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## Standardized Collecting Protocol for Marine Turtle Epibionts

Most epibiont reports are derived from sampling conducted during the monitoring of sea turtle nesting. Scientists working on beaches with sea turtle monitoring programs are those currently providing the most epibiont information. The bilingual collection data sheet provided here was developed from a combination of the turtle monitoring data sheets currently used by the Riverhead Foundation for Marine Research and Preservation (Long Island, New York, USA) and the Programa Universitario para la Protección y Conservación de la Tortuga Marina, Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuario (CUBCA, Guadalajara, Mexico).

These standardized data collecting sheets are general enough to be used for any sea turtle species by any sea turtle research program. Included is a diagram (dorsal and ventral) of a generic marine turtle that can be used to indicate host locations sampled for epibionts. Letter labels can be used to designate body regions. These lettered regions should correspond to appropriately labeled specimen tubes. An editable version of the data collecting sheet provided here is available for download at <http://peabody.yale.edu/collections/invertebrate-zoology/turtle-epibiont-project>.

### *Field Sampling*

Whenever possible preserve specimens collected from different areas on the turtles separately, according to the following body landscape regions:

- Head (A) and neck (B)
- Forelimbs (C) and shoulders (D)
- Hind limbs (E) and cloacal area (F)
- Carapace (G)
- Plastron (H)

Place epibiont specimens in containers labeled according to the turtle and its body landscape region where collected. Do not put in more than the volume of the container can hold. Immediately fill the container to the top with 75% alcohol and seal the lid. Invert the jar a few times to disperse the alcohol throughout the samples. *Note:* Use 75% alcohol to achieve the target of 70% alcohol after wet (water-retaining) specimens are added.

If any DNA extraction is to be done, the alcohol used to fix specimens must be consumable-grade ethyl alcohol. DNA will not be properly

preserved with methyl (“denatured”) alcohol or isopropyl (“rubbing”) alcohol.

It is crucial to mark the date on the data sheets in the format “dd month yyyy” (for example, 05 Nov 2007). This ensures that there can be no confusion as to the precise date. Along with a unique turtle number (specific for a particular year) and the body landscape region, this will uniquely identify the collecting event. Although informally the nesting season can be noted, the day, month and year the sample is collected must always be clear.

### *Labeling Specimens for Research and Museum Curation*

Sort each specimen container by grouping and counting identical taxa. Write the necessary sample identification in pencil or alcohol-resistant ink on a wet-strength paper label placed inside the container. Wet-strength label stock marketed as Resistall can be obtained from University Products, Inc., Holyoke, Massachusetts, USA (<http://www.universityproducts.com/>). Similar products can be substituted. Standard paper stock disintegrates rapidly in fluid preservative and should not be used. Secondary labeling on the outside of the jar can be done for sample identification only, but must not be relied on as the only label. Labels on a plastic jar can become smudged and illegible, and should be avoided when possible.

When sorted, the samples must be distinguishable immediately by a unique identifier, therefore a simple number and letter combination is not sufficient. There have been many instances in which two samples from different years bear the same number, leading to much confusion. A sample identification label should include the year (not the season) when the sample was taken as a prefix, as follows: 2011-1 through 2011-XXX.

The combination of a year and turtle number permits any number to be re-used without confusion. Include an abbreviation for the body landscape region (instead of the letters A to H) on the paper label with the collection number inside the container, to indicate where on the turtle the epibiont came from. This descriptive labeling is more reliable for long-term data preservation because it is not easily confused. Suggested standardized abbreviations include:

Head (H) and neck (N)  
 Forelimbs (FOR) and shoulders (SHD)  
 Hind limbs (HIN) and cloacal area (CLO)  
 Carapace (CAR)  
 Plastron (PLS)

For example, the label “2011–68 CAR” indicates that the specimens in the jar belong to the 68th turtle sampled in 2011 and that the epibionts were removed from the carapace. More detailed notes can be provided on the data sheet.

