

Monitoring studies for ERA validation

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Topics that will be illustrated

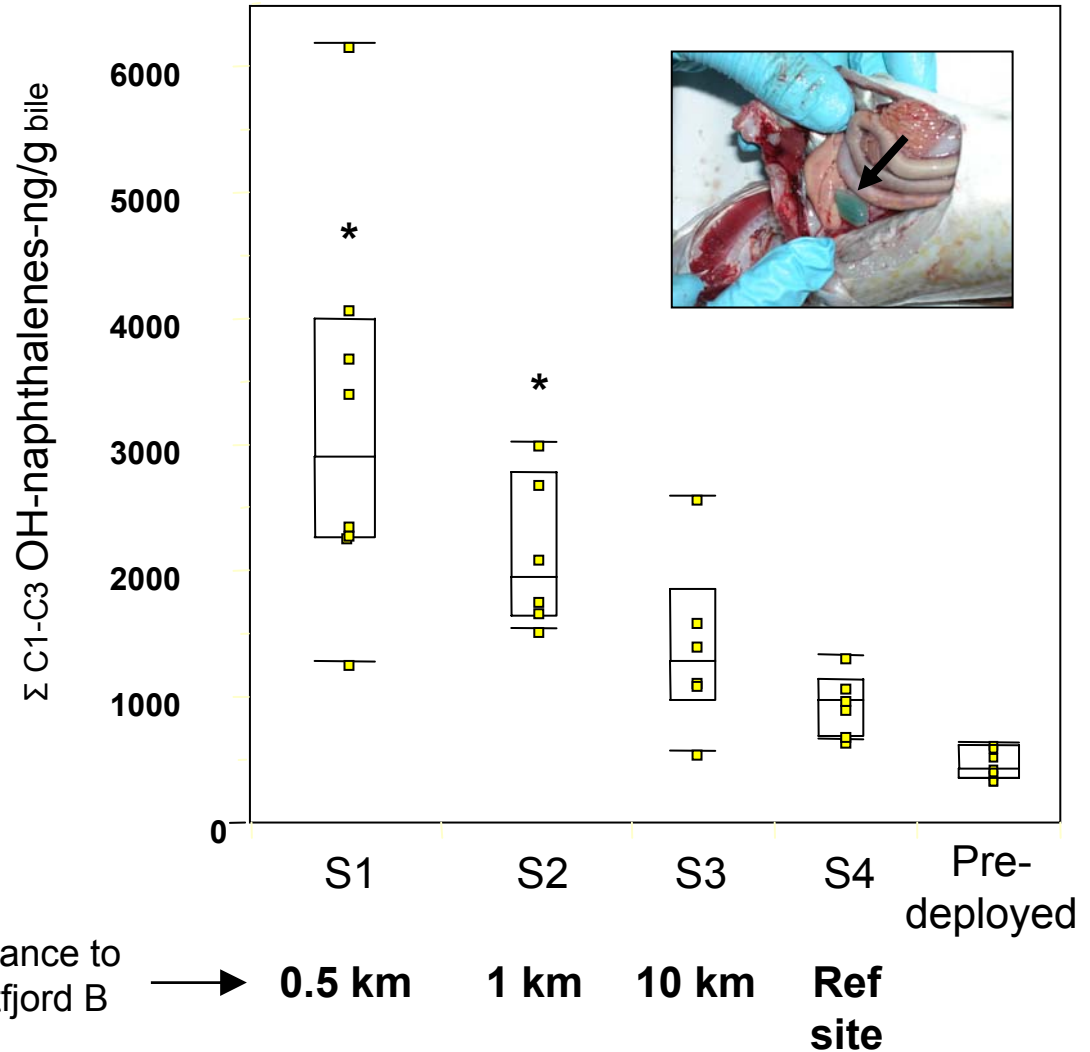
- BECPELAG
- WCM 2003
- Pyrogenic PAH mixture from smelter industry
- Acute discharges (spills)



BECPELAG: C¹⁻³ OH-NPH in caged cod bile



A similar gradient was NOT found in wild fish



Data: E. Aas & G. Jonsson et al (submitted)
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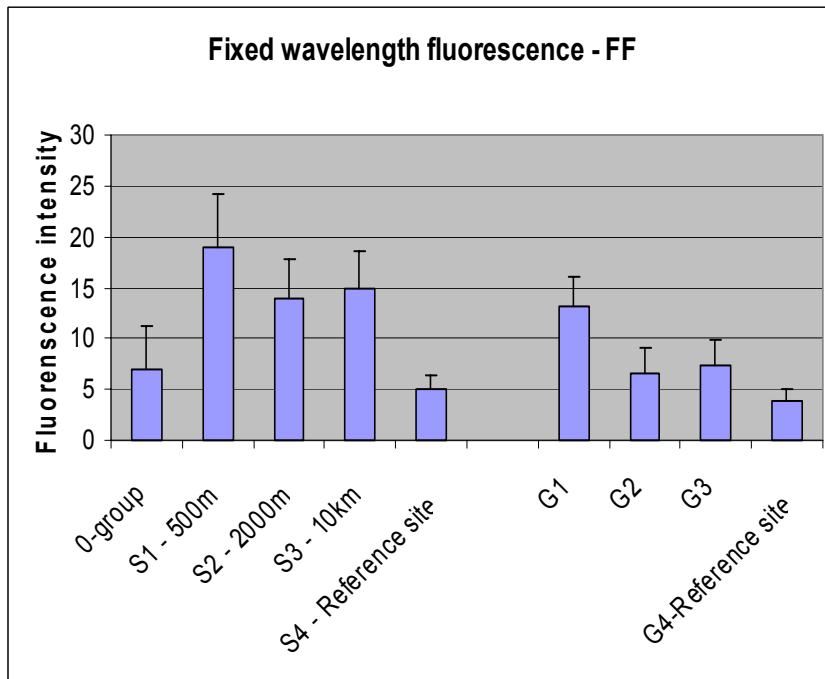
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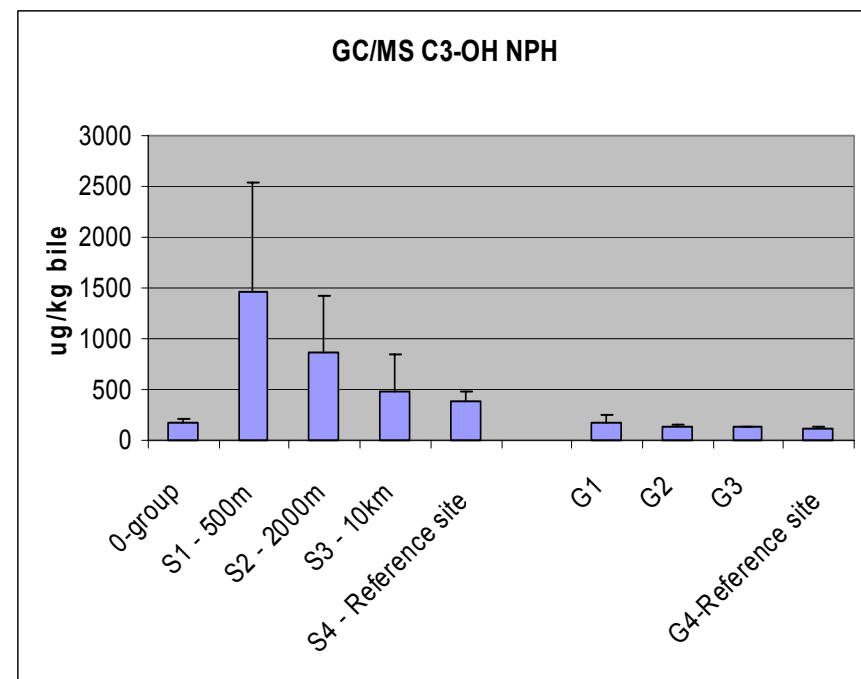
Becpelag workshop 2001

