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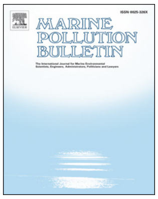

First evidence of explosives and their degradation products in dab (*Limanda limanda* L.) from a munition dumpsite in the Baltic Sea

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ABSTRACT

Corrosion and disintegration of munition shells from the World Wars increase the risk that explosives are released into the marine environment, exposing a variety of organisms. Only few studies investigated contamination of fish with explosives in the field under environmental conditions. Here we present a comprehensive study on the contamination status of dab (*Limanda limanda*) from a munition dumpsite and from reference sites in the Baltic Sea. Bile of 236 dab from four different study sites, including a dumpsite for conventional munitions, was investigated and explosive compounds were detected by high performance liquid chromatography-mass spectrometry. Five explosive compounds were identified, including 2,4,6-trinitrotoluene, 4-amino-2,6-dinitrolouene, and hexahydro-1,3,5-trinitro-1,3,5-triazine. 48% of the samples from the dumpsite contained at least one explosive compound. The results prove that toxic explosive compounds from a dumpsite in the Baltic Sea are accumulated by flatfish and may therefore pose a risk to fish health and human food safety.

1. Introduction

Recent evidence of explosive compounds (explosives including their degradation products) in blue mussels (*Mytilus edulis*) in the Baltic Sea (Strehse et al., 2017) raised the awareness of a possible contamination of marine biota living in the vicinity of dumped munitions. The compounds found in the mussels were the explosive trinitrotoluene (TNT) itself as well as 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT), known to be degradation products of TNT in different aquatic organisms including fish (Lotufo et al., 2010; Talmage et al., 1999; Yoo et al., 2006).

Large parts of the munitions in the seas, including the Baltic Sea, originate from dumping in the aftermath of World Wars I and II (Beck et al., 2018; Beddington et al., 2005), but also all kinds of military operations during and outside wartime contributed to the current contamination (Böttcher et al., 2011). Huge quantities of munitions in the Baltic Sea are located in designated dumpsites, often in coastal areas (Beck et al., 2018), where they were deliberately dumped as a common practice applied until the 1970s (Carton and Jagusiewicz, 2009).

While being exposed to seawater over the years, the munition shells are corroding and the probability of leakage is increasing (Voie and Mariussen, 2017). However, corrosion rates of the shells are depending on various factors such as temperature, salinity, oxygen level and currents (Jurczak and Fabisiak, 2017), leading to huge differences in the corrosion status of munition shells at various locations in the Baltic Sea. Where shells are broken, the explosive compounds leak into the marine environment and may be taken up by marine organisms including fish. Predominant conventional explosives are nitroaromatics, including TNT and 1,3-dinitrobenzene (DNB), as well as nitramines like hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Gledhill et al., 2019).

The toxicity of TNT to fish has been demonstrated in several studies and also the degradation products 2-ADNT and 4-ADNT are toxic, although slightly less compared to TNT itself (Koske et al., 2019; Lotufo et al., 2010; Talmage et al., 1999). Experiments on the bioaccumulation of TNT in fish showed a rapid uptake from the water column, but low bioconcentration factors, probably caused by the low octanol/water partition coefficient of 1.6 (Monteil-Rivera et al., 2009) in combination with the fast biotransformation of TNT in fish. However, the

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Abbreviations: TNT, 2,4,6-trinitrotoluene; 2-ADNT, 2-amino-4,6-dinitrolouene; 4-ADNT, 4- amino-2,6-dinitrolouene; 1,3-DNB, 1,3-dintrobenzene; TNB, 1,3,5-trinitrobenzene; HMX, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine; 2,4-DNT, 2,4-dinitrotoluene; 2,5-DNT, 2,5-dinitrotoluene; 1,4-DNB, 1,4-dinitrobenzene

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bioaccumulation of TNT including all its degradation products is much greater than the bioaccumulation of TNT itself (Belden et al., 2005; Ownby et al., 2005).

