Biomarkers and their application in biomonitoring in the Baltic Sea

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EnviTox webinar

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Hazardous substances – Baltic Sea

Organic

- PCBs
- pesticides, herbicides
- PAH compounds (oil, burning products)
- dioxins and furans
- organometals (e.g., TBTs, TPhT)

- a large amount of other ”emerging” compounds, e.g., surfactants, plasticizers, flame retardants, pharmaceuticals, etc., etc.…

Trace metals

- Pb, Cd, Hg, Cu, Zn, Ni...

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Ca. 80 000 - 100 000 chemical compounds in world seas...

Monitoring?

Hazardous substances – Baltic Sea

- **Pollution load estimates**
  - production
  - use
- **Concentration measurements**
  - sources (e.g., industrial and municipal wastewaters)
  - environment
    - water
    - sediment
    - biota

...BUT THESE DO NOT REVEAL BIOLOGICAL EFFECTS.

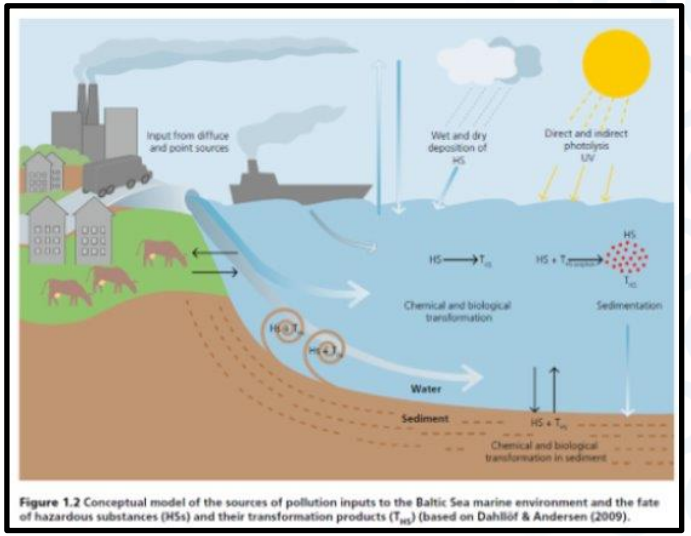

Figure 1.2 Conceptual model of the sources of pollution inputs to the Baltic Sea marine environment and the fate of hazardous substances (HSs) and their transformation products (T_{HS}) (based on Dahlöf & Andersen (2009).

Monitoring of biological effects

- relatively sporadic in the Baltic Sea so far… **BUT developing!**
- ”charismatic” species (large predatory birds, seals…)
- lower food web levels currently not targeted

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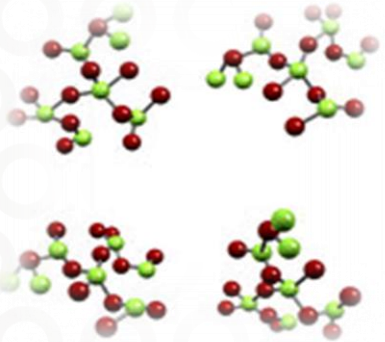
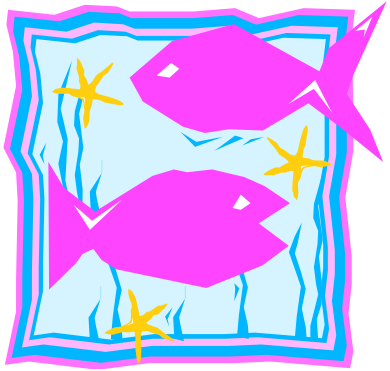
What are biomarkers?

Substance



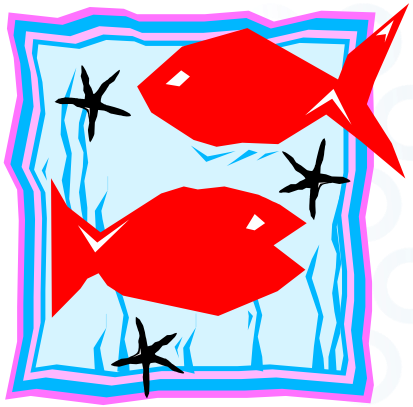
Exposure

Organism



Effect

Change in the organism = biomarker



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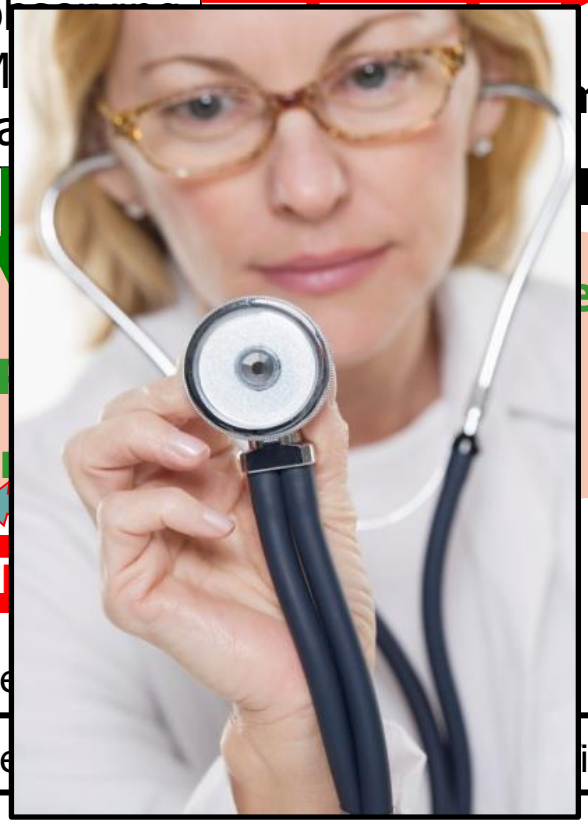
Increasing importance of effects



Biomarkers = HEALTH CHECK

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What are biomarkers?

