Micro- and nanoplastic quantification and effect studies **don’t match**

Tanja Kögel, Ørjan Bjorøy,
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Plastic particles are everywhere and are taken up by aquatic organisms

But only into the gastrointestinal system ?

- Fish fillet (Karami, Golieskardi et al. 2017)
- Fish liver (Collard, Gilbert et al. 2017)
- Blue mussel feet (Kolandhasamy, Su et al. 2018)

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Thresholds for organ entry in aquatic biota

µm

Epithelial cells of the **intestinal wall** of zebrafish, epithelial intestinal barrier of waterflea, **hemolymphe** of mussel, **liver** of zebrafish, **gills** of mitten crabs

(Batel et al., 2016; Rosenkranz et al., 2009; Browne, Dissanayake et al. 2008; Avio, Gorbi et al. 2015; Brennecke et al., 2015)

nm

Hemolymph, stomach, hepatopancreas, ovary and **gills** in a transfer experiment from mussel to crab, **pancreas, gallbladder, heart, brain, eggs** and **eyes** of zebrafish, **blood**, gallbladder, heart, brain and **testis** of Japanese medaka, ovary of water flea, brain of crucian carp, **yolk sac** of Chinese rice fish

(Kashiwada, 2006; Farrell and Nelson, 2013; Mattsson et al., 2017; van Pomeren et al., 2017 ; Cui et al., 2017; Pitt et al., 2018; Chae et al., 2018)

The smaller the particles, the

- more uptake
- into more tissue types
- slower excretion

(Jani et al. 1992; Kashiwada et al. 2006; Browne et al. 2008; Jeong et al. 2016; Mattson et al. 2017 (same surface area); Critchell et al. 2018; Manabe et al. 2011; Farrell and Nelson, 2013; Sussarellu et al., 2016; Van Cauwenberghe et al., 2015;)

What harm do they do?

Systematic review

(submitted)

Micro- and nanoplastic toxicity on aquatic life: Determining factors

Tanja Kögel[1,3], Ørjan Bjorøy[1], Benuarda Toto[2], André Marcel Bienfait[1], and Monica Sanden[1]

Plastic particle toxicity (PPT)

- Crustaceans incl. plankton
- Gastropods – mostly bivalves
- Fish
- Other animals (incl. sea urchins, worms, corals)
- Phytoplankton

Supplemental table 1: PPT on crustaceans

Species	1-999 nm	1-9 µm	10-500 µm	>500 µm	Polymer	Time	Effects and exposure route	Factors	Citatio
Waterflea *Daphnia galeata*	52 nm *5 mg/l*				PS	5 d	Survival and reproduction significantly decreased, low hatching rate. Embryos showed abnormal development, low hatching rate. Adults stored fewer and smaller lipid droplets. Cross-generational transfer of PS NPs.	**Developmental stage**	(Cui et a 2017)
Waterflea *Daphnia magna*	70 nm *0.22-103 mg/l*				PS pristine, aged	3 w	OECD guidelines 2008. Reduced body growth rate and neonate production. Malformations from 30 mg/l. Pristine PS was not lethal, in contrast to PS 5 days pre-incubated with algae.	**Concentration** **Particle condition**	(Besselir et al., 201
	60 nm *2-10 mg/l*				PS	5 h feed 1-2 d tox.	Fed with algae grown with PS. Squashed and torn-out microvilli, no mortality. Toxicity test: Little or no mortality of toxicity from direct exposure to PS.		(Chae et a 2018)
	200 nm *1-80 mg/l*				PS, PS-COOH	2 d	Immobilized to higher extent for PS-COOH (28 – 63% at 20-30 mg/l, 90% at 80 mg/l) than for PS (ca. 8-13% at 20-30 mg/l).	**Concentration** **Particle condition**	(Kim et a 2017)
	50, 500 nm *2.5-14.5 mg/l*	5 µm *2.5-50 mg/l*	10, 15 µm *2.5-50 mg/l*		PS	2 d	Acute toxicity test United States EPA guidelines: 50 nm PS showed significant immobilization.	**Concentration** **Particle size**	(Ma et a 2016)
	88, 110, 300 nm *0.1 -1000 mg/l*				PS-NH$_2$ PS-COOH	6-24 h	PS-COOH NPs incubated in conditioned versus non-conditioned media for 6 h or 24 h elicited an exposure time dependent decrease in EC50 from 36.3 mg/l to 33.7 mg/l and to 9.5 mg/l, respectively. PS-NH$_2$ NPs were more toxic than PS-COOH. Feeding rates decreased in neonates that had been exposed to conditioned NPs.	**Exposure time** **Particle condition**	(Nasser a Lynch, 2016))
	86-125 nm *0.01-1000 mg/l*				PMMA PMMA-PSMA	2 d	Acute toxicity test: Immobilization of daphnia only for PMMA-PSMA.	**Polymer type**	(Booth e al., 2016
	100 nm *0.1-1 mg/l*	2 µm *0.1-1 mg/l*			PS	1 d 3 w	Acute test, OECD 2008: Exposure to 100 nm PS decreased feeding rates. Chronic test: Lower burden in presence of food. Decreased feeding rates for 100 nm particles but not for 2 µm. No significant differences on reproduction.	**Particle size** **Environment**	(Rist et a 2017)
	55 nm, 110 nm *0.4-100 mg/l*				PS-PEI	0.5 h	Toxicity (EC$_{50}$ < 0.77 mg/l) of conc. > 0.4 mg/l, increasing with size. Slightly less sensitive than *Raphidocelis subcapitata.* More sensitive than *Thamnocephalus platyurus.*	**Concentration** **Particle Size** **Species**	(Casado al., 2013
		2 µm *146 mg/l*			PS-COOH fl.	4 h 3 w	Neonates: 4 h, no effect. Adult: 3w, increased mortality after seven days. Differences related to algal concentration. Where ample food is present, MPs have little effect on	**Exposure time** **Environment**	(Aljaibac and

Plastic particle toxicity (PPT) on aquatic biota

PPT effects

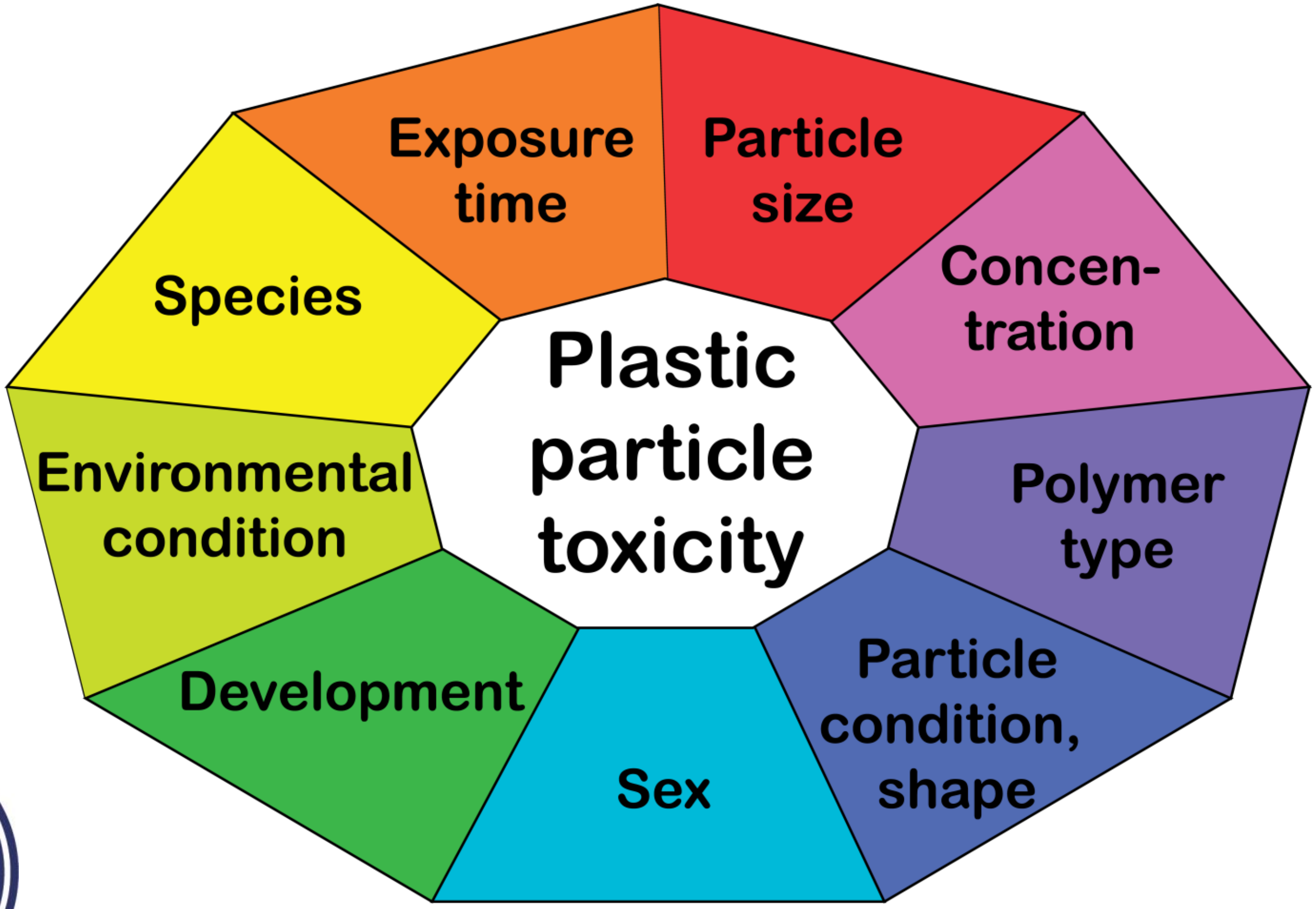
Effects	Reduced body growth or energy	Reduced population growth or survival	Reduced activity	Physiological stress, hormonal disregulation	Cell death, general toxicity	Abberant development	Altered lipid metabolism	Increased body growth or food consumption	Neuropathology	Liver or kidney pathology	Intestinal damage	Affected photosynthesis, chlorophyll	Other
<10 µm													
Crustaceans	9	14	9	4	4	4	1		2		1		3
Gastropoda	8	3	2	6	4	2	1	2	1				1
Fishes	5	1	8	5	3		5	2	2	4	1		3
Animals, other	4	1	1	3	2	4							1
Phytoplankton		7		3	1	1						5	1
sum	26	26	20	21	14	11	7	4	5	4	2	5	9
≥10 µm													
Crustaceans	5	7	3	1			1	2		1			
Gastropoda	2				1	1							2
Fishes	4	1	5	5	1		1	2	2	1	4		
Animals, other	3		1		1	2	1						2
Phytoplankton								1					1
sum	14	8	9	6	3	3	3	5	2	2	4	0	5

73

63

Do not share without consent.

