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Warship wrecks and their munition cargos as a threat to the marine environment and humans: The V 1302 "JOHN MAHN" from World War II

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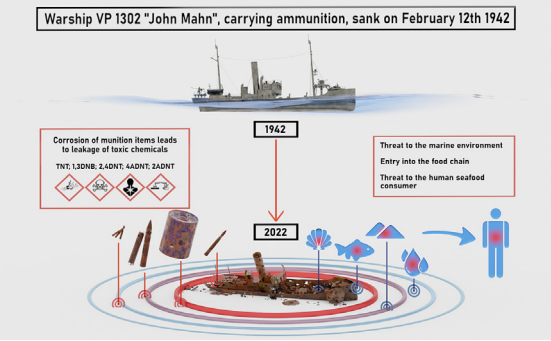
HIGHLIGHTS

- Warship wrecks with munitions on board emerge as point source for environmental risk.
- The World War II ship "Vorpostenboot 1302" ("JOHN MAHN") serves as our case study.
- Energetic chemicals leak out into the marine environment around the wreck.
- Fish and mussels at the wreck take up these toxic and carcinogenetic chemicals.

GRAPHICAL ABSTRACT



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ABSTRACT

In addition to endangering sea traffic, cable routes, and wind farms, sunken warship wrecks with dangerous cargo, fuel, or munitions on board may emerge as point sources for environmental damage. Energetic compounds such as TNT (which could leak from these munitions) are known for their toxicity, mutagenicity, and carcinogenicity. These compounds may cause potential adverse effects on marine life via contamination of the marine ecosystem, and their entry into the marine and human food chain could directly affect human health. To ascertain the impending danger of an environmental catastrophe posed by sunken warships, the North Sea Wrecks (NSW) project (funded by the Interreg North Sea Region Program) was launched in 2018. Based on historical data (derived from military archives) including the calculated amount of munitions still on board, its known location and accessibility, the German World War II ship "Vorpostenboot 1302" (former civilian name - "JOHN MAHN") was selected as a case study to investigate the leakage and distribution of toxic explosives in the marine environment. The wreck site and surrounding areas were mapped in great detail by scientific divers and a multibeam echosounder. Water and sediment samples were taken in a cross-shaped pattern around the wreck. To assess a possible entry into the marine food chain, caged mussels were exposed at the wreck, and wild fish (pouting), a sedentary species that stays locally at the wreck, were caught. All samples were analyzed for the presence of TNT and derivatives thereof by GC–MS/MS analysis. As a result, we could provide evidence that sunken warship wrecks emerge as a point source of contamination with nitroaromatic energetic compounds leaking from corroding munitions cargo still on board. Not only did we find these explosive substances in bottom water and sediment samples around the wreck, but also in the caged mussels as well as in wild fish living at the wreck. Fortunately so far, the concentrations found in mussel meat and fish filet were only in the one-digit ng per

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gram range thus indicating no current concern for the human seafood consumer. However, in the future the situation may worsen as the corrosion continues. From our study, it is proposed that wrecks should not only be ranked according to critical infrastructure and human activities at sea, but also to the threats they pose to the environment and the human seafood consumer.

1. Introduction

Beside the intensive exploitation of the oceans by overfishing and the discharge of hazardous substances by rivers, the seas worldwide are currently threatened by an emerging source of pollution. Several million metric tonnes of all kinds of munitions dumped into the seas during and after the two World Wars (WWI and WWII) are corroding and leaking toxic explosives (such as TNT and its metabolites) (Beck et al., 2019; Appel et al., 2018; Strehse et al., 2017; Böttcher et al., 2011; Carton and Jagusiewicz, 2011; Beddington and Kinloch, 2005) and chemical warfare agents (Bełdowski et al., 2016) into the marine environment. Disposal of the remaining munitions and chemical warfare materials in the North and Baltic Seas was considered the best option following the World Wars (Beck et al., 2018). Other sources of unexploded ordnances (UXOs) are mine belts that were planted to defend the coastlines and shipping routes, shot down fighter aircraft, leftover bombs that were dropped by aircraft to ensure a safe landing at home airports, or ships that were sunken intentionally or in combat (Lotufo et al., 2017; Böttcher et al., 2011; Rodacy et al., 2001). The global total amount of UXOs and discarded military munitions (DMMs) widely distributed in the marine environment is hard to quantify (Beddington and Kinloch, 2005). However, it has been reported that approximately 1.6 metric million tonnes of dumped conventional munitions lie in the German coastal areas of the North and Baltic Seas alone (Böttcher et al., 2011).

