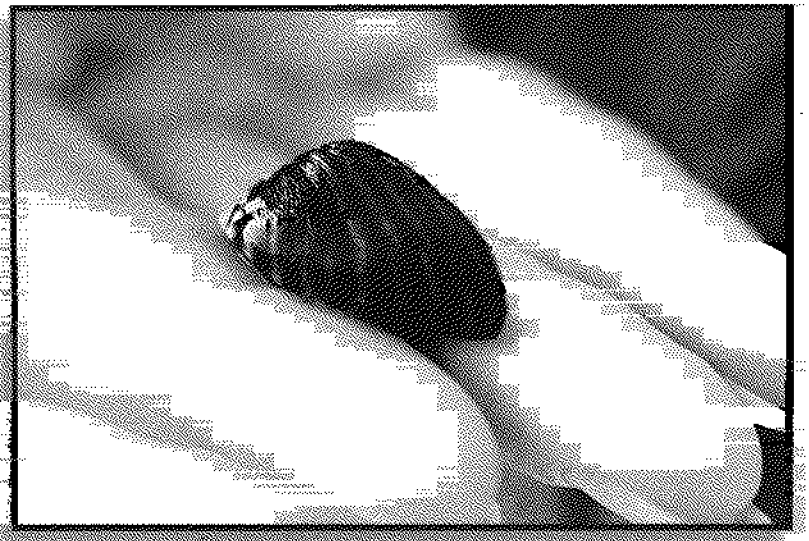
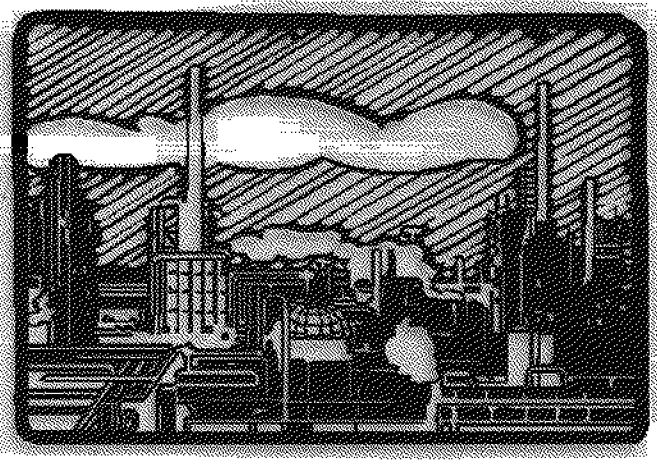
Sampling for Zebra Mussels



in Industrial Facilities



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Charles Ramcharan
Wisconsin Sea Grant

Sampling for Zebra Mussels in Industrial Facilities

Instructions Prepared by Yvonne Allen,
Louisiana State University, Coastal Fisheries Institute

Zebra mussels enter a facility through raw, unfiltered, untreated water drawn from an infested water body. They can enter the plant either as microscopic larvae (veligers) or as larger individuals ready for immediate colonization. The mussels can pose a significant threat to a facility where it is critical that flow is not reduced or impeded. If mussels are allowed to remain undisturbed on structures in the plant, they will grow and may reduce flow efficiencies. A monitoring program may be established to determine the seasonal trends in abundance of both settled mussels and veligers.

There are several areas which need to be addressed when an industrial facility is considering a monitoring program for zebra mussels. In addition to knowing something about the biology of the zebra mussel, you must also consider your plant: architecture, operation, budget and priorities.

This document will provide basic information about the habits of the zebra mussel as they affect industrial facilities. It also outlines quick methods for monitoring both veliger and settled stages within a facility. It is neither intended to give exhaustive information about sampling for zebra mussels, nor does it recommend a specific sampling protocol for all industrial facilities. More detailed information is provided in the references at the end of this publication, and particularly in the Zebra Mussel Information CD produced by the Army Corps of Engineers.

What Harm Can The Zebra Mussel Do?

The primary problem with zebra mussels in industrial facilities is as biofoulers. Zebra mussels attach to surfaces which are submerged in an infested water body. They can affect operation by physical obstruction, by adding weight or adding drag. They can also affect drinking water facilities with taste and odor problems. The critical problem for most facilities is physical obstruction.