Despite the fact that explosive compounds have already been found in different marine biota (Gledhill et al., 2019; Strehse et al., 2017), these substances have not yet been identified in fish sampled from or near dumpsites. In order to provide information on the contamination status of the entire marine food chain with explosive compounds, fish are essential organisms and of particular interest for the human consumer. First approaches with experimentally exposed rainbow trout (*Oncorhynchus mykiss*) revealed that bile and blood plasma are convenient matrices to detect explosive compounds (Ek et al., 2003, 2005). Due to their benthic lifestyle (Hylland et al., 2017), flatfish such as dab (*Limanda limanda*) are particularly exposed to dumped munitions and, thus, represent a promising organism for analysing explosive compounds in fish from the Baltic Sea. This flatfish is a geographically widespread species and considered to be a relatively stationary species sensitive towards environmental stressors. It has been used as a bioindicator species in many studies on the prevalence of diseases, heavy metals (Lang et al., 2017) as well as PAH metabolites in bile fluids (Kammann, 2007) in the Baltic Sea and North Sea. Furthermore, it has been recommended and utilized as monitoring species by different working-groups of the International Council for Exploration of the Seas (ICES, 2012; Lang, 2002; Vethaak and Ap Rheinallt, 1992).

For the first time, we here investigated the contamination status of Baltic flatfish from the close vicinity of a dumpsite for conventional munition. For this purpose, dab from four different study sites in the western Baltic Sea, including a contaminated site, were caught and their bile was analysed for the presence of explosive compounds in order to improve our knowledge about the degree and spatial distribution of fish contamination originating from such dumpsites.

2. Material and methods

2.1. Chemicals

Dimethyl sulfoxide (DMSO, 99.9%), acetonitrile (99.9%), water (15 MΩ cm, HPLC grade) and methanol (MeOH, 99.9%) were obtained from Th. Geyer (Germany). Acetic acid (99%) was purchased from Merck (Germany) and ammonium acetate was obtained from Sigma Aldrich (Germany). Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrolouene (2-ADNT) and 4- amino-2,6-dinitrolouene (4-ADNT) were obtained as reference standards from AccuStandard (USA). 2,4-dinitrotoluene (2,4-DNT) and 2,5-dinitrotoluene (2,5-DNT) were purchased from Neochema (Germany). 1,4-dinitrobenzene (1,4-DNB) was obtained from Sigma-Aldrich (Germany).

2.2. Study sites

All study sites were located in the western Baltic Sea. Kolberger Heide (KH) is a 1260 ha restricted munition dumpsite, approximately 30,000 t of conventional munitions were dumped in this area (Böttcher et al., 2016; Gledhill et al., 2019). Three reference sites were used for comparison: Stoller Ground (SG) located 10 km west of KH, B01 about 25 km northeast of KH and Flensburg Firth (FF). According to the AMUCAD database (EGEOS GmbH, 2019), no actual munition contamination is documented at SG. B01 is located close to the Fehmarn Belt which was contaminated by munition as hundreds of ground mines were dropped there during the war, but attempts were made to clear this important shipping route. FF is declared as a suspicious area for munitions (Böttcher et al., 2011). The locations of the sampling sites are shown in Fig. 1; geographical coordinates are given in Table 2.

2.3. Sampling

Dab were collected during cruises No. 301 (February 2016, KH, FF), No. 311 (February 2017, KH), No. 314 (August 2017, KH, SG) and No. 326 (August 2018, KH, SG) onboard RV Clupea by gillnet fishery at the edges of KH and in FF (fishing for 5–14 h) and by bottom trawling in SG (TV-300 bottom trawl, 15–20 min towing time at 3–4 knots towing speed). Additionally, dab were collected during cruise No. 408 (September 2018, B01) onboard RV Walther Herwig III by bottom trawling (140 ft. bottom trawl, 60 min towing time at 3–4 knots towing speed) in B01.

Only alive dab were sorted from the catches and transferred to tanks containing running seawater of ambient temperature to keep them alive before examination. Fish were weighed, the total length measured and anesthetized by a blow on the head, followed by decapitation prior to the subsequent dissection. The bile was collected by puncture of the gall bladder with disposable needles (0.15 mm × 35 mm) into disposable syringes (1 mL) and transferring it into a glass vial (Agilent, Germany). The biometric data were used to determine the condition factor ($\text{CF} = \text{weight [g]} * 100/\text{length [cm]}^3$) as an indicator of the general fish health status. Otoliths were removed for subsequent age determination according to Maier (1906) and Bohl (1957).

