John Sage Foundation: Project Completion Report

Project Title: Evidence of microplastic ingestion by American lobsters

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Project Background & Rationale:
Modern plastics can last up to 600 years in the marine environment, depending on water conditions, ultraviolet light penetration, and the level of physical abrasion. The introduction of synthetic materials (microplastics) into the marine food web was evaluated by Thompson et al. (2004); these researchers found that microplastics were particularly abundant in subtidal sediments including estuaries, tidal rivers, and creeks. Thus, it would follow that microplastics in the marine environment would also impact species of consequential ecological and economic importance, including the American lobster (*Homarus americanus*).

The American lobster supports one of the most economically successful fisheries in the Gulf of Maine (GoM) and is the overriding economic driver for coastal marine communities (Steneck et al. 2011, McClenachan et al. 2020). Many aspects of the U.S. lobster fishery have changed dramatically over the past few decades, including consecutive increases in traps fished and the average vessel size. Additional changes such as the switch from wooden lathe traps to coated wire mesh traps, combined with other major advances in technology have had major impacts on the fishery, including an increased catch efficiency and effort. As a result, the number of lobster traps being fished at any one time in the GoM exceeds one million and often includes 'ghost trap' gear (ASMFC 2020). Ghost fishing, as defined by Arthur (2014) is '*the ability of fishing gear to continue fishing after all control of that gear is lost by the fisherman'*. Derelict fishing gear damages sensitive habitat and continues to capture both targeted and bycatch species. Marine animals captured in derelict traps may experience starvation, cannibalism, infection, disease, and prolonged exposure to poor water quality (e.g., low dissolved oxygen, Guillory 1993).

Investigating ghost lobster traps, researchers determined that, despite the best efforts to remove derelict fishing gear and create incentives to reduce and minimize these types of deleterious effects, ghost traps are still a pervasive component of this fishery (MA-DMF 2012). One of the other significant findings from this study suggested that, although modern lobster traps are designed with side panels that dissolve (to allow lobsters to escape) if gear is lost, these panels do not always readily degrade as predicted and thus, lobsters remained much longer than anticipated (MA-DMF 2012). Lobsters are also known to chew on manmade objects in their environment when held in captivity, suggesting that lobsters confined in ghost traps may gnaw on the vinyl coating of the wire mesh, as well as bait bags and other parts of the traps. ***Therefore, in this project, we set out to examine the impacts of trap-based microplastics (MPs) of gear stress coupled with the exposure and ingestion of microplastics from the degradation of plastic-based components of the ghost traps (e.g., plastic coating, bait bags, trap tags, etc.).***

In addition to the impacts of microplastics on adult lobsters that can remain as long-term residents in ghost traps, the effects of microplastics may also impact juvenile (sub-legal) lobsters. The life history of American lobsters includes a complex set of egg and larvae developmental stages that are punctuated with a dominant and often long-lived benthic (juvenile and adult) period (see Lawton and Lavalli 1995 for overview). At the final larval stage, postlarvae settle to the bottom and burrow into the substrate, where they will eventually molt

into benthic-dwelling juveniles (Cobb and Wahle 1994) and are known to filter feed during this critical stage. ***It is during this time period that we predict juvenile lobsters may be ingesting microplastics and this is the complementary component that we would like to test.***

We sought to investigate the potential ingestion of microplastics by both adult and juvenile lobsters with the hypothesis that microplastics could be ingested or affect lobsters. Although these kinds of studies have been routinely conducted on marine fish and other commercially important crustaceans like crabs, little work has been done in any species of lobster (Norweigian lobster, Murray and Cowie 2011, Martinelli et al. 2021; American lobster, Potocka et al. 2019; Woods et al. 2020). We aimed to address this gap in our knowledge of anthropogenic impacts on an iconic and economically valuable crustacean.

Objectives:
The original two goals of this project (pre-pandemic) included:

1) Adult lobsters that reside in derelict (ghost trap) gear are subject to potentially higher exposure and ingestion of microplastics. We will test this in the field.*
2) Juvenile lobsters are capable of ingesting microplastics due to their propensity to filter-feed. As a result, these microplastics may be found in some of their tissues including gills and midgut. We will test this in the laboratory.

