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Thalassia testudinum as a potential vector for incorporating microplastics into benthic marine food webs

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ABSTRACT

Seagrasses are among the most productive shallow water ecosystems, serving a diverse assemblage of fish and invertebrates. Tropical seagrass communities are dominated by the turtle grass *Thalassia testudinum*, whose wide, flattened blades host diverse epibiont communities. Amidst its epibionts, *T. testudinum* may also be accumulating microplastics, which are a ubiquitous marine pollutant even in remote locales. To assess the extent of microplastic accumulation, seagrass samples were collected from Turneffe Atoll, which lies offshore but parallel with a major urban center. Seventy-five percent of *Thalassia* blades had encrusted microplastics, with microfibers occurring more than microbeads and chips by a ratio of 59:14. Grazers consumed seagrasses with higher densities of epibionts. Potential mechanisms for microplastic accumulation include entrapment by epibionts, or attachment via biofilms. This study is the first to document microplastics on marine vascular plants, suggesting that macroherbivory is a viable pathway for microplastic pollution to enter marine food webs.

1. Introduction

In the Tropical Western Atlantic, the seagrass *Thalassia testudinum* dominates shallow, soft-bottom coastal waters. These *T. testudinum* meadows are known to enhance ecosystem connectivity between mangrove and coral reef habitats, resulting in higher overall primary productivity and biodiversity in each of these communities (Medina-Gómez et al., 2016). However, seagrass communities are under threat from anthropogenic stress due to coastal development, overfishing of herbivorous fish species, and pollution from runoff or other coastal sources (Honig et al., 2017; Unsworth et al., 2018). Mechanical disturbances from trawling and dredging, as well as obstruction of sunlight from aquaculture shading have also led to large quantities of seagrass habitat loss (Duarte, 2002). Growing threats from these and other anthropogenic stressors in seagrass habitats, including disease, invasive species, algal blooms, and climate change, have led to a global crisis in seagrass ecosystems, with reported cases of temperate and tropical seagrass loss increasing almost tenfold in the last four decades (Orth et al., 2006).

At the same time, there has been a growing awareness and concern surrounding plastic pollution in marine environments. First noted as a potential marine problem in 1971 (Buchanan, 1971; Carpenter and Smith, 1972), plastics have since been observed in marine environments around the globe (Cole et al., 2011). Indeed, plastics reliably constitute 70% of marine litter in some areas (Derraik, 2007). Presently, there is no consensus on the total amount of plastic in the ocean or how much is added/stored annually, though models predict that around 10 million tons of plastics are added each year, with the total amount accumulated by 2025 predicted to be at least 155 million tons (Iñiguez et al., 2016).

Emerging research suggests that microplastic ingestion can be habitat dependent, with fishes in benthic and demersal habitats demonstrating higher rates of plastic consumption than mesopelagic fishes (Baalkhuyur et al., 2018). Additionally, high-energy coastal waters have also been shown to harbor microplastics, with higher concentrations in fine sediments (Ling et al., 2017). This suggests that seagrass ecosystems, which are high-energy, coastal, benthic environments that stabilize fine sediments, may have elevated levels of plastic accumulation. However, the interactions between seagrass blades, which are regularly consumed by a diverse suite of herbivores, and marine microplastics have not yet been examined.

This study investigated the potential for microplastic accumulation and subsequent herbivore consumption on seagrass blades from Turneffe Atoll, Belize. Specifically, *T. testudinum* blades with complex and diverse epibiont communities were examined to determine if microplastics could become encrusted amidst epibionts. To determine whether seagrass herbivores had the potential to consume microplastic debris, surveys were conducted to determine whether herbivorous

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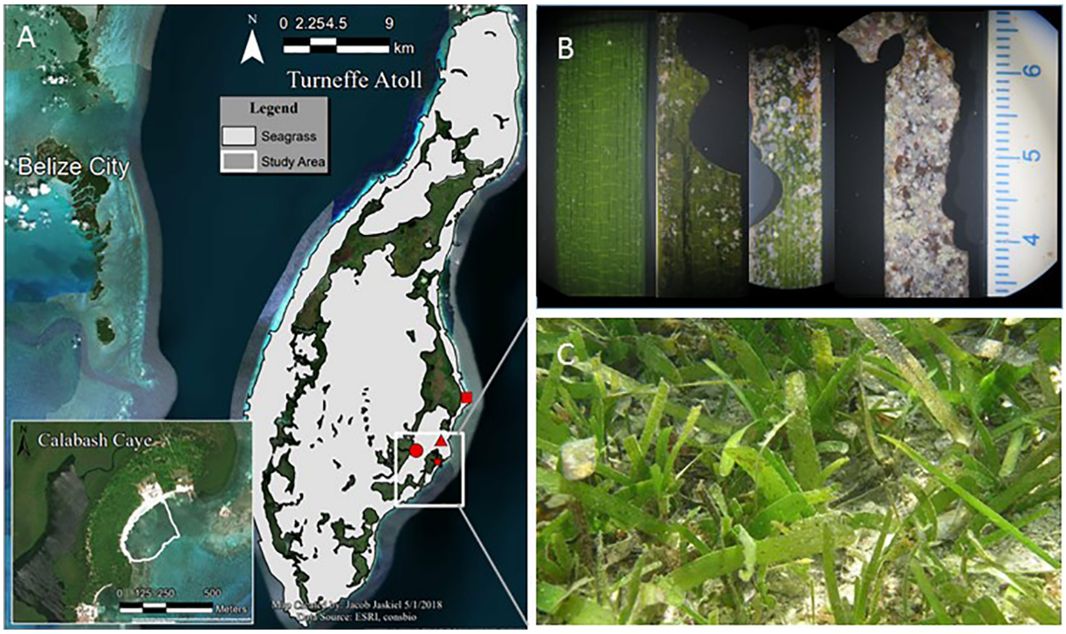

