Zebra and Quagga Mussel Veliger Sampling Protocol
Vertical Tow *
California Department of Fish and Game

*This protocol was adapted from the California Department of Water Resources *Zebra and Quagga Mussel Veliger Sampling Protocol for the State Water Project,* April 29, 2008.

Before collecting plankton samples make arrangements with a laboratory that has the capability to process the samples. Preserve plankton per their specifications.

Zebra and quagga mussels have a planktonic larval lifestage (microscopic, free-swimming in water column) and are called veligers. Veligers range in size from 70-200 microns (µm).

Equipment

- 63-µm plankton tow net (maximum mesh size)
- Rope, 50 meters
- Sample bottles (250 mL – 1L)
- Sample labels
- Sharpie pen
- Alcohol (ethanol or isopropyl, depending on your lab's specifications)
- Plankton Sample Log (on waterproof paper)
- Plankton Sample Datasheet (on waterproof paper)
- Ice chest with crushed ice/blue ice
- Wash bottle(s) filled with tap water
- 5-gal bucket ("wash down" bucket)
- White vinegar, 100% solution (3 to 5 gal or enough to submerge plankton net and tow rope)
- 5-gal bucket with lid ("vinegar decontamination bucket")
- Chlorine solution, 10% bleach (16 to 32 oz or enough to fill spray bottle)
- Spray bottle for chlorine solution (16 to 32 oz)

Field Procedures

Sampling Method – Vertical plankton tow

To optimize the likelihood of capturing veligers if they are present, tows should be made at various locations within a lake/waterbody. It is recommended that 8 to 16 separate tows (depending on the size of the waterbody), of at least 8 meters each in length be made. Sample at a variety of areas, including near boat ramps, open water, near water outflows and inflows, downwind areas, and eddies, or areas where plankton collects (i.e., behind islands, etc). You may also want to sample the entire depth of your water column as vertical distribution of veligers may be variable. To do this, lower the net to 1 meter above the bottom and pull up to the surface. Individual waterbodies (size, depth, productivity, suspended solids, etc.) and equipment (net diameter, mesh size) will vary, so adjust sampling accordingly. Individual tows from the same lake/waterbody can be combined into a single sample jar for laboratory analysis.

To perform a tow, attach rope to the “bridle” (the rope system fixed to the mouth of the net). Gently lower the net into the water to the desired depth. To retrieve, pull rope back in a steady, unhurried, hand-over-hand motion. Note: Do not pull faster than 0.5 m/s (e.g., if the tow distance is 20 m, retrieval should take 40 seconds). Pulling too fast will cause a pressure wave in front of the net that pushes water and plankton away from the mouth of the net, and as such, does not effectively sample the desired volume of water. Record the distance of each tow on the Plankton Sample Datasheet. Rinse net contents into sample bottle (described below) between each tow.

Sample Collection

Label the outside of the sample jar using a permanent marker, such as a Sharpie, with the lake/waterbody, date, and time.

- At the end of each tow, lift the net so that the net opening is above the water surface. Next, lower the net back into the water (keeping the opening above the water surface) and then quickly pull the net straight up; this action will move the collected plankton into the cod-end piece. Repeat this procedure as needed. Note: If sampling from shore, use a 5-gal bucket of water to wash the contents down into the cod-end. Carefully lower the net into the bucket and lift out quickly to wash the organisms down (again, keep the opening of the net out of the water). Repeat as necessary.
- Depending on the configuration of your net, carefully transfer the net contents to the labeled sample jar. Use the wash bottle to gently rinse down any remaining contents into the jar. Try to minimize the amount of rinse water collected in the sample jar so that subsequent tows from the same lake/waterbody can be added.
- Reattach the cod-end piece to the net and repeat the tow.
- Combine the samples for all the tows into one jar, if possible, leaving sufficient room for alcohol.

Sample Preservation

Depending on your lab and the method of sample analysis, preservation method may vary. Know your lab’s protocol prior to collecting samples.

Portland State University analyzes plankton samples using microscopic examination, and requests samples to be preserved in a 70% final alcohol solution. Alcohol can be either 95% non-denatured ethanol or isopropyl (over-the-counter isopropyl is usually 70% alcohol). If using 95% ethanol, samples should be approximately one-quarter plankton/water, and three-quarters alcohol to get a solution that is approximately 70% alcohol. If using 70% isopropyl, you will need a greater proportion of alcohol to water - approximately 85% alcohol to 15% plankton/water. When making several tows keep sample on ice between tows and add alcohol after tows are combined. Samples preserved in this manner can be held and shipped at room temperature and have a shelf life of at least 3 months.