Determining factors for plastic particle toxicity (PPT) on aquatic biota



Plastic particle toxicity

Exposure time

Particle size

Concen-tration

Polymer type

Particle condition, shape

Sex

Development

Environmental condition

Species

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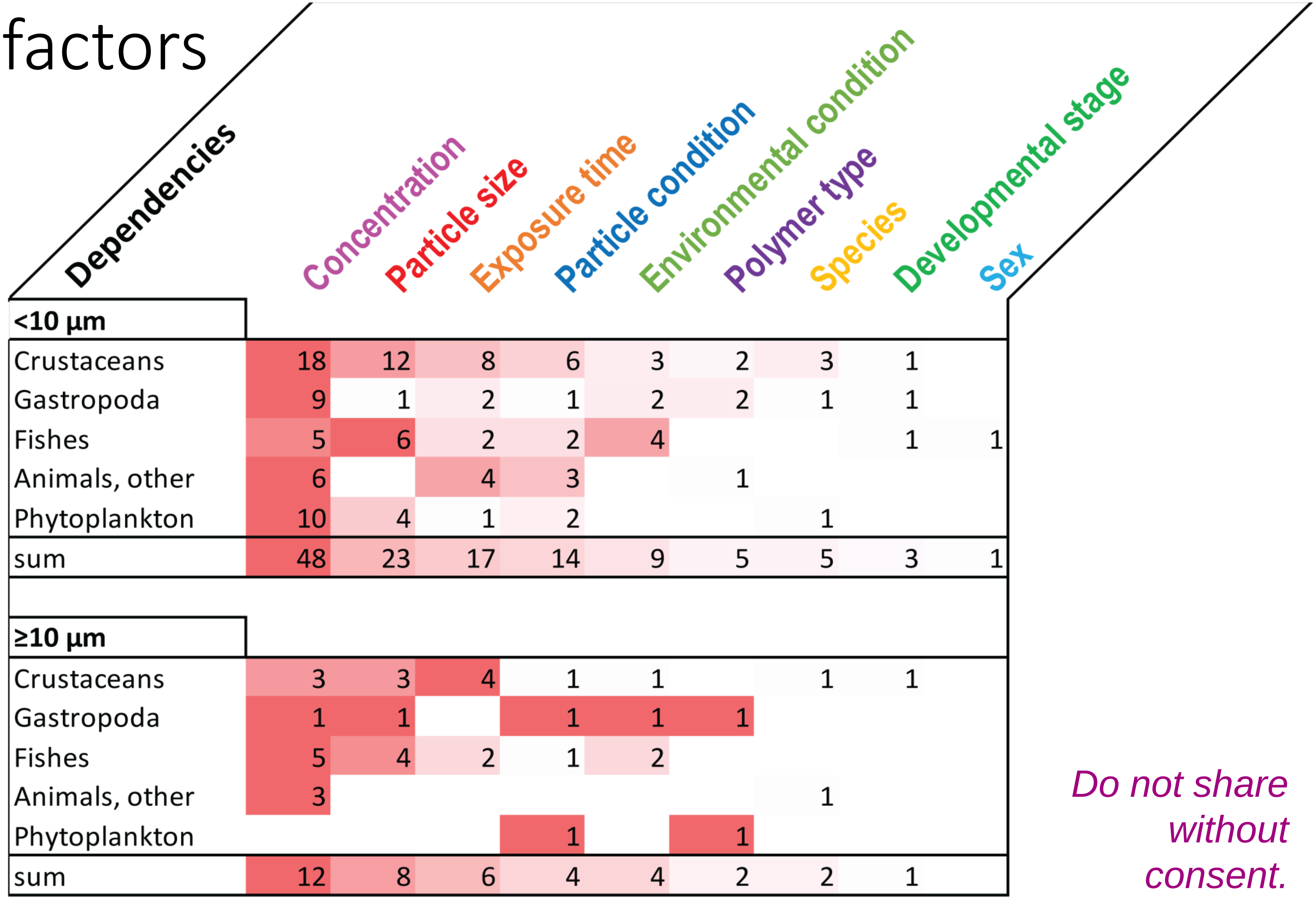
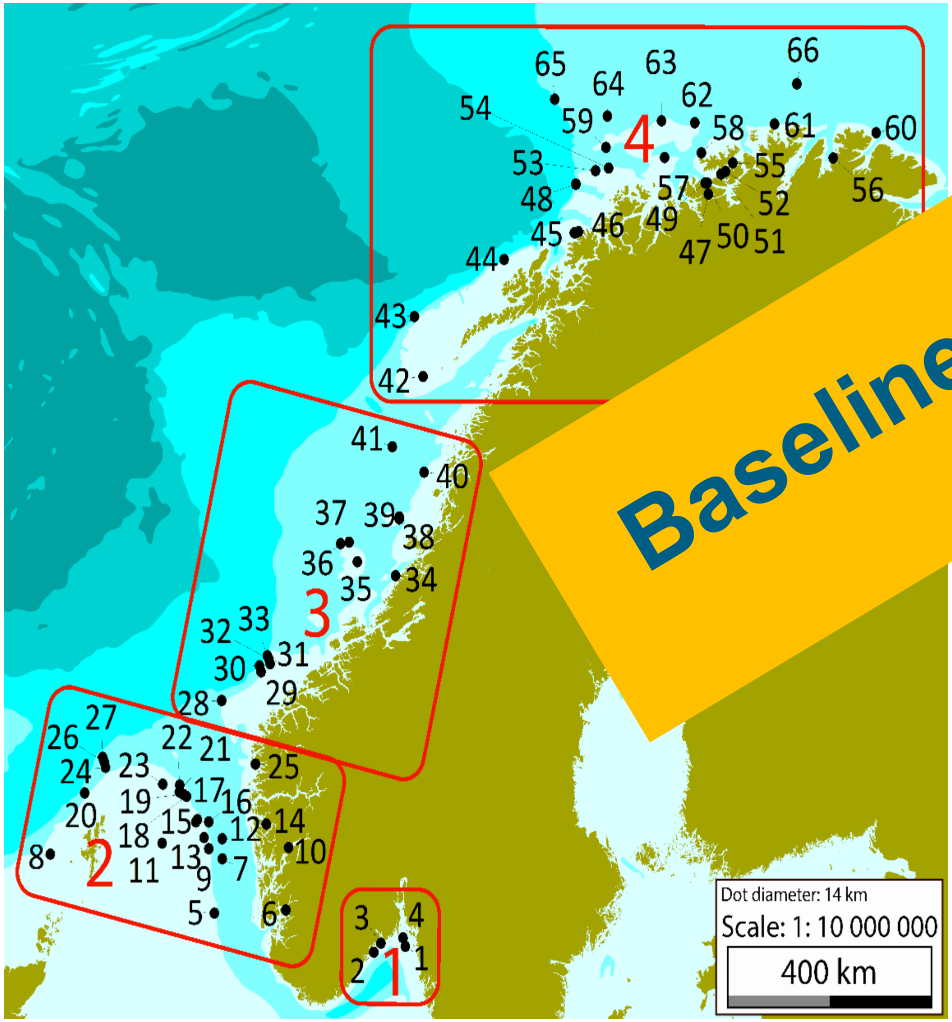
Determining factors



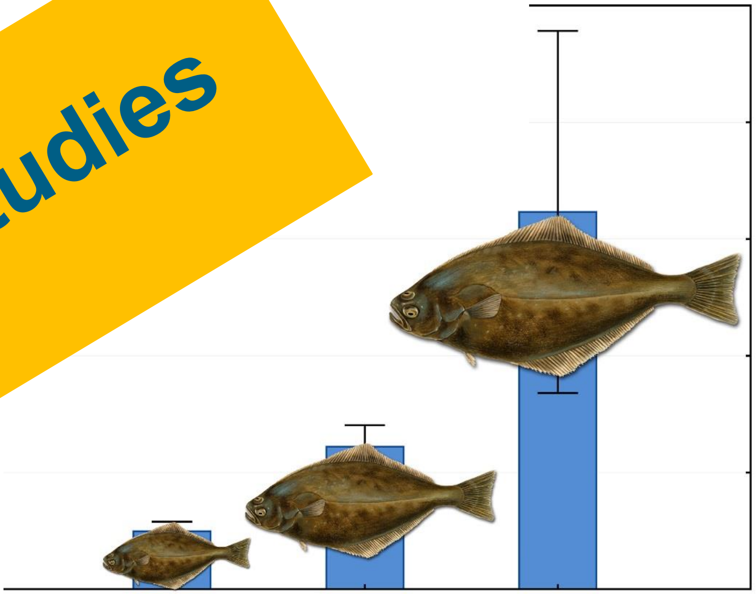
Dependencies	Concentration	Particle size	Exposure time	Particle condition	Environmental condition	Polymer type	Species	Developmental stage	Sex
<10 µm									
Crustaceans	18	12	8	6	3	2	3	1	
Gastropoda	9	1	2	1	2	2	1	1	
Fishes	5	6	2	2	4			1	1
Animals, other	6		4	3		1			
Phytoplankton	10	4	1	2			1		
sum	48	23	17	14	9	5	5	3	1
≥10 µm									
Crustaceans	3	3	4	1	1		1	1	
Gastropoda	1	1		1	1	1			
Fishes	5	4	2	1	2				
Animals, other	3						1		
Phytoplankton				1		1			
sum	12	8	6	4	4	2	2	1	

Catch stations

Mercury concentration



Baseline studies



< 10kg 10–50kg 51-225kg

[Fish weight]

Baseline studies, B. Nilsen et. al., NIFES/IMR

Baseline studies

- Substance concentration
  - Variation with
    - Species/tissue
    - Size
    - Location
    - Season

Risk evaluation

- Long term toxicity
  - EU Maximum level
  - EFSA tolerable intake (TWI/TDI)/Scientific opinion…
  - MOE (margin of exposure, based on BMLD$_{10}$)

Species	**Contaminant**
All fish	Mercur
Oily fish fish liver	Di ts
Shellf	mium/Lead
C	Cadmium
Sm d fish Shellfish	PAH

Risk-based monitoring

What do we measure in the environment?

