Availability and assessment of microplastic ingestion by marsh birds in Mississippi Gulf Coast tidal marshes

Spencer L. Weitzel[a, b, *], Jared M. Feura[b], Scott A. Rush[a], Raymond B. Iglay[a], Mark S. Woodrey[a, b]

[a]Department of Wildlife, Fisheries and Aquaculture, 775 Stone Boulevard, Mississippi State University, Mississippi State, MS 39762, USA

[b]Coastal Research and Extension Center, Mississippi State University, 1815 Popps Ferry Road, Biloxi, MS 39532, USA

[*]corresponding author – weitzel_spencer@yahoo.com

Abstract

Millions of tons of plastic enter the environment every year, where much of it concentrates in environmental sinks such as tidal marshes. With prior studies documenting harm to marine fauna caused by this plastic pollution, the need to understand how this novel type of pollution affects estuarine fauna is great. Yet, research on the fate and uptake of plastic pollutants in estuarine ecosystems is sparse. Therefore, we quantified plastic prevalence and ingestion by two species of resident marsh bird, Clapper Rails (*Rallus crepitans*) and Seaside Sparrows (*Ammospiza maritima*), in coastal marsh ecosystems within Mississippi. We detected microplastics (plastics smaller than 5mm) in 64% of marsh sediment samples, 83% of Clapper Rail and 69% of Seaside Sparrow proventriculus samples. Dominant types of microplastics detected in sediment and bird samples were fibers. This study provides the first evidence of microplastic ingestion by marsh birds and its distribution in coastal marshes within Mississippi.

Keywords Clapper Rail; estuarine; microfiber; pollution; Seaside Sparrow; stomach flushing

Introduction

Plastic pollution in the environment and its subsequent degradation into microplastics has become a mounting global issue (Barnes et al., 2009; Derraik, 2002; Wilcox et al., 2015). Recent findings estimate that 4.8 to 12.7 million metric tons of plastic enter the world's oceans every year, with that number projected to increase (Jambeck et al., 2015). While in the environment, plastics may undergo fragmentation into smaller pieces (Barnes et al., 2009; Wang et al., 2016). These plastic pieces are then transported globally via ocean currents and eventually end up in environmental accumulation zones, or sinks (Cole et al., 2011; Zhang, 2017). Coastal ecosystems, like tidal marshes, are believed to be one such sink for environmental plastic pollutants (Zhang, 2017). In tidal marshes, degradation of larger plastic materials into smaller pieces occurs relatively quickly (Weinstein et al., 2020, 2016).

Various organisms inhabiting estuarine ecosystems directly or indirectly ingest plastic pollutants (Payton, 2017; Waite et al., 2018). However, the current presence, and future persistence of plastics in the environment is not restricted to coastal ecosystems. Numerous studies have documented the ingestion of plastic pieces by a myriad of animal taxa in other marine and aquatic environments (Puskic et al., 2020). One notable taxa shown to ingest large quantities of plastics are seabirds, with predictions estimating 99% of seabird species will have ingested plastic particles by 2050 (Wilcox et al., 2015). In addition to marine-associated birds, freshwater-associated bird species (ducks, geese, and loons) have also been documented to ingest

plastic (Holland et al., 2016). In severe cases, plastic ingestion by birds can lead to mortality, morbidity, and disrupted physiology (Lavers et al., 2014; Lavers et al., 2019).

Along the northern Gulf of Mexico (GoM) plastic pollution has been documented in various coastal ecosystem sinks: barrier islands (Wessel et al., 2019), estuarine beach sediments (Wessel et al., 2016), oyster reefs (Scircle et al., 2020), and in the coastal waters (Mauro et al., 2017). The quantities of microplastics found here are amongst the highest reported in the world (Mauro et al. 2017); however, accumulation rates of this plastic vary widely within the GoM due in part to oceanic currents, winds, and tides (Wessel et al., 2019). Though microplastic pollution in the northern GoM is ubiquitous, higher concentrations have been reported in marine-dominated locations closer to the gulf waters than upriver riverine-dominated locations within an estuary (Wessel et al., 2016). This suggests that the source of these microplastics is the GoM and that they circulate with the oceanic currents and are brought in with the tides. In addition to varying geographically, plastic pollution in the GoM was found to vary temporally as well, with greater accumulation along coastal habitats during the spring and early summer than other periods of the year (Wessel et al., 2019).

Here, we focus on microplastics, which we define as any piece of plastic smaller than 5mm. The objectives of this study are to 1) quantify microplastic distribution and prevalence within tidal marsh sediments of Mississippi, 2) determine possible microplastic ingestion by two resident tidal marsh bird species, Clapper Rail (*Rallus crepitans*) and Seaside Sparrow (*Ammospiza maritima*), and 3) compare amounts ingested by Clapper Rails versus Seaside Sparrows. We also compare the quantities of microplastic pieces ingested by these two species among marsh complexes, how ingested quantities relate to microplastic concentration and availability in surrounding marsh sediment, and how ingestion may vary at further distances of

bird capture location from the GoM. We include distance from the GoM to investigate the potential source of the microplastics – flowing downstream from upriver or being brought in with the tides from the GoM and associated oceanic currents.

