

# GRACE WP3: Field studies and Greenland in situ burning experiment

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# Introduction



- GRACE project: Integrated oil spill response actions and environmental effects
  - WP3: The objective of this work package is to **improve the knowledge** on the **biological impacts of oil spills** and the different oil spill **response methods** in the **northern Atlantic** and the **Baltic Sea**, characterized by **extreme environmental conditions**.

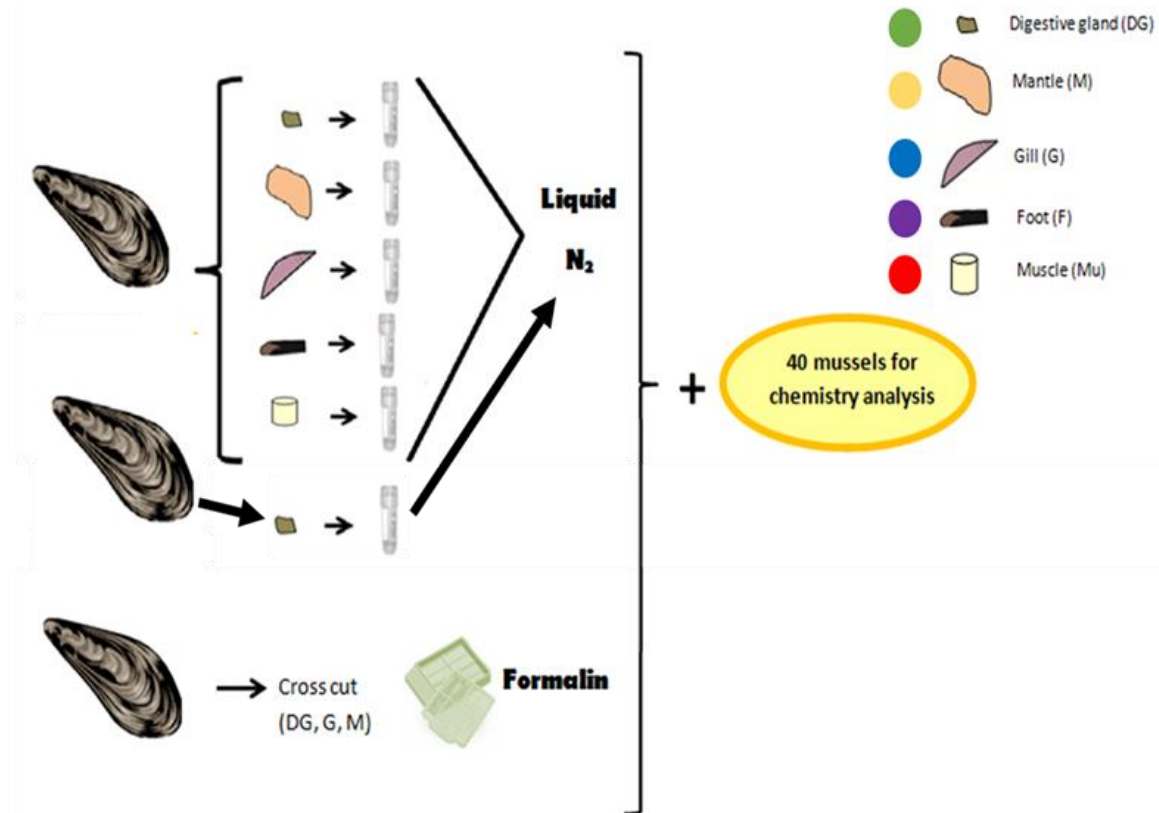


- Establishing biomonitoring programs based on biomarkers (Mussels):
  - Biomarkers **are sensitive to environmental stressors** apart from chemical contaminants, such as temperature, salinity, and dissolved oxygen levels.
  - Regionally relevant **baseline values** and their **natural variability** need to be understood.



Without this knowledge the **risk of incorrect interpretations** is high.

# Materials and Methods



## Chemical tissue burden

PAHs, Metals

## Biochemical endpoints

Antioxidant and Neurotoxicity?

