Elsevier Editorial System(tm) for Marine Pollution Bulletin
Manuscript Draft

Manuscript Number: MPB-D-20-01038

Title: First report from North America of microplastics in the gastrointestinal tract of stranded bottlenose dolphins (Tursiops truncatus)

Article Type: Research Paper

Keywords: microplastics; marine mammals; bottlenose dolphin; estuarine; cetaceans

Corresponding Author: Ms. Francesca Battaglia,

Corresponding Author's Institution:

First Author: Francesca Battaglia

Order of Authors: Francesca Battaglia; Barbara Beckingham; Wayne McFee

Abstract: Microplastics' (MPs) abundance, small size (1 µm - 5 mm), and global distribution render them bioavailable to a variety of organisms directly or by trophic transfer, yet examinations in marine apex predators are currently limited. The present study investigated the occurrence of MPs in the gastrointestinal tract (GIT) of bottlenose dolphins stranded in South Carolina, USA from 2017 to 2018. MPs sized 125 µm - 5 mm were detected in all GITs (n = 7) of stranded bottlenose dolphins. Total MPs ranged between 123 to 422 particles/individual. The most common morphology were fibers (76% of total counts) and common colors were white/clear, black/grey, and blue. This is the first study from North America to quantify MPs in a small coastal cetacean outside Arctic waters and the first specifically in bottlenose dolphins (southeastern United States). Findings and methodology from this investigation can aid future studies examining MP in marine apex predators.

Suggested Reviewers: Amy Lusher
Norwegian Institute for Water Research
amy.lusher@niva.no
Amy Lusher's research interests in microplastics and plastic pollution. Her previous publications include microplastic ingestion in marine mammals, and was among the first in the world to investigate microplastic ingestion in cetaceans.

Maria Cristina Fossi
University of Siena, Italy
fossi@unisi.it
Maria Cristina Fossi is an experienced microplastics researcher that has studied their occurrence in the environment and in marine biota. Her publications include microplastic ingestion in marine vertebrates.

Zhigang Mei
Chinese Academy of Sciences
meizhigang@ihb.ac.cn

Zhigang Mei research interests include marine mammal conservation and ecology, and has previously investigated microplastic ingestion in a species of endangered porpoise.

Jennifer Lynch
NIST and Center for Marine Debris Research, Hawaii Pacific University
Jennifer.lynch@nist.gov
As the Co-Director of the Hawaii Pacific University Center for Marine Debris Research, Jennifer Lynch's research focuses are on quantifying and characterizing plastic marine debris and its sources, fate, transport, and impacts.

Susanne Brander
Oregan State University
branders@oregonstate.edu
Susane Brander's research focus includes microplastics and their potential for bioaccumulation and trophic transfer.

Highlights

First report from North America of microplastics in the gastrointestinal tract of stranded bottlenose dolphins (*Tursiops truncatus*)

F.M. Battaglia[a,]* B.A. Beckingham[b], W.E. McFee[c]

[a]College of Charleston, Graduate Program in Marine Biology, Grice Marine Lab, 205 Fort Johnson Rd., Charleston, SC 29424, USA
[b]College of Charleston, Department of Geology and Environmental Geosciences, 66 George St., Charleston, SC 29424, USA
[c]National Centers for Coastal Ocean Science, National Oceanic and Atmospheric Administration, 219 Fort Johnson Rd., Charleston, SC 29412, USA

Corresponding author:

Francesca Battaglia
Email: battagliafm@g.cofc.edu

*Current affiliation: Coastal Carolina University, Department of Coastal and Marine Systems Science, 100 Chanticleer Dr. E, Conway, SC 29528, USA

- Microplastics in all 7 gastrointestinal tracts of bottlenose dolphins examined
- Microplastics may fragment in cetacean digestive tract
- Fibers dominant morphology observed; fragments, films, and foams also present
- Pathways of exposure and potential impacts on dolphins are still poorly understood

First report from North America of microplastics in the gastrointestinal tract of stranded bottlenose dolphins (*Tursiops truncatus*)

F.M. Battaglia[a,]* B.A. Beckingham[b], W.E. McFee[c]

[a]College of Charleston, Graduate Program in Marine Biology, Grice Marine Lab, 205 Fort Johnson Rd., Charleston, SC 29424, USA
[b]College of Charleston, Department of Geology and Environmental Geosciences, 66 George St., Charleston, SC 29424, USA
[c]National Centers for Coastal Ocean Science, National Oceanic and Atmospheric Administration, 219 Fort Johnson Rd., Charleston, SC 29412, USA

Corresponding author:

Francesca Battaglia
Email: battagliafm@g.cofc.edu

*Current affiliation: Coastal Carolina University, Department of Coastal and Marine Systems Science, 100 Chanticleer Dr. E, Conway, SC 29528, USA

Abstract

Microplastics' (MPs) abundance, small size (1 µm – 5 mm), and global distribution render them bioavailable to a variety of organisms directly or by trophic transfer, yet examinations in marine apex predators are currently limited. The present study investigated the occurrence of MPs in the gastrointestinal tract (GIT) of bottlenose dolphins stranded in South Carolina, USA from 2017 to 2018. MPs sized 125 µm – 5 mm were detected in all GITs (n = 7) of stranded bottlenose dolphins. Total MPs ranged between 123 to 422 particles/individual. The most common morphology were fibers (76% of total counts) and common colors were white/clear, black/grey, and blue. This is the first study from North America to quantify MPs in a small coastal cetacean outside Arctic waters and the first specifically in bottlenose dolphins (southeastern United States). Findings and methodology from this investigation can aid future studies examining MP in marine apex predators.

Keywords: Microplastics; marine mammals; bottlenose dolphin; estuarine; cetaceans

Introduction

As a growing number of publications document the abundance and widespread distribution of plastic in coastal and marine environments, there is mounting concern for the consequential impacts on these ecosystems. Plastic waste can cause physical harm to wildlife both externally, such as suffocation or entanglement, or internally, such as blockages or tears in the gastrointestinal tract (Moore, 2008; Claro *et al.*, 2019). Risks of macroplastic debris have, for instance, been documented for cetaceans (Denuncio *et al.*, 2011; McFee *et al.*, 2014; Currie *et al.*, 2017). Less known are the impacts of smaller, microplastic debris (<5 mm in dimension).

Microplastics (MPs) are categorized by their origin as either primary or secondary. Primary MPs are manufactured small and are used as raw material in industrial processes or as components in production of larger products, while secondary MPs are generated from the fragmentation and degradation of plastic debris in the environment that is exposed to mechanical and chemical abrasion, biological processes, and UV radiation (Cole *et al.*, 2011; Duis and Coors, 2016). Aside from origin, MPs are also described by their shape, color, density, and polymer composition (Galgani *et al.*, 2013), characteristics that are relevant for determining sources, fate, and impacts. MPs are a concern due to their ubiquity, abundance, potential toxicity, and bioavailability to a wide variety of marine organisms (Barnes *et al.*, 2009; Wright *et al.*, 2013; Galloway *et al.*, 2017). Ingestion of MPs has been reported in invertebrates, such as mollusks and crustaceans (*e.g.* Murray and Cowie, 2011; Waite *et al.*, 2018), as well as vertebrates, including fish, sea turtles, seabirds, and marine mammals (*e.g.* Boerger *et al.*, 2010; Lusher *et al.*, 2013; Caron *et al.*, 2018; Duncan *et al.*, 2018; Besseling *et al.*, 2015; Lusher *et al.*, 2015; van Franeker *et al.*, 2018; Hernandez-Gonzalez *et al.*, 2018).

Studies that target MP exposure in cetaceans (*i.e.* whales, dolphins, and porpoises) are very limited due to the large size of these animals, their protected status, and the challenges of obtaining samples in good condition at the time of postmortem analysis. Reports of MPs ingestion in cetaceans have only emerged recently, and only a few individuals from various species are represented (Besseling *et al.*, 2015; Lusher *et al.*, 2015, 2018; van Franeker *et al.*, 2018; Moore *et al.*, 2020). It is thought that the most likely route of exposure to MPs for bottlenose dolphins is through trophic transfer, whereby the MP load is transferred from prey to predator following ingestion. However, recent findings suggest that other incidental ingestion is also a possible route of exposure. Specifically, Xiong *et al.* (2018) and Zhu *et al.* (2019) detected MPs in the gut of a neonate East Asian finless porpoise and a neonate Indo-Pacific humpback dolphin, respectively. These findings suggest that cetaceans may ingest MPs through other behaviors, such as play or exploration of their environment. Determining the extent of exposure is necessary to begin to assess the potential impacts of MP ingestion by cetaceans like the bottlenose dolphin.

The common bottlenose dolphin, *Tursiops truncatus*, is a flagship species in that they can provide an integrative view of the long term health of entire marine ecosystems (Fossi *et al.*, 2012), and a sentinel species, because monitoring their population can provide an early warning for potential health risks to humans (Wells *et al.*, 2004; Bossart, 2011; Reif *et al.*, 2015). The distribution, behavior, and health of the coastal and estuarine resident stocks of South Carolina are well-studied (Zolman, 2002; Hansen *et al.*, 2004; McFee *et al.*, 2006; Houde *et al.*, 2009; Fair *et al.*, 2010; Speakman *et al.*, 2010; Pate and McFee, 2012; McFee *et al.*, 2014), and thus they can be extremely valuable tools for assessing the environmental quality of the region. In South Carolina (SC) waters, bottlenose dolphins are apex predators that feed on a variety of fishes and squid. They are the most common cetacean, with resident estuarine and coastal stocks present year-round, as well as migratory stocks that utilize the resources seasonally (Hayes *et al.*, 2018). They are also extremely important for ecotourism businesses, particularly in Charleston, SC, Hilton Head, SC, and Myrtle Beach, SC (Green *et al*., 2010). Dolphins and humans are regularly interacting and sharing resources, and consequently, dolphins are also frequently exposed to plastic in fishing gear, active or derelict, as well as plastic litter from boats or land-based sources (McFee, 2014; Ragland, 2014).

