Vortex method for separation of forage fish eggs from beach sediment

Addendum to the 2006 revision of
Field Manual for Sampling Forage Fish Spawn in Intertidal Shore Regions

Phillip Dionne
Washington Department of Fish and Wildlife
1111 Washington St SE
Olympia, WA 98501
Phillip.Dionne@dfw.wa.gov

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Introduction

Washington Department of Fish and Wildlife (WDFW) biologists have assessed marine shorelines for evidence of forage fish spawning (presence of eggs) since the 1970's. During this time, the biologists have continued to develop effective and efficient protocols for collecting and identifying forage fish eggs from beaches. The purpose of this document is to describe an alternative method for extracting forage fish eggs from beach sediment samples that increases lab efficiency and egg count accuracy.

The sampling protocols developed prior to 2015 are documented in Moulton and Penttila (2006 revision; https://wdfw.wa.gov/publications/01209/wdfw01209.pdf), which described the process from beach site selection and sediment sample collection through condensing bulk sediment samples to laboratory analysis. As described on pages 24 and 25 of the 2006 field manual, the first step in treating the bulk sample is to sieve the sample through progressively finer sieves (4 mm, 2 mm, and 0.5 mm mesh). Only the material collected in the 0.5 mm sieve is retained for further processing. During the *winnowing* process, the condensed sample material is transferred to a square washbasin where it is covered with a thin layer of water and agitated to suspend and concentrate the lighter material, including eggs above the heavier material. This top layer of lighter material is collected and retained for laboratory analysis (examination of material by microscope) to identify and count the eggs.

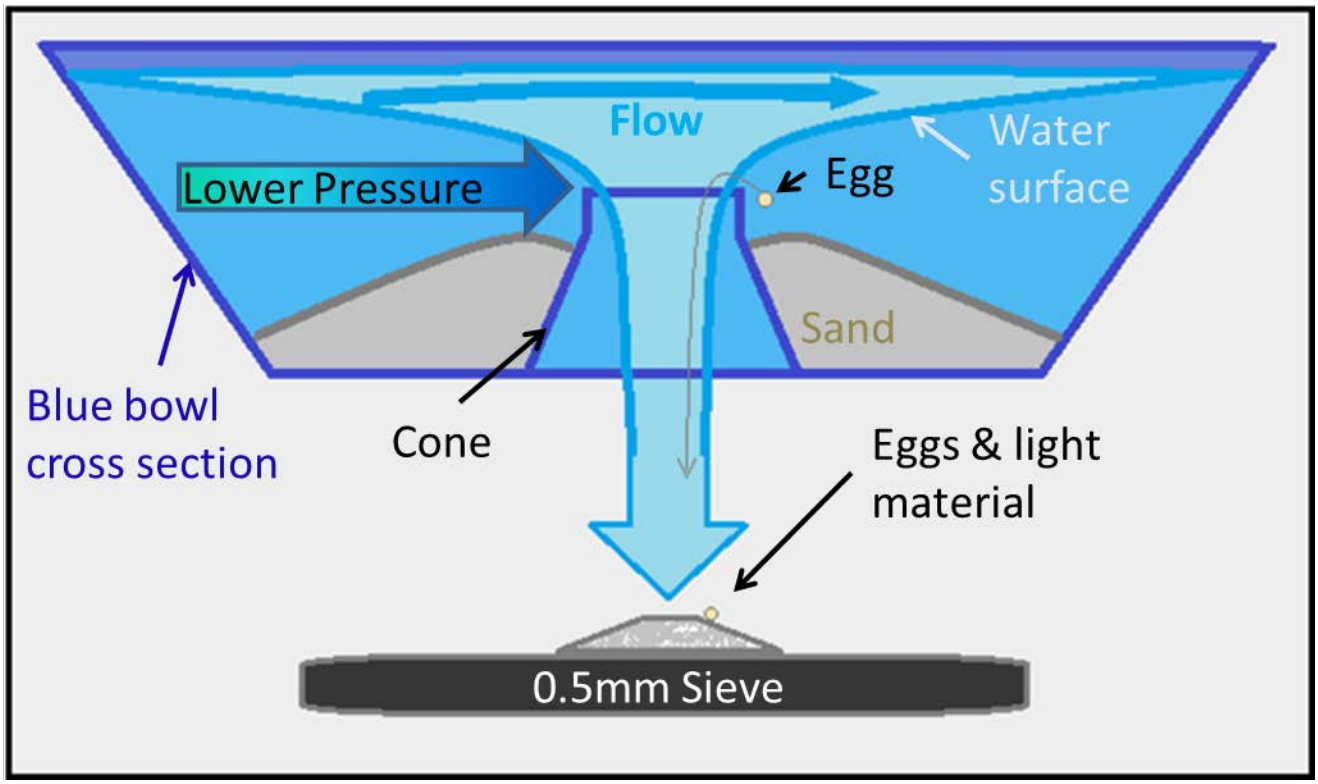
An alternative to the *winnow* method, the *vortex* method, was developed for condensing bulk samples to concentrate eggs. The *vortex* method, like *winnowing*, also follows sieving. The condensed material collected in the 0.5 mm sieve is added to a hydrocyclone device consisting of a circular bowl and a recirculating electric water pump to create a vortex that concentrates the light material. Thus, this step replaces the agitation process described in the 2006 field manual. The *vortex* method resulted in less material to be sorted through in the lab for egg identification. We intend the *vortex* method to be used in place of the *winnowing* method.

