

# BIOLOGICAL EFFECTS OF WWII CHEMICAL WARFARE AGENTS (CWA) MEASURED IN MUSSELS (MYTILUS TROSSULUS) CAGED AT THE MAJOR DUMPING SITE IN THE BALTIC SEA

Raisa Turja, Matthias Brenner, Laura Butrimaviciene, Janina Baršiene, Anu Lastumäki, Kari Lehtonen





## **Outline**

- Bornholm deep major CWA dumping site in the Baltic Sea
  - Background
  - Area
  - Environmental risk
- Mussel caging experiment
  - Caging procedure & sites
  - Anaysed biomarkers
  - Results
- Conclusions & Future activities







## **Background – CWAs in the Baltic Sea**

- Ca. 50 000 t of chemical warfare agents (CWAs) were dumped into the Baltic Sea after WWII.
- MERCW (Modeling of Ecological Risks Related to Sea-Dumped Chemical Weapons) 2006–2008
- **CHEMSEA** (Chemical Munitions Search and Assessment)
  - EU Baltic Sea Region Programme (2011–2014)
    POLAND, FINLAND, GERMANY, SWEDEN, LITHUANIA
  - www.chemsea.eu

CWA chemical	Tons
Mustard gas	25,000
Arsenic oil	7,500
Chloroacetophenone	7,100
Clark I	1,500
Clark II	100
Adamsite	3,900
Phosgene	5,900
Nitrogen mustard	2,000
Tabun	12,000
Lewisite	Not known



## Background - CWAs in the Baltic Sea

 Dumping sites at Bornholm deep and Gotland deep (max. depth 100m).



 Over 500 000 different warfare objects found from the Bornholm dumping site (incl. several wrecks).



Corroded & buried into the sediment

 CWA ammunitions (eg. air bombs and mines), shells and containers have been found in large areas outside the "official" dumping sites.