We hypothesize that resident tidal marsh birds ingest microplastics but that ingestion rates vary with respect to species-specific foraging strategies, as has been found in fish (Peters et al., 2017). Clapper Rails feed on a variety of organisms during the breeding season, predominantly harvesting prey items by probing into muddy marsh sediments. In Mississippi their diet during the breeding season is made up of predominantly fiddler crabs (*Uca* spp.; Rush et al., 2010b, 2020). Fiddler crab species such as *U. rapax* have been experimentally shown to ingest microplastics and subsequently transfer them into their organs, where they can persist for weeks (Brennecke et al., 2015). In Georgia, the third most prevalent food item found in Clapper Rail stomachs were periwinkle snails (*Littorina irrorata*; Oney, 1951), which have also been noted to forage on microplastic surfaces (Weinstein et al., 2016, 2020). Thus, the mode of microplastic intake by Clapper Rails may be indirect, through consumption of prey items which themselves have ingested microplastics, which then results in microplastics moving through the food chain, as has been noted elsewhere (Athey et al., 2020; Cedervall et al., 2012). Seaside Sparrows generally feed on the surface of the marsh platform, where they glean insects (moths, grasshoppers, spiders) and insect larvae from nearby vegetation and the mud surface (Post and Greenlaw, 2020). Therefore, it is possible that they consume microplastics directly when foraging on the marsh surface, potentially mistaking plastic pieces for food items, as noted among many animal taxa which consume macro or microplastics (Cole et al., 2011; Derraik, 2002). We predict microplastic ingestion by Clapper Rails is higher due to the likelihood of

encountering more pieces via probing into the marsh surface rather than ingestion by Seaside Sparrows gleaning prey from the marsh surface.

Materials and methods

Study area

We conducted microplastic sampling among three riverine-dominated estuarine marshes in Mississippi (the Pascagoula, Jourdan, and Pearl Rivers; Fig. 1). The Pascagoula River is the only free-flowing river in the lower 48 United States (Nilsson et al., 2005). The river flows into approximately 6,300ha of estuarine emergent marsh (NOAA, 2010), most of which is protected as a coastal preserve. The majority of the lands along the banks of the Pascagoula River are protected as wildlife management areas and conservation preserves. The population density in this area is 29 persons/km^2 (US Census Bureau, 2019). The Jourdan River borders 1,500ha of estuarine emergent marsh (NOAA, 2010) which is also protected as a coastal preserve. Directly upriver are a housing development and agricultural communities. The population density in this area is 218 persons/km^2 (US Census Bureau, 2019). The lower Pearl River serves as the boundary between Mississippi and Louisiana, with 6,800ha of conserved estuarine emergent marsh (NOAA, 2010) on the Mississippi side alone. Directly upriver from this study site lies large tracts of wildlife management areas, agricultural communities, and a federal government rocket engine test site. The population density in this area is 7 persons/km^2 (US Census Bureau, 2019), but approximately 240km (straight-line distance) upriver from the study site lies the city of Jackson, which has an estimated population of 160,000. We chose these marshes because they are three of the largest riverine-dominated tidal marshes, allowing us to assess microplastic concentrations in estuarine sediments at various points along the rivers as well as between marsh complexes.

Using data from Leggett (2014), we determined the upper-most reaches of each river system where Seaside Sparrows and Clapper Rails were observed and marked a starting point along each river. We then distributed four evenly spaced sampling points between this upstream starting point and downstream where the river meets the GoM in each marsh complex. We placed each sample point at the edge of the marsh where the emergent vegetation met the river. To maintain independence among our bird samples, sample points were placed ≥ 1,000m apart, more than 10x the average home range radius of Clapper Rails and Seaside Sparrows (Rush et al., 2010a; Post and Greenlaw, 2020).

Sediment sample collection & processing

During March 2019, we collected three replicate sediment samples at each of the four sampling locations per marsh (Fig. 1), for a total of 36 sediment samples. Each replicate sediment sample was collected at least one meter apart from one another to maintain independence between samples. All samples were collected within three meters of the marsh edge, to estimate both fine and large scale variability in microplastic prevalence. We used a modified version of Gray et al.'s (2018) sediment sampling approach demonstrated in South Carolina estuaries. For each sample, we placed a 10 x 10cm PVC quadrat over the marsh sediment and collected the top two centimeters of sediment within the quadrat during low tide using a metal spatula. We then placed the sample in a clear Ziploc® bag that was thoroughly pre-rinsed with distilled water prior to sample collection to ensure absence of plastic particles. We brought the sample back to the laboratory and stored it in a freezer until further analysis (within three months of collection).

In the laboratory, sediment processing began with drying samples in a drying oven at 40°C for two days. Once dry, we weighed samples for total dry mass (g) and then sieved each

sample through a series of stacked sieves (4mm, 500µm, 250µm, 125µm, 63µm; modified from Gray et al., 2018). We recorded any obvious plastic pieces retained on sieves and after rinsing to ensure no microplastics were attached, we disposed large plant material. Sample remains on the sieves then underwent density separation by being rinsed into a 1000mL glass beaker with 500mL water and 150g NaCl, and then stirred for five minutes with a metal spatula to agitate the sample and free any trapped microplastics (modified from Gray et al., 2018). The sample was then covered with a ceramic lid and left in solution for 24 hours to allow less dense plastics to separate from denser material and float (Kazmiruk et al., 2018). After 24 hours, the supernatant was poured through a sieve stack (250µm, 125µm, 63µm), and retained material was placed in a 50mL amber glass jar. The amber glass jar was then placed on a hotplate stirrer set at 40°C with 20mL of 30% H_2O_2 for two hours to dissolve organic material (modified from Willis et al., 2017; Gray et al., 2018). If organic material persisted after two hours, an additional 10mL of H_2O_2 was added and the process repeated until almost all of the organic material had dissolved. Once the organic material dissolved, the sample was rinsed into a petri dish and observed under a dissection microscope. The prevalence of microplastics in sediment samples was reported as pieces/g (dry weight) of the marsh substrate from the initial sample.

