



DAIMON Work Package 4: Management strategies for marine

munitions

Lead: Alfred-Wegener-Institute Polar and Marine Research (AWI)

Activity 4.4: Safety risk for humans

Lead: Thünen Institute of Fisheries Ecology (TI-FI)

DAIMON Output 4.4.3: List of disease symptoms in fish, which should be checked for contaminants related to dumped chemical or conventional munitions

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Summary

In the framework of the Baltic Sea projects CHEMSEA, MODUM and DAIMON, carried out in the period 2011-2018, the health status of wild marine fish species collected at dumpsites of chemical munitions/warfare agents (Atlantic cod, *Gadus morhua*) and at a dumpsite of conventional munitions (common dab, *Limanda limanda*) in the Baltic Sea was studied and compared to fish from reference sites considered to be free of dumped munitions. The results revealed the presence of a range of well-known externally and internally visible infectious and non-infectious diseases/pathomorphological changes as well as parasites in fish from the dumpsites and the reference areas, and these conditions are described in the present report.

Besides providing information on diseases/pathomorphological changes recorded, the present report addresses the questions (a) if the diseases and their symptoms identified can be used during routine veterinarian fish quality inspection as indicators of exposure to toxic compounds leaking from dumped munitions and (b) if such findings would warrant a subsequent targeted chemical analysis of toxic munitions compounds in fish in order to minimize potential consumer risks for food safety. The following conclusions are drawn:

- Diseases/pathomorphological changes and parasites recorded cod and dab from the munitions dumpsites and reference areas of the in Baltic Sea are well-known and are not regarded as specific indicators of exposure to or effects of munitions compounds.
- None of the externally visible diseases/pathomorphological or parasites changes occurred at a statistically significant elevated prevalence in the dumpsites compared to the reference sites.
- However, high levels of liver tumors were observed in dab from the dumpsite for conventional munitions in Kiel Bight.
- The approach to screen fish during official controls of competent authorities and own checks
 of food business operators at landing sites for certain disease conditions and to initiate
 subsequent chemical analysis of munitions compounds in fish with symptoms is not
 considered as feasible because of the non-specificity of the diseases/pathomorphological
 changes identified and practical constraints associated with standard official controls of
 competent authorities and own checks of food business operators.

Introduction

Fish disease studies in the Baltic Sea have a long tradition as part of marine environmental monitoring and assessment (Lang 2002, Lang et al. 2017b). Therefore, background data on disease conditions, prevalence and their spatial and temporal variations are available and can be utilized for comparative purposes. The purpose of the present report is to summarise the findings of the projects CHEMSEA¹, MODUM² and DAIMON³ regarding the examination of wild marine fish from dumpsites of both chemical munitions/warfare agents and conventional munitions and from reference sites known or considered to be free of dumped munitions for the presence of gross external and internal disease symptoms. As such, the work done in these projects was the first attempt to study the link between dumped chemical and conventional munitions and fish health over a longer period and in different geographical areas of the Baltic Sea.

Apart from these studies, only few others addressing wild fish health in munitions dumping areas had been conducted. Della Torre et al. (2010, 2013) studied, besides other endpoints, the occurrence of externally visible disease symptoms as well as histopathological changes in liver and spleen tissue of Conger eel (*Conger conger*) and Blackbelly rosefish (*Helicolenus dactylopterus*) from a CWA dumpsite in the Adriatic Sea. The authors recorded gross epidermal and dermal lesions (skin ulcers, ulcerated skin papules) as well as degenerative, inflammatory and proliferative histopathological lesions in skin, liver and spleen in fish from the dumpsite and discussed a possible link of the lesions to exposure of the fish to Yperite or arsenic CWA.