- Biomarkers are efficient **early warning signals** indicating deteriorating environmental conditions and **exposure to hazardous substances**.
- Many biomarkers have strong mechanistic links with **pathology and disease**.
- Biomarkers offer the opportunity to interprete **the levels of hazardous substances** (= environmental concentrations) **in biological terms** (= effects).
- Many biomarkers are **universal,** expressing throughout the biota.
- Biomarkers can be used to make a **health check** on organisms.

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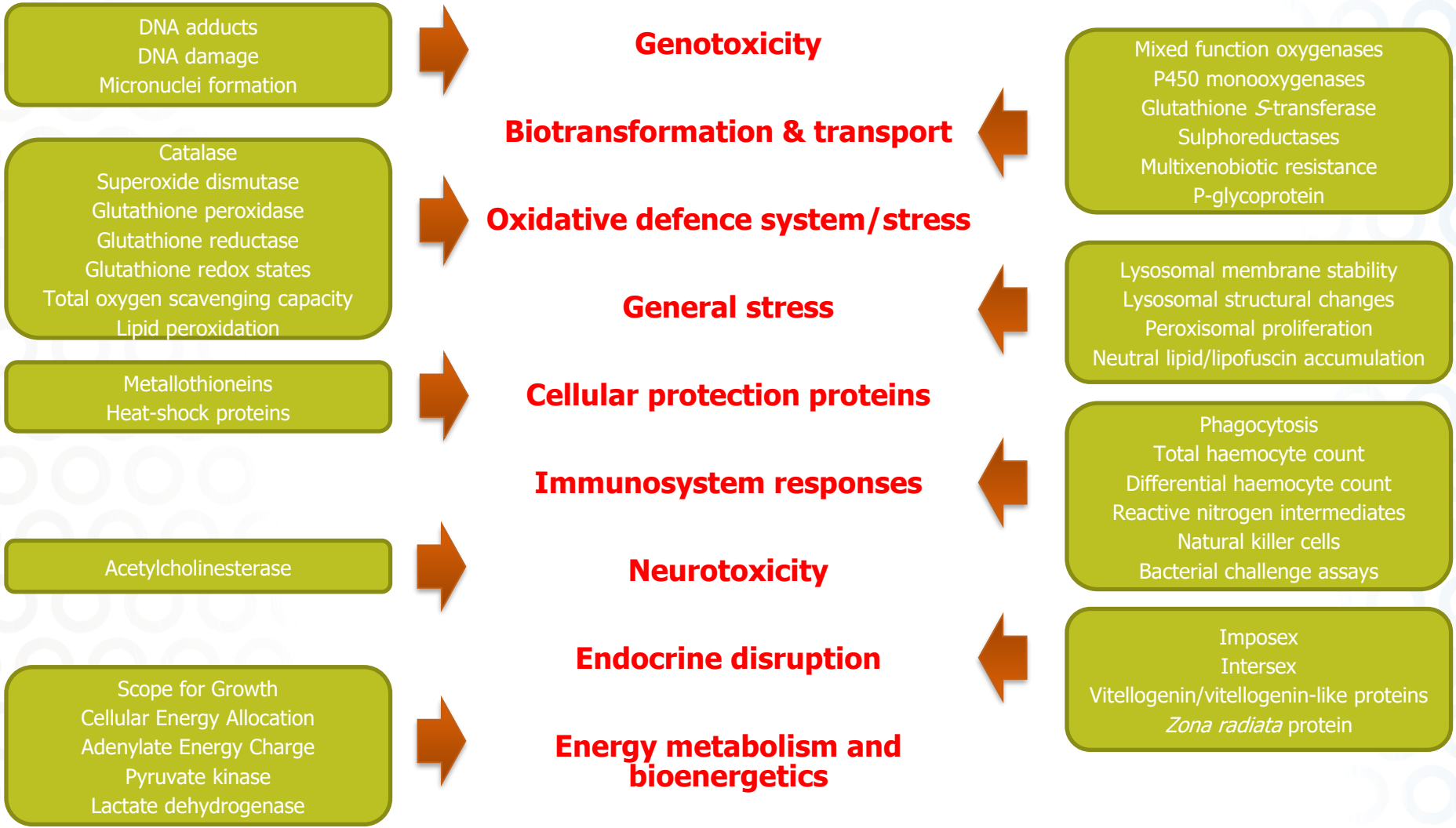
The majority of biomarkers originates from human medicine.


