Investigating the Occurrence of Plastic in Historic Fishes from Galveston Bay Estuary: First-Ever Historic Body Burden Data

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Acknowledgments

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“Every bit of plastic ever made still exists.” (EPA)

Plastics and Common Uses

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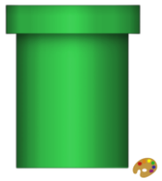
Polyethylene (PE):

- Plastic bags
- Bottles for milk or detergent
- Food packaging

Polyvinyl chloride (PVC):



- PVC pipes and fittings
- Vinyl flooring
- Electrical cables

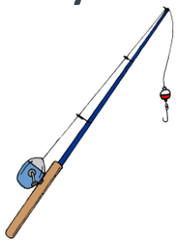
Polyurethane (PU):

- Foam cushions and mattresses
- Car seats
- Insulation panels

Polypropylene (PP):

- Food containers
- Bottle caps
- Packaging materials

Polyamide N-6 (PA):



- Nylon stockings
- Fishing lines
- Toothbrush bristles

Poly(methyl methacrylate) (PMMA):

- Plexiglass and acrylic sheets
- Aquariums
- Signage

Polystyrene (PS):

- Disposable cutlery
- Styrofoam packaging
- Insulation materials

Nylon-66 (N66):

- Textile fibers
- Sports equipment
- Airbags

Polyethylene terephthalate (PET):

- Beverage bottles
- Food containers
- Carpet fibers

Styrene butadiene rubber (SBR):

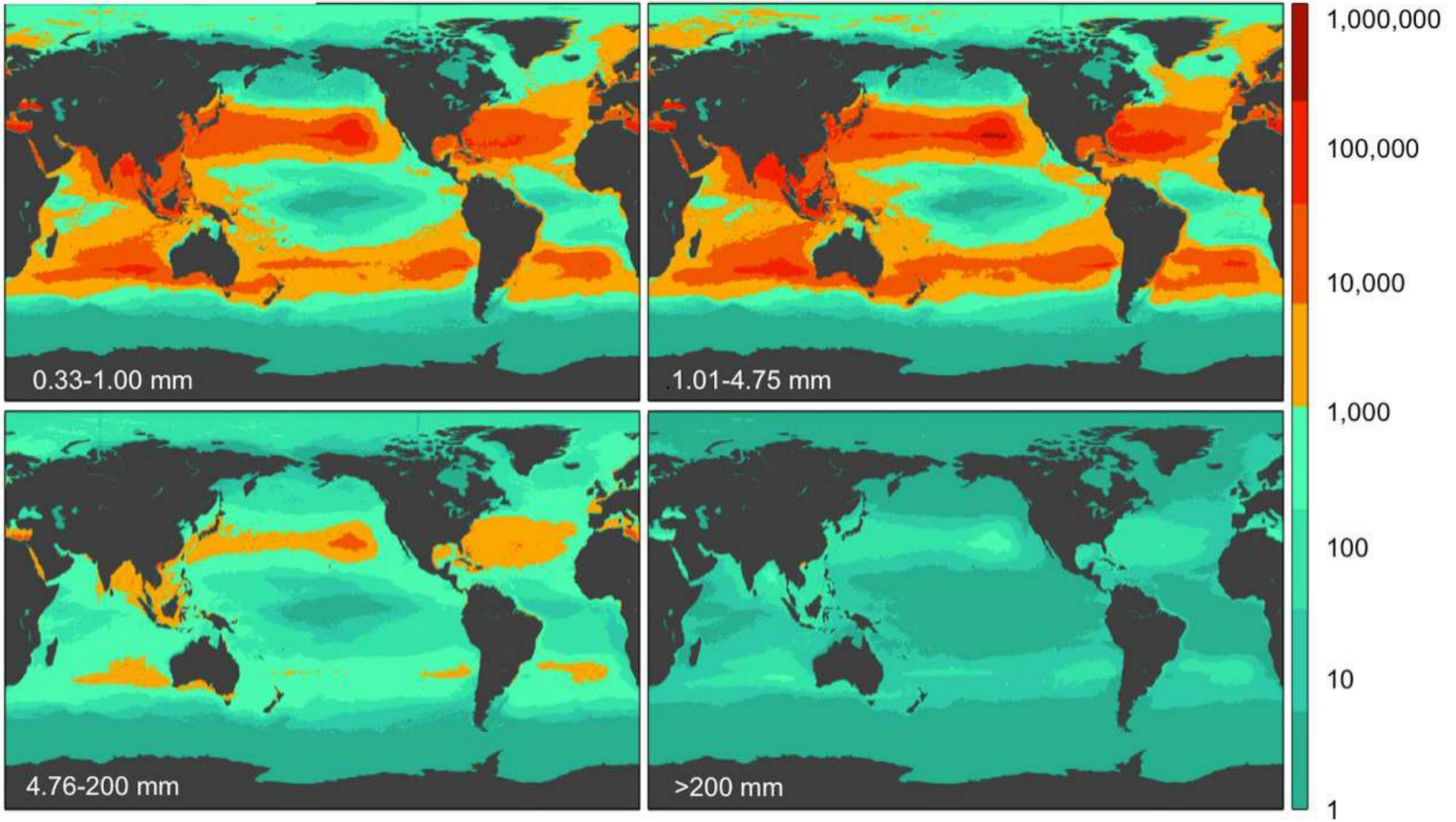
- Tires
- Shoe soles
- Rubber hoses

Polycarbonate (PC):

- Eyeglass lenses
- Safety goggles
- Water bottles

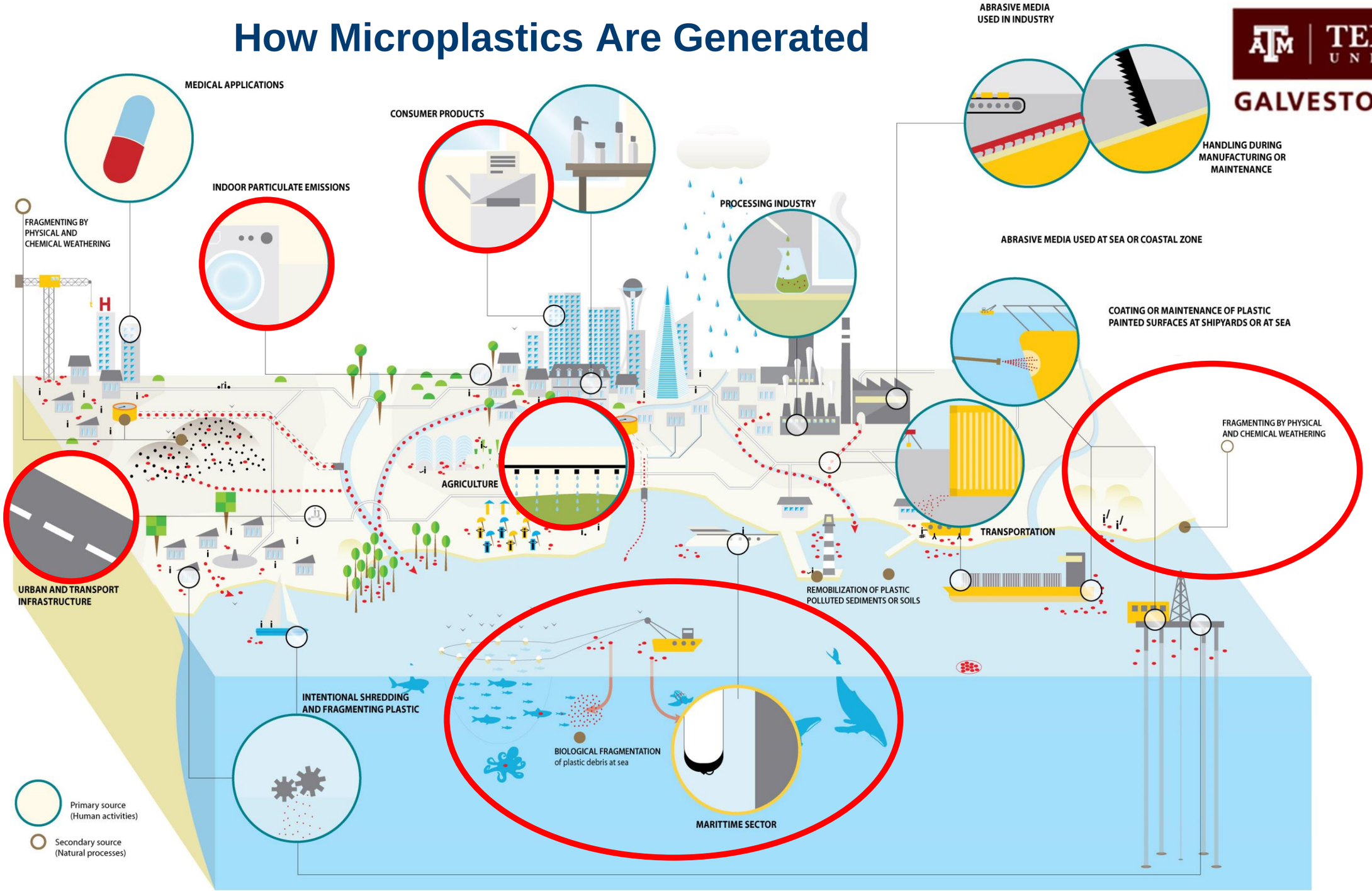
Acrylonitrile butadiene styrene (ABS):

- Lego bricks
- Protective headgear
- Computer keyboard keys



Most plastics in the ocean are very small and easily ingested by organisms

How Microplastics Are Generated



How Plastics Enter the Food Web

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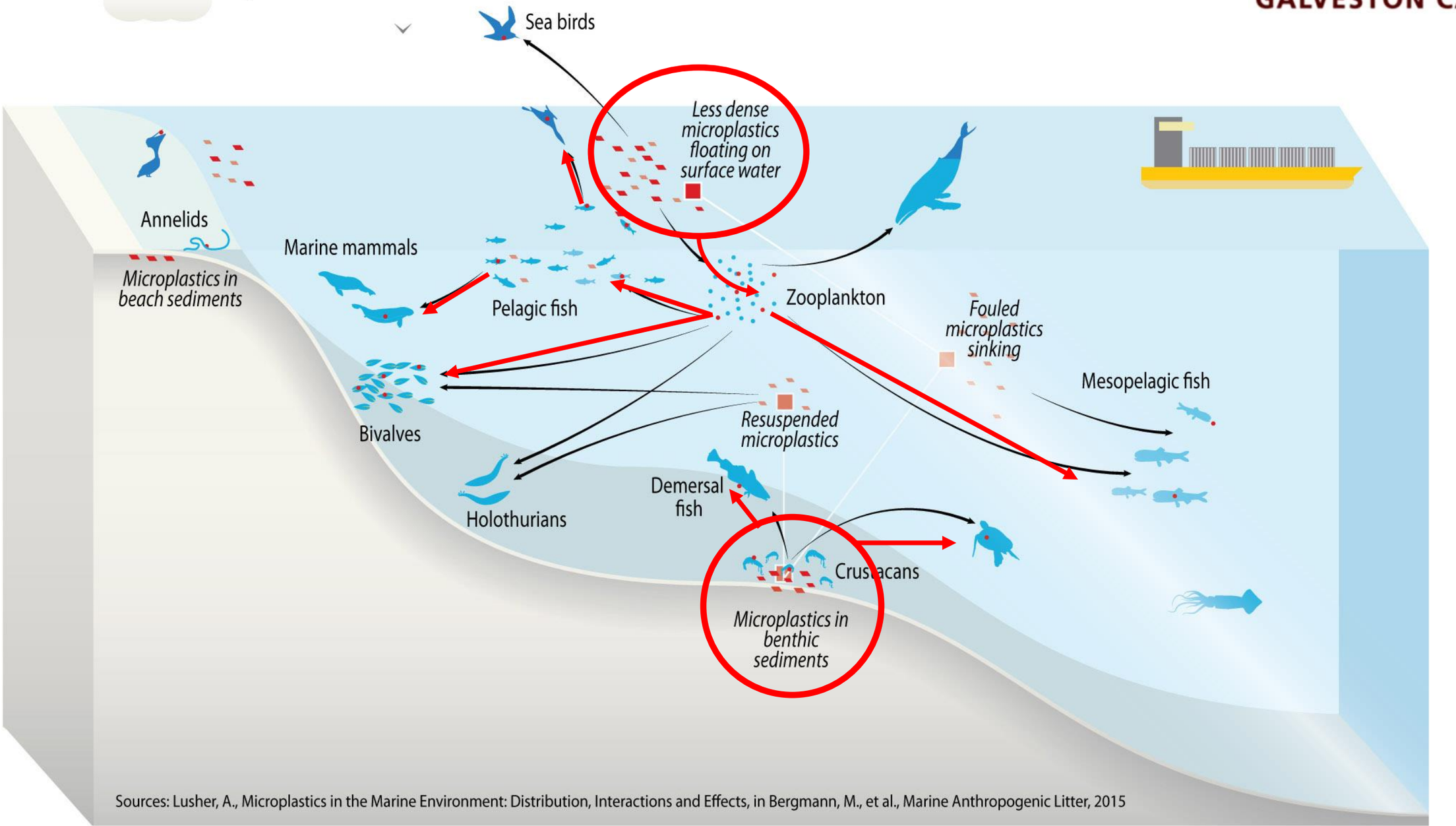

Sources: Lusher, A., Microplastics in the Marine Environment: Distribution, Interactions and Effects, in Bergmann, M., et al., Marine Anthropogenic Litter, 2015

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Why does it matter?

