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

Introduction

The Florida Manatee (*Trichechus manatus latirostris*) is a subspecies of the West Indian manatee (*Trichechus manatus*) and was considered one of the most endangered marine mammals in North America in 2004. Since 1983, the Florida manatee has been under Florida state protection. Due to boat strikes being the main threat to the Florida manatee, the Florida Manatee Sanctuary Act was passed in 1978 that created strict speedboat enforcement regulations and declared the whole state of Florida a manatee refuge (King and Heinen, 2004). Besides boat strikes, Florida manatees are suspectable to plastic pollution (Beck and Barros, 1991).

Over the past decades, an increase in plastic has been used and readily available to the public: 322 million tons are produced every year and, of this amount, it is estimated that 4.8-12.7 million tons will enter the ocean. In fact, 60-80% of all marine debris that enter the ocean are plastics (Beiras et al., 2019; Laist, 1987). Macroplastics, plastics > 5 mm, have caused an increase in the number of Florida manatees dying due to entanglement and ingestion, which causes blockages to the GI tract (Beck and Barros, 1991; Derraik, 2002; Maharana et al., 2020). Beck and Barros (1991) performed 439 necropsies on Florida manatees and found that 4 of the 63 Florida manatees that had ingested plastic died.

Although marcoplastics are a threat to Florida manatees, microplastics also pose a potential danger. Microplastics are plastics < 5 mm that stem from large plastics degrading into smaller fragments (Chatterjee and Sharma, 2019). Microplastics are found in most marine mammals and, unlike macroplastics that obstruct the GI tract, the effects are unknown (Zantis et al., 2021). Barboza et al., (2020) speculates that microplastics can cause “genotoxicity, oxidative stress, changes in behavior, reproductive impairment, mortality, population growth rate decrease, transgenerational effects, among several others.” That being said, microplastics are known to contain toxic contaminants such as halogens, heavy metals, and organic pollutants which come from being manufactured and absorbed from the environment. (Fossi et al., 2012; Veerasingam et al., 2021).

Florida manatees are especially susceptible to microplastics due to their diet. They consume a variety of seagrass species and will sometimes consume small fish and invertebrates living in the seagrass (Hartman, 1971). Microplastics are found to be trapped within the leaves of seagrass, creating a pathway of ingestion by Florida manatees (Sanchez-Vidal et al., 2021). Since the number of macroplastics entering the ocean are increasing, the amount of microplastics in the ocean are also increasing (Shim and Thomposon, 2015); microplastics alone are responsible for 92% of the plastic debris in the world’s oceans (Wang et al., 2008). This creates an increasingly large threat for manatees, as they will ingest a higher concentration of microplastics. As a result, the increase in microplastics throughout the ocean could potentially cause detrimental effects to the Florida manatee such as reduced reproduction, reduced growth, and reduced offspring viability, leading to further population decline (Galloway and Lewis, 2016).

Aims of study

I propose to examine how microplastics, which are poorly understood, affect the Florida manatee due to the hazardous chemicals they contain. The Florida manatee is an important species to study as it is constantly foraging on seagrass and possibly ingesting microplastics. Sanchez-Vidal et al., (2021) found that 1 kg of seagrass has up to 1,470 microplastic pieces. Manatees eat up to 45 kg of seagrass a day, meaning they could potentially be ingesting 66,150 microplastic pieces per day (Whitaker and Hamilton, 2021). Understanding if Florida manatees are ingesting microplastics and the toxins/concentrations they are exposed to can help to understand how these toxins are affecting them, ultimately resulting in conservation methods to save these animals. Since macroplastics are known to cause detrimental effects to Florida manatees, it is imperative to know if microplastics are causing the same, if not worse, effects; in 2020, 637 manatees were found dead: 15 died from human impact including macroplastics while the cause of death for 89 was unknown (Florida Fish and Wildlife Conservation Commission Marine Mammal Pathobiology Laboratory, 2020). This could indicate that microplastics could be causing the death of manatees, making it imperative to study them.

Objectives

The objectives of this study are to:

- Examine the GI tract contents 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, when possible, Florida manatee scat for the presence of microplastics
- Collect microplastics found on seagrass to determine what contaminants are present

This will allow for comparison between contaminants found in microplastics from manatees to contaminants found in microplastics from seagrass beds to determine if Florida manatees are ingesting microplastics while foraging on seagrass.

Material and Methods

A permit will be obtained to collect and work with the samples collected from Florida manatees.

<u>Sample collection</u>

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 °C 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 °C (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 ℃ (Sanchez-Vidal et al., 2021).

Beach walks will be performed in different areas 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.

<u>Sample analysis</u>
Concentrations of microplastics: 3 sperate metal sieves of 4 different sizes will be used for each sample (stomach and intestines, scat, blood, and seagrass leaves). Samples will be placed, in order, into the following size sieves:

- 5 mm
- 1 mm
- .5 mm
- .355 mm

This prevents clogging of the sieves and removes any organic matter and debris other than plastic. Any other large materials left in the sieve will be removed. If microplastics are present, half of the sample will be sent to the Marine Mammal Pathobiology Laboratory to be tested for contaminants, and the other half will be placed in a sealed, sterilized glass jar containing 10% potassium hydroxide (Florida Fish and Wildlife Conservation Commission, n.d.; Hernandez-Gonzalez et al., 2018). In 3 weeks after the organic solution is dissolved, a Buchner Filter and glass microfiber filters will be used to filter the samples with a vacuum pump. The samples will be transferred to Petri dishes and placed in an oven to dry at 50 ℃ for 4 hours. A Leica S8 APO stereoscopic microscope will be used to count the microplastics that were found for each sample and a Carl Zeiss Axiocam ERc5s will be used to take pictures. (Hernandez-Gonzalez et al., 2018) The microplastics will be weighed using a Mettler AE 240 (Schmidt et al., 2018).

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), and di-n-octyl phthalate (DnOP) (Padula et al., 2020; Verla et al., 2019).

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.

After receiving lab results, any toxins and concentrations found in the manatee will be compared with any toxins and concentrations found in the microplastic samples from the seagrass to see if there are similarities.

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Key: https://myfwc.com/research/manatee/rescue-mortality-response/statistics/mortality/categories/

All years: https://myfwc.com/research/manatee/rescue-mortality-response/statistics/mortality/yearly/

2020 data sheet: https://myfwc.com/media/27784/2020yearsummary.pdf