

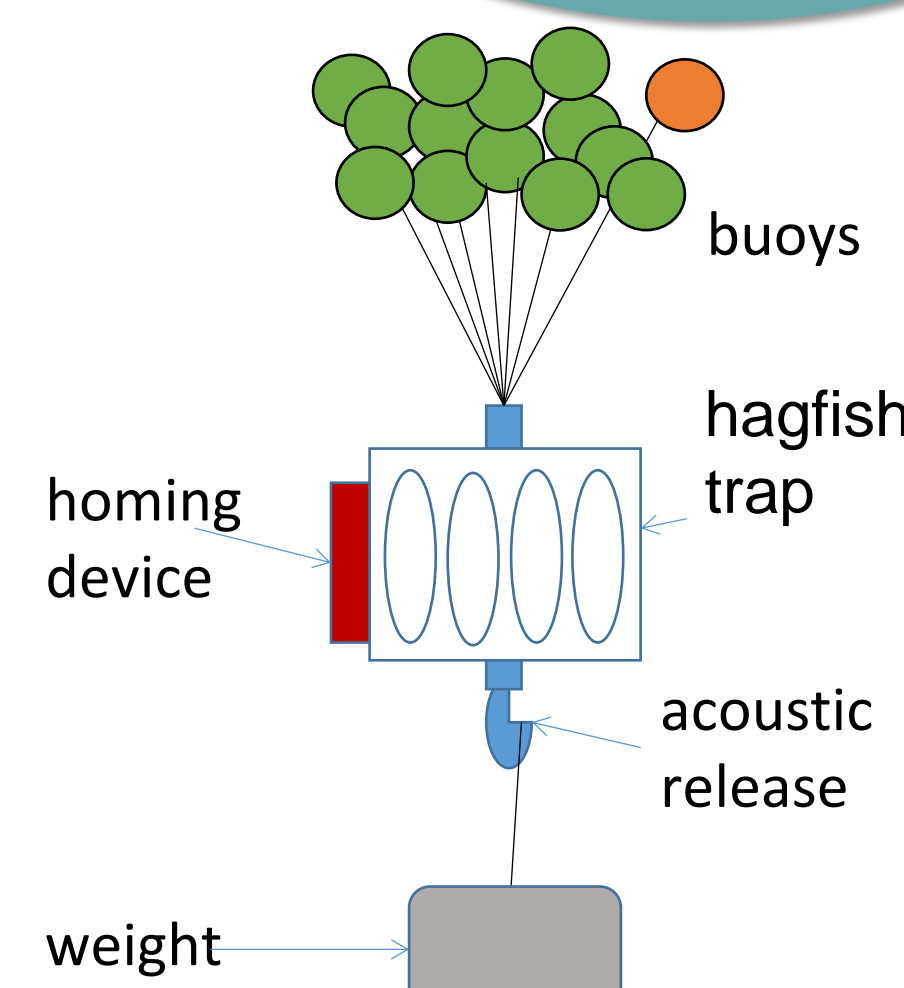
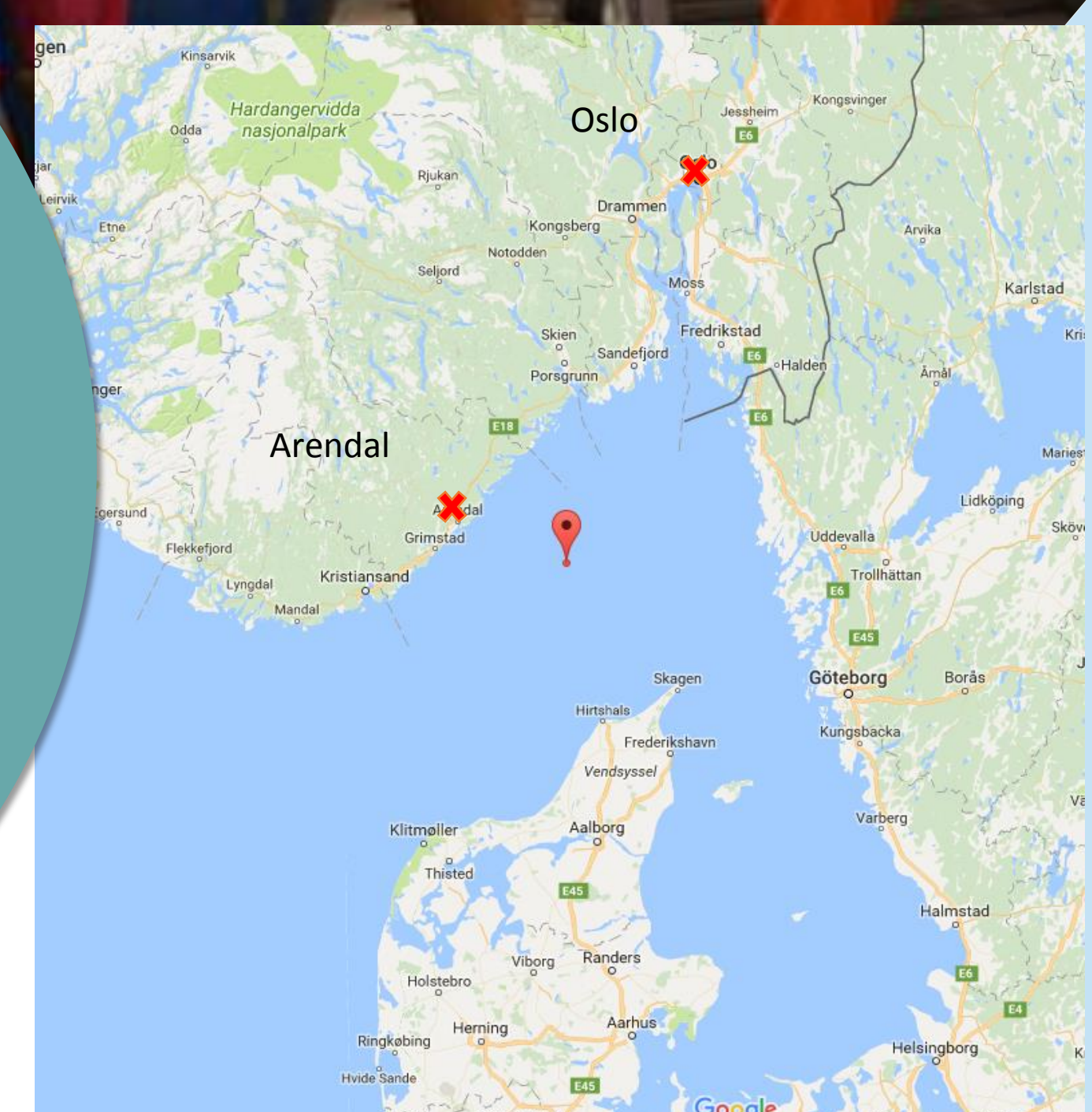
Using the hagfish (*Myxine glutinosa*) to study biological effects of a wreck filled with chemical munitions

The sea bottom of the Skagerrak Strait (North Sea) contains ca. 45,000 tonnes of chemical warfare agents (CWA) dumped after the Second World War. Entire ships loaded with of CWA were intentionally sunk and are still laying on the deep bottoms (ca. 600 m) of the area in different states of deterioration by corrosion. The current status of the CWA in the wrecks is unknown; if released into the environment they may have significant deleterious effects on local marine biota. Within the research programme of the EU Baltic Sea Region Interreg project DAIMON (Decision Aid for Marine Munitions, www.daimonproject.com), one of these wrecks was selected to study the leakage of CWA and their possible biological effects. From the few fish species that inhabit the studied depth range in the region, the hagfish (*Myxine glutinosa*), a sediment-dwelling chordate, was selected as target organism for chemical analyses of CWA in tissues and biological effect studies.

