Premise

Criteria for good bioindicators	Mussels (Mytilus sp.)
Wide geographical range	
Representative of specific monitoring area	
Species are not protected or endangered	
Suitable particle retention time	72 hours [1, 2]
Already used as a bioindicator	
Ability to ingest small and large particles	<1 mm [3]
Sedentary/can be stored in cages	
Invertebrate (less training for handling)	
Can be sampled cost effectively	
Commercially important	
Can be analysed with rapid Nile red screening	
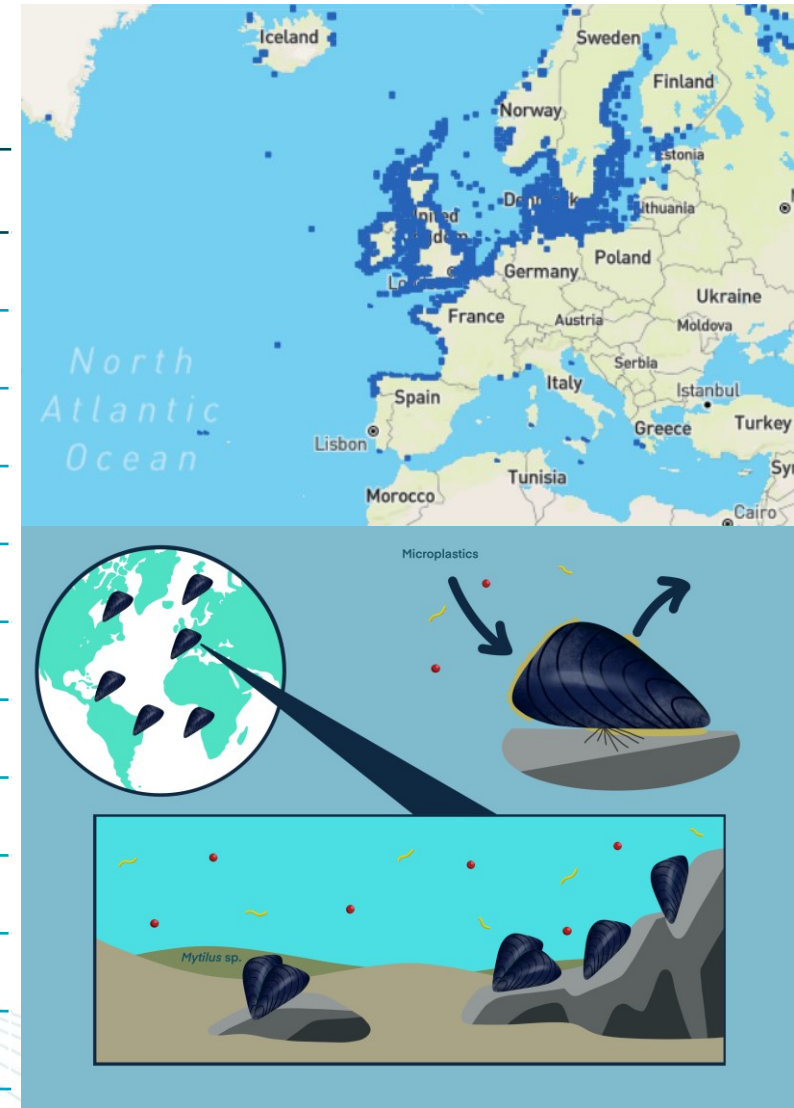
[1]Ward & Kach (2009) DOI: https://doi.org/10.1016/j.marenvres.2009.05.002
[2]Catarino et al. (2017) DOI: https://doi.org/10.1002/etc.3608
[3]Brett & Grooves (1979)

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Sampling

UK (Cefas): 7 stations

France (IFREMER): 15 stations

Spain (IEO): 5 stations

Mussels (n=25 per station)[1] handpicked as part of existing surveys.
November 2022-January 2023.