We compared the two condensing methods, *winnow* and *vortex*, and found the *vortex* method has a higher egg recovery rate than the winnow method (average smelt egg recovery rates, *winnow* method: 59%, *vortex* method: 90%) and results in a smaller volume of material to process in the lab. In light of these improvements in efficiency, we recommend the *vortex* method for condensing bulk samples after sieving. However, before any modifications are made to your sampling program, be advised that careful consideration should be given to potential impacts to results and whether results from the two methods are directly comparable. Please consult with WDFW staff if you would like to discuss compatibility with WDFW data standards.

This document contains a description of the process and system that we have designed and tested. Modifications to the process or system we describe below may alter the efficiency of the system and consequently lead to results that are not comparable with our results. Those who intend to utilize the *vortex* method should obtain training prior to implementation. Biologists using these methods for regulatory surveys must complete the WDFW training. Additional information and resources for training are provided on page 11 of this document.

How it works

- The movement of the water through the bowl creates a vortex resulting in a pressure gradient.
- The material in the water moves from higher pressure at the edge to lower pressure in the middle of the bowl.
- Less dense materials, such as eggs, move towards the center faster than more dense materials.
- The raised cone in the middle of the bowl reduces the amount of sand and other dense material that leaves the bowl.
- The water leaving the blue bowl passes through a 0.5 mm sieve before being returned to the water reservoir.
- The sieve collects only the material that is egg size or greater.



Materials

For more detail, a list of URLs for parts is included on page 12 of this document.

