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Standardizing Sea Turtle Epibiont Sampling: Outcomes of the Epibiont Workshop at the 37th International Sea Turtle Symposium

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I. Introduction. A growing interest in the relationship between sea turtles and their epibionts (*i.e.*, marine organisms that attach to, or dwell on the exterior shell or skin of sea turtles) resulted in a workshop focused on sea turtle epibiosis at the 37th International Sea Turtle Symposium, Las Vegas, Nevada (Pinou *et al.* 2017). The primary objectives of the workshop were to present the current state of knowledge on sea turtle epibiosis research to an interdisciplinary community and to collectively develop a standardized epibiont sampling methodology for improved data sharing among scientists. The talking points of the workshop discussion included: 1.) clarifying the utility of published epibiont lists, 2.) articulating a functional understanding of interspecies connections between sea turtles and epibionts, 3.) framing the need for an open-access, global epibiont database, 4.) developing a repository for collected material accessible to all workers in the field, and 5.) employing hypothesis testing in epibiont research, and 6.) envisioning long-term monitoring plans to advance the study of sea turtle epibiosis. Table 1 lists the titles of talks and presenters.

Contact information for 55 participants was gathered at the workshop and is the basis for a new epibiont listserv. Speakers and registered participants were asked to contribute to a list of relevant and frequently cited review articles, referenced herein. A focal topic of the workshop was to standardize the collecting methodology for sea turtle epibionts. Participants were encouraged to submit their comments and recommendations for improving the existing epibiont data collecting sheet (Lazo-Wasem *et al.* 2011). The modified sea turtle epibiont sampling protocol and data collecting sheet included in this workshop review is in response to participant feedback.

Opening the workshop, John Zardus presented an overview of the current state of knowledge in sea turtle epibiont research by introducing underlying theory and defining fundamental terminology (Fig. 1), explaining how epibionts can inform turtle biology, and describing innovative experiments with epibionts. He explained why epibionts are not all alike, and how lumping them as a single category greatly minimizes the information and understanding they can provide. Identifying variations in epibiont colonization strategies, reproductive modes, and life cycles can potentially lend insight to many aspects of sea turtle biology including regional occurrence, habitat use, health, seasonality, behavior, gender-based patterns, and signals of climate change to name a few. Moving beyond valuable observational studies, the age of experimentation with epibionts has arrived. Experiments can

Titles	Speaker
Summary of the current state of the field for sea turtle epibiont research, discussing major findings and critical studies already conducted	John Zardus, Department of Biology, The Citadel, SC, USA
A tutorial on the characters of sea turtle epibionts that can help improve efficacy in the field	Eric Lazo-Wasem, Peabody Museum of Natural History, Yale University, CT, USA
Introduction to marine diatoms and summary of the recent discoveries of sea turtle-associated diatoms	Roksana Majewska, Uniit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa
Curating Museum Specimens	Eric Lazo-Wasem, Peabody Museum of Natural History, Yale University, CT, USA
Analytical considerations and statistical tools to compliment a global epibiont database	Nathan Robinson, Cape Eleuthera Institute, The Bahamas
Epibionts as indicators of sea turtle habitat use and behavior	Joseph Pfaller, Caretta Research Project, Savannah, GA, USA
Insights into rearing sea turtle epibionts in captivity and future directions	John Zardus, Department of Biology, The Citadel, SC, USA & Francesc Domenech, Marine Zoology Department, University of Valencia, Valencia, Spain

Table 1. Titles of sea turtle epibiont talks at 37th International Sea Turtle Symposium, Las Vegas Nevada, and respective presenters.