[1]Bakir et al. (2020) DOI: https://doi.org/10.3389/fmars.2020.574663
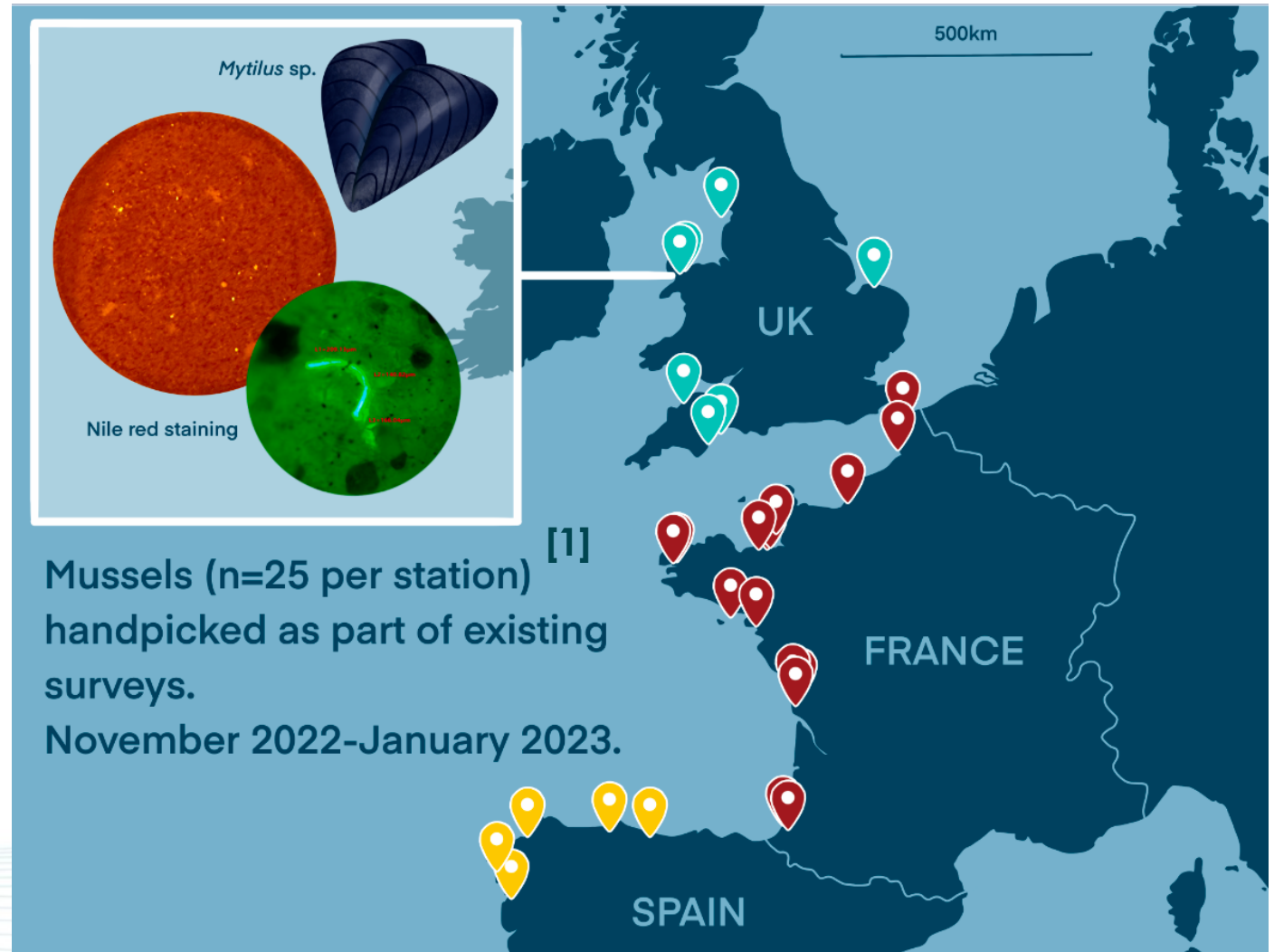
Sample processing

Mussels dissected, **removing byssal threads.**

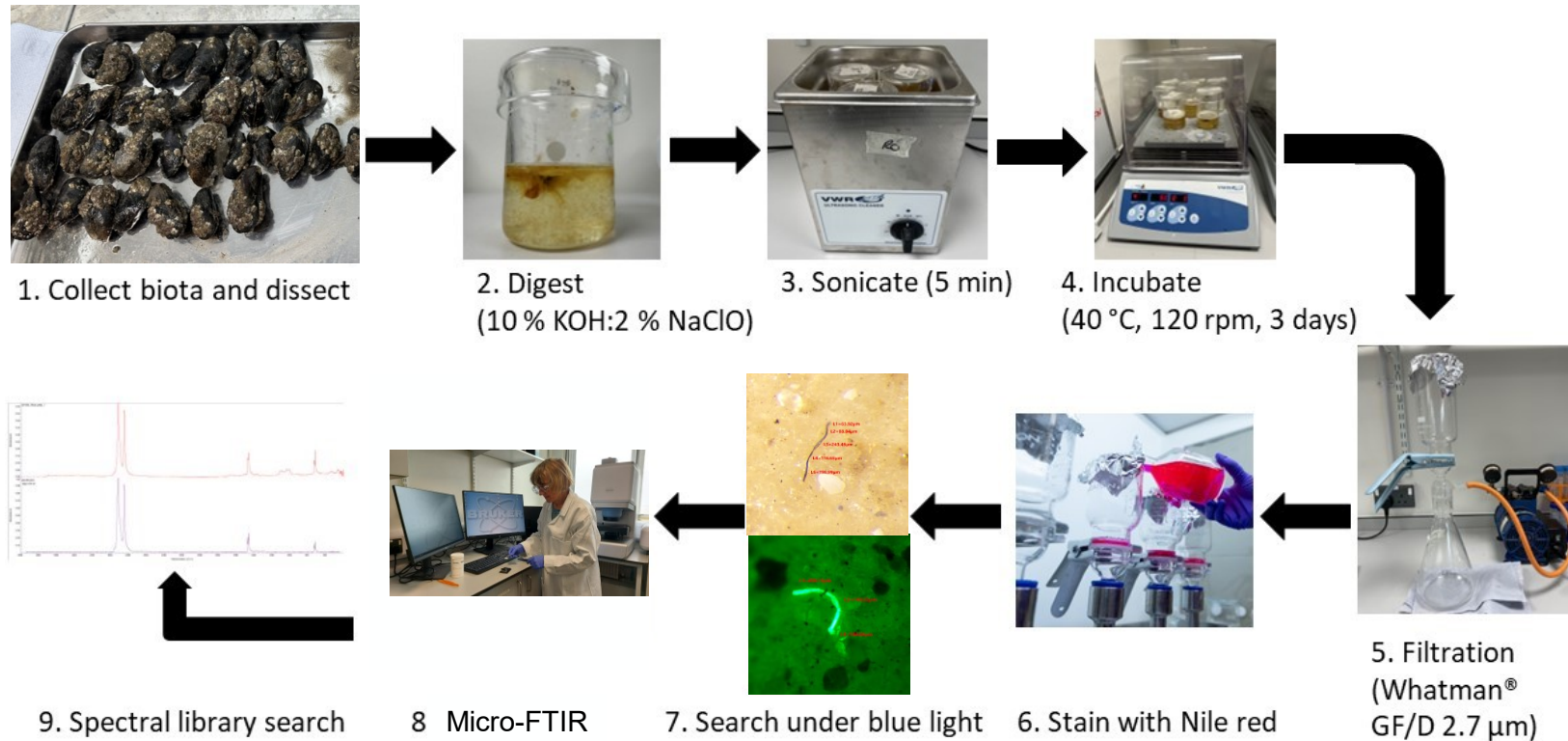Prior to microplastic extraction, tissues were **rinsed with clean water.**

50% of MPs in mussel samples are **adhered to the tissue** surface not ingested [1].