PAH metabolites

Fluorescence

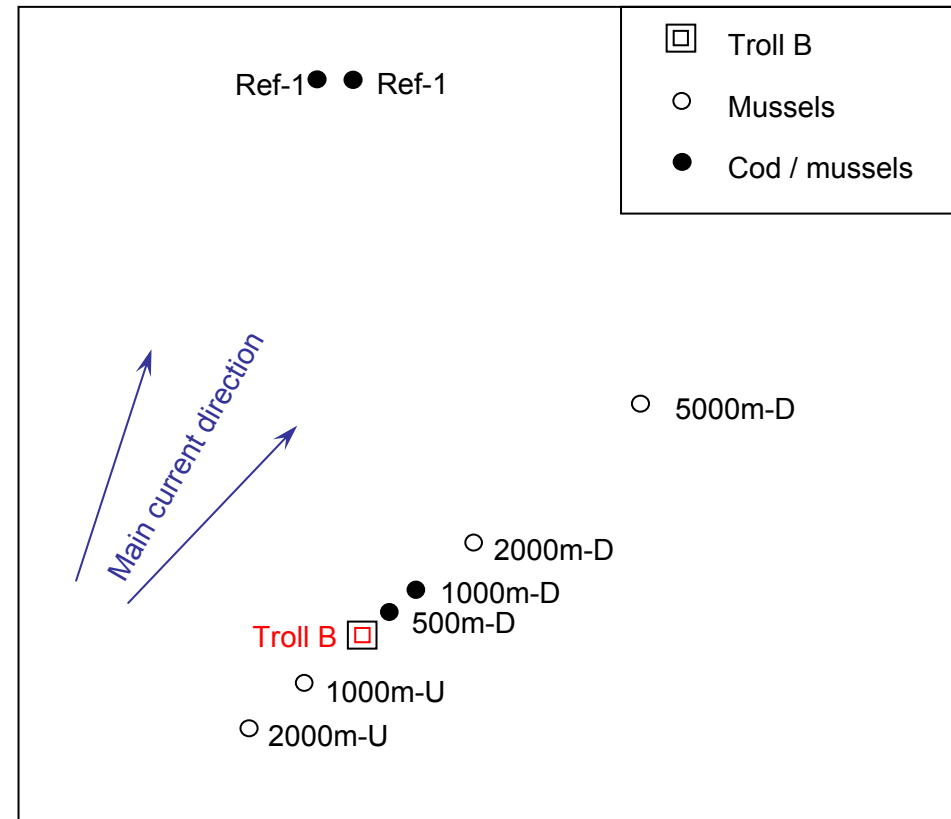


GC/MS

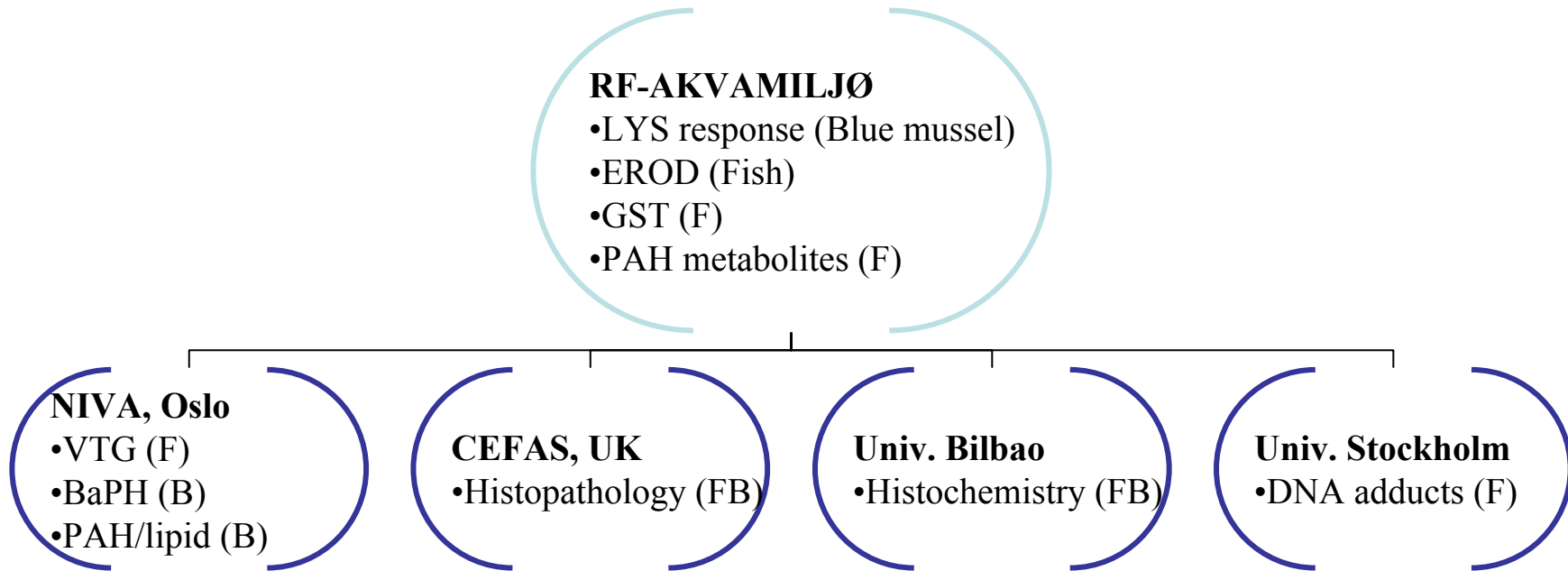


WCM 2003 Experimental work plan

- Transplant fish and mussels
 - Fish farmer, Sotra
 - Mussel farmer, Åfjord
 - Pre-exposure sampling (Aug)
- Caging at 12-15 m depth, total water depth 350 m
- 6 weeks of field exposure (Aug – Oct)



Analytical matrix



Cod (*Gadus morhua*)

- Endocrine disruption
 - [Vitellogenin](#)
- Metabolic detoxification
 - [PAH metabolites](#)
 - [EROD activity](#)
 - [GST activity](#)
- Genotoxicity
 - [DNA adducts](#)



Histological integrity

- [Histopathology](#)
- [Apoptosis in testis](#)
- [Histochemistry](#)

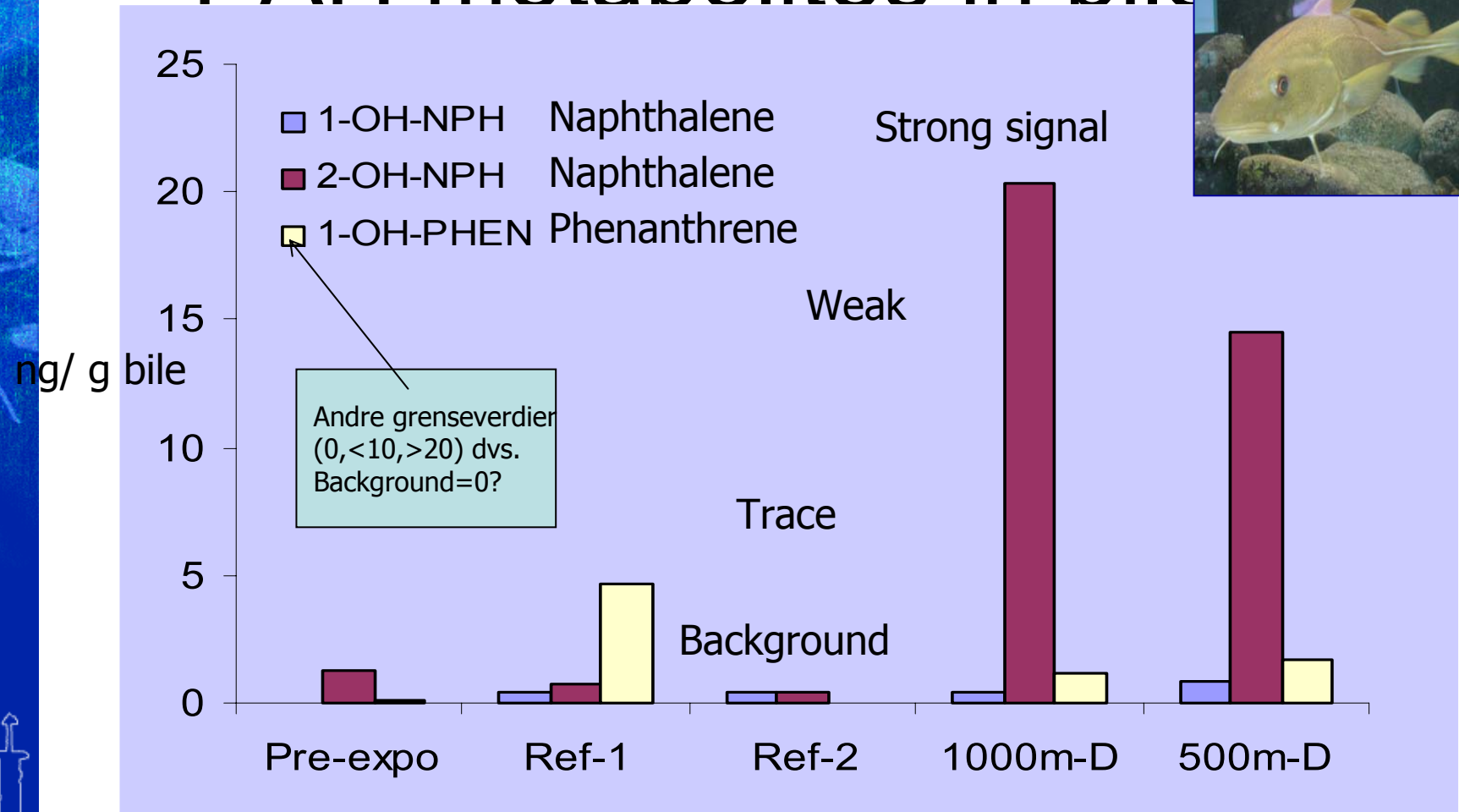
Blue mussel (*Mytilus edulis*)

- General stress
 - [Lysosomal stability](#)
- Metabolic detoxification
 - [BaPH](#)
- Histopathology
 - [Lysosomal degeneration](#)
- Bioaccumulation
 - [PAH body burden](#)