DNA adducts
DNA damage
Micronuclei formation
Genotoxicity
Mixed function oxygenases
P450 monooxygenases
Glutathione S-transferase
Sulphoreductases
Multixenobiotic resistance
P-glycoprotein
Biotransformation & transport
Catalase
Superoxide dismutase
Glutathione peroxidase
Glutathione reductase
Glutathione redox states
Total oxygen scavenging capacity
Lipid peroxidation
Oxidative defence system/stress
General stress
Lysosomal membrane stability
Lysosomal structural changes
Peroxisomal proliferation
Neutral lipid/lipofuscin accumulation
Metallothioneins
Heat-shock proteins
Cellular protection proteins
Immunosystem responses
Phagocytosis
Total haemocyte count
Differential haemocyte count
Reactive nitrogen intermediates
Natural killer cells
Bacterial challenge assays
Acetylcholinesterase
Neurotoxicity
Endocrine disruption
Imposex
Intersex
Vitellogenin/vitellogenin-like proteins
Zona radiata protein
Scope for Growth
Cellular Energy Allocation
Adenylate Energy Charge
Pyruvate kinase
Lactate dehydrogenase
Energy metabolism and bioenergetics

HELCOM CORESET - Recommended indicators for monitoring of biological effects of contaminants

"Pre-core" indicators

- **General stress** caused by a range of contaminants (''early warning''): **lysosomal membrane stability** in fish, bivalves or amphipods;
- Effects caused by **genotoxic** contaminants (''early warning''): induction of **micronuclei** in fish, bivalves or amphipods;
- **Reproductive success** impairments caused by a range of contaminants: **embryo aberrations** in fish (eelpout) or amphipods;
- **General health** status: **Fish Disease Index** based on externally visible fish diseases, macroscopic liver neoplasms, and liver histopathology

"Pre-core" indicators linked to chemical "core" indicators

- **imposex** in marine prosobranch gastropods (→ tributyltin, TBT)
- **PAH metabolites** in fish (→ PAH compounds)

"Candidate" indicators

- **Endocrine disruption: intersex or vitellogenin induction** in male fish
- **Neurotoxicity: acetylcholinesterase activity (AChE)**
- **Biotransformation: ethoxyresorufin-*O*-deethylase activity** (EROD)

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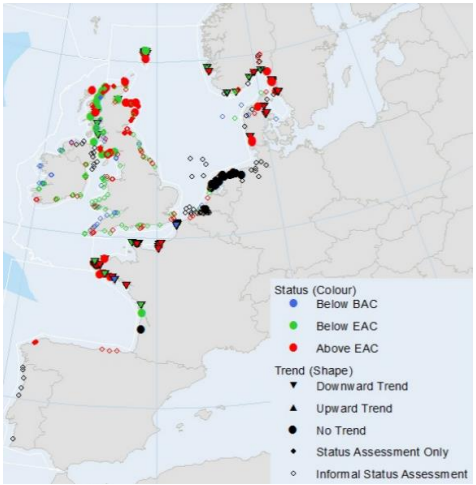
Assessment Criteria

- **EU Directives: Environmental Quality Standards (EQS) for chemicals and biological effects**
- Main question: what effect levels are above EQS?

- First established for chemicals
  - based on toxicity testing using standard laboratory species + safety factors (10-100-1000 x LC_{50}, EC_{50}, NOEC, PNEC…)
  - ecological relevance?

- Assesment Criteria for biological effects
  - Background Assessment Criteria (BAC): normal variability
  - **Ecological Assessment Criteria (EAC): unacceptable effects for biota**
  - **Confounding factors:**
    - species differences, reproductive stage
    - environmental factors, nutritional status, seasonal variability
    - (genetic variability), (physiological adaptation)

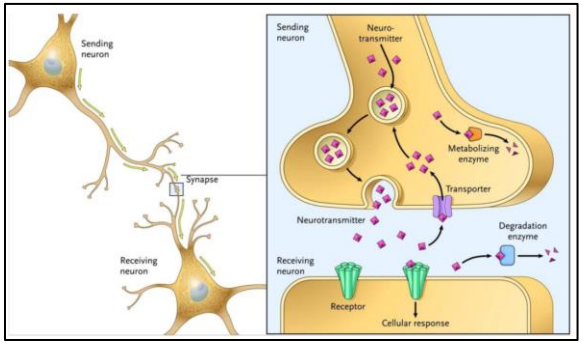
Overview of chemical and biological effect measurements of TBT in sediment and biota



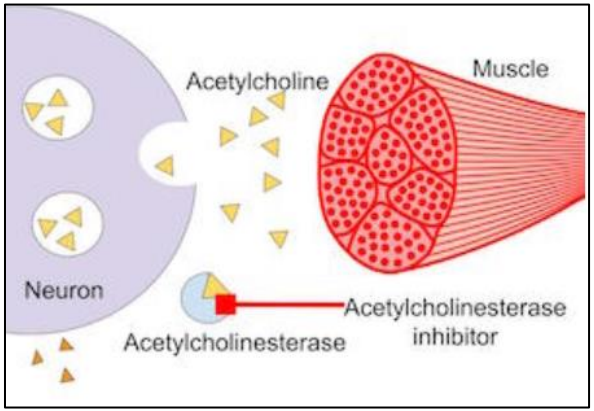
Assessment Criteria – AChE (neurotoxicity)

Table 6.1. Assessment of acetylcholinesterase activity after *in vitro* and *in vivo* exposure of biomonitoring organisms in control laboratory conditions and field studies that have utilized common monitoring species collected from reference locations