Bird sample collection & processing

At each sampling location (n = 4/marsh complex), we attempted to catch three Clapper Rails and three Seaside Sparrows from May to August 2019, during the breeding season when birds are most easily captured. To capture Clapper Rails, we placed a modified mist net (8m long x 60cm high) anchored at the marsh substrate extending vertically in the marsh vegetation. Then, we broadcasted previously recorded Clapper Rail calls from speakers located on both sides of the modified mist net to lure birds into the net where they became entangled (J. Feura, personal

communication). Once captured, we safely removed birds from the net and placed them in a cloth cotton bag to await further processing. To capture Seaside Sparrows, we first located individual sparrows near the sample point using pre-recorded Seaside Sparrow audio recordings. Once we located sparrow(s), we erected a standard 12-meter-long mist net (30mm mesh) near where the bird was found, then had three to four people form a semi-circle around the bird and walked toward the net, "herding" it into the net, where we then safely removed and placed each bird in a separate cloth cotton bag.

After placing a leg band on each individual rail or sparrow, we conducted non-lethal stomach flushing to obtain a sample of the stomach contents (Barrett et al., 2007; Ford et al., 1982). Previous research used stomach flushing successfully to investigate the ingestion of plastics in other bird species (Lavers et al., 2014). Specifically, a lavage tube (4mm outside-diameter, 26cm clear flexible vinyl tube for Clapper Rail, 14-gauge diameter, 7.6cm curved stainless steel rigid veterinary feeding tube for Seaside Sparrow) was moistened with distilled water and carefully inserted into the bird's esophagus until it reached the proventriculus region of the stomach. Then a syringe (40 mL for Clapper Rail, 5mL for Seaside Sparrow) was used to gently pump ambient-temperature distilled water (30mL for Clapper Rail, 2mL for Seaside Sparrow) into the bird's proventriculus through the tube to displace its contents. Once the proventriculus was full of water, the bird was inverted over a collection tray where regurgitated contents and water were collected (Barrett et al., 2007). The bird was then released in the same area where it was caught. In total, each bird was held < 30 minutes from capture until release. Collected samples were immediately rinsed from the collection tray into a sealable Ziploc® bag that was pre-rinsed with distilled water and stored in an enclosed case while in the field.

Samples were transported back to the lab, where they were stored in a refrigerator at 0°C from one to five days prior to additional processing. For each stomach sample collected in the field, we repeated the processing steps noted previously for separating and collecting microplastics from the sediment samples; however, the drying and density separation steps were deemed unnecessary due to the lack of sediment in stomach samples. Since Seaside Sparrows had much less material (specifically fewer shells from *Uca spp.*) in their stomach samples, we opted for using a vacuum pump to pull the sample through a 0.45 µm mixed cellulose ester membrane filter, and then placing the filter in a petri dish to be examined under a microscope.

Enumeration and contamination prevention of microplastic samples

We visually counted microplastics using a 40x magnification dissecting microscope (AmScope Irvine, California), and classified each piece by type: fiber – thin, hair-like strands, fragment – pieces with varying shapes, film – thin, often translucent pieces, or microbead – small, spherical pieces (Sartain et al., 2018) . To minimize sample contamination, we rinsed instruments and containers before and after each processing step with distilled water; however, partway through the study we detected low concentrations of microplastics within the distilled water. We subsequently began pre-filtering the distilled water used in the field and lab for rinsing and processing samples by first pouring it through our finest sieve and ensuring we didn't detect any microplastics in the water after pre-filtering. During lab extraction, cotton white lab coats and clothing, and nitrile gloves were worn while sorting and processing samples. In addition, all samples were kept covered during processing.

To quantify possible contamination of the samples, we performed five controls for the different sample processing methods using both regular and pre-filtered distilled water. We collected all sediment samples before implementing pre-filtration of the distilled water used to

rinse instruments and containers, and we captured all Seaside Sparrows after implementing the pre-filtration process. Therefore, the controls included 1) five controls for the Seaside Sparrow process using pre-filtered distilled water; 2) five controls for the Clapper Rail process using pre-filtered distilled water; 3) and five controls for the Clapper Rail/sediment process without first pre-filtering the distilled water). Controls included conducting all processing steps without any biologic sample present and examining control petri dishes under a microscope and enumerating any microplastics detected. From this procedure, we detected low levels of possible sample contamination. Therefore, to correct all biological sample microplastic counts we subtracted the mean count of microplastics found in the control samples for each type of control (Clapper Rail methodology using pre-filtered water: $\bar{x} = 3$, Clapper Rail/sediment methodology without pre-filtering the distilled water: $\bar{x} = 5$, Seaside Sparrow methodology: $\bar{x} = 2$).

Statistical analyses

We standardized microplastic counts in sediment samples by the dry mass (g) of the sediment sample to obtain a concentration of microplastic pieces per 100g of sediment. To investigate trends and differences in microplastic concentration in the sediment samples at different distances from the GoM along the river and across marsh complexes, we created a set of four candidate generalized linear mixed-effect models in package "glmmTMB" (Brooks et al., 2017) in the statistical programming software R (R Core Team, 2018). We used the concentration of microplastics in the sediment samples as the dependent variable, and (1) a null model, (2) marsh complex, (3) sample point order (1 – 4) – which acts as a proxy for the distance from the GoM along the river, and (4) marsh complex and distance from the GoM as the independent variables in their respective models. As the three repeat sediment samples within a

sample point were not independent, all models included a random-effect variable to control for this non-independence.

To investigate microplastic ingestion by Clapper Rail and Seaside Sparrow, we created a set of 10 candidate generalized linear mixed-effect models. These models examined differences in microplastic counts found in bird stomach samples by species, marsh complex, distance from the GoM, microplastic concentration in the nearby marsh sediment, combinations of these variables, and a null model. Again, as individual bird captures at a sample point may not be independent, we included a random-effect variable in all models to control for this non-independence. We then used Akaike's Information Criterion corrected for small sample sizes (AICc; Akaike, 1974; Burnham and Anderson, 2002) to assess which model(s) from the candidate set best fit the data (ΔAICc < 2), both for the bird ingestion and sediment availability models. We then interpreted the results from the top model(s), but if there was more than one model with a ΔAICc < 2, we model averaged parameter estimates from all models using the function *modavg* in package "AICcmodavg" (Mazerolle, 2019).