In addition, several thousand ships containing considerable amounts of munitions and fuels were lost at sea during WWI and WWII (Knobloch et al., 2013; Monfils, 2005); an estimated 7800 or more ships (both military and commercial) and vessels were sunk during WWII (Monfils, 2005). Likewise, it was common practice to dump redundant munitions together with the ships that carried them to the dumpsite after the wars. As a result, the seas contain several thousands of wrecks, and tons of conventional munitions and chemical warfare agents (Beck et al., 2018; Böttcher et al., 2011; Knobloch et al., 2013; Monfils, 2005).

Shipwrecks have generally been considered hazardous to shipping alone. Hence, their positions and minimum depths were often logged such that wrecks considered to be significantly dangerous were either removed or marked on the nautical charts, and with buoys, to ensure safe navigation. However, as is the case with dumped munitions casings, relevant wrecks that were sunk with munitions onboard have become of research and public interest (Knobloch et al., 2013; Monfils, 2005). A recent report revealed the deterioration of wrecks over time (Landquist et al., 2017). Thus, in addition to the steel components of the vessels (required for stability of the wrecks), the munition casings on these ships are corroding and releasing their toxic chemicals into the marine environment (Beck et al., 2022; Appel et al., 2018; Strehse et al., 2017), thereby posing the same risk as sea-dumped mines, bombs, and torpedo heads to the marine environment and/or the human seafood consumer (Ahvo et al., 2020; Missiaen et al., 2010).

Energetic compounds such as TNT and its derivatives are known for their toxicity, mutagenicity and carcinogenicity (Sabbioni and Rumler, 2007; Bolt et al., 2006; Talmage et al., 1999). Therefore, persistent contamination of the marine ecosystem may cause potential adverse effects to marine life, and directly affect human health via entry into the marine and human food chain, (Maser and Strehse, 2021; Beddington and Kinloch, 2005). To date, very little is known about the bioavailability, accumulation, distribution, metabolism, detoxification, excretion, and in vivo toxic properties of sea-dumped munitions (Lotufo et al., 2016; Ballentine et al., 2015; Rosen and Lotufo, 2007a, 2007b; Nipper et al., 2001). Recent studies have documented the presence of munition compounds in commercially-important fish and mussel species, thus raising the issue of food safety (Koske et al., 2020; Appel et al., 2018; Strehse et al., 2017).

The North Sea is littered with remnant wrecks and munitions from both WWI and WWII (naval and air battles), and the toxic munitions remaining in these deteriorating objects could endanger the marine environment and human seafood consumer (Maser and Strehse, 2021; Monfils, 2005). In view of this, the North Sea Wrecks (NSW) project was launched in 2018 (https://northsearegion.eu/nsw/about/). This project is being funded under priority 3 of the Interreg North Sea Region Program and aims at investigating nationally fragmented data sources on wrecks, cargos, and munitions. As its long-term goal, the NSW aims at providing the tools necessary for authorities, response organizations, economic actors, and other stakeholders to assess and propose solutions for risk mitigation regarding wrecks and munitions in the North Sea.

The present study focused on the German World War II ship "Vorpostenboot 1302" (former civilian name - "JOHN MAHN") as a case study for a comprehensive and scientific-based risk assessment on the leakage and distribution of toxic explosives into the marine environment. This choice was based on relevant criteria such as the amount and type of munitions onboard, the wreck location and depth, distance to the coast or infrastructure, the age and the condition of the wreck and its overall accessibility from the Belgian coast. After visual inspection, the presence of munition compounds in water, sediments, and fish samples was investigated. Monitoring of munition compounds was also performed with caged mussels (*Mytilus edulis*) and passive sampling systems. To the best of our knowledge, this is the first comprehensive study of submerged warships and their potential environmental impact in terms of explosives leaking from corroding munitions.