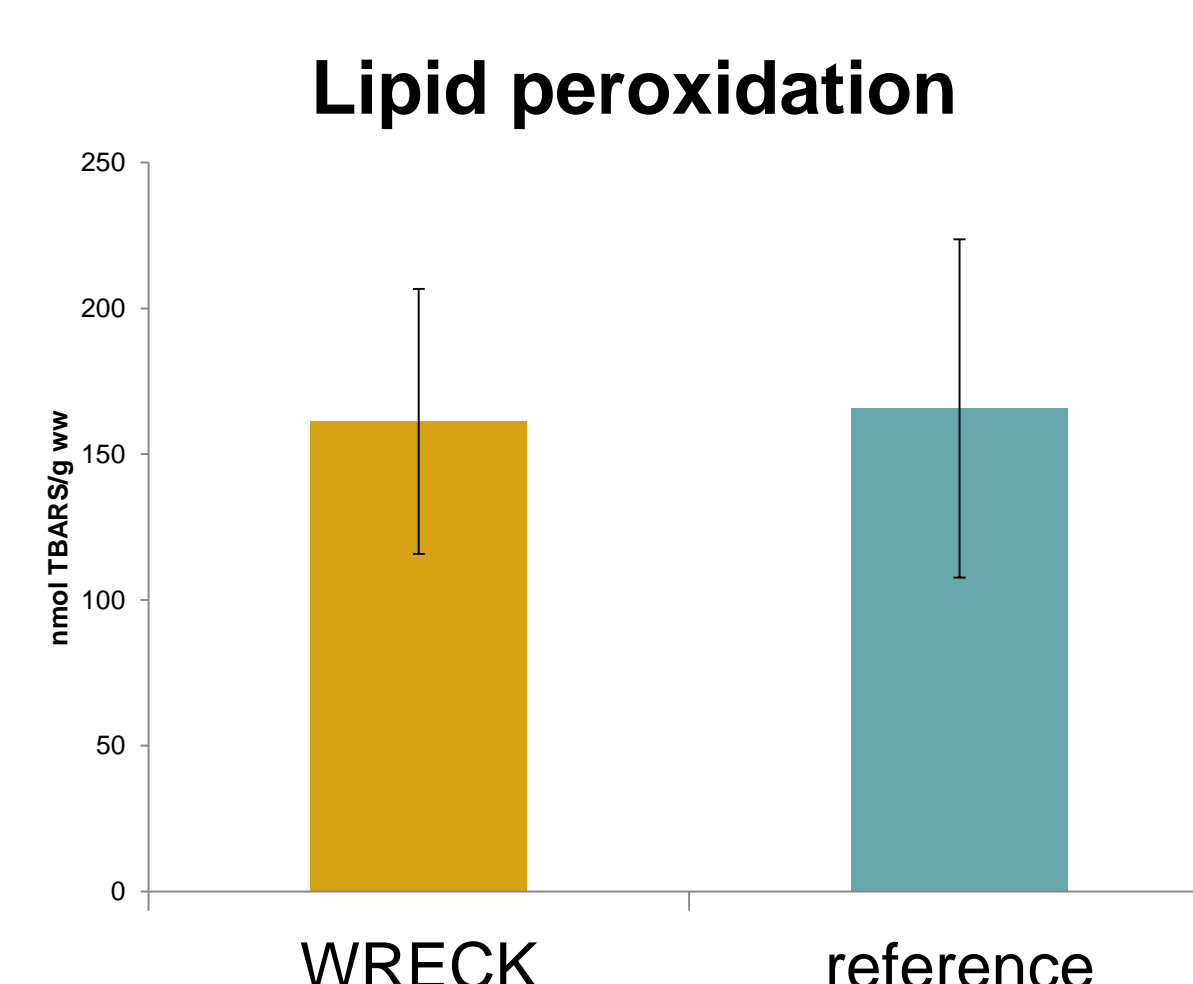
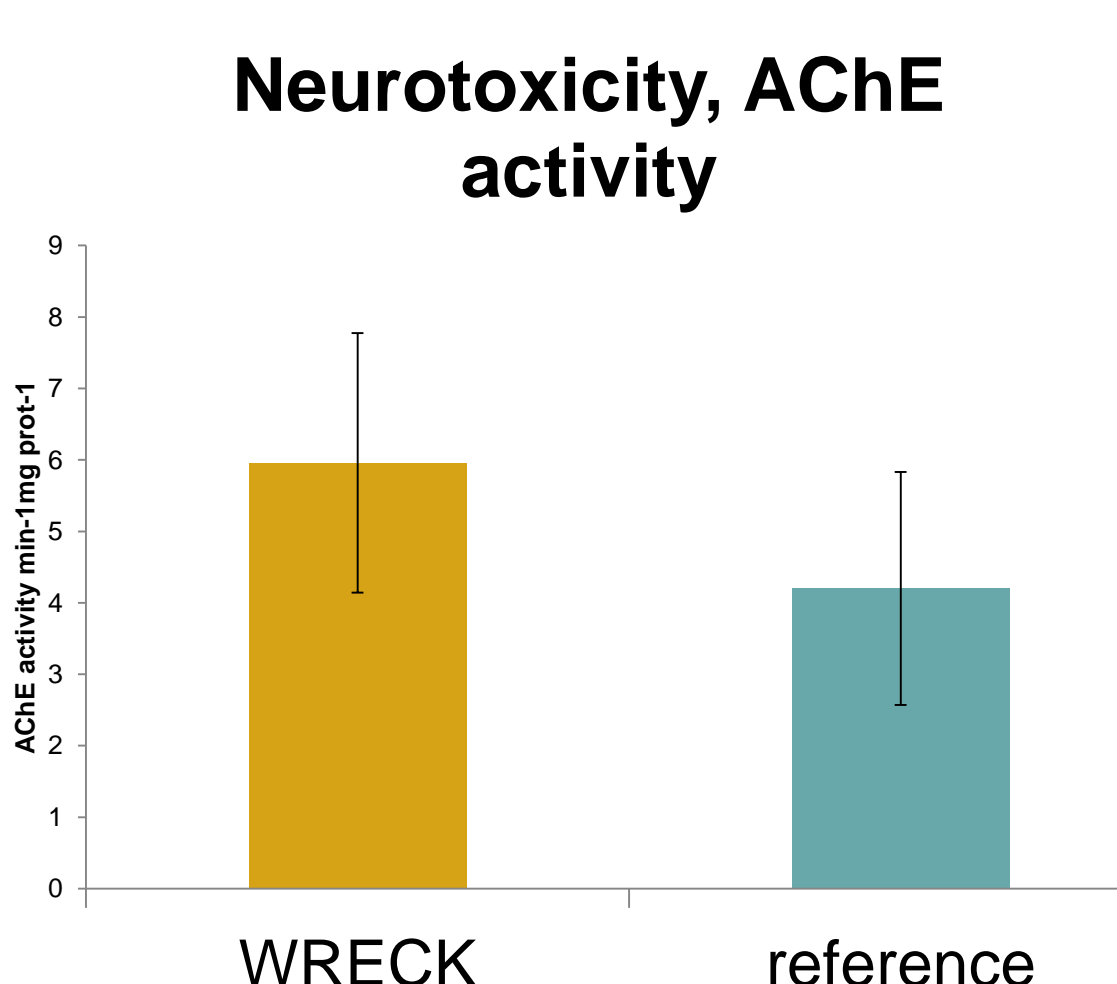
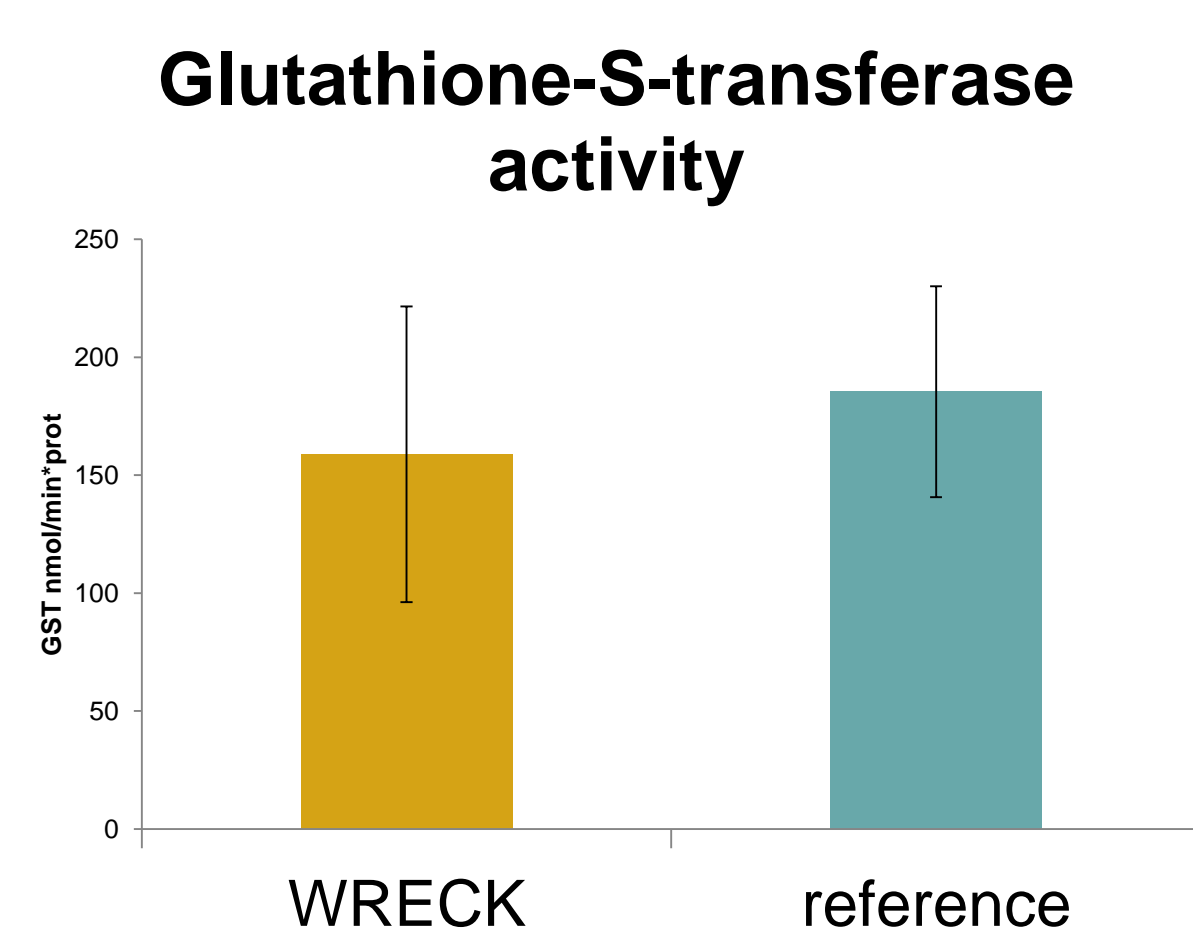
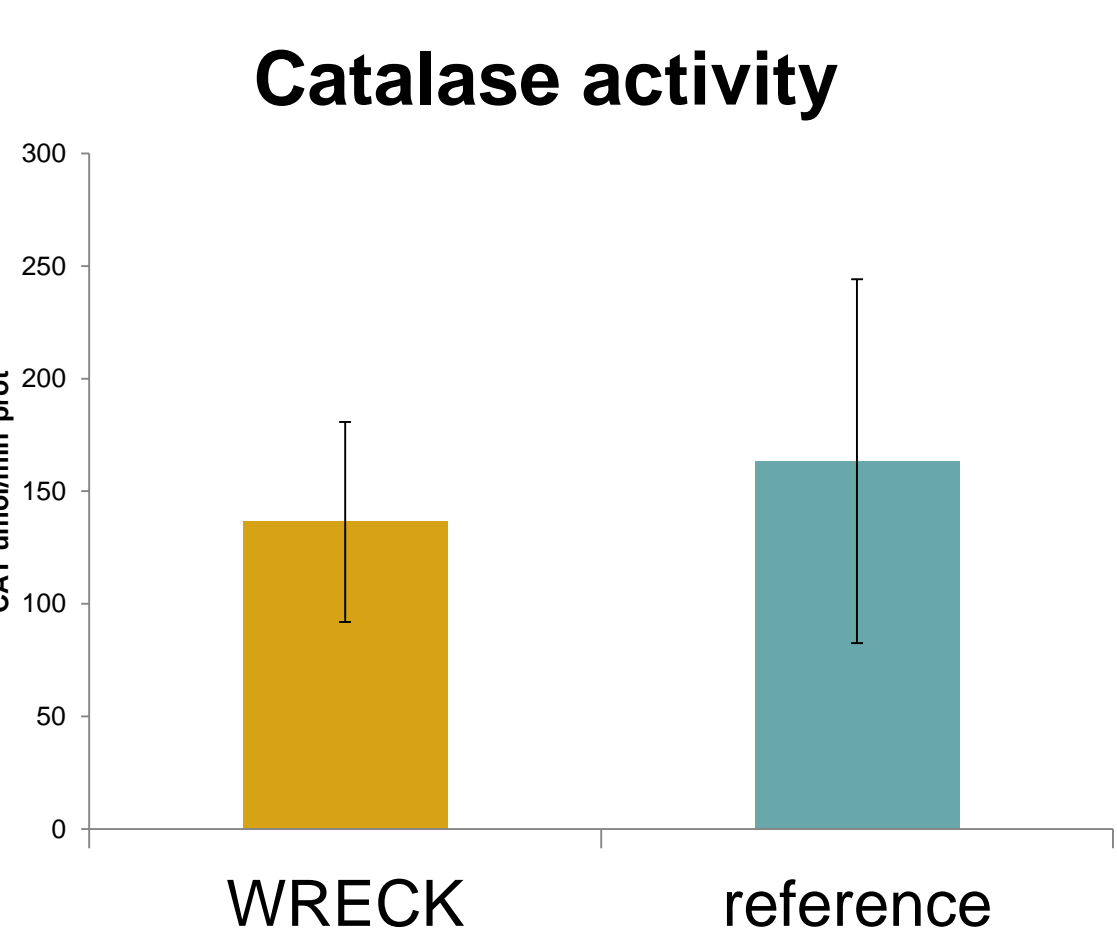
Samples were taken using bait traps near the wreck and from a reference area known to contain no wrecks. Based on this study, the hagfish is regarded as a suitable candidate for ecotoxicological studies of deep marine areas.

Measurement	Method	Liver	Muscle
CWA-related phenylarsenic compounds	LC-MS/MS		X
Liver alterations, infectious agents	Histopathology	X	
Acetylcholinesterase activity (AChE)	Inhibition of the hydrolysis rate of acetylthiocholine		X
Catalase activity (CAT)	Degradation rate of hydrogen peroxide	X	
Lipid peroxidation (LPO)	Amount of thiobarbituric acid reactive substances (TBARS)	X	
Glutathione-S-transferase activity (GST)	Formation rate of the GSH conjugated substrate	X	
Glutathione reductase activity (GR)	Consumption rate of NADPH in the reduction of GSSG to GSH	X	
Superoxide dismutase activity (SOD)	Inhibition % of WST reduction	X	

Established biomarker methods used widely in various fish species were shown here for the first time to be applicable also in hagfish.