Is The Source Water Infested?

NO or NOT YET: Although the source water may not be known to be infested with zebra mussels, it is a good idea for plants establish a low level monitoring program. The first sitings of zebra mussels have often been made by observant plant operators. By knowing the timing of zebra mussel invasion, a plant operator can make a more informed treatment decision.

There are certain minimum water chemistry requirements necessary for survival of zebra mussels. Although adults can tolerate episodes of lower pH and calcium, zebra mussels can not survive in waters where the pH is consistently less than 6.8, and the calcium concentration is less than 10 mg L^{-1} (Army Corps of Engineers. ZMIS. 1996). Adult zebra mussels will likewise tolerate temperatures up to 30°C, but anything above this temperature is eventually lethal (McMahon 1996) Zebra mussels are also primarily found in fresh waters (up to 3 ppt). Mussels found in more saline waters may be false dark mussels (*Mytilopsis leucophaeta*) and not zebra mussels.

YES: If the source water is infested with zebra mussels, the plant drawing water from that source is also infested with zebra mussels. Depending on the operation of the plant, this can have serious effects. Results from a monitoring program will provide basic information which may be used to establish a treatment protocol.

What Areas Should I Be Concerned About?

Zebra mussels present problems in areas with moderately low flow rates (less than three feet per second), and areas with crevices or seams. In pipes, they will tend to find an attachment point where there is a slightly lower flow rate, or an area that offers some protection. One of the most popular areas for settlement seems to be the intake screen. Once one mussel has settled, others will tend to attach there also, forming a clump (druse). This pattern can be particularly problematic in narrow pipes where the settlement of just a few mussels can critically alter flow rates. If flow rates are quite high, chances for successful settlement are greatly reduced. Furthermore, high flow rates will tend to discourage the formation of large druses because they will tend to be blasted off.

Zebra mussels can also pose a problem in non flowing waters. Mussels will readily settle on almost any hard surface. Any equipment (e.g. pumps) which are left in infested waters will probably become colonized by zebra mussels. Mussels may obstruct intake or discharge openings or otherwise interfere with the operation of a piece of equipment. This can be a real danger for any safety equipment which is rarely used or inspected.

Knowing these facts, you can then begin to think about the areas in your facility which are most vulnerable. This information, together with considerations regarding the operation, budget and priorities of your facility will help you to determine which level of monitoring is most appropriate for your situation.

GUIDELINES FOR SAMPLING SETTLED ZEBRA MUSSELS

Presence / Absence of Settled Mussels

Many devices have been designed to standardize and quantify zebra mussel densities, but if the purpose is only to determine whether zebra mussels are present, the following process is simple and straightforward. The best way to check for settled zebra mussels is to look for attachment on strainers, screens, condenser tubes, and any surface which is normally accessible. Very small zebra mussels are barely visible to the unaided eye and will grow through the season. Settled mussels can reach up to 25mm (about 1 inch) after two years of good growth.

It is also a good idea to check for mussels on large, vulnerable surfaces during dewatering. If examination of a large, fixed structure is not possible, or you want to monitor zebra mussel infestation in a different part of the plant, hang a piece of coarse screen, PVC pipe, or cement block directly in an area which receives flow (note that zebra mussels tend to avoid copper and brass). Make sure that the device is not buried in sediment and does not collect a large amount of silt or sediment. Check the device every month or so.

Remember that this is only a tiny surface area compared to the rest of the plant.

If you have access to a permanent floating barge or similar smooth surface, you can also scrape the surface to check for the presence of mussels. Use the scraper described in Figure 1.

