Foraging on sea grass by Florida manatees (*Trichechus manatus latirostris*) poses a potential threat for microplastic ingestion

Ana Noel

UAA – KPC – KBC

Behavioral Ecology of Marine Mammals Research Proposal

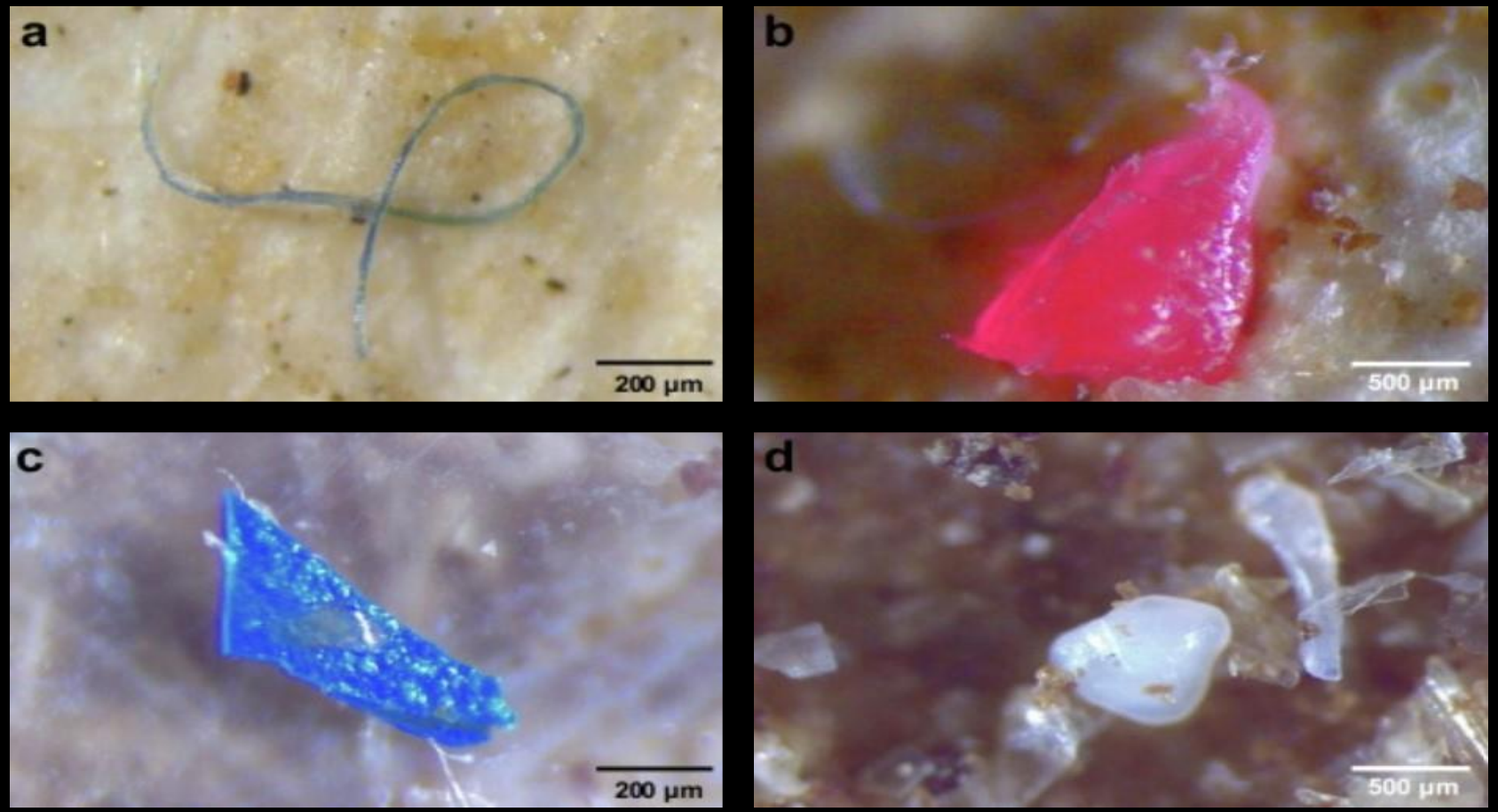
Introduction

- Subspecies of West Indian manatee (*Trichechus manatus*)
- 2004 endangered species
- 1983: Florida state protection
- Boat strikes
- 1978: Florida Manatee Sanctuary Act
- Florida manatee refuge
- Impact of Macroplastics (Beack and Barros, 1991)
  - Ingestion: blockages
  - Entanglement


