Use of Imaging Flow Cytometry (FlowCam) in the study of microplastics

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Microplastic Issues:

1. Detecting, Discriminating and Characterizing MPs
2. Effect of MPs in Marine Systems
Collaborators

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Dr. Patricia Matrai
Senior Research Scientist

Dr. David Fields
Senior Research Scientist

Microplastic fiber uptake, ingestion, and egestion rates in the blue mussel (Mytilus edulis)

Tuesday 8:45 St. Tropaz

Bigelow Laboratory for Ocean Sciences

East Boothbay, Maine

Dr. Patricia Matrai
Senior Research Scientist

Dr. David Fields
Senior Research Scientist

Identifying MPs in North Sea waters – a matter of extraction and detection

Tuesday 17:45-19:30 Poster #111

Biological Institute Helgoland, Germany

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Scientist
What is Flow Cytometry?

Flow Cytometry is a laser based biophysical technology employed in cell or particle counting, providing for the rapid multiparametric characterization of cells or particles.
What is **Imaging Flow Cytometry**?

**Imaging Flow Cytometry** is flow cytometry ... with imaging
FlowCam Image Acquisition

- Flash Illumination
- Flow Cell
- Current Image Frame
- Previous Image Frame
- Optics
- Camera
- Fluid Flow
“Glitter”
Polar Fleece Fragments
Microplastics – Tijuana River National Estuarine Research Reserve

Sample collected by Harry Allen, EPA Region 9
Microplastics — Tijuana River National Estuarine Research Reserve

Sample collected by Harry Allen, EPA Region 9

Note Diatoms
Challenge – Detecting and Discriminate MPs in Aquatic Systems
Identifying MPs in North Sea waters – a matter of extraction and detection

100µm net tow of ~ 34,000 Liters North Sea Water

Claudia Lorenz Poster 111 17:45 – 19:30
Identifying MPs in North Sea waters – a matter of extraction and detection

Diatom
Dinoflagellates
Zooplankton
Microplastic?
Enzymatic Oxidative Digestion  (Löder et al. 2017)

FlowCam used to evaluate efficiency of digestion process:

- Determine amount of sample that can be filtered w/out overloading it with particulate
- Calculate particulate surface area of 1mL sample
- FlowCam captures ‘Area’ of each particle – extrapolate to determine volume of sample that can be handled by a 10mm filter

98.6% reduction of organic material

sodium dodecyl sulfate (SDS)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>protease</td>
<td>32.3%</td>
</tr>
<tr>
<td>cellulase</td>
<td>13.3%</td>
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<tr>
<td>H₂O₂</td>
<td>10.0%</td>
</tr>
<tr>
<td>chitinase</td>
<td>13.3%</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>1.9%</td>
</tr>
<tr>
<td>FlowCam</td>
<td>1.4%</td>
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</tbody>
</table>
Enzymatic Oxidative Digestion process yields non-organics …… Microplastics……

μFTIR Spectroscopy

…… Infared Spectroscopy allows for data on polymer composition, abundance, and size distribution.
Microplastic fiber uptake, ingestion, and egestion rates in the blue mussel (Mytilus edulis)

- 3 Month Feeding study
- Mussels fed a diet of *Rhodomonas saliva*.....

- .....and Polyethlene Microplastic Fibers (Polar Fleece - LL Bean)

Madelyn Woods  MERI
Microplastic fiber uptake, ingestion, and egestion rates in the blue mussel (Mytilus edulis)

FlowCam Images of MP Fibers

Sample Vials

Pink Fiber

Experimental Set Up

Rhodomonas salina

1 Liter
**Method** -

- MPF feeding concentrations from 3 to 30 particles/mL
- Uptake of MPFs and *R. salina* measured by enumerating particle counts over the 72 hour experimental period using FlowCam
- Subsamples counted at intervals of 1, 3, 6, 9, 24 and 72 hours

**Results** -

- *R. salina* uptake rates greatly reduced in mussels exposed to 15 MPF/mL and higher.
- Pseudofeces production showed positive correlation with MPF uptake rates at 30 MPF/mL
  - Up to 70 MPF were counted in a single fecal pellet
  - 300-1000 MPFs accumulated in digestive tract
Take Aways-

- Possible that mussels act as a ‘microplastic sink’ in the Gulf of Maine
- Implications for both wild and cultured mollusc harvesting

If we are we what we eat.......?