2. Materials and methods

2.1. The European Interreg project North Sea Wrecks (NSW)

To get a deeper inside into the problem of sunken warships and their significance in environmental issues, a European Interreg North Sea Region research project was started in 2018 called North Sea Wrecks (NSW). This project is a cooperation between research organizations in Belgium, the Netherlands, Germany, Denmark, and Norway, where each country is investigating several wrecks within their territorial waters and/or EEZs. The main focus was on munitions that are still onboard the sunken wrecks and their possible impact on the marine environment. The criteria for selection were the availability of historical information about the ship and the sinking scenario at site, the accessibility of the wreck to divers, and the suspected amount of munitions items onboard at the time of sinking.

2.2. Historical research in the Federal Archives-Military Archives

The Federal Archives-Military Archives (BArch-MA) in Freiburg (Breisgau, Germany) (www.bundesarchiv.de) is a department of the Federal Archives for protection, indexing and storage of German military records (since 1867). Prior to commencing this study, comprehensive historical research was performed to estimate the location, role in war, original armament, and amount of explosives still onboard on wrecks that were considered questionable. Historical sources such as war diaries, fight reports, as well as contemporary reports about sinking conditions, were included; all data were compared with information from maritime authorities. As a result, the wreck of "Vorpostenboot 1302 (V 1302)" (former civilian name JOHN MAHN) was selected as the research focus. For further details on the sinking of the V 1302 war diaries of the 13th Outpost Flotilla,

the operation "CERBERUS" (Bundesarchiv, 2022), and the prevailing naval structure in the North Sea were consulted.

2.3. Wreck inspection and sampling procedure

The Belgian part of the North Sea is typified by a shallow, tidally dominated, sandbank system with creeping sand waves (Belgische Staat, 2018). The wreck of the V 1302 was first explored by scientific divers on the 26th of February 2019. It lies some 37 km northwest of Zeebrugge at 51° 28,7 N; 02°43,3 O (Lettens, 2002; Gröner et al., 1993) and stands mostly intact and upright between 21 m and 35 m of depth. The wreck is positioned on its keel with a slight slope to starboard side (25°) in east-west direction. Below the former water line close to the bridge a big hole is visible. The deck cabin lies besides the wreck. At the bow is a zone storing anti-aircraft munitions. Depth charges can be found on the stern. The wreck is entangled with nets, trawls and fishing lines (Lettens, 2002). Subsequent dives were performed in May and August of 2019 to conduct a full visual inspection and photographical documentation of the wreck. The wreck site and surrounding areas were mapped in detail using the hull-mounted Kongsberg EM2040 multibeam echosounder of the RV Simon Stevin in August 2019.

On the 16th of March 2020, sediment and water samples were collected near the wreck of the V 1302 using the Simon Stevin and following established sampling procedures (UDEMM, 2019; Gledhill et al., 2019; Lotufo et al., 2017; University of Hawaii, 2014; NNAVAK NW, 2010; Pascoe et al., 2010; Ochsenbein et al., 2008). A low-resolution multibeam image was made for navigation purposes. Next, 12 sampling stations were chosen in a cross-shaped pattern around the wreck, with four stations on both the port and starboard sides of the wreck and two stations near both the stern and bow of the vessel. The intended stations were about 20 m apart. Within one hour, a 1000 cm^2 Van Veen grab with a sampling depth of approximately 15 cm was used to collect a sediment sample at each location. Once on board, the sediments were mixed by hand, stored in a one-liter polyethylene bottle, and frozen at −20 °C. Afterwards, a four-liter Niskin bottle mounted on a carrousel was lowered to one meter above the seabed to collect four water samples – one from each side of the wreck. The water was transferred into one-liter polyethylene bottles and frozen at −20 °C on board. The actual sample coordinates were derived from real-time kinematic positioning (RTK) during each sampling moment; these were later retrieved from the Marine Information and Data Acquisition System of Flanders Marine Institute. All samples were sent to the Institute of Toxicology at Kiel University Medical School (Germany) for chemical analyses of dissolved explosives and their metabolites.