80% of 1655 articles do not take into account plastic <300 µm.

Conkle, J. L., C. D. B. Del Valle, et al. (2018).

Those quantified down to 10 µm:

1. Bergmann et al. 2017 (Barents Sea sediments)

2., 3. Mintenig et al. 2017; Simon et al., 2018 (German and Danish waste water treatment plant effluent

4., 5. Fischer 2017, Pellini et al., 2018 (Fish stomach)

6. Peeken et al. 2018 (Arctic sea ice)

7. Haave et al. 2019 (Bergen fjord)

8. Mani et al. 2019 (River Rhine sediments)

9. Fischer et al. 2019 (Salt, water, sediment)

10., 11. Liu et al., 2019; Olesen et al., 2019 (Sediments of Norwegian urban storm water retention ponds)

12. Vianello et al., 2019 (Indoor air)

13. Bergmann et al. 2019 (Snow)

Microplastics in the city fjord of Bergen

Marine Pollution Bulletin 141 (2019) 501–513

Contents lists available at ScienceDirect

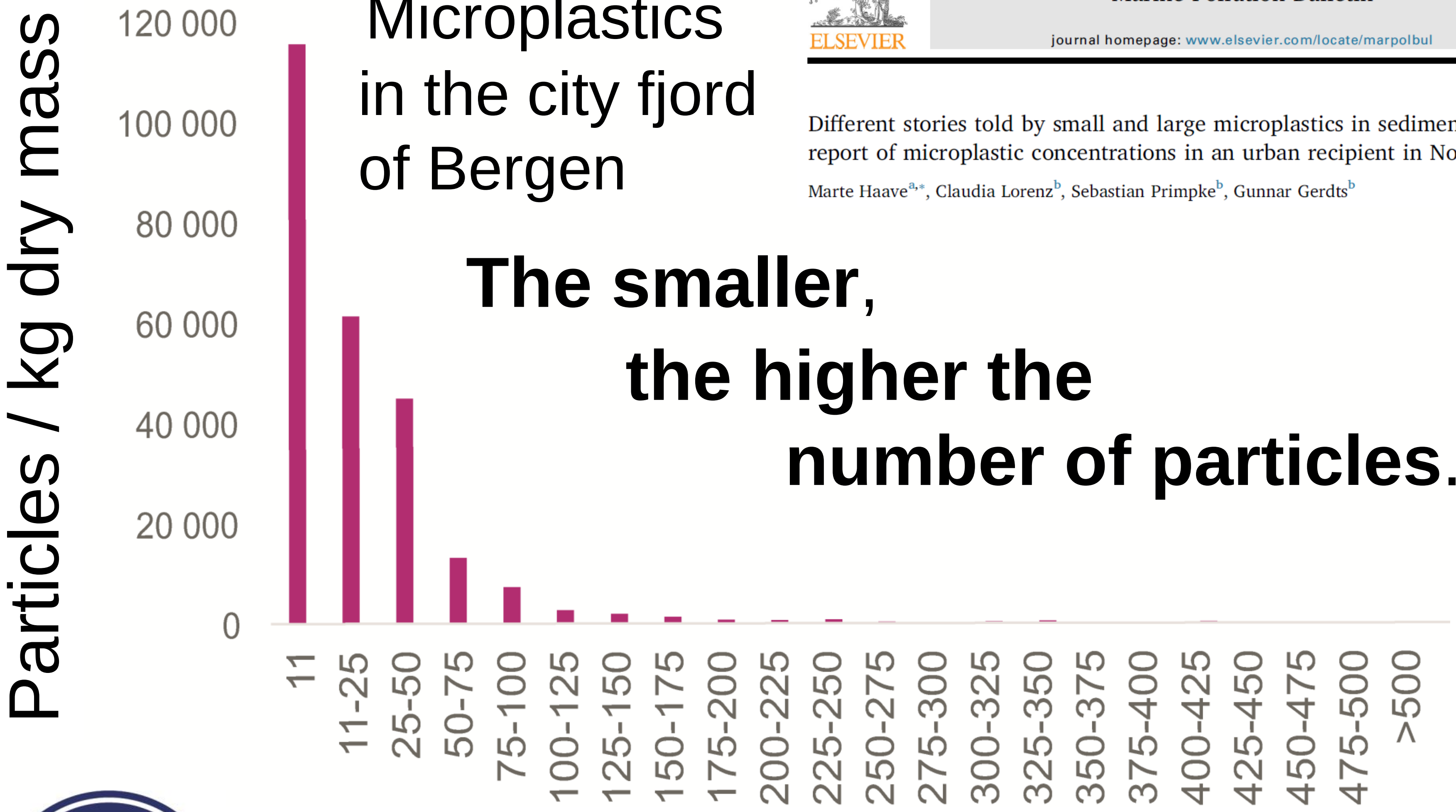
Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Different stories told by small and large microplastics in sediment - first report of microplastic concentrations in an urban recipient in Norway

Marte Haave[a,*], Claudia Lorenz[b], Sebastian Primpke[b], Gunnar Gerdts[b]

The smaller, the higher the number of particles.



µm (mikro-meter)

Do not share without consent.

[redacted]


Most common Manta-Trawl meshsize
250-275
275-300
300-325
325-350
350-375
375-400
400-425
425-450
450-475
475-500
>500
µm

Can micro be extrapolated from nano?

No

→Distribute differently in water.

In the nm-range, gravity plays a minor role, high specific mass plastics - such as PVC – do not sink.

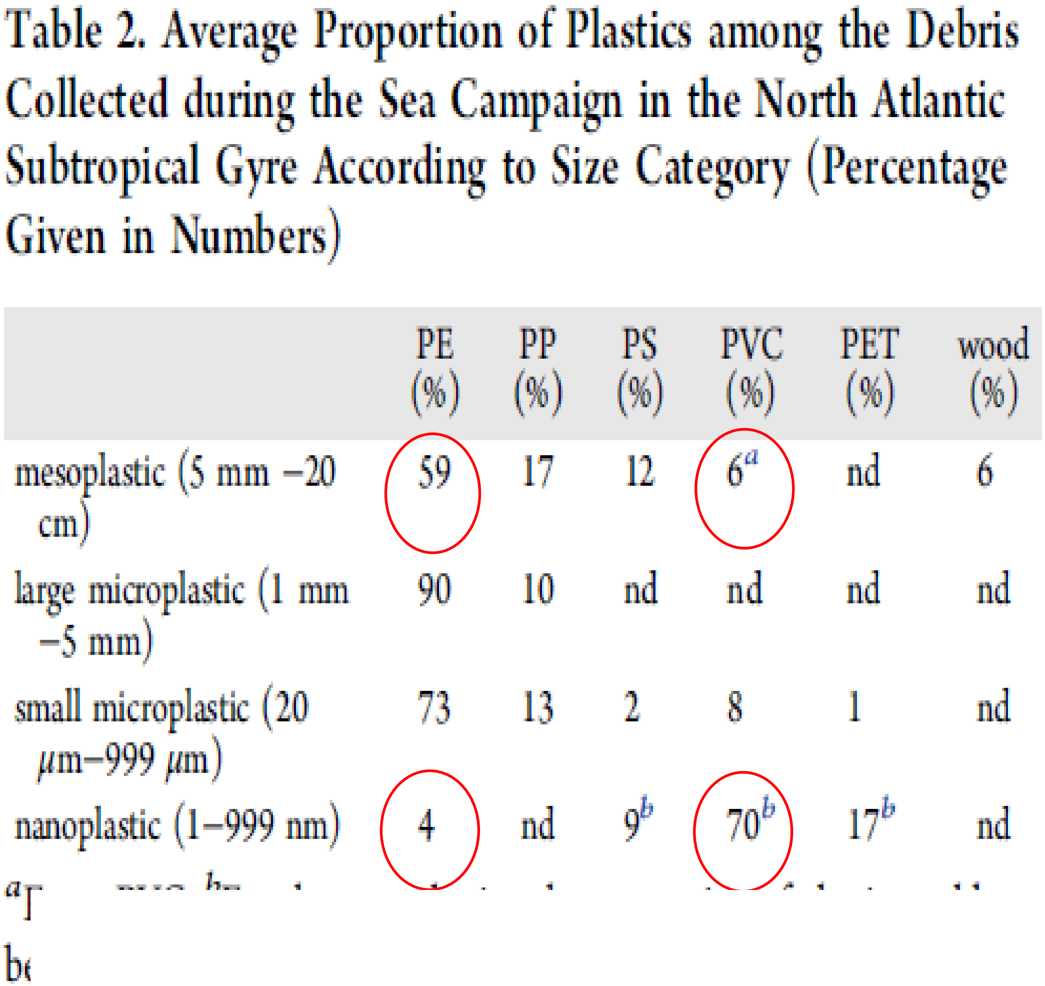
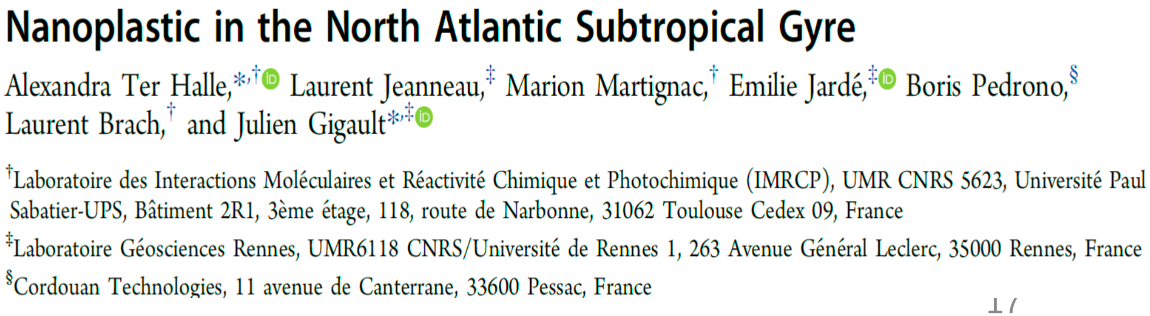


