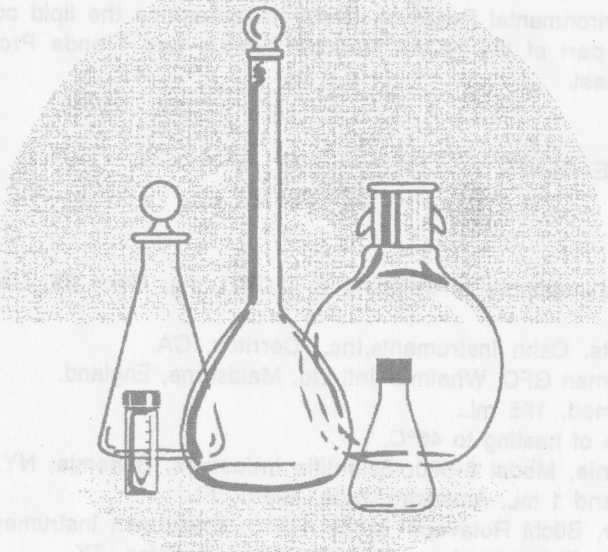
NOAA Technical Memorandum NOS ORCA 71

National Status and Trends Program
for Marine Environmental Quality

Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-1992

Volume II

Comprehensive Descriptions of Complementary Measurements



Silver Spring, Maryland
July, 1993

noaa NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

Coastal Monitoring and Bioeffects Assessment Division
Office of Ocean Resources Conservation and Assessment
National Ocean Service

Tissue Lipid Determination Method

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ABSTRACT

The lipid determination procedure used for the NOAA National Status and Trends Mussel Watch Project Gulf Coast oyster tissue samples is described. Lipid content is operationally described as the weight of sample extracted using dichloromethane.

1. INTRODUCTION

Organic contaminants concentrate in body fat because of their similar chemical properties. Therefore, measurements of lipid content has been used as an aid for normalizing trace organic contaminants in tissue samples for different individuals. The lipid content is operationally defined by the method used to determine it. This section describes the method used by the Geochemical and Environmental Research Group to determine the lipid content, in percent, of oysters analyzed as part of the NOAA National Status and Trends Program Mussel Watch Project in the Gulf Coast.

2. APPARATUS AND REAGENTS

2.1. Equipment

Balance, analytical, capable of weighing to 0.0001 mg, Cahn 29. Cahn Instruments, Inc., Cerritos, CA.
Calibration weights. Cahn Instruments,Inc., Cerritos, CA.
Filter paper, Whatman GFC. Whatman Intl Ltd, Maidstone, England.
Flasks, flat bottomed, 125 mL.
Hot plate, capable of heating to 40°C.
Mixer, Vortex Genie, Model 6-560. Scientific Industries, Bohemia, NY.
Pipettor, 100 µL and 1 mL. American Dade, Miami, FL.
Rotary evaporator, Büchi Rotavapor Model R110. Brinkmann Instruments, Westbury, NY.
Vials, 7 dram, with Teflon-lined cap. Lab Products, Houston, TX.

2.2. Reagents

Methylene chloride (CCl_2H_2) [75-09-2], (pesticide quality or equivalent)
Sodium sulfate (Na_2SO_4) [7757-82-6], ACS Granular, anhydrous (purified by heating at 400°C for 4 hr)

3. PROCEDURE

After the oysters were extracted as described by Wade *et al.* (Volume. IV, this document), the total volume of extraction solvent was determined and recorded. A 20-mL aliquot was removed

and filtered through sodium sulfate which was pre-combusted 400°C for 4 hr, into a 125-mL flask. The aliquot was evaporated to remove the dichloromethane extraction solvent. The residue was dissolved and quantitatively transferred to a 7-dram vial, using dichloromethane. The dichloromethane in the 7-dram vial was evaporated with a stream of nitrogen. The volume of the aliquot was then adjusted to 1.00 mL. The vial was sealed and agitated using the Vortex Genie mixer until the residue was completely dissolved in the dichloromethane.

The analytical balance was calibrated before use with calibration weights. A 1 x 5 cm piece of filter paper was placed on the balance pan and tared. The filter paper was removed and placed on a hot plate that was heated to 40°C. One hundred microliters of the lipid aliquot was removed using a pipettor and placed on the hot filter paper. When the solvent has evaporated from the filter paper, the paper was allowed to cool and reweighed. This procedure was repeated for all samples in the sample set.

4. CALCULATION

The percent lipids is determined using the following formula:

$$\text{Percent lipids} = \left[\frac{[\text{LM (mg)}]\,[\text{TV (mL)}]\,[\text{FV (µL)}]}{[\text{DM (mg)}]\,[\text{AV (mL)}]\,[\text{VW (µL)}]} \right] 100\%$$

where LW is the lipid weight determined in mg, DW is the dry weight (mg) of sample (see Sweet *et al.*, this document), TV is the total volume (mL) of extract, AV is the volume (mL) of aliquot, FV is the final volume (µL) of aliquot, and VW is the volume (µL) weighed .