## Neutral lipids

ORO

## Lipofuscins

Schmorl's reaction

## Lysosomal Structural Changes

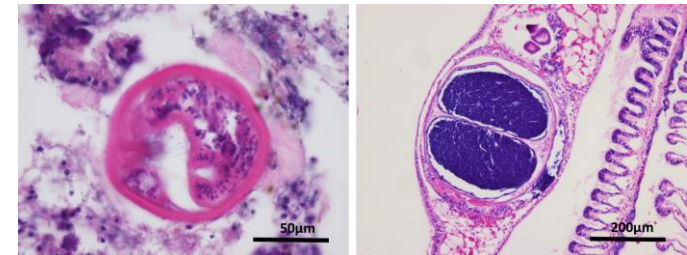
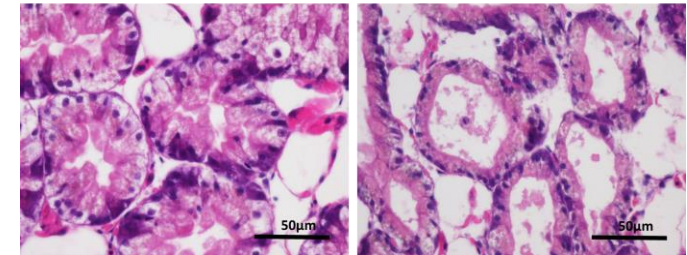
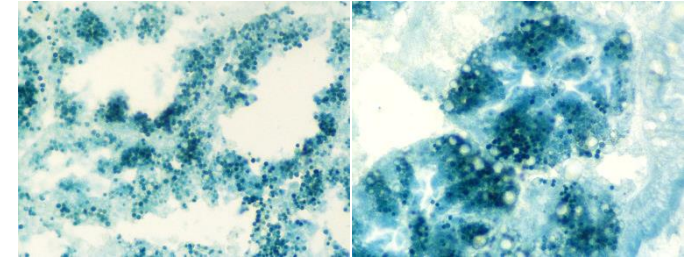
$\beta$ -glucuronidase activity

