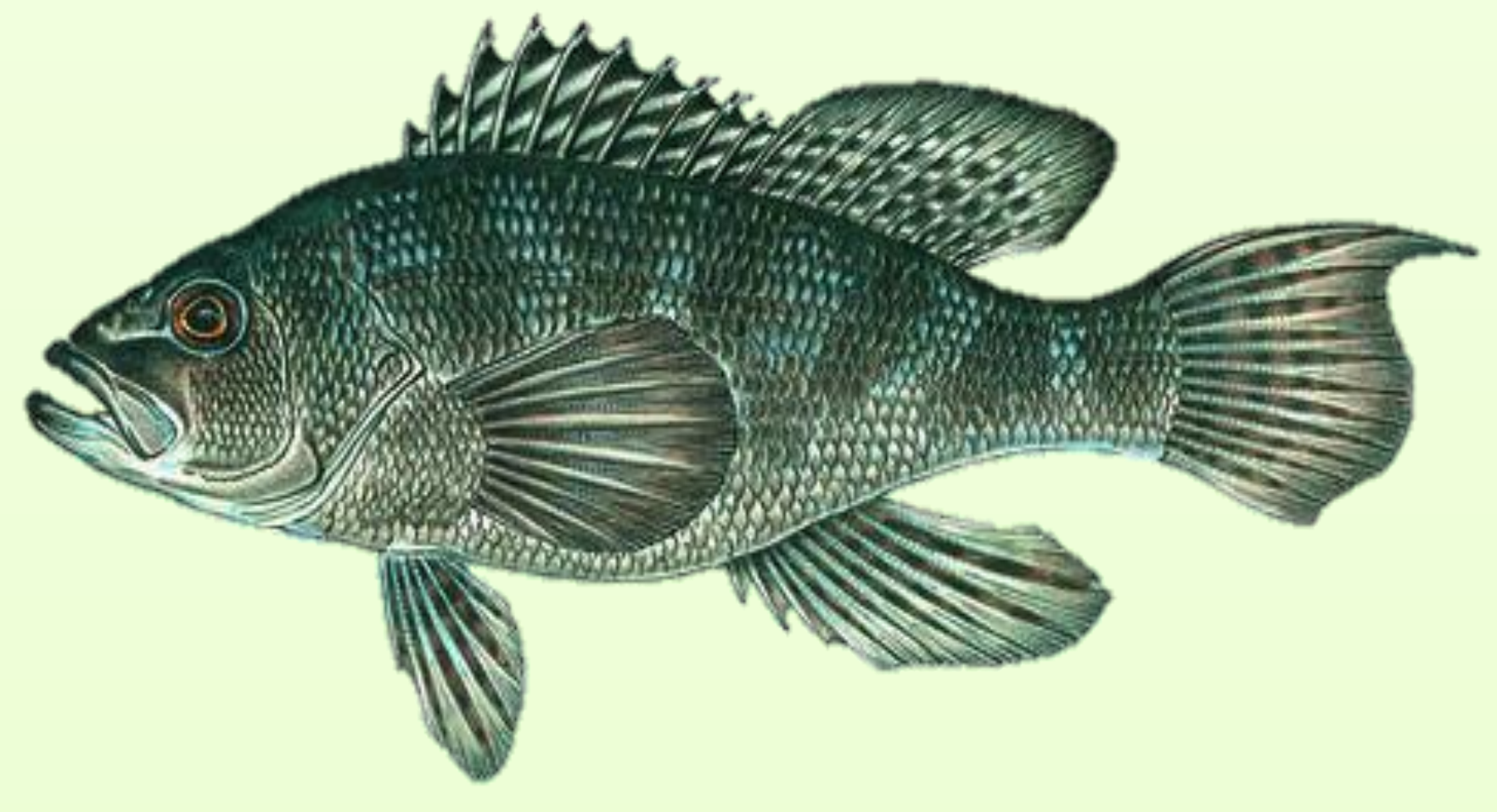
Chemical analysis of ingested plastics and associated organic pollutants in wild-caught black sea bass, *Centropristis striata*

Jincy Joseph[1], Cheyenne Stienbarger[1], Sam Athey[1], Bonnie Monteleone [1], Tony Andrady[2], Wade Watanabe[1], Pam Seaton [1], Alison Taylor [1], Susanne Brander [1,3]
1) University of North Carolina Wilmington, Wilmington, NC 2) North Carolina State University, Raleigh, NC 3) Oregon State University, Corvallis, OR

Introduction

The accumulation of microplastics in oceans, tiny granules of plastic < 5mm in diameter, is one of the major environmental concerns of our age. Due to their large specific surface area and hydrophobic properties, microplastics can absorb numerous organic pollutants. Intake of microplastics by marine organisms may therefore result in introducing toxicants to the organism itself and to the food chain, possibly leading to their bioaccumulation (Lusher *et al*. 2017). Though a number of marine organisms have been found to ingest microplastics, few studies have been performed on commercial fishery species such as black sea bass (*Centropristis striata)*

Here we have studied plastic ingestion by wild-caught black sea bass, an important fishery off the East coast of the United States. Our results showed that out of 102 fish sampled off the coast of North Carolina only three contained macroplastics. In addition, gastrointestinal (GI) tracts were processed to isolate ingested microplastics.and fibers. We are using several spectroscopic and chromatographic techniques to characterize already-detected plastics and to identify the pollutants.