Previous studies have identified estuaries and other coastal areas, particularly urban and industrial coastlines, as MPs hot spots (Isobe *et al.*, 2015; Peters and Bratton, 2016). For instance, recent surveys of Charleston Harbor in SC and the adjacent Cooper, Ashley, and Wando rivers have reported MPs in intertidal sediments, in the sea surface microlayer, and subtidally (Weinstein *et al.*, 2016; Gray *et al.*, 2018; Leads and Weinstein, 2019). Payton *et al.* (2020) also detected MPs in zooplankton in Charleston Harbor, with potential for trophic transfer to zooplanktivorous or filter-feeding fishes. A recent investigation of MP ingestion in spotted seatrout, Atlantic menhaden, striped mullet, spot, and bay anchovy from Charleston Harbor found that 98.9% of fish sampled ingested MPs (≥ 63 μm) with an overall average of 26.9 ± 4.7 of MPs per fish (Parker *et al.*, in review). Spot, bay anchovy, and menhaden are known prey of bottlenose dolphins in SC coastal and estuarine waters (Pate and McFee, 2012). Therefore, there is reason to believe that the bottlenose dolphins living and feeding in SC waterways are exposed to MPs through their diet.

The objective of the present study was to adapt previous methods by Lusher *et al.* (2015) to quantify MPs in the gut of bottlenose dolphins stranded in South Carolina, USA. Given the overlap of bottlenose dolphins' habitat with coastal urban areas, and the detection of MPs in both the environment and lower trophic levels, we suspected that bottlenose dolphins living and feeding in SC coastal waters are vulnerable to MP exposure. We hypothesize that MPs will be present in the gastrointestinal tract of stranded bottlenose dolphins and additionally, that the number of MPs will be positively correlated with the total length of the animal (a proxy for age) and the amount of food material in the gastrointestinal tract (an indication of dietary exposure).

Materials and Methods

Sample Collection

Stranded bottlenose dolphins, among other marine mammals, were reported by the public and the SC Department of Natural Resources to the SC Marine Mammal Stranding Network. When possible, entire animals were retrieved and transported to the National Oceanic and Atmospheric Administration (NOAA), National Ocean Service (NOS) laboratory in Charleston, SC and a full necropsy was conducted. On some occasions, necropsy had to be performed at the site of the stranding. In either case, the sample collection for gastrointestinal tracts (GITs) followed the same protocol described below.

Bottlenose dolphins have three gastric chambers that precede the intestine. A description of the GIT can be found in **Supporting Information** (Fig. SI-1). Prior to removal from the abdominal cavity, the GIT was closed off at the cranial and caudal end by tying a cotton string around the distal end of the esophagus ~5 cm above the opening to the forestomach and at the end of the intestine to prevent any contamination or loss of contents. The stomach and intestines were then cut away with a stainless-steel scalpel and immediately transferred to a sealed plastic bag and stored frozen (-20°C) at the NOS Charleston laboratory until it came time for processing. It was assumed that since the contents of the GIT were never in contact with the plastic bag, contamination of samples during storage would not be an issue. For each necropsy, Level A data were collected which included location of the stranding, external and internal observations, total length (cm), sex, decomposition state, body condition, and any evidence of human interaction. Stranded marine mammals are given a condition code 1 through 5 based on the scaling outlined by Geraci and Lounsbury (2005). Only GITs from relatively fresh stranded animals (code 2 or early 3) were analyzed for MPs. Animals assigned condition codes late 3 and greater, at which point the decomposition is moderate to advanced and sloughing of the stomach lining is observed, were omitted from this study. Additionally, perinates/neonates were omitted due to the assumption that animals not yet weaned and therefore not ingesting MP-contaminated prey would not be exposed to MPs within the size range of interest. A subset of bottlenose dolphins stranded on the SC coastline and estuaries in the years 2017 and 2018 were included in this study.

Gastrointestinal Tract Content Washing and Sieving

The protocol for GIT washing was based on methods used by Lusher *et al.* (2015) with some modifications. Bottlenose dolphin GITs were processed one at a time. The sealed bag containing the stomach and intestines was removed from the freezer and allowed to thaw for approximately 24 hours at room temperature. Once thawed, the three stomach chambers were separated from the intestines and weighed. Beginning with the forestomach, an incision was made and its contents washed with filtered (63 µm mesh screen) tap water into nested stainless steel sieves (12" in diameter, mesh sizes 5 mm, 1 mm, 355 µm, and 125 µm) to separate into three size fractions. Material captured by the 5 mm sieve was visually examined for macroplastic, otoliths, and squid beaks, which were collected and archived for separate diet analysis. The remaining size fractions (1 to 5 mm, 355 µm to 1 mm, and 125 to 355 µm) were rinsed with filtered deionized (DI) water into glass jars labeled with the dolphin's field number, the stomach chamber, and size fraction. Due to their similar function for chemical digestion and smaller volumes relative to the forestomach, the fundic and pyloric chambers were combined, and the pooled contents processed as described above. The empty stomach (all three chambers) was weighed again and the mass subtracted from the full stomach to determine the mass of the combined stomach chamber contents. The intestine was processed similarly to the stomach, however it was practical to examine only a subsample (1/8th of the total mass). Weight of stomach and intestine contents in sections analyzed is provided in Table SI-1.

Strong Base Digestion

Organic material present in the sieved size fractions was eliminated by a strong base digestion using 1M potassium hydroxide (KOH), as recommended by Kuhn *et al.* (2017). A 10M KOH stock solution was prepared by dissolving solid KOH pellets in pre-filtered DI water in a glass container. Under a fume hood, a volume of 10M KOH was added to each size fraction sample to dilute to a 1M solution. Immediately following the addition of KOH, the glass container was covered with aluminum foil, swirled gently by hand, and placed on a hot plate at 55 to 60°C. Digestion times ranged between three to seven days depending on the amount of organic material present in the sample.

Filtration, Density Separation, and Drying

Following base digestion, sample jars were removed from the hot plates and each size fraction was re-sieved to reduce any colloidal material that remained. Material captured by the sieves was rinsed with pre-filtered DI water and then filtered onto a gridded mixed cellulose ester filter (0.45 µm, 47 mm diameter) using a glass and stainless-steel filter-holder unit over a side-arm flask attached to a vacuum pump. The top of the filtering apparatus was covered with a glass fiber filter during filtration to avoid deposition of MPs from the air.

For samples that contained sediment, an additional density separation step using a calcium chloride solution, $CaCl_2$ (1.4 g/cm^3, saturated solution pre-filtered through Whatman© GF/F filter) in a separatory funnel was required prior to filtering the sample. Details of the density separation technique are provided in **Supporting Information**.

After filtration, filters were placed on aluminum dishes, loosely covered, and dried for (<10 minutes at 55°C). Dishes were then removed from the drying oven and covered tightly with aluminum foil. Throughout these steps, the time that sample jars or filters were exposed to air was kept at a minimum by working under a fume hood and keeping samples covered whenever possible.

Microplastic Analysis

MPs in each size fraction were visually identified under a stereomicroscope (Leica 1500, Wetzlar, Germany) equipped with digital camera and image analysis software (Jenoptik, Progres Gryphax V.1.1.8153). The microscope set-up was enclosed in a metal cubicle with roof to minimize airborne contamination from the rest of the lab room. Counting was done methodically using the filter's grid for spatial reference. Suspected MP particles were photographed and the number, color, and morphology (*i.e.*, fiber, fragment, film, foam) of suspected MPs were recorded, using criteria proposed by Hidalgo-Ruz *et al.* (2012) as a guide. Visual criteria are often unreliable for smaller MPs depending on magnification range of microscopes employed. Therefore, each suspected MP was subjected to the hot needle test before it was counted. The hot needle test is a quick, easy, and cost-effective method that has previously been used to identify MPs in biological samples (Vandermeersch *et al.*, 2015). The test consists of heating a stainless-steel hypodermic needle with a flame until it is red hot and then immediately bringing it close to a suspected MP particle. When approached with the hot needle, plastic particles will melt at the edges or curl, while non-plastic material will not (Barrows *et al.*, 2017). A subset of the larger MPs (>1 mm) that were recovered from samples and blanks were analyzed onsite by Fourier-Transform Infrared spectroscopy with Attenuated Total Reflectance (FT-IR-ATR; Bruker Alpha) and another subset delivered to a laboratory for Raman microspectroscopy (Renishaw inVia confocal Raman). Polymer type was identified by matching samples to reference spectra in the instrument library package or Open Specy, an open source polymer spectroscopy library (Cowger *et al.*, 2020). Further details are available in **Supporting Information.**

In order to examine the effectiveness of the sieving process, a subsample of MPs (n = 291) from GITs were measured using image analysis software. Details can be found in **Supporting Information**. A high proportion of measured MPs were found to be outside their intended size fraction since fibers, with width less than the smallest mesh size, can be washed through sieve openings (Table SI-2). As a result, the size fractions were pooled into a single size fraction (125 µm to 5 mm) for reporting results of MP counts in each GIT section.

Contamination Control and Procedural Blanks

Several precautions were taken to minimize contamination while working on processing GIT samples for MP analysis. Before beginning work on a GIT, all equipment was cleaned with natural fiber brushes, rinsed with DI water and rinsed again with acetone. Sample containers were always kept sealed with a screw top lid or covered with aluminum foil. All laboratory surfaces were wiped down with 70% ethanol and all personnel were required to wear a 100% cotton lab coat while in the lab. Water used for washing, rinsing, and preparing chemical reagents was pre-filtered. Preliminary testing to assess the contributions of various potential contamination sources is described in **Supporting Information**.