*Due to the COVID-19 pandemic, we were not able to fully or adequately access many of our research assets or meet many of our research goals over the timeframe for this project. Our group has needed to re-assess the original goals and outcomes encompassing many of our projects given the setbacks, delays, and curtails to student help, and physical facilities. In addition, like many, we have also been affected by supply-chain delays and services which has hindered our timing and capabilities related to this project. Despite these significant challenges, we were still able to accomplish most of the work we set out to do but did have to eliminate Objective-1. However, we still gained a good baseline knowledge for developing the methodologies and insight for providing foundational information for evaluating microplastics in American lobsters.

Project Approach & Methodology:

<u>Lobster source and experimental setup</u>
Lobsters were captured in ventless survey traps set in NH and ME waters and collected and held for us under scientific collection permits (MEDMR ME 2021-18-02 and NHF&G MFD 2011) in flow through floating lobster totes. Lobsters were transported to the Maine Ecology Center at the Wells Reserve where they could be held and processed. A total of 5 lobsters (4 males and 1 female, 50 ± 18.5 mm carapace length) were used for our MP assay trials. Lobsters were allocated into individual 1 L aquaria tanks with seawater (salinity range = 32-35 psu), bio-mechanical filters, and aeration setups and kept in a controlled environmental chamber at 10-11°C with ambient lighting conditions (Fig. 1). Lobsters were also weighed using an Ohaus 400 digital scale (Parsippany, NJ).

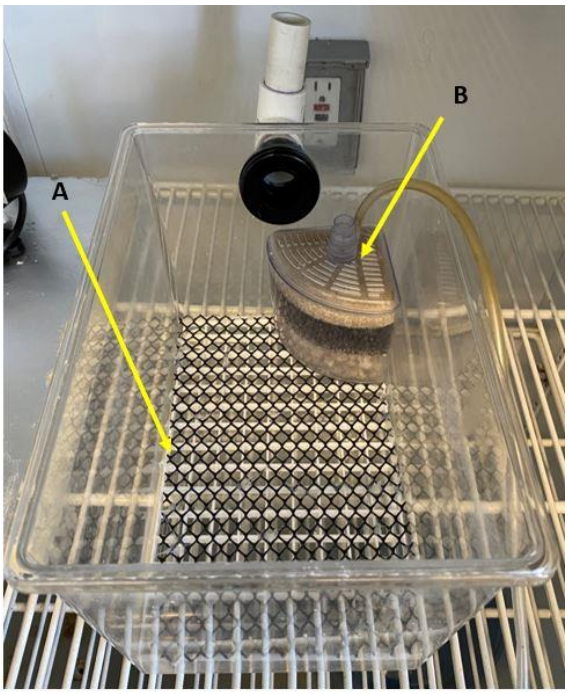


***Fig. 1**. Aquarium setup (4 L) for housing individual lobsters over their 12-week MP exposure trial – (a) false floor to help facilitate collection of detritus, feces, and MPs; (b) bio-mechanical filtration setup.*

Microplastics source and exposure trials

Microplastics for this study, sourced from a commercial lobster trap were obtained from: 1) the plastic wire mesh coating on trap panels shaved off with a knife and; 2) plastic threads from standard trap bait bag (Fig. 2). Plastics were intentionally used from these components of the trap as they represent the most prodigious amount of plastic in commercially manufactured traps. Once the plastics were shaved, they were ground in a pepper mill to reduce them in size to no more than ~2 mm square, which more closely approximates the sizes encountered on nearby shores (Gutzler, personal observation). All lobsters were fed a weekly dose of plastic consisting of 0.5 g of plastic (~52 individual MP/sample) in a solution of 10 mL of seawater (30-35 psu) over a 12-week trial period (i.e., 12 doses of MPs) between June and August, 2021 (Table 1). Frozen mussel (*Mytilus chilensis*) meats (Next Wave Seafood Inc., North East, MD) were thawed and weighed prior to each dosing event, and a plastic slurry was injected into the soft tissue of individual shell-less mussels using a 30 mL syringe (BD, Franklin Lakes, NJ). Mussels were chosen as the vehicle for the plastic slurry as they are both a common lobster prey item and also primary filter feeders, and thus likely to retain any microplastics present in the water. This process is a known vector for microplastic introduction for both molluscs and benthic decapods (Wright et al. 2013).

