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Sea Turtle Field Sampling Methods



Collection of
Tissues for
Mercury Analysis

SEA TURTLE FIELD SAMPLING METHODS

Collection of Tissues for Mercury Analysis

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Biodiversity Research Institute (BRI) is a 501(c)3 nonprofit organization located in Portland, Maine, USA. Founded in 1998, BRI is dedicated toward supporting global health through collaborative ecological research, assessment of ecosystem health, improving environmental awareness, and informing science-based decision making.

May 2022

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1.0 Protocol Overview

This sampling protocol is designed as a guide for the collection and processing of sea turtle tissue samples for the measurement of total mercury. Sample collection following these general protocols will allow comparisons to be made across sampling sites and assist in identifying potential mercury hotspots posing risk to both human and ecosystem health.

2.0 Field Planning and Logistics

Sufficient planning and preparation will ensure that data collected are of the highest quality and will provide accurate information for regional resource managers.

2.1 Permits and Permissions

It is important to obtain all necessary ministry and/or national collection permits and licenses for the collection of sea turtle tissues.

NOTE: All sea turtle species are listed under the *Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)* and require appropriate permits.

3.0 Sea Turtle Egg Sample Collection

3.1 Sampling Overview

Collected samples will provide important information in developing awareness about potential risks associated with mercury pollution. It is important to make sure all samples are collected in a safe and clean manner. Collaborating organizations are asked to record basic information for each sample. Sampling sea turtle eggs is indicative of the female body burden, due to the transfer of methylmercury. Nests should be tracked and returned to after hatching event in order to collect failed eggs.

3.2 Equipment Needed for Sampling

Materials for sea turtle egg tissue sample collection are listed below.

| Item | Purpose |
|------------------------------------|--|
| Small plastic Ziplock style bags | For storing eggs samples |
| Waterproof labels | Sample identification |
| Vials or plastic containers | For protecting egg samples during transport |
| Permanent marker and ballpoint pen | For labeling plastic Ziplock bags |
| Cooler with ice packs | To temporarily store samples in the field |
| Data sheet | To be filled out for every sample collected |
| Map of country/sampling site | Use to mark the location of each sampling site |

3.3 Egg Collection

Standard protocols for the collection of sea turtle eggs are outlined in this section. For each sea turtle egg, there are a series of standard measurements that must be taken and recorded on the sample data sheet.

3.3.1 Sampling Eggs

1. Record sea turtle species and common name and other metadata on the data sheet.
2. Collect two to three nonviable, intact eggs from each sampled nest:
 - o Eggs remaining intact in the nest after the hatching event can be considered failed and can be collected as nonviable.
3. Each sample label must include a nest identifier to link eggs from the same nest
4. Each egg should be placed and stored in its own individual plastic bag with waterproof label
5. While in the field, store samples in a cooler with frozen ice packs
6. **IMPORTANT:** Eggs should be frozen as soon as possible and should remain frozen until analysis.

3.4 Egg Sample Labeling Format

It is imperative that all samples have a unique sample label ID that is correctly and legibly recorded. Be sure to use an alpha-numeric code of species, country, year, nest, and individual number. All sample labels should include:

1. Species (common name and Latin name)
2. Location (latitude, longitude, site, town, county, state, country)
3. Nest identifier
4. Unique identifier
5. Maximum length (mm)
6. Maximum width (mm)
7. Mass (g)
8. Date (example: Mar 18, 2022)

4.0 Sea Turtle Scute Sample Collection

4.1 Sampling Overview

Collected samples will provide important information in developing awareness about potential risks associated with mercury pollution. It is important to make sure all samples are collected in a safe and clean manner. Collaborating organizations are asked to record basic information for each sample. Sampling scutes (keratinized layer) does not impact health of the individual (Bjorndal et al. 2010; Day et al. 2005). Mercury binds with keratin, is stable, and provides information about the chronic exposure of an individual.

4.2 Equipment Needed for Sampling Scutes

Materials for sea turtle scute tissue sample collection are listed below.

| Item | Purpose |
|------------------------------------|--|
| Razor or scalpel | For shaving scute |
| Forceps | For obtaining scute sample and transferring to bag |
| Scrubbing pad | For cleaning turtle carapace |
| Deionized water | For cleaning turtle carapace |
| Alcohol | For sterilizing carapace and instruments |
| Permanent marker and ballpoint pen | For labeling plastic Ziplock bags |
| Plastic Ziplock style bags | To organize and store samples |
| Data sheet | To be filled out for every sample collected |
| Map of country/sampling site | Use to mark the location of each sampling site |

4.3 Scute Collection

Standard protocols for the collection of sea turtle scutes are outlined in this section. For each turtle, there are a series of standard measurements that must be taken and recorded on the sample data sheet.

4.3.1 Sampling Scutes

1. Record sea turtle species and common name and other metadata on the data sheet.
2. Note any flipper tags or other individual identification tags.
3. Clean carapace with water and a scrubbing pad. Then rinse with deionized water and sterilize each area for sampling with isopropyl alcohol.
4. Use a sterilized razor or scalpel and forceps to remove small shavings from multiple scutes
5. **IMPORTANT:** Do not sample scutes with sign of injury or that appear to have a thin keratin layer.
6. **IMPORTANT:** Do not scrape too deeply to avoid injuring the individual or contaminating the sample with non-scute tissue.
7. Using sterilized forceps, transfer scute shavings into individually labelled plastic bags or vials.
8. **IMPORTANT:** Samples should be frozen as soon as possible and should remain frozen until analysis.

NOTE: If possible, collect scute shavings when the female is laying eggs, then return to the same nest or area after hatching occurs to collect nonviable eggs.

4.4 Scute Sample Labeling Format

It is imperative that all samples have a unique sample label ID that is labeled correctly and legibly. Be sure to use an alpha-numeric code of species, country, year, and individual number.

All sample labels should include:

1. Species (common name and Latin name)
2. Any unique identifier information (e.g., flipper tags, PIT tags) if applicable
3. Location (latitude, longitude, site, town, county, state, country)
4. Curved carapace length
5. Curved carapace width
6. Mass (kg) — (if it is possible to weigh the adult turtle)
7. Age and sex
8. Date (example: Mar 18, 2022)

5.0 References

Bjorndal, K.A., Reich, K.J. and Bolten, A.B., 2010. Effect of repeated tissue sampling on growth rates of juvenile loggerhead turtles *Caretta caretta*. *Diseases of aquatic organisms*, 88(3), pp.271-273.

Day, R.D., Christopher, S.J., Becker, P.R. and Whitaker, D.W., 2005. Monitoring mercury in the loggerhead sea turtle, *Caretta caretta*. *Environmental science & technology*, 39(2), pp.437-446.