Procedural blanks were generated and processed alongside each GIT sample. A similar volume of tap water was passed through the clean nested sieves and each sieve rinsed with an equal volume of filtered DI water as its corresponding sample into glass jars. Blank jars were treated to all subsequent processing steps in the same manner as samples and counts in blanks were subtracted based on morphology and color from their corresponding sample. Airborne blanks were created for each dolphin by placing a gridded mixed cellulose ester filter on an aluminum dish and leaving it exposed to the air on the lab bench alongside sample processing. While executing the protocols for this study, there was potential for airborne contamination during two steps: (1) while washing the GIT in the lab sink and (2) while counting MPs under the microscope. Samples were otherwise covered with either aluminum foil or a glass fiber filter. MPs from airborne blanks were counted under the stereomicroscope and subjected to the hot needle test for verification. MPs observed in airborne blanks were not subtracted from samples because procedural blanks inherently captured any potential input from this source; rather airborne blanks served to inform that the contamination contribution from the air was relatively stable on the different days that the washes/counts took place.

Statistical Analysis

At the onset of this investigation, standard protocols for quantifying MPs in the gut of stranded stranded cetaceans were absent in the literature, with only a couple of studies to use as reference (*i.e.* Lusher *et al.*, 2015; Besseling *et al.*, 2015). As considerable time was spent in the early stages of this study on consolidating and adapting various methods in the literature, sample size for the present study was limited to GITs from seven individuals (n = 7). Total counts were reported for each individual dolphin as well as for each section of GIT for each dolphin. Mean, median, and range for each MP morphology, and percent composition were reported for MPs belonging to nine broad color categories:

white/clear, black/grey, blue, red/pink, yellow, orange, green, purple, and brown/tan. Kendall's Tau was calculated (RStudio, R 3.6.0) to test for a correlation between total number of MPs and total length, number of MPs in the stomach and mass of the stomach contents, and number of MPs in the intestine subsample and mass of the intestine subsample contents. Wilcoxon ranked sum tests were calculated (RStudio, R 3.6.0) to compare the number of MPs in each GI section.

Results

Bottlenose Dolphin Sample Characteristics

Seven dolphin GITs (n = 7) were analyzed for MPs in this study, and are listed in Table 1 by field number with information on stranding location and date, sex, length, and stranding code. The seven dolphins selected are among the 101 bottlenose dolphins that stranded on the SC coast between 2017 and 2018 (48 and 53, respectively) of which 68 dolphins were assigned codes 2-3 (fresh dead and moderate decomposition) and thus could be considered for MP analysis. Four GITs were from animals stranded within an estuarine habitat, and three GITs were from animals stranded on a coast facing the Atlantic Ocean (Fig. 1). Dorsal fin pictures were submitted to the National Marine Mammal Foundation (NMMF) for possible identification in *FinBase* (Adams *et al.*, 2006), but none matched known individuals in the database. No evidence of human interaction for any of the dolphins was observed at the time of necropsy. No macroplastics (>5 mm) were observed in any of the stomach and intestine samples from bottlenose dolphins. Some dietary items were found in samples, however detailed diet analysis was outside the scope of the current work. Otoliths and squid beaks were manually removed from samples for future diet analysis, but polychaete jaws were also observed when samples were analyzed under the microscope. Kuhn *et al.* (2017) reported that polychaete jaws were among the natural materials digested by KOH, however they seemed to be resistant to the processing in the present study. It is thought that concentration, time, and heat applied to samples played a role, but this was not further investigated.

Procedural Blanks

MP counts reported in the results of this study are adjusted counts yielded from subtracting MPs in procedural blanks from their corresponding sample. Despite the precautions taken to minimize contamination, MPs were found in all procedural blanks. The largest source identified was tap water used to wash GIT contents onto sieves (see **Supporting Information**). A double-layer stainless steel screen was inserted into the sink faucet aerator to reduce this source of contamination, but some fibers may pass through. Level of suspected MPs in tap water was in the range of concentrations reported elsewhere (Koelmans *et al.*, 2019). In a few cases, MP counts by morphology and color in blanks were greater than what was found in the sample, leading to a count of -1 or -2 and sample counts in this case were reported as zero. A total of 55 to 119 MPs were counted across blanks when GI compartments and size fractions are combined for individuals. The number of MPs counted in blanks can be reported as a percentage of the total counts in corresponding samples in order to compare the level of blank contamination to recommendations by Provencher *et al.* (2017) that blank counts should ideally be less than 10% of sample counts. In the present study, total blank counts were calculated to be 16% to 37% of total sample counts (mean ± SD = 22.8 ± 7.3%). MPs observed in blanks belonged to only two morphologies: fibers and fragments, with fibers being the vast majority (98%). There were no films or foams observed in blanks. Only seven of nine color groups were observed in blanks, with orange and brown/tan not represented (Fig. SI-1). White/clear was the most frequently observed color in procedural blanks (62%).

Total Number of Microplastics

MPs were present in all dolphin GITs and in every stomach and intestine section analyzed in this study (100% occurrence). The number of MPs within an individual, blank-corrected, ranged from 123 to 422 and on average there were 280.6 ± 113.0 MPs per dolphin (Table 2). An estimated 3600-4500 pieces of white foam were found in the forestomach sample from dolphin field number SC1820. These pieces likely originated from larger pieces of foam that may have broken apart during digestion while the dolphin was alive, or during postmortem processing of the stomach for MP analysis, and thus is represented by “1” for analyses.

There were no significant differences in number of MPs among the three GIT sections ($p>0.1$, Fig. 4A, 4B). The number of MPs within the forestomach ranged from 16 to 151, while the number of MPs in the fundic+pyloric stomachs ranged from 47 to 245 (Table 2). The number of MPs within the intestine sample (1/8th of total mass) ranged from 45 to 134 (Table 2). Neither the total length of the animal (Fig. SI-6) nor the mass of stomach contents (Figure 3; $z = 0.7596$, $p = 0.4475$, tau = 0.2928) explained the variance among MP counts. However, there was a significant positive correlation between number of MPs and the mass of contents in the intestine subsample (Figure 3; $z = 2.2787$, $p = 0.0227$, tau = 0.7807).

Microplastic Morphology, Color, and Polymer Identification

Four MP morphologies were observed in samples: fibers comprised the greatest percentage of MPs, followed by fragments, films, and foams (example photographs of each type are provided in Figures SI-3-5). Size analysis of MPs across GIT compartments in one individual, SC1730, found that the longest dimension was significantly smaller ($p<0.0001$) in the intestine than the forestomach and fundic+pyloric compartments (Tukey’s pair-wise comparison; see **Supporting Information**). Nine color categories were identified based on the MPs counted in samples. Overall, the greatest proportion of MPs were white/clear

(66.6%), followed by black/gray (12.6%), and blue (9.1%). The remaining four colors were observed in less abundance: red/pink (3.4%), yellow (3.4%), orange (1.8%), brown/tan (1.7%), green (1.0%) and purple (0.4%) (Fig. 4A). White/clear was not only the most common color observed overall but was also consistently the most common color observed across individuals (Figure 4B).

Twelve MPs (≥500 µm) were selected from various samples for analysis by FT-IR-ATR spectroscopy with useful spectra only acquired for five due to relatively low instrument sensitivity. A white/clear film and a brown fragment produced spectra resembling polyamine powdered resin glue used as a weather and water-resistant wood glue, a white foam resembled low-density polyethylene foam mixed with a paraffin wax, a yellow fragment matched to polypropylene, and a white fiber matched to polyethylene. A black fiber from dolphin field number SC1732 produced a spectrum resembling that of carbon black (a component of tires and other rubber materials) as seen in the study by Leads and Weinstein (2019), but there was no match in the instrument spectral library. Additionally, fifteen suspected MPs isolated from the intestine of SC1820 in the 1-5 mm size fraction and four suspected MPs from the corresponding blank were analyzed by Raman microspectroscopy. All white/clear fibers analyzed (five in sample and two in blank) and one black fiber in the blank were identified as polyethylene terephthalate (PET). The other spectra, all for colored fibers or fragments, were not resolved due to fluorescence masking. Details on match quality, including spectra and photographs of analyzed particles are provided in the **Supporting Information**.

Discussion

Comparison of Microplastics Abundance and Size to Other Studies

The present study confirmed that bottlenose dolphins are exposed to MPs and the number of MPs ranged from 123 to 422 per individual. MPs were observed throughout the GIT, marked by their presence in all sections (forestomach, fundic+pyloric, and intestine). This suggests that MPs can travel through the digestive tract of cetaceans and be egested. The identification of MPs throughout the digestive tract has also been reported by other recent studies in marine mammals (Lusher *et al.*, 2018; Nelms *et al.*, 2019; Moore *et al.*, 2020). While macroplastic debris was not observed in GITs, there was evidence to suggest that plastics were fragmenting. One such example is the thousands of pieces of polyethylene foam found in one individual (Fig. SI-6). In addition, MPs in the intestine sample of dolphin field number SC1730 were significantly smaller than those found in the forestomach and fundic+pyloric sections. Although a preliminary observation, this result may infer that digestive processes break MPs down further into smaller pieces. Besseling *et al.* (2015) also remark on the possibility of fragmentation of particles occurring during passage of the gastrointestinal tract or sample processing. Further research is needed to understand how non-digestible particles such as plastics traverse the GIT of cetaceans.

Studies specific to cetaceans that can be compared to the results in the present study are currently limited (Table 3). A common finding across all studies, present included, is the presence of MPs in the GIT of all individuals examined. The finding that MPs are ingested by cetaceans of various species and inhabiting different bodies of water illustrates the widespread MP contamination of coastal marine food webs.

