Monitoring methodologies for accidental spill of HNS at sea: integrated approach

IRIS

General background: Toxicity classification of HNS carried by ships

- Evaluated by the IMO/GESAMP working group
  - MARPOL 73/78 convention establishing profiles for classifying hazard of HNS
  - 1995 – harmonization with OECD guidelines
  - Revised 2002 annex II - Globally Harmonised System (GSH) with new hazard profiles

Column	A Bioaccumulation	B Aquatic toxicity	C & D Toxicity to human beings	E Interference with others uses of the sea

  - 2007 new revision with four categories

Category	X	Y	Z	OS
Hazard	+++	++	+	-

The facts …

- Relatively high chemical sea traffic in European waters
  - ☹ Environmental hazards
- Scarce ecotoxicological data for the marine environment
- Current monitoring based mainly on chemistry
- Need for complementary monitoring methodologies

Issues raised for HNS spill at sea

- New chemicals released on market: problem to implement methods for all of them
- In case of spill, rapid dilution → below chemical detection limits
- Which methods can we propose in complement?
- Added value of biological effect assessment ?
- Are the methods currently evaluated in e.g. ICES(WGBEC)/OSPAR (JAMP) applicable ?
- Can they be applied for the monitoring of remediation i.e. post-spill ?
- Communication of the results to regulators: how can they be integrated as a basis for decision-making ?

PRAGMA – A pragmatic and integrated approach for the evaluation of environmental impact of oil and chemical spilled at sea: input to European guidelines

EU DG-ENVIRONMENT agreement number 07.030900/2005/429172/SUB/A5

www.iris.no/pragma

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Status PRAGMA

- Technical implementation completed & final reporting to EU DG-Environment issued beginning January 2008

➔Fuel oil and Styrene

➔Appraisal of methodolgies

➔Laboratory pilot exposures

RESPIL – Response means to chemicals spilled at sea and environmental damage

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Cedre

CNRS/UBO
UMR 6539
LEMAR

EU DG-ENVIRONMENT grant agreement 07.030900/2006/448357/SUB/A3

RESPIL

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Status Respil

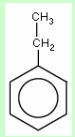
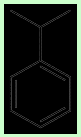
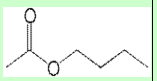
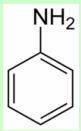
- On going – will benefit from the outcome of PRAGMA

➔Select HNS carried by ships in bulk and posing potential risks for environment (large volume, frequency, toxic…)

➔Laboratory exposures

➔Field (mesocosm) exposures

Selection of HNS in Respil

			GESAMP										
Nom	Chemical structure	SEBC Code	A	B	C	D	E	Mar-pol	Solubility g/100mL	Trafic Rank	Half-life in solution	BCF estimated from log kow	Acute Aquatic toxicity LC_{50}
Ethyl benzene		**FE**	0	**3**	1	I	XX	Y	**0,015**	**41**	Half-life in marine mesocosm (Wakeham et al., 1983): •Spring (8-16℃): **20 days** •Summer (20-22℃): **2.1 days** •Winter (3-7℃): **13 days**	**Low** in aquatic organisms. BCF: 2.16	•*Daphnia magna* (24 h): **2.2** mg/L •Mysid shrimp (96 h): 5.1 mg/L
Cumene		FE	T	**3**	1	I	X	Y	**0,0074**	80	Half-life in an aerobic freshwater sediment/water test system (Williams et al. 1993): **2.5 days**	**Slight potential** to bioaccumulate in fish. BCF: 356	•*Daphnia magna* (24 h): **4.8** mg/L •Mysid shrimp (96 h): 1.2 mg/L
n-Butyl acetate		**FED**	0	**2**	0	I	X	Y	**0,70**	68	Half-life at 20℃ (HYDROWIN model US EPA, 2000):•at pH 9 **11,4 days** •at pH 8 **114 days** •at pH 7 **3,1 years**	**Unlikely** to be bioaccumulated. BCF for fish: 14	•*Daphnia magna* (24 h): **72,8** mg/L •Brine shrimp (24 h): 150 mg/L
Aniline		**FD**	0	**3**	2	II	XX	Y	**3,4**	**24**	**Short** half-life (i.e., up to a few weeks)	**Low** bioaccumulation potential	•*Daphnia pulex* (48 h): **0,1** mg/L

General objectives

- To propose alternative methods to classical monitoring techniques for accidental spill
- To perform pilot studies using these methods
  - Biomarkers
  - Other ”new”/emerging techniques
- Integration in environmental practises & policy

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Brief overview of EU-projects

- Accidental spill at sea
- Environmental status
- Sentinel species
- Biological effects
- (Bio)sensing
- Pilot study
  - Laboratory and ”field” (mesocosm)
  - exposure and recovery

➡ Methods recommendation

➡ Contingency plan

➡ Communication and decision-making process

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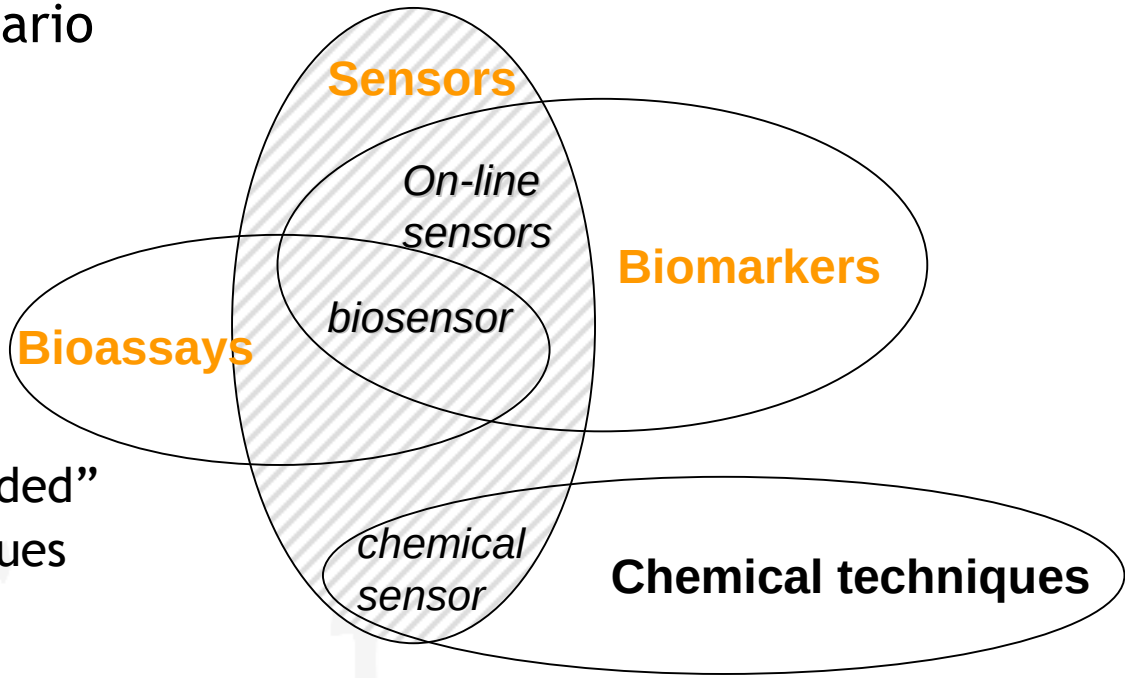
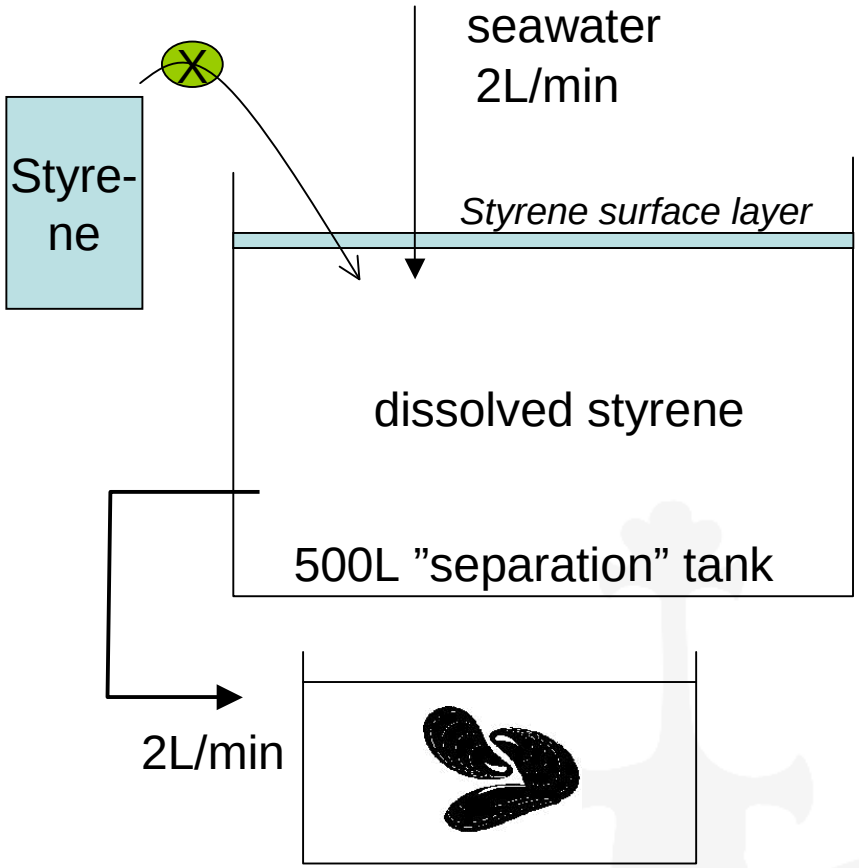
Some key features…

