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Evidence of microplastics from benthic jellyfish (*Cassiopea xamachana*) in Florida estuaries

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ABSTRACT

Plastic pollution is a concern in many nearshore ecosystems, and it is critical to understand how microplastics (plastics < 5 mm in length) affect nearshore marine biota. Here, we report the presence of microplastics in the benthic, upside-down jellyfish (*Cassiopea xamachana*) across three estuaries in south Florida. Microplastics were recovered from *Cassiopea* using an acid digestion, then enumerated via microscopy, and identified using micro Fourier-transform interferometer (μFTIR) analysis. Out of 115 specimens analyzed, 77% contained microplastics. Bell diameter and number of plastics per individual varied significantly across locations with the highest plastic densities and bell diameter observed in individuals from Big Pine Key, followed by Jupiter, and Sarasota. μFTIR analysis confirmed that synthetic microfibers were the dominant microplastic measured at all three locations and may indicate *Cassiopea* as potential sinks of microplastic. *Cassiopea* may be used as bioindicators of microplastic contamination in the future, allowing for potential plastic pollution mitigation.

1. Introduction

Anthropogenic debris has become ubiquitous in all marine environments, with plastic being the largest component of marine litter (Auta et al., 2017; Bergmann et al., 2015). Coastal marine ecosystems are especially susceptible to plastic pollution (Barnes et al., 2009), with plastic debris occurring by means of both nearshore terrestrial runoff (e.g., stormwater and/or wastewater discharge) (Reisser et al., 2013; Lebreton et al., 2017; Ling et al., 2017) and wash-up along the shoreline from marine sources (Barnes et al., 2009). For instance, coastal sites in the Pacific were found to have 4–27 times more plastic debris than offshore subsurface waters (Desforges et al., 2014). Coastal shorelines receiving both marine and terrestrial inputs become regions for debris accumulation and thus likely "hot spots" for plastics.

Microplastics, a subset of plastics that are < 5 mm in length, are increasingly found in nearshore systems (Auta et al., 2017) and have been discovered in many marine species (Laist, 1997). Typically, microplastics are either created as microbeads for personal care products or nurdles for pre-production plastic, are the byproduct of the degradation of larger macroplastics (Auta et al., 2017), or are textile fibers from laundering clothes, all of which can eventually flow offshore (Rochman, 2018). More recently, a growing body of literature indicates that microplastics can exert strong negative health effects on nearshore aquatic organisms (Kühn et al., 2015; Lusher et al., 2016). Specifically, microplastic uptake by nearshore biota, such as zooplankton, shellfish, fish, and crabs, has been found to: 1) reduce the amount of food consumed by individual organisms (via physical impaction of the gut) driving subsequent weight loss (Ogonowski et al., 2016); 2) reduce growth rates (Jeong et al., 2016; Ogonowski et al., 2016; Watts et al., 2015); 3) compromise immune systems (Avio et al., 2015; Canesi et al., 2015; Greven et al., 2016); 4) affect reproductive systems and output (Cole et al., 2015; Jeong et al., 2016; Rochman et al., 2014; Sussarellu et al., 2016); and 5) cause mortality (Ogonowski et al., 2016; Oliveira et al., 2013; Rist et al., 2016). This can result in additional impacts to nearshore food webs as there is an indication that microplastics can be conserved as they move up trophic levels (Wright et al., 2013; Botterell et al., 2019). Further, hydrophobic organic pollutants, including pharmaceuticals, polychlorinated biphenyls (PCBs), phthalates, flame

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retardants, and pesticides, can readily adsorb to microplastics. Once ingested, these chemicals have the potential to cause endocrine disruption to marine biota (Rochman et al., 2014, 2013). Despite uncertainties regarding the ecological risks of microplastics, its accumulation is a globally recognized threat that requires actions of assessment and control (Bonanno and Orlando-Bonaca, 2018). It is well known that invertebrate monitoring is useful for indicating the prevalence of pollutants (Boening, 1999; Gesteira and Dauvin, 2000; Bayen et al., 2004; Du Châtelet et al., 2004; Epstein et al., 2016; Templeman and Kingsford, 2010; Iliff et al., 2019; McKenzie et al., 2020) and there are ongoing efforts to identify bioindicator species for microplastics (Li et al., 2019; Macali and Bergami, 2020) in coastal marine environments.

One group of coastal marine and estuarine organisms that has been underrepresented in microplastics literature is gelatinous zooplankton (hereafter referred to as jellyfish). A paucity of literature on jellyfish encounters with microplastics is surprising given that jellyfish are exceedingly abundant in many ecosystems, are consumed by a variety of organisms, and the spatial-temporal dynamics of pelagic jellyfish, largely controlled by wind and currents, could reflect the distribution of plastics (Macali and Bergami, 2020; Hays et al., 2018). Jellyfish provide important ecosystem services in coastal marine habitats including habitat provisioning, prey for charismatic megafauna (e.g., sea turtles), and contributing to carbon and macronutrient dynamics (Sweetman et al., 2014; Sweetman and Chapman, 2015). Despite the ubiquity of microplastics and the known ecological roles of jellyfish in marine environments, the ecotoxicological effects of microplastics on jellyfish are relatively unknown. While Costa et al. (2020) reported that microplastic ingestion affects *Aurelia* sp. ephyrae jellyfish health, impairing both their survival and behavior, Sucharitakul et al. (2020) did not detect physiological or histological harm to *Aurelia aurita* medusae following microbead ingestion. A recent study conducted on mauve stingers (*Pelagia noctiluca*), the most abundant jellyfish species in the Mediterranean Sea adjacent to Ponza Island, Italy, indicated that macroplastics were trapped in 20% of the jellyfish collected (N = 20) (Macali et al., 2018). In this same study, plastic debris was retrieved from the gastrovascular cavities, indicating ingestion, or in their oral lobes, indicating potential entanglement. In either case, the microplastics could be readily consumed by gelatinovores and other secondary consumers becoming biomagnified in the marine food web (Macali et al., 2018).

