British Columbia Forage Fish Spawning Monitoring: Citizen Science Methodology

Prepared by: Mount Arrowsmith Biosphere Region Research Institute

October 2018
Revised: July 2021

MARRI

VANCOUVER ISLAND UNIVERSITY

TABLE OF CONTENTS

1.0 MATERIAL CHECKLIST

1.1 Fieldwork Checklist

Sample Collection

- ☐ Data sheets/clipboard
- ☐ Pencils
- ☐ Sample site document for designated area
- ☐ Tide tables (current and previous day)
- ☐ Camera
- ☐ Thermometer
- ☐ Measuring tape x2
- ☐ GPS unit (optional)
- ☐ Scoop (500 mL)
- ☐ 4 L Sample containers
- ☐ Sample tags
- ☐ Sediment Grain Card

Sample Processing

Sieving Process

- ☐ 5 gallon bucket with holes in the bottom
- ☐ Sieves – 4.0 mm, 2.0 mm, 0.5 mm
- ☐ Water buckets
- ☐ Water pitcher (optional)
- ☐ Plastic tub(s)
- ☐ Sample jar(s)
- ☐ Nylon brush
- ☐ Hose for water

Vortex Process

- ☐ 68 litre tote
- ☐ Bilge pump with hose and quick connectors
- ☐ Nylon stocking and an elastic
- ☐ Blue Bowl with stands
- ☐ 0.5 mm sieve
- ☐ 12 V marine battery
- ☐ Shims
- ☐ Turkey baster
- ☐ Big plastic spoon
- ☐ Small plastic spoon
- ☐ Rubber spatula
- ☐ Wash bottle (optional)
- ☐ Stockard's solution
- ☐ MSDS Sheet for Stockard's solution

1.2 Laboratory Checklist

- ☐ Dissecting microscope
- ☐ Petri dishes
- ☐ Small spoon
- ☐ Pipette
- ☐ Fine point forceps
- ☐ Corresponding datasheets
- ☐ Vials
- ☐ Stockard's solution
- ☐ MSDS Sheet for Stockard's solution

2.0 HOW TO SAMPLE – STEP BY STEP

2.1 Site Assessment

1. Assess the area based on sediment type, with Pacific sand lance preferring medium sandy sediments 0.25 mm to 0.5 mm, with spawning also documented in coarse sand and fine pebble sediments 1.0 mm to 7.0 mm in diameter. Surf smelt prefer a sand and pea gravel combination, 1.0 mm to 7.0 mm. The landward boundary of the spawning area is the 'high tide mark', typically identified by a seaweed line, and the seaward boundary is where there is a change in sediment type, becoming larger in size, or is simply at a lower elevation if there is no change in sediment type. *Note: See section 6.0, Best Practices, for images of preferable sediment types.*
2. Lay out the 30 m measuring tape through the middle of the suitable substrate for forage fish spawning habitat/activities.
3. Many of the sampling sites in your area are likely to have been previously visited. Check the *Sample Site* document that corresponds to the area. If the beach location is in the document, you can follow the landmark description in order to return to the previously sampled site.
4. Lay the 30 m measuring tape down in the same location where the last sampling activities took place. Be sure to lay the 30 m measuring tape below the highest tide mark, which could be either the last high tide or second effective high tide line (see section 2.2.1 for descriptions of 'last high tide' and 'second effective high tide') – this means that the landmark distance will differ from that of the previous sample. Ideally, the measuring tape will be laid approximately 0.5 m to 1 m <u>in elevation</u> below the foreshore features (log line, dune grass, etc.). It is important to lay the measuring tape below the highest water mark, usually identified by a line of beach wrack (washed up seaweed), because the forage fish could not move further up the beach then what the recent high tides would allow.
5. In the event that the landmark location does not have ideal sediment, as it is subject to change with wind and wave action, identify a nearby location along the beach that has the suitable sediment type. Lay out the measuring tape approximately 0.5 m to 1 m <u>in elevation</u> below the foreshore features (log line, dune grass, etc.).

2.2 Filling out the Forage Fish Spawning Beach Survey Datasheet

2.2.1 Location & High Tide Events

1. Use the *Location Code* document for Vancouver Island, fill out the regional district, as well as the municipality and/or electoral area that the sample site falls within. For the beach code use the first letter of each word in the name of the beach or the first two letters of the beach's name if it is only one word. For example, the code for 'Community Park Beach' would be 'CPB', whereas the code for 'Morningside Beach' could be 'MOR'.
2. Review Fisheries and Oceans Canada (DFO) management areas to note the fisheries management area in which you are sampling in.
3. In the case for those locations that have sample sites established, use the document that showcases each of the sites; it has the location and DFO areas noted for each beach.
4. Finally, use the tide tables that you have printed off for that day and region to identify the 'Last High Tide', referring to the most recent high tide event, and 'Second Effective High Tide', which refers to a high tide that occurred the previous day that reached an elevation greater than or equal to the last high tide. Record the date, time, and elevation of each respective event, if applicable.

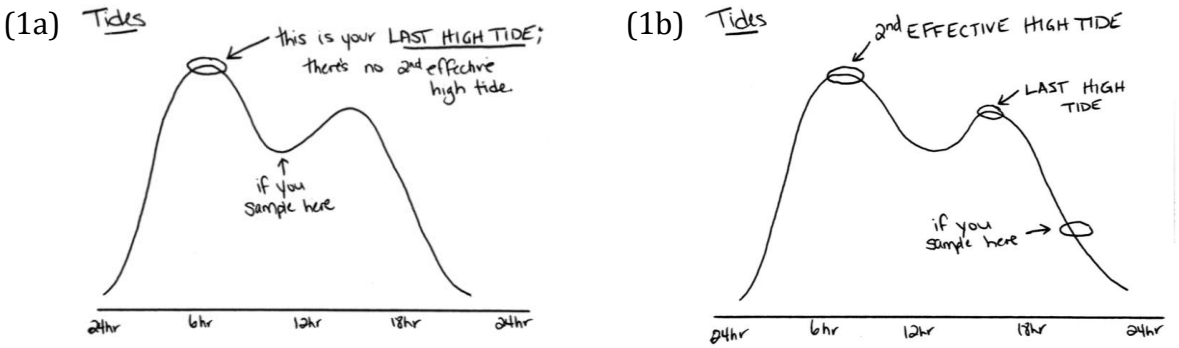


Figure 1. Explanation of tides that occurred prior to the time of sampling: (1a) last high tide, and (1b) second effective high tide.

2.2.2 Samplers

5. Record the sampler's name(s) and affiliated organization, if applicable.
6. Record the date and time of the sample collection, as well as the identification number/name of the camera that you are using to take photos.

2.2.3 Current Conditions

7. Use the closest weather station to your location to record the air temperature, wind direction, and wind speed. Remember that winds are named after the direction they are coming from. *Note: Weather Underground (wunderground.com) is a useful website/app to use. Additionally, any locally accurate weather stations for your area can be used to record the current conditions.*
8. Using the thermometer, record the temperature of the water at the deepest depth you are able to reach safely; be sure that the water you place the thermometer in is not stagnant (i.e., tide pools).

