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SPECIAL ISSUE-LETTER

Microplastic contamination in Corpus Christi Bay blue crabs, *Callinectes sapidus*

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Scientific Significance Statement

Plastic materials have been observed in marine and coastal ecosystems around the world and while their full effects are not completely understood, they negatively impact a variety of organisms. Invertebrates have been observed with plastic in their guts, but it is unknown if blue crabs, which are an important U.S. commercial and recreational fishery, consume these materials. This article reinforces the importance of quality control, proper methodology, and material confirmation in microplastic studies and provides evidence that blue crabs in Corpus Christi Bay, TX ingest microplastic fibers and particles.

Abstract

Microplastic pollution has been observed in marine environments around the world and has the potential to negatively impact marine organisms if ingested. Blue crabs (*Callinectes sapidus*) are susceptible to this pollution because they feed in sediment where dense plastics accumulate. Microplastic ingestion by blue crabs was assessed in Corpus Christi Bay, TX. Crab stomachs were extracted and digested using a hydrogen-peroxide based tissue destruction method followed by material confirmation using microattenuated total reflectance Fourier transform infrared spectroscopy (μ-FTIR). From the 39 blue crabs sampled, 28 fully synthetic fragments and fibers and 24 semisynthetic fibers were found within their stomachs. After correcting for possible contamination, 36% of collected blue crabs contained fully synthetic fragments and fibers and semisynthetic fibers with an estimate of 0.87 items per crab. This study demonstrates the need for further studies that assess the impacts of plastic ingestion on blue crabs.

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Author Contribution Statement: E.N.W. and J.L.C. worked together and contributed to the initial preparation of the manuscript. E.N.W. proposed the initial research question of whether or not blue crabs were exposed to and contaminated by microplastics. Both E.N.W. and J.L.C. refined and developed the sampling methods, lab methodology, and sample analysis. E.N.W. conducted the field sampling and processing of blue crabs according to developed methods. E.N.W. and J.L.C. analyzed the results and wrote the initial paper together. N.L. effort focused on revising the original manuscript, where he played a vital role in addressing comments, reviewing data, and general editing.

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Blue crabs are common in the Gulf of Mexico and western Atlantic Ocean where they are the target of several large recreational and commercial fisheries ($219 million annually in the U.S.) (National Marine Fisheries Service 2016). They serve as prey for many organisms (fish, rays, and larger invertebrates) (Hovel and Lipcius 2001) and are also opportunistic omnivores that feed on plants, animals, detritus, and carcasses when available (Laughlin 1982). Their benthic foraging habits, opportunistic feeding strategies, and proximity to sources of anthropogenic litter increase their likelihood of exposure to microplastics. This is particularly true for denser plastics that are more likely to accumulate in sediment, like polyvinyl chloride (PVC), and buoyant plastics that are fouled by biofilms and settle out of the water column, such as polyethylene (PE) or polypropylene (PP) (Wright et al. 2013). Due to their highly opportunistic feeding strategies, blue crabs may be unable to distinguish between their natural foods and plastics, such as when it is biofouled or entangled in other substrates, and could even preferentially target them (Graham and Thompson 2009; Murray and Cowie 2011).

Marine invertebrates around the world have been observed with microplastics in their stomachs and tissue, with concentrations as high as 57.2 plastic items per organism (Table 1) confirmed by Fourier transform infrared spectroscopy (FTIR) analysis. Ingested microplastics observed in these studies vary in shape, color, and material, and are likely correlated with the organism's location and feeding habits. Studies on the Norway lobster (*Nephrops norvegicus)* and the Chinese Mitten crab (*Eriocheir sinensis*) found that 83% of the sampled lobsters and 13% of the sampled crabs were contaminated with microplastics (Table 1). In both studies, the majority of recovered plastics consisted of clear balled fibers that were observed to match those originating from fisheries (nets, ropes, fishing line) (Wójcik-Fudalewska et al. 2016) or had similar μ-Raman spectroscopy spectra (Murray and Cowie 2011). Another study that investigated blue mussels, *Mytilus edulis*, found that microplastic concentrations were positively correlated with the organism's proximity to human populations (Li et al. 2015).

