Explosives compounds from sea-dumped relic munitions accumulate in marine biota

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Highlights:

- Munition compounds were detected in >98% of organisms collected throughout the southwest Baltic Sea, at a median level of 6 pmol/g (approximately 1 ng/g)
- Tissue content of TNT, ADNT, and DANT were significantly elevated in a munitions dumpsite at Kolberger Heide
- TNT was rarely detected in fish, whereas the transformation product compounds ADNT and especially DANT were nearly ubiquitous
- ADNT and DANT were higher in fish viscera than muscle, suggesting reduced risk to seafood consumers, although fish muscle from Kolberger Heide was more contaminated than elsewhere

Abstract

Relic munitions are a hazardous legacy of the two world wars present in coastal waters worldwide. The southwest Baltic Sea has an especially high prevalence of unexploded ordnance and dumped munition material, which represent a large potential source of toxic explosive chemicals (munition compounds, MC). In the current study, diverse biota (plankton, macroalgae, tunicate, sponge, mollusc, echinoderm, polychaete, anemone, crustacea, fish) were collected from the Kiel Bight and a munitions dumpsite at Kolberger Heide, Germany, to evaluate the potential bioaccumulation of explosives and their derivatives (2,4,6-trinitrotoluene, TNT; 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene, ADNT; 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene, DANT; 1,3-dinitrobenzene, DNB; and 1,3,5-trinitro-1,3,5-triazinane, RDX). One or more MCs were detected in >98% of organisms collected throughout the study region (n=178), at a median level of 6 pmol/g (approximately 1 ng/g) and up to 2 x 10^7 pmol/g (TNT in Asterias rubens collected from Kolberger Heide). In most cases, TNT and its transformation product compounds ADNT and DANT were significantly higher in biota from the munitions dumpsite compared with other locations. Generally, DNB and RDX were detected less frequently and at lower concentrations than TNT, ADNT, and DANT. In commercially important fish species (plaice, flounder) from Kolberger Heide, TNT and ADNT were detected in 17 and 33% of samples, respectively. In contrast DANT was detected in every fish sample, including those outside the dumpsite. Dinitrobenzene was the second most prevalent MC in fish tissue. Fish viscera (stomach, kidney, liver) showed higher levels of DANT than edible muscle flesh, with highest DANT in liver, suggesting reduced risk to seafood consumers. This study provides some of the first environmental evidence for widespread bioaccumulation of MC in a coastal marine food web. Although tissue MC content was generally low, corrosion of munition housings may lead to greater MC release in the future, and the ecological risk of this exposure is unknown.

Keywords: Ocean dumping, underwater munitions; TNT; trinitrotoluene; RDX; DNB; dinitrobenzene; bioaccumulation; Kolberger Heide

1. Introduction

Nearly a century of ocean dumping and global wars in the 1900s have left coastal marine systems littered worldwide with relic munitions. European waters are particularly impacted by the legacy of the two World Wars (WWI and WWII), and numerous intentional dumpsites exist consisting each of as much as 1 million metric tons of munition material (e.g., Beaufort's Dyke; Beddington and Kinloch, 2005). German waters of the North and Baltic Seas have at least 15 documented munitions dumpsites and extensive munitions-contaminated regions, together comprising some 1.6 million metric tons of munitions (Böttcher et al., 2011).

The majority of relic munitions in marine waters contain conventional explosives, but not chemical warfare agents (Beck et al., 2018, and references therein). For WWII-era munitions, the predominant explosives used were TNT (trinitrotoluene), RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), and in some German munitions, DNB (dinitrobenzene) (Haas and Thieme, 1996). These explosive chemicals can exhibit marked toxicity to biological receptors. Effects include genotoxicity, carcinogenicity, and cytotoxicity (Juhasz and Naidu, 2007; Lotufo et al., 2013; Talmage et al., 1999).

For some compounds such as TNT, structurally-similar transformed metabolites (i.e., for TNT: aminodinitrotoluenes and diamino-nitrotoluenes) can also exhibit toxic effects (Lotufo et al., 2013; Koske et al., 2019). The aminated transformation products of TNT can be formed microbially (Spain, 1995) or within organs of higher organisms, especially the liver (Ownby et al., 2005; Koske et al., 2020a,b). The precursor TNT compound has limited potential for bioaccumulation due to rapid excretion and transformation (Lotufo et al., 2009; Lotufo et al., 2016). In contrast, the aminated transformation product compounds are accumulated to a greater degree, and non-extractable TNT residues can also represent a major fraction of the accumulated TNT (Ownby et al., 2005; Belden et al., 2005; Lotufo et al., 2016).

Explosive compounds and their transformation products (referred to generally as "munition compounds", MC) have only rarely been detected in environmental samples (as reviewed by Beddington and Kinloch, 2005; Beck et al., 2018). The comprehensive literature review of Lotufo and colleagues (2017) illustrates the lack of environmental MC data, including water, sediments, and biota. In most cases, only the most highly exposed organisms had detectible MC levels. For example, one study found tissue TNT content on the order of 100-1000 µg/g tissue in a featherduster worm, star coral, and sea urchin collected directly from the housing of a breached bomb (Porter et al., 2011). At a marine munitions dumpsite in Hawaii, MC were also detected at levels of 30 – 140 ng/g in shrimp (*H. ensifer*), but these were at or below the analytical detection limit of 250 ng/g (Koide et al., 2016). In a preliminary study in the Baltic Sea, Gledhill and colleagues (2019) measured MC in a few biota samples (*Alga, Asteroidea, Tunicata*) and found concentrations generally less than 100 µg/kg tissue. This suggests that the low rate of MC detection in field-collected biota samples may be due to analytical sensitivity, not lack of exposure and uptake.

Due to their toxic effects, particularly sub-lethal, MC have the potential to cause important deleterious impacts on coastal marine ecosystems. Although there is little evidence thus far of widespread MC release from underwater munitions or uptake by marine biota, corrosion thresholds suggest that the

period of greatest release may only begin in modern times. Therefore, exposure of coastal marine biota to toxic chemicals from relic munitions is likely to increase.

The aim of the current work was to examine MC content in biota samples collected ad hoc during a munitions research project in the southwest Baltic Sea. Here, recently developed, high-sensitivity analytical methods were applied to detect trace MC content in biotic tissue. These data provide insight into potential uptake into coastal marine food webs, and the risk of toxic MC eventually reaching human seafood consumers.

2. Methods

2.1 Study site

Samples in the current study were collected throughout German waters of the southwest Baltic Sea, during seven research cruises in 2017 during the project UDEMM ("Environmental monitoring for the remediation of munitions in the sea"). Most of the samples were collected at a munitions dumpsite at Kolberger Heide, which is closed to marine traffic (Fig 1b). This dumpsite is estimated to contain some 30000 tons of munitions (Kampmeier et al., 2020), including a wide variety of types such as bombs, mines, torpedoes, and grenades. Exposed explosives solids have also been frequently observed (e.g., Beck et al., 2019), which may be from original dumping (Wichert, pers. comm.), or residual material from low-order (incomplete) detonation during blow-in-place clearance activities at the site in early 2009 (Pfeiffer, 2009). Mussels transplanted to the Kolberger Heide dumpsite accumulated TNT as well as ADNT and DANT over several months (Strehse et al., 2017; Appel et al., 2018; Maser and Strehse, 2020).

2.2 Sample collection and processing

Within the Kolberger Heide dumpsite exclusion zone, samples were hand-collected by scientific divers. Large wracks of living seaweed were collected into plastic bags to also retain associated faunal communities. Mussels and sea stars were collected individually. Outside the exclusion zone, benthic samples were collected with a Van Veen grab and sieved (0.5 mm mesh size) to collect benthic epi- and infauna. Fauna were sorted and identified to at least phylum level to assess approximate trophic interactions. One fish and one sea star were collected near Dänisch Nienhof by an angler. At control (i.e., non-Kolberger Heide; non-KH) and Kolberger Heide (KH) sites, demersal fish samples (plaice and flounder) were collected with benthic trawls. At the munitions dumpsite, trawls were conducted along the boundaries of the exclusion zone.