2.2.4 Sediment Sample Collection

9. Record your beach station number, sample number, and time at which you are sampling. *Note: Sample areas are only 30 m by 5 m, therefore there is a possibility that more than one sample can be taken from a single beach station.*
10. If you do not have a GPS unit, record the UTM coordinates of your location from the *Sample Site* document. Be sure to note if where the sample is being collected is different then what is identified in the document and provide some direction as to how it varies.
11. If you do have a GPS unit, go to the 15 m mark along your 30 m measuring tape and record the UTM coordinates.
12. Using the 'Field Observation Sampling Codes', found on the back of the datasheet, choose the <u>dominant</u> beach sediment type. If there is a mix of sediments on the beach, identify which sediment type is dominant in the 30 m by 5 m sampling area. It can be noted in the comments section at the bottom of the datasheet if there was a mix of sediment sizes. The sediment type should be approximated using the sediment grain size card.
13. Using the 'Field Observation Sampling Codes', identify the character of the backshore, which refers to how impacted the area above the beach station is as a result of human development.
14. With the second tape measure, measure the width of the potential forage fish spawning habitat. The width typically stretches from the highest tide mark (either last high tide or second effective high tide), usually determined by a line of washed up seaweed, approximately 0.5 m in elevation below the foreshore features (log line, dune grass, etc.), down to the area that has a notable change

in sediment. *Note: If the beach has completely uniform sediment from top to bottom, the width will extend only a few metres in vertical elevation.*

Figure 2. Identifying the 'width' of the beach that is suitable for forage fish spawning activities in the upper intertidal zone.

15. The length of the potential forage fish spawning habitat is referring to the length of beach that contains the suitable sediment. In order to keep this simple, record if the extent of the beach with suitable sediment is greater than (>) or less than (<) 100 m. *Note: If the length of potential spawning beach is greater than 300m it is a good idea to establish a second beach station at this site.*
16. Record the 'Landmark Object' that you have chosen to measure your 30 m measuring tape from. The object must be a permanent, unmovable object at the top of the beach, along the backshore.
17. You will measure the distance between the 30 m measuring tape, at the 15 m mark, and the chosen landmark. Be sure that the measurement is perpendicular to the measuring tape. Record this measurement in the 'Landmark Distance (m)' column. *Note: Sometimes the landmark does not line up with the 15 m mark. Therefore, be sure to measure the distance of the landmark to the measuring tape perpendicularly. Record in the comment section where on the measuring tape the landmark was measured from and what end of the beach the 0m mark is located. For example, 'the landmark was measured from the 24 m mark and the 0m mark was at the west end of the beach.'*

18. Using the 'Field Observation Sampling Codes', record how shaded the sample site is. *Note: This measurement considers a seasonal and daily average for the site.*
19. There are two sediment samples that can be collected:
    a. A 'Bulk' ("B") sample is a 4 L sediment sample that is collected when no embryos are evidently present at the site. This is the most common type of sample to be collected.
    b. A 'Scoop' ("S") sample requires the collection of approximately 500 mL of sediment. This method is used when embryo masses are visible on the beach. This will ensure species identification and minimal collection of embryos, reducing the overall impact.
20. Each site requires six photos to be taken, including one of the completed sample tag, one of the sediment next to an object for size comparison (use the sediment grain card), one of the beach backshore, beach right, beach foreshore, and beach left. Ensure that you move, as necessary, to get representative photos of the foreshore and backshore. If multiple samples are collected at a single beach station only the photo of the sample tag and sediment are required for each subsequent sample. *See section 2.3 for how to complete a sample tag.*
21. Finally, include any additional comments regarding the site(s), or objects/wildlife you observed at the site in the "Comments" section at the bottom.

2.3 Sample Collection

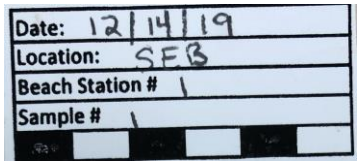


Figure 3. Sample tag.

1. Fill out a sample tag, including the date, location (beach code), sample station, and sample number.
2. You will need a 4 L plastic container, a filled out sample tag, and the 500 mL scoop.
3. Your sample area is 30 m by 5 m – therefore, it runs down the entire length of the 30 m measuring tape and 2.5 m on either side of it, towards the foreshore and backshore.
4. Place the sample tag into the 4 L sample container. The sample tag will follow the sample from this point forward, all the way to the lab analysis.
5. Using the scoop and container, you will collect 4 L of sediment from the sample area, identifying the most ideal sediments along the 30 m measuring tape to collect. Be sure to collect a representative sample, spreading out along the measuring tape where the sediment is being collected; collect approximately half of the sample from above the measuring tape and the other half from below. *Note: This is biased sampling. Density counts are not being determined from this sampling method, simply presence and non-detection.*

Figure 4. Sampling requires the collection of a 4 L sediment sample: (4a) and (4b) depict a sample container that is not filled to 4 L, while (4c) and (4d) show what a full sample container should look like.

6. Rinse the scoop <u>after every sample</u> collected to avoid cross contamination between samples.

Note: If bulk samples cannot be processed immediately, they should be stored in 0.5°C to 7°C, such as a fridge, for up to 7 days; this will aid in reducing the rate of decomposition and embryo mortality.

2.4 Sample Processing

It is possible to complete the next steps either in the field or back at the office and/or laboratory, each requiring specific equipment:

2.4.1 Sieving in the Field

1. You will need a 5-gallon bucket with holes drilled in the bottom, three stackable sieves (4.0 mm, 2.0 mm, and 0.5 mm), a water bucket, a wash bucket, and a sample jar.
2. First, ensure that the sieves are stacked in the appropriate order from largest to smallest with the smallest being on the bottom. Then place the sieves on top of the 5-gallon bucket and add the sample into the top sieve. Transfer the sample tag into the sample jar and ensure that this stays with the sample throughout the rest of the processing.
3. This is a two-person job – the first person will be collecting water to pour over the sample while the second person is responsible for shaking the sediment through the sieves.
4. Once the sample is fully washed through the sieves, transfer the sample from the 0.5 mm sieve into the wash bucket, this is the sample that you will process using the vortex method. *Note: The sediment left in the 4.0 mm and 2.0 mm sieves can be disposed.*
5. Ensure you clean the sieves using the nylon brushes, and rinse out the buckets after each sample, avoiding cross contamination between samples.

Figure 5. Research assistants sieving sediment samples.

2.4.2 Sieving at the Office/Laboratory

6. Follow the same method as stated above in '*Sieving in the Field*,' but instead of one person collecting water to pour over the sample, a hose can be used to wash the sample through while the other person shakes the sieves.
7. Ensure that you have collected the sample from the 0.5 mm sieve in a wash bucket, as well as clean the sieves and 5-gallon bucket between each of the sieving events.

2.4.3 Vortex Method

Note: Vortex method instruction discussed below is adapted from WDFW's published methods (Dionne, 2015), which can be found here: https://wdfw.wa.gov/publications/02022.

8. Ensure the nylon stocking is secured around the bilge pump using an elastic.
9. Fill the 68 L tote with 3 to 4 buckets of water, the hose, or until it is half full.

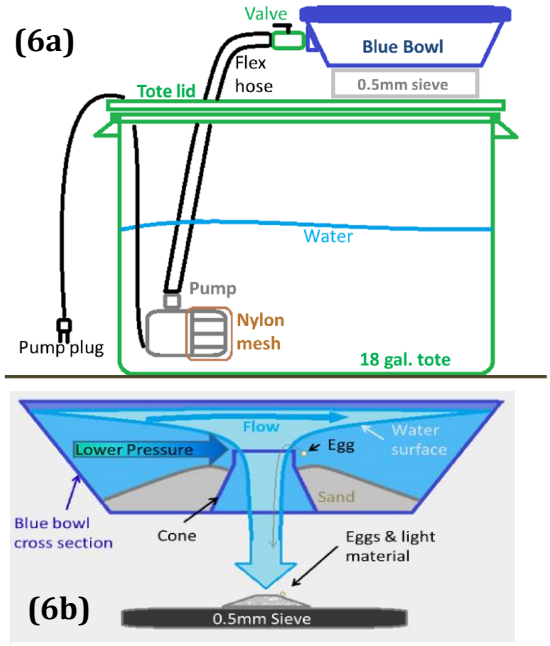


Figure 6. Vortex method explanation: (6a) vortex kit set-up and (6b) how the vortex is generated (Dionne, 2015).