Results

We detected microplastics in 64% (n = 23) of sediment samples (median = 10.0 microplastic pieces/100g in sediment samples containing any microplastics, range = zero – 194.0; Table 1). Microplastic fibers dominated samples (98%), with only 2% of detected microplastics being fragments. The highest ranked models (ΔAICc < 2) to describe microplastic concentration in sediment samples were 1) the null model and 2) the model that incorporated distance from the GoM as the predictor variable (Table 2). As the null model was the top model, we conclude that microplastic concentration did not vary among any of the stated factors.

We collected stomach flush samples from 35 Clapper Rails and 36 Seaside Sparrows across all marsh complexes. We detected microplastics in 83% (n = 29) of Clapper Rail and 69% (n = 25) of Seaside Sparrow stomach samples. The median count of microplastic pieces per stomach sample for individuals that contained microplastics was 6 (SD = 7.2) for Clapper Rail and 2 (SD = 2.7) for Seaside Sparrow (Fig. 2). Fibers were the dominant type of microplastics detected in Clapper Rail (99%) and Seaside Sparrow (98%) stomach samples. The models which best fit these data to describe the microplastic counts found in bird stomach samples were 1) the model that incorporated a combination of species and the concentration in the nearby sediment; 2) the model that assessed only differences in ingestion counts by species; and 3) the model that incorporated a combination of species and the marsh complex the bird was captured in (Table 3). According to model averaged parameter estimates, Seaside Sparrow had fewer microplastic pieces on average in their stomach contents than Clapper Rail (-1.11, 95% C.I. -1.40 – -0.81 fewer pieces on average). Although the concentration of microplastics in the paired sediment samples was included as a covariate in the top model, parameter estimates suggest that microplastic ingestion did not differ by the concentration of microplastics in the nearby sediment (0.00, 95% C.I. 0.00 – 0.01). In addition, birds caught in the Pascagoula River and Hancock County marshes showed no difference in microplastic ingestion amounts when compared to birds caught in the Jourdan River marshes (0.32, 95% C.I. -0.02 – 0.67 and 0.31, 95% C.I. -0.02 – 0.65, respectively).

Discussion

This study provides the first evidence of microplastic ingestion by resident tidal marsh birds. Our model selection and parameter estimate results support our hypothesis that the quantity of microplastics ingested is greater in Clapper Rails than Seaside Sparrows. However,

since the stomach volume varies between the two species, future studies should attempt to account for this when making direct comparisons of the total microplastic load between each species. Additionally, since Clapper Rails regularly regurgitate pellets to offload crab and snail shells (Meanley, 1962) whereas Seaside Sparrows do not, the pellets may act as an additional avenue to offload ingested plastics. Therefore, the quantity of microplastics detected in the stomach samples may represent the ability of the two species to pass the microplastics, rather than solely the variation in amounts ingested. Although the top model also included the concentration of microplastics in the nearby sediment, surprisingly this parameter estimates showed no change in predicted microplastic counts found in bird stomach samples with varying microplastic concentrations in the nearby marsh sediment. We suspect this relationship may not have been adequately evaluated due to the high variability in microplastic concentrations in the sediment samples, the overall low counts of microplastics detected in the stomach samples, and small sample sizes.

In addition to providing evidence of microplastic ingestion by marsh birds, this study also provides the first evidence of microplastic prevalence, concentration, and variation within and between tidal marsh sediments along the Mississippi Coast. Although microplastics were prevalent in our sediment samples, we didn't find any fine scale (i.e., within-point variability) or broad scale (i.e., between points within a marsh or between marshes) differences in their concentration in marsh sediment along a riverine gradient within a single marsh complex or between marsh complexes. We believe our distribution of points was too limited, due to being restricted to the occurrence of Clapper Rails and Seaside Sparrows, to detect a possible difference in microplastic concentration in sediment sample points closer to the GoM versus upriver, as found by Wessel et al. (2016). Although we didn't find a trend in sediment

microplastic concentrations, these findings are consistent with previous studies which have investigated microplastic concentration in tidal sediments along the southeastern United States, finding large variability within and among sites (Gray et al., 2018; Yu et al., 2018).

The use of stomach flushing to investigate microplastic loads in marsh birds has its utility, but there are also some important limitations to acknowledge. We recognize that microplastic counts obtained from performing stomach flushing may not fully represent the microplastic load in the rest of the digestive system, as some plastic pieces may linger in the stomach after stomach flushing (Sileo et al., 1990). In addition, the effectiveness of the stomach flushing process to obtain a regurgitant sample for microplastic investigation may be affected by whether the bird species regularly regurgitates food or not. For example, Lavers et al. (2014) used stomach flushing on seabirds which regularly regurgitate food for their chicks, whereas Clapper Rails and Seaside Sparrows do not. When stomach and gut content samples are necessary as part of understanding a population decline or developing a plan for conserving a species, the use of non-lethal stomach flushing can be a preferred method when dealing with species of conservation concern, where receiving federal and state authorization for collecting individual birds is difficult, as well as inadvisable. Although stomach flushing is just one of several ethical and approved alternative non-lethal techniques to sample avian diets (Fair et al., 2010), previous studies have also used fecal sampling as a non-lethal method to estimate microplastic loads in birds (Bessa et al., 2019). However, due to the inability to locate fecal samples in the marsh and impracticality of holding a captured live bird until it produces a fecal sample, we chose not to pursue this method. Furthermore, non-lethal stomach flushing allows for flexible and adaptable study designs for other hypothesis-driven studies on microplastics to meet calls for increasing the rigor of microplastics research, rather than relying on opportunistic

sampling events which may be less robust for statistical inference to a larger population (Provencher et al., 2020).

