NORMAN cross working group on passive sampling
Passive sampling in support of chemical monitoring in biota for the Water Framework Directive

Biota monitoring and WFD: vision and remaining challenges

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Why EQS for Biota in the WFD?

The WFD requires biota EQSs to protect:

- 1. Humans from adverse effects resulting from the consumption of chemical-contaminated aquatic food (fish, molluscs, crustaceans, etc.).
- 2. Predators and top predators, such as birds and mammals, from risks of secondary poisoning
- 3. Benthic and pelagic predators (e.g. predatory fish) that may also be at risk from secondary poisoning.

At present, biota standards developed for birds and mammals are assumed to be sufficiently protective for benthic and pelagic predators.

- A standard would be required if there was a risk of secondary poisoning of predators (e.g. mammals or birds) from eating contaminated prey (QSbiota,secpois), or a risk to humans from eating fishery products (QSbiota, hh food).
- The triggers for deriving a QSbiota, hh food are dominated by **hazard properties**, whereas a QSbiota sec pois is triggered by the possibility of **accumulation** in the food chain in conjunction with hazard properties.

The lowest standard calculated for the different objectives of protection will normally be adopted as the overall quality standard for that compartment.

Context: Implementation of EQS biota for bioaccumulative PS/PHS (Dir 2013/39/EU)

TL 5

QSbiota, sec pois

QSbiota, hh

TL 4

Hg
HCBD
HBCDD
Dicofol

Whole fish

HCB
PBDE
PFOS
PCDD/F + PCB-dl
Heptachlor and heptachlor epoxide

Fish filet

1 < TL ≤ 3

B[a]P
Fluoranthene
PCDD/F + PCB-dl

QSwater, sec pois

HBCDD
Dicofol

QSwater, hh food

PFOS
Heptachlor and heptachlor epoxide
B[a]P
Fluoranthene

backcalculation

backcalculation

Why EQS for Biota in the WFD?

Further reasons

- Need for an accumulating matrix for avoiding **sensitivity problems** in water analysis, especially in marine waters
- Need for an accumulating matrix for **trend monitoring** and verification of non-deterioration principle (together with sediments)
  - Integrating WFD monitoring with Marine Convention monitoring in coastal waters

EQS derivation according to Technical Guidance Document (TGD)

EQS derived for «European fish»:

- Trophic Level (TL) = 4
- Lipid content = 5%
- Dry weight =26%

CIS Guidance Document No: 27
Technical Guidance For Deriving Environmental Quality Standards. Rev 2018