Organism	Tissue	Reference location or control conditions	Sampling season or month	Bottom temperature or temperature range (°C)	BAC AChE 10th percentile (activity nmol min^{-1} mg^{-1} protein)	EAC (activity nmol min^{-1} mg^{-1} protein)	Reference
Invertebrates							
Mytilus galloprovincialis	Gills	Wild mussels Mediterranean Sea in Spain	May–June	15–25	15	10	J. A. Campillo-Gonzalez (pers. comm.)
Mytilus galloprovincialis	Gills	Caging in field Mediterranean Sea—Carteau, France	Seasonal cycle	14–25	29	20	Bodin *et al.* (2004)
Mytilus edulis	Gills	Wild mussels Atlantic Ocean (NW Portugual)	Seasonal cycle	-	26	19	L.Guilhermino (pers. comm.)
Mytilus edulis	Gills	Wild mussels Atlantic Ocean (Loire estuary)	Seasonal cycle	-	30	21	Bocquené *et al.* (2004)
Vertebrates							
Plathichthys flesus	Muscle	French Atlantic Ocean (Seine Bay)	-	15	235	165	Burgeot *et al.* (2001)
Plathichthys flesus	Muscle	French Atlantic Ocean (Ster estuary, Brittany)	-	15	335	235	Evrard *et al.* (2010)
Limanda limanda	Muscle	French Atlantic Ocean (Seine Bay)	-	15	150	105	Burgeot *et al.* (2001)
Mullus barbatus	Brain	Mediterranean Sea SE Spain (Màlaga-Almeria)	October	14	75	52	C. Martínez-Gómez (pers. comm.)
Mullus barbatus	Muscle	Mediterranean Sea (France, Spain, Italy)	*In situ*	18	155	109	Burgeot *et al.* (1996a); Bocquené (pers. comm.)



Neurotoxic chemicals inhibit AChE activity



Biomarkers in marine monitoring

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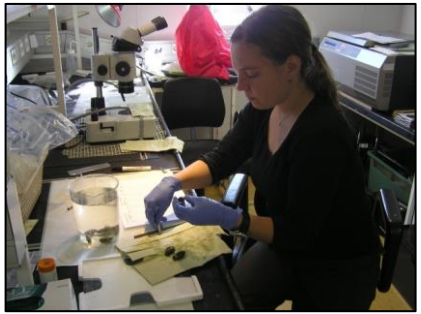
Mussels in biomonitoring

Genus *Mytilus*

- *M. edulis, M. galloprovinicialis, M. trossulus, M. californiensis, M. chilensis*
  - wide geographical distribution
  - sessile (attached to the substrate)
  - long-living
  - tolerant to environmental changes
  - relatively easy to handle
  - filter large amounts of water ---> exposure to contaminants in water and particles



Mussels in biomonitoring

OSPAR Commission Monitoring of the Northeastern Atlantic

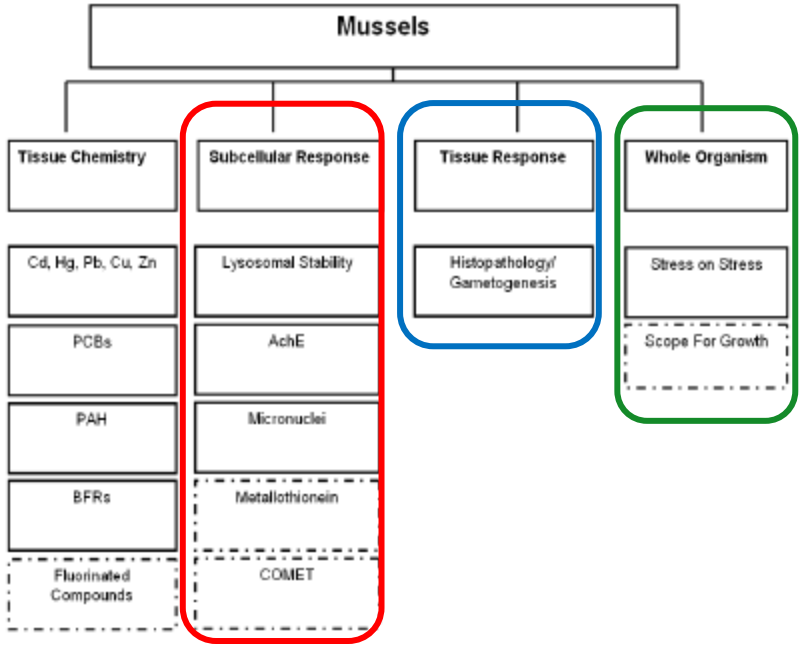


Figure 2.3. Methods included in the mussel component of the integrated monitoring framework. Solid lines, core methods; broken lines, additional methods. PCBs, polychlorinated biphenyls; PAH, polycyclic aromatic hydrocarbon; BFRs, brominated flame retardants; AChE, acetylcholinesterase.

