California Department of Fish and Wildlife

Quagga/Zebra Mussel Plankton Tow Sampling Protocol

Purpose of Sampling:

Plankton tow sampling is a form of early-detection monitoring for quagga and/or zebra mussel veligers, the planktonic, larval life stage, whereby small organisms (plankton) are collected by pulling a fine-mesh net through the water column (referred to as a "tow"). The plankton collected is then analyzed in a laboratory for the presence of veligers using cross-polarized light microscopy (CPLM) and/or DNA using polymerase chain reaction (PCR) analysis. To optimize the potential for detecting veligers, if present, plankton tows should follow a standardized sampling method, sample a large volume of water, and target the months (water temperatures) and locations where veligers are most likely to occur. Of equal importance, samples must be preserved and handled properly in order to maintain their integrity so 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 at www.wildlife.ca.gov/mussels.

When and Where to Sample:

Water Temperature

Plankton monitoring is typically conducted when water temperatures are between 9°C - 18°C (48°F - 64°F), when spawning is occurring. In warmer regions, where water temperatures remain within this range throughout the year, mussels can spawn year round. It is recommended tows be conducted monthly when temperatures are conducive to spawning.

Locations

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).

Depth

To increase the probability of capturing veligers if they are present, tows from depths of 15 meters are recommended.

Number of Sites and Number of Tows

The number of sites within a waterbody should be based on the size of the waterbody, but a minimum of three sites is recommended. A **minimum** total volume of 1000 liters of water should be filtered through the net per site. Therefore, the number of tows needed at each site should be determined by the diameter of the net used and the depth of each tow. Based on the diameter of the net, corresponding plankton net area (m^2) (Table 1, Appendix B), and the depth of each tow, the number of tows needed per site to filter 1000 liters can be calculated using the equation provided in Appendix B.

Summary of Sampling Recommendations

Parameter	Recommendation
Water temperature	9°C - 18°C (48°F - 64°F)
Locations	Around floating structures, marinas, inlets and outlets, coves, down-wind areas and eddies
Depth	0 – 15 m (0 – 50')
Number of sampling sites per waterbody	Variable; based on size of waterbody, minimum of 3
Number of tows per sampling site	Variable; based on depth and net size
Total volume sampled	Minimum 1000 liters (264 gallons) per site

Disclaimer: recommendations of equipment and supplies by brand or vendor are made only for the convenience of the user. Recommendations are not an endorsement and equipment or supply items of other brands that are offered by vendors may work just as well.

Equipment and Supplies:

- Plankton tow net – 63 or 64 micron mesh size
  - 8 inch diameter (WildCo part number 426-A28 recommended)
  - 12 inch diameter (Aquatic Research Instruments simple plankton net recommended)
- Tow rope – 100 foot minimum with 1 or 5 meter graduation marks
- Ballast weight – optional, use if needed
- Collection/sample bottles – plastic wide mouth 250 or 500 mL capacity
- Sample labels – Environmental Sampling Supply 2 X 3 inches, part no. 0203-5000 recommended (labels are sometimes provided with a bottle order)
- Ink pen/pencil
- Plankton Sample Datasheets; Appendix D (for internal data collection/management)
- CDFW Shellfish Health Lab sample submission/chain of custody (COC) form; Appendix E (for samples being submitted CDFW's Shellfish Health Lab)
- Notebook/ notepad
- Sharpie-type marker
- Hand calculator
- Spool for tow rope
- Carabineer
- Eighteen (18) gallon Rubbermaid tote with lid – 23.9 X 15.9 X 16.5 inch
- White vinegar (approximately 5% acetic acid)
- Household bleach (approximately 6% hypochloride)
- Spray bottle 32 oz. (grey Spraymaster type recommended)
- Measuring cup with graduations for milliliters or ounces
- Zip lock bags – 1 gallon
- Ruler with 1 mm graduations
- Non-denatured ethanol (200 proof)
- Baking soda, 4% solution in distilled water (W/V)
- pH paper (Whatman type CF pH range 4.5 – 10 recommended)
- Blue ice or gel packs
- Cooler – large enough to retain all samples
- Boat

Optional Equipment and Supplies:

- Bucket, 1-5 gallons
- Tools and tool box
- Camera
- Depth finder
- Multi-parameter water quality meter
- GPS unit
- Write-in-the-rain paper
- Clip board
- Cell phone
- Personal floatation devices
- First aid kit
- Fire extinguisher
- Batteries, all size

Equipment Preparation Prior to Collection

1. Decontaminate nets and related equipment before use. The decontamination protocol is provided in Appendix A.
2. If necessary affix a ballast weight to the net assembly.
3. Options for marking the tow rope:
    A. Measure the tow rope in 1 or 5 meter intervals
    B. Using a Sharpie type marker or labeling tape mark the rope at 1 or 5 meter intervals (markers can bleed or run during the decontamination process).
    C. Or, electrical shrink wrap can be used to mark the rope at 1 or 5 meter intervals.
        a. To do this obtain electrical shrink wrap slightly larger than the rope's diameter
        b. Cut the shrink wrap in inch segments
        c. Measure and mark the rope with a pen at 1 or 5 meter intervals
        d. Slide the appropriate number of shrink wrap segments on the rope
        e. Place one over each marked meter
        f. Heat the shrink wrap with a blow torch or hair dryer (the heat will shrink the wrap in place)
4. It is highly recommended that the tow rope be loaded onto a spool.
5. Blue ice / gel packs need to be frozen.
6. A refrigerator must be available for storage after collection.
7. Prepare 4% baking soda solution per Appendix B.

Vertical Tow Protocol

Note: A minimum of 1000 liters should be filtered from a given site. See Appendix B for example calculations.

1. If using a net with a valve, make sure the valve is closed; lower the net off the side of the boat perpendicular to the surface of the water.
    - Lower the net 15 meters or 1 meter above the bottom, whichever is deeper.
2. Count the graduation marks and record the depth of the net. Depth distance information is needed to determine the volume of water sampled.
3. **Do not allow the net to contact the bottom of the water body.** Touching the bottom will clog the net. If this happens, draw the net back up to the surface and thoroughly wash all of the material off. Do not dispense any of the bottom material into the sample bottle.
4. Pull the net up at a rate of about ½ meter per second. Pulling at a faster rate will create a wave in front of the net that will reduce filtering efficiency and may also damage veligers.
5. As the net is drawn towards the surface, maintain vertical alignment so that the center axis of the net is perpendicular to the surface of the water.
6. 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 wash any material clinging to the inner surface of the net into the cod end. Do not submerge the bridle ring while dipping the net.
7. Depending on how the cod end is configured, dispense or decant the tow material into the sample bottle.
   Repeat steps 1-7 until a minimum of 1000 liters of water has been filtered through the net.
8. Label the bottle with the waterbody, site name, date/time and name of collector, preservation type, analysis type, and agency.
9. Complete the Plankton Sample Datasheet in Appendix D for internal collection/maintenance of field data.
10. Complete the Lab Submission Form located at the end of Appendix E for all samples being submitting to CDFW's Shellfish Health Lab. This form is not required for samples submitted to external labs.
11. Place the bottle in a cooler with gel packs or blue ice.
12. Continue to the next site.

Samples must remain chilled to prevent degradation. Samples should be preserved in the parking lot per the preservation protocol found in Appendix C.

Horizontal Tows

Vertical tows are preferred over horizontal tows. However, horizontal tows may be required when sampling shallow water.

1. If the water is stagnant or the flow rate is slow, the net can be pulled in a horizontal direction with the net below the surface. A ballast weight may have to be attached to keep the net submerged.
2. The total length of the tow can be determined using the graduation marks on the tow rope.
3. See Appendix B for example calculations.
4. Complete the Plankton Sample Datasheet in Appendix D for internal collection/maintenance of field data.
5. Complete the Lab Submission Form located at the end of Appendix E for all samples being submitting to CDFW's Shellfish Health Lab. This form is not required for samples submitted to external labs.

