

Fall 12-17-2019

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Recommended Citation

LeRoy, Kaylin and Boneillo, George, "Microfiber concentrations in sand from nesting sea turtle beaches in Costa Rica, South Carolina, and Florida." (2019). *Honors Theses*. 358.
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**Microfiber concentrations in sand from nesting sea turtle beaches in Costa Rica, South
Carolina, and Florida.**

By

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Marine Science

Submitted in Partial Fulfillment of the
Requirements for the Degree of Bachelor of Science
In the HTC Honors College at
Coastal Carolina University

Fall 2019

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Abstract

Microplastics are an increasing threat to marine environments and the organisms that inhabit them. Since plastic pollution in the ocean is prominent throughout the globe, microplastic is washing ashore, affecting important sea turtle nesting sites. This study quantified microfibers in the sand of various sea turtle nesting sites around the world. Sand samples were collected in Myrtle Beach (South Carolina), Destin (Florida) and the Pacuare Reserve (Costa Rica). The microfibers were retrieved using a super saline solution and then filtered under a hood. Filters were then counted using microscopy. Microfibers were found at every sampling site, with the lowest surface concentration in Myrtle Beach (52 pieces/50g sand), and the highest surface concentration in Destin (869 pieces/50g of sand). The average amount of microfibers per 50 grams of sand was 80 ± 39 in Myrtle Beach, 709 ± 183 in Destin, and 142 ± 69 in Costa Rica. Because plastic has a higher specific heat than sand, these findings demonstrate the potential threat to incubation temperatures and the sex ratio of sea turtle hatchlings.

Introduction

Plastic has become a staple in the modern world due to its use in packaging, storing, or transport of goods in a cost-efficient way (Andrady 2011). While this is good economically, the over production of plastic has devastating effects on the environment, specifically the ocean's ecosystems. There are many possible ways for plastics to enter the ocean, such as the increase of fishing, whether it be recreational or commercial in which the gear used is plastic. As well as beach litter ending up in the ocean, and through river runoff directly into the oceans (Andrady 2011). Other sources can be linked to sewage systems and wind as well (Gall and Thompson 2015). It is unknown exactly how much plastic enters the ocean every year, it is estimated that approximately 8 to 12.7 million metric tons of plastic enters the ocean every year (Jambeck et al.

2015). Plastics found in the marine environment are listed as one of the largest threats to marine life (Gall and Thompson 2015).

The plastic that enters the ocean decomposes at a slow rate, most of which take years to decompose. The decomposition of plastics can occur in many ways, with the most efficient mechanism being photodegradation, which is from UV solar radiation (Andrady 2011). While this is a relatively efficient method of decomposition, the rate significantly decreases when these plastics are in the ocean. (Andrady 2011). This is due to the ocean having relatively lower temperatures and oxygen concentration. With this degradation process, many of these plastics end up breaking down into much smaller pieces, many becoming microplastics.

There are two categories of microplastics, one category is plastics that are deliberately produced to be microplastics for items such as cosmetic products, and those who have broken down from larger pieces to become microplastics (Beckwith and Fuentes 2018). Microplastics are defined as pieces of plastic that are less than 5mm and over 333 micrometers and, are not visible to the naked eye (Beckwith and Fuentes 2018). The impact of microplastics on marine organisms can be directly sourced to ingestion by organisms and, toxins that come from the plastic itself. Marine life such as mussels will filter out and ingest the plastics from the water, and in return bioaccumulation will occur when other organisms eat them (Yu et al. 2016). These organisms in return are accumulating plastic and the toxins that are in the particles as well.