Davies and Vethaak (2013)

Seasonal variability in biomarkers

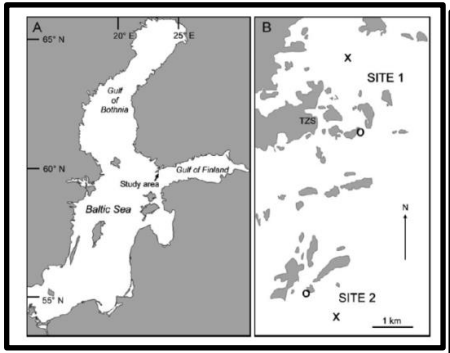
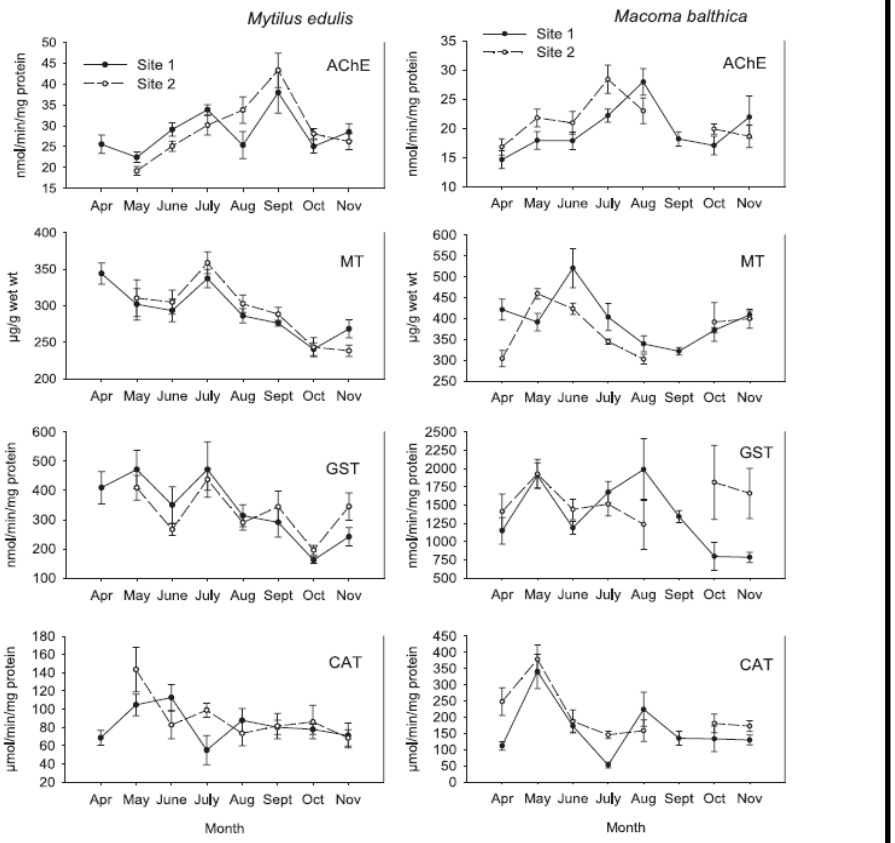


Table 1
Seasonal variations in temperature, salinity, and/or oxygen saturation and Secchi depth in (A) surface water at the sampling sites of *Mytilus edulis* and (B) near bottom water at the sampling sites of *Macoma balthica*

Month	Temperature (°C)		Salinity		Oxygen saturation (%)	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
(A) *Mytilus edulis* sampling						
Apr	0.5	nm	5.4	nm	nm	nm
May	7.6	8.1	5.1	5.1	4.0	6.0
June	10.3	8.6	5.5	5.5	5.0	7.9
July	19.5	18.5	5.3	5.4	2.8	3.4
Aug	16.1	16.4	5.4	5.3	4.5	4.9
Sept	15.8	15.8	5.6	5.6	3.0	3.5
Oct	12.5	12.5	5.4	5.6	6.0	7.5
Nov	5.1	5.7	6.0	6.1	3.0	3.5

Month	Temperature (°C)		Salinity		Secchi depth (m)	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
(B) *Macoma balthica* sampling						
Apr	1.9	nm	6.4	nm	50	nm
May	4.4	4.4	4.9	6.2	67	74
June	4.1	3.8	6.3	6.4	62	60
July	10.8	9.1	5.7	5.6	64	66
Aug	6.4	6.0	6.3	6.5	49	50
Sept	15.8	nm	5.4	nm	85	nm
Oct	11.0	10.8	6.1	6.2	65	70
Nov	6.5	7.0	6.0	6.3	80	71

The depths of *M. balthica* sampling sites were 36 m (Site 1) and 33 m (Site 2). nm—not measured.

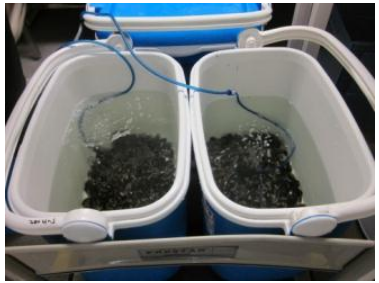


ig. 2. Seasonal variations in AChE activity (mean ±S.E.) in the gill (*M. edulis*) or foot (*M. balthica*) tissue, and the levels of MT and activities of GST and AT in the digestive gland tissue of the study species at the study sites.