5. QUALITY CONTROL

Duplicate samples for percent lipid percent determinations are analyzed using approximately 10% of the samples. Duplicate analyses agreed with a 10% relative percent difference or better.

6. CONCLUSIONS

The lipid content of oysters is operatively defined. The values are normally between 2 to 20%. The lipid content can be useful in comparing contaminant concentrations of oysters with different lipid contents.

7. ACKNOWLEDGEMENTS

Thanks to the GERG laboratory personnel C. Frazier, Y. Yu, and G. Salata who do the tedious weighing of lipid extracts.

Lipid Weight Determination Procedures Followed by Battelle Ocean Sciences

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ABSTRACT

This document describes the procedures followed by Battelle Ocean Sciences to determine bivalve lipid weights for the years 1988 through 1993 of the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Mussel Watch Project.

1. INTRODUCTION

This document summarizes the procedures followed to determine and calculate lipid content in bivalve tissues for East and West Coast samples.

2. SAMPLE PROCESSING

This lipid weight determination procedure is an element of the Battelle tissue extraction procedure. The procedure presented below has been used since 1988 of the Mussel Watch Project.

2.1. Equipment

Aluminum foil
Balance, Mettler AC 100 or equivalent
Flask, Erlenmeyer, 500-mL with graduation marks
Pipette, 10-mL, Class A
Weighing pans, aluminum, baked at 120°C for 24 hr

2.2. Analytical procedures

Tissues were extracted according to protocols presented in Peven *et al.* (Volume IV, this document). The centrifuged extracts were combined in an 500-mL Erlenmeyer flask and the total volume recorded. A 10-mL aliquot was removed from the flask using a Class A glass pipette, the extract was evacuated into a pre-weighed aluminum weighing pan, and the pan covered with aluminum foil. After standing for approximately 24 hr at room temperature the pan was examined to ensure that the solvent (dichloromethane) has completely evaporated. The pan is then re-weighed, and the weight recorded.

2.3. Calculation

The lipid weight is calculated using the following formula:

$$\text{Total lipid weight (g)} = \frac{\text{Extracted sample volume (mL)}}{\text{Aliquot volume (mL)}} \text{ [Aliquot dry wt (g) - tare wt (g)]}$$

$$\text{Lipid weight (g/g)} = \frac{\text{Total lipid weight (g)}}{\text{Sample dry weight (g)}}$$

3. CONCLUSIONS

The lipid weight determination procedures described above were used by Battelle for the Mussel Watch Project. These same procedures are currently in use.

One hundred microliters of CH HPLC I-std solution and 50 µL of Tissue AH HPLC I-std solution were added to the bottle and the contents swirled thoroughly.

One hundred microliters of CH HPLC I-std solution was added to each of the CH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

Fifty microliters of Tissue AH HPLC I-std solution was added to each of the low-level AH analyte-calibration solutions from the Extraction section, and the contents mixed on a Vortex Genie for 2 sec at setting 4-6.

The AH/CH EXTRACT was further processed as described in the Precleanup Section 5.4.

5.2. Extraction of samples for CHs and lipid

Tissues were customarily analyzed in sets of twelve samples each. In addition to the 8-9 regular tissue samples in each set, there may be one or more method blank samples, one spiked blank sample, one matrix spike sample, one reference tissue sample, one field blank sample, and one duplicate tissue sample. For composite samples larger than 3.0 g, 0.5 g were used for Dry Weight Determination, 0.2 g were taken for triglyceride analysis, and 3 g were extracted and the extract used for CHs and lipid. For samples of between 2.5 - 3.0 g, 0.5 g was used for Dry Weight Determination. The remainder of the sample was extracted and the extract used for CHs and lipid. No triglyceride analysis was performed. For samples of between 1.5 - 2.5 g, 0.5 g were used for Dry Weight Determination, the remainder was extracted, and the total extract used for CHs. No triglyceride or lipid analyses were performed. For samples smaller than 1.5 g, the total amount was extracted and all of the extract used for CH analysis. No other analysis was performed.

Using a spatula, and being careful to place the sample on the bottom and not the sides, the appropriate amount of tissue (see above) weighed to the nearest 0.01 g was placed in a labeled 100-mL centrifuge tube with a Teflon-lined foil cap.

For samples larger than 1.5 g, approximately 0.5 g were set aside for Dry Weight Determination, and the remaining portion was stored in a freezer.

Thirty-five milliliters of dichloromethane was added to each tube, except for the field blank.

The field blank was prepared at this step in the analytical procedure by washing down the empty sample container three times with 10-mL aliquots of dichloromethane, adding the combined washes to a labeled 100-mL centrifuge tube with Teflon lined foil cap, then adding 5 mL of dichloromethane.

One hundred microliters of CH I-std solution was added to each tube.

One thousand microliters of CH spike solution was also added to the tube for the spiked blank and/or matrix spike.

Two CH analyte-calibration solutions were prepared by adding to each of two GC vials labeled "CH Spike Vial" in addition to the sample number, 1000 µL of CH spike solution and 100 µL of CH I-std solution.