© David Schrichte

© Save the manatee club

(King and Heinen, 2004)



(Barboza et al., 2020)

(Lehner, 2015)

Microplastics

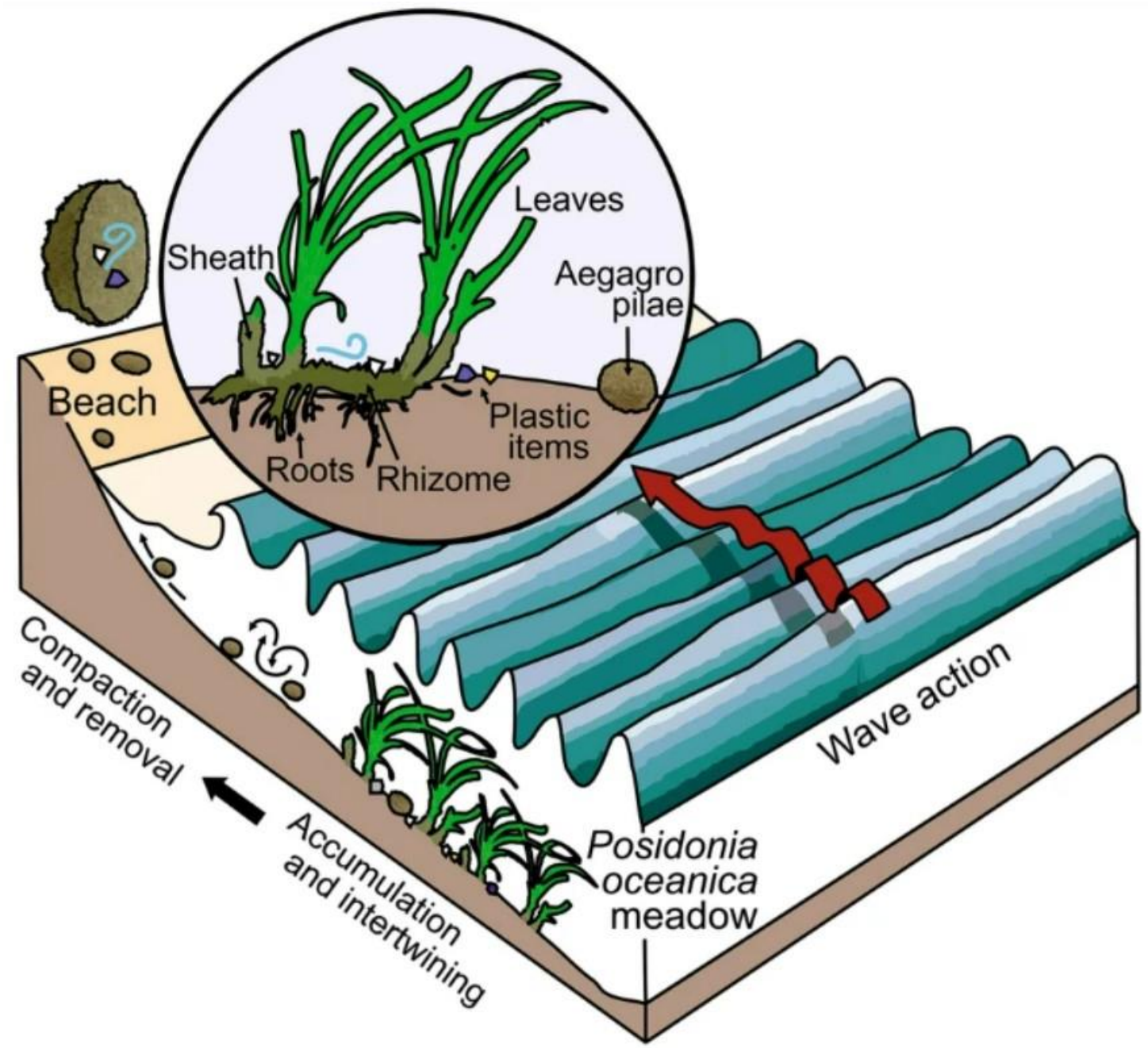
< 5 mm

Macroplastics → microplastis

(Chatterjee and Sharma, 2019)

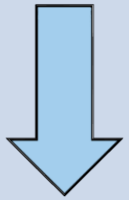
Microplastics cont.