Unlike their pelagic counterparts, benthic jellyfish, to the best of our knowledge, have yet to be evaluated for microplastics. Benthic jellyfish are relatively sessile animals that live on the seafloor and are largely comprised of upside-down jellyfish from the genus *Cassiopea*. Benthic jellyfish (hereafter referred to as *Cassiopea*) are globally distributed in nearshore habitats including coral reefs, mangrove forests, seagrass beds, and lagoons, and may be particularly abundant in areas adjacent to high human population densities, possibly due to elevated nutrient concentrations (Stoner et al., 2016, 2011). In one such urban marine environment in Queensland, Australia, environmental contaminants were detected in higher concentrations in *Cassiopea* tissue than in surrounding ambient seawater (Templeman and Kingsford, 2010). *Cassiopea* have recently gained attention for their potential to serve as bioindicators of environmental contaminants such as trace metals (Templeman and Kingsford, 2010; Templeman and Kingsford, 2015; Epstein et al., 2016) and herbicides (McKenzie et al. 2020). Benthic organisms such as *Cassiopea* may be susceptible to microplastics, as some microplastics are more prevalent in shallow coastal sediments due to hydrodynamic forcing, whereby buoyant microplastics may be pushed onshore (Claessens et al., 2011). Further, polyvinyl chloride (PVC), nylons, and polyethylene terephthalate (PET) are more prone to sinking (Auta et al., 2017), which may make these microplastics more abundant in benthic environments (Wright et al., 2013). *Cassiopea* are unique in that they spend their adult lives (as medusae) in an upside-down orientation in which their oral arms extend into the water column to better expose endosymbiotic algae living in their tissues to light (Stoner et al., 2011). These algae, in turn, provide over 100% of the animal's energy requirements (Freeman et al., 2017, 2016), though *Cassiopea* also obtain food heterotrophically (Jantzen et al., 2010), and can ingest zooplankton at up to 50 pulses per minute (Larson, 1997). As a result of passive feeding by *Cassiopea*, as well as its relatively sessile nature and unique body orientation, it is likely that *Cassiopea* either ingest or incorporate microplastics. Due to the unique features of *Cassiopea*, their bioaccumulative capacity, and their unusual life history, we hypothesize that benthic jellyfish may play an important role by acting as “sinks” of microplastics, potentially serving as bioindicators of microplastic contamination.

To this end, the objective of this study was to evaluate microplastics associated with benthic jellyfish and provide baseline information on whether evaluating jellyfish from several locations may yield spatial variation in microplastic densities and composition, potentially indicating areas with heavy microplastic contamination. Implications of this work may allow resource managers to identify plastic “hot spots” (Karlsson et al., 2017) and evaluate possible point sources of contamination. Further, if large amounts of microplastics are found in *Cassiopea*, it is possible that benthic jellyfish may affect nearshore food webs if they are preyed on by secondary consumers.

2. Methods

2.1. Study locations and jellyfish sampling

Cassiopea xamachana jellyfish were collected in three small semi-enclosed shallow, estuarine embayments in South Florida, USA between April 2018 and July 2019. *Cassiopea xamachana* were identified based on their unique oral arms and appendages (shown in Fig. 1); further details of morphological features are described in previous work identifying this species (Stoner et al., 2016; Freeman et al., 2017). Forty-one *Cassiopea* were collected from Coon Key, Sarasota, FL (27°19′13.5″N 82°34′14.4″W), 40 *Cassiopea* from Big Pine Key, FL (24°39′07.5″N 81°22′29.5″W), and 34 from Dubois Park in Jupiter, FL (26°56′27.4″N 80°04′20.6″W) (Fig. 2). In total, we collected 115 *Cassiopea* with bell diameters ranging between 5.0 and 18.5 cm (Fig. 3A). Samples were all collected from 5 to 100 cm depth depending upon tides. The Coon Key site within Sarasota Bay is semi-enclosed by a concrete seawall and red mangroves (*Rhizophora mangle*), and the benthos is sandy and dominated by seagrass (*Thalassia testudinum*) and unidentified red macroalgae algae (Rhodophyta). Coon Key and Sarasota Bay receive marine water from the Gulf of Mexico and fresh water from smaller tributaries. The Big Pine Key site is a shallow lagoon enclosed in a mangrove (*R. mangle*) island with benthos of fine muddy sediments mixed with sand. Big Pine Key receives saltwater from the Gulf of Mexico and the Atlantic; freshwater inputs are typically limited to rainfall events. The Dubois Park site in Jupiter is a muddy sand tidal lagoon lined with red (*R. mangle*) and black (*Avicennia germinans*) mangroves, located approximately 200 m south of the Jupiter Inlet. Dubois Park and the Jupiter inlet receive marine waters from the Atlantic Ocean and fresh water from rainfall, smaller tributaries, and the Loxahatchee River. Water temperatures in these three regions range from 19 to 30 °C and salinities 11–36 ppt (NOAA 2019; Loxahatchee River District Riverkeeper, n.d.).

Cassiopea were hand-collected from the benthos using nitrile butadiene rubber (NBR) gloves, carefully wiping off any attached sediment from each animal. Bell diameters of *Cassiopea* were measured by quantifying the length of the bell from rhopalia to rhopalia (illustrated in Fig. 1) following methods outlined in Stoner et al. (2011). Once *Cassiopea* were collected, samples were stored in Polyethylene-Low Density (LDPE) Ziplock bags, placed on ice, and stored individually in the laboratory at −20 °C to minimize tissue decay and preserve sample quality until further processing. Neither NBR nor LDPE was detected in any samples, including blanks (see below).