10. Put the tote lid back on and feed the bilge pump through the smaller hole, ensuring that the alligator clips and the flex hose stick out and the pump is fully submerged.
11. Rest the 0.5 mm sieve over the larger hole and place the blue bowl on top of that. Make sure that the sieve and blue bowl are as level as possible – use shims to level it if necessary.
12. Connect the bilge pump's hose to the blue bowl. Before you attach the battery clamps to the 12V battery, ensure that the valve on the blue bowl is open – it should never be closed when the pump is running, it will cause the hose to burst. Refer to section 5.0 to build a vortex kit of your own if you do not have one.
13. Attach the battery clamps to the battery and allow the blue bowl to fill with water. *Note: Always connect the positive cable first, followed by the negative cable.*
14. Add the sediment sample to the blue bowl. The water should be approximately 1 to 2 cm from the top of the bowl after the sediment has been added.
15. Add the entire sample to the blue bowl, ensuring that you rinse out the wash bucket as well. *Note: If you have a very large sample that will over flow the raised centre be sure to process the sample in multiple portions.*
16. Once the sample is in the bowl, use the spatula and small spoons to agitate the sediment starting at the centre and moving the sediment towards the outer rim, for 3 minutes – this will release the lighter materials, such as embryos and organic matter. These lighter materials will be carried by the water vortex through the raised centre and be collected in the sieve below.
17. After agitation, let the water run for another minute, allowing the vortex to collect any of the last material.

Figure 7. Depicting how to agitate the sediment in the blue bowl.

18. When you are ready to shut off the bilge pump, you will need to close the valve attached to the blue bowl and disconnect the battery clamp simultaneously. Closing the valve will ensure that sediment doesn't get sucked back into the bilge pump. *Note: Always remove the negative cable first, then the positive cable.*
19. Using the baster, collect the sediment that is directly beside the raised centre of the blue bowl. This action will ensure that any final organic materials that didn't make it over the rim will be included in the sample.
20. Wash the final sample that was collected in the 0.5 mm sieve, into a sample jar. *Note: Try to limit the amount of water entering into the sample jar.*
21. If your coordinating group (MABRRI, Peninsula Stream Society, etc.) asks you to add Stockard's solution to your sample, do so at this time.
    a. Using a pipette, do your best to remove the top layer of water in the sample jar.
    b. In a well ventilated area and wearing gloves and safety glasses, add enough Stockard's solution to cover the sediment sample.
    c. The sample can then be stored at room temperature until your coordinating group is able to pick up the sample. *Note: Your coordinating group may request you do not add Stockard's solution. Be sure to check in with them with regards to their preference.*
22. Between samples, wash the sieves with a nylon brush and rinse out the blue bowl.

23. When finished, rinse all of the equipment, including the nylon stocking. Additionally, rinse the bilge pump in fresh water. This is especially important if you used salt water to process the samples. The salt water will take a toll on the equipment over time.

3.0 LABORATORY ANALYSIS

Note: It is up to the citizen scientists whether or not they would like to take on the task of laboratory analysis. If not, ensure that they are passed onto the coordinating group (MABRRI, Peninsula Stream Society, etc.) to be analyzed in the laboratory for embryos.

1. Each sample should be analyzed separately, ensuring no cross contamination.
2. Using a small spoon, take a very small amount of your sample and spread it thinly in a petri dish. Creating a single layer, rather than a thick layer of sediment, along the bottom of the petri dish is the best technique and will reduce the possibility of missing embryos.
3. Comb through the entire sample using the dissecting microscope.
4. Whenever you think that you have found an embryo, use a pipette to transfer it gently into another petri dish.
5. Be sure to separate all of the embryos that you find from the sediment for further analysis. If there are embryos present, you will document:
   [1] the species,
   [2] the number of each species, and
   [3] the alive to hatched/dead ratio of each species.

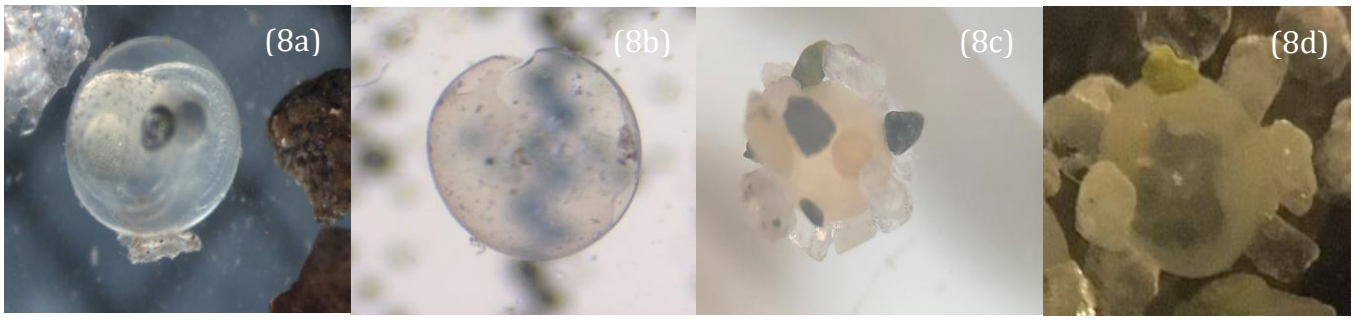


Figure 8. Embryos that are alive vs hatched/dead. Figures (8a) and (8b) show surf smelt embryos alive and hatched, respectively (Brian Koval, Peninsula Stream Society, 2019). Figures (8c) and (8d) show Pacific sand lance embryos alive and hatched, respectively.

   If there are more than 100 embryos, you are only required to stage the first 100. All of this data is to be documented on the data sheet.
6. After analysis, the embryos that are found should be transferred to vial and preserved in Stockard's solution. Be sure you are in a ventilated area and wearing gloves and safety glasses when using the Stockard's solution. You will pipette the solution from the bottle into the sample vial with the embryos; you only need enough solution to cover the embryos.

3.1 Species

There are four potential fish species that you are likely to see when sampling, including:

3.1.1 Pacific Sand Lance (Ammodytes personatus)

- Embryos are 0.8 mm to 1.0 mm in diameter
- Have multiple sand grains attached
- Not completely round
- Milky colour
- There is 1 large oil droplet in the yolk

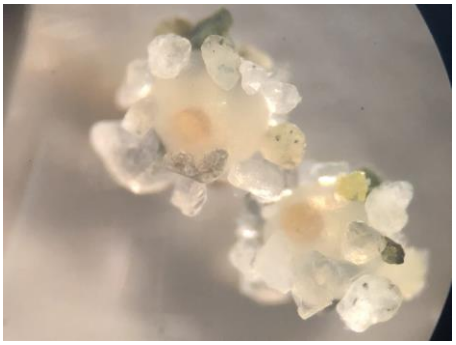


Figure 9. Pacific sand lance embryos.

3.1.2 Surf Smelt (Hypomesus pretiosus)

- Embryos are 1.0 mm to 1.2 mm in diameter
- The embryo will only be attached to the sediment at a single point, where the membrane has ruptured and folded back, exposing an adhesive attachment point, called the 'peduncle'
- Non-self-adhesive – they do not attach to other embryos

Figure 10. Surf smelt embryos (Brian Koval, Peninsula Stream Society, 2019).

3.1.3 Rock Sole (Lepidopsetta bilinear)

- Perfect sphere
- Very transparent
- Does not attach to sediment – no attachment sites
- Non-adhesive

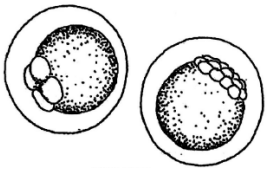


Figure 11. Rock sole embryos (Moulton & Penttila, 2006).

3.1.4 Pacific Herring (Clupea pallasii)

- Embryos are 1.3 mm to 1.5 mm in diameter
- Almost entirely spawn on marine vegetation
- They have distinct shell attachment sites
- Often found in layers or clumps

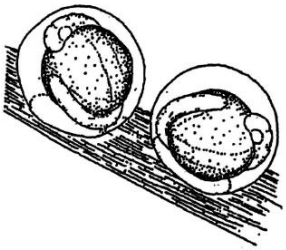


Figure 12. Pacific herring embryos (Moulton & Penttila, 2006).

3.2 Embryo Validation Process

Whenever embryos are detected in samples, they will need to be confirmed by an expert prior to submission of results to the database. Be sure to provide your coordinator with all of the materials associated with the sample, including datasheet and photos. Once the coordinator has all the required materials and photos of the embryos, they will send these off for confirmation to an expert in either British Columbia or Washington State. Your coordinator will keep you informed with regards to the results of your samples.

4.0 DATA MANAGEMENT

4.1 Submitting Data to Sampling Coordinator

The coordinating groups will be gathering and compiling all of the data collected by the citizen scientists for submission to the Strait of Georgia Data Centre. Be sure to provide the coordinator(s) with all the materials associated with the sample(s) you collected, including datasheet(s) and photos.

Note: It is good practice to keep a copy of the data you collected. Be sure to either take a photo of the submitted datasheet or send a photo/scan of the datasheet to your coordinator.

4.2 Strait of Georgia Data Centre

The coordinating groups will submit all compiled data to the Pacific Salmon Foundation's Strait of Georgia Data Centre, an open-access database hosted by the University of British Columbia. All data with regards to forage fish in the Salish Sea will be stored in this database, allowing for anyone interested in the data to access it. You can access the database from this link: http://sogdatacentre.ca/.

5.0 BUILDING A VORTEX METHOD UNIT

5.1 Materials Required

- ☐ 68 litre tote with lid
- ☐ Water bucket
- ☐ Blue bowl concentrator
- ☐ An adjustable hose valve
- ☐ 750 to 1000 GPH submersible electric water pump
- ☐ Alligator clips
- ☐ Nylon stockings and an elastic
- ☐ 60 cm length of ¾" corrugated hose
- ☐ ¾" male hose fitting
- ☐ 2 x ¾" hose clamps
- ☐ Quick connect hose fittings
- ☐ 0.5 mm Sieve
- ☐ Wash tub
- ☐ Shims
- ☐ Rubber spatula
- ☐ Large plastic spoon
- ☐ Small plastic spoons
- ☐ Baster

- ☐ Nylon brush
- ☐ Wash bottle (optional)
- ☐ Metal hangers (optional)
- ☐ 12 V marine battery

5.1.1 For construction

- ☐ Box cutter
- ☐ Permanent marker
- ☐ Electrical tape

5.2 Material Preparation

5.2.1 Body of the Vortex

1. Cut two holes in the tote's lid: one smaller one in the top corner for the flex hose and battery clamps to come out of and one larger round one that the 0.5 mm sieve will sit on. You will have to customize this hole to ensure that your sieve will not fall through. *Note: It is suggested you draw the holes with a marker prior to cutting, ensuring that the holes will not be too big.*

Figure 13. Holes cut into the tote lid for the vortex kit (Dionne, 2015).

5.2.2 Bilge Pump Preparation

2. Connect the bilge pump to one end of the corrugated hose using one of the hose clamps.
3. Insert the ¾" male hose fitting into the other end of the corrugated hose and secure it with the second hose clamp.
4. Add one of the quick connector fittings to the male hose fitting on the corrugated hose.
5. Using pliers, attach the alligator clamps to the bilge pump cables. Be sure to connect the clamps to the appropriate cables.
6. Stretch the nylon stocking over the pump's water intake and secure in place with an elastic. The stocking ensures that if any embryos are lost in the tote, they will not pass through the pump and into the blue bowl, potentially cross contaminating samples if multiple are being processed. *Note: Be sure the nylon stocking is tight so the stocking does not get sucked in when the pump is turned on.*

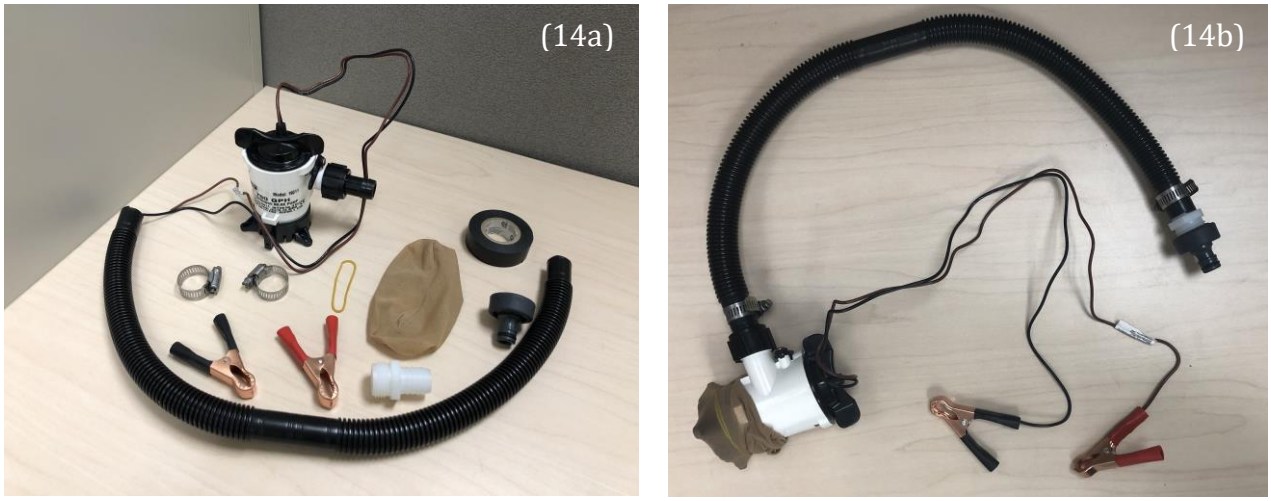


Figure 14. Bilge pump preparation: (14a) identifies the materials that will be required to prep the bilge pump and (14b) depicts how the bilge pump should look when put together.

