DePaul Discoveries

Volume 13
Article 15

2024

Microplastic Accumulation in the Marsh Periwinkle (Littoraria irrorata)

Sarah Keaton
DePaul University, skeaton1@depaul.edu

Hannah Lukhard
DePaul University, hlukhard@depaul.edu

Eli Windy
DePaul University, ewindy@depaul.edu

Manar Hasan
DePaul University, mhasan19@depaul.edu

Recommended Citation

Keaton, Sarah; Lukhard, Hannah; Windy, Eli; and Hasan, Manar (2024) "Microplastic Accumulation in the Marsh Periwinkle (Littoraria irrorata)," *DePaul Discoveries*: Volume 13, Article 15.
Available at: https://via.library.depaul.edu/depaul-disc/vol13/iss1/15

Microplastic Accumulation in the Marsh Periwinkle (Littoraria irrorata)

Acknowledgements

We offer special thanks to Alexandra Krak for her guidance and support throughout this project. This research was initiated as part of a project for the Bio 318 course Field Studies in Marine and Estuarine Biology. We would like to thank the Department of Biological Sciences at DePaul University for funding this research opportunity and the staff members from the South Carolina Department of Natural Resources for providing critical support that allowed us to conduct this research.

Microplastic Accumulation in the Marsh Periwinkle (*Littoraria irrorata)*

Sarah Keaton*

Department of Biological Sciences

Hannah Lukhard*

Department of Biological Sciences

Eli Windy

Department of Environmental Sciences and Studies

Manar Hasan

Neuroscience Program

Timothy Sparkes, PhD; Faculty Advisor

Department of Biological Sciences

ABSTRACT Contamination of oceans by microplastics (<5 mm) currently poses a major threat to aquatic and terrestrial ecosystems worldwide. Recent attention towards this issue has raised questions about the extent to which microplastics have accumulated in the environment and has led to an increase in studies on the effects of microplastics in various organisms. However, levels of contamination in protected natural areas are still largely unexplored, yet can offer an important empirical perspective on the issue. In addition, little is known about the potential effects of microplastics on animal behavior in the field. This research was conducted within the protected ACE Basin National Estuarine Research Reserve (NERR, South Carolina, USA). In this habitat, the marsh periwinkle (*Littoraria irrorata*) is a primary consumer in the salt marsh and serves as an indicator of the health of the ecosystem. We examined the pattern of microplastic accumulation in the foot and intestines of marsh periwinkles (n = 60) in relation to their availability in the habitat (water column, sediment). We also examined the relationship between microplastics and behavior (microhabitat use). We found that periwinkles accumulated microplastics in a non-random manner, relative to the habitat, and that this pattern was generally consistent between the foot and intestines. Microplastic abundance was also similar for periwinkles collected on the sediment and those collected on the cordgrass indicating that there was no obvious effect of microplastics on microhabitat use. There was no detectable relationship between microplastics and periwinkle size and no consistent effect of distance from the estuary on microplastics.

**skeaton1@depaul.edu, hlukhard@depaul.edu, tsparkes@depaul.edu*

Research Completed in Winter 2022

INTRODUCTION

Modern production and consumption of plastic has changed the global profile of waste production, resulting in large-scale environmental accumulation. It is estimated that plastics make up about 80% of marine litter, with between 5 and 13 million metric tons of plastic ending up in oceans annually (Jambeck et al., 2015). The majority of environmental plastic pollutants can be categorized as microplastics (MPs), defined as any small plastic particle measuring less than 5 millimeters in dimension. They can be introduced into the environment either directly or from the fragmentation of larger plastic debris. Common polymers of MPs in marine systems are polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyamide, and polycarbonate (Bajt, 2021).

Compared to larger plastic particles, microplastics pose unique hazards to marine habitats and their organisms. A significant concern is the direct effect of their ingestion on marine organisms, as these particles have been found to accumulate in gill appendages and soft tissues (Gray et al., 2018). In addition to acting as a mechanical hazard, microplastics also have the potential for absorption into the circulatory system via the translocation of polystyrene microspheres, a phenomenon that has been observed in rodents, humans, and mussels (Browne et al., 2008). Their accumulation at higher trophic levels has the potential to disrupt entire food chains. This poses a growing threat to many commercially and recreationally important species and humans.