2.4. Biomonitoring with blue mussels (Mytilus edulis) and passive sampling systems

Blue mussels (*Mytilus edulis*) were collected from a reference site, the offshore windmill farm Belwind, on the 17th of March 2020. They were plucked from pylon C5 (51°40′ N; 2°48′ E) at about 10 m depth by scientific divers. Mussels with a shell length of 6 to 8 cm were chosen, put into nylon nets and kept cool in a flow-through system fed by the ships' seawater pump. The next day, two passive samplers (Chemcatcher®) were added to each net and the bags were placed on the wreck at 30 m depth by divers. The use of passive samplers allows a time-averaged determination of energetic compounds within the water column over time. Mussels were placed along a transect over the entire length of the wreck and labelled as M1/PS1 – M4/PS4. After four months, divers collected the mussel nets that were planted with the passive samplers. Mussels were dissected in the ship's laboratory; the tissues were snap-frozen and stored in liquid nitrogen vapour on board and then transported at −80 °C to the laboratory on land for GC–MS/MS analysis. The passive sampler membranes were cooled on ice, transported to the laboratory and stored at +4 °C until use.

2.5. Fishing and fish preparation

Fish (pouting; *Trisopterus luscus; a* sedentary species that stays locally at their wrecks) were caught using sports fishing gear. Twenty specimens above 25 cm long were caught at the wreck site. Fish were anesthetized by a blow on the head, followed by decapitation and subsequent dissection. The bile was collected from gall bladder with disposable needles (0.15 mm × 35 mm) and syringes (1 mL), and transferred into cryo vials before snap-freezing in liquid nitrogen. Approximately 20 g skin free muscle tissue was dissected from the fish filet, snap-frozen in liquid nitrogen and stored in 15 mL Falcon tubes at −20 °C for chemical analysis.

2.6. Chemical laboratory analyses

2.6.1. Materials and chemicals

For calibration, trinitrotoluene (98.9 % purity, 1 mg/mL, in acetonitrile (ACN):methanol (MeOH) 50:50), 1,3-dinitrobenzene (97.0 % purity, 1 mg/mL, in ACN:MeOH 50:50), 2,4-dinitrotoluene (98.3 % purity, 1 mg/mL in ACN:MeOH 50:50), 4-amino-2,6-dinitrotoluene (98.4 % purity, 1 mg/mL, in ACN:MeOH 50:50) and 2-amino-4,6-dinitrotoluene (97.8 % purity, 1 mg/mL, in ACN:MeOH 50:50) were purchased from AccuStandard, New Haven, USA. For spiking, Isotopically-labelled TNT ($^{13}C7$, 99 %; $^{15}N3$, 98 %, 1 mg/mL in benzene, wetted with >33 % H_2O) was purchased from Cambridge Isotope Laboratories, Inc., Andover, USA. Acetonitrile (UHPLC-grade, purity ≥99.97 %) was purchased from Th. Geyer (Renningen, Germany) and used without further purification. CHROMABOND Easy polystyrene-divinylbenzene-copolymer reversed-phase solid phase extraction columns 80 μm, 3 mL/200 mg and 1 mL/30 mg (Macherey Nagel, Düren, Germany) were used. Chemcatcher passive sampler with HLB-L receiving phase discs and PES diffusion limiting membranes were obtained from T.E Laboratories Ltd. (Tullow, Ireland) and activated according to the manufacturer's specifications. Ultrapure water (18.2 MΩ cm) was prepared on site with a Veolia ELGA Purelab Flex system. β-Glucuronidase type H-1 from *Helix pomatia* was used (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany).

2.6.2. Water sample preparation

Water samples were prepared by an adapted method according to the UDEMM Best Practice Guide (UDEMM, 2019). One-liter seawater was transferred into an EVA infusion bag (ICU Medical, Inc., San Clemente, CA, USA) with 25 ng $^{13}C^{15}N$-TNT as internal standard and allowed to flow through an unconditioned 3 mL Chromabond Easy SPE column, in the absence of light at 4 °C. The effluent was discarded. Columns were dried i.vac (0.5 h) and eluted with 4 mL ACN. The eluate was concentrated to 600 μL using a RVC 2–25 CDplus rotary vacuum concentrator (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) and stored in 1.5 mL amber vials at −20 °C.

