



Invasive Mussel eDNA Sampling Protocol

Invasive Species Program

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Overview

The CDFW Invasive Mussel eDNA Sampling Protocol describes CDFW-recommended methods and procedures for collecting invasive mussel environmental DNA (eDNA) samples in ambient waters. Use of these methods is recommended but not generally required. However, following the described procedures (including collection and submission of all described quality control samples and sample labeling) is required for any samples submitted to the CDFW Genetics Research Laboratory for analysis.

eDNA sampling can be used to detect DNA of target species in aquatic environments. This protocol describes two methods by which eDNA samples are collected: plankton tows, and with the Smith-Root Citizen Scientist Sampler.

It is recommended to collect water quality measurements in conjunction with eDNA sampling including water temperature, salinity, dissolved oxygen, pH, and sampling depth. Please refer to the CDFW Water Sampling and Water Quality Measurements Protocol for further instructions regarding water quality measurements and data collection.

Plankton Tow eDNA Sampling Protocol

Introduction

Plankton tow sampling is the recommended method to collect invasive mussel eDNA from reservoirs, creeks, and other aquatic environments with sufficient depth. This method can filter a larger volume of water than the traditional eDNA filter method and is therefore more likely to capture any eDNA present.

The method for collecting plankton tow samples for eDNA is derived from plankton tow sampling for veliger detection. Additional sampling precautions and quality control procedures are implemented to prevent cross-contamination of samples.

Only a small aliquot from the collected plankton tow samples will be used for eDNA processing at the lab. The remaining plankton tow samples should be preserved and retained until negative eDNA results are returned from the lab.

Preparation, Equipment, and Supplies

1. Please review the CDFW Plankton Tow Sampling Protocol for instructions on plankton tow sampling and equipment preparation.
2. Ensure that plankton tow nets, bottles, and other field equipment have been properly decontaminated to avoid eDNA cross contamination. See [Appendix A](#) for further instructions on decontamination.
3. Assign all field equipment a unique identifying code. See [Appendix B](#) for further instructions.

Before heading into the field, prepare your eDNA sampling equipment and supplies to streamline collection in the field. Store and pack your supplies in a decontaminated space. [Everything on this list should be new or decontaminated](#). For this process, you'll need:

- Bottle of non-denatured 200 proof ethanol
- Plastic tote
- Sharpie
- Cooler
- Falcon tube for cooler blank
- Ziploc bag for plankton samples
- Ice packs

Plus, the following for each waterbody you plan to sample:

- One 2-gallon Ziploc bag containing:
 - Falcon tubes: one for each eDNA sample, plus 1 more for the net blank
 - Nitrile gloves
 - Two 250ml Nalgene bottles OR one 500mL bottle filled with tap water for the net blank
 - 250ml Nalgene bottles (one for each plankton sample)
 - Whirl-Pak bags: one for each eDNA sample, plus one for the net blank
 - Optional: disposable pipettors
- Plankton net and cod end
- Garbage bag

Preparation process

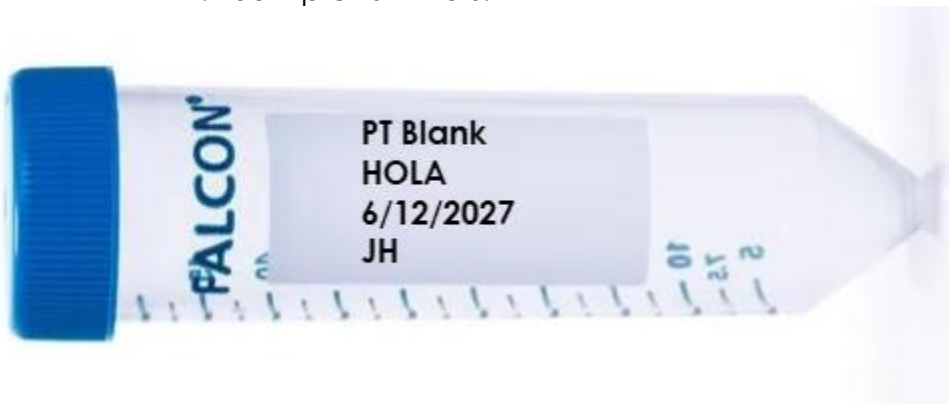
With clean hands, fill each falcon tube with 25mL of non-denatured 200 proof ethanol. For the cooler blank, add 25 mL of tap water as well. Screw the cap tightly onto each falcon tube.