CHEMSEA, MODUM and DAIMON studies in dumpsites of chemical munitions/warfare agents located in the deep basins of the Baltic Sea focused on the Baltic cod (*Gadus morhua*) because of its

³ DAIMON: Decision Aid for Marine Munitions, 2016-2019 (BSR Programme, Interreg) www.daimonproject.com

¹ CHEMSEA: Chemical Munitions Search & Assessment, 2011-2013 (BSR Programme, Interreg)

² MODUM: Towards the Monitoring of Dumped Munitions Threat, 2013-2016 (NATO SfP)

ecological and economical relevance (Lang et al. 2017b). DAIMON studies on effects of dumped conventional munitions were carried out in a shallow coastal area (Kolberger Heide) located in Kiel Bight, western Baltic Sea, with the common dab (*Limanda limanda*) as target species. Dab is the most abundant flatfish species in the area, it is relatively stationary, and its diseases are well-known from long-term fish disease monitoring (Lang 2002, Lang et al. 2017a).

The aims were not only to generate data on the prevalence of disease symptoms/pathomorphological changes in the dumpsites and the reference areas, but also to study fish from the dumpsites for the occurrence of specific disease symptoms directly linked to substances originating from munitions that can, thus, be used as indicators of exposure to and effects of toxic munitions compounds, for instance during official controls of competent authorities and own checks of food business operators in landed and marketed fish.

Methods applied

During the CHEMSEA, MODUM and DAIMON projects, fish from dumpsites of chemical and of conventional munitions and from reference sites considered to be free of dumped munitions in the Baltic Sea were examined for grossly visible external and internal disease symptoms (including parasites) (for details see Lang et al. 2017b).

In total, 20 research cruises were carried out onboard the research vessels Walther Herwig III (2011-2018) and Clupea (2015-2018). The cruises with RV Walther Herwig III largely focused on studies in CWA dumpsites, while cruises with RV Clupea focused on studies in a conventional munitions dumpsite (Kolberger Heide, Kiel Bight).

Methods applied during external disease examination and diagnosis were largely based on internationally standardized methods for fish disease surveys developed by the International Council for the Exploration of the Sea (ICES) and through the BEQUALM programme (<u>www.bequalm.org</u>) for marine environmental monitoring programmes (Bucke et al. 1996, Lang and Mellergaard 1999, Lang 2002). Also studies on liver histopathology were carried out according to established guidelines (Feist et al. 2014, www.bequalm.org).

Results

Tables 1 and 2 provide information on the common grossly visible diseases/pathomorphological changes and parasites in cod and dab, respectively, recorded during the projects CHEMSEA, MODUM and DAIMON, as well as on their causes and average prevalence recorded.

Diseases/pathomorphological changes and parasites in Baltic cod (*Gadus morhua*)

Common externally visible diseases/pathomorphological changes and parasites of Baltic cod are skin ulcerations (haemorrhagic, acute, chronic, healing, healed stages), fin rot/erosion (acute, healing, healed stages), epidermal hyperplasia/ papilloma, skeletal deformities (lordosis/scoliosis, vertebral compression, pugheadedness) and pseudobranchial swelling (pseudotumours, X-cell disease). Gross **www.daimonproject.com 3**

externally visible parasites are *Cryptocotyle lingua*, *Lernaeocera branchialis* and *Loma morhua* (see Tab. 1 and Plate I). The most conspicuous internal parasites are nematode larvae in the body cavity/on the liver belonging to the family Anisakidae. The causes of the diseases and the average prevalence of these disease and parasites as recorded during the above-mentioned projects in the years 2011-2018 are shown in Tab. 1.

It has to be noted that especially for skin ulcerations and skeletal deformities, long-term data from the Baltic Sea show that there has been marked spatial and temporal variation in prevalence. For instance, in the 1990s, an exceptionally high prevalence of around 25 % was recorded in the western Baltic Sea which decreased thereafter to lower levels. The same was true for skeletal deformities, for which a considerably higher prevalence of around 15 % was observed in certain areas in the 1990s (Lang, unpublished data). Reasons have not been resolved.

The results reveal that there were no major differences in the prevalence of the diseases/pathomorphological changes and parasites affecting cod between the CWA dumpsites and the reference sites (Tab. 1) (Lang 2017b). Also for liver histopathology, there was no indication of effects in the CWA dumpsites compared to the reference sites (Faber 2014).