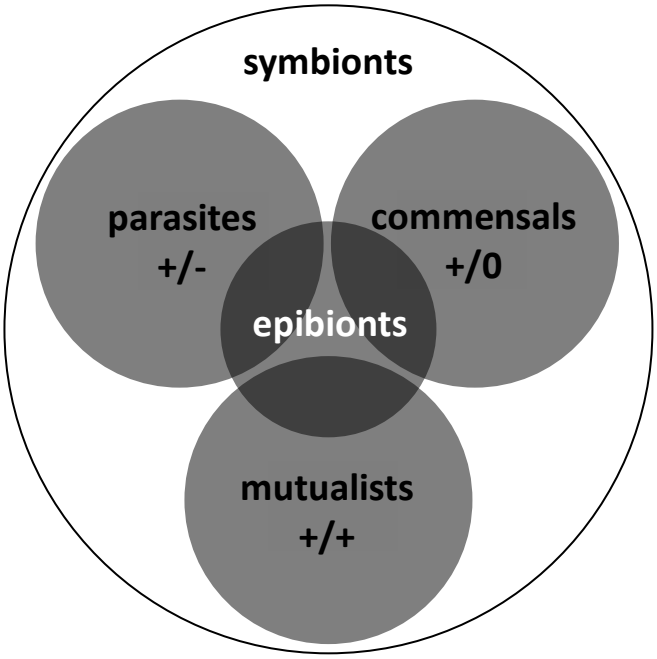

Figure 1. Venn-style diagram delineating the relationship of epibionts (*i.e.*, organisms that live externally in association with a host partner or basibont) to different categories of symbionts. Plus, minus, and zero symbols indicate beneficial, deleterious, and neutral associative outcomes respectively for the symbiont (left of the hyphen) and the host (right of the hyphen). Epibionts can possess any life mode, the operative factor that unifies them is that they live external to the host. All organisms together in an epibiotic association comprise a single communal entity the holobiont, which forms an ecological unit.

be performed in isolated laboratory settings, controlled laboratory settings or in the field. Epibionts reared in the laboratory can be studied using flumes and flow tanks while recruitment panels and grow-out racks in the field can give information on environmental factors. Molecular genetic studies are a potentially powerful tool to explore phylogenetic relationships among epibionts (*e.g.,* Zardus *et al*. 2014; Ewers-Saucedo *et al.* 2017) and biogeographic patterns related to migrations of their hosts (*e.g.,* Pinou *et al.* 2013).

The workshop continued with Eric Lazo-Wasem, who presented a summary of epibiont lists published in a variety of different studies and gave an overview of the more common epibionts and how they are distinguished, focusing in particular on the Atlantic and Pacific epibiont species. A guide to the common epibionts is available at http://peabody.yale.edu/collections/invertebrate-zoology/turtle-epibiont-project. Images or slides may be used freely, but please acknowledge E.A. Lazo-Wasem, Yale Peabody Museum.

Roksana Majewska's presentation introduced participants to epibiotic diatom collection methods and future research directions with newly discovered sea turtle-associated diatoms, and highlighted their potential as indicators of sea turtle health and behavior. Diatoms, unicellular microalgae enclosed within their silica cell walls inhabit every ocean and every continent. Although both diatoms and sea turtles have been known to scientists for centuries, until very recently, the epizoic diatoms growing on sea turtles have been completely unknown. The latest studies showed that diatoms are universally present on all seven sea turtle species often reaching very high abundances (Majewska *et al.* 2015a, 2017a; Robinson *et al*. 2016). She continued by explaining how a diatom biofilm is formed on sea turtles, what its potential negative and positive impacts are to the host animal, and how it could provide insights into sea turtle ecology and their overall well-being. According to recent observations, sea turtle diatom communities are composed of both truly epizoic (*i.e.,* requiring the animal substratum to develop and survive) and possibly sea turtle-specific (*i.e.,* existing only on sea turtles) diatoms as well as opportunistic species that join the biofilm when a sea turtle visits various localities (Frankovich *et al.* 2015, 2016; Majewska *et al.* 2015b, 2017b,c, 2018; Riaux-Gobin *et al.* 2017). This has led to two major conclusions: a) the fate of the unique and largely undiscovered turtle-associated microbes is inevitably linked to the fate of sea turtles and our conservation efforts (Majewska 2018), b) further investigations and routine epizoic diatom collection may constitute a valuable support to the current practices and recommendations for sea turtle epibiont research. As diatom collection is simple and relatively inexpensive, we encourage scientists working with sea turtle monitoring programs to include the collection of diatoms into their sampling routine. The standard protocol for diatom sampling is given in the Revised Collecting Protocol For Marine Turtle Epibionts section IIB.

Eric Lazo-Wasem offered guidelines on preserving and shipping specimens to colleagues and museums, and this methodology is provided in the Revised Collecting Protocol For Marine Turtle Epibionts section IIC and IID. He also initiated a call for data aggregation and best practices in an effort to standardize collection methodology and improve communication among workers in the field. Collaborative monitoring is essential to gleaning a more global understanding of the relationship between sea turtles and their epizoites. A global database increases the scale of analysis and helps make epibionts indicators of sea turtle biology through illustrating geospatial distribution and illuminating causal factors affecting turtle health. In an effort to achieve a common methodology, a sea turtle epibiont collecting protocol and sampling sheet are provided in the Revised Collecting Protocol For Marine Turtle Epibionts sections IIA and IIF.

Following prior discussions on the importance of developing a global database to aggregate worldwide epibiont data, Nathan Robinson continued the conversation by stressing the need to move away from simple 'lists' of epibiont abundance and diversity. There is now a distinct need to develop and begin testing specific hypotheses in relation to sea turtle epibiosis if we hope to keep moving the field forward. Furthermore, the published literature already provides sufficient data to move in this direction. Lastly, he provided a quick demonstration of two freely available tools: PAST and EstimateS that can provide meaningful statistical analyses for epibiont data. PAST is suited to multivariate analyses comparing community structure, while EstimateS provides rarefaction curves that indicate the appropriate sample size needed to inform researchers once sufficient sampling has occurred to appropriately represent the biodiversity of epibionts at a given site.