Table 2. Average Proportion of Plastics among the Debris Collected during the Sea Campaign in the North Atlantic Subtropical Gyre According to Size Category (Percentage Given in Numbers)

	PE (%)	PP (%)	PS (%)	PVC (%)	PET (%)	wood (%)
mesoplastic (5 mm –20 cm)	59	17	12	6^a	nd	6
large microplastic (1 mm –5 mm)	90	10	nd	nd	nd	nd
small microplastic (20 μm–999 μm)	73	13	2	8	1	nd
nanoplastic (1–999 nm)	4	nd	9^b	70^b	17^b	nd



Nanoplastic in the North Atlantic Subtropical Gyre

Alexandra Ter Halle,*[†] Laurent Jeanneau,[‡] Marion Martignac,[†] Emilie Jardé,[‡] Boris Pedrono,[§] Laurent Brach,[†] and Julien Gigault*[‡]

[†]Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique (IMRCP), UMR CNRS 5623, Université Paul Sabatier-UPS, Bâtiment 2R1, 3ème étage, 118, route de Narbonne, 31062 Toulouse Cedex 09, France

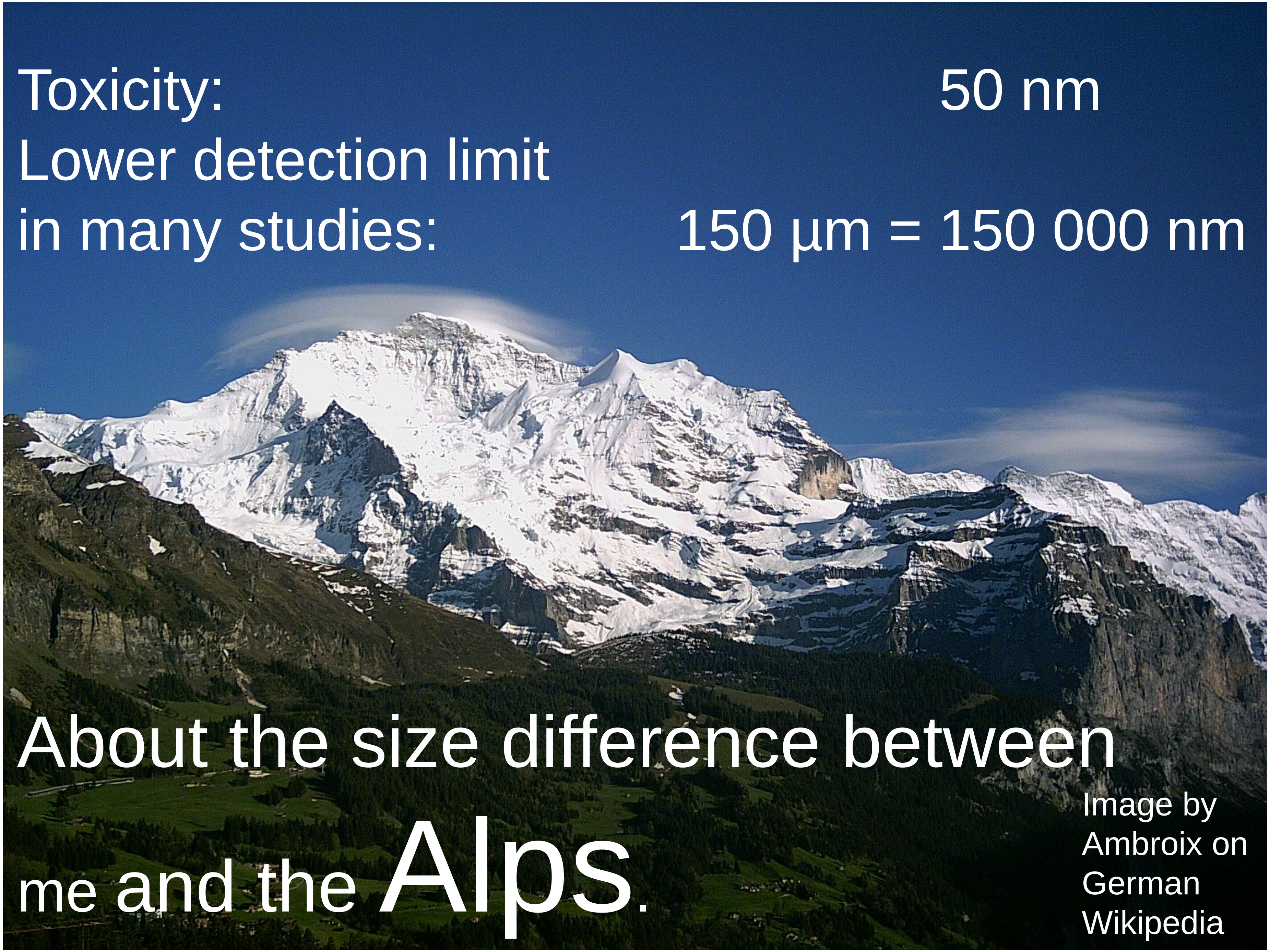
[‡]Laboratoire Géosciences Rennes, UMR6118 CNRS/Université de Rennes 1, 263 Avenue Général Leclerc, 35000 Rennes, France

[§]Cordouan Technologies, 11 avenue de Canterrane, 33600 Pessac, France

Most MP
< ½ mm

Most MP
>½ mm

Haave et al. 2019

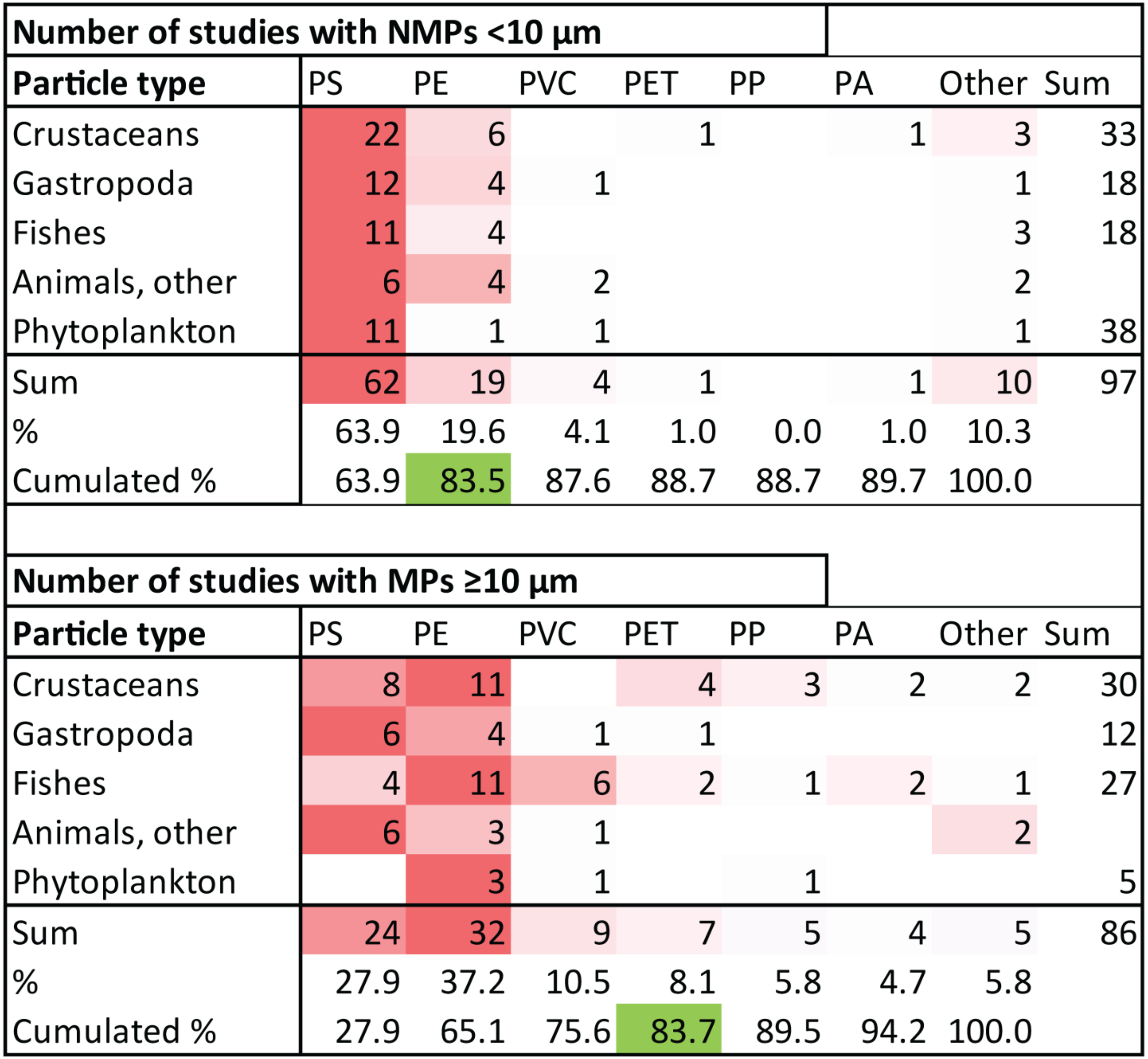


Toxicity: 50 nm

Lower detection limit in many studies: 150 µm = 150 000 nm

About the size difference between me and the Alps.

Image by Ambroix on German Wikipedia



Number of studies with NMPs <10 µm

Particle type	PS	PE	PVC	PET	PP	PA	Other	Sum
Crustaceans	22	6		1		1	3	33
Gastropoda	12	4	1				1	18
Fishes	11	4					3	18
Animals, other	6	4	2				2	
Phytoplankton	11	1	1				1	38
Sum	62	19	4	1		1	10	97
%	63.9	19.6	4.1	1.0	0.0	1.0	10.3	
Cumulated %	63.9	83.5	87.6	88.7	88.7	89.7	100.0	

Number of studies with MPs ≥10 µm

Particle type	PS	PE	PVC	PET	PP	PA	Other	Sum
Crustaceans	8	11		4	3	2	2	30
Gastropoda	6	4	1	1				12
Fishes	4	11	6	2	1	2	1	27
Animals, other	6	3	1				2	
Phytoplankton		3	1		1			5
Sum	24	32	9	7	5	4	5	86
%	27.9	37.2	10.5	8.1	5.8	4.7	5.8	
Cumulated %	27.9	65.1	75.6	83.7	89.5	94.2	100.0	

pe

European plastic converter demand by polymer types in 2017

Data for EU28+NO/CH.