As this study is the first to investigate microplastic ingestion by marsh birds, we provide a few suggestions here to consider during future similar studies. First, efforts to increase sample size across broader geographic areas should be a primary focus. We recognize the limitations of our relatively small sample sizes across a relatively limited geographic area. We caution readers to be judicious in extrapolating results beyond Mississippi. Second, since our sample size was small and counts of microplastics in stomach samples generally low, we recognize that the methods used to correct biological samples for laboratory and field contamination may affect calculations of the proportion of birds and sediment in which microplastics were present. Third, due to the high spatial and individual variability in sediment and stomach sample microplastic counts detected in our samples, we recommend variability be accounted for as part of a sampling and/or study design, particularly when designing future sampling frameworks to monitor microplastics availability and ingestion across and within tidal marsh ecosystems. Fourth, comparing amounts and types of prey items found in individual stomach samples may help to elucidate an important relationship between prey consumed and varying microplastic loads. Despite these shortcomings, we believe that this study provides sufficient evidence of baseline microplastic ingestion by two resident tidal marsh bird species in Mississippi.

Although this research significantly increases our understanding of the uptake of microplastics by marsh birds in tidal ecosystems, additional work is needed to determine both the acute and chronic effects of microplastic ingestion on tidal marsh vertebrates, understand the transfer of microplastics through estuarine food webs, and for understanding the ultimate fate of environmental plastic pollution. More broadly, this study adds to the growing body of literature

on the prevalence of microplastic ingestion by animal taxa exposed to microplastics found throughout the environment. We believe that as plastic pollution in the environment is projected to increase (Jambeck et al., 2015), the ingestion of these plastics by species inhabiting tidal marsh ecosystems may increase as well.

Acknowledgements

We thank Anna Gamblin and Utsaha Gurung for their help with field and laboratory work. We are grateful to Amanda Sartain, Eric Sparks, Caitlin Wessel, and many others who helped familiarize us with microplastic sample processing. Lastly, we are especially thankful for the many volunteers that helped us with field work throughout the study. This publication is a contribution of the Mississippi Agricultural and Forestry Experiment Station and based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project (JMF, MSW). Additional funding support included National Oceanic and Atmospheric Administration [Awards #NA17NOS4200045, #NA18NOS4200063] to the Mississippi Department of Marine Resources' Grand Bay National Estuarine Research Reserve (MSW), Mississippi Department of Marine Resources [Contract #8200025414] to Mississippi State University (MSW), and Mississippi Department of Environmental Quality – Gulf Environmental Benefit Fund [Grant #335414] to Mississippi State University (JMF, MSW, RBI, SLW). This work was conducted under Mississippi State University Institutional Animal Care and Use committee protocol IACUC-19-134, federal bird banding permit #23835 (SAR), Mississippi Department of Marine Resources special use permit #SRP-020-19, and Mississippi Department of Wildlife, Fisheries, and Parks state scientific collecting permit. Two anonymous reviewers provided valuable comments helping to improve the quality of this manuscript.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19, 716–723.

Athey, S.N., Albotra, S.D., Gordon, C.A., Monteleone, B., Seaton, P., Andrady, A.L., Taylor, A.R., Brander, S.M., 2020. Trophic transfer of microplastics in an estuarine food chain and the effects of a sorbed legacy pollutant. Limnol. Oceanogr. Lett. 5, 154–162. https://doi.org/10.1002/lol2.10130

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. B Biol. Sci. 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Barrett, R.T., Camphuysen, K., Anker-Nilssen, T., Chardine, J.W., Furness, R.W., Garthe, S., Hüppop, O., Leopold, M.F., Montevecchi, W.A., Veit, R.R., 2007. Diet studies of seabirds: A review and recommendations. ICES J. Mar. Sci. 64, 1675–1691. https://doi.org/10.1093/icesjms/fsm152

Bessa, F., Ratcliffe, N., Otero, V., Sobral, P., Marques, J.C., Waluda, C.M., Trathan, P.N., Xavier, J.C., 2019. Microplastics in gentoo penguins from the Antarctic region. Sci. Rep. 9, 14191. https://doi.org/10.1038/s41598-019-50621-2

Brennecke, D., Ferreira, E.C., Costa, T.M.M., Appel, D., da Gama, B.A.P., Lenz, M., 2015. Ingested microplastics (>100μm) are translocated to organs of the tropical fiddler crab *Uca rapax*. Mar. Pollut. Bull. 96, 491–495. https://doi.org/10.1016/j.marpolbul.2015.05.001

Brooks, M.E., Kristensen, K., Koen, J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inglated Generalized Linear Mixed Modeling. R Journal 9, 378–400.

Burnham, K.P., Anderson, D.R., 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York, USA.

Cedervall, T., Hansson, L.A., Lard, M., Frohm, B., Linse, S., 2012. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. PLoS One 7, e32254. https://doi.org/10.1371/journal.pone.0032254

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Derraik, J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44, 842–852. https://doi.org/10.1016/S0025-326X(02)00220-5

Fair, J., Paul, E., Jones, J., 2010. Guidelines to the use of wild birds in research. Ornithological Council, Washington, D.C. https://doi.org/10.2307/1369129

Ford, H.A., Forde, N., Harrington, S., 1982. Non-destructive methods to determine the diets of birds. Corelia 6, 6–10.