Table 1.1 Current EQS_{biota} and basis for derivation

Substance	EQS_{biota} ($\mu g\ kg^{-1}$ wet weight (ww))	Matrix	Protection goal	Driving data	Assessment factor
Brominated diphenyl ethers	0.0085	Fish	Human health via consumption of fishery products	Mice dietary toxicity $BMDL_{10}$ for BDE-99 = 9 $\mu g\ kg^{-1}$ bw = internal daily dose of 4.2 ng kg^{-1} bw d^{-1} (using longest human half-life (1442 days)	30
Fluoranthene	30	Crustaceans and molluscs	Human health via consumption of fishery products	0.2 mg.kg^{-1} d^{-1}, chronic oral (gavage) rat study used to calculate a virtually safe dose (VSD) of $5x10^{-4}$ mg kg^{-1} d^{-1}.	VSD representing oral exposure associated with a 10^{-4} excess lifetime cancer risk based on the read-across between benzo[a]pyrene and fluoranthene
Hexachloro-benzene	10	Fish	Human health via consumption of fishery products	WHO-EHC guidance value for neoplastic effects of 0.16 µg kg bw^{-1} d^{-1}	Based on a person weighing 70 kg (acceptable daily intake of 1.12 µg hexachlorobenzene d^{-1}) and an average fish consumption of 115 g d^{-1}
Hexachloro-butadiene	55	Fish	Secondary poisoning	Chronic NOAEL mice = 0.2 mg kg^{-1} bw d^{-1}	Conversion factor = 8.3 (kg bw/kg $food^{-1}$.d^{-1}) = 1.66 mg kg $food^{-1}$ Assessment factor = 30
Mercury and its compounds	20	Fish	Secondary poisoning	365 day NOEC rhesus monkey growth 0.22 mg kg^{-1} food	10, due to the large number of NOECs available for methyl mercury
PAHs Benzo[a]pyrene	5	Crustaceans and molluscs	Human health via consumption of fishery products	Maximum levels for foodstuffs for benzo[a]pyrene: - 0.005 mg.kg^{-1} ww for crustaceans and molluscs	Maximum levels given for "fresh" (other than smoked) aquatic resources. No assessment factor applied.
Dicofol	33	Fish	Secondary poisoning	*Falco sparverius* Reproduction NOEC = 1 mg kg^{-1} feed ww	30
PFOS	9.1	Fish	Human health via consumption of fishery products	Cynomolgus monkey 183d NOAEL = 0.03 mg kg^{-1}	90
Dioxins and dioxin-like compounds	0.0065 TEQ_{2005}	Fish, crustaceans and molluscs	Human health via consumption of fishery products	Maximum levels given for foodstuffs content of the sum of DL-compounds (PCDDs, PCDFs and DL-PCBs)	
HBCDD	167	Fish	Secondary poisoning	Japanese Quail reproduction NOEC = 5 mg kg^{-1} feed	30
Heptachlor and heptachlor epoxide	6.7×10^{-3}	Fish	Human health via consumption of fishery products	2 year mice oral study – cancer, non-threshold approach Slope factor : 9.1 $(mg.kg^{-1}.d^{-1})^{-1}$ used to calculate a VSD of 1.1 10^{-7} mg kg^{-1} d^{-1}	VSD representing oral exposure associated with a 10^{-4} excess lifetime cancer risk.

Need for a EU harmonisation of biota monitoring: the WFD-CIS Guidances

https://ec.europa.eu/environment/water/water-framework/facts_figures/guidance_docs_en.htm

Aims:

- Harmonise protocols of biota monitoring in terms of sampling and analysis (*CIS Guidances N° 25 - Chemical Monitoring of Sediment and Biota; N° 33 - Analytical Methods for Biota Monitoring*)
- Make classification of quality status comparable among MS (*CIS Guidance N° 32 - Biota Monitoring*) in terms of:
  - Monitoring strategy
  - Species selection
  - …..

First open issue:
Minimise natural and monitoring variability

Time integrated exposition: the role of fish age

- Fish lenght: relationship lenght vs age
- Fish age: relationship fish age vs concentrations

How many sites and samples for a statistical assessment?

- Need to define the statistical power
- Possibility to Pooling samples

Constraint: Frequency of sampling: (directive 2013/39, art 8b.3: minimum requirement: once per year). It is difficult to imagine that most MS will decide higher frequency

First open issue:
Minimise natural and monitoring variability

Suggested Options in Guidance 32

- Apply normalisation
  - To lipids for hydrophobic compounds
  - To dry weights for Hg and PFOS

Does normalisation work in reducing variability?

EU implementation experiences in Normalisation

- Normalisation to lipids for hydrophobic compounds is able to reduce inter-species and intra-species variability (see e.g. Fliedner et al., 2016 and 2018)

- Normalisation to dry weights or protein for Hg and PFOS has negligible effects (see e.g. Fliedner et al., 2016; Valsecchi et al. *Env Toxicol Chem*, 2020).