- Effects unknown (Zantis et al., 2021)
- Absorb toxins (Fossi et al., 2012)
  - Halogens
  - Heavy metals
  - Organic pollutants
- Manatees feed on seagrass (Hartman, 1971)
  - Ingestion of small fish and invertebrates
- 1 kg = 1,470 microplastic pieces (Whitaker and Hamilton, 2021)
- Manatees eat 45 kg = 16,150 microplastic pieces (Whitaker and Hamilton, 2021)

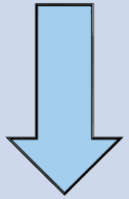


(Sanchez-Vidal et al., 2021)

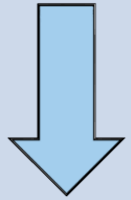
Potential negative effects of consuming microplastics



Reduced reproduction



Reduced growth



Reduced offspring viability

(Galloway and Lewis, 2016)

Industrial Waste
House Hold Waste

Plastic Waste

- Polyvinyl chloride
- Polyethylene terephthalate
- Polystyrene
- Nylon

Fragmentation

- UV Light
- Low Temperature

Primary Microplastics

- Personal Care Products
- Laundering of synthetic clothes

Carrier of the pollutants

Secondary Microplastics

- Plastic bags
- Bottles

Toxic Components

- Dyes
- Heavy Metals
- Chemicals
- Organic Pollutants

Attached to

Health Risks

- Retardation in Growth
- Infertility
- Blockage of body tracts

Adverse effect to aquatic life

Aquatic Animals

Food Chain

Health Risks

- Cancer
- Oxidative Stress
- Impaired Immune Response

(Gola et al., 2021)

Aims of study

Determine if microplastics are present in Florida manatees

Determine what toxins/concentrations are affecting Florida manatees

Understand how microplastics are affecting Florida manatees

Conservation of Florida manatees

First detailed review of microplastics in marine mammals, showing that microplastics are present in most organisms

Sample collection methods:

Digestive tracts from dead animals

Scats from live animals

However, significant methodological variance among studies: standardization is required

Microplastic ingestion pathways:

Direct: Intake of seawater

Indirect: trophic transfer from prey

(Zantis et al., 2021)

Objectives

Examine	the GI tract content of deceased Florida manatees to determine the amount of microplastics in the stomach and intestines
Examine	the blood and tissues of deceased Florida manatees to determine the amount of microplastics in these tissues
Analyze	the blood and tissues of Florida manatees to determine if there is evidence of toxins such as halogens, heavy metals, and organic pollutants
Examine	Florida manatee scat, if available, for the presence of microplastics
Collect	microplastics found on seagrass to determine what contaminants are present
Compare	microplastics from manatees to contaminants found in microplastics from seagrass beds to determine if Florida manatees are ingesting microplastics while foraging on seagrass

Methods- Sample Collection

A permit will be obtained to work the Florida manatees and samples

Tissue collection: A collaboration with Florida Fish and Wildlife will be established to obtain the contents of the stomach, intestines, tissues, and blood from necropsied Florida manatees (Florida Fish and Wildlife Conservation Commission, n.d.). The blood samples will be refrigerated at 4 ℃ and the other samples will be placed in 70% ethanol after collection (Dierauf, 1994; Hernandez-Gonzalez et al., 2018).

Scat collection: Manatee scat will be collected near the surface with a 150 µm mesh net immediately after defecation. 100% ethanol will be used to preserve samples at -20 ℃ (Carol et al., 2019)

Seagrass leaves collection: Loose leaves will be collected when washed ashore. In the lab, the leaves will be dried out for several days in low humidity conditions at 25 ℃

Timeline: Beach walks will be performed once every 2 weeks for 3 years in order to find deceased manatees that have washed up on the beach. Scat and seagrass samples will be collected every month for 3 years.