Black sea bass
https://www.mass.gov/service-details/learn-about-black-sea-bass

Attenuated total reflection in conjunction with Fourier transform infrared spectroscopy (FTIR-ATR), and Raman spectroscopy were used to identify types of plastics. The identification of pollutants was achieved by solvent extraction and subsequent gas chromatography-mass spectrometry (CG/MS) analysis. Tissue analysis will also be performed to understand the leaching of pollutants from the plastics. The extracts will be purified using Agilent bond elute Enhanced matrix removal (EMR-Lipid) and will be analyzed with GC/MS-MRM (multiple reaction monitoring) method. We anticipate that our results from the analytical studies will contribute to the current knowledge on the plastic pollution in *C. striata* and will inform both fishery management and potential concerns regarding human consumption of black sea bass that have ingested plastics.

Objectives:

- ➢ Characterize the polymer type and identify associated pollutants of macroplastics obtained from wild-caught sea bass samples using FTIR-ATR, Raman spectroscopy, and GC/MS
- ➢ Isolate microplastics from GI tracts of wild-caught sea bass using KOH digestion, followed by a series of filtrations

Approach and Experimental Methods

<u>Field Sampling</u>**:** Sea bass were collected from two sites off the coast of North Carolina from Beaufort, NC and Oak Island, NC (Figure 1)

<u>Wild sea bass processing:</u> Fish were dissected and GI tracts were examined for macroplastics. To isolate adhering pollutants, an extraction with 20:80 acetone:hexane was performed.

Figure 1. Sampling sites for wild black sea bass

For isolating microplastics:

- GI tracts were digested with 10 % KOH at 50 ^{o}C for 72 hours
- The digestate was passed through a series of filters; 1mm, 64 µm, and 5 µm (Figure 2)
- The contents obtained in each steps were examined under Zeiss dissecting microscope (ZDS)
- Measures were taken to mitigate contamination

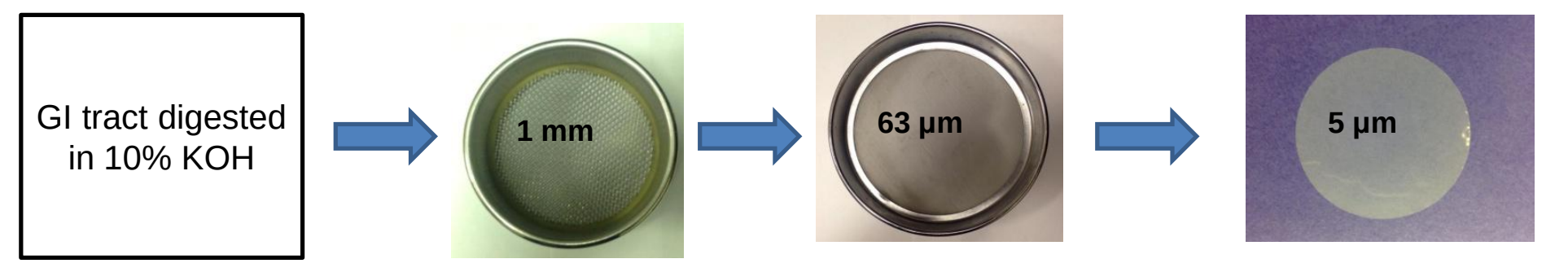


Figure 2: Schematic of filtration process to isolate microplastics. KOH digested GI tracts were filtered through 1 mm stainless steel filter, 63 µm stainless steel filter, and 5 µm polycarbonate filter