- Plastic degradation takes over 500 years
- Plastic is a principial threat to biological life
- Bioaccumulation in organisms that humans consume
- Direct environmental and human health concerns

Modes of Uptake

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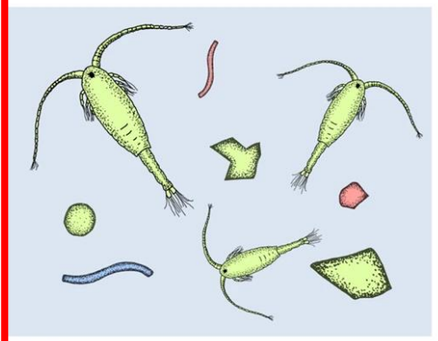
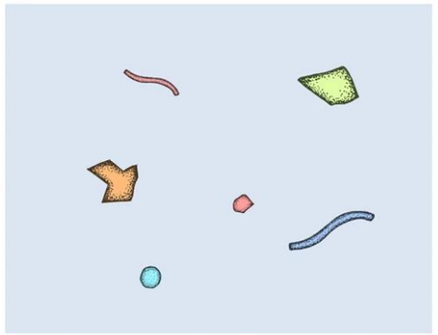
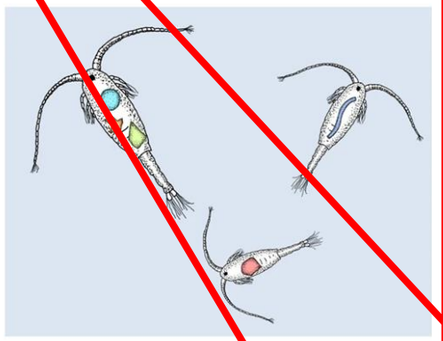
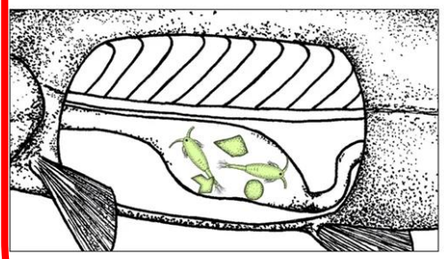
Active uptake

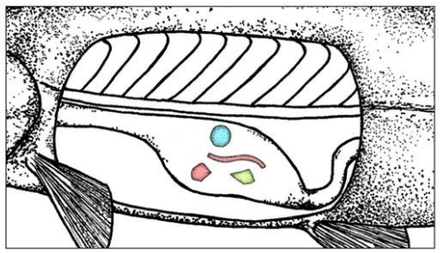
Passive uptake

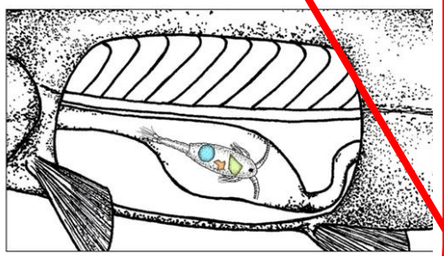








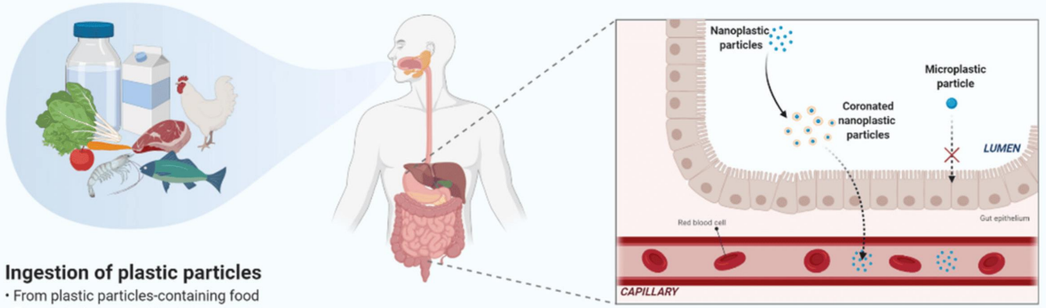




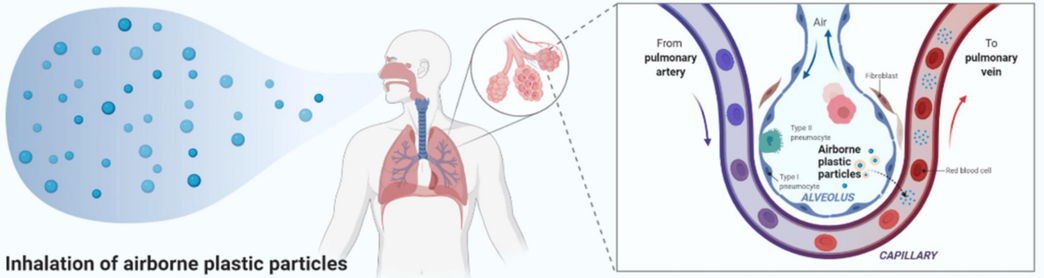
Confusion with food

Accidental ingestion (while feeding/drinking)

Transfer with the Food chain

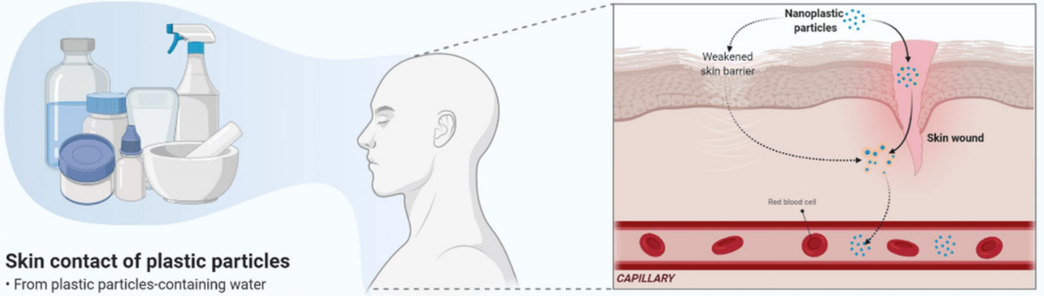


Ingestion of plastic particles
- From plastic particles-containing food
- From plastic particles-containing drinks



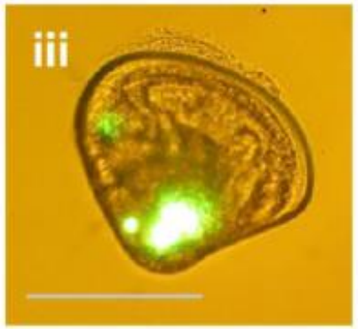
Inhalation of airborne plastic particles
- Indoor from synthetic textiles
- Outdoor from contaminated aerosol from ocean waves, airborn fertilizer particles from drief wastewater treatments, or atmospheric fallout

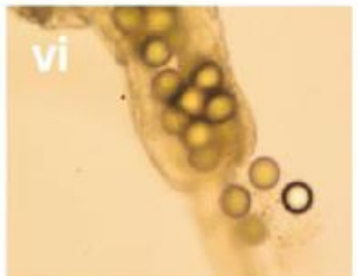
Factors that affect the absorption of plastic particles in the lungs:
- Hydrophobicity
- Surface charge
- Surface functionalization
- Surrounding protein coronas
- Particle size

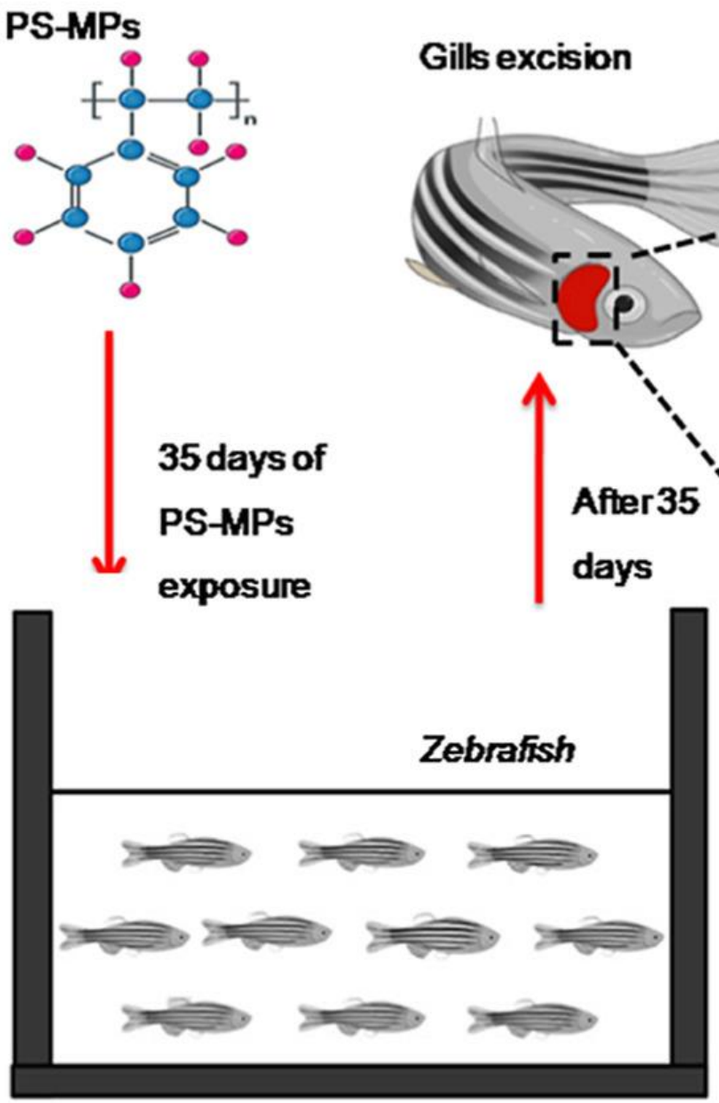


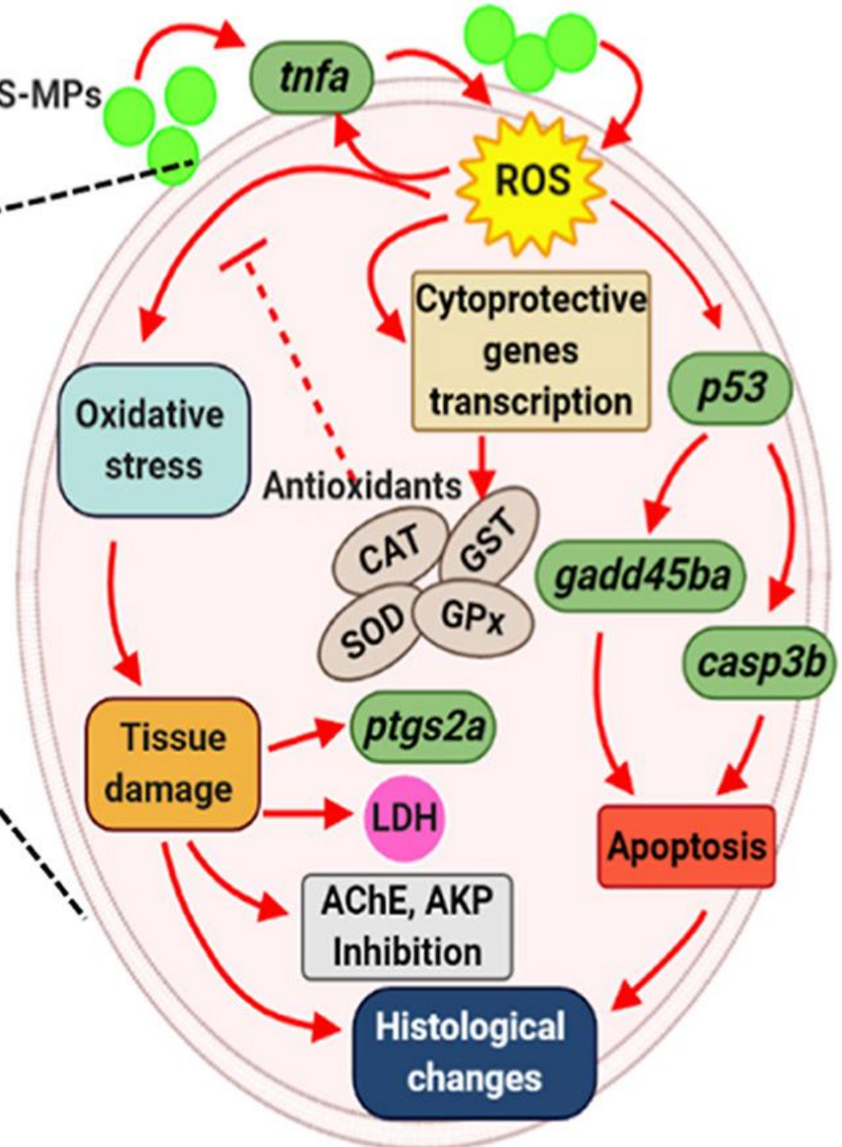
Skin contact of plastic particles
- From plastic particles-containing water
- From plastic particles-containing health and beauty products

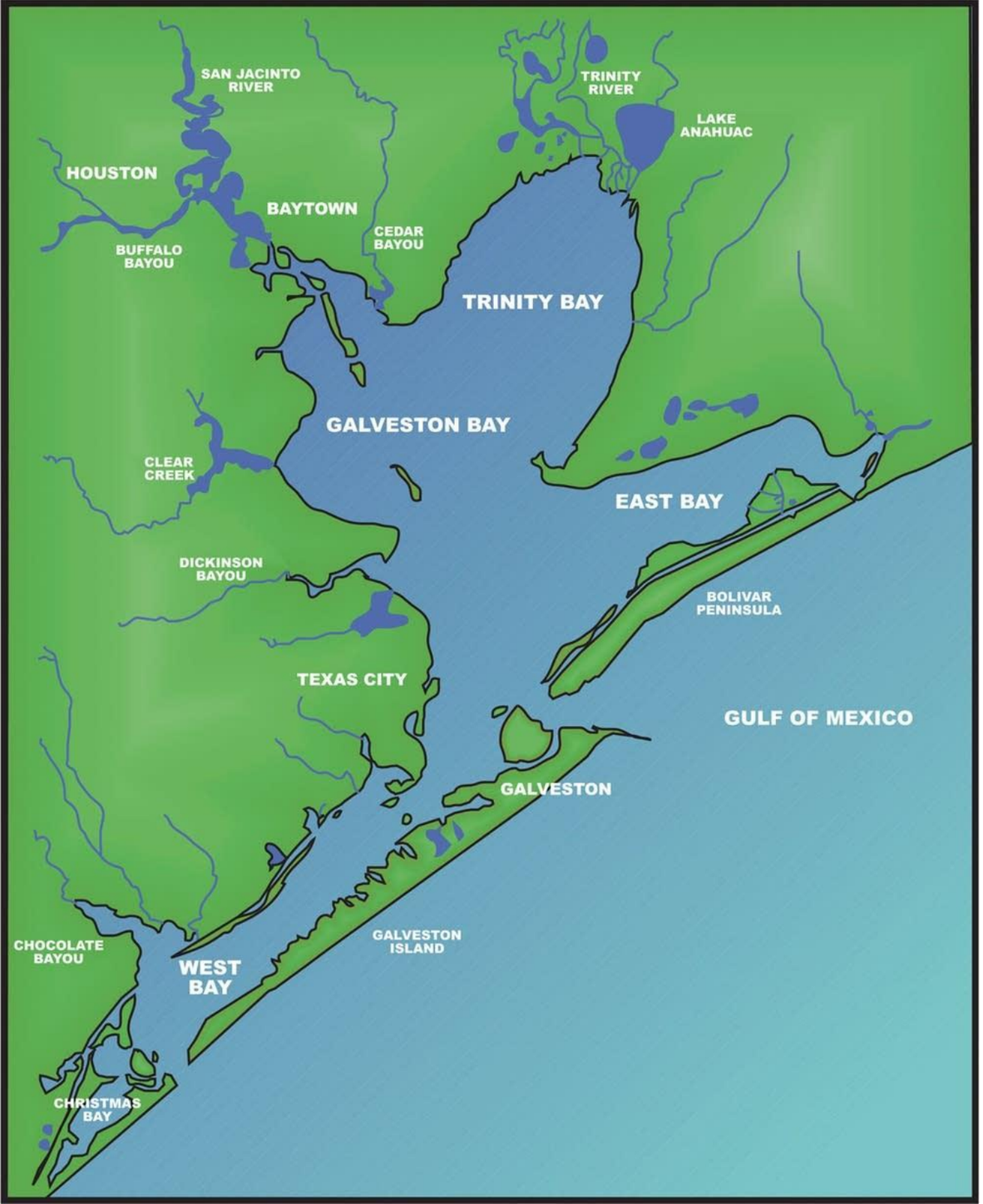
Biological Effects











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Research Objective:

Quantify the body burden and investigate the trend of plastic accumulation in gulf menhaden/bay anchovies (Clupeiformes), striped mullet (Mugiliformes) and sand/spotted sea trout (Acanthuriformes) over the last 60+ years in Galveston Bay Estuary System.

Hypotheses:

H_1: Body burden of plastics will increase over time in all three species of fish in this study.

H_2: Microplastics become detectable in the environment in GBES fishes ~ 1970.