Joseph Pfaller presented a summary on the development and application of his Conceptual Model of Epibiosis (Frick & Pfaller 2013), a framework for understanding "why we see what we see." He explained that turtles that spend time in different habitats (*e.g.*, neritic vs oceanic) or different regions (*e.g.*, tropical vs temperate)

will develop characteristic epibiont communities. He suggested that because of this, we can use epibionts as indicators of sea turtle habitat use and behavior. He also suggested that we can apply this conceptual framework to develop more hypotheses-driven approaches to epibiont research moving forward. His presentation finished with a discussion of the understudied roles that epibionts play in the lives of sea turtles and that sea turtles play in the lives of their epibionts. This raised discussion about the definition of an epibiont, and whether the term should include parasitic forms. Pfaller explained that parasitic forms should be considered epibionts, but that they occupy a unique position on the cost-benefit continuum such that they evoke a high cost on the host turtle by directly consuming host tissue and potentially acting as intermediate disease vectors. Some epizoic organisms may serve as intermediate hosts to other associates of sea turtles, for example *Ozobranchus* leeches and the sea turtle fibropapilloma virus, and the ectoparasitic copepod *Balaenophilus manatorum* that has gained recent scrutiny and subsequent support as an intermediate host (Lazo-Wasem *et al.* 2007; Crespo-Picazo *et al.* 2017), overall cautioning about ignoring connections among partners and expanding our understanding of external sea turtle epizoites, internal endozoites, and endoparasites.

The workshop closed with a discussion of papers examining the rearing of epibionts, experimental studies, and transmission models that can be used to expand understanding through laboratory studies controlling for environmental variables (Zardus *et al.* 2008; Alfaro-Nunez *et al.* 2014, 2017; Domenech *et al.* 2017). John Zardus and Francesc Domenech shared their experiences and challenges with developing and implementing such laboratory studies and stressed the importance of creating the appropriate environmental conditions for rearing epibionts. A discussion of this work lead to the ethical considerations associated with testing transmission and settlement models on live sea turtles.

Closing comments by participants that require further discussion at future meetings led to the question of whether epibionts should be removed at all? Many workshop attendees were interested in knowing if the removal of epibionts negatively impacted the turtle, and if removal of some embedded obligate barnacles causes sores and/or blistering? Given the lack of studies in this area, such questions could not be universally answered. However, barnacles that embed deeply in the skin (*e.g., Stephanolepas muricata*) and leave bleeding open wounds upon removal (Flint *et al.* 2009) are probably best left in place. Certainly those that penetrate through shell (*e.g.*, *Chelolepas cheloniae*), should not be removed. Shell blistering may be a consequence of the natural resolution of barnacle wounding, either through loss of barnacles or their overgrowth by turtle tissue.

The workshop culminated with participant feedback on the current epibiont data collecting sheet proposed by Lazo-Wasem *et al.* (2011) which covers sea turtle monitoring data but only a small section dedicated to epibiont sampling. Participants overwhelmingly called for a revised data sheet, dedicated to sampling sea turtle epibionts, and that includes more data on environmental variables such as ocean (*i.e.*, water temperature) and geographic setting (*i.e.*, cove, open ocean). They also asked for the development of a sampling method that will account for underestimates and imperfect epibiont identification. We address these comments in the following way. First, improving imperfect identification of epibionts can only be accomplished by saving vouchers, or entire collections deposited in museums for further examination and species validation. Lazo-Wasem *et al.* (2011) is a valuable field guide for sea turtle epibiont identification, and another regional field guide will soon be available (Lazo-Wasem *et al.* in-prep). Second, sampling underestimates are addressed in the Epibiont Sampling Sheet (Appendix I) with a query field asking if and where epibionts were observed, followed by a table for recording number of sampling tubes collected by turtle body region, and a column for indicating if this body region sampling is complete. To simplify sampling efforts in the field, the sampling sheet is double sided, with the first page dedicated to the sea turtle host and epibiont collection, and the second side devoted to field information that can be added later. We include a version of the sampling sheet in English and Spanish to facilitate international collaboration, and invite contributions for additional languages to be sent to Eric Lazo-Wasem who will add these to the Yale-Peabody Museum of Natural History Turtle Epibiont Project website (http://peabody.yale.edu/collections/invertebrate-zoology/turtle-epibiont-project).