The number of MPs enumerated in GITs of individual cetaceans varies among published studies, however, all previous studies observed notably fewer MPs than the present study. Lusher *et al.* (2018) examined the GITs from 21 cetaceans (stranded or bycatch between 1990 and 2015) from Ireland for MPs, including two bottlenose dolphins, and plastic particles (0.3 to 16.7 mm) ranging from 1 to 88 MPs/individual. Moore *et al.* (2020) provided the first measure of MP ingestion in the beluga whale (*Delphinapterus leucas*). From sampling the stomach, intestine, and feces, they estimated abundance ranged from 18 to 147 MPs/individual. To date, this is the only study aside from the present originating from North American waters. Previous studies that only analyzed part of the GIT (i.e. stomach or intestine only) also showed lower detections than the present study when the same GI section is compared. Hernandez-Gonzalez *et al.* (2018) examined the stomach contents of 35 common dolphins (*Delphinus delphis*) stranded on the Galician coasts of Spain from 2005-2010 and found 3 to 41 MPs/individual. The present study found a range of 67 to 304 MPs/individual in stomach contents. Xiong *et al.* (2018) investigated MP ingestion in seven bycaught East Asian finless porpoises (*Neophocaena asiaeorientalis sunameri*) from 2015 in the intersection between the Yellow Sea and Bohai Sea of China by examining their intestinal contents and found 10 to 32 MPs/individual. Again, the total and range was less than what was measured in the intestine for bottlenose dolphins in this study (45 to 134 MPs/individual), especially considering Xiong *et al.* (2018) inspected the entire intestine while only a 1/8th subsample was used by this study. Zhu *et al.* (2019) also found MPs within a subsample of the intestinal tracts from three stranded humpback dolphins (*Sousa chinensis*) from Guangxi Beibu Gulf, China. Based on analysis of their subsamples, they estimated 2, 30, and 45 MPs for the calf, adult female, and adult male humpback dolphin, respectively. These estimates are close to what was observed by Xiong *et al.* (2018) in finless porpoises, but still less than what was measured in bottlenose dolphins in the current study.

The higher number of MPs detected in the GITs of stranded bottlenose dolphins in SC relative to other species from previous studies may be explained at least in part by differences in methodologies (Provencher *et al.*, 2017). However, there are other aspects of the dolphins' movements, diet, and behavior that may also help explain the results. For example, difference in marine debris ingestion was found in two species of dolphins depending on feeding niche at the sea bottom versus in near-surface habitat (Di Beneditto and Ramos, 2014). Bottlenose dolphins move freely throughout the SC coastal waterways, including the harbors, bays, and sounds and the rivers that feed into them (Gubbins, 2002). As a result, they are feeding and socializing in areas characterized by high commercial and recreational boating and fishing, tourist activities, and land and urban development (McFee, 2014; Ragland, 2014), encountering plastic pollution, equipment, and structures. Although dolphins do not drink seawater, they may be exposed to MPs from point sources upriver, such as wastewater effluent (Conley *et al.*, 2019), MPs in run-off from roads, such as tire wear particles (Leads and Weinstein, 2019), or MPs generated from the breakdown of plastic litter in the environment (Weinstein *et al.*, 2016). Even members of the coastal migratory stock are in proximity to sources as they pass within two kilometers of the coast. Furthermore, dolphins are generalist predators that feed on a wide variety of prey. In a recent study on five fish species commonly found in Charleston, SC, researchers found that 99% of sampled fish had MPs in their gut (Parker et al., in review). All five species included in the study are common prey items of bottlenose dolphins feeding in the area based on diet analysis from stranded animals (Pate and McFee, 2012). The work that has been conducted in recent years in South Carolina, mainly in Charleston, has contributed to our understanding of the distribution and abundance of MP pollution in the area and the exposure to various species at different trophic levels, which aids in understanding the exposure in the ecosystem's top level predators, such as the bottlenose dolphin.

Correlation of Microplastic Abundance to Total Length and Digestive Contents

Although sample size in the present study was limited to seven individuals, we preliminarily tested for associations between MP abundance and dolphin size and digestive contents. Total length, measured from the tip of the upper rostrum to the fluke notch, is used as a proxy for determining age class. Denuncio *et al.* (2011) and Puig-Lozano *et al.* (2018) both found that occurrence of marine debris ingestion was higher in younger animals. However, length of animal may also be associated with more GI volume or surface area to retain MP. MP abundance in the GIT of stranded bottlenose dolphins was not found to have a correlation with total length. Hernandez-Gonzalez *et al.* (2018) also found no correlation between MPs in stomach contents and total length in the common dolphin. In the present study, neonate/perinate bottlenose dolphins were purposely excluded because it was assumed that animals not hunting for prey would not be exposed to MPs. Interestingly, the study by Xiong *et al.* (2018) included one neonatal (<73 cm) porpoise, whose intestines contained comparable numbers of MPs, comprised of fibers, fragments, and foams, to intestines of adults the study. Furthermore, Zhu *et al.* (2019) also detected MPs within a humpback dolphin calf. These findings put into question the validity of the assumption that newborns still not yet weaned do not ingest MPs, and suggests other incidental ingestion sources. A larger sample size of stranded dolphins that includes all age classes such as neonate/perinate, juvenile, subadult, and adult (as distinguished by total length or other aging techniques) along the southeastern U.S. coast may provide more insight to this question.

Furthermore, it was hypothesized that the number of MPs within the stomach and intestine are correlated with the wet weight of the contents, assuming that MPs are obtained primarily from diet. While the mass of stomach contents was not correlated with its MP abundance, the mass of the intestine subsample contents was found to have a positive correlation. This may simply be due to random chance resulting from a limited sample size of seven animals, since the correlation was not particularly strong, or it could be a result of digestion of contents leading to a lower water content and therefore stronger relationship between mass and number of MPs.

Comparison of Microplastic Morphology and Color to Other Studies

MPs within the GIT of stranded bottlenose dolphins in the present study were of four different morphologies: fibers, fragments, films, and foams. A fifth morphology that has been observed in some other studies is spheres (*e.g.*, Hernandez-Gonazalez *et al.*, 2018, Moore *et al.*, 2020); however, spheres were not observed in any of the seven dolphin GITs in this study.

Consistent with findings in other marine biota, including cetaceans, microfibers were in highest relative abundance (76.1% of MPs). A review of studies on MPs in aquatic organisms by de Sá *et al.* (2018) found that fibers and fragments are the most commonly reported types in field surveys. In cetaceans, Lusher *et al.* (2018) found that fibers were the most common (83.6%) while the remaining items were classified as fragments (16.4%). Interestingly, white fibers, which were by far the most abundant type of MP observed in the present study, were found by Lusher *et al.*, (2018) to be a tiny proportion of both total fibers (0.6%) and total MPs (0.5%). However, this may be a result of their method of using a white glass microfiber filter, which can hinder visual detection of transparent or white fibers. Furthermore, Hernandez-Gonzalez *et al.* (2018) found that 96.6% of MPs recovered from the stomach contents of common dolphins in Spain were fibers. Microfibers made up the largest percentage of total MPs in the intestines from East Asian finless porpoises (Xiong *et al.*, 2018) and humpback dolphins (Zhu *et al.*, 2019) from China coastlines (70% and 70.3%, respectively), which is consistent with the findings in the present study, however by contrast, microfibers were not the dominant morphology in all individuals of East Asian finless porpoises (range 43.8 to 93.3%). Finally, fibers comprised about half (49%) of the morphology observed in beluga whales sampled by Moore *et al.* (2020). Additionally, fibers were the dominant morphology found in fish species known to be common prey items for bottlenose dolphins in coastal SC waters (Parker *et al.*, in review). Fibers in coastal waters may originate from treated or untreated wastewaters, stormwaters, or from the degradation of plastics in the environment (Conley *et al.*, 2019; Weinstein *et al.*, 2016; Naik *et al.*, 2020).

The color categories observed in this study were very similar to the nine different colors of MPs identified by Lusher *et al.* (2018) but differed in that gray was a separate color from black and brown/tan was not observed. Of the nine color categories for MPs identified in the present study, white/clear was by far the most observed color, even after blank-correction, although white/clear fibers were also the most abundant MP observed in procedural blanks. This finding is not unique to this study; in fact, this has led some investigators to omit white fibers from consideration altogether (Dekiff *et al.*, 2014; Goldstein and Goodwin, 2013; van Cauwenberghe *et al.*, 2013; Hernandez-Gonzalez *et al.*, 2018). White/clear is commonly reported as the most or one of the most abundant colors in MP studies, such as in northern fur seals (Donohue *et al.*, 2019), southern ocean fur seals (Eriksson and Burton, 2003), north Pacific pelagic predatory fish (Choy and Drazen, 2013), riverine fish (Roch and Brinker, 2017), and estuarine fish in Charleston Harbor, SC (Parker *et al.*, in review). However, it should be considered that some reagents used for digestion methods, such as KOH used in this study and nitric acid by other studies (Caron *et al.*, 2018) can cause discoloration of some polymers possibly resulting in overrepresentation of white/clear MPs in the sample. At the low concentration (1M) utilized by this study, Kuhn *et al.* (2017) found that a wide range of polymer types in different shapes and forms were not affected. However, it is uncertain how degradation in the environment as well as in the digestive tract of bottlenose dolphins can affect these results. As noted by Caron *et al.* (2018), other factors that affect whether color is retained include the chemical composition of color agents in the plastic particles and the manufacturing technique with which these color agents were incorporated (i.e. dispersion vs. dissolution).

Aside from white/clear, black and blue were the other most commonly observed colors in the present study (12.6% and 9.1%, respectively). The most commonly observed colors by Lusher *et al.* (2018) in cetaceans were blue (29.3%), grey (18.2%), and black (16.9%). If black and grey are combined as they are in the present study, black/grey becomes the second most abundant color, before blue, just as the present study found. In the study by Hernandez-Gonzalez *et al.* (2018) on common dolphins (white omitted), blue (45.3%) and black (24.6%) also appeared more frequently than other colors. Finally, the most prevalent color for MPs found in finless porpoises (Xiong *et al.*, 2018) was reported to be blue, while blue was the third most abundant color found in bottlenose dolphins, after white/clear and black. Color of MPs is important to note because it may provide insight as to what organisms feeding directly on MPs are likely to detect or select (Provencher *et al.*, 2017), and thus which colors (and therefore also additive dyes) are more likely to enter the food web and potentially be transferred to apex predators like the bottlenose dolphin.

