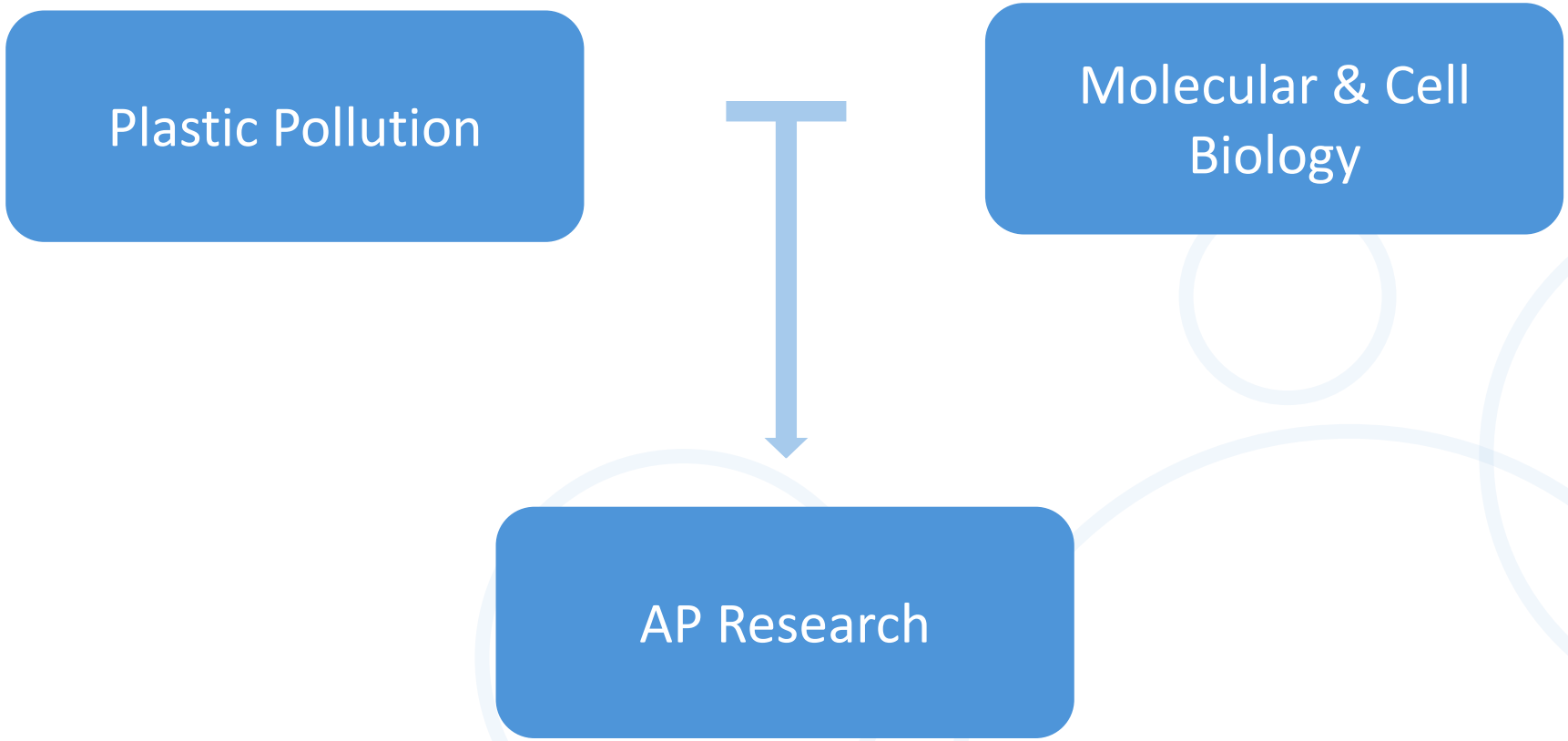
Characterizing the Diatom Communities of Freshwater Polyethylene Terephthalate and Polypropylene Plastispheres

Selin Filiz

Personal Interest



Plastic Pollution

1970s

First reported

60%

In landfills, natural environment

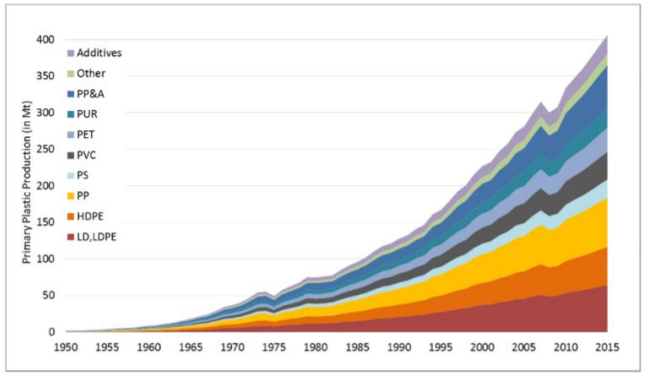


fig. S2. Global primary plastics production (in million metric tons) according to polymer type from 1950 to 2015.

"near permanent contamination of the natural environment with plastic waste is a growing concern" (Geyer et al., 2017)

(Andrady, 2011; Du et al., 2022; Zettler et al., 2013)

Plastic Pollution

- Entanglement, ingestion publicized
- Microplastics, POPs

Unknown: The "Plastisphere"

"composition of the biofilm community … remains to be investigated …" (Eich et al., 2015)

"studies of plastic-associated microbial communities are lacking …" (Zettler et al., 2013)

"very little is known about the communities of microbes …"
(Amaral-Zettler et al., 2017)

(Kirstein et al., 2019)

The Plastisphere

The distinct biofilm community that selectively attaches to plastic surfaces

1. Pioneer organisms
2. EPS → Secondary colonizers
3. Community succession

Diatoms

(Dey et al., 2022; Zettler et al., 2013; Bamford et al., 2023; Davidov et al., 2020)

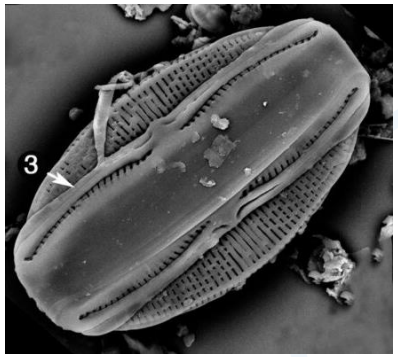
Diatoms

- Unicellular phytoplankton
- Single cells or chains of cells
- **Frustule**: Cell wall of hydrated glass
- **Raphe**: Slit in frustule for movement

Centric

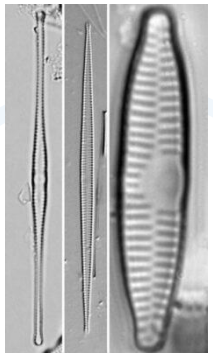
Coscinodiscus

Pennate



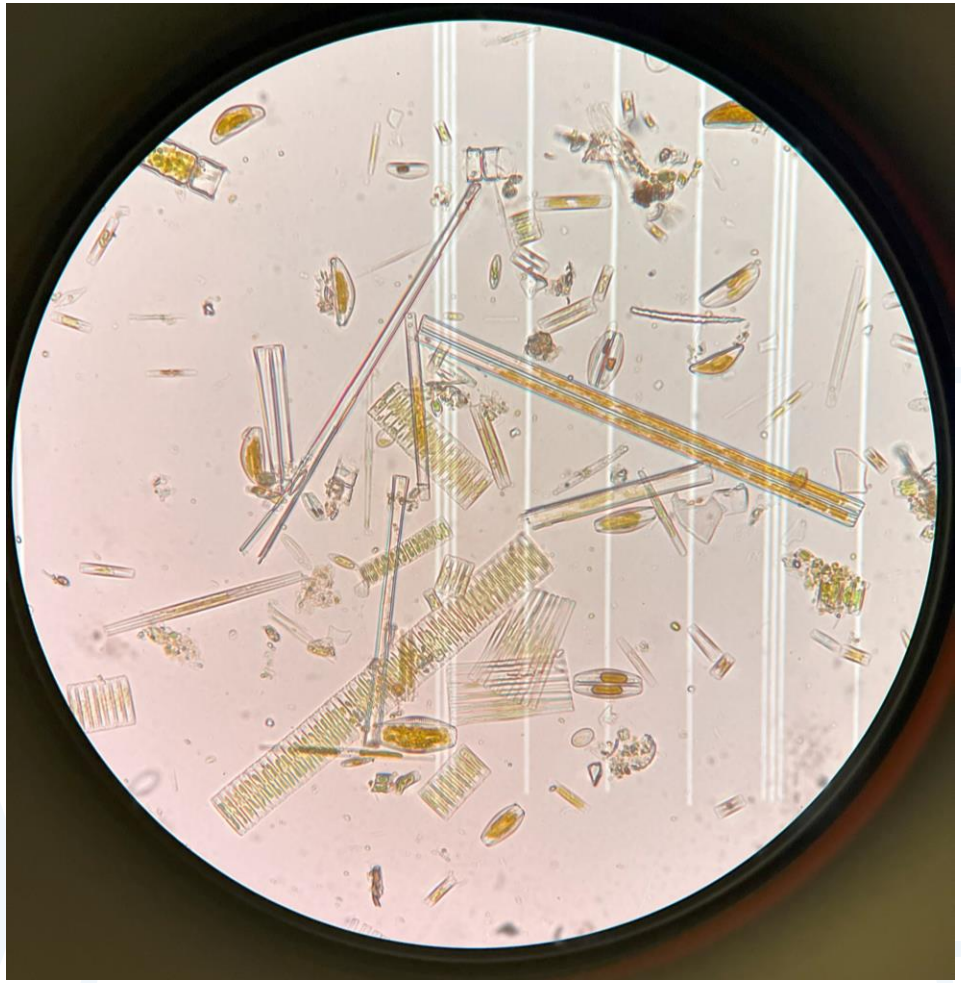
Amphora

Raphid



Fragilaria

Araphid



*400x magnification

(Virginia, 2009; Fu et al., 2022; Spaulding et al., 2021)

Diatoms

Supporting

40% of primary production
40% of carbon sequestration

Regulating

Regulate carbon, nutrients, protect microorganisms

Provisioning

Diatomaceous earth
Nanotech, drug-delivery

Cultural

“revolutionized the connection of science and art”

(B-Béres et al., 2022)

Gaps

GAP #1

- Lack of plastisphere research

GAP #2

- “the consequences of plastics in freshwaters remains poorly known” (Azevedo-Santos et al., 2021)

GAP #3

- Plastispheres reflect geography: none in Pacific Northwest (Amaral-Zettler et al., 2017)

Research Goal

Morphologically characterize and compare the composition of diatom communities in **polyethylene terephthalate** (PET) and **polypropylene** (PP) plastispheres in **freshwater** environments over a period of **five weeks**.

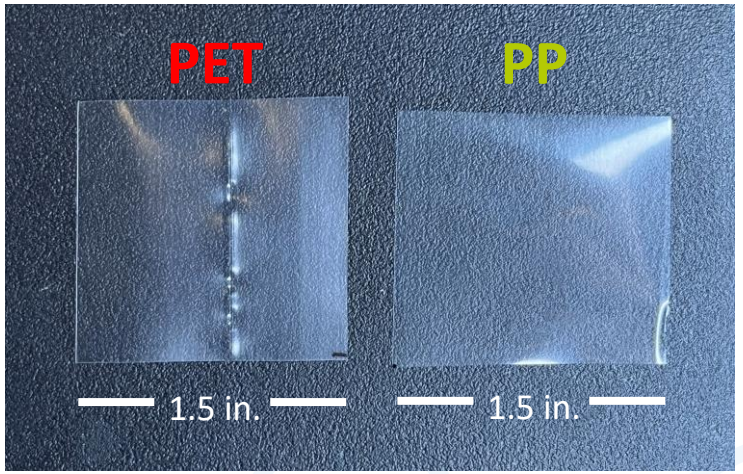
Quasi-Experimental

1. Experiment Installation
2. Sampling
3. Crystal Violet Assay (CV)
4. Diatom Characterization (DC)
5. Analysis

(Eich et al., 2015)

Experiment Installation

- **PET**: Clear Ziploc bags
- **PP**: Clear binder pockets



PET

6 bags x 20 squares

PP

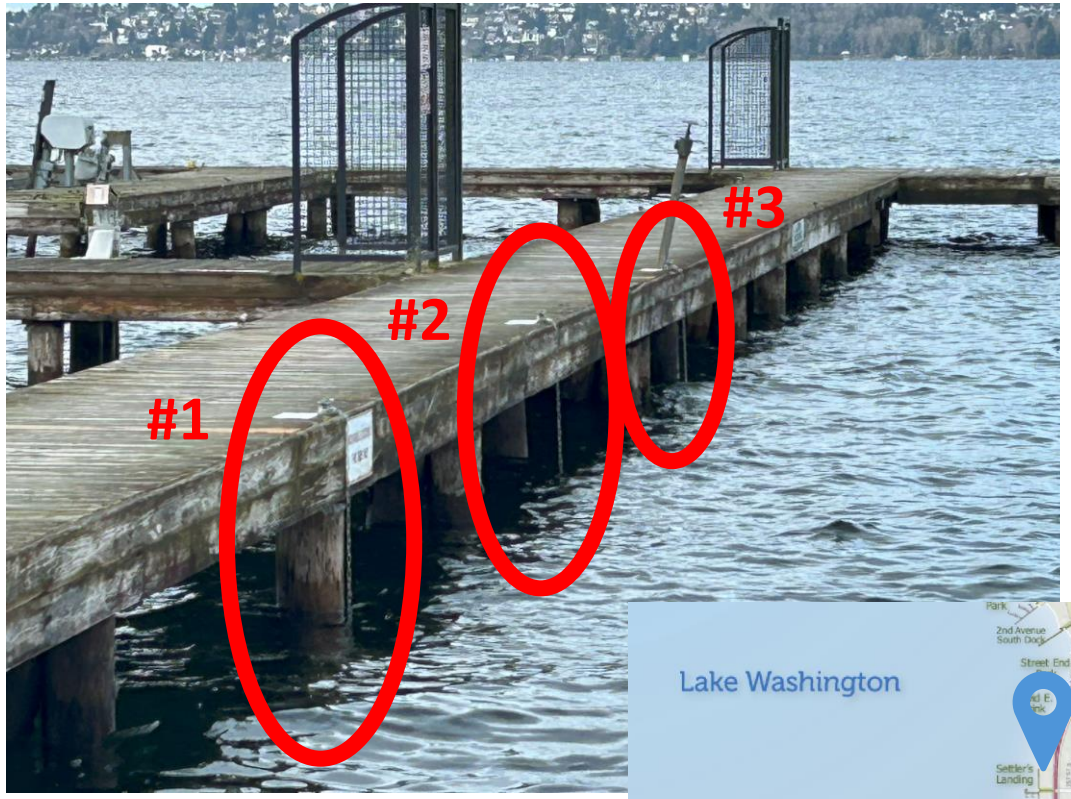
6 bags x 20 squares

By Sampling Time

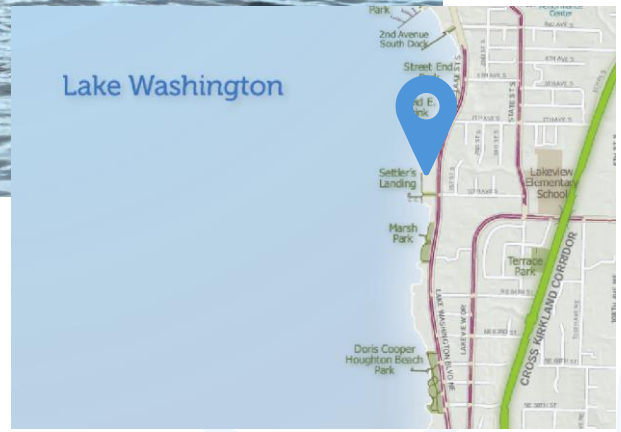
x3

(Geyer et al., 2017)

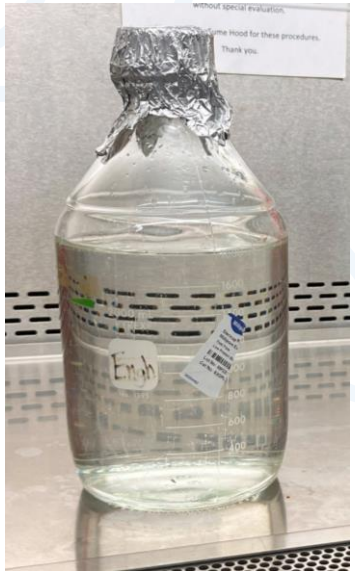
Experiment Installation



Settler's Landing
~10.187 °C



1.8 L SFW



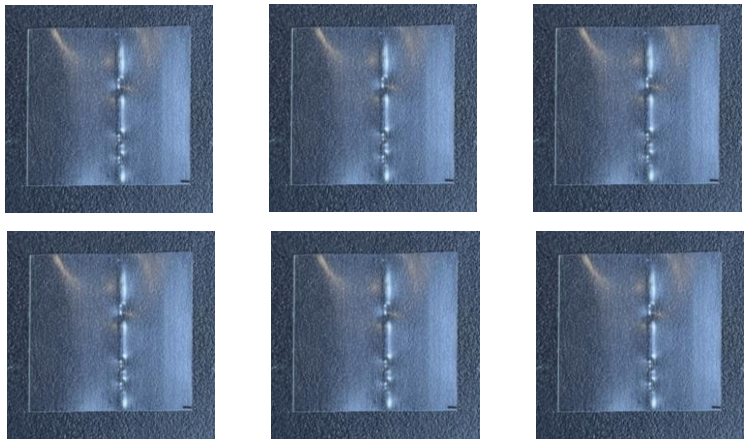
(City of Kirkland Parks Department)

Sampling

WK3, WK4, WK5

PET

SRS

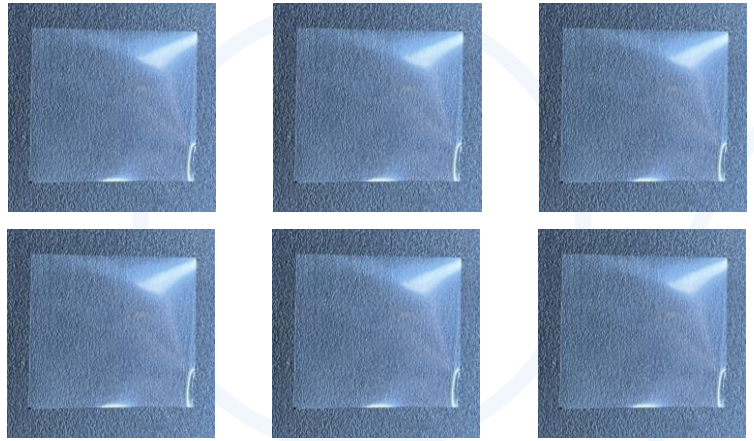


CV

DC

PP

SRS



CV

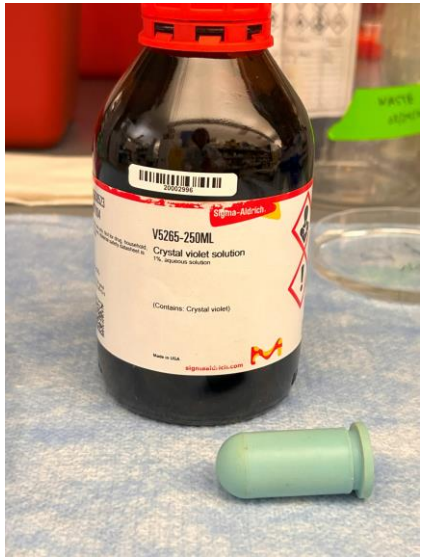
DC

PLYS

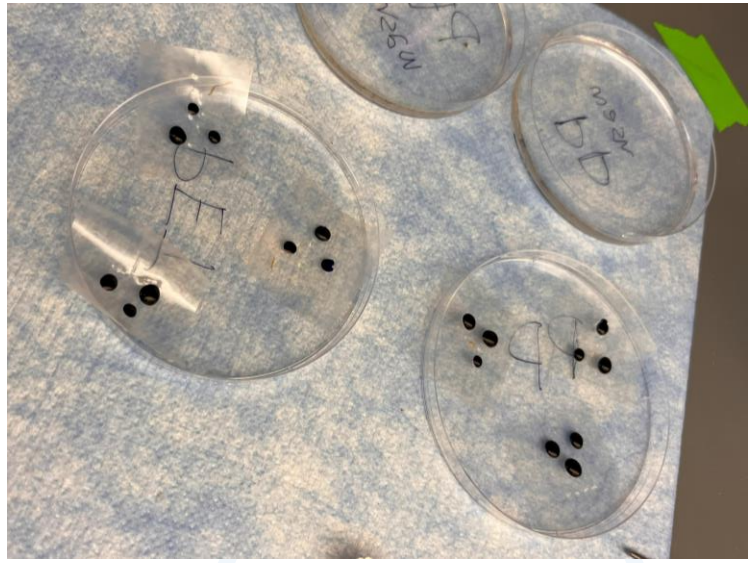
DC

Crystal Violet Assay (CV)

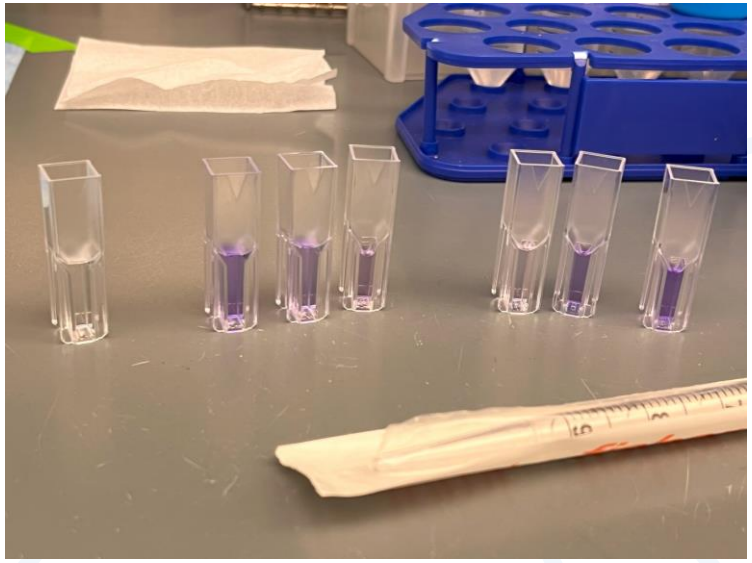
(Lobelle & Cunliffe, 2011)



Crystal Violet Stain (1% w/v)

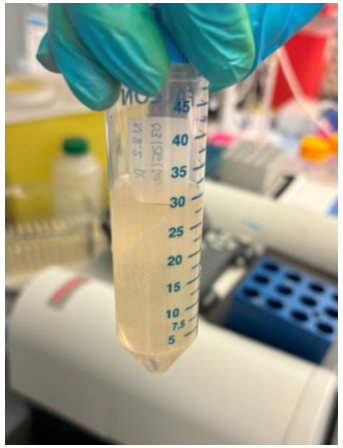


3 Drops



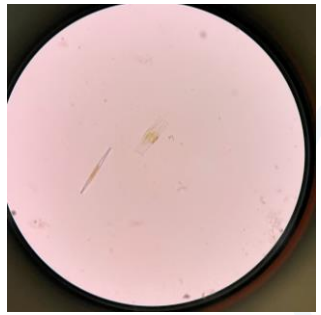
Optical density measured at 595 nm

Diatom Characterization (DC)



Isolating, concentrating biofilm

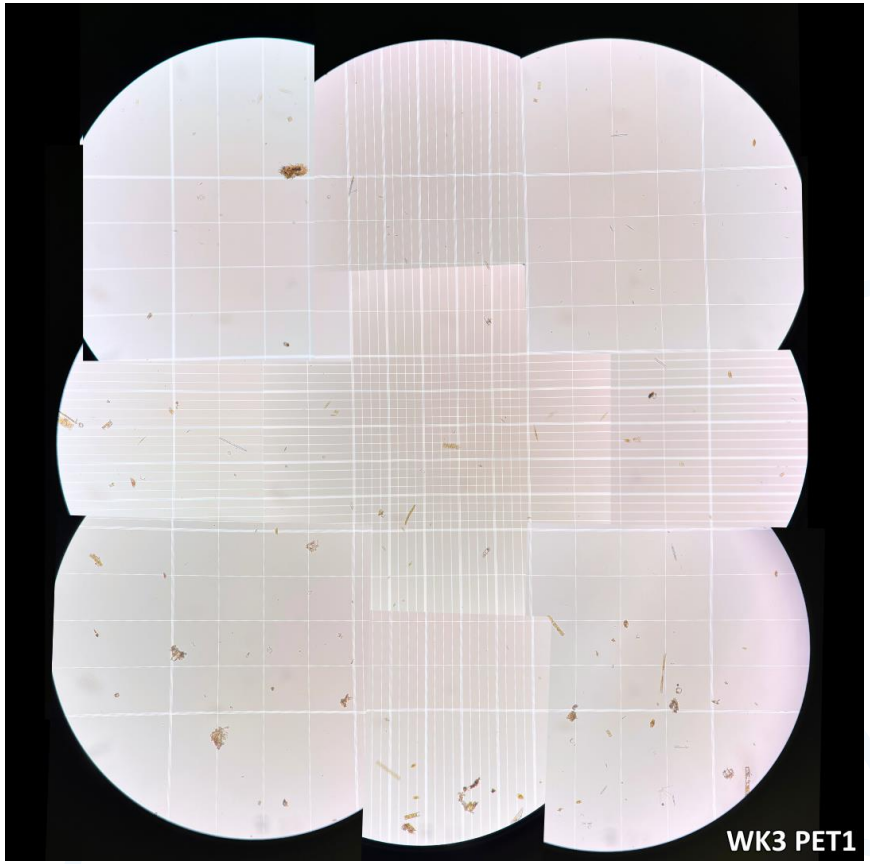
PET 4.3



PP 5.3

PLYS 5.3

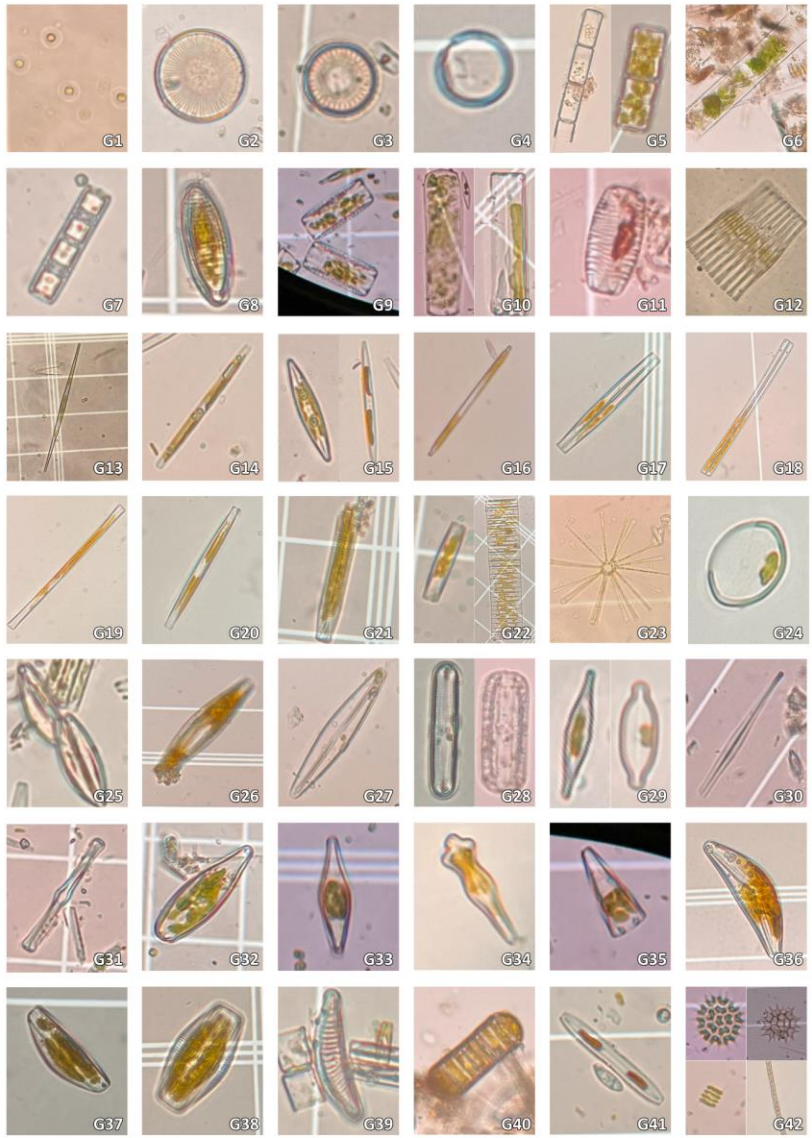
400x mag.



Hemocytometer, 100x mag.

Analysis

Diatoms of North America

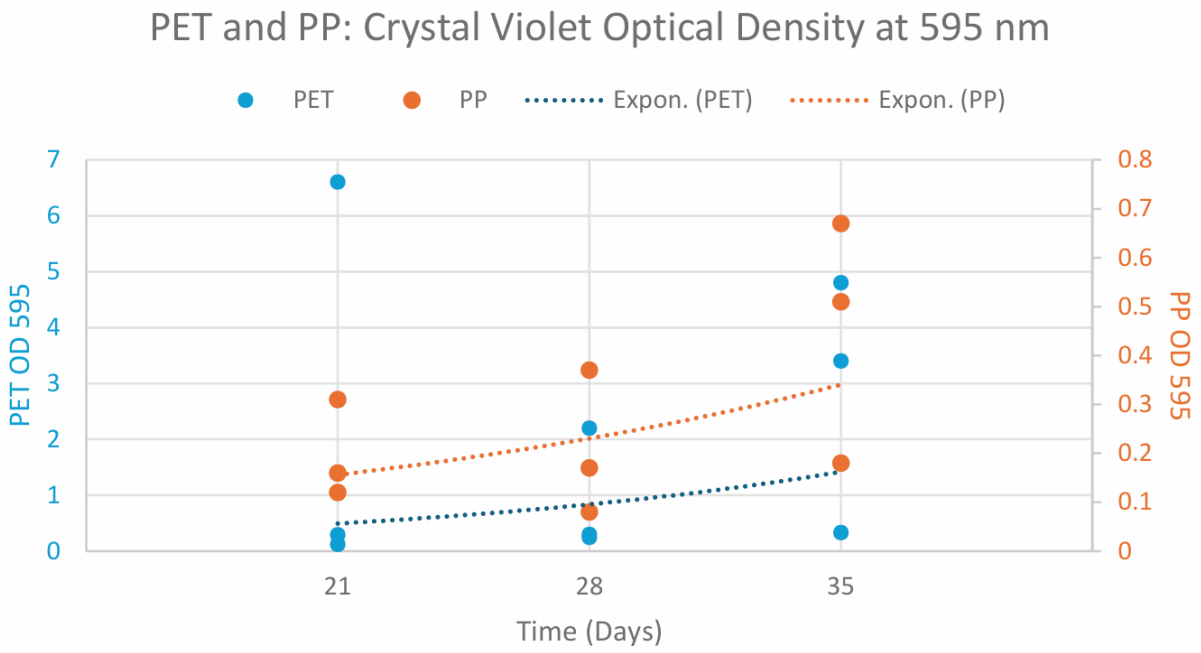


Diatom Groups Based on Morphological Features

Group	Category	Morphology	Genus
G1	Centric	Small, circular/rectangular frustule.	
G2	Centric	Circular frustule with radial striae organized into bundles. Spines on valve margin.	*Stephanodiscus* spp.?
G3	Centric	Circular frustule with radial striae. May have aerolae in center.	*Stephanocyclus* spp.? *Lindavia* spp.? *Cyclotella* spp.?
G4	Centric	Circular frustule. No visible striae.	*Stephanocyclus* spp.? *Lindavia* spp.? *Cyclotella* spp.?
G5	Centric	Rectangular frustules. May grow in long colonies linked at valve face.	*Melosira* spp.?
G6	Centric	Rectangular frustule, diagonal symmetry. Mantle edge is recurve. Long external tubes at mantle edge.	*Trieres* spp.? *Guinardia* spp.?
G7	Centric	Rectangular frustule. May grow in colonies.	*Aulacoseira* spp.?
G8	Araphid	Ovoid frustule with transapical striae. May grow in chain colonies.	*Pseudostaurosira* spp.?
G9	Araphid	Rectangular frustule. Ribs with along both longitudinal sides.	*Diatoma* spp.?
G10	Araphid	Rectangular frustule.	*Diatoma* spp.?
G11	Araphid	Rectangular frustule that swells in center. Transapical striae.	*Diatoma* spp.?
G12	Araphid	Frustule narrows to slender point. Cells form band-shaped colonies.	*Fragilaria* spp.? *Tabellaria* spp.? *Microtabella* spp.?
G13	Araphid	Frustule gradually tapers to a slender point. Fusiform.	*Fragilaria* spp.? *Ulnaria* spp.?
G14	Araphid	Slender, rectangular frustule with transapical chloroplasts.	*Nitzschia* spp.? *Synedra* spp.?
G15	Araphid	Pointed ellipsoidal frustule. Transapical or lateral chloroplasts.	*Synedra* spp.?
G16	Araphid	Ellipsoidal frustule. Fusiform.	*Synedra* spp.?
G17	Araphid	Rectangular frustule that swells in center. Longitudinal channel. Fusiform.	*Synedra* spp.? *Thalassionema* spp?
G18	Araphid	Rectangular frustule. Longitudinal channel. Fusiform.	*Synedra* spp.? *Thalassionema* spp?
G19	Araphid	Rectangular frustule. Fusiform.	*Synedra* spp.? *Thalassionema* spp?
G20	Araphid	Rectangular frustule. Fusiform.	*Synedra* spp.? *Thalassionema* spp?
G21	Araphid	Rectangular frustule with striae extending across central canal.	*Synedra* spp.?
G22	Araphid	Rectangular frustule. May grow in zig-zag chains.	*Synedra* spp.?
G23	Araphid	Forms stellate colonies, attached by mucilage pads at end. Symmetrical to apical axis.	*Asterionella* spp.?
G24	Symmetric Biraphid	Ovoid frustule with raphe, interrupted in center.	*Amicula* spp.?
G25	Symmetric Biraphid	Ovoid frustule with raphe.	*Caponea* spp.?
G26	Symmetric Biraphid	Ellipsoidal frustule, striae reach towards longitudinal canal (do not continue across entire valve).	*Biremis* spp.? *Pinnularia* spp.?
G27	Symmetric Biraphid	Ellipsoidal frustule, longitudinal ribs appear to have slits with a small central area. Forms a point at valve ends.	*Craticula* spp.? *Frustulia* spp.?
G28	Symmetric Biraphid	Oblong frustule with transapical striae. Longitudinal canals around raphe.	*Muelleria* spp.? *Neidiopsis* spp.? *Pinnularia* spp.?
G29	Symmetric Biraphid	Ellipsoidal frustule coming to an abrupt point.	*Navicula* spp.? *Kobayasiella* spp.? *Mastogloia* spp?
G30	Symmetric Biraphid	Frustule comes to a slender point. Fusiform.	
G31	Symmetric Biraphid	Pennate diatom. Swells in center.	
G32	Asymmetric Biraphid	Rounded frustule tapering to a point on one end.	*Gomphonema* spp.?
G33	Asymmetric Biraphid	Rounded frustule tapering gradually to a point on both ends.	*Gomphonema* spp.?
G34	Asymmetric Biraphid	Pennate diatom, frustule swells at center, headpole is broad and ends abruptly in a point.	*Gomphonema* spp.?
G35	Asymmetric Biraphid	Triangular frustule.	*Rhoicosphenia* spp.?
G36	Asymmetric Biraphid	Frustule is wedge shaped, striae extend to raphe.	*Amphora* spp.? *Cymbella* spp.?
G37	Asymmetric Biraphid	Frustule is wedge shaped, striae extend to central canal(s).	*Amphora* spp.? *Cymbella* spp.?
G38	Asymmetric Biraphid	Ellipsoidal frustule with interrupting central canal.	*Amphora* spp.? *Cymbella* spp.?
G39	Asymmetric Biraphid	Crescent frustule with striae.	
G40	Nitzschioid	Oblong frustule with transapical striae.	*Denticula* spp.?
G41	Nitzschioid	Pennate diatom, frustule narrows towards center. Has a raphe, transapical chloroplasts.	*Tryblionella* spp.?
G42	Other	These organisms appeared multiple times among samples but were unidentifiable.	

Crystal Violet Assay (CV)

Crystal Violet Biofilm Quantification: Optical Density at 595 nm												
Week	**Date**	**Days**	**PET1**	**PET2**	**PET3**	**PET Mean**	**PET SD**	**PP1**	**PP2**	**PP3**	**PP Mean**	**PP SD**
0	3/20/2024	0 (Control)	0.8	0.41	N/A	N/A	N/A	0.25	0.24	N/A	N/A	N/A
3	3/25/2024	21	0.29	0.12	6.6	2.3366667	3.693133	0.12	0.16	0.31	0.196667	0.100167
4	4/1/2024	28	0.25	2.2	0.3	0.9166667	1.11168	0.08	0.37	0.17	0.206667	0.148436
5	4/8/2024	35	4.8	3.4	0.33	2.8433333	2.286402	0.18	0.51	0.67	0.453333	0.249867



PET p-values

Two-Sample t-test, α = 0.05

WK3 — 0.5801 — WK4 — 0.2837 — WK5

WK3 — 0.8516 — WK5

PP p-values

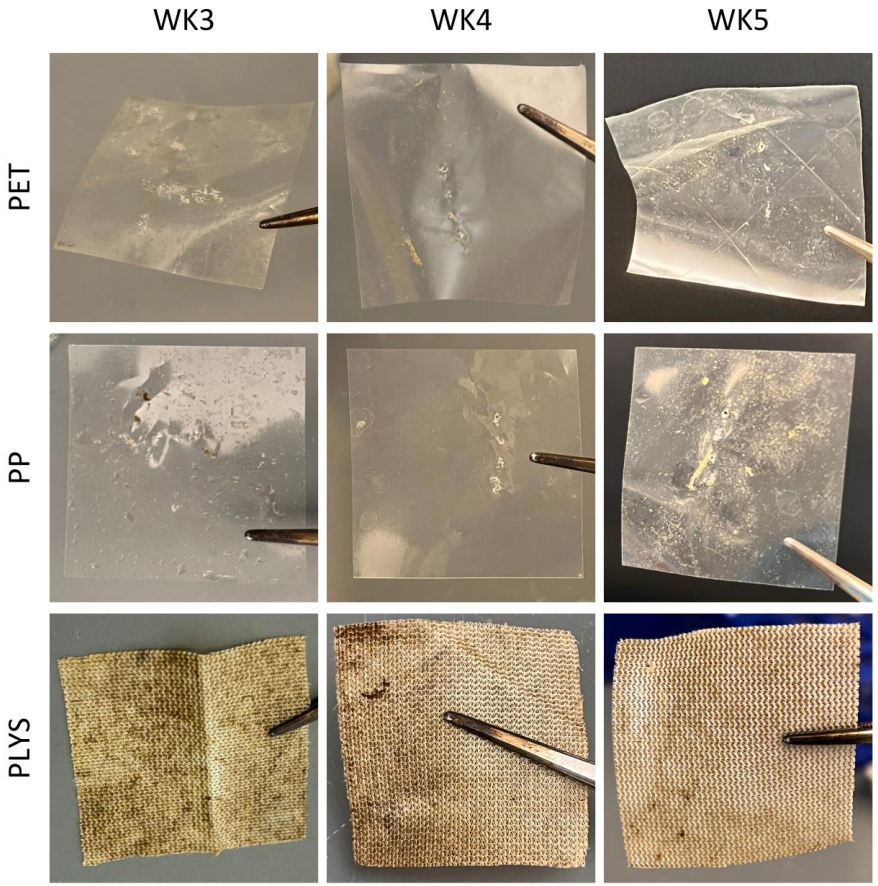
Two-Sample t-test, α = 0.05

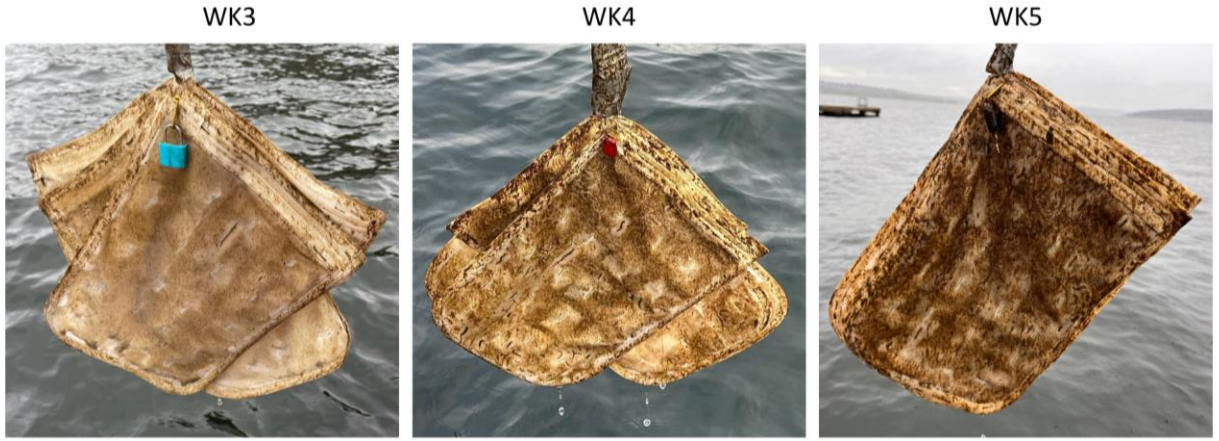
WK3 — 0.9282 — WK4 — 0.231 — WK5

WK3 — 0.2099 — WK5

Variable

Crystal Violet Assay (CV)

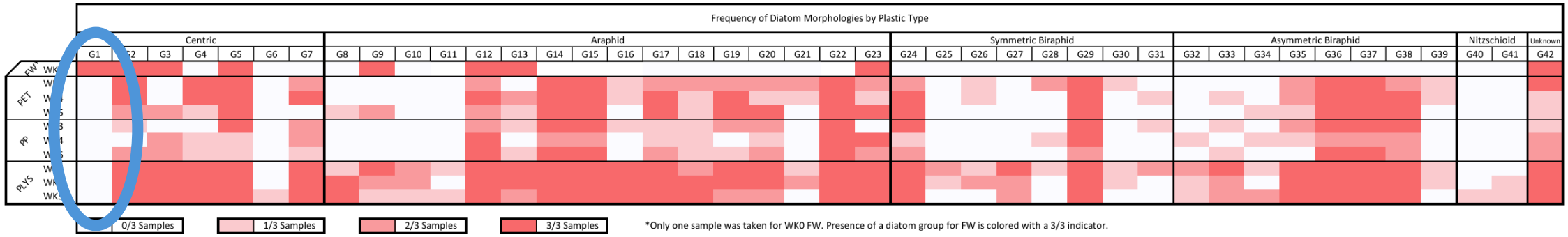


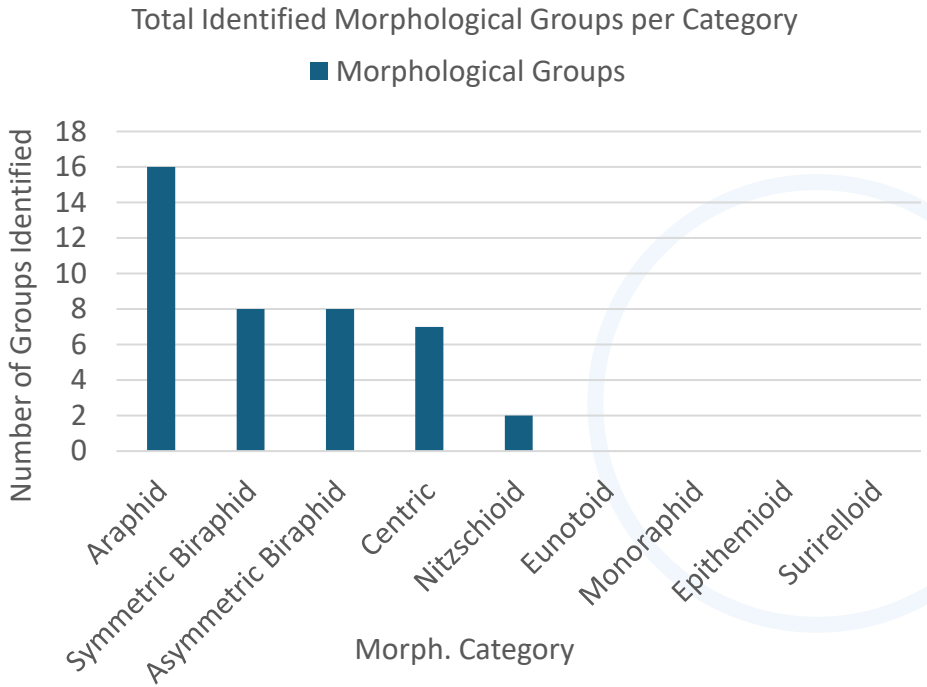


Anecdotal evidence:
Increase in biofouling

Diatom Characterization (DC)

1849 Images





Supports Dudek et al. (2020)

FW: Centric, Araphid

Diatom Characterization (DC)

	Total		
	2/3	3/3	(3/3)/(Total) %
FW	N/A	8	19.51%
PET	12	9	21.95%
	6	11	26.83%
	4	13	31.71%
PP	5	9	21.95%
	7	4	9.76%
	8	6	14.63%
PLYS	5	24	58.54%
	6	23	56.10%
	6	20	48.78%

WK3 → WK4 → WK5 (PP)

WK3 → WK4 → WK5 (PLYS)

Diversity: % of 3/3 morph. groups present

PLYS, PET, PP
Most → least diverse

Texture may play a role in diversity

Time may select for diatoms

Diatom Characterization (DC)

	Total		
	2/3	3/3	(3/3)/(Total) %
	N/A	8	19.51%
	12	9	21.95%
	6	11	26.83%
	4	13	31.71%
	5	9	21.95%
	7	4	9.76%
	8	6	14.63%
	5	24	58.54%
	6	23	56.10%
	6	20	48.78%

FW

WK3
WK4
WK5

PET

PP

PLYS

Diversity: % of 3/3 morph. groups present

PLYS, PET, PP
Most → least diverse

Texture may play a role in diversity

Time may select for diatoms

Diatom Characterization (DC)

No significant difference in plastics

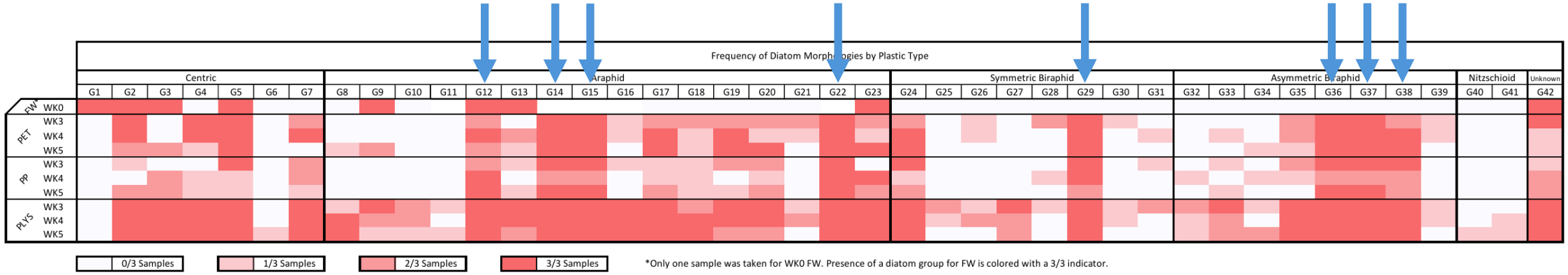
		Diatom Diversity by Plastic Type																	
		Centric			Araphid			Symmetric Biraphid			Asymmetric Biraphid			Nitzschioid			Total		
FW	WK0	2/3	3/3	(3/3)/(Total) %	2/3	3/3	(3/3)/(Total) %	2/3	3/3	(3/3)/(Total) %	2/3	3/3	(3/3)/(Total) %	2/3	3/3	(3/3)/(Total) %	2/3	3/3	(3/3)/(Total) %
		N/A	4	57.14%	N/A	4	25.00%	N/A	0	0.00%	N/A	0	0.00%	N/A	0	0.00%	N/A	8	19.51%
PET	WK3	1	3	42.86%	7	3	25.00%	2	1	12.50%	2	2	25.00%	0	0	0.00%	12	9	21.95%
	WK4	4	0	0.00%	1	6	37.50%	0	2	25.00%	1	3	37.50%	0	0	0.00%	6	11	26.83%
	WK5	2	1	14.29%	2	7	43.75%	0	2	25.00%	0	3	37.50%	0	0	0.00%	4	13	31.71%
PP	WK3	1	1	14.29%	3	3	18.75%	0	2	25.00%	1	3	37.50%	0	0	0.00%	5	9	21.95%
	WK4	2	0	0.00%	2	3	18.75%	0	1	12.50%	3	0	0.00%	0	0	0.00%	7	4	9.76%
	WK5	2	0	0.00%	3	4	25.00%	2	0	0.00%	1	2	25.00%	0	0	0.00%			14.63%
PLYS	WK3	0	5	71.43%	2	11	68.75%	2	3	37.50%	1	5	62.50%	0	0	0.00%	5	24	58.54%
	WK4	0	5	71.43%	3	12	75.00%	2	2	25.00%	1	4	50.00%	0	0	0.00%	6	23	56.10%
	WK5	0	5	71.43%	3	9	56.25%	1	2	25.00%	2	4	50.00%	0	0	0.00%	6	20	48.78%

*Only one sample was taken for WK0 FW. Presence of a diatom group for FW is included in the 3/3 column.

Texture is a likely confounding variable

Stronger relationship between PLYS and diatoms?

Diatom Characterization (DC)



Araphid

- G12: *Fragilaria* spp.? *Tabellaria* spp.? *Microtabella* spp.?;
- G14: *Nitzschia* spp.? *Synedra* spp.?;
- G15: *Synedra* spp.?;
- G22: *Synedra* spp.?

Symmetric Biraphid

- G29: *Navicula* spp.? *Kobayasiella* spp.? *Mastogloia* spp?

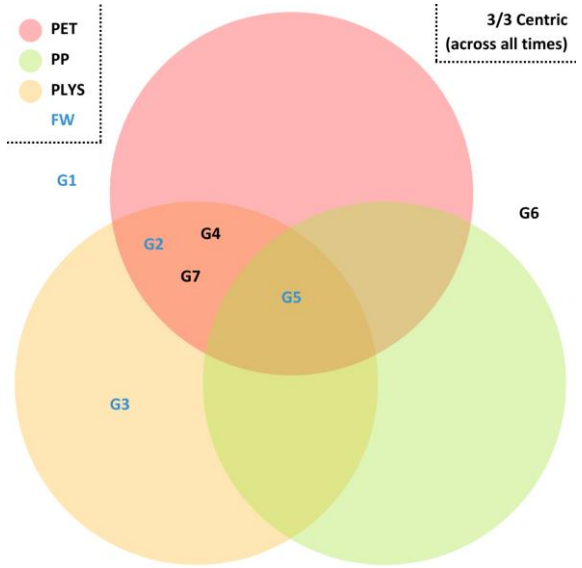
Asymmetric Biraphid

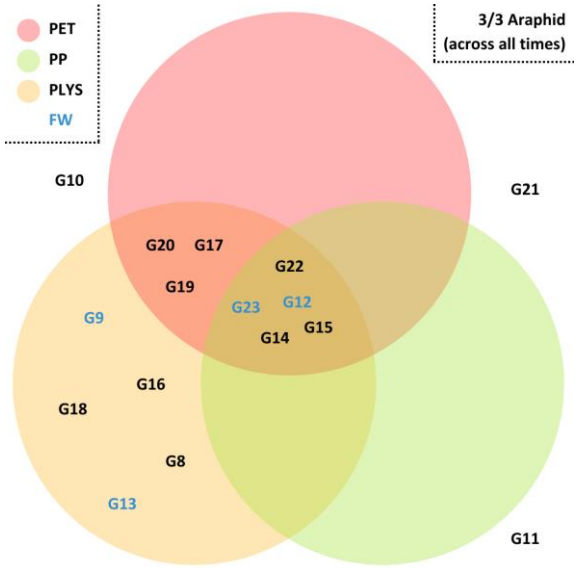
- G36: *Amphora* spp.? *Cymbella* spp.?;
- G37: *Amphora* spp.? *Cymbella* spp.?;
- G38: *Amphora* spp.? *Cymbella* spp.?

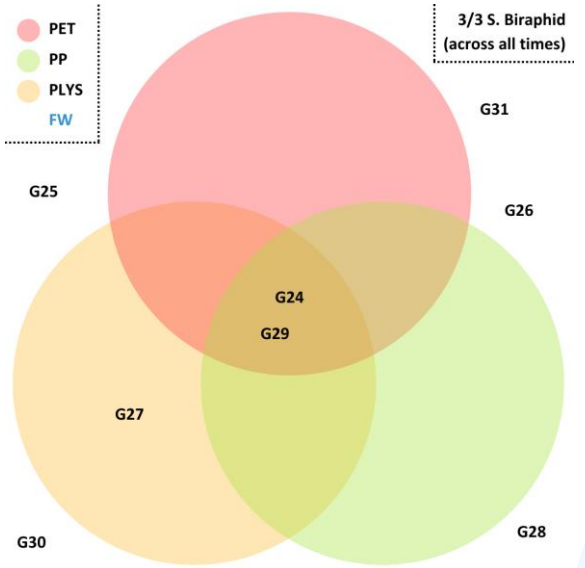
Substrate unspecific **core** of species

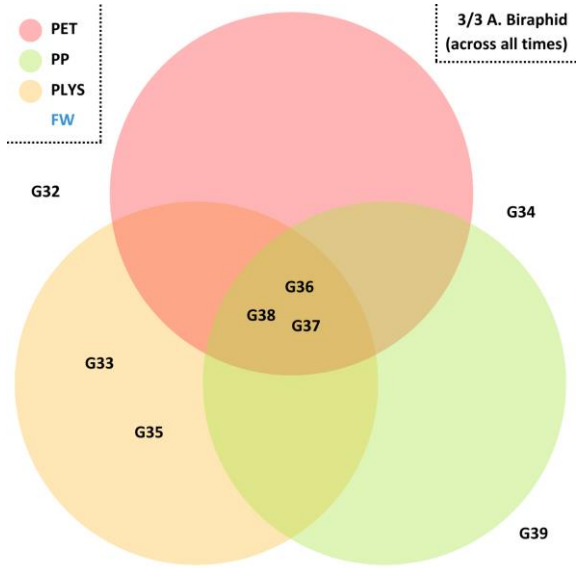
(Kirstein et al., 2015; Zettler et al., 2013)

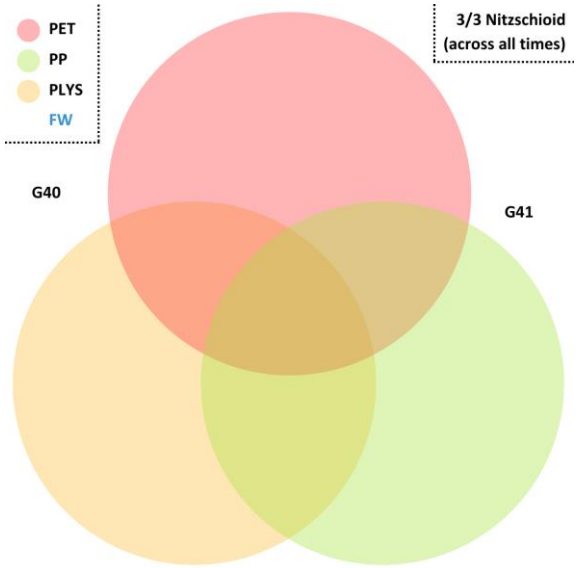
Diatom Characterization (DC)











No groups unique to PET, PP

Implications

Biofouling → Density
Removes nutrients, photo-oxidative degradation unlikely, shifts biogeochemical cycles

Core group, divides → New ecological niche
Plastisphere impact evolves over time, affects biodiversity

(Andrady, 2011; Fu et al., 2022; Virginia, 2009; B-Béres et al., 2022; Zettler et al., 2013; Amaral-Zettler et al., 2015; Bamford et al., 2023)

Limitations

General

- Uncontrolled variables: temp, salinity, pH, texture, etc

Crystal Violet

- Imprecision: Pasteur pipette, 5 mL wash
- Varied PET texture

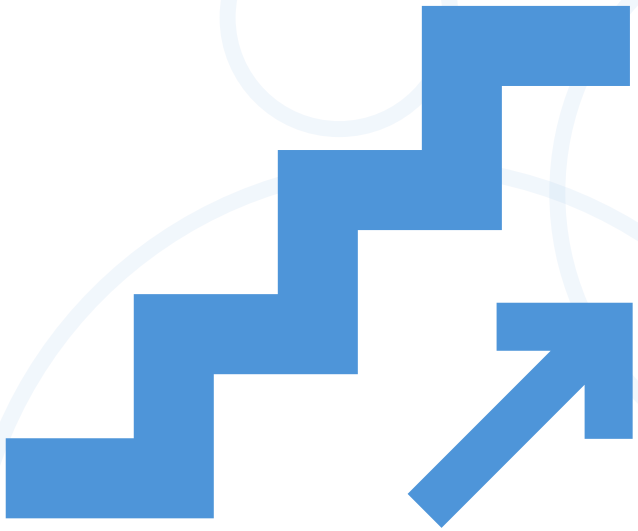
Diatom Characterization

- Potentially missing plastic-specific microbes
- Inexperience, lack of resources → bias

(Dey et al., 2022; Kirstein et al., 2015)

Next Steps

- Thorough **biofilm quantification** method
- Pressure wash to **isolate plastic-specific** (Kirstein et al., 2015)
- Isolate plastic type variable in **controlled environment**
- **Genetic sequencing, machine learning** to identify species
- Studies of **several months**
- **Education** with diatoms



Acknowledgements

Randy Huberman

International Community School

ISB

Nicci Osborn

Kirkland Parks and Community Services

City of Kirkland

Public Works

Expert Adviser: Dr. Jacob J. Valenzuela

Institute for Systems Biology

King County

Department of Natural Resources and Parks

DEPARTMENT OF ECOLOGY

State of Washington

References

Amaral-Zettler, L. A., Zettler, E. R., Slikas, B., Boyd, G. D., Melvin, D. W., Morrall, C. E., Proskurowski, G., & Mincer, T. J. (2015). The biogeography of the Plastisphere: Implications for policy. *Research Communications, 13*(10), 541-546. https://doi.org/10.1890/150017

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin, 62*(8), 1596-1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Azevedo-Santos, V. M., Brito, M. F. G., Manoel, P. S., Perroca, J. F., Rodrigues-Filho, J. L., Paschoal, L. R. P., Goncalves, G. R. L., Wolf, M. R., Blettler, M. C. M., Andrade, M. C., obile, A. B., Lima, F. P., Ruocco, A. M. C., Silva, C. V., Perbiche- Neves, G., Portinho, J. L., Giarizzo, T., Arcifa, M. S., & Pelicice, F. M. (2021). Plastic pollution: a focus on freshwater biodiversity. *Ambio, 50*(7), 1313-1324. https://doi.org/10.1007/s13280-020-01496-5

Bamford, N. C., MacPhee, C. E., & Stanley-Wall, N. R. (2023). Microbial primer: an introduction to biofilms – what they are, why they form, and their impact on built and natural environments. *Microbiology, 169*(8). https://doi.org/ 10.1099/mic.0.001338

B-Béres, V., Stenger-Kovács, C., Buczkó, K., Padisák, J., Selmeczy, B. G., Lengyel, E., & Tapolczai, K. (2022). Ecosystem services provided by freshwater and marine diatoms. *Hydrobiologia, 850*(12/13), 2707-2733. https://doi.org/10.1007/s10750-022-04984-9

City of Kirkland Parks Department (Cartographer). (2018). *2018 Parks Trails Guide* [Map]. City of Kirkland. https://www.kirklandwa.gov/files/sharedassets/public/v/1/parks-amp-comm-services/online-parks-guide/pdfs/2018parks_trails-guide_1map-reduced.pdf

Davidov, K., Iankelevich-Kounio, E., Yakovenko, I., Koucherov, Y., Rubin-Blum, M., & Oren, M. (2020). Identification of plastic-associated species in the Mediterranean Sea using DNA metabarcoding with Nanopore MinION. *Scientific Reports, 10*(1), 1-12. https://doi.org/10.1038/s41598-020-74180-z

Dey, S., Rout, A. K., Behera, B. K., Ghosh, K. (2022). Plastisphere community assemblage of aquatic environment: plastic-microbe interaction, role in degradation and characterization technologies. *Environmental Microbiome, 17*(1), 1-21. https://doi.org/10.1186/s40793-022-00430-4

Du, Y., Liu, X., Dong, X., & Yin, Z. (2022). A review on marine plastisphere: biodiversity, formation, and role in degradation. *Computational and Structural Biotechnology, 20*, 975-988. https://doi.org/10.1016/j.csbj.2022.02.008

Dudek, K. L., Cruz, B. N., Polidoro, B., & Neuer, S. (2020). Microbial colonization of microplastics in the Caribbean Sea. *Limnology & Oceanography Letters, 5*(1), 5-17.https://doi.org/10.1002/lol2.10141

Eich, A., Mildenberger, T., Laforsch, C., Weber, M. (2015). Biofilm and diatom succession on polyethylene (PE) and biodegradable plastic bags in two marine habitats: early signs of degradation in the pelagic and benthic zone?. *PLoS ONE, 10*(9). https://doi.org/10.1371/journal.pone.0137201

Fu, W., Shu, Y., Yi, Z., Su, Y., Pan, Y., Zhang, F., & Brynjolfsson, S. (2022). Diatom morphology and adaptation: current progress and potentials for sustainable development. *Sustainable Horizons, 2*(100015). https://doi.org/10.1016/j.horiz.2022.100015

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances, 3*(7), 1-5. https://doi.org/10.1126/sciadv.1700782

Kirstein, V. I., Wichels, A., Gullans, E., Krohne, G., & Gerdts, G. (2019). The Plastisphere - Uncovering tightly attached plastic "specific" microorganisms. *PLoS ONE, 14*(4), 1-17. https://doi.org/10.1371/journal.pone.0215859

Lobelle, D., Cunliffe, M. (2011). Early microbial biofilm formation on marine plastic debris. *Marine Pollution Bulletin, 62*(1), 197-200. https://doi.org/10.1016/jmarpolbul.2010.10.013

Spaulding, S. A., Potapova, M. G., Bishop, I. W., Lee, S. S., Gasperak, T. S., Jovanoska, E., … Edlund, M. B. (2021). Diatoms.org: supporting taxonomists, connecting communities. *Diatom Research, 36*(4), 291–304. https://doi.org/10.1080/0269249X.2021.2006790

Virginia, E. A. (2009). The life of diatoms in the world's oceans. *Nature, 459*, 185-191. https://doi.org/10.1038/nature08057

Zettler, E. R., Mincer, T. J., & Amaral-Zettler, L. A. (2013). Life in the "plastisphere": microbial communities on plastic marine debris. *Environmental Science and Technology, 47*(13), 7137-7146. https://doi.org/10.1021/es401288x

Thank you.
Questions?