Fig. 2. *(Left): Standard double-parlor lobster trap (meter stick for scale); note that the green color is a plastic coating that is dipped onto the wire mesh during the manufacturing process; (Middle): Example of green plastic shred (removed from trap) along with a lobster trap bait bag (orange) which was also used as part of our MP source; (Right): MP ground into smaller sizes (inside circle) for use in our lobster assays.*

Lobsters were given 3-4 days to consume mussel meat after each feeding. False floors installed on the bottom of each aquarium tank ensured that unconsumed mussel, plastics, and waste would be isolated and could be collected. Lobster tanks were cleaned, siphoned, and replaced with new seawater weekly and all siphoned contents were stored in labeled 50 mL tubes.

Lob ID	Total Mussel Meat Fed (g)	# MP Doses	Lobster Weight (g)	CL (mm)	Plastic Fed (g)	Plastics Fed (% of bodyweight)
4	74.55	12	102	50	1.67	1.64
5	68.50	12	104	52	1.67	1.61
6	76.55	12	91	62	1.67	1.84
7	49.60	7	94	51	0.98	1.04
8	57.05	10	24	34	1.40	5.94
AVG	**65.25**		**83**	**50**	**1.48**	**2.41**

Table 1. *Summary information for lobsters (n = 5) that were used in our pilot MP study. Lobsters were held for a total of 12 weeks and fed 1x, weekly.*

Microplastic analyses

At the completion of each trial, all lobsters were euthanized in an ultracold freezer for 20 minutes, after which, each lobster was dissected for histological examination. Briefly, selected tissues (hepatopancreas, gut, gills) were removed, placed in labeled tissue cassettes, and flash frozen before being shipped to EMSL Analytical, Inc. (Cinnaminson, NJ) for subsequent analyses. (Table 2, Fig. 3). We also visually assessed gut contents of each lobster for any signs of plastic residuals.

Fig. 3. *Histological tissue cassettes of lobster samples processed in preparation for MP analyses.*

Sample ID	Description	Date/Time Sampled
7-HEP	American Lobster (hepatopancreas tissue)	10/28/2021
7-GUT	American Lobster (gut tissue)	10/28/2021
5-HEP	American Lobster (hepatopancreas tissue)	11/8/2021
5-GUT	American Lobster (gut tissue)	11/8/2021
4-HEP	American Lobster (hepatopancreas tissue)	11/9/2021
4-GUT	American Lobster (gut tissue)	11/9/2021
8-HEP	American Lobster (hepatopancreas tissue)	11/12/2021
8-GUT	American Lobster (gut tissue)	11/12/2021
6-GIL	American Lobster (gill tissue)	11/1/2021
6-GUT	American Lobster (gut tissue)	11/1/2021

Table 2. *Histological tissue cassettes inventory of lobster samples processed in preparation for MP analyses.*

A total of 10 tissue samples were submitted for analysis. Each sample was removed from the plastic histology container and placed into a clean glass vial, weighted, and placed into a drying oven set to 60°C until no further weight loss was noted (~7 hours). Samples were stored in a desiccator for an additional 24 hours to further remove moisture without damage to the potential microplastic particles. Dried samples were subjected to a Wet Peroxide Oxidation (WPO) preparation consisting of an aqueous solution of hydrogen peroxide (30%) and ferrous oxide (0.05M) and brought to 75°C until the tissue was dissolved (~30 minutes). The solution was passed through a 500µm stainless steel sieve in order to separate the suspension into two size fractions.

The large fraction (retained in the sieve) was analyzed by stereomicroscopy (Nikon, DF Microscope) and polarized light microscopy (Zeiss, Universal Petrographic Microscope) for potential microplastic particles. All suspect plastic particles were separated for analysis by Raman spectrometry using a Horiba, XploRA Plus. The small fraction (<500µm) was passed through 0.8µm filters and analyzed by polarized light microscopy and Raman spectrometry for microplastic particles.