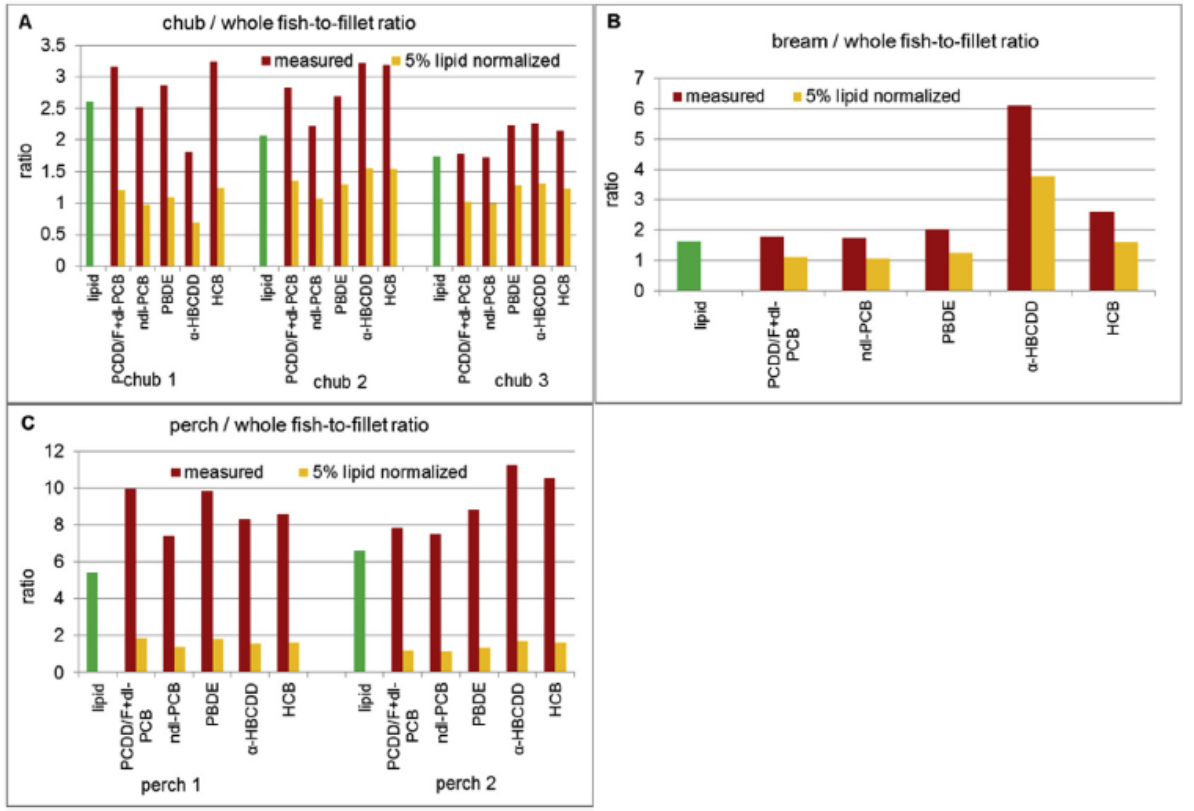


Fig. 5. Whole fish-to fillet ratios of measured and 5% lipid normalized concentrations of PCDD/F+dl-PCB, ndl-PCB, PBDE, α-HBCDD and HCB in chub (*Squalius cephalus*, chub 1: n = 9; chub 2: n = 11, chub 3: n = 8), bream (*Abramis brama*, n = 8), and perch (*Perca fluviatilis*, perch 1: n = 10; perch 2: n = 8) sampled 2015 at Kelheim/Danube.

Second dilemma: To cut or not to cut (Fillet or whole fish?)

First Options:

- Choose the matrix according to the protection goals
  - Different analytes should be monitored in different fish tissues of the same sample

QSbiota, sec pois

Hg
HCBD
HBCDD
Dicofol

Whole fish

QSbiota, hh

HCB
PBDE
PFOS
PCDD/F + PCB-dl
Heptachlor and heptachlor epoxide

Fish filet

  - Hardly to manage and expensive
  - Looking for alternative solutions

Second dilemma: To cut or not to cut (Fillet or whole fish?)

Alternative options

- Analysing only fillet
  - Garantees continuity with most monitoring programs for trend monitoring
  - Is consistent with the current specifications of food regulation
  - BUT Underestimates risks to top predators
- Analysing only whole fish
  - Is the most conservative option (as risks toward human health are overestimated).
  - WF homogeneisation is the simplest option
  - BUT bigger fish are difficult to homogenise as a whole

Second dilemma: To cut or not to cut (Fillet or whole fish?)