Sample Identification

1. Samples need to be marked for identification when received at the Shellfish Health Lab. Adhesive labels should be used and information should be recorded with permanent ink. Ethanol used for preservation will cause ink to run; therefore, ethanol must be kept off any labels or identification markings. It is recommended that bottles be marked with a waterbody and site name (use of abbreviations is ok), preserved, and then have the label, with more detail, placed on each bottle.
2. **Include a lab sample submission/chain of custody (COC) form with all shipments and deliveries.**
   A copy of the CDFW Shellfish Health Lab submission form is included in this document at the end of Appendix E. Important information to include is: date of collection, the collector's name, waterbody name, description of locations, GPS data or waypoint, total tow depth, water depth, net hoop diameter, time and means of preservation, and both storage condition and storage location prior to shipment.

CDFW Regional Scientist Contacts

For the current list of CDFW's Regional Quagga/Zebra Mussel Scientists and their contact information, please visit CDFW's quagga/zebra mussel webpage at www.wildlife.ca.gov/mussels, or download the contact list here: http://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=4955.

Appendices

A. **Decontamination protocol for equipment used to collect plankton samples for quagga and zebra mussel larvae detection analysis**

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

C. **Plankton tow preservation protocol for the detection of quagga and zebra mussel veliger larvae**

D. **Plankton sample datasheet**

E. **Sample submission guidelines and sample submission form**

Appendix A

Decontamination protocol for equipment used to collect plankton tow samples for quagga and zebra mussel larvae detection analysis

After the tow samples have been collected from a water body all equipment coming into contact with the water must be decontaminated prior to use elsewhere. For thorough decontamination, equipment will have to be soaked in an acetic acid solution (vinegar) and then sprayed with a 10% bleach solution. The vinegar dissolves the veliger's shell but will not denature DNA. The bleach will denature DNA but will not dissolve shells. Therefore, the vinegar must be used before the bleach so DNA will be exposed to the denaturing bleach. Vinegar and bleach can present safety hazards if not used properly. Material Safety Data Sheets (MSDS) are included at the end of this appendix for both vinegar and bleach. Heed all MSDS precautions and follow all MSDS procedures, practices, safeguards and requirements when using vinegar and bleach.

Protocol:

1. Place items to be decontaminated in the 18 gallon Rubbermaid tote.
2. Fill the tote with enough household vinegar to completely cover all of the 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, a 10% bleach solution can be prepared in a Rubbermaid tote or a similar type of container and used to soak items for 15 minutes following the vinegar soak.
7. After the bleach treatment, thoroughly rinse all of the items off with tap water and allow them to air dry.

The vinegar can be reused multiple times. It's recommended that vinegar be poured back into the original container for storage. The vinegar should be periodically checked with pH test strips to make sure the pH level remains at approximately 2 to 3.

The Clorox Company
1221 Broadway
Oakland, CA 94612
Tel. (510) 271-7000

Material Safety Data Sheet

I Product: CLOROX REGULAR-BLEACH

Description: CLEAR, LIGHT YELLOW LIQUID WITH A CHARACTERISTIC CHLORINE ODOR

Other Designations	Distributor	Emergency Telephone Nos.
Clorox Bleach EPA Reg. No. 5813-50	Clorox Sales Company 1221 Broadway Oakland, CA 94612	For Medical Emergencies call: (800) 446-1014 For Transportation Emergencies Chemtrec (800) 424-9300

II Health Hazard Data

DANGER: CORROSIVE. May cause severe irritation or damage to eyes and skin. Vapor or mist may irritate. Harmful if swallowed. Keep out of reach of children.

Some clinical reports suggest a low potential for sensitization upon exaggerated exposure to sodium hypochlorite if skin damage (e.g., irritation) occurs during exposure. Under normal consumer use conditions the likelihood of any adverse health effects are low.

Medical conditions that may be aggravated by exposure to high concentrations of vapor or mist: heart conditions or chronic respiratory problems such as asthma, emphysema, chronic bronchitis or obstructive lung disease.

FIRST AID:

Eye Contact: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses, after first 5 minutes. Continue rinsing eye. Call a physician.

Skin Contact: Wash skin with water for 15-20 minutes. If irritation develops, call a physician.

Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, call a physician. Do not give anything by mouth to an unconscious person.

Inhalation: Remove to fresh air. If breathing is affected, call a physician.