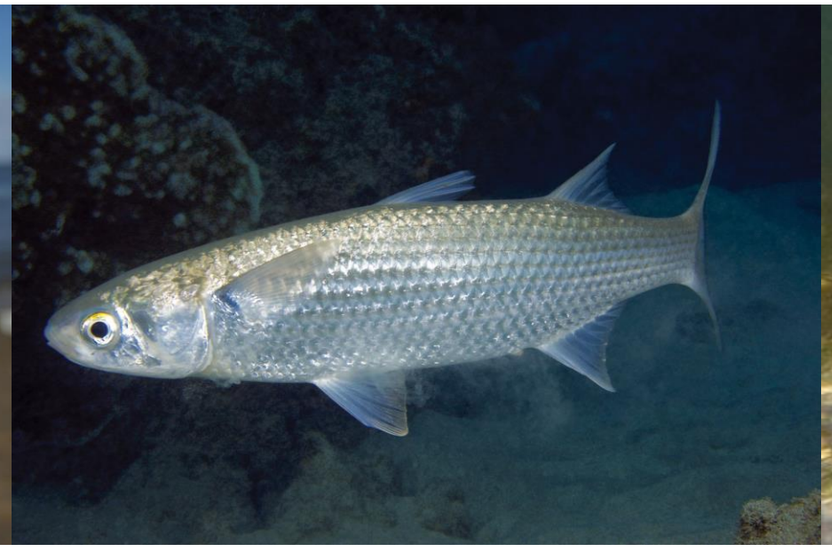
H_3: Body burden of plastics will be influenced by feeding mode and trophic level.

H_4: Trend of plastic body burden in these fish will correlate with global plastic production.

Why These Fish?

Gulf Menhaden:

- Filter feeder / small prey
- Base of marine food webs
- Support some of the largest processing industries in the USA - > $170 million in 2016



Striped Mullet:

- Filter and suction feeder
- One of the most abundant and preyed upon fish

Spotted Sea Trout:

- Suction feeder / larger prey
- Higher trophic level
- On our dinner plate

Texas Parks and Wildlife Department's list of Species of Greatest Conservation Need include many species which prey on Clupeiformes and Mugiliformes (e.g. blue fin tuna, red snapper and black tip sharks)

Methods: Research Design

- Samples were chosen from the TAMU Biodiversity Research & Teaching Collections based on size, year and location
- Limited availably of individuals, dates and sizes constrained the study
- Samples collected between 1958 and 2021
- Body masses and length ranging between 5-30g and 2-20cm
- Usable Sample Size :

Trout	-	N = 27
Mullet	-	N = 24
Menhaden	-	N = 23

- Collected and ran >40 individuals from each species, but samples older than 1960's were not digestible and therefore not quantifiable

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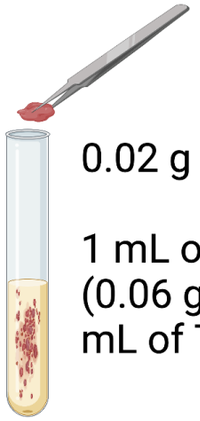
Sample Collection:

TAMU Biodiversity Research & Teaching Collections: College Station

The geographic coverage of specimens in the Collection of Fishes includes 71 countries, and all 7 continents.

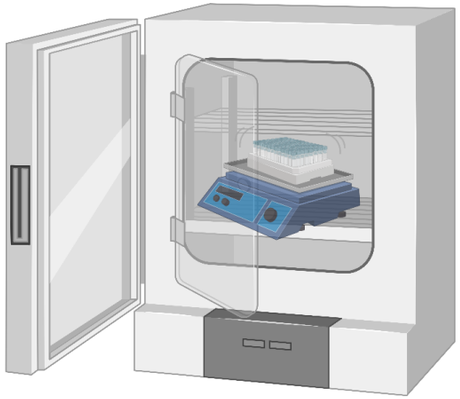
The Collection of Fishes contains over 875,697 individuals.

Methodology



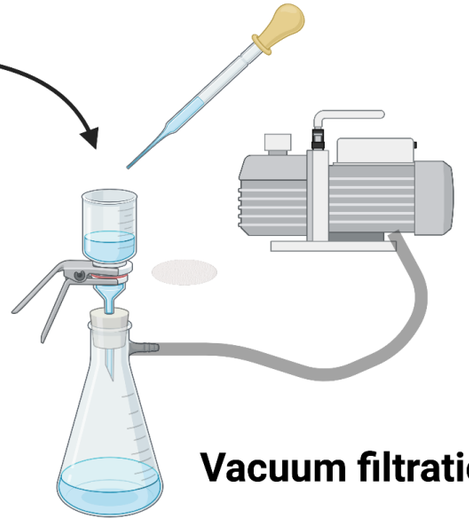
0.02 g dry weight tissue

1 mL of Pez
(0.06 g pancreatin/
mL of Tris buffer, pH 8)



Protein digestion

40°C Temperature
24 hr digestion

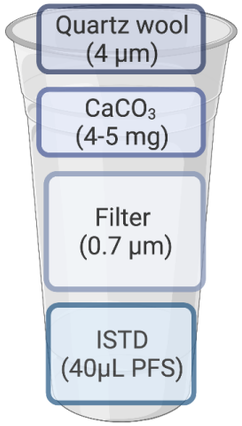


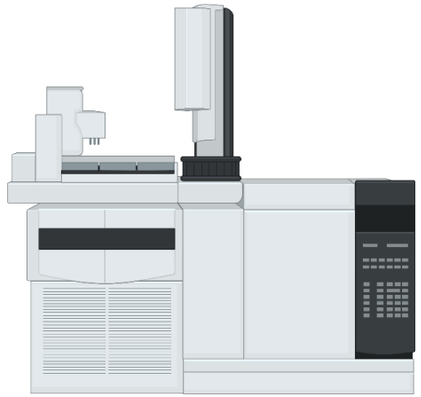
Vacuum filtration

Filter solution over 0.7 µm
filter (GF/F)

Dry filter
36°C, 24 hr

Pyrolysis cup





Py-GCMS
Triple Quad

Methods: Muscle Digestion Process

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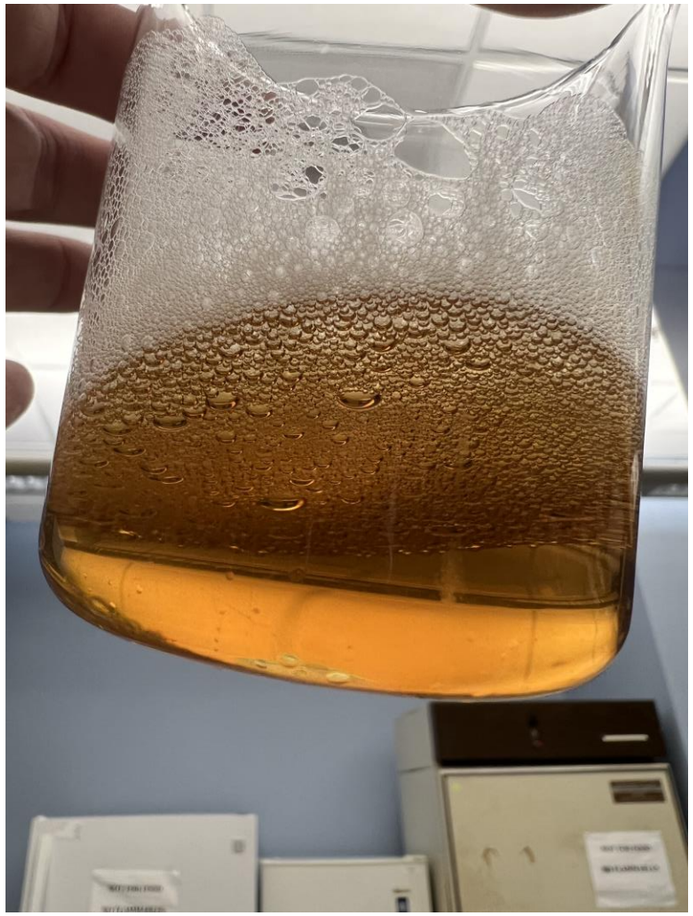
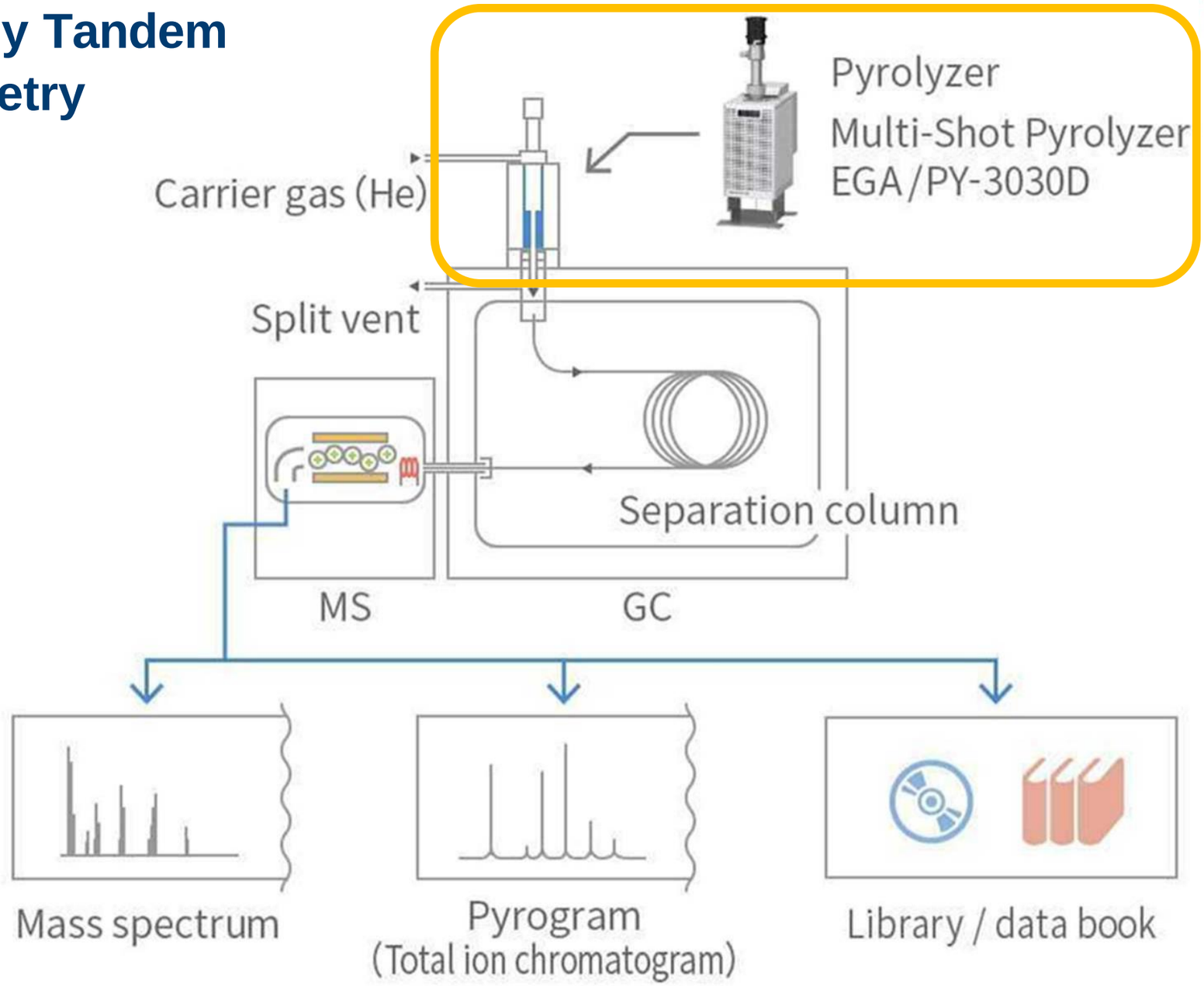
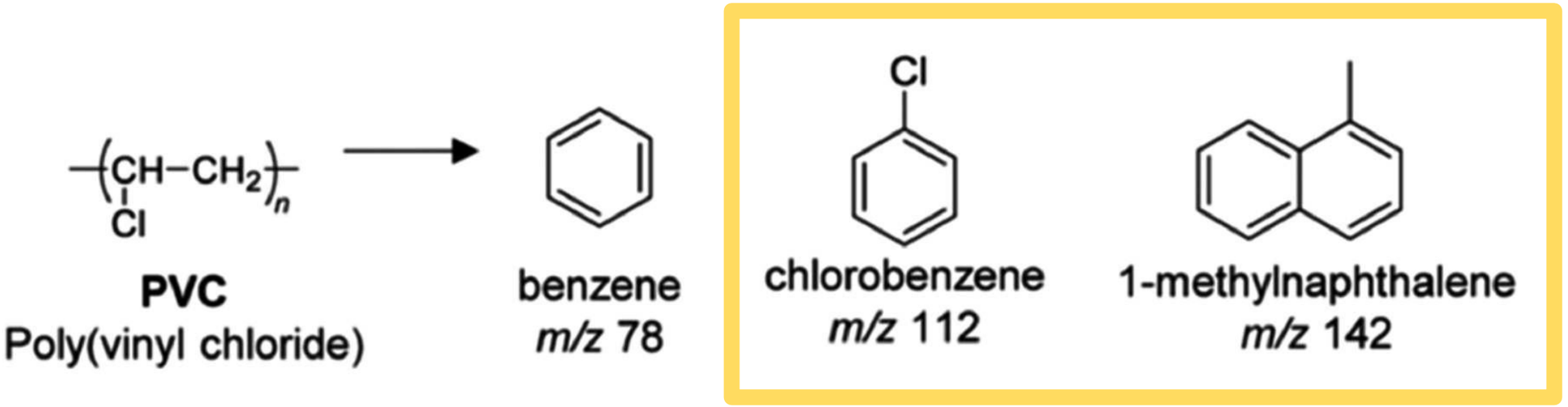
Filtered Enzyme + Muscle Tissue = Digested Muscle Slurry



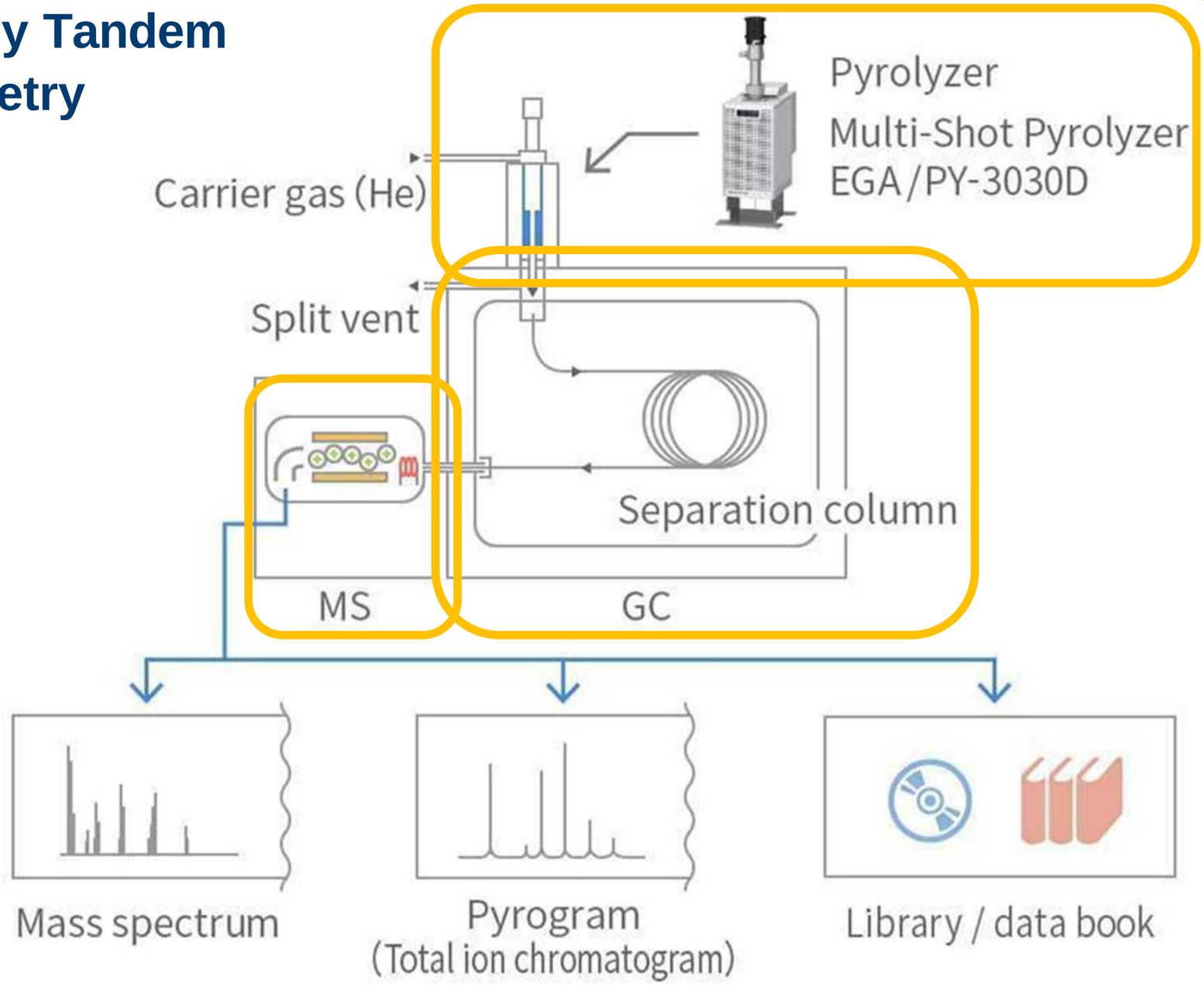
Pyrolysis Gas Chromatography Tandem Mass Spectrometry