Bivalve flesh was removed from the shell, and the shell was discarded. Except for fish, the entire soft tissues of organisms were homogenized and analyzed. Fish length and weight were measured after collection, and they were then dissected to isolate liver, kidney, stomach, and edible muscle (skinless). Muscle flesh was sampled from all fish, and viscera were only measured in eight of the 12 fish from control sites. All fish but one were females. In total, 178 unique specimens were investigated, for a total of 235 samples including different organs dissected from the fish.

2.3 Munition compound analysis

Sample processing and analysis followed that described by Gledhill et al. (2019) and are briefly described here. High purity water (MQ, 18.2 MΩ cm^{-1}; Milli Q, Millipore) and LCMS grade acetonitrile (ACN;

Optima, Fisher Scientific) were used throughout. Standards were made from commercially available mixed standard solutions in ACN (Composite Explosive Mixture ASM-8330-R-0.5X, 0.5 mg mL^{-1}, Amchro, Germany) and kept at −20 °C. Working standards were prepared in 67:33 MQ: ACN (v:v).

Biota samples were frozen at −20 °C until analysis. Approximately half of the samples (106 of 235) were lyophilized, while the other half were processed wet in order to prevent loss of other volatile analytes. For dry samples, tissue was ground to a coarse powder using a stainless-steel grinder for large samples or glass rod for small samples. Between 1 and 1300 mg of dry samples were extracted with different volumes of ACN depending on available mass: < 100 mg, 100 – 500 mg, and >500 mg were extracted with 1, 5, and 10 mL ACN, respectively. For wet samples, tissue was ground using a stainless-steel grinder. Between 0.04 and 6.8 g wet tissue were extracted, again with differing volumes depending on available mass: < 0.3 g, 0.3 – 3 g, and >3 g were extracted with 1, 5, and 10 mL ACN, respectively. Samples were sonicated in an ultrasonic bath (Pallsonic; Allpax, Germany) for 15 min at room temperature. Extracts were filtered using 0.2 µm polytetrafluorethylene syringe filters (Whatman GD/X). Extraction blanks were performed in the same manner as samples for the three different extraction solvent volumes.

Extracts were diluted to 33% ACN and stored at -20°C until analysis. Extracted MC were separated on an Acclaim Explosives E2 high performance liquid chromatography (HPLC) column and analyzed by electrospray ionization mass spectrometry (ESI-MS; Thermo Fisher Q-Exactive) following Gledhill and colleagues (2019). Tissue MC content is reported here in molar units to allow direct comparison of TNT and its transformation product compounds ADNT and DANT.

2.4 Statistical tests

Wilcoxon signed rank tests were performed on KH versus non-KH pairs where the number of samples with detectible MC content was greater than four. Sample sites considered “KH” were constrained to the marine traffic restricted zone where the density of dumped munitions is especially high (Kampmeier et al., 2020). Non parametric Kruskal-Wallis rank sum tests were performed on fish tissue MC content followed by pairwise comparisons using Wilcoxon rank sum test with continuity correction (P value adjustment method: BH; Benjamini and Hochberg, 1995). Statistical analysis was carried out in R (R Core Team, 2017), and figures were produced using the package ggplot2 (Wickham, 2009).

2.5 Quality parameters

Extraction blank levels were uniformly low, leading to a very low, sub-picomolar limit of detection (Table S1). Detection limits shown in Table S1 represent the upper estimate, given that analyte concentrations were variable due to varying sample mass and eluent volume. A greater limitation of the current study is the relatively poor reproducibility of triplicate extractions (median RSD 27-45 %; Table S1). The within sample variability observed in the current study is partly related to the wide range in sample concentrations, as MC levels near the detection limit have higher inherent uncertainty (Table S1); indeed, many of the replicated samples had very low concentrations or even undetectable levels of the target MC (e.g., TNT was only detected in about a third of the replicated samples). However, poor precision of MC analysis has been observed in spiked samples. For example, in a rigorous intercomparison exercise with spiked tissue samples, precision was on the order of 5-25% (intralaboratory) and 10-40% (interlaboratory; Craig et al., 2019).

To our knowledge, no certified reference materials are available for organic explosives in biota tissue to verify the accuracy of the extraction procedure. One laboratory intercomparison has been performed with spiked tissues (Craig et al., 2019), although it is not clear if added MC behave similarly to accumulated MC, and the apparent abundance of non-extractable TNT residues (Ownby et al., 2005; Belden et al., 2005; Lotufo et al., 2016) suggests that extraction of accumulated MC reflects only a fraction of the body burden in wild exposed organisms.

3. Results

3.1 Munition compound content in biota

At least one of the MC measured in the current study was detected in at least one individual of every organism class sampled (Table 1) In fact, of the 178 unique specimens analyzed, 175 (98.3%) had detectable levels of one or more of the target MC (no MC could be detected in one mollusc sample and two polychaetes; SI Table S2). Tissue MC content varied widely, spanning 7 orders of magnitude (between 0.1 and $2.6x10^6$ pmol/g), with a median content of 6.1 pmol/g. In general, TNT, ADNT, and DANT were detected at higher levels and more frequently than RDX and DNB (Fig. 2).

Variable numbers of each organism class were analyzed. The groups that had the highest number of analyses were macroalgae (n=37), mollusc (n=22), echinoderm (n=55), and fish (n=24; Table 1 and Fig. 2). Within these groups, fish had the lowest overall MC content (maximum 800 pmol/g in liver tissue; Table 2). Macroalgae, mollusc, and echinoderm had MC content as high as 10^5 pmol/g, and generally showed decreasing MC content in the order TNT>ADNT>DANT>RDX=DNB. Many of these samples had one or more analytes that were below detection (see "detects," Table 1), which limited the statistical comparison. Among the fish tissues, DANT and DNB were most frequently detected, followed by ADNT and finally TNT and RDX, which were rarely detected (Fig. 3).

A limited and variable number of samples were collected of the other organisms, but they are included here for comparison. Only three plankton samples were collected, and DANT and RDX were the only MC detected in the few samples (Fig 2). The tunicate, sponge, anemone, and crustacean samples all contained measurable levels of all MC, except that no RDX was detected in the four tunicate samples. Collectively, these organisms also contained some of the highest levels of TNT, ADNT, and DANT observed in the dataset (up to 10^4 pmol/g). However, too few samples (<10 total of each species) were collected to make statistical comparisons. Polychaetes were only collected at non-KH sites and contained low and similar levels of all five target MC (0.37 – 13 pmol/g).

3.2 Isomer ratios

Isomer ratios were calculated for samples in which both isomers of ADNT and DANT were detected (Fig. 4). A single isomer was detected in 28 of 130 samples for ADNT and 33 of 209 samples for DANT, so no ratio was calculated in those samples. The ratios of 4-ADNT to 2-ADNT in the samples were between 1 and 4.5, except for one mollusc sample at 0.4 and one macroalgae sample at 6.2. Approximately 36% of the samples had 4-ADNT to 2-ADNT ratios between 1 and 1.5. Ratios of 2,4-DANT to 2,6-DANT showed a wider range, varying between 0.02 and 68, and were more evenly distributed at ratios below 2.5. Of the 13 samples with ratios greater than 5.5, eight were from fish tissues.