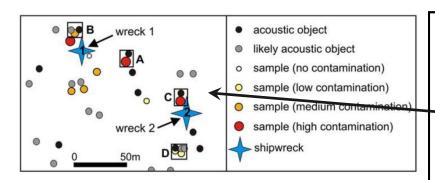


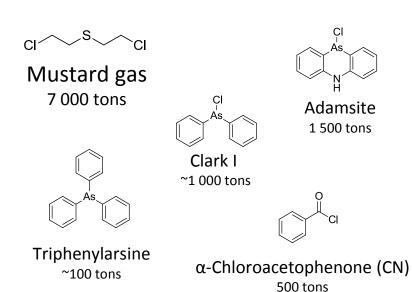


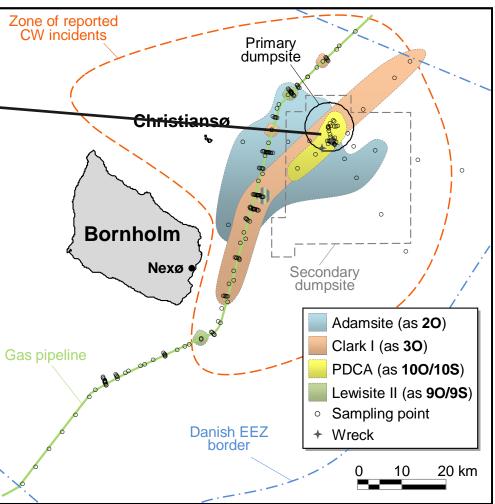
# **Dumping sites**



# **Bornholm dumping site**





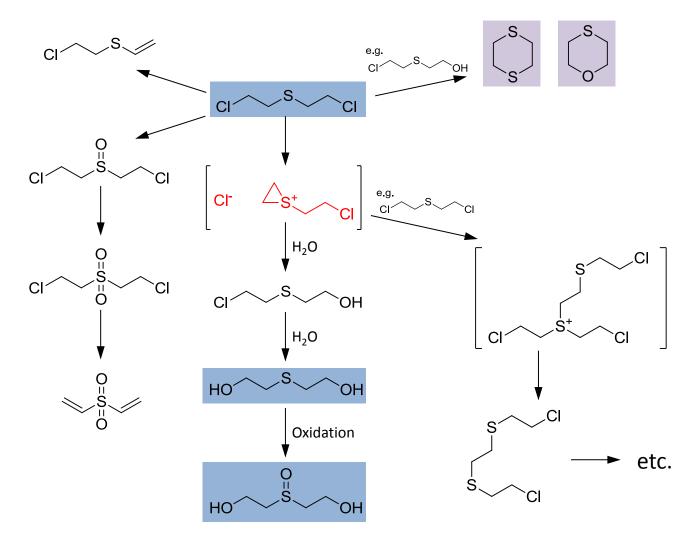


# Mustard gas degradation

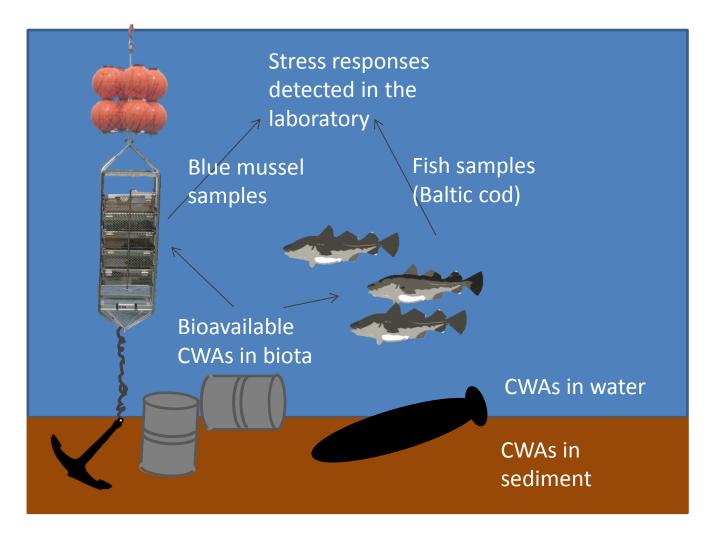
#### **Lump of mustard gas**



Sanderson & Fauser, DMU-75-00061B, University of Aarhus, 2008.



# **Environmental risk**

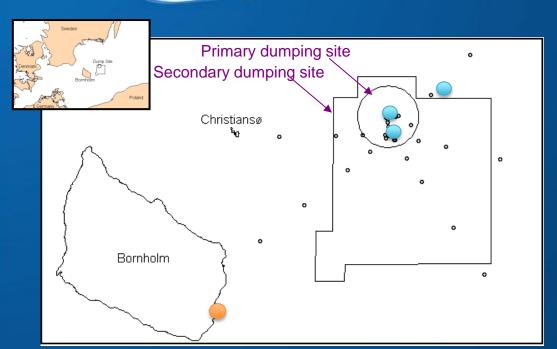








# Mussel caging experiment



- May August 2012
- Collection site of the mussels: "start" and "End"
- Caging: 3 stations, 2 depths (35m ja 65m)
- 400 mussels per cage
- Temperature, salinity and oxygen sensors attached to the cages.

- Mussel cages
- Collection site





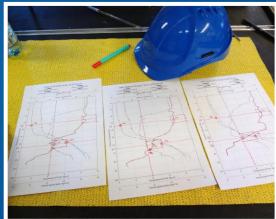






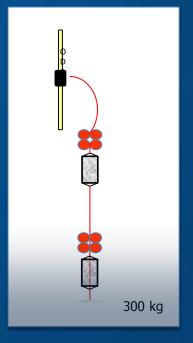
# Mussel caging experiment











Surface:

Temp 18°C, Sal 7.5 ppm

35m:

Temp 10°C, Sal 7.5 ppm

65m:

Temp 4°C, Sal 11 ppm

- Low mortality in all cages.
- Good oxygen conditions at 65m (6.3-4.9 mL/L).
- Cages were also equipped with POCIS passive samplers.
- Samples for all biomarker and chemical analysis as planned.





#### **Measured biomarkers**



- Genotoxic & sytoxicity
  - Micronuclei and other nuclear abnormalities
- Neurotoxicity
  - AChE
- General stress
  - Antioxidant enzyme activities
  - Biotransformation
  - Lysosomal membrane stability (Neutral Red retention)
  - Histological markers
  - Bioenergetics (CEA) and condition index





