DAIMON – EcoTox Toolbox (Kurz)

Entscheidungshilfe zur Beurteilung der Auswirkungen versenkter Munition in der Ostsee

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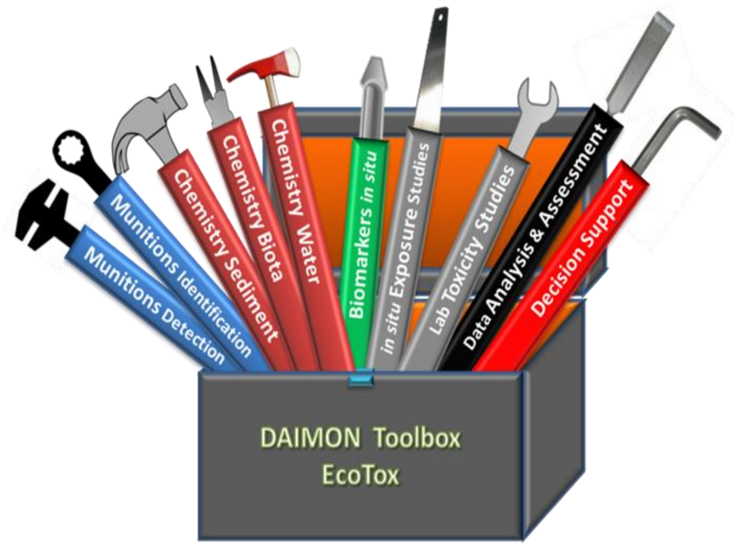
Image by JPI Oceans ©FFI

EcoTox Toolbox, was ist das?



Eine Box voller Tools = Methoden

- Munition detektieren und identifizieren
- Analyse von relevanten Substanzen
- Analyse von biologischen Effekten
- Datenanalyse und Bewertung
- Entscheidungshilfe

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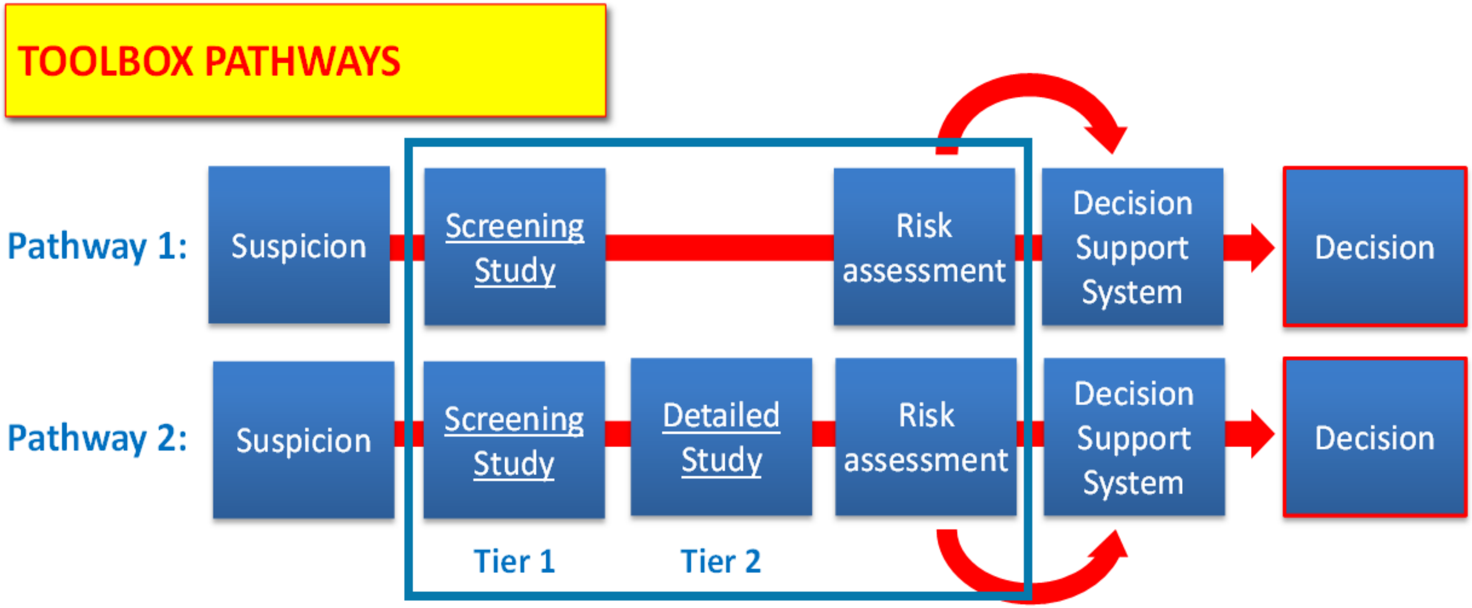
Zweistufiges Konzept