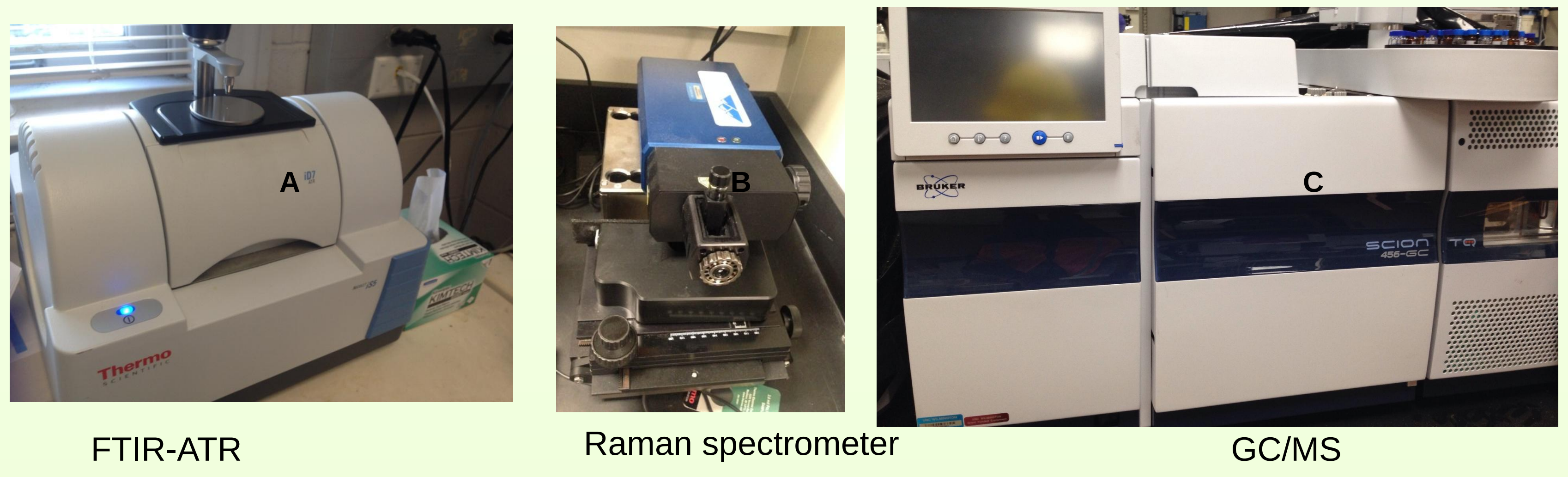


Figure 3: Instruments used for the chemical analysis of plastics and pollutants A) FTIR-ATR-Thermo scientific Nicolet iS5- iD7 ATR , B) Sierra 785 Raman spectrometer C) GC/MS: Bruker Scion TQ 456 GC equipped with a capillary column (Rtx-1701 w/Integra-Guard)

Results and Discussion

<u>Spectroscopic characterization of macroplastics</u>: Out of 102 samples dissected, only three