While using ocean water samples is popular for quantifying the microplastics, beach sand samples are beneficial for quantifying the long-term accumulation of microplastics (Yu et al. 2016). The microplastics that accumulate in the sand can indirectly affect the organisms that live on or in these beach habitats and can affect the composition of these coastal environments (Beckwith and Fuentes 2018, Yu et al. 2016). For instance, sea turtles use beaches as nesting

grounds every year. Since the eggs are incubated in the sand, the sex of the turtles is influenced by the sand temperature. Microplastics have higher specific heat capacity than sand, so if there is a high concentration of microplastics in the sand, this can affect the temperature at which the sea turtle eggs incubate as well as the duration of incubation for the turtles (Beckwith and Fuentes 2018, Duncan et al. 2018). For turtles, warmer incubation temperatures result in females, and cooler incubation temperatures result in males (Mrosovsky 1987). There is a pivotal temperature at which the turtle eggs incubate where approximately half of the nest will be female, and half male, which for loggerhead sea turtles occur around 29.0 degrees Celsius (Mrosovsky 1987).

In this study, the concentration of microfibers at sea turtle nesting beaches were analyzed and quantified to evaluate the likely impact of microfibers on nesting beaches. To do this, sand samples from sea turtle nesting beaches in Costa Rica, South Carolina, and Florida were collected from the surface and analyzed to determine approximate microfiber concentrations in various areas and depths on these beaches. At these beaches, various species of sea turtles such as the loggerhead, green, and leatherback nest near the dunes.

Field Methods:

The study area consisted of sand samples from sea turtle nesting sites, including Myrtle Beach, SC as well as Destin, Florida and the Pacuare Reserve in Costa Rica (Figure 1). Myrtle Beach, SC as well as Destin, Florida are nesting sites for Green (*Chelonia mydas*) and Loggerhead (*Caretta caretta*) sea turtles. The Pacuare Reserve in Costa Rica is an important nesting site for leatherback sea turtles (*Dermochelys Coracia*), with a mean density of 142 nests per km (Rivas et al 2015). At each sampling site, sand samples were taken using a metal shovel from the top 1-5cm layer of sand and placed into a glass or aluminum container. The Florida samples were collected at three different locations on the beach. Dune, Swash, and wrack

samples were collected and placed into glass containers. The South Carolina samples were taken at two locations on the beach, the dune and swash.

In Costa Rica, there is a 6km beach starting where the Pacuare river flows into the ocean. Sand samples were taken on the beach at 1km increments, sample 6 was taken closest to the river (0km), and sample 0 was furthest away (6km). These samples were placed in aluminum foil pouches and taken back to the lab for extraction and analysis. There were three additional samples taken in Costa Rica, one surface sample (0cm), one sample at mid-depth (35cm), and one sample at 70cm depth. This depth was chosen because it is the approximate sea turtle nesting depth. An ocean water sample was also collected in Costa Rica, filtered, and evaluated for microfibers.

Separation Method:

The extraction methods that were utilized come from Besley et al. 2017. A density separation method was used to separate the microplastics from the sand. To do this, a super-saturated salt solution was made by dissolving 200g of salt per liter of water from the MilliQ filtration system, to ensure the least amount of contamination in the samples. Then, a heated magnetic stirrer was used to stir the mixture at a low speed until all of the salt dissolved. Once all of the salt dissolved, the mixture was filtered under a hood to remove any impurities or contamination.

Once the sand samples were taken back to the lab, they were dried for 48 hours at 50 degrees Celsius. After the drying period ended, 50g of sand was measured out, then mixed with 250mL of the saturated salt solution. The samples were left alone to settle for five minutes and were covered in aluminum foil or a lid to prevent any atmospheric contamination. After the mixture settled, the water above the sand layer was transferred to the filtration system. The

samples were filtered through a 0.45 micrometer filter. The equipment will be rinsed thoroughly with deionized water after each filtration to minimize sample loss. The filters will then be placed in a petri dish for further analysis. This extraction process was repeated until the counts were under a threshold of 10-40 pieces per filter (Figure 2). The fibers were quantified using a microscope. Blank samples were also run, meaning that the super saturated solution was filtered without being mixed with sand first. This was done in order to determine if the solution was a possible source of fibers to the samples.