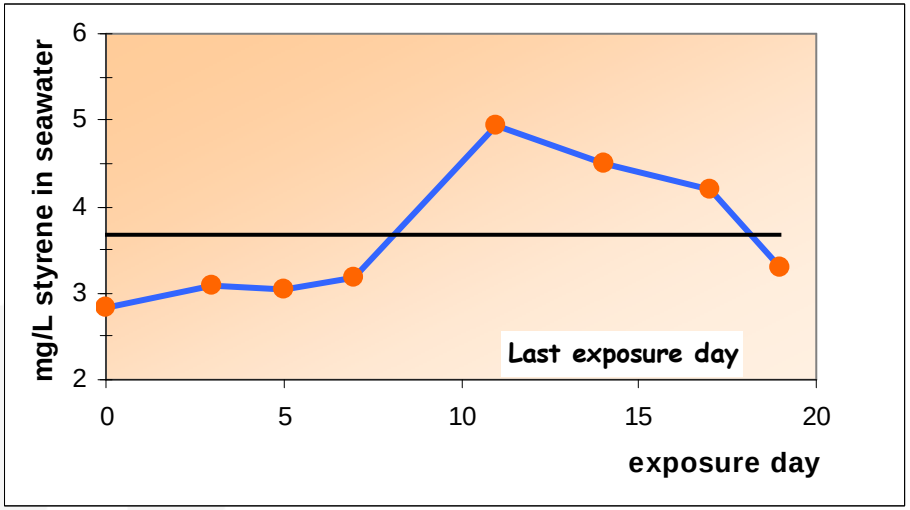
- ”Realistic” exposure scenario
- Chemistry
  - Water, body burden
- Biomarker
  - Prioritize simple methods
  - ”Effect” markers
  - ICES/OSPAR ”recommended”
  - Other promising techniques
- Bioassay with larvae
- (Bio)sensor
  - Cost-effective screening assessment level



Conceptual monitoring framework

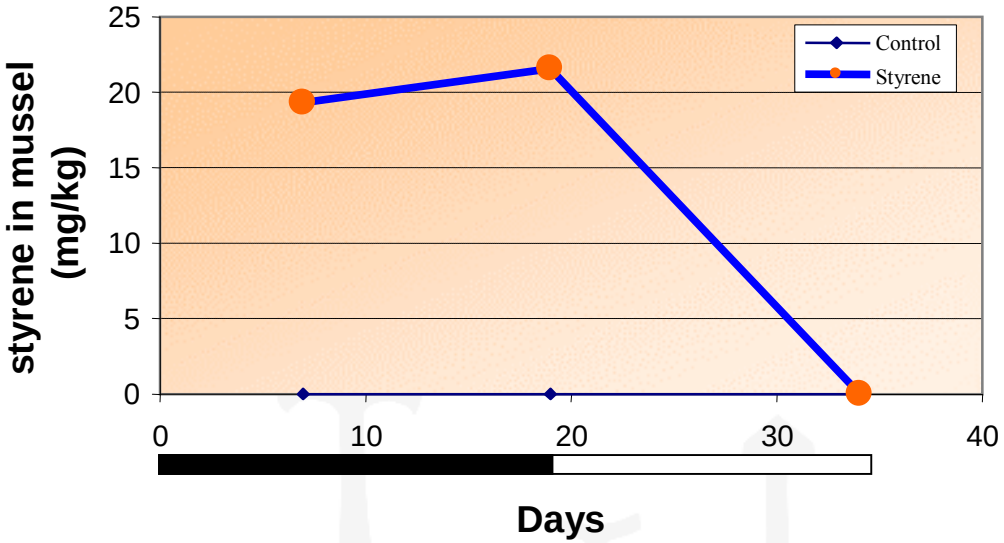
Exposure scenario (styrene)





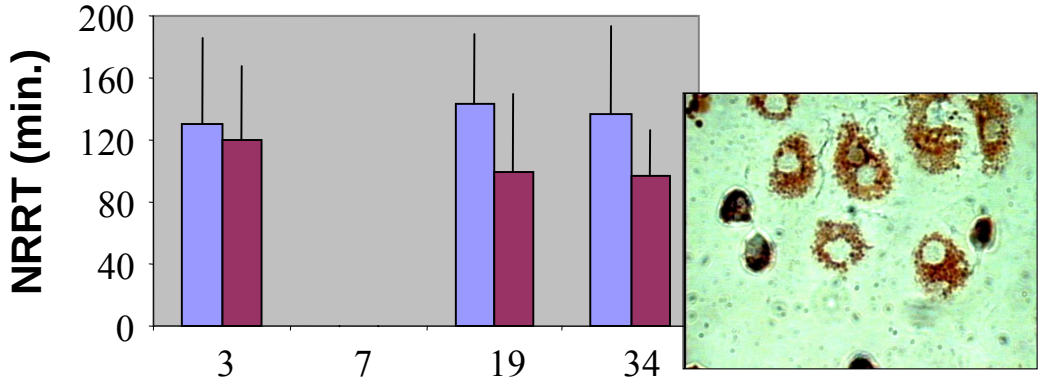
Bioaccumation

Average BCF=6.3

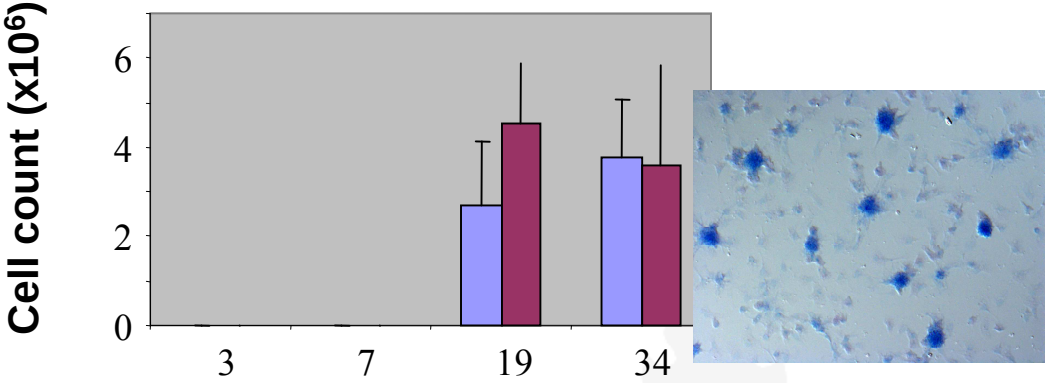


➔ Measurable body burden but relatively low bioconcentration compared to for example PAH