Uptake of microplastics has potential health and economic implications for fisheries and the humans that rely on them. Microplastics can negatively impact species through a variety of lethal and sublethal effects, including choking, pseudo-satiation, maiming, reduced fitness, and the alteration of behavior (Laist 1987, 1997; Gregory 2009; Wright et al. 2013; Ivar do Sul and Costa 2014). Plastics also frequently contain additives or sorbed chemicals and metals from the environment that can leach into the organism upon uptake (Teuten et al. 2009; Browne et al. 2013; Vedolin et al. 2018) and transfer between trophic levels (Browne et al. 2008; Batel et al. 2016). It is also possible for microplastics to transfer to humans when the entire organism's soft tissue is consumed (Li et al. 2015) or the edible parts of the organisms overlap with contaminated tissue, as is the case with bivalves or soft-shell crabs. Bivalves sold in U.K. and Chinese markets were found to contain microplastics in concentration ranging from 0.9 to 10.5 microplastics per gram of tissue (Li et al. 2015, 2016). While it is unknown what effects ingested microplastics have on humans, bivalves are not the only contaminated seafood we consume (Table 1) and other fisheries likely face similar exposure to microplastics.

Blue crabs are an economically important fishery in coastal Texas as well as many fishing ports in the U.S. They also serve as a prey item for larger fish and invertebrates. Despite the likelihood of their exposure and position in the food web, ingestion of microplastics by blue crabs has not been characterized. As such, the goal of this study was to determine whether microplastic ingestion by blue crabs was comparable to other marine invertebrates so as to assess the need for further studies on chemical leaching from plastics and their accumulation rates in this economically important fishery. We answered this question by analyzing the microplastic contamination in the stomachs of blue crabs collected from Corpus Christi Bay, TX using chemical digestion techniques and micro Fourier transform infrared spectroscopy (μ-FTIR).

Methods

Materials

Equipment used for this method included the vacuum filtering apparatus with cellulose acetate membrane filters (47 mm diameter and 0.8 μm pore size, Advantec), a Thermo Nicolet iS10 FTIR equipped with a mercury cadmium telluride infrared detector and a iN5 Microscope with a germanium crystal for attenuated total reflectance, and a Meiji Technology EMZ-8TR stereomicroscope. Chemicals used included high performance liquid chromatography (HPLC) grade acetone (Fisher Scientific), HPLC grade hexane (Fisher Scientific), and 30% by volume hydrogen peroxide (H_2O_2) (Sally's Beauty Supply store). All H_2O_2 was prefiltered at 0.8 μm, stored in a refrigerator at 4°C in a clean amber glass bottle when not in use, and replaced after 30 d to maintain concentration.

Microplastic contamination in Corpus Christi blue crabs

Microplastic ingestion by blue crabs was assessed by collecting specimens from three sites around Corpus Christi Bay. A total of 39 blue crabs were collected (12 from Site A, 15 from Site B, and 12 from Site C) using lines baited with raw chicken. Raw chicken used as bait was not tested for microplastic contamination prior to use. However, the baited lines were closely monitored so that crabs were captured immediately upon attacking the chicken, limiting it as a potential source of contamination. They were then placed in a in a hard-plastic cooler with a PE exterior and PP interior and transported back to the lab. Travel time from the sampling location to the lab varied from 15 to 45 min. Upon returning to the lab, the length, mass, and sex of the crabs were recorded. Blue crabs were then chilled to numb their senses and euthanized humanely before their stomachs were collected and individually placed into

Table 1. Summary of microplastic contamination in invertebrates: experimental evidence of microplastic contamination, detailing the organisms, location, quantity of plastics, percent of individuals contaminated, and the method used to extract the plastics.