Auf ein Screening (Schritt 1) folgt eine detaillierten Studie (Schritt 2) soweit notwendig.



Wer wendet die Toolbox an?

- forschendes/überwachendes Labor (**ANWENDER**)

  „Ich bin neu in dem Thema und suche nach erprobten Methoden und Konzepten, auf die ich mich berufen kann."

- verantwortliche Behörde, Organisation, Politiker (**ENTSCHEIDER**)

  „Ich möchte meine nachgeordnete Behörde anweisen, ein STV-Monitoring durchzuführen und brauche eine Grundlage, an der man sich bei diesem Auftrag orientieren kann."

- **EXPERTIN**, die „nur" einzelne Methoden oder „nur" die Strategie aus der Toolbox nutzen möchten.

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Was ist drin in der Toolbox?

- **Strategie** – wie setze ich die Methoden ein und wie gehe ich mit den Ergebnissen um?
- **Fact Sheets** – 40 einzelne Methoden

Fact sheets auf www.thuenen.de

Fischereiökologie

- **Aktuelles und Service**
- **Arbeitsbereiche**
  - Meeresumwelt
    - Leitstelle Umweltradioaktivität in Fisch
    - Chemische Spurenenanalytik
    - MSRL und Integrierte Bewertung
    - Fischkrankheiten
    - Meeresmüll
    - Munition im Meer
      - DAIMON Ecotox Toolbox
  - Biodiversität und Wanderfische
  - Aquakultur
- **Projekte**
- **Publikationen**
- **Personal**
- **Geräte und Ausstattung**

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DAIMON Toolbox Fact Sheet Table

ALL Fact Sheets

1: Munitions detection and identification

1.1: Munition detection procedure with a hydroacoustic and magnetometry equipment

1.2: Identification and visual inspection of detected munitions-like objects

1.3: Munitions identification via Neutron Activation Analysis (NAA)

2: Hazardous substances

2.1: Chemical analysis of CWA-related compounds in sediment with LC-MS/MS

2.2: Chemical analysis of CWAs and degradation products in sediment with GC-MS/MS

2.3: Chemical analysis of conventional munitions in sediment with GC-MS/MS

2.4: Chemical analysis of CWA-related compounds in pore water with LC-MS/MS

2.5: Chemical analysis of CWA-related phenylarsenic chemicals in bile

2.6: Chemical analysis of CWA-related phenylarsenic chemicals in cut fillet

2.7: Chemical analysis of CWA-related phenylarsenic chemicals in fish liver

2.8: Chemical analysis of CWA-related phenylarsenic chemicals in fish gills

2.9: Chemical analysis of CWA-related phenylarsenic chemicals in mussel soft tissue

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2.9: Chemical analysis of CWA-related phenylarsenic chemicals in mussel soft tissue

2.10: Extraction of explosives and metabolites from fish bile

2.11: Analysis of explosives and metabolites via HPLC-QQQ-MS

3: Biological effects

3.1: Sampling of wild fish

3.3: Homogenisation of fish liver and mussel digestive gland tissues

3.4: Homogenisation of fish muscle and mussel gill tissues

3.5: Fulton's Condition Factor (CF) in Fish

3.6: Condition Index (CI)

3.7: Hepatosomatic Index (HSI) in Fish

3.8: Glycogen – accumulation of primary energy reserve in mussels

3.9: Hematology – blood glucose level

3.10: Lipid peroxidation

3.11: Superoxide dismutase activity

3.12: Catalase activity

3.13: Glutathione peroxidase activity

3.14: Glutathione reductase

3.15: Glutathione S-transferase activity

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Fact sheet 3.17

DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category: Biological Effects

Toolbox components: Disease/Pathology, Carcinogenicity

Fact Sheet 3.17: Fish liver histopathology (LH)

Authors: Thomas Lang and Katharina Straumer, Thünen Institute of Fisheries Ecology

What is it?