Suggested Options in Guidance 32

- Analyse only fillet and convert data to whole fish
  - Conversion factors for fillet-to-whole fish contaminant levels are available only for certain compounds:
    - Hg and PCB (see Guidance 32)
    - PBDE, PFOS, HCB, HBCDD, PCDD/F (Fliedner et al.2018; Rüdel et al. 2020; Valsecchi et al, 2020)
    - Are they independent on fish species?

EU experiences in Fillet to whole fish conversion

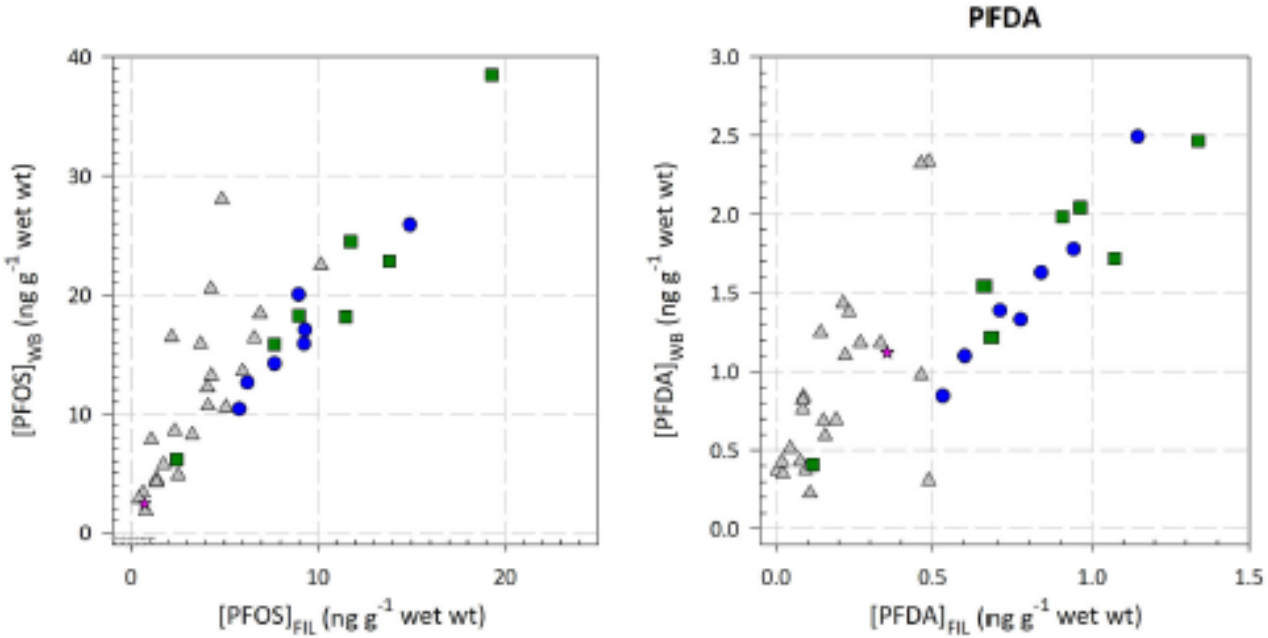
Table 3 Fillet-to-whole fish conversion factors and equations for priority substances (for all fish, $n=36$, and for 3–4 years/3–5 years fish, $n=20$)

Priority substance	Conversion factor (or equation) fillet-to-whole fish (for all fish, $n=36$) (significance level, one tailed)	Conversion factor fillet-to-whole fish (for 3–4 years/3–5 years fish, $n=20$) (significance level, one tailed)
PCDD/F + dl-PCB	$\ln(\text{conc}_{\text{whole fish}}) = 1.56 + 0.63 * (\ln \text{conc}_{\text{fillet}})$ ($p<0.0001$)	5.3 ($p<0.0001$)
HCB	3.7 ($p<0.0001$)	3.6 ($p<0.0001$)
PBDE	5.2 ($p<0.0001$)	5.4 ($p<0.0001$)
HBCDD	1.7 ($p<0.0001$)	1.8 ($p=0.030$)[a]
PFOS	2.6 ($p<0.0001$)	2.7 ($p<0.0001$)
Mercury	$\ln(\text{conc}_{\text{whole fish}}) = -0.031 + 0.949 * (\ln \text{conc}_{\text{fillet}})$ ($p<0.0001$)	0.81 ($p<0.0001$)