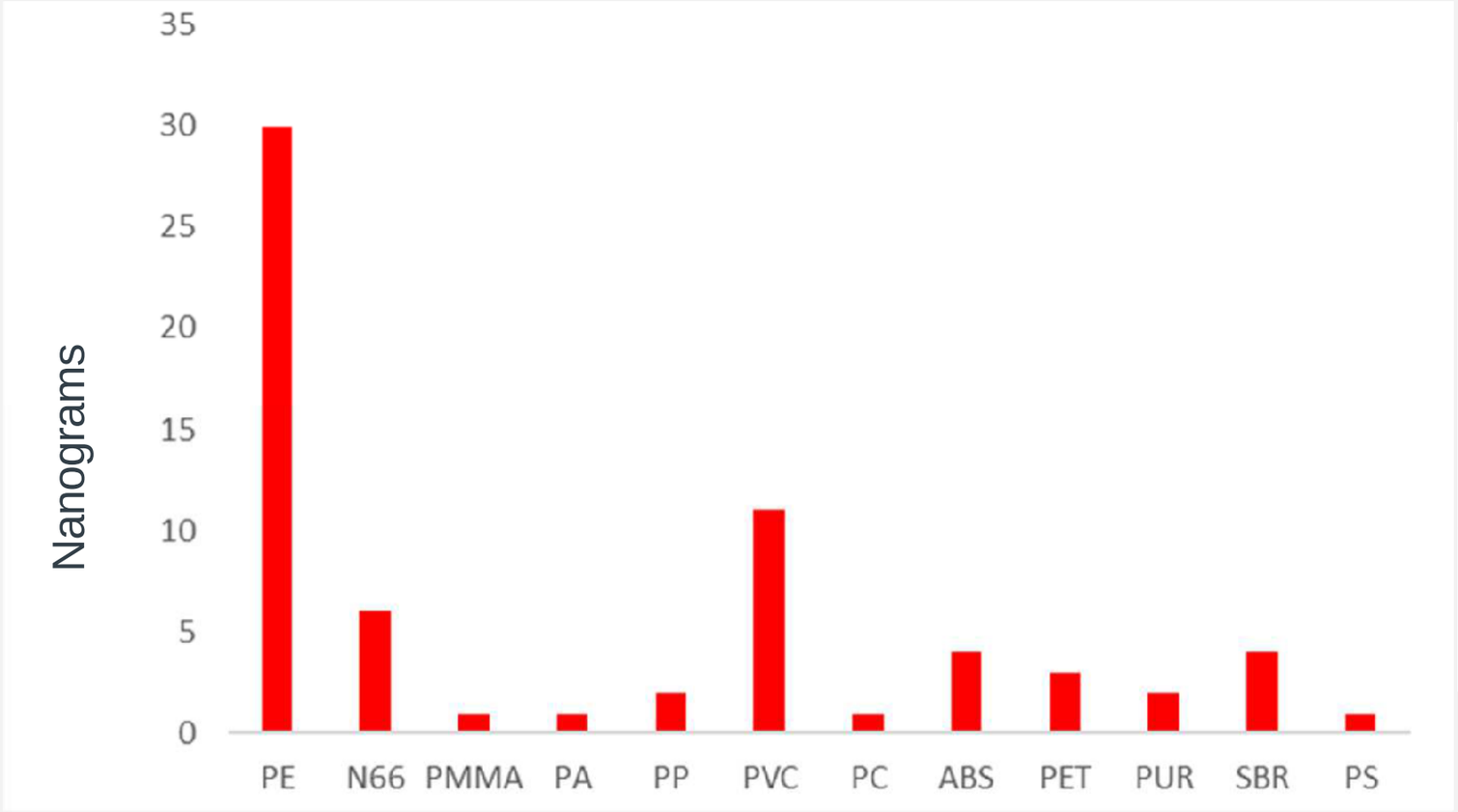
Methods: Products of Pyrolysis Products of PVC

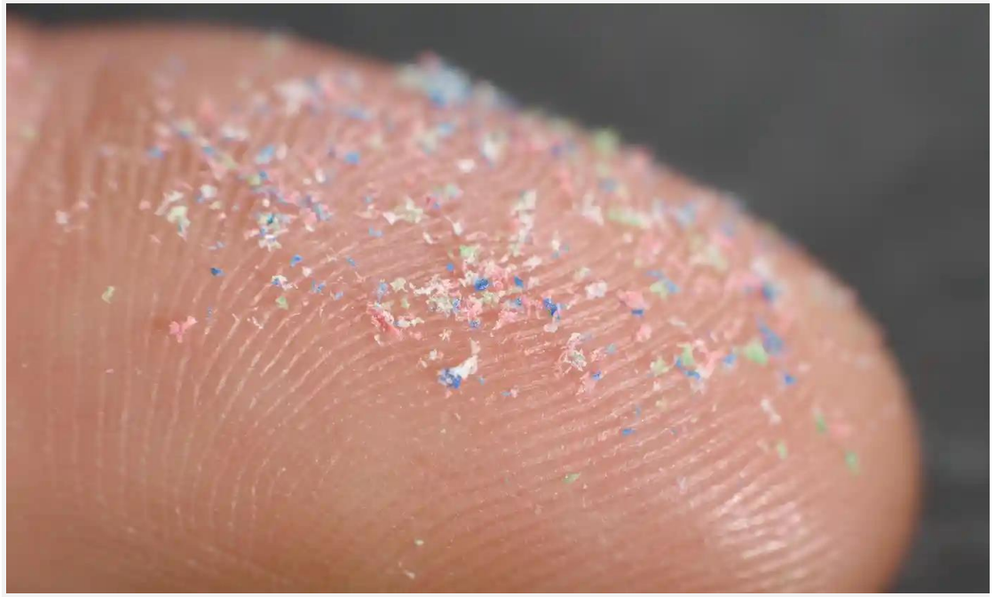


Pyrolysis Gas Chromatography Tandem Mass Spectrometry



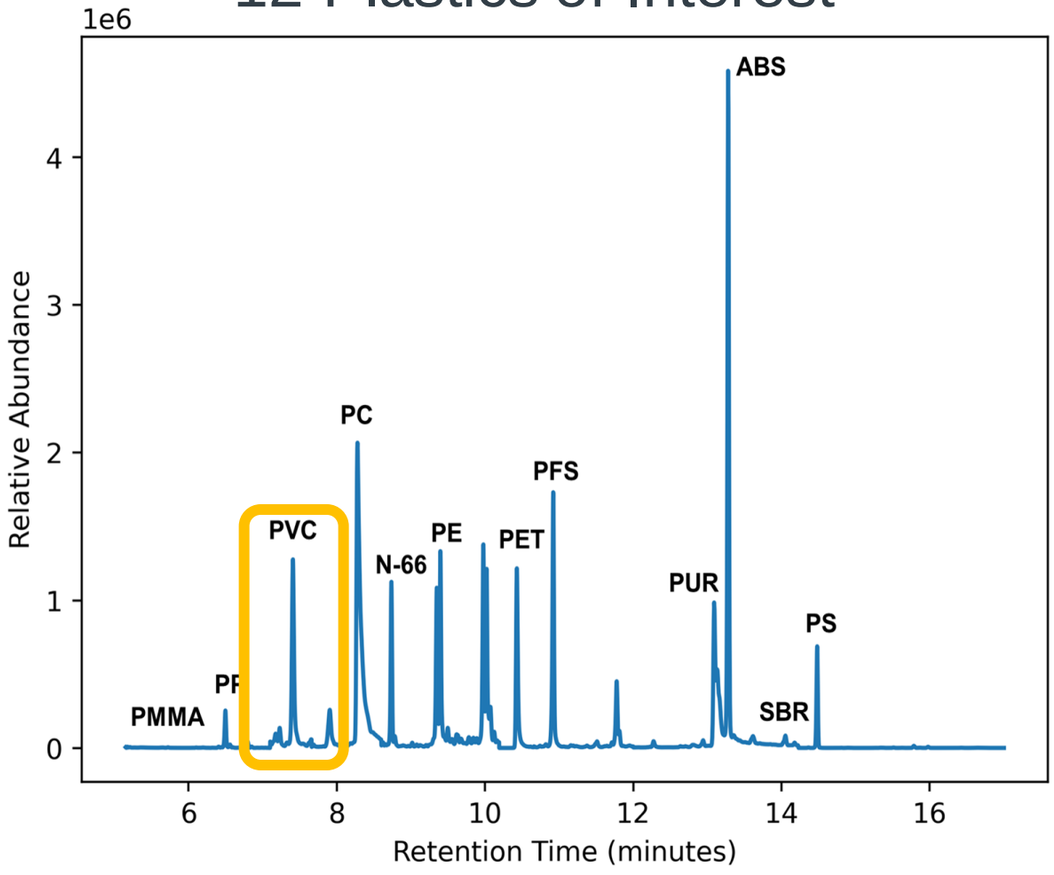
Method Limits of Detection:
Plastics are detectable at > 0.4 ng



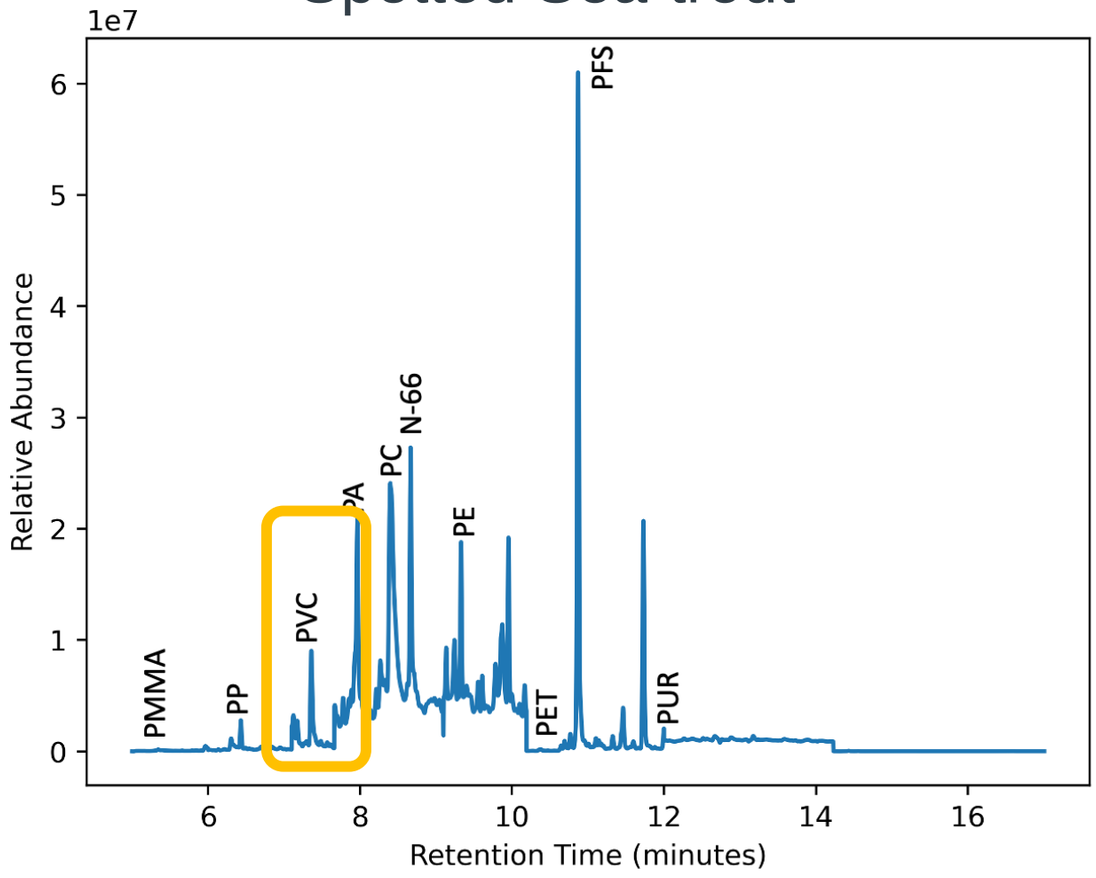


Methods: Standard and Trout Chromatogram

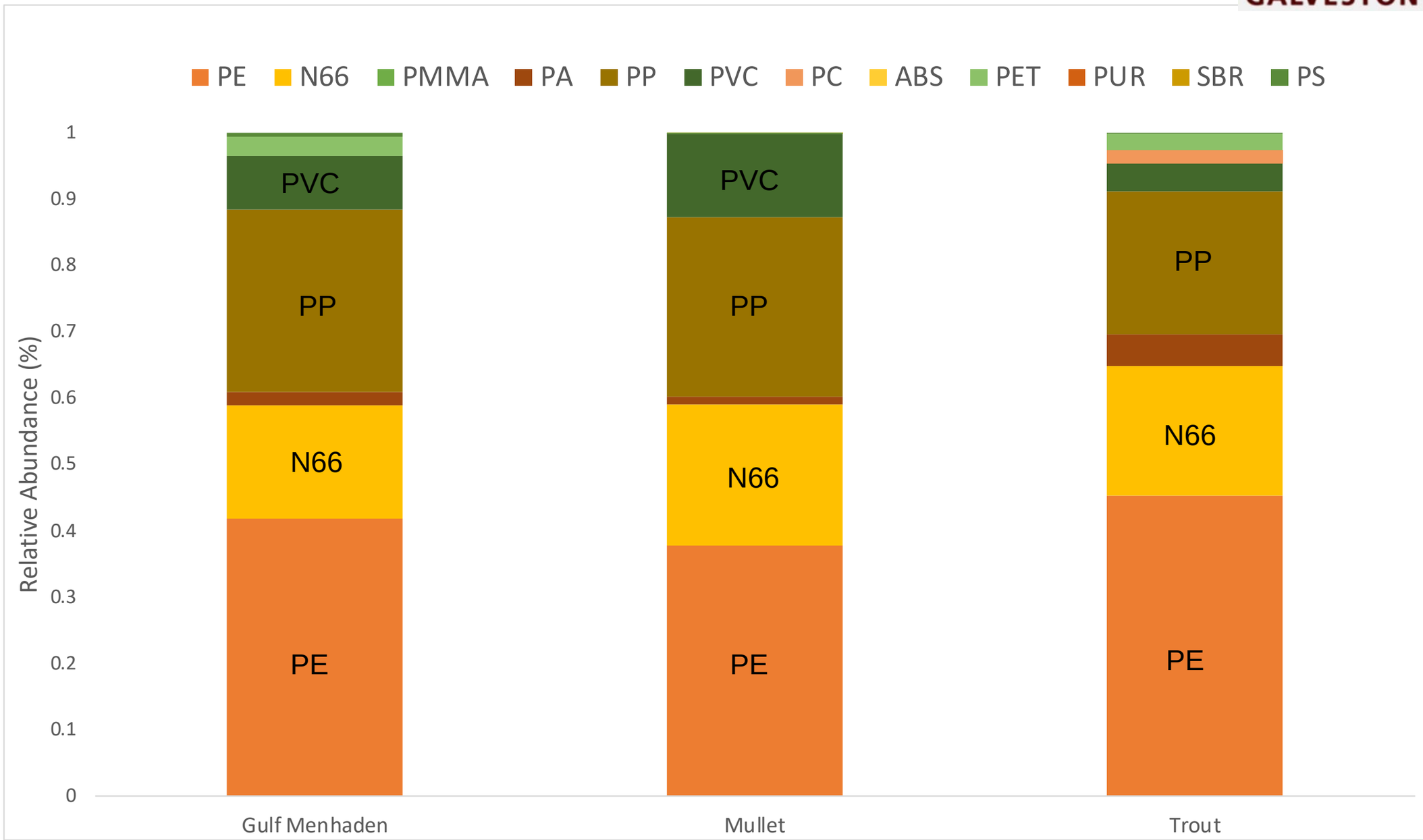
12 Plastics of Interest



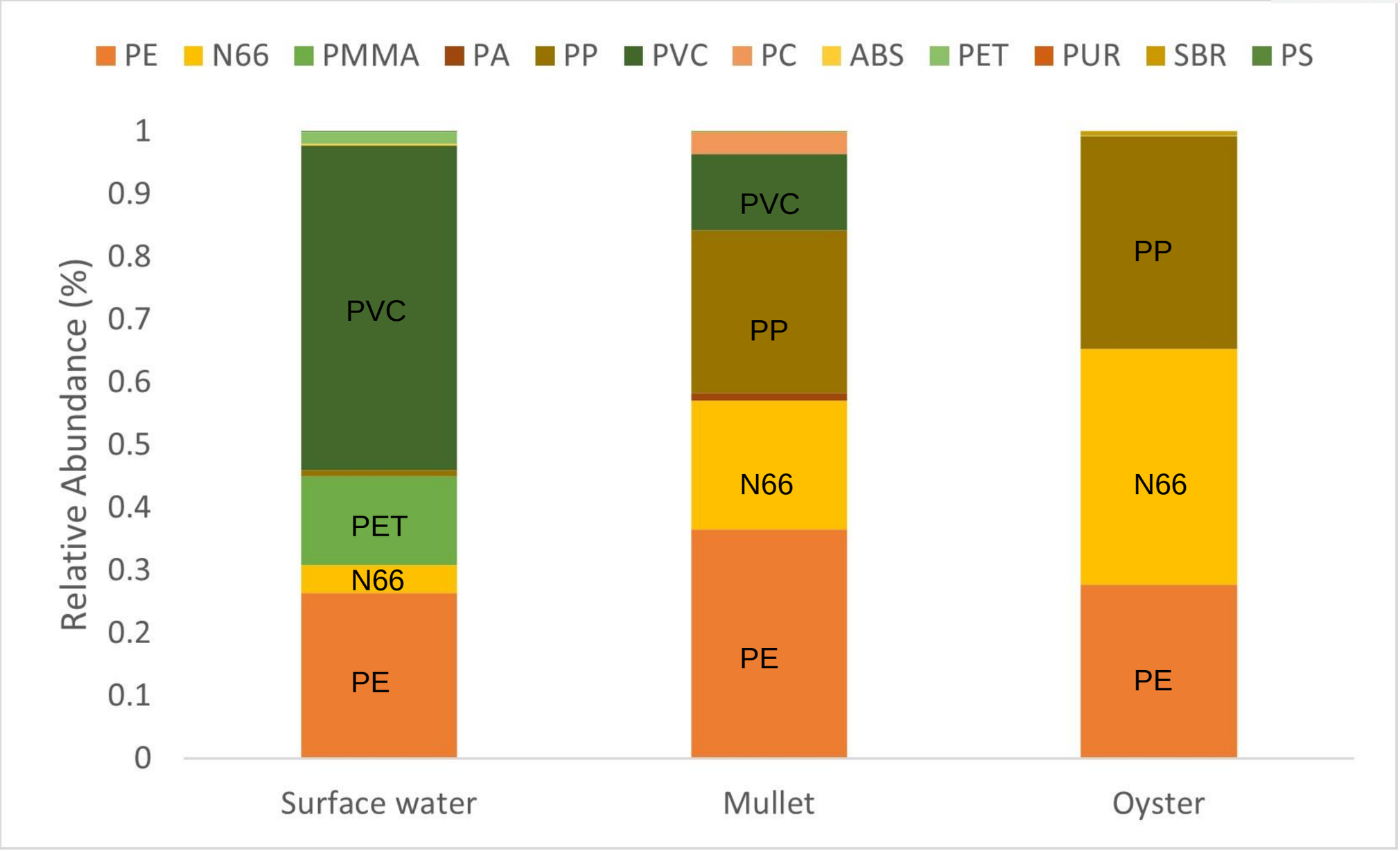
Spotted Sea-trout



Results: Relative Abundance

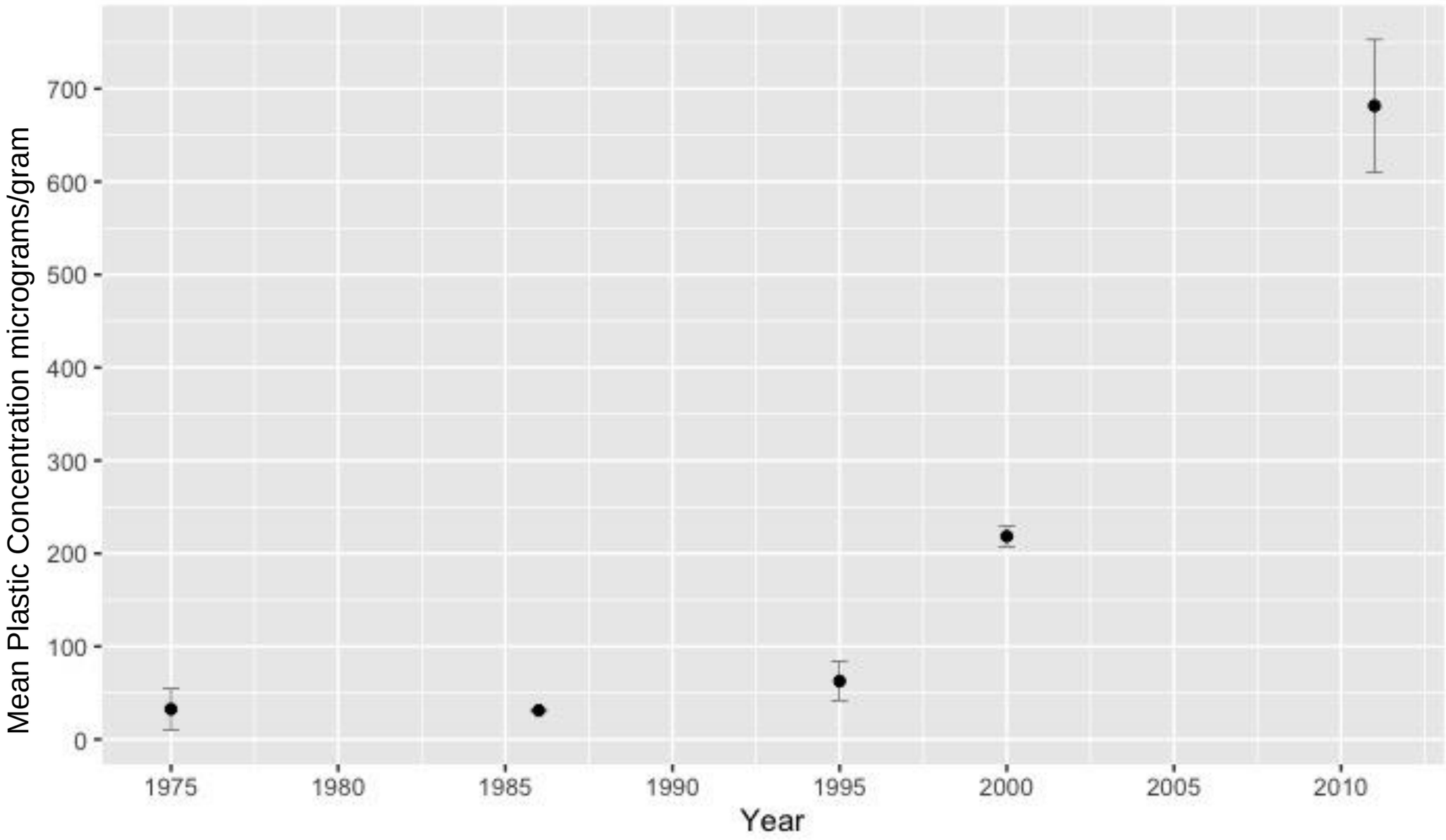


Results : Environmental vs Biological Abundance



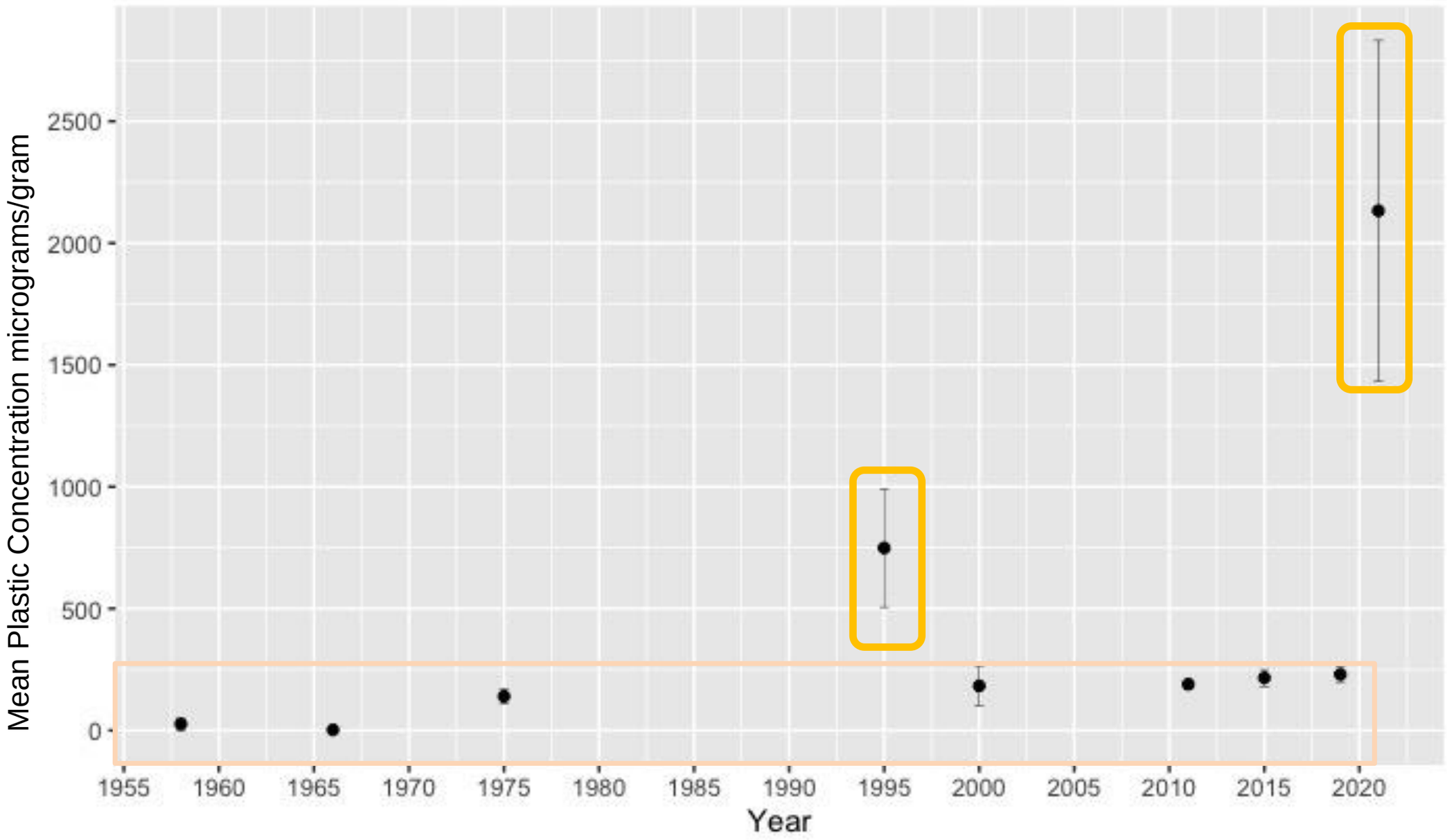
Acknowledgments:
Marcus Warton
Asif Mortuza

Results: Gulf Menhaden Historic Plastic Burden



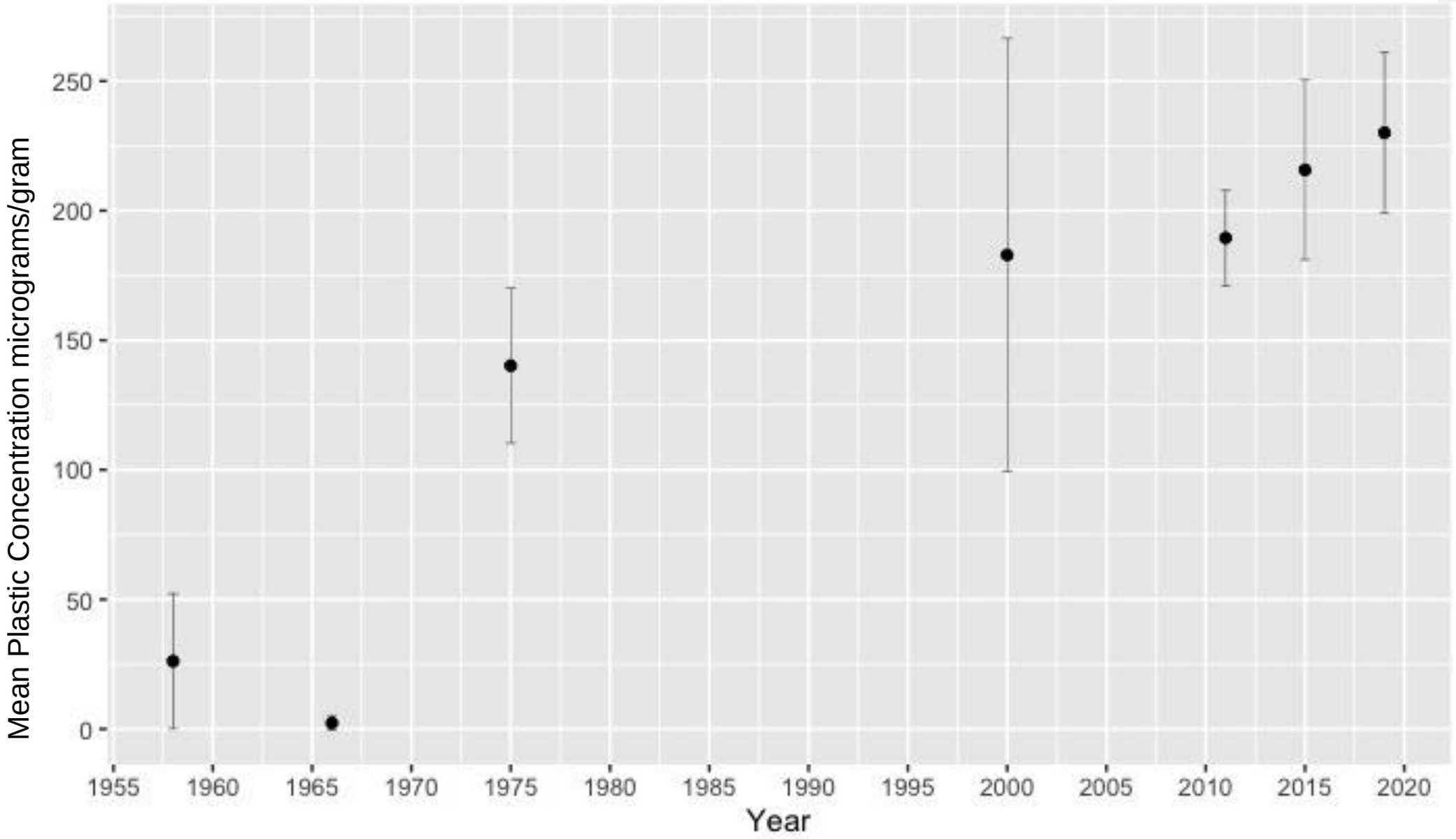
n = 2-5
N = 23

Results: Spotted Sea Trout Historic Plastic Burden

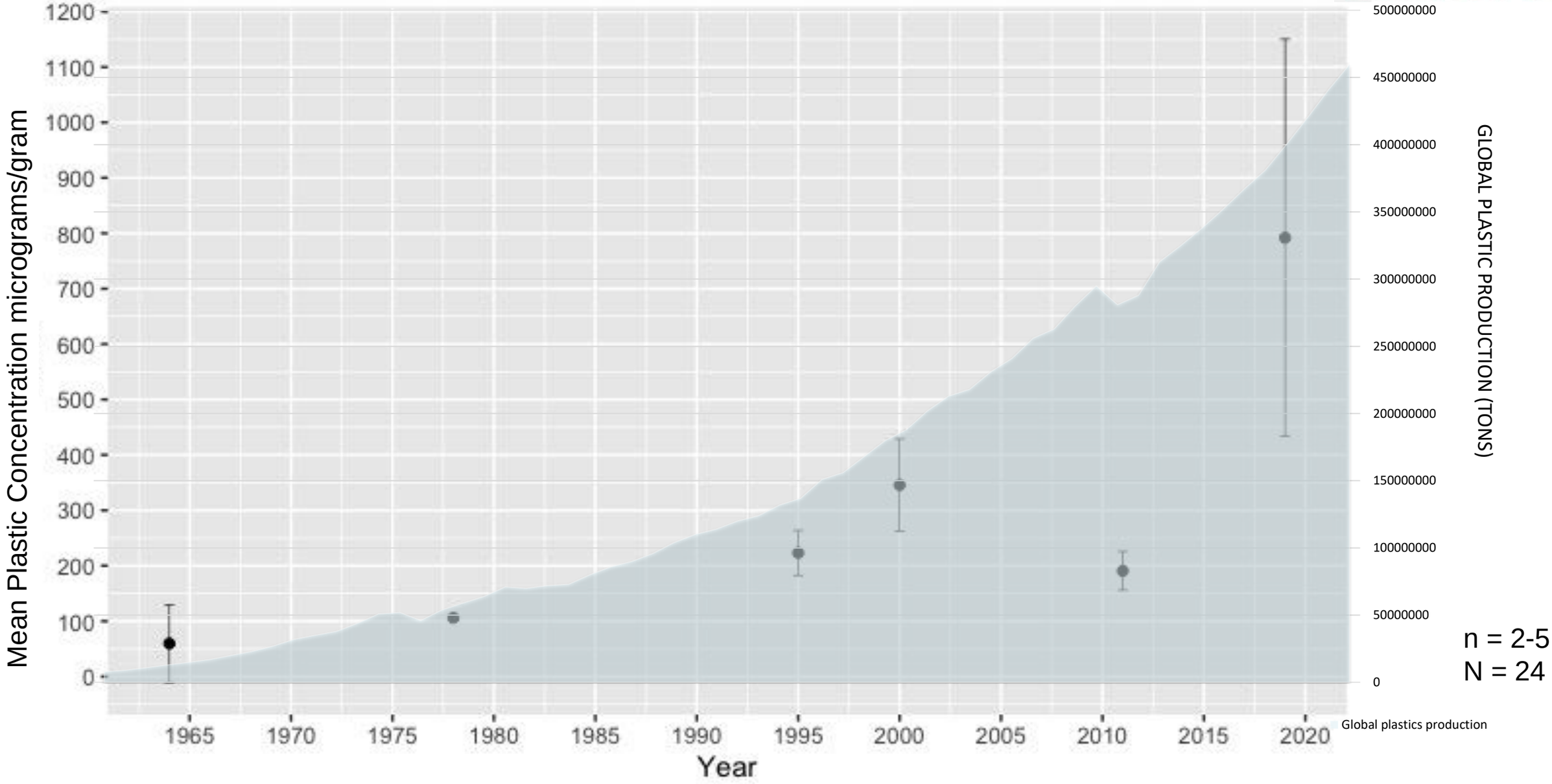


n = 2-5
N = 27

Results: Spotted Sea Trout Historic Plastic Burden



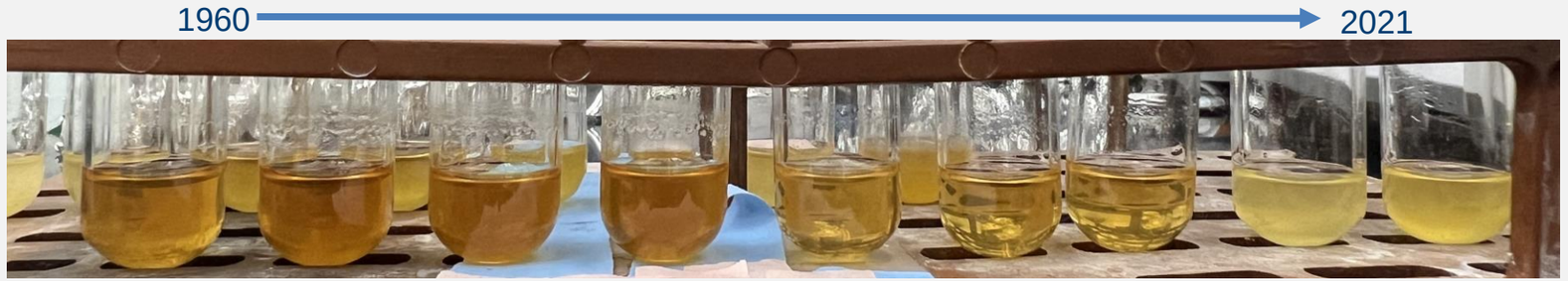
Results: Striped Mullet Historic Plastic Burden



n = 2-5
N = 24

Discussion

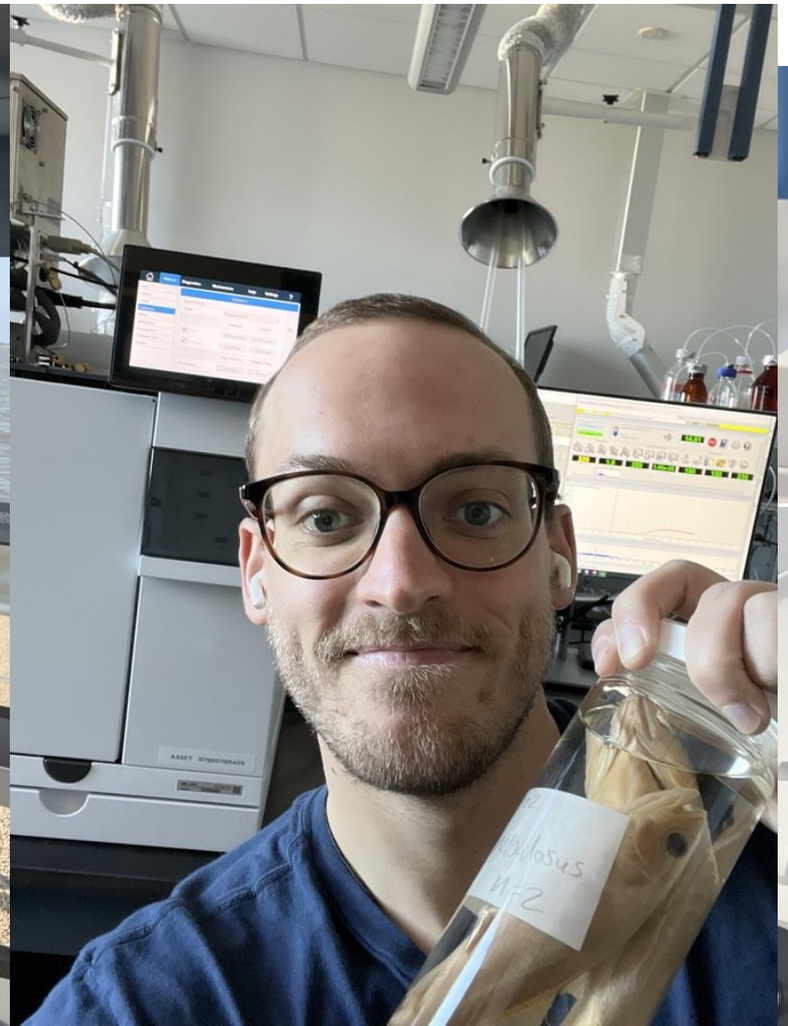
- Increasing trend in plastics found in all 3 Orders of fish
  - Hypothesis is supported
- Microplastics were detected in the environment in GBES fishes as far back 1970
  - Collecting these data prior to 1958 is difficult and not feasible on a large scale
  - Hypothesis is supported
- Relative abundance of plastics in this study seem to be independent of trophic level and feeding mode in GBES fishes
  - Hypothesis is not supported
- Body burden of all plastics exponentially increases over time in the Galveston Bay Estuary System in these fish
  - Hypothesis that trend of plastic accumulation follows the global production of plastics is supported

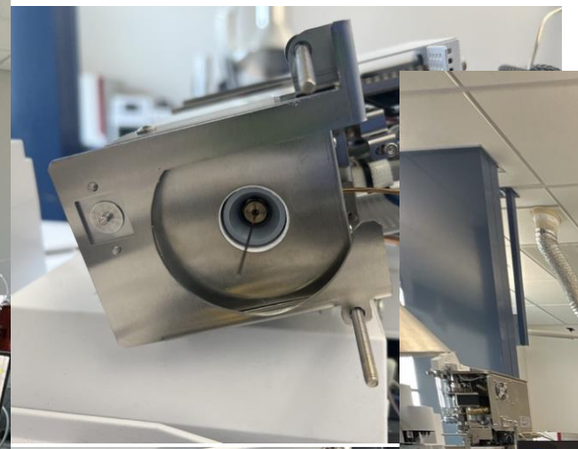


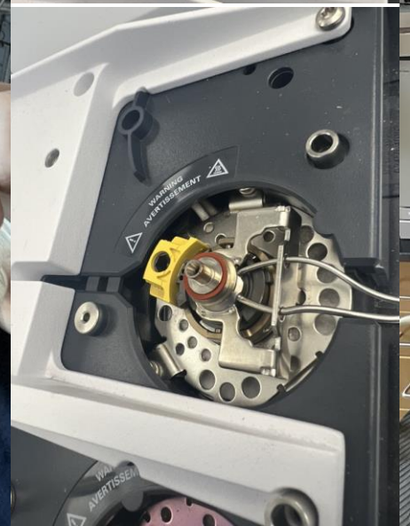
Conclusions

- Importance of investigating the past to understand the present to determine potential for environmental risks.
- Importance of collections like the Biodiversity Research and Teaching Collections in College Station
- This method is novel and allows the sensitive measurement of plastic in many complex matrices.
- The environmental and biological concentrations this methods produces grants a pivotal start point for toxicological studies and modeling plastic transport investigate how body burden and environmental exposure effects biological life.

Questions?







References

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- Cole, Matthew & Lindeque, Penelope & Fileman, Elaine & Halsband, Claudia & Goodhead, Rhys & Moger, J. & Galloway, Tamara. (2013). Microplastic Ingestion by Zooplankton. Environmental science & technology. 47. 10.1021/es400663f.

