Health Status of Cod (*Gadus morhua*) at Dumpsites for Chemical Warfare Agents in the Baltic Sea

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Aim of the study:

- to assess the health status of cod at known and suspected chemical warfare agents (CWA) dumpsites and in reference areas of the Baltic Sea
- by applying a batterie of physiological, pathological, cellular and subcellular health indicators
Some words about cod (*G. morhua*) in the Baltic Sea:

- Two cod stocks in the Baltic Sea:
  - western stock
  - eastern stock

- Borderline between stocks: ~ Bornholm

- Eastern stock spawn in summer in the Bornholm Basin and in the Gotland basin (mainly because of favourable salinity)

- Western stock spawns in early spring in the Arkona Sea and further west

- Fishery: approx 70,000 t/year
Cod sampling areas

- Bornholm Basin CWA
- Gdansk Deep CWA
- Gotland Basin CWA
- Little Belt CWA
- Main dumping site

western stock  eastern stock
Sampling:
- May 2012: Gotland Deep and reference areas
- Sept. 2013: Bornholm Basin and reference areas

Fishing: pelagic and bottom trawling

Platform: FRV Walther Herwig III (DE)
Vertical distribution of cod: impact of hydrography (T, S, O₂)

Bornholm Basin (B13)
- Water depth: 95 m
- Fishing: 40-60 m

Gdansk Deep (B15)
- Water depth: 105 m
- Fishing: 60-80 m

Gdansk (B09, ref.)
- Water depth: 60 m
- Fishing: bottom

Fishing depth Dec. 2011
Health indicators:

**Fitness**
- Condition factor (CF)
- Liver somatic index (LSI)
- Spleen somatic index (SSI)
- Gonadosomatic index (GSI)

**Disease & pathology**
- Gross diseases and parasites
- Liver histopathology
- Head kidney pathology
- Lysosomal membrane stability
- Differential blood cell counts

**Cyto-/genotoxicity**
- Morphological alterations in red blood cells

**Neurotoxicity**
- Acetylcholinesterase (AChE) inhibition

**Oxidative stress**
- Antioxidant defence enzymes (e.g., catalase, glutathione reductase)
Cod fitness indices

Results: some significant differences between sampling years; CF higher in western cod (B01, B11, B10), GSI higher in eastern cod (B13, B09); no significant differences between CWA areas (B13, B14, B15) and reference area (B09)
### Externally visible diseases of cod

<table>
<thead>
<tr>
<th>Disease/parasite</th>
<th>Aetiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin ulcerations</td>
<td>Bacterial</td>
</tr>
<tr>
<td>Skeletal deformities</td>
<td>Multifactorial</td>
</tr>
<tr>
<td>Pseudobranchial swelling</td>
<td>Parasitic (Amoeba-like)</td>
</tr>
<tr>
<td>Fin rot/erosion</td>
<td>Bacterial</td>
</tr>
<tr>
<td>Epidermal hyperplasia/papilloma</td>
<td>Viral, skin</td>
</tr>
<tr>
<td><em>Cryptocotyle lingua</em></td>
<td>Digenean trematode, skin</td>
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<tr>
<td><em>Loma branchialis</em> (= <em>morhua</em>)</td>
<td>Microspora, gills</td>
</tr>
<tr>
<td><em>Lernaeocera branchialis</em></td>
<td>Copepode, gill chamber</td>
</tr>
</tbody>
</table>

Examinations based on standardised methodologies for fish disease monitoring (ICES, BEAST project)
Results: some significant differences in the FDI between sampling years and between areas (only 2011); highest value in fish from the Bornholm CWA dumpsite (B13) in 2011 but not in 2012; overall, no clear indication of effects of CWA on the status of external diseases
# Liver diseases/histopathology in cod

<table>
<thead>
<tr>
<th>Lesion category</th>
<th>Aetiology</th>
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<tbody>
<tr>
<td>Non-specific lesions</td>
<td>Multiple causes (e.g., infections)</td>
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<tr>
<td>Early toxicopathic non-neoplastic lesions</td>
<td>Various contaminants</td>
</tr>
<tr>
<td>Foci of cellular alteration (tumour pre-stages)</td>
<td>Carcinogenic compounds</td>
</tr>
<tr>
<td>Benign tumours</td>
<td>Carcinogenic compounds</td>
</tr>
<tr>
<td>Malignant tumours</td>
<td>Carcinogenic compounds</td>
</tr>
</tbody>
</table>