1. Using the sharpie, label each falcon tube with the appropriate sample or blank label, the date and your initials. The following examples are for Hodges Lake:
 - a. Cooler Blank
 - i. "Cooler Blank"
 - ii. Date
 - iii. Sampler's initials.



b. Plankton Tow Net Blank

- i. "PT Blank"
- ii. Waterbody code
- iii. Date
- iv. Sampler's initials.



c. eDNA sample

- i. 4 letter code for the waterbody, then unique 2-digit number for your monitoring site
- ii. Date
- iii. Sampler's initials.



2. Place the cooler blank directly into the cooler, without a whirl-pak bag. Place all other falcon tubes into the 2-gallon Ziploc bag.
3. Label each plankton sample bottle with the appropriate sample label (e.g. PT-HOLA-01), the date and your initials, then place empty plankton sample bottles into the 2-gallon Ziploc bag.
4. Add nitrile gloves, the tap water, and the Whirlpak bags into the Ziploc bag. Add plastic pipettors if desired.
5. Place 2-gallon Ziploc bag, plankton net and cod end, and garbage bag into the plastic tote.
6. Place ice packs and an additional Ziploc bag into the cooler. This bag will hold plankton sample bottles once collected and prevent water from the plankton samples from potentially contaminating the eDNA samples.
7. Repeat this process for each waterbody you plan to sample. At the end, you should have a series of 2-gallon Ziploc bags and plankton nets in your tote.
8. Once the 'clean' tote is prepped, pack all your supplies for surface surveys, water quality collection and plankton sample preservation in the 'dirty' tote.

Sample Collection

Cooler Blank

This is done at the beginning of the sampling day to track any contamination that may come from a sample leaking into the cooler.

1. Wearing nitrile gloves, pour 25 ml of tap water and 25 ml of ethanol into a Falcon tube.
2. Place in the cooler. Do **not** place cooler blank into a WHIRL-PAK sampling bag.

Start of Waterbody Sampling

When sampling in the field, you will have three storage locations for supplies at each waterbody: the clean tote for decontaminated supplies, an 'interim' trash bag for storage of supplies that have contacted that waterbody, and a dirty tote for supplies that have contacted multiple waterbodies. Once you arrive at your monitoring site, you'll start by collecting your eDNA samples. Bring your plankton net, trash bag, the waterbody's corresponding 2-gallon Ziploc bag and your cooler to the sampling site.

Plankton Tow Net Blank

This is done before starting sampling at a waterbody. It determines if there is any eDNA on the net from the last time it was used (that wasn't removed during decontamination). Collect your net blank before touching the net to the dock or the water.

1. Pour 500mL of tap water through the cleaned and decontaminated plankton tow net.
2. Carefully pour or pipette 25 ml of water from the cod end into a falcon tube. If pipetting, do not reuse pipettes. The falcon tube blank should already contain 25 ml of ethanol.
3. Place the blank in a Whirl-Pak sampling bag and into the cooler.

Plankton Sampling

Refer to the CDFW Plankton Tow Sampling Protocol for full plankton sampling procedures. Samples need to be preserved as soon as possible upon collection to avoid eDNA degradation and false negative results.