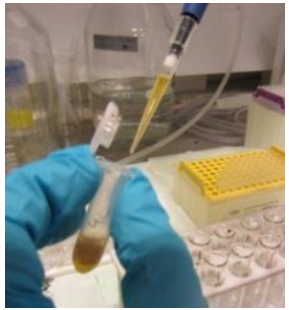
The mussel caging method







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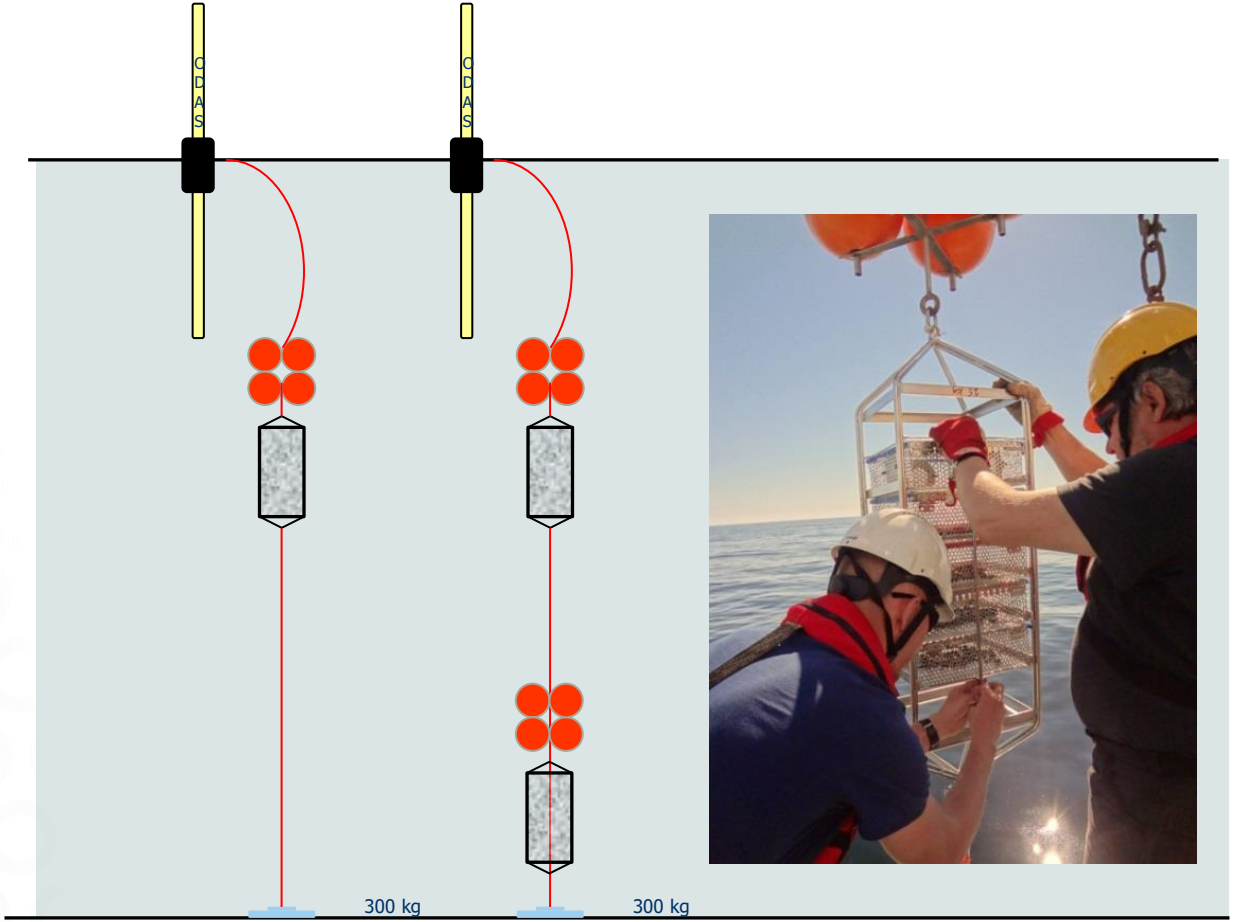
Mussel caging

- avoiding genetic differences and physiological adaptation of organisms at the monitored sites
- background data similar in all caged mussels
- well-defined exposure times and sites

→ a better control over the processes → more comparable data



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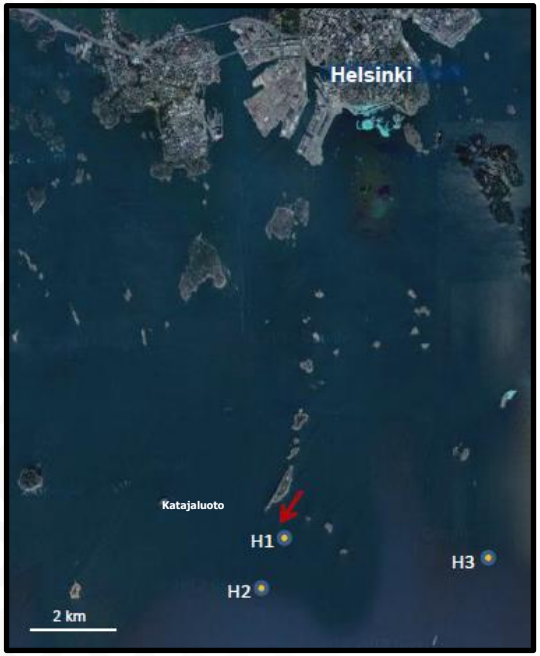
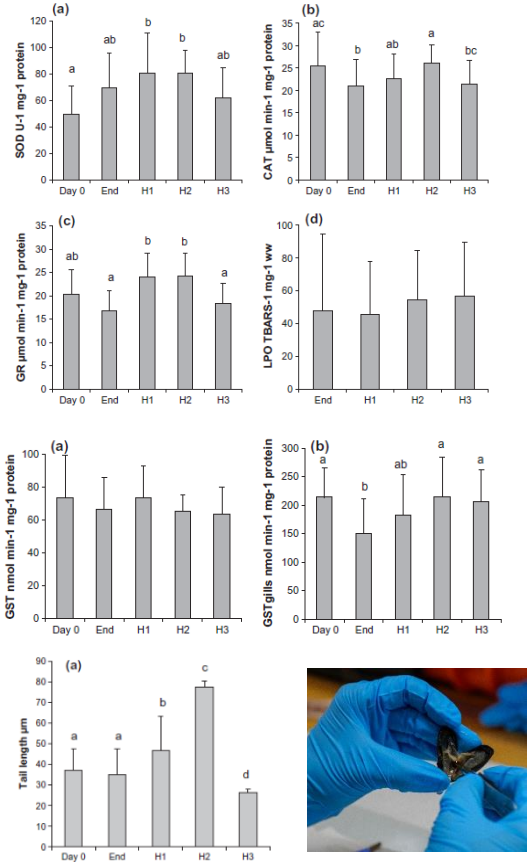
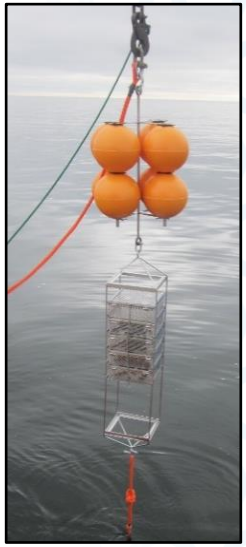
Biomonitoring off Helsinki WWTP