2.6.3. Sediment sample preparation

Sediment samples were extracted as described in Bünning et al. (2021). In brief, 100 g wet sediment was mixed with 250 mL ultrapure water, spiked with 25 ng $^{13}C^{15}N$-TNT, shaken for 80 min and sonicated for 15 min. Samples were then centrifuged at 4500 rpm (10 °C) for 15 min (J2-HS centrifuge, Beckman Coulter GmbH, Krefeld, Germany), filtered through a 595 1/2 pleated filter and applied onto SPE-columns using a mild vacuum. Columns were dried for 30 min i.vac., eluted with 4 mL ACN, concentrated to 600 μL and stored at −20 °C in 1.5 mL amber vials.

2.6.4. Passive sampler preparation

Passive samplers (Chemcatcher®) adsorb chemicals over a period of time by flow through diffusion membranes. The receiving phase disc and diffusion limiting membrane were retrieved from the passive sampler and separated, dried separately for 30 min i.vac., and each eluted for 1 h in 20 mL ACN at 230 rpm on an Unimax 1010 shaker (Heidolph, Schwabach, Germany). The eluate was concentrated to 600 μL and measured directly with GC–MS/MS.

Table 1
GC–MS/MS programs for splitless and large volume injections.

Parameter	Splitless	Large volume Injection
Injector	Split − /splitless	Programmable temp. Vaporization
Inlet liner	Quartz wool	Quartz wool
Injection volume	1 μL	5 μL
Injection temp.	230 °C	70 °C, (0.18 min, 50 mL × min^{-1}), 5 °C/s to 240 °C (1.5 min, no split), 240 °C (5 min, 200 mL/min^{-1})
Column flow	1.5 mL × min^{-1}	1.2 mL × min^{-1}
Oven temp.	100 °C (0.20 min), 30 °C/min to 220 °C (0.30 min), 80 °C to 280 °C (1 min)	100 °C (1 min), 35 °C/min to 220 °C (0.7 min), 70 °C to 280 °C (1 min)
Total run time	6.25 min	6.99 min
Transfer line temp.	250 °C	250 °C
Ion source temp.	300 °C	300 °C
Ionization method	EI	EI

2.6.5. Blue mussel and fish filet sample preparation

Blue mussels were processed as described in Bünning et al. (2021). The method for fish filet was adjusted accordingly. Mussel flesh and fish filets were freeze-dried for 48 to 72 h. Lyophilized samples were homogenized using an IKA A11 basic analytical mill (IKA Werke GmbH & Co. Kg, Staufen im Breisgrau, Germany), and aliquots of 500 mg were weighed into light-proof 5 mL tubes. Two mL of ACN and 10 μL of a 100 ng/mL $^{13}C^{15}N$-TNT solution was added as internal standard. Samples were vortexed for 60 s, sonicated for 15 min, and centrifuged at 4100 rpm (4 °C) for 15 min. Supernatants were transferred into 25 mL graded flasks, diluted with ultrapure water and applied onto unconditioned Chromabond Easy SPE-columns using mild vacuum. Columns were then dried i.vac for 30 min, and samples were eluted with 4 mL ACN, concentrated to 600 μL and stored at −80 °C in 1.5 mL amber vials.

2.6.6. Fish bile preparation

One hundred μL of bile was added to a 1.5 mL microreaction tube containing 3600 units of *β*-glucuronidase from *Helix pomatia* dissolved in 400 μL sodium acetate buffer (pH 4.8), and 0.5 ng $^{13}C^{15}N$-TNT. For fish with lesser volumes of bile, the amounts obtained were pooled into volumes of 100 μL and processed as indicated above. Samples were incubated at 37 °C for 20 h in a Thermomixer Compact (Eppendorf, Hamburg, Germany). After cooling to room temperature, the samples were loaded onto 1 mL Chromabond Easy columns (preconditioned with 300 μL ultrapure water, 600 μL MeOH, and 300 μL ultrapure H_2O). The microreaction tubes were rinsed three more times with 500 μL of ultrapure water each, which was also given onto the columns. Columns were dried i. vac. for 15 min, followed by elution with 5-times 50 μL ACN. The eluate was transferred into 1.5 mL amber glass vials with 250 μL glass inserts and stored at −80 °C.

Table 3
Method specific limits of detection (LoD) and quantification (LoQ).