Source: PlasticsEurope Market Research Group (PEMRG) and Conversio Market & Strategy GmbH

PP
19.3%

Food packaging, sweet and snack wrappers, hinged caps, microwave containers, pipes, automotive parts, bank notes, etc.

OTHERS
19%

Hub caps (ABS); optical fibres (PBT); eyeglasses lenses, roofing sheets (PC); touch screens (PMMA); cable coating in telecommunications (PTFE); and many others in aerospace, medical implants, surgical devices, membranes, valves & seals, protective coatings, etc.

PE-LD
PE-LLD
17.5%

Reusable bags, trays and containers, agricultural film (PE-LD), food packaging film (PE-LLD), etc.

PE-HD
PE-MD
12.3%

Toys, (PE-HD, PE-MD), milk bottles, shampoo bottles, pipes, houseware (PE-HD), etc.

PVC
10.2%

Window frames, profiles, floor and wall covering, pipes, cable insulation, garden hoses, inflatable pools, etc.

PUR
7.7%

Building insulation, pillows and mattresses, insulating foams for fridges, etc.

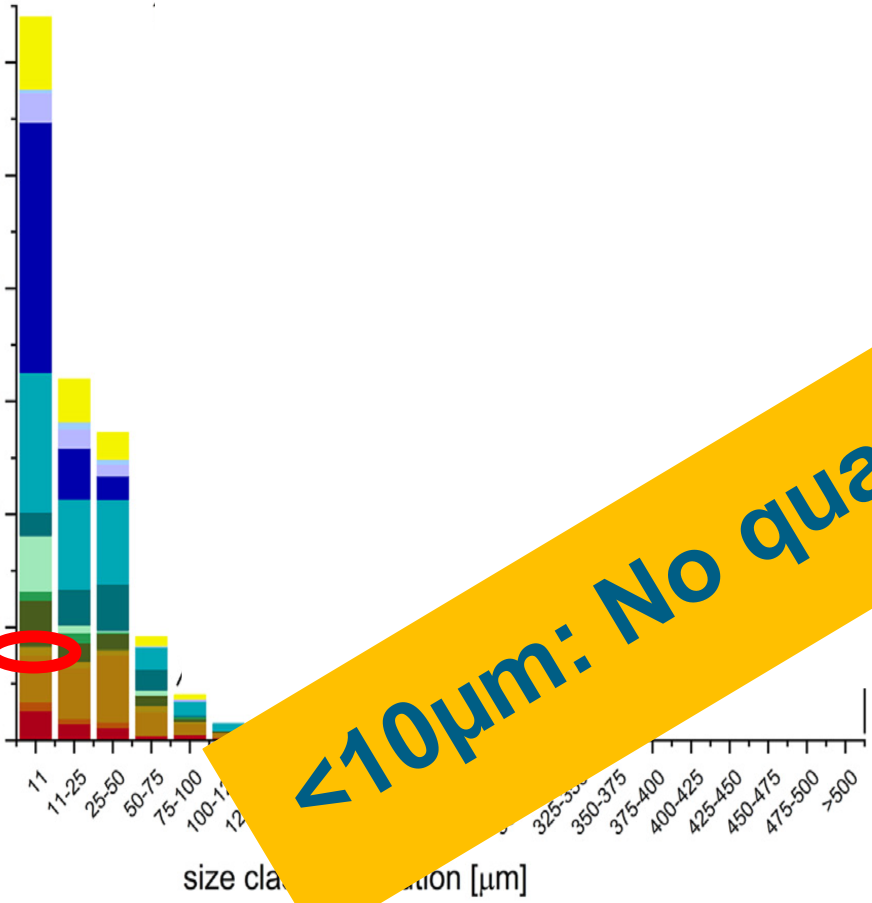
PET
7.4%

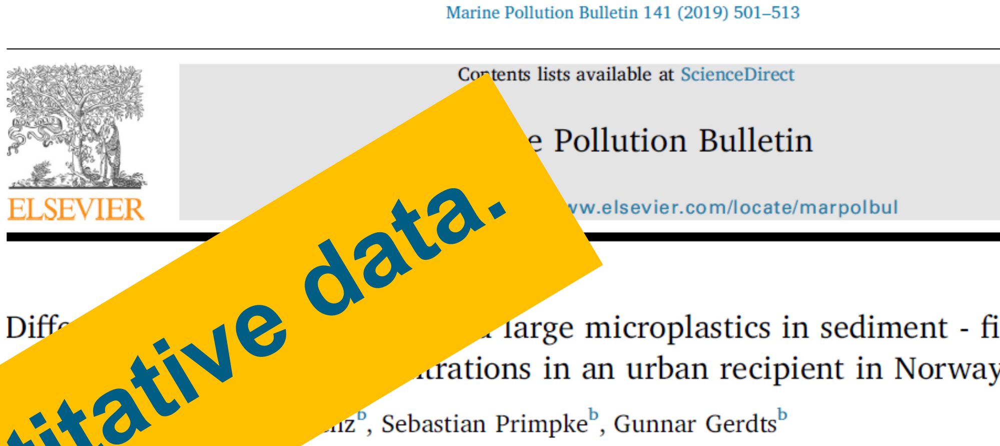
Bottles for water, soft drinks, juices, cleaners, etc.

PS, EPS
6.6%

Eyeglasses frames, plastic cups, egg trays (PS); packaging, building insulation (EPS), etc.

Plasticseurope.org





Marine Pollution Bulletin 141 (2019) 501–513

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Diff large microplastics in sediment - fi

rations in an urban recipient in Norway

nz[b], Sebastian Primpke[b], Gunnar Gerdts[b]

<10µm: No quantitative data.

Baseline studies

- Substance concentration
  - Variation with
    - Species/tissue
    - Size
    - Locati
    - S

Need quantification!

Risk evaluation

- Long term toxicity
  - EU Maximum level
  - EFSA tolerable intake (TWI/TDI)/Scientific opinion…
  - MOE (margin of exposure, based on BMLD_{10})

Chemical characterization with two complementary quantitative methods

- µ Fourier Transformation Infrared Spectrometry/Microscopy (µ-FTIR) Agilent Cary 620/670
    - Focal plane array
    - Detection limit: 3 µm / 10 µm
    - Information on particle size and shape

- Pyrolysis-gas chromatography/mass spectrometry (py-GC/MS) - Orbitrap
    - Information about mass
    - Faster
    - Particle size through pre-fractionation, incl. nano if mass > LOD (<0.5 µg, ca. one 100 µm particle)

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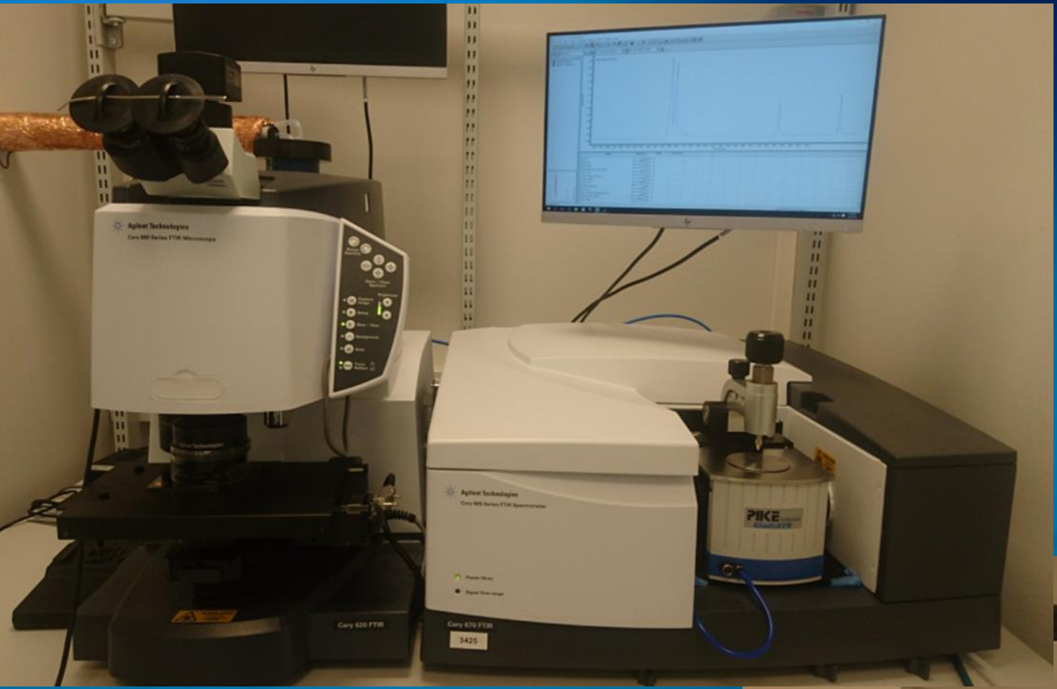
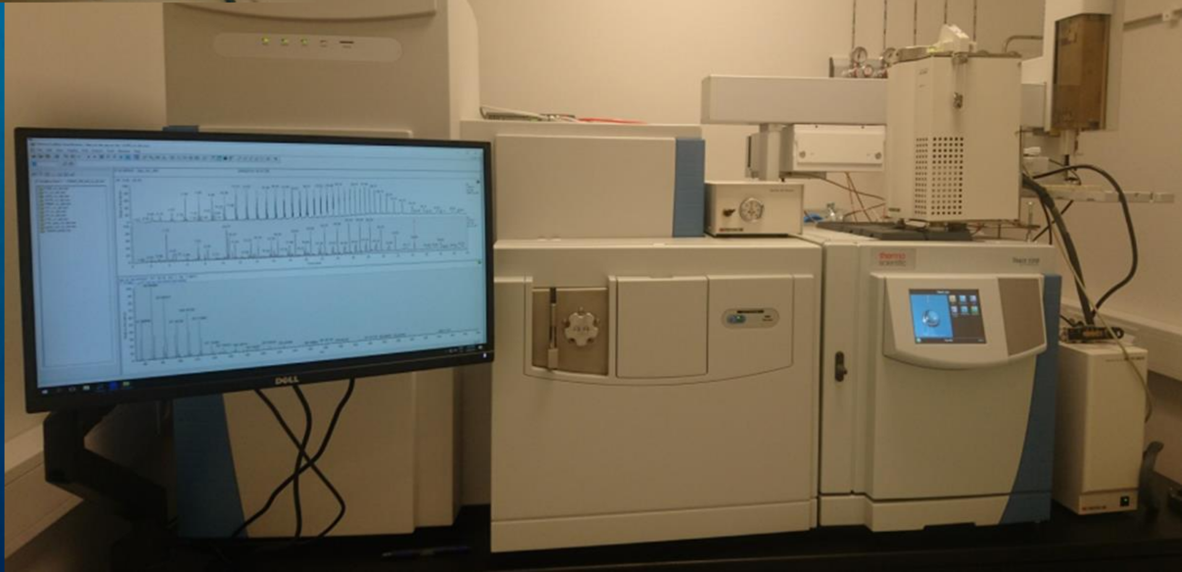
IMR instruments for microplastic analysis