The Bureau of Reclamation lab requires samples for PCR analysis to be preserved in a final solution of 25% non-denatured ethanol. With less alcohol, samples must be kept chilled, shipped overnight on ice, and be processed within days.

Dilutions for final solutions and different alcohol percentages can be calculated at http://www.restrictionmapper.org/dilutioncalc9.htm .

Shipping Samples

Ethanol (ethyl alcohol) and isopropyl alcohol are class 3 hazardous material. They may be shipped via ground transportation in limited quantities when proper packed and labeling. Consult you carrier for guidance.

Data Recording

Maintain a log sheet of samples (Plankton Sample Log), and complete a datasheet for each sample (Plankton Sample Datasheet). Fill datasheets out while sampling to ensure accuracy. For the sample log, record the date, location, and time each sample was collected. For individual samples, record the net diameter, mesh size, and distance (i.e., depth) of each tow. Remember to include the units of measurement used. This information may be used to calculate the actual water volume sampled.

Provide sampling data and results to the California Department of Fish and Game quagga contact within your region.

Cleaning and Storing Equipment

To prevent cross-contamination and reduce the risk of spreading zebra and quagga mussels, one plankton net, rope, bucket, etc., is used per site. All sampling gear (including net, rope, wash bottles, buckets, etc) that comes into contact with the water should be soaked in vinegar for 24 hours (an absolute minimum of 4 hours), and rinsed. As an additional precaution, equipment can be sprayed with or soaked in chlorine solution for 5 minutes, and then thoroughly rinsed with clean tap water (the bleach is corrosive so rinse thoroughly with clean tap water). Vinegar can be reused. Dispose of the contaminated rinse water away from the waterbody. The vinegar solution can be reused multiple times. The chlorine solution should be discarded after 24 hours.

> Vinegar Solution:
> 100% white table vinegar (5% acetic acid solution).
>
> Chlorine Solution:
> 10% solution of household bleach (5.25% sodium hypochlorite). To make the bleach solution, add 1.5 cups of household bleach to 1 gal of water. The bleach solution must be fresh (less than 24 hours old).

Disassemble net and hang to dry. Routinely inspect the net for damage or wear and repair or replace if necessary.

Boat Decontamination

If trailering a boat to a different waterbody, please decontaminate before transporting.

After loading onto the trailer,

- Run the engine for 5-10 seconds to blow out excess water and vegetation from internal drive, then turn off engine.
- Remove aquatic plants and animals from water intake grate, steering nozzle, watercraft hull and trailer.
- Rinse watercraft and equipment with high pressure hot water (140+ ºF) OR dry everything for at least 5 days

Plankton Sample Log
California Department of Fish and Game

Collection Information	
Collector(s):	Affiliation:
Contact information (email or phone # if not DFG):	

Samples

Date	Location	Time	Comments

Return datasheets to the appropriate California Department of Fish and Game Regional office.

Plankton Sample Datasheet

California Department of Fish and Game

(One datasheet per sample)

Collection Information	
Date:	
Waterbody:	
Location (GPS or site description):	
Collector(s):	Affiliation:
Contact information (email or phone # if not DFG):	

Net	
Mesh size*:	
Net diameter*:	

Tows

#	Location description	Distance/depth of tow*	Comments

Sample Identification Number (optional)
#

Sample Analysis
Shipped to:
Comments:

*Be sure to include units of measurement

Return completed datasheets to the appropriate California Department of Fish and Game Regional office.

DFG Regional Office Contacts for Quagga Mussel Monitoring

Region 1 – Northern Region
Counties: Del Norte, Humboldt, Lassen, Mendocino, Modoc, Shasta, Siskiyou, Tehama and Trinity
601 Locust Street, Redding, CA 96001
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Region 2 – North Central Region
Counties: Alpine, Amador, Butte, Calaveras, Colusa, El Dorado, Glenn, Lake, Nevada, Placer, Plumas, Sacramento, San Joaquin, Sierra, Sutter, Yolo and Yuba
1701 Nimbus Road, Rancho Cordova, CA 95670
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Region 3 – Bay Delta Region
Counties: Alameda, Contra Costa, Marin, Napa, Sacramento, San Mateo, Santa Clara, Santa Cruz, San Francisco, San Joaquin, Solano, Sonoma, and Yolo
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Region 4 – Central Region
Counties: Fresno, Kern, Kings, Madera, Mariposa, Merced, Monterey, San Benito, San Luis Obispo, Stanislaus, Tulare and Tuolumne
1234 E. Shaw Avenue, Fresno, CA 93710
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Region 5 – South Coast Region
Counties: Los Angeles, Orange, San Diego, Santa Barbara and Ventura
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Region 6 – Inland Deserts Region
Counties: Imperial, Inyo, Mono, Riverside and San Bernardino
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