I° Case Study: different German water bodies and different fish species (From Rüdel et al. *2020*)

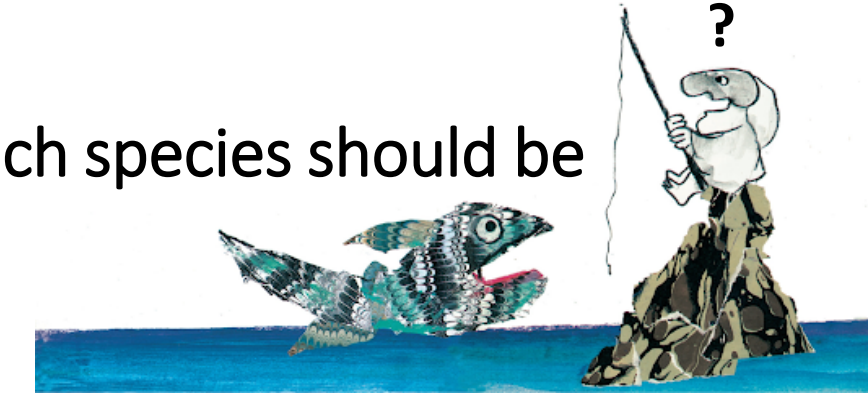
II° Case Study: Different subalpine lakes and different fish species:

Different colors are different fishes.
ANCOVA: The effect of species (qualitative variable) was significant ($p = 0.043$)
(From Valsecchi et al. *Env Toxicol Chem*, 2020)



Third open issue: Which species?

Guidance 32: no specific recommendation about which species should be sampled



Agreed characteristics (see also Guidance 25):

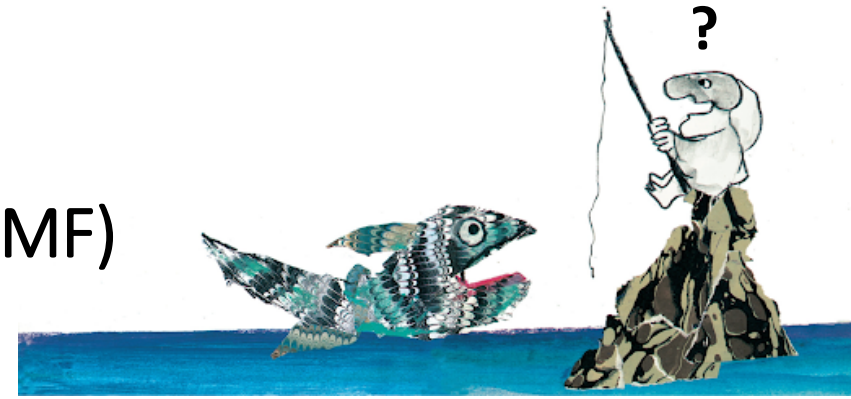
- Samples must be **representative** of the population and be able to be obtained every year without negative impacts on local populations.
- Avoiding migrant and protected species (the case of eels)
- Continuing the existing biota monitoring programmes (again the case of eels…)
- Close to EU fish Trophic Level 4

Open questions:

- Locally or regional representative? How about inter-basin comparability in status classification?
- Only autochtonous?
- Species of food interest?
- ……?

Third open issue: Which species?

