APPENDIX IX

AED LABORATORY OPERATING PROCEDURE FOR MEASUREMENT OF TOTAL LIPIDS USING MODIFIED BLIGH-DYER METHOD

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1. OBJECTIVE

This document defines a procedure based on a modification of the method reported by Bligh and Dyer (1959). This procedure is used to analyze marine tissues for total lipid content.

2. MATERIALS

Solvents

Methanol - Baxter Pesticide Grade
Chloroform - Baxter Pesticide Grade (ethanol free)
Deionized water

Glassware

TurboVap tubes, 25ml scintillation vials, and 50ml centrifuge tubes muffled at 450 degrees F for 6 hours.

Equipment

Mayer N-Evap Analytical Evaporator
Zymark TurboVap Evaporator
Sorvall RC2-V Centrifuge
Kinematica Homogenizer with 12mm tip.

3. ANALYTICAL PROCEDURE

All Trophic Transfer samples were stored at -20 degrees c immediately after collection and thawed just prior to analysis. Solvent ratios in the following procedure are expressed in the order: **chloroform/methanol/water**.

3.1) For lobster muscle, place 10g wet homogenized tissue in a 50ml centrifuge tube. For lobster hepatopancreas and Nereis tissue place 5g wet homogenized tissue in a tared 25ml scintillation vial.

3.2) Calculate the amount of water that is in the sample by using the formula:[grams wet x (1 - dry/wet ratio)] = (ml)water. The (ml)water is used to calculate the appropriate amounts of chloroform and methanol to add to the centrifuge tube to obtain a **solvent volume ratio of 1/2/0.8**. Thus, to calculate the amount of chloroform needed for 4ml of water in the sample, multiply 4ml x 1.25 = 5ml chloroform and 2 x chloroform = 10ml methanol. The ratio of chloroform/methanol/water in the centrifuge tube or vial is now 5/10/4 or **1/2/0.8** . Add the appropriate amounts of chloroform and methanol to the centrifuge tube and blend with a 12mm polytron tip for 60 seconds.

3.3) Add an additional volume of chloroform to the centrifuge tube/vial that is equal to the amount used in step 2. Blend for 30 seconds.**(Solvent volume ratio 1/1/0.4)**

3.4) Add an additional volume of water to the centrifuge tube/vial that is equal to the amount calculated in step 2. Blend for 30 seconds. **(Solvent volume ratio 1/1/0.9)**

3.5) Cap the tube/vial and centrifuge for 10 minutes. Draw off the chloroform and dispense it into a turbovap tube for muscle tissue or a 25ml scintillation vial for hepatopancreas and Nereis tissue.

3.6) Rinse all transfer tools with small portions of chloroform, collecting the washes in the centrifuge tube or scintillation vial.

3.7) Add an additional volume of chloroform equal to 2 times the amount used in step 2 to the remaining tissue in the centrifuge tube or vial. Blend for 30 seconds. **(Solvent volume ratio 1/1/0.9)**

3.8) Cap the tube/vial and centrifuge for 10 minutes. Draw off the chloroform and transfer to the turbovap tube. Rinse transfer tools with small portions of chloroform into the tube or vial.

3.9) Repeat steps 7 and 8 except shake manually instead of using the Polytron.

3.10) If the extract is cloudy or contains an emulsion, pass it through a layer of sodium sulfate and collect. Repeat as needed to clarify extract. Rinse apparatus with small portions of chloroform.

3.11) For muscle tissue, volume reduce the extract under a nitrogen stream in the turbovap tube to 1ml then transfer to a 25ml scintillation vial and blow to dryness under nitrogen in an N-Evap evaporator. For hepatopancreas and Nereis tissue extracts (which are already in a 25ml scintillation

vial) reduce to dryness in the N-Evap evaporator.

3.12) Place the uncapped scintillation vial in an oven at 100 degrees c for 1 hour then allow the vial to cool in a desiccator for 15min and weigh.

3.13) Calculate the weight percent of total lipid in the sample using the formula: ((g)lipid / (g)dry sample weight) * 100 = percent lipid.

4. REFERENCES

Bligh, E.G. and W.J. Dyer. 1959. Canadian Journal of Biochemistry and Physiology, 37(8), pp. 2-8.