Polymer Identification

A shortcoming of the primary methods used in this study (visual identification and hot needle test) is that the polymer type is not revealed. Polymer type is an important quality to report because it gives insight into the potential sources of contamination as well as the physical and chemical properties of the particles, such as density and chemical additives, which dictate their fate and impacts in marine ecosystems. Furthermore, these techniques can confirm whether a particle identified as plastic through visual detection is in fact plastic (avoid false-positives) or identify MPs that may elude visual detection (avoid false-negatives). In the investigation by Moore *et al.* (2020) in beluga whales, of the 350 suspected MP particles identified via visual detection (but not using hot needle test), 81 (23%) were confirmed plastic through FT-IR and approximately 192 (55%) were determined semi-synthetic. Natural materials, such as rubber or cotton, can be blended with plastics (e.g. textiles) or used as starting materials to create semi-synthetic polymers. Semi-synthetic MPs may also be a concern in the environment (Remy *et al.*, 2015). Natural and semi-synthetic materials should not react under hot needle test employed in the present study. More research is needed to evaluate how polymer typing can contribute to a greater understanding of pathways of exposure and potential risks to aquatic ecosystems.

Conclusion

Bottlenose dolphins are long-lived marine apex predators in South Carolina coastal and estuarine ecosystems. MPs are widely distributed globally and are increasingly being recognized as an emerging contaminant of concern. Only a handful of studies have been conducted to document MP exposure by higher trophic level biota and are mainly restricted to European (*e.g.*, Lusher *et al.*, 2015, 2018; Hernandez-Gonzalez *et al.*, 2019; Nelms *et al.*, 2019) and Chinese (Xiong *et al.*, 2018; Zhu *et al.*, 2019) waters. Lusher *et al.* (2018) call for more international data on MP ingestion by marine mammals. To our knowledge, the present study is the first originating from the western Atlantic Ocean and southeastern United States. Consistent with previous findings elsewhere in small coastal cetaceans, MPs, primarily microfibers, were detected in the GIT of all individuals and GI sections examined, but the number of MPs per individual was greater than what has been previously observed. At this time, a lack of standard methods for extracting, isolating, and enumerating MPs in the gut of cetaceans hinders study cross-comparisons (Provencher *et al.*, 2017). Although methods were developed contemporaneously, the present study does agree with the protocols recommended for wider adaptation in Claro *et al.* (2019). Harmonization for future monitoring work as well as research into potential toxicological concerns will improve understanding of whether MPs pose a threat to marine apex predators such as bottlenose dolphins.

CRediT authorship contribution statement

Francesca Battaglia: Conceptualization, Methodology, Formal Analysis, Investigation, Visualization, Writing- Original draft preparation, Funding Acquisition. **Barbara Beckingham:** Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision. **Wayne McFee:** Conceptualization, Methodology, Resources, Data Curation, Writing- Review & Editing, Supervision

Declaration of competing interest

None.

Acknowledgements

The authors would like to acknowledge Dr. Robert Young at Coastal Carolina University, the SC Stranding Network Coordinator, and the volunteers of the SC Marine Mammal Stranding Network, as well as the SC Department of Natural Resources for response, post-mortem analyses, and the collection of samples and data for the dolphins utilized in this study. Dr. Carlos Carrero and Dr. Jorge Moncada at Auburn University are thanked for Raman microspectroscopy analysis. In addition, the authors would like to acknowledge Dr. Leslie Hart at the College of Charleston and Dr. John Kucklick at the National Institute of Standards and Technology for assistance with conceptualization and writing the Master's thesis from which this study draws. Thanks to Todd Speakman at the National Marine Mammal Foundation for attempting photo-identification of the dolphins examined in this study. NOAA Disclaimer: The scientific results and conclusions, as well as any opinions expressed herein, are those of the authors and do not necessarily reflect the views of NOAA or the Department of Commerce. The mention of any commercial product is not meant as an endorsement by the Agency or Department. This research was partially funded by the College of Charleston, the NOS/NOAA Marine Debris Program, and Coastal Carolina University. Funding was provided by the Graduate Program of Marine Biology, The Graduate Student Association, and The Graduate School at the College of Charleston.

TABLES

Table 1. Descriptions of stranded *T. truncatus* from South Carolina with GITs analyzed for microplastics.

Field #	**Stranding Date (MM/DD/YY)**	**Location**	**Sex**	**Total Length (cm)**	**Condition Code***
SC1704	01/18/17	Isle of Palms, Charleston County	F	245	2
SC1730	06/07/17	Daniel Island, Berkeley County	M	210	3
SC1732	06/16/17	Seabrook Island, Charleston County	F	194	3
SC1751	12/17/17	Isle of Palms, Charleston County	F	235	2
SC1802	01/10/18	Chechessee River, Beaufort County	F	222	3
SC1820	03/29/18	Kiawah Island, Charleston County	M	167	3
SC1828	04/25/18	Seabrook Island, Charleston County	M	256	3

*Code 2 = fresh dead; code 3 = moderately decomposed

Table 2. Total numbers of microplastics by morphology as measured for each section of individual bottlenose dolphin GITs and descriptive statistics for combined GI counts. Numbers are blank-corrected.

Field #	**GI Tract Compartment**	**Total MPs**	**Fibers**	**Fragments**	**Films**	**Foams**
SC1704	Forestomach	77	64	5	0	8
	Fundic+Pyloric	59	52	5	1	1
	Intestine	120	94	13	13	0
	Combined GI	**256**	**210**	**23**	**14**	**9**
SC1730	Forestomach	95	83	10	2	0
	Fundic+Pyloric	207	206	1	0	0
	Intestine	101	101	0	0	0
	Combined GI	**403**	**390**	**11**	**2**	**0**
SC1732	Forestomach	31	24	4	2	1
	Fundic+Pyloric	47	46	0	1	0
	Intestine	45	45	0	0	0
	Combined GI	**123**	**115**	**4**	**3**	**1**
SC1751	Forestomach	57	47	10	0	0
	Fundic+Pyloric	245	13	7	225	0
	Intestine	120	115	0	5	0
	Combined GI	**422**	**175**	**17**	**230**	**0**
SC1802	Forestomach	16	14	2	0	0
	Fundic+Pyloric	51	49	2	0	0
	Intestine	134	129	2	2	1
	Combined GI	**201**	**192**	**6**	**2**	**1**
SC1820	Forestomach	70	65	0	4	1*
	Fundic+Pyloric	80	46	10	6	18
	Intestine	59	54	5	0	0
	Combined GI	**209**	**165**	**15**	**10**	**19**
SC1828	Forestomach	151	120	4	27	0
	Fundic+Pyloric	153	82	23	48	0
	Intestine	46	46	0	0	0
	Combined GI	**350**	**248**	**27**	**75**	**0**
Mean ± S.D.		280.6 ± 113.0	213.6 ± 87.9	14.7 ± 8.5	48.0 ± 84.4	4.3 ± 7.3
Median		256	192	15	10	1
IQR		201-403	165-248	6-23	2-75	0-9

*An estimated 3600-4500 pieces of white foam were found in forestomach of SC1820. These pieces likely originated from larger pieces of foam that may have broken apart during digestion while the dolphin was alive, or during post-mortem processing of the stomach for microplastic analysis, and thus is represented by "1" for analyses.

Table 3. Summary of findings regarding microplastic ingestion in toothed-whales (odonotocetes). Some studies only measured within the stomach (S) or intestine (I), and thus the total number and range for the current study are broken down into S and I for easier comparison. The entire GIT was analyzed by Lusher *et al*. (2015, Lusher *et al*. (2018), and Moore *et al*. (2020).

Study	**Species**	**N**	**Range**	**Size Range**	**Morphologies Observed**	**% of Total MPs**
Present	Bottlenose dolphin (*T. truncatus*)	7	123 – 422 (S: 67 – 304; I: 45 – 134)	125 µm – 5 mm	Fibers Fragments Films Foams	76.1 5.3 17.1 1.5
Lusher *et al.* (2015)	True’s beaked whale (*M. mirus*)	1	NA	118 µm – 1 mm	Fibers Fragments Films	79.5 18.2 2.3
Lusher *et al.* (2018)	Various odontocetes (including 2 *Tt*)	21	1 - 88	300 µm – 16.7 mm	Fibers Fragments	83.6 16.4
Hernandez-Gonzalez *et al.* (2018)	Common dolphin (*D. delphis*)	35 (S)	3 - 41	290 µm – 4.92 mm	Fibers Fragments Beads	96.6 3.16 0.24
Xiong *et al.* (2018)	East Asian finless porpoise (*N.a. sunameri*)	7 (I)	10 - 32	125 µm – 5 mm	Fibers Fragments Sheets Foams	70.1 13.4 14.9 1.5
Zhu *et al.* (2019)	Humpback dolphin (*S. chinensis*)	3 (I)	2 - 45	200 µm – 4.8 mm	Fibers Fragments Flakes	70.3 ~20 ~10
Moore *et al.* (2020)	Beluga (*D. leucas*)	7	18 - 147	<5 mm	Fibers Fragments (Included one sphere)	49 51