Quantifying Abundance of Settled Mussels

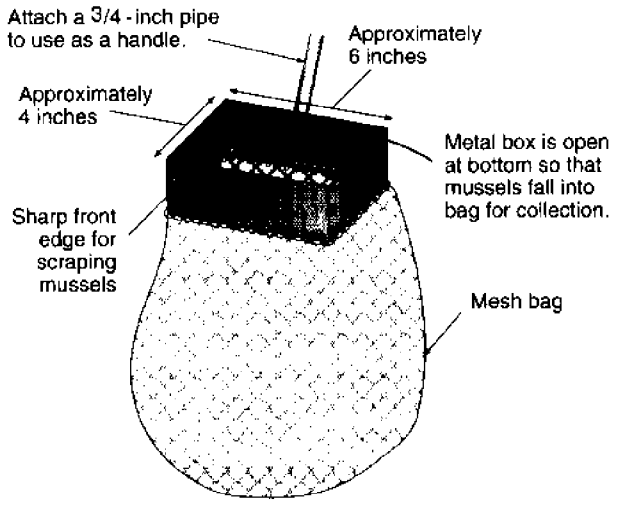
In order to compare the severity of the infestation to other areas, you can also report the number of settled mussels for a given area. To do this, measure the area scraped and counts all zebra mussels scraped form this area. Results are reported as the number of mussels per area (e.g. number per meter squared). The abundance of zebra mussels can, however be extremely patchy. Zebra mussels will often form dense clumps around a preferred settlement location while ignoring the surrounding surface. Also, bear in mind that the effects of an infestation of 100,000 mussels can be very different depending on whether the mussels are 2mm or 20mm in length. Obviously, the greatest problems will be caused by large mussel populations which begin to impact flow.

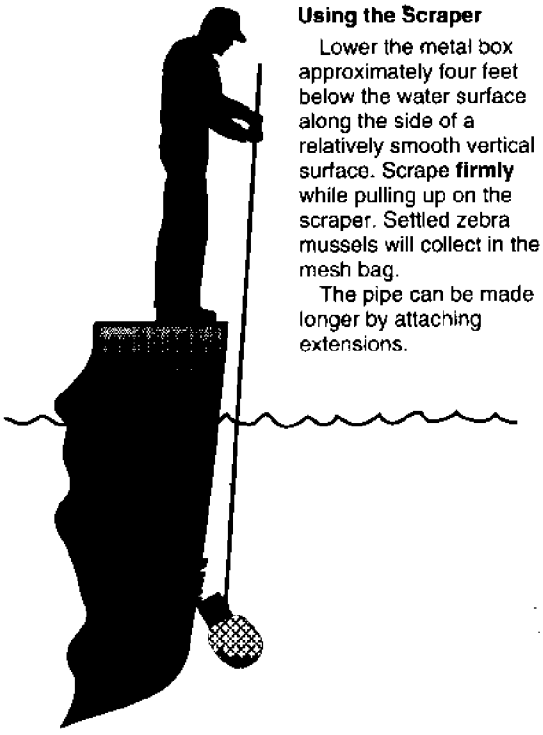
FIGURE 1

Metal Box Scraper

Box is modified from a metal index card box with the lid and bottom removed. Attach a mesh bag (1/16-inch to 1/4-inch mesh).

Drill holes near the bottom and attach the box using heavy thread. Attach a 3/4-inch pipe to the box to serve as a handle.





Using the Scraper

Lower the metal box approximately four feet below the water surface along the side of a relatively smooth vertical surface. Scrape **firmly** while pulling up on the scraper. Settled zebra mussels will collect in the mesh bag.

The pipe can be made longer by attaching extensions.

GUIDELINES FOR SAMPLING ZEBRA MUSSEL LARVAE

Zebra mussel larvae (veligers) are free floating, microscopic organisms. If the source water is infested, veligers are entering the plant through raw, unfiltered, unheated water. If the veliger is large enough at the time that it passes through the plant, it may settle and attach to any suitable surface it finds. Once attached, it may remain there and grow or it may become dislodged and move further through the system.

Regular veliger monitoring will help you establish the pattern and abundance of veligers coming through the system. You may then choose to treat after a seasonal peak in veliger abundance, because newly settled zebra mussels can be very vulnerable to treatment. This approach will ensure that there is no accumulation of mussels in the plant.

If the source water has not been identified as having zebra mussels, you can use the following technique to look for veligers as a part of a regular monitoring program.

