Evaluation of Microplastic Loading in Diamondback Terrapin and their Associated Habitats: Sediment Pilot Study

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Introduction

- Microplastics are a globally emerging contaminant of concern.
- Low-lying coastal marshes serve as sinks and filters for terrestrial waste, putting species using these habitats at risk.
- The Diamondback Terrapin (*Malaclemys terrapin*) is the only endemic estuarine-dwelling turtle in North America and is potentially at risk for microplastic exposure or ingestion.
- An extensive literature search was conducted (Tables 1 and 2) to develop a study design, with the goal of evaluating marsh sediments in estuarine habitats.
- As part of a pilot study conducted in March of 2024, sediment samples were collected and are currently undergoing processing using the following study design.
- Results of this pilot study will refine final sampling and processing protocols implemented in a larger-scale study.

Table 1 Sources for field sample collection study design.

Source	Habitat Type	Core Diameter	Number of Cores	Sample Depth
Alvarez-Zeferino et al. 2020	Beach shoreline	19-cm	10 per site	5-cm
Jenkins and Branco 2020	Salt marsh	9-cm	3 per plot	1-cm
Khan and Prezant 2018	Salt marsh	7.62-cm	3 per plot	10-cm
Klein et al. 2015	Riverine shoreline	Unknown	30-40 per site	2- to 3-cm
Lloret et al. 2021	Estuarine salt marsh	9-cm	3 to 4 per site	2-cm
Pinheiro et al. 2022	Mud flat to high marsh	4.7-cm	6 per zone	5-cm

Table 2 Sources for sediment processing protocols. Asterisks in Organic Digestion column indicate use of Fenton's reagent.

Source	Habitat Type	Sieve Range	Organic Digestion?
Alvarez-Zeferino et al. 2020	Beach shoreline	1.13 to 5-mm	Yes
Beckwith and Fuentes 2018	Beach shoreline	63 to 125-µm	No
Hidalgo-Ruz et al. 2012	Beach shoreline	38-µm to 4.75-mm	No
Khan and Prezant 2018	Salt marsh	250-µm to 4-mm	No
Klein et al. 2015	Riverine shoreline	63 to 630-µm	Yes
Lloret et al. 2021	Estuarine salt marsh	250-µm to 5-mm	Yes*
Lo et al. 2018	Sandy beaches to mud flats (1:1)	250-µm to 5-mm	Yes*
Willis et al. 2017	Riverine estuary	63-µm to 4-mm	Yes*
Zhou et al. 2020	Sandy to muddy	5 to 50-µm	No

Methods

Pilot Study Field Methods

- Metal and aluminum were used whenever possible to reduce potential for plastic or microplastic contamination; samplers wore brightly colored apparel so fibers could be easily identified and removed during laboratory processing.
- Three sites were selected to assess variation in potential point source microplastic loading, with decreasing proximity to point sources from east to west (Figure 1).
- Two plot types: shoreline and random (Figure 2)
  - The initial shoreline plot located at a randomly determined distance from the vessel and subsequent equidistant-spaced plots were used (3 total shoreline plots per site).
  - The random plot locations were determined by walking 5-10 minutes into the marsh (3 total random plots per site).
- Three 5-cm deep cores were collected from each plot and stored in aluminum tins for transportation back to lab.