5.2.3 Blue Bowl Concentrator Preparation

7. Add the second quick connector fitting to the blue bowl concentrator.
8. Prepare 'legs' for the blue bowl by cutting the metal hangers with wire cutters and folding the ends of the metal hangers back. *Note: The blue bowl concentrators come with 'legs', however they are typically too small and result in the blue bowl slipping off of the sieve. Therefore, it is recommended to build these longer 'legs' to ensure the sample being processed does not slip off.*

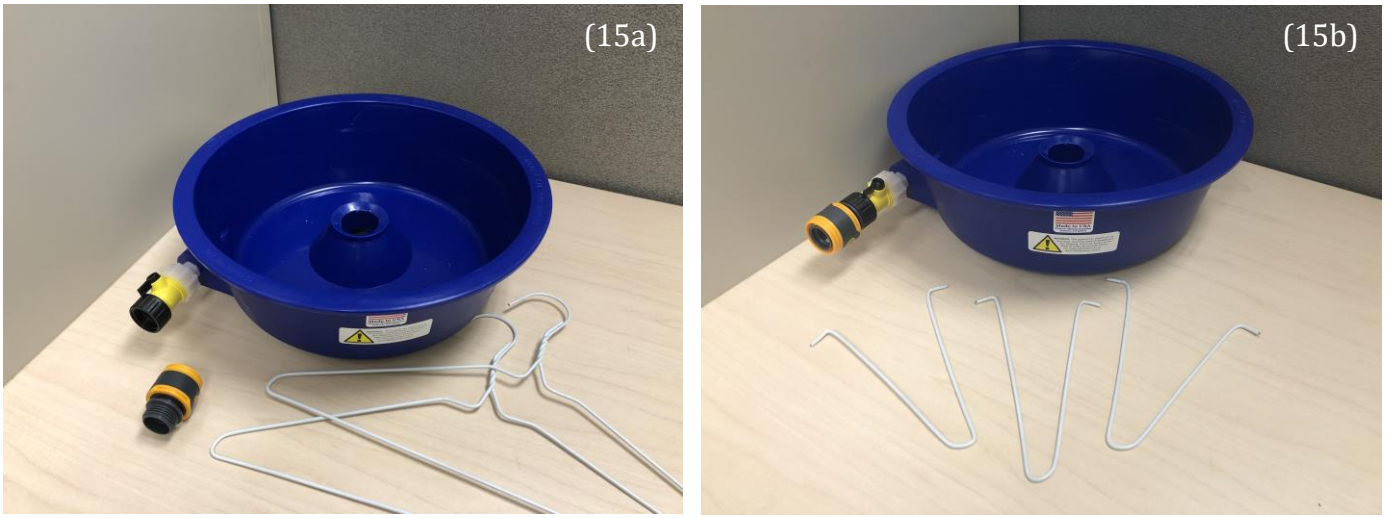


Figure 15. Blue bowl concentrator preparation: (15a) identifies the materials that will be required to prep the blue bowl and (15b) depicts how the blue bowl should look when put together.

5.3 Assembly

9. On a level surface, fill the tote half full of water, place the bilge pump in the tote, and secure the tote's lid, feeding the alligator clamps and corrugated hose through the "pump hole."
10. Set the 0.5 mm sieve over top of the "water return hole" and stack the blue bowl on top of the sieve, extending the 'legs' so it sits level.
11. Attach the corrugated hose to the blue bowl using the quick connector fittings. The final product should appear like Figure 16.
12. Follow the sediment processing details under the "*Sample Processing – Vortex Method*."

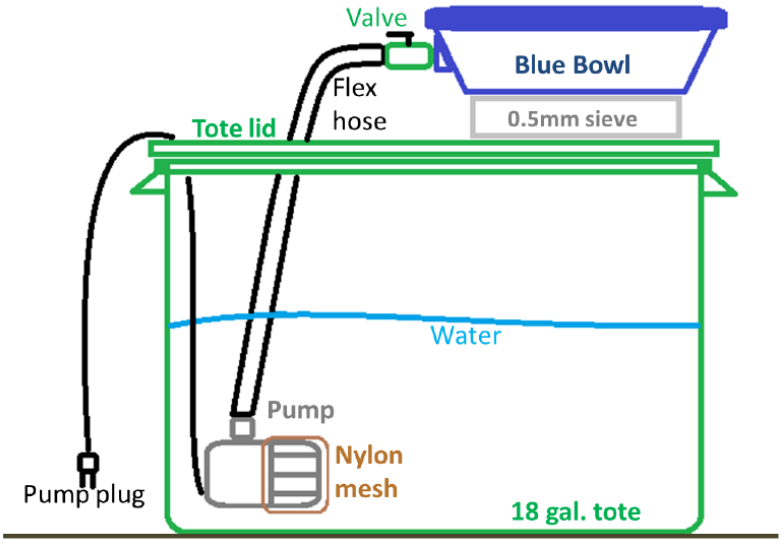


Figure 16. How to set up a vortex kit set-up (Dionne, 2015).

6.0 BEST PRACTICES

6.1 Identifying Suitable Sediment

When selecting spawning habitat, different species of forage fish have different preferences for different sediment types. It is not uncommon that there is a lot of broken shell material mixed in with the sand and gravel. Do not try to avoid the shell materials as forage fish embryos can attach to the shells, as they are the same size as their favourable sediments.

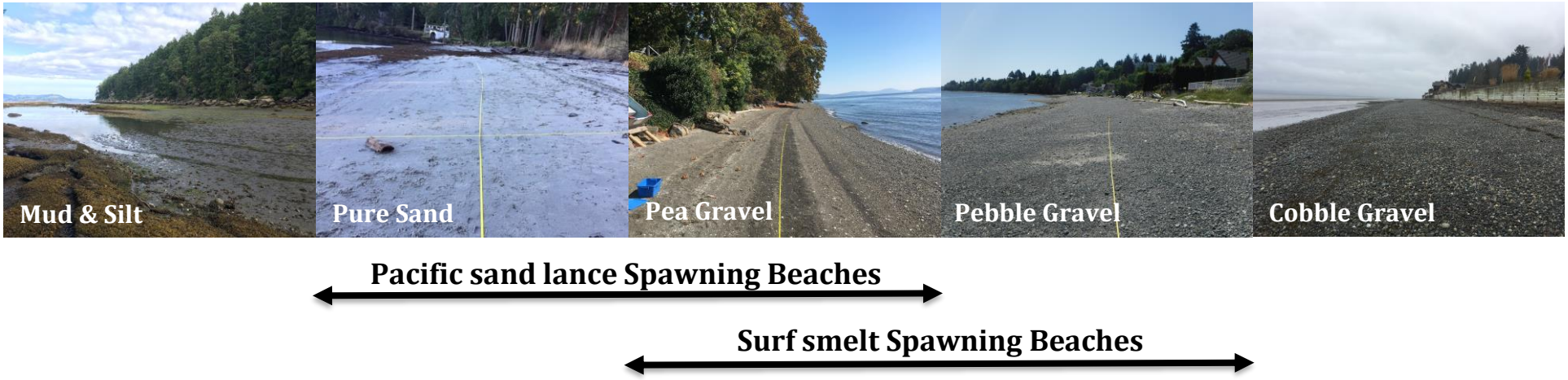


Figure 17. Identifying which beaches are preferable for Pacific sand lance and surf smelt to use for spawning.

6.1.1 Suitable Sediment: Pacific Sand Lance (PSL)
Pacific sand lance spawn from November to mid-February and prefer medium sand 0.25 mm to 0.5 mm in diameter, with spawning also documented in coarse sand and fine pebble sediments from 1.0 mm to 7.0 mm in diameter.