µFTIR Microscope
Agilent Cary 620/670

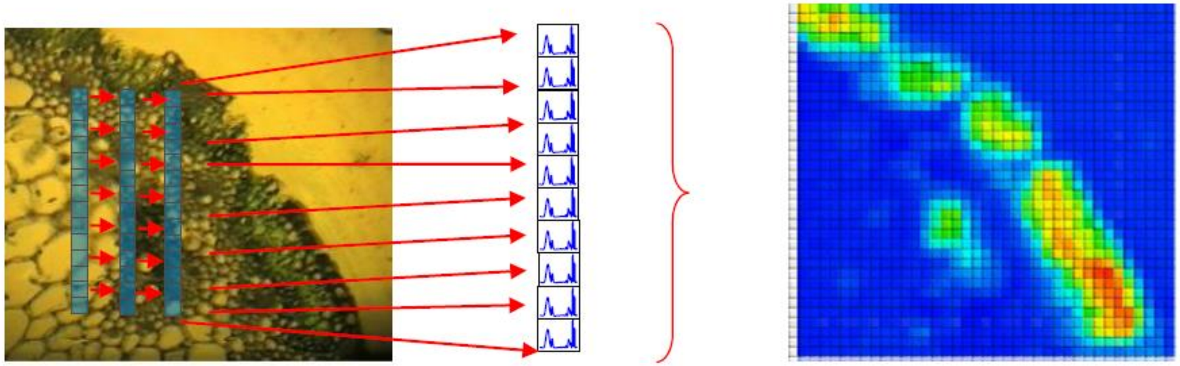
Pyrolysis-GC/MS Orbitrap
Thermo QExactive



FTIR Microscope Measurement Modes:

3: Linear array Mapping

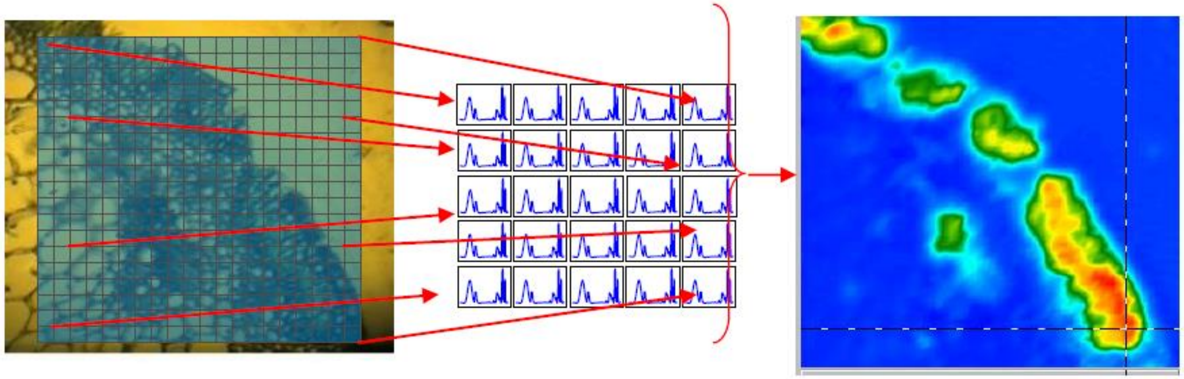
Acquisition of spectra by a row (1x16) of detectors. Faster than single point mapping, but still much slower than FPA imaging



Focal Plane Array

4: FPA Imaging

With an FPA detector, up to 16384 spectra can be recorded **simultaneously** in a single measurement.

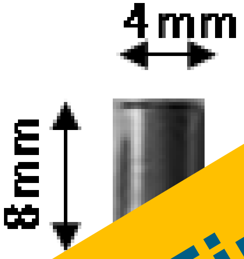


N_2-Cooled FPA detector – one manufacturer in Santa Barbara/USA. Based on military technology

Applied by Agilent (Cary620) and Bruker (Hyperion3000)

Py-GC/MS for plastic polym

Pyrolysis cup:



First results: Fish meal and mesopelagic biota: ppm plastic content

Pyrolysis → G ography → Mass spectrometry

Plastic degrades to smaller com-ponents → gass

Separation according to volatility and polarity (retention time)

Ionization, degradation. Mass spectrum (m/z).

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thermo scientific

APPLICATION NOTE 1064

Pyrolysis-GC-Orbitrap MS - a powerful analytical tool for identification and quantification of microplastics in a biological matrix

Authors
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[2]Institute of Marine Research,
Bergen, Norway

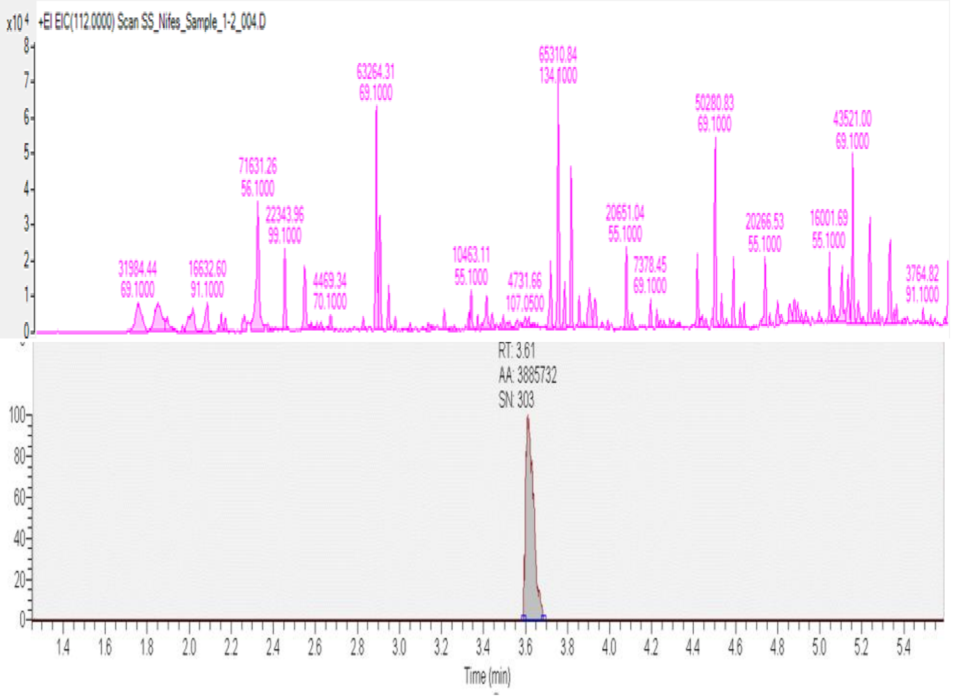
https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-10643-gc-ms-microplastics-biological-matrix-an10643-en.pdf

Py-GC/MS

Exploring the selectivity with QExactive high resolution MS, e.g. chlorobenzene from PVC. Screening for the best quantifiers/qualifiers

(Top): Single quad pyrogram

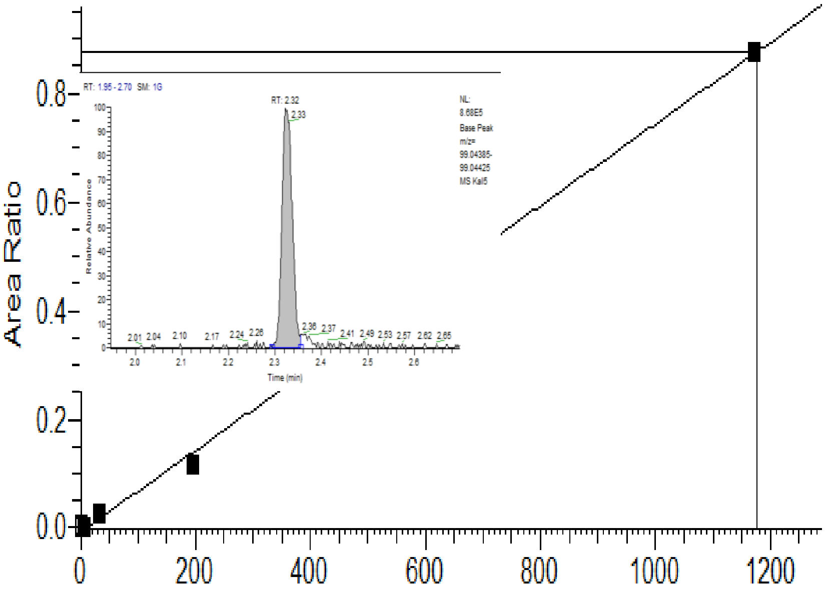
(Bottom): QExactive pyrogram



Boundaries of sensitivity e.g. PMMA (dissolved) linear calibration curve down to < 5 ng OC. Restraining factor: calibration standards for solid polymers