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. https://doi.org/10.1016/j.marpolbul.2018.01.030

Holland, E.R., Mallory, M.L., Shutler, D., 2016. Plastics and other anthropogenic debris in freshwater birds from Canada. Sci. Total Environ. 571, 251–258. https://doi.org/10.1016/j.scitotenv.2016.07.158

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771. https://doi.org/10.1017/CBO9781107415386.010

Kazmiruk, T.N., Kazmiruk, V.D., Bendell, L.I., 2018. Abundance and distribution of microplastics within surface sediments of a key shellfish growing region of Canada. PLoS One 13, 1–16. https://doi.org/10.1371/journal.pone.0196005

Lavers, J.L., Bond, A.L., Hutton, I., 2014. Plastic ingestion by Flesh-Footed Shearwaters (*Puffinus carneipes*): implications for fledgling body condition and the accumulation of plastic-derived chemicals. Environ. Pollut. 187, 124–129. https://doi.org/10.1016/j.envpol.2013.12.020

Lavers, J.L., Hutton, I., Bond, A.L., 2019. Clinical pathology of plastic ingestion in marine birds and relationships with blood chemistry. Environ. Sci. Technol. 53, 9224–9231. https://doi.org/10.1021/acs.est.9b02098

Leggett, A.H., 2014. Distribution, abundance, and habitat associations of breeding marsh birds in Mississippi tidal marsh. Thesis. University of Georgia, Athens, Georgia, USA.

Mauro, R.D., Kupchik, M.J., Benfield, M.C., 2017. Abundant plankton-sized microplastic particles in shelf waters of the northern Gulf of Mexico. Environ. Pollut. 230, 798–809. https://doi.org/10.1016/j.envpol.2017.07.030

Mazerolle, M.J., 2019. AICcmodavg: model selection and multimodel inference based on (Q)AIC(c). R package version 2.2-2.

Meanley, B., 1962. Pellet casting by King and Clapper Rails. The Auk 79, 113. https://doi.org/10.2307/4082461

Nilsson, C., Reidy, C.A., Dynesius, M., Revenga, C., 2005. Fragmentation and flow regulation of the world's large river systems. Science 308, 405–408. https://doi.org/10.1126/science.1107887

NOAA, 2010. 2010 C-CAP Regional Land Cover [dataset]. National Oceanic and Atmospheric Administration (NOAA) Office for Coastal Management, Charleston, South Carolina, USA. https://coast.noaa.gov/digitalcoast/data/

Oney, J., 1951. Fall food habits of the Clapper Rail in Georgia. J. Wild. Manage. 15, 106–107. https://doi.org/10.2307/3796779

Payton, T.G., 2017. Microplastic in the estuarine food web of Charleston Harbor, SC. University of Charleston, South Carolina, USA.

Peters, C.A., Thomas, P.A., Rieper, K.B., Bratton, S.P., 2017. Foraging preferences influence microplastic ingestion by six marine fish species from the Texas Gulf Coast. Mar. Pollut. Bull. 124, 82–88. https://doi.org/10.1016/j.marpolbul.2017.06.080

Post, W., Greenlaw, J.S., 2020. Seaside Sparrow (*Ammospiza maritima*), version 1.0, in: Rodewald, P.G. (Ed.), Birds of the World. Cornell Lab of Ornithology, Ithaca, New York, USA.

Provencher, J.F., Covernton, G.A., Moore, R.C., Horn, D.A., Conkle, J.L., Lusher, A.L., 2020. Proceed with caution: The need to raise the publication bar for microplastics research. Sci. Total Environ. 748, 141426. https://doi.org/10.1016/j.scitotenv.2020.141426

Puskic, P.S., Lavers, J.L., Bond, A.L., 2020. A critical review of harm associated with plastic ingestion on vertebrates. Sci. Total Environ. 743, 140666. https://doi.org/10.1016/j.scitotenv.2020.140666

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Rush, S.A., Gaines, K.F., Eddleman, W.R., Conway, C.J., 2020. Clapper Rail (*Rallus crepitans*), version 1.0, in: Birds of the World. Cornell Lab of Ornithology, Ithaca, New York, USA.

Rush, S.A., Mordecai, R., Woodrey, M.S., Cooper, R.J., 2010a. Prey and habitat influences the movement of Clapper Rails in northern Gulf Coast estuaries. Waterbirds 33, 389–396. https://doi.org/10.1675/063.033.0317

Rush, S.A., Olin, J.A., Fisk, A.T., Woodrey, M.S., Cooper, R.J., 2010b. Trophic relationships of a marsh bird differ between gulf coast estuaries. Estuaries and Coasts 33, 963–970. https://doi.org/10.1007/s12237-010-9281-6

Sartain, M., Wessel, C., Sparks, E., 2018. Microplastics sampling and processing guidebook. Mississippi State, Mississippi, USA.

Scircle, A., Cizdziel, J.V., Tsinger, L., Anumol, T., Robey, D., 2020. Occurrence of microplastic pollution at oyster reefs and other coastal sites in the Mississippi Sound, USA: impacts of freshwater inflows from flooding. Toxics 8, 35. https://doi.org/10.3390/toxics8020035

Sileo, L., Sievert, P.R., Samuel, M.D., Fefer, S.I., 1990. Prevalence and characteristics of plastic ingested by hawaiian seabirds, Proceedings of the Second International Conference on Marine Debris, 2-7 April 1989, Honolulu, Hawaii, USA.