Fig. 1. A. Satellite image of Calabash Caye Field Station on Turneffe Atoll, Belize with seagrass beds of Turneffe Atoll represented by light gray shading. Calabash seagrass area is outlined in white on the left inset map. Quadrat harvest sites are indicated by shapes, with the 3 closest to Calabash outlined in the white box. The circle is the Calabash Patch Reef Lagoon, star is the Calabash Patch Reef, triangle is Oceana Sandflats, and the small square is the Soldier Caye Conservation Zone B. Images of *Thalassia testudinum* blades displayed by rank epibiont score, from clean blades (1, left) to fully encrusted (4, right). Bite marks by piscine herbivores are visible on blades 2–4. C. Photo from the Calabash Caye seagrass flats, showing a typical seagrass bed, with blades of all epibiont ranks growing adjacently. Photographs by R. Branconi.

fishes had higher consumption rates on seagrass blades with or without epibionts. It was hypothesized that if seagrass blades with high abundances of epibionts were preferentially consumed, then encrusting microplastics would be inadvertently consumed as well. The consumption of microplastics by herbivores would represent a previously undocumented pathway through which microplastics are introduced into lower trophic levels of coastal food webs.

2. Methods

2.1. Field collections

Samples of *T. testudinum* were collected from the Calabash Patch Reef (0.073 km^2) on December 19th, 2017, on Turneffe Atoll Marine Reserve in Belize (17.2828° N, 87.8116° W, Fig. 1A). A total of 16 seagrass blades were hand-harvested along the seagrass beds adjacent to the island at a depth of 1-2 m for detailed microscopic examination. Blades were assigned a rank score based on their epibiont percent coverage: Blades with < 25% coverage were ranked a one, 25–50% coverage were ranked a two, 50–75% were ranked a three, and > 75% coverage were ranked a 4 (Fig. 1B). Of the blades collected, 3 blades were ranked 1, 1 blade was ranked 2, 5 blades were ranked 3, and 7 blades were ranked 4. Samples were then sealed and preserved in 50 mL Falcon tubes with 10% formalin and transported to Boston University for later stereomicroscopy analysis.

2.2. Examination of seagrass blade epibionts

Blades of *T. testudinum* were examined, photographed, and analyzed using a Leica DFC550 stereomicroscope and Leica Application Suite V4.9.0. The presence of microplastics on the blades was recorded along with the shape as either microfibers, microbeads, or other microfragments. To prevent any contamination of the samples, the microscope and glass surfaces were cleaned using Kimtech Kimwipes Delicate Task Wipers and 95% ethanol between each imaging session and each sample analysis. Care was taken to avoid any potential opportunity for clothing fibers to interact with blades, so as to prevent microfiber transfer. Each blade was removed from formalin and allowed to dry before being analyzed. Plastics identified were recorded with an ID number to prevent recounting of the same plastic in multiple images.

2.3. Examination of parrotfish feeding preferences

To analyze the relationship between epibiont density and parrotfish feeding, samples were collected from four sites on Turneffe Atoll, including Oceana Sandflats, Calabash Patch Reef Lagoon, Calabash Patch Reef and the Soldier Caye Conservation Zone (Fig. 1). At each site, seagrasses from two randomly-thrown 0.25m^2 gridded quadrats were completely harvested, and seagrass density estimated to the nearest 5%. The 8 quadrats together yielded 483 blades, which were then analyzed for epibiont density rank. The number of piscine grazing marks per blade was recorded.

2.4. Statistical analysis

All statistical analyses were conducted using Systat v13.2, unless otherwise noted. All data met test assumptions. To examine the relationship between epibiont rank and piscine grazing marks, results were analyzed using a one-way ANOVA. A one-way ANOVA was also used to analyze the relationship between epibiont rank and microplastic densities. A paired *t*-test was used to analyze the mean microbeads per blade versus mean microfibers per blade. Column statistics for mean, SE, maximum and minimum were derived using Graphpad Prism, which was also used to generate figures. Map of seagrass area on Turneffe Atoll was generated with ArcGIS.

3. Results

Microplastics ranged in colors and sizes with beads typically being a bright blue color, while fibers were more varied, including black, blue, and red (Fig. 2). Many were found to be embedded or encrusted within the epibiont matrix (Fig. 2). Of the 16 blades, 12 (75%) were found to