Sample Collection and Analytical Considerations for Microplastics Studies

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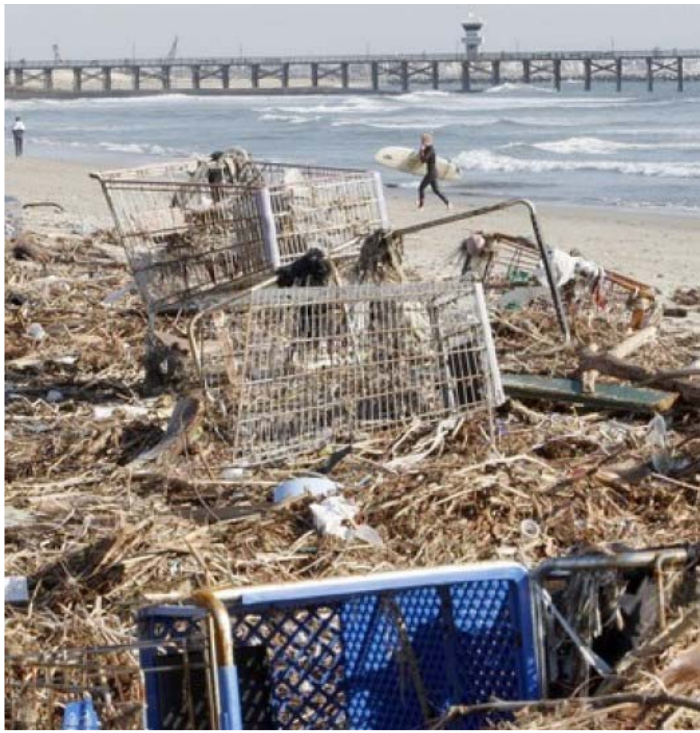


Stormwater discharge, Los Angeles, CA

US EPA Region 9 - TMDL

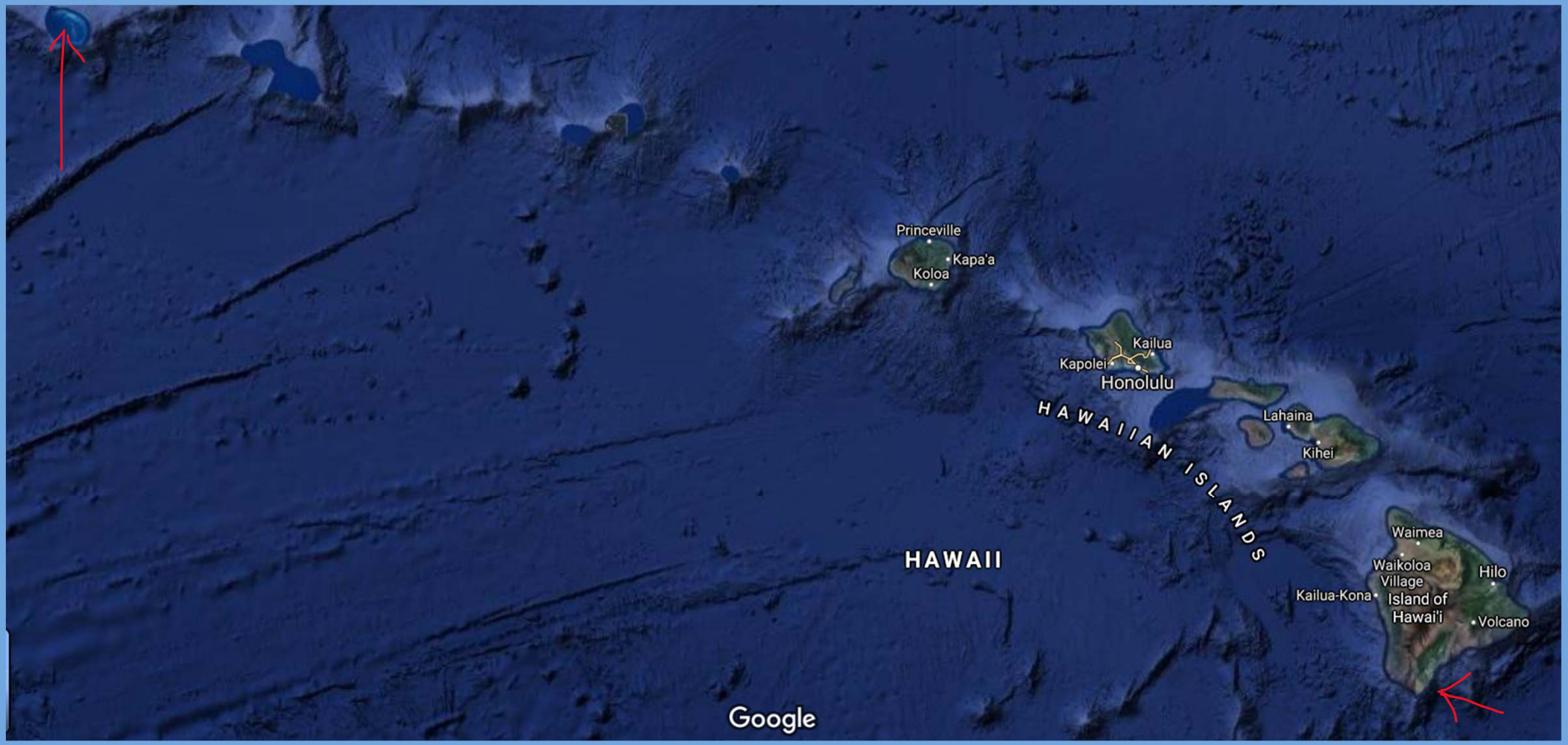
EPA identified two waterbodies that were not included in Hawaii's 303(d) List.