Diseases/pathomorphological changes and parasites and parasites in Baltic dab (*Limanda limanda*)

Tab. 2 and Plate II show the major gross diseases/pathomorphological changes and parasites of dab from the western Baltic Sea (Kiel Bight) recorded in the course of the DAIMON project in the period 2016-2018. Common and well-known externally visible diseases of dab are lymphocystis, epidermal hyperplasia/papilloma, skin ulcerations (acute, healing, healed stages), fin rot/erosion (acute, healing, healed stages), skeletal deformities (lordosis/scoliosis, vertebral compression, pugheadedness) and X-cell gill diseases. The most common gross parasite of dab from the western Baltic Sea is *Cryptocotyle lingua*, whilst *Stephanostomum baccatum*, *Lepephtheirus pectoralis* and *Acanthochondria cornuta* occur, but are more rare. With a few exceptions, the prevalence of the diseases and parasitic infestations are generally low in the Baltic Sea.

The most conspicuous internal disease recorded was liver anomalies, mainly consisting of liver nodules of different sizes. According to standard guidelines for fish disease surveys (Bucke et al. 1996, Feist et al. 2014), all liver nodules >2 mm are quantified, fixed and subjected to histological confirmation. The results reveal that a high percentage of fish collected directly outside the conventional munitions dumpsite Kolberger Heide (Kiel Bight) was affected by liver tumours (benign and malignant) and their precursor stages (foci of cellular alteration, FCA). The prevalence was significantly higher compared to dab with identical demographic characteristics from clean reference sites in the same geographical regions (western Baltic Sea) (Straumer et al., in preparation). It can, thus, not be excluded that agents emitted from conventional munitions may be causally involved in the pathogenesis. As a matter of facts, TNT and some of its metabolites as well as RDX are regarded as potential carcinogenic toxicants (Furedi et al. 1984, Parker et al. 2006, Smets et al. 2007, EPA 2014, 2018), and these substances were found in different compartments (water, sediment, benthic biota and fish) in the dumpsite Kolberger Heide (UDEMM, unpublished data, Goldenstein et al., in preparation).

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For the externally visible diseases/pathomorphological changes and parasites, there were no differences between fish from the dumpsite of conventional munitions and the reference sites in Kiel Bight (Straumer et al., in prep.).

Table 1: Common diseases, pathomorphological changes and parasites of cod (*Gadus morhua*) in the Baltic Sea recorded during the projects CHEMSEA, MODUM and DAIMON 2011-2018 (Lang *et al.* 2017b modified)

Disease/pathomorphological changes and parasites	Type of disease/ aetiological agent	Average prevalence over all sampling areas
Skin ulcerations (acute/healing stages)	Bacterial	5 %
Skin ulcer pre-stages (haemorrhagic ulcers)*	Bacterial	4 %
Epidermal hyperplasia/ papilloma	Viral (likely)	2 %
Fin rot/erosion (acute/healing stages)	Bacterial	3 %
Skeletal deformities (lordosis, scoliosis, pugheadedness)	Multifactorial	2 %
Pseudobranchial swelling/pseudotumour (X- cell disease)	Amoeba-like parasite (likely)	1 %
Cryptocotyle lingua	Parasitic trematode (Digenea) (metacercariae)	15 %
Lernaeocera branchialis	Parasitic copepod	2 %
Loma morhua*	Parasitic microsporidia	>63 %
Nematode larvae in the body cavity*	Parasitic nematode (Anisakidae)	34 %

*: Included because often monitored in combination with externally visible diseases

Table 2: Common diseases, pathomorphological changes and parasites of dab (*Limanda limanda*) in the Baltic Sea recorded during the DAIMON project 2016-2018