Estuaries are critical for providing ecosystem services such as coastal protection, carbon sequestration, and nutrient cycling. Considered "nurseries of the sea," numerous animal species rely on estuaries for breeding and nesting. They also hold unparalleled economic and commercial value, with 47% of the United States' gross domestic product coming from estuary regions in 2021 (Rouleau et al., 2021). However, coastal areas are also generally more susceptible to microplastic pollution, as pollution patterns mirror pathways of natural sediment accumulation, where most material is deposited at the mouths of rivers and near the coastline. Estuarine environments have shown a median concentration of microplastic particles almost 4 times as high as that of deep-sea environments (Harris, 2020). With estuaries being valuable to the environment and economy, there is a pertinent need to address microplastic pollution to protect the health of estuarine ecosystems and their associated species.

This study was conducted in Bennett's Point, South Carolina. Bennett's Point lies within the convergence of the Ashepoo, Combahee, and Edisto Rivers, which together form the ACE Basin. The ACE Basin is one of the largest undeveloped estuaries on the East Coast in the South Carolina Lowcountry. Salt marshes, which fill the space between the brackish estuarine waters and dry land, comprise approximately 66% of South Carolina's wetland habitats. Still, microplastics are known to have already been accumulating in their sediments for several decades (Lloret et al., 2021). To protect these valuable wetlands, the National Estuarine Research Reserve System (NERR), a network of 30 coastal sites, is dedicated to the effective, science-based management of coastal and estuarine environments. Each reserve is funded by the National Oceanic and Atmospheric Administration (NOAA) and provides national guidance, monitoring research, education, stewardship, and training programs to help reserves address the challenges facing estuaries and their communities. As most microplastic studies are conducted in polluted areas, our study aims to instead investigate how microplastics can accumulate in organisms located in more protected areas to present a different empirical perspective on the topic.

Our primary goal was to explore the current state of microplastic pollution in the marsh periwinkle snail, *Littoraria irrorata,* a primary consumer of the salt marshes of coastal South Carolina. To achieve this objective, we investigated the relationships between microplastics in *L. irrorata*, and microplastics in the sediment and water column of their habitat across varying distances from the Ashepoo River. We also examined the relationship between MP accumulation and behavior (microhabitat use). Here, we provide a basis for investigating the largely unknown effects of microplastic pollution on *L. irrorata* in natural areas protected at the state and federal level. By exploring the current state of microplastic pollution in the primary consumers of these protected salt marshes, we can gain insight into the health of these salt marsh habitats as a collective. This also contains further implications for the future of these protected areas, as well as potential policy decisions regarding how to best go about addressing these issues.

METHODS

Sample collection

The region of salt marsh examined here is located at Bennetts Point on the Ashepoo River approximately 13 km from the ocean and 105 km from Charleston, SC. The salt marsh has an abundance of smooth cordgrass *Sporobolus alterniflorus* (formerly *Spartina alterniflora),* which the periwinkles consume along with the fungus colonizing the cordgrass (Silliman and Newell, 2003). Samples were collected from the salt marsh (32.554688" N, - 80.474218" W) in an area that was adjacent to the gravel road (Lighthouse Lane, Figure 1). The road runs diagonally from the tree line to the estuary. Collections occurred at low-tide during the early afternoon in mid-December, 2022. Weather conditions were 54°F with overcast skies and no precipitation. A grid system was created consisting of 5 horizontal transects along the farthest part of the path, labeled A through E, each 5 meters apart. At each horizontal transect location, 6 vertical transects, labeled 1 through 6, were extended out into the marsh. These points were also all 5 meters apart. This resulted in a 20 x 25 meter grid of salt marsh made of 30 total collection points (Figure 1).