1. From the cod end, pour the plankton sample into the 250 mL Nalgene bottle with the corresponding label.
2. Cap the bottle and mix the sample by inverting the bottle three times.
3. Aliquot sample for eDNA analysis:
 - a. Carefully pour or pipette 25 ml of the mixed plankton sample into a 50 mL falcon tube. If pipetting, do not reuse pipettes. The falcon tube should already have 25 mL of non-denatured ethanol inside.
 - b. Cap and mix the tube by inversion for 30 seconds.
 - c. Place the Falcon tube into a Whirl-Pak sampling bag and into the chilled cooler.
 - d. If a cooler is not available, the eDNA sample should be stored in a dark location out of the sunlight and placed in a chilled container as soon as possible.
 - e. eDNA samples should be frozen upon return from the field.
4. Place the plankton sample bottle with remaining sample water into the cooler, inside the Ziploc bag in the cooler.
5. Pack up the 2-gallon Ziploc bag with the used supplies and seal it. Place the plankton net and sealed Ziploc bag inside the 'interim' trash bag.
6. If driving to an additional site at this waterbody, keep plankton net and Ziploc bag in trash bag when you return to the car and place trash bag in a location separate from the 'dirty' and 'clean' totes in the car. Use contents of trash bag for all eDNA sampling at that waterbody.

Plankton Sample Preservation

Repeat the full sample collection process for every waterbody you plan to sample. All plankton samples can be placed in the same Ziploc bag in the cooler.

***Note:** Plankton tow samples should be retained until a negative eDNA result is returned.

Smith-Root Citizen Scientist Sampling Protocol

Introduction

The Smith-Root Citizen Scientist eDNA sampling collection method is used at locations where the plankton tow eDNA collection method cannot be feasibly done. This includes locations where the water is too shallow for a plankton tow such as a shallow creek. Water is vacuumed from the aquatic environment and passed through a filter in the filter house. The filter collects eDNA from the water and will be analyzed for the presence of invasive mussel DNA at the lab.

Equipment and Supplies

- Smith-Root citizen scientist sampler
- Smith-Root 5 um self-preserving filters
- Nitrile gloves
- Tap water for filter blank (~200 ml)

If not sampling directly from a stream or other water source:

- 1 L bottle or large Whirl-Pak sampling bags for water sample collection
- DNA Away™ or 10% bleach for cleaning

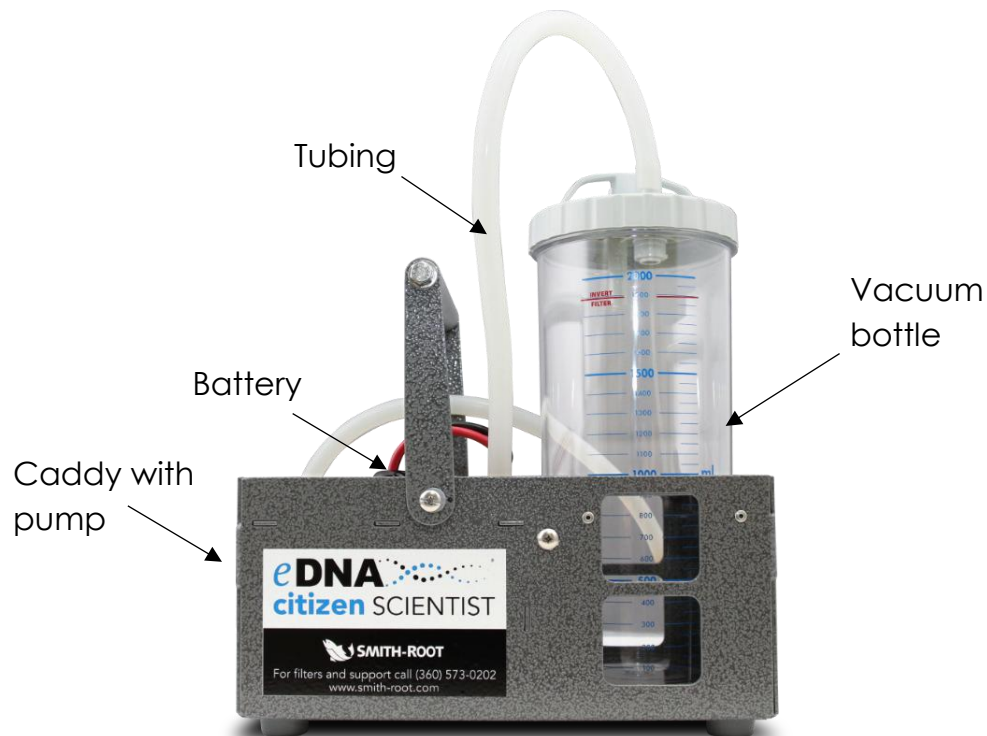


Figure 1. Components of a Smith-Root Citizen Scientist eDNA sampler.



