Microplastics and Microbiomes: How Ingestion of Weathered Microplastics Impacts Freshwater Fathead Minnows

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(Micro)plastic materials

<5 mm plastic particles consisting of a wide array of polymers

Polyester	**Pajama pants, fleece jacket**
Polyethene (PE)	Plastic bags, six-pack rings, straws
Polypropylene (PP)	Bottle caps, netting, rope
Polystyrene (PS)	Food containers, utensils, foam cups
Poly(vinyl chloride) (PVC)	Plastic film, bottles, cups

MP examples from Andrady (2011)

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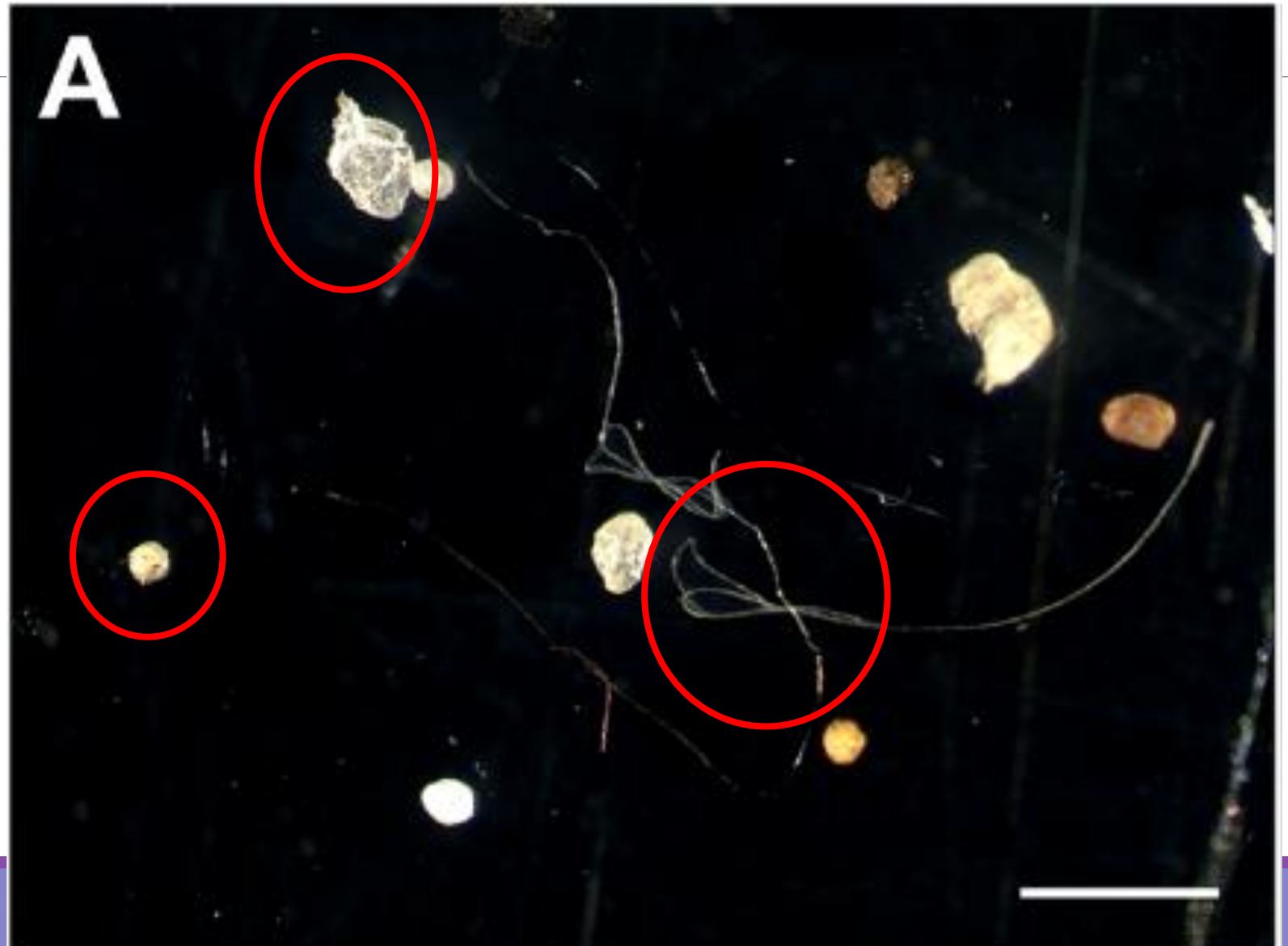
Poly(vinyl chloride) (PVC)	**Building materials**

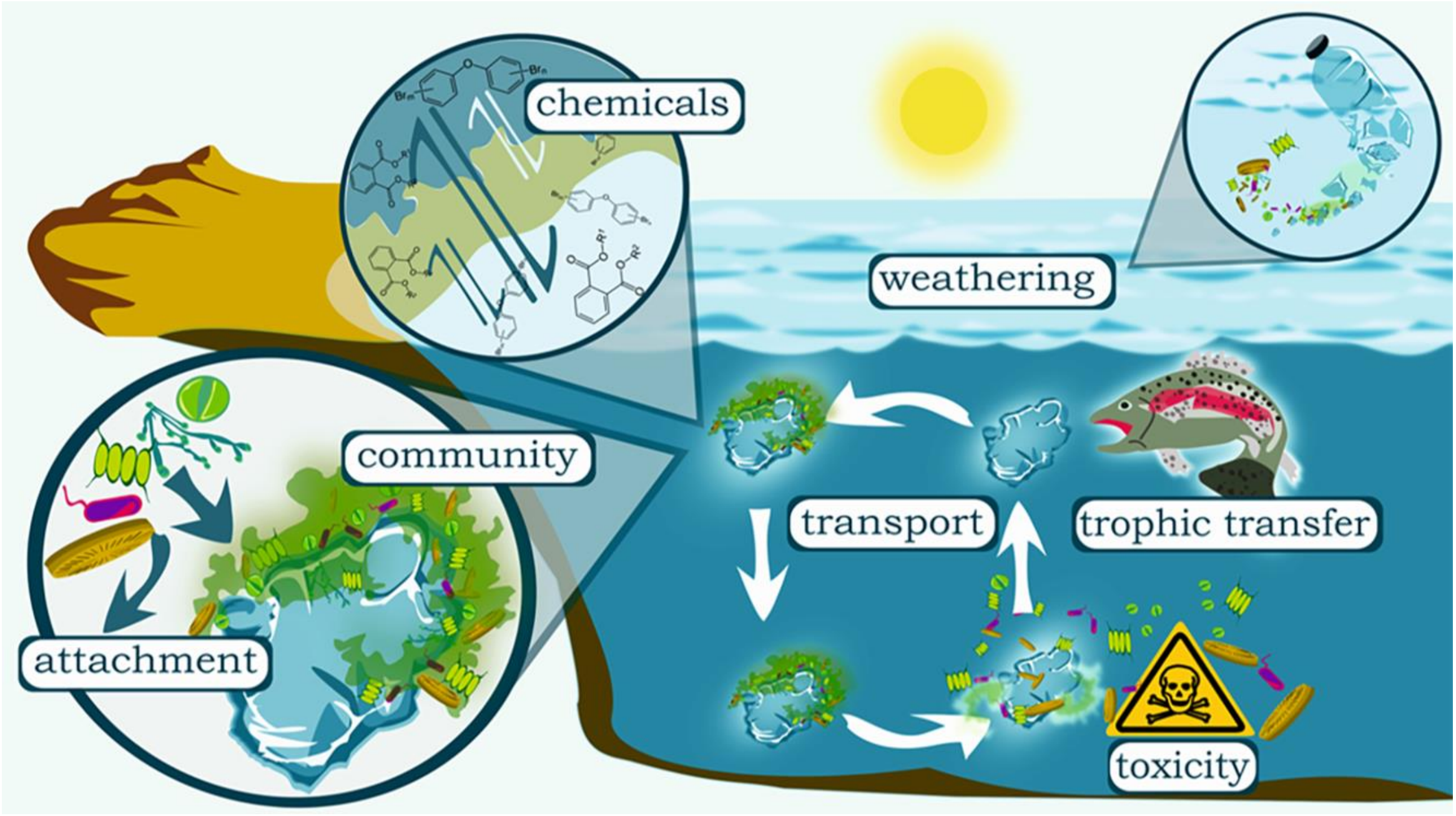
MP examples from Andrady (2011)

Microplastics

Both primary (beads, pellets) and secondary (fibers, fragments) sources



Wagner et al. (2014)



Rummel et al. (2017)

Microplastics in the Environment:

Global freshwater environments contain 10^{-2} to 10^{4} particles/m^3 (Adam et al., 2019)

- 10^{-5} to 10 particles/L
- Most sizes > 330 µm particles

North and South American freshwater environments contain 0.16 to 3,437.94 particles/m^3 (Sarijan et al., 2021)

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Microplastics in the Great Lakes Region

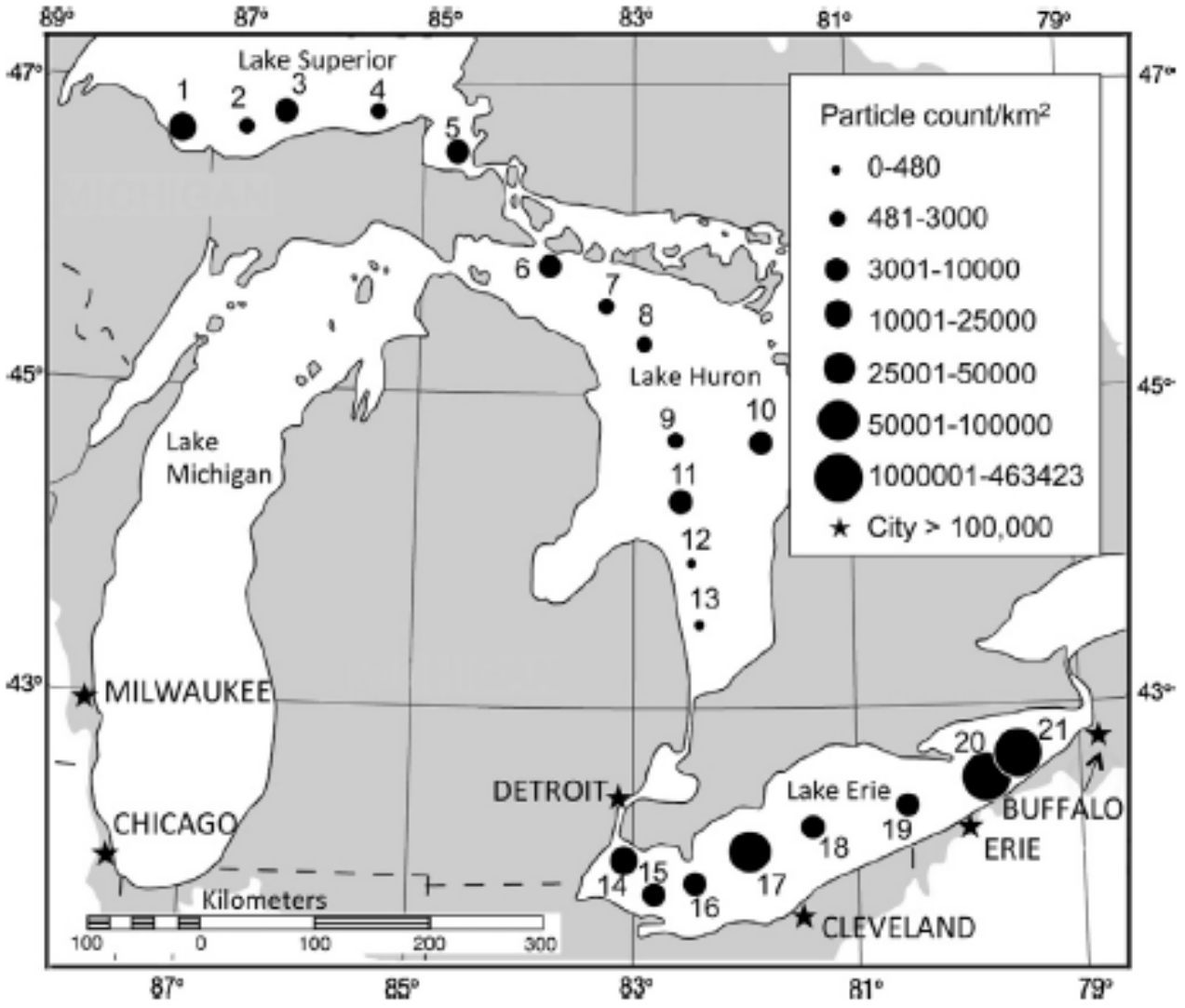
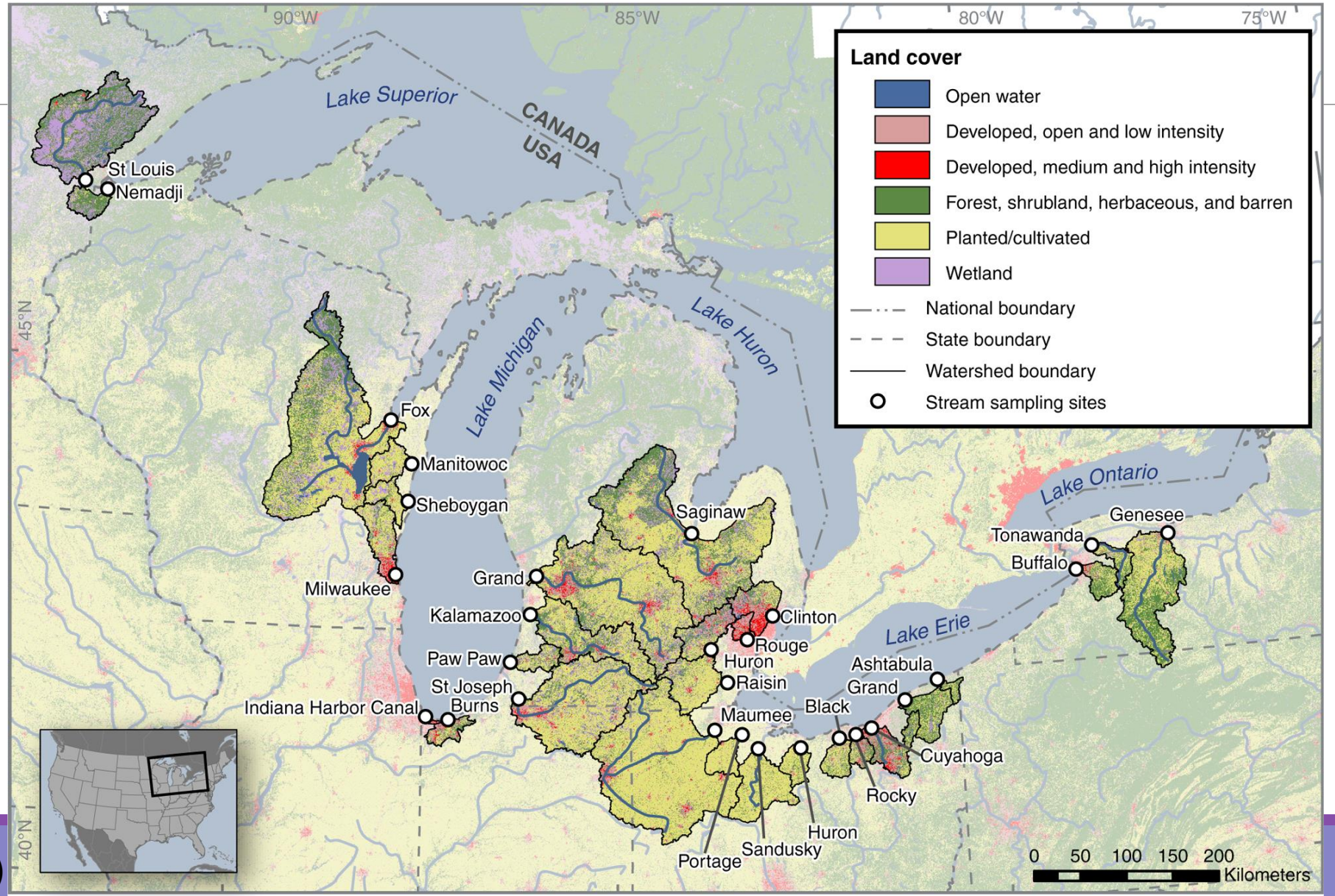


Fig. 1. Distribution of plastic particles by count for 21 samples collected in three of the Laurentian Great Lakes.

Eriksen et al. (2013)

Microplastics in the Great Lakes Region



Baldwin et al. (2016)

Microplastics in Biota: Great Lakes Fishes

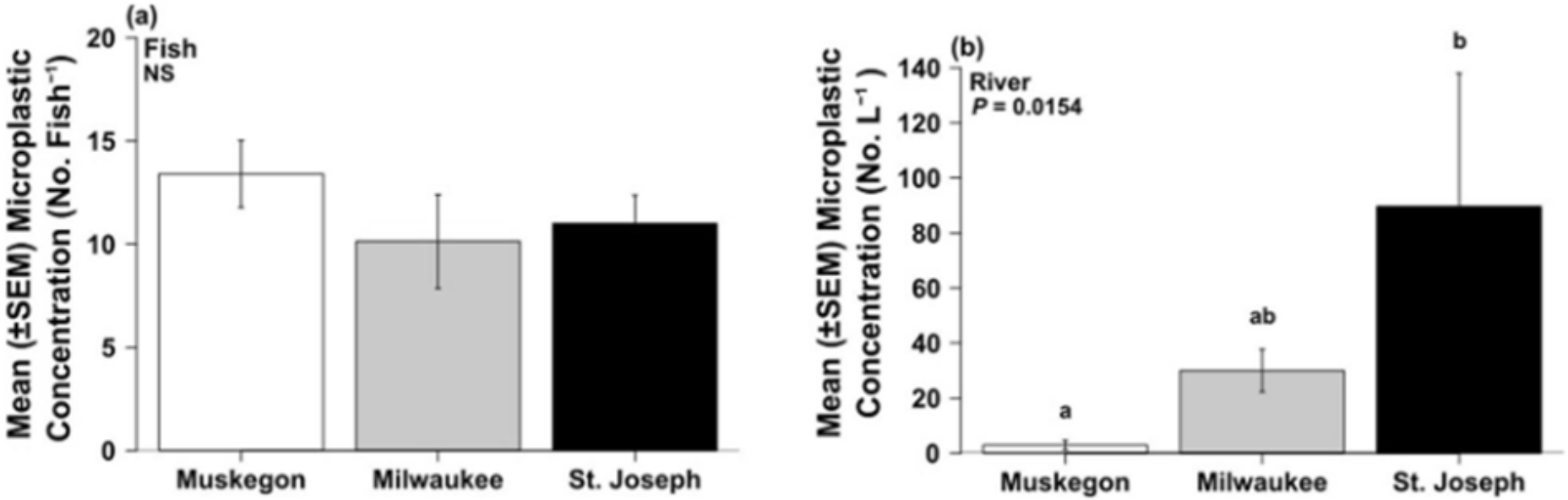
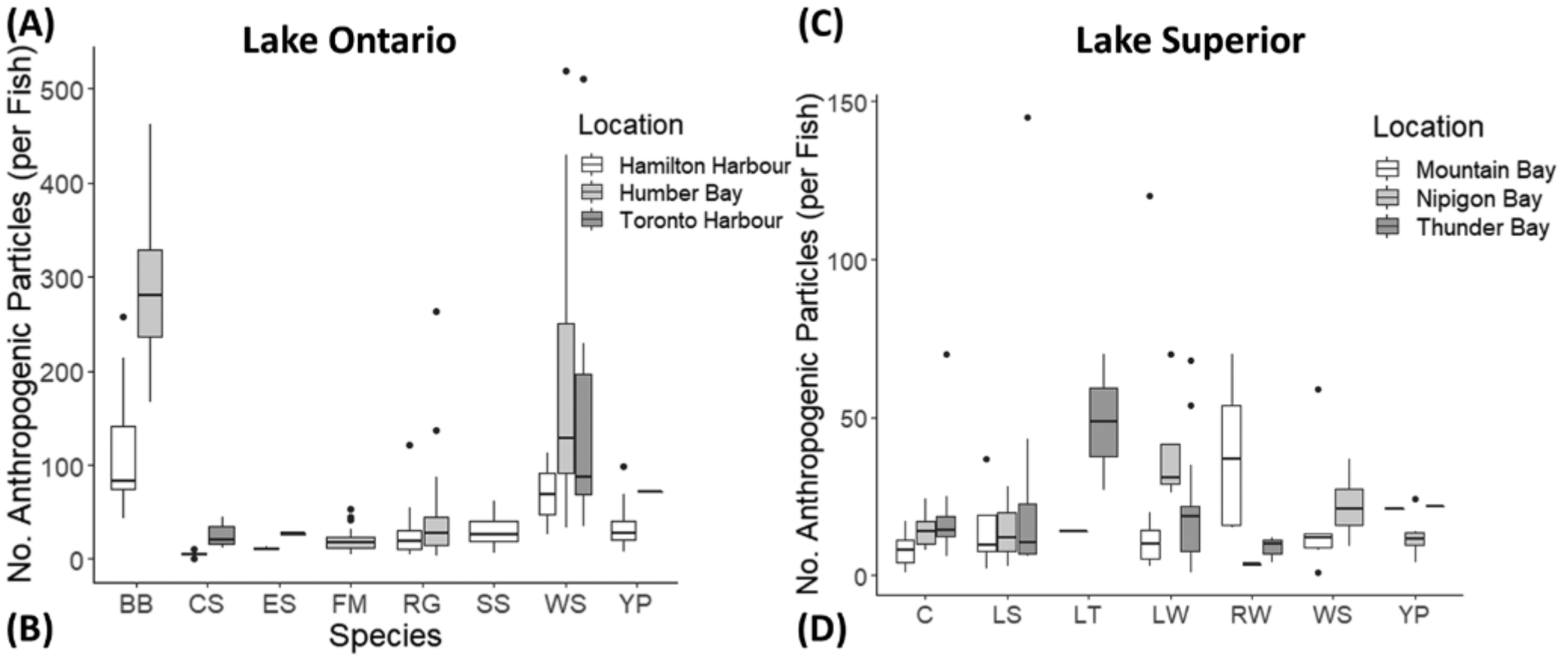


Figure 2. Mean microplastic concentration in fish (a) and mean microplastic surface water concentration (b) between Muskegon, Milwaukee, and St. Joseph Rivers during summer 2016. Letters indicate significant difference between sites at $P < 0.05$.

McNeish et al. (2018)

Microplastics in Biota: Great Lakes Fishes

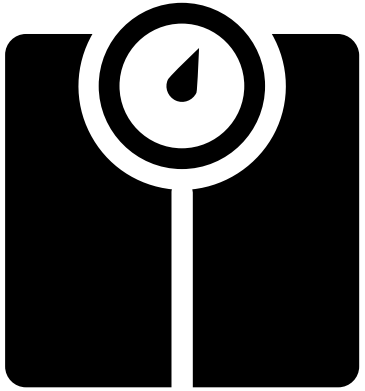


Munno et al. (2021)

Microplastic Effects:

Physical:

Cause blockages



Nutritional:

Replaces food

Toxicity:

Adsorbs contaminants

Microplastic Effects: Microbiome

An assemblage of microbes associated with a host organism

- Bacteria – our study focus
- Fungi
- Algae

May be found in or on organisms':

- Skin
- Mouth
- Gastrointestinal tract

Biotic and abiotic factors

Fish lifestyle

Fish genotype

Fish physiology

Fish immuno system

Transient community member

Fish environment

Fish gut microbiota

- Change in composition (phylogenic), Change in microbial metabolism, Change in genes expression level

Host

- Changes in phenotype

Growth, Health, Performance, Energy uptake, Feed efficiency, Feed conversion rate

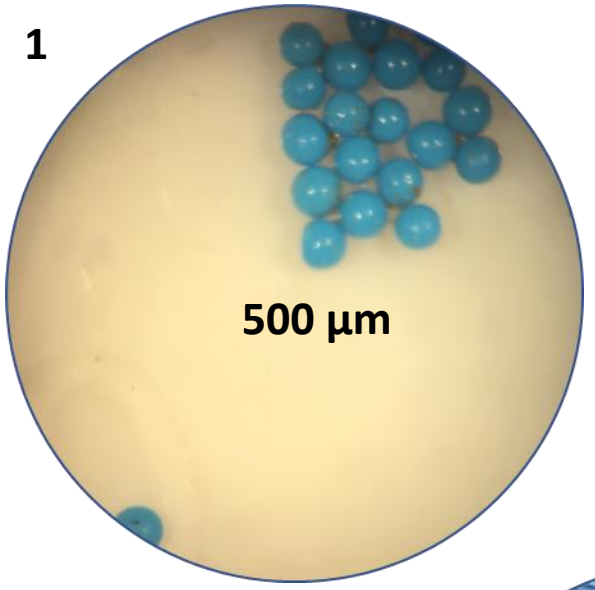
Ghanbari et al. (2015)

Study Design:
Objectives & Hypotheses

1. To measure the impact on sublethal endpoints including growth, condition factor, and hematocrit.
   a) All three endpoints will decrease in treatment groups compared to controls.
2. To investigate changes in gut microbial communities.
   a) There will be differences in diversity and community composition between treatment groups and the controls.
3. To assess expression of representative immune and stress related liver genes.
   a) There will be a change in expression in *cyp1a*, *gsta*, and *ncf2*

Study Design: Microplastic Incubation

1



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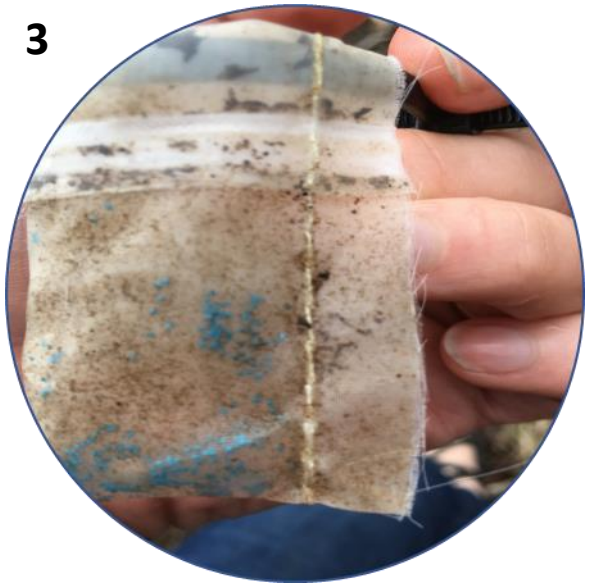

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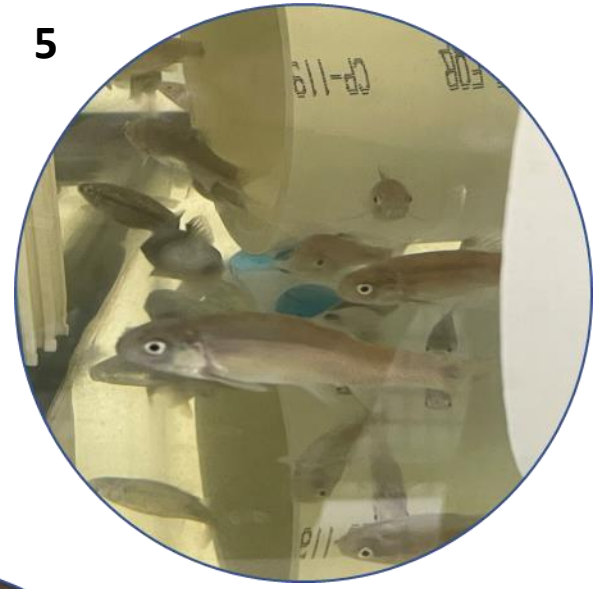

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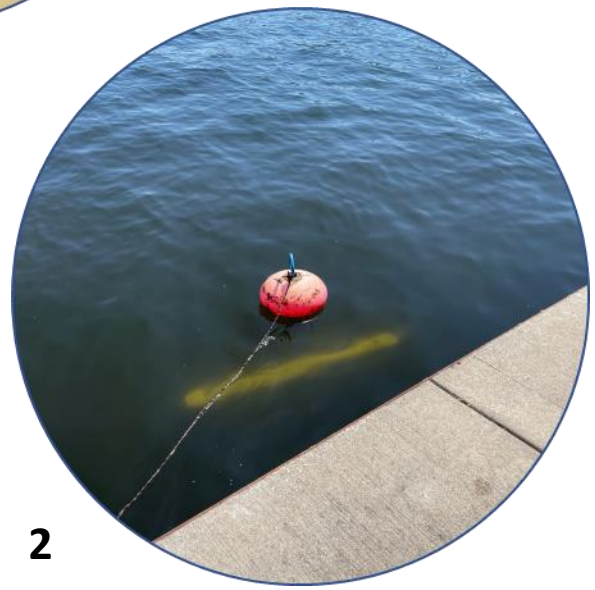

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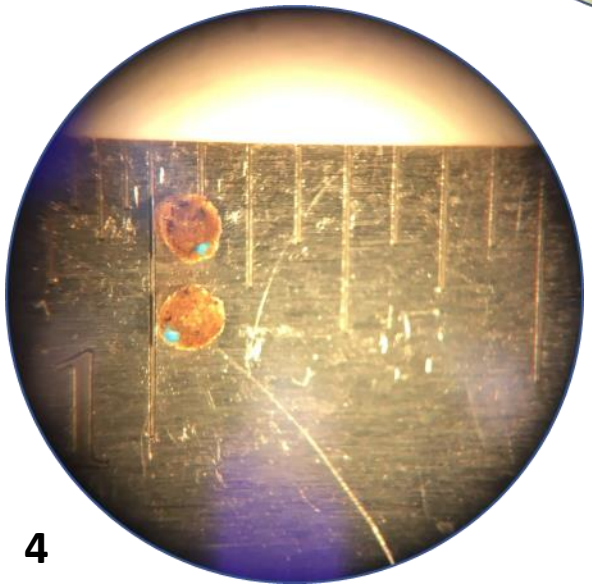
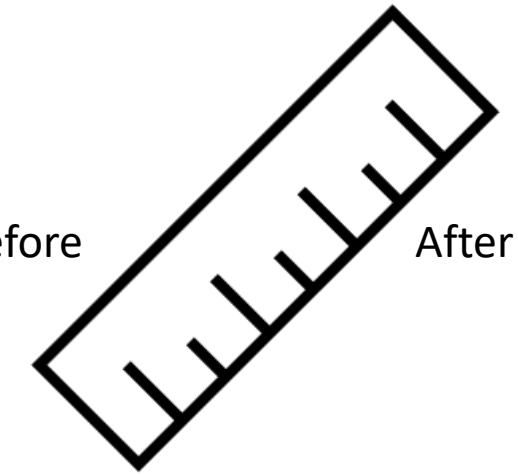


Study Design: Ingestion Test

- 28-day chronic exposure
- Adult fathead minnows
- Even numbers males and females
- 16 fish food pellets per fish per day
- Flow-through – Muskegon Lake water

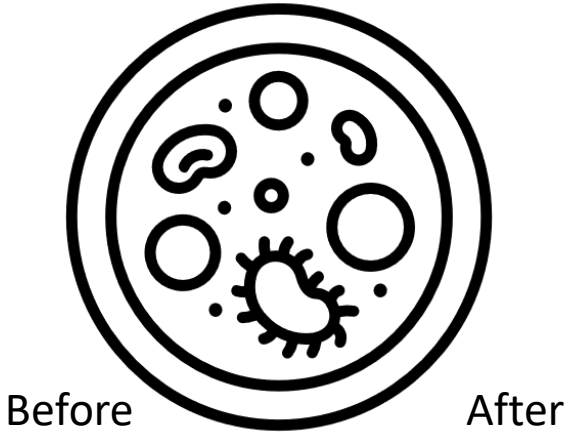
	Microplastic	**Non-Microplastic**
Control	0	16
Low	4	12
High	16	0

Methods:
Sample and Data Collection



Weigh and measure
Growth & condition factor

Blood sample
Hematocrit

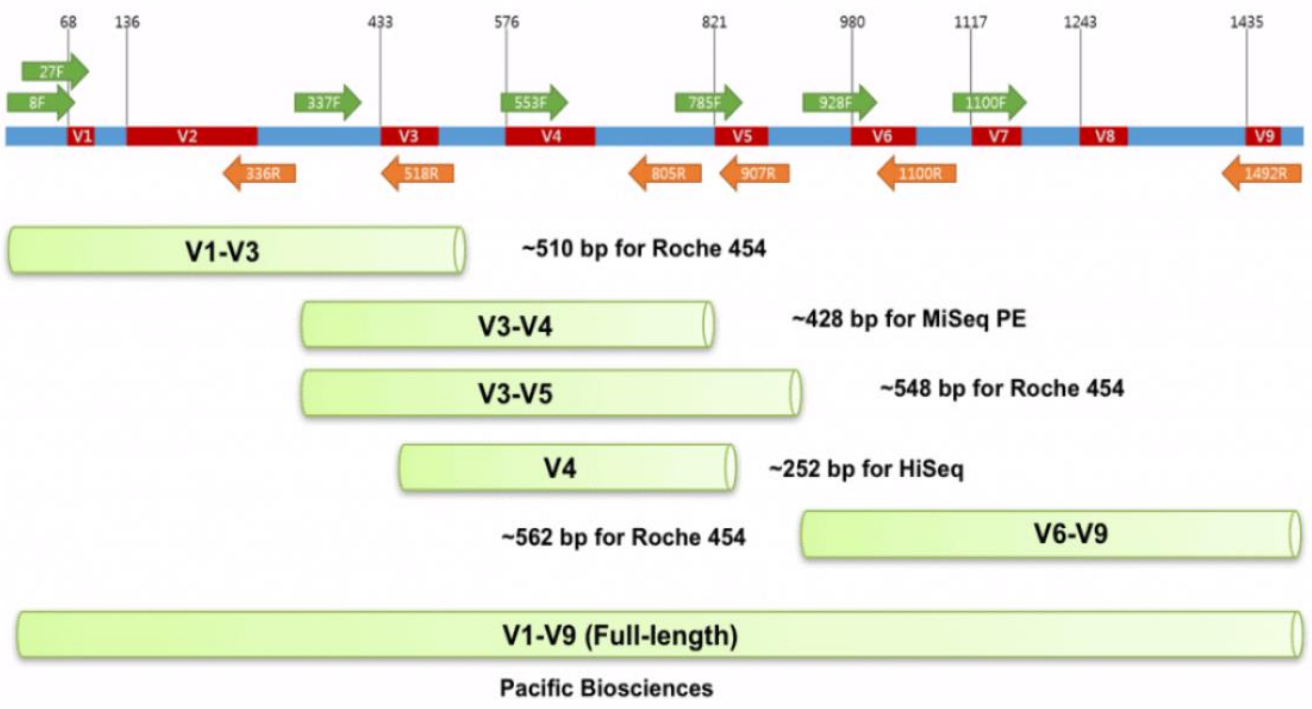


Fecal and gut samples
Microbial community
16S amplicon sequencing

Liver tissue
Gene expression
qPCR

Methods:
Gut Microbial Community

- 16S region of bacterial genome
- Amplified and sequenced the V4 region
- Used the dada2 pipeline for sequence processing
- Designated ASVs = amplicon sequence variants

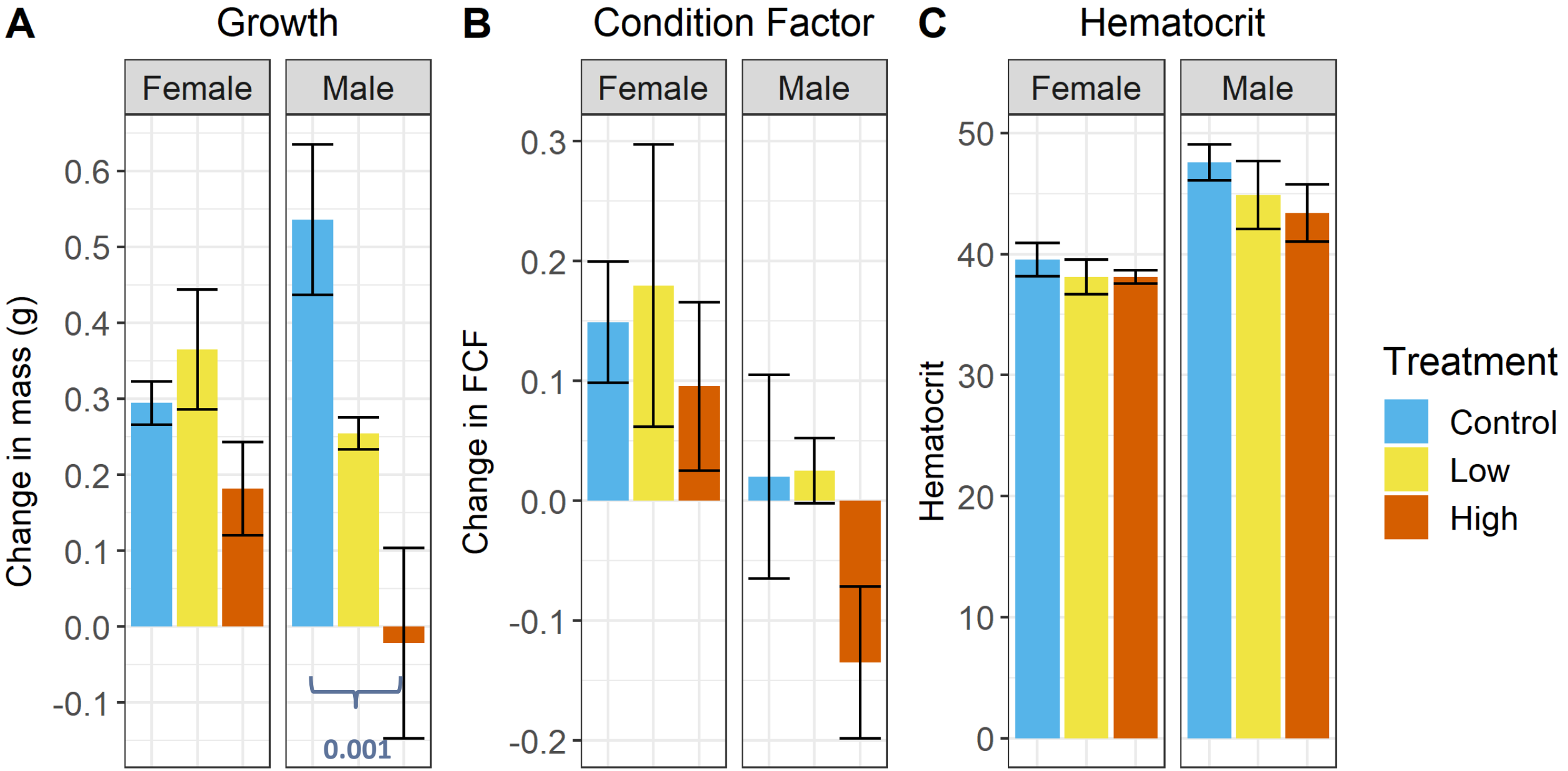


Source: https://help.ezbiocloud.net/16s-rrna-and-16s-rrna-gene/

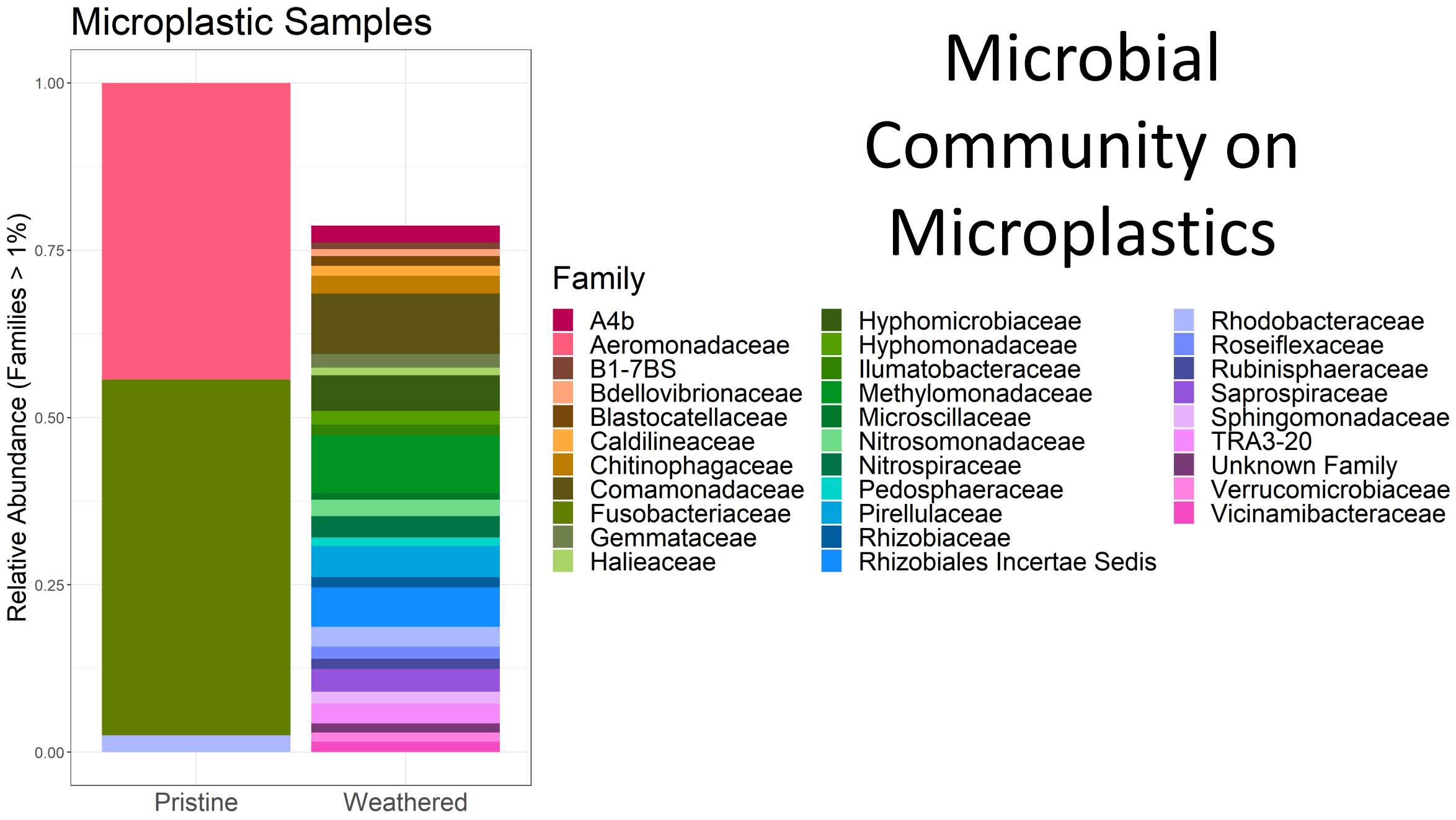
Methods:
Gene Expression

- Extracted RNA from the livers of the male replicates
- Compared five replicates from each treatment
- RNA amplification and relative quantification
- Difference between reference gene (*ef1a*) and target genes (*cyp1a, gsta, ncf2*)
- Assessed relative gene expression

Health Endpoints

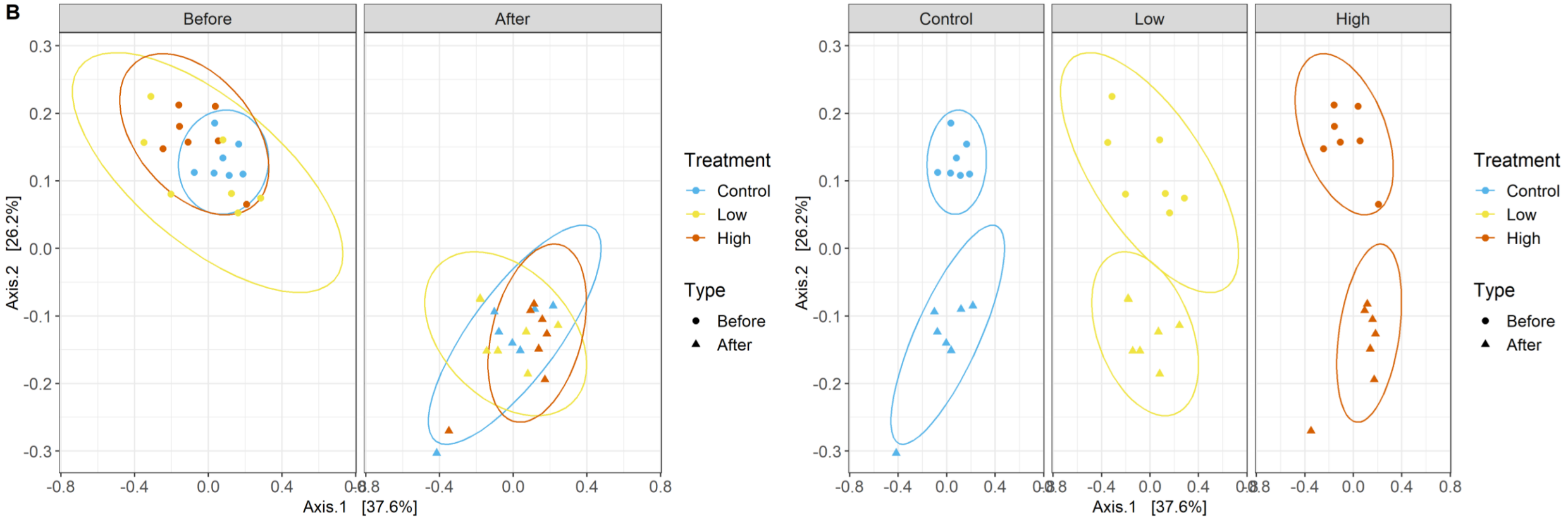


Microbial Community on Microplastics

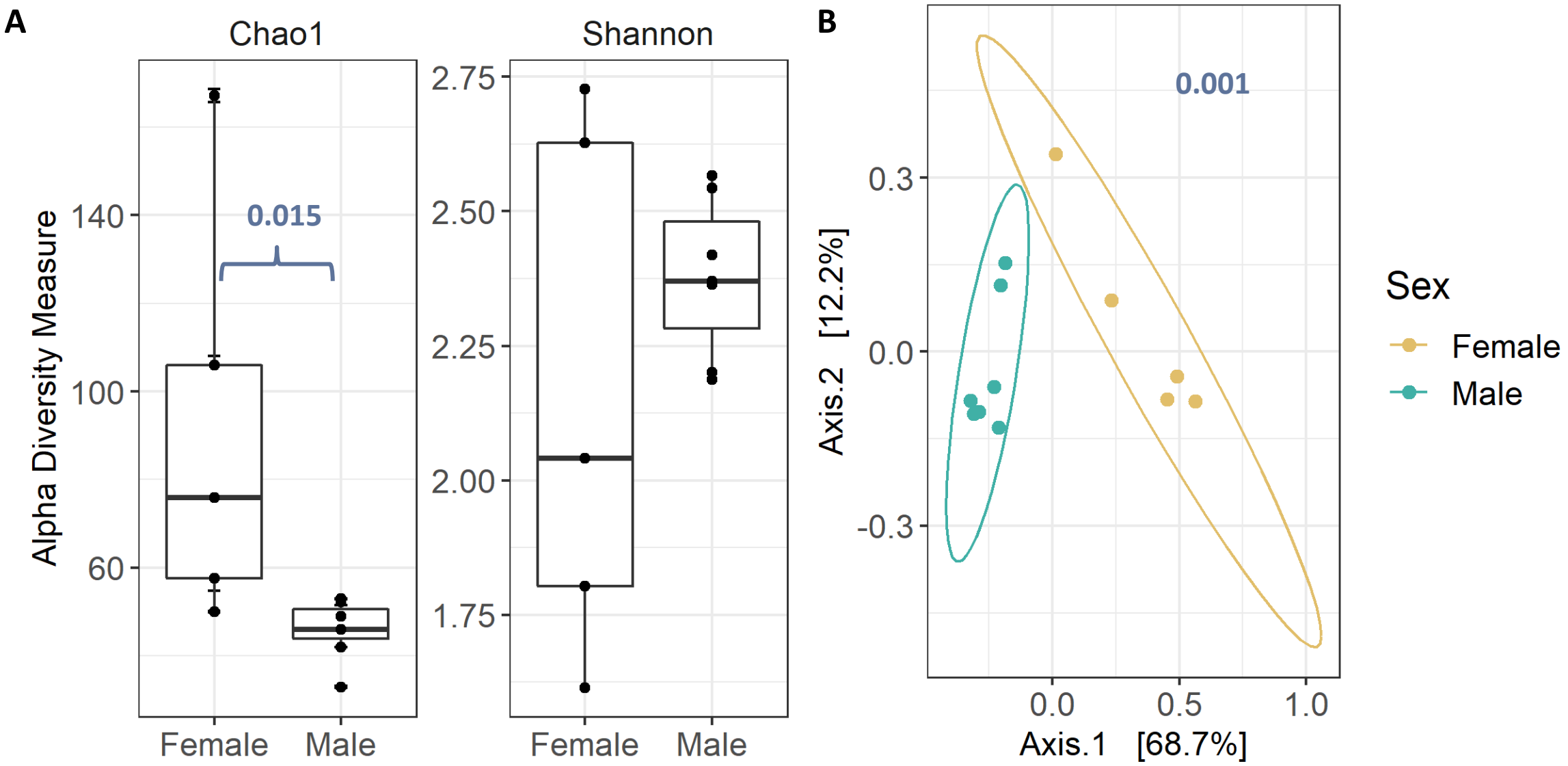


Females By Treatment vs. Over Time:

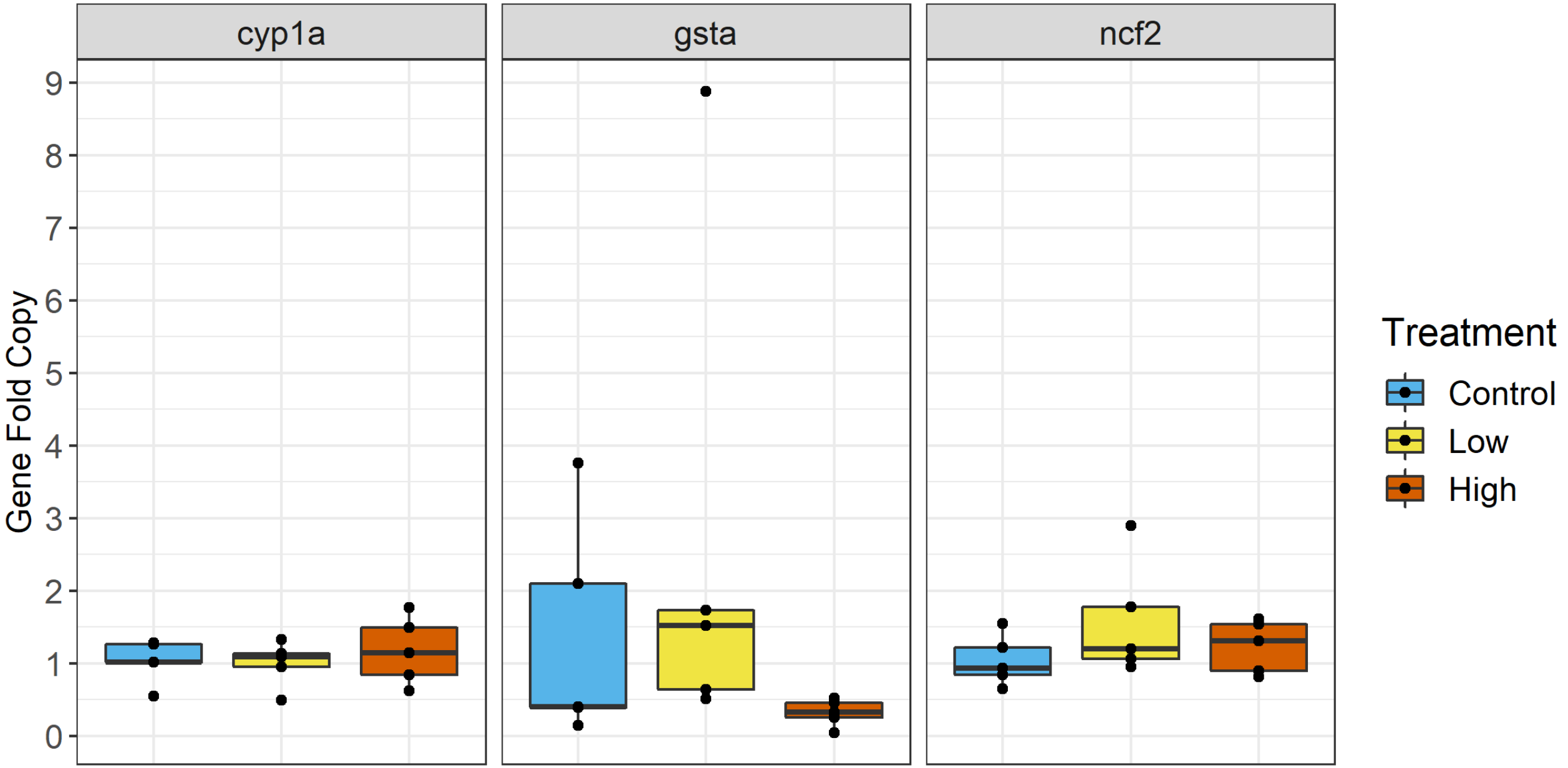
Tank collected fecal pellets



Sex Effects


A
Chao1
Shannon
Alpha Diversity Measure
140
100
60
0.015
2.75
2.50
2.25
2.00
1.75
Female
Male
Female
Male
B
0.001
0.3
0.0
-0.3
Axis.2 [12.2%]
0.0
0.5
1.0
Axis.1 [68.7%]
Sex
Female
Male

Gene Expression



Conclusions

- The decline in growth in males is in line with other studies, but effects are likely sex, species, and polymer dependent
  - Microplastic exposure may leave organisms or populations vulnerable to other stressors such as climate change or water quality

- There are limited studies on microplastic impacts on gut microbial community
  - We saw clear differences by sex and over time, which may outweigh the impacts of microplastic ingestion

- The effects of microplastics on gene expression has been variable in the literature
  - This was measured after chronic exposure; if there were mild, acute stress effects, these may have been compensated for over the course of the study

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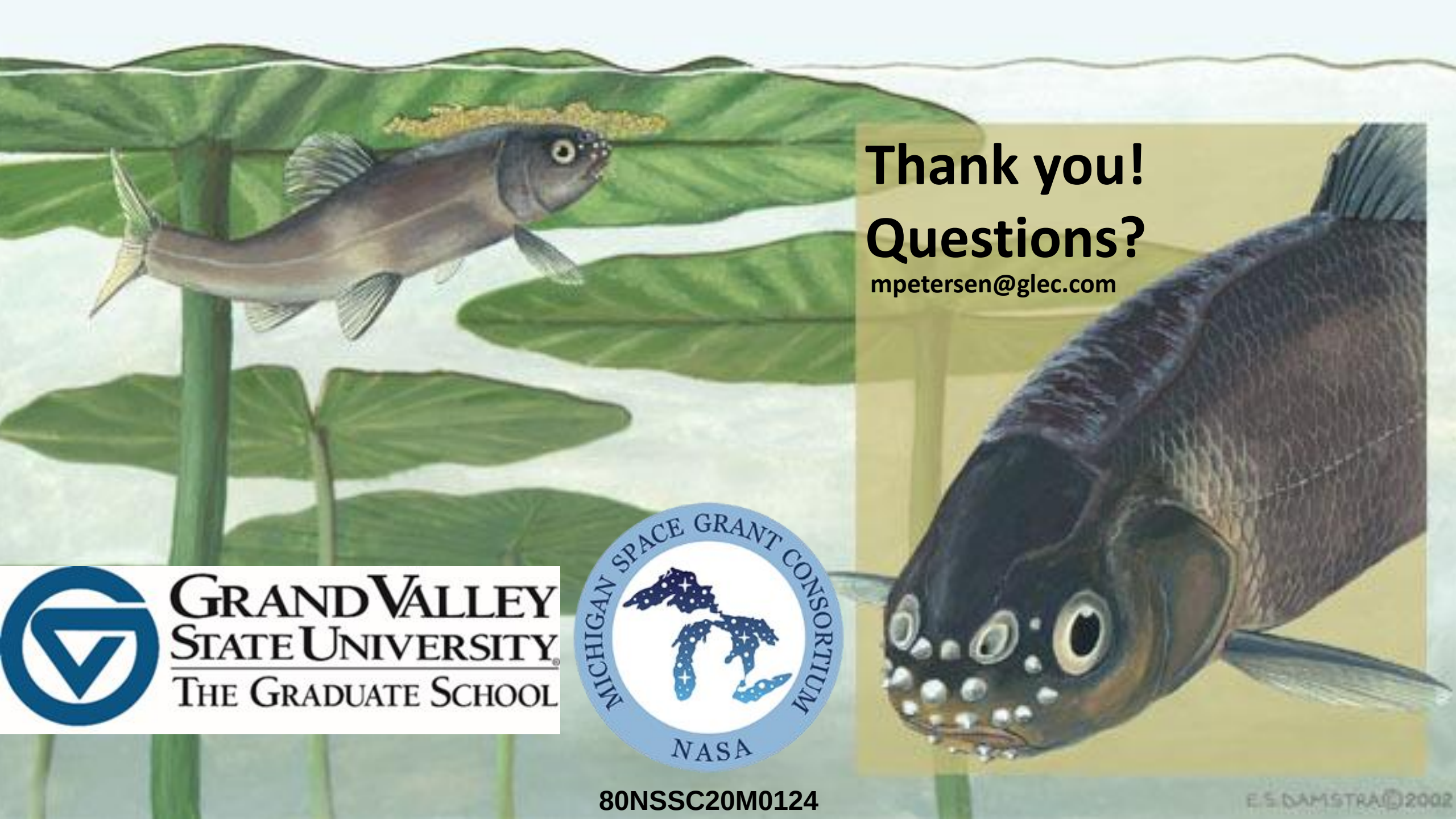
Future Work

- Replicated exposures
  - Reproductive endpoints
  - Full lifecycle
- Increased sample size
  - Test each sex
- Additional gene expression
  - Female samples
  - Other target genes or tissues
- Additional polymers, types, etc.
  - Environmentally collected microplastics



Thank you!
Questions?

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