## Lysosomal Membrane Stability

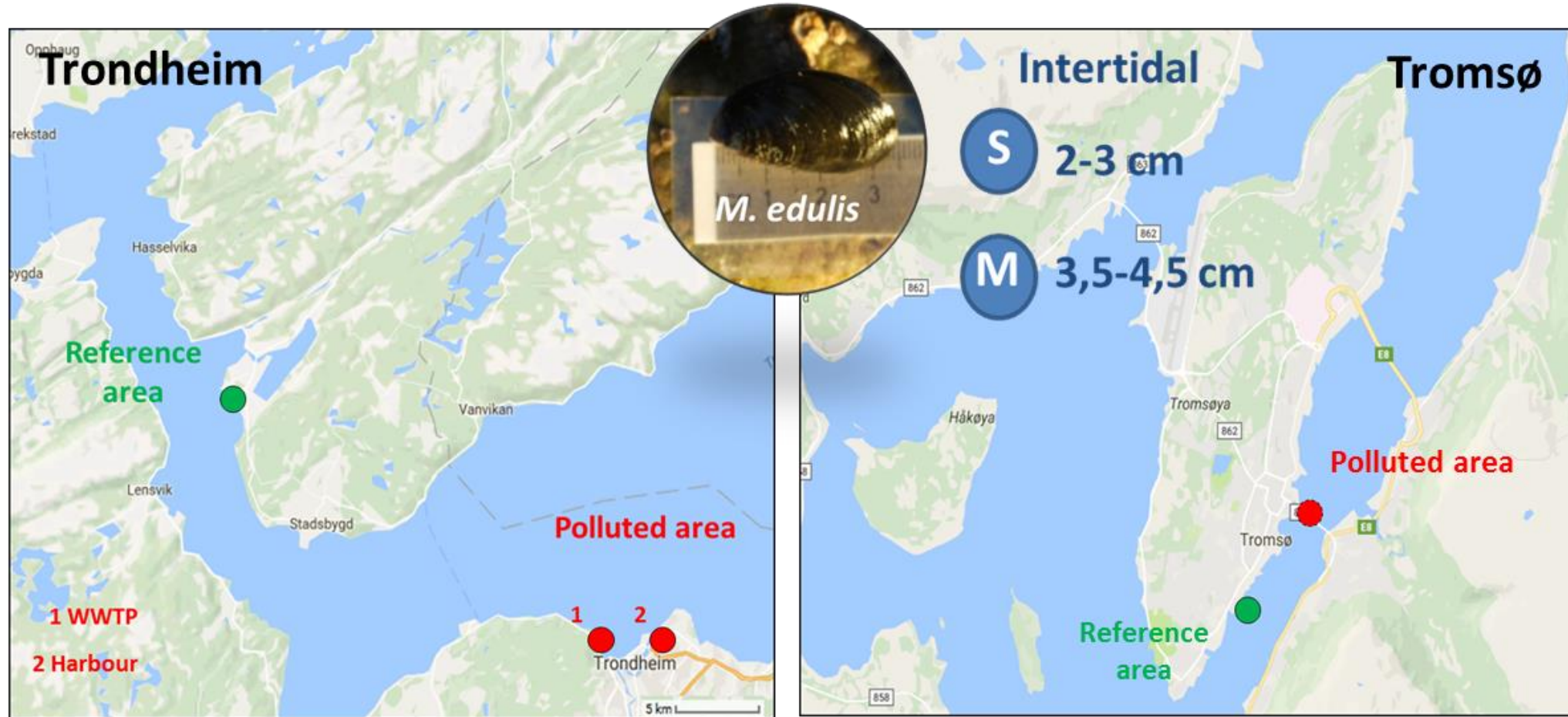
Hexosaminidase activity

## Histopathology

Hematoxylin/Eosin



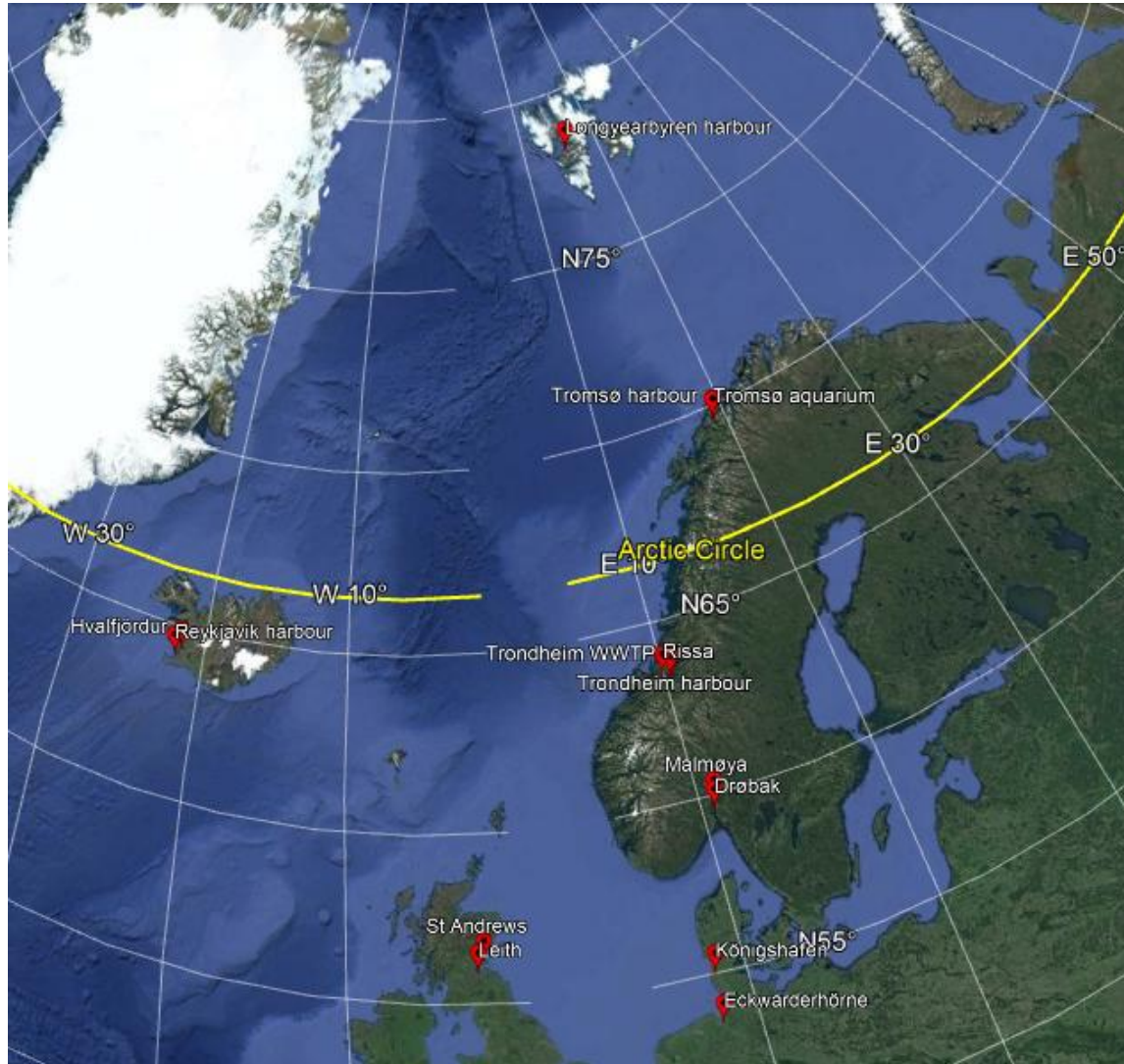
# North Atlantic Ocean: pilot study



# Highlights

- Relatively **high PAH** concentrations in **Trondheim** reference site that were **not found again in 2017**. Even **though biomarkers did not indicate chemical stress**.
- **Medium sized mussels in Tromsø** reference site relatively **high PAH** concentration and some biomarker responses (**tissue-level**) **similar to the harbor**.
- **Differences between pristine sites** (VvNL, gamete developmental stage, parasitic burden) probably related to **environmental factors**.
- **WWTP different** to other sites in Trondheim (reserve storage, parasitic burden, gamete developmental stage)
- **No consistent differences** between **sizes**, although certain **differences in PAH** accumulation were found in **Tromsø**.

# North Atlantic Ocean



-Pristine and polluted sites  
(Svalbard: only pristine)

-Intertidal mussels (Oslo harbor  
and Svalbard: subtidal mussels)

-Two sizes: small (2-3cm) and  
medium (3.5-4.5cm)

# Highlights

- **No clear PAH accumulation patterns between sizes.**
- **No consistent differences between sizes in biomarker response.**
- **Differences between pristine sites (VvNL, gamete developmental stage, tissue-level biomarkers, parasitic burden) probably related to environmental factors.**
- Overall the **battery of biomarkers is useful to discriminate chemically disturbed sites** from pristine sites at different latitudes.
- **First time** in which such a **broad battery** of biomarkers is applied in a **large scale latitudinal sampling** in the **Northern Atlantic Ocean.**



# Ongoing research

- Complete biomarker analysis of all the sampling points
- Biochemical biomarkers
- Chemical analysis of metals in soft tissues
- Identification of species

# Baltic Sea



## Localities:

Kiel and Tvärminne

## Seasons:

Spring (2017)

Summer (2016)

Autumn (2016)

## Sampled:

Subtidal mussels

# Remote sensing

- Mapped Level-3 MODIS daily data at 4 km resolution (Aqua and Terra satellites) were downloaded from the NASA Ocean Color.
- Smoothed seasonal cycle (October 2002-October 2018)
  - **Chl-*a***
  - **Particulate organic carbon**
  - **Sea surface temperature**