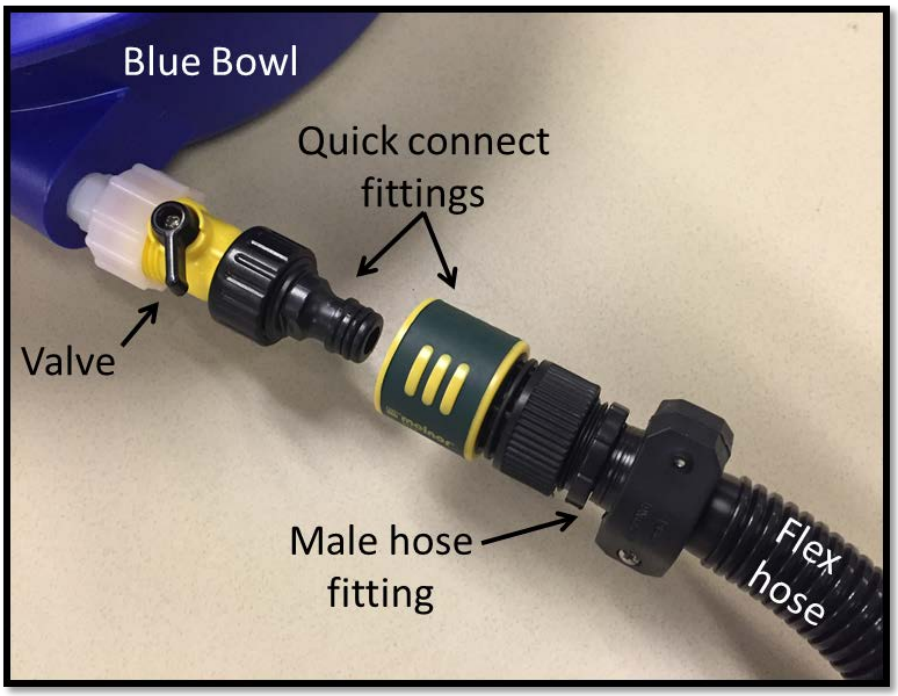
One 18 gallon tote with lid
One blue bowl gold concentrator
One 750 to 1000 gph submersible electric water pump
One, two foot length of ¾" flex hose
One, ¾" hose clamp
One, ¾" male thread hose end kit
One adjustable hose valve
One quick connect hose fittings kit with female thread
One, 0.5 mm sieve (this can be the same sieve used to sieve the bulk sample)
Three shims
One, 250 to 1000 ml wash bottle
One rubber spatula
One plastic spoon
One large pipette or turkey baster
Sample jars

Tools for assembly:
Screw driver
Metric ruler
Permanent marker
Box cutter

Optional: The unit can be configured with a bilge pump and 12 volt battery to allow for use at locations where electricity is not available.

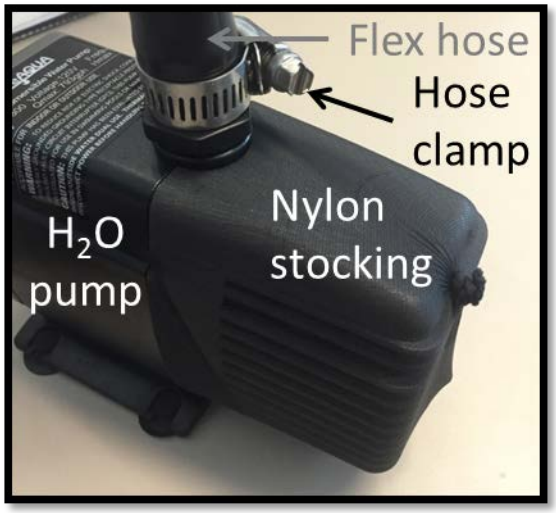
Assembly

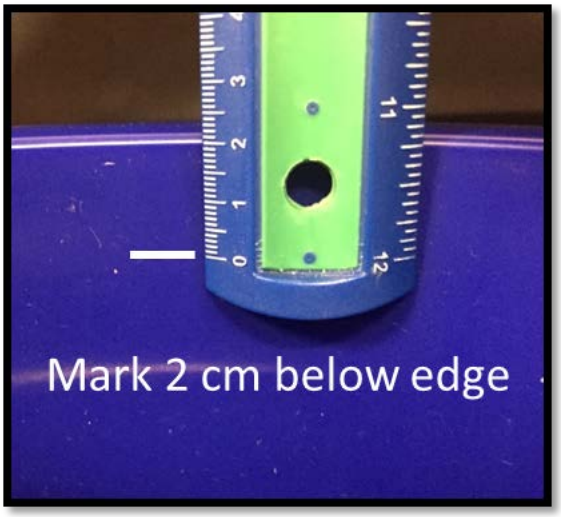
1. First assemble the pump with the flex hose, hose clamp, male hose end, adjustable valve and one side of the quick connect hose fitting. Attach the other side of the quick connect hose fitting to the blue bowl.



2. Use a nylon stocking or pantyhose to stretch over the water intake of the pump to act as a filter and ensure that any eggs that may inadvertently fall into the water reservoir are not passed though the pump to other samples.