II. Revised Collecting Protocol for Sea Turtle Epibionts. The data collection sheet provided here is revised from Lazo-Wasem *et al.* (2011) in response to feedback from the 2017 sea turtle epibiont workshop (Pinou *et al.* 2017). Here we present a data sampling sheet that adds query fields associated with environmental conditions pertinent to sea turtle epibionts and eliminated fields associated with nest monitoring. The data sampling sheet addresses underestimation of sampling diversity by standardizing collecting effort. In this collecting protocol we include an observation period to acknowledge the presence or absence of epibionts on eight regions of the sea turtle body landscapes (*i.e.*, head, neck, forelimbs, shoulders, hindlimbs, cloacal area, carapace, and plastron). When permitted, we encourage the use of photography to document positioning and orientation of epibionts. We limit opportunistic field sampling and ask that collectors indicate the number of tubes collected per body region, and report whether sampling was complete (exhaustive) or not. We expect high diversity of epibionts from turtles with high epibiont loads, with many sample tubes collected accordingly, whereas diversity and tube numbers will likely be low in sea turtles with few epibionts or from individuals that are incompletely sampled. We also provide a method for collecting diatoms for future study.

The revised sampling sheet includes an English and Spanish version (Appendix I). It calls for a team-based field approach decoupling data collection of sea turtle monitoring programs and epibiont sampling so that sea turtle and epibiont data sheets are separate. This epibiont data sampling sheet is general enough to be used for any sea turtle species and by any epibiont research program interested in sea turtle epibiont diversity. The "collector name" and "sampling number" spaces in the header permit field projects to be organized by a unique identifying event. The epibiont sampling sheet includes a schematic diagram (dorsal and ventral) of a generic marine turtle to record where epibionts were observed. The collector uses the table to indicate whether or not these areas were sampled completely and standardized abbreviations to designate body regions are used. An editable version of the epibiont sampling sheet, is available in English and Spanish for download at http://peabody.yale.edu/collections/invertebrate-zoology/turtle-epibiont-project.

II A. Sampling Epibionts from Sea Turtles. Preserve specimens collected from different regions of the turtles separately according to guidelines on the sampling sheet. Prepare sampling tubes prior

to going into the field, and make sure to label collecting tubes and provide an internal label of durable paper according to the turtle ID and the following body landscape regions:

Head (H) & Neck (N)
Forelimbs (FOR) & Shoulders (SHD)
Hindlimbs (HIN) & Cloacal Region (CLO)
Carapace (CAR)
Plastron (PLS)

Upon encountering a turtle begin by first evaluating where epibionts are present and then noting these locations on the schematic diagram of the dorsal and ventral views. There is a space on the sheet to indicate special notes. Place epibiont specimens in labeled containers according to the turtle ID number and body landscape region. Use the table on the epibiont sampling sheet to indicate the number of tubes collected for each body region, and in the appropriate column indicate if all epibionts of each body region were collected (C) or if the collecting was incomplete (IC). Limit epibiont samples to no more than 66% of the container volume. Immediately fill containers to the top with 75% alcohol, and seal lids. The internal label should be written in pencil because alcohol can smear ink unless an archival pen is used. Invert the container a few times to disperse the alcohol throughout the epibiont samples. *Note*: An alcohol concentration of 75% or higher is used because the target, after adding wet (water retaining) specimens is 70% alcohol or more.

If DNA is to be extracted from the samples, the alcohol used to fix the specimens must be consumable grade ethyl-alcohol. Do not use alcohol labelled as "laboratory grade" as it will contain chemicals added specifically to make the fluid non-consumable; DNA will not be properly preserved with methyl ("denatured") alcohol or isopropyl ("rubbing") alcohol. In some regions it is possible to find grain alcohol, in concentrations of 150 or 190 proof, at retail liquor stores. Although this can be expensive, it ensures the proper alcohol type for preserving samples destined for molecular work.

It is crucial to mark the date on the sampling sheets in the format "dd month yyyy" (for example, 05 Nov 2007). This assures that there can be no confusion as to the precise date. This date along with a unique turtle number (specific for a particular year) and body landscape region will serve to uniquely identify the collecting event. Although informally the season can be referred to, it must *always be clear what day, month, and year is sampled*.

II B. Sampling diatoms from nesting sea turtles

Diatoms for culturing (live samples)

Materials:

wide mouth plastic tubes with caps, *e.g.*, 50 ml Falcon tubes (recommended), two per sea turtle
toothbrushes (hard), two per sea turtle
filtered seawater (filter pore diameter ≤ 0.22 µm), *ca.* 25 ml per sample
parafilm (sealing material)
waterproof marker for labeling

Procedure:

Separate (clean, single-use) toothbrushes must be used to collect samples from different sea turtle individuals and their different body parts (carapace and skin). Diatom biofilm should be collected by vigorously brushing/scrubbing the sea turtle carapace/skin (neck and at least one flipper) with a hard toothbrush. Each time the toothbrush should be rinsed with the prepared filtered seawater by placing the toothbrush head inside the tube and shaking it energetically. Brushing and rinsing should be repeated several times. The carapace sample should turn greenish or brownish, with visible pellets of biofilm gathering at the bottom. The skin sample may turn either greenish-brown or grey, with visible pellets at the bottom. Tubes should be closed, sealed, and labelled appropriately. They can be kept in the refrigerator (or other cool and dark place) for up to 7 days before the isolation and transfer to the culture medium.