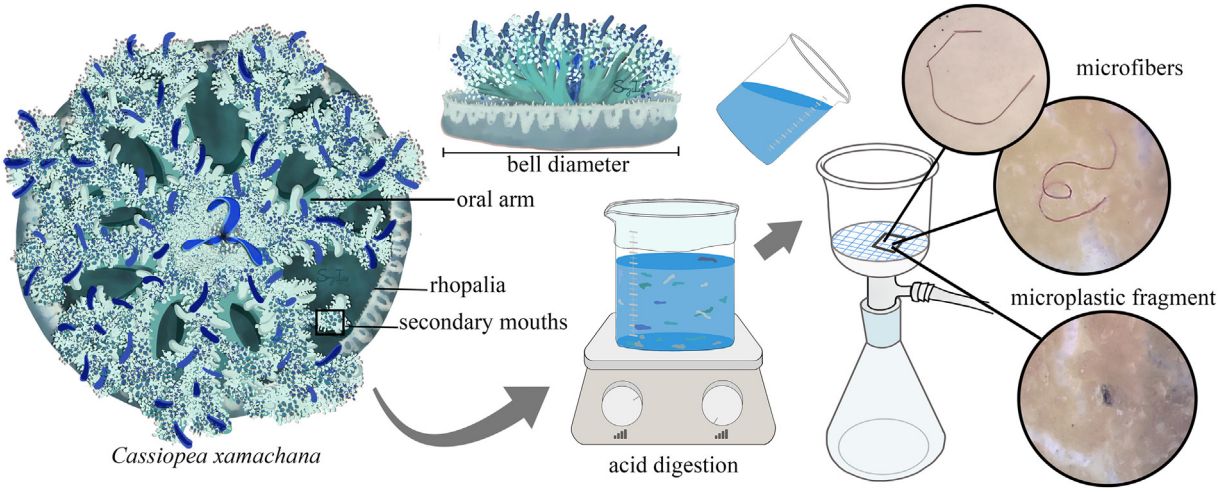

Fig. 1. Diagram of benthic upside-down jellyfish *Cassiopea* and illustrated methods to isolate microplastics.

2.2. Sample processing and data analysis

In the laboratory, *Cassiopea* were thawed at room temperature before examination in a sterile fume hood. To minimize the risk of environmental contamination of samples, for all analyses, cotton laboratory coats were worn, and the use of plastic material was avoided whenever possible. To minimize any contamination, all laboratory material used during sample processing was cleaned with prefiltered deionized water before use. *Cassiopea* were carefully transferred into glass beakers, where the contents of the LDPE bags were transferred into the glass beaker and then rinsed using the deionized water to capture any residual mucus, tissue, and/or microplastics that might have adhered to surfaces. Following modified protocols outlined in Claessens et al. (2013), individual *Cassiopea* were digested in 250-ml glass beakers filled with a 20% pre-filtered, warm nitric acid solution (HNO_3, analytical reagent grade, Fisher Chemical) for 48 h. The volume of solution used was standardized based on *Cassiopea* bell diameter using the following equation, where X = bell diameter of each individual jellyfish, 8.7 is the bell size standardized to 100 ml of solution, and 0.2 is 20% nitric acid solution used:

$$X\,(100)/8.7 = \text{total solution (ml)}\,(0.2)$$

Following the 48 h initial digestion, samples were boiled (100 °C) for 2 h and filtered, while still warm (80 °C) in a sterile fume hood. The resulting mixture was then immediately vacuum filtered over gridded 0.45-μm, 70-mm diameter mixed-cellulose ester filter papers. Samples were loosely covered with aluminum foil during this process to prevent any contamination of samples from atmospheric microplastic particles. During the digestion of *Cassiopea*, procedural blanks were also run without invertebrate tissues in parallel with samples containing the digestion solutions. Particles retained on the filter were visually inspected under a stereomicroscope with a polarizer attached (AmScope trinocular 2×–225×, 4× magnification). Photographs of all potential debris were recorded, the shape and color of each sample noted, and the maximum length of each item manually measured (mm) using ImageJ software following protocols outlined in Sun et al. (2017).

Visual classification of any particles found was carried out using established criteria (Lusher et al., 2014). For particles to be classified as anthropogenic debris, they had to (1) be homogeneously colored, (2) be shiny and not matte, (3) have no cellular organic structures visible, (4) be equally thick throughout their length, and (5) had to have three-dimensional bending (Lusher et al., 2016). Particles were assigned to four particle type categories: fragment (e.g., pieces of broken apart plastic), pellet (microbeads), film (such as plastic bags), and microfibers. When debris did not satisfy one or more of these requirements, a hot point test (using a hot needle to ascertain whether the debris in question was plastic or non-synthetic) was used (Roch and Brinker, 2017).

Due to budgetary constraints a subset (n = 11) of microplastic samples was haphazardly selected for micro Fourier-transform interferometer (μFTIR) analysis (Hook and Kahle, 1996). The subset was randomly chosen within each site for a range of bell sizes. The purpose of using μFTIR was not to systematically identify the composition of all microplastics encountered, but to verify that a subset of particles isolated from jellyfish were from an anthropogenic source. Individual μFTIR results were compared against FTIR library matches at Microvision Laboratory in Chelmsford, MA. μFTIR results were given a Hit Quality Index (HQI) to assess the quality of the match between the unknown sample and a reference database of polymer spectra. While HQI does not determine the absolute chemical structure, it does provide a known reference to the polymers common between the unknown sample and the reference database. This allows for the identification of the type of polymer present, not its specific source product. For samples identified as cellulosic materials, polarized light microscopy and a previously published methodology by Comnea-Stancu et al. (2017) were used to differentiate between natural and man-made cellulosic structures.

Separate independent sample *t*-tests were used to test for differences in the incidence of microplastic per sample ($p \leq 0.05$) between *Cassiopea* samples containing microplastics and procedural controls at each site allowing for greater confidence in our sample collection and processing techniques. A series of analyses of variance (ANOVA) tests and Tukey significance tests were then run to determine differences in *Cassiopea* bell size, microplastic size, and microplastics detected per location. Relationships between *Cassiopea* bell size and plastics (size and counts) were determined using simple linear regressions. All statistics were conducted using R v 3.6.

3. Results

Independent sample t-tests confirmed that procedural controls had significantly fewer microplastics present, with a total of 11 microfibers retrieved from 11 filters (1 microfiber per filter). There were 115 procedural control filters evaluated in total, (controls from Sarasota: 0.12 ± 0.33 plastics detected, $t = 5.4$, $df = 80$, $p \leq 0.001$; controls from Jupiter: 0.06 ± 0.24 plastics detected, $t = 5.9$, $df = 66$, $p \leq 0.001$; controls from Big Pine Key: 0.10 ± 0.30 plastics detected, $t = 7.8$, $df = 78$, $p \leq 0.001$).