III Hazardous Ingredients

Ingredient	Concentration	Exposure Limit
Sodium hypochlorite CAS# 7681-52-9	5 - 10%	Not established
Sodium hydroxide CAS# 1310-73-2	<1%	2 mg/m^3 [1] 2 mg/m^3 [2]

[1]ACGIH Threshold Limit Value (TLV) - Ceiling

[2]OHSA Permissible Exposure Limit (PEL) – Time Weighted Average (TWA)

None of the ingredients in this product are on the IARC, NTP or OSHA carcinogen lists.

IV Special Protection and Precautions

No special protection or precautions have been identified for using this product under directed consumer use conditions. The following recommendations are given for production facilities and for other conditions and situations where there is increased potential for accidental, large-scale or prolonged exposure.

Hygienic Practices: Avoid contact with eyes, skin and clothing. Wash hands after direct contact. Do not wear product-contaminated clothing for prolonged periods.

Engineering Controls: Use general ventilation to minimize exposure to vapor or mist.

Personal Protective Equipment: Wear safety goggles. Use rubber or nitrile gloves if in contact liquid, especially for prolonged periods.

KEEP OUT OF REACH OF CHILDREN

V Transportation and Regulatory Data

DOT/IMDG/IATA - Not restricted.

EPA - SARA TITLE III/CERCLA: Bottled product is not reportable under Sections 311/312 and contains no chemicals reportable under Section 313. This product does contain chemicals (sodium hydroxide <0.2% and sodium hypochlorite <7.35%) that are regulated under Section 304/CERCLA.

TSCA/DSL STATUS: All components of this product are on the U.S. TSCA Inventory and Canadian DSL.

VI Spill Procedures/Waste Disposal

Spill Procedures: Control spill. Containerize liquid and use absorbents on residual liquid; dispose appropriately. Wash area and let dry. For spills of multiple products, responders should evaluate the MSDS's of the products for incompatibility with sodium hypochlorite. Breathing protection should be worn in enclosed, and/or poorly ventilated areas until hazard assessment is complete.

Waste Disposal: Dispose of in accordance with all applicable federal, state, and local regulations.

VII Reactivity Data

Stable under normal use and storage conditions. Strong oxidizing agent. Reacts with other household chemicals such as toilet bowl cleaners, rust removers, vinegar, acids or ammonia containing products to produce hazardous gases, such as chlorine and other chlorinated species. Prolonged contact with metal may cause pitting or discoloration.

VIII Fire and Explosion Data

Flash Point: None

Special Firefighting Procedures: None

Unusual Fire/Explosion Hazards: None. Not flammable or explosive. Product does not ignite when exposed to open flame.

IX Physical Data

Boiling point ..... approx. 212°F/100°C
Specific Gravity (H_2O=1) ..... ~ 1.1 at 70°F
Solubility in Water ..... complete
pH ..... ~11.9

DATA SUPPLIED IS FOR USE ONLY IN CONNECTION WITH OCCUPATIONAL SAFETY AND HEALTH DATE PREPARED 08/09

Fisher Science Education
6771 Silver Crest Road, Nazareth, PA 18064 (800) 955-1177
Emergency Number: (800) 255-3924

Material Safety Data Sheet

Section 1 – Chemical Product and Company Identification

Catalog Numbers: S25623
Product Identity: Distilled White vinegar 5%

Chemical Family: Not Applicable
Synonyms: No Information Available
Recommended Use: Laboratory chemicals

Manufacturer's Name: AquaPhoenix Scientific, Inc., 9 Barnhart Dr., Hanover, PA 17331, (866) 632-1291
Emergency Contact Number (24hr): Chemtel (800) 255-3924

Issue Date: 01/03/07
Revision Date: 02/19/12, 08/03/12

Section 2 – Hazard Identification

Emergency Overview: If ingested give large quantities of water. Get medical attention. Wash areas of contact for at least 15 minutes.
Appearance: Clear, colorless liquid **Odor:** Vinegar-like
Target Organs: Eyes, skin, respiratory system, teeth.
Potential Health Effects/ Routes of Exposure:
Eyes: Causes irritation, redness, pain, tearing.
Skin: Causes irritation, redness and pain.
Ingestion: May cause irritation of the digestive tract.
Inhalation: Not likely to be a hazard by inhalation.
Chronic Effect / Carcinogenicity: None (IARC, NTP, OSHA)
Aggravated Medical Conditions No information Available.
These chemicals are considered hazardous by OSHA.
See section 11 for toxicological information. See section 12 for potential environmental effects.