# Chemical burden in soft tissues

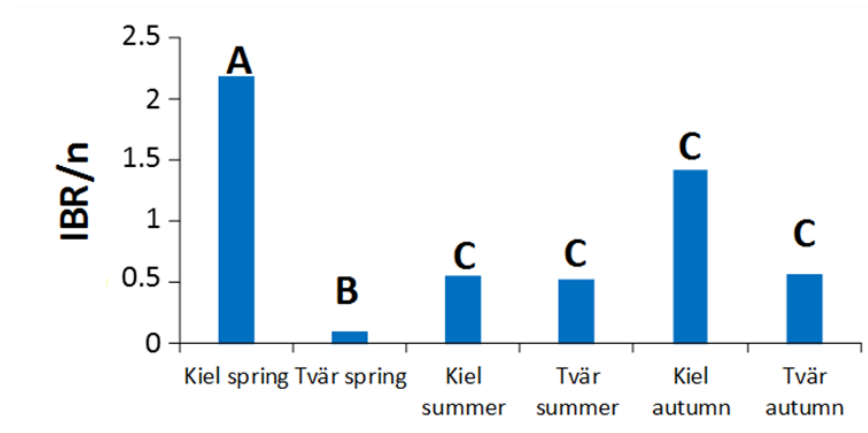
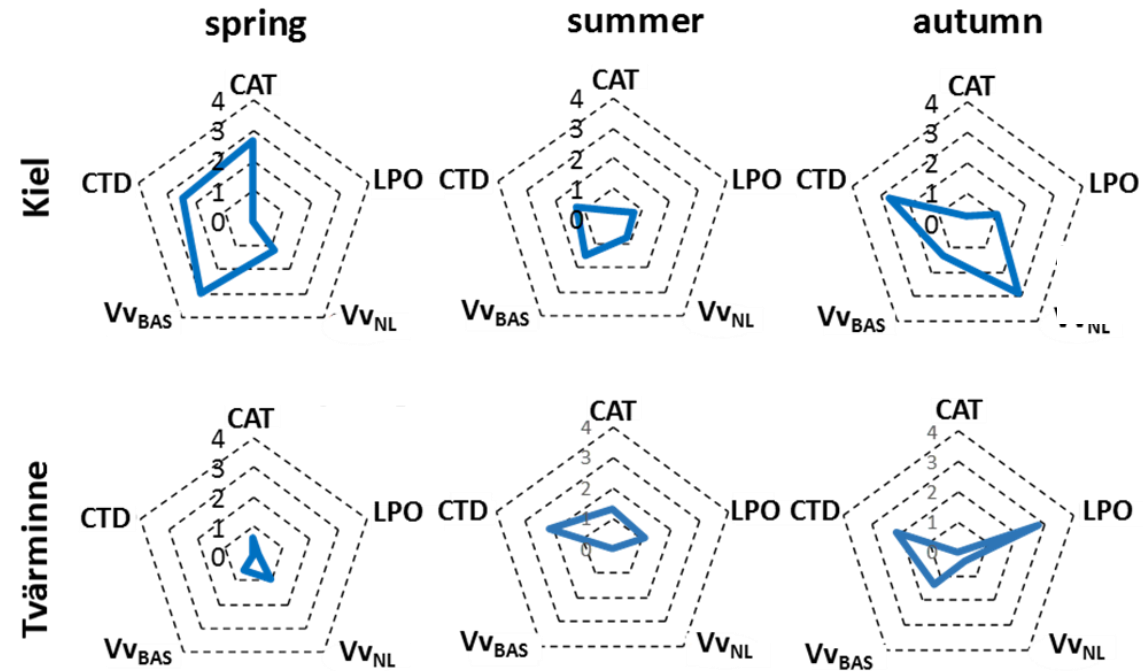
- Chemical burden in tissue:
  - 15 PAH analyzed
  - 33 metals analyzed