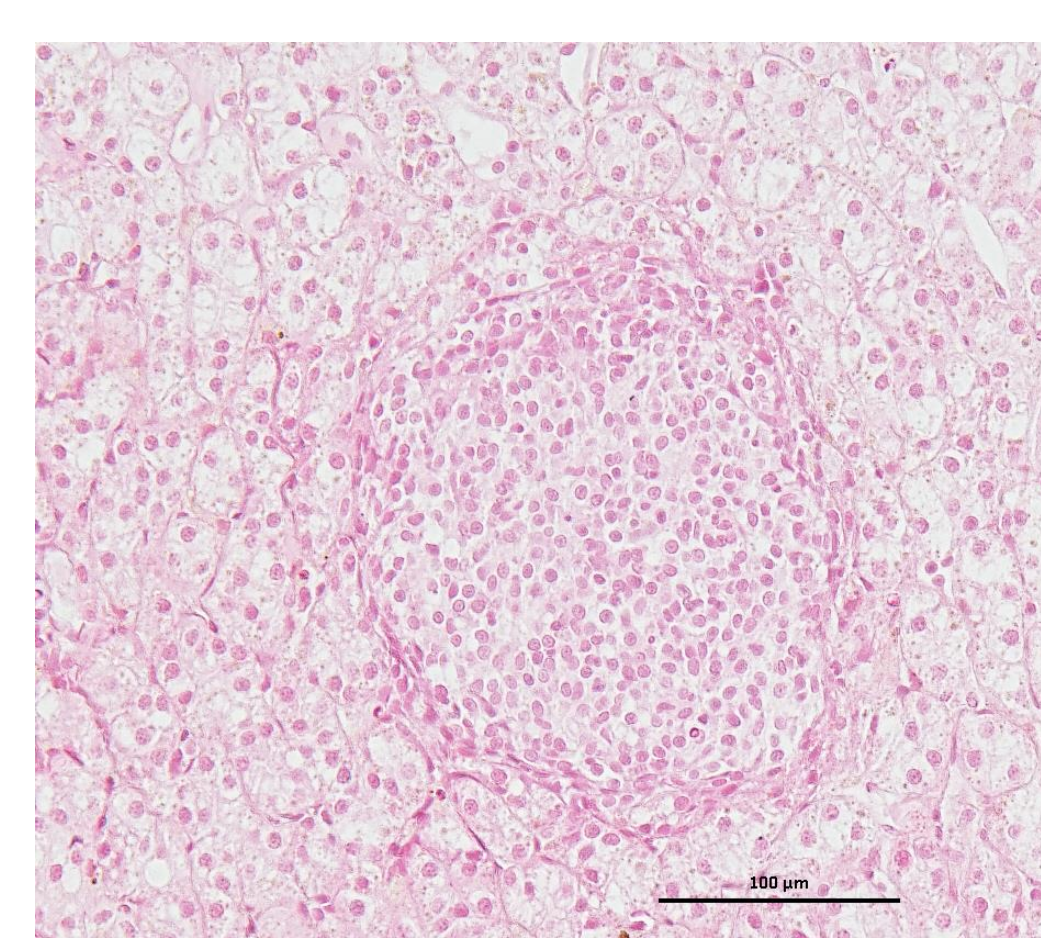


Hagfish in trap bottle (left) and hagfish liver sections showing liver alterations (right, analysis by FishVet Group).



Challenges with studying Skagerrak CWA wrecks:

- Deep waters, few suitable species for biomonitoring
- Wreck areas unknown → difficult to find a clean reference area
- More research on chemical contamination and mapping of CWA wrecks is needed



Granulomas were found in multiple samples. Each sample contains more than one granuloma in difference sizes. The basic cause is non-distinctive as no reason (e.g. parasites) has been identified. Analysis TI-FI.

Key observations:

- Results from the chemical analysis indicated the **presence of oxidized forms of CWA-related phenylarsenic compounds** in most of the muscle samples.
- Histopathological analysis showed five samples with moderate or pronounced presence of nodules (foci of cellular alteration, FCA), with **no significant difference** between samples collected near the wreck and at the reference position. No infectious agents (bacteria or fungi) or tumours were found (analysis by FishVet Group Norge AS & TI-FI).
- Only **minor differences** in the measured biochemical biomarker responses could be observed between the groups
- More information on the biology of hagfish and the natural variability of their biomarkers is needed to distinguish true effects of hazardous substances.