US Census Bureau. 2019. American community survey 5-year demographic and housing Estimates [dataset], Table DP05. https://data.census.gov/cedsci/

Waite, H.R., Donnelly, M.J., Walters, L.J., 2018. Quantity and types of microplastics in the organic tissues of the eastern oyster *Crassostrea virginica* and Atlantic mud crab *Panopeus herbstii* from a Florida estuary. Mar. Pollut. Bull. 129, 179–185. https://doi.org/10.1016/j.marpolbul.2018.02.026

Wang, J., Tan, Z., Peng, J., Qiu, Q., Li, M., 2016. The behaviors of microplastics in the marine environment. Mar. Environ. Res. 113, 7–17. https://doi.org/10.1016/j.marenvres.2015.10.014

Weinstein, J.E., Crocker, B.K., 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, 1632–1640. https://doi.org/10.1002/etc.3432

Weinstein, J.E., Dekle, J.L., Leads, R.R., Hunter, R.A., 2020. Degradation of bio-based and biodegradable plastics in a salt marsh habitat: Another potential source of microplastics in coastal waters. Mar. Pollut. Bull. 160, 111518. https://doi.org/10.1016/j.marpolbul.2020.111518

Wessel, C.C., Lockridge, G.R., Battiste, D., Cebrian, J., 2016. Abundance and characteristics of microplastics in beach sediments: insights into microplastic accumulation in northern Gulf of Mexico estuaries. Mar. Pollut. Bull. 109, 178–183. https://doi.org/10.1016/j.marpolbul.2016.06.002

Wessel, C., Swanson, K., Weatherall, T., Cebrian, J., 2019. Accumulation and distribution of marine debris on barrier islands across the northern Gulf of Mexico. Mar Pollut. Bull. 139, 14–22. https://doi.org/10.1016/j.marpolbul.2018.12.023

Wilcox, C., Van Sebille, E., Hardesty, B.D., 2015. Threat of plastic pollution to seabirds is global, pervasive, and increasing. Proc. Natl. Acad. Sci. 112, 11899–11904. https://doi.org/10.1073/pnas.1502108112

Willis, K.A., Eriksen, R., Wilcox, C., Hardesty, B.D., 2017. Microplastic distribution at different sediment depths in an urban estuary. Front. Mar. Sci. 4, 1–8. https://doi.org/10.3389/fmars.2017.00419

Yu, X., Ladewig, S., Bao, S., Toline, C.A., Whitmire, S., Chow, A.T., 2018. Occurrence and distribution of microplastics at selected coastal sites along the southeastern United States. Sci. Total Environ. 613–614, 298–305. https://doi.org/10.1016/j.scitotenv.2017.09.100

Zhang, H., 2017. Transport of microplastics in coastal seas. Estuar. Coast. Shelf Sci. 199, 74–86. https://doi.org/10.1016/j.ecss.2017.09.032

Tables and Figures

Table 1. Microplastic concentrations in sediment samples across three riverine-dominated marsh complexes in coastal Mississippi. Microplastic concentrations are normalized to represent pieces per 100g of dry sediment. Lower sample point numbers indicate being closer to where the river meets the Gulf of Mexico (GoM) increasing (up to 4) as you move distally from the coast within each marsh complex.

Marsh complex (West to East)	**Sample point (distance from the GoM)**	**Median microplastic concentration /100g sediment**	**Standard deviation (+/-)**
Hancock County	1	3	6.8
Coastal Preserve	2	0	2.9
	3	5	3.2
	4	104	89
Jourdan River	1	4	5
Marshes	2	16	29.4
	3	0	5.8
	4	2	4.2
Pascagoula River	1	16	14
Coastal Preserve	2	1	3.2
	3	0	7.5
	4	0	11

Table 2. Model selection table of candidate models for sediment concentrations of microplastics per 100g of sediment collected along an upstream gradient (distance from GoM) across three riverine-dominated marsh complexes in coastal Mississippi.

Model	AICc	ΔAICc	w_i
Null model	445.1	0.0	0.63
Distance from GoM.	446.5	1.4	0.31
Marsh complex	450.3	5.2	0.05
Marsh complex and distance from GoM.	452.0	6.9	0.02

GoM. = Gulf of Mexico. AICc, ΔAICc, and w_i represent metrics of model fit where AICc is the Akaike Information Criterion corrected for small sample sizes (Akaike, 1974; Burnham and Anderson, 2002), ΔAICc is the difference in AICc between each model and the top model, and w_i represents each model's weight among the candidate models used in model averaging parameter estimates.

Table 3. Model selection table of candidate models for the amount of microplastics found in Clapper Rail (*Rallus crepitans*) and Seaside Sparrow (*Ammospiza maritima*) stomach samples with varying amounts of microplastics in the nearby sediment, along an upstream gradient (distance from GoM), and across three riverine-dominated marsh complexes in coastal Mississippi.

Model	AICc	ΔAICc	w_i
Species + sediment	450.8	0.0	0.48
Species	451.8	1.0	0.30
Species + marsh complex	452.7	1.9	0.19
Species + distance from GoM.	458.2	7.4	0.01
Species + marsh complex + sediment + distance from GoM.	458.5	7.7	0.01
Species + marsh complex + distance from GoM.	458.7	7.9	0.01
Sediment	515.5	64.7	0.00
Null model	519.9	69.1	0.00
Marsh complex	522.4	71.6	0.00
Distance from GoM.	524.1	73.3	0.00

GoM. = Gulf of Mexico. AICc, ΔAICc, and w_i represent metrics of model fit where AICc is the Akaike Information Criterion corrected for small sample sizes (Akaike, 1974; Burnham and Anderson, 2002), ΔAICc is the difference in AICc between each model and the top model, and w_i represents each model's weight among the candidate models used in model averaging parameter estimates.