2.4. Bile preparation

For the extraction of explosive compounds from bile samples, 25 µL of each bile was transferred into reaction tubes filled with 1 mL of ice-cold acetonitrile. Subsequently, 5 µL of the internal standard 1,4-dinitrobenzene (1,4-DNB, 10 ng/µL) was added to the dilution, and the samples were thoroughly mixed. The reaction tubes were centrifuged at 6000 rpm (4 °C, 10 min) and the supernatant was in each case transferred into a 1.5 mL amber vial. The supernatant was reduced under a stream of nitrogen to a volume of 150 µL. The remaining volume was transferred into a new 200 µL amber vial and further reduced to a final volume of 50 µL, which was used for HPLC-MS analysis. The samples were stored at −20 °C until analysis. The entire extraction procedure was carried out under minimized light to reduce the influence of photodegradation.

2.5. HPLC-MS

5 µL of the final bile extract were injected on a column in an Agilent 1290 Infinity High-Performance-Liquid-Chromatograph coupled to an AB Sciex QTrap 5500 Triple Quadrupole/Ion-Trap Mass Spectrometer (HPLC-MS). Gradient separation was achieved using an Acclaim Explosives E2 Column (Thermo Fisher Scientific) kept at 22 °C.

Ionization was conducted in negative atmospheric pressure chemical ionization (APCI) mode. Explosives and selected metabolites were detected via a multiple reaction monitoring (MRM) mode based on characteristic MS/MS transitions, previously optimized using commercially available standard substances. Characterisation of unknown compounds was achieved in scan-mode of the linear ion trap by selecting nitroaromatic compounds using a neutral loss scan targeting the loss of nitro-groups (m/z 46), followed by an enhanced product ion scan, producing a sensitive mass spectrum of the nitro-containing molecule.

Abundances of explosive compounds were quantified via the internal standard 1,4-DNB and corrected for response factors. Response factors were determined using an external calibration covering the expected range of concentrations. The method used is described in detail by Koske et al. (2019). Limits of detection (LOD) and limits of quantitation (LOQ) for explosive compounds were calculated according to DIN 32645 (2008) and are listed in Table 1.

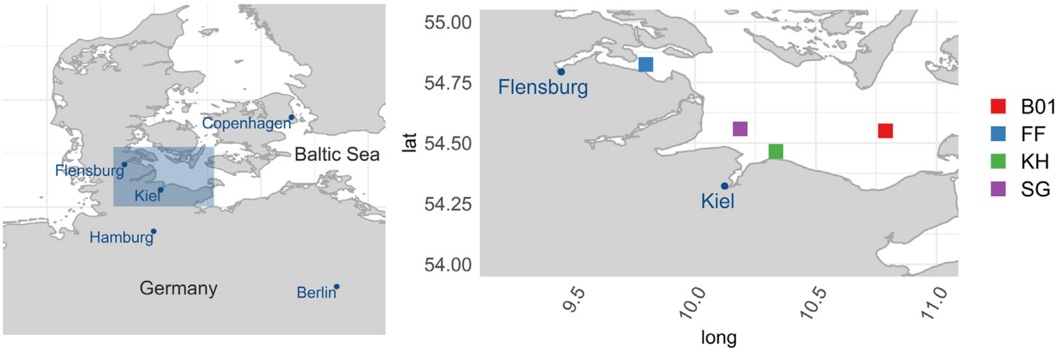

Fig. 1. Sampling sites of dab in the western Baltic Sea. Samples were taken at the Kolberger Heide dumpsite (KH) and at three different reference sites, B01, Flensburg Firth (FF) and Stoller Ground (SG) close to the German coastline.

Table 1
Detection limits (LOD) and quantification limits (LOQ) obtained for different explosive compounds, calculated according to DIN 32645 (2008). Abbreviations: HMX, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine; TNT, 2,4,6-trinitrotoluene; 2,4-DNT, 2,4-dinitrotoluene; 2,5-DNT, 2,5-dinitrotoluene; 2-ADNT, 2-amino-4,6-dinitrolouene; 4-ADNT, 4- amino-2,6-dinitrolouene.