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Microlitter extraction

Two **harmonised** protocols for individual and pooled sample (20 g) analysis.

1. Collect biota and dissect
2. Digest (10 % KOH:2 % NaClO)
3. Sonicate (5 min)
4. Incubate (40 °C, 120 rpm, 3 days)
5. Filtration (Whatman® GF/D 2.7 μm)
6. Stain with Nile red
7. Search under blue light
8. Micro-FTIR
9. Spectral library search

Controls for contamination

- Cotton lab coats
- Clean nitrile gloves
- Working under a hood
- Filtered water and reagents
- Lab controls/blanks

UK
Average: 1.48 ± 1.66 (primarily white/clear fibres)

France
Average: **Data pending**

Spain
Average: **Data pending**

Method comparison
(Bootstrap percentile method)

Are pooled samples and analysis of individual mussels comparable?

Per individual
Cefas: 24 individuals
IFREMER: 21 individuals (3 reps of 7).

In total, Cefas **5 items** and IFREMER found **76.6** (extrapolated to account for analysing fewer mussels). Equating to **15.3 times higher** concentrations.

Per gram
Cefas mass (per 7 mussels): 33.19
IFREMER: 13.43 g.

Cefas used **2.47 times more tissue** with an average contamination per gram of **32.9 times less** than IFREMER.

Method comparison
(Bootstrap percentile method)

Results not directly comparable without first exploring these differences.

Table 1: Summary of comparison data between UK and France for ML per individual and per gramme

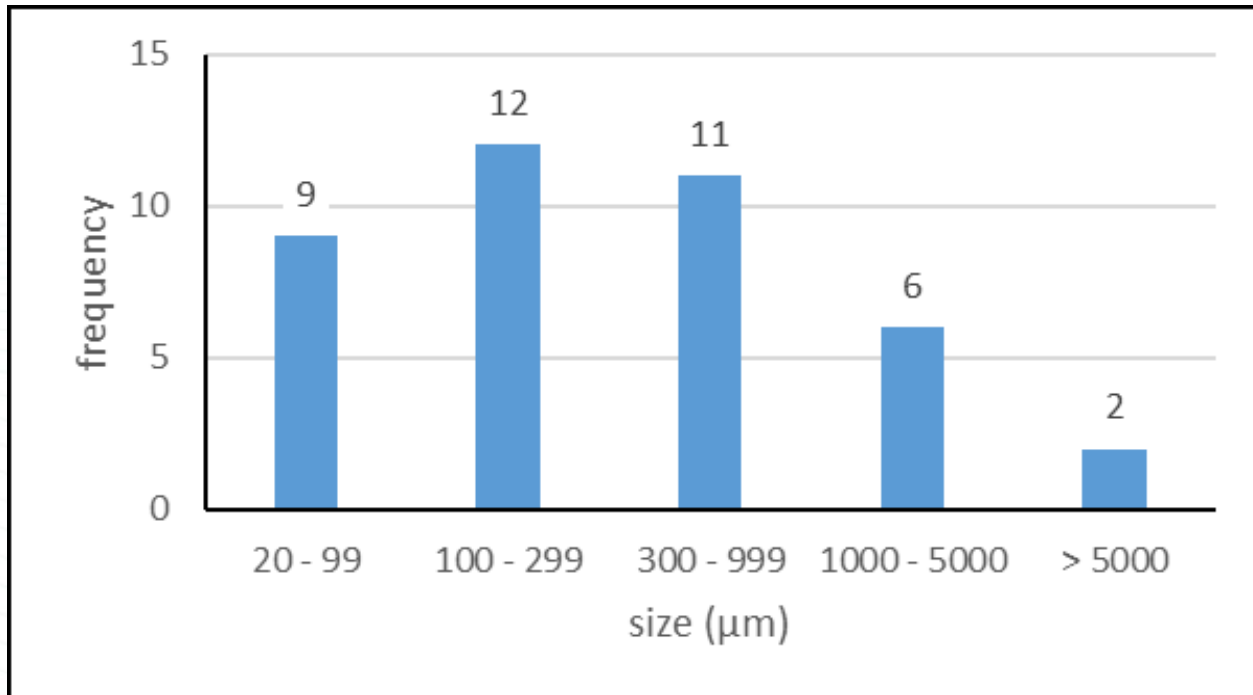|	Mean per ind	95% CI		ML per gramme	95% CI
UK	0.21	(0, 0.63)		0.051	(0, 0.14)
France	3.19	(2, 5)		1.676	(1.01, 2.67)