Explosive	Splitless			PTV-large volume		
	LOD [fg/μL]	LOQ [fg/μL]	R^2	LOD [fg/μL]	LOQ [fg/μL]	R^2
1,3-DNB	333	1099	0.9444	32	105	0.9644
2,4-DNT	77	254	0.9968	10	33	0.9934
TNT	152	502	0.9879	47	155	0.9878
4-ADNT	95	314	0.9951	8	26	0.9959
2-ADNT	103	341	0.9943	11	37	0.9919

Method-specific detection limits (LODs) were determined as described by Bünning et al. (2021), using solvent standards according to the EUR 28099 EN calibration standard method. Volumes applied to columns are 1 μL and 5 μL for the splitless and the PTV large volume methods, respectively. The limit of quantification (LOQ) was set at 3.3 times the detection limit.

2.6.7. GC–MS/MS analysis

A Thermo Scientific TRACE 1310 gas chromatograph, coupled to a TSQ 8000 EVO triple quadrupole mass spectrometer with electron ionization source was used in secondary reaction monitoring (SRM) mode. The GC was equipped with a TraceGold TG-5MS amine 15 m × 0.25 mm × 0.25 μm column (Thermo Fisher Scientific Inc., Waltham, MA, USA). For water-, sediment- and passive sampler samples, splitless injections on a split − /splitless-injector were performed on quartz wool injection port liners (4 mm × 6.5 mm × 78.5 mm, Thermo Fisher Scientific Inc., Waltham, MA, USA). Injections of biota samples were carried out on a programmable temperature vaporization (PTV)-injector with packed quartz wool liners (2 mm × 2.75 mm × 120 mm, Thermo Fisher Scientific Inc., Waltham, MA, USA)). Helium served as carrier gas for the GC, and Argon as collision gas for the mass spectrometer (both Alphagaz, purity 99.999 %). Spectra were recorded and analyzed in Chromeleon 7.2 (Thermo Fisher Scientific Inc., Waltham, MA, USA). GC–MS/MS programs developed for this study are presented in Table 1, retention times and (SRM) transitions are shown in Table 2.

Method-specific detection limits (LODs, Table 3) were determined as described in Bünning et al. (2021) using solvent standards according to the EUR 28099 EN calibration standard method. The limit of quantification (LOQ) was set at 3.3 times the detection limit.

Table 2
Retention times for the splitless (Rt SL) and PTV-large volume injection (Rt LVI) methods.

Compound	Rt SL [min]	Rt LVI [min]	Molecular mass [g × mol^{-1}]	Transition [m/z]		CE [eV]
1,3-Dinitrobenzene	2.43	3.20	168.11	Q	122.0 > 75.0	12
				q	168.0 > 75.0	20
				q	168.0 > 122.0	8
2,4-Dinitrotoluene	2.77	3.52	182.13	Q	165.0 > 63.1	22
				q	165.0 > 90.1	16
				q	165.0 > 118.1	8
Trinitrotoluene	3.41	4.09	227.13	Q	210.0 > 164.1	6
				q	164.0 > 90.1	10
				q	108.0 > 76.1	12
$^{13}C^{15}N$-Trinitrotoluene	3.41	4.09	237.06	Q	220.1 > 173.1	6
				q	220.1 > 203.1	8
				q	189.1 > 82.1	10
4-Amino-2,6-dinitrotoluene	4.22	4.85	197.15	Q	197.0 > 180.1	6
				q	180.0 > 163.1	8
				q	163.0 > 78.0	14
2-Amino-4,6-dinitrotoluene	4.42	5.07	197.15	Q	197.0 > 180.1	6
				q	180.0 > 133.0	6
				q	180.0 > 67.0	12

Quantitative (Q) and qualitative (q) secondary reaction monitoring transitions (m/z) of explosives and internal standard $^{13}C^{15}N$-TNT are given.

3. Results

3.1. Historical data from the "Vorpostenboat 1302" (former civilian name "JOHN MAHN")

In the project North Sea Wrecks (NSW) a historical research was conducted in the Federal Archives-Military Archives (BArch-MA) in Freiburg, Germany, to select a suitable ship wreck as a case study for potential environmental pollution from munition-containing wrecks in the North Sea. Based on relevant criteria such as the age and condition of the wreck, the amount and type of munitions onboard, the wreck location and depth as well as the distance to the coast or infrastructure (the latter criteria were important for determining accessibility to the wreck site), the "Vorpostenboot V 1302" at the Belgian coast was selected.