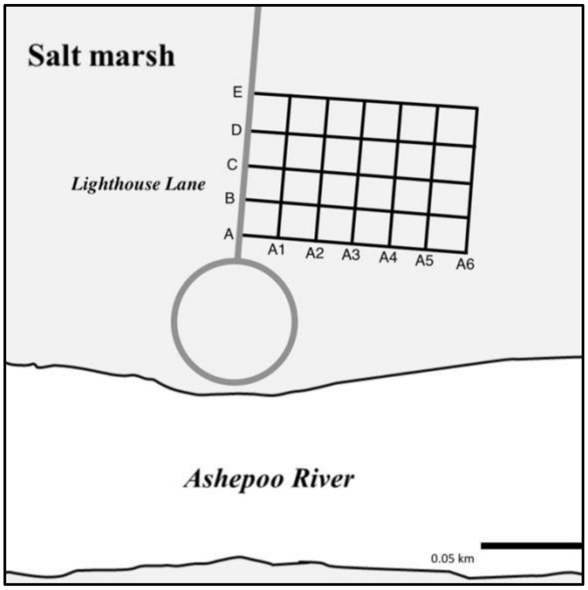


Figure 1. Representation of the 20 x 25 meter grid used for data collection off of Lighthouse Lane

L. irrorata (n = 60) were captured in pairs at each of the collection sites; one from the sediment and one from a cordgrass stalk. *L. irrorata* were randomly selected within arm's reach at each collection point. Sediment samples (n = 10) of approximately 15-30 ml were collected in falcon tubes from points A2 through E2 and A5 through E5. Water column samples (n = 5) of approximately 15-30 ml were collected in falcon tubes from the water column at points A3 through E3, while disturbing the surrounding sediment as little as possible. *L. irrorata* were labeled individually based on the microhabitat use (sediment, cordgrass) and location of collection (A1-E6). The length (mm) of the longest distance across the shell of each snail was recorded using calipers.

Microplastic (MP) identification

L. irrorata were euthanized in ethanol and dissected using a dissecting microscope. The foot and intestines of each snail were removed and preserved separately in ethanol. One slide was prepared of each foot and intestine, and each was examined for MPs for a standardized amount of time (7 minutes per slide). Foot samples were prepared by separating the foot from the operculum and observing both on the same slide. Intestine samples were prepared by flattening the tissue and adding ethanol to the slide. Three replicates were created for each sediment and water sample, and these slides were also examined for MPs for approximately 7 minutes per slide. When creating water sample slides, sediment in the collection tube was allowed to settle to the bottom of the tube and excluded from the water used to create slides. All MPs were categorized into 1 of 5 types based on physical traits: films, filaments, fragments, pellets, and others (Calcutt et al., 2018). The total number of MPs identified in each slide was recorded as well as the number of each type. To minimize inter-rater bias, we included internal checks on the identification of MPs (i.e., we overlapped individual data collectors with sample types).

RESULTS

All of the samples examined contained microplastics. The most abundant microplastics were films, fragments, and filaments (Figure 2, 3).

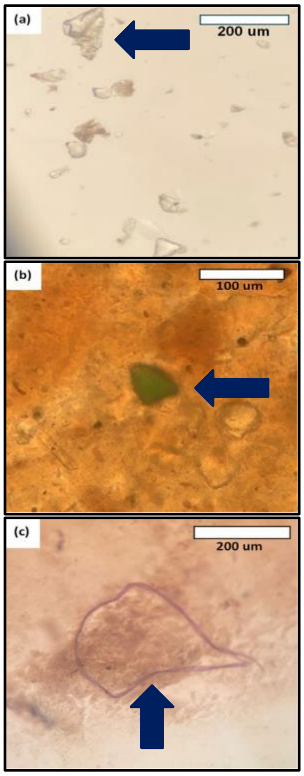


Figure 2. The most abundant microplastics recovered from the samples (arrows point to MP's). Transparent film from the water column (a), green fragment from the sediment (b), and purple filament from the intestines (c). Scale bars are estimates based on typical MP sizes.

The pattern of allocation of MPs in the periwinkles and the habitat is shown in Figure 3. Overall, the pattern was not independent of sample type (contingency chi^2: chi^2 = 175.4, df = 12, $p < 0.001$). As can be seen, the pattern of accumulations between the foot and intestines was relatively consistent with films being the most abundant type of MP present. In contrast, the most abundant MPs in the sediment were fragments and filaments were the most abundant in the water column.