3.3 Statistical comparisons

Organisms from the KH munitions dumpsite generally had higher MC content than those from other locations. Organisms with sufficient sample sizes (i.e., number of organisms with measurable MC) for statistical comparison are indicated in Fig. 2 and 3 by the asterisk or "ns" symbols. Macroalgae from KH had higher levels of TNT ($p<0.01$), ADNT ($p<0.05$), and DANT ($p<0.05$). Molluscs from KH were higher in TNT and ADNT ($p<0.01$), and echinoderms from KH had higher levels of TNT ($p<0.01$) and DANT ($p<0.05$). Sample sizes were insufficient to compare MC content in common seastars (*A. rubens)*, except for the largest specimens (>7 cm diameter) which had higher DANT content in KH ($p<0.05$; SI Fig. S1). Ratios of 4-ADNT to 2-ADNT were significantly higher only for macroalgae in KH. No differences were evident for DANT isomer ratios.

When all the fish tissues were pooled, only DANT was significantly higher in samples from KH ($p<0.01$; Fig. 2). Within the different fish tissues, DANT content in muscle and kidney were higher in samples from KH ($p<0.001$ and $p<0.01$, respectively; Fig. 3). Comparison among the tissue types showed that kidney tissue in KH was significantly higher in DANT than muscle ($p<0.01$), and liver was elevated relative to all other tissues ($p<0.001$). At non-KH sites, liver DANT content was significantly higher than muscle or stomach ($p<0.01$).

4. Discussion

4.1 Uptake of munition compounds and bioconcentration factors (BCF)

One or more MC were detected in >98% of organisms collected throughout the study region, suggesting widespread exposure to MC contamination. The high rate of detection is consistent with Porter and colleagues (2010) in Vieques, Puerto Rico, who found one or more MC in every biota sample tested (although all samples in that study were collected from surfaces of munitions objects). The MC content in biota is generally low, on the order of 1 – 10 pmol/g, approximately 1000-fold lower than observed in laboratory experiments (usually nmol/g or higher; e.g., Ballentine et al., 2015; Belden et al., 2005; Cruz-Uribe et al., 2007).

Lower levels of MC are expected in field-collected biota given the much lower concentration of dissolved MC in seawater compared with experimental treatments. Bioconcentration factors (the ratio of the chemical content in tissue to the concentration in the surrounding water, with units of ml/g; BCF) for field samples are far more variable than in experiments, but not drastically different. Bottom water dissolved MC concentrations in the southwest Baltic Sea are on the order of 1 – 1000 pmol/L (Beck et al., in Greinert, 2019). Taking 100 pmol/L as a reasonable dissolved concentration, the tissue content measured in the current study gives median BCF values for TNT, ADNT, DANT, RDX, and DNB of 79, 105, 130, 9.4, and 17 ml/g, respectively. The BCF range is very large, around 1 ml/g at the low end, and up to 2 x 10^7 ml/g at the high end (i.e., the MC content in Fig. 2 divided by 0.1 pmol/ml). The highest BCF values were observed for TNT, ADNT, and DANT, and were observed in multiple organism types.

Median BFC values in the current study are consistent with those from laboratory experiments, with nitroaromatics up to 10s of ml/g, and approximately an order of magnitude higher than nitramines such as RDX (Ballentine et al., 2015; Lotufo and Lydy, 2007; Lotufo, 2017, and references therein). BCF values up to several hundred ml/g have been reported for TNT in green algae and fish viscera (453 and 338 ml/g,

respectively; Talmage et al., 1999). High BCF values were also reported for crab eggs (485 ml/g; Ballentine et al., 2015). Consistency between the median BCF measured in field samples, and those from experiments suggests that MC uptake by biota in the environment is represented reasonably well by laboratory studies. Biota MC body burdens may be a good indicator of exposure to water column levels, as indicated by increased MC levels in mussels located closer to munitions objects (Maser and Strehse, 2020). However, the variation of BCF over some seven orders of magnitude in natural samples suggests that either exposure of some organisms is far greater than expected (e.g., given the exponential increase in dissolved MC concentration near munitions objects (Beck et al., 2019)), or that yet unknown environmental factors lead to greatly increased uptake.

4.2 Detection across organism classes and trophic interactions

Biota specimens collected in this study do not necessarily reflect direct trophic interactions in the Baltic Sea food web. Nonetheless, the sampled organisms do represent a range of trophic levels and feeding strategies, and there are likely trophic linkages among them. Plankton and macroalgae are primary producers; tunicates, sponges, and molluscs are filter feeders and detritovores; crustaceans, echinoderms, anemones, and polychaetes include detritovores, grazers, and predators; plaice and flounder are predators. Trophic transfer of MC would likely result in greatest enrichment of fish, but this is not the case (Fig. 2). Indeed, macroalgae should be at the lowest trophic level, and yet had a very high rate of MC detection (Table 1) and some of the highest observed levels of TNT, ADNT, and RDX (Fig.2).

Macroalgae can accumulate and transform TNT (Ballentine et al., 2015), although the transformation products may represent a small fraction of the accumulated TNT (<20%; Cruz-Uribe et al., 2007). In the current study, ADNT in macroalgae was up to 60-fold higher than TNT (average 7.1), suggesting that transformation product compounds are also likely accumulated from external dissolved sources. Future work to determine the ratios of TNT and transformation product compounds in the water column will help clarify the question of ex situ versus in situ transformation, and there are important outstanding knowledge gaps on uptake pathways (dissolved vs. particulate vs. diet) in natural systems.

The lack of correlation between tissue MC content and approximate trophic level may also result from excretion of MC by the organisms. Lotufo et al. (2016) showed nearly complete depuration of TNT and aminated transformation products from sheepshead minnows and mussels within 24 and 48 h, respectively. Non-extractable residues (determined in that study using radiotracers) represented a higher mass of the TNT and derivatives, and was depurated less rapidly (>200 h). Rosen and Lotufo (2007) also showed that MC uptake in mussels reached steady state rapidly and was eliminated within hours, limiting the likelihood of trophic transfer.

Rapid depuration is consistent with the observation that concentrations in mussels transplanted to the KH munitions dumpsite apparently reached steady state levels and did not continuously accumulate MC (Appel et al., 2018). Furthermore, in the current study, no correlation was observed between MC content in *A. rubens* and the organism size (SI Fig. S1). This suggests that MC did not accumulate over the organism lifetime, although this is complicated by the tendency of *A. rubens* to shrink when food availability is poor (Budd, 2008).

Similarly, in fish tissues, none of the MC showed covariation with the size of the individual. This implies that the MC burden observed in Baltic Sea biota may reflect only days of prior exposure, which is also consistent with the higher levels of TNT and ADNT observed in organisms near the MC source at KH compared with other sites (Fig. 2). The transformation product compound DANT is depurated more slowly, and similar levels in fish across all sites (Fig. 2) may indicate longer-term exposure and retention of the less rapidly depurated compound.

Lack of increase across trophic levels or with size suggests that MC content in biota primarily reflects recent proximity to source rather than long-term accumulation. This has encouraging implications for environmental remediation, since it implies that MC contamination in biota is likely to decline rapidly if the source is reduced or eliminated. Alternatively, MC levels in live seafood may be reduced by holding in uncontaminated waters to allow depuration.

It is possible that some of the MC content measured in whole organisms, especially the very high values observed in some *A. rubens* specimens, may reflect external contamination by explosives particles rather than accumulation in tissue. However, particulate explosive contamination cannot explain the concomitant high levels of TNT transformation products also observed in *A. rubens* (SI Fig. S1), and such particles are less likely to have affected the other organisms.

4.3 Accumulation of munition compounds in fish

Of the organisms sampled in this study, fish represent the great exposure risk to seafood consumers. TNT and RDX were not detected in most fish tissue samples (Fig. 3). ADNT was detected with higher frequency, especially in fish from KH, and DANT and DNB were detected in all fish tissue types and virtually all specimens. Fish tissue MC content showed no relationship with individual fish length or weight (not shown).