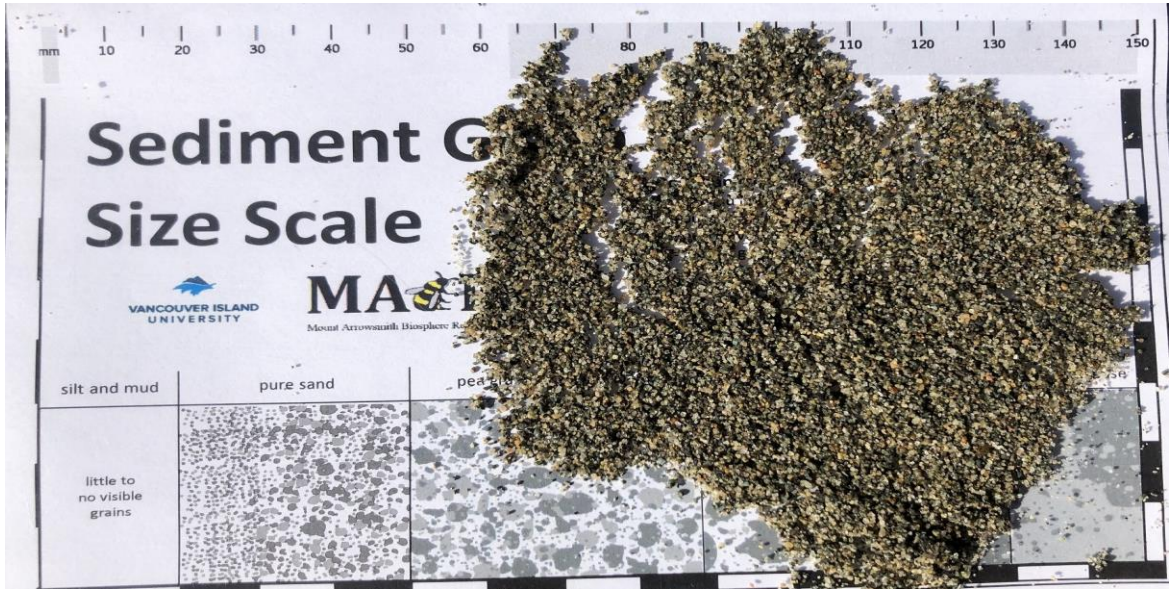


Figure 18. Pure sand: preferable sediment for PSL spawning (to scale).

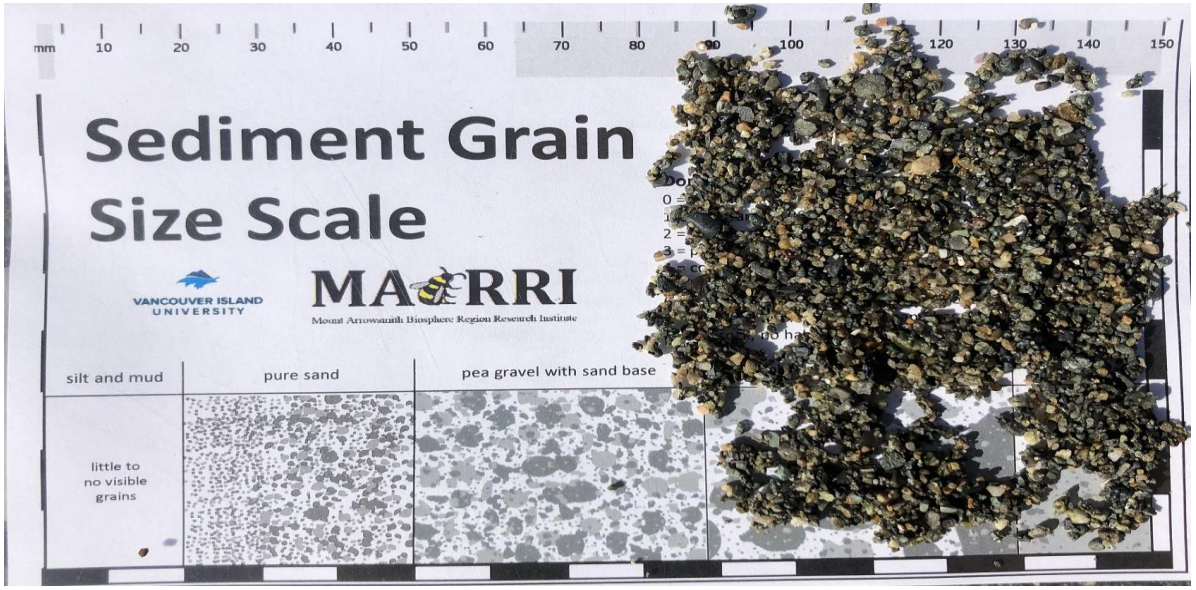


Figure 19. Pea gravel with sand base: preferable sediment for PSL spawning (to scale).

6.1.2 Suitable Sediment: Surf Smelt (SS)
Surf smelt spawn have been found to spawn year-round in coarse sand to fine pebble sediment mixes ranging from 1.0 mm to 7.0 mm in diameter.

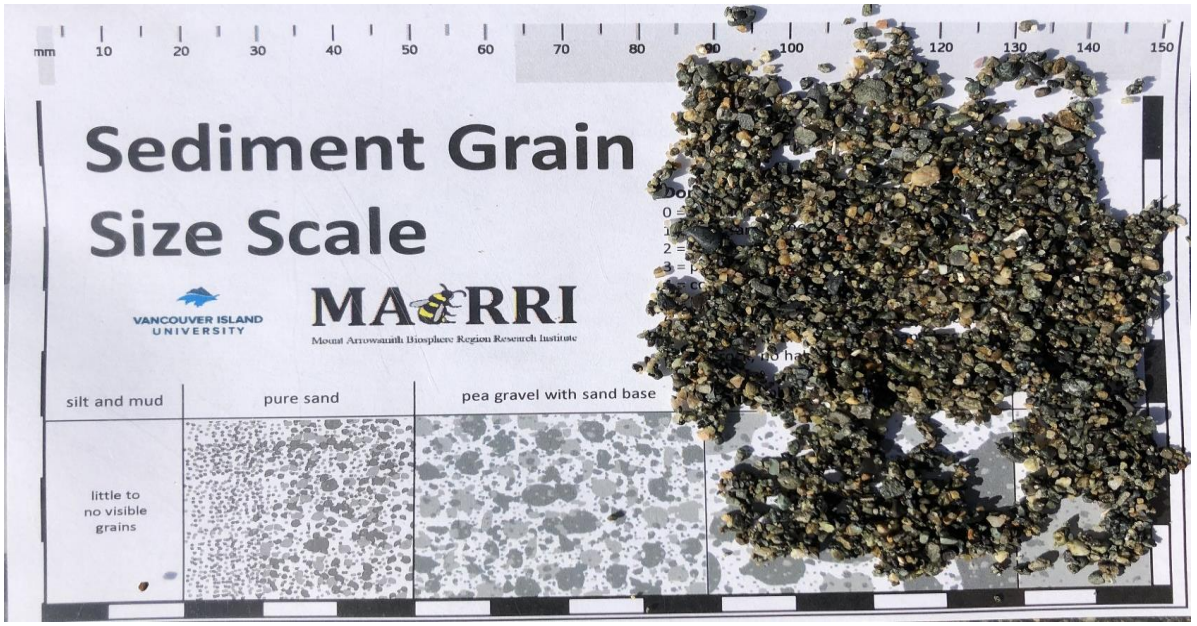


Figure 20. Pea gravel with sand base: preferable sediment for SS spawning (to scale).