Class of studied organism	Organism	Location	Microplastic contamination	Individuals with plastics (%)	Method used	Tissue analyzed in study	Method of verification	Reference
Crustacea	*Aristeus antennatus*	Northwest Mediterranean Sea	n.d	39.2	Manual dissection and 10% KOH	Gut and stomach	ATR-FTIR	Carreras-Colom et al. (2018)
	Callinectes sapidus	Corpus Christi Bay, Gulf Coast	0.44–0.72 items per individual	25.6	30% H_2O_2	Stomach	ATR-FTIR	This study
	Chionoecetes opilio	Chukchi and Bering seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue and gills	FTIR	Fang et al. (2018)
	Crangon crangon	Southern North Sea (UK, France, Belgium, and The Netherlands)	1.23 ± 0.99 items per individual	63	HNO_3: $HCLO_4$	Soft tissue	Hot-needle test	Devriese et al. (2015)
	Emerita analoga	California coast	0.65 ± 1.64 items per individual	35	Manual dissection	Digestive tract	ATR-FTIR	Horn et al. (2019)
	Eriocheir sinensis	Baltic Sea (Poland), coast of Portugal	n.d	13	Manual dissection	Stomach	Visual identification	Wójcik-Fudalewska et al. (2016)
	Euphausia pacifica	Northeast Pacific Ocean, Canada	0.059 items per individual	n.d	HNO_3	Ingestion	n.d	Desforges et al. (2015)
	Lepas spp.	Northeast Pacific Ocean	1.35 items per individual	33.5	Manual dissection	Stomach and intestinal tract	Raman spectroscopy	Goldstein and Goodwin (2013)
	Maja squinado	Celtic Sea	1.39 ± 0.79 items per individual	42.5	Manual dissection	Stomach	FTIR	Welden et al. (2018)
	Neocalanus cristatus	Northeast Pacific Ocean, Canada	0.029 items per individual	n.d	HNO_3	Ingestion	n.d	Desforges et al. (2015)
	Nephrops norvegicus	Clyde Sea	n.d	83	Manual dissection	Stomach	Raman spectroscopy	Murray and Cowie (2011)
	Orchestoidea tuberculata	Playa Quintay, Chile coast	0.071 items per mg of feces	n.d	20% KOH	Ingestion	Visual identification	Carrasco et al. (2019)
	Pandalus borealis	Chukchi and Bering seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue and gills	FTIR	Fang et al. (2018)