Substance	LOD [ng/mL bile fluid]	LOQ [ng/mL bile fluid]
HMX	0.4	2.4
RDX	1.2	4.4
TNT	2	7.6
2,4-DNT	13.2	46.4
2,5-DNT	9.2	33.2
2-ADNT	3.2	12.4
4-ADNT	2.8	10.8

2.6. Statistical analysis

Statistical analyses were carried out using R Version 3.6.2 (R Core Team, 2019). The correlation between concentration of 4-ADNT in bile and the age of fish from Kolberger Heide was tested using Pearson's product moment correlation coefficient.

3. Results

A total of 236 dab were examined in this study (biometric data in Table 2) and individual bile samples were taken for chemical analysis. On average, the dab from the munition dumpsite KH were the largest and oldest fish (mean values 30.2 cm and 5.4 years, resp.), while fish from the reference site SG were the smallest and youngest (24.8 cm; 3.2 years). At all study sites, more female than male dab were caught. Dab from FF showed comparable biometric characteristics (29.1 cm; 5.1 years) as dab from KH. Dab from B01 (25.7 cm; 3.6 years) were comparable with those of SG. The mean values of the condition factor (CF) at all four study sites were in the range of 1.0 to 1.2.

Bile samples from three different reference sites in the vicinity of Kiel Bight (B01, FF, SG) and samples taken from the edges of the Kolberger Heide munition dumpsite (KH) were analysed for explosive compounds. No explosive compounds were detected in 94 samples from SG (Table 3). Seven samples from FF were analysed and one sample contained 1.24 ng/mL HMX. Of B01, 20 samples were analysed, three of them containing either 4-ADNT or HMX. 9.35 ng/mL HMX were measured in one sample, resulting in an extrapolated mean of 0.47 ng/mL per sample with a correspondingly great range, as shown in Table 3. In total, 115 bile samples were taken at KH, and HMX, RDX, TNT, 2-ADNT as well as 4-ADNT were detected (Table 3). The mean concentration of 4-ADNT (17.06 ng/mL) was considerably higher than that of 2-ADNT (1.60 ng/mL). The maximum concentration overall measured in all samples from KH was 141 ng/mL 4-ADNT. The highest single 2-ADNT concentration measured was 32.5 ng/mL bile.

When comparing the proportions of bile contamination in the two study sites with the biggest sample sizes, a clear difference between the reference site SG and the munition dumpsite KH becomes obvious, as shown in Table 3. No explosive compounds were found in bile samples from SG, or rather their concentrations were below the detection limit. In contrast, 2-ADNT was detected in 12% and 4-ADNT in 45% of the dab bile samples from KH. 2% of the samples from KH contained TNT. In summary, 55 individual samples of a total of 115 samples from KH contained at least one explosive compound, which corresponds to a contamination rate of 48%.

To test for a possible correlation between the concentration of explosive compounds in bile and the age of fish from Kolberger Heide, 4-ADNT was used as example since this compound was the most predominant found. The correlation between 4-ADNT concentration and fish age ($r = 0.06$) was poor and not significant.

A detailed look at the concentrations of the explosive compounds measured in bile from KH, shown in Fig. 2, reveals a much more pronounced contamination with 4-ADNT in contrast to 2-ADNT. Not only more samples contained 4-ADNT, but also the concentrations were considerably higher compared to 2-ADNT. In 11 samples from KH, 2-ADNT as well as 4-ADNT was detected. The TNT concentrations measured in two samples were lower than all measured concentrations of 2-

Table 2
Biometric data of dab from study sites B01, Stoller Ground (SG), Kolberger Heide (KH) and Flensburg Firth (FF). Geographical coordinates of the sampling stations are given as latitude and longitude for a point or rectangle. Total length, condition factor (CF) and age are expressed as mean values ± standard deviation.

