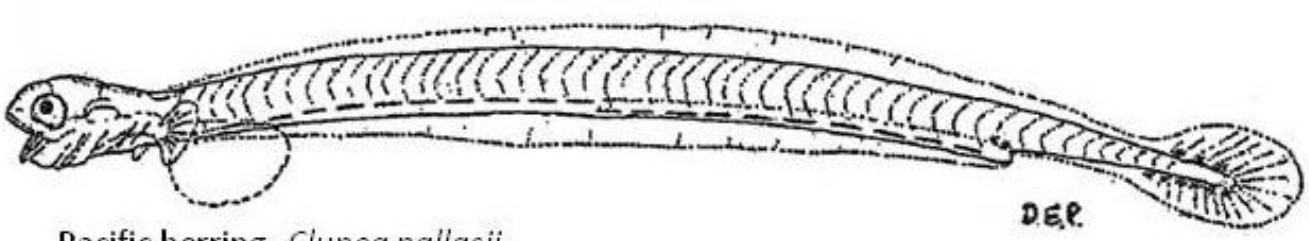
Newly Hatched Larva

Surf Smelt Embryological Stage Categories

Two-week Summer Incubation Time line



Identification Guide to Larval Forage Fishes of Puget Sound

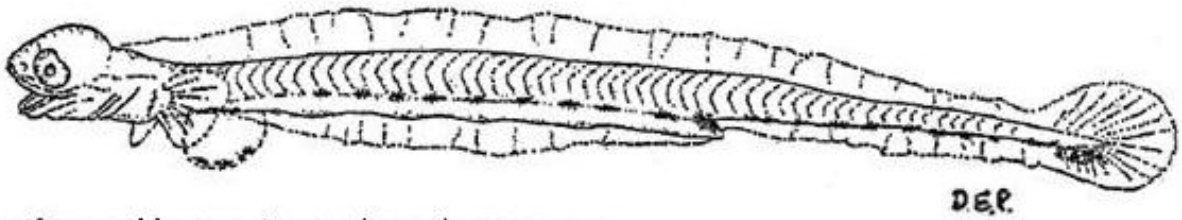


Pacific herring, *Clupea pallasii*

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

Surf smelt, *Hypomesus pretiosus*

1. Head-vent distance about 80% of standard length, 17-20 ventral gut spots
2. Ventral chromatophores in a single row, with 2 parallel rows above it on the anterior ¾ of the gut
3. About 50 myomeres between the pectoral fins and vent
4. Yolk sac markedly behind pectoral fins, ventral surface with many tiny chromatophores

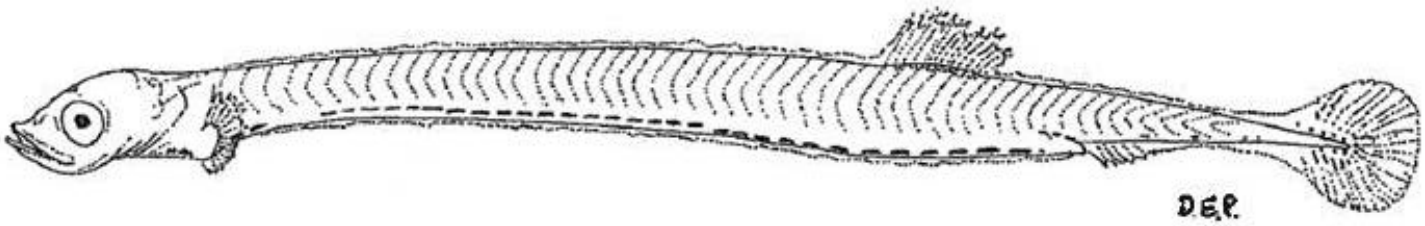


Pacific sand lance, *Ammodytes hexapterus*

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

All figures approximately 15 times natural size.

Larval Forage Fishes of Puget Sound

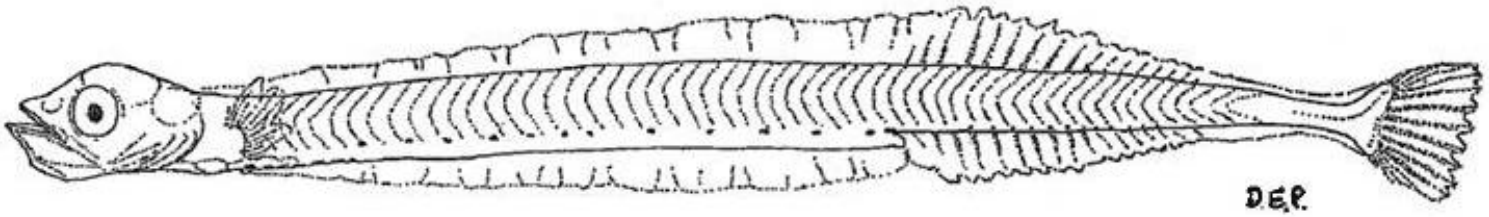


Pacific herring, *Clupea pallasii*

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

Surf smelt, *Hypomesus pretiosus*

- Row of chromatophores on either side of gut, overlapping with single row of chromatophores on ventral midline of gut
- Rayless adipose fin present



Pacific sand lance, *Ammodytes hexapterus*

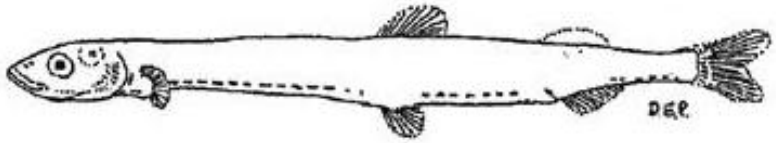
- Widely spaced row of chromatophores on either side of gut
- Wide membranous fins continuous on dorsal and ventral midlines, fin rays appearing posterior to anus

All figures approximately 10-12 times natural size.

Identification of Post-Larval Forage Fishes of Puget Sound

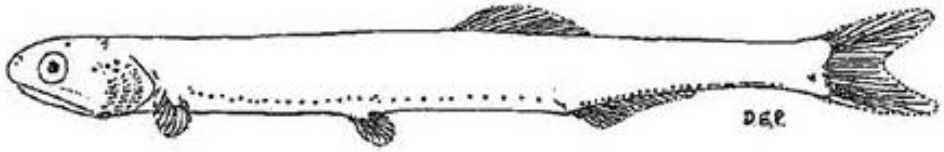
Pacific herring, *Clupea pallasii*

- Insertion of dorsal fin posterior to pelvic fins
- Rows of ventral chromatophores distinct only anterior to pelvic fins



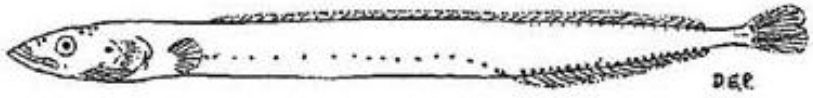
Surf smelt, *Hypomesus pretiosus*

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



Northern Anchovy, *Engraulis mordax*

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



Pacific sand lance, *Ammodytes hexapterus*

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

Fishes of this stage are 30-35 mm in standard length and semi-transparent. All figure are pictured 3-4 times their natural size.

Forage Fish Spawn Sample Analysis Page ____ of _____

Recorder_____

Sample Location Day Month Year

Beach Number	Sample Number	Species	1 cell to morula	Blastula	Gastrula	1 / 2 - 1 coil	1 coil	1½ coil	>1½ coil	Late eyed	Dead	# Eggs	% Dead	Est. # broods	Comments

Version 1.0, July 2011