Asteroidea	*Asterias rubens*	Chukchi and Bering seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue	FTIR	Fang et al. (2018)
	Ctenodiscus crispatus	Chukchi and Bering seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue	FTIR	Fang et al. 2018
	Hymenaster pellucidus	Rockall trough, North Atlantic Ocean	0.48 ± 0–9.10 ± 4.21 items per individual	0–40	Enzymatic digestion	Soft tissue	FTIR	Courtene-Jones et al. (2019)
	Hymenaster pellucidus	Rockall Trough, North Atlantic Ocean	1.153 ± 0.278 items per individual	40	Enzymatic digestion	Soft tissue	ATR-FTIR	Courtene-Jones et al. (2017)
	Leptasterias polaris	Chukchi and Bering seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue	FTIR	Fang et al. (2018)
Ophiuroidea	*Ophiomusium lymani*	Rockall Trough, North Atlantic Ocean	1.96 ± 0.66 to 3.43 ± 1.35 items per individual	25–80	Enzymatic digestion	Soft tissue	FTIR	Courtene-Jones et al. (2019)
	Ophiomusium lymani	Rockall Trough, North Atlantic Ocean	1.582 ± 0.448 items per individual	73.7	Enzymatic digestion	Soft tissue	ATR-FTIR	Courtene-Jones et al. (2017)
	Ophiura sarsii	Chukchi and Bering Seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Whole organism	FTIR	Fang et al. (2018)
Holothuroidea	*Apostichopus japonicus*	Coastal waters of China	9.88 ± 6.81 to 0.4 ± 0.37 items per individual	n.d	30% H_2O_2 and 10% KOH	Coelomic fluid	FTIR	Mohsen et al. (2019)
Gastropoda	*Cerithidea cingulata*	Persian gulf	17.7 ± 0.3 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Naji et al. (2018)
	Colus jeffreysianus	Rockall Trough, North Atlantic Ocean	0.678 ± 0.044 items per individual	28.6	Enzymatic digestion	Soft tissue	ATR-FTIR	Courtene-Jones et al. (2017)
	Euspira nana	Chukchi and Bering Seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue (excluding foot)	FTIR	Fang et al. (2018)
	Latisipho hypolispus	Chukchi and Bering Seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue (excluding foot)	FTIR	Fang et al. (2018)
	Retifusus daphnelloides	Chukchi and Bering Seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue (excluding foot)	FTIR	Fang et al. (2018)
	Thais mutabilis	Persian Gulf	3.7 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Naji et al. (2018)
Polychaeta	*Arenicola marina*	North Sea Coast (France, Belgium, The Netherlands)	1.2 ± 2.8 items per g of tissue	100	HNO_3	Whole organism	Raman spectroscopy	Van Cauwenberghe et al. (2015)
Bivalvia	*Alectryonella plicatula*	China	4.3–57.2 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2015)
	Amiantis purpuratus	Persian Gulf	6.8 ± 1.8 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Naji et al. (2018)
	Amiantis umbonella	Persian Gulf	6.9 ± 2.3 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Naji et al. (2018)

(Continues)

Table 1. Continued

Class of studied organism	Organism	Location	Microplastic contamination	Individuals with plastics (%)	Method used	Tissue analyzed in study	Method of verification	Reference
	Astarte crenata	Chukchi and Bering Seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue	FTIR	Fang et al. (2018)
	Crassostrea gigas	Atlantic Ocean (France)	0.47 ± 0.16 items per g of tissue	n.d	HNO_3	Soft tissue	Raman spectroscopy	Van Cauwenberghe and Janssen (2014)
	Crassostrea sp.	Coastal China	2.93 items per individual	84	30% H_2O_2 and 10% KOH	Soft tissue	FTIR	Teng et al. (2019)
	Cyclina sinensis	China	4.3–57.2 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2015)
	Macoma tokyoensis	Chukchi and Bering Seas continental shelf	0.04–1.67 items per individual	n.d	10% KOH	Soft tissue	FTIR	Fang et al. (2018)
	Meretrix lusoria	China	4.3–57.2 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2015)
	Mytilus edulis	China	1.5–7.6 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2016)
	Mytilus edulis	Halifax Harbor, Canada	34–178 items per individual	100	30% H_2O_2	Soft tissue	Visual identification	Mathalon and Hill (2014)
	Mytilus edulis	Coastal waters of the United Kingdom	1.1–6.4 items per individual	43	30% H_2O_2	Soft tissue	FTIR	Li et al. (2018)
	Mytilus edulis	Germany (Mussel Farm)	0.36 ± 0.07 items per g of tissue	n.d	HNO_3	Soft tissue	Raman spectroscopy	Van Cauwenberghe and Janssen (2014)
	Mytilus edulis	South west coast of the United Kingdom	1.43–7.64 items per individual	88	10% KOH	Soft tissue	FTIR	Scott et al. (2019)
	Mytilus edulis	North Sea Coast (France, Belgium, The Netherlands)	0.2 ± 0.3 items per g of tissue	100	HNO_3	Soft tissue	Raman spectroscopy	Van Cauwenberghe et al. (2015)
	Mytilus edulis and *Perna viridis*	Coastal Waterrs of China	0.77–8.2 items per individual	n.d	30% H_2O_2	Soft tissue	ATR-FTIR	Qu et al. (2018)
	Mytilus galloprovincialis	Ionean Sea (Mediterranean Sea)	0.8 ± 0.2 to 0.9 ± 0.2 items per individual	46.25	30% H_2O_2	Soft tissue	FTIR	Digka et al. (2018)
	Mytilus galloprovinicialis	China	4.3–57.2 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2015)
	Patinopecten yessoensis	China	4.3–57.2 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2015)
	Perna perna	Santos Estuary, Brazil	n.d	75	HNO_3	Whole organism	Polarized light microscopy	Santana et al. (2016)
	Pinctada radiata	Persian Gulf	3.9	n.d	30% H_2O_2	Soft tissue	FTIR	Naji et al. (2018)