fish contained macro plastics. One fish has ingested a plastic tape and there were three fragments (small, medium, and large) of the tape, sample ID: BT3 (Figure 4a). Second one was a fluorescent plastic bait, sample ID: YP18 (Figure 4b), third was a foamy material and it also appeared to be part of a bait, sample ID: YP45 (Figure 4c)

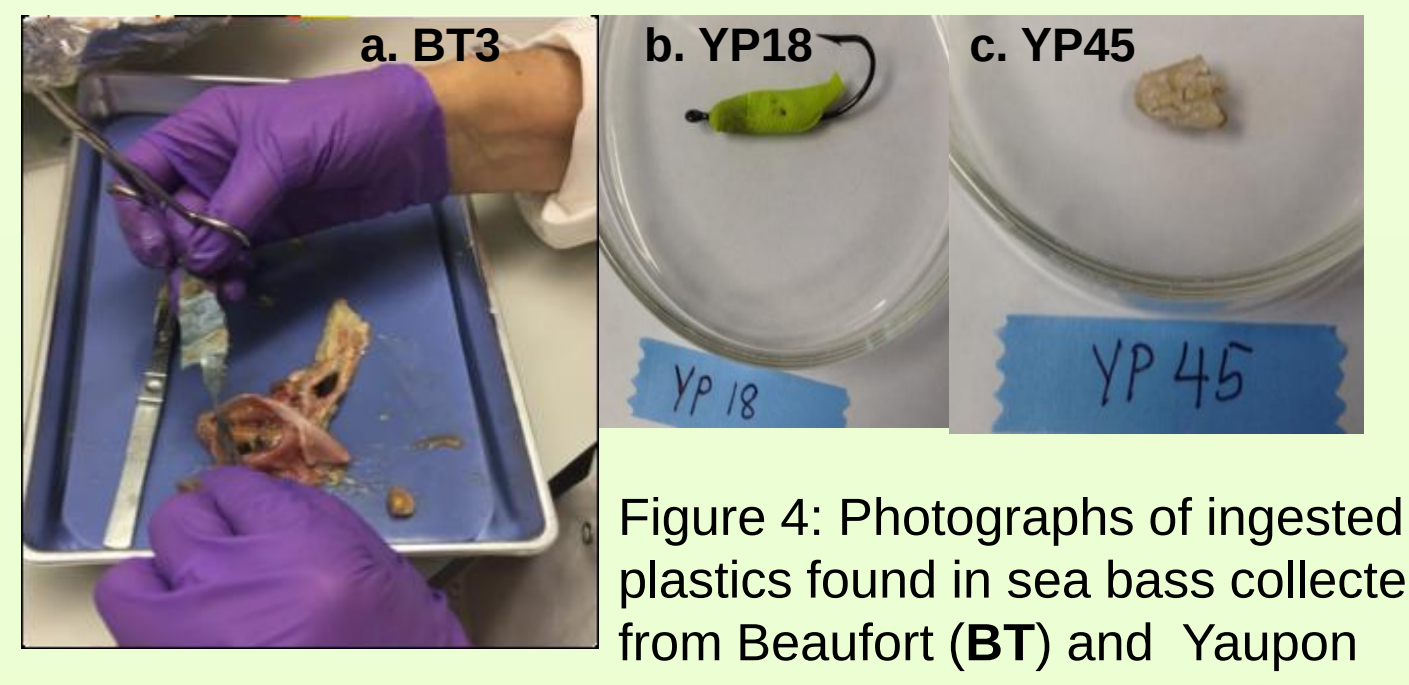
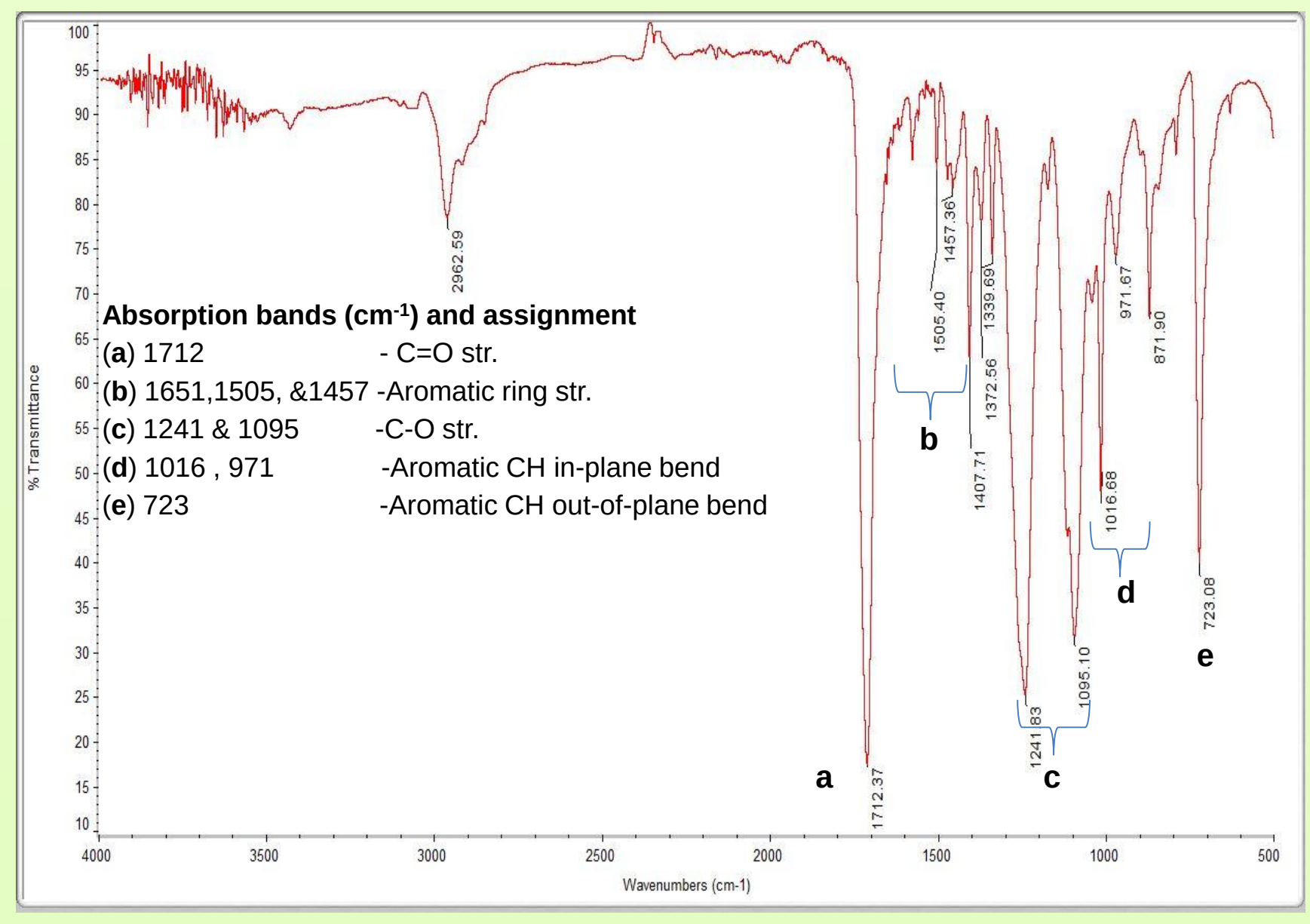