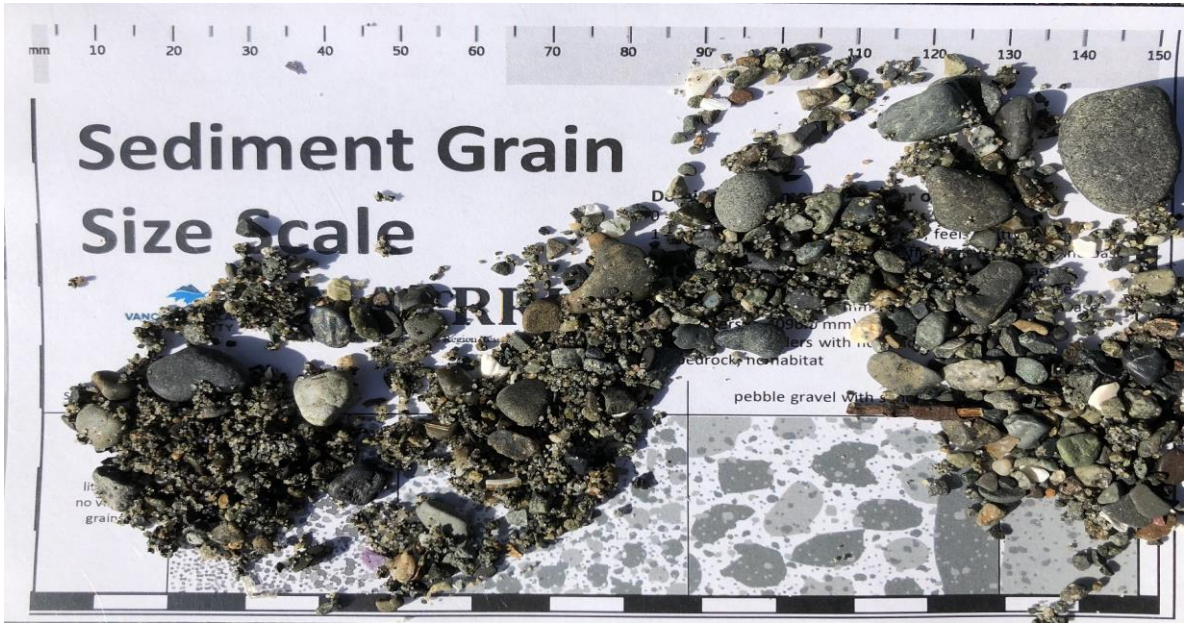


Figure 21. Pebble gravel with sand base: preferable sediment for SS spawning (to scale).

6.1.3 Unsuitable Sediments

Some of the sediments that beaches consist of are not suitable for Pacific sand lance and surf smelt to spawn on. Large cobble not ideal; although forage fish embryos can attach to this larger sediment, they are less likely to survive. Additionally, mud and silt is too fine and compact, making it difficult for respiration to occur and increasing the likelihood of embryo smothering.

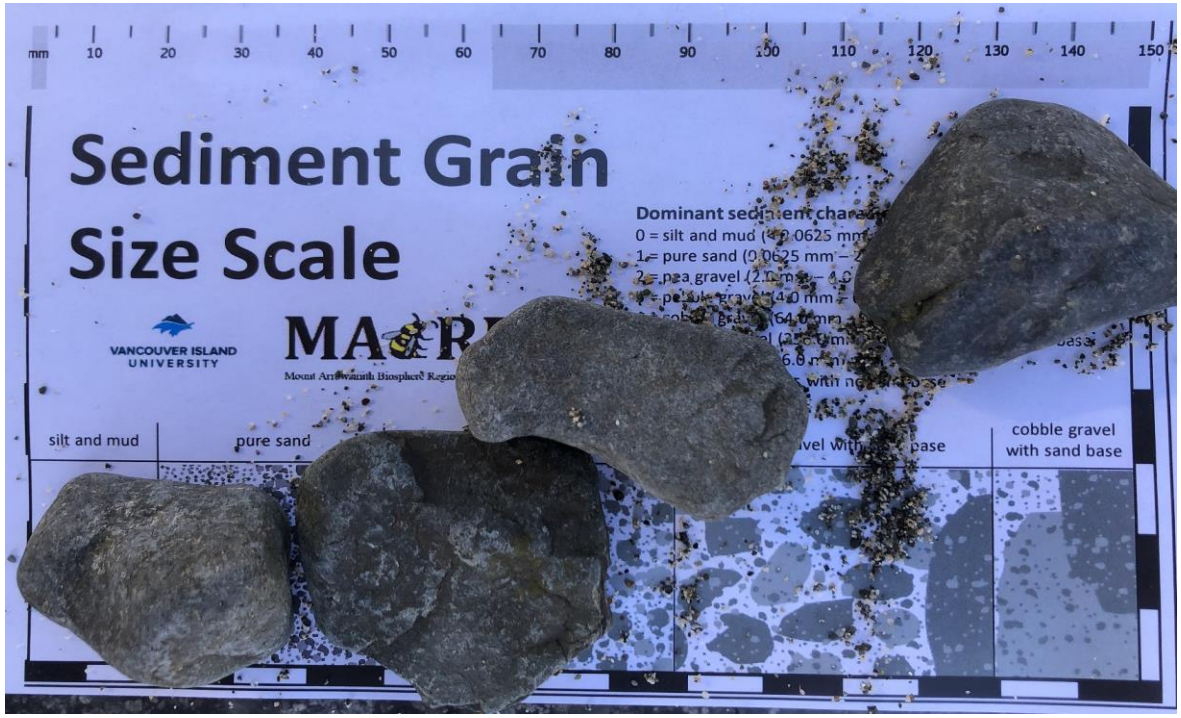


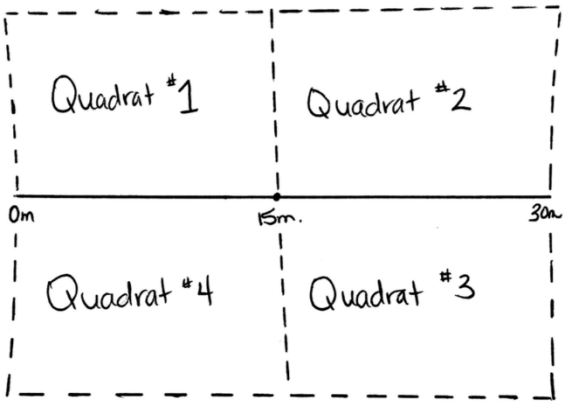
Figure 22. Cobble gravel with a sand base: unsuitable sediment for PSL and SS spawning (to scale).

6.2 Forage Fish Sampling Methods

A few things to consider when sampling:

- Select the most ideal looking sediment. Sampling those sediments that are approximately 0.2 mm to 7.0 mm in diameter will provide you with the greatest potential of capturing a spawning event. This is biased sampling, but these protocols are used to determine where and when surf smelt and/or Pacific sand lance are spawning in British Columbia. Therefore, the data collected will indicate presence or non-detection.
- When investigating a beach's sediment composition, be sure to move some of the top layer of larger sediment or seaweed out of the way to determine if:
    - the ideal sediment identified on the surface is a few centimeters thick and not just a small layer situated overtop larger cobble sediments; or,
    - the not ideal sediment (i.e., larger cobble) identified does not have a layer of ideal sediment below it before moving on to the next beach. *Note: When sampling you can move the top layer of sediment and scoop the ideal sediment below. This can be done because when spawning occurs, the wave action moves the embryos around, allowing them to settle down under the larger sediment.*
- When collecting the sediment, do your best to get a representative sample of the entire sample area (30 m by 5 m). A simple way to do this is to mark the 15 m mark along the 30 m measuring tape and envision that your area is divided into 4 equal quadrats, each 15 m by 2.5 m (see below). By doing this you can collect approximately 25% of your sample from each section.