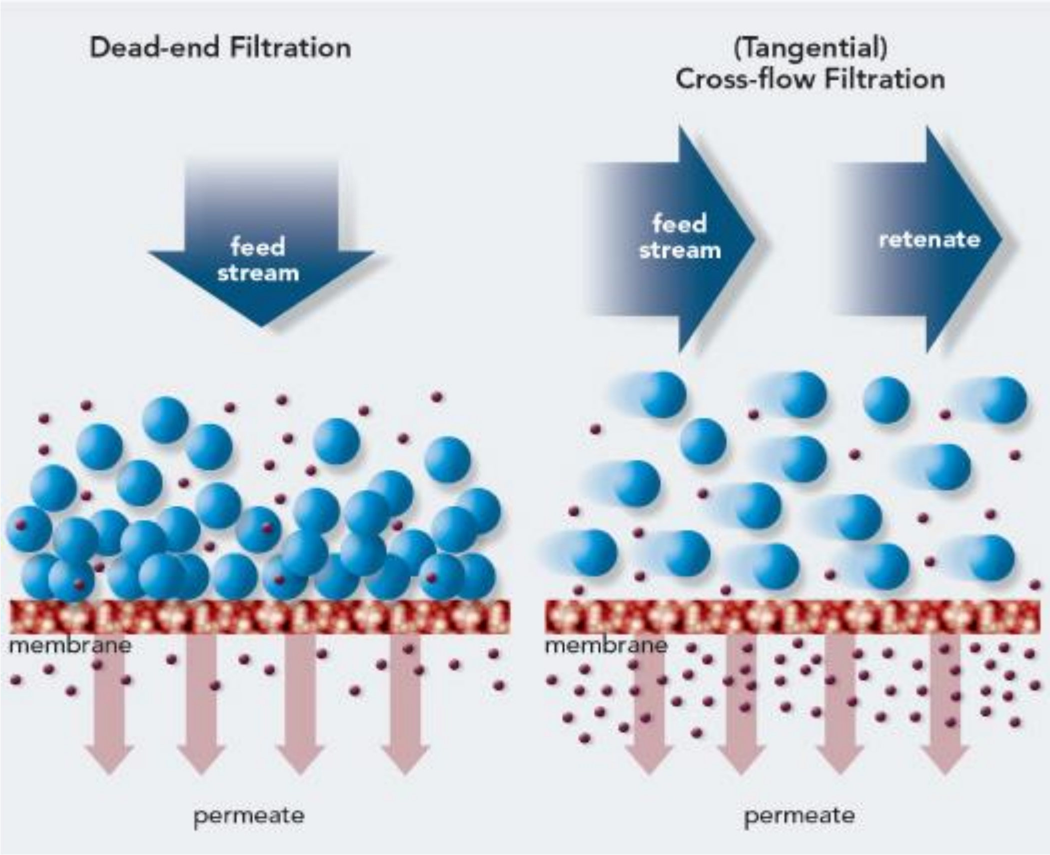
PMMA

$Y = -0.00840355 + 0.000750918 * X \quad R^2 = 0.9987 \quad W: Equal$



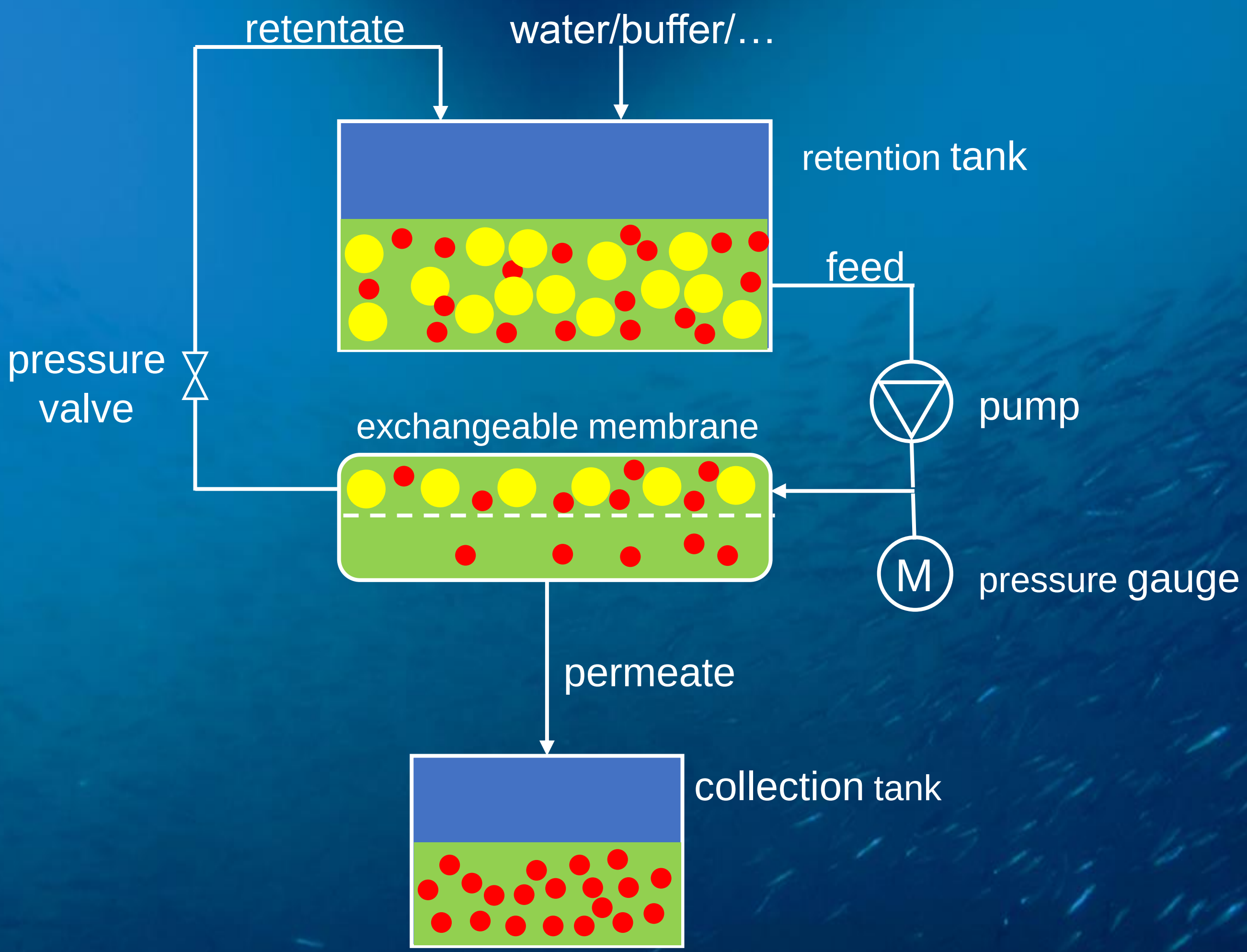
Size fractionation

Traditional
Filtration (>10 µm)
vs.
Crossflow (<10 µm)

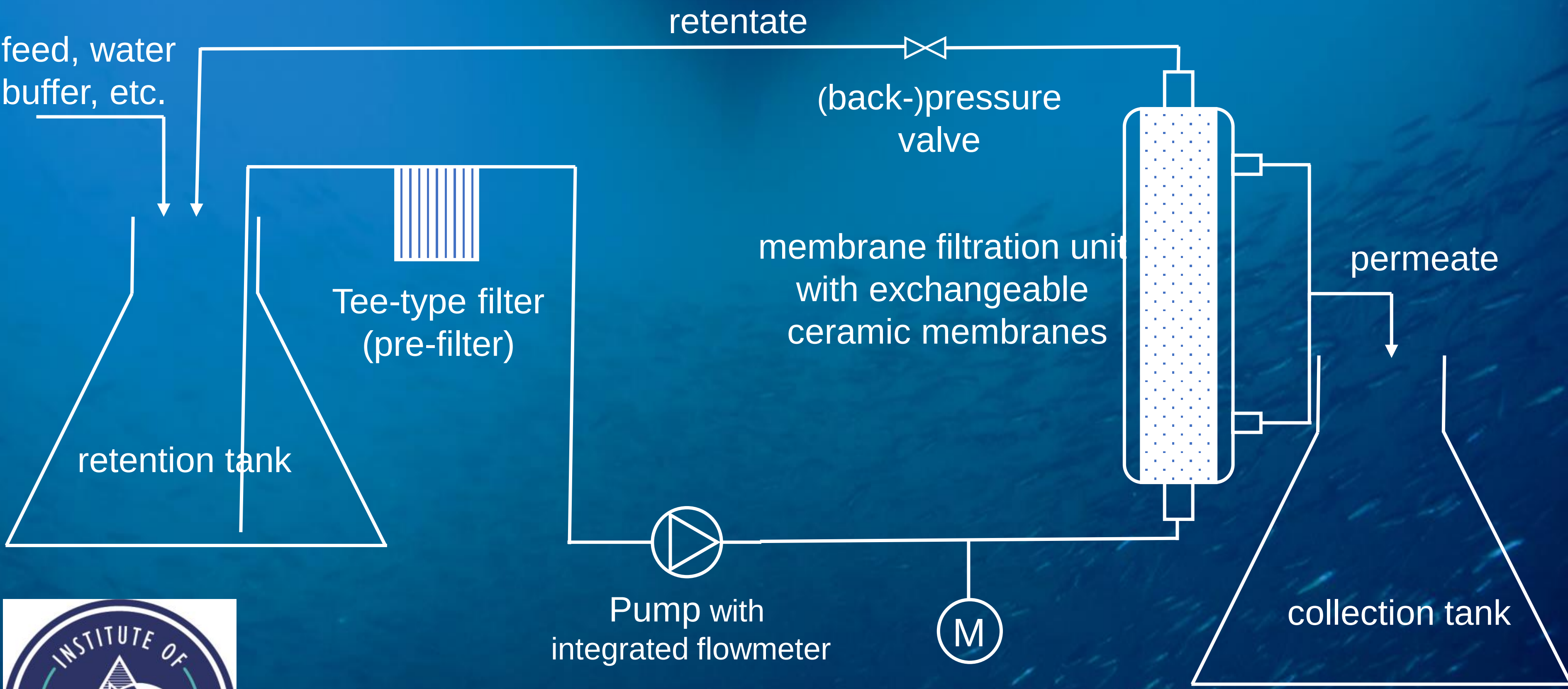


Source: www.winebusiness.com

Nanoplastic crossflow filtration



Prototype at IMR



retentate

feed, water buffer, etc.