Collecting a Veliger Sample

Materials Needed to Collect a Veliger Sample

- Bucket marked to a known volume (e.g., a 5 gallon pail)
- 64 micron plankton net affixed with a collecting cup. The net may be ordered from Wildco (1-800-799-8301; Product # 426-A28; approx. $80)
- Some device to hang the plankton net while you take the sample.
- Wash bottle
- Sample bottle (250 ml Nalgene bottle is recommended)
- 95% Ethyl or isopropyl alcohol — if the sample will be preserved for later analysis
- Materials to label the sample bottle with the following information: location of the sample in the plant, adjacent water body, volume of water filtered, mesh size of net, preservative used (see Table 1 for example)
- Data sheet to record collection information and water chemistry parameters (see Table 2)
- Stopwatch
- Hose to direct water into bucket or plankton net
- Materials to measure other water chemistry characteristics

Procedure for Collecting a Veliger Sample

A. Identify vulnerable locations in the plant. Think about the places in the plant which might be most vulnerable to zebra mussel infestation. Do these areas receive raw, untreated, unheated water? If so, these areas are vulnerable to settling by zebra mussel larvae. If the water is heated or has had chemicals added, the zebra mussel veligers will *probably* be killed. To establish a baseline pattern of veliger abundance, sampling should be done about once every two weeks when the water temperature is greater than 8°C.

B. Connect a hose to the point where you will take the sample.

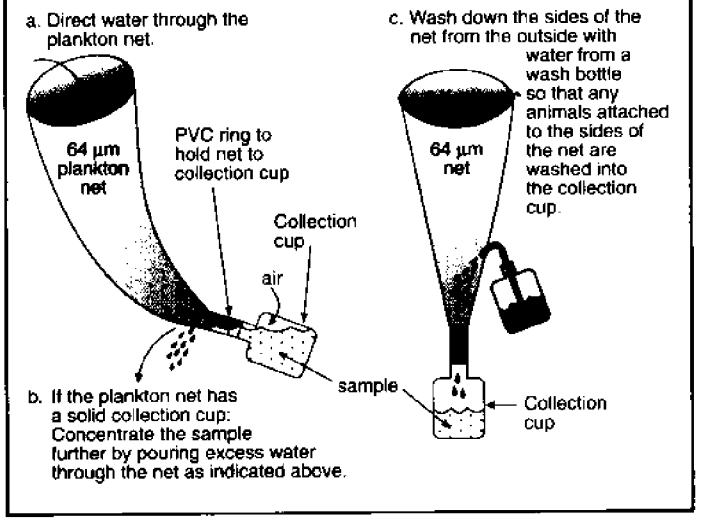
C. Open a tap or valve and establish a reasonable flow rate (1-2 minutes per five gallons is reasonable). If the flow rate is too high, you will blast the organisms against the mesh of the plankton net and they may be damaged and hard to identify.

D. Once the desired flow rate is set, direct the water into the bucket and time how long it takes to fill a known volume. Make the appropriate calculations to determine how long it will take to a complete sample. We have routinely filtered 100 liters (26.5 gallons) of water, and this seems to give a good sample. For example, if it takes 2 minutes to fill 5 gallons, it takes 0.4 minutes per gallon. 26.5 gallons/sample x 0.4 minutes/gallon = 10.6 minutes / sample which is 10 minutes, 36 seconds.

E. Direct the water flow through the net and take the sample (Figure 2a).

F. Wash down the outside of the net with water so all the animals are concentrated in the collection cup at the bottom of the net. If the plankton net has a solid plastic collection cup at the end, you have to concentrate the sample further so that all the sample is in the cup (see technique below - Figure 2b and 2c) If the plankton net has a mesh window in the collection cup at the end, the entire sample will be in the collection cup.

FIGURE 2



G. When the entire sample is concentrated in the collection cup, carefully unscrew the cup and pour the concentrated sample into a sample bottle. Rinse the collection cup several times to make sure all the sample removed from the cup. If you want to preserve this sample, fill the sample bottle with alcohol so that the final concentration is approximately 70% alcohol and 30% sample.

H. Record collection information and water quality parameters each time you take a sample. It is useful to know

water temperature, pH, conductivity and some measure of calcium (hardness, alkalinity, $CaCO_3$) at the time took the sample (refer to Table 2).