(23a)



(23b)

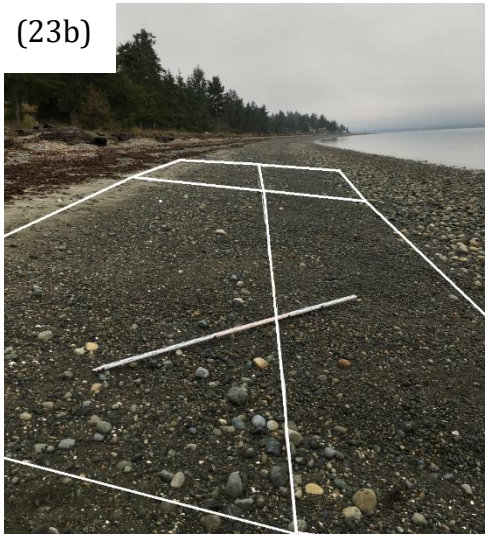


Figure 23. An example of how to visualize the sampling area to collect the representative sample: (23a) breaking the 30 m by 5 m into quadrats, and (23b) the quadrats drawn out on a beach.

Note: If a portion of your sample area has non-ideal sediment (bedrock, large cobble, mud, or silt), do your best to get an equal amount of sediment from each of the ideal sections.

6.3 Sample Processing

A few things to consider when processing your sample(s):

- When transferring the sample from the sieve to the wash tub in the field, pour the water through the back of the sieve and slowly rotate the sieve to ensure the entire sample has been transferred.

    Note: When using a hose, it is still easiest to spray the sieve from the backside to transfer the entirety of the sample into the wash tub.

- When sieving in the field, you can use a water pitcher instead of a large water bucket to reduce physical ailments, as well as have better control of the water flow.
- If you still retain a very large sample after you have processed your sample through the sieves, it is best to process the sample in multiple 'batches' through the vortex kit; this will enable the greatest amount of the preferential sediments to be collected.
- When you have completed both processing steps, sieves and the vortex kit, and you are transferring your sample into a jar, it is best to first scoop the bulk of the sediment into the jar with a small spoon (rather than using water to coerce your sample into the jar). If you use water from the start, there is the potential that your jar will overflow, which may result in losing some of your sample.
- Be sure to clean all your gear between samples (i.e., sieves, blue bowl, buckets, baster, spoons, etc.) to prevent cross contamination.
- If you use salt water to process your sample, be sure to rinse all of your gear with fresh water as soon as possible, including flushing the bilge pump.

6.4 Laboratory Analysis

A few things to consider when analyzing your sample(s):

- Drawing grid lines or a set of circles on the bottom of the petri dish can aid in distinguishing where in the petri-dish you have already looked
- When putting sediment into the petri dish to analyze, be sure to only put in a very small amount to make a single layer of sediment. If sediments are stacked on top of one another, processing them takes longer and it is less likely that you will see the embryos mixed in amongst the sediment.
- Look for movement in the petri dish when moving it around. The embryos sway in the water when the dish is moved around, while the sediment will remain steady.

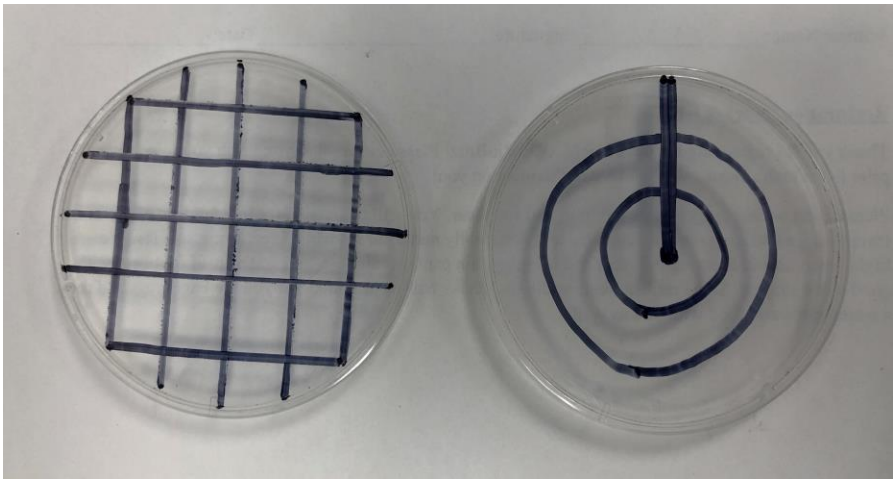


Figure 24. Petri dishes with grid lines and circles to aid in analysis process.

- When you think you have found an embryo, use the forceps to gently squeeze it – this will ensure that it is not a rock or a piece of plastic.
- If the sample is not in preservative, be sure to keep it in a cool place (i.e. fridge) and analyze your samples within seven days.

6.5 How to Safely Use Stockard's Solution

Although Stockard's solution is more potent than ethanol, there are some benefits to using it. It is recommended that Stockard's solution be used because ethanol can bleach and desiccate the embryos, making species identification difficult. Additionally, when the embryos are preserved in Stockard's solution than can be stored for years and can be used for educational purposes.

Although it is preferred that the preservative be used by the coordinating group, there will be a few instances when it will be preferable that citizen scientists are the ones to administer the Stockard's solution. Therefore, there are a few things to consider when using it:

- Ensure to have the MSDS sheet on hand
- Recommended to have WHMIS training
- Be sure to be in a well ventilated space (ex. outside) and wearing gloves and safety glasses when using
- Use a pipette to transfer the Stockard's solution into the jar or vial in a controlled fashion
- When filling the sediment or embryos, only add enough solution to fully cover them
- Provide all Stockard's solution to the coordinating group to ensure that proper waste disposal protocols are followed.

7.0 REFERENCES

A variety of documents and resources were used to produce this manual, including:

Dionne, P. (2020). Personal communication.

Dionne, P. (2015). Vortex method for separation of forage fish eggs from beach sediment. Washington Department of Fish and Wildlife, 1-14.

Fisheries and Oceans Canada (DFO). (2002). Surf smelt: Stock status report B6-09(2002). Retrieved from https://waves-vagues.dfo-mpo.gc.ca/Library/345799.pdf

Koval, B. (2019). Images of embryos.

Meisel Rock Products. (2015). Sand and gravel uses. Retrieved from http://www.meiselrockproducts.com/sand-and-gravel-uses/

Moulton, L.L. & Penttila, D.E. (2006). San Juan County forage fish assessment project: Field manual for sampling forage fish spawn in intertidal shore regions. Retrieved from the Washington Department of Fish & Wildlife.

Robards, M.D., Piatt, J.F., & Rose, G.A. (1999). Maturation, fecundity, and intertidal spawning of Pacific sand lance in the northern Gulf of Alaska. *Journal of Fish Biology, 54,* 1050-1068.

Robinson, C. (2020). Personal communication.

Swanpark. (2019). White pea gravel. Retrieved from http://swanpark.site/white-pea-gravel/

Thuringer, P. (2003). Documenting Pacific sand lance (Ammodytes hexapterus) spawning habitat in Baynes Sound and the potential interactions with intertidal shellfish aquaculture. Retrieved from https://www.for.gov.bc.ca/tasb/slrp/marine/south_island/baynes/docs/sandlance/baynes_sandlance_%20draftreport.pdf

Thuringer, P. (2017). Personal communication.

Thuringer, P. (2020). Personal communication.