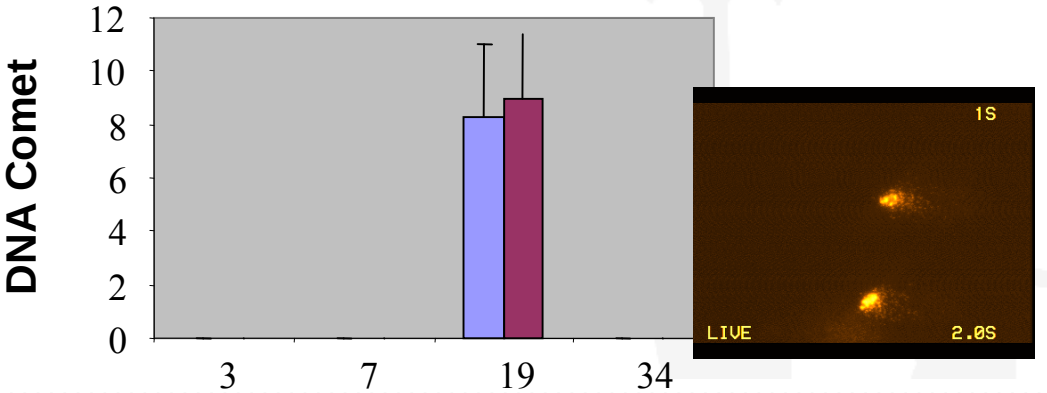
Stress responses in hemolymph cells



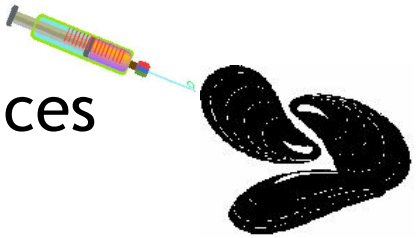
➔ trend for NRRT decrease with time



➔ higher cell numbers at day 19, recovered



➔ no diffences



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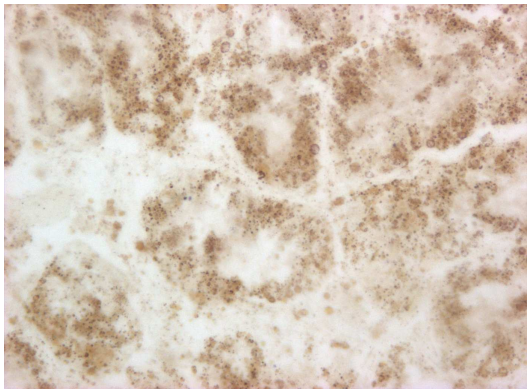
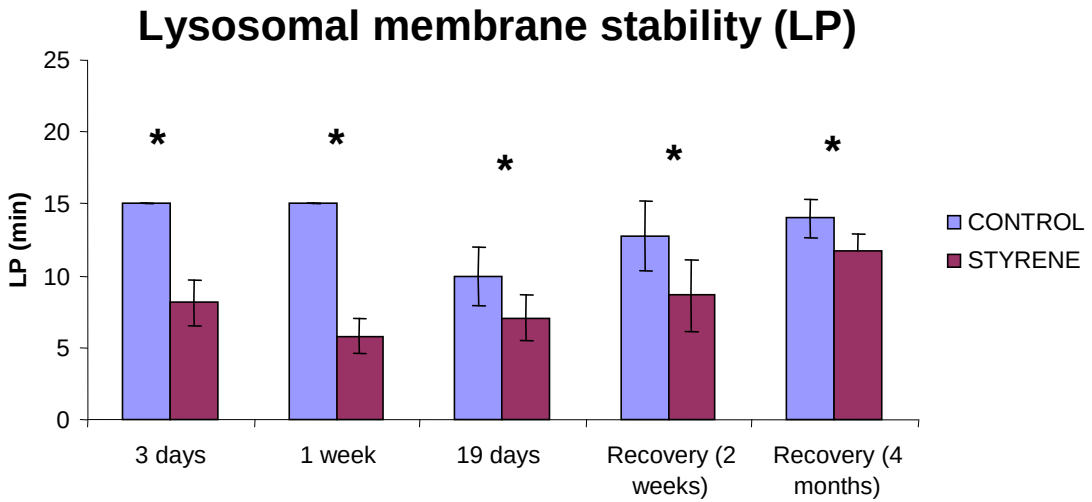
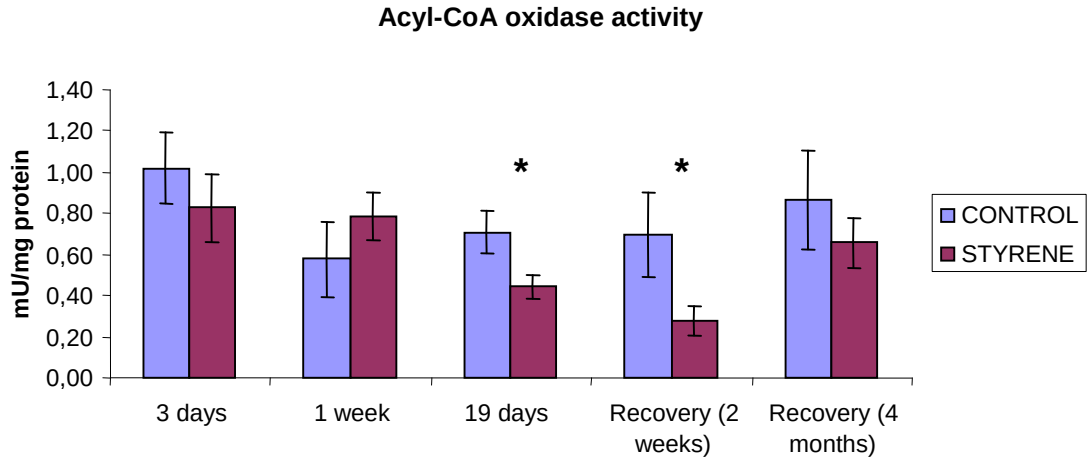
Histological markers





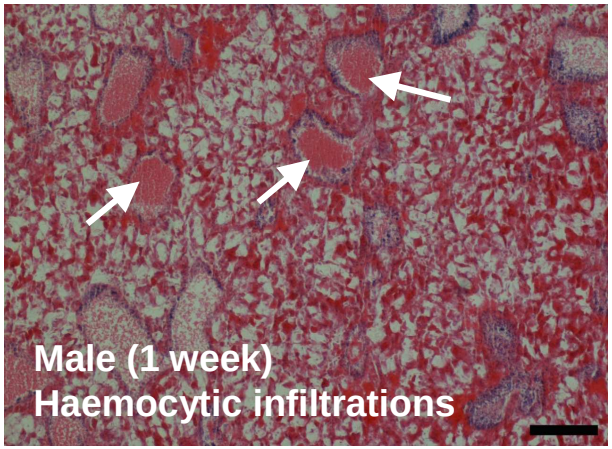


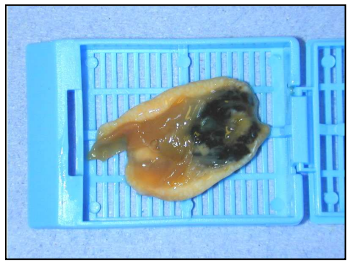
➔ Positive LP responses at all sampling time, including recovery

➔ AOX decreasing at end of exposure and 2 weeks recovery.

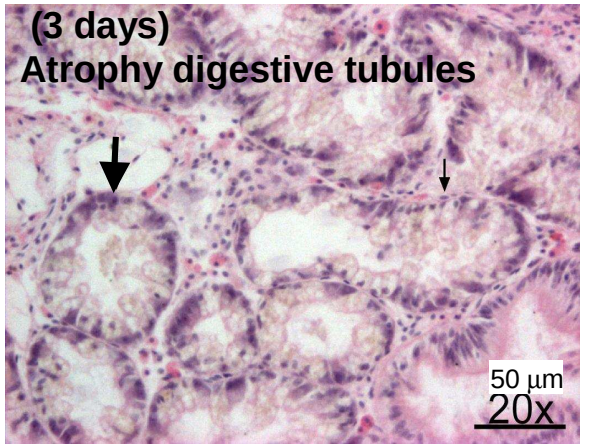
Histopathological assessment

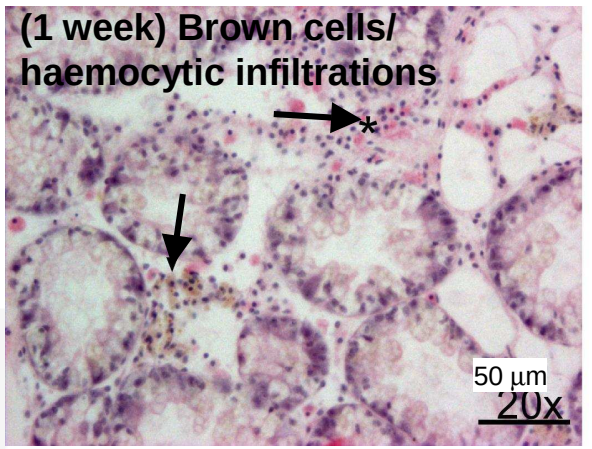
GONAD





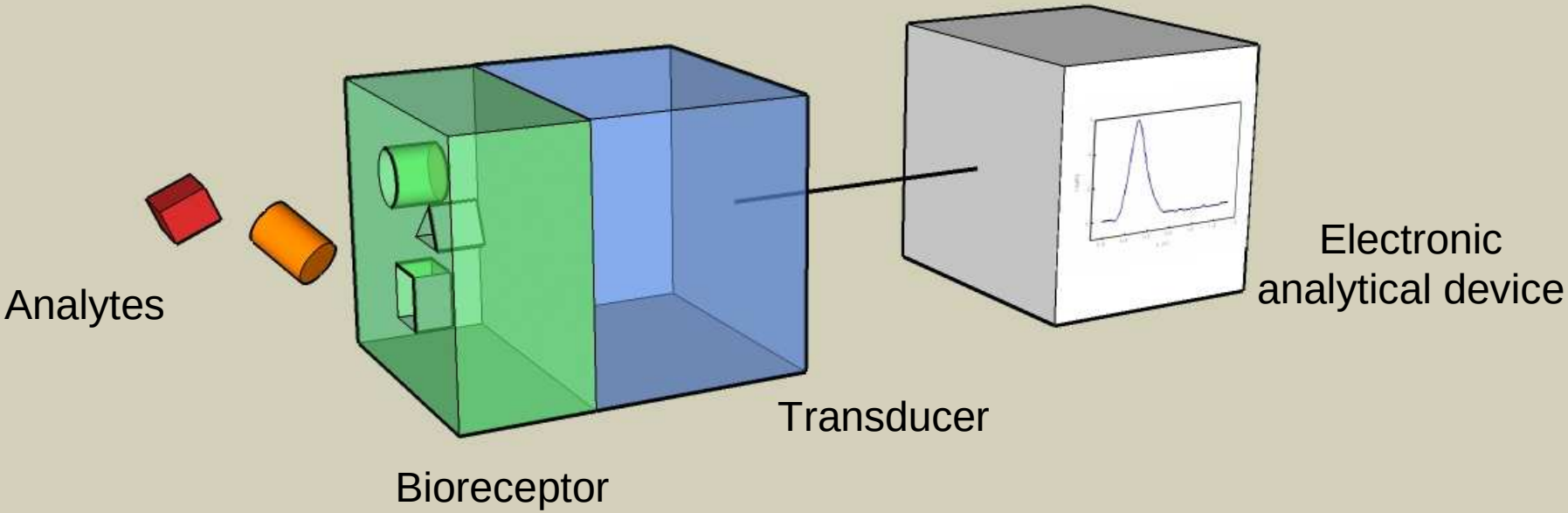
DIGESTIVE GLAND





- Gonad: no major differences
- DG: high prevalence of brown cells and atrophic tubules during exposure (>3days) and at recovery (2w)

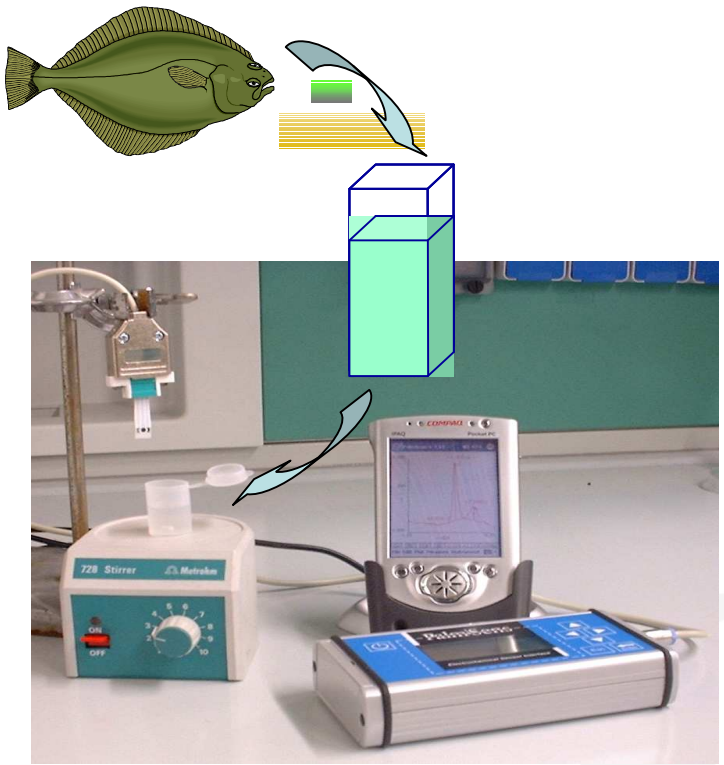
Configuration and characteristics of a biosensor