- The mussels sent to IFREMER may have been **smaller**, accounting for the smaller mass.
- **Loss of intervalvular liquid** during defrosting, will affect weight.
- IFREMER may be able to **detect more small MPs**, which are typically more abundant[1], than Cefas.
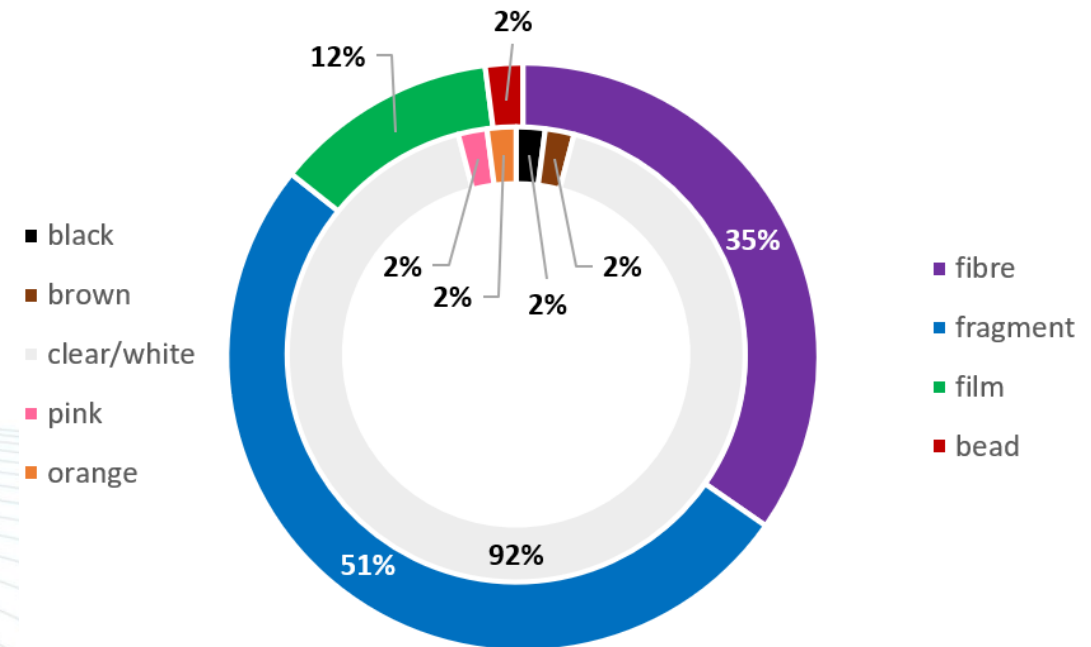
[1] Lindeque et al. (2020) DOI: https://doi.org/10.1016/j.envpol.2020.114721

UK Results

- 22 of 175 mussels (13%) with microlitter/ 6 (3%) with microplastics.
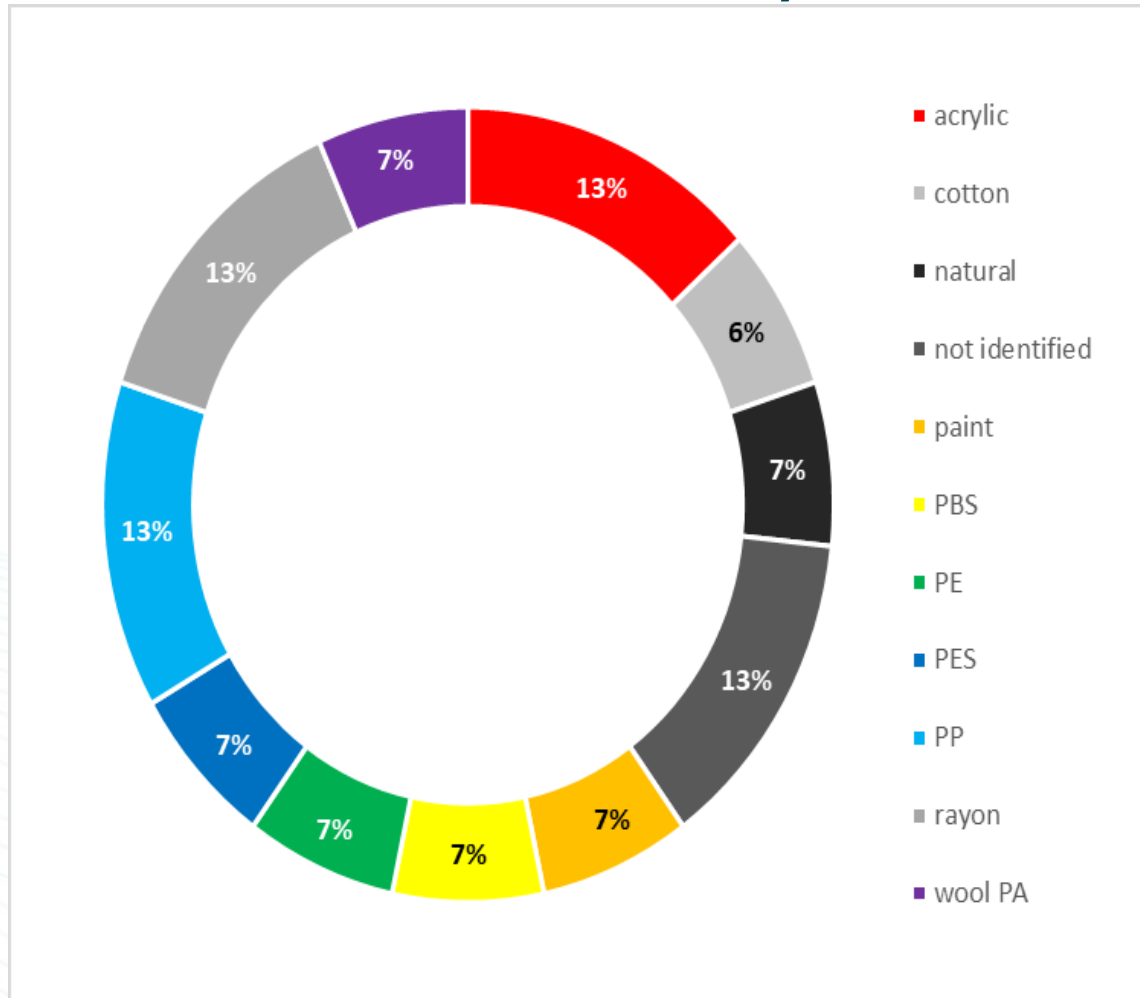- 49 microlitter items collected.