For the blanks and each sample of 2.5 g or more, two wide-mouth 250-mL bottles with Teflon-lined caps were prepared: one bottle was labeled "CH EXTRACT", in addition to the sample number; the other bottle was labeled "Lipid EXTRACT", in addition to the sample number. Each bottle was tared. For each sample smaller than 2.5 g, one wide-mouth 250-mL bottle with a Teflon-lined cap was assembled and labeled "CH EXTRACT" in addition to the sample number.

Twenty-five grams of sodium sulfate was added to each sample.

The sample in the tube was macerated/extracted for 0.5 min with a Tissumizer at setting 80. The speed was reduced to setting 60 and maceration continued for 1.5 min. Care was taken to avoid spattering the tissue.

The Tissumizer probe was rinsed with approximately 5 mL of dichloromethane and the washings collected in the tube.

The sample was centrifuged for 5 min at 2,000 rpm and the extract decanted into the bottle labeled "CH EXTRACT", in addition to the sample number. Care was taken not to transfer any solids.

Thirty-five milliliters of dichloromethane was added to each sample tube, and the three steps immediately above were repeated, combining the second extract with the first.

The sodium sulfate/sample mass was washed by adding 10 mL of dichloromethane to the tube and then swirling the tube to thoroughly mix the contents. The washings were decanted into the bottle labeled "CH EXTRACT", being careful to leave all solids in the tube. The bottle was recapped.

Fifty microliters of CH HPLC I-std solution was added to the bottle, and the contents swirled to thoroughly mix the contents.

One hundred microliters of CH HPLC I-std solution was added to each of the CH analyte-calibration solutions from the Extraction section, and the solution mixed on a Vortex Genie for 2 sec at setting 4-6.

For the blanks and samples of 2.5 g or more, the bottle containing the total CH EXTRACT was weighed and approximately 30 ml of the CH EXTRACT was transferred into the bottle labeled "Lipid EXTRACT" in addition to the sample number. The bottles were capped, and the bottle containing the Lipid EXTRACT was reweighed.

The lipid and CH extracts were further processed as described in Sections 5.3 and 5.4 respectively.

5.3. Concentration of lipid extracts

Six to eight boiling chips were added to the bottle containing the Lipid EXTRACT, and, using a steam table, the extract volume was reduced to 15-20 mL; 7.5 cc of diatomaceous earth filtrate was added to the bottle and swirled.

A tared pear-shaped flask was placed in the filtering container of the vacuum filtration apparatus and the Büchner funnel positioned so that the tip of the funnel was just below the mouth of the flask. A filter paper was placed in the funnel.

The vacuum source was activated and the filter paper wetted with dichloromethane. A seal formed between the funnel and the paper when the paper could no longer be moved. The Lipid EXTRACT was decanted from the bottle into the Büchner funnel.

The bottle was washed with approximately 5 mL of dichloromethane and the washings decanted into the funnel. This step was repeated twice.

Using the rotary evaporator, the dichloromethane was evaporated from the extract in the flask. The temperature of the water bath was set at 35-40°C. The solvent was evaporated at as fast a rotation as possible without splashing the sample. When water condensed on the outside of the flask, the flask was lowered into the bath, being careful not to boil the extract. If boiling occurred, the flask was quickly lifted out of the water bath and either the temperature of the bath lowered or air was bled into the system to stop the boiling.

When the dichloromethane evaporated and any remaining liquid was thick and oily, the vacuum was broken, the flask was removed, and placed in a drying oven at 50°C for 2 hr.

The flask was cooled in a desiccator, and weighed. The weight of the dried lipid was then calculated.

Using the weights of the CH EXTRACT bottle and the Lipid EXTRACT bottle, the fraction (F_x) of the total CH EXTRACT that was used for the Lipid EXTRACT was calculated as follows:

$$F_x = \frac{\text{Wt. of Lipid extract and bottle - Wt. of bottle}}{\text{Wt. of total CH extract and bottle - Wt. of bottle}}.$$

Finally, the percent lipid content of the original sample was calculated, as follows:

$$\text{Percent Lipid} = \frac{\text{Wt. of lipid}}{(F_x \text{ extract taken})\ (\text{Orig. sample wt.})}\ 100\%$$

5.4. Pre-cleanup of AH/CH and CH extracts

A cleanup column was prepared by adding a 10-15-mm plug of glass wool to a chromatography column and tamping it down with a glass rod, then adding 10 cc of alumina, followed by 20 cc of silica gel and 5 cc of sand.

Fifty milliliters of dichloromethane was added slowly to the column, and allowed to drain into a waste container. This step was repeated once. The tip of the column was then rinsed with dichloromethane.

Another wide-mouth 250-mL bottle, labeled "AH/CH" or "CH" in addition to the sample number, was placed under the column. The extract of samples prepared in either Sections 5.1 or 5.2 was slowly decanted into the column and allowed to drain into the bottle.

The first bottle was washed with approximately 5 mL of dichloromethane, and the washings slowly decanted into the column. This step was repeated twice, and the washings allowed to drain into the bottle.

The column wall was washed down with 35 mL of dichloromethane, and the washings allowed to drain into the bottle.