Figure 2. A Smith-Root filter pouch containing the filter housing and the snorkel.

Equipment Preparation Prior to Collection

1. Before heading to the field, ensure the sampler is fully charged. There is a charging cord included for the battery.
2. Ensure that the vacuum bottle has been decontaminated before use and between waterbodies to prevent cross contamination. To decontaminate, soak the vacuum bottle for 15 minutes in bleach solution (10%) and rinse three times with water.

Sample Collection

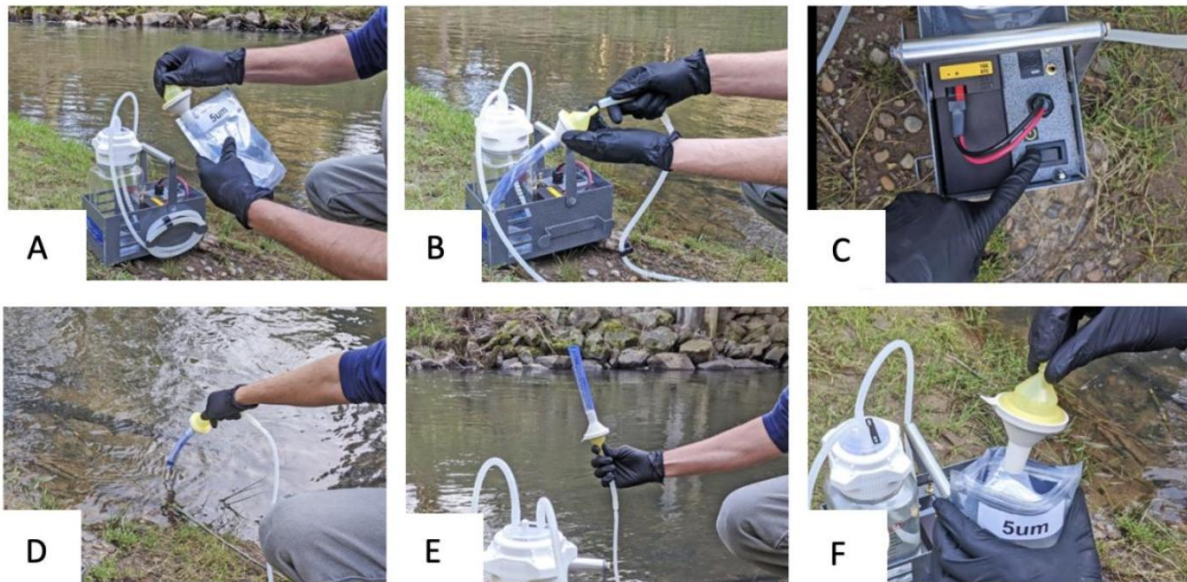


Figure 3. Overview of sample collection using the Smith-Root Citizen Scientist. Figure from “eDNA Sampling Protocol: Smith-Root Citizen Scientist sampler”.

Water collection

Skip this step if sampling directly from a stream or other water source (as in Figure 3D).

Wearing gloves, rinse sample bottle three times with ~10 ml of sample water; discard rinse water offshore or away from sampling site. Fill bottle with 1 L of sample water. If bottles are limited, large Whirl-Pak sampling bags can be used instead.

Field Blank

Before beginning sampling at a waterbody, filter ~200 ml of tap water through a clean filter.

1. Wearing gloves, open an individually wrapped Smith-Root filter pouch.
2. Without touching the snorkel, attach it to the smooth end of the filter housing (**Figure 3A**). This can be done by holding the sides of the filter housing bag to attach the snorkel. The included tweezers in the filter pouch can be discarded
3. Attach the sampler hose to the ridged end of the filter (**Figure 3B**).
4. Turn on the vacuum pump (**Figure 3C**).
5. Insert the snorkel into the field blank container.
6. When ~200 mL has been filtered, invert the filter housing so the snorkel is facing the sky.