### *Shipment of Specimens*

Be sure to travel with a copy of the permit authorizing the collection of the epibionts and a letter or permit indicating that the specimens can legally be transported out of the originating country. Samples to be transported by aircraft (in personal baggage or as air cargo) must not be in ethyl alcohol, which is a violation of International Air Transport Association (IATA) regulations. Instead, samples should be carefully decanted through a small screen, the screen rinsed with shipping fluid (see below for a description) back into the container, and additional shipping fluid added to fill the jar.

To seal the jar, wrap a small amount of Teflon® tape (available from hardware and plumbing supply stores) around the threads and close the jar tightly. Seal the outside of the lid by wrapping it with white or black electrical tape pulled tight on itself and cut cleanly. These steps will insure no leakage.

The shipping fluid to use is Carosafe®, a proprietary product marketed by Carolina Biological Supply Co., Burlington, North Carolina, USA (<http://www.carolina.com/>). This fluid is safe for shipping specimens that may ultimately be the subject of molecular work. Dilute the concentrated fluid (9 parts water to 1 part concentrate). Because it is not a hazardous material, there is no fear that material will be confiscated. It is imperative, however, to have a copy of the proprietary information in the box with the specimens, along with note to Transportation Security Administration (TSA) inspectors that reads as follows:

To: TSA Inspectors

These preserved scientific specimens are being shipped in Carosafe® liquid, a nonhazardous material as defined by current IATA regulations, and thereby is allowable as a shipping solution. Its primary ingredient is propylene glycol, a US FDA-approved food additive. If specimen containers are opened for inspection, they must be resealed tightly and placed upright to prevent leakage; if specimens dry out they will lose their scientific value.

Thank you,

Name: \_\_\_\_\_

Title: \_\_\_\_\_

Be sure to include a technical description, or Material Safety Data Sheet (MSDS) of the Carosafe® liquid (or other acceptable shipping fluid) with the samples.

### *Specimen Deposition and US Reporting (Importation)*

As soon as possible after arrival at the receiving institution, the shipping fluid must be decanted from the sample jar through a 0.5 mm mesh standard screen (to catch tiny epibionts) and the screen rinsed with 75% nondenatured alcohol. Add more alcohol to make sure that the sample container is filled to the top, sufficient to dilute any remaining shipping fluid.

Within 180 days a US Fish and Wildlife Service 3-177 Import/Export Permit must be filed with the US FWS regional office or through the US FWS Electronic Declarations (eDecs) website (<https://edecs.fws.gov/>); forward a printed copy once electronic approval is received.

This filing, although mandatory, does not need to take place during import and export. Researchers should be prepared to remind inspectors at the time of import of the 180-day reporting period, and it is important to carry a copy of the relevant page of the US Code of Federal Regulations (Title 50, Part 14, “Importation, Exportation, and Transportation of Wildlife” [50 CFR 14.64(b)(3)]) that describes the reporting rules.

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## Epibiont Data Collecting Sheet

Field number (Número de campamento) \_\_\_\_\_ Date and time (Fecha y hora) \_\_\_\_\_

Beach number (Número de la playa) \_\_\_\_\_

City/Town(Ciudad/Pueblo) \_\_\_\_\_ County (Municipal) \_\_\_\_\_

Latitude and longitude (Latitud y longitud)

\_\_\_\_\_ / \_\_\_\_\_ degrees (grados)/decimals (decimales)

Host species (Especie) \_\_\_\_\_

Positive (Positivo)       Probable (Probable)       Unsure (Inseguro)

Nearest body of water (Cuerpo de agua mas cercana) \_\_\_\_\_

Offshore (Fuera de la costa)       Inshore (Costa)

Water temperature (Temperatura del agua) \_\_\_\_\_ Air temperature (Temperatura del aire) \_\_\_\_\_

Cloacal temperatura (Temperatura cloacal) \_\_\_\_\_

### *Human Interaction (Información sobre la interacción humana)*

Fishery interaction (Interacción pescadera):