<u>Vinyl content in lobster traps</u>

In addition, we also made calculations for the amount of plastic coating present in a lobster trap. A 4.5” x 4.5” square section of standard 1.5” vinyl-dipped wire trap mesh was weighed using an Ohaus 400 digital scale, then all the vinyl coating was removed using a razor blade, and the bare wire was reweighed to determine the weight of the vinyl coating. We then measured

the area of mesh present in a standard double-parlor commercial lobster trap and used the weight of vinyl from the subsample to extrapolate the weight of vinyl wire coating present on a full trap.

Results & Findings:

Analytical MP analyses

Overall, we did not find any evidence of MP presence in any of the tissues that were processed (Tables 3, 4; Figs. 4, 5). Below are the summary results from those analyses.

Sample ID	Description	Microplastics
7-HEP	American Lobster (hepatopancreas tissue)	No Microplastics Detected
7-GUT	American Lobster (gut tissue)	No Microplastics Detected
5-HEP	American Lobster (hepatopancreas tissue)	No Microplastics Detected
5-GUT	American Lobster (gut tissue)	No Microplastics Detected
4-HEP	American Lobster (hepatopancreas tissue)	No Microplastics Detected
4-GUT	American Lobster (gut tissue)	No Microplastics Detected
8-HEP	American Lobster (hepatopancreas tissue)	No Microplastics Detected
8-GUT	American Lobster (gut tissue)	No Microplastics Detected
6-GIL	American Lobster (gill tissue)	No Microplastics Detected
6-GUT	American Lobster (gut tissue)	No Microplastics Detected

Sample Preparation		Sample Weight		
Sample	Vial	Wet wt. (g)	Dry wt. (g)	Dry wt. (%)
7-HEP	1	1.00784	0.20167	20.01
7-GUT	2	0.87349	0.17744	20.31
5-HEP	3	0.83970	0.16441	19.58
5-GUT	4	0.91805	0.18725	20.40
4-HEP	5	0.83982	0.18065	21.51
4-GUT	6	1.04815	0.20848	19.89
8-HEP	7	0.20882	0.04026	19.28
8-GUT	8	0.27570	0.05222	18.94
6-GILL	9	0.24648	0.06774	27.48
6-GUT	10	0.71351	0.15348	21.51

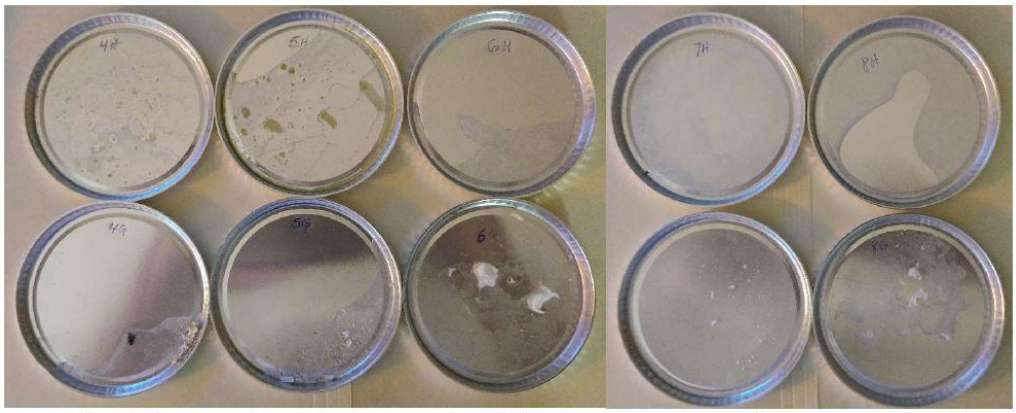
Table 3. *(Top): Summary of MP tissue result for 10 samples of lobster tissue taken from 5 individual lobsters; (Bottom): Determination of sample dry weight for calculation of MP concentration.*