Across all sites and samples, 88 individuals (77%) contained microplastics. Spatial differences in microplastics were also detected, with

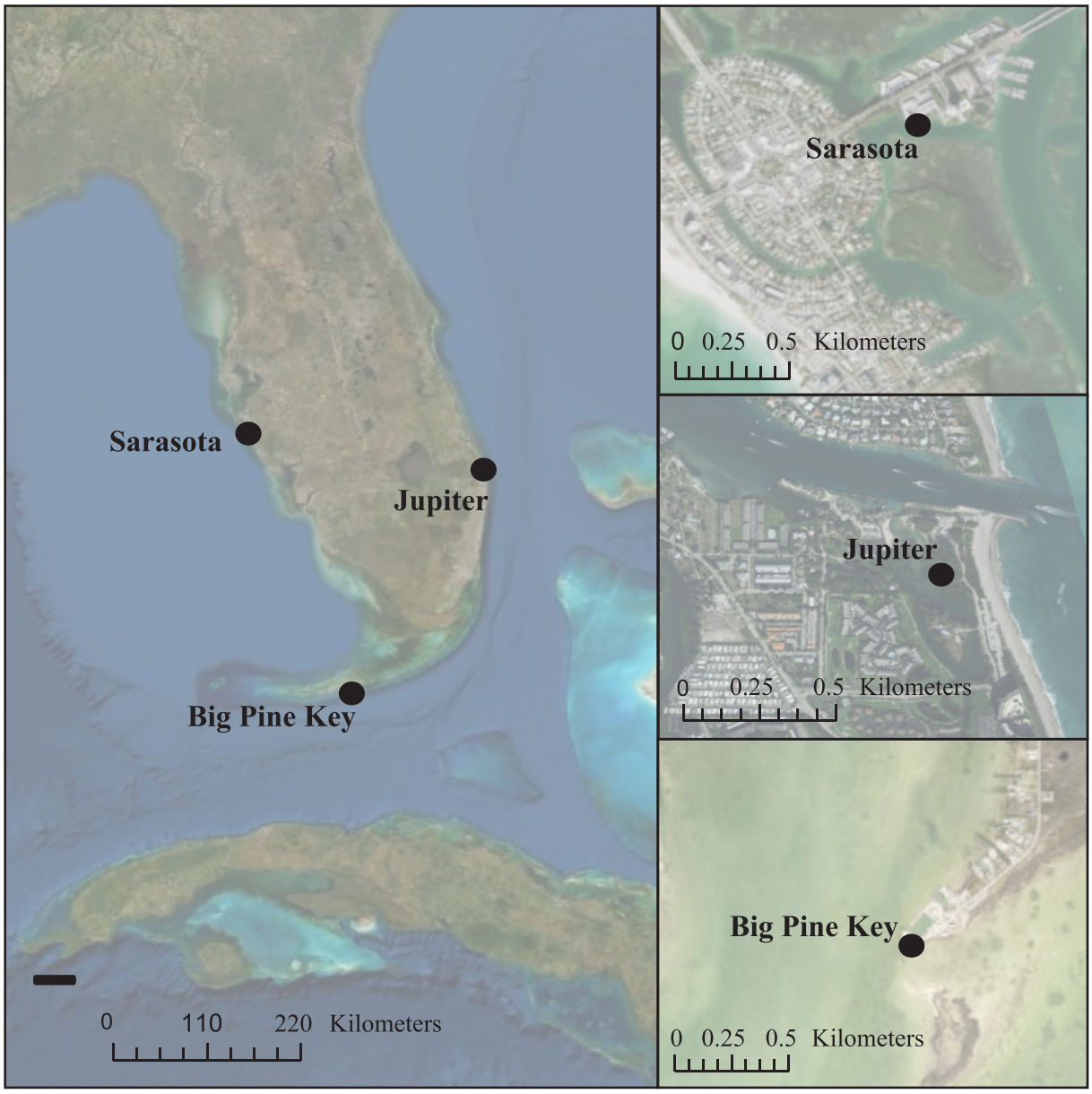

Fig. 2. Map showing *Cassiopea* sample locations in Florida, USA. Close-up panels show specific sites: Sarasota (top right), Jupiter (middle right), and Big Pine Key (lower right).

the number of plastics detected per individual differing significantly by location (ANOVA: df = 2, F = 11, $p \leq 0.001$). *Cassiopea* collected from Big Pine Key had significantly higher plastic densities per individual than from Sarasota and Jupiter (Tukey's, $p \leq 0.001$). In total, 66% of *Cassiopea* from Sarasota contained microplastics, 74% of *Cassiopea* from Jupiter, and 90% of *Cassiopea* from Big Pine Key. Microplastics detected increased from a mean (± standard deviation) of 1.1 ± 1.1 per individual in Sarasota to 1.3 ± 1.2 per individual in Jupiter to 2.4 ± 1.8 per individual in Big Pine Key (Fig. 3B). Plastic size did not significantly differ between locations (ANOVA: df = 2, F = 2.2, p = 0.11) and in total ranged 0.008–6.6 mm across the locations (Fig. 3C).

Bell diameters significantly differed between locations (ANOVA: df = 2, F = 22, $p \leq 0.001$; Tukey's $p \leq 0.03$), increasing from a mean (± standard deviation) in Sarasota of 9.4 ± 2.7 cm to 11.9 ± 2.4 cm in Jupiter to 13.6 ± 3.2 in Big Pine Key (Fig. 3A). Pearson bivariate correlation between the number of plastics detected per individual and *Cassiopea* bell size diameter (across all sites) was significant (Pearson's R = 0.32, n = 115, $p \leq 0.001$). The relationship was weak with an adjusted $R^2 = 0.09$, but the overall trend was a greater number of plastics detected in *Cassiopea* with a larger bell size (Fig. 4A). In contrast, plastic size did not appear to relate to *Cassiopea* bell size (Fig. 4B, Pearson's $R < 0.01$, n = 115, p = 0.99, and adjusted $R^2 < 0.01$). Although *Cassiopea* were significantly larger in Big Pine Key, there were no discrete spatial patterns between the number of plastics detected and bell size (Pearson's $R \leq 0.22$, n = 34–41, $p \geq 0.17$ per location) or plastic size and bell size (Pearson's $R \leq 0.17$, n = 34–41, $p \geq 0.75$ per location).