Photo: S. Brander

Figure 4: Photographs of ingested plastics found in sea bass collected from Beaufort (**BT**) and Yaupon reef (**YP**) a) plastic tape b) fish bait c) foamy material

The ATR-FTIR spectra of large plastic tape fragment and the absorption bands are shown in figure 5. The spectra of the rest of the plastic tape fragments also identical to the large fragment (Figure 6). Based on the characteristic bands and comparing to the reference compound (Figure 6), the polymeric structure of the ingested plastic tape is assigned to poly (ethylene terephthalate) (Figure 7).



Absorption bands (cm^{-1}) and assignment
(**a**) 1712 - C=O str.
(**b**) 1651,1505, &1457 -Aromatic ring str.
(**c**) 1241 & 1095 -C-O str.
(**d**) 1016 , 971 -Aromatic CH in-plane bend
(**e**) 723 -Aromatic CH out-of-plane bend

Figure 5: FTIR-ATR spectrum of large fragment from the plastic tape (BT3 large). Letters represent the characteristic vibration used to identify the polymer

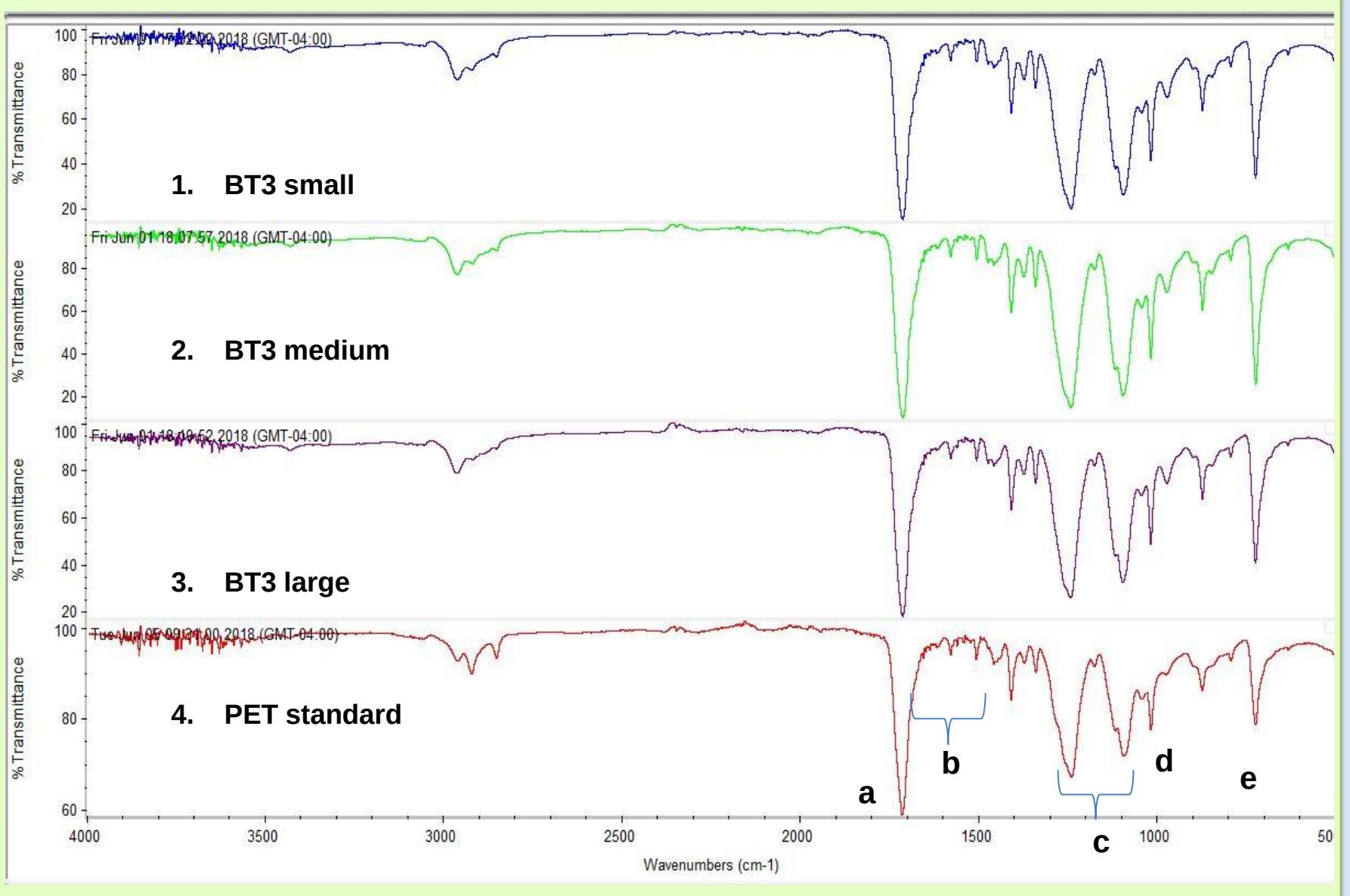


Figure 6: FTIR-ATR spectra of plastic tape fragments.BT3 (1) small, (2) medium (3) large and (4) PET standard. Letters represent the characteristic vibration used to identify the polymer