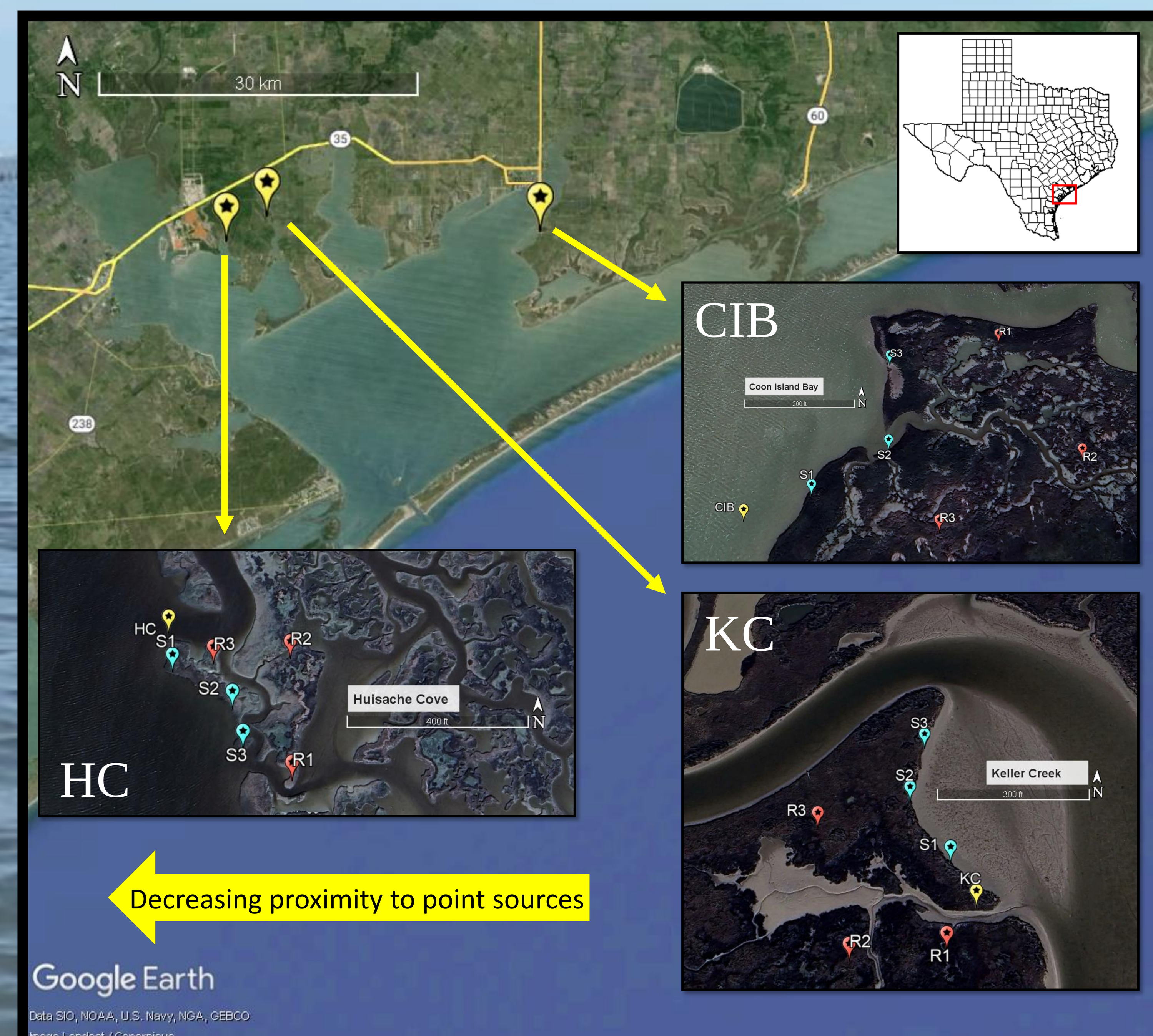

Figure 1 Pilot study field survey area.

Figure 2 Shoreline plot and sediment core (inset) distribution.

Laboratory Sample Processing Methods

- Methods adapted from Lloret et al. (2021; Figure 3).
- Samples were initially dried under a fume then placed in an oven at < 60°C. Drying times and weights were recorded.
- Samples will undergo an initial sieving process through 5-mm, 250-µm, and 100-µm sized sieves, then dried again.
- Density separation with 40-ppt NaCl solution will be used to remove denser materials followed by another drying phase.
- Organic digestion using Fenton's reagent (Iron (II) and hydrogen peroxide) will be used to remove organic material.
- A final dry weight will be used to calculate the total percentage of the sample that is comprised of microplastics.