(back-)pressure valve

membrane filtration unit with exchangeable ceramic membranes

permeate

Tee-type filter (pre-filter)

retention tank

collection tank

Pump with integrated flowmeter

pressure gauge

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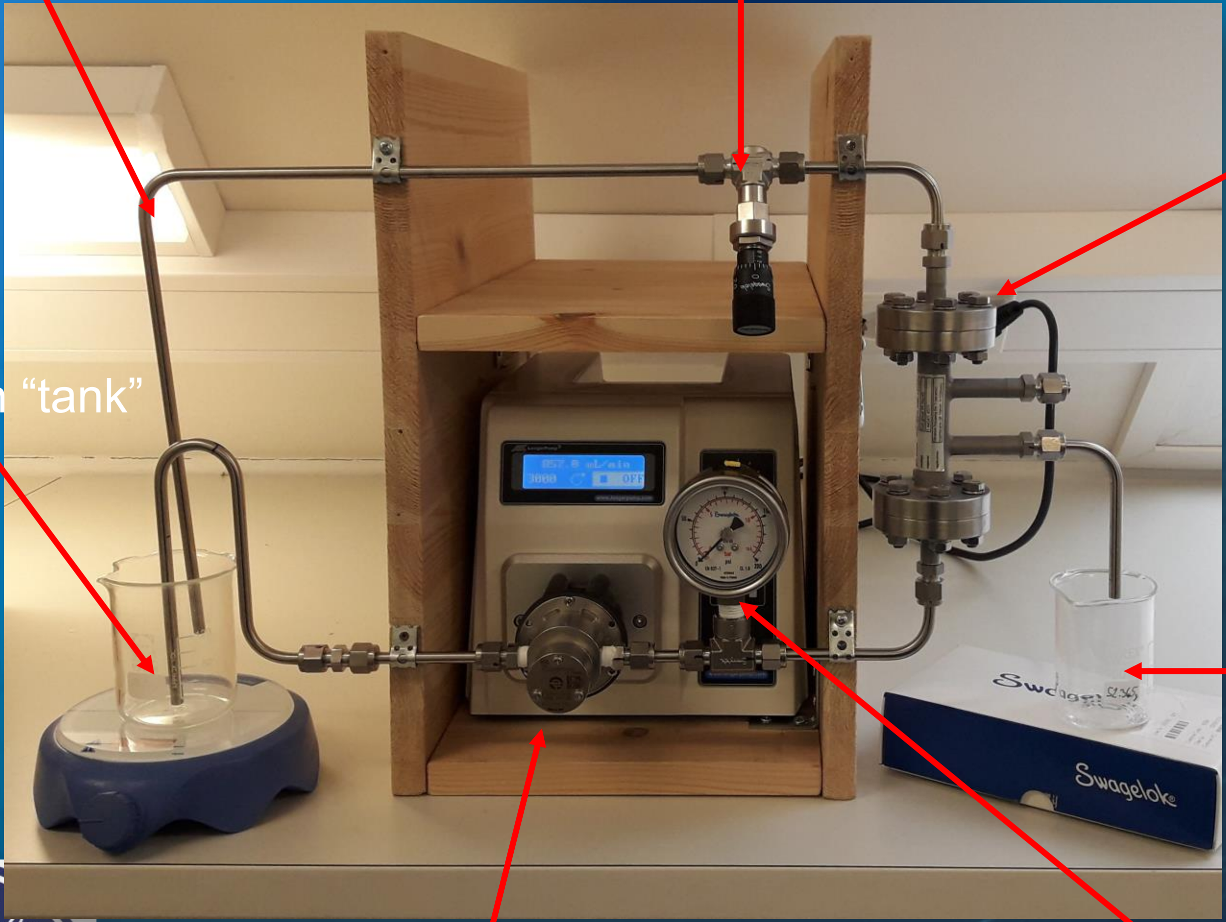


Swagelok stainless steel tubing (1/4 in)

Backpressure valve

Stainless steel membrane housing

Retention "tank"

Filtrate/Permeate

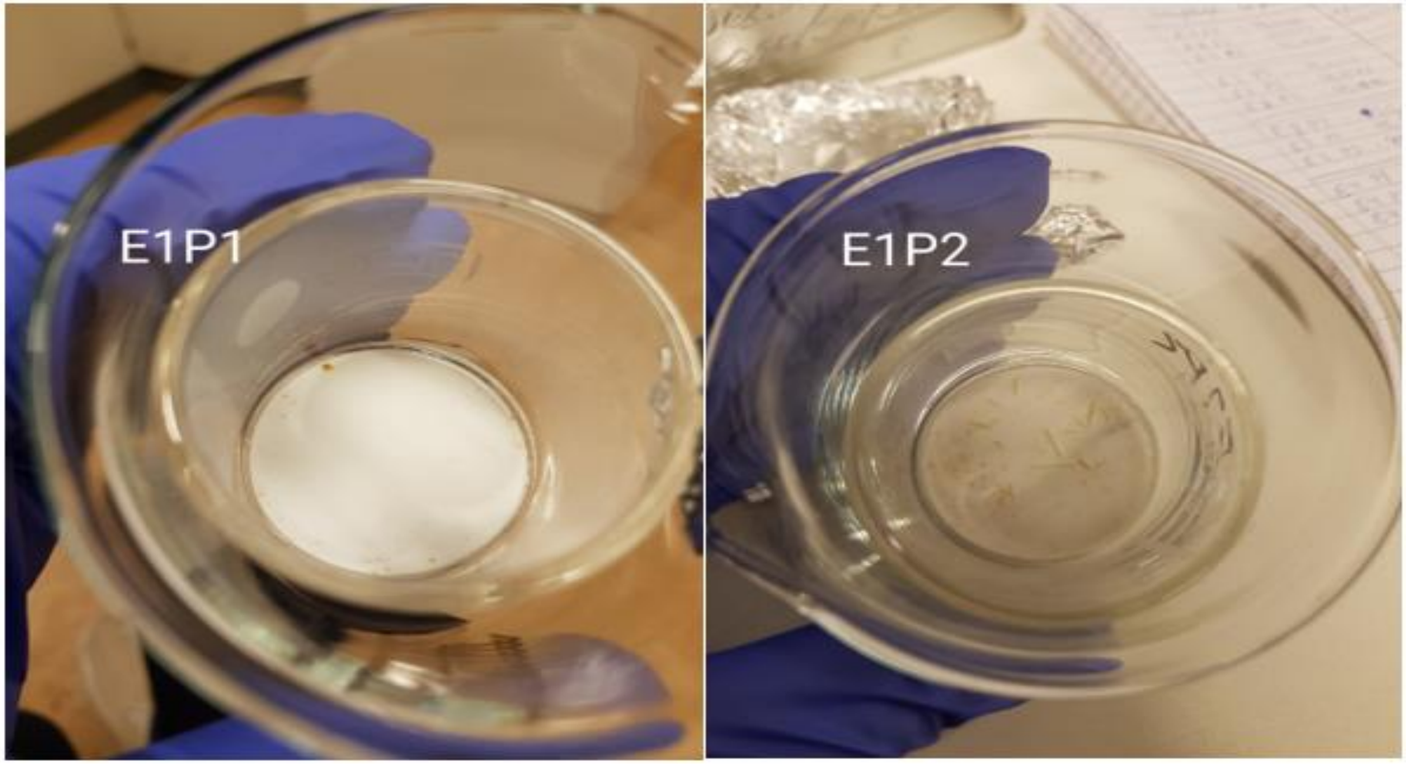
Micro gear pump (Longer LP-WT3000-1FB)
~90-900 mL/min, max. ~14 bar

Pressure gauge
Working pressure ~2-12 bar

Problems

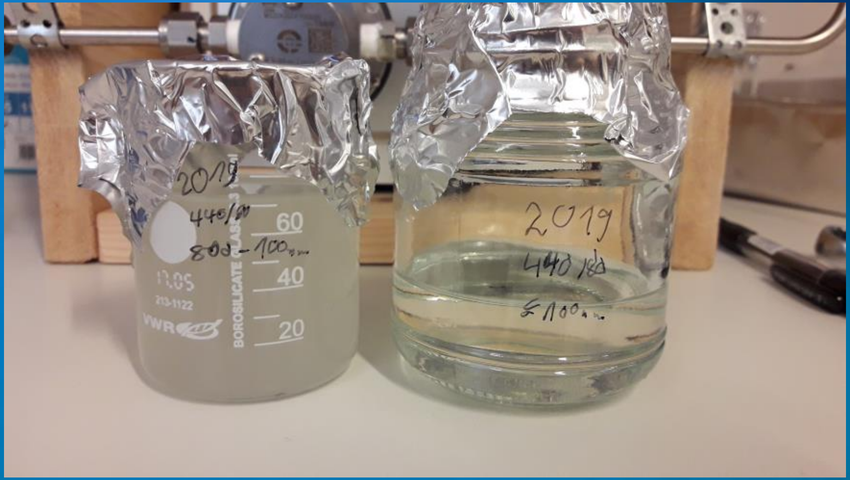
- Quality of samples varies → overload anodisc (fishbones)
- Processing in «siMPle» (out of memory) - we need to distribute sample to larger area on anaodic as compared to Ålborg / Jes Vollertsen
- Quality of FTIR data matches not well with database. We are in the process of adding our own standards
- We lack «our» natural particles: fishbone, exoskeleton etc. Necessary for better hits.
- A lot of FTIR signal in samples. Fishbones? Fatty esters? Contamination? FTIR Spektra similar to EVA, but ain't.
- Py-GC/MS out of order because of leakage. Error search with technichian from Thermo ongoing. Maybe need to send to Germany.
- Challenging to assmble standards for the lower part of the calibration curve.

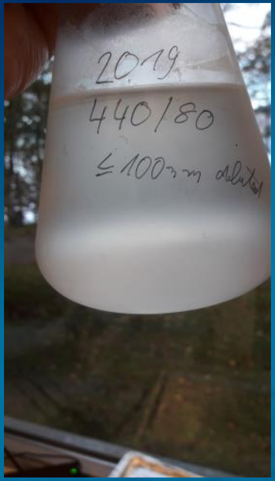
Extraction challenges



Two similar samples. Note the fish bones after tissue degradation.
(Picture: Thomas Næsheim, IMR)

Filtration challenges nm range





1) Filtration of KOH/Tween-digested salmon muscle in the nano-fraction possible, but very time-consuming
2) Originally clear filtrate can become cloudy again (coagulating proteins or fatty acids?)

→Adding high conc. NaCl, and lipase?

→Adding ethanol?

Involved in microplastics quantification @HI

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