7. Continue to run the vacuum pump for at least one minute to air dry the filter (**Figure 3E**).
8. Remove the snorkel from the filter housing and discard the snorkel.
9. Place the filter housing back in the pouch (**Figure 3F**). Remove as much air as possible before sealing the pouch.
10. Label pouch with:
 - a. "Field Blank"
 - b. Waterbody code
 - c. Date
 - d. Sampler's initials
 - e. Example for Hodges Lake:



11. Store pouch in a chilled cooler. If a cooler is not available, the sample should be stored in a dark location out of the sunlight and placed in a chilled container as soon as possible.
 - a. Smith-Root pouches can be stored in the same cooler as eDNA plankton samples.
 - b. Samples should be frozen upon return from the field for long-term storage.
12. Discard the filtered water in the collection vessel. Filtered water should be discarded away from sample collection area.

Filtering

1. Wearing gloves, open an individually wrapped Smith-Root filter pouch.
2. Without touching the snorkel, attach it to the smooth end of the filter housing (**Figure 3A**). This can be done by holding the sides of the pouch to

attach the snorkel. The included tweezers included in the filter pouch can be discarded .

3. Attach the sampler hose to the ridged end of the filter (**Figure 3B**).
4. Turn on the vacuum pump (**Figure 3C**).
5. Insert the snorkel into the sample water (**Figure 3D**).
6. When 2 L have been filtered (or when filter clogs), invert the filter housing so the snorkel is facing the sky.
7. Continue to run the vacuum pump for at least one minute to air dry the filter (**Figure 3E**). Record final volume of water filtered in field data sheet.
8. Remove the snorkel from the filter housing and discard the snorkel.
9. Place the filter housing back in the pouch (**Figure 3F**). Remove as much air as possible before sealing the pouch.
10. Label pouch with:
 - a. 4 letter code for the waterbody, then unique 2-digit number for your monitoring site
 - b. Date
 - c. Sampler's initials
 - d. Example for Hodges Lake:



11. Store pouch in a chilled cooler. If a cooler is not available, the sample should be stored in a dark location out of the sunlight and when feasible placed in a chilled container as soon as possible.
 - a. Smith-Root pouches can be stored in the same cooler as eDNA plankton samples.
 - b. Samples should be frozen upon return from the field.
12. Discard the filtered water in the collection vessel. Filtered water should be discarded away from sample collection area.

Cleaning the Sampler

1. Thoroughly wipe down the Smith-Root sampler after each field day with a wet paper towel.

Additional Resources

1. Demonstration video for the Smith-Root citizen scientist sampler: <https://youtu.be/BupozR1fnlg>
2. Smith-Root citizen scientist sampler manual: <https://www.smith-root.com/support/downloads/edna-citizen-scientist-sampler-manual>

Data Recording and Reporting

Every eDNA sample and associated data must be recorded on a datasheet. In addition, a Laboratory Submission form must be completed for every sample submitted to the Fisheries Genetics Lab for analysis. All data will be entered into a data reporting system, and the datasheets will be retained on-site.

Sample Submission

eDNA samples should be shipped to the lab **as soon as possible**. All eDNA samples should be frozen prior to shipping. Samples should be shipped in a hard sided cooler with ice packs using next-day delivery services. Please ship frozen samples to the CDFW Genetics Lab:

Dr. Bryan Barney
CDFW Fisheries Genetics Research Laboratory
1415 North Market Blvd, Suite 9
Sacramento, CA 95834

Appendices

- Appendix A Decontamination Protocol
- Appendix B eDNA Best Practices
- Appendix C Genetics Lab Submission Form

Appendix A Decontamination Protocol

All equipment that comes in contact with the water must be decontaminated to prevent eDNA cross contamination and spread of AIS.

General Field Equipment Decontamination

The following items can be sprayed down with a 10% bleach solution and allowed to sit for at least 15 minutes. Rinse well afterwards.