Microplastics retrieved from *Cassiopea* were examined using both microscopy and spectral analysis (Fig. 5) and grouped by color and shape. Using μFTIR on a sub-set of microplastic samples confirmed that all of the particles analyzed can from a source outside of the marine environment, and confirmed the presence of both synthetic (e.g. rayon) and natural (e.g. cotton) microfibers (Table 1). Of the 11 particles analyzed (Table 1), 4 were polyester fibers, 3 were mixed/rayon/synthetic fibers, 2 were natural fibers, 1 nitrocellulose fragment, and 1

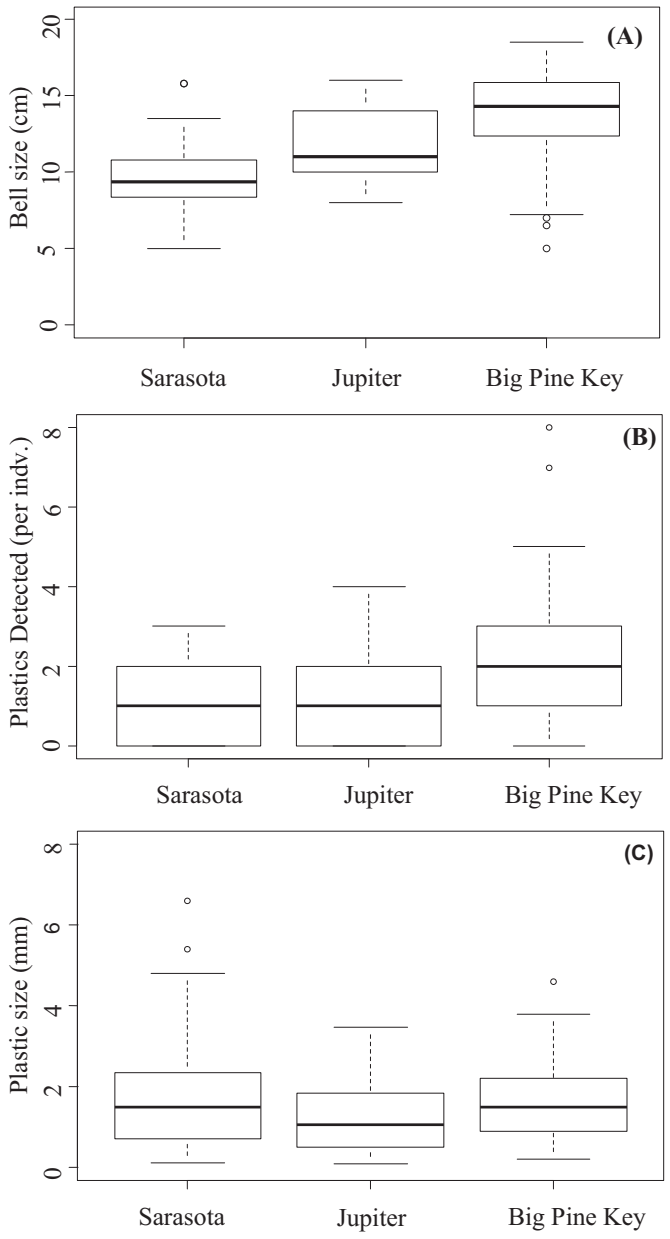

Fig. 3. Boxplots showing minimum whiskers, first quartile box, median line third quartile box, and maximum whisker and outlier circles of (A) bell size, (B) number of plastics detected, and (c) plastic size per *Cassiopea*.

polyurethane fragment. Fig. 5A is an example of the μFTIR output showing the rayon > 80%, nylon, polyester standard (reference # FB393) in gray and microfiber samples (in red) collected from *Cassiopea* in Sarasota (Fig. 5A). Fig. 5B is the PLM image confirmation of the rayon > 80%, nylon, polyester standard (reference # FB393) and Fig. 5C is the same sample under polarized light. The majority of microplastics found across all locations were black, blue or red (Fig. 6A). We found no patterns or significant differences in color abundance by location (ANOVA, all $p > 0.5$) (Fig. 6A). Of the 181 total plastics retrieved, 94% were microfibers and comprise dominant microplastics measured at all three locations (Fig. 6B).

4. Discussion

Following a growing body of literature indicating microplastic contamination in marine biota (reviewed in Lusher et al., 2016), as well as evidence of plastic ingestion by pelagic jellyfish (Macali et al., 2018), we hypothesized that benthic jellyfish might act as bioindicators of microplastic pollution in nearshore systems. Nearly 80% of *Cassiopea* sampled here across three estuaries contained microplastics. The use of nitric acid to dissolve organic matter will destroy some fraction of plastics in the samples (Desforges et al., 2015), thus, results are typically a conservative estimate of microplastic presence and density. Here, the μFTIR results confirmed the presence of both synthetic and natural fibers, so it is likely that a small subset of the microfibers quantified in this study were natural fibers not completely dissolved in the acid digestion process. Regardless, the dominance of microfibers, both synthetic and natural, indicate anthropogenic inputs (e.g., wastewaters contaminated from washing textiles and/or contents of stormwater runoff; Browne et al., 2011; Reisser et al., 2013; Lebreton et al., 2017; Ling et al., 2017). The dominance of microfibers reported here is consistent with other studies showing microfiber ubiquity in the marine environment (Barrows et al., 2018; Mishra et al., 2019). Interestingly, Big Pine Key, Florida had the largest percentage of individuals containing microplastics (90%) and the highest density (2.4 ± 1.8 microplastics) per individual. In sum, this study compiles the largest dataset on microplastic prevalence in jellyfish that we are aware of and demonstrates resounding evidence that benthic jellyfish in Florida's estuaries do contain microplastic.