The correction with Trophic Magnification Factors (TMF)



This could allow

- correcting between different fish at different TL
- correcting data collected in lower taxa such as mollusks and crustaceans, saving data from previous campaigns (e.g. mussel watch), allowing caging

Critical points:

- Need for experimental measurements of Stable Isotopes for TL derivation. The use of literature-based TL (e.g. from FishBase) is often ineffective
- Choice of the correct baseline (e.g. pelagic or littoral?) to derive TL from SIA
- Choice of the correct TMF: site-specific or regional TMF?

EU implementation experiences in TMF correction

List of requirements for getting good TMF data

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Environmental Policy & Regulation

Practical Advice for Selecting or Determining Trophic Magnification Factors for Application Under the European Union Water Framework Directive

Karen A Kidd,† Lawrence P Burkhard,‡ Marc Babut,§ Katrine Borgå,‖ Derek CG Muir,# Olivier Perceval,†† Heinz Ruedel,‡‡ Kent Woodburn,§§ and Michelle R Embry‖‖*

Rüdel *et al. Environ Sci Eur* (2020) 32:138
https://doi.org/10.1186/s12302-020-00404-8

Environmental Sciences Europe

Selection of correct TMF values for biota monitoring under WFD

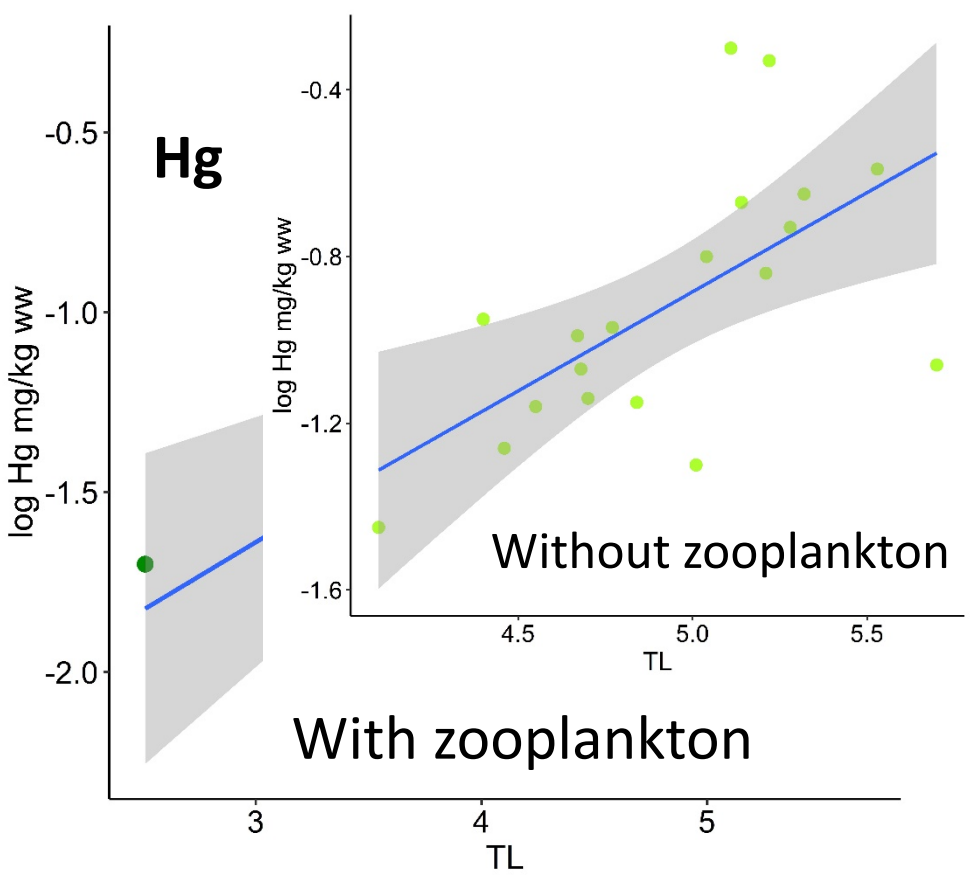
RESEARCH Open Access

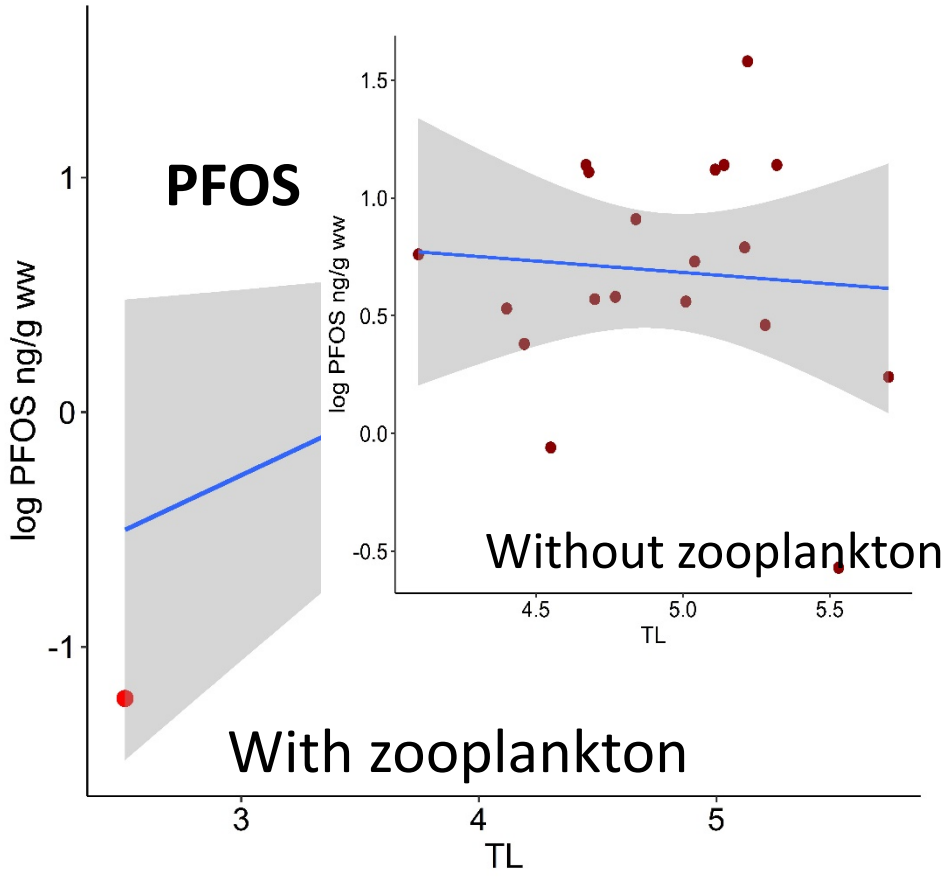
Selection and application of trophic magnification factors for priority substances to normalize freshwater fish monitoring data under the European Water Framework Directive: a case study

Heinz Rüdel[1*], Verena Kosfeld[1,2], Annette Fliedner[1], Georg Radermacher[1], Christian Schlechtriem[2], Anja Duffek[3], Caren Rauert[3] and Jan Koschorreck[3]

EU implementation experiences in TMF correction: Lake Mergozzo, Italy

Mazzoni et al., Water **2020**, 12, 1591;





Using invertebrates for Biota Monitoring

- It is the mandatory option for PAHs; possible option for PCDD/F and dioxin-like
- For the other PS and PHS, TL correction *via* TMF allows to use lower taxa such as mollusks and crustaceans
- It would allow to continue Mussel Watch-like programs in coastal waters for trend monitoring
- Invertebrates can be (more or less) easily transplanted or caged (active monitoring)
- Passive vs Active monitoring has been widely discussed in Guidances 25 and 32
  - A warning is needed for transplanting allochtonous species

EU implementation in invertebrate monitoring: Caging invertebrates in French rivers in a Tier approach