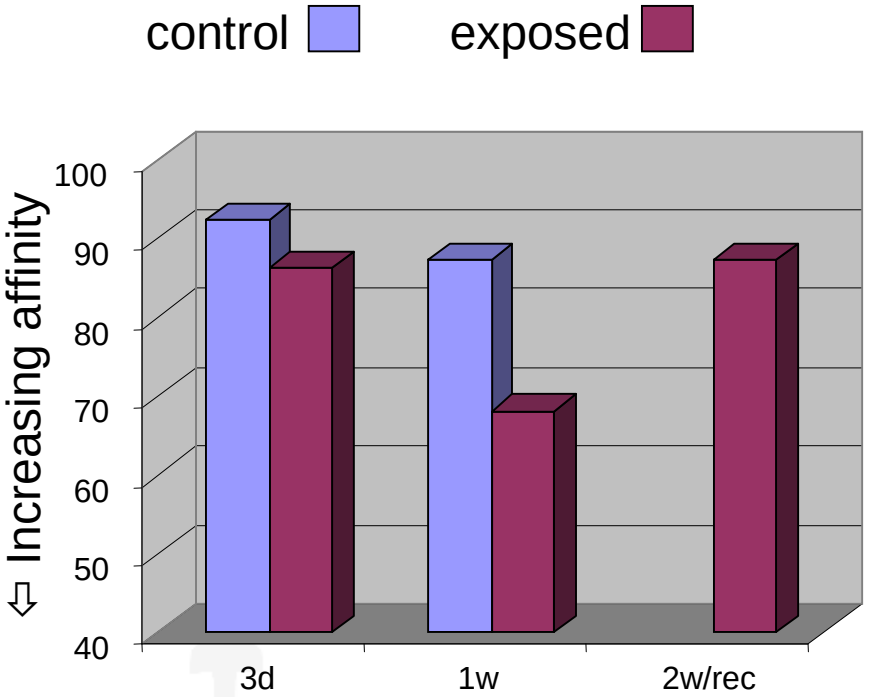


- Minimum sample preparation
- Fast readings
- Small
- Portable

↳ well suited to complement analytical methods for environmental monitoring

Biosensor screening: dna biosensor (”genosensor”) in fish bile samples





- Positive geno-sensing response during exposure
- No differences during recovery

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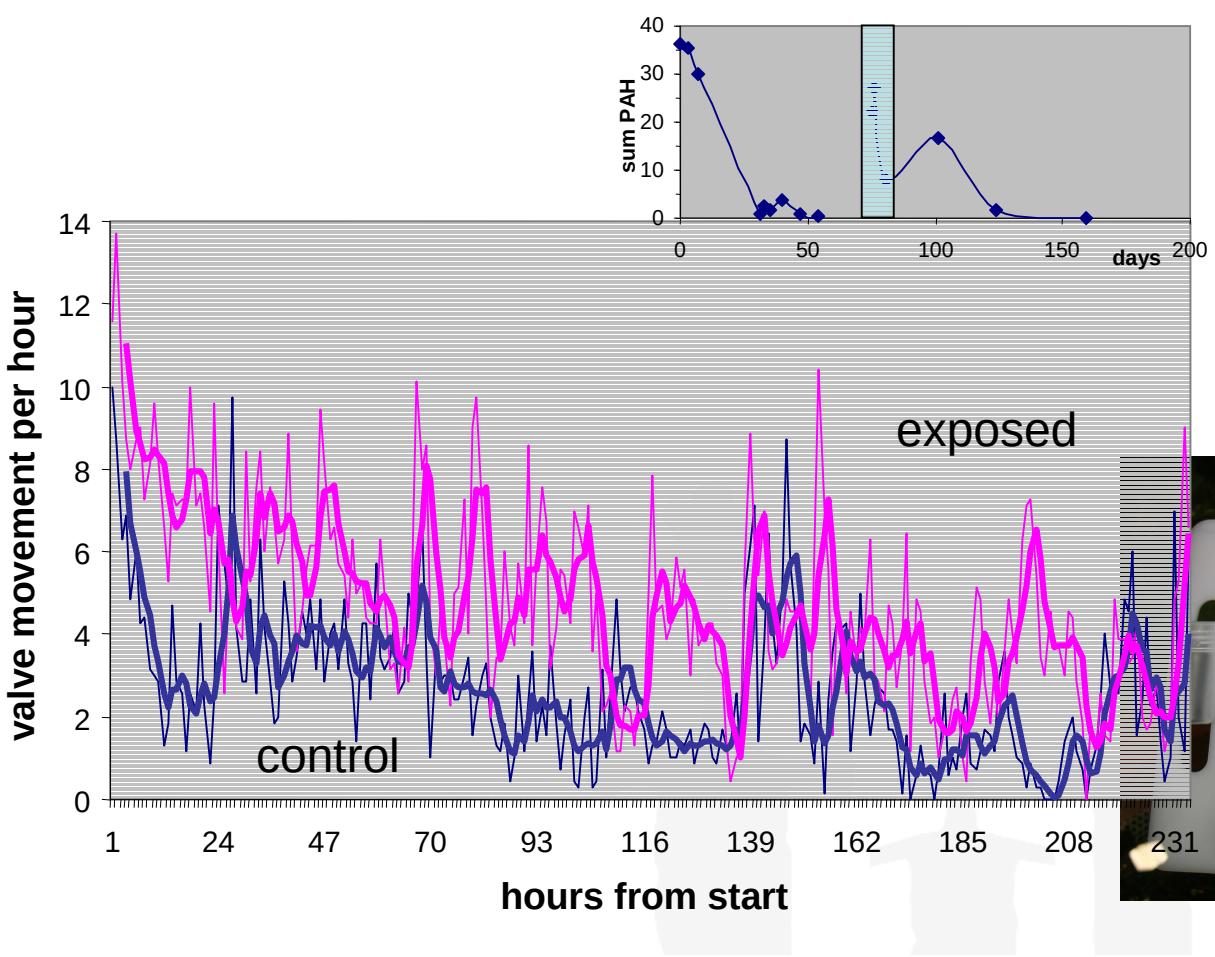
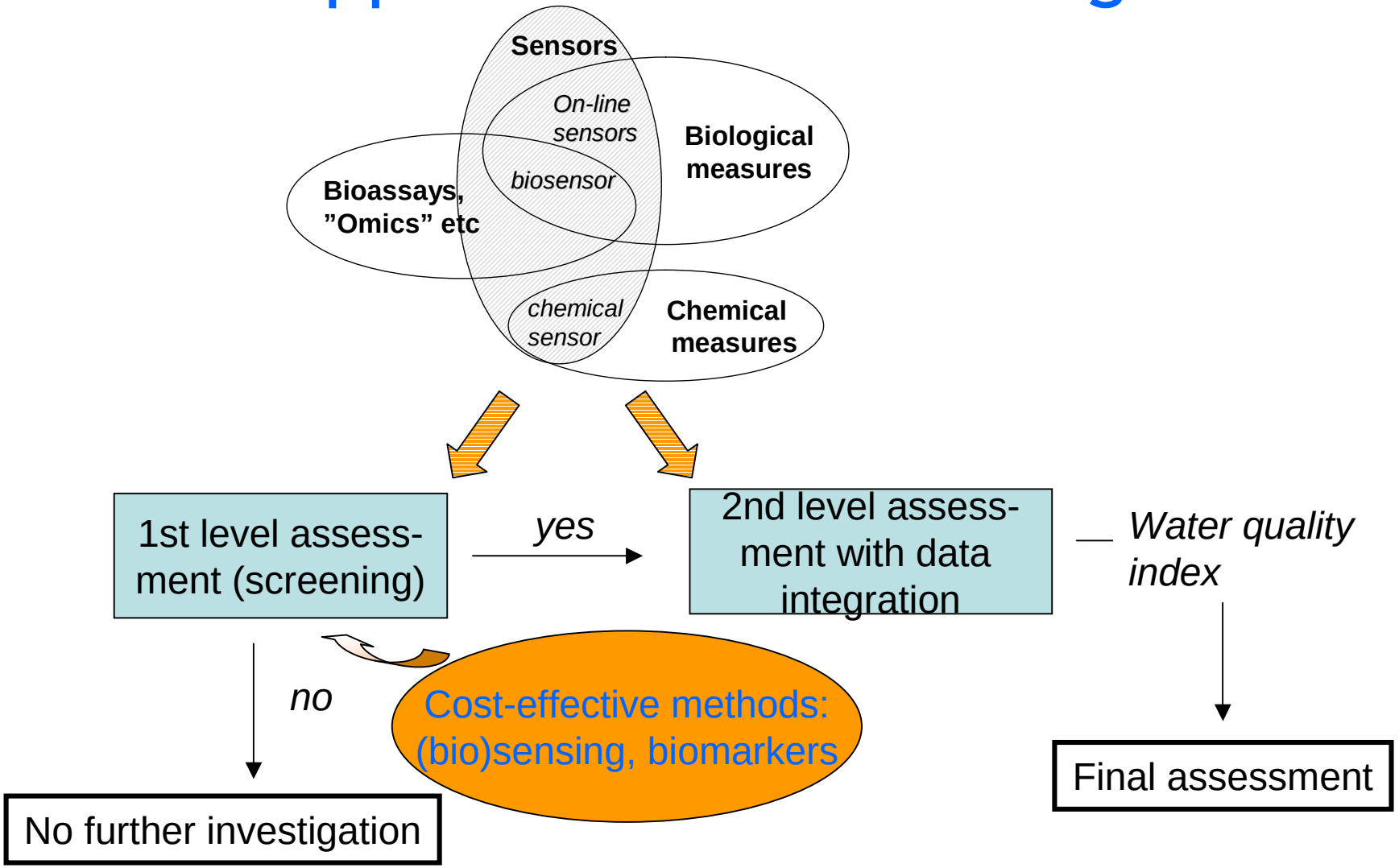
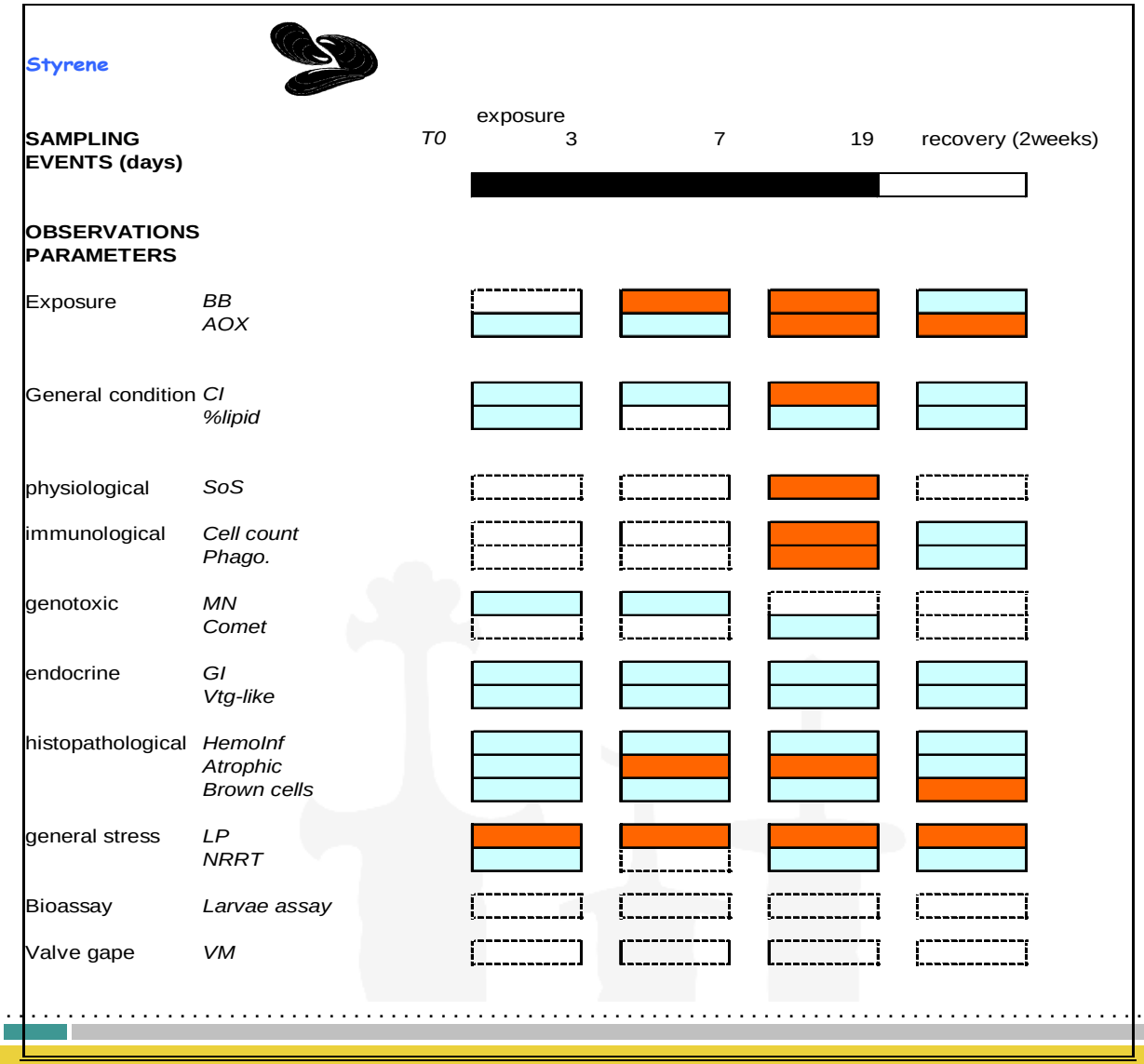
Sensing – valve gape movement (fuel oil)