(Sanchez-Vidal et al., 2021)

Soils Mechanics Industries

Methods: concentrations of microplastics

- 3 sperate metal sieves of 4 different sizes
  - 5 mm metal sieve
  - 1 mm sieve
  - .5 mm sieve
  - .355 mm sieve
  - Prevents clogging
  - Removes any organic matter and debris other than plastic
  - Large materials left in the sieve will be removed

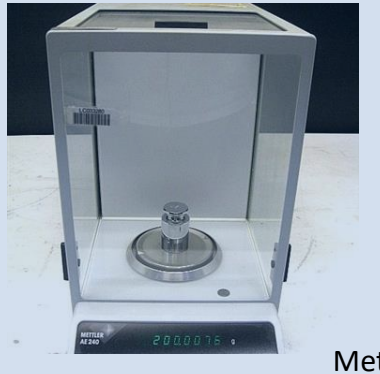
(Hernandez-Gonzalez et al., 2018)

Methods: concentrations of microplastics

Millipore Sigma

Leica Microsystems

World Didac



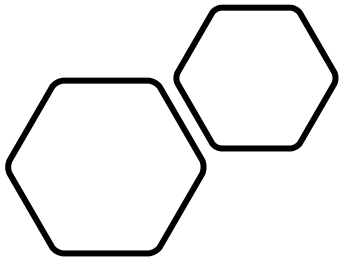
Mettler Toledo

- If microplastics present
  - Half sent to Marine Mammal Pathobiology Laboratory
  - Half placed in sealed, sterilized glass jar with 10% potassium hydroxide
  - 3 weeks: organic solution is dissolved
  - Buchner Filter and glass microfiber filters will be used to filter the samples with a vacuum pump.
  - Samples dried in Petri dish in oven to dry at 50 ℃ for 4 hours.
  - Leica S8 APO stereoscopic microscope used to count microplastics
  - Photographed with Carl Zeiss Axiocam ERc5s
  - Weighed with Mettler AE 240 (Schmidt et al., 2018)

(Hernandez-Gonzalez et al., 2018)

Methods- sample analysis

Toxins tested for include:



- Iron (Fe)
- Manganese (Mn)
- Aluminum (Al)
- Lead (Pb)
- Copper (Cu)
- Silver (Ag)
- Zinc (Zn)

- Polyaromatic hydrocarbons (PAHs)
- Organochlorine pesticides (OCPs)
- Polychlorinated biphenyls (PCBs)
- Dimethyl phthalate (DMP)
- Diethyl phthalate (DEP)
- Benzyl butyl phthalate (BBP)
- Dibutyl phthalate (DBP)
- Diethyl hexyl phthalate (DEHP)
- Di-n-octyl phthalate (DnOP)

Tissue and microplastic analysis: blood and tissue samples will be sent to the Marine Mammal Pathobiology Laboratory to determine the presence of microplastics and which, if any, of the above toxins are present and the concentrations of each. Microplastic samples from the stomach and intestines, scat, and seagrass will also be sent to the Marine Mammal Pathobiology Laboratory to test for toxins/concentrations.

(Padula et al., 2020; Verla et al., 2019)

Questions?

Ana Noel · (608) 513-3773 · amnoel@alaksa.edu

San Diego Zoo Wildlife Alliance

References

Barboza, L.G.A., Lopes, C., Oliveira, P., Bessa, F., Otero, V., Henriques, B., Raimundo, J., Caetano, M., Vale, C. and Guilhermino, L. (2020). Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. *Science of the total environment*, *717*, 134625.

Beck, C. A., and Barros, N. B. (1991). The impact of debris on the Florida manatee. *Marine Pollution Bulletin*, *22*(10), 508-510.

Beiras, R., Tato, T., and López-Ibáñez, S. (2019). A 2-Tier standard method to test the toxicity of microplastics in marine water using *Paracentrotus lividus* and *Acartia clausi* larvae. *Environmental toxicology and chemistry*, *38*(3), 630-637.

Carroll, E.L., Gallego, R., Sewell, M.A., Zeldis, J., Ranjard, L., Ross, H.A., Tooman, L.K., O'Rorke, R., Newcomb, R.D. and Constantine, R. (2019). Multi-locus DNA metabarcoding of zooplankton communities and scat reveal trophic interactions of a generalist predator. *Scientific Reports*, *9*(1), 1-14.

Chatterjee, S., and Sharma, S. (2019). Microplastics in our oceans and marine health. *Field Actions Science Reports. The Journal of Field Actions*, (Special Issue 19), 54-61. Derraik, J. G. (2002). The pollution of the marine environment by plastic debris: a review. *Marine pollution bulletin*, *44*(9), 842-852.

Dierauf, L. A. (1994). Pinniped forensic, necropsy and tissue collection guide.