Section 3 – Composition, Information on Ingredients

Acetic Acid, CAS# 64-19-7, 5% v/v
Water, purified, CAS# 7732-18-5, 95% w/v

Section 4 – First Aid

Eyes: Immediately flush eyes with water for at least 15 minutes. Get medical assistance immediately.
Skin: Flush with water for 15 minutes. Get medical assistance if irritation develops.
Ingestion: DO NOT induce vomiting. Dilute with water or milk. Get medical assistance.
Inhalation: Remove to fresh air. Give artificial respiration if necessary. If breathing is difficult, give oxygen.
Notes to Physician Treat symptomatically.

Section 5 – Fire Fighting Measures

Flash Point: No information Available **Autoignition Temperature:** No information Available
Explosion Limits Upper No Information Available **Lower** No Information Available

Extinguishing Media: Any means suitable for extinguishing surrounding fire.
Unsuitable Extinguishing Media: No information available
Fire & Explosion Hazards: Not considered to be a fire or explosion hazard
Fire Fighting Instructions / Equipment: Use normal procedures. Use protective clothing. Use NIOSH-approved breathing equipment.
Hazardous Combustion Products: No information Available.
Sensitivity to mechanical impact No information available.
Sensitivity to static discharge No information available.
Specific Hazards Arising from the Chemical: No information available
NFPA Rating: (estimated) Health: 2; Flammable: 0; Reactivity: 0

Section 6 – Accidental Release Measures

Personal Precautions Use personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing. Remove from all sources of ignition.
Environmental Precautions Should not be released into environment.
Methods for Containment and Clean Up Soak with inert material. Keep in suitable and closed containers for disposal. Always obey local regulations.

Section 7 – Handling and Storage

Handling: Wash hands after handling. Avoid contact with skin and eyes. Wear personal protective equipment.
Storage: Keep container tightly closed. Store in a cool, dry, well-ventilated area. Protect from freezing.

Section 8 – Exposure Controls, Personal Protection

Acetic Acid, CAS# 64-19-7, ACGIH TLV: 25mg/m3, OSHA PEL: 25mg/m3
Water, purified, CAS# 7732-18-5, ACGIH TLV: NA, OSHA PEL: NA
Engineering Measures/ General Hygiene: Normal ventilation is adequate
Personal Protection Equipment: Skin Protection: Chemical resistant gloves.
Eye/Face Protection: Safety Glasses or goggles. **Respiratory Protection:** Normal ventilation is adequate

Section 9 – Physical and Chemical Properties

Appearance/Physical State: Clear, colorless liquid
Odor: Vinegar-like
Boiling Point: 117-118C
Melting Point: 16.6C
Vapor Density: 2.07
Evaporation Rate: No information Available
pH: Acidic
Flammability: No Information Available
Solubility: Infinite
Relative Density: No Information Available
% Volatility: No Information Available
Specific Gravity: No Information Available
Vapor Pressure: No Information Available
Flash Point: No information Available
Coefficient of water/oil distribution: Not Available
Odor Threshold: Not Available
Decomposition Temperature: No Information Available
Partition Coefficient n-octanol/water: Not Available
Molecular Weight: 60.05

Section 10 – Stability and Reactivity

Chemical Stability: Stable under normal conditions of use and storage.
Incompatible Materials: Strong bases
Conditions to Avoid: No information Available
Hazardous Decomposition Products: irritating fumes
Hazardous Polymerization: Does not occur
Hazardous Reactions: None under normal processing.

Section 11 – Toxicological Information

Routes of Exposure/Symptoms/Corrosiveness – See Section 2
LD50 orl-rat: 3310 mg/kg (Acetic Acid)
LC50 inhalation-rat: 5620 ppm/ 1hr. (Acetic Acid)
Irritation: No information Available

Toxicologically Synergistic: No Information Available
Chronic Exposure
Carcinogenicity No known carcinogenic chemicals.
Sensitization No information available.
Mutagenic Effects not mutagenic in AMES test.
Reproductive Effects Experiments have shown reproductive toxicity effects on laboratory animals for acetic acid.
Developmental Effects (Immediate/Delayed) No information available.
Teratogenicity No information available.
Other Adverse Effects No information available.
Endocrine Disruptor Information No information available.