A tiered approach for monitoring



Summary of responses in mussel exposed to styrene

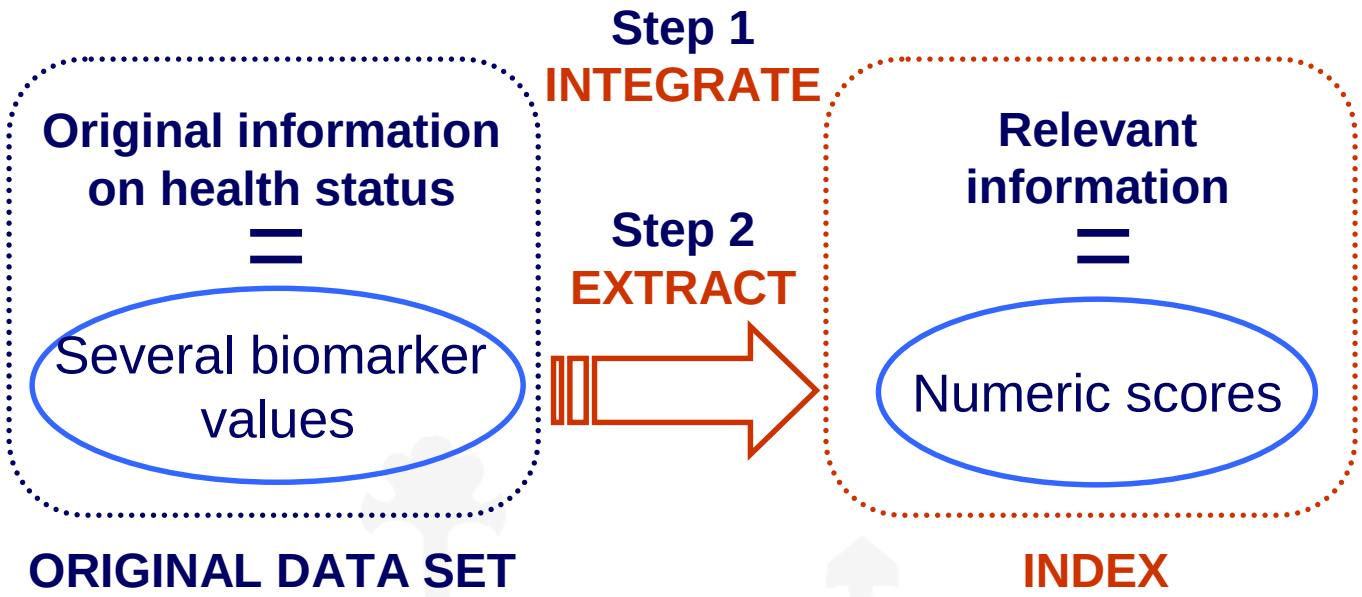


Integration process

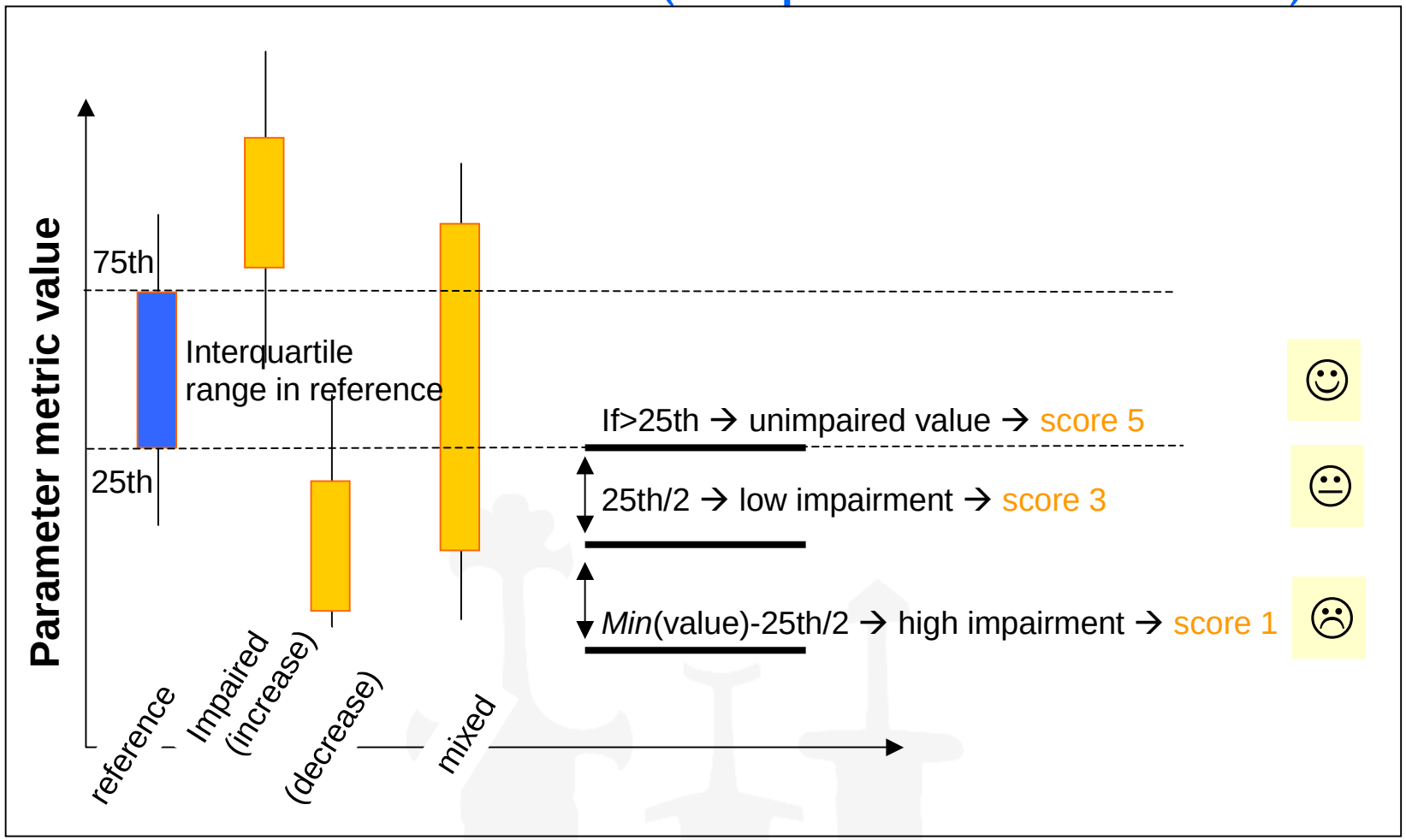
- How can we use the information from biological effects in a sound and pragmatic way for environmental status ?
- General requirements set in projects
  - Provide an easy-to-understand summary of the situation
  - Easy to vizualize
  - Use existing analytical methodologies

