NYKOS

New Knowledge on Sea Disposial

Biological effects assessment of a mine discharge into a Norwegian fjord using field transplanted mussels

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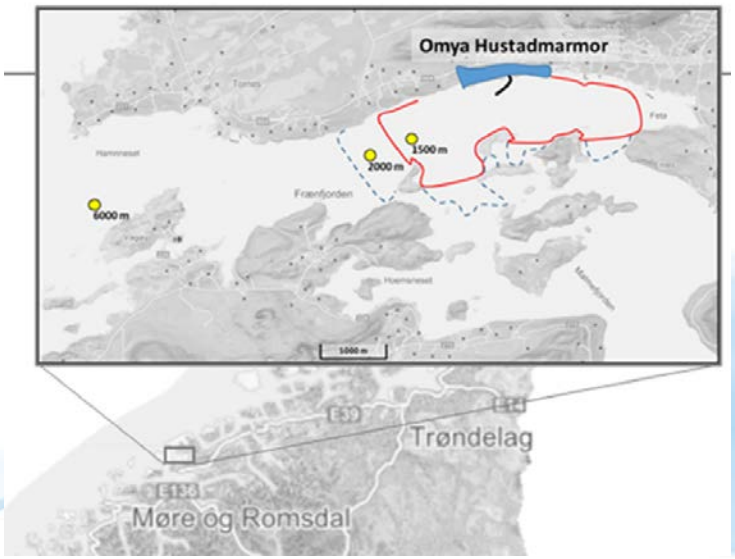
Study site

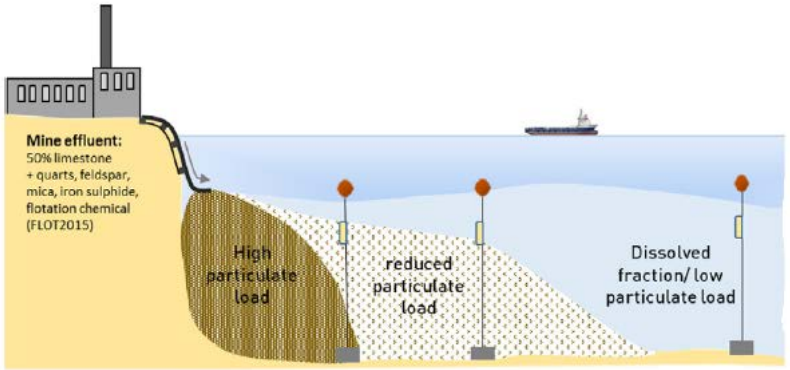
- Omya Hustadmarmor, Elnesvågen located on the West coast of Norway releases fine particulates from the processing of limestone into the Frænfjord.

- The tailings:
  - 50% limestone ($CaCO_3$), which mostly comprises of small particles (<20 µm diameter).
  - Other: quarts, feldspar, mica and iron sulfides
  - Production chemicals, flotation chemical (FLOT2015).

- Discharge by pipeline since 1978 and an impacted zone around the discharge outlet of 1 to 2 km is evident, with low abundance of benthic marine life



Field survey



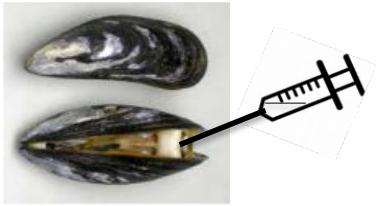
- Mussels (*Mytilus edulis*) from outer Oslofjord, held at Solbergstrand at 8 ± 1°C.
- Transported to site overnight.
- Fjord water temperature 10 ± 1°C.
- Day 0 mussels
- 18-20 m deep.
- 8 week exposure (April – June).
- Mussel collected by diver, transported overnight to NIVA lab.
- Fine film on mussels at two closest stations.

Sampling

Length and weight (w.w.) from all sampled mussels

15 mussels → Condition Index = (soft tissue d.w./shell d.w.) x100

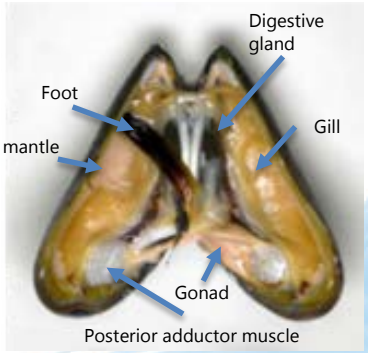
20 mussels → Stress on stress



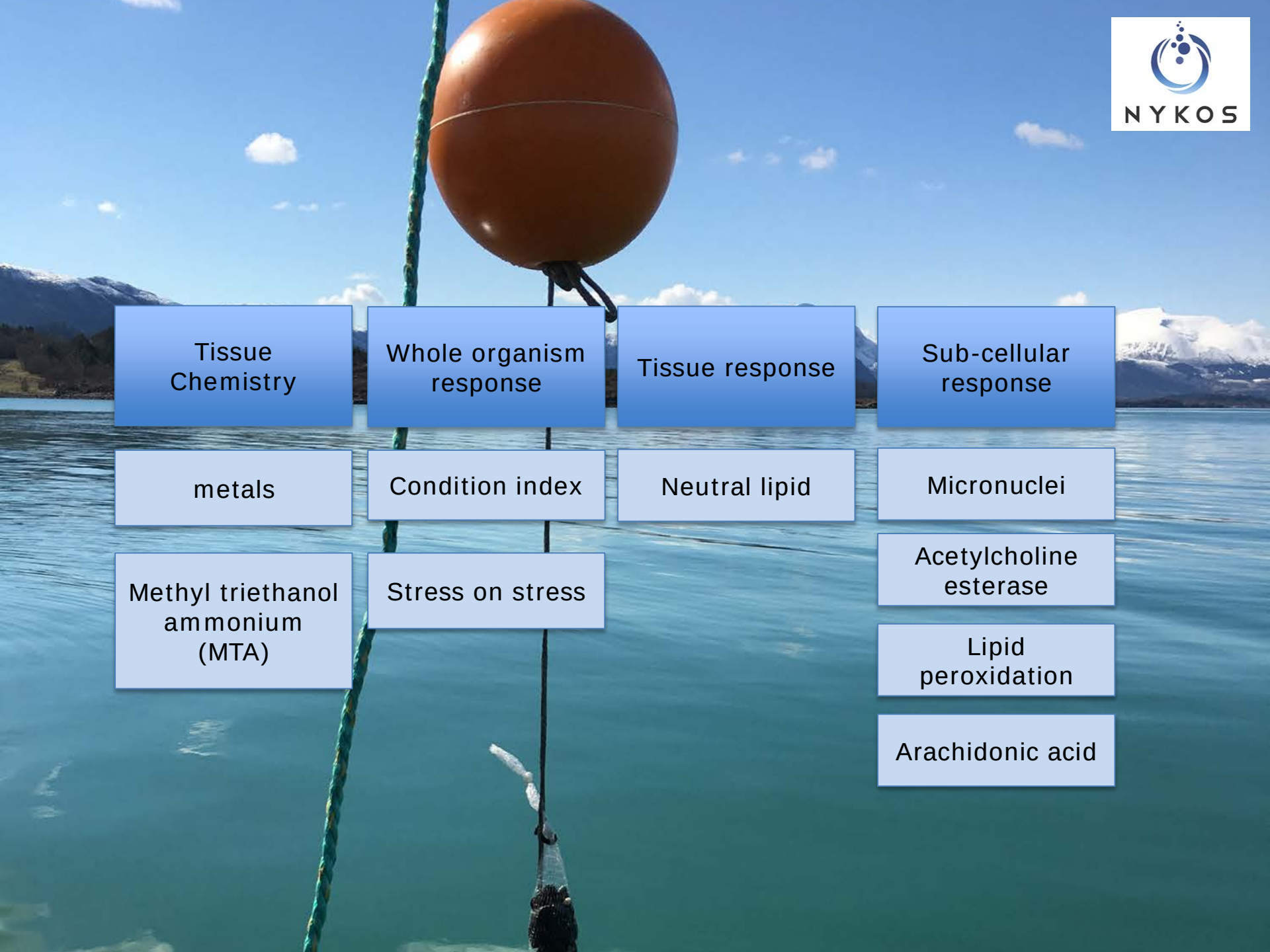
15 mussels → Haemolymph removed, smeared on microscope slide and fixed for micronuclei assessment

→ Gill tissue removed and snap frozen in liquid nitrogen, stored at -80°C.

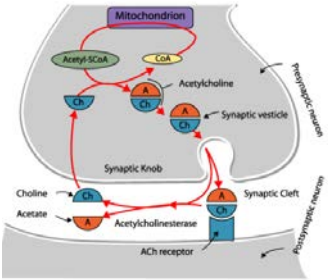
→ Digestive gland removed, snap frozen in liquid nitrogen and stored at -80°C.

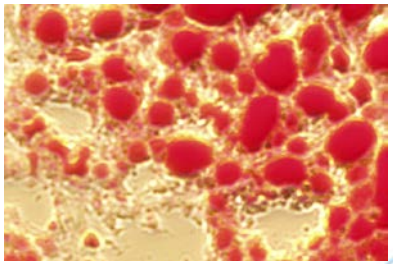


15 mussels → Whole soft tissue removed and pooled 5 mussels in 3 pools. Frozen for chemical analysis


NYKOS
Tissue Chemistry
Whole organism response
Tissue response
Sub-cellular response
metals
Condition index
Neutral lipid
Micronuclei
Acetylcholine esterase
Methyl triethanol ammonium (MTA)
Stress on stress
Lipid peroxidation
Arachidonic acid

Biomarker suite





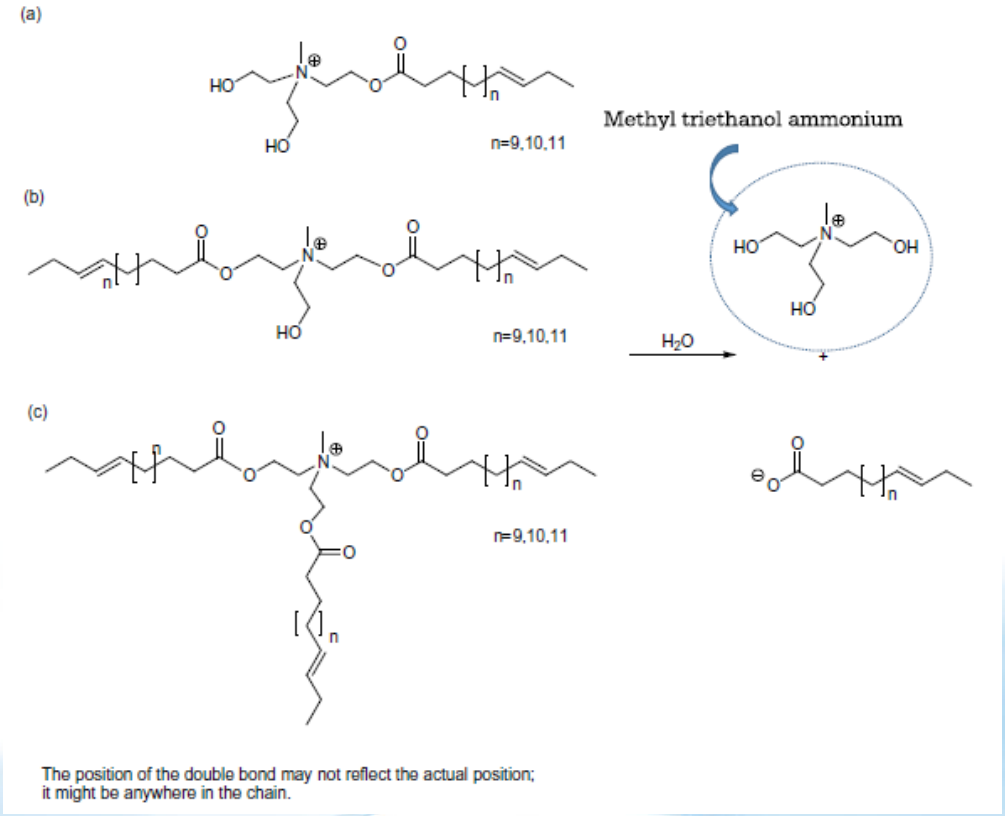
- Condition Index (CI): general fitness, physiological status.
- Stress-on-stress (SoS): general health status.
- Micronuclei formation (MN): chromatin-containing structures. MN frequency provides evidence of DNA breakage and spindle dysfunction and reveals a time integrated response to complex mixtures of contaminants. (genotoxic response).
- Acetylcholine esterase (AChE) inhibition: an essential neurotransmitter enzyme. Inhibition can have effects on CNS (neurotoxicity).
- Lipid peroxidation (LPO): oxidative deterioration of cellular membranes, which can alter membrane fluidity and permeability or attack other intracellular molecules. Lipid peroxidation bi-product MDA (malondialdehyde) reflects contaminant induced oxidative damage.
- Neutral lipid (NL): organic contaminants linked with changes in the metabolism of neutral lipids resulting in accumulation inside lysosomes of the digestive gland.
- Stress hormone (Arachidonic Acid (ARA)) measured with HRMS. Anti-inflammatory substance produced by mussels under stress

Chemical analysis

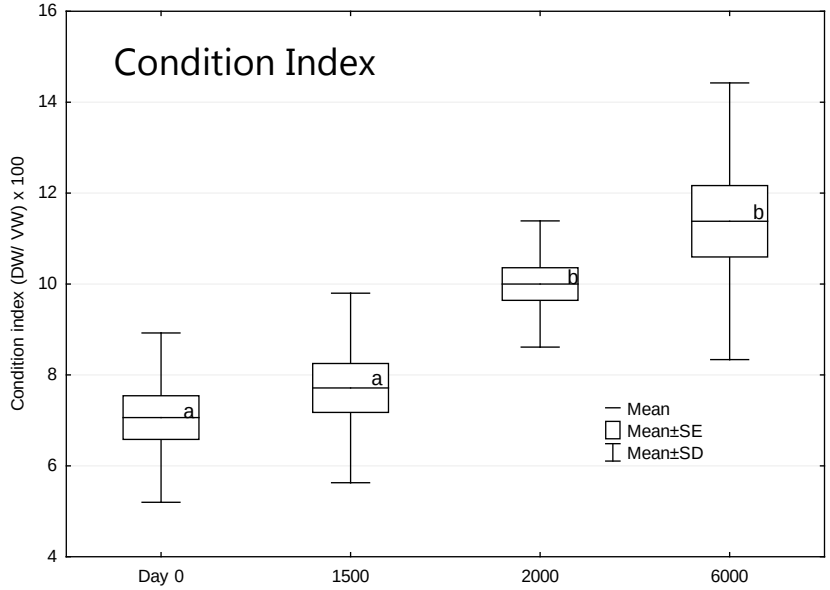
- ICP-MS (Al, Ag, As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sn, Zn)
- UPLC-HRMS
  - Methyl triethanol ammonium (MTA) the main transformation product of FLOT2015.

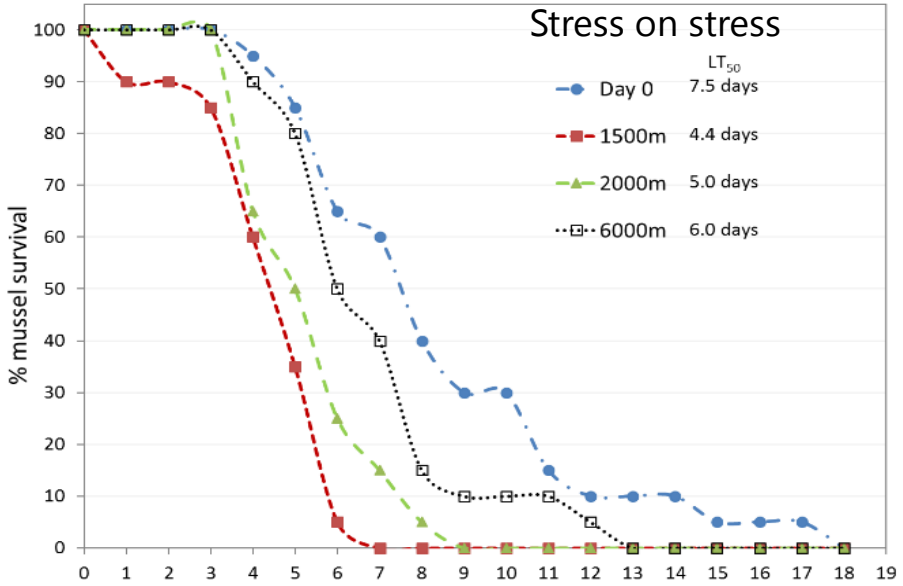
FLOT2015:

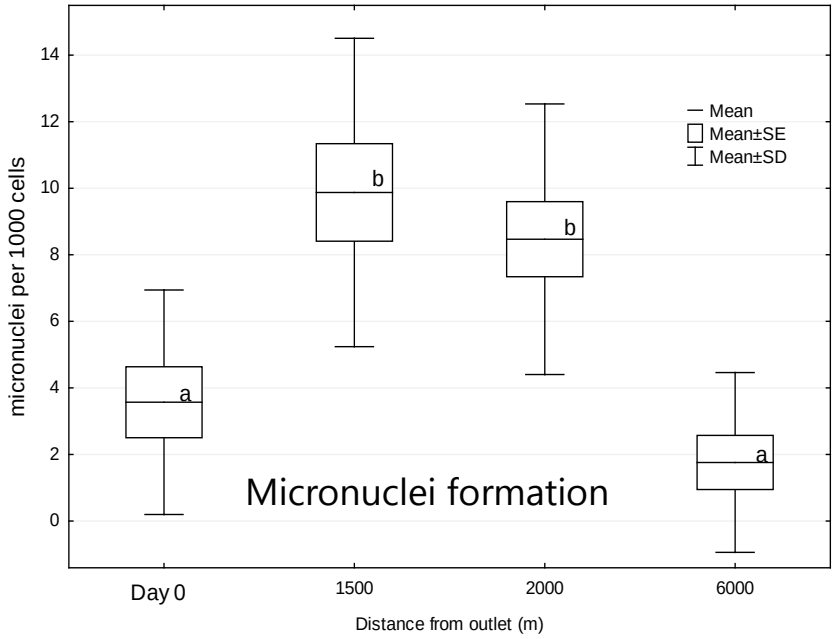
- formed by a mixture of unsaturated fatty acids, mono-, di, tri-esters with a methyl triethanol ammonium moiety (functional group)
- In water, it hydrolyses, breaks the ester linkage producing fatty acids and MTA

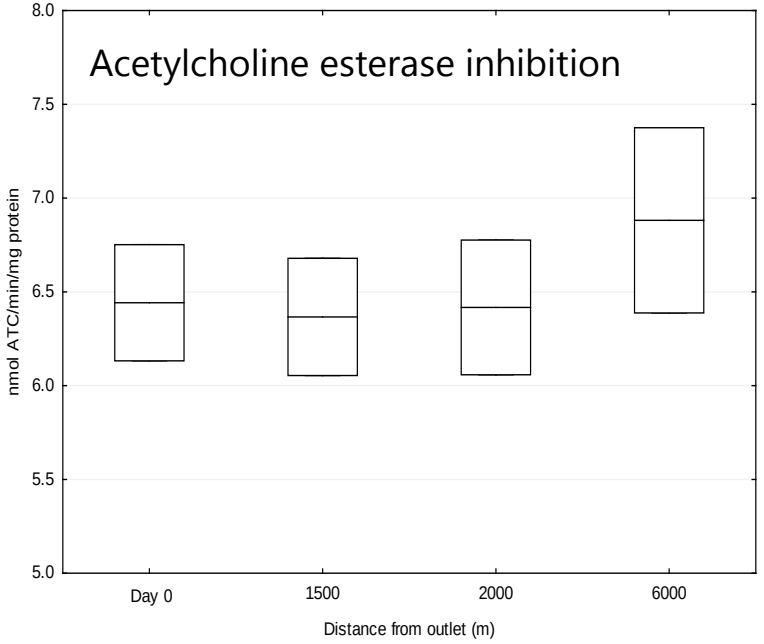


Results: biomarker

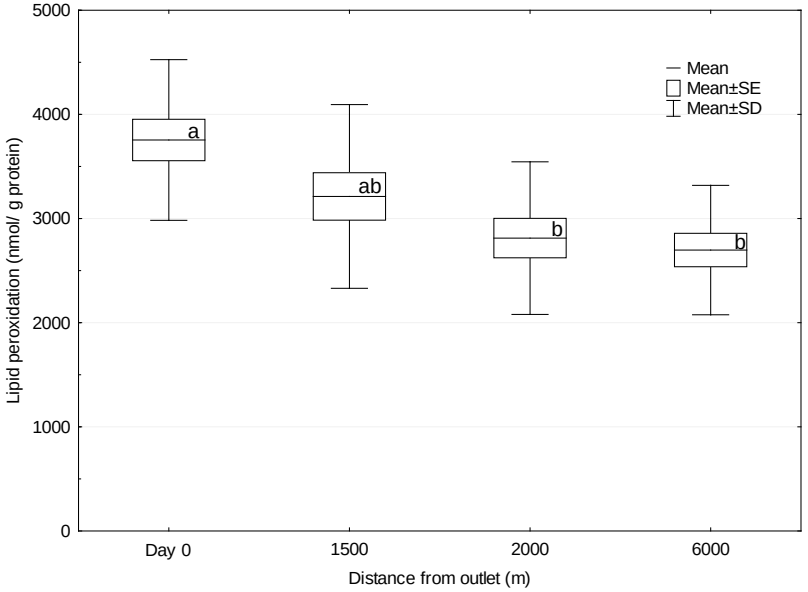


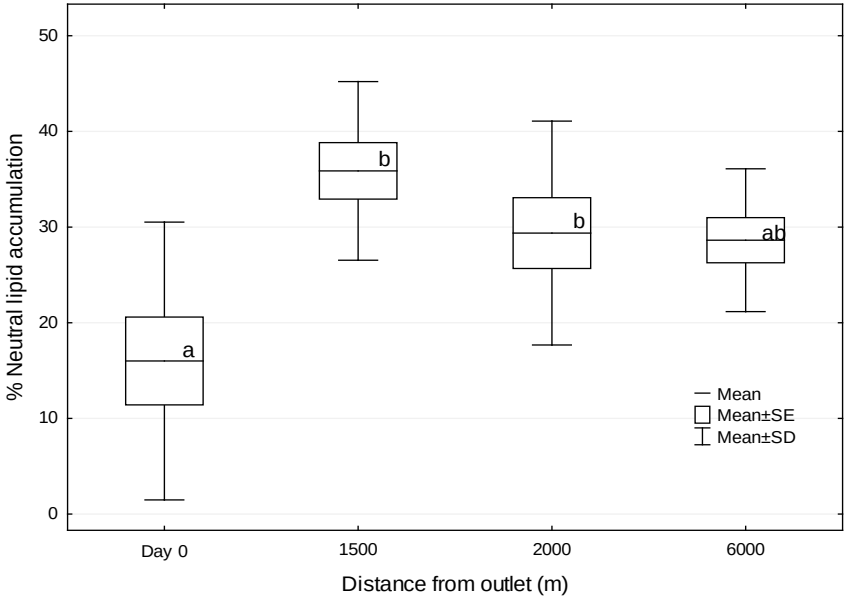


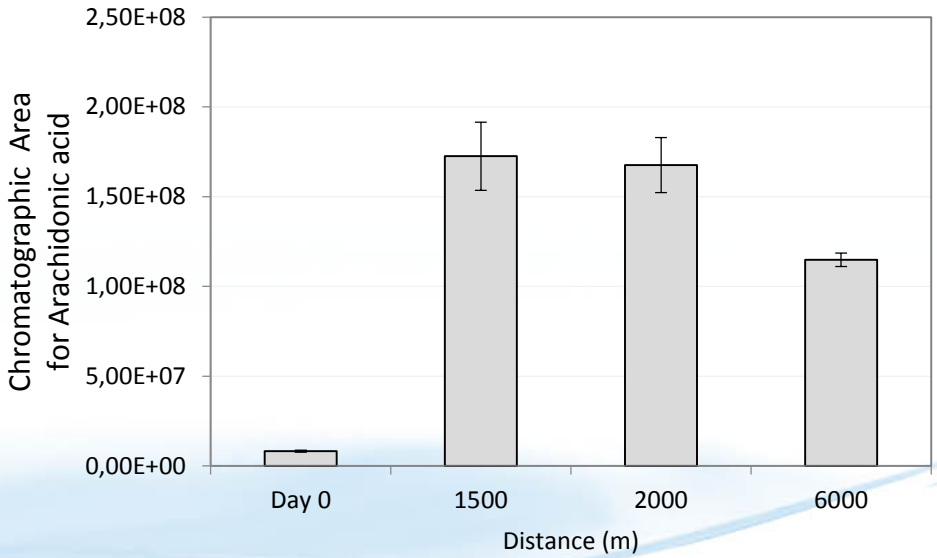




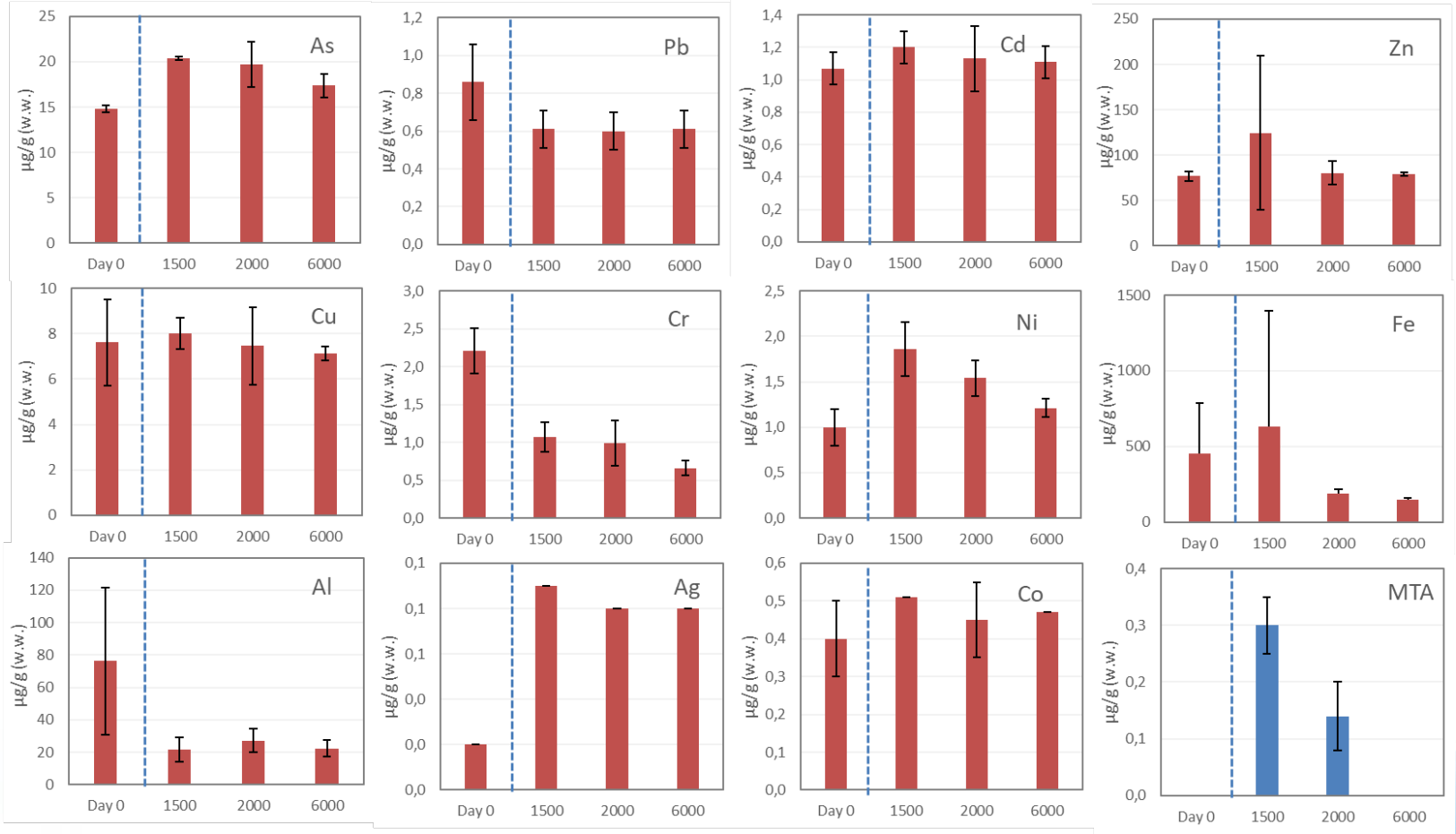
Results: biomarkers







Results: chemical analysis



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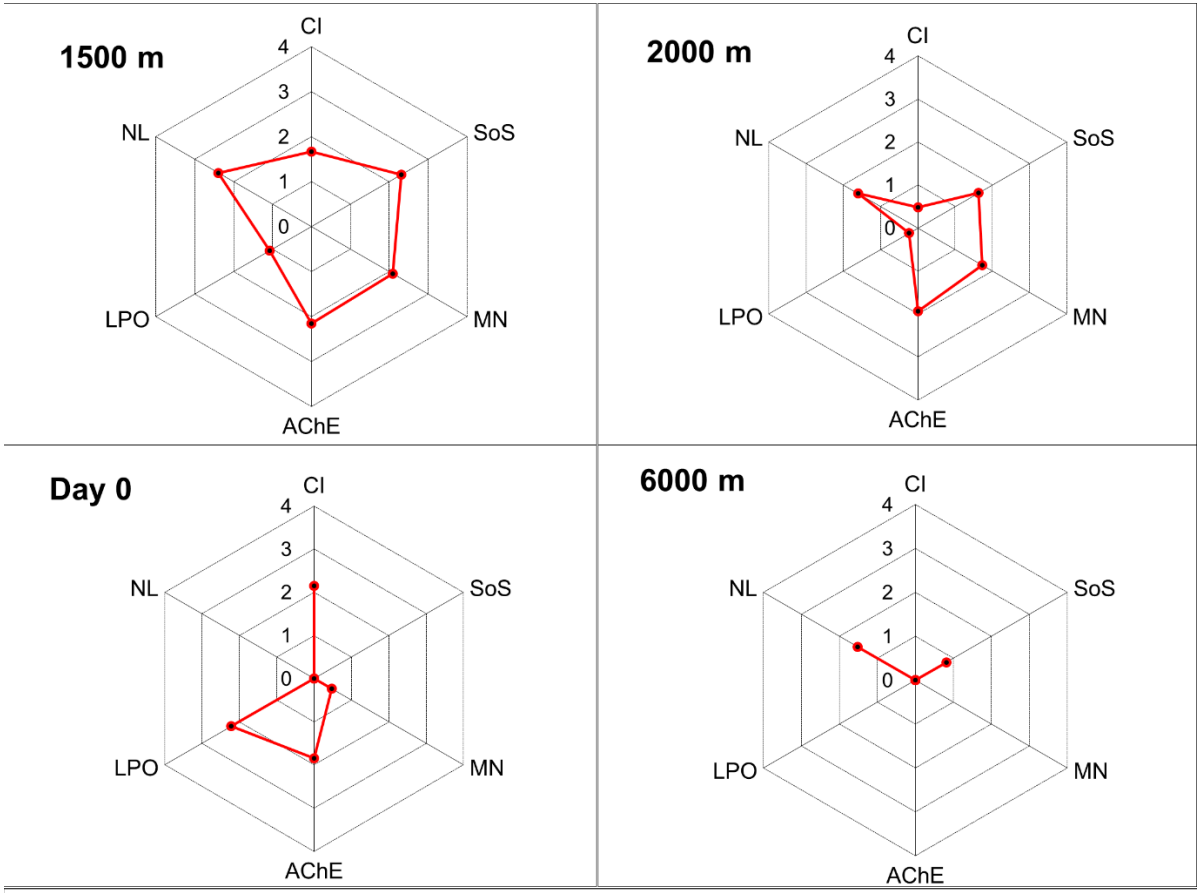
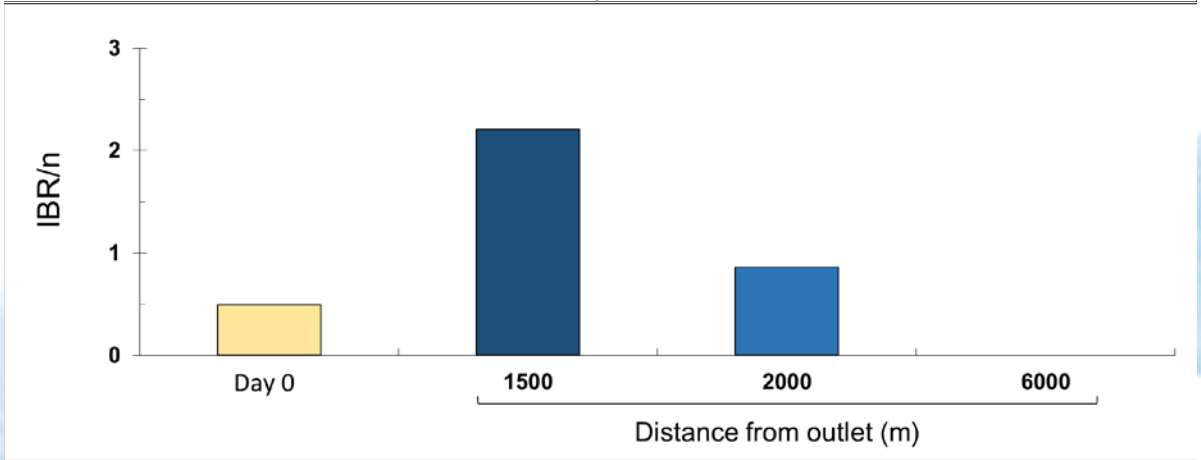
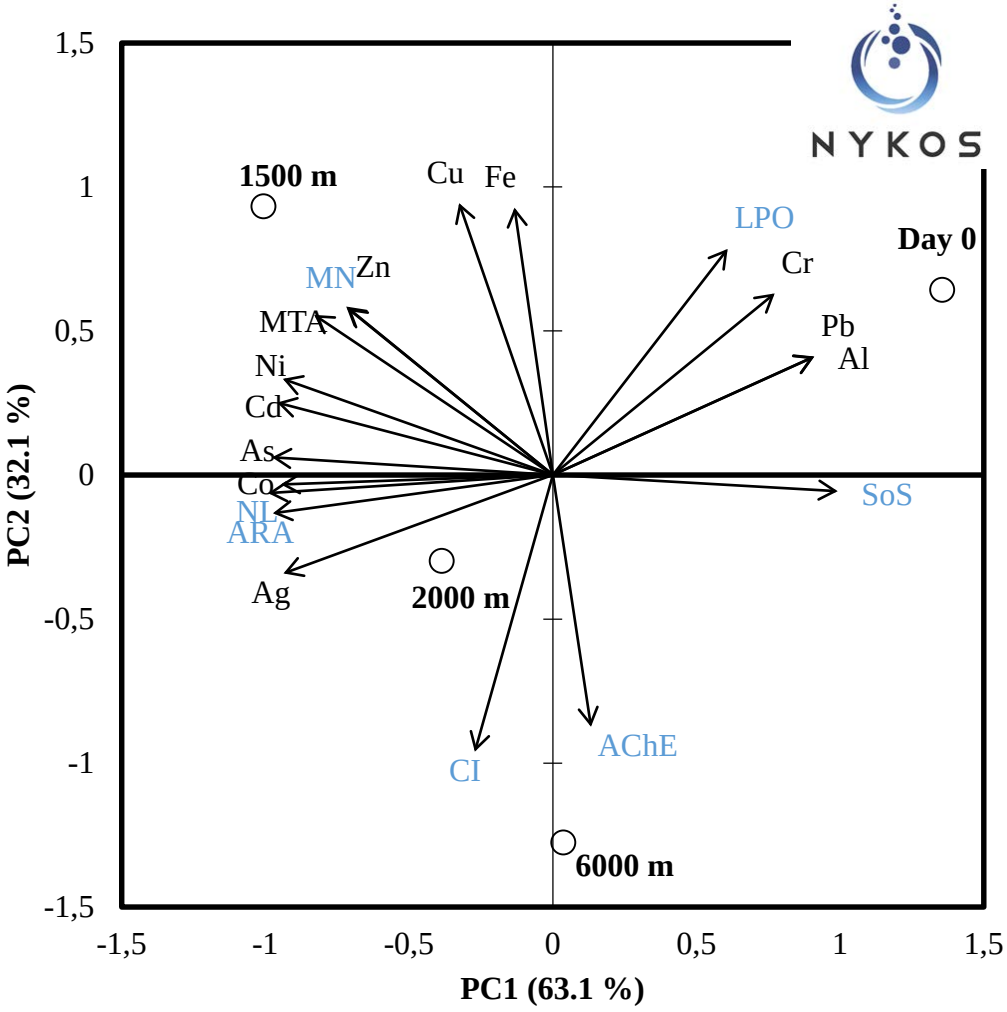

1500 m
2000 m
Day 0
6000 m
CI
SoS
MN
AChE
LPO
NL
0
1
2
3
4


IBR/n
0
1
2
3
Day 0
1500
2000
6000
Distance from outlet (m)

Principal Component Analysis (PCA):

to discriminate the main variables responsible for the variance in the chemical body burden and biological effects data

- Clear spatial differentiation between mussel groups
- PC1 63% of variance and separation between day 0 and field exposed mussels
- Day 0 (high SoS and LPO) associated with metals Cr, Pb, Al
- PC2, 32% of variance and separated field exposed mussels
- 1500 m mussels most environmentally stressed BIOMARKERS (higher MN, NL, ARA, lower AChE, CI and SoS)
- Chemicals: MTA, Zn, Fe, Ni



Group	CI	SoS	MN	AChE	LPO	NL	ARAC	chemicals
DAY 0	low	high	low	lower	high	low	low	Cr, Pb, Al
6000m	high	high	low	higher	low	mid	mid	
2000m	mid	low	high	lower	low	mid	high	MTA, As, Ni
1500m	low	low	high	lower	high	high	high	MTA, Zn, Cu, Fe, Ni, As, Cd

Conclusion

- Clear biological responses observed in mussels closest to the discharge outlet (1500 m and 2000 m:

Group	CI	SoS	MN	AChE	LPO	NL	ARAC	chemicals
DAY 0	low	high	low	lower	high	low	low	Cr, Pb, Al
6000m	high	high	low	higher	low	mid	mid	
2000m	mid	low	high	lower	low	mid	high	MTA, As, Ni
1500m	low	low	high	lower	high	high	high	MTA, Zn, Cu, Fe, Ni, As, Cd

- Concentrations of MTA, a chemical marker for the esterquat based flotation chemical FLOT2015 used at Hustadmarmor, was detected in whole mussels up to 2000m from the discharge outlet.

- This confirms exposure of the mussels to the mine tailing discharge and links tailing exposure (including metal concentrations) with the biological effects observed.

Acknowledgements

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An integrative biological effects assessment of a mine discharge into a Norwegian fjord using field transplanted mussels

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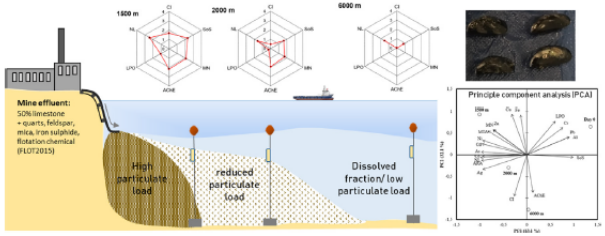
HIGHLIGHTS

- Transplanted mussels used to discover biological effects of suspended mine tailings
- Biomarkers and chemical body burden measured in field transplanted mussels
- Detection of MTA a chemical marker for flotation chemical (FLOT2015) in mussels
- Link between MTA, metals and biomarker responses with distance from discharge point
- IBR and PCA linked chemical exposure from the tailings with biomarker response

GRAPHICAL ABSTRACT




Thank you for your attention