Size

Form

UK Results

Polymer identification

Legend:
- acrylic
- cotton
- natural
- not identified
- paint
- PBS
- PE
- PES
- PP
- rayon
- wool PA

Chart values: 13% (acrylic), 6%, 7%, 13%, 7%, 7%, 7%, 7%, 13%, 13%, 7%

In the UK, 15 of the 49 items identified were analysed by μ-FTIR.
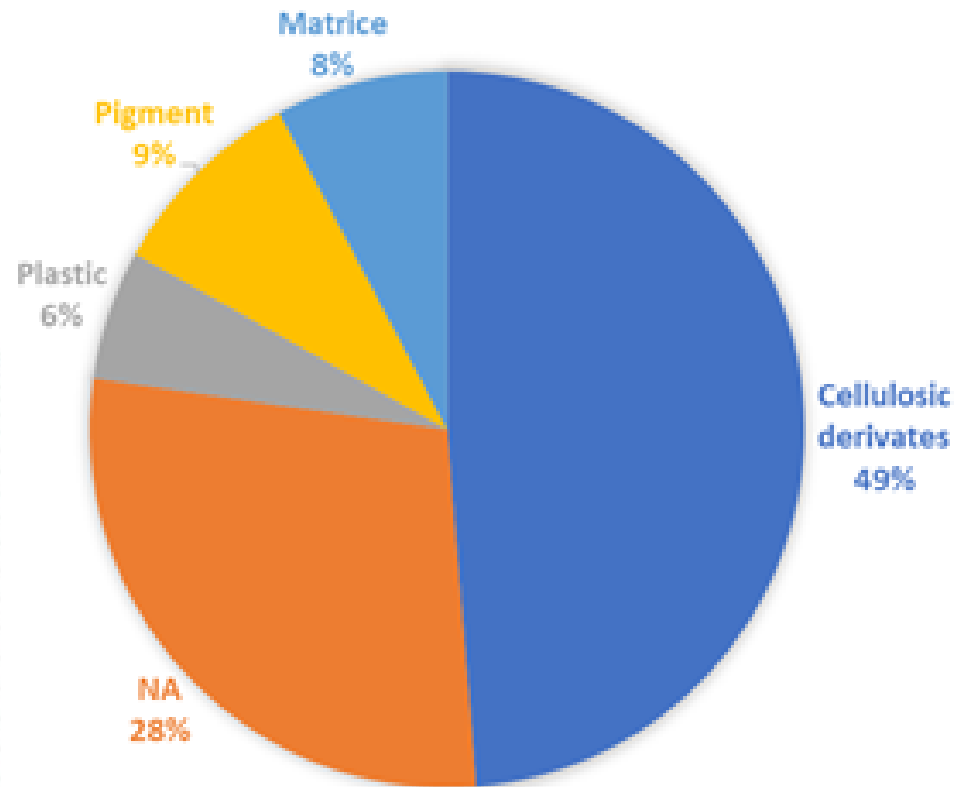
The most common materials were **acrylic** (plastic), **polypropylene** (plastic), and **rayon** (semi-synthetic).

Some items did not match library spectra and were assumed to be natural.

FR Results

- 92 MPs in 350 mussels = 26% with microlitter

Polymer identification

Matrice 8%

Pigment 9%

Plastic 6%

NA 28%

Cellulosic derivates 49%

In FR, using Raman spectroscopy, 15 plastic particles were detected with a wide variety of polymers : PDMS, PE, PAN, PP, PS, PU, Polyester...

In 15 fibers, the most common materials were Cellulosic derivates, pigment, and synthetic (plastic).

Microlitter per individual (UK)

Hot spot and cold spot at **Menai Strait**.

Need to look at local inputs and other environmental factors.

Mean contamination per individual: **0.269 ± 1.228**.

Map labels:
- 0.448 ± 0.827 — Foulney
- 0.080 ± 0.277 — Menai Strait (East & West)
- 0.680 ± 2.810
- 0.280 ± 1.021 — Blakeney
- Torridge
- 0.115 ± 0.326
- 0.208 ± 0.833 — Lyme Bay
- Fishcombe
- 0.000 ± 0.000
- UK
- 300km

Microlitter per individual (France)

Hot spot at **Le Passage**.

Mean contamination per individual: **0.257 ± 0.564**.

Map labels and values:
- Pointe de Saint Quentin: 0.037 ± 0.064
- Pointe aux oies: 0.000 ± 0.000
- Villerville: 0.185 ± 0.321
- Chausey: 0.139 ± 0.137
- Roche du Port: 0.122 ± 0.107
- Elron rive gauche: 0.533 ± 0.503
- Plage St Enogat: 0.111 ± 0.192
- Le Passage: 1.500 ± 1.500
- Baie Arguenon: 0.000 ± 0.000
- Houat: 0.104 ± 0.100
- Pointe de Chemoulin: 0.375 ± 0.650
- Longe de Boyard: 0.033 ± 0.058
- Bouchots de Charente: 0.500 ± 0.866
- Galon d'Or: 0.000 ± 0.000
- Estacade de Capbreton: 0.486 ± 0.578
- Port de Bayonne: 0.000 ± 0.000

FRANCE

300km

Microlitter per individual (Spain)

Ferrol
0.233 ± 0.252

Pravia
1.916 ± 0.509

Muros
1.026 ± 1.074

Ribadesella
1.144 ± 0.288

Vigo
0.083 ± 0.144

SPAIN

300km

Hot spots at **Ribadesella, Pravia** and **Muros**.