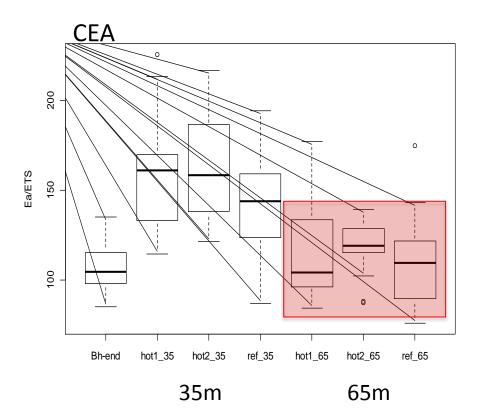


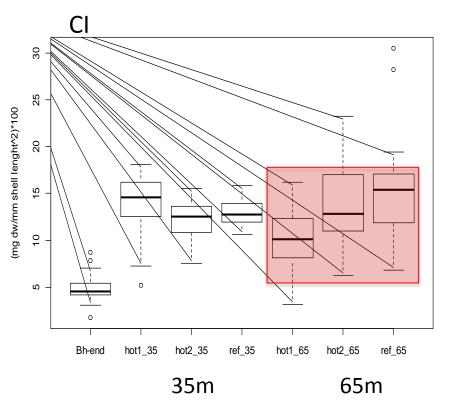




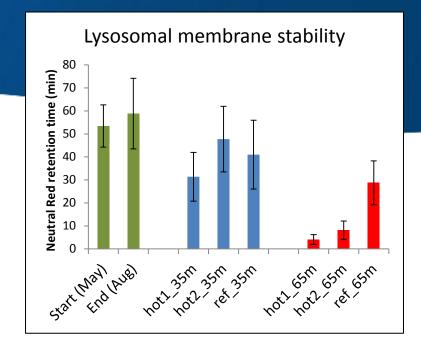


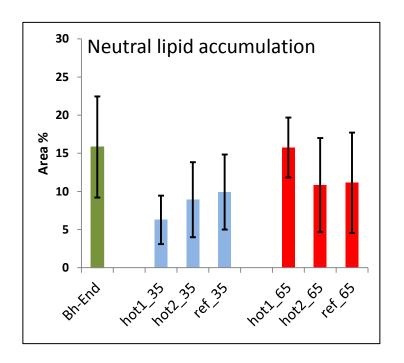
- Cellular energy allocation and condition index
  - Station hot1\_65m: available energy, but low condition index.
  - Food conditions at 35m.

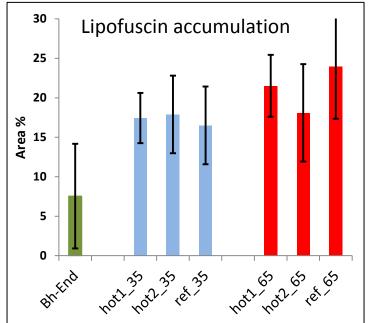




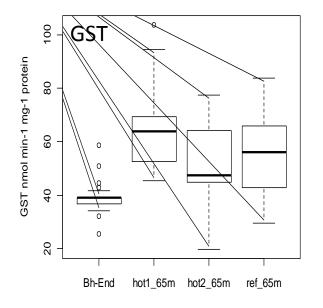
- Histochemical parameters
  - Lysosomal membrane stability
  - Lipofuscin is an end metabolic product of peroxidation processeses.
  - Neutral lipid is indicator of lipidosis induced by toxic organic chemicals

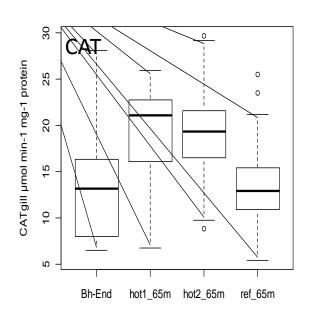


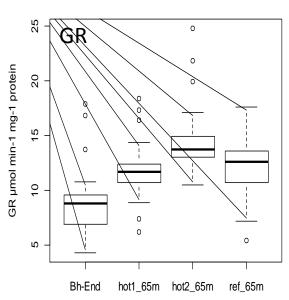




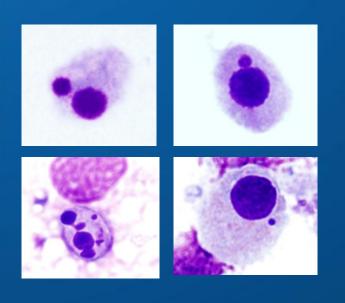
- AChE no significant differences!
- GST, CAT, GR activities (65m cages).







- Genotoxic and cytotoxic responses
  - Fragmented & apoptotic cells at hotspot sites.



#### Micronuclei and other nuclear abnormalities 70 60 Abnormalities/1000 cells 50 BN ■ 8-shaped FR+AP 30 ■ NB+NBf 20 BNb MN 10 0 Bh-End hot1 hot2 ref 65m Start (May) 65m 65m (Aug)





#### **Conclusions & Future activities**

- Highest biomarker responses at all studied biological levels were detected at 65m hotspot sites (especially hot1\_65m).
- Results from chemical analysis (sediments and mussels)
- Laboratory exposure experiments
  - Sediment and sediment extract (Daphnia)
  - Selected CWA chemicals (blue mussels)
- Modeling leakage effects on marine biota & risk assessment























Johann Heinrich von Thünen-Institut