Yes (Sí)     No (No)       Cannot be determined (No se puede determinar)

Evidence of boat collision (Evidencia de golpes de barcos):

Yes (Sí)     No (No)       Cannot be determined (No se puede determinar)

### *Measurements (Medidas)*

[all measurements and weights in metric units (todos métricos)]

Straight carapace length (Largo recto del caparazón) \_\_\_\_\_

Straight notch to notch (Recto marca a marca) \_\_\_\_\_

Curved carapace length (Largo curvo del caparazón) \_\_\_\_\_

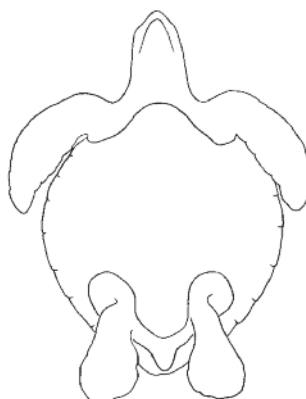
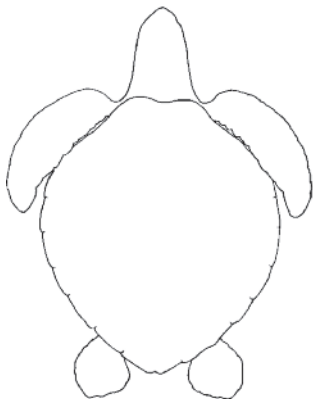
Curved notch to notch (Curvo marca a marca) \_\_\_\_\_

Straight maximum carapace width (Ancho máximo recto del caparazón) \_\_\_\_\_

Curved maximum carapace width (Ancho curvo del caparazón) \_\_\_\_\_

Plastron length across mid-line (Largo del plastrón, a travéz de la línea media) \_\_\_\_\_

Length of tail extending beyond carapace, *D. coriacea* only (Largo de la cola extendida más allá del caparazón) \_\_\_\_\_ Weight (Peso) \_\_\_\_\_



Tags present (¿Etiquetas presente?)

Yes (Sí)     No (No)

If yes, enter identification number for both PIT and metal tags

(Si sí, el Número de identificación para PIT y "metal tags"):

\_\_\_\_\_ Left fore flipper (Aleta izquierda)

\_\_\_\_\_ Right fore flipper (Aleta derecha)

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Blood drawn? (¿Sacaron sangre?)     Yes (Sí)     No (No)

Date (Fecha) \_\_\_\_\_ Time (Hora) \_\_\_\_\_ Initials (Iniciales) \_\_\_\_\_

Photos taken? (¿Tomaron fotos?)     Yes (Sí)     No (No)

\_\_\_\_\_  
Turtle (Tortuga)

\_\_\_\_\_  
Nest (Nido)

\_\_\_\_\_  
Track (Rastro)

Number of eggs laid (Número de huevos puesto) \_\_\_\_\_

Number of eggs collected (Número de huevos colectados) \_\_\_\_\_

Egg translocation notes (Ficha de sembrado) \_\_\_\_\_

Date (Fecha) \_\_\_\_\_ Time (Hora) \_\_\_\_\_ Nest number (Número de nido) \_\_\_\_\_

Location of nest (Ubicación del nido en el corral) \_\_\_\_\_

| Subnest<br>(Subnido) | Column<br>(Columna) | Row<br>(Hilera) | Number of eggs buried<br>(Huevos sembrados) |
|----------------------|---------------------|-----------------|---|
| _____                | _____               | _____           | _____                                       |
| _____                | _____               | _____           | _____                                       |
| _____                | _____               | _____           | _____                                       |

Eggs damaged? (¿Huevos dañados?)

Eggs damaged during transportation (Huevos dañados en el transporte) \_\_\_\_\_

Eggs damaged while buried (Huevos dañados en el sembrado) \_\_\_\_\_

Observer (Observador) \_\_\_\_\_

Epibionts collected? (¿Epibióticas colectados?)     Yes (Sí)     No (No)

Notes (Notas):