Concentrations of ADNT and DANT were higher in fish organ tissue, especially DANT in liver (Fig. 3). Mariussen and colleagues (2018) showed that TNT is excreted by salmon through the gall bladder, and that TNT transformation products accumulate in bile. The high liver MC concentrations observed in the current study match with recent work showing detectible MC concentrations in dab (*Limanda*) bile, and higher concentrations in fish from KH compared with other sites (Koske et al., 2020b). This is consistent with previous work showing that TNT and transformation product compound accumulation during aqueous exposure was higher in fish viscera than muscle (Ownby et al., 2005). Preferential accumulation of MC in fish viscera compared with muscle suggests a reduced risk to seafood consumers.

4.4 ADNT and DANT isomer ratios

There is extensive evidence for regioselective nitro-reduction of TNT, with 4-ADNT and 2,4-DANT metabolites favored to varying degrees depending on aerobic vs. anaerobic pathways (Barrows et al., 1997; Preuss et al., 1993; Elovitz and Weber, 1999). Marine mesocosm experiments showed 4-ADNT to 2-ADNT ratios in marine organisms (phytoplankton, macroalgae, crab, and molluscs) generally between 1.1 and 1.2, but as high as 9.4 in crab eggs (Ballentine et al., 2015). In a field bio-monitoring experiment at the KH site, only 4-ADNT was detected in mussels exposed for several months (no 2-ADNT, DANT, or TNT; Appel et al., 2018; Maser and Strehse, 2020). In three subsequent deployments, TNT, 2-ADNT, 4-

ADNT and 2,4-DA-6-NT were all detected in the exposed mussels (Maser and Strehse, 2020). The ratio of mean 4-ADNT to 2-ADNT in one deployment was 1.3 (Strehse et al., 2017), while subsequent deployments showed ratios approaching 4 (Maser and Strehse, 2020).

In the current study, most 4-ADNT to 2-ADNT ratios were between 1 and 2.5, similar to the previous studies referenced above. There was no significant difference in 4-ADNT to 2-ADNT ratios among organism types, which as proposed by Ballentine and colleagues (2015), suggests a preferential biotransformation pathway to 4-ADNT that is not organism specific. Only macroalgae showed significantly higher 4-ADNT to 2-ADNT ratios in KH compared with other sites ($p<0.01$; Fig. S2). Transformation potential was demonstrated in in vitro experiments with flatfish liver extract, with 4-ADNT to 2-ADNT ratios reaching a maximum of 2.6 (Koske et al., 2020a). In the current study, a single ADNT isomer was detected in only three liver samples at levels between 1.1 and 1.3 pmol/g (4-ADNT once, and 2-ADNT twice).

The median ADNT and DANT isomer ratios in the current study were similar (1.5, and 1.6, respectively), although DANT ratios reached higher levels (13 samples with DANT ratio above 6 compared with 1 sample above 6 for ADNT; Fig. 4). 2,4-DANT to 2,6-DANT ratios in samples from KH were not significantly different from samples collected at other sites. DANT isomer ratios in whole mussels and fish in incubation experiments have been reported as high as 22 (Lotufo et al., 2016). The highest DANT ratios in the current study, as high as 68, were observed in fish liver samples, which were also the samples representing most of the ratios above 6. The higher ratios observed in the current study in liver samples suggests a transformation mechanism in liver tissue that strongly favors the 2,4-DANT isomer. Given the lack of detectable TNT or ADNT in liver tissue, transformation in fish liver appears to progress rapidly to DANT.

4.5 Ecological and human health risk

The accumulation of MC in natural biota populations represents a clear potential risk to marine organism health. This is further indicated by the apparent higher incidence of liver lesions and tumors in demersal fish populations near the KH munitions dumpsite (Koske et al., 2020b). The environmental BCF values estimated in the current study are similar to those observed in laboratory experiments, but absolute MC tissue content is several orders of magnitude lower and water concentrations (ng/L) are much lower than water quality criteria (WQC) for acute and chronic effects (see comprehensive summary in Lotufo et al., 2017). Limited experimental data mean that WQC are available for fewer MC in marine waters, but they are generally lower compared with freshwater by a factor of 5 or so. Therefore, marine organisms may be more susceptible to MC toxicity than indicated by available data, and this may further be affected by environmental conditions. Because of these uncertainties, more work is necessary to evaluate toxic effects of MC release from relic munitions on organisms in natural marine settings.

Accumulation of MC in biota poses a potential risk to seafood consumers. Among the sampled organisms, only molluscs and fish are potentially consumed as seafood. The US Environmental Protection Agency defines an oral reference dose (RfD) for TNT of 0.5 µg/kg body weight/d, and an Oral Cancer Slope Factor (CSF) for carcinogenic risk of 30 per µg/kg body weight/d, based on very limited animal exposure studies (EPA IRIS, 1993). The RfD is an estimate of the daily exposure level that is likely to have no increase in negative effects during a lifetime, whereas the CSF is the increase in lifetime cancer risk per unit dose. For a 70 kg individual, 35 µg TNT would need to be consumed daily to reach the RfD.

Taking TNT* as the sum of tissue TNT, ADNT, and DANT content, the current study found median tissue levels of less than 5 ng/g TNT*. To reach the RfD, these levels would require daily consumption of about 7 kg seafood, far higher than average seafood consumption in Germany (~ 14 kg/y, or 38 g/d; Fisch-Informationszentrum e. V., 2020). These levels represent a chronic daily intake (CDI) of 2.7×10^{-6} mg/kg/d, giving a carcinogenic risk of 0.08×10^{-6} (CDI multiplied by CSF). This is lower than the generally accepted limit of 1×10^{-6}, suggesting negligible carcinogenic risk due to TNT exposure from seafood consumption. However, this should be taken carefully given that the CSF is based on very limited data from non-human individuals. The maximum measured TNT* was 2.5, 0.13, and 0.009 µg/g in mollusc tissue, fish viscera, and fish muscle respectively. Consumption of only mollusc flesh at these levels would exceed the RfD, and only under the unlikely conditions that the most contaminated organisms are eaten continuously over a consumer's lifetime. Fish viscera tissue is closer to a level of concern, but the most contaminated fish muscle measured in the current study would not be expected to pose a chronic health risk to seafood consumers.

5. Conclusions

This study provides some of the first extensive evidence of MC accumulation in field-collected biota. Higher MC levels were observed in biota within the major munitions dumpsite at KH where unequivocal chemical release from munitions objects has been shown (Beck et al., 2019). Non-quantitative and systematic observations from scientific divers and visual observations with towed cameras and AUVs at this site indicate that the abundant munitions objects may act as an artificial reef, hosting higher densities of organisms at the point of greatest exposure risk. The current study provides a strong link between chemical emission from relic munitions and uptake of these chemicals throughout the food web. No evidence was observed for trophic biomagnification, and lack of covariation with organism size suggests depuration processes regulate tissue MC content, consistent with previous experimental studies. This has encouraging implications for environmental management, as it suggests that remediation and removal of the munitions objects would likely lead to rapid decrease in the tissue burden of MC in marine biota. The current study provides only a preliminary assessment of ecological exposure to chemicals from underwater munitions, and a more comprehensive study is necessary to confirm the extent and regional differences of exposure. There are important outstanding questions, especially regarding the pathway of exposure (food or water) and toxicological effects associated with the exposure.