Ruditapes philippinarum	China	4.3–57.2 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2015)
Scapharca subcrenata	China	4.3–57.2 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2015)
Sinonovacula constricta	China	4.3–57.2 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2015)
Tegillarca granosa	China	4.3–57.2 items per individual	n.d	30% H_2O_2	Soft tissue	FTIR	Li et al. (2015)

n.d, not determined.

clean 50 mL glass scintillation vials. Stomachs were then processed using the microplastic extraction method outlined in "Microplastic extraction method" section.

The cooler was not considered a source of contamination for this experiment as the focus of the study was the microplastics within the collected blue crabs' stomachs. Microplastics generated or encountered during transportation would need to be ingested by the blue crabs to appear as contamination in the results. Given the short duration blue crabs were exposed to the plastic cooler and the stress/duress of transport, we deemed the risk of contamination from this step to be minimal. This is further discussed in "Assessment of microplastics in Corpus Christi blue crabs" section.

Microplastic extraction method

Methods for the extraction of microplastics from soft-tissue were adapted from Li et al. (2015). Blue crab stomachs were isolated and placed in a clean 50 mL centrifuge tube, loosely sealed with a cap, and dried at 40°C for 7 d. Then the stomachs were gently crushed with a glass stirring rod to increase the tissue surface area. To ensure no materials remained on the rod, it was rinsed three times into the centrifuge tube with 2 mL of 30% H_2O_2, for a total of 6 mL. The glass stirring rod was then visually inspected under a stereomicroscope to ensure no materials remained attached. Samples were then loosely recapped and digested overnight at 20°C before the addition of another 2 mL of 30% H_2O_2 followed by gentle swirling for 15 s. This digestion step was repeated twice, using a total of 6 mL more over 72 h, before the centrifuge tubes digested at 20°C for a final 48 h. Next, the centrifuge tubes were heated at 40°C in a hot water bath for 2 h before vacuum filtration through a 0.8 μm cellulose acetate membrane filter. The filtering apparatus and now-empty centrifuge tube were inspected under a microscope to ensure the complete transfer of material. Filters were then visually inspected under a stereomicroscope for suspected microplastic materials.

Microplastic identification and analysis

Suspected microplastic particles and fibers (ranging in diameter from 10 to 400 μm) extracted from blue crabs were analyzed using μ-FTIR on a Thermo Nicolet iS10 FTIR equipped with a mercury cadmium telluride infrared detector and a iN5 Microscope with a germanium crystal for attenuated total reflectance. Sample spectra were collected with 256 scans at a resolution of 8 cm^{-1} over the range of 650–4000 cm^{-1}. Backgrounds were measured before each sample run and all collected spectra were compared to the "Forensic Comprehensive," "HR sprouse polymers by ATR," "ICHEM Nicodom ATR, ATR 100 Specta Dema Library," and "Hummel polymer sample library" databases for identification. Samples that positively matched the database (> 65% confidence) were included in the results and made available on Dryad (Waddell et al. 2019).