EMSL ID:	362103387-0001		
Sample ID:	7-HEP		
Description:	American Lobster (hepatopancreas tissue)		
Amount Analyzed:	0.02001(kg)	LOQ (Particles/kg):	99.95
Preparation Parameters	Value	Units	Comments
Sub-sample (prepared):	0.02001	(kg)	A
Effective Filter Area:	1070	(mm2)	
Field Area:	535	(mm2)	
No. Fields Analyzed:	1	(No.)	
Area Analyzed:	535	(mm2)	
Limit of Quantitation:	99.95	(Particles/kg)	
Particle Size Range (µm)	Concentration (Particles/kg)	Percent in Range	Comments
<1	<LOQ	N/A	B
1 - 5	<LOQ	N/A	B
5 - 10	<LOQ	N/A	B
10 - 50	<LOQ	N/A	B
50 - 100	<LOQ	N/A	B
100 - 500	<LOQ	N/A	B
500 - 1000	<LOQ	N/A	B,C
1000 - 5600	<LOQ	N/A	C
>5600.0	<LOQ	N/A	C, D
Total Microplastics	<LOQ	Min. Diam. = 0um	Max. Diam. = 0um

Comments: LOQ = Limit of Quantitation

A) Parameters used in the preparation of the sample.
B) Particles observed by microscopic analysis.
C) Particles observed by sieve separation and stereo microscopic analysis.
D) Particles larger than the generally accepted definition of microplastics.
Sample volume based upon filtration rate and suspended particle concentration.

Table 4. *Example MP summary analysis for an individual lobster (ID 7), by selective particle size sorting and methodology.*



Sample 4 Sample 5 Sample 6 Sample 7 Sample 8

Figure 4. *Images of the large material collected on the sieve during sample preparation. The material is composed of tissue remains, calcium carbonate and salt. No microplastics were observed in the large (>500µm) particle fraction.*

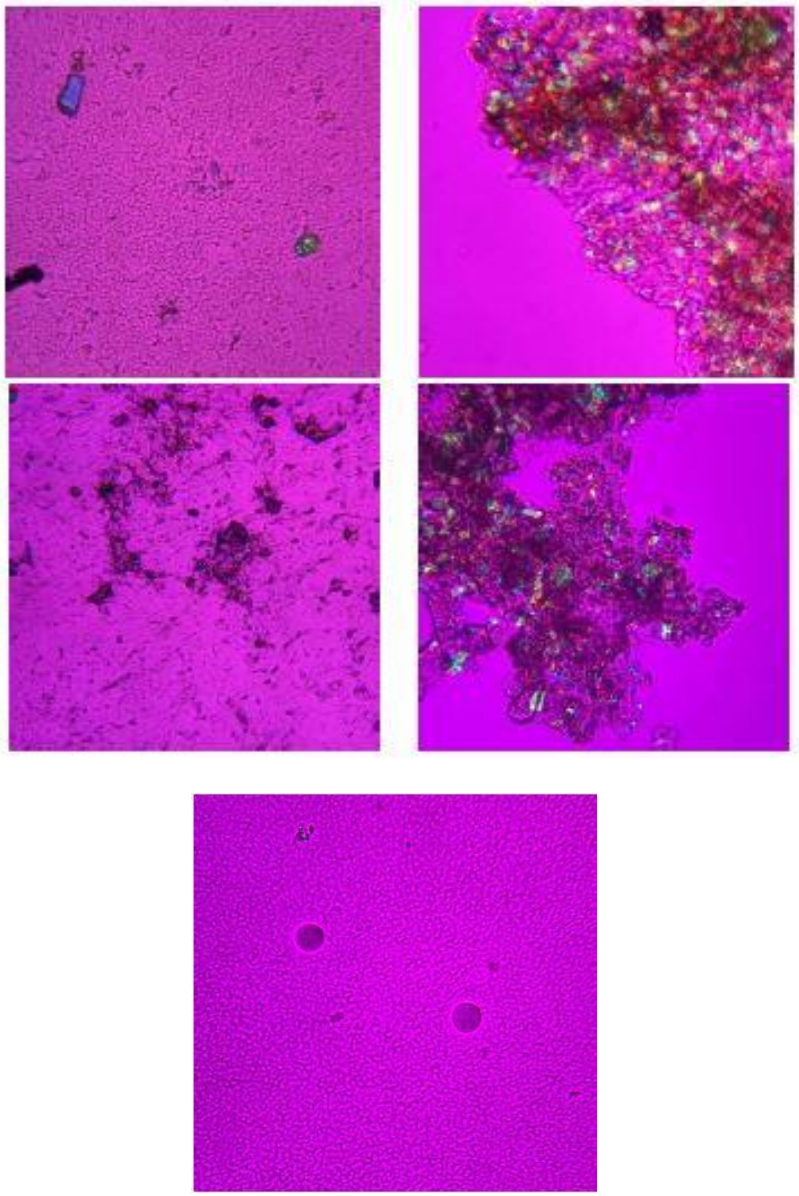


Figure 5. *Selected polarized light images (PLM) for tissues showing tissue remains and calcium carbonate residues: (Top 2 panels): Images from Lobster-4, gut tissue; (Middle 2 panels): Images from Lobster-5, hepatopancreas tissue; (Bottom panel): PLM image showing polystyrene spheres used for calculation of percent recovery during the quality control process. Methodological protocol targeted a concentration of 6,176 particles/mL; measured concentration yielded 6,272 particles/mL (±10%).*

Gross MP observations

Although the microscopic analysis component of this study did not result in any apparent MP presence in tissue, we did in fact positively identify at least one case of lodged plastic material within the gut of one of our five lobsters (Fig. 6). Additionally, we also observed and documented cases where MPs were evident in feces or still attached to semi-consumed mussel fragments (Fig. 7). **Therefore, despite the absence of MPs at the tissue histological level, we still have evidence for some MPs being passed and processed by lobsters in this study.**

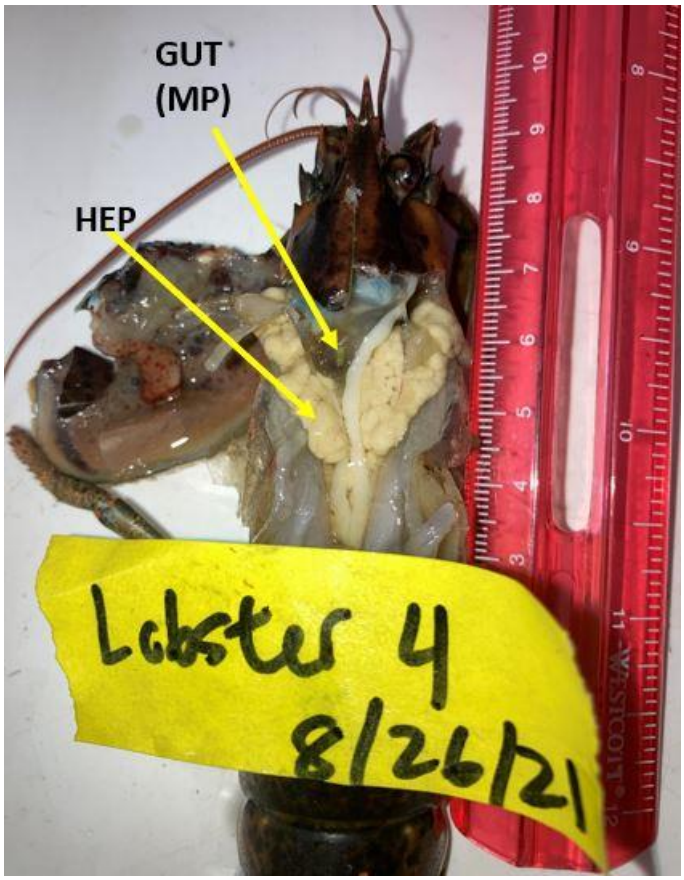


Figure 6. *Gross anatomical observations for one of our dissected lobsters from our MP study; Arrow for (GUT, MP) indicates pieces of semi-digested MP within the gut of Lobster-4; Additional arrow shows the hepatopancreas, a major organ of interest for this study.*

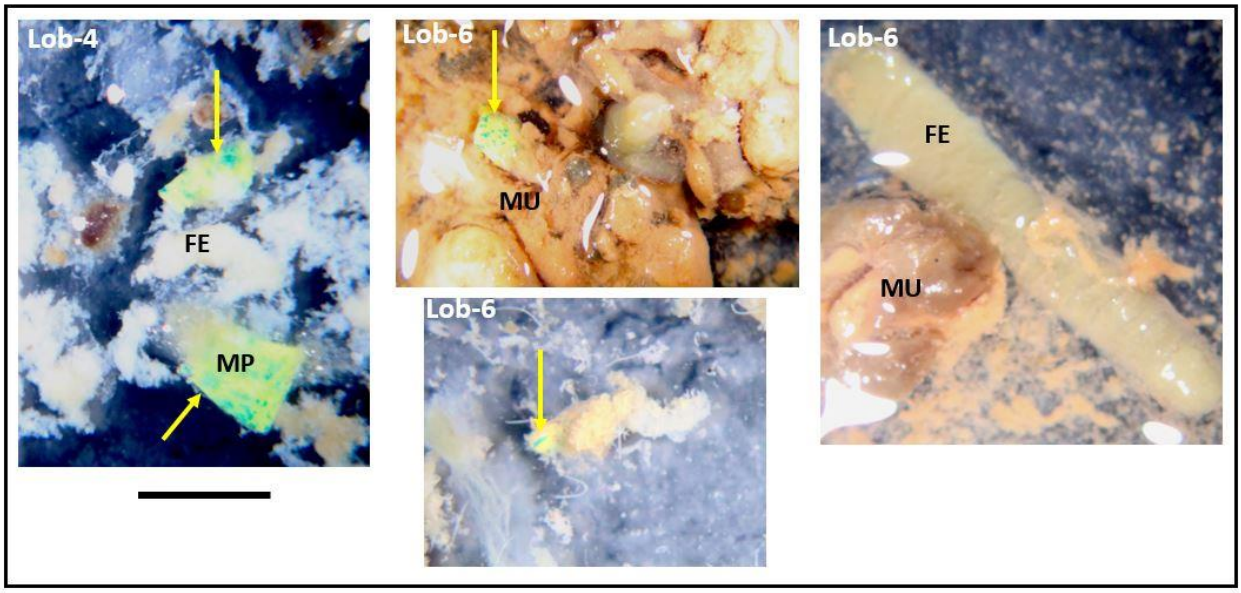


Figure 7. *Dissecting scope images (0.8x) for samples of lobster slurry (siphoned from holding tanks) that include feces (F) and residual mussel (MU) tissue for two lobsters, 4 and 6 from this study. Green-shaded areas are microplastics (MP); scale bar = 2.5 mm.*

Vinyl content of lobster traps

A 4.5" x 4.5" section of standard 1.5" vinyl-dipped wire trap mesh contained 7.3 g of vinyl coating, or 0.36 g/in^2 of mesh area (inches are used rather than centimeters for measurements in this section, as traps are built using inches as standard dimensions). A standard commercial double-parlor lobster trap (48" long by 22" wide by 14" tall) was determined to contain a total mesh area of ~3820 in^2 after accounting for cutouts for twine trap heads and escape gaps. Consequently, we estimate each such trap to contain 1375 g of vinyl coating. This should be taken as a conservative estimate, as it does not take into account sections of the trap where mesh may be doubled up for reinforcement or additional framing. Trap size and configuration varies widely depending on the preference of individual fishermen, but to the best of our knowledge, this is the first attempt of any sort to quantify the amount of plastic potentially introduced to the environment by a single trap.

Discussion & Impacts:

Microplastics (MP) will continue to be a ubiquitous and pervasive presence impacting marine environments at all scales. While water and sediment are the preeminent sinks of MPs, marine organisms are often the end point for MP bioaccumulation through both their physiological (e.g., respiration) and biological (e.g., eating and digestion) processes (Cole et al. 2011, Wright et al. 2013, Ribeiro et al. 2019). Based on our observations, experiments, and analyses, it is clear that lobsters will not avoid food items containing MP and will ingest any MP found within their prey. The histological results from this study did not indicate retention of MP within any of the body tissues we tested; however, the presence of MP within the gut and feces of study lobsters demonstrates that MP are, in fact, a possible threat to lobster health. For example,