**GOOD ENVIRONMENTAL STATUS LEVELS OF POLLUTANTS FOR  
THE STUDIED AREAS**

# Highlights

- Mussels from **Tvärminne** are **conditioned** by **the winter period** when sea surface is covered by **ice** and there is **lack of sunlight**.
- **Food supply** seemed to be the critical factor influencing **gametogenic developmental stage** in both sampling sites, which causes **important confounding effect in biomarker responsiveness**
- **Biomarker** responsiveness changes in **different ways** depending on the **biological complexity level/biomarker**.
- In general **tissue-level biomarkers are less influenced by ecological variables**. Although in **Tvärminne** the more demanding **energetic trade-off** caused by **reproduction+hibernation** caused tissue-level alterations.

# Integrated Biomarker Response index



# Scientific publication

- Submitted to STOTEN as:

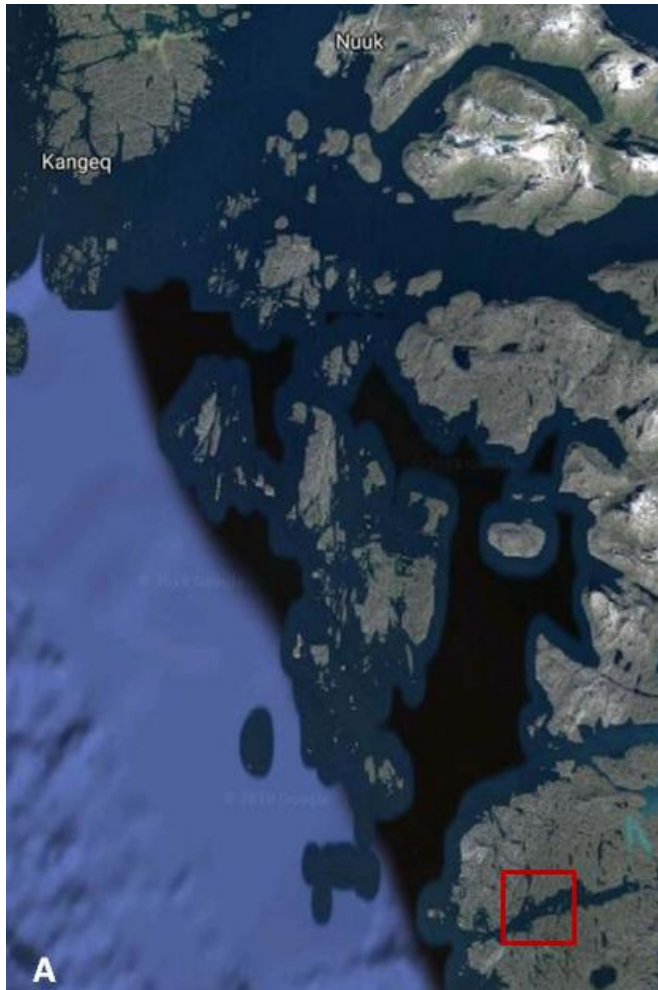
## INFLUENCE OF SEASON-DEPENDING ECOLOGICAL VARIABLES ON BIOMARKER BASELINE LEVELS IN MUSSELS (*Mytilus trossulus*) FROM TWO BALTIC SEA SUBREGIONS

Denis Benito, Aino Ahvo, Jari Nuutinen, Dennis Bilbao, Jon Saenz, Nestor Etxebarria, Xabier Lekube, Urtzi Izagirre, Kari K Lehtonen, Ionan Marigomez, Beñat Zaldibar and Manu Soto

# Greenland *in situ* oil burning

- **On-shore** (NAN crude oil) and **off-shore** (IFO180) *in situ* oil burnings
  - **Intertidal mussels** transplanted to **cages** two day before burning.
  - Mussels **sampled 3 days after burning**.
  - Mussels **transported** to Nuuk in **air** at ambient temperature (3-4 hours).
  - Whole mussels (**no dissection**) **frozen** at -80°C in Nuuk.
  - **Dissection** of **whole frozen** mussels in PiE (UPV/EHU)
  - Part of **digestive gland** and **gills** sent to **SYKE** for biochemical biomarkers