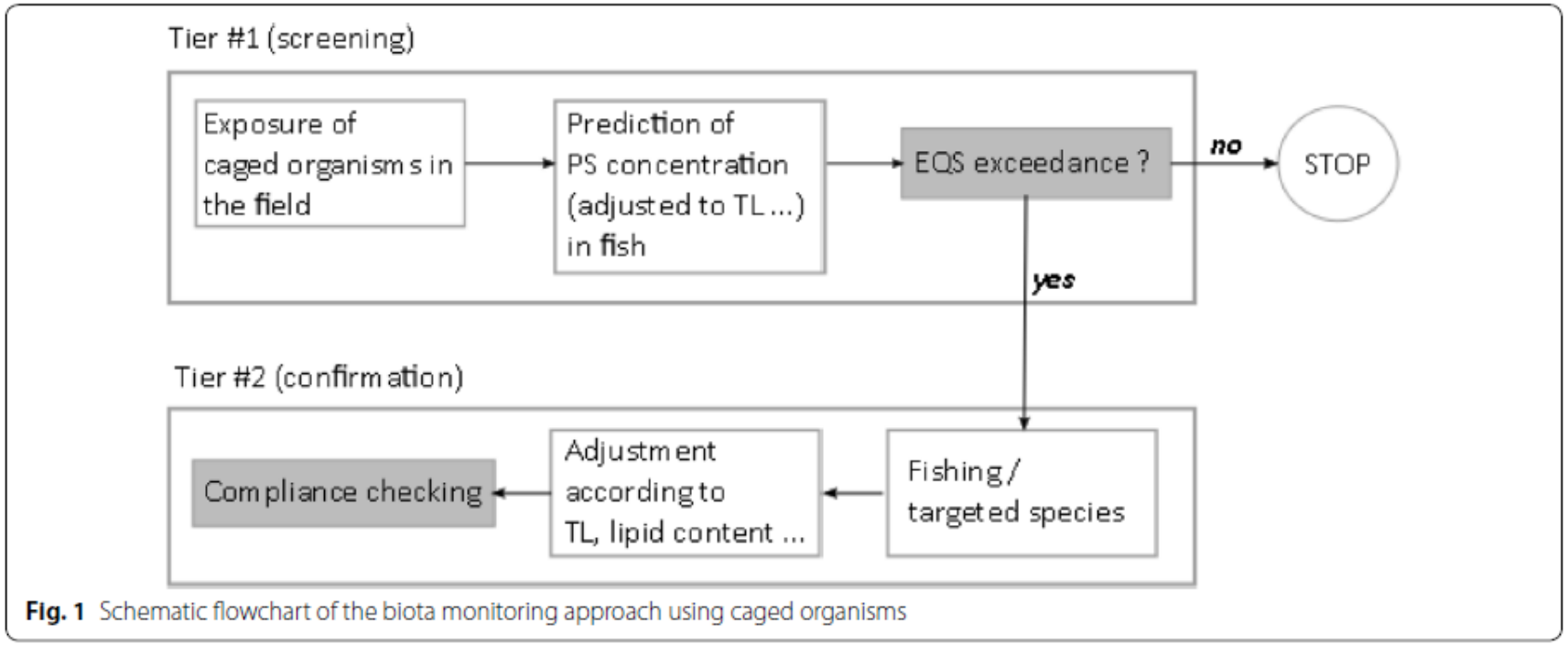


Fig. 1 Schematic flowchart of the biota monitoring approach using caged organisms

A dataset was implemented by monitoring PFOS in caged gammarids exposed at 15 sites in French rivers, and in fish from the same sites. Isotopic ratios ($\delta^{13}C$ and δ^{15} N) were also measured in gammarids and fish. The proposed tiered approach was efficient. (from Babut *et al. Environ Sci Eur* (2020) 32:131)

The way forward…

- There is no perfect solution for every situation
- Sharing the implementation experiences among the MS
- The supporting role of applied research in data discussion for a critical evaluation of the different options (hopefully funded)
- Testing solutions in small areas and short term monitoring
- Exploring alternative ways… Passive sampling?

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TMF

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ILLUSTRATIONS from E. Luzzati, Punch and the magic fish; H. Bosch, The Garden of Earthly Delights Triptych - Museo del Prado; C. Chaplin, Modern Times