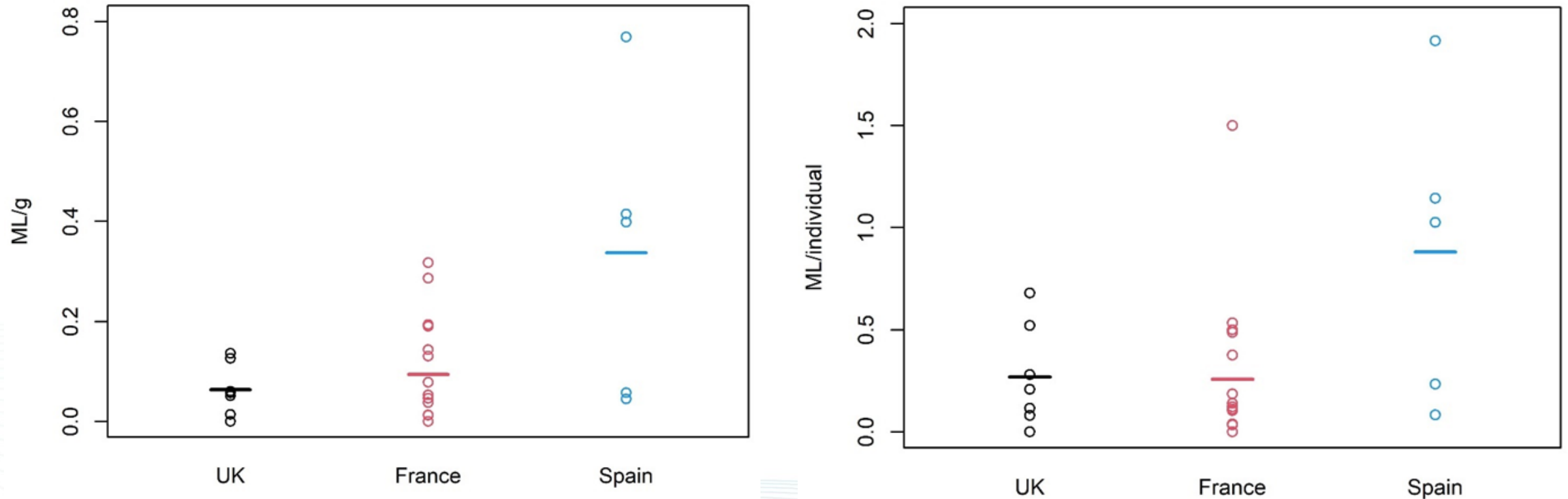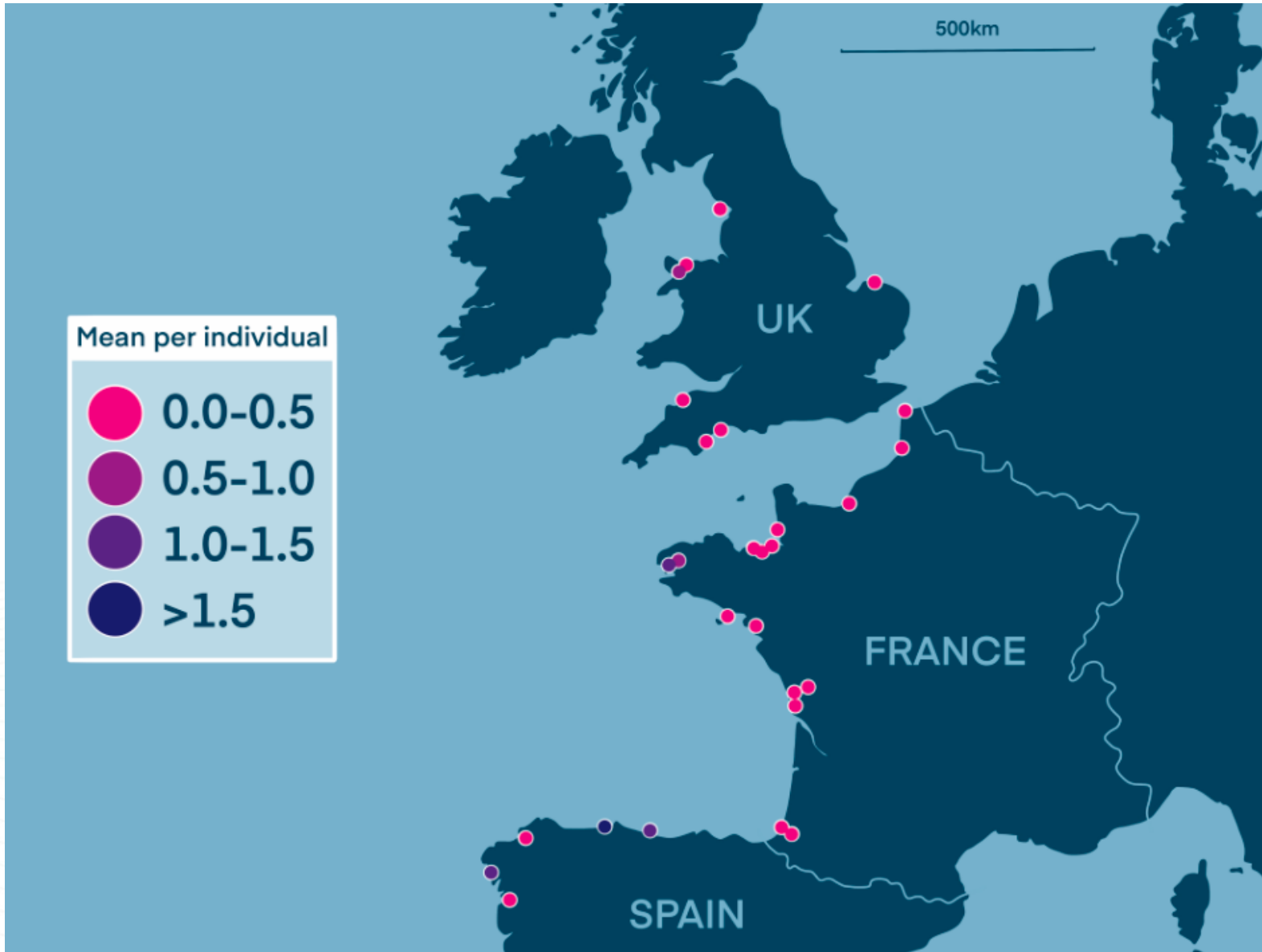Mean contamination per individual: **0.881 ± 0.837**.