Diatoms for standard diatom analysis (morphological and molecular examination)

Materials:

wide mouth plastic tubes with caps, *e.g.*, 50 ml Falcon tubes (recommended), two per sea turtle
toothbrushes (hard), two per sea turtle
ethanol (at least 70%), *ca.* 25 ml per sample
parafilm (sealing material)
ethanol-proof marker for labeling

Procedure:

Separate (clean, single-use) toothbrushes must be used to collect samples from different sea turtle individuals and their different body parts (carapace and skin). Diatom biofilm should be collected by vigorously brushing/scrubbing the sea turtle carapace/skin (neck and at least one flipper) with a hard toothbrush. Each time the toothbrush should be rinsed with the prepared ethanol by placing the toothbrush head inside the tube and shaking it energetically. Brushing and rinsing should be repeated several times. The carapace sample should turn greenish or brownish, with visible pellets of biofilm gathering at the bottom. The skin sample may turn either greenish-brown or grey, with visible pellets at the bottom. Tubes should be closed, sealed, and labelled appropriately.

II C. Labeling Specimens for Research and Museum Curation.

Sort each specimen tube by grouping sampled landscape regions by host turtle. Multiple tubes should be numbered consecutively for each body landscape of a turtle. At this point epibionts can be stored until the researcher is ready to sort and identify epibionts in each container for a specific turtle sampled. When a researcher is ready, select a container (linked to a body region for a specific turtle), and sort organisms by grouping and counting identical taxa. At this point each group of taxa will require a new container. Write the necessary sample identification in pencil or alcohol-resistant ink on wet-strength paper bearing the necessary sample identification and place INSIDE the container. Wet-strength label stock can be obtained from University Products Inc, marketed under the proprietary name "Resistall." Similar products or high-quality paper, *i.e.*, 100% cotton fiber paper "executive stationary" can be substituted. Standard paper stock (*e.g.*, standard printer paper) 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 will inevitably become smudged and illegible, and should be used only for as an aid for organizing samples, and never as the primary label technique.

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) and host number of when a sample was taken as a

prefix, as follows: 2018-1 through 2018-XXX. The combination of a year and turtle number permits any number to be re-used without confusion. Include the abbreviation for the body landscape region 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)
Hindlimbs (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.

II D. 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.

Two options exist for transporting specimens. Regardless of method used, it is important to have copies available of any necessary collecting and export permits; the former allows the collecting to take place, and the latter allows transport from the originating country to another country. If shipping services are available (air cargo) specimens can be packed for shipping, but strict rules apply regarding the shipment of hazardous materials. Several years ago, regulating authorities relaxed the rules about hazardous shipping, to allow small amounts of hazardous material to shipped, providing specific rules are adhered to. The general rules state that if no container has more than 30 ml of free alcohol, and no single box contains more than 500 ml, the material can be considered non-hazardous. For full details consult a number of detailed accounts by searching for the rules spelled out under the title "A180 Special Provision." Note that most commercial shippers are aware of the various regulations and can provide assistance in this regard.

The second option involves changing the fluid from a hazardous material to one that is not, thereby making the material safe for either shipping or hand-carrying in personal baggage. General procedures are outlined below:

Epibiont samples can be transported in carry-on luggage by adhering to the following guidelines. Samples to be transported via 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 off 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 in hardware or plumbing supply stores) around the threads and securely tighten the lid; if possible use lids that have some sort of liner, so that as the lid is tightened compression against the liner will make a positive seal. Seal the outside of the lid by wrapping it with white or black electrical tape pulled tight on itself and cut cleanly. An alternative is to use Parafilm® (http://www.bemis.com/na/products/parafilm-floratape/parafilm-lab) which is widely available at science supply companies. These steps will insure minimal leakage.

If required, the ethanol-preserved diatom samples can be centrifuged to pellet the collected microalgae and supernatant (ethanol) can be removed. The diatom material can then be transported as air-dried pellets.