Quality control and contamination assessment

All glassware and utensils were washed with detergent and subsequently rinsed with deionized water prior to use. Glassware was muffled at 500°C for 4 h and covered with aluminum foil after cooling. Utensils and glassware were inspected under a microscope prior to use. Laboratory contamination of samples was assessed using a method blank. This consisted of a precleaned empty vial that was identically processed alongside the samples at a rate of one for every three samples for a total of 13 blanks. Each method blank was exposed to the same conditions and manipulation as its paired samples, and was, once processed and filtered, left open for the duration of the microscope sorting of the paired samples. Therefore, the method blanks were exposed to the same laboratory conditions for the same duration as the three samples they were paired with. Contaminants observed in method blanks were then used to establish limits of detection (LOD) (De Witte et al., 2014). This was calculated as the mean plastic contamination from method blanks +3x standard deviation (SD) for each item, by its color, shape (fiber, particle, or film), and material type. The materials found in the blanks were pooled and applied to all samples, not just the samples paired with particular blanks. Corrective action was only taken if an item of the same color, shape, and material was found in both the method blank and samples. For example, if blue polyester fibers were found in a method blank and its associated samples, corrective action was taken. However, if red polyester fibers were found in a method blank, but only blue polyester fibers were found in the associated samples, no corrective action was taken for the blue polyester fibers. This approach provides a conservative method to assess microplastic materials found in our samples.

Results

Assessment of microplastics in Corpus Christi blue crabs

A total of 39 blue crabs were collected from around Corpus Christi Bay. Their stomachs contained 157 suspected microplastics (126 fibers, 29 fragments, and 2 films). After μ-FTIR analysis, 52 items were confirmed as synthetic or semisynthetic, which included 49 fibers, 2 fragments, and 1 film (Table 3). The fiber materials were cellulose/rayon blend (24, 49%), polyester (15, 31%), acrylic/acrylic blends (9, 18%), and polystyrene (1, 2%) (Table 3). The fragments were identified as polyethylene terephthalate and polycarbonate while the film was identified as a phenoxy resin.

LOD were calculated from the contamination observed on the method blanks. However, method blank #6 was lost during analysis, preventing its inclusion in the pooled method blank calculations. In total, method blanks contained 19 clear fibers, 17 clear/white/yellowed fragments, 2 black fragments, 1 blue fiber, 1 turquoise fiber, and 1 red fiber (Table 2). Both the red and turquoise fiber were identified as polyester while the blue fiber was identified as a cellulose blend. Black fragments were identified as cellulose while all clear/white/yellowed fragments found in method blanks were identified as polystyrene. Clear fibers found in method blanks were identified as either polyester, polystyrene, or cellulose. LOD

Table 2. Summary of microplastics observed on 12 method blanks and calculated LODs used to correct microplastics and semisynthetic fibers observed in sampled blue crabs.

Blank #	Clear/white/yellowed polystyrene fragments (no. of items)	Clear (PS, PE, and cellulose) fibers (no. of items)	Black cellulose fragment (no. of items)	Red polyester fiber (no. of items)	Turquoise polyester fibers (no. of items)	Blue cellulose fibers (no. of items)
1	3.00	1.00	0.00	0.00	0.00	0.00
2	1.00	1.00	0.00	0.00	0.00	0.00
3	3.00	1.00	0.00	0.00	0.00	0.00
4	3.00	2.00	0.00	0.00	0.00	0.00
5	1.00	2.00	0.00	0.00	0.00	0.00
6	Lost	Lost	Lost	Lost	Lost	Lost
7	1.00	0.00	1.00	0.00	0.00	0.00
8	3.00	2.00	1.00	1.00	0.00	0.00
9	1.00	0.00	0.00	0.00	0.00	0.00
10	0.00	1.00	0.00	0.00	0.00	0.00
11	0.00	4.00	0.00	0.00	1.00	0.00
12	0.00	4.00	0.00	0.00	0.00	0.00
13	1.00	1.00	0.00	0.00	0.00	1.00
Sum	17.00	19.00	2.00	1.00	1.00	1.00
Mean	1.42	1.58	0.17	0.08	0.08	0.08
SD	1.240112	1.311372	0.389249	0.288675	0.288675	0.288675
LOD	5.137004	5.51745	1.334415	0.949359	0.949359	0.949359

Table 3. Summary of synthetic and semisynthetic items observed in the stomachs of 39 blue crabs sampled from Corpus Christi Bay with and without LOD corrections.