Our results agree with previously documented cases of microplastics in benthic invertebrates, including bivalves, crustaceans, gastropods, and cnidarians (Davidson and Dudas, 2016; Ding et al., 2019; Rotjan et al., 2019; Santana et al., 2016; Sun et al., 2017; Thushari et al., 2017; Van Cauwenberghe et al., 2015). However, the lack of standardized methods and reporting in microplastics studies has made it difficult to compare direct values determined from the various methods. Previous studies have used a combination of various digestion methods (Hidalgo-Ruz et al., 2012), have not reported species names (Sun et al., 2017), and reported results in various units. The only other reports of microplastics in wild jellyfish document a 34% average encounter rate of microplastic in planktonic jellyfish (Sun et al., 2017), lower than the 77% encounter rates measured here. Further, Sun et al. (2017) measured microplastics non-discriminately across unidentified pelagic species collected offshore. The mechanisms driving the difference in microplastics encountered by pelagic jellyfish and benthic jellyfish are unclear, but this disparity is likely due to differences between habitats and life cycles. Since buoyant microplastics are typically pushed onshore they tend to accumulate in shallow benthic environments (Claessens et al., 2011; Wright et al., 2013). Our results are consistent with the prevalence of microfibers in benthic organisms (Wright et al., 2013), and the polyethylene detected here using μFTIR corresponds to the microfibers identified in coastal sediments in the northern Gulf of Mexico (Wessel et al., 2016). Collectively, this suggests that pelagic jellyfish might not be as regularly exposed to the same microplastics in the water column. Furthermore, microplastic pollution may be more prolific in areas adjacent to high human population densities (Browne et al., 2011; Henry et al., 2019). Since *Cassiopea* are particularly abundant in urban coastal areas (Stoner et al., 2016, 2011), it is likely that *Cassiopea* are exposed to more microplastics on the benthos than their pelagic counterparts.

Spatial variation also played a role in driving differences in microplastic exposure to *Cassiopea*. Surprisingly, the estuaries adjacent to higher human population densities and activity (Sarasota Bay and Dubois Park), which would presumably accumulate plastic debris (Browne et al., 2011; Rochman, 2018) were found to have fewer microplastics per jellyfish than the less urbanized estuary in the Florida Keys. In this study, Sarasota Bay would have been the most likely area experiencing anthropogenic inputs due to the high population density within that area (1474 ± 3 people/km^2), followed by Jupiter (1161 ± 1 people/km^2), where the particular area sampled is adjacent to a popular recreational swimming lagoon. The highest occurrence and density of plastics were found in samples from Big Pine Key that is farther from areas of heavy anthropogenic use (185 ± 17 people/km^2)

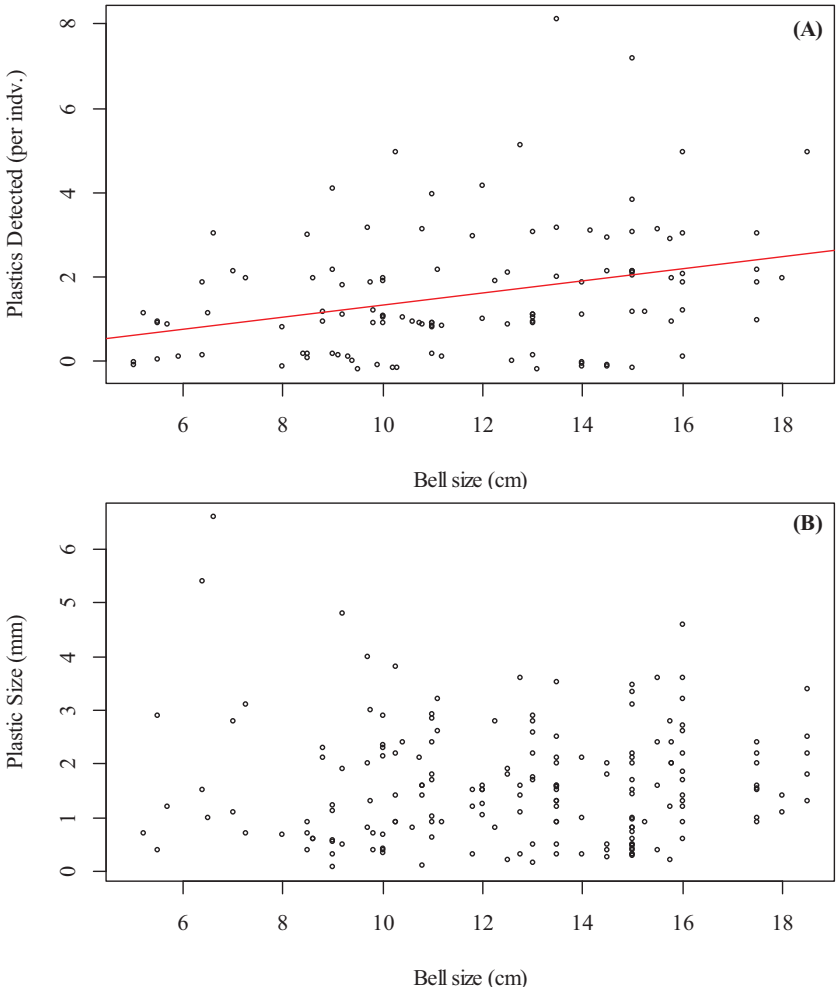

Fig. 4. (A) number of plastics and (B) plastic size per *Cassiopea* bell size (Pearson's R = 0.32, n = 115, p ≤ 0.001 shown in red).

(U.S. Census Bureau, 2018). This result is surprising given that other studies have documented patterns of trace metal accumulation in *Cassiopea* reflecting differences in the extent of adjacent urban areas (Templeman and Kingsford, 2010). It is likely that microplastics are not evenly distributed, becoming concentrated in areas with ideal hydrodynamic conditions (Macali and Bergami, 2020). Yet these three study sites are strikingly similar in their features, all lacking adjacent obvious stormwater or wastewater outflows which are typically associated with microfibers (e.g., Browne et al., 2011; Henry et al., 2019).One possible explanation is that higher plastics detected from Big Pine Key Florida might be the result of damage caused by Hurricane Irma. On September 10th, 2017 Hurricane Irma made its first landfall in the U.S. at Cudjoe Key, Florida (~12 km south of Big Pine Key), causing extensive damage and lingering debris fields in coastal waters (Tomiczek et al., 2020). Hurricanes can increase the transfer of terrestrial debris from land to sea, leading to a higher density of microplastic pollution in coastal waters (Lattin et al., 2004; Thompson, 2005). These conditions may have contributed to the amount and composition of microplastics available for jellyfish. For instance, synthetic microfibers were the only microplastics identified from Jupiter and Sarasota, whereas both microfibers and fragment debris such as paint chips (potentially from hurricane debris), were among some of the microplastics identified at Big Pine Key. Moving forward, we suggest that future studies consider distance to microplastic source points (e.g., hurricane impact sites, landfills, or stormwater or wastewater outflows). It should be noted that while we observed the highest density of microplastics from Big Pine Key, we also obtained the largest jellyfish from Big Pine Key. Though we only observed a weak correlation between the density of plastics and *Cassiopea* bell size (across all sites), it would not be surprising if larger jellyfish with increased surface area encounter more microplastics. Although our data does not directly identify a mechanism that explains the heterogeneous spatial distribution of microplastics in *Cassiopea*, further study is warranted on traits of *Cassiopea*, both intrinsic (e.g., size, arm length) and extrinsic (e.g., microhabitat, current patterns), that relate to microplastic incidence.