Studies on fish liver histopathology have frequently been applied to identify effects of contaminants at the cellular and tissue level (Malins at al. 1985 a,b, Hinton and Lauren 1990, Vethaak and ap Rheinallt 1992, Bucke and Feist 1993, Hinton 1994, Vethaak and Wester 1996, ICES 1997, Myers et al. 1998 a,b, Stehr et al. 1998, Lang 2002, Stentiford et al. 2003, Feist et al. 2004, Lang et al. 2006, Fricke et al. 2012, Faber 2014, Lang et al. 2017). Since the liver is the main metabolic organ and is involved in detoxification of environmental contaminants, it is particularly suitable as target organ for histopathological studies.

Liver histopathology is amongst the techniques recommended for monitoring biological effects of contaminants (Feist et al. 2004, OSPAR 2007, Davies and Vethaak 2012) and studies are carried out under national and international monitoring programmes (EU Marine Strategy Framework Directive, OSPAR Coordinated Environmental Monitoring Programme, HELCOM Baltic Sea monitoring).

Technical guidelines and quality assurance procedures for studying fish liver histopathology in the context of monitoring have been published and implemented, largely through activities of the International Council for the Exploration of the Sea (ICES) (ICES 1997, Feist et al. 2004). An international quality assurance programme (BEQUALM) has been established that addresses, amongst other biological effects techniques, also liver histopathology (www.bequalm.org).

What does it tell you?

The occurrence of fish liver pathology is considered as an indicator of habitat quality and environmental health, reflecting the impact of stressors, including hazardous substances, on fish health. (Vethaak & ap Rheinallt 1992, ICES 1997, Lang 2002, Feist et al. 2004, Lang et al. 2017).

Some of the known and well-documented liver pathologies are regarded as contaminant-specific indicators. For instance, the occurrence of neoplastic liver lesions (liver tumours and their pre-stages) are a well-documented indicator of exposure to carcinogenic contaminants. Other types of liver lesions are non-specific (e.g., inflammatory lesions) and reflect general stress conditions if they occur at a prevalence higher than normal.

projects CHEMSEA and DAIMON. In particular neoplastic pathologies are a suitable indicator to identify and assess effects of carcinogenic munitions compounds. However, because the indicator may also respond to non-munitions hazardous compounds, it is not recommended to use it in isolation, but in concert with other biological effects indicators and chemical measurements.

Type of Indicator (tick box)

☐ non-specific stress indicator
☒ specific for groups of contaminants incl. CWA or explosives
☐ CWA-specific indicator
☐ specific for substances related to explosives (e.g. TNT)

How to measure it?

Methods for fish disease surveys, including studies on liver histopathology, have largely been developed and repeatedly intercalibrated through ICES activities and through the fish disease component of the BEQUALM programme (www.bequalm.org) (ICES 1997, Feist et al. 2004, Lang et al. 2017).