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Author contributions

AJB: Conceptualization, Formal Analysis, Writing – original draft; MG: Formal Analysis, Methodology, Writing – review & editing; MK: Resources, Investigation; CF: Resources, Writing – review & editing; CS: Project administration, Funding acquisition; JG: Project administration, Funding acquisition, Writing – review & editing; EPA: Resources, Funding acquisition, Writing – review & editing

Supporting information

- Table with analytical quality assessment parameters
- Table with complete MC dataset
- Figure with *A. rubens* MC content
- Figure with isomer ratios

References

Appel, D., Strehse, J. S., Martin, H. J., & Maser, E. (2018). Bioaccumulation of 2, 4, 6-trinitrotoluene (TNT) and its metabolites leaking from corroded munition in transplanted blue mussels (M. edulis). Marine pollution bulletin, 135, 1072-1078.

Ballentine, M. L., Tobias, C., Vlahos, P., Smith, R. W., and Cooper, C. (2015). Bioconcentration of TNT and RDX in coastal marine biota. Arch. Environ. Contam. Toxicol. 68, 718–728. doi: 10.1007/s00244-014-0104-9

Barrows, S. E., Cramer, C. J., Truhlar, D. G., Elovitz,M. S., andWeber, E. J. (1997). Factors controlling regioselectivity in the reduction of polynitraromatics in aqueous solution. Environ. Sci. Technol. 30, 3028–3038. doi: 10.1021/es960004x

Beck, A. J., Gledhill, M., Schlosser, C., Stamer, B., Böttcher, C., Sternheim, J., ... & Achterberg, E. P. (2018). Spread, behavior, and ecosystem consequences of conventional munitions compounds in coastal marine waters. Frontiers in Marine Science, 5, 141.

Beck, A. J., van der Lee, E. M., Eggert, A., Stamer, B., Gledhill, M., Schlosser, C., & Achterberg, E. P. (2019). In situ measurements of explosive compound dissolution fluxes from exposed munition material in the Baltic Sea. Environmental science & technology, 53(10), 5652-5660.

Beck et al., Greinert, J. (2019). Practical Guide for Environmental Monitoring of Conventional Munitions in the Seas-Results from the BMBF funded project UDEMM “Umweltmonitoring für die Delaboration von Munition im Meer” Version 1.1.

Beddington, J., and Kinloch, A. (2005). Munitions Dumped at Sea: A Literature Review. London, UK: Imperial College Consultants.

Belden, J. B., Ownby, D. R., Lotufo, G. R., and Lydy, M. J. (2005). Accumulation of trinitrotoluene (TNT) in aquatic organisms: Part 2 - Bioconcentration in aquatic invertebrates and potential for trophic transfer to channel catfish (Ictalurus punctatus). Chemosphere 58, 1161–1168. doi: 10.1016/j.chemosphere.2004.09.058

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B, 57, 289-300.

Böttcher, C., Knobloch, T., Rühl, N.-P., Sternheim, J., Wichert, U., and Wöhler, J. (2011). Munitionsbelastung der Deutschen Meeresgewässer – Bestandsaufnahme und Empfehlungen (Stand 2011). Meeresumwelt Aktuell Nord- und Ostsee, 2011/3. Hamburg; Rostock: Bundesamt für Seeschifffahrt und Hydrographie (BSH).

Budd, G.C. 2008. *Asterias rubens* Common starfish. In Tyler-Walters H. and Hiscock K. (eds) Marine Life Information Network: Biology and Sensitivity Key Information Reviews, [on-line]. Plymouth: Marine Biological Association of the United Kingdom. [cited 07-05-2020]. Available from: https://www.marlin.ac.uk/species/detail/1194

Craig, H. D., Jenkins, T. F., Johnson, M. T., Walker, D. M., Dobb, D. E., & Pepich, B. V. (2019). Method development and laboratory intercomparison of an RP-HPLC-UV method for energetic chemicals in marine tissues. Talanta, 198, 284-294.

Cruz-Uribe, O., Cheney, D. P., and Rorrer, G. L. (2007). Comparison of TNT removal from seawater by three marine macroalgae. Chemosphere 67, 1469–1476. doi: 10.1016/j.chemosphere.2007.01.001

Elovitz, M. S., and Weber, E. J. (1999). Sediment-mediated reduction of 2, 4, 6- trinitrotoluene and fate of the resulting aromatic (poly)amines. Environ. Sci. Technol. 33, 2617–2625. doi: 10.1021/es980980b

EPA. Integrated Risk Information System (IRIS). 1993. 2,4,6-Trinitrotoluene (TNT) (CASRN 118-96-7). Last Revised 1993. www.epa.gov/iris/subst/0269.htm (accessed 26.03.2021)

Fisch-Informationszentrum e. V. 2020. Fisch Wirtschaft: Daten Und Fakten 2020. ISSN 1438-5376. https://www.fischinfo.de/index.php/verbraucher/broschueren (accessed 26.03.2021)

Gledhill, M., Beck, A. J., Stamer, B., Schlosser, C., & Achterberg, E. P. (2019). Quantification of munition compounds in the marine environment by solid phase extraction–ultra high performance liquid chromatography with detection by electrospray ionisation–mass spectrometry. Talanta, 200, 366-372.

Haas, R., and Thieme, J. (1996). Band 2: Explosivstofflexikon. Bestandsaufnahme von Rüstungsaltlastverdachtsstandorten der Bundesrepublik Deutschland, 2. Erweiterte Auflage, Umweltbundesamt [Inventory suspected armament Contam. Fed. Repub. Ger. Second Ext. Ed. Fed. Envi. 378.

Juhasz, A. L., and Naidu, R. (2007). Explosives: fate dynamics, and ecological impact in terrestrial and marine environments. Rev. Env. Contam Toxicol. 191, 163–215. doi: 10.1007/978-0-387-69163-3_6

Kampmeier, M., van der Lee, E. M., Wichert, U., & Greinert, J. (2020). Exploration of the munition dumpsite Kolberger Heide in Kiel Bay, Germany: Example for a standardised hydroacoustic and optic monitoring approach. Continental Shelf Research, 198, 104108.

Koide, S., Silva, J. A. K., Dupra, V., and Edwards, M. (2016). Bioaccumulation of chemical warfare agents, energetic materials, and metals in deep-sea shrimp fromdiscarded military munitions sites off PearlHarbor. Deep. Res. Part II Top. Stud. Oceanogr. 128, 53–62. doi: 10.1016/j.dsr2.2015.03.002

Koske, D., Goldenstein, N. I., & Kammann, U. (2019). Nitroaromatic compounds damage the DNA of zebrafish embryos (Danio rerio). Aquatic Toxicology, 217, 105345.

Koske, D., Goldenstein, N. I., Rosenberger, T., Machulik, U., Hanel, R., & Kammann, U. (2020a). Dumped munitions: New insights into the metabolization of 2, 4, 6-trinitrotoluene in Baltic flatfish. Marine Environmental Research, 160, 104992.

Koske, D., Straumer, K., Goldenstein, N. I., Hanel, R., Lang, T., & Kammann, U. (2020b). First evidence of explosives and their degradation products in dab (Limanda limanda L.) from a munition dumpsite in the Baltic Sea. Marine pollution bulletin, 155, 111131.

Lotufo, G. R., Chappell, M. A., Price, C. L., Ballentine, M. L., Fuentes, A. A., Bridges, T. D., et al. (2017). Review and Synthesis of Evidence Regarding Environmental Risks Posed by Munitions Constituents (MC) in Aquatic Systems. ERDC/EL TR-17-17, SERDP ER-2341.

Lotufo, G. R., and Lydy, M. J. (2005). Comparative toxicokinetics of explosive compounds in sheepshead minnows. Arch. Environ. Contam. Toxicol. 49, 206–214. doi: 10.1007/s00244-004-0197-7

Lotufo, G. R., Belden, J. B., Fisher, J. C., Chen, S. F., Mowery, R. A., Chambliss, C. K., et al. (2016). Accumulation and depuration of trinitrotoluene and related extractable and nonextractable (bound) residues in marine fish and mussels. Environ. Pollut. 210, 129–136. doi: 10.1016/j.envpol.2015.11.049

Lotufo, G. R., Lydy, M. J., Rorrer, G. L., Cruz-Uribe, O., & Cheney, D. P. (2009). Bioconcentration, bioaccumulation and biotransformation of explosives and related compounds in aquatic organisms. Ecotoxicology of explosives, 135, 156.

