Invasive Mussel Plankton Tow Monitoring Protocol

Plankton tow sampling is a form of early-detection monitoring for the larval life stage of invasive mussels (veligers), whereby a fine mesh net is pulled through the water column (referred to as a "tow"). The plankton is then analyzed in a laboratory to visually identify veligers using cross-polarized light microscopy (CPLM). If organisms suspected to be invasive mussel veligers are found, DNA is extracted from those organisms, and molecular analysis is used to confirm visual identification. To optimize the potential for detecting veligers, if present, plankton sampling should follow a standardized sampling method, sample a large volume of water, and target the months (water temperatures) and locations in a waterbody where veligers are most likely to occur. Of equal importance, samples must be preserved and handled properly to maintain their integrity so that analysis yields accurate results.

To enhance early detection, monitoring for adult mussels should be conducted along with plankton tow sampling. Monitoring for adult mussels can be conducted through monthly inspections of artificial substrate samplers and by surveying surfaces of shoreline, multiple habitat types, and structures located in high use areas. Separate protocols for these methods are available on the CDFW Invasive Species Program Invasive Mussels webpage.

When, Where, and How to Sample

Water Temperature

CDFW recommends plankton sampling every 14 days when water temperatures are optimal for mussel spawning (16°C-30°C (61°F-86°F)).

Selecting Sampling Sites Within a Waterbody

Veliger distribution can be highly localized. Therefore, sampling should occur at multiple sites throughout the waterbody to increase the potential for detection. Sampling sites should include areas of high use and likely sites of mussel introductions, such as around docks, boat launch ramps, floating restrooms, marinas, at inlets and outlets of the waterbody (mouths of tributaries; dams), and in downwind areas and eddies (which can be identified by accumulation of leaves, pollen, and debris on the surface of the water).

Number of Sites

The number of sites sampled within a waterbody should be based on the size of the waterbody, but a minimum of three sites is recommended. Conduct only one tow per site. Depending on the number of samples to be submitted to the lab, samples from multiple sites may be composited into one sample bottle.

Tow Volume

The volume of water to be filtered per sample can vary depending on the planned number of samples to be submitted to the lab, the depth and size of the waterbody, and the turbidity of the water, with special consideration for dense algae. As a rule of thumb, aim to filter at least 1000 liters of water per sample bottle. Based on the diameter of the net, corresponding plankton net area (m^2) (Table 1, Appendix A), and the depth of each tow, the number of tows needed to filter 1000 liters can be calculated using the equation provided in Appendix A. The tow volume may need to be reduced in shallow waterbodies or in waterbodies with high algal densities. If possible, avoid sampling areas with high algal densities, or reduce the total sample volume if high densities of algae cannot be avoided. Samples that are cloudy with sediment or green algae are difficult, if not impossible to effectively analyze in the lab. Discard samples that are contaminated with sediment and resample. Reduce the number of tows composited per bottle in areas with dense algae.

Type of Tow

Vertical tows should be collected whenever possible to increase the probability of capturing veligers if they are present. Tows should sample the entire water column beginning 1 meter from the bottom.

Horizontal tows should only be used if vertical tows are not possible, such as in very shallow or flowing water.

Disclaimer: **References to equipment and supplies by brand or vendor are made only for the convenience of the reader and are not endorsements or recommendations of products.**

Equipment and Supplies

Plankton Tows:

- Plankton tow net –63- or 64-micron mesh size
    - 8-inch diameter (e.g., Wildco part number 426-A28)

    - 30 cm diameter (e.g., Aquatic Research Instruments simple plankton net)
- Tow rope – 30 meter minimum with 1- or 5-meter graduation marks
- Spool for tow rope
- Ballast weight, if needed
- Sample bottles – plastic wide mouth 250- or 500-mL capacity
- Ruler with 1 mm graduations
- Sample labels (e.g., Environmental Sampling Supply 2 X 3 inches, part no.
    - 0203-5000)
- Permanent marker
- Boat
- Calculator
- Blue ice/gel packs and cooler
- Sample preservative (Appendix B)
    - Non-denatured ethanol (200 proof)
    - Baking soda, 4% solution
    - Distilled water (W/V)
- pH paper (e.g., Whatman type CF pH range 4.5 – 10)
- If submitting samples to CDFW lab for analysis:
    - Plankton Sample Datasheets
    - Lab sample submission/chain of custody (COC) form

Decontamination (Appendix C):

- 18-gallon plastic tote with lid (sized to accommodate net and other equipment)
- White vinegar (approximately 5% acetic acid)
- Household bleach (approximately 6% hypochlorite)
- Spray bottle 32 oz.

- Measuring cup with graduations for milliliters or ounces
- Gallon plastic resealable bags

Equipment Preparation Prior to Sampling

1. Decontaminate nets and related equipment before use (Appendix A).
2. If necessary, affix a ballast weight to the net assembly.
3. Mark the tow rope in 1- or 5-meter intervals with permanent marker or electrical shrink wrap. Test to confirm marks are not removed by the decontamination process.
4. Attach the tow rope to the spool and wind it onto the spool.
5. Prepare 4% baking soda solution (Appendix B).