Results:

Microfibers were found at every sampling site. The Myrtle Beach samples contained the least amount of fibers per 50g of sand, with an average of 80 ± 40 (Figure 3). The Myrtle Beach swash sample contained 108 fibers per 50g of sand, while the dune sample contained 52 fibers per 50g of sand (Figure 4). The counts decreased with every repeated filter, as represented in figure 2. Destin had the highest amount of fibers, with an average of 709 ± 184 fibers per 50g of sand (Figure 3). The swash had a total of 508 fibers, the wrack contained a total of 750 fibers and the dune had a total of 869 fibers per 50g of sand (Figure 5).

The Pacuare Reserve had an average of 142 ± 69 fibers per 50g of sand (Figure 3). Station 0 contained 55 fibers, Station 1 contained 100 fibers, Station 2 had 90 fibers, station 3 contained 103 fibers, Station 4 contained 102 fibers, Station 5 contained 261 fibers, and Station 6 contained 70 fibers, all per 50g of sand (Figure 6). The surface sample taken (0cm) contained a total of 307 fibers, while the sample at 35 cm contained 42 fibers, and the 70cm sample contained 32 fibers per 50g of sand (Figure 7).

Discussion

All samples from every site contained microfibers. While all of the samples are higher counts than in studies mentioned above, the Myrtle Beach samples most closely compared to the study done by Beckwith and Fuentes in 2018. Myrtle Beach had the lowest fiber concentration, the swash site was the higher concentration of the two sampling locations on this beach. Since the swash sample had higher concentrations than the dune sample, this could indicate that in Myrtle Beach the main source of microfibers comes from the ocean. The swash zone is where the waves break, and a lot of mixing occurs there, so the fibers are likely to get trapped in the sand at this location. For the dunes, the source of the fibers is more likely to be atmospheric contamination.

The Destin samples had the opposite outcome from Myrtle Beach. The dune sample was higher than the swash, with the wrack sample in between the two. With the dune sample being higher at this location, this could impact sea turtle nests since the dunes are primarily where nesting occurs on the beach (Beckwith and Fuentes 2018). While all of the counts at this location were high, since the swash contained the least amount of plastic the ocean is not likely to be the main source of fibers at this location. While the ocean is likely supplying fibers to the beach, this may not be the main process that is making these counts so high. The samples were taken at a heavily populated area, where there are large amounts of human activity happening. The dune sample was taken near a public beach access point, meaning the fibers could have been sourced from clothing, towels, beach chairs, umbrellas, shoes, trash, or any other items that are taken to a beach. At the particular beach, there were beach chairs and towels lined up from the wrack to close to the dunes, meaning the sources listed above are likely impacting the whole beach. When the averages found at this location are higher than any other studies done, even the study done by

Beckwith and Fuentes, which also took place on the Northern Gulf of Mexico. However, it was found that the western most sites in Florida contained the most microplastics, which could explain why this site has such high concentrations (Beckwith and Fuentes 2018). An explanation for this could be the loop currents in the Gulf of Mexico are in close proximity to the western most sites, which could possibly supply more microplastic to the shore (Beckwith and Fuentes 2018).

The Pacuare samples contained fairly consistent values, excluding an outlier at the second sampling site. The values found at this site were similar to those found near the Bohai sea in China (Yu et al 2017), excluding the outlier at the second sampling site. This could be due to a difference in morphology of the beach at this point, which changed the way the fibers were trapped on this portion of the beach. It is unlikely for this beach to have the same sources of fibers as the other sampling sites, due to the lack of tourism. While there are not as many tourists coming through this area, people do walk on this beach to monitor leatherback sea turtle nesting. The results of the depth profile that was done show minimal fibers at sea turtle nesting depth. The few fibers that were found at depth are a possible result of contamination when retrieving the samples. These findings are similar to the Yu et al study, where less fibers were found at depth than at the surface. This could be because the top layer of sand directly interacts with ocean water, which could be a source of fibers to the sand (Yu et al 2017). Since the top layer of sand was so highly concentrated, the fibers could be insulating the sand at nesting depth, which could result in raised incubation temperatures. These samples were collected in plastic tubes rather than the foil pouches, which could have influenced the results.