3. Use a ruler and a permanent marker to make a mark 2 cm below the inner edge of the blue bowl at several locations around the bowl.



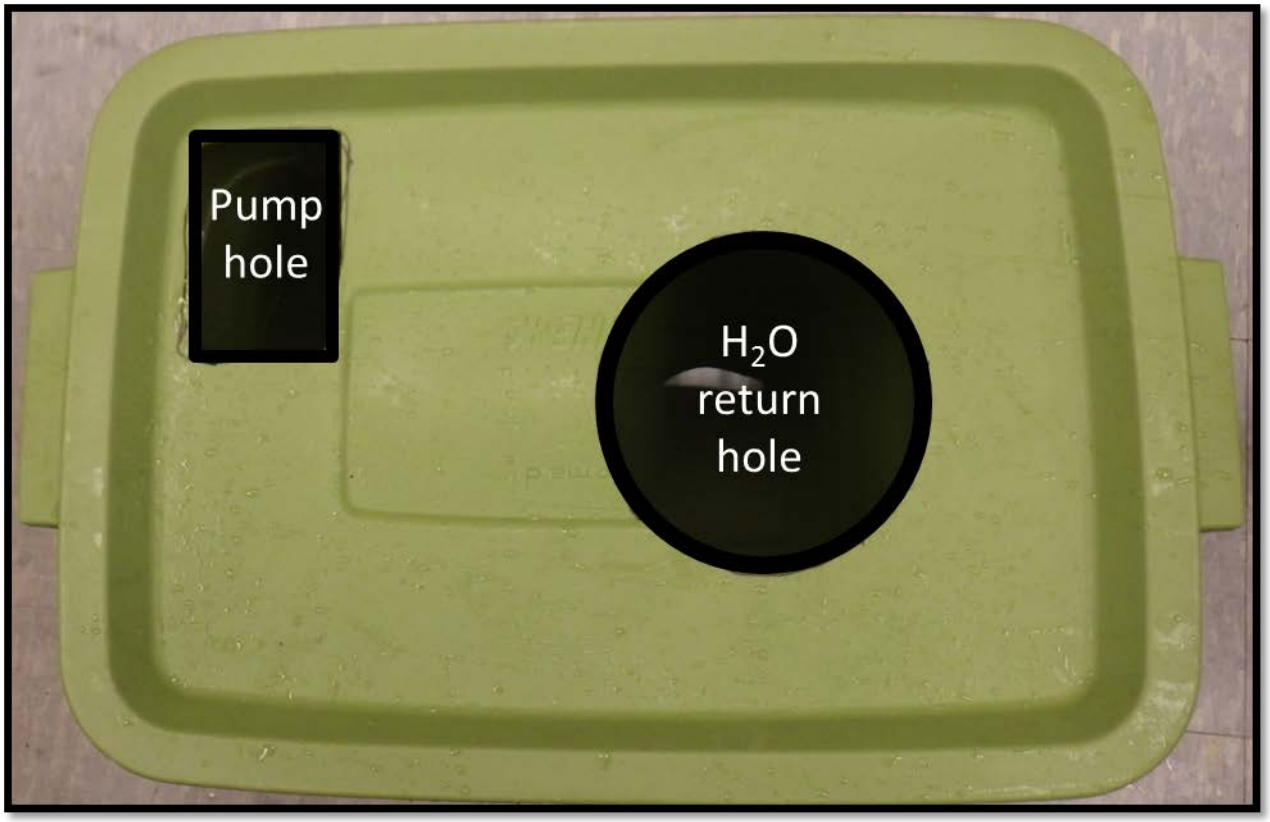


Assembly

4. Next, modify the tote lid by cutting two holes; one for the pump and one for water to return after passing through the blue bowl and the sieve.

The pump hole should be large enough for the pump to pass through and should be located so that the flex hose can be easily connected to the blue bowl without kinking.

The water return hole should be smaller than the outer diameter of your sieve so that the sieve can rest on the lid without falling through the hole. Sieves are generally 8” to 12” in diameter.



