

APPENDIX

Protocol for gravimetric lipid analysis:

Thimble preparation (48 hours prior to extraction)

1. label Whatman cellulose thimbles (use lead pencil) with desired fish ID #s
2. place thimbles in drying oven @ 60C.
3. pre-drying of thimbles is essential and should be done for at least 48 hrs!

Tissue preparation (48 hours prior to extraction)

1. Locate carcass of interest in Dr. Wilbur's -80C freezer
2. Allow carcass to thaw at room temperature for ~5 minutes
3. Label bottom of polystyrene pans with id#s of fish to be extracted
4. Place pan on analytical balance and tare
5. Weigh fish and record on data sheet
6. Dissect fish to remove liver and viscera
7. Weigh liver and viscera and record on data sheet
8. Add remaining carcass to tared pan.
9. Record wet weight (WWT) of carcass on datasheet
10. Place pan with carcass in drying oven at 60C
11. Dry carcass for 48 hours.

Day of extraction:

1. Fill each 125ml flask to $\frac{3}{4}$ level with clean petroleum ether
2. Retrieve (quickly & one at a time) labeled thimbles from oven
3. Measure DWT of thimble. Should be done as quickly as possible (within 5 sec of removing thimble from oven)
4. Set thimble aside
5. Retrieve (quickly & one at a time) dried tissue samples from oven
6. Using forceps, carefully transfer dried tissue into a tared pan on balance. Record DWT
7. Transfer dried tissue into thimble with matching fish id#
8. Place thimble containing tissue into Soxhlet chamber
9. Turn on cold water faucet to start flow through condensers
10. Plug in heater control box and adjust setting to '2.5'
11. Note start time and check extractors frequently
12. After 4 hours, unplug heater and allow solvent to cool
13. Remove thimbles from chambers using tongs, and place in drying oven
14. Discard used solvent into waste canister (in fire cabinet underneath fume hood)
15. Allow extracted samples to dry at 60C for 48 hours
16. After 48 hours, measure DWT of thimble + tissue
17. Return thimble to drying oven for later ash determination

BIOGRAPHICAL SKETCH

Susanna Leah Holst was born on July 18, 1977 in Lansdale, Pennsylvania. She went to Colgate University in Hamilton, New York for two years, then in her junior year she participated in the Sea Education Association program during her first semester and went to study abroad in Australia at James Cook University of Northern Queensland the following semester. She transferred her credits from Colgate to James Cook University, where she earned her Bachelor of Science degree in Marine Biology in April 1999. She worked at the National Undersea Research Center in Key Largo, Florida for a year on a coral reef monitoring study before she enrolled at the University of North Carolina at Wilmington in January 2001 for the Master of Science in Marine Science program. Ms. Holst graduated in July of 2003.