Examining a Veliger Sample

There are three levels of detail you can work at when looking for zebra mussel veligers:

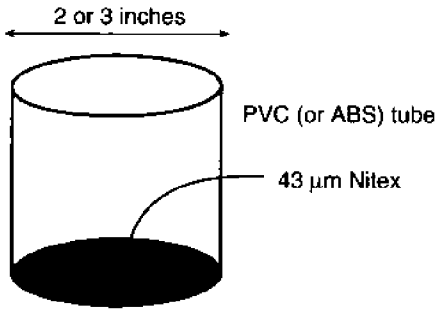
1. Presence / Absence - Scan the entire sample to determine whether veligers are present
2. Counting a sample with few veligers - Work through the entire sample and count all the veligers present in the sample to determine veliger density.
3. Counting a sample with many veligers - Examine a fraction of the entire sample to determine veliger density.

Examining a sample at the first two levels is fairly straight forward. The entire sample is examined. A bit more detail and equipment is necessary for analysis at the third level.

Materials Needed to Examine a Veliger Sample

- counting tray marked with rows to avoid counting the same items twice. Glass and some other type of acrylic will work, disposable plastic Petri dishes do not work.
- sieve to concentrate the sample (see Figure 3)
- fine probe
- dissecting microscope with maximum magnification of at least 40x, and two polarizing filters
- wash bottle
- tally counter
- data sheet

FIGURE 3



How To Make A Sieve :
Cut some PVC or ABS pipe into about 2½ inch sections. Epoxy a small piece of 43 micron Nitex to the bottom, sealing well. This is quick and cheap but may come apart because no glue, even epoxy, bonds well to PVC or ABS pipe. The netting will also wear out. Be prepared to replace periodically.

Procedure For Examining The Entire Sample Volume (Levels 1 and 2)

A. Pour the preserved sample through the 43 µm sieve. Make sure that the sample bottle is thoroughly rinsed out and the entire sample is concentrated in the sieve. This step will remove any preservative, so you will not have to deal with this while you examine the sample.

B. Carefully backwash the sample into a counting tray or beaker. If the sample does not have very much debris or sand, you may be able to examine the whole sample at once. If there is a lot of sand or debris, you will have to examine it in parts or subsample.

C. Veligers tend to lie on the bottom of a glass petri dish, but some may also be caught on the surface tension. Use a few drops of soapy water to break the surface tension and allow all the veligers to lie on the bottom.

D. Use cross polarized light to see the veligers under the microscope more clearly. This requires a substage light on your microscope. One polarizing filter is attached to the microscope objective lens, and another polarizing filter is placed between the substage light and the sample. Rotate the upper polarizing filter until the background is dark. Figure 4 shows what zebra mussel veligers look like under normal and cross-polarized light. It is easy to confuse veligers with ostracods which are common microscopic crustaceans in freshwater. Ostracods are distinguished most easily from veligers by shape. Ostracods are more kidney bean shaped while veligers are either round or D-shaped with a prominent straight hinge. *Corbicula* veligers can be distinguished from zebra mussel veligers based on size. *Corbicula* veligers are much larger at the D-stage. Refer to Johnson (1995) for more details on identifying veligers under cross-polarized light.

E. It can be important to note whether the veligers are present as whole or half shells. Use the probe to manipulate the veliger onto its side to determine if both halves of the shell are present.

F. If you want to keep the sample after counting, wash the sample back into the sample bottle and add preservative.

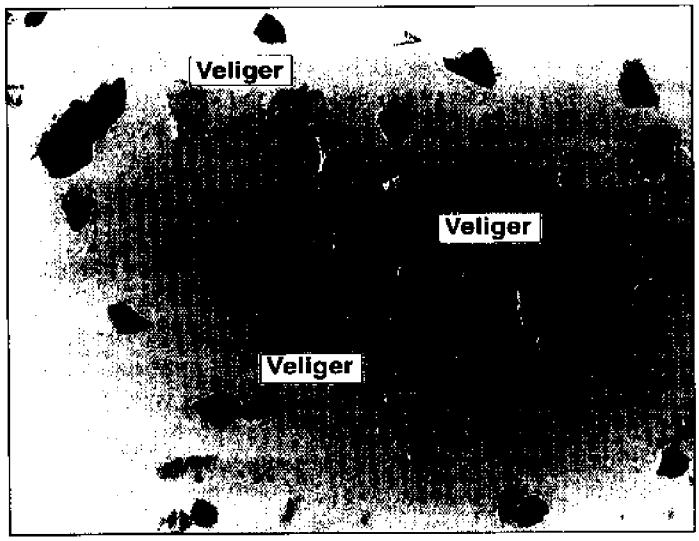
Examining a Fraction of the Veliger Sample (Subsampling)