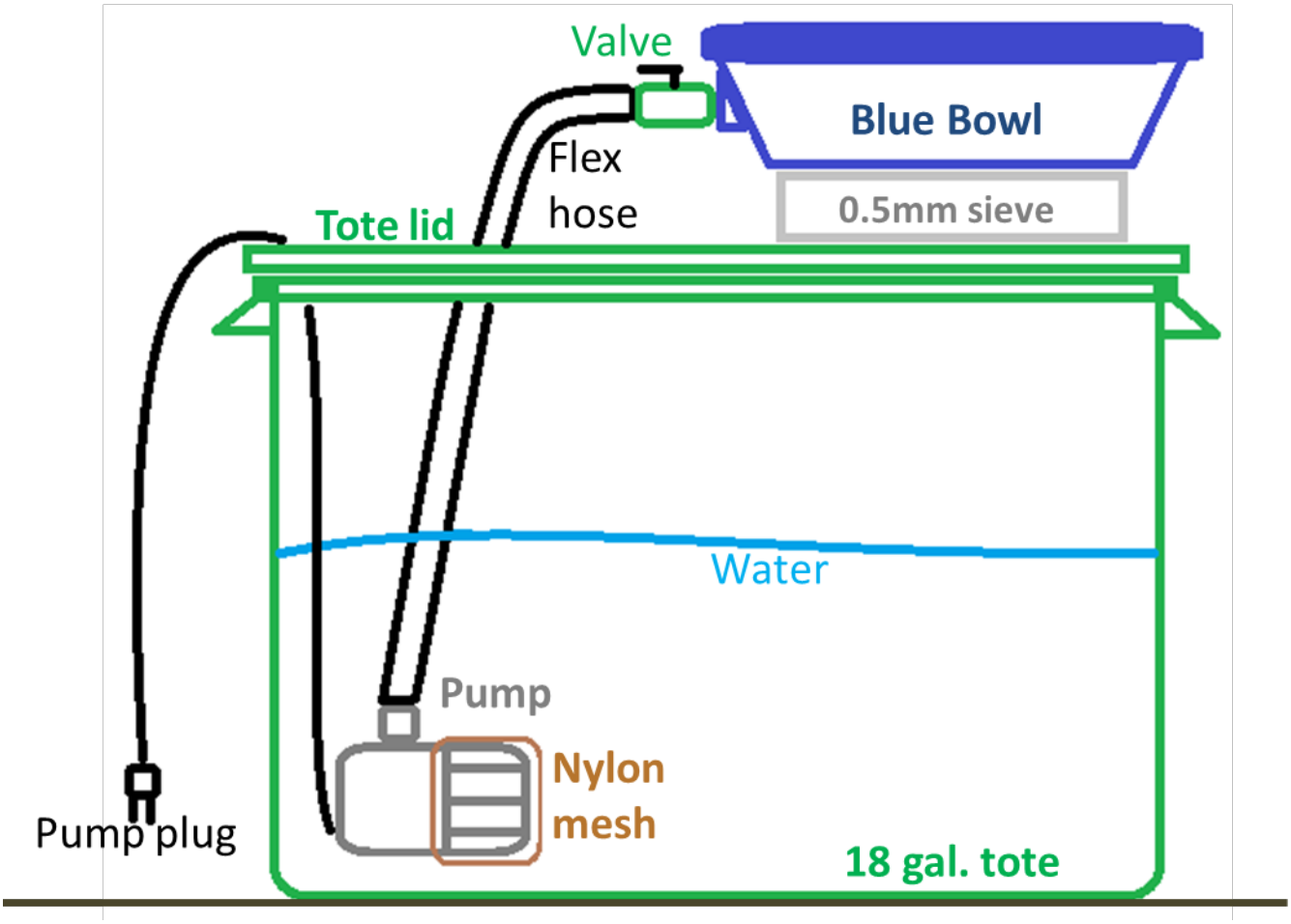
Set up

1. Remove any equipment stored within the tote and place the tote on a relatively level surface.

2. Add enough water to the tote so that the pump will be covered by several inches of water when connected.

3. Attach the tote lid, place the 0.5 mm sieve over the water return hole, place the blue bowl on top of the sieve, and connect the pump to the bowl.