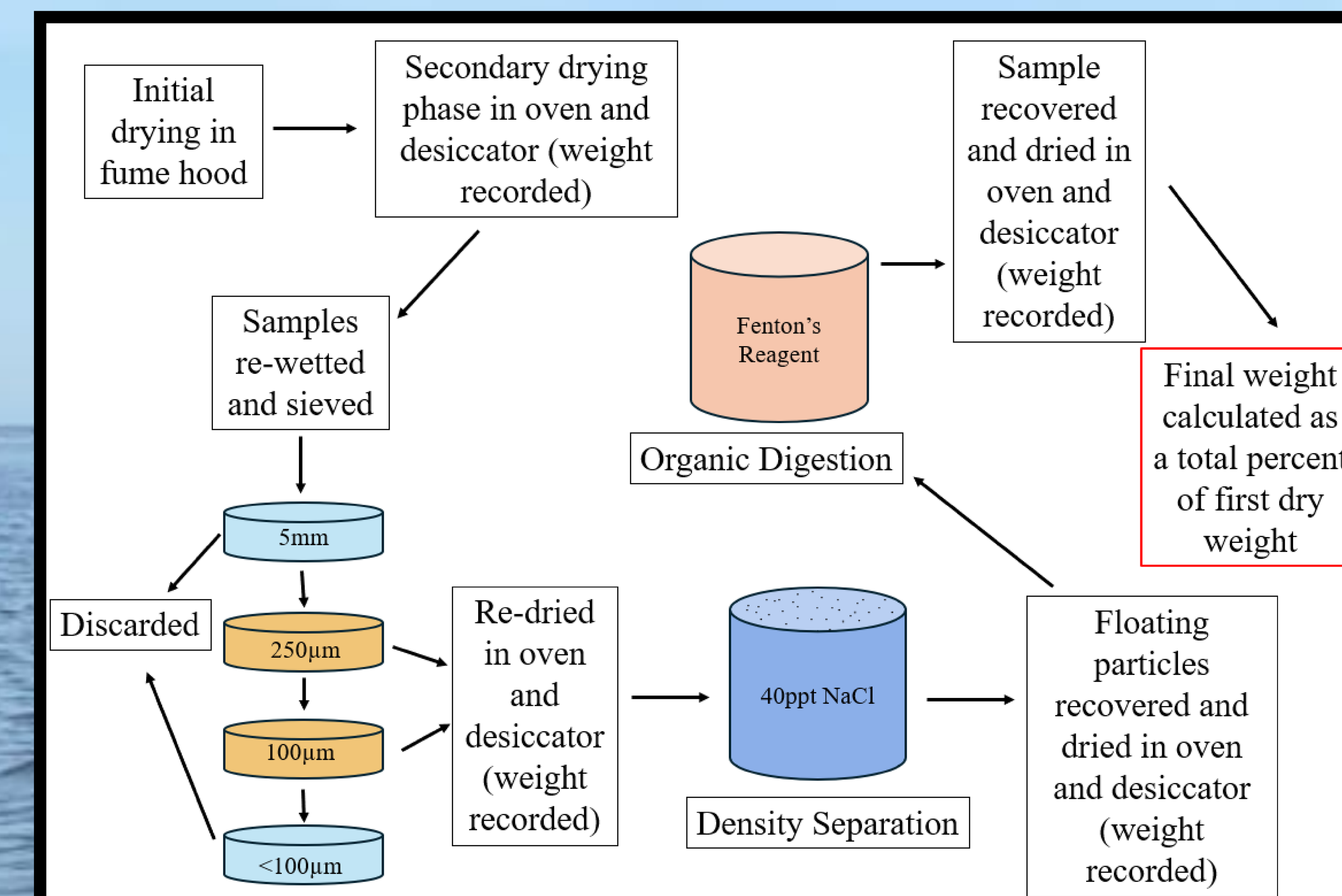

Figure 3 Flowchart of Laboratory Methods

Future Plans

- Once protocols are refined, additional samples will be collected at new sites in Matagorda and San Antonio Bay as part of a larger study.
- Resulting data will be used to quantify and visualize the gradient of microplastic sediment loading across the bay(s).

Acknowledgements

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- Sampling was conducted under permissions outlined in IACUC protocol #0224.001.R0 and TPWD Scientific Permit for Research #SPR-0504-383

MATAGORDA BAY MITIGATION TRUST

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