Diseases/pathomorphological changes and parasites	Type of disease/ aetiological agent	Average prevalence over all areas
Lymphocystis	Viral	11 %
Epidermal hyperplasia/ papilloma	Viral	2 %
Skin ulcerations (acute/healing stages)	Bacterial	2 %
Fin rot/erosion (acute/healing stages)	Bacterial	<1 %
Hyperpigmentation	Unknown	<1 %
Skeletal deformities (lordosis, scoliosis, pugheadedness)	Multifactorial	<1 %
X-cell gill disease	Amoeba-like parasite (likely)	<1 %
Stephanostomum baccatum	Parasitic trematode (Digenea) (metacercariae)	<1 %
Cryptocotyle lingua	Parasitic trematode (Digenea) (metacercariae)	47 %*
Lepeophtheirus pectoralis	Parasitic copepod	<1 %
Acanthochondria cornuta	Parasitic copepod	<1 %
Macroscopic liver neoplasms >2 mm	Carcinogenic contaminants	9 %

*: Only quantified in Kiel Bight

Food Safety

Responsibilities

According to EU Regulation (EC) 178/2002, the food business operators have primary legal responsibility for ensuring food safety. If a food business operator considers or has reason to believe that a food which he/she has imported, produced, processed, manufactured or distributed is not in compliance with the food safety requirements, he/she shall immediately initiate procedures to withdraw the food in question from the market where the food has left the immediate control of that initial food business operator and inform the competent authorities thereof. Where the product may have reached the consumer, the operator has effectively and accurately to inform the consumers of the reason for its withdrawal, and if necessary, the operator has to recall products

already supplied to the consumer when other measures are not sufficient to achieve a high level of health protection.

The official food control authorities of the EU Member States enforce food law and monitor and verify the relevant requirements of food law fulfilled by food business operators at all stages of production, processing and distribution. For that purpose, they maintain a system of official controls and other activities as appropriate to the circumstances, including public communication on food safety and risk, food safety surveillance and other monitoring activities covering all stages of production, processing and distribution. Member States also lay down the rules on measures and penalties applicable to infringements of food and feed law.

Food controls

Wild fish species intended for the food chain have to be safe for human consumption and have to be conform to freshness criteria. According to Food Regulation (EC) No 853/2004 of the European Parliament and of the Council, the provisions of specific hygiene rules to fishery products (Section VIII) have to be mandatorily fulfilled. These requirements include the primary production (fishing) of live fishery products with a view to being placed on the market and to the associated operations on board of fishing vessels, including slaughter, bleeding, gutting, removing fins, refrigeration and wrapping, and in addition with a view to the transport and storage of fishery products.

Special emphasis is given to requirements concerning parasites: Food business operators must ensure that fishery products have been subjected to a visual examination for the purpose of detecting visible parasites before being placed on the market. Fishery products that are obviously contaminated with parasites must not be placed on the market. Furthermore, food business operators placing fishery products on the market that intended to be consumed raw or marinated, salted and any other treated fishery products, if the treatment is insufficient to kill the viable parasite, must ensure that the raw material or finished product undergo a freezing treatment in order to kill viable parasites that may be a risk to the health of the consumer. For parasites other than trematodes, the freezing treatment must consist of lowering the temperature in all parts of the product to at least minus 20 °C for not less than 24 hours or minus 35 °C for not less than 15 hours.

Food business operators must ensure, depending on the nature of the product or the fish species, that fishery products placed on the market for human consumption meet the food safety standards for fishery products. Besides microbiological criteria these are organoleptic properties to ensure that fishery products comply with freshness criteria, limits with regards to histamine and to total volatile nitrogen. Furthermore, fishery products that may harbor risks for toxins being harmful to human health must not be placed on the market.

In addition, further requirements given in special regulations have to be considered. For this reason, the maximum levels of certain contaminants in fishery products, such as lead, mercury, cadmium, Dioxins and PCBs, polycyclic aromatic hydrocarbons, have to be controlled to ensure high levels of public health. They are laid down in Regulation (EC) No 1881/2006. National monitoring programs for inorganic and organic contaminants and residues in fishery products and other foodstuffs have been established by food control authorities for several years.