4. Add water to the bowl to aid in determining if it is near level. Use the shims to level the bowl if needed by placing them under the edge of the sieve.



Sample processing

Note: Before each sample is processed, the blue bowl and sieve should be rinsed and the pump should be run briefly with the valve open while disconnected from the blue bowl to avoid any possible cross contamination between samples.

Once your vortex unit is setup and the bulk sample has been sieved to retain the sediment in the 0.5 mm sieve, you are ready to run the sample through the vortex.

1. Open the valve about ½ way and turn on the power to the pump.

   The pump should not be left on with the valve closed as the hose may rupture.

2. Use the valve to adjust the flow as needed to ensure that water is not overflowing the outer edge of the blue bowl. A vortex will form draining through the center of the bowl.

3. Add up to about 60 oz. of the sieved sediment to the bowl. The rubber spatula and wash bottle may be used to help add the sediment to the bowl.

   If you have more sediment you may need to divide the sample and repeat the process.

4. Once the sediment has been added, open the valve all the way, or until the water is about 1 to 2 cm from the edge of the bowl. You should aim to keep the water level within about 2 cm of the edge of the bowl for steps 5 and 6 of the sampling process.

   It is common for the water level to drop after you add sediment due to the decreased water velocity caused by the rough surface of the sediment, so be prepared to adjust the valve.

Sample processing

5. Using a sturdy plastic spoon or the spatula, stir the sediment from the middle to the edge of the bowl by sliding the spoon down the edge of the cone, across the bottom of the bowl, then up the side.

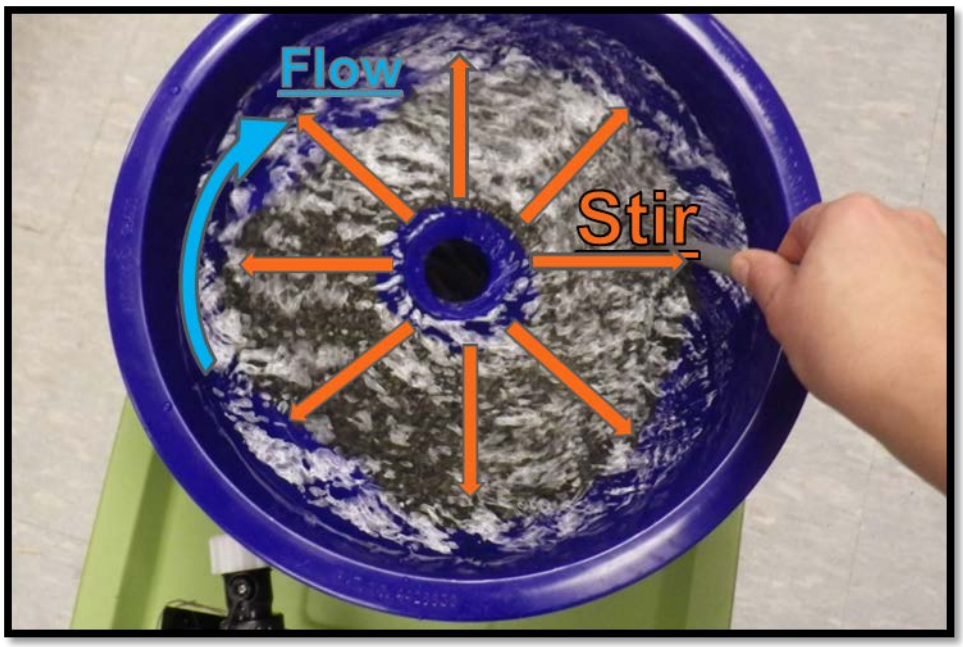
   A plastics spoon is preferred over metal because it will not scratch the surface of the bowl. Scratches may affect the flow of water and may create areas where sediment or eggs could be trapped.

   Move around the perimeter of the bowl as you stir while paying special attention to areas where the sediment has piled up or accumulated around the cone. This will help suspend eggs and ensure that they aren't being buried under the sand.