Section 12 – Ecological Information

Ecotoxicity: Acetic Acid has high biochemical oxygen demand, and a potential to cause oxygen depletion in aquatic systems.
Persistence and Degradability: Expected to be biodegradable **Mobility:** No Information Available
Bioaccumulation/ Accumulation: No Information Available

Section 13 – Disposal Considerations

Chemical waste generates must determine whether a discarded chemical is classified as a hazardous waste. Comply with all local, state, and federal regulations.

Section 14 – Transport Information

DOT – Not Regulated

Section 15 – Regulatory Information (not meant to be all inclusive)

OSHA Status: These chemicals are considered hazardous by OSHA.
Canada DSL: This chemical is listed on Canada's DSL list.
TSCA: These chemicals are listed on the TSCA Inventory.
SARA Title III Section 313: Not Applicable
RCRA Status: Not Applicable
CERCLA Reportable Quantity: Acetic Acid – 5000lbs.
WHMIS: Not-controlled

Section 16 – Additional Information

Disclaimer: The information on this MSDS applies to this specific material as supplied. It may not be valid for this material if it is used in combination with any other materials. It is the user's responsibility to determine the suitability and completeness of this information for his own particular use. No warranty is implied regarding the accuracy of the data or the results to be obtained form the products use.

Appendix B

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 with 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 1.1 = 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's 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)	0.01square meters	100 meters
8 inches (20 cm)	0.03 square meters	33.4 meters
12 inches (30 cm)	0.07 square meters	14.3 meters
20 inches (50 cm)	0.20 square meters	5.3 meters

- Example: A 30 cm net is used to collect 3 x 20 meter 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 C

Plankton tow preservation protocol for the detection of quagga and zebra mussel veliger larvae

Objective: Preserve the integrity of veliger shells and tissues in plankton tow samples so that veligers are amenable to PCR and CPLM analyses.

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 tow.
4. Divide the measurement in step 1 by 0.76.
5. The quotient is the level to which absolute ethanol is added.
6. The sample is now preserved. Store the sample under refrigeration conditions until shipping.

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 be 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.

Note: Samples must remain chilled. All samples should be placed in a cooler with gel or blue ice packs immediately after collection so they do not warm up and begin to degrade. Do not freeze the samples. Freezing damages shells and reduces detection sensitivity. Samples need to be preserved as soon as possible after collection (no more than 3 hours after collection).

Appendix D
Plankton Sample Datasheet

Collection Information		
Waterbody:	Date:	Collector:
Collector Affiliation:	Phone #:	

Net Information			
Mesh Size (µm):	Net Diameter (cm):	Net #:	Reel #:

Calculations for volume: V = (area of net)(total depth in m)(1000L/m^3) feet to meter x .3048

8in net V= (.03m^2)(total depth in m)(1000L/m^3) 12in net V= (.07m^2)(total depth in m)(1000L/m^3)

Tows

Sample ID: ____________ Total # of Tows: ______________________________ Preservation: pH Buffer: ____/.95 = ______

Ethanol:____/.76 =

Total Depth of Tows: Volume = Time: ______

Location Description	V/H Tow	Tow Depth (m)	Water Q. Depth (m)	Temp °C	pH	HDO %	HDO mg/l	Turb. NTU	Sp. Cond. (µS/cm)

Sample ID: ____________ Total # of Tows: ______________________________ Preservation: pH Buffer: ____/.95 = ______

Ethanol:____/.76 =

Total Depth of Tows: Volume = Time: ______

Location Description	V/H Tow	Tow Depth (m)	Water Q. Depth (m)	Temp °C	pH	HDO %	HDO mg/l	Turb. NTU	Sp. Cond. (µS/cm)

Sample ID: ____________ Total # of Tows: ______________________________ Preservation: pH Buffer: ____/.95 = ______