EPA has identified the waterbodies of Kamilo Point Beach on Hawaii and Tern Island in French Frigate Shoals NWHI as impaired by trash and requiring Total Maximum Daily Loads (TMDLs) under Clean Water Act, Section 303(d).

The Islands Of Hawaii Hold One Of The Dirtiest Places In The World

Kamilo Point shows just how dire the world's plastic pollution problem really is.

https://www.huffpost.com/entry/kamilo-beach-hawaii-dirtiest-beach-america_n_58e99a38e4b05413bfe3792d


Princeville
Kapa'a
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HAWAIIAN ISLANDS
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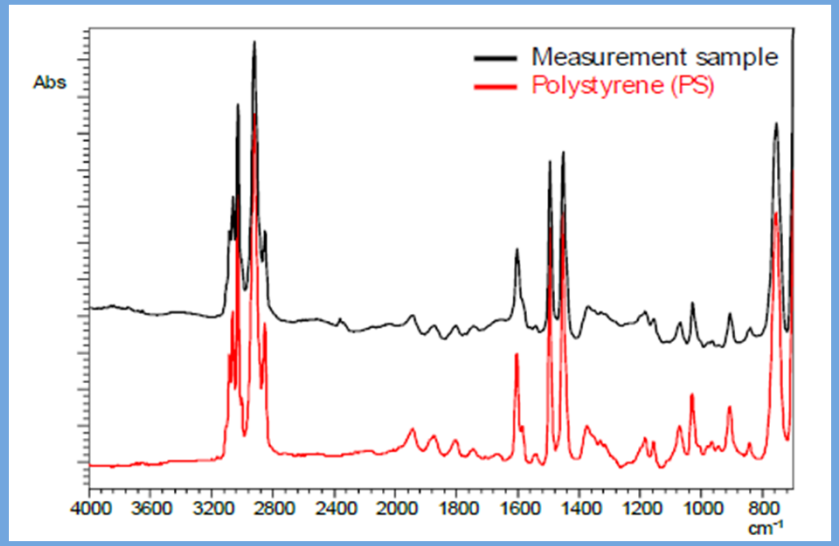
Waimea
Waikoloa Village
Hilo
Kailua-Kona
Island of Hawai'i
Volcano
Google

Tern Island - Papahanaomokuakea, Marine National Monument

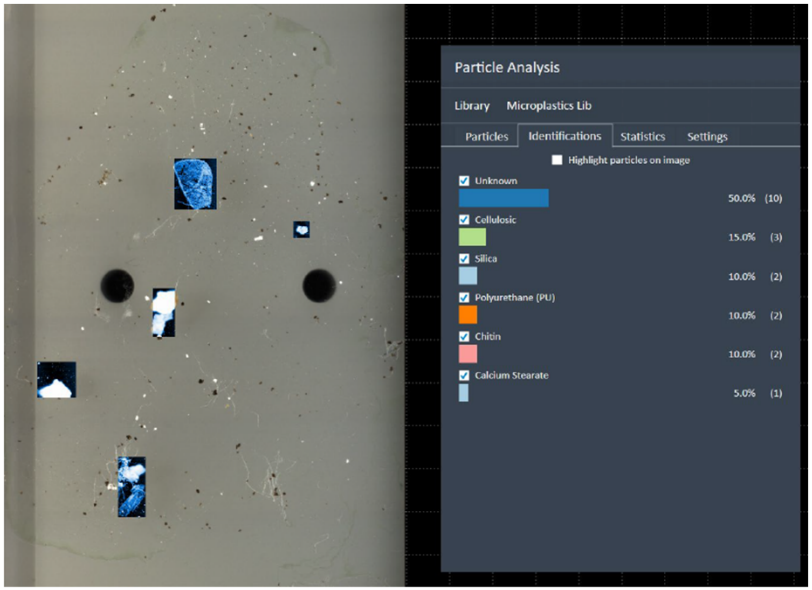
Sampling/Extraction/Identification & Analytical methods remain variable

- Sampling approach – Neuston nets 333um allowed particles to be missed, also shed particles into the samples
- Extraction challenges – Peroxide (WPO), KOH, enzymatic digestion is time-consuming
- Polymer identification
  - Microspectroscopy (Raman/FTIR)
  - Py-GCMS – emerging technique
- Quality Control and Standardized Methods are important



EPA Region 9 & ASTM International D19

- Began a microplastics method development in "all waters" and funded significant research (June 2016).
- Multiple partner organization to develop standardized methods and practices for identifying microplastics in water.
  - Published methods for collection and sample preparation
  - Pending: reference sample preparation, analysis with Pyrolysis-GCMS and FTIR microspectroscopy.
  - Partners include: CA Department of Public Health, Pima County Wastewater, Agilent Technologies, Shimadzu Corp., East Bay MUD, LA County Sanitation and Frontier Analytical (Japan)

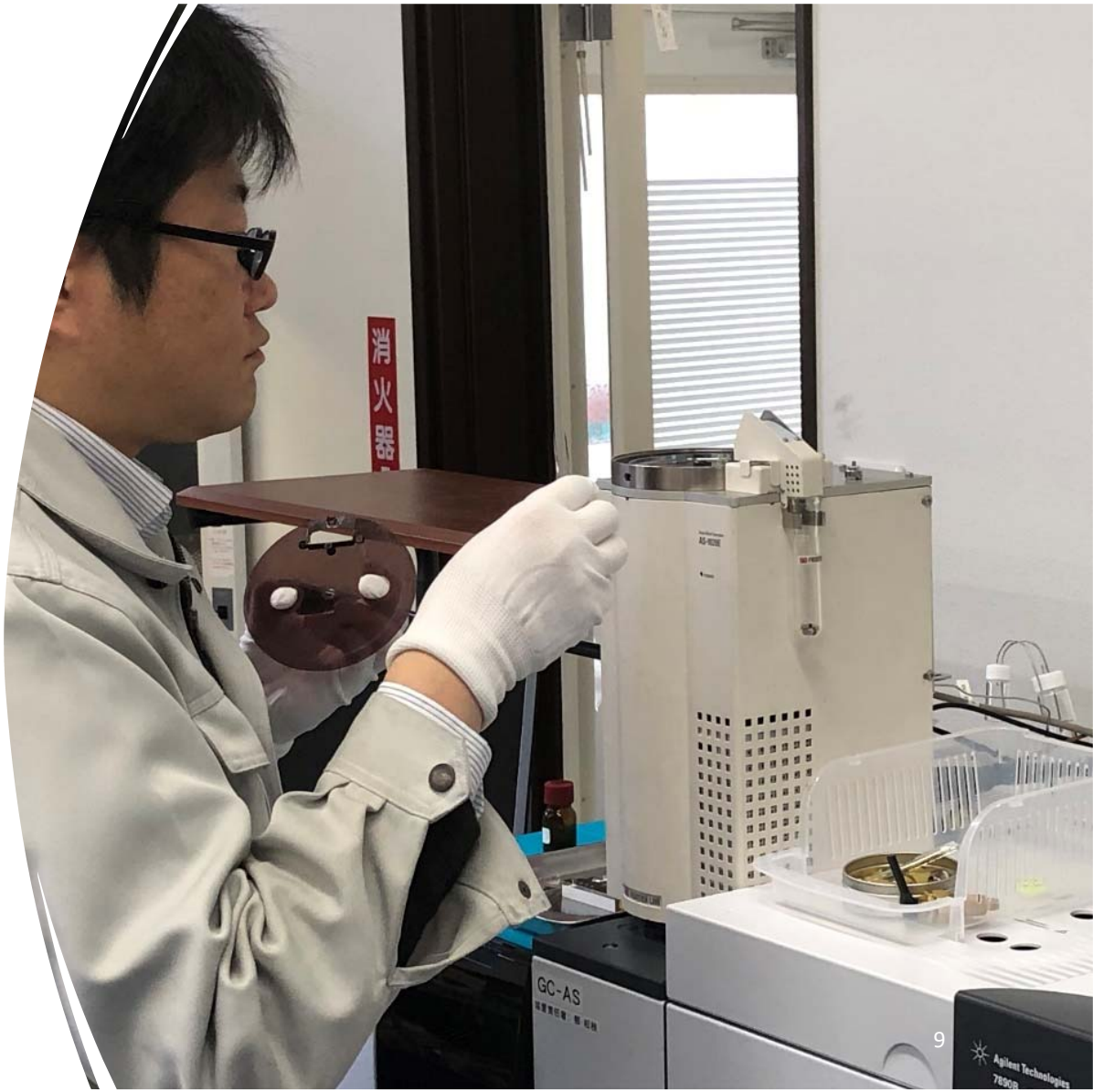


Agilent LDIR

- The Laser Direct Infrared Imaging detector provides compositional analysis of substances using spectral matching
- This helps us get positive plastic counts by polymer type with lots of supporting information.
- Rapid automation – simultaneous analysis

Mass-based Methods – Pyrolysis/GCMS (Frontier Analytical)

- Flash pyrolysis (between 500-600°C) of a sample yields pyrolyzates which evolve into the GC column.
- A mass spectrometer detects the pyrolyzates and produces a pyrogram
- Plastic polymer contents are identified by peak height and retention time and their mass is estimated.



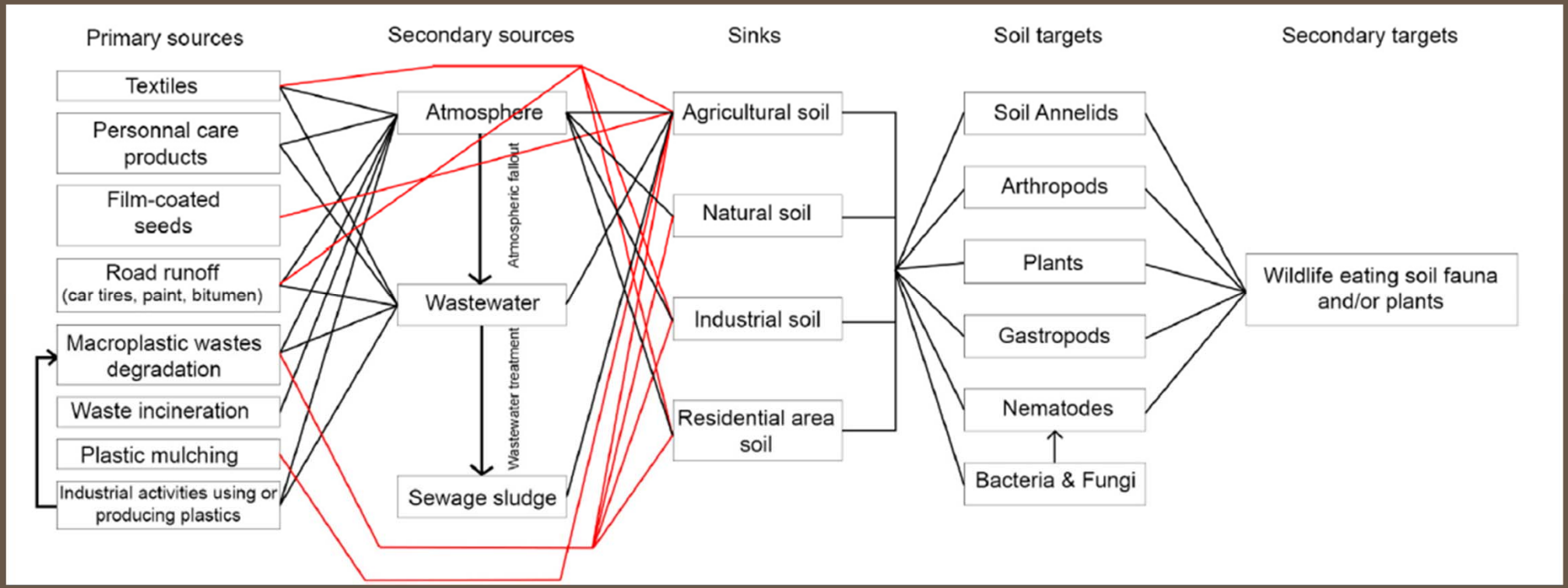
Sample Planning – Sampling Study Design

- Commonly, studies are focused on QC and lab methods and less so on environmental variability
- Environmental variability is often considered far more significant than analytical variability. Sampling design is essential for managing environmental variability.
- Typical sampling studies use inferential statistics – commonly t-tests and ANOVA.
- Sometimes data are transformed to achieve fit.

Data Quality Objectives for Sampling

1. Every effort requires: a study goal or problem statement
   - Decision-based or estimation-based goal?
2. A suitable conceptual model
3. A meaningful metric and suitable analytical method
   - Is this a count-based study or mass-based study? What is the Detection Limit?
4. A study boundary statement
5. An analytical approach
   - a decision rule or estimation approach
6. A desired level of confidence in the results
   - often including an analysis of decision error
7. A sampling plan that demonstrates how each supports the conclusion

Guidance on Systematic Planning using the Data Quality Objectives Process; EPA/QA G-4 (2006)
https://www.epa.gov/sites/production/files/2015-06/documents/g4-final.pdf
Also, see Interagency Field Manual for the Collection of Water-Quality Data; USGS Open File Report 00-213 (2000)



Example Conceptual Model

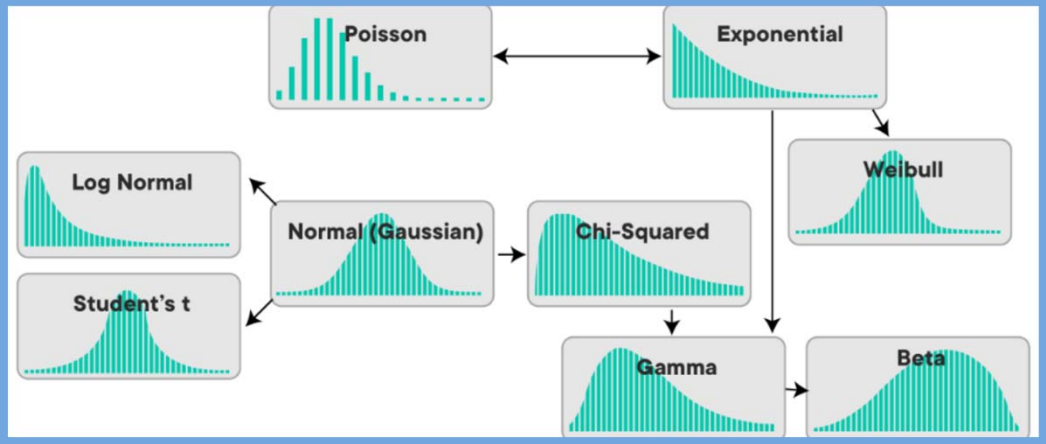
- Land-based environmental exposure estimation conceptual model from (Jaques & Prosser, STOTEN 2021)

Dennis Helsel's "Zombies" of Data Analysis

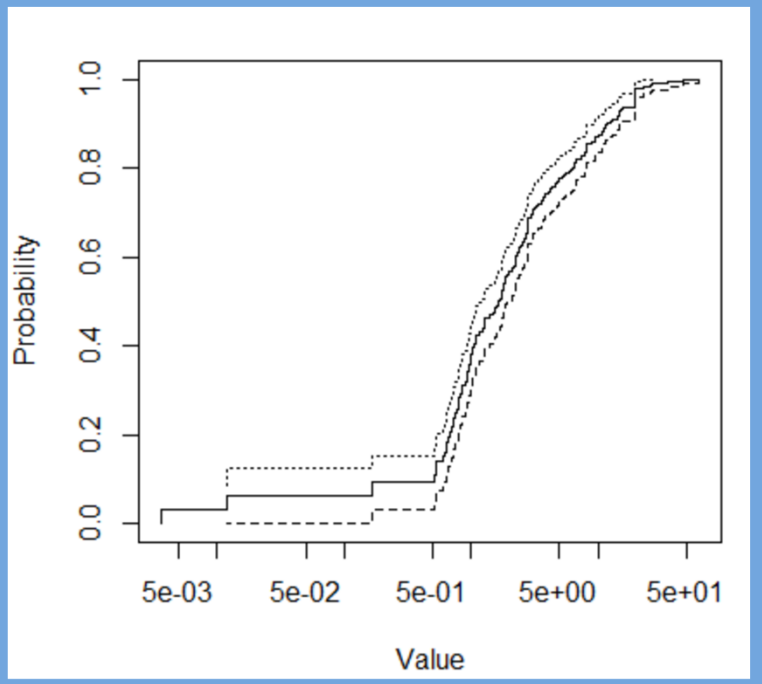
- Using t-tests and ANOVA – using traditional methods is not advised, likely false negatives (missing differences when they are really there)
- Sample sizes are too small – 30 samples is still not enough for traditional methods
- Rejecting outliers without reason – these maximum or minimum results may represent a condition worth exploring
- p-hacking – over reliance on the p-value as a threshold, combined with other issues you may miss the signal
- Using excel for statistical analysis

Graphing the data is critical to understanding it. Recommend inter-quantile plots and distribution analysis.

Models for Continuous Environmental Data



Parametric



Non-parametric (with non-detects)

A Word about Analytical Approach

- **Is it a decision-based study or estimation-based study?**
- **Most studies are small requiring estimation-based approaches in lieu of hypothesis testing.**
  - **Caution against using hypothesis testing for small samples with high variability**
- **Do not substitute or omit non-detects, include outliers.**
- **Emphasize distribution analysis, consider non-parametric methods.**
- **For estimation-based (n<30): Use the Upper Confidence Limit or Prediction Limit.**
- **For decision-based (n>30): Consider Inferential Statistical Methods but ensure assumptions are met.**

Statistical Methods in Water Resources; USGS Techniques and Methods 4-A3 (2020)

Summary – Wastewater Data "count"

- Samples were collected from both Tertiary & Secondary systems.
- Samples were repeated at a different time of year (Condition A vs. B).
- Raw results follow a lognormal distribution but with low confidence and big gaps in the frequency histogram.
- Use flow-adjusted concentrations from data set to smooth the data – these also have more meaning for the study design (MPv or MP/m^3).

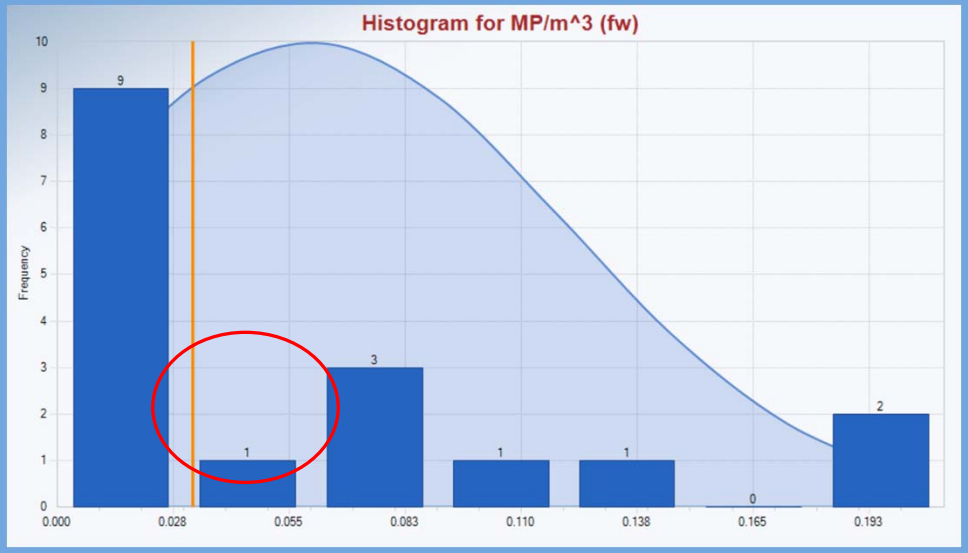
Data courtesy of San Francisco Estuary Institute, D. Lin and R. Sutton

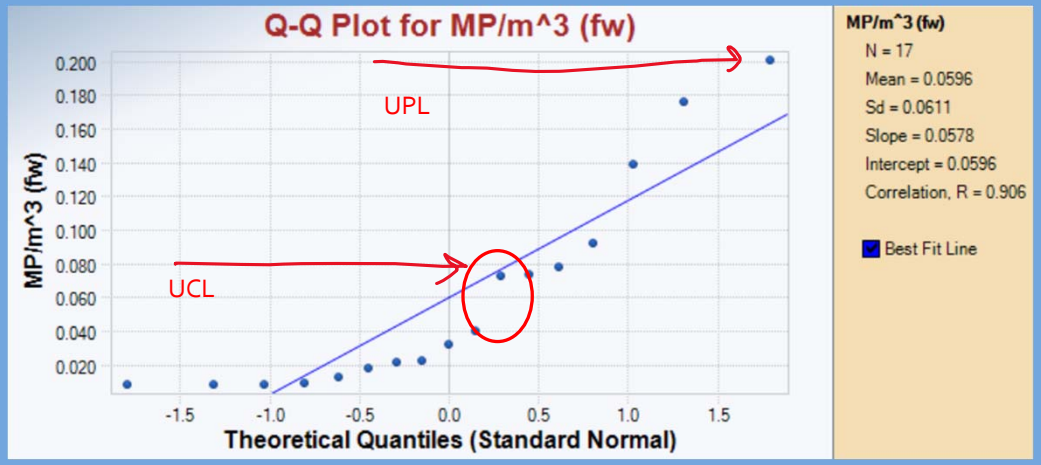
Sutton et al. 2019. Understanding Microplastic Levels, Pathways, and Transport in the San Francisco Bay Region. SFEI-ASC Publication 950

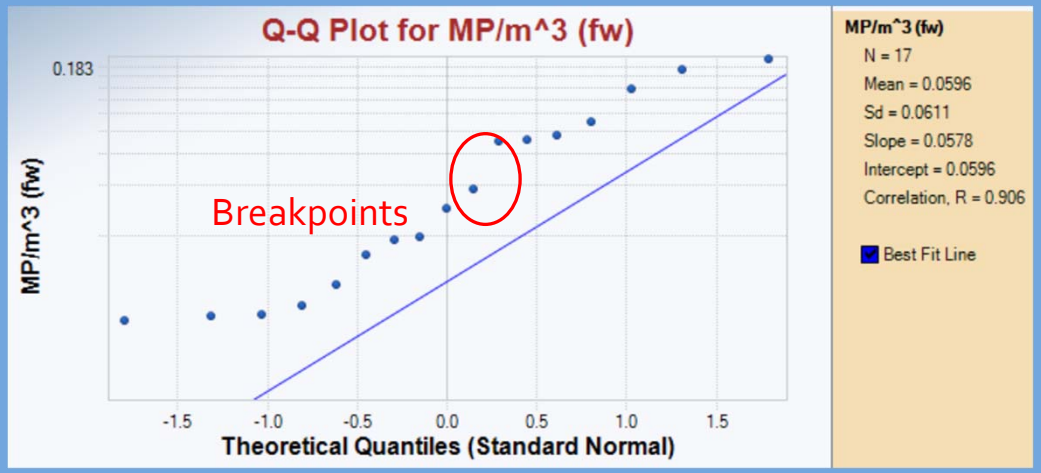
Wastewater data - pooled

- Combined (pooled) SFEI data is best represented by a lognormal or gamma distribution.
  - Recommended Bootstrapped 95% UCL (predicted median) is 0.143 (lognormal) or 0.101 (Gamma – preferred).
  - The Bootstrapped 95% UPL (estimated 95% quantile) or 0.196 (Gamma).
  - Inter-quartile (Q-Q) plot though indicates a significant "break point" which suggests it is worth reconsidering the concept that there are no significant differences among factors in the sample.

Distribution analysis of pooled data (n=17)





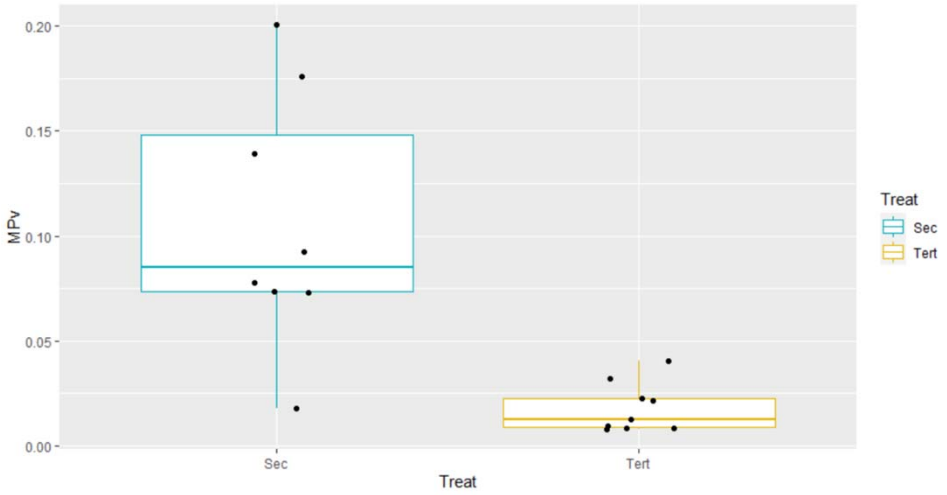


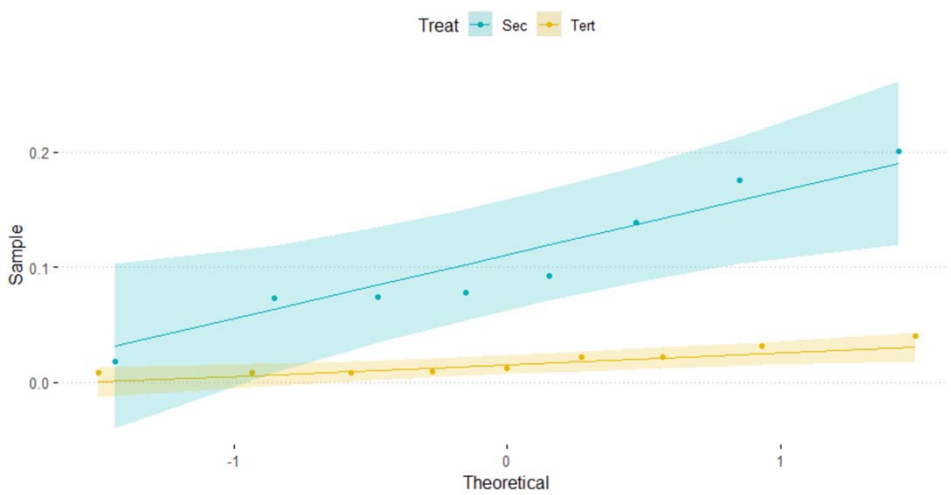
Break points (circled) in the distribution – use caution as factors may play a role in observed differences

Split out Tertiary vs. Secondary

- Side-by-side (between factor) Q-Q plot suggests NOT combining secondary and tertiary data as there appears to be an observable difference
- We calculated the 95% (Bootstrap) UPLs for Tertiary and Secondary
  - Assume both are lognormal (tertiary also fits normal distribution but not recommended – predicts some results <0)
- Secondary: 0.399
- Tertiary: 0.0515 – lower than "sec"
  - significant chance that single results from secondary will be between 0.0515 and 0.399

Secondary vs. Tertiary Boxplots, Q-Q Plots

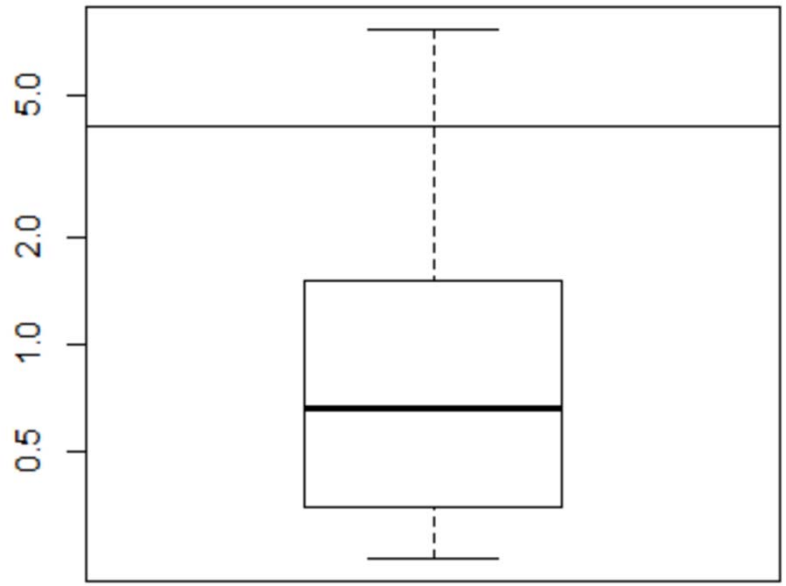


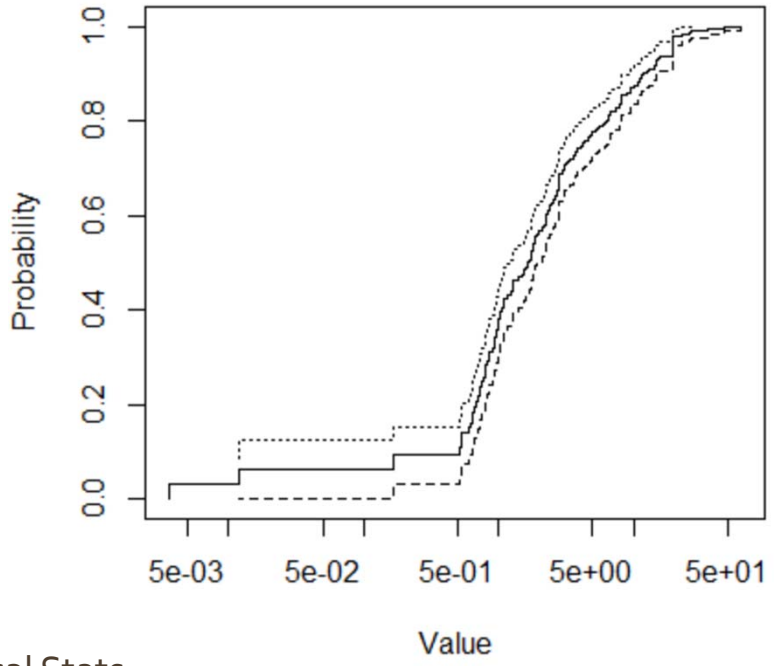


Using UCLs/UPLs for Future Work

- Will any samples from the next study have a flow-weighted concentration (MP/m^3) > 0.196?
  - For overall – poor predictor.
- Will any samples from the study have a flow-weighted concentration exceeding 0.399?
  - For Secondary – better predictor.
- Will any future samples have a flow-weighted concentration exceeding 0.0515?
  - For Tertiary – best predictor.

BPA in Fish Tissue – methods for censored data

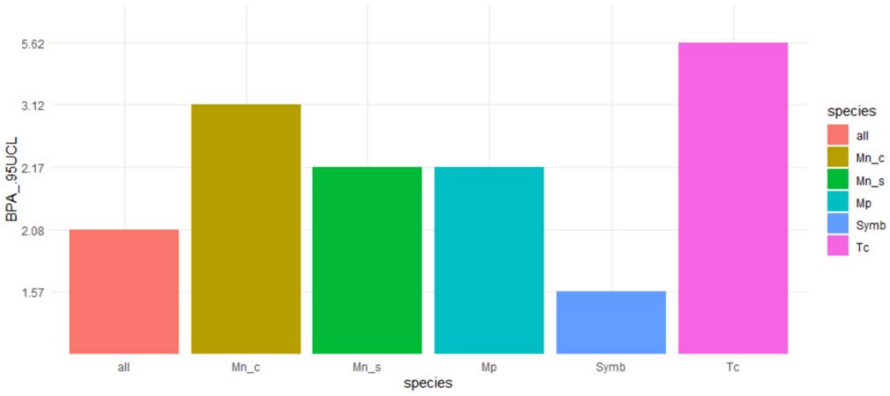




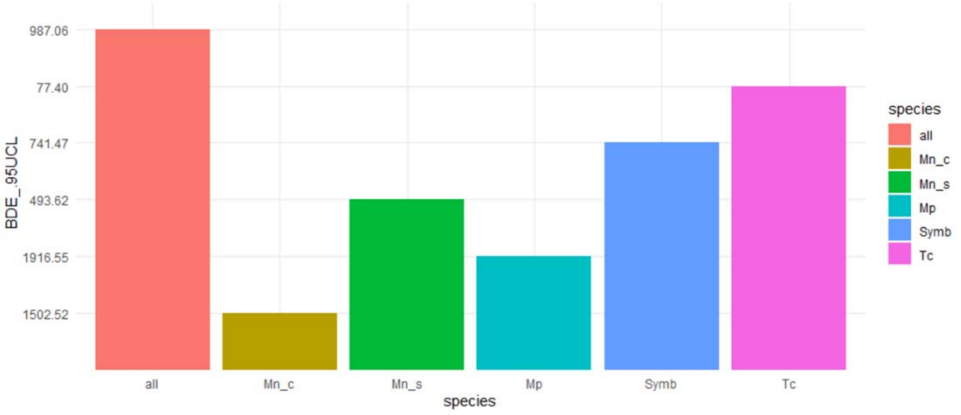
See R Scripts NADA and NADA2, Dennis R. Helsel, Practical Stats.
Data from Gassel & Rochman. 2019. Environmental Pollution. 248: 1000-1009

Mass Data with Non-Detects

- With small data sets there is also a high likelihood of Non-Detects. This is common with Mass Data.
- There are common methods of fitting distributions with NDs.
- All data is used to generate Bootstrapped UCLs.



BPA UCLs in Myctophid Tissue (by species)



BDE UCLs in Myctophid Tissue (by species)

Analytical support provided by C. McCoy, Weston Solutions.

Designing Microplastic Field Studies

- **Integrating plastic particle analysis with existing water monitoring**
  - Oceans and estuaries, rivers, lakes and source waters
  - Includes point-source and non-point source monitoring
  - Repeatable and of high quality
- **SFEI SF Bay 2018-19**
- **SCCWRP-UCR SoCal Bight 2020-21**

Future Applications of Microplastic Sampling and Monitoring

- Future applications may include:
  - environmental health/risk assessment
  - impact on sanitation
  - role in food safety
  - role in other adverse human health outcomes
  - managing plastic in a community – effectiveness of plastic waste management measures
  - managing waste – corporate liability and opportunity
  - corporate and/or industry image – public relations

Where & when does my decision apply?

Monitoring vs. One-time?

Does size/shape matter?

What's my expected variance – priors?

Count vs. Mass?

SoCal Bight 2013

- Trash Monitoring Sites in the SoCal Bight
- Include Riverine, Epibenthic and Benthic sampling sites
- Visual observations of macrodebris
- Estimates amounts by factor using Confidence Intervals
- Data is robust for trend detection (between monitoring periods)

Regional Monitoring Program: Trash and Marine Debris: SCCWRP Technical Report 928