Vertical Tow

1. If the cod end of the net has a valve, close the valve.
2. Slowly lower the net into the water, perpendicular to the surface of the water, until you just feel the cod end touch the bottom, or until you reach the end of your rope. When you feel the cod end touch the bottom, gently pull the rope back up approximately 1 meter so the net does not touch the bottom.
3. Never let the mouth of the net hit the bottom of the waterbody, as sediment will get into the net clogging the fine mesh and making sample analysis difficult or impossible. If a tow contains sediment simply discard it, rinse the bottle and net at the surface of the water, and repeat the tow.
4. Make note of the rope marking, which either indicates actual depth, or represents an incremental measurement to be counted as the rope is retrieved.
5. Pull the net up at a consistent rate of about 0.5 meters per second and counting the increments. Pulling at a faster rate will create a wave in front of the net that will reduce filtering efficiency and may also damage veligers.
6. As the net is drawn towards the surface, maintain vertical alignment so that the rope remains perpendicular to the surface

of the water.

7. After the net is drawn above the water line slowly dip the net in and out of the water several times while maintaining vertical alignment to consolidate the net contents by flushing plankton off the inner surface of the net, down to the cod end. Do not submerge the mouth of the net while dipping the net.
8. Depending on how the cod end is configured, dispense or decant the plankton sample into the sample bottle.
9. Record the length/volume of the tow.
10. Select another sampling site and repeat steps 1-9 until a minimum of 1000 liters of water has been filtered through the net.
11. Label each sample bottle with an adhesive label and permanent ink. Include at minimum waterbody name, sample ID, date collected, and method of preservation. Ethanol will cause ink to run, so avoid contact with the label.
12. Complete the Plankton Sample Datasheet (Appendix D) for internal collection/maintenance of field data.
13. Place the bottle in a cooler with gel packs or blue ice until preservative is added.
14. Preserve per Appendix B within 3 hours of collection and keep refrigerated.
15. At the time of shipping preparation, complete the Laboratory Submission Form for all samples being submitted to CDFW's lab for analysis. This form is not required for samples submitted to external labs.

Horizontal Tow

If the water is stagnant or the flow rate is slow:

1. Calculate the required length of the horizontal tow. See Appendix B for example calculations. The actual length of the tow can be determined using the graduation marks on the tow rope.
2. Pull the net horizontal to the surface of the water, with the mouth of the net below the surface but not touching the bottom. A ballast weight may have to be attached to keep the net submerged.
3. Consolidate the net contents, label the sample, and preserve as described in steps 6-15 per instructions for the vertical tow.

If the water is flowing:

1. Measure the flow velocity in meters per second.
2. Place the mouth of the net facing upstream so the water flows into the net.
3. Keep the net in the water until approximately 1000 L has run through it. This can be calculated with the formula: Seconds = 1 m^3/(area of net m^2)(flow velocity m/s)
4. Consolidate the net contents, label the sample, and preserve and as described in steps 6-15 per instructions for the vertical tow.

Data Recording and Reporting

Every plankton tow and associated data must be recorded on a datasheet. In addition, a Laboratory Submission form must be completed for every sample submitted to the lab for analysis. Send datasheets, or copies of them, to the appropriate CDFW regional contact. All data will be entered into a data reporting system and the datasheets will be retained on-site.

Datasheets are available at:

CDFW Plankton Sampling Datasheet

CDFW Regional Scientists

CDFW Invasive Mussel Regional Contacts.

Appendices

A. **Reagent preparation and plankton tow calculations**

B. **Plankton tow preservation protocol**

C. **Decontamination protocol for plankton tow sampling**

Appendix A

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Reagent preparation and plankton tow calculations

A. Conversions

- To convert feet to meters multiply by 0.3048
- To convert inches to centimeters multiply by 2.54
- To convert cubic meters to liters multiply by 1000
- Conversions if a measuring cup is used:
    - 1 ounce = approximately 30 milliliters
    - 1 cup = 8 ounces
    - 1 cup = approximately 250 milliliters

B. Preparation of a 4% baking soda (sodium bicarbonate) solution

- Use the following formula to prepare a 4% by weight (W/V) solution: desired volume in ml x 0.04 g baking soda = grams of baking soda to add
- Example: to make a 1-liter solution of 4% baking soda solution, add 40 grams of baking soda to 1000 milliliters (1 L) of deionized water. A standard 28 mm soda bottle cap holds about 5 grams of baking soda and ½ teaspoon of baking soda is about 3 grams. These values can be used to prepare a solution that is approximately 4% baking soda. For example, adding a level soda bottle capful of baking soda to a 250 ml Nalgene container that is approximate ½ full of water would provide a solution of baking soda close enough to 4% that it could be used to adjust the pH of plankton tow samples per the protocol described in Appendix A.