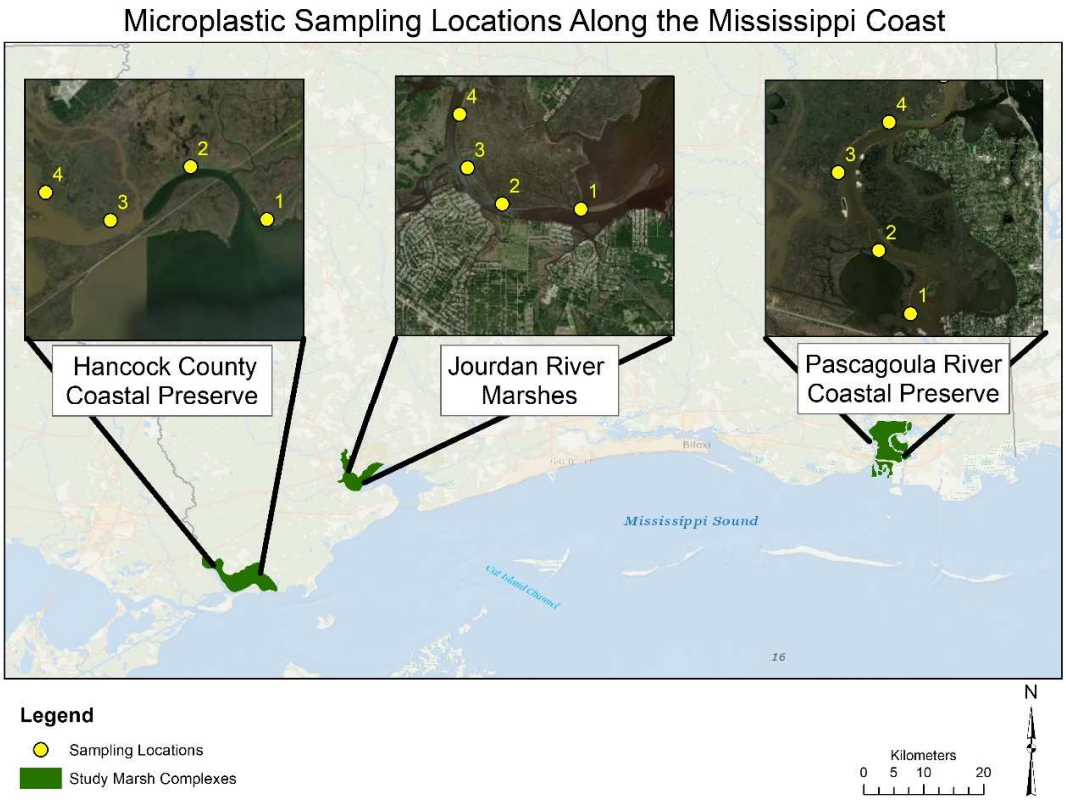

Figure 1. Map showing the three marsh complexes along the Mississippi Coast where we performed microplastic sampling. The inset maps show the four sampling points along each study marsh complex where sediment was collected, and where birds were captured within 100m of each sampling point. The lower Pearl River marshes are labeled the Hancock County Coastal Preserve in the above figure.

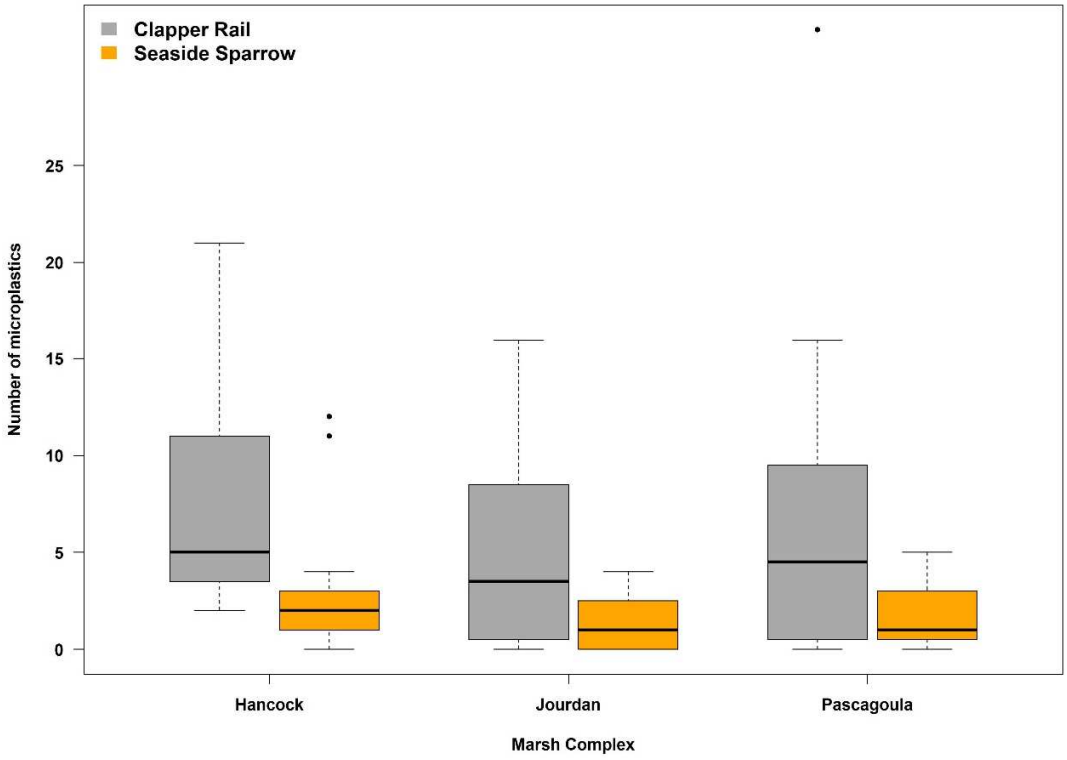

Figure 2. Box-and-whisker plot showing the distribution of microplastic quantities found in stomach samples of Clapper Rail and Seaside Sparrow within three riverine-dominated marshes along the coast of Mississippi. The lower Pearl River marshes are labeled 'Hancock' in the above figure. The box ends represent the first and third quartile of the data whereas the bold horizontal line within the box represents the median. The whiskers represent the maximum and minimum values for each group within 1.5x the interquartile range, with more extreme values represented by single points.