One important caveat of this study is that the entire animal was dissolved and filtered, so it is impossible to determine if the microplastics were present in the stomach, in the surrounding manubrium, or attached to the intricate oral arms of the animal. Thus, our data do not confirm that the *Cassiopea* were ingesting the microplastics observed. It is possible that microplastics become entrapped in the delicate ornate oral arms, entangled in the mucus cassiosome structures (see Ames et al., 2020), or assimilated during jellyfish pulsation (Ohdera et al., 2018). However, other studies demonstrate that cnidarians, including pelagic jellyfish, ingest plastics (Hall et al., 2015; Macali et al., 2018; Rotjan et al., 2019). This, along with hundreds of secondary heterotrophic feeding mouths (Ohdera et al., 2018) indicates the possibility that *Cassiopea* also consume microplastics. Regardless of how *Cassiopea* encounter microplastics, the occurrence and density of microplastics observed in *Cassiopea* reveals the nature of microplastic contamination in their environment. For instance, the high number of microfibers found in *Cassiopea* across all three sites is comparable to previous work indicating that microfibers are the most common microplastic in benthic deposit feeders (Wright et al., 2013). Additionally, *Cassiopea*

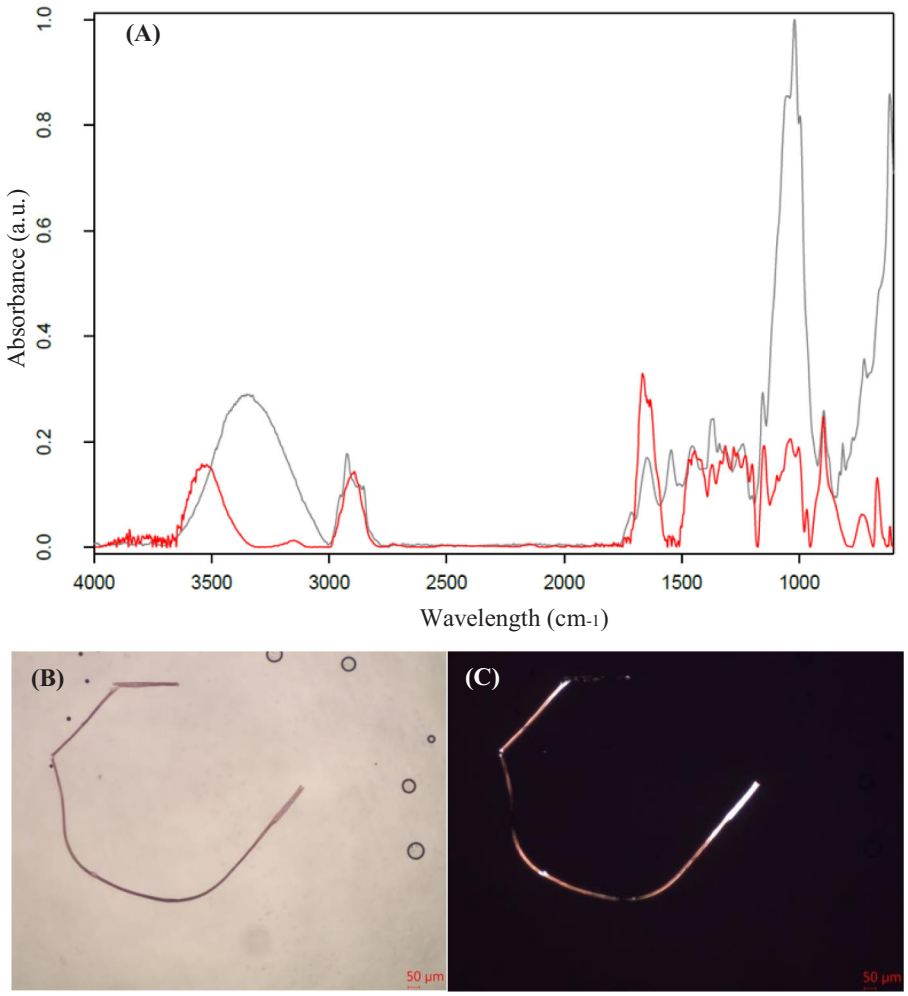

Fig. 5. Microfiber sample collected in Sarasota showing (A) FTIR results where gray is the rayon > 80%, nylon, and PTE mix standard (Beltissimo mixed yarn rayon > 80%, nylon and PTE mix) and red is the sample wavelength, (B) PLM image, and (C) is the same PLM under polarized light.

may reject some microplastic particles (as observed in holothurians (Graham and Thompson, 2009)), or selectively ingest them (as observed in corals (Hall et al., 2015)). If *Cassiopea* mirror the selective foraging behavior observed in other cnidarians, they are likely to accumulate specific types of microplastics (Karlsson et al., 2017). The dominance of black and blue microfibers measured here are consistent with other studies in marine sediments and waters (Gago et al., 2018), whereas white, clear, and blue microplastics are more commonly ingested by benthic organisms (Wright et al., 2013). It is imperative that further work explore feeding behavior of benthic jellyfish in the presence of microplastics to ascertain whether *Cassiopea* actively consume microplastics, and what effects, if any, they have on *Cassiopea* feeding behavior and physiology.