FIGURES

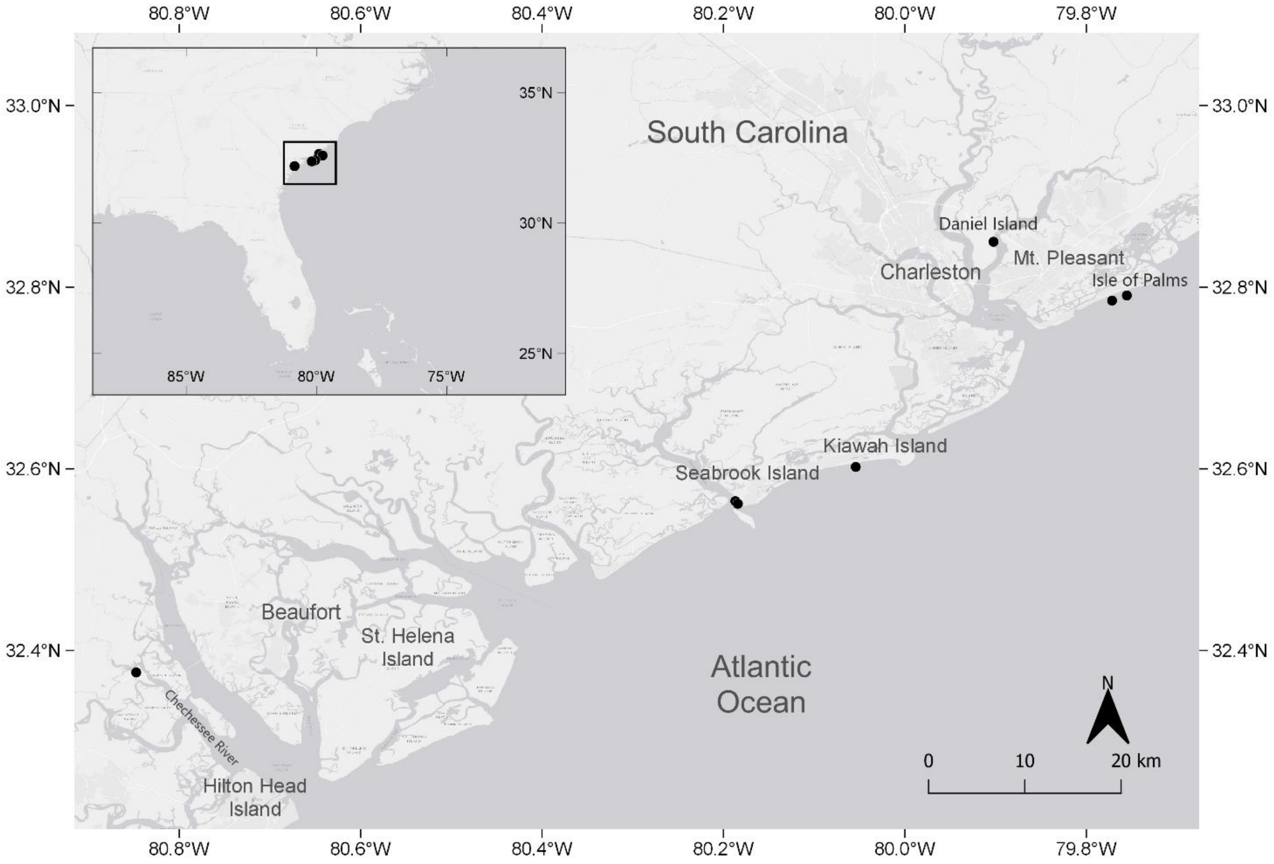

Figure 1. Map of stranding locations for bottlenose dolphins (n = 7).

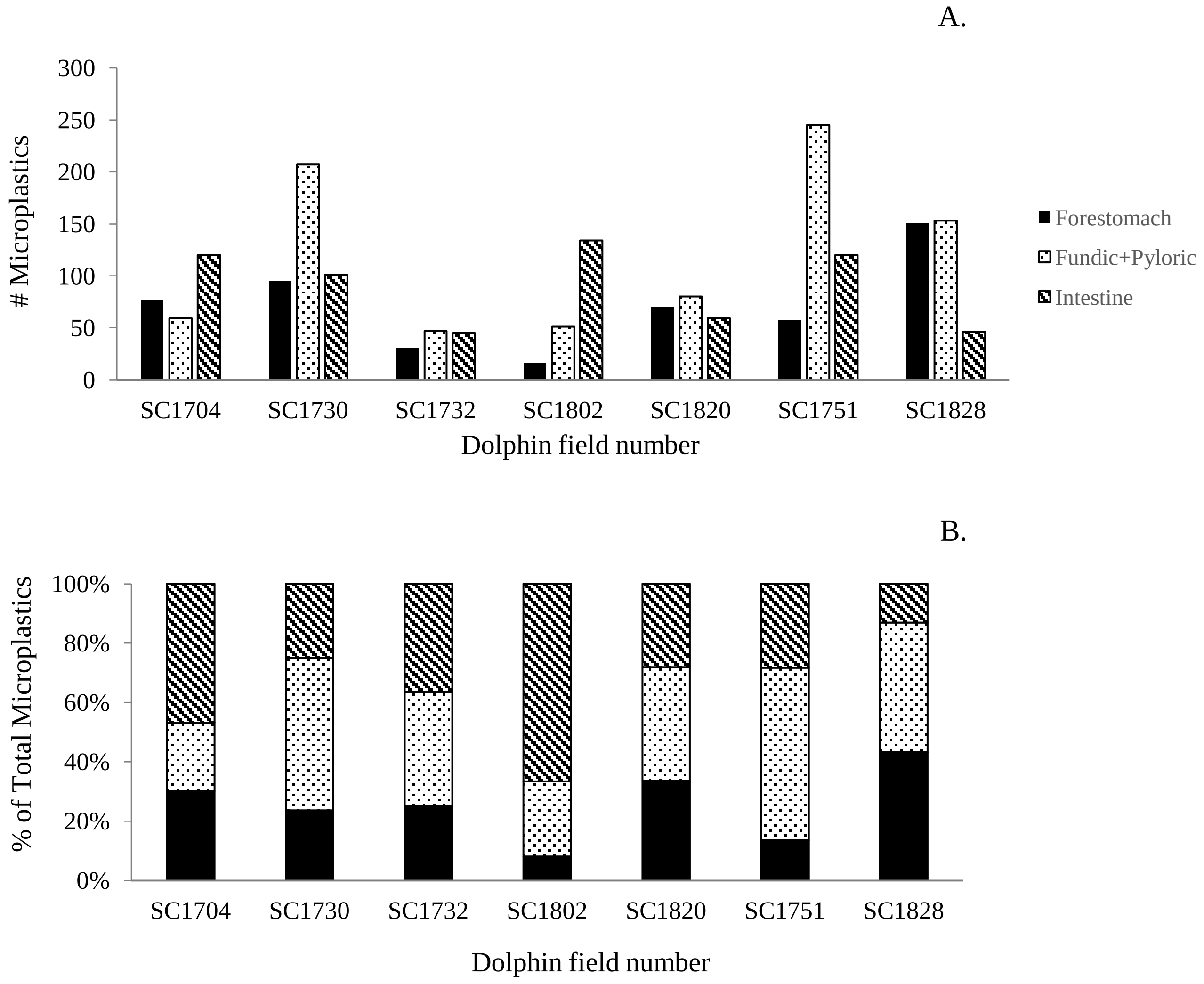

Figure 2. Total number of microplastics for individual stranded bottlenose dolphins in GI section (A); Relative percentages of total microplastics for each GI section within individual dolphins (B). Intestine data represents only 1/8th of the total intestine mass.

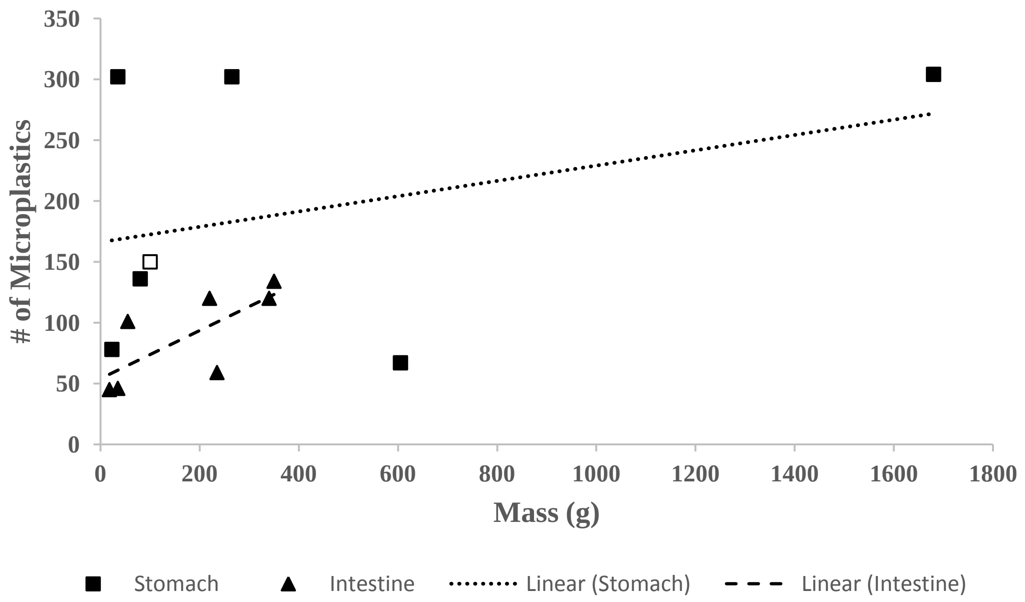

Figure 3. Total number of microplastics as a function of mass of GI compartment contents. A significant correlation was found between the number of microplastics and the digestive contents of the intestine subsample (p = 0.0227). The unfilled point denotes the uncertainty in the microplastic count for SC1820 stomach due to a large abundance of foam pieces.