Lotufo, G. R., Rosen, G.,Wild, W., and Carton, G. (2013). Summary Review of the Aquatic Toxicology of Munitions Constituents, ERDC/EL TR-13–R-18.

Lotufo, G. R., Belden, J. B., Fisher, J. C., Chen, S. F., Mowery, R. A., Chambliss, C. K., et al. (2016). Accumulation and depuration of trinitrotoluene and related extractable and nonextractable (bound) residues in marine fish and mussels. Environ. Pollut. 210, 129–136. doi: 10.1016/j.envpol.2015.11.049

Lotufo, G. R. (2017). Toxicity and bioaccumulation of munitions constituents in aquatic and terrestrial organisms. In Energetic Materials (pp. 445-479). Springer, Cham.

Mariussen, E., Stornes, S. M., Bøifot, K. O., Rosseland, B. O., Salbu, B., & Heier, L. S. (2018). Uptake and effects of 2, 4, 6-trinitrotoluene (TNT) in juvenile Atlantic salmon (Salmo salar). Aquatic Toxicology, 194, 176-184.

Maser, E., & Strehse, J. S. (2020). “Don’t Blast”: blast-in-place (BiP) operations of dumped World War munitions in the oceans significantly increase hazards to the environment and the human seafood consumer. Archives of toxicology, 94(6), 1941-1953.

Ownby, D. R., Belden, J. B., Lotufo, G. R., and Lydy, M. J. (2005). Accumulation of trinitrotoluene (TNT) in aquatic organisms: part 1 – Bioconcentration and distribution in channel catfish (Ictalurus punctatus). Chemosphere 58, 1153–1159. doi: 10.1016/j.chemosphere.2004.09.059

Pfeiffer, F. (2009). Bericht über die in-situ-Begleituntersuchungen zur Munitionssprengung in der Ostsee vom 18.2.2009.

Porter, J. W., Barton, J. V., and Torres, C. (2011). “Ecological, radiological, and toxicological effects of naval bombardment on the coral reefs of Isla de Vieques, Puerto Rico,” in Warfare Ecology: A New Synthesis for Peace and Security, eds. G. E. Machlis, T. Hanson, Z. Špiri´c, and J. E. McKendry (Dordrecht: Springer Netherlands), 65–122.

Preuss, A., Fimpel, J., and Diekert, G. (1993). Anaerobic transformation of 2,4,6-trinitrotoluene (TNT). Arch.Microbiol. 159, 345–353. doi: 10.1007/BF00290917

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rosen, G., and Lotufo, G. R. (2007). Toxicity of explosive compounds to the marine mussel, Mytilus galloprovincialis, in aqueous exposures. Ecotoxicol. Environ. Saf. 68, 228–236. doi: 10.1016/j.ecoenv.2007.03.006

Strehse, J. S., Appel, D., Geist, C., Martin, H. J., & Maser, E. (2017). Biomonitoring of 2, 4, 6-trinitrotoluene and degradation products in the marine environment with transplanted blue mussels (M. edulis). Toxicology, 390, 117-123.

Talmage, S. S., Opresko, D. M., Maxwell, C. J., Welsh, C. J., Cretella, F. M., Reno, P. H., et al. (1999). Nitroaromatic munition compounds: environmental effects and screening values. Rev. Environ. Contam. Toxicol. 161, 1–156. doi: 10.1007/978-1-4757-6427-7_1

Wickham, H. (2009) ggplot2: elegant graphics for data analysis. Springer New York.

Table 1. Summary data for MC in biota tissue, including average and range of measured tissue MC content and the number of samples in which MC were detected.

		TNT (pmol/g)				Sum ADNT (pmol/g)				Sum DANT (pmol/g)				RDX (pmol/g)				DNB (pmol/g)			
	n	Aver-age[1]	Range	Detects[2]		Aver-age[1]	Range	Detects[2]		Aver-age[1]	Range	Detects[2]		Aver-age[1]	Range	Detects[2]		Aver-age[1]	Range	Detects[2]	
KH																					
Plankton	1	n/a	n/a	0	0%	n/a	n/a	0	0%	97	n/a	1	100%	n/a	n/a	0	0%	n/a	n/a	0	0%
Macroalgae	23	2500	(0.34 - 17000)	21	91%	3500	(5.3 - 24000)	23	100%	17	(0.4 - 71)	20	87%	10	(0.13 - 57)	14	61%	49	(0.86 - 290)	18	78%
Tunicate	4	960	(220 - 1700)	2	50%	6400	(16 - 17000)	4	100%	34	(7.5 - 54)	4	100%	n/a	n/a	0	0%	19	(1.3 - 54)	3	75%
Sponge	2	19000	n/a	1	50%	22000	n/a	1	50%	25	(1.7 - 49)	2	100%	0.6	n/a	1	50%	85	n/a	1	50%
Anemone	1	10000	n/a	1	100%	66000	n/a	1	100%	6700	n/a	1	100%	30	n/a	1	100%	0.5	n/a	1	100%
Polychaete	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Crustacean	4	3800	(9.3 - 7600)	2	50%	2800	(33 - 9600)	4	100%	3800	(12 - 15000)	4	100%	8.8	n/a	1	25%	6.8	(1.9 - 17)	4	100%
Mollusc	8	1300	(5.7 - 8200)	7	88%	1500	(14 - 3700)	8	100%	58	(5.3 - 280)	8	100%	1.2	(0.19 - 2.9)	5	63%	16	(0.34 - 68)	5	63%
Echinoderm	41	97000	(0.22 - 2400000)	36	88%	29000	(0.2 - 520000)	29	71%	180	(1.3 - 2500)	40	98%	1.8	(0.18 - 16)	13	32%	190	(0.38 - 2700)	22	54%
Fish (mus-cle)	12	4.2	(3.2 - 5.2)	2	17%	0.8	(0.69 - 1.2)	4	33%	12	(5.9 - 20)	12	100%	0.7	(0.54 - 0.9)	3	25%	1.4	(0.94 - 2.5)	11	92%
non-KH																					
Plankton	2	n/a	n/a	0	0%	n/a	n/a	0	0%	180	(59 - 310)	2	100%	3.9	n/a	1	50%	n/a	n/a	0	0%
Macroalgae	14	5.5	(0.36 - 15)	10	71%	6.4	(1.2 - 11)	14	100%	0.8	(0.22 - 1.4)	10	71%	1.2	(0.11 - 2.3)	7	50%	0.4	(0.19 - 1)	4	29%
Tunicate	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Sponge	2	n/a	n/a	0	0%	5.2	(5 - 5.3)	2	100%	1.1	n/a	1	50%	1.7	(1.1 - 2.3)	2	100%	n/a	n/a	0	0%
Anemone	1	n/a	n/a	0	0%	4.6	n/a	1	100%	n/a	n/a	0	0%	0.7	n/a	1	100%	n/a	n/a	0	0%
Polychaete	17	3.8	(0.46 - 7)	9	53%	1.9	(0.61 - 5.7)	9	53%	4.7	(0.47 - 13)	10	59%	0.9	(0.28 - 1.8)	5	29%	1.2	(0.37 - 2.7)	5	29%
Crustacean	5	5.0	(1.1 - 7.6)	3	60%	3.7	n/a	1	20%	3.3	(1.3 - 5.5)	4	80%	0.5	n/a	1	20%	1.7	(0.74 - 2.7)	2	40%
Mollusc	14	7.4	(0.66 - 21)	12	86%	150	(0.21 - 1600)	11	79%	18	(2.6 - 46)	7	50%	2.6	(0.26 - 9.6)	6	43%	2.0	(0.65 - 6.9)	8	57%
Echinoderm	14	2.1	(0.47 - 6.5)	6	43%	0.7	(0.27 - 0.93)	3	21%	3.8	(0.59 - 9.9)	12	86%	1.3	(1.2 - 1.3)	2	14%	1.6	(0.21 - 4.3)	6	43%
Fish (mus-cle)	12	0.9	n/a	1	8%	0.9	n/a	1	8%	8.9	(1.6 - 20)	11	92%	42	n/a	1	8%	3.0	(0.37 - 12)	12	100%

[1] Where MC were detected in only one sample, this is the measured value, not the average

[2] "Detects" refers to the number of samples in which the MC was detected. The percentage of collected samples in which MC were detected is also indicated.