The presence of microplastics in benthic jellyfish is further evidence of unforeseen anthropogenic impacts in marine environments – plastic accumulation in organisms. It is not only the microplastics themselves, but any associated persistent organic pollutants that pose a threat to marine life. Some microplastics are known to readily absorb persistent organic pollutants, which can have deleterious effects and be transferred through food chains and harm other marine life (Hirai et al., 2011; Mato et al., 2001; Teuten et al., 2007). Polyvinyl chloride (PVC), for example, increases the bioavailability of benzo(*a*)pyrene with toxic

Table 1
Unknown sample (n = 11) identification from µFTIR by sampling location.

Location	Unknown sample	Hit Quality Index	Reference material (database)
Sarasota	Polyester (PET) fiber	454	Jacket material (IVENIX STD)
	Rayon fiber	379, 340	Rayon typical of clothing (FB412, FB412)
	Natural and synthetic fiber blend	854	Cotton 95%, spandex 5% (IVENIX standard)
	Synthetic cellulose-based fiber	143	Rayon > 80%, nylon (FB393)
Jupiter	Natural fiber	268	Wool fiber (MicroVision Standard)
	Polyester (PET) fiber	323	Mixed synthetic fiber medical wipe (IVENIX standard)
	Polyester (PET) fiber	607	Glove stitching (IVENIX standard)
Big Pine Key	Nitrocellulose material	528, 398, 314	Car enamel, nail lacquer, nitrocellulose-based Celolesk (AD071, NIC10864, C 1037)
	Polyurethane material	419, 300, 260	Wood stain, fuchsite, pigment blue 32 (AC328, NIC07643, 69458-70-4)
	Natural fiber	812	Cotton (FB405, FB405)
	Polyester (PET) fiber	523	Glove stitching (IVENIX standard)

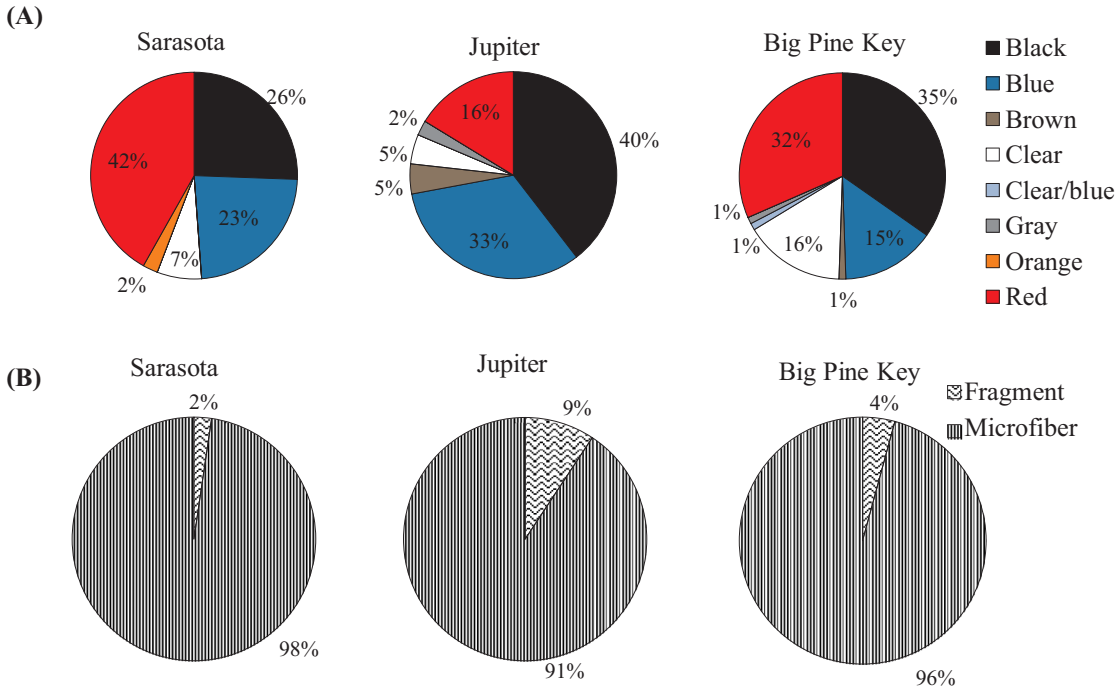

Fig. 6. Composition of plastics (% of total) at each sample location by (A) color and (B) shape. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

effects on benthic invertebrates (e.g., Gomiero et al., 2018). Although we did not detect PVC here in the μFTIR subset, the effects of PVC on co-pollutant absorbance in benthic invertebrates is disconcerting. Therefore, it is imperative that further in depth studies explore feeding behavior of benthic jellyfish in the presence of various microplastics to ascertain whether *Cassiopea* actively consume microplastics, and what effects, if any, different microplastics have on *Cassiopea* feeding behavior and physiology. Furthermore, the prevalence of microplastics is likely to have physiological consequences as well as larger-scale impacts on ecosystem functioning. We suggest a focus on the physiological impacts of plastic contamination in *Cassiopea* to resolve implications on a broader ecosystem scale. For instance, *Cassiopea*'s predators include sea turtles (*Dermochelys coriacea* (Arai, 2005)), fireworms (*Hermodice carunculata* (Stoner and Layman, 2015)), and butterflyfish (*Chaetodontidae*; Stoner, in prep.). Thus, future research must consider both the foraging behavior of *Cassiopea* on microplastics and how the physiological and behavioral impacts of ingesting plastics (e.g., gut obstruction and potential starvation) will evoke larger trophic level effects. Although we do not yet know the full implications of microplastics in *Cassiopea*, we have demonstrated the presence of microplastics in benthic jellyfish across three different estuaries in Florida. Our findings support that microplastic contamination is likely ubiquitous in the nearshore marine environment and we suggest *Cassiopea* as a bioindicator of microplastic contamination.

CRediT authorship contribution statement

Samantha M. Iliff: Data curation, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Eliza R. Wilczek:** Data curation, Investigation, Methodology, Writing - review & editing. **Rachel J. Harris:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - review & editing. **Ryan Bouldin:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - review & editing. **Elizabeth W. Stoner:** Conceptualization, Data curation, Methodology, Formal analysis, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

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.

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