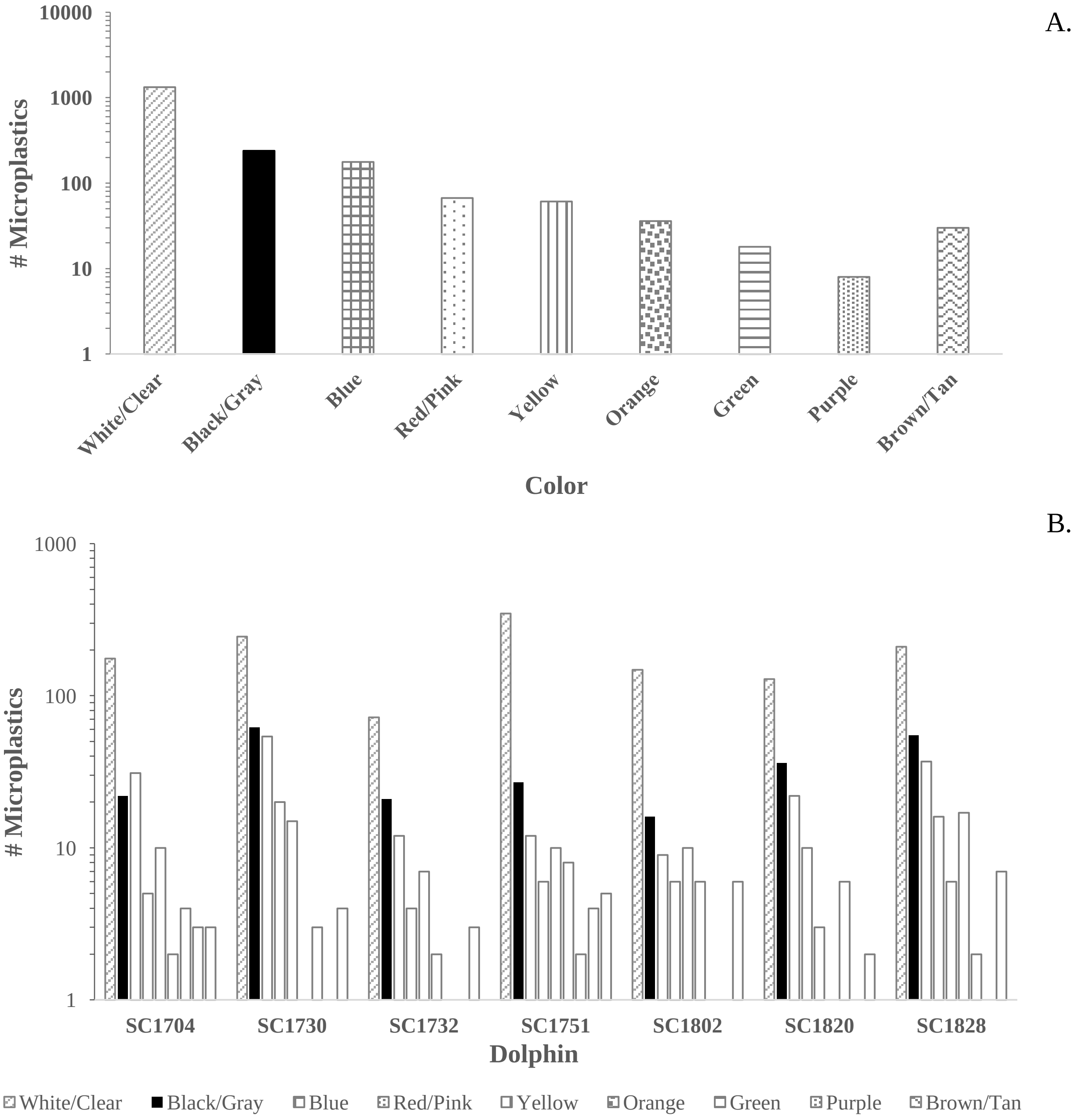

Figure 4. Total numbers of microplastics (n = 1,964) split into nine color categories (A) and total numbers of microplastics from each color category as observed in individual dolphins (B). Note log y-axis scales.

References

Adams, J.D., Speakman, T., Zolman, E., and Schwacke L.H., 2006. Automating image matching, cataloging, and analysis for photo-identification research. *Aquatic Mammals*, 32(3), 374- 384.

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364, 1985–1998.

Barrows, A.P., Neumann, C.A., Pieper, C., Berger, M.L., Shaw, S.D., 2017. Guide to Microplastics Identification, a Comprehensive Methods Guide for Microplastics Identification and Quantification in the Laboratory. Marine & Environmental Research Institute, Blue Hill, ME.

Besseling, E., Foekema, E.M., Van Franeker, J.A., Leopold, M.F., Kuhn, S., Rebolledo, E.L.B., Hesse, E., Mielke, L., IJzer, J., Kamminga, P., Koelmans, A.A., 2015. Microplastic in a macro filter feeder: Humpback whale *Megaptera novaeangliae*. *Mar. Pollut. Bull*. 95 (1), 248-252.

Boerger, C.M., G.L. Lattin, S.L. Moore, and C.J. Moore. 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Mar. Pollut. Bull*. 60 (12), 2275-2278.

Bossart, G. D. 2011. Marine mammals as sentinel species for oceans and human health. *Vet. Pathol.* 48, 676–690.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: Sources and sinks. *Environ. Sci. Technol.* 45(21), 9175–9179.

Caron, A.G.M., Thomas, C.R., Berry, K.L.E., Mottie, C.A., Ariel, E., Brodie, J.E., 2018. Ingestion of microplastic debris by green sea turtles (*Chelonia mydas*) in the Great Barrier Reef: Validation of a sequential extraction method. *Mar. Pollut. Bull.* 127, 743-751.

Choy, C.A., and Drazen, J.C., 2013. Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific. *Mar. Ecol. Prog. Ser.* 485, 155-163.

Claro, F., Fossi, M.C., Ioakeimidis, C., Baini, M., Lusher, A.L., McFee, W., McIntosh, R.R., Pelamatti, T., Sorce, M., Galgani, F., Hardesty, B.D. 2019. Tools and constraints in monitoring interactions between marine litter and megafauna: Insights from case studies around the world. *Mar. Pollut. Bull.* 141, 147-160.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. *Mar. Pollut. Bull.* 62(12): 2588-97.

Conley, K., Clum, Al, Deepe, J., Lane, H., and Beckingham, B. 2019. Wastewater treatment plants as a source of microplastics to an urban estuary: Removal efficiencies and loading per capita over one year. *Water Res.* X. 3, 100030.

Cowger, W, Gray, A, Hapich, C, Rochman, C, Lynch, J, Primpke, S, Herodotou, O. 2020. Open Specy. https://wincowger.shinyapps.io/spectra/. Accessed May 2020.

Currie, J.J., Stack, S.H., McCordic, J.A., Kaufman, G.D. 2017. Quantifying the risk that marine debris poses to cetaceans in coastal waters of the 4-island region of Maui. *Mar. Pollut. Bull.* 121, 69-77.

Dekiff, J. H., Remy, D., Klasmeier, J., and Fries, E., 2014. Occurrence and spatial distribution of microplastics in sediments from Norderney. *Environ. Pollut.* 186, 248–256.

Denuncio, P., Bastida, R., Dassis, M., Giardino, G.M., Gerpe, M., Rodriguez, D., 2011. Plastic ingestion in Franciscana dolphins, *Pontoporia blainvillei* (Gervais and d'Orbigny, 1844), from Argentina. *Mar. Pollut. Bull.* 62 (8), 1836–1841.

de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? *Sci. Tot. Environ.* 645, 1029-1039.

Di Beneditto, A.P.M., and Ramos, R.M.A., 2014. Marine debris ingestion by coastal dolphins: What drives differences between sympatric species? *Mar. Pollut. Bull*. 83(1), 298-301.

Donohue, M.J., Masura, J., Gelatt, T., Ream, R., Baker, J.D., Faulhaber, K., and Lerner, D.T., 2019. Evaluating exposure of northern fur seals, *Callorhinus ursinus*, to microplastic pollution through fecal analysis. *Mar. Pollut. Bull.* 138, 213-321.

Duncan, E.M., Broderick, A.C., Fuller, W.J., Galloway, T.S., Godfrey, M.H., Hamann, M., Limpus, C.J., Lindeque, P.K., Mayes, A.G., Omeyer, L.C.M., Santillo, D., Snape, R.T.E., and Godley, B.J., 2019. Microplastic ingestion ubiquitous in marine turtles. *Glob. Change Biol*. 25, 744-752.

Duis, K. and Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environ. Sci. Eur*. 28, 2.

Eriksson, C. and Burton, H., 2003. Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. AMBIO 32, 380-384.

Fair, P.A., Adams, J., Mitchum, G., Hulsey, T.C., Reif, J.S., Houde, M., Muir, D., Wirth, E., Wetzel, D., Zolman, E., McFee, W., Bossart, G.D., 2010. Contaminant blubber burdens in Atlantic bottlenose dolphins (Tursiops truncatus) from two southeastern US estuarine areas: Concentrations and patterns of PCBs, pesticides, PBDEs, PFCs, and PAHs. *Sci. Tot. Environ.* 408(7), 1577-1597.

Fossi, M.C., Panti, C., Guerranti, C., Coppola, D., Giannetti, M., Marsili, L., Panti, C., de Sábata, E., Clo, S., 2012. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale *(Balaenoptera physalus*). *Mar. Pollut. Bullet*. 64, 2374-2379.

Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., *et al.*, 2013. "Guidance on Monitoring of Marine Litter in European Seas," in *EUR – Scientific and Technical Research Series – ISSN 1831-9424 (Online)*, eds G. Hanke, S. Werner, F. Galgani, J. M. Veiga, and M. Ferreira (Luxembourg: Publications Office of the European Union)

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. *Nat. Ecol. Evol.* 1(5), 0116.

Geraci, J.R., and Lounsbury, V.J., 2005. *Marine Mammals Ashore: A Field Guide for Strandings*. National Aquarium in Baltimore.

Goldstein, M.C., Goodwin, D.S., 2013. Gooseneck barnacles (*Lepas* spp.) ingest microplastic debris in the North Pacific Subtropical Gyre. *PeerJ* 1:e184.

Gray, A.D., Wertz, H., Leads, R.R., Weinstein, J.E., 2018. Microplastic in two South Carolina estuaries; occurrence, distribution, and composition. *Mar. Pollut. Bull*. 128, 223-233.

Green, M. A., McFee, W. E., Levine, N. 2010. A GIS analysis of coastal development and trends in bottlenose dolphin strandings in Charleston, SC: Implications for coastal marine spatial planning. U.S. Department of Commerce, NOAA Technical Memorandum NOS NCCOS 124. 56 pp.

Gubbins, C., 2002. Use of home ranges by resident bottlenose dolphins (*Tursiop truncatus*) in a South Carolina estuary. *J. Mamm*. 83(1), 178-187.

Hansen, L.J., L.H. Schwacke, G.B. Mitchum, A.A. Hohn, R.S. Wells, E.S. Zolman and P.A. Fair 2004. Geographic variation in polychlorinated biphenyl and organochlorine pesticide concentrations in the blubber of bottlenose dolphins from the U.S. Atlantic coast. *Sci. Total Eviron.* 319, 147-172.