- Striped Mullet. Photo credit: Keoki Stender www.marinelifephotography.com

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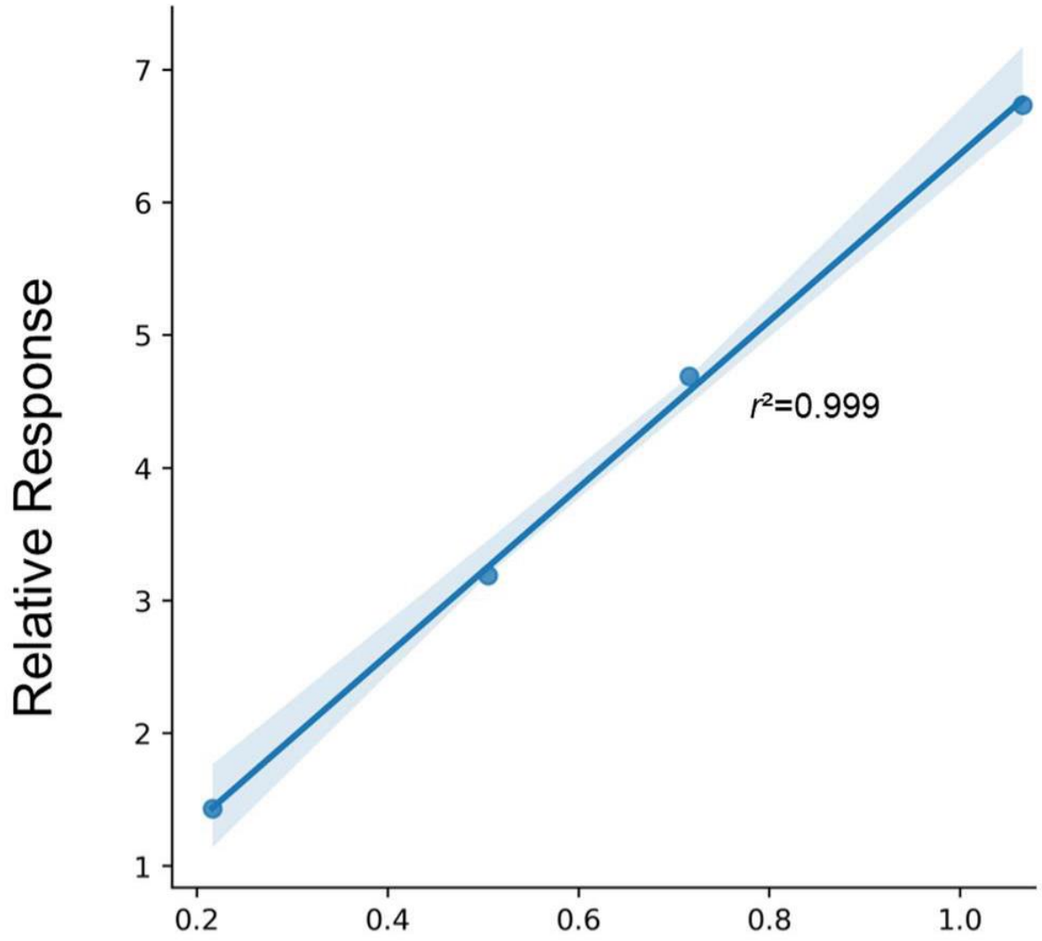
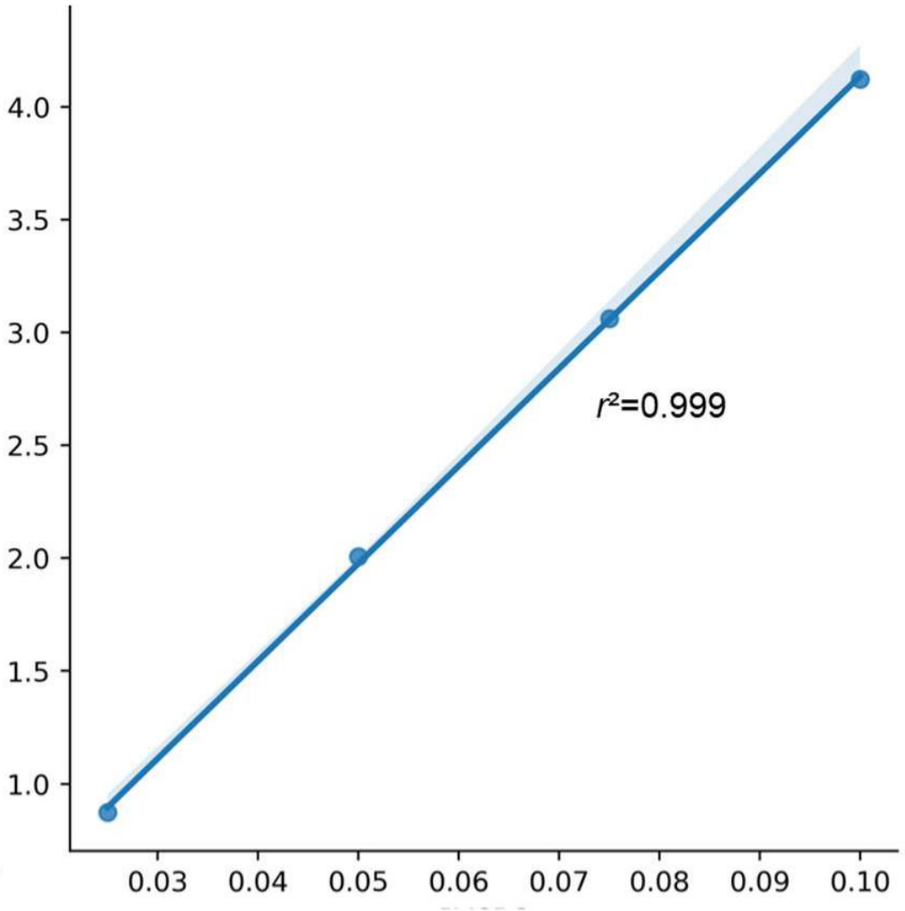
PE

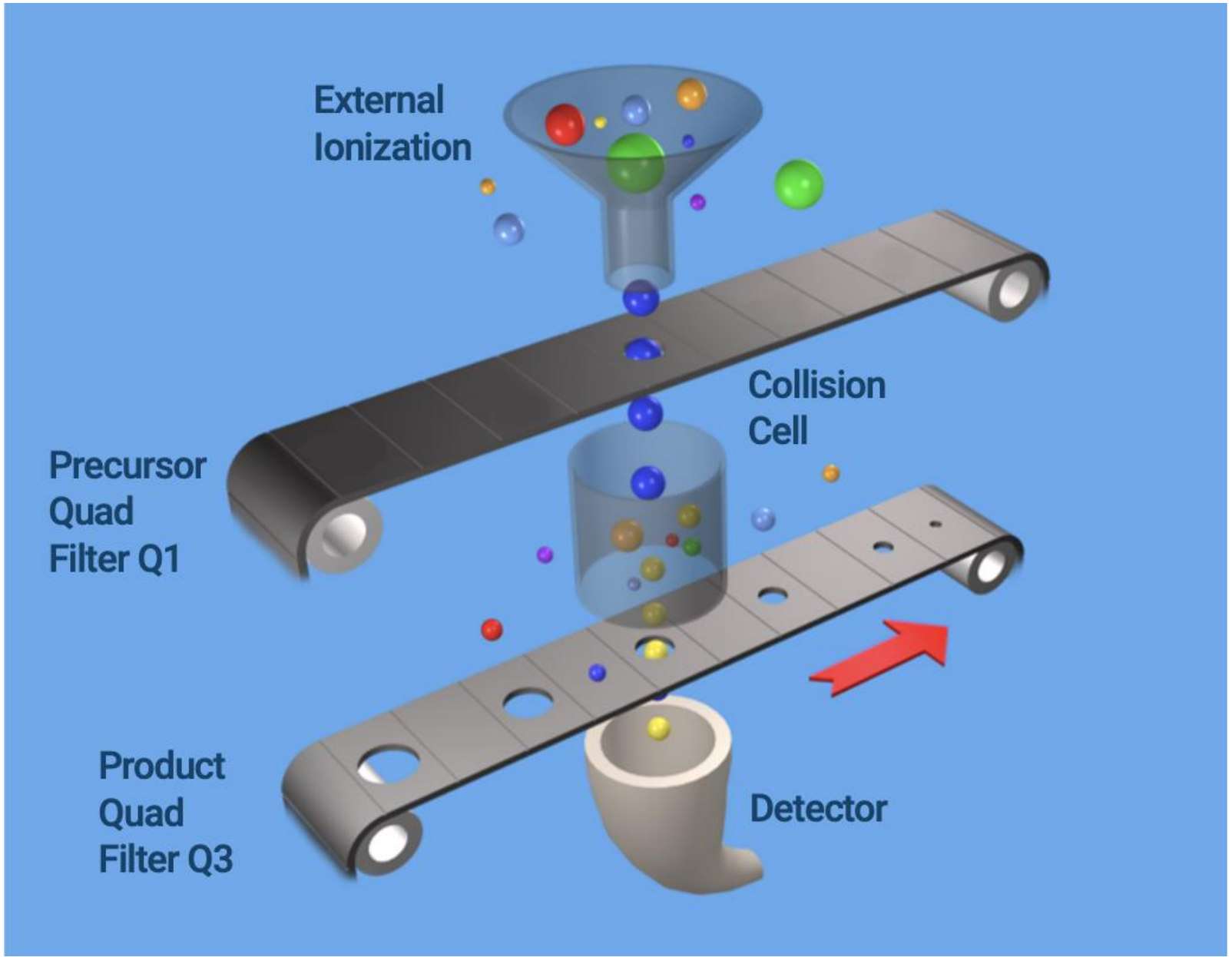


PVC



Relative Concentration
(Concentration of plastic to internal standard 40ug PFS)

Conceptual Model of TQMS Selectivity



Type of plastic material		Common uses
1	**Polyethylene**	• Plastic bags and bin bags • Food containers • Computer hardware casing • Playground fixtures and equipment
2	**Polypropylene**	• Carpeting, rugs and upholstery • Laboratory equipment • Automotive parts • Medical devices
3	**Polyvinyl-chloride**	• Plumbing products, • Electrical cable insulation, • Clothing • Medical tubing
4	**Polyethylene Terephthalate**	• Bottles • Foods containers • Polyester clothing • First-aid blankets
5	**Polystyrene**	• Food and liquid containers • Building insulation • Packaging materials • CD cases

Source: Dalberg analysis, Jambeck & al (2017), The American Chemistry Council (2018), PlasticsEurope (2018)

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Preserved vs Frozen Tissue Samples

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1960 → 2021

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Top Ten Items Over 25 Years

RANK	DEBRIS ITEM	NUMBER OF DEBRIS ITEMS	PERCENTAGE OF TOTAL DEBRIS ITEMS
1	CIGARETTES/CIGARETTE FILTERS	52,907,756	32%
2	FOOD WRAPPERS/CONTAINERS	14,766,533	9%
3	CAPS, LIDS	13,585,425	8%
4	CUPS, PLATES, FORKS, KNIVES, SPOONS	10,112,038	6%
5	BEVERAGE BOTTLES (PLASTIC)	9,549,156	6%
6	BAGS (PLASTIC)	7,825,319	5%
7	BEVERAGE BOTTLES (GLASS)	7,062,199	4%
8	BEVERAGE CANS	6,753,260	4%
9	STRAWS/STIRRERS	6,263,453	4%
10	ROPE	3,251,948	2%
	TOP TEN TOTAL DEBRIS ITEMS	**132,077,087**	**80%**
	TOTAL DEBRIS ITEMS WORLDWIDE	**166,144,420**	**100%**

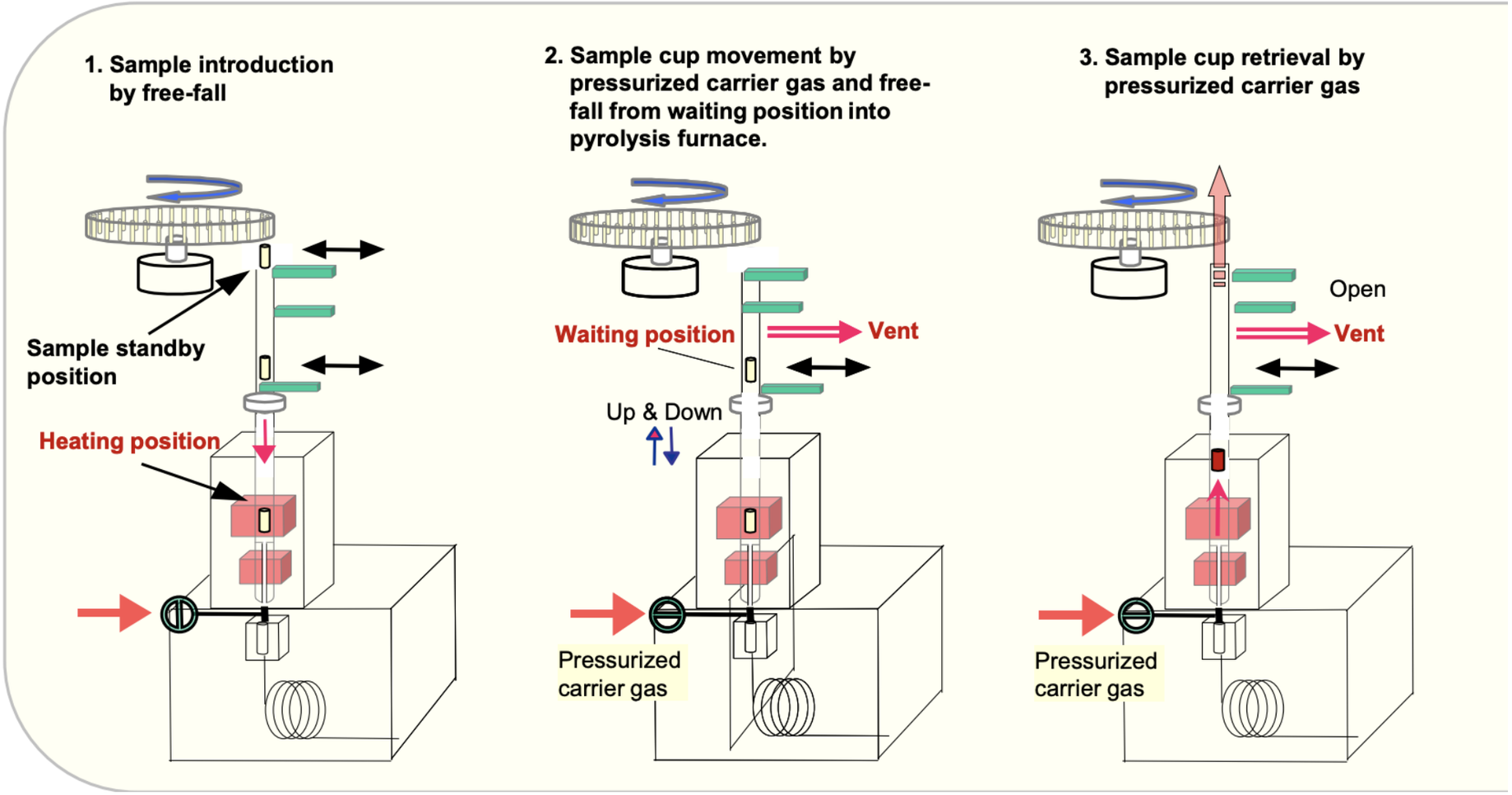
SOURCE: OCEAN CONSERVANCY/INTERNATIONAL COASTAL CLEANUP

Figure 1. Data collected over 25 years of coastal clean-up projects world- wide reveals that the most common intems are made of plastic. Source: Ocean Conservancy. Tracking Trash: 2011 Report.

Most common items from beach clean-ups are plastics

Pyrolysis

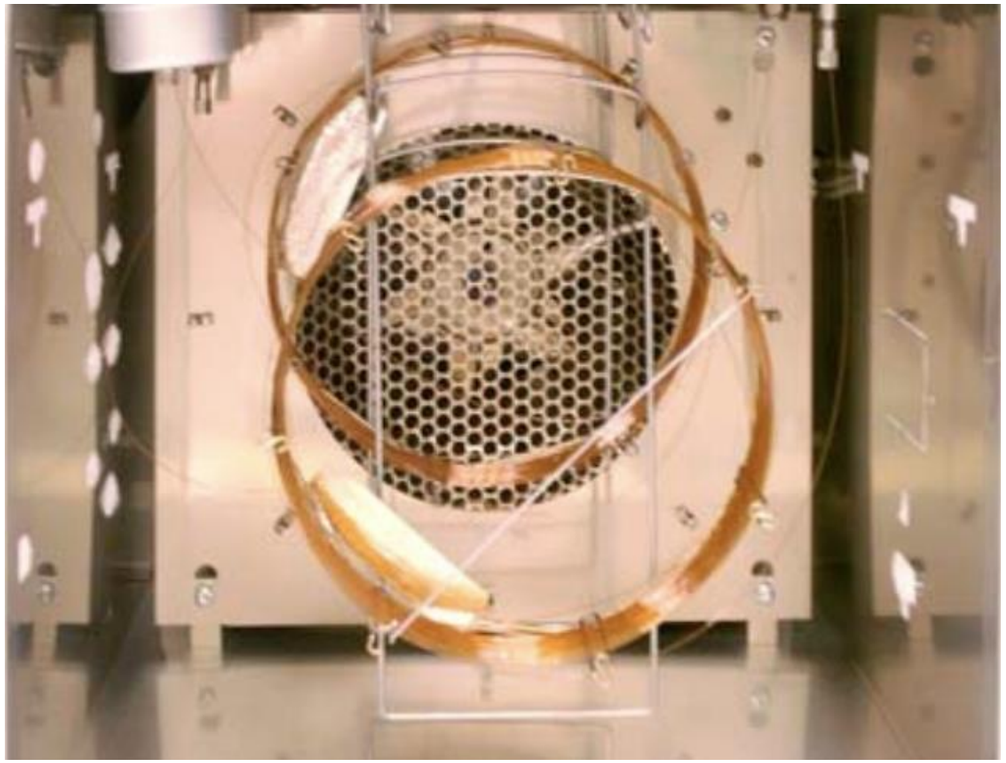
STEP ONE



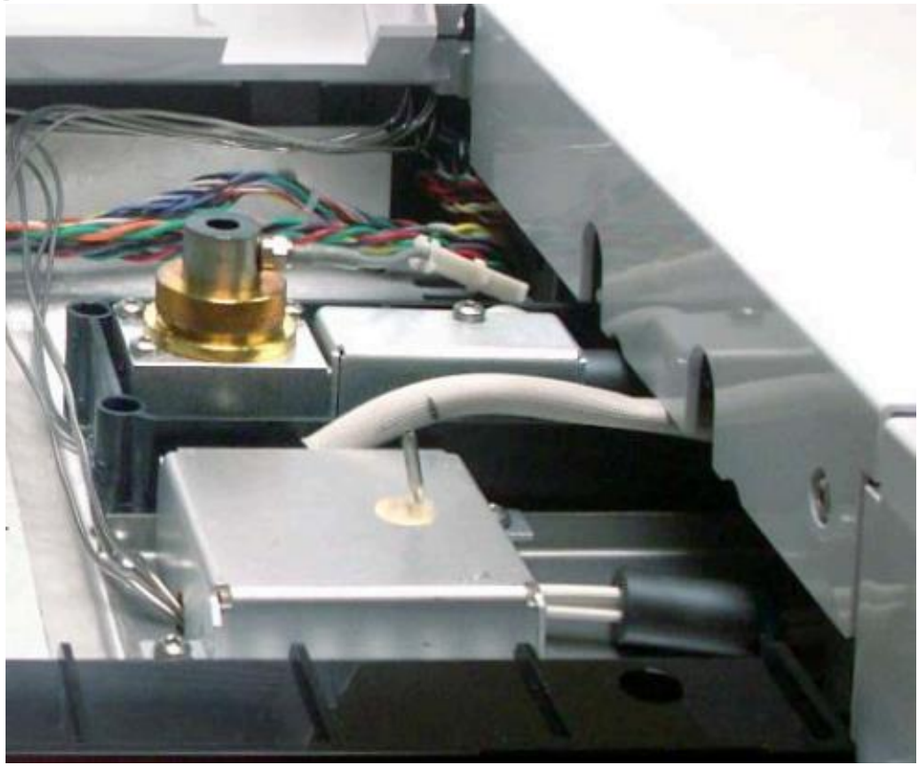
Gas Chromatography

STEP TWO

GC column and oven- Separates the sample into individual compounds as it travels through the column