attempts at digesting MP have been shown to cause diminished nutritional reserves in other invertebrates (Wright et al. 2013). While finding MP in the feces shows that MP can be passed through the gut and excreted, different size fragments from those used in this study may not be so easily dealt with. It is not unreasonable to expect that smaller MP could pass into other tissues or larger MP fragments may be unable to be excreted and could be harmful within the digestive system, as has been seen in other species (Wright et al. 2013, Watts et al. 2014, Ribeiro et al. 2019). Alternatively, these small MP pieces may bio-accumulate over time in such tissues as gonad, hepatopancreas, and gut, as has been reported in other studies (e.g., Martinelli et al. 2021); however, the initial goal of this study was to investigate acute effects of MP, although we suspect that over time MP would likely accumulate in such lobster tissues.

Our use of trap materials as MP sources was designed to be environmentally relevant. Nearly 3 million traps are fished in the Gulf of Maine each year (ASMFC 2020), and the Gulf of Maine Lobster Foundation estimates approximately 175,000 traps are lost (i.e., becoming ghost or derelict lobster gear) each year (see http://www.gomlf.org/gear-grab/). Taking our estimate of 1.3 kg of vinyl coating per trap as a conservative baseline (not including other synthetic components of the trap, such as bait bags, fasteners, or rope), this equates to 227.5 tons of vinyl alone introduced to the ocean each year. Our experimental results demonstrate the potential for ingestion of MP materials sourced from decaying lobster traps through a realistic environmental pathway, mediated by filter-feeding bivalves that are then consumed by lobsters. Consequently, the possible negative effects of fishing gear loss must be expanded from direct interactions such as the death or injury of animals in ghost traps to include environmental effects such as becoming a source of MP.

Although many studies address the economic and management challenges of derelict fishing gear (Arthur et al. 2014), no study to-date with American lobster has sought to investigate the long-term effects of MP ingestion nor the behavioral dynamics associated with protracted exposure of lobsters inside derelict lobster gear. While these are questions that deserve more research and attention, we do know that short-term ingestions of MP by other crustaceans do in fact have an impact on behavior and other life-history traits. For example, Tosetto et al. (2016) reported that short-term consumption of MP by common beachhoppers (sand fleas, *Platorchestia smithi*) had a negative effect on activity, weight gain, and overall survival. Other studies using copepods demonstrated changes in fecundity, egg size, and survival (Lee et al. 2013, Cole et al. 2015). In short, more research is needed to obtain useful data as to the concentrations of MP that are deleterious to such organisms (including lobsters) and over varying time frames and for what functions and behaviors may be affected.

The analytical analyses that were employed for this study were comprehensive and reflect many of the most technology-savvy methods (e.g., Raman spectroscopy) in use today for the detection of MP at the tissue and nano-particle level. That being the case, we also advocate for alternative, less expensive DIY techniques. For example, the Systematic Identification of MicroPLastics in the Environment (siMPle) developed by Aalborg University, (Denmark) and the Alfred Wegener Institute (Germany) is an application for the fast detection of microplatic materials in environmental samples. This free software compares the IR spectra of the sample

with each reference spectra in the database, then assigns a material to them along with a probability score (see: https://simple-plastics.eu/). Continued development of such accessible tools will allow scientists and others the ability to monitor organisms over a wider spatial range and possibly more frequently.

Our key recommendation for future research with respect to lobsters and MP include:

Recommendations for future research:

1) Designing a more comprehensive longer-term laboratory-based study that addresses the bioaccumulation of MP in lobster tissue and organs.
2) Conducting a coast-wide survey of lobsters of all life-stages (larvae, juveniles, adults) to identify and map the presence of MP
3) Exploring in-situ behavioral and physiological consequences of MP ingestion by lobsters that include but may not be limited to movement and swimming capabilities, weight, respiration, mating, fecundity, and susceptibility to disease
4) Utilizing inexpensive, but novel technologies for DIY MP assessments
5) Educating end-users and concerned citizens as to the potential impacts of MP on lobsters

The results of this Sage-funded work were fruitful, productive, and engaging with others in the lobster fishery, and we look forward to sharing our results with those important end-users as we continue to learn and address environmental change and impacts to the single most important fishery in North America and Maine, the American lobster.

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