Color	Shape	Material	Material category	Fully and semisynthetic microplastics (no. of items)	Blank corrected microplastics (no. of items)
Green	Fragment	Polycarbonate	Fully synthetic	1	1
Gray	Film	Phenoxy resin	Fully synthetic	1	1
Clear	Fragment	Polyethylene terephthalate	Fully synthetic	1	1
	Fiber	Cellulose/rayon blend	Semi-synthetic	5	0
		Polyester	Fully synthetic	5	0
		Polystyrene	Fully synthetic	1	0
		Acrylic	Fully synthetic	3	3
Blue	Fiber	Cellulose/rayon blend	Semi-synthetic	15	10
		Polyester	Fully synthetic	6	5
Black	Fiber	Cellulose/rayon blend	Semi-synthetic	4	4
		Polyester	Fully synthetic	2	2
		Acrylic	Fully synthetic	1	1
Red	Fiber	Acrylic	Fully synthetic	5	5
Purple	Fiber	Polyester	Fully synthetic	1	1
Turquoise	Fiber	Polyester	Fully synthetic	1	0

were calculated for the materials found in the method blanks, rounding to the nearest whole number, and were determined to be 6 for clear fibers, 5 for clear/white/yellowed polystyrene fragments, and 1 for red polyester fibers, blue cellulose/rayon blend fibers, and turquoise polyester fibers. The polystyrene contamination was likely from the petri dishes that were used to store filters. μ-FTIR analysis identified only one polystyrene fragment in samples, but due to blank contamination, it was not included in the final calculations. There were no PP fragments or fibers identified in crab stomachs, so their transport in the cooler did not result in sample contamination. Clear polyester, polystyrene, and cellulose fibers, turquoise polyester fibers, and red polyester fibers were excluded from the adjusted final results as all of the fibers of each of those types were found in quantities below the LOD. Samples with blue cellulose/rayon blend fibers in quantities greater than the calculated LOD (1 fiber per sample) were included in the final results for semisynthetic fibers (Table 3).

After accounting for laboratory contamination, 20 fully synthetic fragments and fibers were recovered, consisting of 8 polyester fibers, 9 acrylic/acrylic blend fibers, 1 polycarbonate fragment, 1 polyethylene terephthalate fragment, and 1 phenoxy resin film. There were 14 fibers composed of a cellulose/rayon blend after accounting for method blank corrections. Based on LOD corrected results, 10 of 39 blue crabs (25.6%) had fully synthetic fragments and fibers within their stomach, for an average of 0.51 fully synthetic objects per blue crab. When including the semisynthetic cellulose/rayon fibers with the fully synthetic fibers, 14 of the 39 blue crabs (35.9%), equating to 0.87 fully and semisynthetic microplastics per blue crab. To reduce confusion, any mention of microplastics in the discussion unless explicitly stated otherwise includes both fully and semisynthetic materials.

Discussion

This study is the first to assess microplastic contamination in blue crabs and found that 35.9% of the sampled organisms contained microplastics and synthetic fibers in their stomach. Microplastics have been observed in invertebrates around the world, including decapods like *Nephrops norvegicus* and *Eriocheir sinensis* (Table 1). Given the highly opportunistic feeding habits of blue crabs (Laughlin 1982) and the proximity of sampled organisms to a coastal population center, microplastic contamination was expected.