6. Stir for 1 to 3 minutes, and then allow the bowl to run undisturbed for about 10 seconds before turning off the pump and closing the valve.

   It is important to close the valve quickly after turning off the pump to avoid material being sucked back into the hose.

7. Once the water has settled, examine the sediment in the area immediately around the cone for eggs. If eggs are observed, skim them off with a spoon, or suck them up with a pipette or turkey baster and add them to the sample jar.



Sample processing

8. Remove the blue bowl from the sieve and with the aid of a wash bottle, rinse the material captured by the sieve into a sample jar.

9. Once the material from the sieve is in the sample jar, strain off as much water as possible (being careful not to lose eggs), cover the sample material with preservative and insert the appropriate sample label before securing the lid to the sample jar.

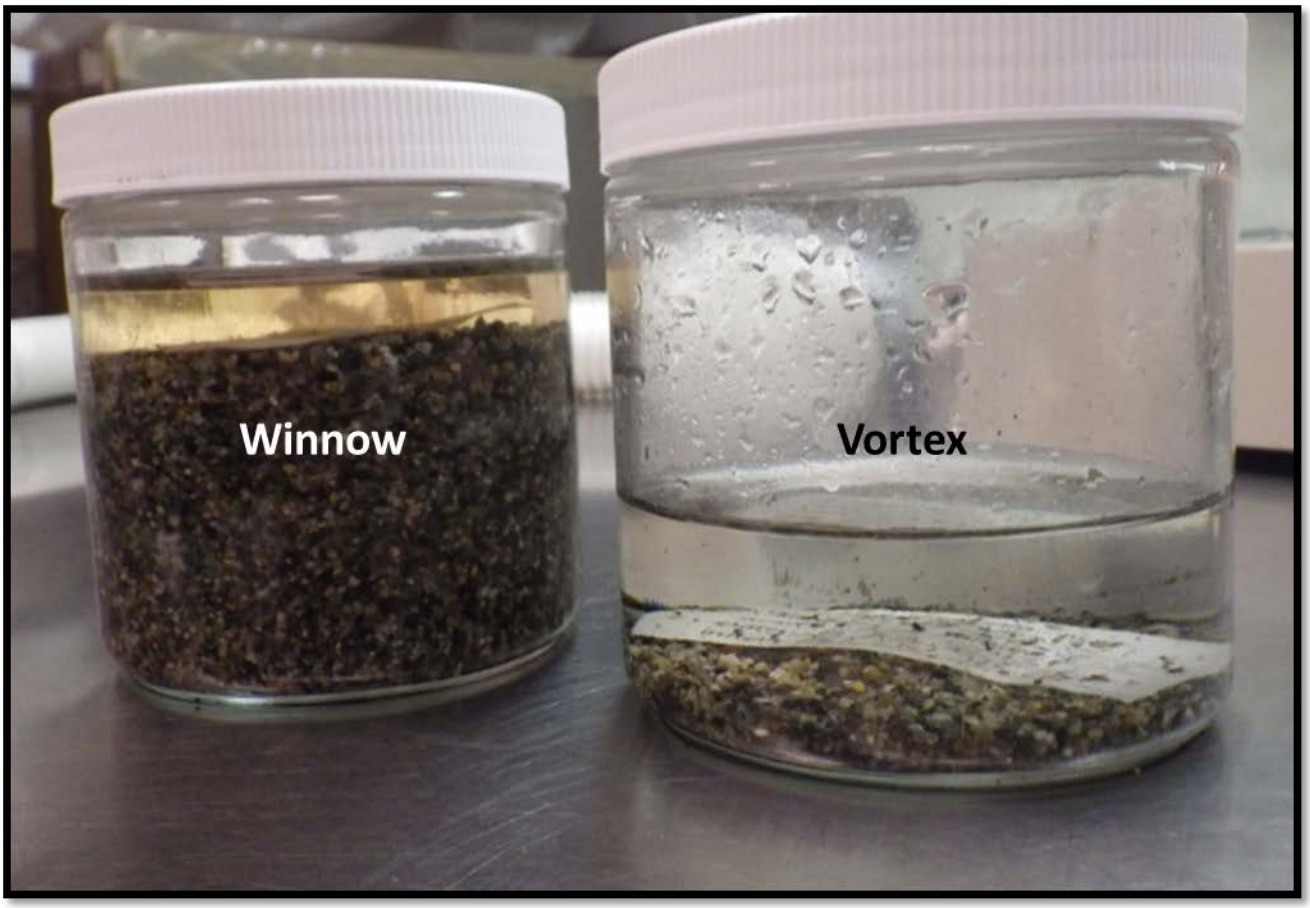
   The sample is now ready for lab processing.