Sampling site	Coordinates	Sex m/f	Total length [cm]	CF [g/cm^3]	Age [year]
B01 (n = 20)	54°33,04N 10°47,31E	5/15	25.7 ± 3.1	1.01 ± 0.09	3.60 ± 1.20
SG (n = 94)	54°32,87N - 54°33,52N 10°09,94E - 10°11,23E	26/68	24.8 ± 2.9	1.03 ± 0.14	3.20 ± 1.09
KH (n = 115)	54°27,28N - 54°27,97N 10°19,28E - 10°20,56E	12/103	30.2 ± 3.2	1.10 ± 0.15	5.37 ± 1.36
FF (n = 7)	54°49,47N 09°47,81E	0/7	29.1 ± 2.3	1.16 ± 0.11	5.14 ± 0.83

Table 3

Mean concentrations [ng/mL] of different explosive compounds measured in dab bile from four study sites in the vicinity of Kiel Bight: Reference sites (B01, FF, SG) and munition dumpsite (KH). The range (minimum and maximum value) is given in brackets. For LOD (limit of detection) see Table 1. Percentage of bile contaminated with explosive compounds is calculated based on the sample size per area.

Area	Sample size	HMX [ng/mL]	HMX contamination per area	RDX [ng/mL]	RDX contamination per area	TNT [ng/mL]	TNT contamination per area	4-ADNT [ng/mL]	4-ADNT contamination per area	2-ADNT [ng/mL]	2-ADNT contamination per area
B01	20	0.47 (0–9.35)	5%	< LOD	0%	< LOD	0%	0.59 (0–6.92)	10%	< LOD	0%
FF	7	0.18 (0–1.24)	14%	< LOD	0%	< LOD	0%	< LOD	0%	< LOD	0%
SG	94	< LOD	0%	< LOD	0%	< LOD	0%	< LOD	0%	< LOD	0%
KH	115	0.07 (0–1.99)	5%	0.12 (0–3.73)	5%	0.06 (0–3.87)	2%	17.06 (0–141)	45%	1.60 (0–32.5)	12%

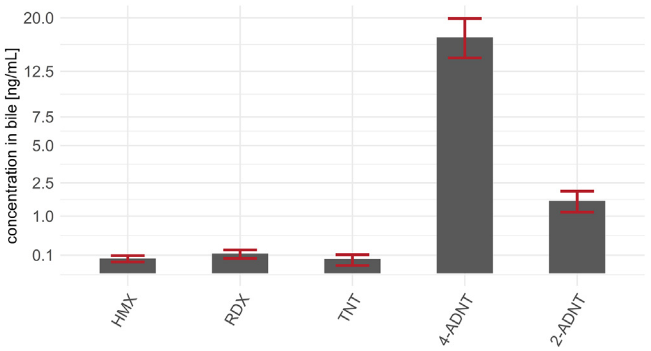

Fig. 2. Concentrations of explosive compounds (ng/mL) found in dab bile from the Kolberger Heide dumpsite (KH). Bars represent the mean concentration for each compound. Error bars (red) represent the standard error of the mean (SEM), sample size $n = 115$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ADNT and 4-ADNT.

4. Discussion

The aim of the present study was to investigate the degree of contamination with explosive compounds in fish caught near a dumpsite for conventional munition and to improve the knowledge about the spatial distribution of this contamination. Different reference sites in the surrounding area of the dumpsite should be investigated to identify a possible spread of the contamination in fish, caused, for example, by the distribution of the compounds via the water phase or by migration of the fish themselves.

In total, 55 out of 115 bile samples (48%) from dab caught at the Kolberger Heide dumpsite contained one or more explosive compounds. This high degree of contamination is in striking contrast to the low contamination level of bile samples from the three reference sites. The uptake of explosive compounds, like TNT, 2-ADNT and 4-ADNT, by marine biota at this dumpsite has already been shown in an earlier study that used blue mussels as biomonitoring system (Strehse et al., 2017). Since mussels are usually attached to solid surfaces and are filter-feeding organisms, their body burden strongly depends on the water concentrations of explosive compounds in the surrounding area. In comparison, fish caught at the edges of the dumpsite probably passed through a larger area and, therefore, integrate the munition contamination present at various points in the vicinity – this could be both inside and outside the dumpsite.