### Examples of non-specific liver lesions

- **Myxozoa**
- **Nematoda**
- **Infiltration/Regeneration**
- **Macrovesicular Steatosis**

**Results:** Non-specific liver lesions with no clear link to pollution are dominating, neoplastic lesions (tumours and pre-stages) are very rare.
Liver diseases/histopathology in cod: Liver Disease Index

Results: some differences in LDI between areas and sampling years; no significant differences between CWA areas (B13, B14, B15) and reference area (B09)
Results LMS-Test: Differences of the reference site (B09) are significant for both peak 1 and 2 (Kruskal-Wallis-Test, p<0.05, n=60)
Results Dumping sites: Tissue less dense, haematopoietic cell (HC) cluster small with less number of cells and higher number of histopathological alterations

Results reference area: Dense tissue, cluster of HC surrounding blood vessels, less alterations
Results: Significantly more alterations at dumping site B13 compared to reference site B09 (Kruskal-Wallis-ANOVA-Test, p<0.05, n=30).
**Results:** significant differences in geno-/cytotoxicity levels between areas and sampling years; indication of elevated levels in CWA areas (B13, B14, B15) compared to the reference area (B09)
Enzymatic biomarkers in cod: oxidative stress, neurotoxicity

**Results:** Some significant differences in GR activity between dumpsite (B13, B13 dump) and reference area (B09); no differences in GST, CAT and AChE (data from Dec. 2011)
Fish Health Index A integrates 5 health parameters with 3 grades each:

a) Condition factor  
b) Liver somatic index  
c) External diseases  
d) Liver histopathology  
e) Lymphocyte ratio

\[
{\text{FHI}} = (a_{1,2,3} + b_{1,2,3} + c_{1,2,3} + d_{1,2,3} + e_{1,2,3}) \times 5^{-1}
\]

FHI range: 1 (good) – 3 (bad)

Results: some significant differences in FHI-A values between cod from the reference area (B09) and other areas. However, no indication that health of cod from CWA areas (B13, B14, B15) is significantly affected.
Single health parameters measured in cod reveal differences between CWA dumpsites and reference areas.

Responses were stronger on tissue and cell level and less pronounced on individual or population level (early warning?)

However, when combining all health parameters into a simple Fish Health Index (FHI-A/FHI-B), the results do not indicate major differences in health status of cod from CWA dumpsites compared to cod from reference areas.

A possible reason for the inter-annual variability could be changes in hydrographic conditions between 2011 and 2012 (influx) that may have affected the general health status or the vertical distribution of cod.
Hydrography: $O_2$ concentrations in the Bornholm Basin

Oxygen deficiency in Oct./Nov. 2011

But: Oxygen increase in Feb. 2012

Results: strong variations (seasonal/annual) in hydrographic conditions (e.g. $O_2$ concentrations in deep water layers), largely depending on water influx from the west
**Vertical distribution of cod: impact of T, S and O\textsubscript{2}**

**Results:** Cod in the Bornholm Basin prefer salinities of >11 PSU and oxygen concentrations of > 1 ml/L. However, they can be found even in deep water layers < 1 ml/L O\textsubscript{2} (e.g. June 2007 and 2008); if O\textsubscript{2} concentrations are higher, fish aggregate at the bottom (e.g. April 2008).
Health status of cod in CWA areas: Conclusions (2)

- There is evidence that cod prefers deep water layers even at low $O_2$ concentrations and may, thus, get in direct contact with dumped munitions and CWA, increasing the risk of adverse health effects.

- However, due to the inconsistent pattern and the marked inter-annual variability of responses measured so far, there is no clear picture yet regarding the extent of such effect.

- Data on more parameters (biological and chemical) and over a longer period of time are required for a more conclusive assessment of acute and chronic CWA effects.

- In a variable environment like the Baltic Sea, it cannot be excluded that health risks posed by CWA vary on the short and the long term and, thus, regular monitoring and assessment of CWA risks should be considered.
Thanks for your attention and support!