There are many possible sources of error when studying microfibers. Atmospheric contamination is hard to avoid, especially when sampling. In order to avoid this problem in the

lab, every procedure was done under the hood. There also could have been errors when counting the filters under the microscope, as some of the samples had so many clustered together deciphering them was a challenge. Although the super saline solution that was used for separation was Milli-Q water that was filtered, the salt in the water as well as the water itself could have been a potential source of extra fibers in the samples.

These findings present a likely impact on important sea turtle nesting beaches, while also highlighting the severity of the global plastic problem. It has been discovered that sea turtle incubation time is decreasing, due to increase nesting temperatures, which result in an increase in females (Reneker and Kamel 2016). These increasing temperatures can be linked to climate change, since record high temperatures are being recorded. Further research on the impact of these microfibers on the sand temperature should be done in order to identify the impact microfiber concentrations have on incubation temperature for sea turtles. Studies such as this should be expanded to other important nesting beaches around the world, in order to better understand the magnitude of the problem globally.



Figure 1. Sampling sites.

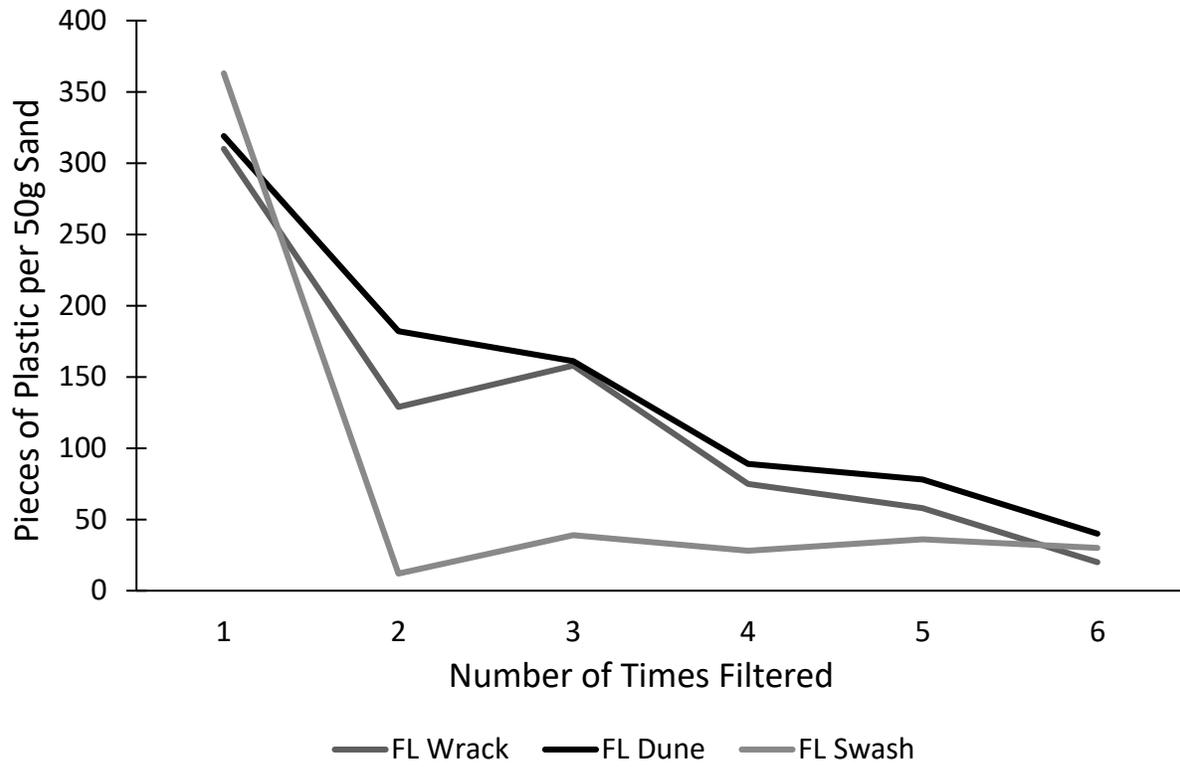


Figure 2. Plastic counts for each time the Destin wrack, dune, and swash samples were filtered.