Degradation products of TNT accounted for most of the contamination in bile samples from the Kolberger Heide, as 12% 2-ADNT and 45% 4-ADNT but only 2% TNT were found in the samples. This finding raises the question of whether the degradation products were only formed in the fish or already in the water, in the sediment or in the prey before uptake. TNT degradation usually implies the reduction of the nitro groups and can take place via abiotic or biotic processes (Beck et al., 2018). Therefore, it is likely that both TNT and TNT degradation products such as 2-ADNT or 4-ADNT are present in the water column and can be taken up by fish. This was confirmed by the analysis of water samples taken from Kolberger Heide near a pile of sea mines containing several TNT degradation products, including 2-ADNT and 4-ADNT (Gledhill et al., 2019). Recently, an in-situ study determined the dissolution flux of TNT at Kolberger Heide with 0.0047–0.277 mg/cm^2/day (Beck et al., 2019), proving that explosive compounds are released into the water column and are, thus, also available for fish. These findings suggest that the dab from Kolberger Heide caught in this study were temporarily exposed directly to TNT, 2-ADNT and 4-ADNT via the surrounding water. Furthermore, the possibility of ingestion of contaminated sediment by dab should not be excluded as an additional source of exposure.

In addition, Gledhill et al. (2019) have demonstrated that macroalgae, sea stars and ascidians take up TNT and its metabolites in the KH dumpsite. The concentrations measured in these benthic organisms ranged up to 24 µg/g dry weight. Since sea stars, molluscs and crustaceans are among the most important food resources of dab (De Clerck and Torreele, 1988), it is very likely that fish will also ingest these substances via the food web. However, due to the low bioconcentration potential of TNT in fish (Ownby et al., 2005), the accumulation of explosive compounds via the food chain appears to be less decisive than direct uptake through water or sediment (Lotufo et al., 2017).

Obviously, at the reference site SG no fish was found to contain explosive compounds despite the fact that, for instance, the distance between SG and KH is only about 10 km. Usually dab tend to stay in their territory which can be demonstrated by specific fish disease prevalence patterns (Diamant and McVicar, 1990; McVicar et al., 1988). However, it is also possible that dab swim further distances (Damm et al., 1991; Temming, 1989). Therefore, it cannot be ruled out that dab leave the Kolberger Heide and this could also explain why two dab caught in the reference area B01 contained 4-ADNT. In addition, the presence of munitions from military operations in the areas B01 and FF (see Section 2.2) may also explain the explosive compounds detected in the respective samples. In summary, it is likely that dab contaminated with explosive compounds are spread in the larger vicinity of Kolberger Heide, however, the compounds can no longer be detected in the bile after a short time due to the rapid biotransformation (Belden et al., 2005).

The evidence that a prolonged exposure of fish in the direct proximity of munitions leads to a higher concentration of explosive compounds in bile has also been demonstrated in a cage experiment with dab in the Kolberger Heide (Straumer et al., unpublished data). Analysis of bile samples after three weeks of exposure of the dab in the cages in direct vicinity of dumped munitions revealed that concentrations of 2-ADNT (range 20.9–358.8 ng/mL), 4-ADNT (range 148.9–1291.6 ng/mL) and TNT (range 0.0–6.8 ng/mL) in the bile were considerably increased compared to concentrations detected in the wild dab from KH addressed in the present study (Straumer et al., unpublished data).

Since biotransformation reactions of xenobiotics in fish are mainly catalysed by enzymes of the cytochrome P450 (CYPs) superfamily (Schlenk et al., 2008), important transformation reactions of explosives are also taking place in the liver. In this respect, the degradation of TNT in fish has been demonstrated in in vivo exposure studies (Lotufo et al., 2010; Mariussen et al., 2018), and in vitro experiments with liver samples also revealed the metabolization of TNT specifically in dab (Koske et al., 2020). In addition to biotransformation in fish themselves, photolysis also plays a decisive role for the degradation of TNT in seawater (Luning Prak et al., 2017). This means that the presence of TNT degradation products cannot be narrowed down to just one source.