Scoring system as decision-making tool

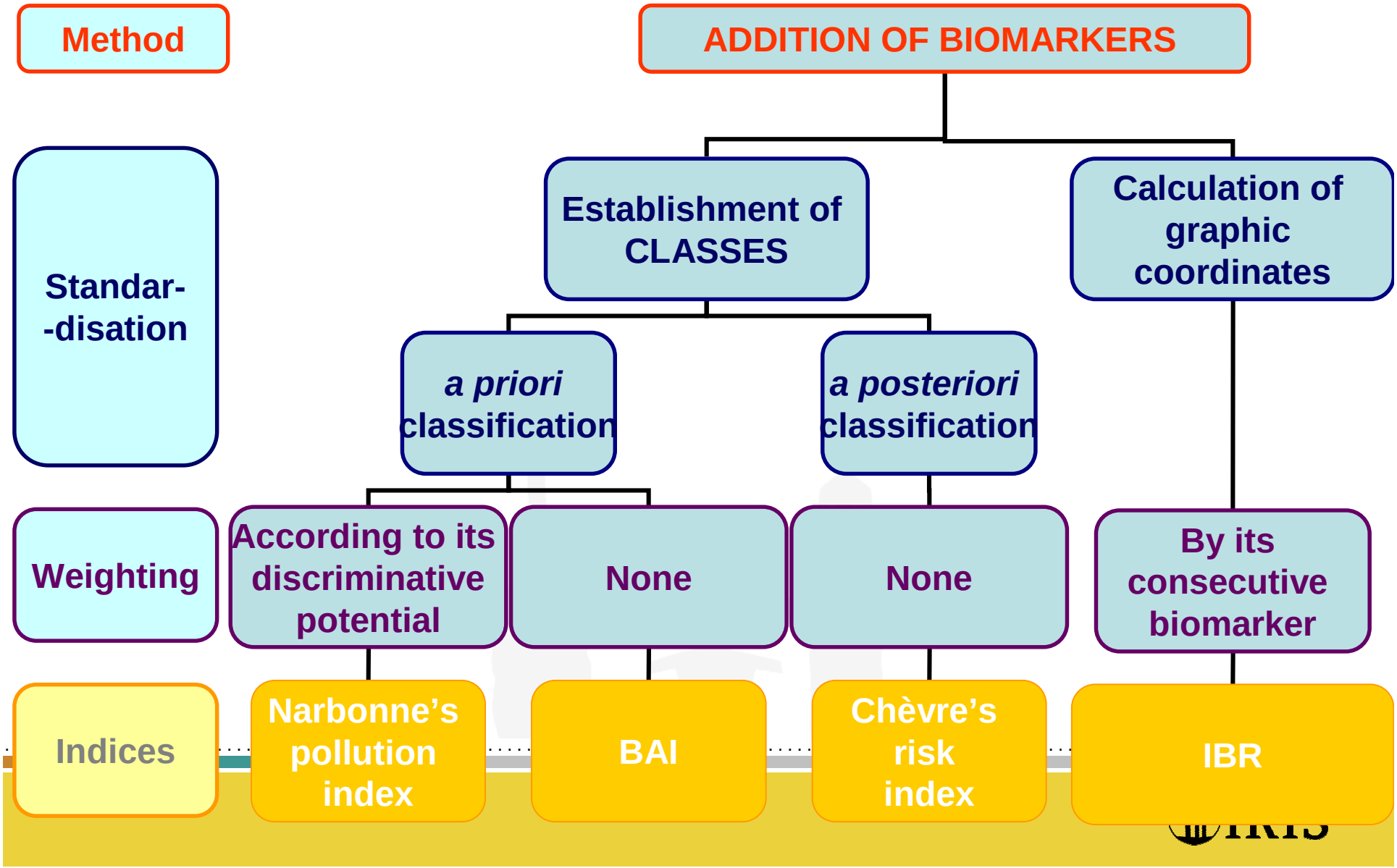
General Principles :



Multimetric index (adapted from US EPA)



Relevant quality indices reviewed



IBR – Integrated biomarker response

Station/Group	B1	B2	B3	B4
k0	11.7	47.1	156.7	476.8
k2	11.8	48.6	162	470.9
w2	11.2	51.2	165.2	458.6
w3	11	55.4	176.3	435.8
p2	13.6	56.3	168.4	434.7
p4	12.1	49.4	126.8	171.6

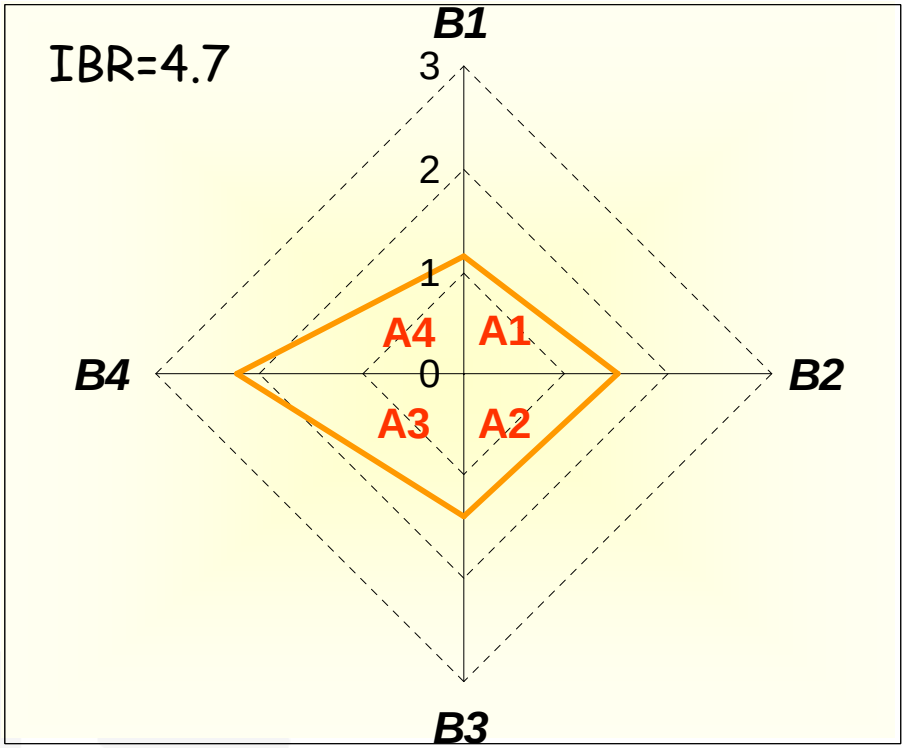
w3 11 55.4 176.3 435.8

↓

S 1.15 1.50 1.39 2.21

↓

$IBR = \Sigma Ai$



(*modified from Beliaeff & Burgeot,2002*)

EU-PRAGMA Original data matrix – **Styrene**
mean estimates of 11 general condition (GH) and general stress parameters -

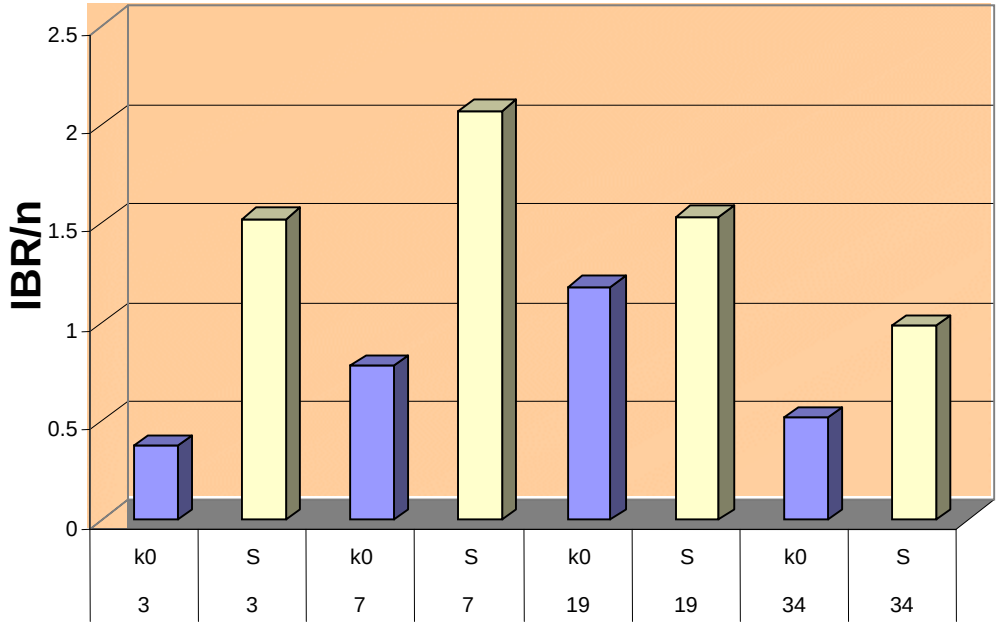
IBR calculation sheet - Since the number of biomarkers (n) used varied with time, the values of iBR are given as IBR/n