Ethanol:____/.76 =

Total Depth of Tows: Volume = Time: ______

Location Description	V/H Tow	Tow Depth (m)	Water Q. Depth (m)	Temp °C	pH	HDO %	HDO mg/l	Turb. NTU	Sp. Cond. (µS/cm)

Samples preserved to 20% with 200 proof non-denatured ethanol, buffered with 5 ml of a 4% baking soda solution per 100 ml ☐ Time:__________

Appendix E

Sample submission guidelines and submission form

Note: The California Department of Fish and Wildlife (CDFW) Shellfish Health Laboratory (SHL) is located at the UC Davis Bodega Marine Laboratory. As per the instructions below, samples need to be mailed to the Bodega Marine Laboratory where they will be routed to the Shellfish Health Laboratory. Samples may also be hand delivered to the Shellfish Health Lab per the instructions below.

Authorized Submissions:

Samples submitted to the Bodega Marine Laboratory SHL are usually collected by CDFW personnel or individuals working with CDFW personnel. The SHL accepts samples from any California State, out-of-state, or federal personnel qualified to collect samples. The SHL will also accept samples from water management personnel and academic institutions. Laboratory capacity is limited. First priority will be given to CDFW submissions. Compromised samples will not be tested. It is recommended that sample collection follow the **CDFW Quagga/Zebra Mussel Plankton Tow Sampling Protocol.**

Sample Delivery Options:

Properly preserved and maintained plankton tow samples collected for lab analysis may be either hand delivered or shipped to the SHL. Include a sample submission form with each set of samples. Make sure samples are clearly marked for identification. Samples should be delivered or shipped to the SHL within 1 week of collection.

Contact Information:

Contact Jim Snider at the SHL for any questions regarding quagga/zebra mussel testing.
Phone: (707) 785-2066
Email: James.Snider@wildlife.ca.gov

Hand Delivered Samples:

Hand delivered samples should be transported in a cooler and maintained at refrigeration temperature during transport. Samples may be hand delivered during normal business hours; Monday through Friday, 9:00 am to 5:00 pm. The lab is closed on weekends and holidays. Call Jim Snider prior to delivery to make sure personnel will be available to receive samples. Arrangements may be made for afterhours deliveries, contact Jim Snider for arrangements.

Shipping Samples:

Shipped samples should be packaged in a styrofoam packer (or a similar type cold packer) contained secondarily in a cardboard box. Use gel packs to keep samples chilled. Do not use wet ice. The Bodega Marine Lab (BML) shipping and receiving department is open Monday through Thursday and closed on Fridays, weekends, and holidays. All freight must be received no later than Thursday in any given week. Samples should be shipped for next day delivery. Samples that are held over the weekend by the courier service will be considered compromised and will not be tested. Samples collected late in the week may be held over the weekend if properly preserved and refrigerated and shipped the following week.

Location:

The location of the BML can be found at:

http://maps.google.com/maps/myplaces?hl=en&ll=38.31905,-123.055509&spn=0.090101,0.153637&ctz=420&t=m&z=13

The CDFW Shellfish Health Lab is located in rooms N307 and N310. Entrance to the BML is gated. The gate closes at 5:00 pm.

Shipping Address:

Bodega Marine Laboratory
Shellfish Health
Attention: Jim Snider
2099 Westside Road
Bodega Bay, CA 94923

Reporting Results:

Results will be reported in letter or memo format and will be emailed to designated contacts.

Laboratory Fees:

Currently there is no fee for quagga/zebra mussel plankton tow testing at the SHL.

CDFW Shellfish Health Laboratory Submission Form
Quagga/Zebra Mussel Plankton Tows

Name: _____
Agency: _____ Title: _____
Phone #: _____ Email: _____
Mailing Address: _____
Waterbody: _____
Site Location: _____

Was the sample preserved at the time of collection with baking soda and 20% absolute ethanol and stored at refrigeration temperature as per Appendix A: Plankton tow preservation protocol for the detection of quagga and zebra mussel veliger larvae in this document?

☐ Yes ☐ No If no, please specify the preservation method used:

Plankton Net Diameter (include units): _____
Plankton Net Mesh Size (include units):

Sample No.	Collection Date	Sample Description	Indicate Horizontal or Vertical Tow (H or V)	Total Tow Depth in Container (indicate feet or meters)