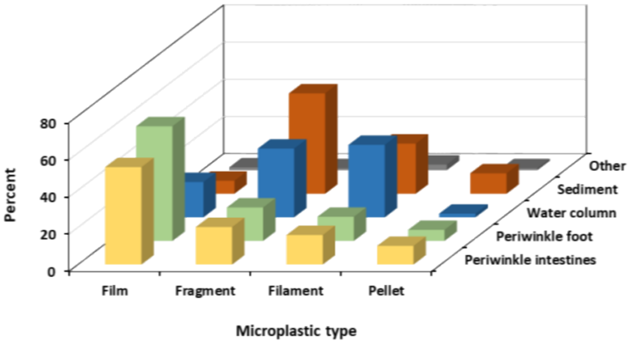


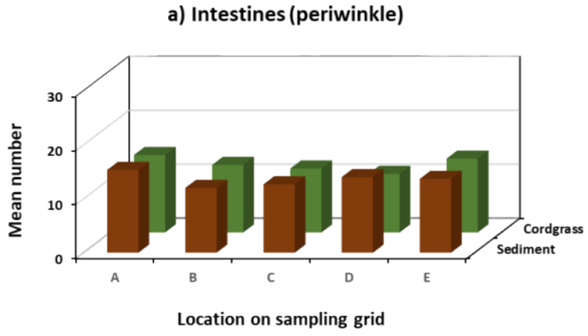
Figure 3. Accumulation patterns of microplastics from the four sample types

Periwinkle size and microplastic abundance

A linear regression analysis of *L. irrorata* shell length and total MP abundance showed that there was no detectable relationship between size and MP abundance ($R^2 = 0.02$, $p = 0.3$, $n = 60$).

Microplastic abundance and behavior

We compared total MP abundance between *L. irrorata* captured on the cordgrass and those captured on the sediment to determine the relationship between MP accumulation and behavior (Figure 4). For this analysis, sample values were averaged within a grid row (i.e., A-E) then analyzed using a paired t-test on the average values. The results are shown in Figure 4. There was no detectable difference in MP abundance between periwinkles collected on the sediment and those collected on the cordgrass (intestines: $t = 2.4$, df = 4, $p = 0.08$; foot: $t = 1.2$, df = 4, $p = 0.3$).



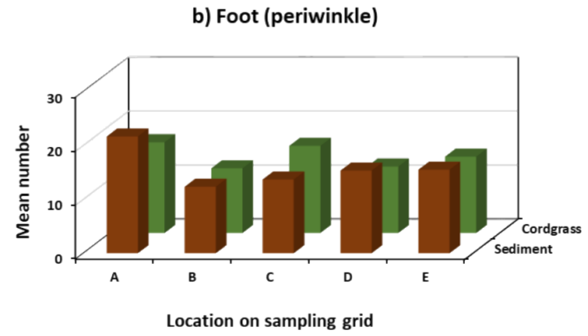


Figure 4. Relationship between microplastic abundance and behavior (microhabitat use) of periwinkles.

Microplastic abundance and grid location

The relationships between grid location and MP accumulation in periwinkles are also shown in Figure 4. For this analysis, grid locations within a row were used as replicates. There was not a consistent effect of grid location (A-E) on MP accumulation (one-way ANOVA: cordgrass-intestine, F = 1.7, df = 4,25, p = 0.2; sediment-intestine, F = 1.1, df = 4,25, p = 0.4; cordgrass-foot, F = 1.9, df = 4,25, p = 0.1). The sediment-foot samples showed an effect of distance from the estuary on MP accumulation (F = 8.2, df = 4,25, p < 001, row A is closest to the estuary), but this relationship was not present when the foot and intestine samples were combined. The combined foot and intestines samples showed significant overall effects, but the patterns were not related to distance from the estuary in a simple manner (Figure 5, cordgrass, F = 3.6, df = 4,25, p = 0.02; sediment, F = 7.5, df = 4,25, p < 0.001).

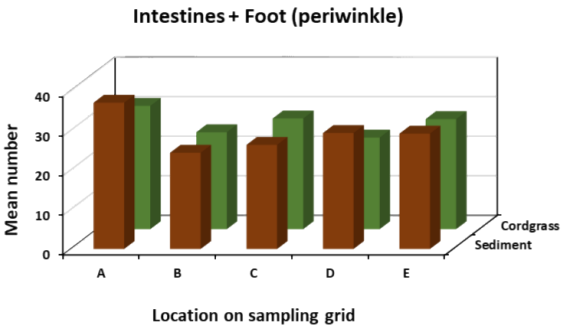


Figure 5. Microplastic abundance and relation to grid location for the combined foot and intestine samples. The relationships for intestines and foot are shown in Figure 4.

