Technical Assistance

Geoduck Tissue Sample Preparation and Homogenization: Standard Operating Procedure



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Prepared by: Washington State Department of Health under a cooperative agreement with the Agency for Toxic Substances and Disease Registry.

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Title Geoduck tissue sample preparation and homogenization

Scope

This document describes the procedure for grinding Geoduck samples. Geoduck samples are usually analyzed on a wet weight basis. Liquid nitrogen is added to the cubed up geoduck tissue in the Robot Coupe Blixer to aid in finely grinding the samples.

Status

This document is considered current standard operating procedure of the Office of Environmental Health, Safety and Toxicology when management approval is documented by signature below. This Standard Operating Procedure is effective on the date of approval signature and supersedes all previous versions.

Approved

Dan Alexanian

Manager
Title

Date

Historical File

Signature/Initials

Date

I. Equipment and Apparatus

- A. Stainless steel knives cleaned according to Ecology lab SOP.
- **B.** Cutting boards cleaned according to Ecology lab SOP.
- **C.** Aluminum foil
- **D.** Robot Coupe Food Processor– cleaned according to Ecology lab SOP.
- **E.** Stainless steel bowls and cup cleaned according to Ecology lab SOP.
- F. Cleaning tools dilute soap solution, brushes, squirt bottle with 18 $M\Omega$ -cm water (DI water), squirt bottle of rinse acetone, squirt bottle of rinse methanol.

II. Sample Preparation

A. Dissection

- 1. Remove one geoduck from cooler.
- 2. Rinse geoduck (over sink) with DI water (using squirt bottle).
- **3.** Note sample identification number on tracking sheet.
- **4.** Weigh the whole geoduck and enter weight (g) on tracking sheet.

- **5.** Cut away shell from tissue (on cutting board covered with aluminum foil).
- **6.** Cut "edible" muscle from shell (if still attached). Treat as "edible tissue".
- 7. Cut gutball away from siphon/mantle. Set gutball aside for trim processing.
- **8.** Trim non-edible tissue from siphon/mantle and discard.
- **9.** Take siphon/mantle to water bath area for skinning.
- **10.** Trim any edible meat from gutball and combine with "edible tissue" sample previously set aside. Trim non-edible tissue from gutball and discard.
- **11.** Weigh gutball and enter the weight on tracking sheet. Place individual gutballs into a small zip-loc bag or a bowl. Five gutballs from the same sample site are placed in a large zip-loc bag or a bowl for the composite sample.
- **12.** Repeat steps 1-11 with all geoducks from same sample location or composite samples.
- 13. Discard foil, clean knives and cutting boards.
- **14.** Keep shell for aging.

B. SKINNING

- **1.** Prepare DI water bath to ~ 200 degrees F (near boiling).
- 2. Place one geoduck neck/mantle in water bath for 10 seconds.
- **3.** Remove from water bath with tongs or strainer.
- **4.** Pull outerskin from neck and mantle using fingers or forceps (discard skin).
- **5.** Split siphon and rinse with DI water to remove detritus.
- **6.** Weigh siphon/mantle and enter the weight on tracking sheet.
- **7.** Repeat steps 1-6 with all geoducks from same sample location or composite samples.
- **8.** Discard water bath, clean pot and utensils with soap and water. Rinse with acetone or methanol.

C. CHOPPING

1. Place geoduck tissue on cutting board. (Note: no need to dice gutball tissue).

- 2. Cut siphon/mantle into dice sized cubes.
- **3.** Place cube tissue into a small zip-loc bag or a bowl. Five geoducks from the same sample site are placed in a large zip-loc bag or a bowl for the composite sample.
- **4.** Tissue is ready to be homogenized.
- **5.** Discard foil. Clean knives and cutting boards.

D. GRINDING/HOMOGENIZATION

- 1. Place either gutball or edible tissue into Robot Coupe Blixer. The edible tissue is process separately from the gutball unless the whole geoduck is being process as one sample.
- 2. Multiple geoducks of the same sample and tissue type can be placed in Robot Coupe until 3/4 full.
- **3.** Pour liquid nitrogen over geoduck until covered (~2 cups).
- 4. Cover Robot Coupe
- 5. Press the "on" button.
- **6.** Turn off after 60 seconds.
- **7.** Ensure that geoduck tissues is homogeneously ground (if not, adding more liquid nitrogen and /or blending may be necessary)
- **8.** Using a spatula, place ground tissue into two 8-ounce ICHEM jars (one for metals analysis and the other for archive).
- **9.** Discard remaining homogenate and clean Robot Coupe before processing the next geoduck sample.

E. RINSATE

1. At least one time per day, run a sample of DI water through the process and collect for laboratory analysis.

III. Documentation

A. A bench sheet is required for each sample batch.

IV. Safety and Health

- **A.** Always use personal protective equipment.
- **B.** Use ear protection when grinding.
- **C.** Acetone or methanol rinse equipment under a hood.
- **D.** DO NOT use acetone or other organic solvents on the Robot Coupe plastic cover or on plastics; it will warp. If a solvent is necessary for cleaning, use methanol or ethanol alcohol.