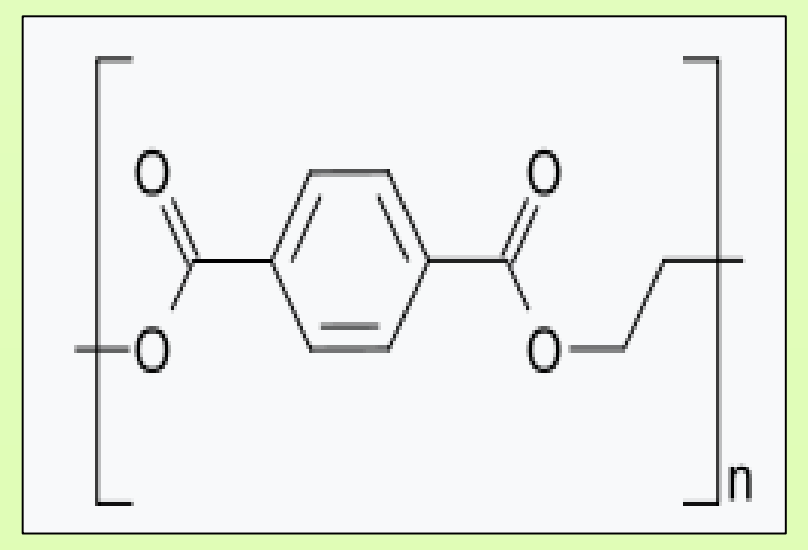
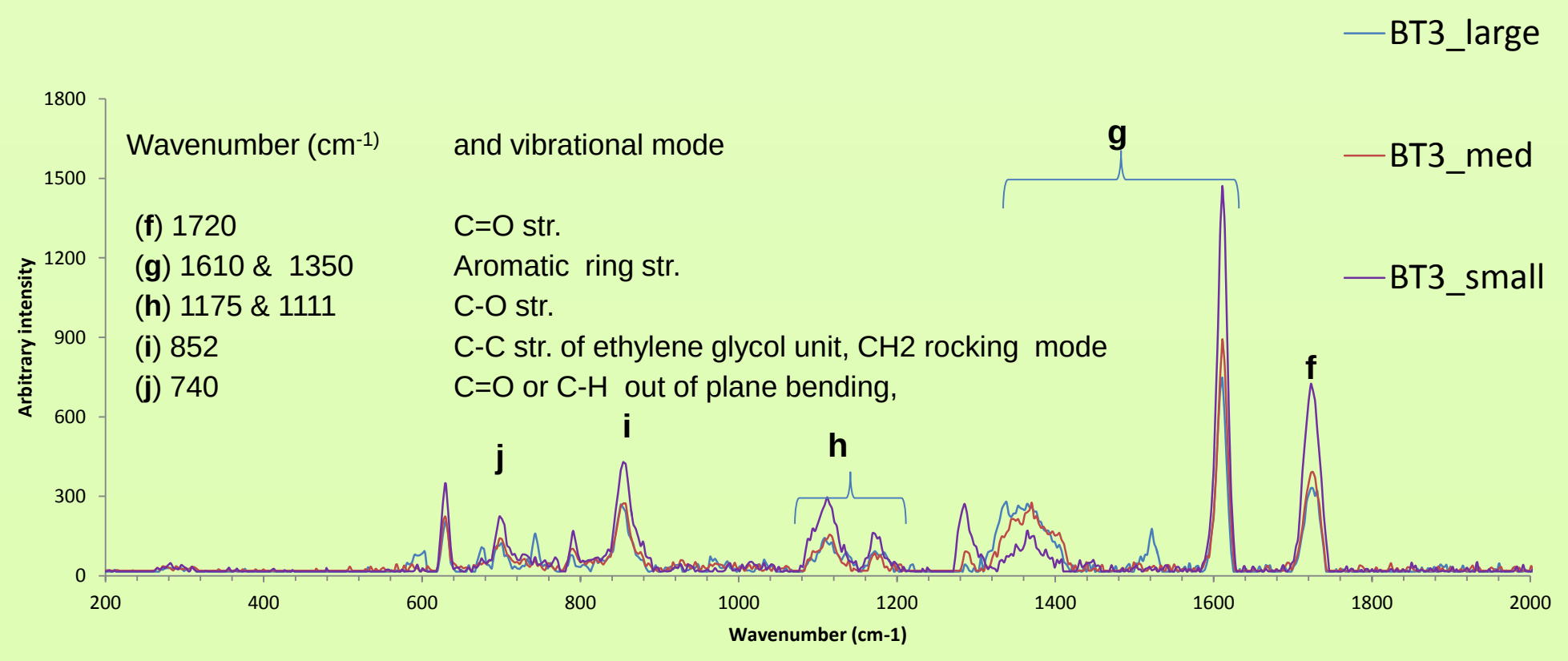


Figure 7: Structure of Poly(ethylene terephthalate) (PET)



Wavenumber (cm^{-1}) and vibrational mode
(**f**) 1720 C=O str.
(**g**) 1610 & 1350 Aromatic ring str.
(**h**) 1175 & 1111 C-O str.
(**i**) 852 C-C str. of ethylene glycol unit, CH_2 rocking mode
(**j**) 740 C=O or C-H out of plane bending,

Figure 8: Raman spectra of plastic tape fragments.BT3 small, medium, and large. Letters represent the characteristic vibration used to identify the polymer.

Additional evidences for the structure of BT3 samples obtained from Raman spectra. All the fragments showed similar spectra (Figure 8) and perfectly matches with poly (ethylene terephthalate) standard (Figure 9). The major vibrations and assignments are shown in figure 8 and 9.