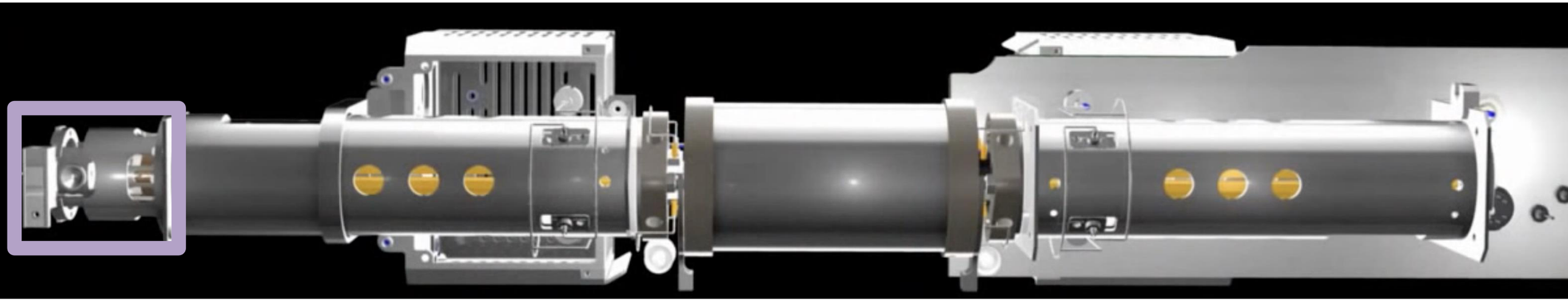


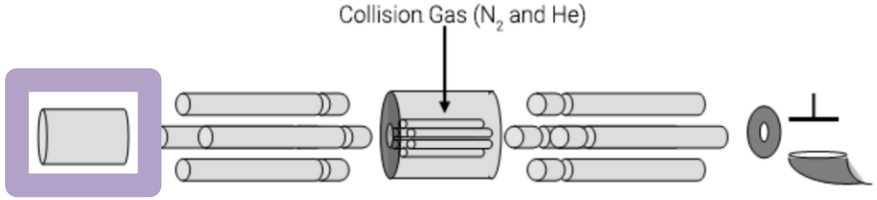
Detector- Separated compounds enter the detector and an electrical signal proportional to the amount of compound detected is generated.



MS TQ

STEP THREE



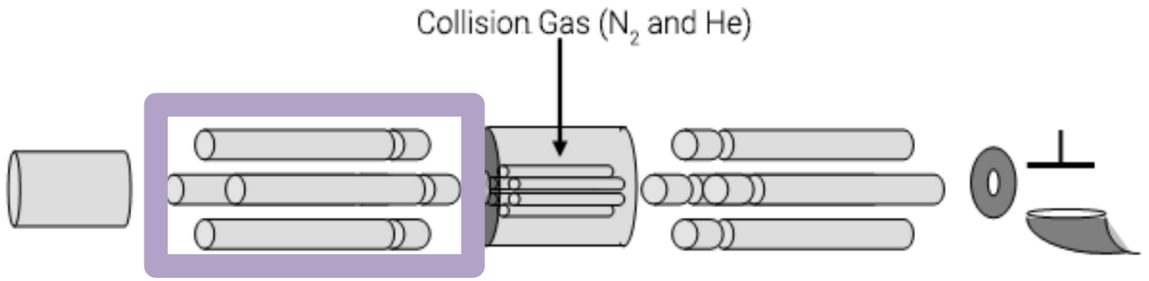


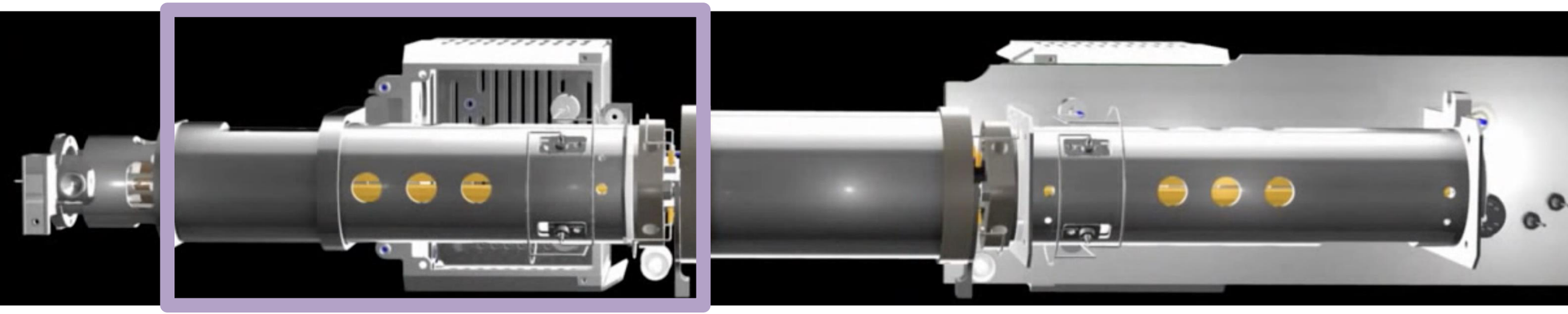
Ion source:

Sample gets blasted with high-efficiency electron ionization source that ionize the products of pyrolysis.

MS TQ

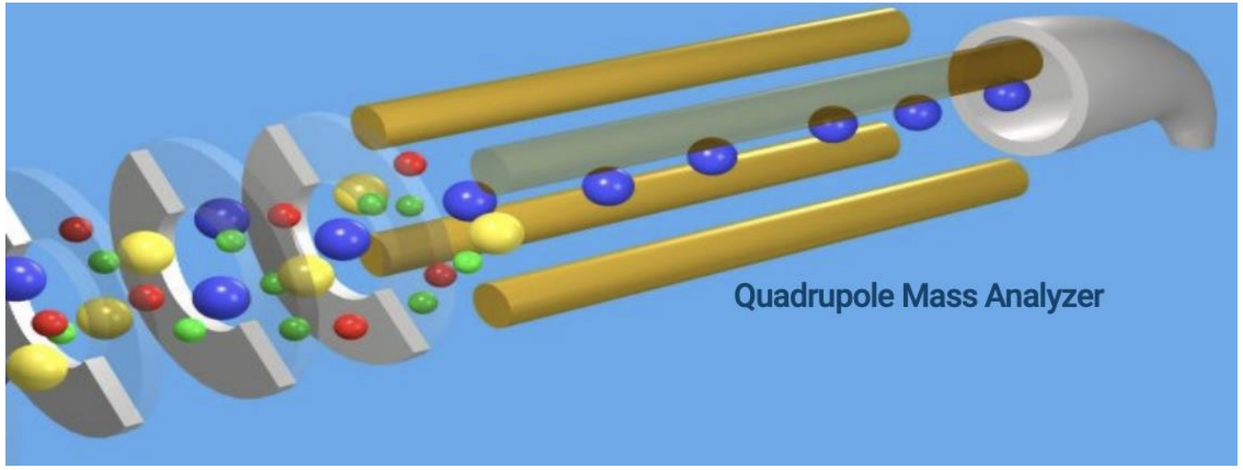
STEP THREE





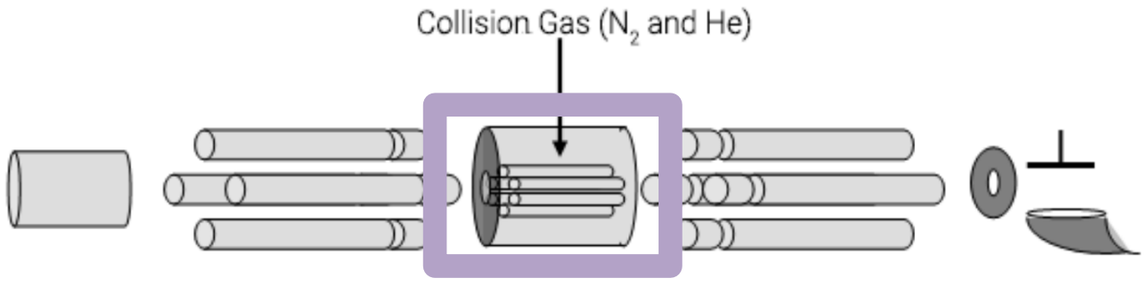
Quadrupole 1

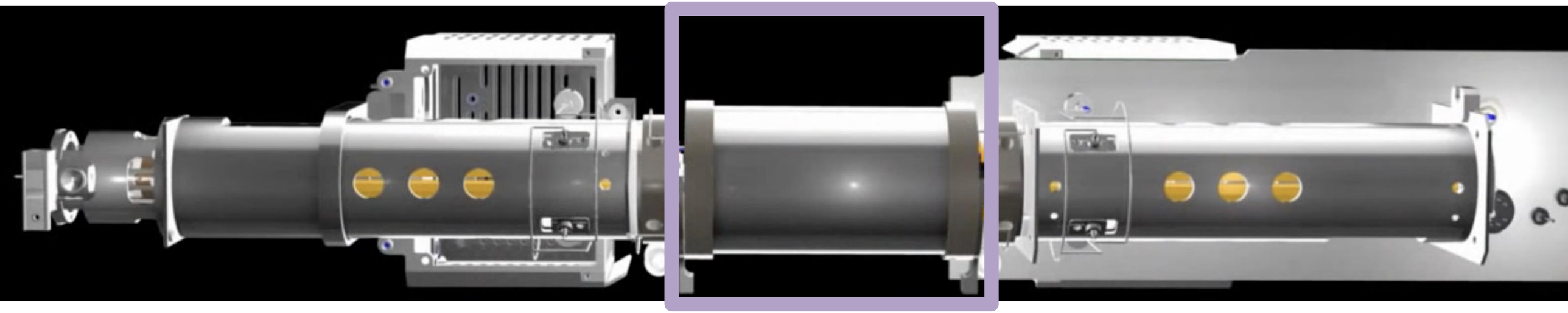
1) Removes matrix/column bleed ions and most neutrals
2) Helium ions and neutrals are thrown off course
3) The electron ionization creates lots of meta stable helium. These move randomly and have a good chance of reaching the detector…if not for collision.
4) Filters for specific masses of precursor (parent) ions



MS TQ

STEP THREE





Collision Cell – Hexapole

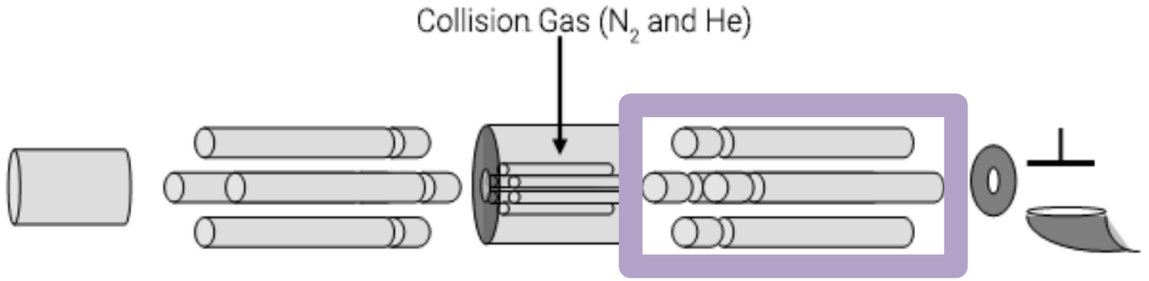
5) Small amounts of helium are added to nitrogen to quench meta stable helium

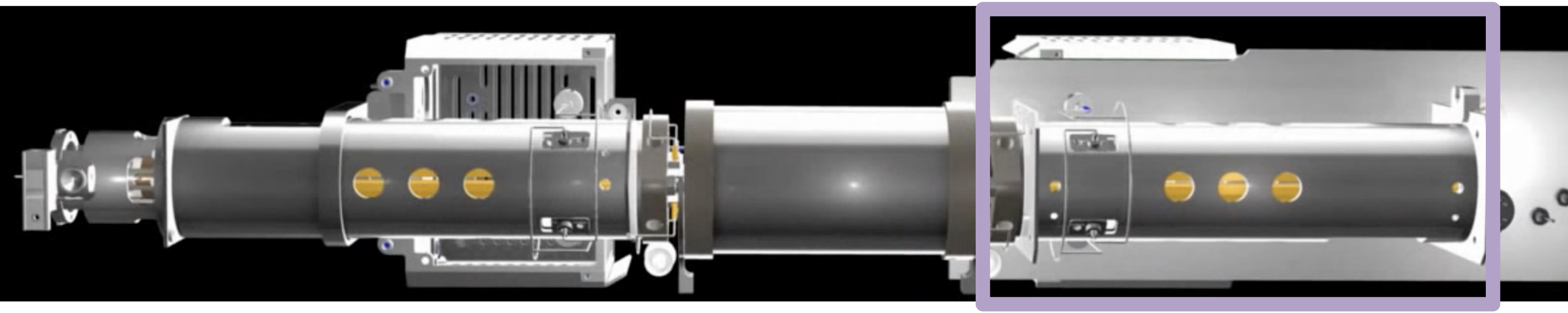
6) Helium ions and neutrals are thrown off course

7) Sample compounds collide with high energy H and N ions and fragment into daughter ions

MS TQ

STEP THREE





Quadrupole “2”- Q3

8) Selects for product (daughter) ions by mass filtration

9) Enables isolation of multiple daughter ions from a single parent ion

Mass Spectra Example

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