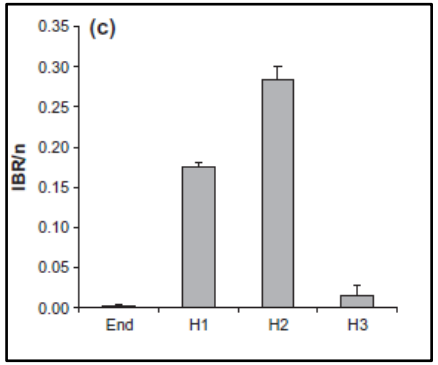
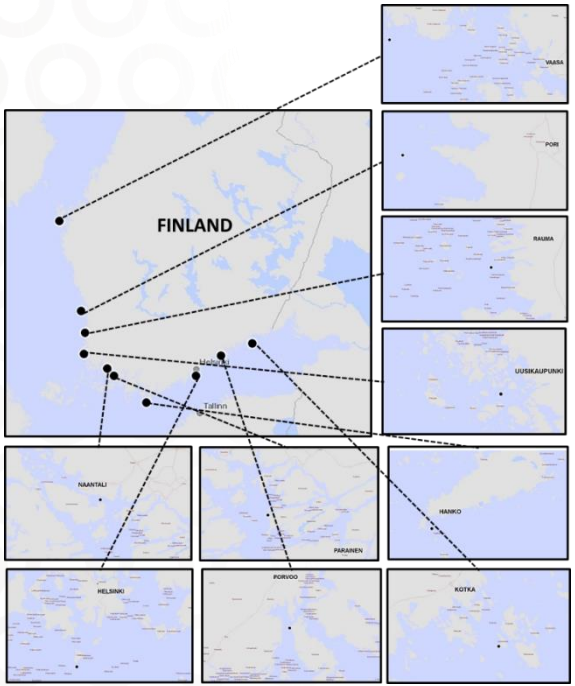
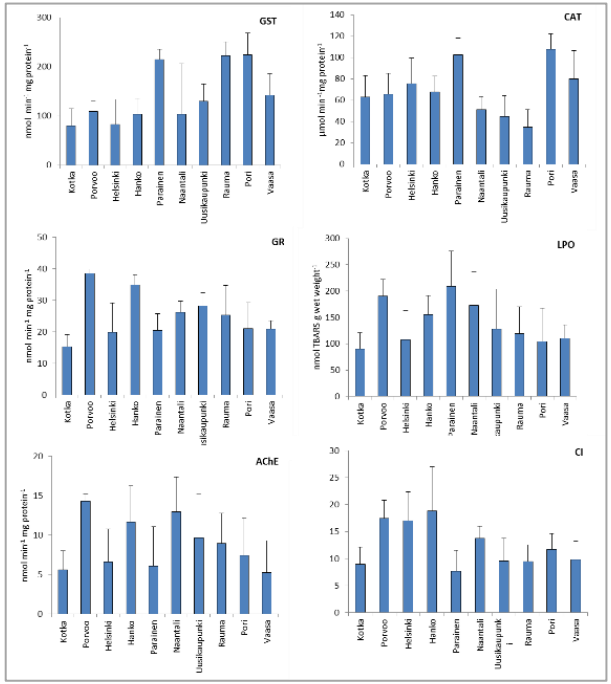


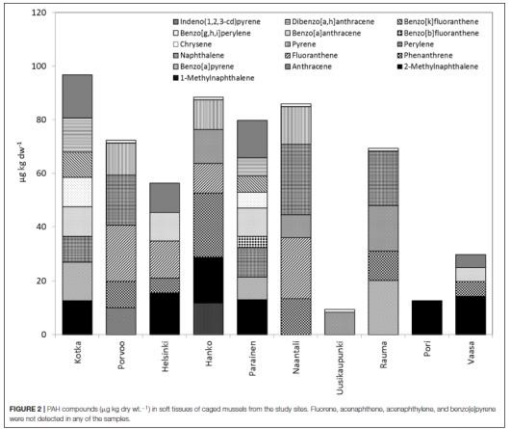
Table 4
Concentrations of pharmaceuticals in passive samplers (POCIS) at mussel caging sites H1–H3. Values are given as the average ng/POCIS of three extractions except for H1 where the average of two extractions is given ± standard deviation, not detected (nd).