If there are many veligers present in the sample, you may want to try to subsample the concentrated veliger sample.

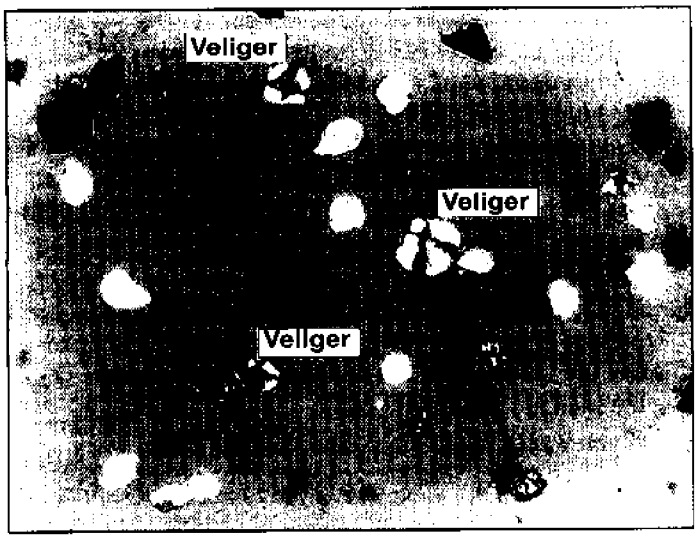
A good subsample is a *representative fraction* of the entire sample. Typically, when you count using subsamples, you must count several subsamples to get an accurate count. To determine the proper subsample volume to count, you must do some statistical calculations. The calculations consider the amount of sample you have counted, the typical amount of variation you encounter in a sample, and the level of confidence you are willing to accept in your density estimate. I generally refer to a 100L total sample where 3-5 subsamples are taken and variation between subsamples is very low. It is, however, a good rule of thumb to make sure that you have either greater than 30 veligers per subsample in a minimum of three subsamples, or that you have counted 10-20% of the total sample.

FIGURE 4

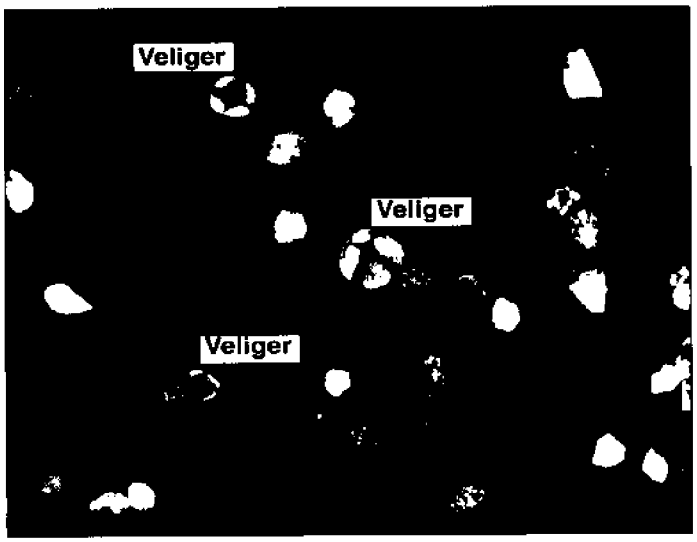
Zebra Mussel Veligers



Transmitted Light and No Filter



Transmitted Light and Half Polarization
(shows cross)



Transmitted Light and Full Polarization
(showns cross)

All photos:
Magnification 30X obj, 3.3 eyepiece = 99x

Additional Materials Needed to Subsample

- marked beaker (do not use the factory markings, these are only approximate)
- Hensen-Stempel pipette (with 1ml and 2ml attachments [Hensen-Stempel pipettes are commercially available from Wildco (800-799-8301) Product #1806-D50. These come with two removable spools - one for a 1 ml subsample and one for a 2 ml subsample.]