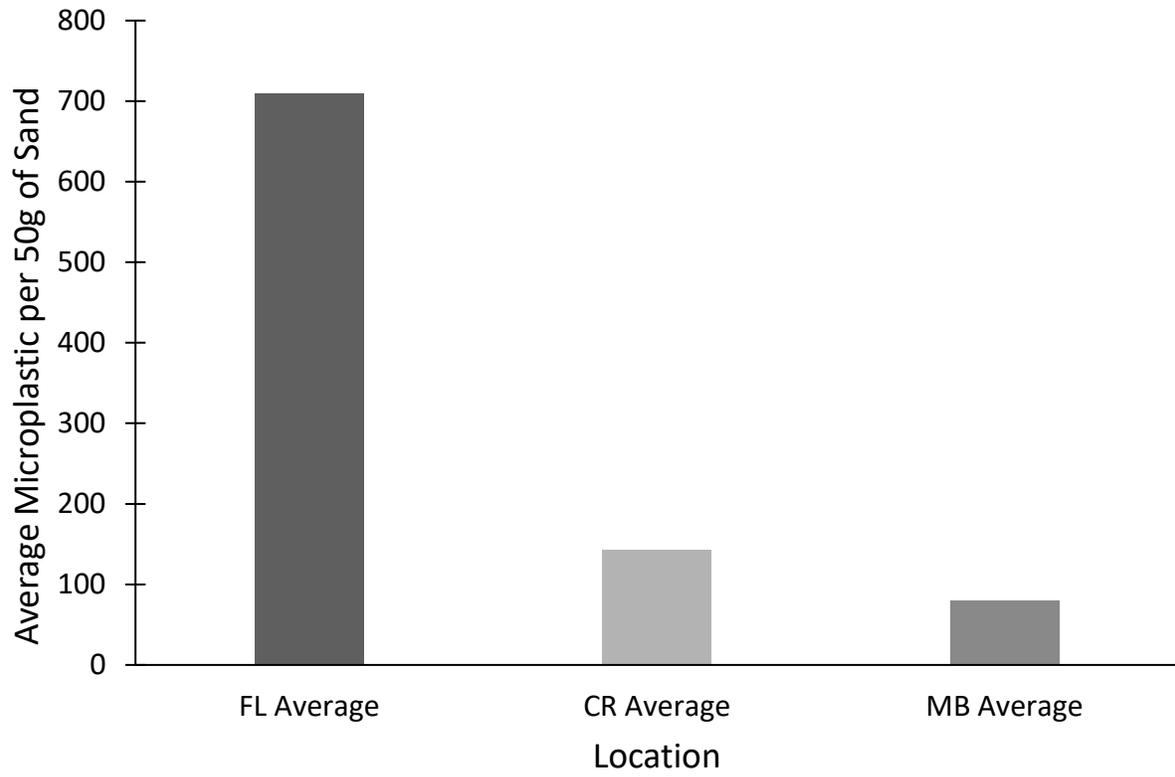


Figure 3. Average microfiber concentration from each sampling site.