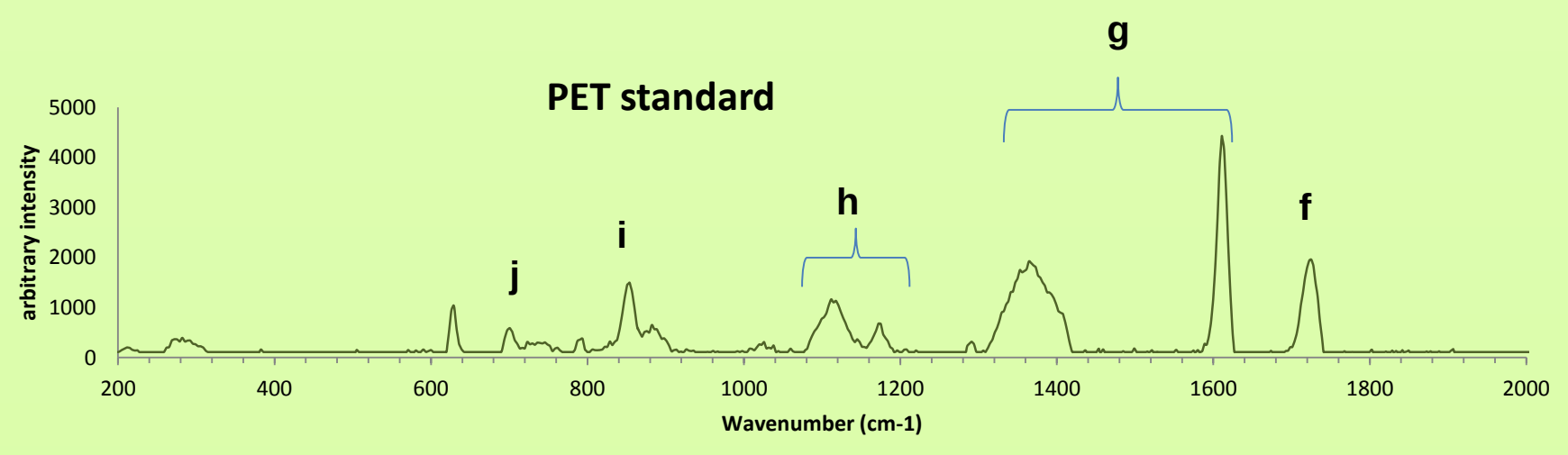
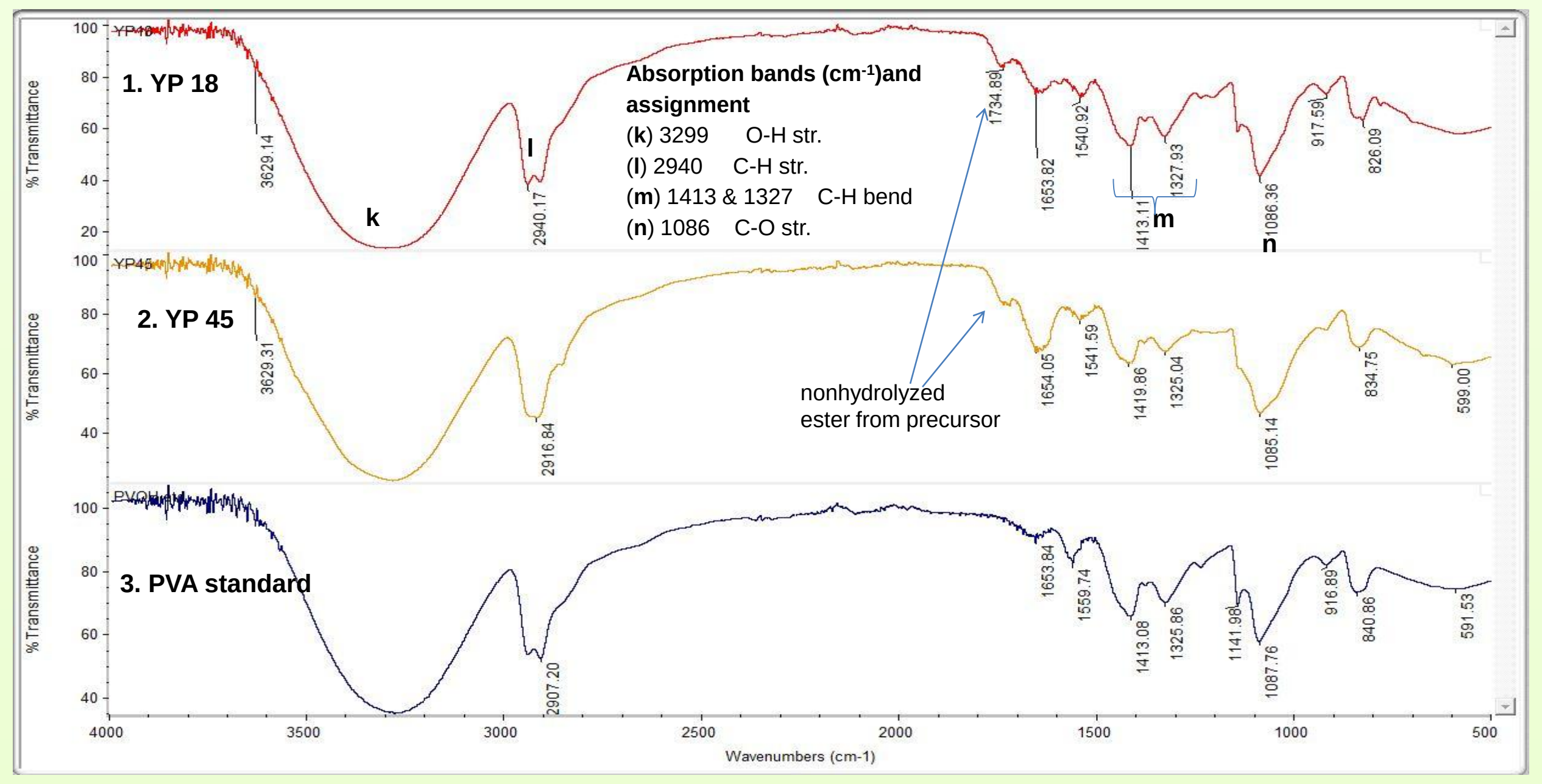


Figure 9: Raman spectra of PET standard. Letters represent the characteristic vibration used to identify the polymer.

Similarly, the structures of the other two macroplastics were also identified by FTIR-ATR. Both spectra shows similar absorption bands(Figure 10) and the structure of the polymer is identified as polyvinyl alcohol (PVA)(Figure 11). Though the structure of PVA lacks a carbonyl group, both spectra shows a very weak band around 1734 cm^{-1} and it indicate the presence of nonhydrolyzed acetate groups remaining in PVA from its precursor polyvinyl acetate (Qui *et al.* 2013).



Absorption bands (cm^{-1})and assignment
(**k**) 3299 O-H str.
(**l**) 2940 C-H str.
(**m**) 1413 & 1327 C-H bend
(**n**) 1086 C-O str.