Procedure For Subsampling (Level 3)

A. Pour the preserved sample through the 43 µm sieve. Make sure that the sample bottle is thoroughly rinsed out and the entire sample is concentrated in the sieve.

B. Backwash the concentrated sample thoroughly from the sieve into the marked beaker.

C. Bring the sample to a known volume with a wash bottle.

D. Mix sample carefully with the Hensen-Stempel pipette so that no sample is spilled (see Figure 5 below) bring the sample into suspension by creating a vortex with a circular motion of the pipette, then break the vortex by moving the pipette in a "X" motion so that the sample is randomly distributed. Try to avoid hitting the sides or bottom of the beaker with the pipette because zebra mussel veligers are very delicate. Quickly withdraw a subsample.

E. Introduce the subsample into the counting tray. Rinse off the pipette with a wash bottle to make sure veligers do not adhere to the pipette.

F. Count all individuals in the sample or subsample and note the total number on the data sheet.

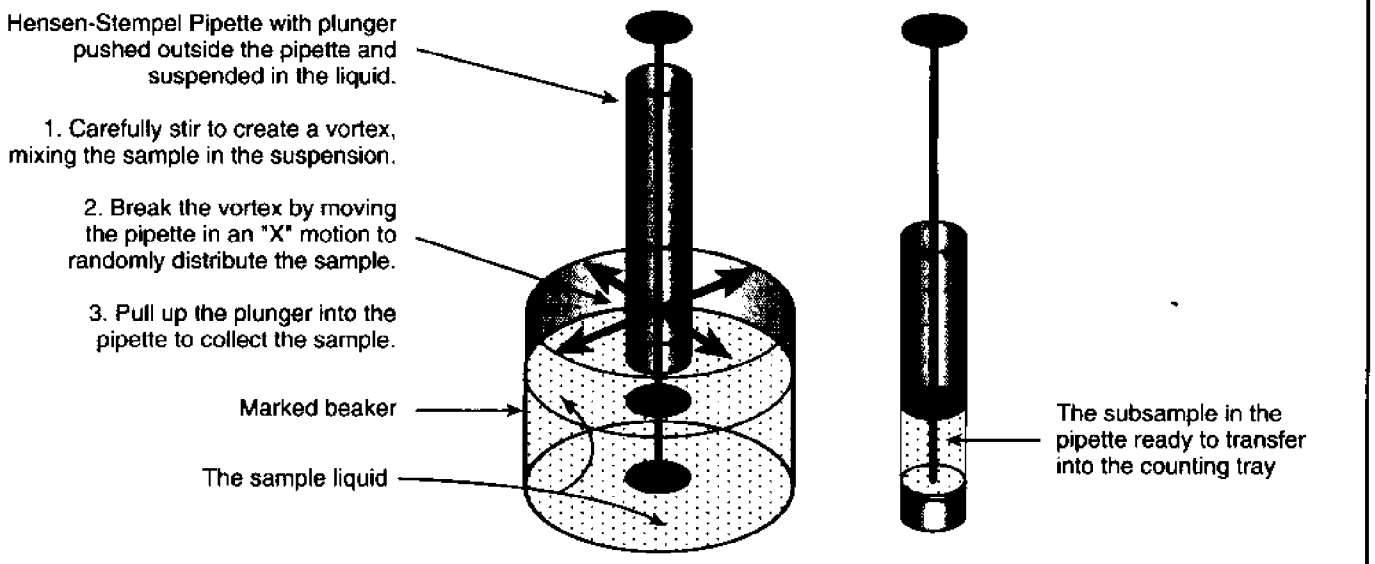
G. After counting, wash the entire subsample into a holding dish.

H. Repeat this procedure for each subsample.

I. When all subsamples are counted, concentrate the entire sample (including all subsamples) into the sieve, wash them back into the sample bottle, and add preservative.