Statistical analysis

Figure 1: Station means of ML per gramme (left) and per individual (right) for each country. Also shown is the overall mean (short, horizontal line).

Hot spots differ between regions, but generally concentrations are similar.

Discussion
A global context

Mean per gram (range):
UK: 0.06 ± 0.26 (0–2.7)
France: 0.09 ± 0.17 (0–0.65)
Spain: 0.32 ± 0.35 (0–1.04)

Fits within the bounds of previous studies on mussels (not shop bought). But are generally low in a global context.

Figure 1. The overall microplastics per gram (MPs/g) content for mollusks illustrated in a log base 10log10log10 scale. Points represent mean MPs/g values for the studies, where reported. Whiskers represent the reported ranges of MPs/g.
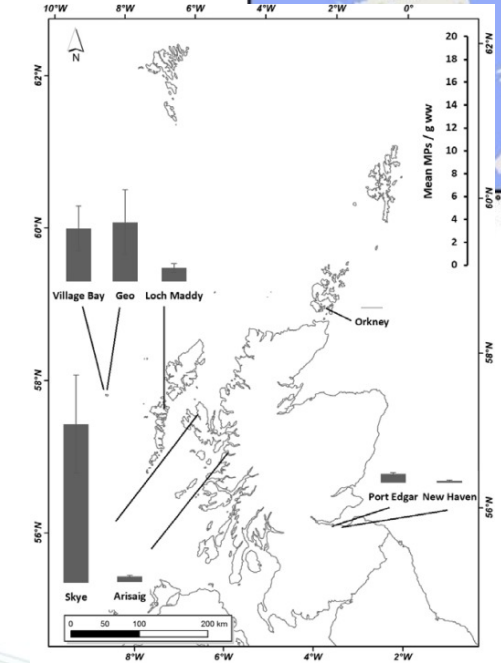

[1]

[1] Danopoulus et al. (2020) DOI: https://doi.org/10.1289/EHP7171
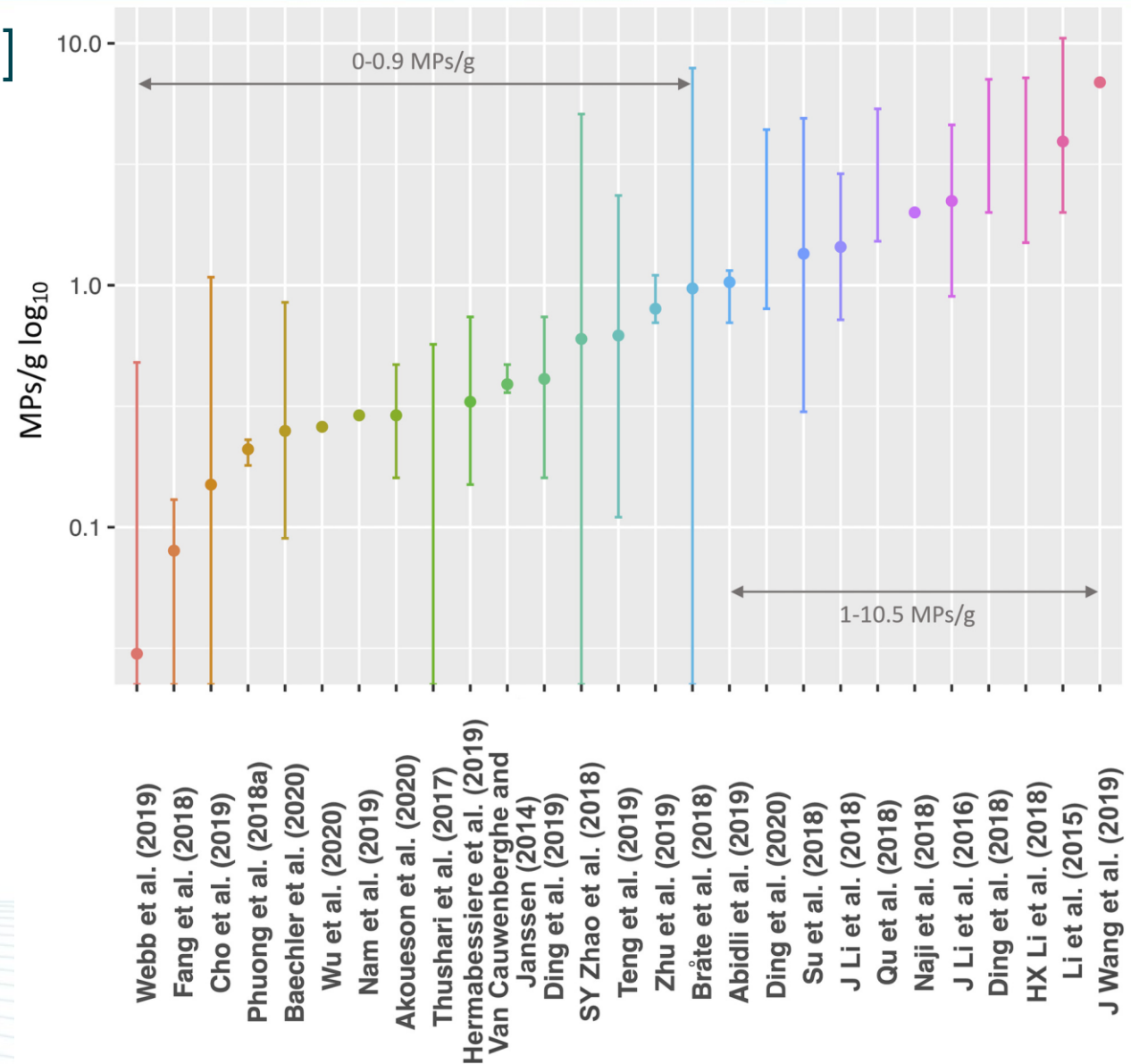
Discussion
UK context – *Mytilus edulis*