At present, there are no additional investigations in fishery products in place with respect to contaminants related to dumped chemical or conventional munitions in the Baltic Sea. If there are evident risks of an ongoing corrosion process of munitions in defined regions of the Baltic Sea or elsewhere, additional substances such as RDX and TNT should be taken into account of the established monitoring programs and specific studies of fishery products, respectively. In such cases, the outcome of a finalized monitoring programme of dumped chemical or conventional munitions residues in bivalve molluscs or other suitable fish or shellfish species located in production areas of the North Sea could provide helpful information on the contamination levels.

Such bivalve molluscs or mussels have been successfully used as biomonitoring systems since several years for different compounds or contaminants. Also for the detection of TNT and its metabolites, bivalve molluscs had been used. Typical metabolites of the TNT are the compounds 1,3-Dinitrobenzene, 2,4-Dinitrotoluene, 2,6-Dinitrotoluene, 4-Amino-2,6-Dinitrotoluene and 2, Amino-6-4-Dinitrotoluenen which can occur caused by biological degradation in the environment. By filtration high amounts of water, these compounds can be accumulated in the mussels. The chemical analysis of the mussels enables the detection and quantification of the contaminants. Considering the time of exposure, this form of biomonitoring gives information on enrichment rates of the contaminants in biological tissue. One big advantage of the biomonitoring with mussels is that it can easily be adapted to temporal and spatial demands, e.g. to get information on long- and short-term trends or to check special locations.

Fish disease controls

Compared to fish raised in aquaculture for human consumption, there are no legal requirements for veterinary controls of living wild-caught fish species concerning fish diseases and its causing agents. In the first instance, fish with abnormalities like skin ulcerations or deformations for example are rejected before being placed on the market because of the fact that they are unsuitable for human consumption. In determining whether any food is unsafe, regard shall be made to the intended use of the food and at each stage of production, processing and distribution, and to the information provided to the consumer, including information on the label, or other information generally available to the consumer concerning the avoidance of specific adverse health effects from a particular food or category of foods.

Discussion

The diseases, pathomorphological changes and parasites recorded in cod and dab in the course of the CHEMSEA, MODUM and DAIMON projects are well known and their occurrence has repeatedly been reported before (Dethlefsen and Watermann 1982, Draganik et al. 1994, Kosior et al. 1997, Mellergaard and Lang 1999, Lang 2002, ICES 2006, Weirup 2015, Lang et al. 2017a,b). Their presence is generally regarded as a natural phenomenon, as long as the disease prevalence does not markedly exceed natural background conditions. However, if the disease prevalence is increased above natural background levels, this is regarded as an indication of the impact of environmental stressors on fish health. Anthropogenic hazardous substances are amongst the environmental stressors wild fish in the Baltic Sea are faced with and may, thus, affect fish health, either by causing toxic effects leading to disease or by causing stress leading to suppression of the natural immune mechanisms against diseases. This may ultimately result in a significant increase in disease prevalence (Lang 2002).

For none of the diseases, pathomorphological changes and parasites identified in cod and dab in the above-mentioned projects and reported herein, a direct and specific cause-effect relationship to chemical warfare agents or substances originating from conventional munitions has been documented so far (Lang et al. 2017b). As a matter of fact, it appears questionable that such a link exists, because the diseases have repeatedly been found in the same fish species, as well as in other species, from various geographical regions in and outside the Baltic Sea known to be free of dumped munitions. However, we cannot exclude the possibility that chemical warfare agents and/or substances leaking from conventional munitions are toxic to exposed fish and may act similar to **www.daimonproject.com**

other contaminants causing diseases in fish (e.g., neoplastic pathologies may be caused by various carcinogenic substances). For instance, DAIMON data indicate that the prevalence of liver tumours in dab from the dumpsite for conventional munition in Kiel Bight (Kolberger Heide) exposed to TNT is significantly increased compared to dab from reference sites (Straumer et al., unpublished data). As described above, munitions compounds may act as environmental stressors, adversely affecting the immune system of fishes exposed and their resistance towards disease agents and may, thus, lead to a measurable increase in prevalence of diseases and/or infestation with parasites. Both pathways (toxic effects, non-specific immunosuppression) can be regarded as realistic scenarios associated with chronic or acute leakages of compounds from dumped munitions. It can, thus, be concluded that surveillance of the health status of fish from dumpsites and reference areas is a useful tool for monitoring and assessment of environmental risks posed by dumped munitions.