DISCUSSION

A previous study done examining microplastics in South Carolina estuaries found that microplastic concentration in the region are comparable to concentrations of microplastics in estuaries globally (Gray et al., 2018). Our study showed that microplastics were common both in the periwinkles and habitat of the salt marsh. MP abundance was not related to periwinkle size, which likely indicates that *L. irrorata* do not accumulate more MPs with age in the intestines and foot. This result is consistent with the interpretation that microplastics either pass through the intestines and feet over time, or are accumulated elsewhere in the body.

The abundance of MPs in *L. irrorata* found on cordgrass showed no correlation to that of those found on the sediment, suggesting that *L. irrorata* behavior was not associated with MP presence in these samples. The impacts of MPs are likely complex and dependent on the type of behavior examined. For example, a recent study on the common periwinkle (*Littorina littorea*) showed that toxic leachates from microplastics inhibited vigilance and antipredatory behavior when exposed to predatory crabs (Seuront, 2018). Further studies are needed to determine the potential impacts of MPs on the suite of behaviors expressed by *L. irrorata*.

The types of MPs found to accumulate in *L. irrorata* were observed to differ from those found to accumulate in the sediment and water column samples. Most notably, films were much more abundant in *L. irrorata* than in their environment, while fragments and filaments were more common in the environment. These findings tell us that *L. irrorata* do not accumulate MPs in direct relation to their availability in their habitat and suggests that *L. irrorata* are more likely to accumulate certain types of MPs.

We found that films were more likely to be accumulated by periwinkles than other microplastics. One study conducted in South Carolina salt marshes showed that *L. irrorata* exhibited grazing preferences for biofilms on polystyrene strips over biofilms on polyethylene and polypropylene strips (Weinstein et al., 2016). Thus, it is possible that periwinkles exhibit preferences for films due to the combined effects of polymer type and biofilm composition. This mechanism could potentially explain the higher abundance of films in the intestines but likely does not explain the accumulation of films in the foot unless there is an association between the two. This type of association has been shown in other periwinkles (*L. littorea*, *L. obtusata*) where the MP's found in mucus trails are correlated with the accumulation patterns in both the foot and intestines (Gutow et al., 2019). However, our results contrast with a recent study on *L. littorea* in Germany, which showed that microplastic fragments were the most common type of microplastic present in the organisms (Polt et al., 2023).

The results showed that although microplastics were common both in *L. irrorata* and the habitat, the way that microplastics accumulate and influence behavior may be complex. Future studies that examine these relationships in more detail would likely yield valuable insights into the impacts of microplastics on ecosystems in nature. The current state of MP accumulation in *L. irrorata* is crucial for understanding contamination in the rest of the food chain, as the natural predators of *L. irrorata* include various shore birds, the diamondback terrapin (*Malaclemys terrapin*), Atlantic mud crab (*Panopeus herbstii*) and the blue crab (*Callinectes sapidus*), a very important commercial species. In addition, future studies that examine the specific chemical makeup of these microplastics using analytical techniques such as nuclear magnetic resonance spectroscopy (NMR), Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, and Pyrolysis - gas chromatography - mass spectrometry (Py-GC/MS) could result in a more precise chemical identification of the plastics found in these organisms, as well as provide insight into the origins of these microplastics. This methodology for identifying the polymer types of microplastics has been successfully used in several previously published studies (Gnoffo & Frache, 2023; Liu et al., 2024; Merrill et al., 2023; Peez et al., 2019).

ACKNOWLEDGEMENTS

We offer special thanks to Alexandra Krak for her guidance and support throughout this project. This research was initiated as part of a project for the Bio 318 course *Field Studies in Marine and Estuarine Biology*. We would like to thank the Department of Biological Sciences at DePaul University for funding this research opportunity and the staff members from the South Carolina Department of Natural Resources for providing critical support that allowed us to conduct this research.

AUTHOR CONTRIBUTIONS

HL, SK, EW and MH designed the original experiment and collected the data during the Bio 318 course. HL and SK ran the statistical analysis, wrote and edited the manuscript, and revised the manuscript based on comments received from the reviewers.