A proven, effective shipping fluid is Carosafe, a proprietary fluid marketed by U.S. based Carolina Biological Supply Company, Burlington, North Carolina, USA (www.carolina.com). Carosafe is a solution of propylene glycol, a food additive and stabilizer that has proven safe for shipping specimens that may ultimately be the subject of molecular work. The product comes concentrated, and for use it is diluted (9 parts water, 1 part Carosafe Concentrate). Propylene glycol is not classified as a hazardous material, and therefore can be used without fear of the material being confiscated. It is imperative, however, to have a copy of the proprietary information Material Safety Data Sheet (MSDS) in the box with the specimens, and note to TSA inspectors that reads as follows:

To: TSA Inspectors
These preserved scientific specimens are being shipped in Carosafe liquid, a non-hazardous material as defined by current IATA regulations and thereby is allowable as a shipping solution. Its primary ingredient is Propylene Glycol, a U.S. 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

II E. Specimen Deposition and U.S. Reporting (Importation). If entering the U.S. with specimens, one must strictly adhere to the wording on the Customs Declaration regarding the transport of "animals." Although the intent is to prevent the import of agricultural pests or pathogenic organisms, the wording nevertheless simply refers to "animals." As such, the correct response is to acknowledge that specimens are being transported. This may be deemed unimportant once an explanation is given as to the nature of the animals in question, or it may prompt a required visit to US Department of Agriculture inspectors who will question you regarding the material. As none of the specimens in transit are protected by CITES (Convention on International Trade of Endangered Species), there should be no requirement to have the contents inspected by an officer of the U.S. Fish and Wildlife Service (USFWS).

A USFWS 3-177 Import/Export Permit must be filed with the USFWS regional office that reflects the point of entry (*e.g.*, New York, Miami, etc.); although paper copies are accepted, the USFWS strongly advises scientists to use the Electronic Declarations (eDEC) available at the USFWS website (https://edecs.fws.gov/).

This filing, although mandatory, does not need to take place during import or export; however, wildlife inspectors vary in their acceptance and application of the 180-day reporting period. Much aggravation can be reduced by filing immediately upon bringing specimens into the country or upon receipt of shipped specimens. 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 U.S. Code of Federal Regulations (Title 50, Part 14, "Importation, Exportation, and Transportation

of Wildlife" [50 CFR 14.64(b)(3)]) that describes the relevant reporting rules.

Be aware that Transportation Security Agency (or similar) inspectors, particularly outside the U.S., may not possess zoological training, and small round objects (barnacles) sealed in bags may superficially represent pharmaceutical products and narcotics. It is best to bring a copy of a well-illustrated article depicting epibionts to show to inspectors if you are questioned about the contents of your samples.

As soon as possible after arrival at the receiving institution the shipping fluid must be decanted from the sample jar through a 0.5mm mesh standard screen (to catch tiny epibionts) and the screen rinsed with 75% non-denatured alcohol. Return rinsed specimens to the container and add sufficient alcohol to ensure the sample container is filled completely to dilute any residual shipping fluid.

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The following list of references include citations from the above text plus other relevant work for readers interested in the subject

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APPENDICES

Two page "Epibiont Sampling Sheets" in English and in Spanish (translated by Eliamar Gonzalez - gonzalez137@connect.wcsu.edu).

COLLECTOR _____ *SAMPLING NUMBER* _____

Epibiont Sampling Sheet (ENGLISH)

HOST INFORMATION (injury information on back)

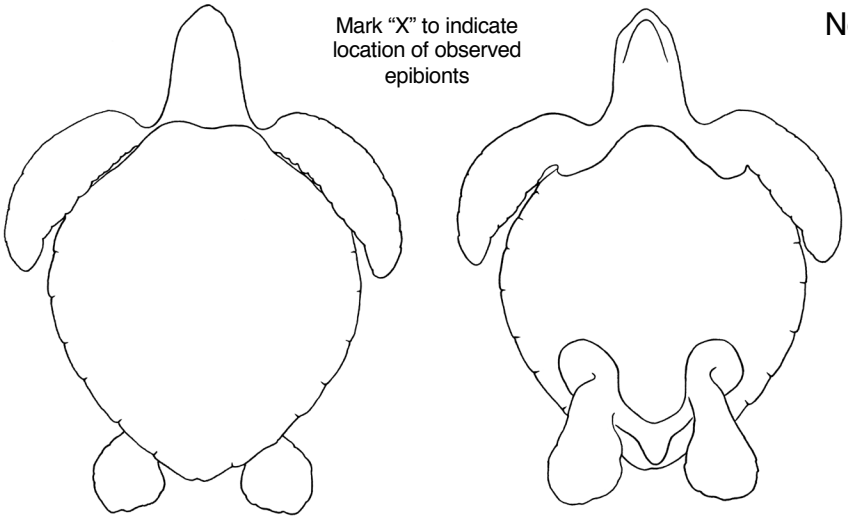
Turtle species: _____ ☐ Positive ☐ Probable ☐ Unsure

Morphometrics: SCL _____ SCW _____ CCL _____ CCW _____

Tags: ☐ Present ☐ Absent LFF _____ RFF _____ LRF _____ RRF _____

State of turtle: ☐ Free-roaming ☐ Nesting ☐ Stranded [☐ Alive ☐ Dead ☐ Cold-stunned]

EPIBIONT INFORMATION Epibionts observed: ☐ Present ☐ Absent



Notes:

Epibionts collected: ☐ All ☐ Some ☐ None Diatoms collected: ☐ Yes ☐ No

BODY REGION	COLLECTED?	COMPLETE?	NUMBER OF CONTAINERS
Head (H)	☐ Yes ☐ No	☐ Yes ☐ No	
Neck (N)	☐ Yes ☐ No	☐ Yes ☐ No	
Shoulders (SH)	☐ Yes ☐ No	☐ Yes ☐ No	
Front flippers (FF)	☐ Yes ☐ No	☐ Yes ☐ No	
Rear flippers (RF)	☐ Yes ☐ No	☐ Yes ☐ No	
Inguinal (ING)	☐ Yes ☐ No	☐ Yes ☐ No	
Carapace (C)	☐ Yes ☐ No	☐ Yes ☐ No	
Plastron (P)	☐ Yes ☐ No	☐ Yes ☐ No	

COLLECTOR _____ *SAMPLING NUMBER* _____

FIELD INFORMATION DATE _____ TIME _____

Latitude / Longitude: _____ / _____

Site/Beach _____ City/Town _____ Country _____

Habitat: ☐ Beach [☐ Nesting ☐ Stranded] ☐ Inwater [☐ Nearshore ☐ Open Ocean]

Description _____

Capture method: ☐ Beach patrol ☐ Hand-capture ☐ Fishing gear [☐ Net ☐ Hook/line]

Description _____

Estimated time between turtle capture and epibiont survey/collection: _____

Temperature: Water _____ Air _____ Sand _____

Rainfall: Date of most recent rainfall (>5cm) _____ Amount _____

INJURY INFORMATION Injuries: ☐ Present ☐ Absent

Body part: ☐ H ☐ N ☐ SH ☐ LFF ☐ RFF ☐ LRF ☐ RRF ☐ ING ☐ C ☐ P

Description _____

Missing appendages: ☐ Yes ☐ No Description _____

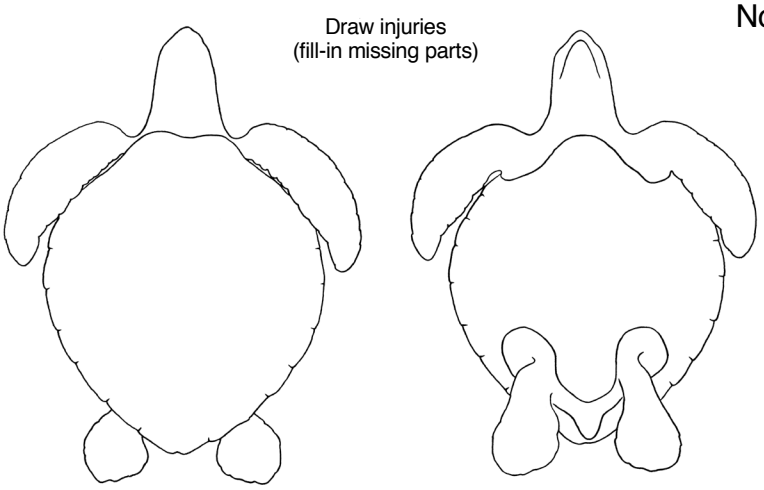
Lesions: ☐ Yes ☐ No Description _____

Tumors: ☐ Yes ☐ No Description _____

Hooks or fishing line: ☐ Yes ☐ No Description _____

Cracked carapace or plastron: ☐ Yes ☐ No Description _____

Draw injuries
(fill-in missing parts)



Notes:

INVESTIGADOR _____ NÚMERO *DE MUESTREO* _____

Hoja de Muestras de Epibiontes (ESPAÑOL)

INFORMACIÓN DEL HOSPEDADOR (información de lesiones en la parte trasera)

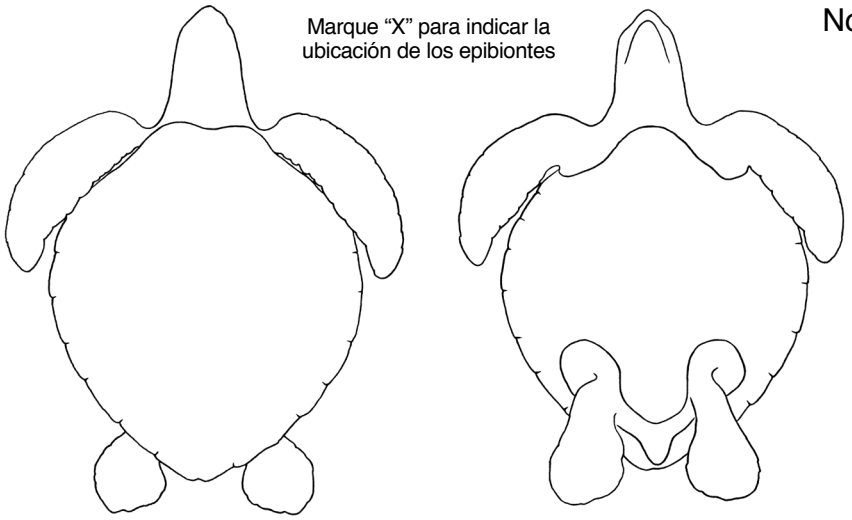
Especie de tortuga:_____ ☐ Positivo ☐ Probable ☐ Inseguro