- Cooler
- Ice Packs
- Totes holding used field gear

Plankton Tow Net Decontamination

Plankton tows nets require a 2-step decontamination process to support both cross-polarized light microscopy and DNA 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.

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.

Equipment and Supplies

- 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

Procedure

1. Place items to be decontaminated in an 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 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 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. Once bleach is diluted, it is only effective for 24 hours. Any leftover bleach should be discarded.

Plankton Tow Bottles and Falcon Tubes

Plankton tow bottles and Falcon tubes can be cleaned, decontaminated, and reused for the next sampling event.

1. To clean plankton tow bottles and Falcon tubes, scrub and rinse the bottles / tubes and caps with dish soap and water. This step is done to remove residual debris and ethanol from the bottle / tube. Note: Ethanol and bleach should not be mixed as they will form chloroform gas.
2. Once the bottles / tubes and caps have been cleaned, add them to a 10% bleach solution bath and let them soak for 10 minutes. The bleach solution can be reused but should be disposed after 24 hours.
3. Rinse the bottles and caps at least three times with water.

Plankton tow bottles and Falcon tubes that are not cleaned and decontaminated with a 10% bleach solution are not suitable for eDNA sampling and should not be used until they have been decontaminated.

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](#)

Additional Resources:

[CDFW Aquatic Invasive Species Decontamination Protocol 2022](#)

Appendix B eDNA Best Practices

This Appendix summarizes Best Practices in the field and in the office to minimize cross contamination of eDNA.

Order of Monitoring

eDNA sampling at a monitoring site should be done first before taking water quality measurements and performing other monitoring surveys such as surface surveys and artificial substrates. If applicable, eDNA sampling should always begin at the most upstream site.

Labeling of Plankton Tow Equipment

Field equipment used for eDNA sampling must be labeled with a unique identifier. This information will be recorded on the eDNA datasheet.

Equipment that should be labeled includes:

- Plankton tow net, rope and cod end
- Bag that holds plankton net

In Field Equipment Storage

Once field equipment has come into contact with a waterbody (e.g., plankton net) or with the samples (e.g., cooler), it is considered “contaminated” because it may have come into contact with mussel DNA. Contaminated field equipment will need to be isolated from unused field equipment to minimize cross-contamination. Have a garbage bag or separate tote box ready to hold contaminated field equipment until the end of the day. If possible, store the contaminated tote box and equipment physically away from clean equipment. (For example, clean equipment in the cabin of the vehicle and dirty equipment in the cargo area).

Equipment Storage

Field equipment associated with eDNA sampling must be stored in a space that is free of mussel DNA, termed a “clean area”. The clean area should have no invasive mussel specimens, displays, positive mussel samples, used field equipment or any sample potentially contaminated with invasive mussel DNA. Once a clean area has been designated, spray and wipe down surfaces with a bleach solution to denature DNA. Decontaminate all eDNA field equipment before storing it in the clean area. Field equipment to store in the clean area includes plankton tow nets, cod ends, rope, filters, any reagents used in the field, falcon tubes, field blanks, coolers, and nitrile gloves.

It is also recommended to design and implement a strict protocol to ensure the clean area remains uncontaminated. Examples of this could include:

- Installing plastic floor mats if your clean area is in a carpeted space so that the floor can be decontaminated with bleach spray
- Implementing a one-way workflow to prevent contaminated supplies from entering the clean area
- Bringing a change of clothes to change into after performing field work to eliminate the possibility of transferring eDNA from your clothes to the clean area.
- Having a physical barrier to the clean area (door, stanchion) with a DNA Clean Area advisory sign.

Appendix C Genetics Lab Submission Form

CDFW Genetics Research Laboratory Submission Form
Invasive Mussel Monitoring eDNA Samples

Name:

Agency:

Phone #:

Email:

Mailing Address:

Plankton sample or Filter?	Collection Date	Sample ID	Waterbody	Monitoring Site

Complete for Plankton Samples:

Was the plankton sample preserved at the time of collection with absolute ethanol to 50% and stored at freezer temperature?

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

Plankton Net Diameter (include units):

Plankton Net Mesh Size (include units):