The accumulation of explosive compounds in fish was also investigated by Ek et al. (2006) using European flounder (*Platichtys flesus*) exposed to cleaved artillery shells for 8 weeks. Neither TNT nor its degradation products were detected in bile and blood plasma of these fish. Extensive surveys for detecting explosive compounds in biota were conducted at Vieques Island in Puerto Rico, a former naval training area, and high concentrations of TNT, 1,3-DNB, TNB and RDX in the mg/kg range were detected in tube worms, corals and sea urchins (Porter et al., 2011). Not every explosive compound was detected in each sample, but in addition TNB was detected in dusky damselfish (*Stegastes adustus*), sampled at the same site. Other fish sampled in the same area, including groupers, snappers and parrotfish, did not show detectable concentrations of explosive compounds (Porter et al., 2011). Other studies investigated contamination in various biota samples with explosive compounds in areas affected by munitions in the USA, Hawaii and Norway. Up to 10 µg/kg HMX were detected in mussels and snails (Rossland et al., 2010), high concentrations of TNB (7800 µg/kg) were detected in bent-nosed clam (*Macoma nasuta*), and 3-nitrotoluene (up to 460 µg/kg) and nitrobenzene (up to 320 µg/kg) were measured in starry flounder (*Platichthys stellatus*) at Jackson Park Housing Complex, USA (Lotufo et al., 2017). Few deep-sea shrimp (*Heterocarpus ensifer*) sampled at a sea-disposal site on Hawaii (HI-05) contained 4-ADNT (up to 45 µg/kg) (Koide et al., 2016). Goatfish samples taken from "Ordnance Reef" (HI-06), Hawaii, contained explosive compounds including TNB, 2,4-DNT, HMX and high concentrations of RDX (1600 µg/kg) (Lotufo et al., 2017; UH, 2014). These results show that marine organisms living in the vicinity of munition dumpsites accumulate explosive compounds, making the degree of contamination shown here appear realistic. However, the variations in the identified explosive compounds in biota samples between the different studies result not least from the different analytical methods (Beck et al., 2018) and are therefore difficult to compare.

An explanation for the difference in biometric data between sampling sites are the different fishing methods. Due to the selectivity of the gillnet for larger fish, which was used in KH and FF, larger and, thus, mainly older fish were caught there. As female become larger than male dab (Rijnsdorp et al., 1992), proportionally more female fish were caught in KH and FF compared to SG and B01. According to the CF, dab from KH were fitter than fish from SG and B01. However, the mean CF values from all four sampling sites were similar to the CF of dab from the North and Baltic Seas described in other studies (Htun-Han, 1978; Lang et al., 2017) and are subject to seasonal changes (Ortega-Salas, 1980; Saborowski and Buchholz, 1996). We, therefore, assume that the dab used in this study had a common CF at each study site. A correlation between the amount of explosive compounds in the bile and the age of the fish from Kolberger Heide could not be determined in the present study using the example of 4-ADNT.

The detection of explosive compounds in fish from the Baltic Sea shown here is in line with the findings of the same chemicals in mussels and other marine biota (Gledhill et al., 2019; Strehse et al., 2017) at the same site. This raises concerns about contamination of the marine food chain up to larger organisms such as fish. Furthermore, the genotoxicity of TNT and its degradation products in fish (Koske et al., 2019) poses a potential risk for long-term effects in fish living near munition dumpsites. In order to determine the amount of explosive compounds that fish may accumulate over time, measurements of liver or filet samples are recommended, as bile is excreted periodically.

5. Conclusion

We have shown the degree of contamination with explosive compounds in bile samples from wild dab caught in the close vicinity of the Kolberger Heide munition dumpsite. In comparison, bile samples from a nearby reference site, where no munitions were directly disposed, did not show any contamination associated with munitions. Therefore, we have proven that chemicals released from dumped munition are taken up by fish in the Baltic Sea. To our knowledge, this is the first study showing a broad contamination of fish caused by dumped conventional munitions in the Baltic Sea. The rapid biotransformation of explosive compounds in fish leads to a close local correlation of the results. The determination of explosive compounds in the bile of dab opens the possibility to detect the same compounds in potentially contaminated other fish species important to the human consumer. The same analytical method may also be applied to fish muscle samples after the extraction method has been adapted. Further studies on the detection of explosive compounds in fish will help to obtain an overall picture of the contamination status of fish exposed to dumped munitions and to achieve a targeted management of munition dumpsites.

CRediT authorship contribution statement

Daniel Koske: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization.**Katharina Straumer:**Conceptualization, Methodology, Validation,

Investigation, Resources, Writing - original draft, Writing - review & editing.**Nadine I. Goldenstein:**Methodology, Validation, Formal analysis, Investigation, Writing - original draft.**Reinhold Hanel:**Writing - review & editing, Supervision, Funding acquisition.**Thomas Lang:**Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.**Ulrike Kammann:**Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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