Figure 10: FTIR-ATR spectrum of 1) YP18 (fluorescent fish bait), 2) foamy fragment,3) PVA standard. Letters represent the characteristic vibration used to identify the polymer

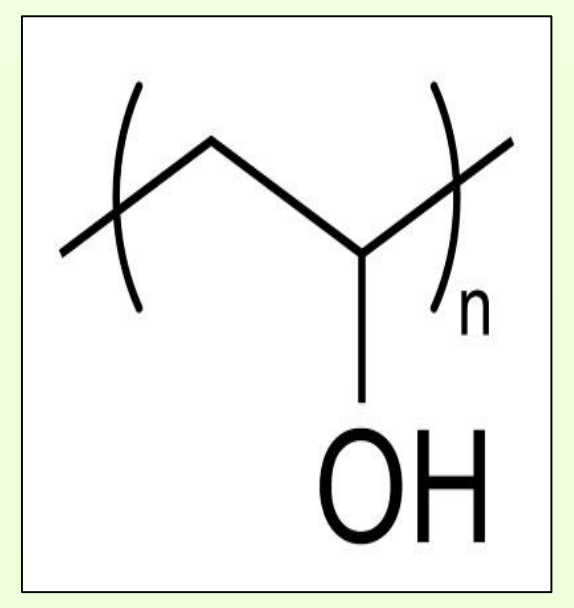


Figure 11: Structure of Poly(vinyl chloride) (PVA)

Chemical methods are necessary to identify the polymer of the ingested plastic as most of the time the recovered plastic lacks the standardized resin codes. We show that the polymer of the ingested plastic tape is PET and the polymer of fish bait and other microplastic fragment is PVA

<u>Microplastics :</u> Our preliminary analysis identified over 60 particles (Figure 12) and the classification was based on color, shape, and morphological properties. Based on the shape, we grouped them into several types and the percentage of each type is shown in pie chart (Figure 13). Our results showed approximately 60 % as fibers, which is comparable to what others have been observed. Further analyses are required for the unambiguous identification of collected particles as microplastics.

Figure 12: Photographs of possible microplastics collected from the digestive tracts of wild-caught sea bass A) green particle, 63 um filter, 3x magnification. B) yellow strand, 1 mm filter, 1x magnification. C) light blue fiber, 5 um filter, 4x magnification. D)transparent film, 1 mm filter, 1x magnification, E) fragment,

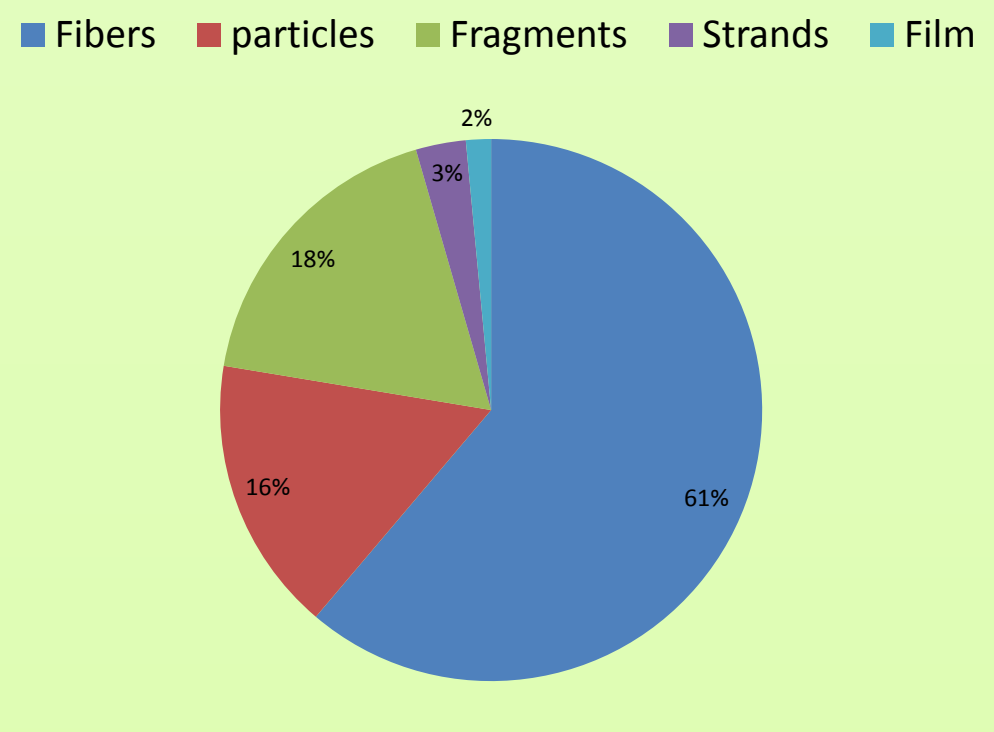


Figure 13: Pie chart showing the percentage of each type of anthropogenic particles.

Conclusions and future directions

In this work, we investigated plastic ingestion in wild-caught black sea bass and our results showed three macroplastics and more than 60 possible microplastics. Chemical characterization of ingested macroplastics revealed the polymer composition as PET and PVA. Preliminary experiments on pollutants identified a few phthalates and sulfonyl compounds as adsorbed pollutants on ingested plastics. Leaching of chemicals into fish tissue will be investigated using GC/MS -MRM method.

References

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