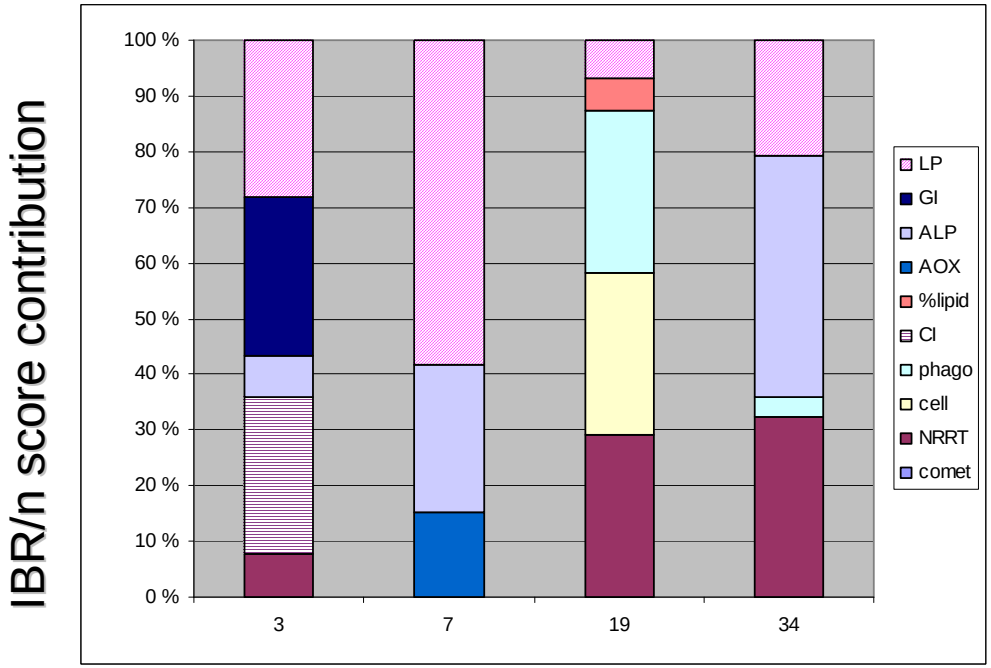
	sampling	group	comet	NRRT	cell	phago	CI	%lipid	AOX	ALP	GI	LP	MN	styrene bb
day	**3**	k0	-	130	-	-	0.624748	0.915774	**1.018771**	**13.84535**	**2**	**15**	-	
		S	-	120	-	-	0.585926	0.910636	**0.825532**	**15.57385**	**1.5**	**8.15**	-	
	7	k0	-	-	-	-	0.497533	-	**0.577453**	**13.76551**	**1.368421**	**15**	-	0.020
		S	-	-	-	-	0.547427	-	**0.788501**	**17.63642**	**1.736842**	**5.8**	-	19.30
	19	k0	8.2932	143	2.71	0.315577	0.495639	1.008355	**0.708785**	**16.34418**	**1.315789**	**10**	-	0.020
		S	9.0	100	4.541111	1.1	0.543603	1.086201	**0.442874**	**14.28322**	**1.631579**	**7.05**	-	21.63
	34	k0	-	137	3.750661	1.000036	0.422045	0.883554	**0.69744**	**11.49431**	**1.578947**	**12.75**	-	0.013
		S	-	96.66667	3.605556	1.1	0.490323	0.858129	**0.275474**	**16.91201**	**1.8**	**8.625**	-	0.018
		m	8.63	121.11	3.65	0.87	0.53	0.94	0.67	14.98	1.62	10.30		
		s	0.48	19.28	0.75	0.38	0.06	0.09	0.23	2.01	0.23	3.56		
		CV	6 %	16 %	21 %	43 %	12 %	9 %	35 %	13 %	14 %	35 %		

Comet=DNA strand break (hemocyte); NRRT=hemocyte neutral red retention time; cell=hemocyte cell counts; phago= Phagocytosis in hemocyte; CI=Whole condition index; %lipid=%total lipid; AOX=peroxisome proliferation; ALP=Vtg-like proteins; GI=Gonadic index; LP=lysosomal membrane stability (histology); MN=Micronuclei (hemocyte);

Styrenebb= Styrene body burden in whole organism

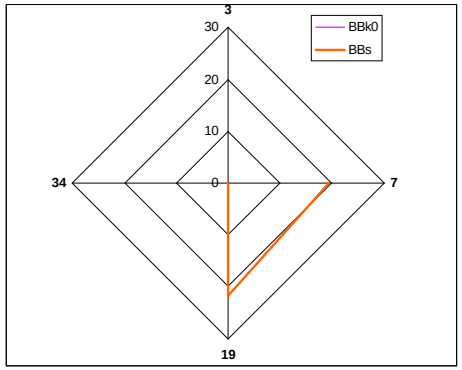
”Weighted IBR” (IBR/n) calculation



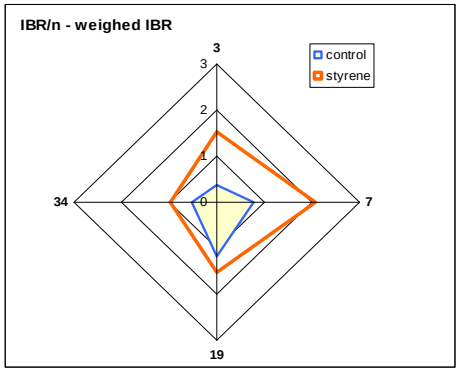


Exposure days

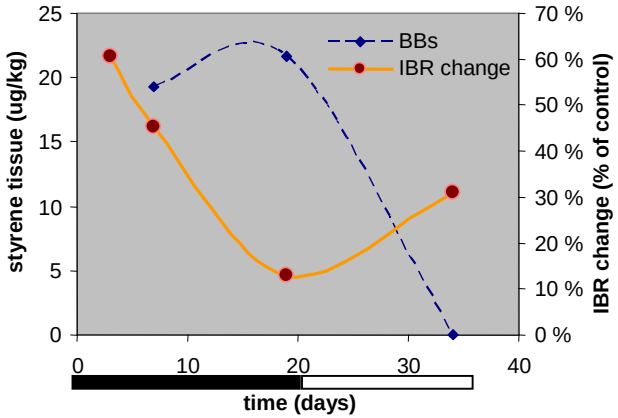
K0=control
S=Styrene



Styrenebb



IBR/n



IBR change expressed as
(IBRh-IBRk)/sum(IBRh:IBRk)

EU-PRAGMA Original data matrix – **Fuel**
mean estimates of 11 general condition (GH) and general stress parameters -

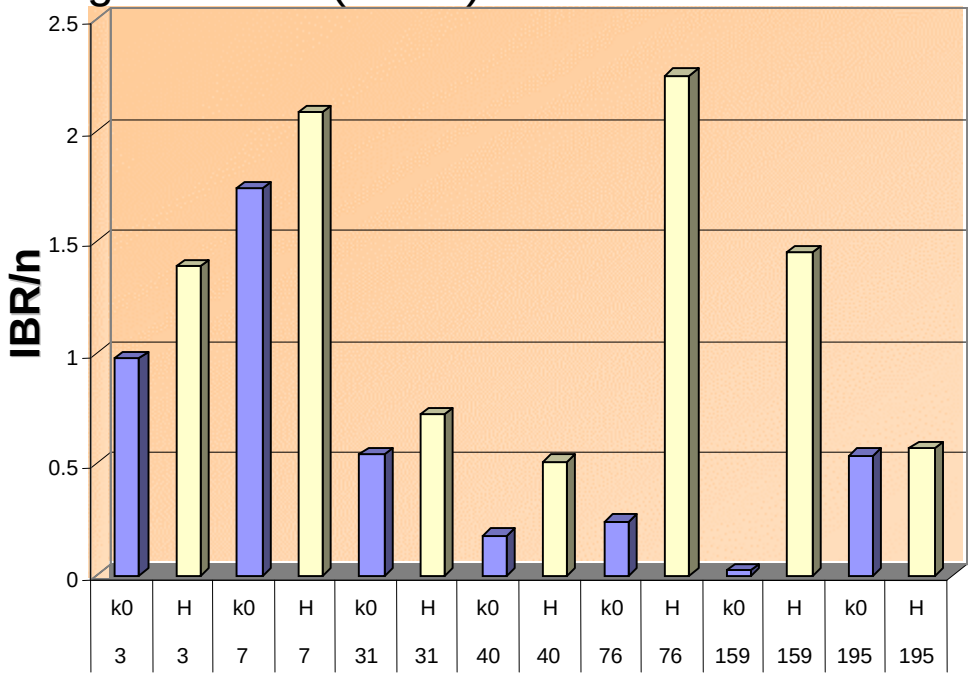
IBR calculation sheet - Since the number of biomarkers (n) used varied with time, the values of iBR are given as IBR/n