Morfometria: SCL _____ SCW _____ CCL _____ CCW _____

Marcas: ☐ Presente ☐ Ausente LFF_____ RFF_____ LRF_____ RRF_____

Estado de la tortuga: ☐ Libre ☐ Anidando ☐ Varada [☐ Viva ☐ Muerta ☐ Aturdida por frío]

INFORMACIÓN DE LOS EPIBIONTES Epibiontes observados: ☐ Presente ☐ Ausente



Notas:

Epibiontes colectados: ☐ Todos ☐ Algunos ☐ Ninguno Diatomeas colectadas: ☐ Sí ☐ No

REGIÓN DEL CUERPO	¿COLECTADOS?	¿COMPLETOS?	NÚMERO DE CONTENEDORES
Cabeza (H)	☐ Sí ☐ No	☐ Sí ☐ No	
Cuello (N)	☐ Sí ☐ No	☐ Sí ☐ No	
Hombros (SH)	☐ Sí ☐ No	☐ Sí ☐ No	
Aletas anteriores (FF)	☐ Sí ☐ No	☐ Sí ☐ No	
Aletas posteriores (RF)	☐ Sí ☐ No	☐ Sí ☐ No	
Inguinal (ING)	☐ Sí ☐ No	☐ Sí ☐ No	
Caparazón (C)	☐ Sí ☐ No	☐ Sí ☐ No	
Plastrón (P)	☐ Sí ☐ No	☐ Sí ☐ No	

INVESTIGADOR _____ NÚMERO *DE MUESTREO* _____

INFORMACIÓN DE CAMPA FECHA _____ HORA _____

Latitud / Longitud: _____ / _____

Sitio/Playa _____ Ciudad/Pueblo _____ País _____

Habitat: ☐ En playa [☐ Anidando ☐ Varada] ☐ En agua [☐ Costa ☐ Océano abierto]

Descripción _____

Método de captura: ☐ Patrulla en playa ☐ Captura manual ☐ Equipo de pesca

Descripción _____ [☐ Red ☐ Gancho/Línea]

Tiempo estimado entre la captura de al tortuga y la recolección de los epibiontes: _____

Temperatura: Agua _____ Aire _____ Arena _____

Precipitación: Fecha de la precipitación más reciente (>5cm) _____ Cantidad _____

INFORMACIÓN DE LESIONES Lesiones: ☐ Presente ☐ Ausente

Región del cuerpo: ☐ H ☐ N ☐ SH ☐ LFF ☐ RFF ☐ LRF ☐ RRF ☐ ING ☐ C ☐ P

Descripción _____

Amputación: ☐ Sí ☐ No Descripción _____

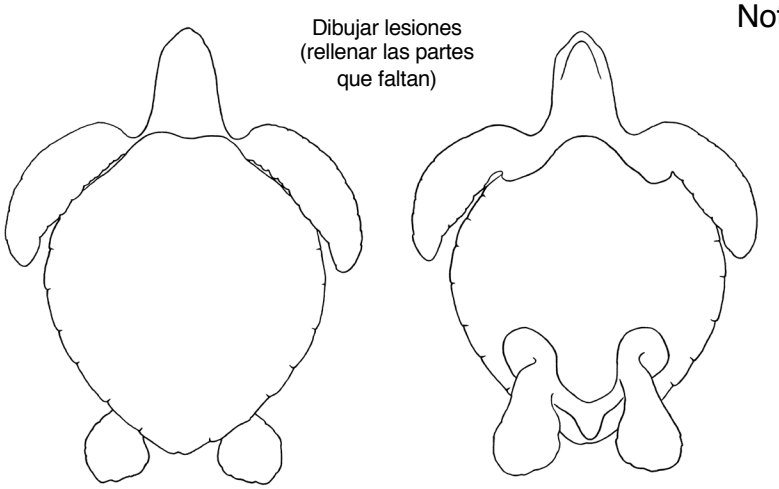
Heridas: ☐ Sí ☐ No Descripción _____

Tumores: ☐ Sí ☐ No Descripción _____

Ganchos o línea de pesca: ☐ Sí ☐ No Descripción _____

Caparazón o plastrón agrietado: ☐ Sí ☐ No Descripción _____

Dibujar lesiones (rellenar las partes que faltan)



Notas:

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