C. Preparation of a 10% bleach (sodium hypochlorite) solution

- Use the following formula to prepare a 10% bleach solution: **total volume of solution desired x 0.1 = volume of bleach to add**
- Example: Add 50 milliliters of bleach to 450 milliliters to prepare a 10% bleach solution (V/V). A measuring cup can be used to measure the bleach and water at a 1:10 proportion. It is recommended that the bleach solution be prepared in a 32 oz. Spraymaster (gray) spray bottle. The gray bottle will help protect the bleach from degradation.

D. Determination of a vertical tow volume in liters

- To determine a vertical tow volume, multiply the area of the plankton net hoop by the total depth of all the tows in the sample bottle and then multiply by 1000. Round the value to 2 significant figures.

Area of the net hoop (m^2) x tow depth (m) x 1000 liters/m^3 = total tow volume (L)

Table 1. Plankton net diameter and the corresponding area (m^2) of the net hoop, used to determine the minimum tow depth required to achieve a 1000-liter tow volume.

Net Diameter	Area of Plankton Net Hoop (m^2)	Minimum Tow Depth to get 1000 Liters Total Volume
5 inches (13 cm)	0.01 m^2	100 m
8 inches (20 cm)	0.03 m^2	33.4 m
12 inches (30 cm)	0.07 m^2	14.3 m
20 inches (50 cm)	0.20 m^2	5.3 m

Example: A 30 cm net is used to collect 3 x 20 m tows. All 3 of the tows are dispensed into the sample collection bottle.

0.07 m^2 x 60 m x 1000 L/m^3= 4200 liters of source water represented in the bottle

E. Determination of horizontal tow volume in liters

- It is difficult to determine horizontal volume. An estimate can be made in the same way vertical tow volume is calculated. That is, the length of the tow in meters multiplied by the hoop area in square meters then multiplied by 1000 L/m^3.

Horizontal tows do not account for veliger depth distribution and there is often a lot of sediment in horizontal tows. For these reasons horizontal tows are discouraged.

Appendix B

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Plankton tow preservation protocol

Plankton tow samples must be preserved to maintain the integrity of veliger shells and tissue for accurate cross polarized light microscopy and PCR analyses. Preservation should occur within 3 hours of collection, and the preserved sample must remain chilled until analyzed. Do not freeze samples.

Summary: Add 5 ml of a 4% (W/V) baking soda solution per 100 ml plankton tow sample then bring the volume to 20% absolute ethanol (V/V).

Protocol:

1. After tows have been poured into the collection bottle, mark the level with a Sharpie and measure the height of the liquid using a ruler with millimeter graduations.
2. Divide the height measurement by 0.95
3. The quotient is the level to which the 4% baking soda solution is added.

   This will be a relatively small quantity. A small cup should be used to pour the solution into the bottle.
4. Divide the measurement in step 1 by 0.76.
5. The quotient is the level to which absolute ethanol is added.

   The sample is now preserved. Store and ship the sample under refrigeration conditions.

Note: After the addition of baking soda and ethanol the pH of the sample should be 8.0 or slightly higher. The pH can be measured in the field with pH test strips. If the pH is below 8.0, add more baking soda solution. The pH of the sample will also be measured in the laboratory at the time of analysis and reported with results. A pH below 8.0 at the time of analysis means that more baking soda solution should have been added at the time of preservation.

Example preservation calculations:

Tow samples are collected and dispensed into a 250 ml Nalgene container. The tow sample level is measured at 65 mm.

65 mm / 0.95 = 68.4 mm (~ 68 mm)

mark 68 mm on the bottle and add the baking soda solution to this level.

65 mm / 0.76 = 85.5 mm (~ 86 mm)

mark 86 mm on the bottle and add absolute ethanol to this level.

Appendix C

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Decontamination protocol for plankton tow sampling

All equipment that comes into contact with the water must be decontaminated. Samples must use a 2-step decontamination process to support both cross-polarized light microscopy and PCR analysis. This process requires an acetic acid (vinegar) bath to dissolve veliger's shells, followed by application of a 10% bleach solution to denature DNA. Failure to follow this two-step decontamination protocol may result in inaccurate results.

Vinegar and bleach present safety hazards if not used properly. Material Safety Data Sheets (MSDS) are included, and all procedures, practices, safeguards, and requirements therein should be followed.

Protocol:

1. Place items to be decontaminated in the 18-gallon tote.
2. Fill the tote with enough household vinegar to completely cover all items.
3. Soak the items in vinegar for a minimum of 2 hours (24 hours is preferred).
4. After soaking in vinegar thoroughly rinse the items in tap water.
5. Spray the items with a 10% bleach solution and allow the items to sit for 15 minutes.
6. Alternatively, prepare a 10% bleach solution in a tote and soak items for 15 minutes.
7. After the bleach treatment, thoroughly rinse all the items in tap water and allow them to air dry.

The vinegar can be reused multiple times. Its acidity should be periodically tested with pH test strips to make sure the pH does not rise above 3. It is recommended that vinegar be poured back into the original container for storage.

The Material Safety Data Sheets for the chemicals used can be accessed through the following links:

Clorox Bleach MSDS

Distilled White Vinegar MSDS

200 Proof Non-Denatured Ethyl Alcohol MSDS