Besides providing information on diseases, pathomorphological changes and parasites recorded in wild fish from munitions dumpsites, the present report addresses the questions (a) if the diseases and their symptoms found in the projects can be used during routine food inspection of offical controls of competent authorities and own checks of food business operators of marketed fish as indicators of exposure of the fish to toxic compounds leaking from dumped munitions and (b) if such findings would warrant subsequent targeted chemical analysis of toxic munitions compounds in order to exclude potential consumer risks and effects on human food safety. Based on experience made and data available it is concluded that this approach is not suitable for the time-being for a number of reasons:

- So far, no gross externally visible diseases/pathomorphological changes have been identified that show a clear cause-effect relationship with munitions compounds.
- It is unlikely that fish with conspicuous disease symptoms will be found during offical controls of competent authorities and own checks of food business operators, because such fish will be discarded by fishermen at sea and will not be landed and marketed.
- Even if single fishes with disease symptoms are found on the market, the role of contaminants as aetiological agents would be unclear so that targeted chemical analysis for munitions-related compounds would not be reasonable.
- Although the DAIMON project for the first time generated data revealing the uptake of chemical compounds originating from dumped chemical and conventional munitions in wild fish, it is still not clear which of these compounds are suitable for environmental and food safety monitoring. Furthermore, analytical methods applicable in routine chemical environmental and veterinary monitoring still have to be developed and validated.

Conclusions and recommendations

In the projects CHEMSEA, MODUM and DAIMON, a number of diseases/pathomorphological changes and parasites were identified in fish from dumpsites of chemical warfare agents (cod) and dumpsites of conventional munitions (dab). However, the same conditions were also recorded in reference areas considered to be free of dumped munitions. Therefore, it is concluded that none of the disease conditions recorded can be regarded as a specific indicator of exposure or effects. Because of the non-specificity of the diseases/pathomorphological changes recorded, it can further be concluded that -currently- screening for the diseases during offical controls of competent authorities and own checks of food business operators in order to identify fish exposed to munitions compounds is not an appropriate approach to minimise consumer risks.

It appears more appropriate that the geographical origin of the fish/seafood marketed is more specifically documented for the official controls and own checks of food business operators so that fish from munitions dumpsites with known emissions of toxic compounds can easily be identified to initiate appropriate actions or –as a ultimate facility- can be excluded from the market.

Regarding the chemical analysis of toxic munitions compounds in seafood it is concluded that there is a clear need for more research aiming at a better understanding of the metabolism, uptake, accumulation and biomagnification of munitions compounds in marine organisms, with an emphasis on accumulation in the edible portion of marine organisms regarding food safety.

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Plate I: Common grossly visible diseases/pathomorphological changes and parasites of Baltic cod (*Gadus morhua*) recorded during the MODUM, CHEMSEA and DAIMON projects (Lang et al. 2017b; reprinted with permission from the publisher)

	©2004 BFAFi
Acute skin ulceration	Healing skin ulceration
	75 - 76 - 36 - 3
Healed skin ulceration	Acute fin rot/erosion
Epidermal hyperplasia/papilloma on the skin	Skeletal deformity (compression of the spine)
20 30 35 	1 2 3 4 5 6 7 8 9 11 12 13 14 15 16 11 C
Skeletal deformity (lordosis and scoliosis)	Skeletal deformity (pugheadedness)

Plate I: (cont.)

Pseudobranchial swelling (X-cell pseudotumour) in the oral cavity, upper jaw	<i>Lernaeocera branchialis</i> (Copepoda) in the gill chamber
<i>Cryptocotyle lingua</i> (Digenea, metacercaria) in the skin	Nematode larvae (Anisakidae) on the liver surface
Loma sp. (Microspora) in the gills	

Plate 2: Common grossly visible diseases/pathomorphological changes and parasites of common dab (*Limanda limanda*) recorded during the DAIMON project



Plate 2: (cont.)