# Greenland on-shore in situ oil burning



## Reference area buoys

RA 1m depth

RB 4m depth

## On-shore *in situ* burning buoys

OSBI 1m depth

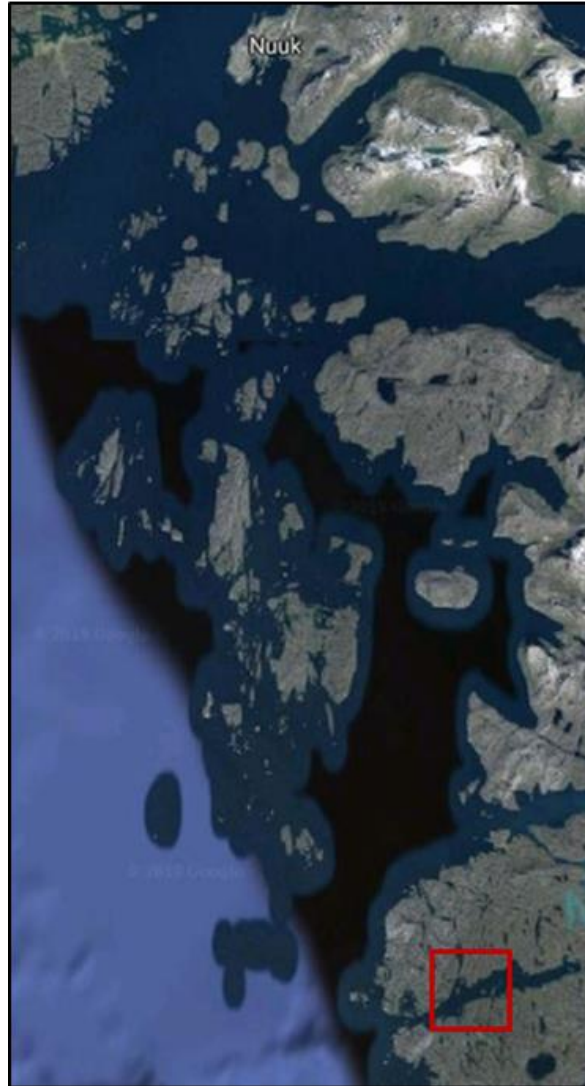
OSBIV 4m depth



# Major finds

- Differences regarding **PAH burden** in tissues in the **C10-C25 THC**s, can be associated to the **presence of diesel-like** compounds, especially at **1 m depth**.
- **Tissue-level** biomarkers and **histopathology** could not be assessed because of **lack of sample quality**.
- **Oil burning** under the present conditions did **not exert** any **oxidative stress** in mussels.
- Upon exposure to in situ burning of the oil spill on shore **VvNL remained unchanged**.
- The **response profile** of **cellular biomarkers** at **1m depth** (lysosomal size reduction and membrane destabilization, and slight lipofuscin accumulation) corresponds to the one that **could be expected after short term exposure to petroleum hydrocarbons**.

# Greenland off-shore *in situ* oil burning



## Reference area buoys

RA

1m depth

RB

4m depth

## Off-shore *in situ* burning area

Station 088 (FIRE)

1m depth

4m depth

8m depth

## Non-transplanted mussels (shore)

081

083

086

088

# Major finds

- **THC levels** systematically **higher** in mussels caged at the **depth of 1 m in 088 and 089** stations.
- **Tissue-level** biomarkers and **histopathology** could not be assessed because of **lack of sample quality**.
- **Higher oxidative stress** levels at **higher depths**. **Exposure to chemical residues of the burning event caused a decrease in enzyme activity in 088** station.
- Stations **081** and **086** showed **elevated CAT and GST** levels what is **common** in cases when the **stress is not overwhelming**.
- **Lysosomal enlargement** was observed at the burning site station **088 at 1 m depth**.
- **Depth** affected **lysosomal** biomarkers

# THANK YOU FOR YOUR ATTENTION!