Notes for lab processing

The laboratory procedures described in the field manual by Moulton and Penttila (2006) describe the process of further winnowing and reducing the sample prior to analysis with a dissecting microscope.

We have found that the volume of material retained after processing with the vortex method is typically so small that no additional winnowing or reduction is necessary. Instead, the entire preserved sample can generally be inspected for eggs in a standard 10 cm petri dish in just two or three batches.

For samples with a high volume of material in the condensed sample, it may be appropriate to apply the additional condensing process described in the field manual laboratory procedures.



Additional Resources

For training, consultation, or more information about WDFW forage fish studies, please contact Phillip Dionne at: *Phillip.Dionne@dfw.wa.gov; 360-902-2641*

Sampling protocols, identification guides, maps and other materials are available online at: *wdfw.wa.gov/conservation/research/projects/marine_beach_spawning/*

Field Manual: (https://wdfw.wa.gov/publications/01209/wdfw01209.pdf)
Moulton, L., and D. E. Penttila. "San Juan County forage fish assessment project: Field Manual for sampling forage fish spawn in intertidal shore regions"; First Edition; March 2001 (revised 2006) *San Juan County Marine Resource Committee and Northwest Straits Commission, La Conner, WA.* (2006).

Acknowledgments: Special thanks Ned Pittman for assembling the first prototype for the vortex method, to Kira Kranzler for photos and organizing methods testing, and to Dan Penttila, Shannon Miller, and the numerous Washington Conservation Corps interns for participating in the methods testing.

Parts vendors

The use of product brand names, images, vendor names and web addresses for the sources or descriptions of materials are included for convenience to aid in the identification of the materials used by WDFW in the development of these methods and do not represent an endorsement of the vendor or the product by the WDFW or its staff. Alternate products and/or vendors are likely available. We apologize for out of date or inactive links.

18 gallon tote: https://www.homedepot.com/p/18-Gal-Roughneck-Tote-RMRT180001/302148847

Blue bowl (includes hose valve): http://www.blackcatmining.com/mining-equipment/blue-bowl.cfm

750 – 1000 gph water pump: http://www.ebay.com/itm/Active-Aqua-Submersible-Water-Pumps-Aquarium-Reservoir-Fountain-Pond-Hydroponics-/111476699981

¾” flex hose: http://www.blackcatmining.com/mining-equipment/flex-hose.cfm

¾” quick–connect hose connection (with or without valve): http://www.amazon.com/Gilmour-2939Q-Premium-Complete-Quick-Connect/dp/B000E1AHVW

¾” male thread hose repair kit: http://www.tacomascrew.com/Products/Couplers-Connectors/Gilmour-01M-Garden-Hose-Repair-Ends?CAWELAID=120168600000024660&CAGPSPN=pla&catargetid=120168600000026509&cadevice=c&gclid=CKD8kczP6sYCFZJgfgod9PMKiw

0.5 mm sieve: https://www.fishersci.com/shop/products/fisherbrand-u-s-standard-stainless-steel-sieves-8-in-dia-2-in-d/0488110q
A 1/50 inch fine mesh sieve is an alternative: http://www.goldfeverprospecting.com/keclsc.html

Shims: http://www.homedepot.com/p/Unbranded-8-in-Composite-Shim-Bundle-of-12-SHM1-12-TW/202807695

Rubber spatula: http://www.amazon.com/Farberware-Color-Silicone-Spoon-Spatula/dp/B005GT01KE