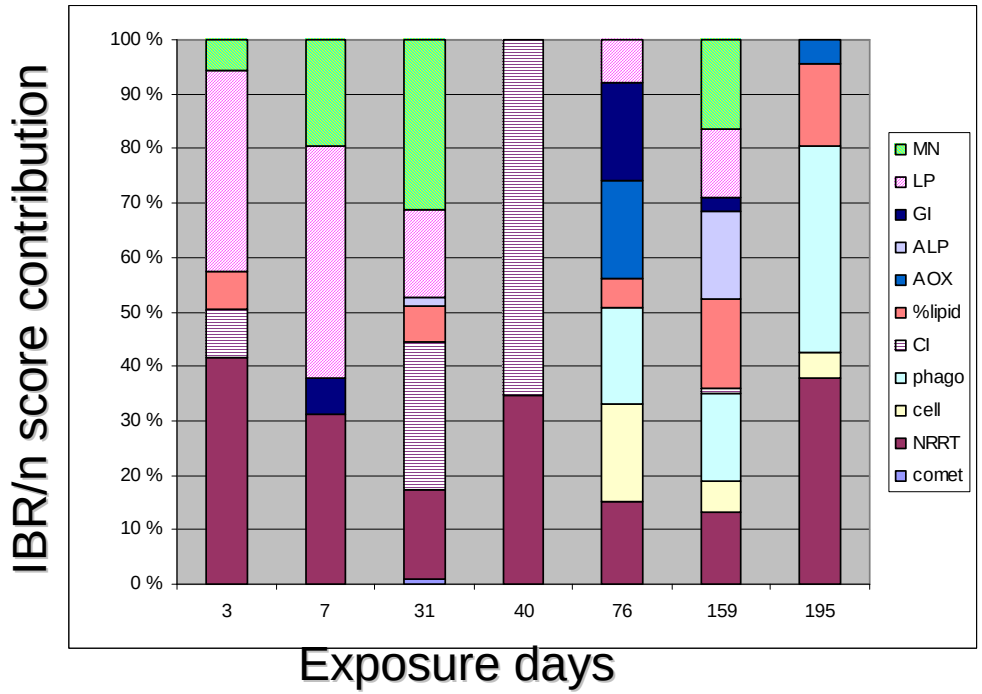
	sampling	group	comet	NRRT	cell	phago	CI	%lipid	AOX	ALP	GI	LP	MN	PAH bb
day	**3**	k0	-	153	-	-	0.4042	1.054	**1.114241**	**18.6678**	**1.7**	**14.1666667**	**1.1**	-
		H	-	140	-	-	0.3661	1.178	**1.045949**	**9.109531**	**1.894737**	**10**	**1.2**	-
	7	k0	-	150	-	-	0.4240	-	**1.36614**	**75.02142**	**1.65**	**15.625**	**0.75**	2.0
		H	-	113	-	-	0.4592	-	**1.161463**	**42.72605**	**1.45**	**7.15**	**1**	18004.9
	31	k0	8.301	133	-	-	0.4999	0.848	**0.865135**	**13.08885**	**1.2**	**12.1875**	**0.625**	1.5
		H	8.396	97	-	-	0.3881	0.973	**0.562671**	**14.14573**	**1.947368**	**7.0625**	**0.875**	9279.3
	40	k0	7.205	133	-	-	0.5215	0.927	-	-	-	-	-	3.2
		H	6.177	80	-	-	0.4060	0.771	-	-	-	-	-	11621.4
	76	k0	11.708	150	1.789	1.000	0.2245	0.786	**0.42247**	**14.48597**	**2.222222**	**13.25**	-	0.0
		H	10.804	78	3.311	2.137	0.2258	0.981	**0.719463**	**13.79524**	**1.45**	**9.5**	-	15341.1
	159	k0	-	150	2.261	1.000	0.2465	0.555	**0.707873**	**2.032841**	**1.421053**	**15**	**0.6**	1.1
		H	-	93	2.763	1.495	0.2119	0.726	**0.665958**	**18.48642**	**1.166667**	**10.25**	**1.375**	3200.5
	195	k0	-	157	3.768	1.000	0.2283	0.667	**0.843312**	**22.48598**	**1.5**	**10.9375**	-	66.6
		H	-	139	4.353	1.149	0.2577	0.814	**0.95534**	**4.83821**	**2.05**	**11.25**	-	1431.3
		m	8.76	126.22	3.04	1.30	0.35	0.86	0.87	20.74	1.64	11.36	0.94	
		s	2.11	28.23	0.96	0.45	0.11	0.17	0.27	19.93	0.33	2.80	0.28	
		CV	24 %	22 %	31 %	35 %	32 %	20 %	31 %	96 %	20 %	25 %	30 %	

Comet=DNA strand break (hemocyte); NRRT=hemocyte neutral red retention time; cell=hemocyte cell counts; phago= Phagocytosis in hemocyte; CI=Whole condition index; %lipid=%total lipid; AOX=peroxisome proliferation; ALP=Vtg-like proteins; GI=Gonadic index; LP=lysosomal membrane stability (histology); MN=Micronuclei (hemocyte);

PAHbb= PAH body burden in whole organism

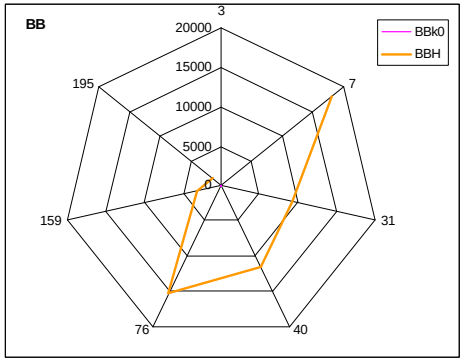
”Weighted IBR” (IBR/n) calculation



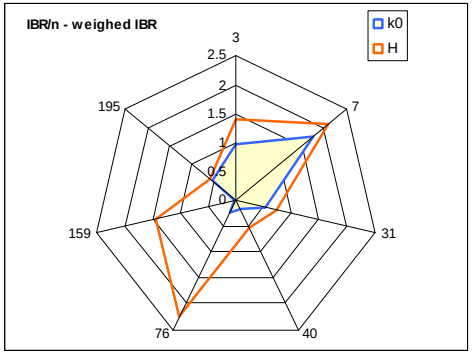


Exposure days

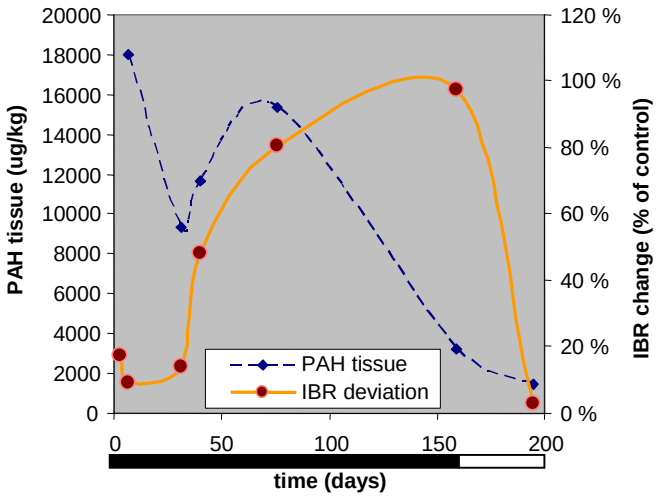
K0=control
H=fuel



PAHbb

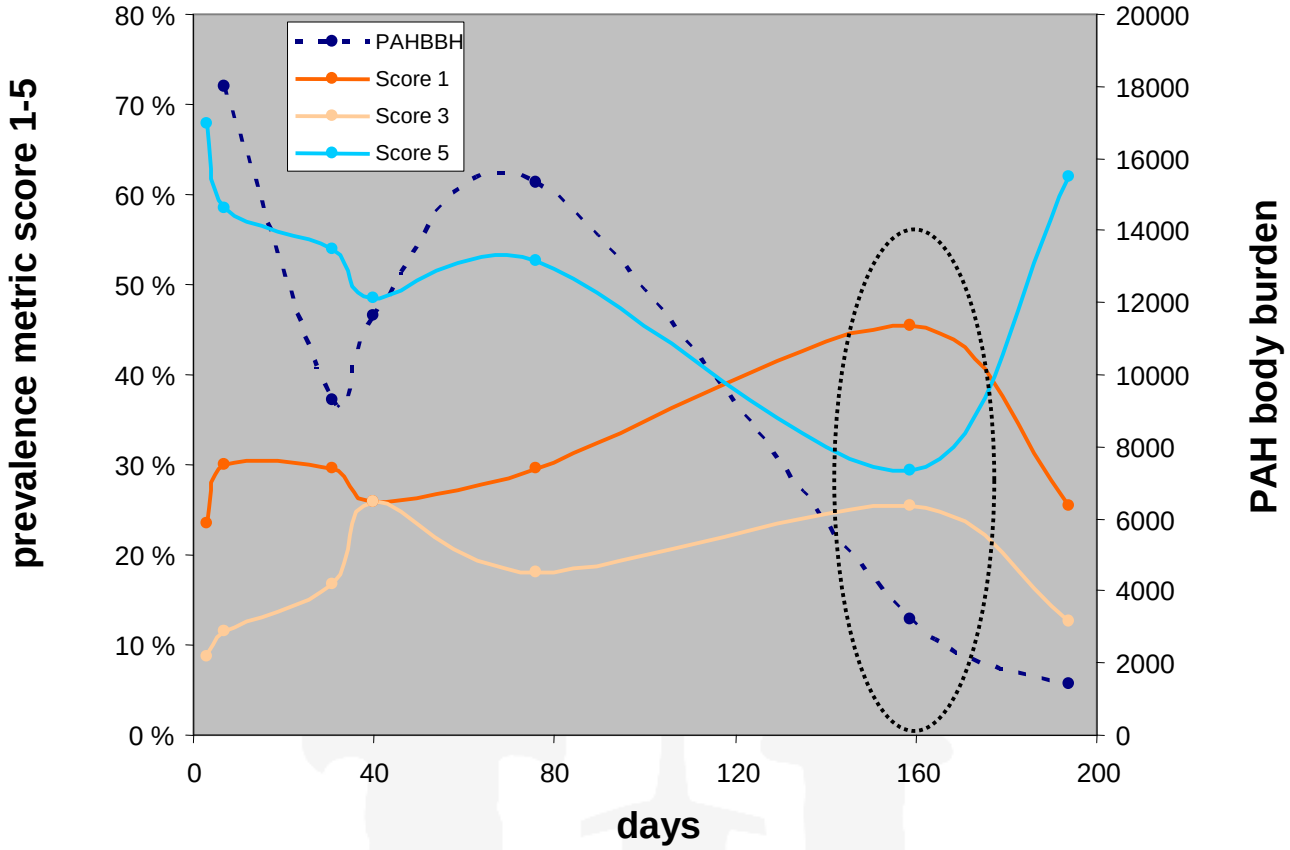


IBR/n



IBR change expressed as
(IBRh-IBRk)/sum(IBRh:IBRk)

Multimetric index (based on CI, NRRT, %lipid, AOX, ALP, LP and GI)



IRIS

Conclusion

- Methodologies based on biological effects can be used to assess water quality status following spill of HNS carried by ships
- Short acute exposure may have prolonged effects on marine biota
- Should explore the feasability of using sensing techniques at least at screening level
- For decision-making, integration into a simple environmental index may provide valuable information

Acknowledgements

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Total E&P Norge as

Basque Government # ETORTEK - IMPRES PROJECT 03-07

Internal initiative