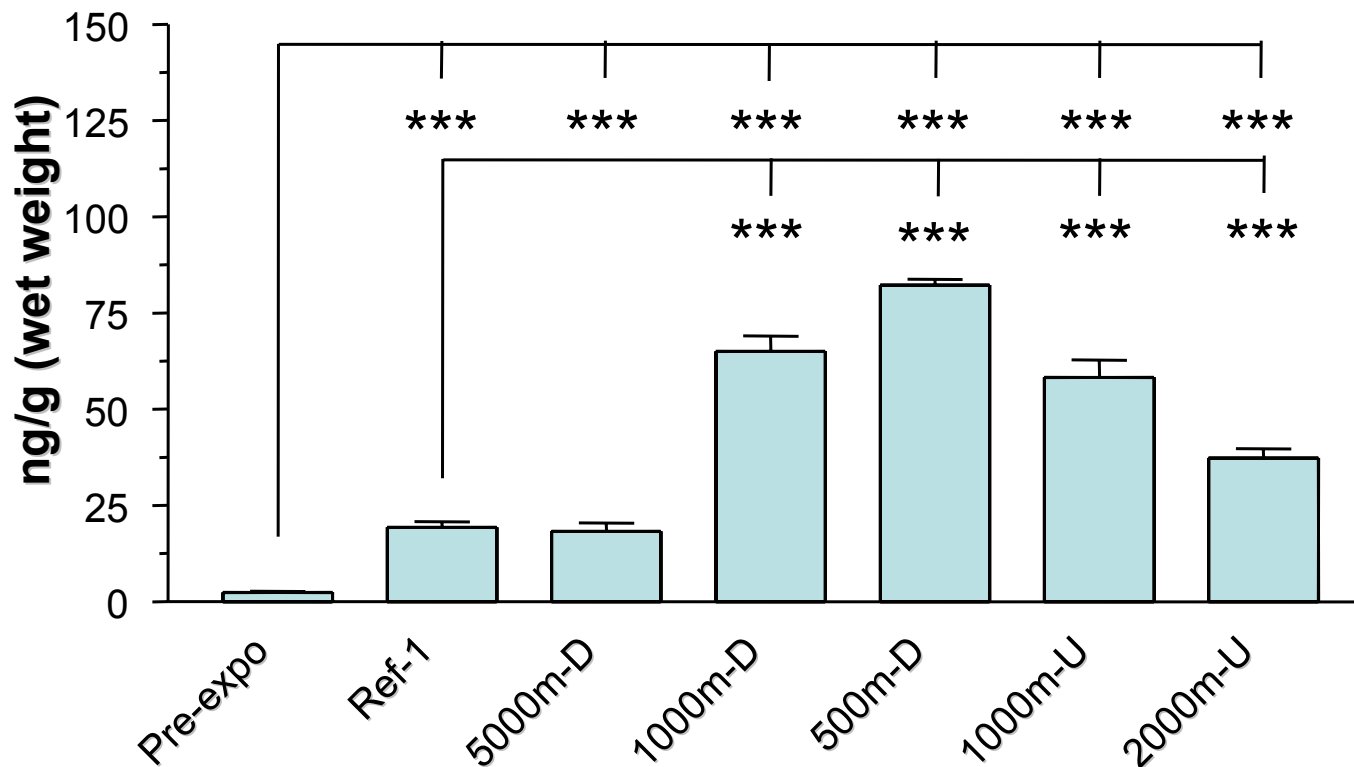
WCM2003

PAH metabolites in bile



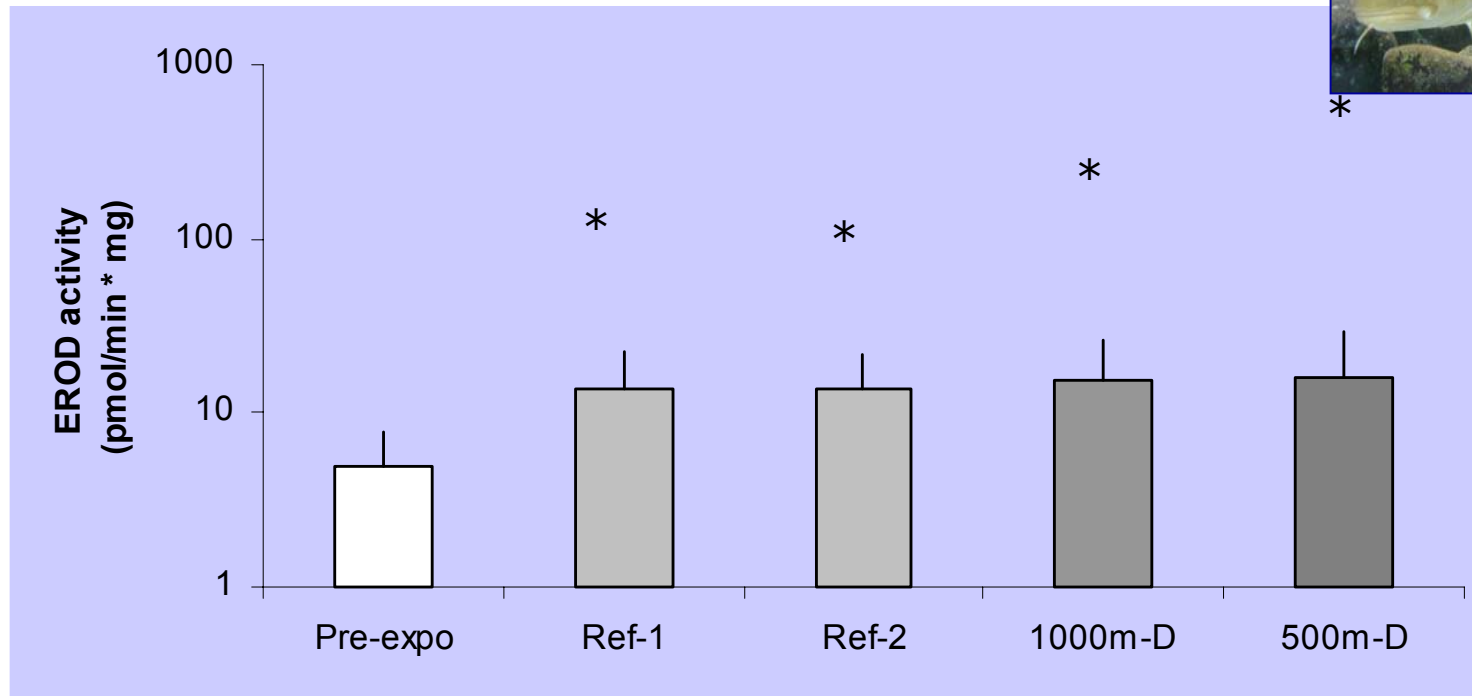
WCM 2003

Body burden of C2-phenanthrene



WCM 2003

Hepatic EROD activity



Low normal 1-5

High normal 15-20

Weak induction 200% of control average

Strong induction >400%

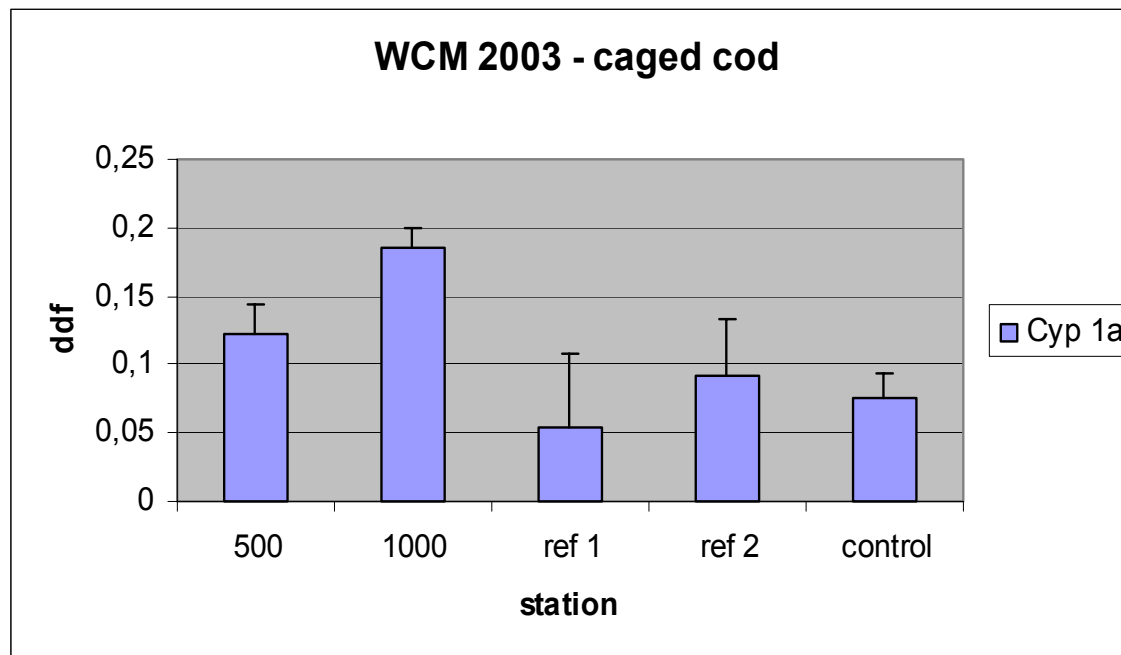
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WCM 2003