Florida Fish and Wildlife Conservation Commission. *Necropsy*. Florida Fish and Wildlife Conservation Commission. https://myfwc.com/research/manatee/rescue-mortality-response/carcass/necropsy/

Florida Fish and Wildlife Conservation Commission Marine Mammal Pathobiology Laboratory. (2020). Final Manatee Mortality Table by County, 01/01/2020 to 12/31/2020. Florida Fish and Wildlife Conservation Commission. https://myfwc.com/research/manatee/rescue-mortalityresponse/statistics/mortality/yearly/

References cont.

Fossi, M. C., Panti, C., Guerranti, C., Coppola, D., Giannetti, M., Marsili, L., and Minutoli, R. (2012). Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (*Balaenoptera physalus*). *Marine pollution bulletin*, *64*(11), 2374-2379.

Galloway, T. S., and Lewis, C. N. (2016). Marine microplastics spell big problems for future generations. *Proceedings of the national academy of sciences*, *113*(9), 2331-2333.

Gola, D., Tyagi, P. K., Arya, A., Chauhan, N., Agarwal, M., Singh, S. K., and Gola, S. (2021). The impact of microplastics on marine environment: a review. *Environmental Nanotechnology, Monitoring & Management*, 100552.

Hartman, D. S. (1971). *Behavior and ecology of the Florida manatee, Trichechus manatus latirostris (Harlan), at Crystal River, Citrus County*. Cornell University.

Hernandez-Gonzalez, A., Saavedra, C., Gago, J., Covelo, P., Santos, M. B., and Pierce, G. J. (2018). Microplastics in the stomach contents of common dolphin (*Delphinus delphis*) stranded on the Galician coasts (NW Spain, 2005–2010). *Marine pollution bulletin*, *137*, 526-532.

King, J. M., and Heinen, J. T. (2004). An assessment of the behaviors of overwintering manatees as influenced by interactions with tourists at two sites in central Florida. *Biological Conservation*, *117*(3), 227-234.

Laist, D. W. (1987). Overview of the biological effects of lost and discarded plastic debris in the marine environment. *Marine pollution bulletin*, *18*(6), 319-326.

Maharana, D., Saha, M., Dar, J.Y., Rathore, C., Sreepada, R.A., Xu, X.R., Koongolla, J.B. and Li, H.X. (2020). Assessment of micro and macroplastics along the west coast of India: abundance, distribution, polymer type and toxicity. *Chemosphere*, *246*, 125708.

References cont.

Padula, V., Beaudreau, A. H., Hagedorn, B., and Causey, D. (2020). Plastic-derived contaminants in Aleutian Archipelago seabirds with varied foraging strategies. *Marine Pollution Bulletin*, *158*, 111435.

Sanchez-Vidal, A., Canals, M., de Haan, W. P., Romero, J., and Veny, M. (2021). Seagrasses provide a novel ecosystem service by trapping marine plastics. *Scientific reports*, *11*(1), 1-7.

Schmidt, N., Thibault, D., Galgani, F., Paluselli, A., and Sempéré, R. (2018). Occurrence of microplastics in surface waters of the Gulf of Lion (NW Mediterranean Sea). *Progress in Oceanography*, *163*, 214-220.

Shim, W. J., and Thomposon, R. C. (2015). Microplastics in the ocean. *Archives of environmental contamination and toxicology*, *69*(3), 265-268.

Veerasingam, S., Ranjani, M., Venkatachalapathy, R., Bagaev, A., Mukhanov, V., Litvinyuk, D., Mugilarasan, M., Gurumoorthi, K., Guganathan, L., Aboobacker, V.M. and Vethamony, P. (2021). Contributions of Fourier transform infrared spectroscopy in microplastic pollution research: A review. *Critical Reviews in Environmental Science and Technology*, *51*(22), 2681-2743.

Verla, A. W., Enyoh, C. E., Verla, E. N., and Nwarnorh, K. O. (2019). Microplastic–toxic chemical interaction: a review study on quantified levels, mechanism and implication. *SN Applied Sciences*, *1*(11), 1-30.

Wang, F., Wong, C. S., Chen, D., Lu, X., Wang, F., and Zeng, E. Y. (2018). Interaction of toxic chemicals with microplastics: a critical review. *Water research*, *139*, 208-219.

Whitaker, J. O., and Hamilton, W. J. (2021). *Mammals of the Eastern United States*. Cornell University Press.

Zantis, L. J., Carroll, E. L., Nelms, S. E., and Bosker, T. (2021). Marine mammals and microplastics: A systematic review and call for standardization. *Environmental Pollution*, *269*, 116142.