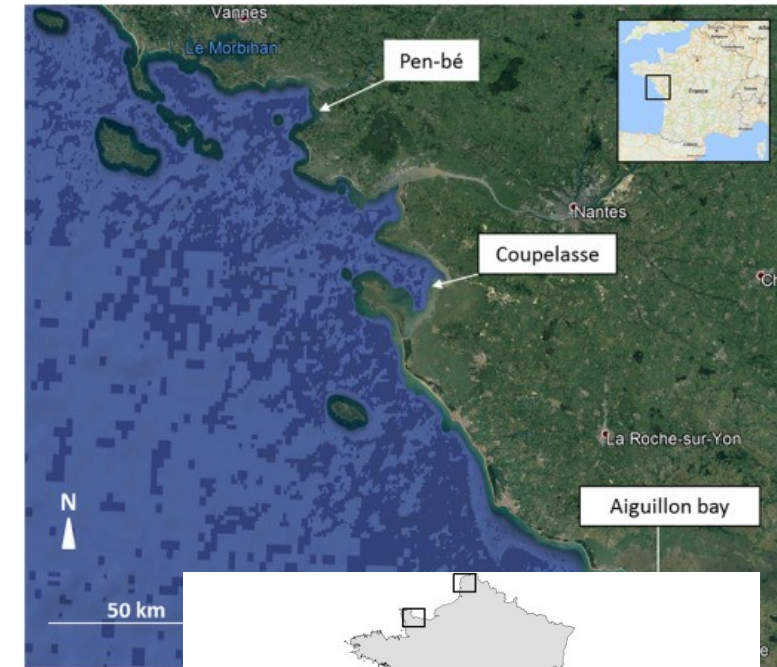| UK	Mean per individual (range)
Present study	0.3 (0–14)
Scott et al. (2019)	1.43-7.64 (NR) - meso included
Li et al. (2018) [1]	Not reported (NR) (1.1-6.4)
Catarino et al. (2018) [2]	3.2 (NR)
Courtene-Jones et al. (2017)	Per g reported only 1.1-4.4 (NR)

[1] Li et al. (2018) DOI: https://doi.org/10.1016/j.envpol.2018.05.038
[2] Catarino et al. (2018) DOI: https://doi.org/10.1016/j.envpol.2018.02.069

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Discussion
French context – *Mytilus edulis*

[1]

[2]

France	Mean per individual (range)
Present study	0.3 (0–3)
Hermabessiere et al. (2019) [2]	0.76 (NR)
Phuong et al. (2018a) [1]	0.6 (NR)
Phuong et al. (2018b)	Per g reported 0.23 (NR)

[1] Phuong et al. (2018a) DOI: https://doi.org/10.1016/j.marpolbul.2017.10.054
[1] Hermabessiere et al. (2019)
DOI: https://doi.org/10.1016/j.envpol.2019.04.051
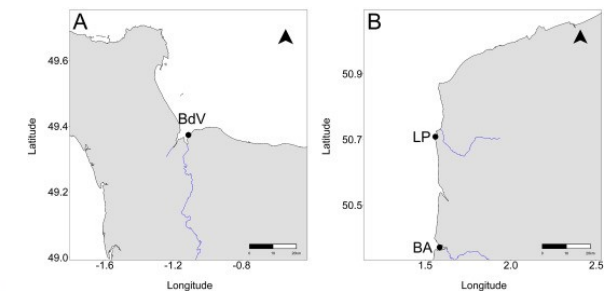
Discussion
Spanish context – *Mytilus* spp.

Spain	Mean per individual (range)
Present study	0.9 (0–2.4)
Reguera et al. (2019) [1]	Per gram reported only 1.6-2.6 (NR)

[1] Reguera et al. (2019) DOI: https://doi.org/10.3989/scimar.04927.05A

Next steps

- Report

- UK to analyse non-fluorescent particles which may have been missed by Nile red (e.g., fibres, dark coloured particles, semi-synthetic material)

- FR goes further MP analyses

- Feed into OSPAR on bioindicator from µP

- Publish? (individual countries and/or comparison study)

Thank you for listening!

Happy to take questions.

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