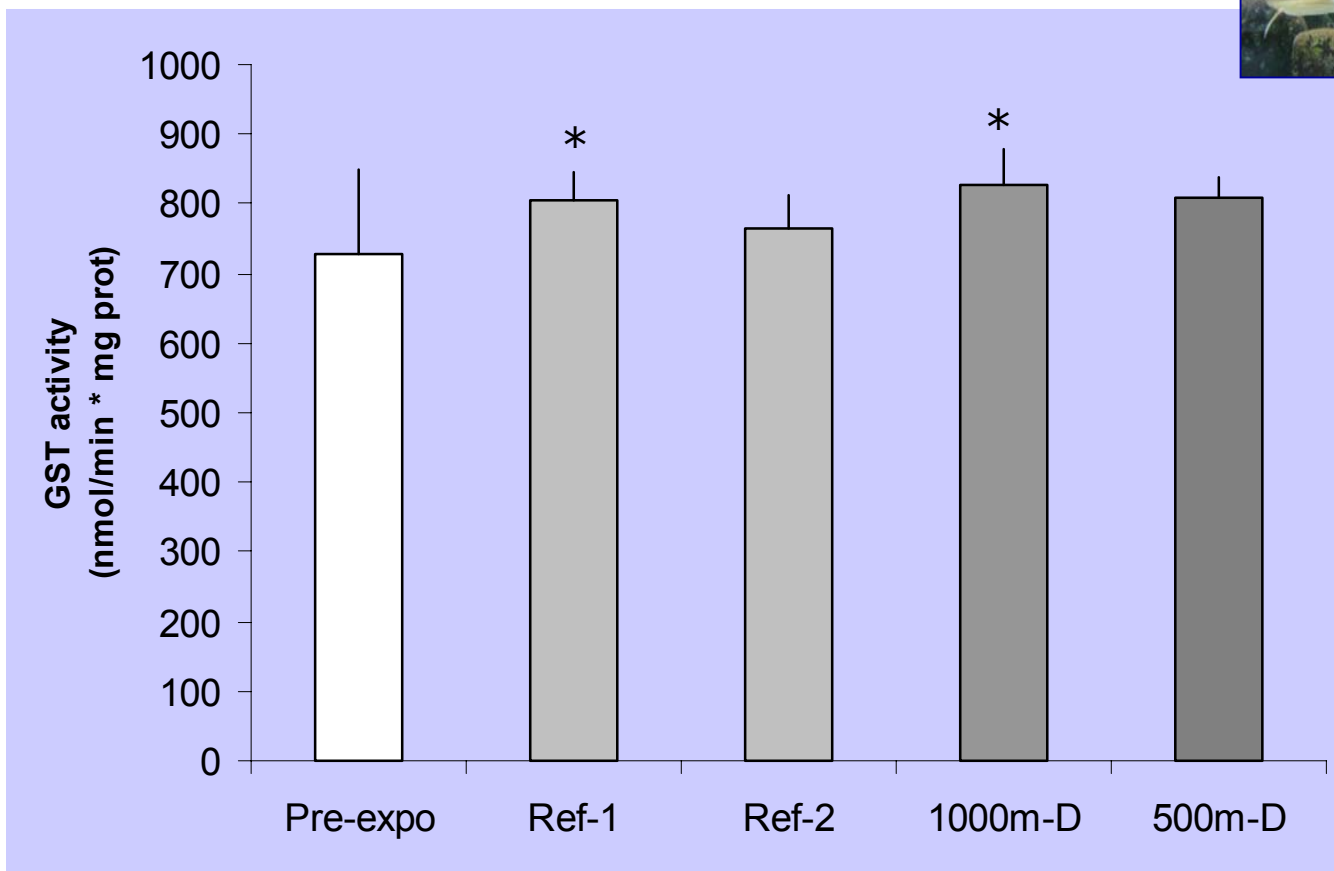
Additional Biomarker measurement

- Detoxification enzyme
 - Cyp 1a (ELISA)



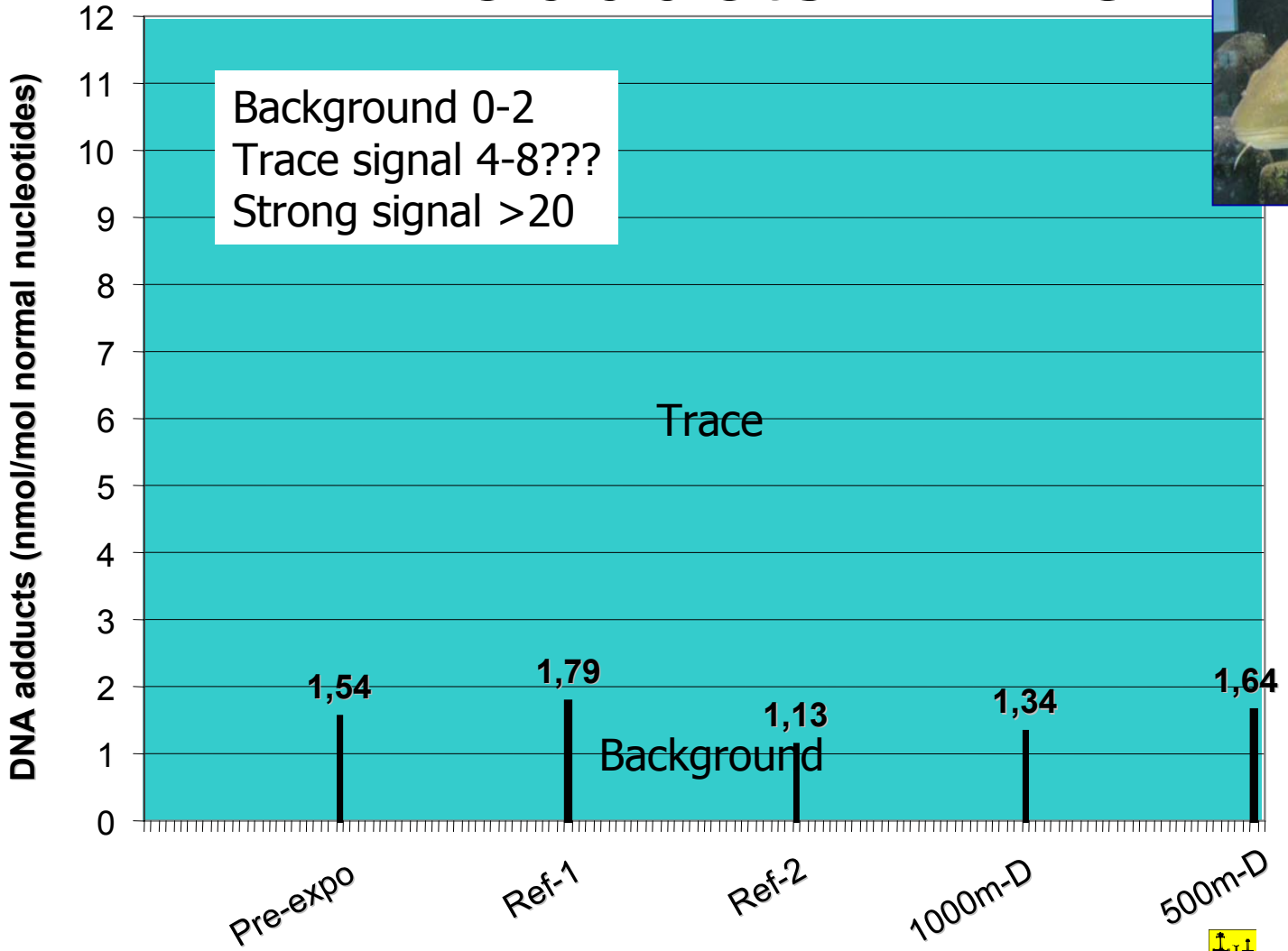
WCM2003

Hepatic GST activity



WCM 2003

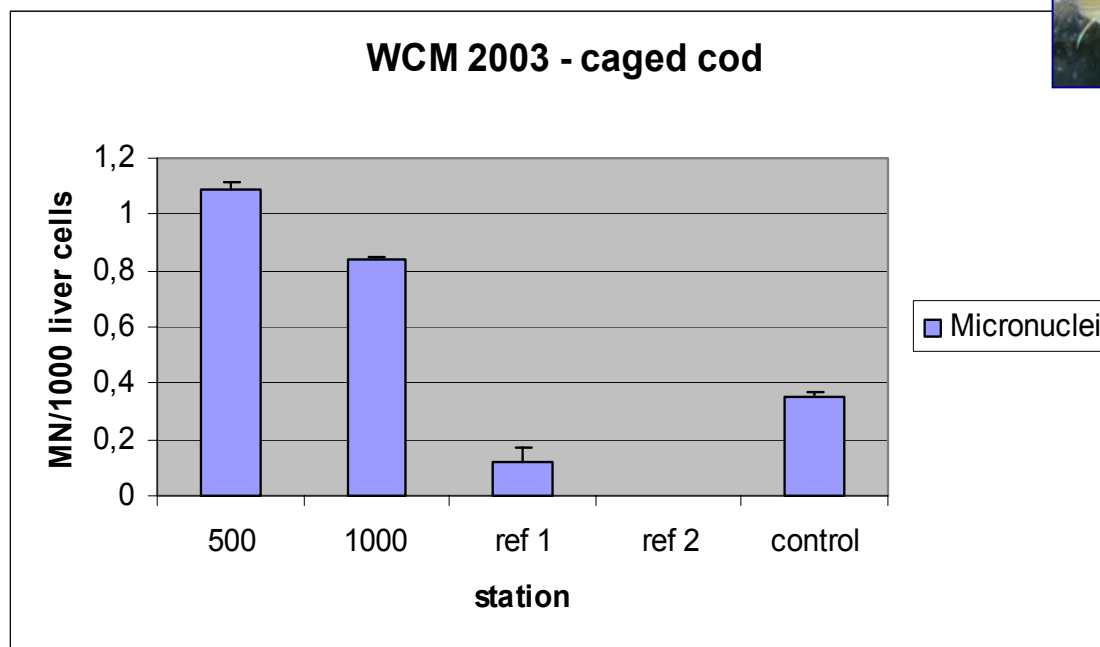
DNA adducts in liver



WCM 2003

Additional Genotoxic effect

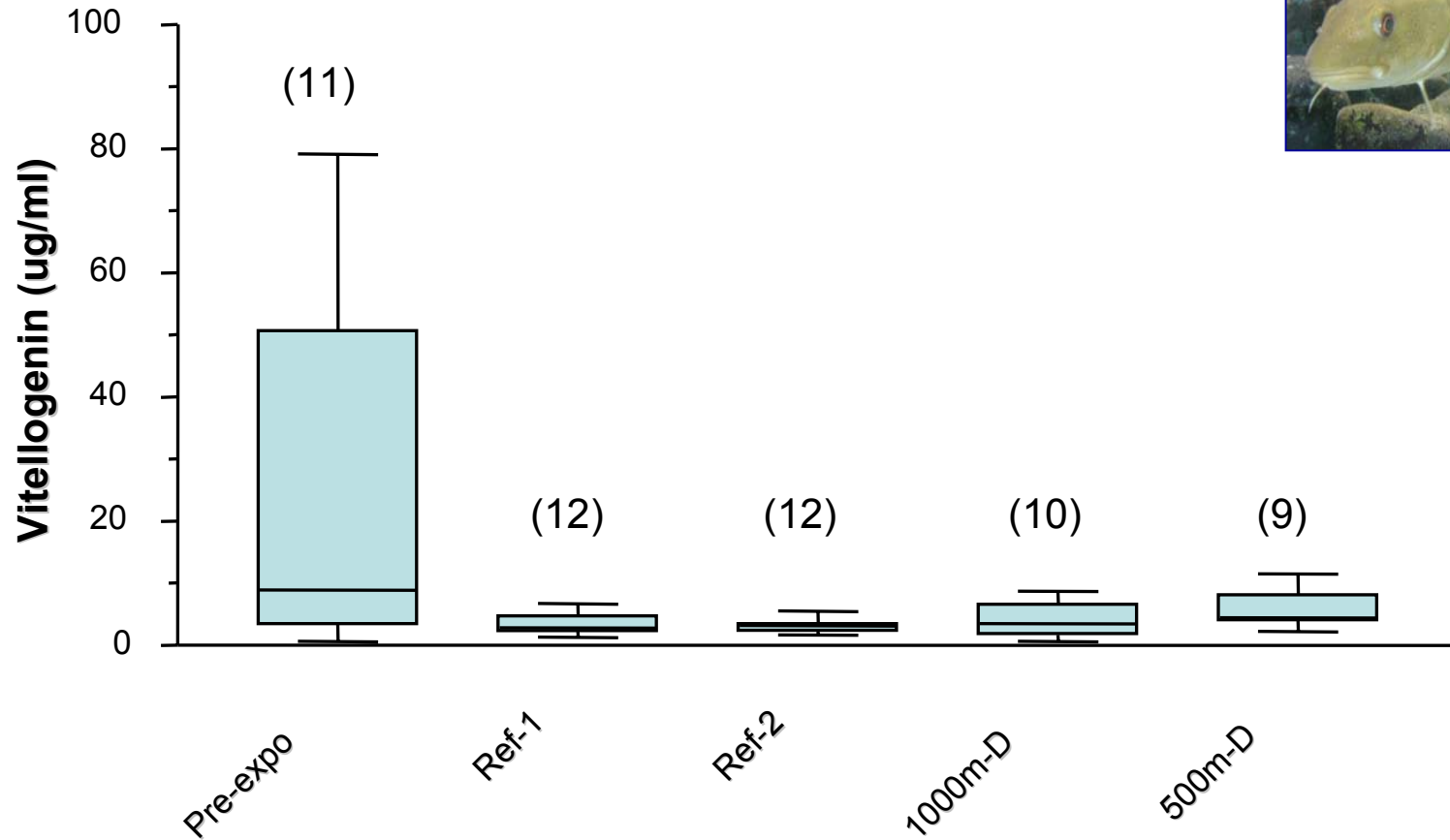
- Irreversible chromosome disturbance
 - Micronuclei



Janina Barsiene, Univ. Vilnius

WCM 2003

Vitellogenin (vtg) in blood plasma



WCM 2003

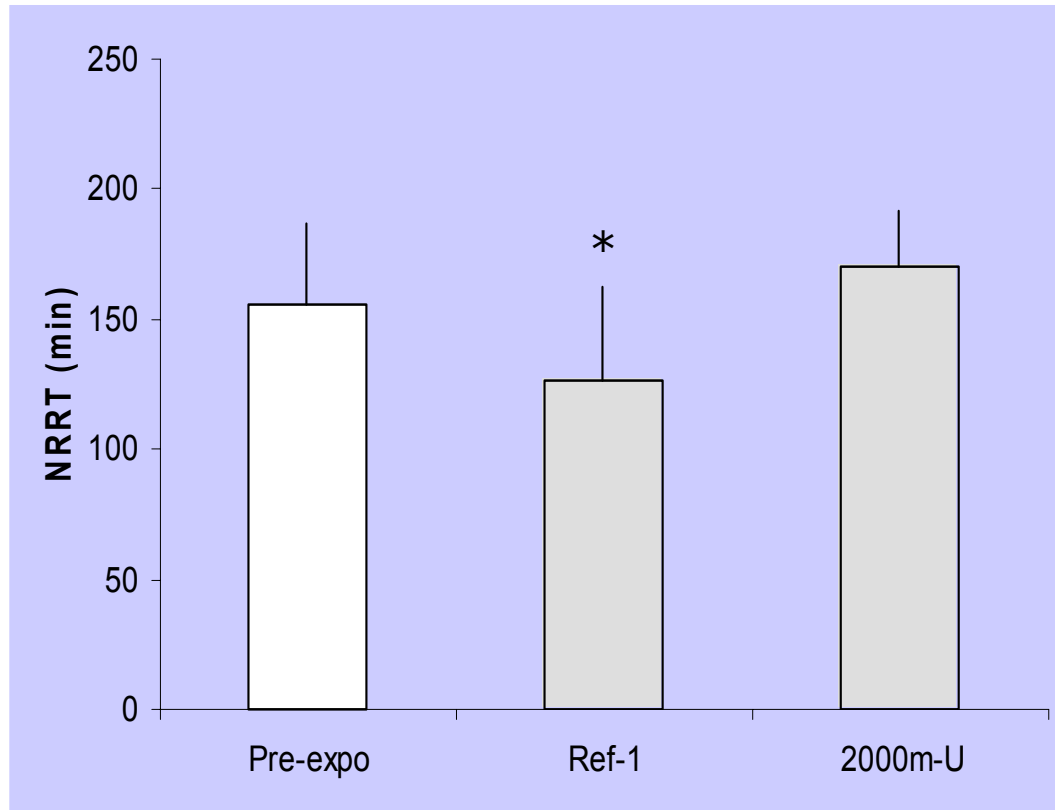
Histopathology

- Observed effects were very small
- Question about relevance of these methods for only a month exposure



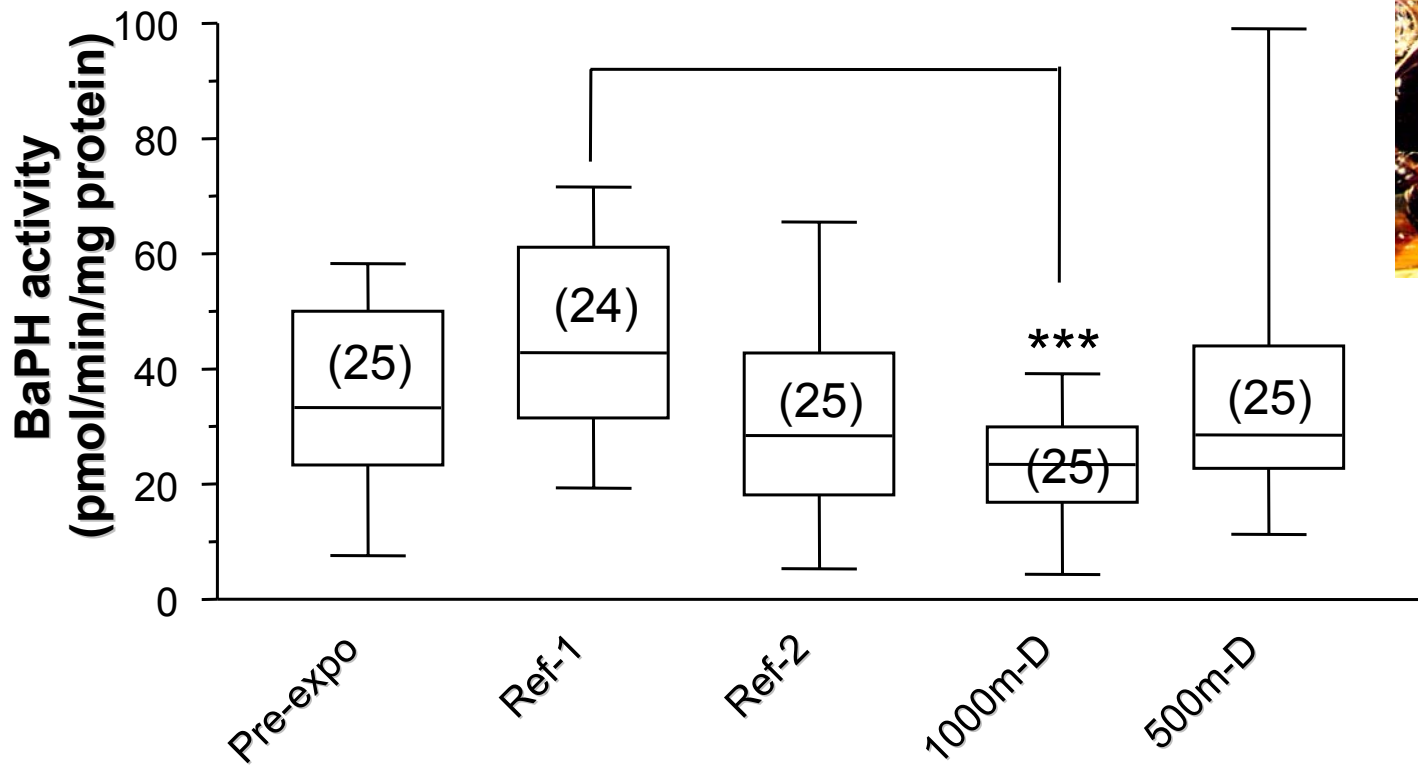
WCM 2003

Lysosomal stability in haemocytes (onboard screening, reduced sample set)



WCM 2003

Benzo[a]pyrene hydroxylase activity in digestive gland tissue

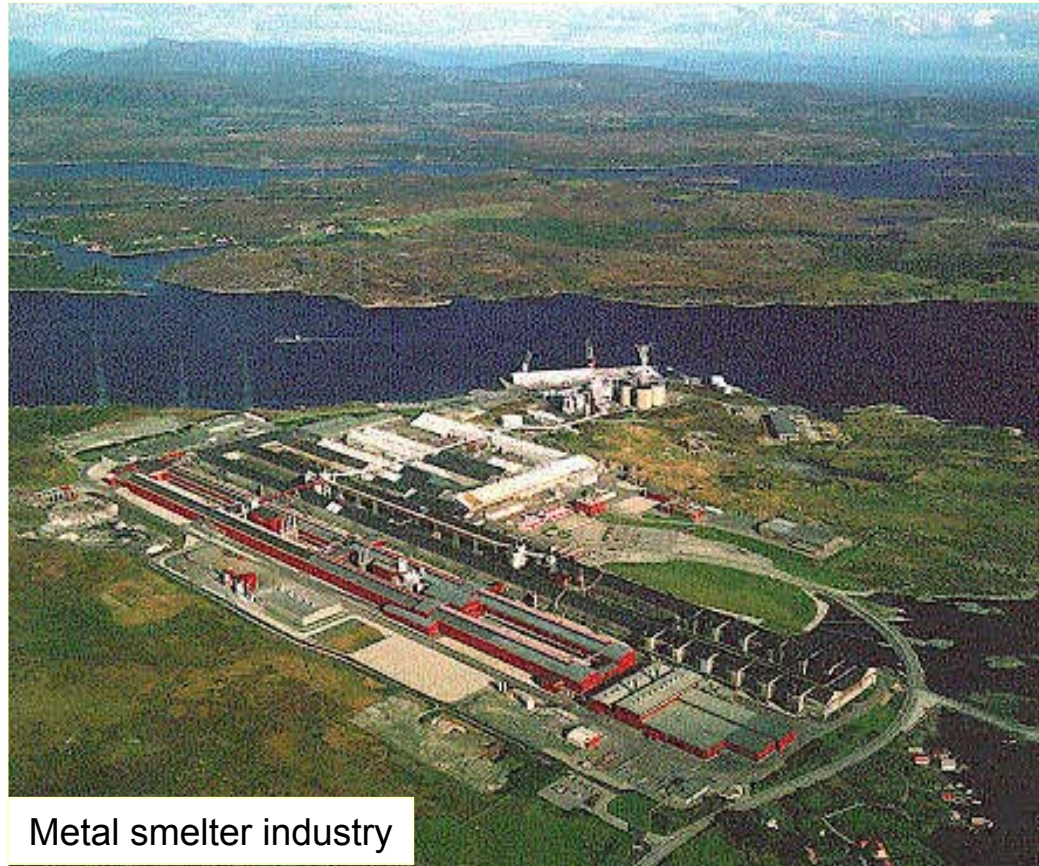


Main findings of WCM2003

- Results confirm that organisms were successfully exposed to the discharge plume!
- Low exposure and effect levels at the Troll field compared to the Statfjord field (BECPELAG)
 - Good results for the environmentalist
- Potential for improvements:
 - Planning the right timing of field work during the year (weather condition, work on board)
 - Techniques with cage moorings
 - Clean original biological material is important
 - Choice of reference locations



Other case – pyrogenic PAH mixture



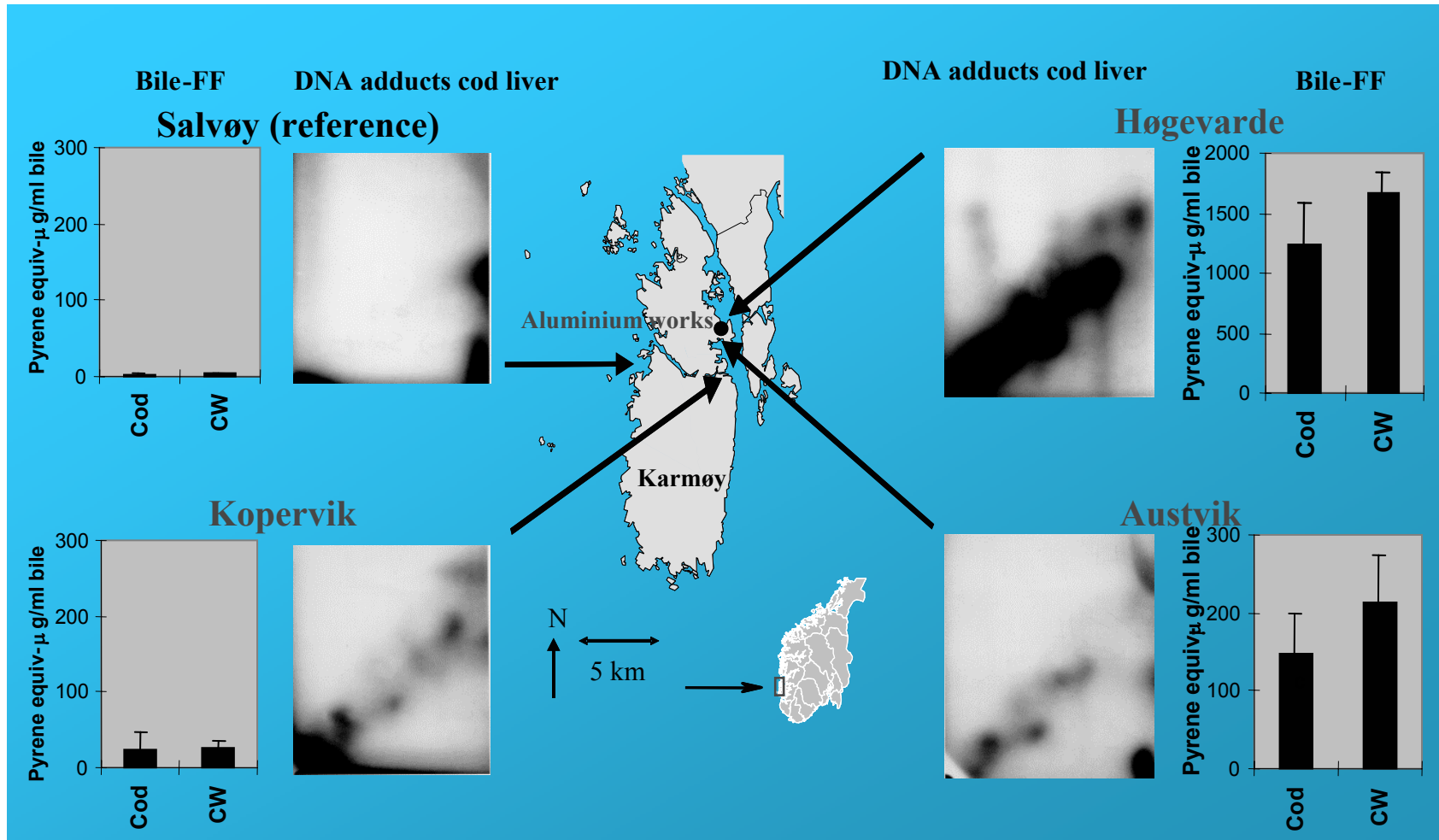
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Karmsundet



DNA adducts in cod detected by ^{32}P -postlabelling and PAH metabolites in bile detected by fixed wavelength fluorescence (FF) in cod and corkwing wrasse caught in the vicinity of an aluminum works. Note the different scale for FF at Høgevarde.

BEEP – example of our genotoxicity results



Alkaline Unwinding assay - evaluation

Advantages

Frozen tissue

Any kind species

Any kind of tissue

(liver, digestive gland, gills, blood)

Very small amounts of tissue

(approx. 20 mg)

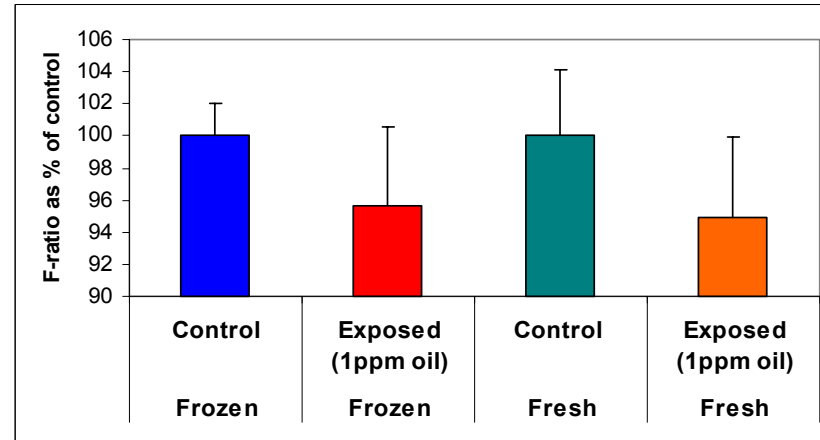
Relatively fast (30 samples/day)

Includes few steps and minimal reagent requirements

Disadvantages

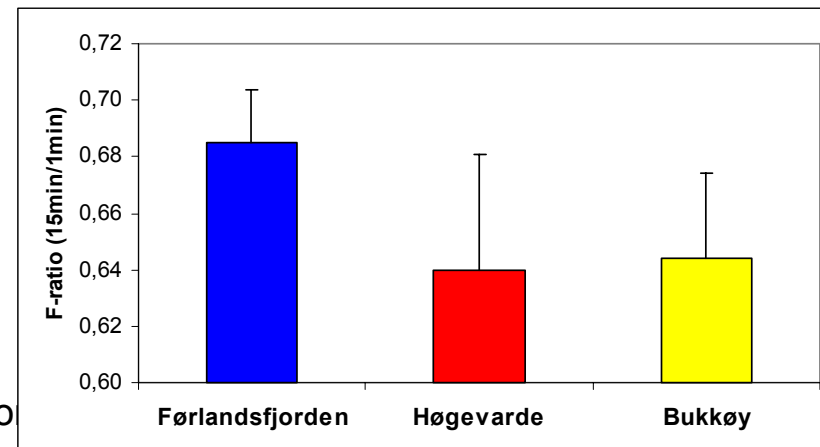
Seems to be relatively insensitive

(continuing evaluation)



**Alk. Unwinding assay
on wrasse liver
(pH 11.85)**

Monitoring studies for validation
(data and ill.: B.K. Larsen)



Oil spill

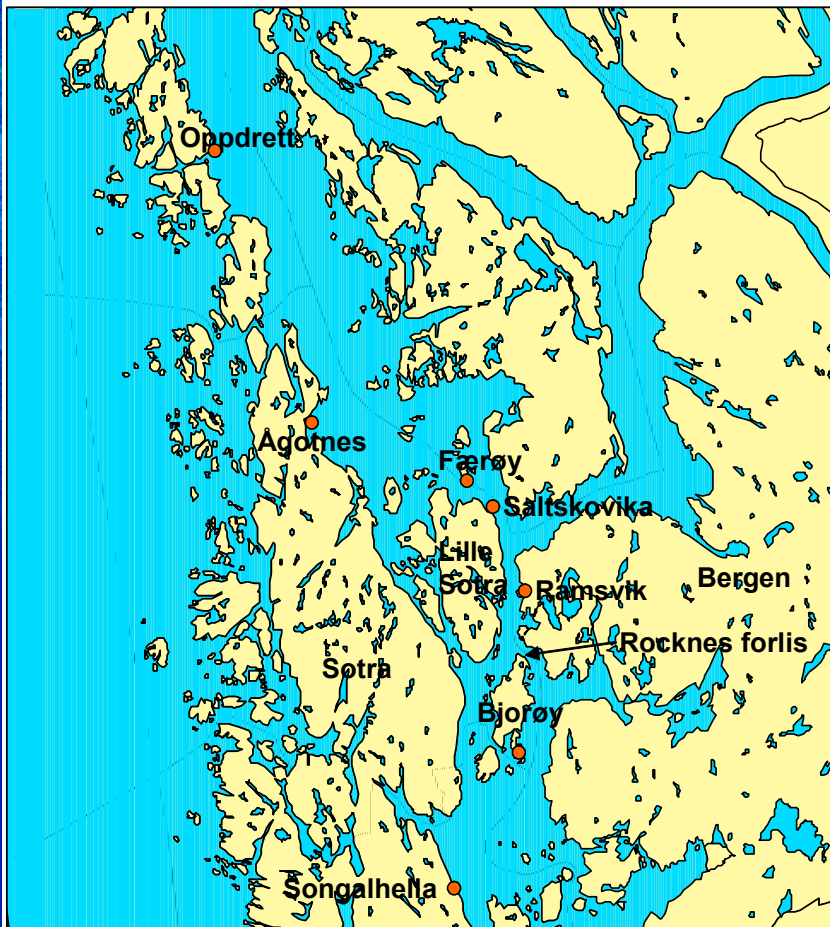
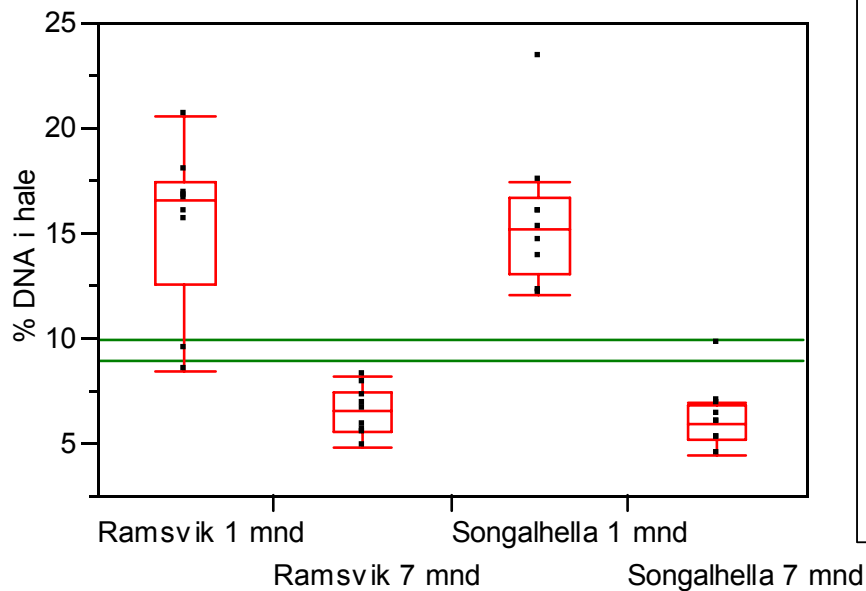
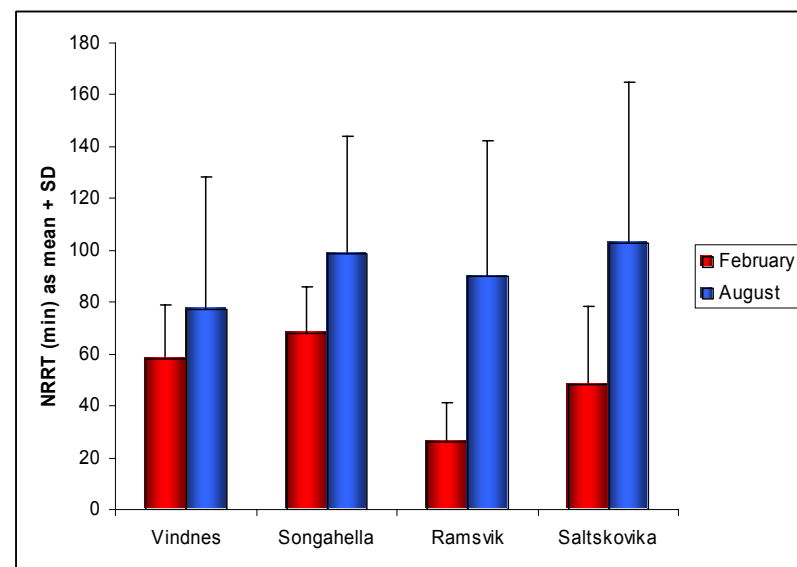


Foto: Asbjørn Økland, VG

Results ship wreckage



Comet assay



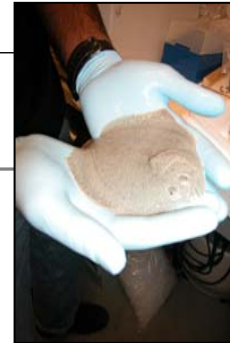
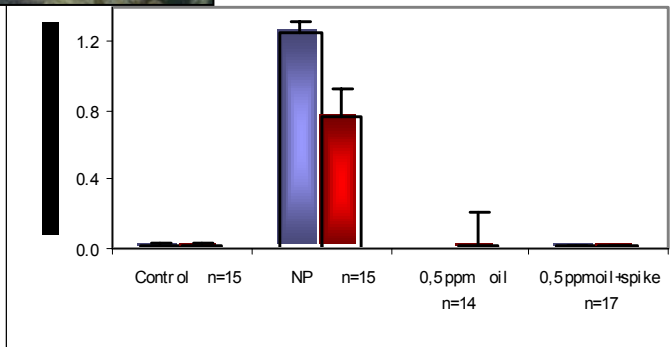
Lysosomal stability

Stavanger workshop 1 & 3

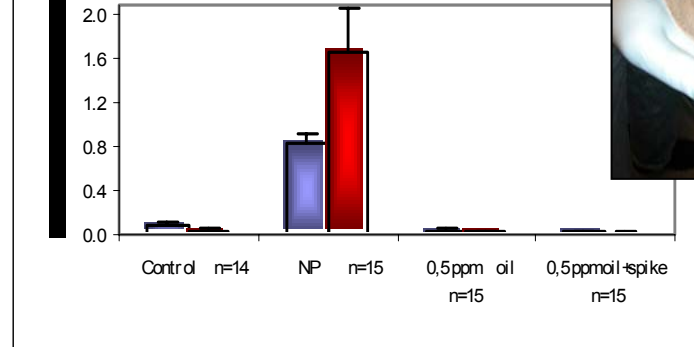
Plasma vtg/zrp in juvenile cod and turbot



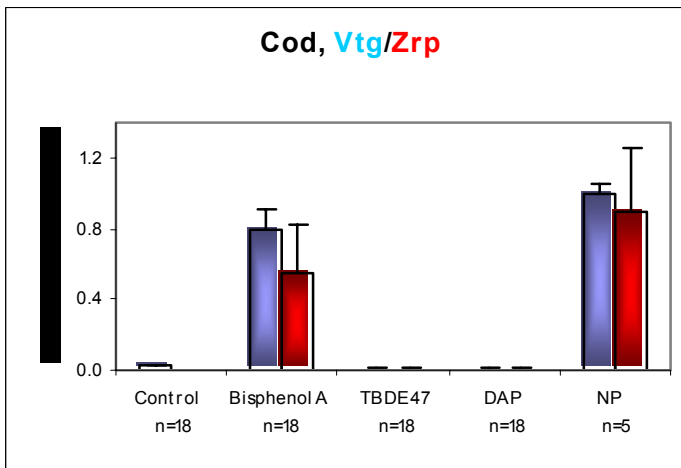
Cod, Vtg/Zrp



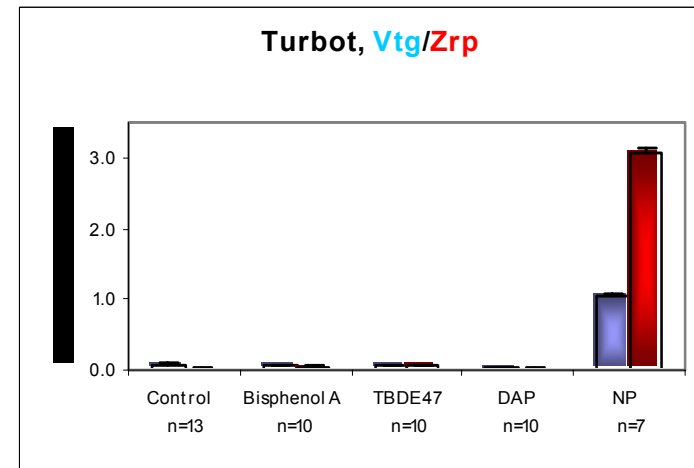
Turbot, Vtg/Zrp

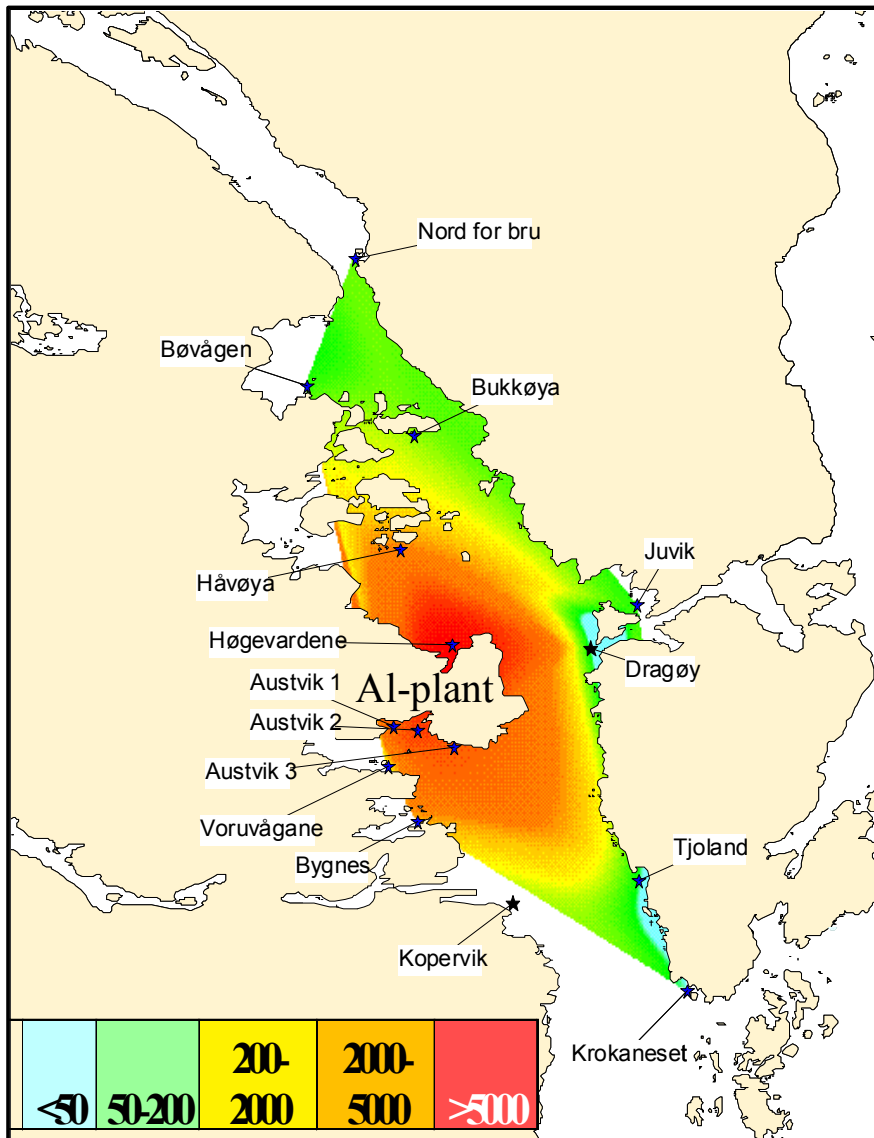


Cod, Vtg/Zrp



Turbot, Vtg/Zrp





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