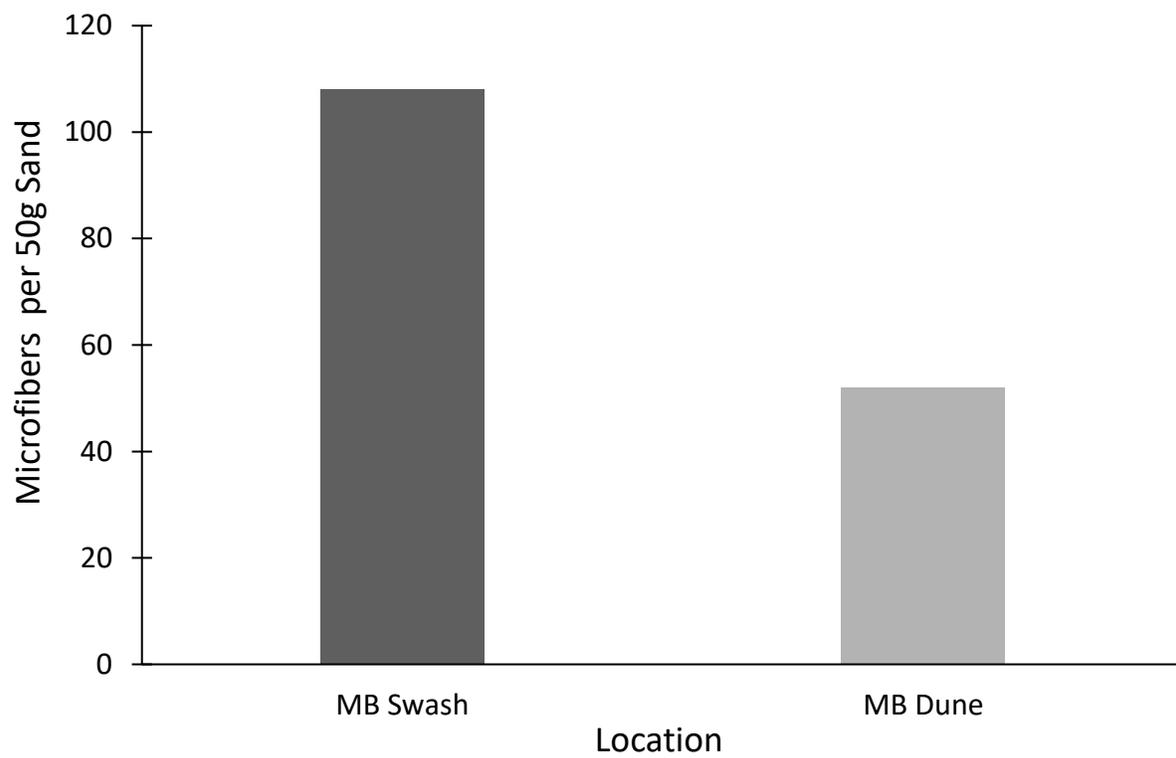


Figure 4. Average microfiber concentrations from Myrtle Beach, South Carolina at the swash and dune.