Pharmaceutical	H1	H2	H3
Diclofenac	25 ± 8	33 ± 1	nd
Ibuprofen	12 ± 1	nd	nd
Ketoprofen	20 ± 9	nd	nd
Naproxen	39 ± 10	12 ± 3	nd
Metoprolol	40 ± 6	25 ± 4	8 ± 2
Bisoprolol	39 ± 3	19 ± 4	5 ± 1
Acebutolol	8 ± 1	4 ± 1	nd
Venlafaxine	25 ± 5	14 ± 2	nd
Carbamazepine	232 ± 15	232 ± 7	147 ± 5
∑Pharmaceuticals	440	339	160

Biomonitoring on the Finnish coast 2018-19: mussel caging







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Biomonitoring on the Finnish coast 2018-19: integrated assessment

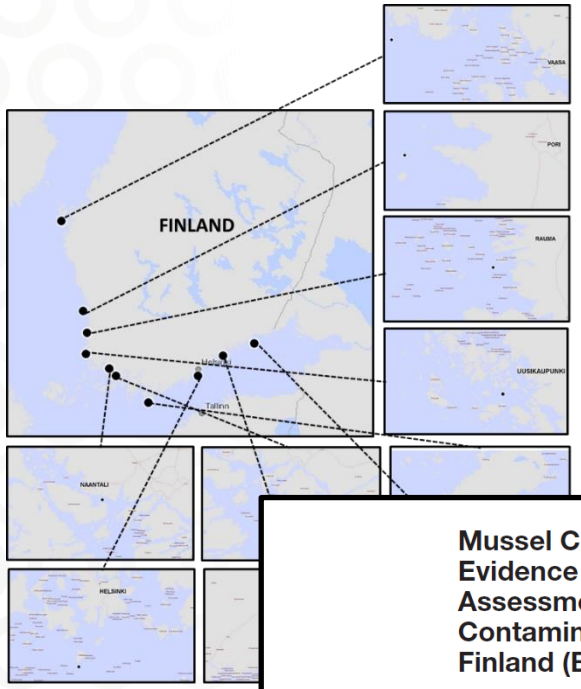

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TABLE 3 | Elaborations with levels of hazard assigned to the different LOEs and the final WOE.

Site	Chemical characterization	Bioavailability	Biomarkers	Benthic communities	Near-bottom oxygen	Eutrophication	Weight of Evidence integration	
Kotka	HQ: 0.284 Absent -	HQ: 63.417 Major BaP–DBahA–BkF; PER	HQ: 4.229 Moderate CAT–GST	HQ: 67.174 Major	Absent	Major	MODERATE	
Porvoo	HQ: 0.311 Absent -	HQ: 63.030 Major ANT–FLU; PER	HQ: 4.642 Moderate GST-LPO-CAT; GR	HQ: 46.078 Moderate	Slight	Major	MODERATE	
Helsinki	HQ: 2.271 Slight 100% Zn	HQ: 14.842 Slight -;-	HQ: 2.517 Moderate -; GST	HQ: 31.326 Slight	Absent	Major	SLIGHT	
Hanko	HQ: 0.28 Absent -	HQ: 29.925 Moderate -; 1-MetNAPH	HQ: 2.714 Moderate GR-GST; -	HQ: 46.377 Moderate	Absent	Moderate	SLIGHT	
Parainen	HQ: 1.7 Slight 100% Zn	HQ: 59.329 Major BbF–BaP; PER	HQ: 2.008 Slight LPO;-	HQ: 48.291 Moderate	Absent	Major	MODERATE	
Naantali	HQ: 2.829 Moderate	HQ: 80.710 Major FLU; PER–Sn	HQ: 2.402 Moderate GST-CAT; -	HQ: 49.020 Moderate	Absent	Major	MODERATE	
	Q: 1.985 ight	HQ:2.42 Moderate CAT-GST; -	HQ: 9.520 Absent	Slight	Moderate	SLIGHT		
	Q: 64.589 ajor PER–BaP	HQ: 2.125 Slight CAT; -	HQ: 33.676 Slight	Absent	Moderate	MODERATE		
	Q: 0 sent	HQ: 0 Absent -;-	HQ: 50.986 Moderate	Absent	Slight	SLIGHT		
	Q: 4.296 ght	HQ: 1.0 Slight -;-	HQ: 59.938 Moderate	Absent	Slight	SLIGHT		

ical characterization of seawater (showing the percentage of the parameter contributing most to the HQ), bioavailability (parameters showing neters showing moderate or major effects), and benthic communities.

Mussel Caging and the Weight of Evidence Approach in the Assessment of Chemical Contamination in Coastal Waters of Finland (Baltic Sea)

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Edited by:
Francois Galgani, Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), France

Contamination status of coastal areas of Finland (northern Baltic Sea) markedly affected by anthropogenic activities (harbors, shipyards and maritime activity, industry, municipal and agricultural inputs, legacy contamination) was assessed for the first time using the weight of evidence (WOE) approach. The key element of the study was the

Thank you for your attention!