Previous studies found contamination in crustaceans ranged from 0.04 to 2.26 microplastics per organism in up to 83% of samples collected (Table 1). In some invertebrates, like *Mytilus edulis* and *Cerithidea cingulata*, the values were one to two orders of magnitude greater than crustaceans (Table 1). Our results are within the range of those found in other studies examining crustaceans (Table 1) but are low when compared to other classes of organism. This could be due to lower blue crab microplastic exposure, differences in organismal feeding strategies, and geographical location. Differences between results may also reflect variation in methodology, either using different digestants (such as HNO_3 or KOH) or different methods for quality control (Table 1).

This study only targeted stomach tissues and ignored other susceptible organs like the gills which would be exposed to

plastics and fibers in the water column (Watts et al. 2014). While not included in the results of this study, fibers were observed tangled within blue crab sample gills. With bivalves, microplastic studies involve digestion of the whole organisms, with the most common digestants being either 30% H_2O_2, 10% KOH, or HNO_3 (Li et al. 2015; Van Cauwenberghe et al. 2015; Fang et al. 2018). Some digestants, particularly HNO_3, can degrade plastic polymers like nylon, which may affect their results (Van Cauwenberghe et al. 2015). The digestant (30% H_2O_2) did not degrade PVC, PP, polystyrene, PE, and acrylic during preliminary testing. However, 30% H_2O_2 was unable to adequately digest the shell or gills of the blue crabs which restricted the tissues studied. Other studies assess the microplastic contamination throughout the whole organism and not just through one tissue or route of exposure, as was done in this study, which may account for differences in observed contamination (Table 1).

Quality control methods are vital to microplastic study validity. Contamination was accounted for in this study using method blanks to establish a LOD for items based on their color, material, and shape (De Witte et al., 2014). By correcting for contamination found in blanks, this LOD method generates a conservative estimate for the materials found in samples. If the quantity of a specific microplastic observed in the sample is less than the LOD, it is assumed to be from contamination and not included in the results. Of the 28 items identified in this study as fully synthetic polymers, only 20 were included after correcting for contamination. Similarly, only 14 of the 24 semisynthetic fibers were included in the results after correction.

At present, there is no agreed upon quality control method in microplastic research. Common quality control methods employed in microplastic studies include establishing LODs as described by De Witte et al. (2014), employing preventative methods like reducing the exposure time of samples and regularly cleaning and inspecting equipment (Teng et al., 2019), or establishing blanks to correct samples (Digka et al. 2018; Naji et al. 2018). Similarly, the way contamination is accounted for in the results, be it employing a correction, subtraction, or fully removing corresponding items observed in sample contamination, can lead to large differences in the final reported values (Santana et al. 2016; Digka et al. 2018). Indeed, the method employed in this study, correcting the results only if they were below the LOD, resulted in over a third (34.6%) of the identified microplastics being excluded from the results.

Fully synthetic fibers, polyester and acrylic, made up 85% of the synthetic items recovered, after accounting for corrections. This is consistent with fibers observed in other studies (Salvador Cesa et al. 2017) and their prevalence in modern textiles (Mishra et al. 2019). Additionally, these acrylic and polyester fibers have densities greater than water at 1.09–-1.20 g cm^{-3} and > 1.35 g cm^{-3}, respectively (Sundt et al. 2015). This indicates that they would precipitate and accumulate in sediment under low water turbulence conditions, where blue crabs feed. The semisynthetic, cellulose/rayon fibers are also denser than water (~ 1.5 g cm^{-3}). These fibers are important to include in research as they are chemically modified (Hartmann et al. 2019) and may also contain chemical additives (dyes, plasticizers, flame retardants, etc.) or sorbed contaminants (Bakir et al. 2014).

Studies have found that contaminants on plastics can transfer to organisms after consumption (Browne et al. 2013; Bakir et al. 2014). However, this process and its potential impacts, if any, are currently an important focus within this research field. Collection of those data would inform approaches to fisheries management for and regulations of microplastic pollution. But, the logical first step is to document and characterize microplastic ingestion. This study adds blue crabs to the growing list of fisheries that are susceptible to ingestion of microplastic pollution.

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