"n/a": not applicable

Table 2. Summary data for MC in fish tissues, including average and range of measured tissue MC content and the number of samples in which MC were detected.

		TNT (pmol/g)				Sum ADNT (pmol/g)				Sum DANT (pmol/g)				RDX (pmol/g)				DNB (pmol/g)			
	n	Average[1]	Range	Detects[2]		Average[1]	Range	Detects[2]		Average[1]	Range	Detects[2]		Average[1]	Range	Detects[2]		Average[1]	Range	Detects[2]	
KH																					
Muscle	12	4	(3.2 - 5.2)	2	17%	1	(0.69 - 1.2)	4	33%	12	(5.9 - 20)	12	100%	1	(0.54 - 0.9)	3	25%	1	(0.94 - 2.5)	11	92%
Liver	12	7	n/a	1	8%	1	(1.1 - 1.3)	3	25%	170	(46 - 490)	11	92%	1	n/a	1	8%	1	(0.99 - 1.5)	4	33%
Stomach	12	11	n/a	1	8%	5	(2.4 - 7.1)	6	50%	19	(6 - 43)	12	100%	2	n/a	1	8%	3	(0.9 - 4.2)	6	50%
Kidney	12	n/a	n/a	0	0%	7	(2.3 - 14)	4	33%	36	(6.1 - 130)	12	100%	2	n/a	1	8%	3	(1.4 - 4.3)	8	67%
non-KH																					
Muscle	12	0.9	n/a	1	8%	0.9	n/a	1	8%	8.9	(1.6 - 20)	11	92%	42.0	n/a	1	8%	3.0	(0.37 - 12)	12	100%
Liver	8	n/a	n/a	0	0%	n/a	n/a	0	0%	200	(9.1 - 800)	8	100%	1.9	n/a	1	13%	1.1	(0.91 - 1.2)	2	25%
Stomach	8	n/a	n/a	0	0%	n/a	n/a	0	0%	8.2	(4.7 - 11)	8	100%	n/a	n/a	0	0%	3.3	(1.4 - 5.9)	8	100%
Kidney	8	n/a	n/a	0	0%	1	n/a	1	13%	14	(9.8 - 19)	8	100%	n/a	n/a	0	0%	3	(1.5 - 3.9)	4	50%

[1] Where MC were detected in only one sample, this is the measured value, not the average

[2] "Detects" refers to the number of samples in which the MC was detected. The percentage of collected samples in which MC were detected is also indicated.

"n/a": not applicable

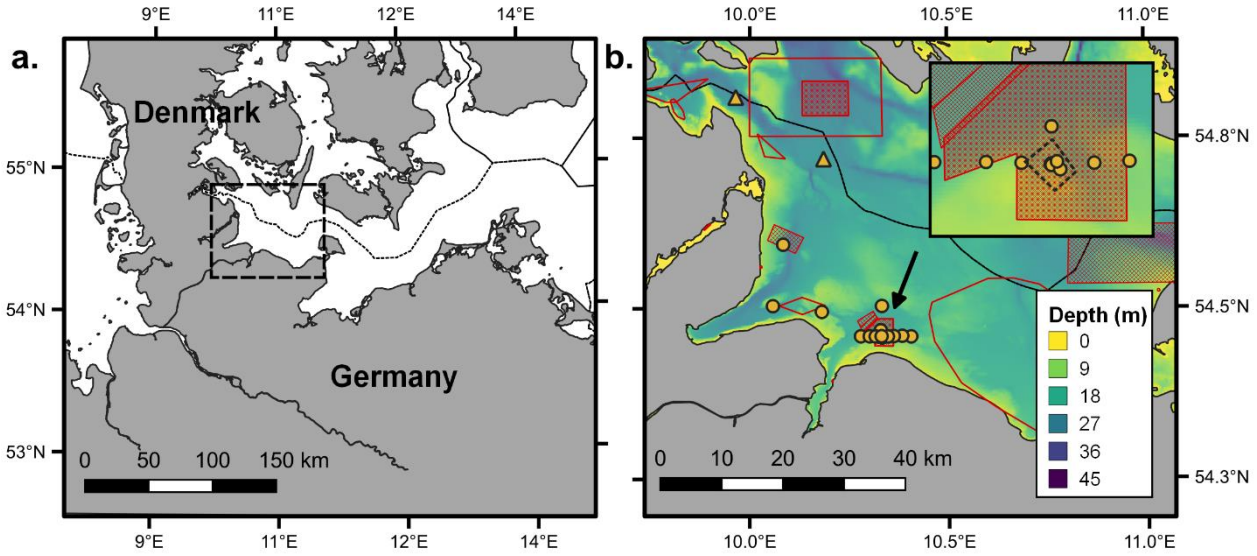

Figure 1. Study site. Panel b shows a close-up of the study region outlined by the dashed box in panel a. Dotted lines indication national EEZ boundaries. Samples were collected at locations indicated by the orange dots. Orange triangles indicate "control" fish sampling sites. Red boxes in panel b indicate known munitions hotspots: stippled areas are munitions dumpsites, cross-hatched areas are known to contain munitions, and open areas are strongy suspected to contain munitions (Böttcher et al., 2011; amucad.org). The arrow and inset in panel b indicate the Kolberger Heide dumpsite, with the marine traffic restricted zone outlined by the dashed black line. Bathymetry data in panel b are made available by EMODnet (emodnet.eu).