Hayes S.A., Josephson, E., Maze-Foley, K., Rosel, P.E., Byrd, B., Chavez-Rosales, S., Col, T.V.N., Engleby, L., Garrison, L.P., Hatch, J., Henry, A., Horstman, S.C., Litz, J., Lyssikatos, M.C., Mullin, K.D., Orphanides, C., Pace, R.M., Palka, D.L., Soldevilla, M., Wenzel, F.W., 2018. TM 245 US Atlantic and Gulf of Mexico Marine Mammal Stock Assessments - 2017. NOAA Tech Memo NMFS NE-245; 371

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

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environ. Sci. Technol.* 46(6), 3060-3075.

Houde, M., Pacepavicius, G., Darling, C., Fair, P.A., Alaee, M., Bossart, G.D., Solomon, K.R., Letcher, R.J., Bergman, A., Marsh, G., Muir, D.C.G., 2009. Polybrominated diphenyl ethers and their hydroxylated analogs in plasma of bottlenose dolphins (*Tursiops truncatus*) from the United States east coast. *Environ. Toxicol. Chem*. 28(10), 2061-2068.

Isobe, A., Uchida, K., Tokai, T., and Iwasaki, S., 2015. East Asian seas: A hot spot of pelagic microplastics. *Mar. Pollut. Bull*. 101(2), 618-623.

Koelmans, A.A., Nor, N.H.M., Hermsen, E., Kooi, M., Mintenig, S.M., and De France, J. 2019. Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. *Water Research*, 155:410-422.

Kuhn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., and van Franeker, J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Mar. Pollut. Bull*. 115(1-2), 86-90.

Leads, R,R. and Weinstein, J.E., 2019. Occurrence of tire wear particles and other microplastics within the tributaries of the Charleston Harbor Estuary, South Carolina, USA. *Mar. Pollut. Bullet*. 145, 569-582.

Lenz, R., Enders, K., Stedmon, C.A., Mackenzie, D.M.A., Nielson, T.G., 2015. A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement. *Mar. Pollut. Bull.* 100(1), 82-91.

Lusher, A.L. McHugh, M., and Thompson R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull*. 67(1-2), 94-99.

Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, O'Connor, I., Officer, R., 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: The True's beaked whale *Mesoplodon mirus*. *Environ. Pollut.* 199, 185-191.

Lusher, A.L., Hernandez-Milian, G. Berrow, S., Rogan, E., O'Connor, I., 2018. Incidence of marine debris in cetaceans stranded and bycaught in Ireland: Recent findings and a review of historical knowledge. *Environ. Pollut.* 232, 467-476.

McFee, W.E., Hopkins-Murphy, S.R., and Schwacke, L.H., 2006. Trends in bottlenose dolphin (*Tursiops truncatus*) strandings in South Carolina, USA, 1997-2003: Implications for the Southern North Carolina and South Carolina Management Units. Journal of Cetacean Research and Management 8(2), 195-201.

McFee, W., 2014. Report on the Entanglement of Marine Species in Marine Debris with an Emphasis on Species in the United States. National Oceanic and Atmospheric Administration Marine Debris Program Silver Spring, MD. 28 pp

McFee, W.E., Speakman, T.R. Balthis, L., Adams, J.D., Zolman, E.S., 2014. Reproductive seasonality of a recently designated bottlenose dolphin stock near Charleston, South Carolina, U.S.A. *Mar. Mamm. Sci*. 30(2), 528-543.

Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environ. Res*. 108, 131-139.

Moore, R.C., Loseto, L., Noel, M., Etemadifar, A., Brewster, J.D., MacPhee, S., Bendell, L., Ross, P.S., 2020. Microplastics in beluga whales (*Delphinapterus leucas*) from the Eastern Beaufort Sea. *Mar. Pollut. Bull.* 150, 110723.

Murray, F., and Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). *Mar. Pollut. Bullet*. 62, 1207-1217.

Naik, R.A., Rowles, L.S., Hossain, A.I., Yen, M., Aldossary, R.M., Apul, O.G., Conkle, J., Saleh, N.B. 2020. Microplastic particle versus fiber generation during photo-transformation in simulated seawater. *Sci. Tot. Environ.* doi: 10.1016/j.scitotenv.2020.139690

Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. *Environ. Pollut*. 238, 1–9.

Payton, T.G., Beckingham, B.A., Dustan, P. 2020. Microplastic exposure to zooplankton at tidal fronts in Charleston Harbor, SC USA. *Estuar. Coast. Shelf Sci*. 232, 106510. doi: 10.1016/j.ecss.2019.106510

Parker, B.W., Beckingham, B.A., Ingram, B.C., Ballenger, J.C., Weinstein, J.E., Sancho, G. (*In review)* Microplastic and tire wear particle occurrence in fishes from an urban estuary: influence of feeding characteristics on exposure risk. *Mar. Pollut. Bull.*

Pate, S.M., and McFee, W.E., 2012. Prey species of bottlenose dolphins (*Tursiops truncatus*) from South Carolina waters. *Southeastern Naturalist*. 11(1), 1-22.

Peters, C.A., and Bratton, S.P., 2016. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. *Environ. Pollut*. 210, 380-387.

Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Bravo Rebolledo, E.L., Hammer, S., Kuhn, S., Lavers, J.L., Mallory, M.L., Trevail, A., van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: A review and recommendations for standardization. *Anal. Methods*, 9, 1454-1469.

Puig-Lozano, R., Bernaldo de Quiros, Y., Diaz-Delgado, J., Garcia-Alvarez, N., Sierra, E., De la Fuente, J., Sacchini, S., Suarez-Santana, CM., Zucca, D., Camara, N., Saavedra, P., Almunia, J., Rivero, M.A., Fernandez, A., Arbelo, M., 2018. Retrospective study of foreign body associated pathology in stranded cetaceans, Canary Islands (2000-2015). *Environ. Pollut*. 243, 519-527.

Ragland, J., 2014. Report on the occurrence and health effects of anthropogenic debris ingested by marine organisms. National Oceanic and Atmospheric Administration Marine Debris Program. Silver Spring, MD. 19 pp

Reif, J.S., Schaefer, A.M., and Bossart, G.D. 2015. Atlantic bottlenose dolphins (*Tursiops truncatus*) as a sentinel for exposure to mercury in humans: Closing the loop. *Vet. Sci*. 2(4), 407-422.

Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G., Lepoint, G. 2015. When microplastic is not plastic: The ingestion of artificial cellulose fibers by macrofauna living in seagrass macrophytodetritus. *Environ. Sci. Technol.* 49, 11158-11166.

Roch, S., and Brinker, A., 2017. Rapid and Efficient Method for the Detection of Microplastic in the Gastrointestinal Tract of Fishes. *Environ. Sci. Technol.* 51 (8), 4522-4530.

Speakman, T., S.M. Lane, L.H. Schwacke, P.A. Fair and E.S. Zolman. 2010. Mark-recapture estimates of seasonal abundance and survivorship for bottlenose dolphins (*Tursiops truncatus*) near Charleston, South Carolina, USA. J. Cetacean Res. Manage. 11(2): 153-162.

Van Cauwenberghe, L., Vanreusel, A., Mees, J., and Janssen, C.R., 2013. Microplastic pollution in deep sea sediments. *Environ. Pollut*. 182, 495-499.

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C.R., Marques, A., Granby, K., Fait, G., Kotterman, M.J.J., Diogene, J., Bekaert, K., Robbens, J., Devriese, L., 2015. A critical view on microplastics quantification in aquatic organisms. *Environ. Res*. 143, 46-55.

van Franeker, J.A., Bravo Rebolledo, E.L., Hesse, E., Ijsseldijk, L.L., Kühn, S., Leopold, M., Mielke, L., 2018. Plastic ingestion by harbour porpoises *Phocoena phocoena* in the Netherlands: establishing a standardised method. *Ambio*. 47, 387–397.

Waite, H.R., Donnelly, M.J., and Walters, L.J. 2018. Quantity and types of microplastics in the organic tissues of the eastern oyster *Crassostrea virginia* and Atlantic mud crab *panopeus herbstii* from a Florida estuary. *Mar. Pollut. Bull.* 129, 179-185.

Weinstein, J.E., Crocker, B.K., and Gray, A.D., 2016. From macroplastic to microplastic: Degradation of high-density polyethylene, polypropylene, and polystyrene in a salt marsh habitat. *Environ Toxicol. Chem*. 35(7), 1632-1640.

Wells, R.S., Rinehart, H.L., Hansen, L.J, Sweeney, J.C., Townsend, F.I., Stone, R., Casper, D.R., Scott, M.D., Hohn, A.A., and Rowles, T.K. 2004. Bottlenose dolphins as marine ecosystem sentinels: Developing a health monitoring system. *Ecohealth*. 1, 246-254.

Wright S.L., Thompson R.C., Galloway T.S., 2013. The physical impacts of microplastics on marine organisms: a review. *Environ Pollut.* 178, 483–492.

Xiong, X., Chen, X., Zhang, K., Mei, Z., Hao, Y., Zheng, J., Wu, C., Wang, K., Ruan, Y., Lam, P.K.S., and Wang, D., 2018. Micropalstics in the intestinal tracts of East Asian finless porpoises (*Neophocoena asiaeorientalis sunameri*) from Yellow Sea and Bohai Sea of China. *Mar. Pollut. Bull.* 136, 55-60.

Zolman, E.S., 2002. Residence patterns of bottlenose dolphins (*Tursiops truncatus*) in the Stono River estuary, Charleston County, South Carolina, U.S.A. *Mar. Mamm. Sci*. 18, 879-892.

Zhu, J., Yu, X, Zhang, Q., Li, Y., Tan, S., Li, D., Yang, Z., and Wang, J., 2019. Cetaceans and microplastics: First report of microplastic ingestion by a coastal delphinid, *Sousa chinensis. Sci. Tot. Environ.* 659, 649-654.

***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: