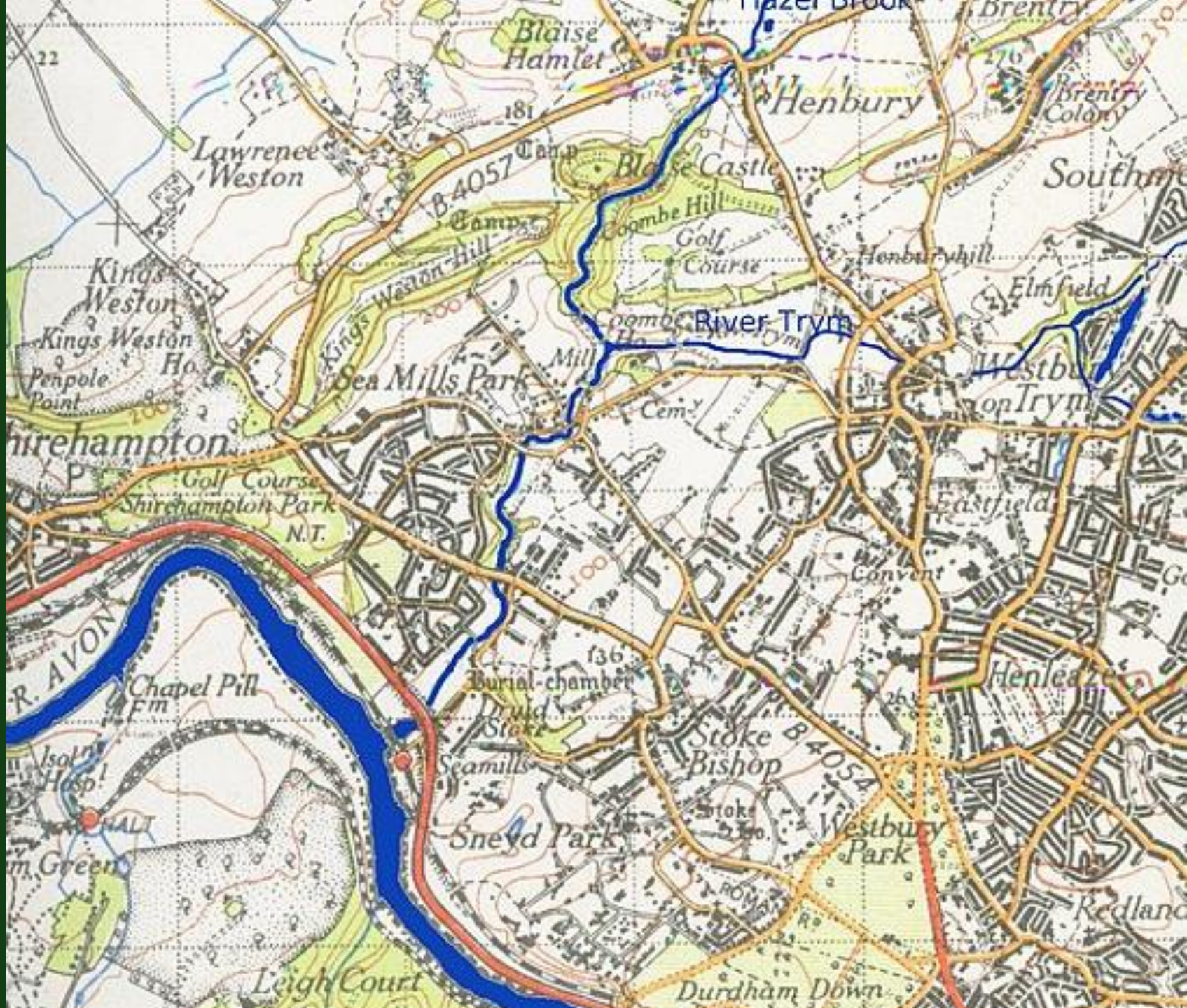


Trout in the Trym

Group 6



Trout in the Trym (NGO)

- A grassroots, volunteer led community group.
- Aim to 'restore trout to the Trym' by cleaning up the area from pollution.



Background information

- The river Trym flows south into the River Avon, with a tributary known as the River Hazel Brook
- Wessex Water manages multiple Combined Storm Overflows (CSOs) along the course of the Trym



Why is it important?



Visible litter

- Investigating the microplastic pollution in the area between the Trym River and the Hazel Brook
- Hazel Brook water originates in Cribbs Causeway from a pond with waste
- Environmental consequences on wildlife and human exposure
- Investigating multiple locations to find the source
- Consistent monitoring to monitor interventions and progress

Hypotheses and research questions

Aim of research: To explore the microplastics concentration and the possible causes of this along the River Trym.

Do CSOs have an impact on the microplastics in the river?

Hypothesis 1: There will be more microplastics found downstream than upstream of a CSO.

Does the car park (and roads) impact microplastics in the river?

Hypothesis 2: There will be more microplastics found downstream of the car park than upstream.

How does discharge impact microplastic concentration along the river?

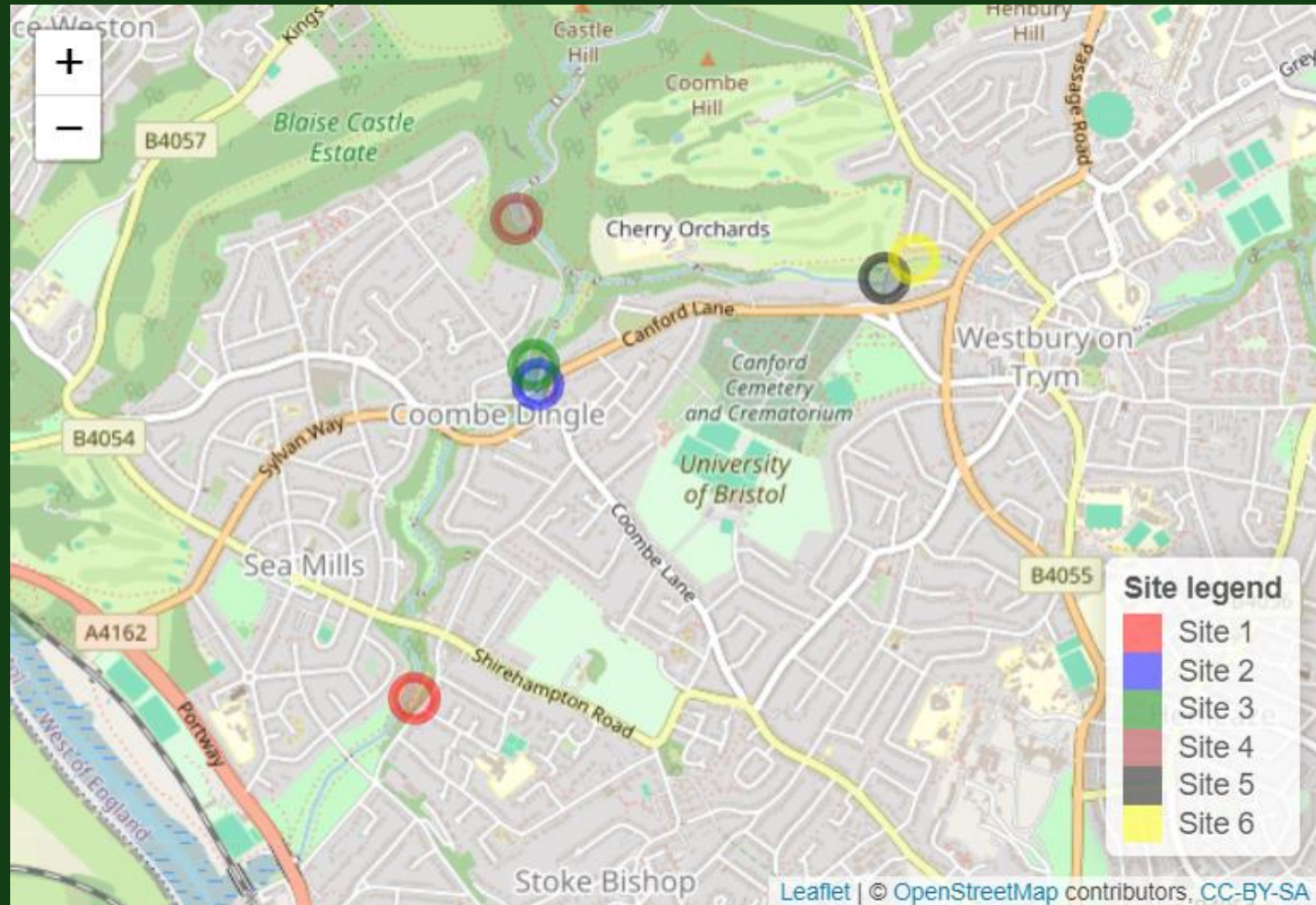
Hypothesis 3: Sites with a higher discharge will have a higher concentration of microplastics.

How does the water chemistry vary between the sites?

Hypothesis 4: Sites downstream from CSO will have higher EC, and lower DO, and sites with more microplastics will have a lower pH.

Field sites

- **Site 1:** downstream, approaching mouth to the Avon
- **Site 2:** downstream of car park
- **Site 3:** upstream of car park
 - **Site 4:** Hazelbrook tributary
- **Site 5:** downstream of CSO on the other tributary
- **Site 6:** upstream of CSO on the other tributary



Sampling strategy



- Purposive sampling technique
- Worked our way upstream
- Water samples not sediment samples
- Samples and probe readings collected from the center, upstream of discharge
- Flowmeter for velocity

Sampling procedure

1. Microplastic contamination samples
 - Standardised depths
 - 8 repetitions
2. Probe measurements
 - 3 repetitions
3. Flow velocity -> discharge
 - Segmented channel width
 - Flow metre
 - $Q = AV$



Lab work

Selective fluorescence staining method using Nile Red

Procedure:

1. Plastic-free laboratory water
2. Ultrasonic Sonicator bath

Glass & ceramic pressure filter unit



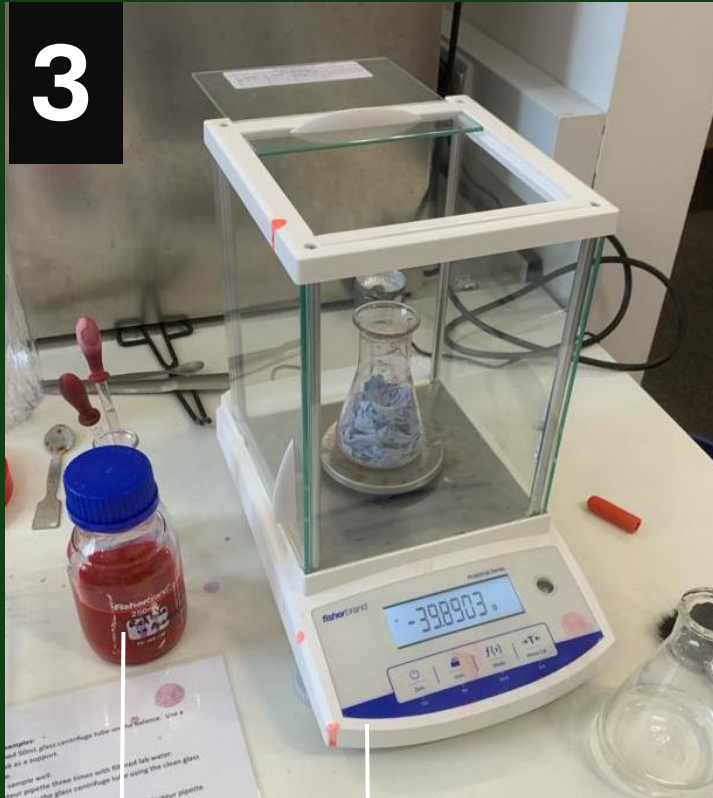
Ultrasonic sonicator bath

3. Adding the NR

4. Mixing and filtering

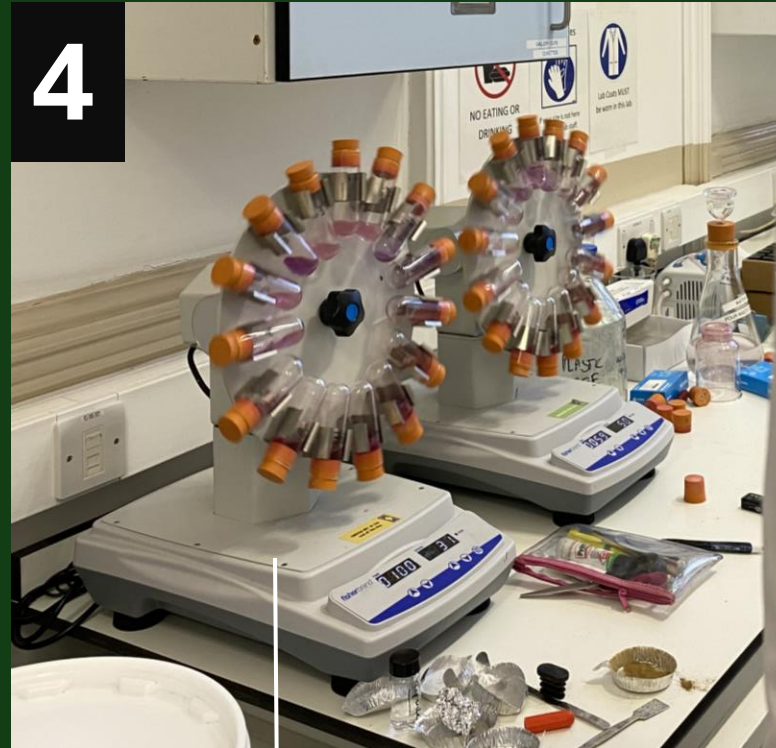
5. Photographing using Crime-lite

Camera on stand

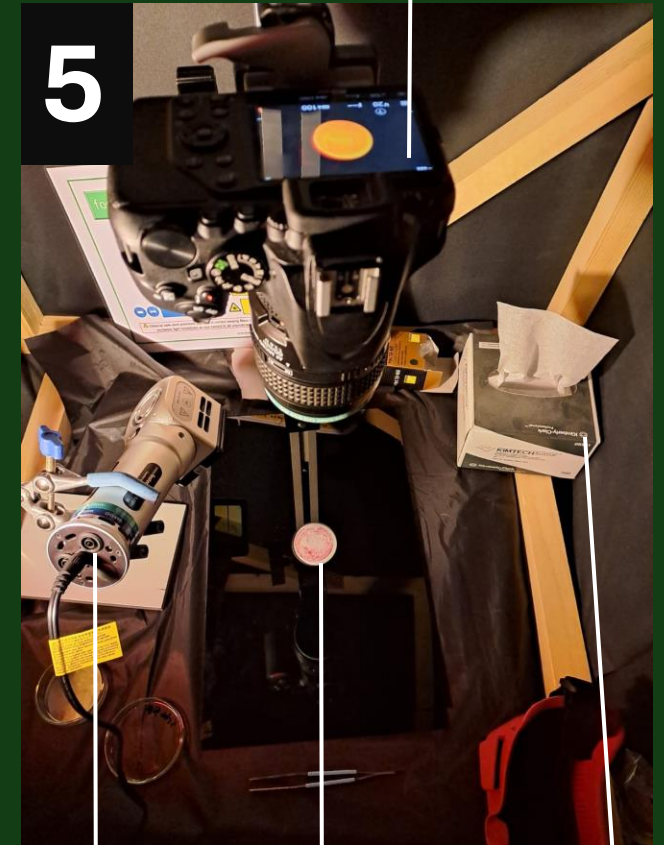


Nile Red dye

Analytical balance



Rotating mixer



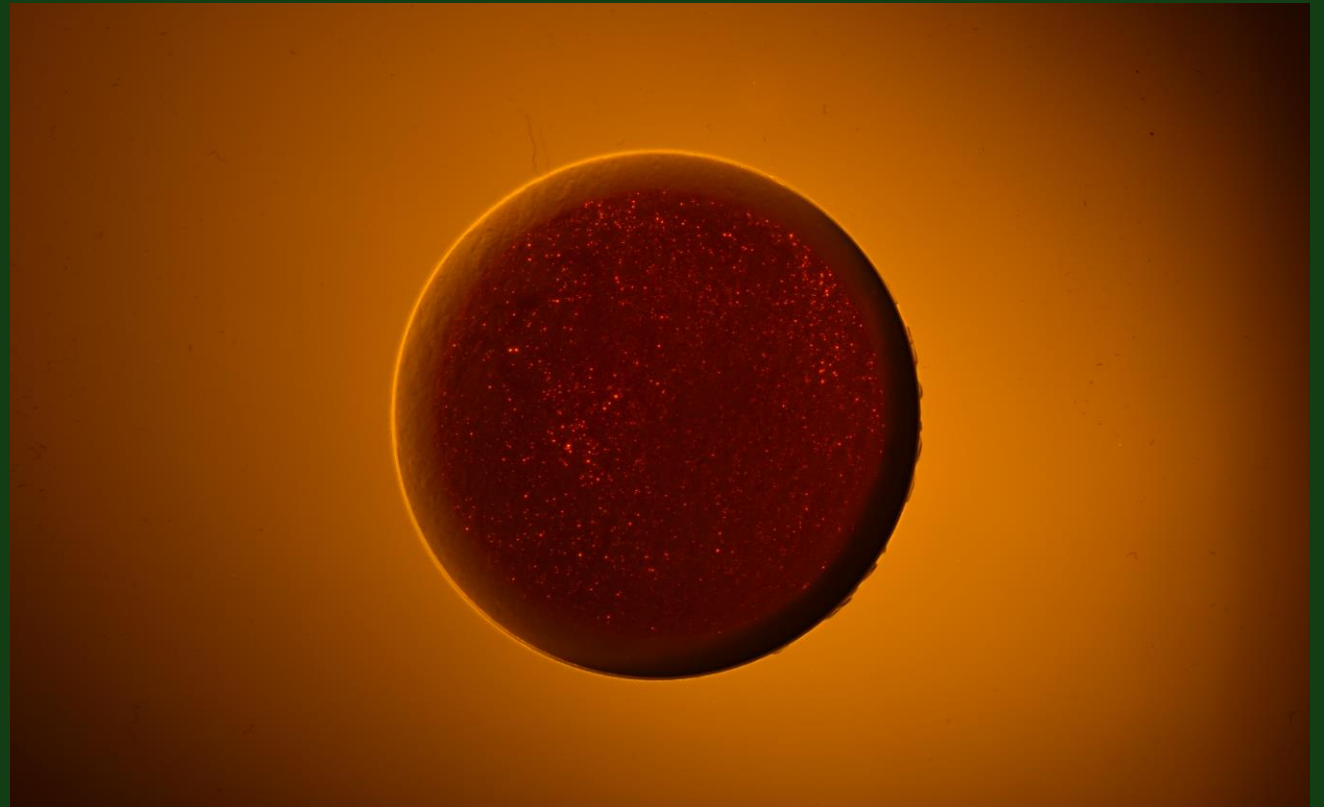
Crime-lite

Filter paper with sample

Lens wipes

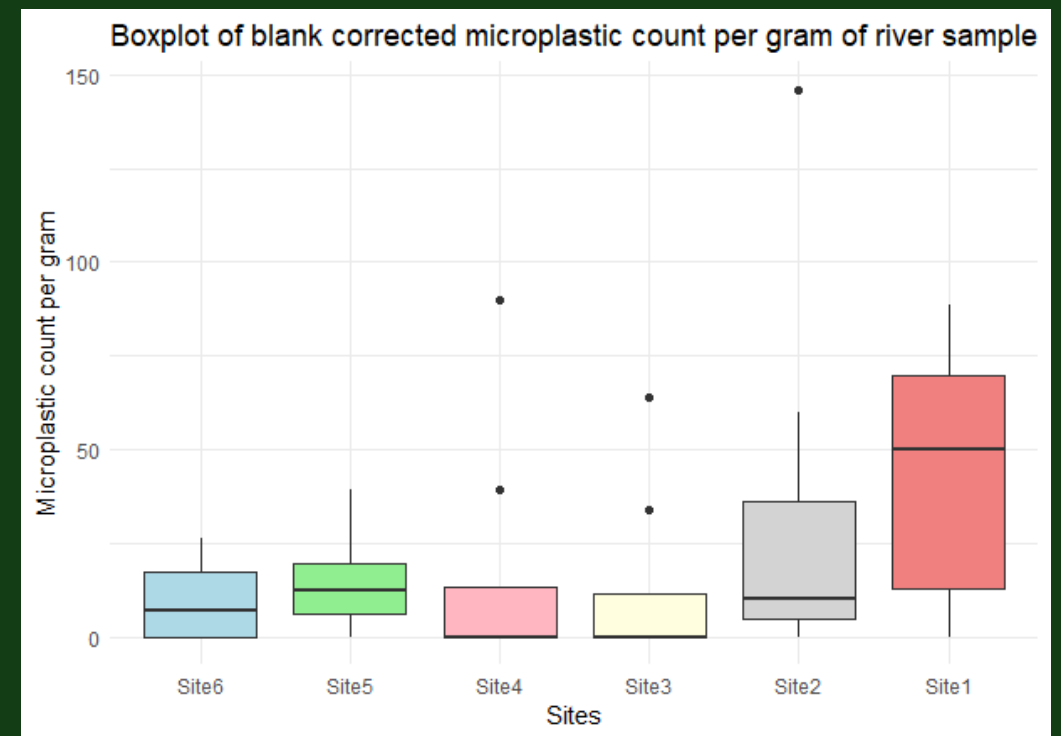
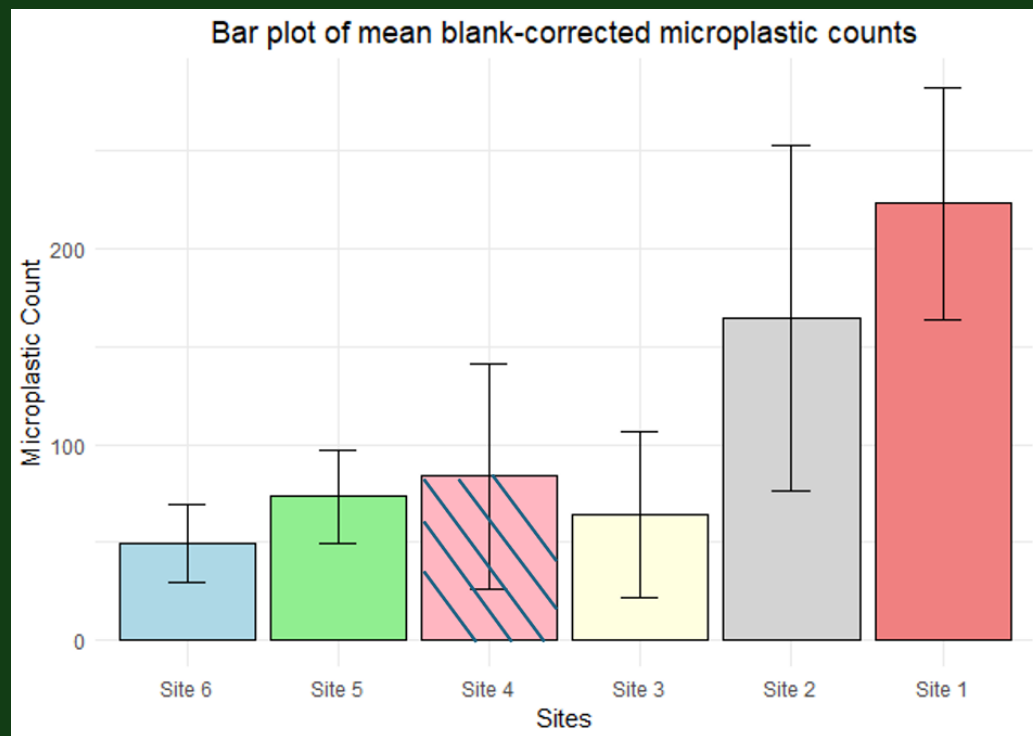
Analysis

- Microplastic count
- Water chemistry analysis
- Microplastic per gram comparisons
- Discharge vs microplastics
- Analysis implications



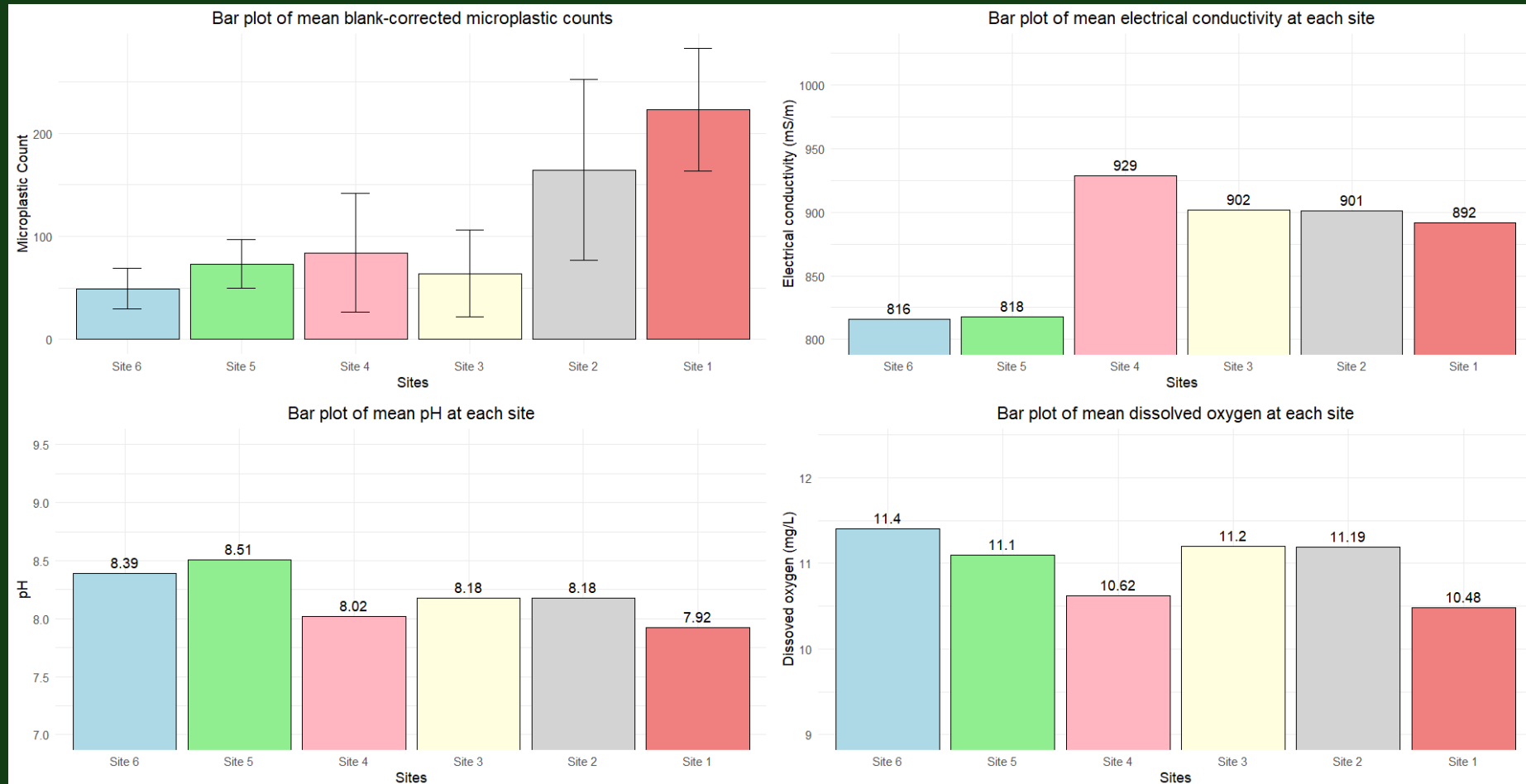
Microplastic count

- Strong downstream trend
- Inflated values of sites downstream of pollution source
- Error bars show higher variance at sites with higher microplastic count
- Most sights have some microplastic free recordings



Water chemistry analysis

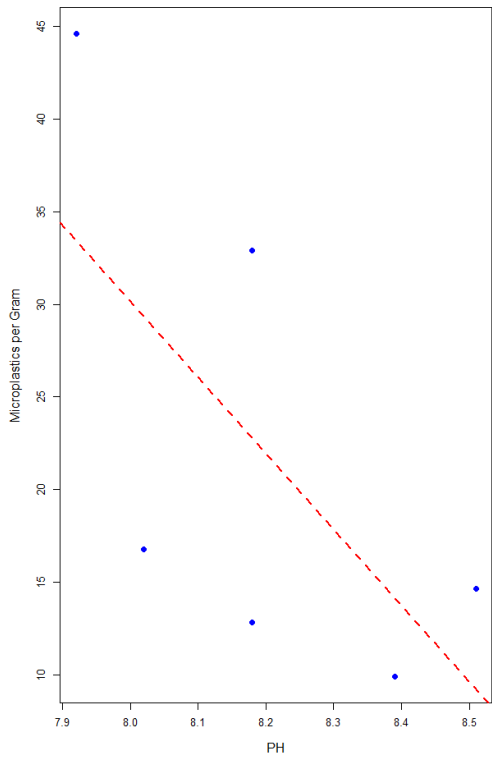
- Visual representations and statistical tests show no correlation in EC and microplastics
- Very weak negative correlation between DO and microplastic with 20% of variance being explained by DO
- Slight correlation with high microplastics at low pH which will be explored further



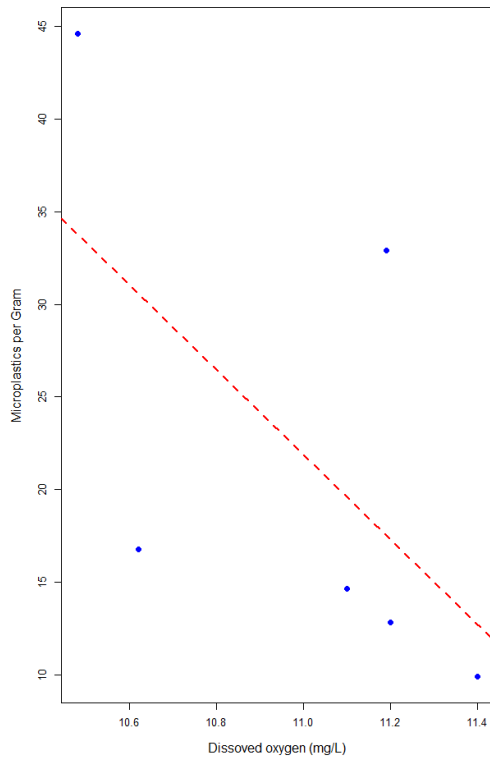
Water chemistry analysis

- Few PH anomalies
- No statistical similarity between pH mean and microplastic mean.
- Weak negative correlation between pH and microplastic at 29.5%

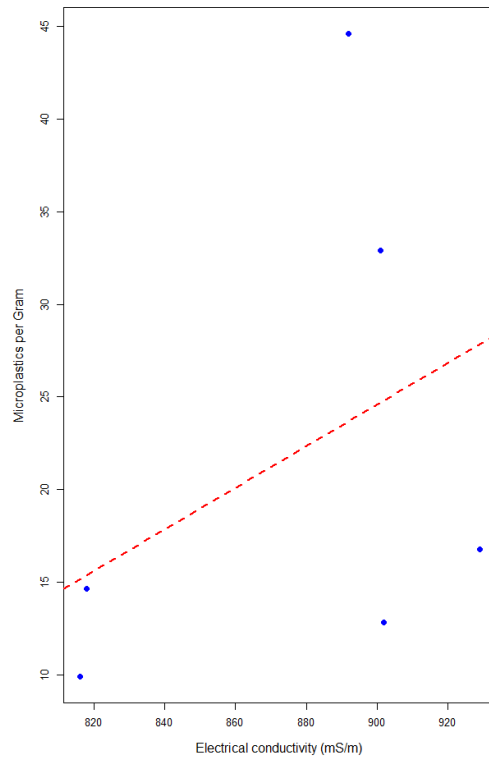
Relationship between PH and Microplastics



Relationship between DO and Microplastics



Relationship between EC and Microplastics



Welch Two Sample t-test

```
data: main_dataset$microplastics_per_gram and main_dataset$PH
t = 2.4491, df = 5.0026, p-value = 0.05798
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.679214 28.154214
sample estimates:
mean of x mean of y
 21.9375    8.2000
```

```
lm(formula = microplastics_per_gram ~ PH, data = main_dataset)
```

Residuals:

1	2	3	4	5	6
11.190	10.141	-9.959	-12.554	5.442	-4.261

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	358.64	191.69	1.871	0.135
PH	-41.06	23.37	-1.757	0.154

Residual standard error: 11.54 on 4 degrees of freedom

Multiple R-squared: 0.4356, Adjusted R-squared: 0.2945

F-statistic: 3.087 on 1 and 4 DF, p-value: 0.1537

Discharge vs microplastics

- Linear regression models shows 73.5% of microplastics per gram variance is influenced by discharge.
- T test shows there is no significant difference between the means of the two variables (p value = 0.0112).

```
lm(formula = microplastics_per_gram ~ discharge, data = main_dataset)
```

Residuals:

1	2	3	4	5	6
0.9584	7.8916	-11.2142	0.4399	3.0746	-1.1503

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	6.646	4.903	1.355	0.2468
discharge	2115.441	548.396	3.858	0.0182 *

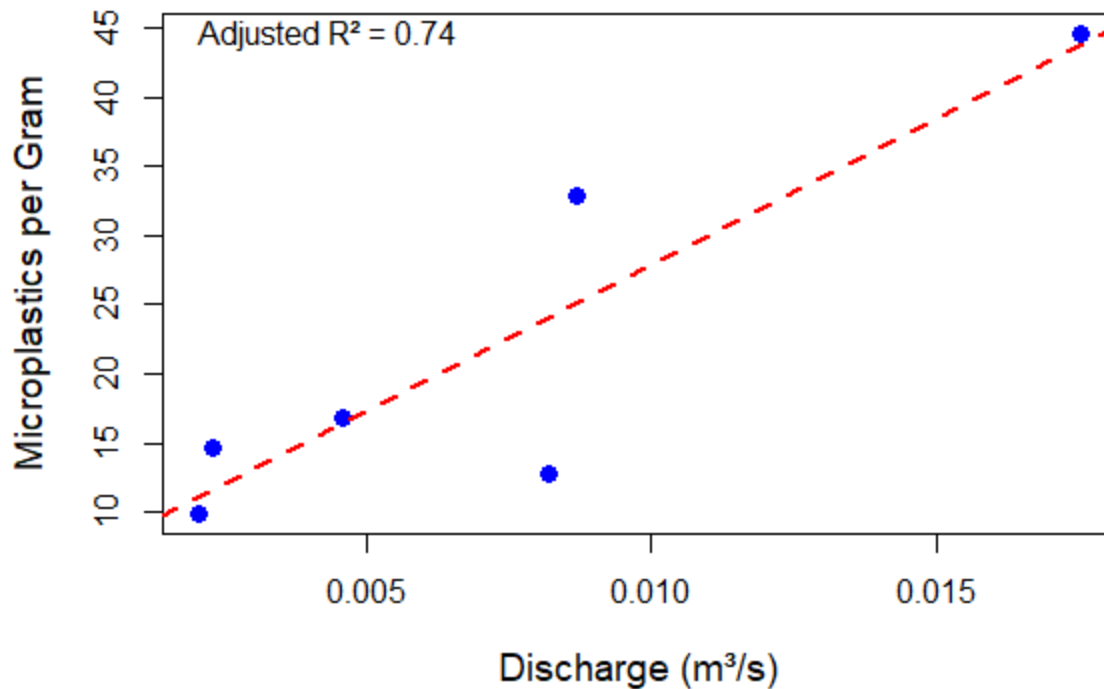
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 7.07 on 4 degrees of freedom

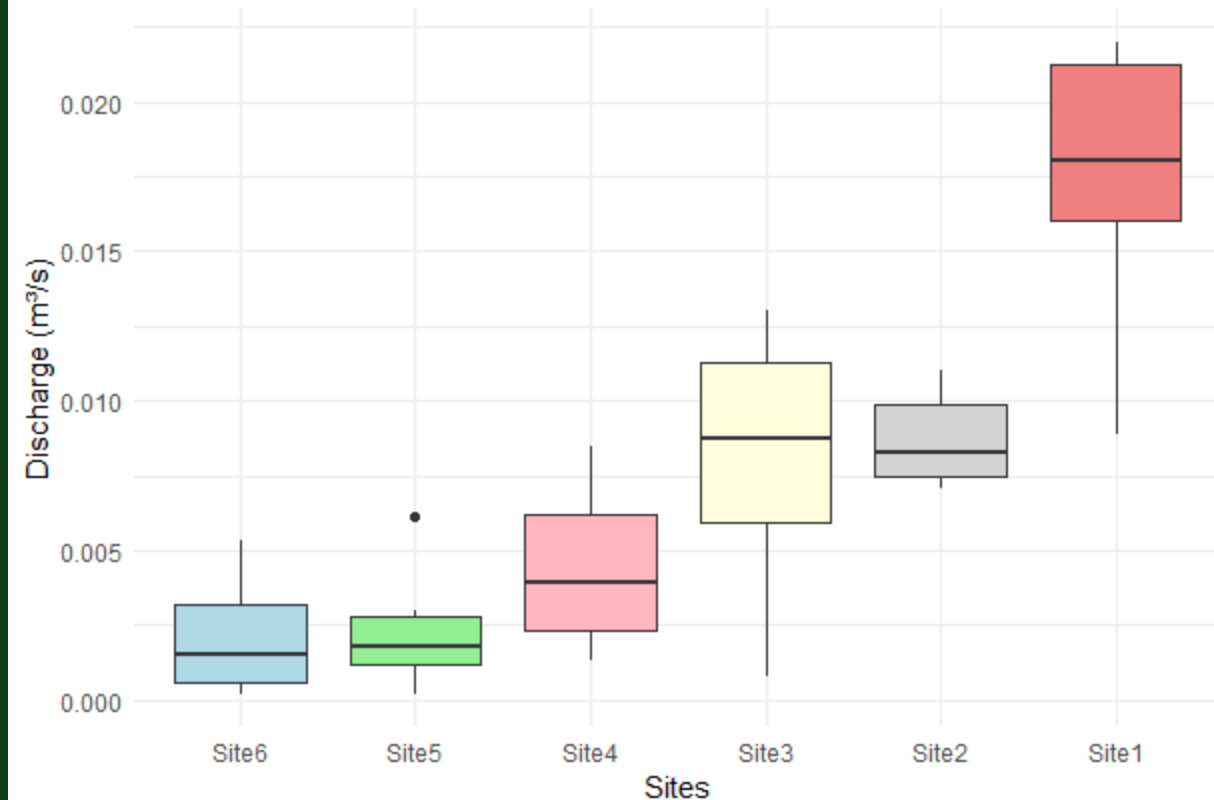
Multiple R-squared: 0.7881, Adjusted R-squared: 0.7352

F-statistic: 14.88 on 1 and 4 DF, p-value: 0.01819

Relationship between Discharge and Microplastics

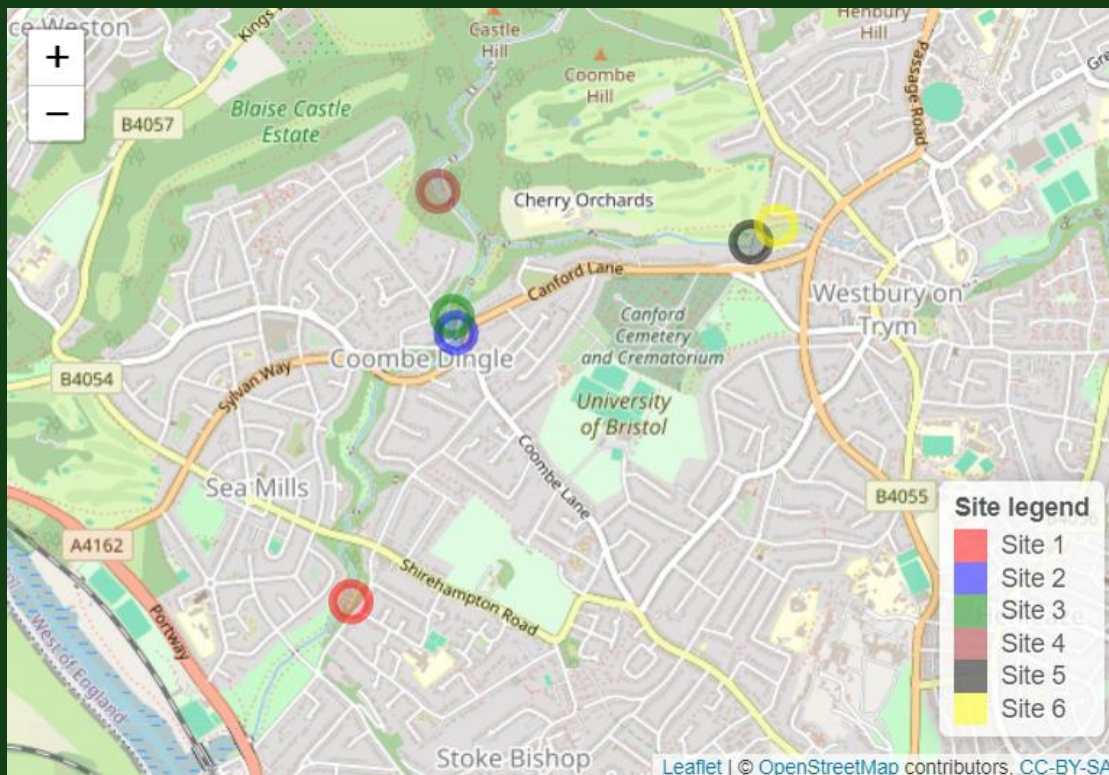


Boxplot of River Discharge from 6 Sites along the River Trym



Microplastic per gram site comparisons

- Tukey test shows very little correlation
- Site 1 and site 6 correspond the least which has the biggest distance between them
- This confirms downstream trend
- T test between site 2 and 3 shows means are significantly different showing car park effect (p value = 0.327)



Tukey multiple comparisons of means
95% family-wise confidence level

Fit: aov(formula = Values ~ Site, data = your_data_long)

\$Site	diff	lwr	upr	p adj
Site2-Site1	-11.725	-57.1196	33.6696	0.9709118
Site3-Site1	-31.825	-77.2196	13.5696	0.3105702
Site4-Site1	-27.850	-73.2446	17.5446	0.4574140
Site5-Site1	-29.975	-75.3696	15.4196	0.3754570
Site6-Site1	-34.750	-80.1446	10.6446	0.2228194
Site3-Site2	-20.100	-65.4946	25.2946	0.7714243
Site4-Site2	-16.125	-61.5196	29.2696	0.8940644
Site5-Site2	-18.250	-63.6446	27.1446	0.8342756
Site6-Site2	-23.025	-68.4196	22.3696	0.6572992
Site4-Site3	3.975	-41.4196	49.3696	0.9998214
Site5-Site3	1.850	-43.5446	47.2446	0.9999960
Site6-Site3	-2.925	-48.3196	42.4696	0.9999607
Site5-Site4	-2.125	-47.5196	43.2696	0.9999920
Site6-Site4	-6.900	-52.2946	38.4946	0.9974121
Site6-Site5	-4.775	-50.1696	40.6196	0.9995614

Secondary Data Collection - Microplastics

We gathered a variety of secondary data, all of which included studies identifying microplastics as plastic particles measuring under 5000µm ([GESAMP, 2015](#)).

We also tried to find studies which used water samples instead of sediment as the microplastic sampling method.

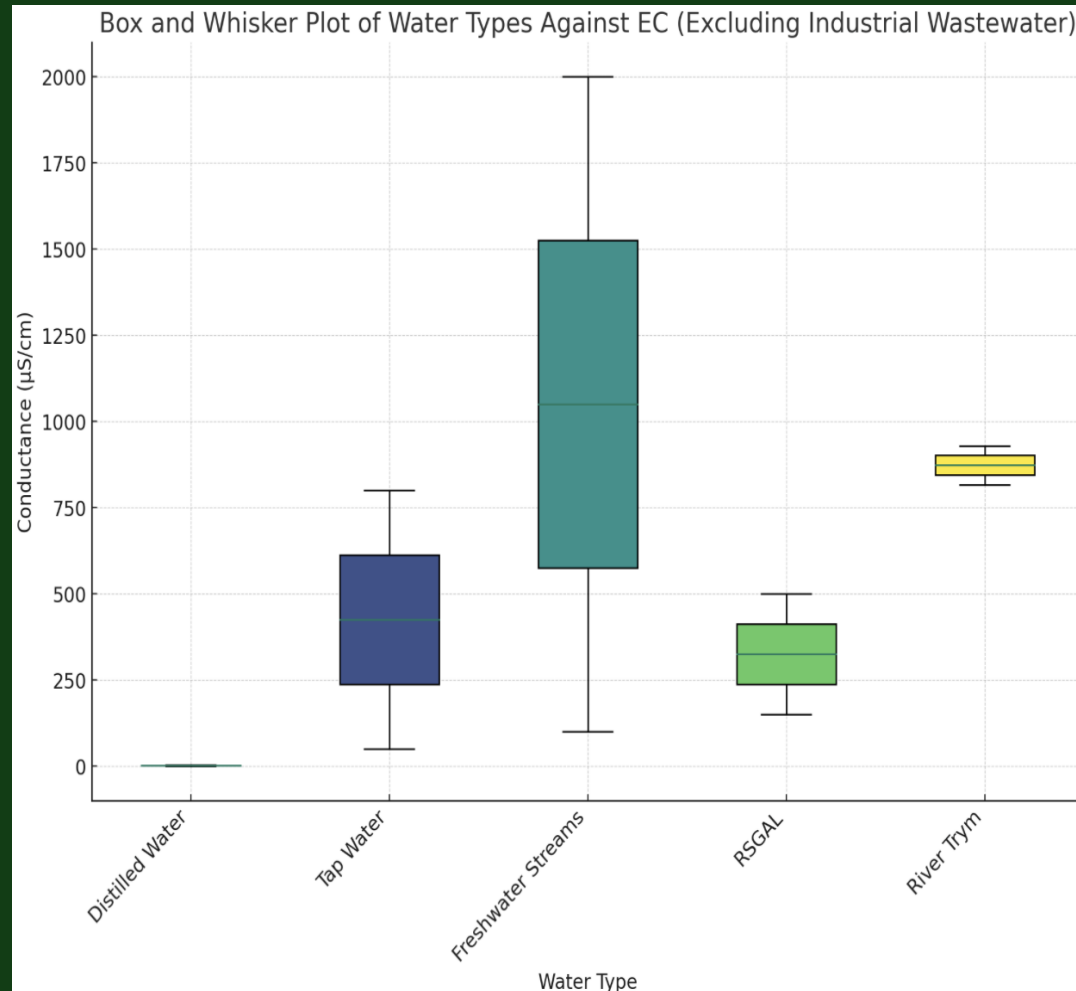
Figure Label	Reference	Size of mesh filter used	Concentration in MP/g	Landscape	Comments on Study
A	Blair et al., 2019	11 µm	0.141-0.432 MP/g	Freshwater urban river	Research of an urban river, River Klein, Glasgow
B	Tibbetts, et.al 2018	63-250 µm	0.092 MP/G	Urbanised catchment area	High concentration reflects urbanized, populated catchment area
C	Dikareva & Simon, 2019	63-500 µm	0.303MP/g	Small streams, New Zealand	Urbanization gradient study across a catchment
D	Group 6 Avon Project, 2023	0.22 µm	10.44MP	Ashton Brooke small stream, Bristol	Average of 2 points in a small stream, flowing in and out a reservoir
E	Horton <i>et al.</i> , 2016	1.2 µm	1.41 MP/g	Urban freshwater lake	Urban Freshwater lake in the UK
F	Harley-Nyang et al., 2022	1.2 µm	37.7-286.5 MP/g	Inflow at wastewater treatment works	Field and laboratory study using density separation and infrared spectroscopy
G	Trout on the Trym	1.2 µm	21.9 MP/G	River Trym	Urban River

Secondary Data Collection - EC

Here we compared the values of EC in different types of water.

RSGAL – Rivers supporting good aquatic life (SWT, n.d)

All other values are taken from (Atlas Scientific, 2022)



Water Type	Conductance Range (µS/cm)
Distilled Water	0.5-3
Tap Water	50-800
Freshwater Streams	100-2,000
Rivers supporting good Aquatic life	150-500
Industrial Wastewater	10,000
River Trym	816-929

Discussions

Analysis implications

- Small negative correlation between pH and microplastics.
- No correlation between DO and EC with microplastics.
- Strong downstream trend shows added pollution from CSO's and car park.
- Large increase in microplastics upstream and downstream from car park.
- Strong positive statistically significant correlation between discharge and microplastic per gram.



Discussions

Limitations in the **lab**

- Contamination (lab coats, plastic equipment, airborne/dust)
- Nile red (NR):**
- Potential for biogenic staining (algae, lignin)
 - Favours the detection of hydrophobic samples



Limitations in the **field**

- Sudden, unexpected change in river discharge
- Hand-held flow metre

Limitations in the **analysis**

- Counting microplastics manually lead to a large uncertainty in results, had to change method



Discussions

Suggestions for future work

- Using specialised equipment e.g. FTIR – identify polymer types – find sources
- Impacts of the microplastics for wildlife and environment
- Investigate the impact of CSOs on microplastic concentration before and after storm overflow events
- Further investigate the link between DO and Microplastics



Conclusions

Hypothesis 1: There will be more microplastics downstream than upstream of a CSO.

✓ *Accept – significant increase in microplastics directly downstream from CSO (site 5) compared to upstream (site 6)*

Hypothesis 2: There will be more microplastics found downstream of the car park than upstream.

✓ *Accept – significant increase in microplastics downstream from the car park (site 2) compared to upstream (site 3)*

Hypothesis 3: Sites with a higher discharge will have a higher concentration of microplastics.

✓ *Accept – statistically significant increase in microplastics in sites with higher discharge*

Hypothesis 4: Sites downstream from CSO will have higher EC, and lower DO, and sites with more microplastics will have a lower pH.

No correlation found between EC and microplastics, and inconclusive results regarding DO being in lower concentration downstream of CSOs, and a slight negative correlation between microplastics and pH

Questions?

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