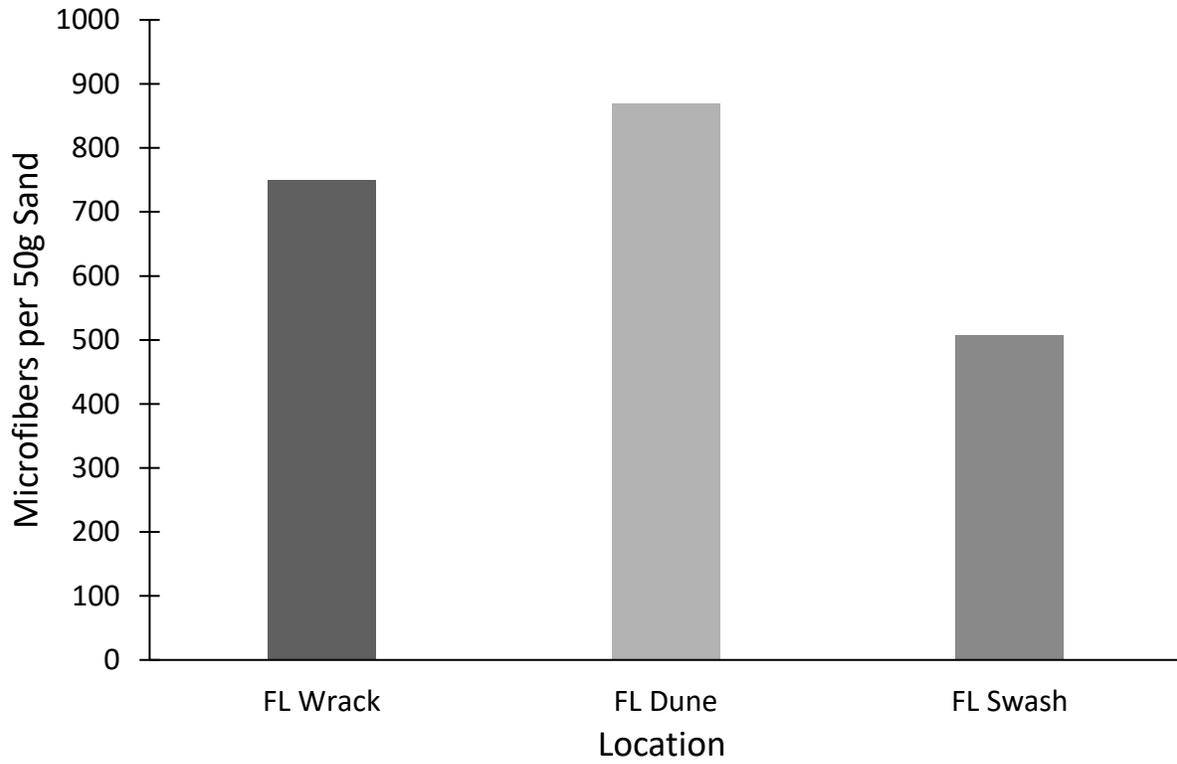


Figure 5. Average microfiber counts from Destin, Florida at the wrack, dune, and swash.

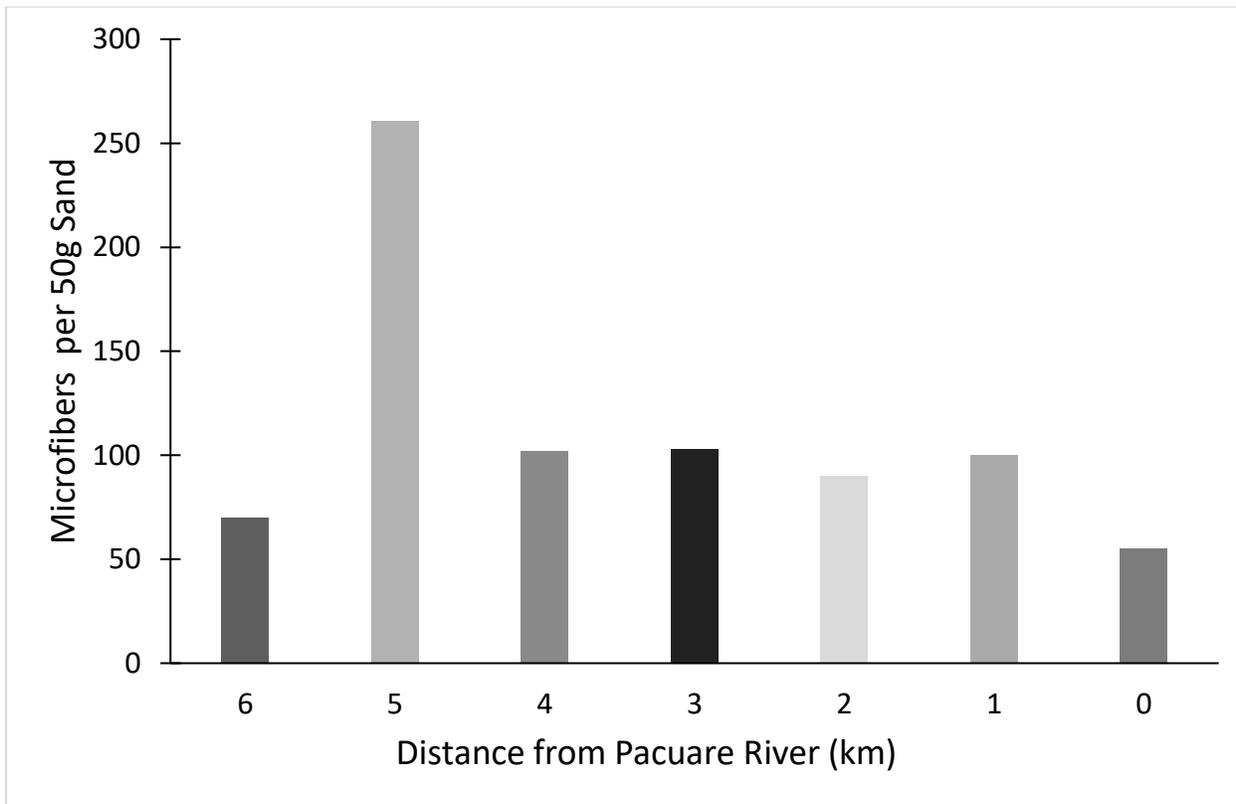


Figure 7. Average microfiber concentrations from the Pacuare Reserve in Costa Rica, at 1km distances starting at the Pacuare River.