J. To calculate the density of veligers per liter based on the subsamples, follow the equation provided on the sample data sheet (Table 2). In this equation, dilution volume means the volume from which the subsample is withdrawn.

FIGURE 5



For more information on zebra mussel sampling, consult:

Claudi, R. and Mackie, G.L. 1993. Practical Manual for Zebra Mussel Monitoring and Control. Lewis Publishers. Boca Raton, FL.

Johnson, L.E. 1995. Enhanced early detection and enumeration of zebra mussel (*Dreissena* spp.) veligers using cross-polarized light microscopy. Hydrobiologia 312: 139-146.

Marsden, J.E. 1992. Standard Protocols for Monitoring and Sampling Zebra Mussels. Illinois Natural History Survey Biological Notes 138. Contact: Illinois Natural History Survey; Natural Resources Building; 607 East Peabody Dr.; Champaign, IL 61820; Ph: (217) 333-6880.

McMahon R.F. 1996. The Physiological Ecology of the Zebra Mussel, *Dreissena polymorpha*, in North America and Europe. American Zoologist 36: 339-363.

Nalepa, T.F. and Schlosser, D.W. 1993. Zebra Mussels: Biology, Impacts, and Control, Lewis Publishers, Boca Raton, FL.

U.S. Army Corps of Engineers Waterways Experiment Station. 1992. Hand-Held Sampler for Zebra Mussel Collection. Zebra Mussel Research Technical Notes ZMR-1-04. U.S. Army Corps of Engineers Waterways Experiment Station; 3909 Halls Ferry Road; Vicksburg, MS 39180.

U.S. Army Corps of Engineers Waterways Experiment Station. 1996. Zebra Mussel Information System. CD-ROM. Contact: Michael J. Grodwitz, U.S. Army Engineer Waterways Experiment Station; CEWES-ER-A; 3909 Halls Ferry Road; Vicksburg, MS 39180; Ph: (601) 634-2972; Fax: (601) 634-2398; email: grodowm@ex1.wes.army.mil.

TABLE 1

Zebra Mussel Veliger Sample
Plant Name: ______________
Sample Date: ____/____/____
Sample Location: ______________
Adjacent Water Body: ______________
Volume Filtered: ______________
Preservative: ______________
Mesh Size: ______________

Zebra Mussel Veliger Sample
Plant Name: ______________
Sample Date: ____/____/____
Sample Location: ______________
Adjacent Water Body: ______________
Volume Filtered: ______________
Preservative: ______________
Mesh Size: ______________

Zebra Mussel Veliger Sample
Plant Name: ______________
Sample Date: ____/____/____
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Mesh Size: ______________

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Preservative: ______________
Mesh Size: ______________

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Volume Filtered: ______________
Preservative: ______________
Mesh Size: ______________

Zebra Mussel Veliger Sample
Plant Name: ______________
Sample Date: ____/____/____
Sample Location: ______________
Adjacent Water Body: ______________
Volume Filtered: ______________
Preservative: ______________
Mesh Size: ______________

TABLE 2

Zebra Mussel Veliger Data Sheet						
Sample Date: / /		Time (24h):		Sample collected by:		
Plant Name and Location:						
Adjacent Body of Water:				River Mile:		
Location within the plant: (use a separate sheet for each location)						
Water Chemistry						
Temp(^{O}C):		pH:				
DO (mg L^{-1}):		Conductivity (µmhos):				
Salinity(‰):		Secchi (if applicable):				
Total Hardness (mg/L):		Other:				
Veliger Sample						
Volume filtered (L):		Preservative used:				
Mesh Size (µm):		Preservative concentration:				
Net Diameter (if applicable):						
Counting Information						
Date counted: / /				By:		
Subsample Number:	1	2	3	4	5	Total
Dilution Volume (ml):						NA
Subsample Volume (ml):						
# Whole Veligers						
# ½ Veliger Shells						/ 2 = whole
# Corbicula						
Calculate veliger density: {[(total number of veligers counted)/(total volume of subsample analyzed)]*dilution volume}/volume filtered = # veligers / liter						
Comments/Problems:						