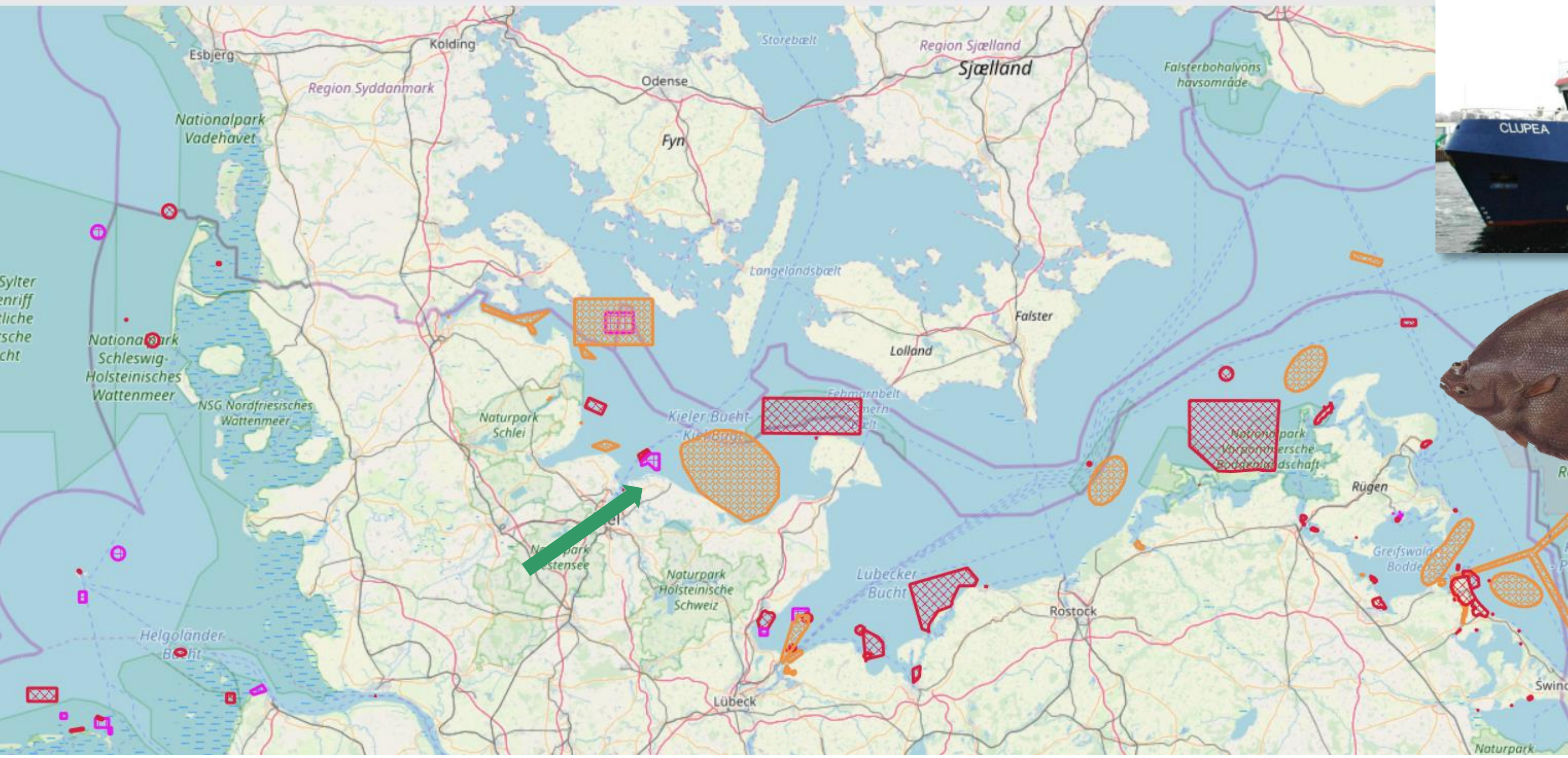
Technical guidelines for measuring liver histopathology as part of biological effects monitoring are available from ICES publications and from the Coordinated Environmental Monitoring Programme (CEMP) and Joint Assessment and Monitoring Programme (JAMP) of the OSPAR Commission (Feist et al. 2004, OSPAR Commission 2007, Davies and Vethaak 2012). These standardised methods are applied routinely by countries bordering the Baltic Sea and North Sea as well as adjacent areas.

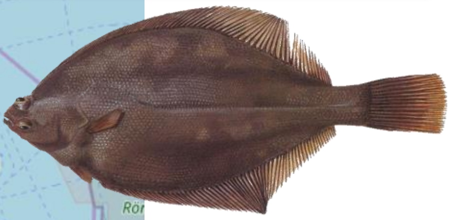
The method consists of sampling of liver tissue from a defined number of fish (e.g., 30-50 specimens per sampling area, Feist et al. 2004), fixation in 10 % neutral buffered formalin, histological processing (embedding, cutting, staining etc.) and microscopic analysis. Detailed information addressing all relevant methods is provided by Feist et al. (2004). This includes guidelines for lesion diagnosis (diagnostic key) and a categorisation of lesions according to the groups listed in Tab. 1.

General sampling requirements for fish liver histopathology are identical with those detailed in Fact Sheet 3.16 for externally visible fish diseases and Fact Sheet 3.25 for macroscopic liver neoplasms.

Species: Methodologies and diagnostic criteria involved in the monitoring of liver histopathology and for macroscopic liver neoplasms have largely been developed based on studies with flatfish species, in Europe mainly the flatfish species common dab (*Limanda limanda*) and European flounder (*Platichthys flesus*), but can also be adapted to other flatfish species, e.g., plaice (*Pleuronectes platessa*), and also to bottom-dwelling roundfish species, such as cod (*Gadus morhua*) (Faber 2014) or eelpout (*Zoarces viviparus*) (Fricke et al. 2012). In North American monitoring programmes, flatfish species such as winter flounder (*Pleuronectes americanus*), English sole (*Pleuronectes vetulus*), starry flounder (*Platichthys stellatus*), and rock sole (*Lepidopsetta bilineata*) have been widely used for biological effects studies due to their susceptibility to contaminants and their propensity to develop toxicopathic liver lesions (Myers *et al.* 1998b).

Fallbeispiele – Kolberger Heide





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Fallbeispiele – Anwendung der EcoTox Toolbox

Frage: Geht in der **Kolberger Heide** Gefahr von versenkter Munition für das Ökosystem (insb. Fische) aus?

- Ist in dem Gebiet **Munition vorhanden** (chemisch, konventionell)?
- Lassen sich Komponenten der Munition **chemisch nachweisen**?
- Zeigen **biologische Tests eine Wirkung** auf das Ökosystem an?

Ausgewählte Fact Sheets:

3.1: Sampling of wild fish
2.10: Extraction of explosives and metabolites from fish bile
2.11: Analysis of explosives and metabolites via HPLC-QQQ-MS
3.16: Externally visible fish diseases (EVFD)
3.17: Fish liver histopathology
3.26: Micronucleus Assay

Fallbeispiel 1

Kolberger Heide	ja	nein	Methode	Fact Sheet
Munition vorhanden? (konventionell)	x		Sonar, ROV, Kamera, Dokumente, Amucad.org	1.1.-1.3
Chemisches Screening Fisch	x		TNT-Metaboliten in Fischgalle	2.10, 2.11
Fisch in situ Biomarker 1		x	Äußerlich sichtbare Fischkrankheiten	3.16
Fisch in situ Biomarker 2	x		Lebertumore in Fisch	3.25
Fisch in situ Biomarker 3	x		Micronucleus Assay	3.26

ERGEBNIS: Versenkte konventionelle Munition ist vorhanden und TNT-Metaboliten konnten im Fisch nachgewiesen werden. Zwei von drei Biomarkern zeigen Effekte an. => Fisch ist vermutlich durch Munition negativ beeinflusst.

Fazit

- Mit der Toolbox hat man eine **einfache und effiziente Strategie**, um ein Meeresgebiet zu bewerten.
- Die Methodensammlung (**Fact Sheets**) umfasst zahlreiche anwendungsbereite Methoden die empfohlen und erprobt sind durch die DAIMON-Gruppe.
- Die Toolbox zeigt, dass **chemische und biologische Untersuchungen Hand in Hand** gehen müssen.
- Die Ergebnisse aus der Toolbox können ins **DSS** einfließen.
- Wir haben alle Voraussetzungen für ein **Monitoring von STV / Explosivstoffen im Meer.**

Download

DAIMON Ecotox Toolbox Text mit der Strategie

DAIMON Ecotox Toolbox Fact Sheets mit den Methoden
www.thuenen.de/de/fi/arbeitsbereiche/meeresumwelt/munition-im-meer/daimon-ecotox-toolbox/

**DAIMON SOPs - **Weiterentwicklung der Fact Sheets
https://www.daimonproject.com/standard-operation-procedures-sops.html

www.daimonproject.com