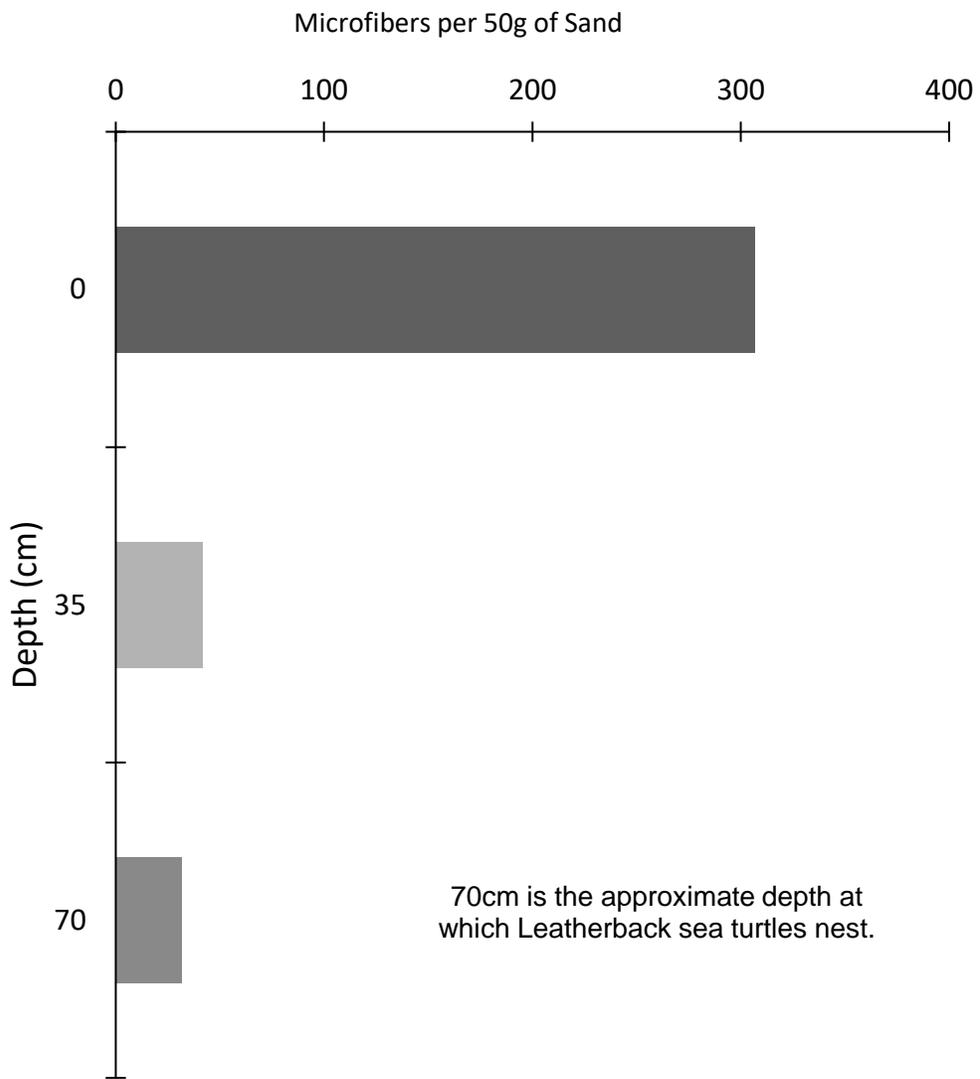


Figure 8. Total fiber concentrations for surface (0cm), mid (35cm), and bottom (70cm) depths at the Pacuare Reserve in Costa Rica.

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