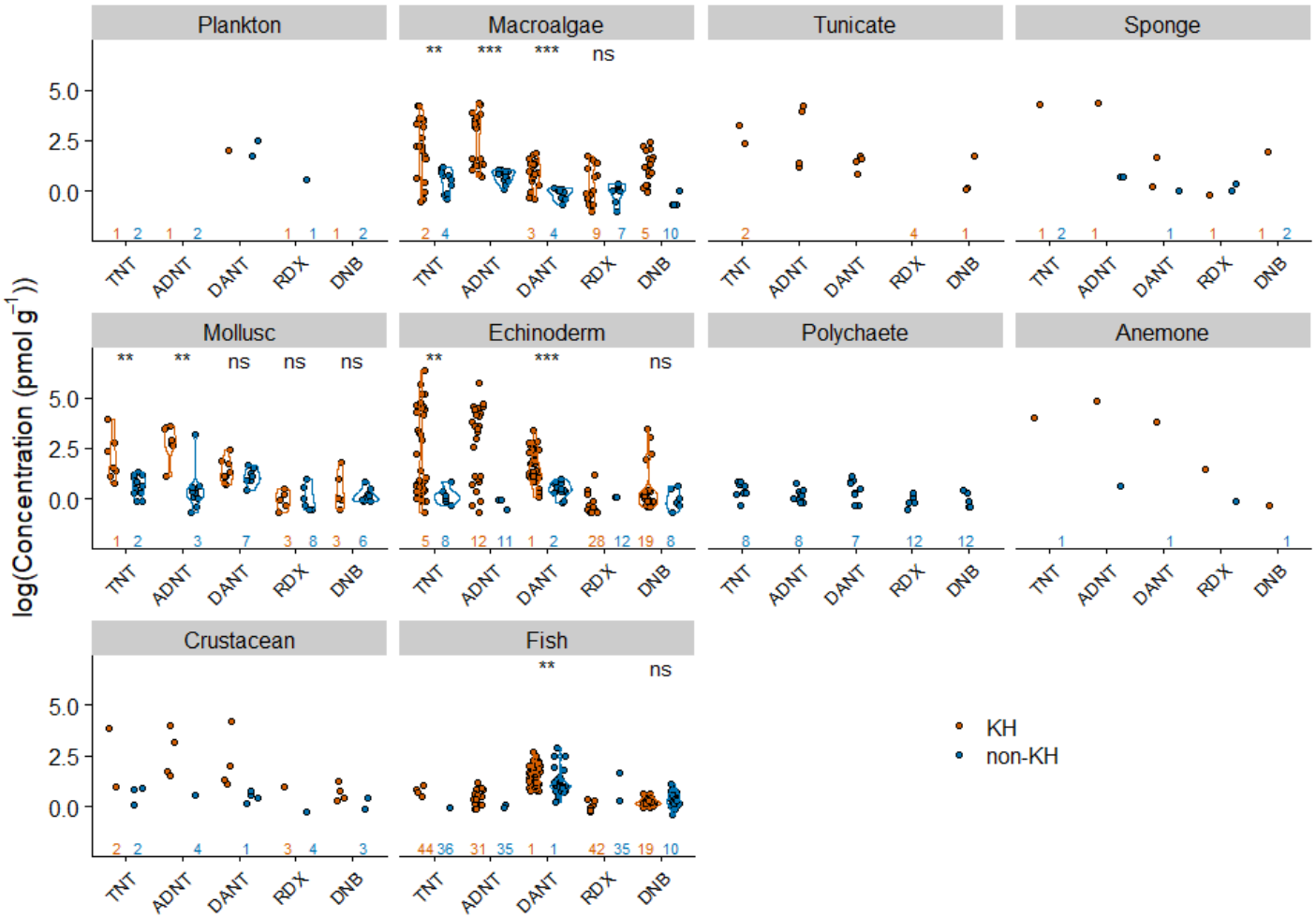

Figure 2. Tissue MC content in biota from the southwest Baltic Sea. "KH" refers to individuals collected within or near the Kolberger Heide munitions dumpsite; "non-KH" refers to those collected in other locations. ADNT and DANT are reported as the sum of their isomers. Values for individual samples are shown as dots, violin plots are added for site-organism combinations where n>4. The numbers of samples for each site-organism combination where compounds were not detected is indicated at the base of the graph (also see Table 1 for the detection rate). Results of analysis of statistical differences between sites (Wilcoxon signed rank test performed on KH versus non-KH pairs where n per organism at both sites >4) are indicated above the data: *- p<0.001, ** - p<0.01, *** - p<0.05 , ns - p>0.05.

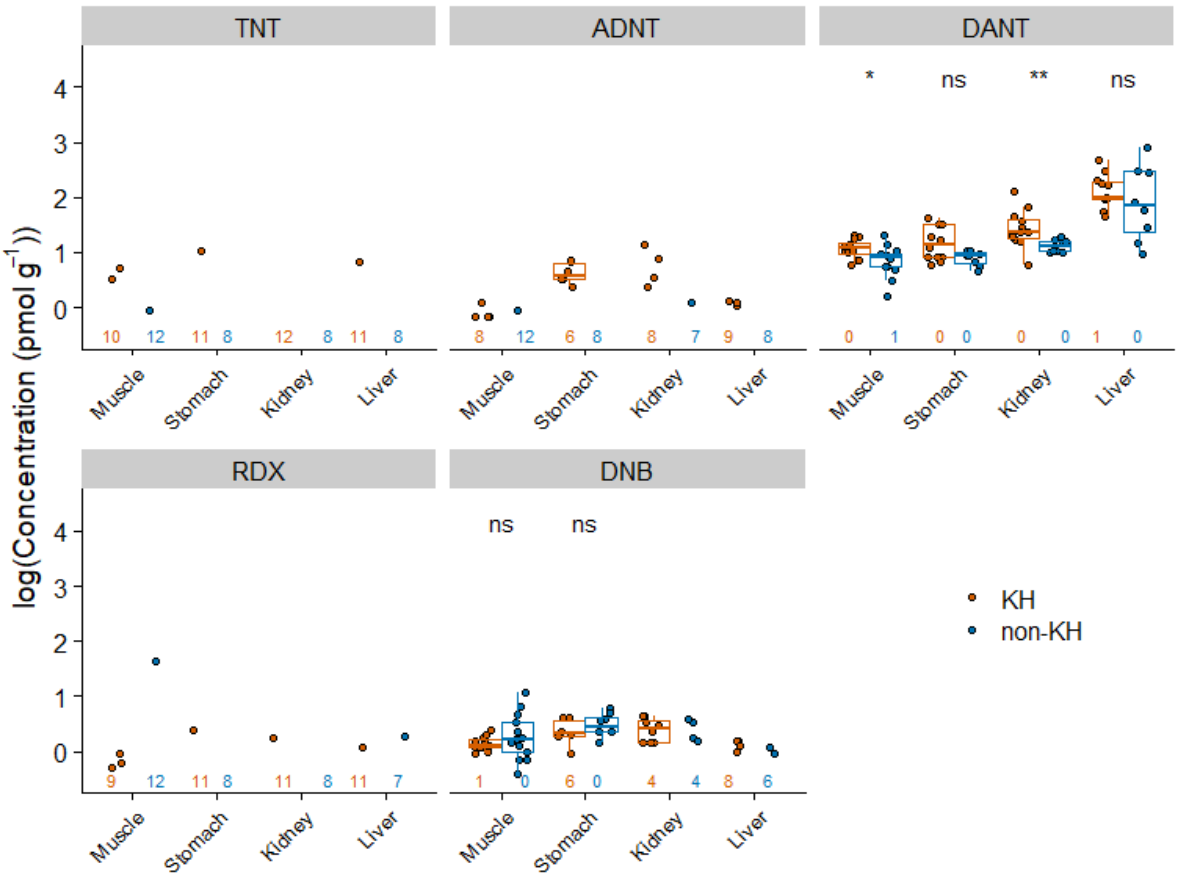

Figure 3. Munition compound content in different fish tissues. Symbols as described in Fig. 2.

25

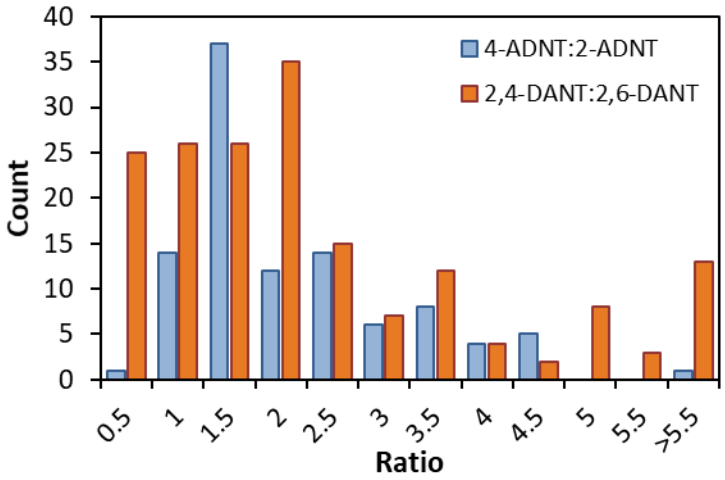

Figure 4. Histograms of ADNT (blue) and DANT (orange) isomer ratios in all samples where both isomers were detected.