REFERENCES

Bajt, O. (2021). From plastics to microplastics and organisms. *FEBS Open Bio*, *11*(4), 954–966. https://doi.org/10.1002/2211-5463.13120

Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M., & Thompson, R. C. (2008). Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environmental Science & Technology*, *42*(13), 5026–5031. https://doi.org/10.1021/es800249a

Calcutt, J. Nussbaumer, A., Sluka, R. (2018). Guidelines for sampling microplastics on sandy beaches. http://www.arocha.org/wpcontent/uploads/2018/01/Microplastic-sampling- protocol.pdf

Gnoffo, C., & Frache, A. (2023). Identification of plastics in mixtures and blends through pyrolysis-gas chromatography/mass spectrometry. *Polymers*, *16*(1), 71. https://doi.org/10.3390/polym16010071

Gray, A. D., Wertz, H., Leads, R. R., & Weinstein, J. E. (2018). Microplastic in two South Carolina estuaries: Occurrence, distribution, and composition. *Marine Pollution Bulletin*, *128*, 223–233. https://doi.org/10.1016/j.marpolbul.2018.0 1.030

Gutow, L., Bartl, K., Saborowski, R., & Beermann, J. (2019). Gastropod pedal mucus retains microplastics and promotes the uptake of particles by marine periwinkles. *Environmental Pollution*, *246*, 688–696. https://doi.org/10.1016/j.envpol.2018.12.0 97

Harris, P. T. (2020). The fate of microplastic in marine sedimentary environments: A review and synthesis. *Marine Pollution Bulletin*, *158*, 111398. https://doi.org/10.1016/j.marpolbul.2020.1 11398

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*(6223), 768–771. https://doi.org/10.1126/science.126035252

Liu, S., Wang, C., Yang, Y., Du, Z., Li, L., Zhang, M., Ni, S., Yue, Z., Yang, K., Wang, Y., Li, X., Yang, Y., Qin, Y., Li, J., Yang, Y., & Zhang, M. (2024). Microplastics in three types of human arteries detected by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). *Journal of Hazardous Materials*, *469*, 133855. https://doi.org/10.1016/j.jhazmat.2024.133 855

Lloret, J., Pedrosa-Pamies, R., Vandal, N., Rorty, R., Ritchie, M., McGuire, C., Chenoweth, K., & Valiela, I. (2021). Salt marsh sediments act as sinks for microplastics and reveal effects of current and historical land use changes. *Environmental Advances*, *4*, 100060. https://doi.org/10.1016/j.envadv.2021.100 060

Merrill, G. B., Hermabessiere, L., Rochman, C. M., & Nowacek, D. P. (2023). Microplastics in marine mammal blubber, melon, & other tissues: Evidence of translocation. *Environmental Pollution*, *335*, 122252. https://doi.org/10.1016/j.envpol.2023.22

Peez, N., Janiska, M.-C., & Imhof, W. (2019). The first application of quantitative 1H NMR spectroscopy as a simple and fast method of identification and quantification of microplastic particles (PE, PET, and PS). *Analytical and Bioanalytical Chemistry*, *411*(4), 823–833. https://doi.org/10.1007/s00216-018-1510- z

Polt, L., Motyl, L., & Fischer, E. K. (2023). Abundance and distribution of microplastics in invertebrate and fish species and sediment samples along the German Wadden Sea Coastline. *Animals*, *13*(10), 1698. https://doi.org/10.3390/ani13101698

Rouleau, T., Colgan, C.S., Adkins, J., Castelletto, A., Dirlam, P., Lyons, S., and Stevens, H. (2021). The economic value of America's estuaries: 2021 update. Washington: Restore America's Estuaries. http://www.estuaries.org/economics/2021- report

Seuront, L. (2018). Microplastic leachates impair behavioural vigilance and predator avoidance in a temperate intertidal gastropod. *Biology Letters*, *14*(11), 20180453. https://doi.org/10.1098/rsbl.2018.0453

Silliman, B. R., & Newell, S. Y. (2003). Fungal farming in a snail. *Proceedings of the National Academy of Sciences*, *100*(26), 15643–15648. https://doi.org/10.1073/pnas.2535227100

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. *Environmental Toxicology and Chemistry*, *35*(7), 1632–1640. https://doi.org/10.1002/etc.3432