The JOHN MAHN was built in 1927 at the REIHERSTIEG shipyard in Hamburg (Germany) as a robust fishing vessel (under the construction number 580). With a range of >6000 nautical miles, it was suitable for embarking on fishing trips to the North Atlantic. Until 1932, it sailed as SD 131 JOHN MAHN, as a fishing vessel from March 9, 1932 to September 28, 1939 (under the registration BX 221). In 1939, the German Navy confiscated the ownership of JOHN MAHN, converted it to a "Vorpostenboot" and put it into service as the V 1302 with the 13th "Vorpostenflottille". (Fig. 1).

On February 12, 1942, it was assigned with the special task of serving as a navigation point and anti-air defense for the heavy cruiser Prinz Eugen and battleships Scharnhorst and Gneisenau that were evacuating from Brest to German ports during Operation Cerberus. For this purpose, V 1302 "JOHN MAHN" and the sister "Vorpostenboot" V 1303 were fully armed before deployment. While V 1303 anchored at a fixed point, V 1302 was in a dynamic position (about 300 m south to the former). Research in the diary of V 1302 "JOHN MAHN" in the Federal German Military Archive in Freiburg revealed clearly the fight situation and the location where it sank (51°28′ N; 2°41′ E), after having been attacked by British aircraft on February 12th, 1942 (Figs. 2 and 3). The damage observed on the wreck (Fig. 4) is also consistent with the battle report.

The wartime records show that part of the anti-aircraft ammunition was used during a short but intensive fight, while the depth charges and much of the other munitions were not used and should still be lying on or around the wreck. From the battle report on fired munitions, the remaining load on board can be estimated both quantitatively and qualitatively. Although some uncertainties remain, this estimation is of high importance to the risk assessment tool that is being developed by the NSW project. According to the war diary and the battle report, only the forward Anti-Aircraft (AA) gun (2 cm) was fired. The consumption of ammunition (based on timeframe, reaction time, distance to the aircrafts, and firing rate of the guns), allowed calculation of the amount of remnant on board the V 1302 wreck. Overall, almost one metric ton of nitroaromatic explosives is expected to be still onboard the V 1302 wreck (Table 4).

3.2. Occurrence of munitions compounds around the wreck

3.2.1. Energetic compounds in the surrounding water

Chemical analysis of water samples taken at 30 m depth near the wreck was performed using high resolution GC–MS/MS, to estimate the release of explosive chemicals from the wreck (Fig. 5A). Unfortunately, the passive sampler from the stern (PS4) of the wreck was lost during the sampling procedure.

In the water samples, 2,4,6-trinitrotoluene (TNT – the parent compound; mean = 6.3 ng/L) was the most abundant energetic compound, while the TNT metabolites 1,3-dinitrobenzene (1,3-DNB; mean = 0.7 ng/L), 2-amino-4,6-dinitrotoluene (2-ADNT; mean = 1.3 ng/L) and 4-amino-2,6-dinitrotoluene (4-ADNT; mean = 1.0 ng/L) were comparatively less abundant. This hints at the metabolism of TNT in the wreck or its vicinity. The diamino metabolite 2,4-diamino-6-nitrotoluene (2,4-DNT) has not been detected (below the limit of detection; LOD; Fig. 5A). Moreover, higher concentrations of TNT (approximately fivefold) were recorded at the starboard side (W1), compared to the other two sampling positions - the stern (W2) and the port side (W3).

3.2.2. Energetic compounds in the surrounding sediment

Low concentrations of energetic compounds were also detected in the sediment samples around the V 1302 wreck (Fig. 5B). Concentrations in all 12 samples (S1 - S12; selected in a cross-shaped pattern around the wreck) were comparable (in the single-digit ng/kg range), although samples from the starboard and port sides (S4 and S7, respectively) recorded TNT concentrations in the low two-digit ng/kg range. It is noteworthy that all measurements were recorded at trace level. As a result, even two to threefold differences between low two-digits and single-digit numbers

Fig. 1. Model of the transition from civilian "JOHN MAHN" to the military "Vorpostenboot V1302".
The three-dimensional model (designed with the software "Blender") was based on blueprints, archived documents and photos of similar ships.

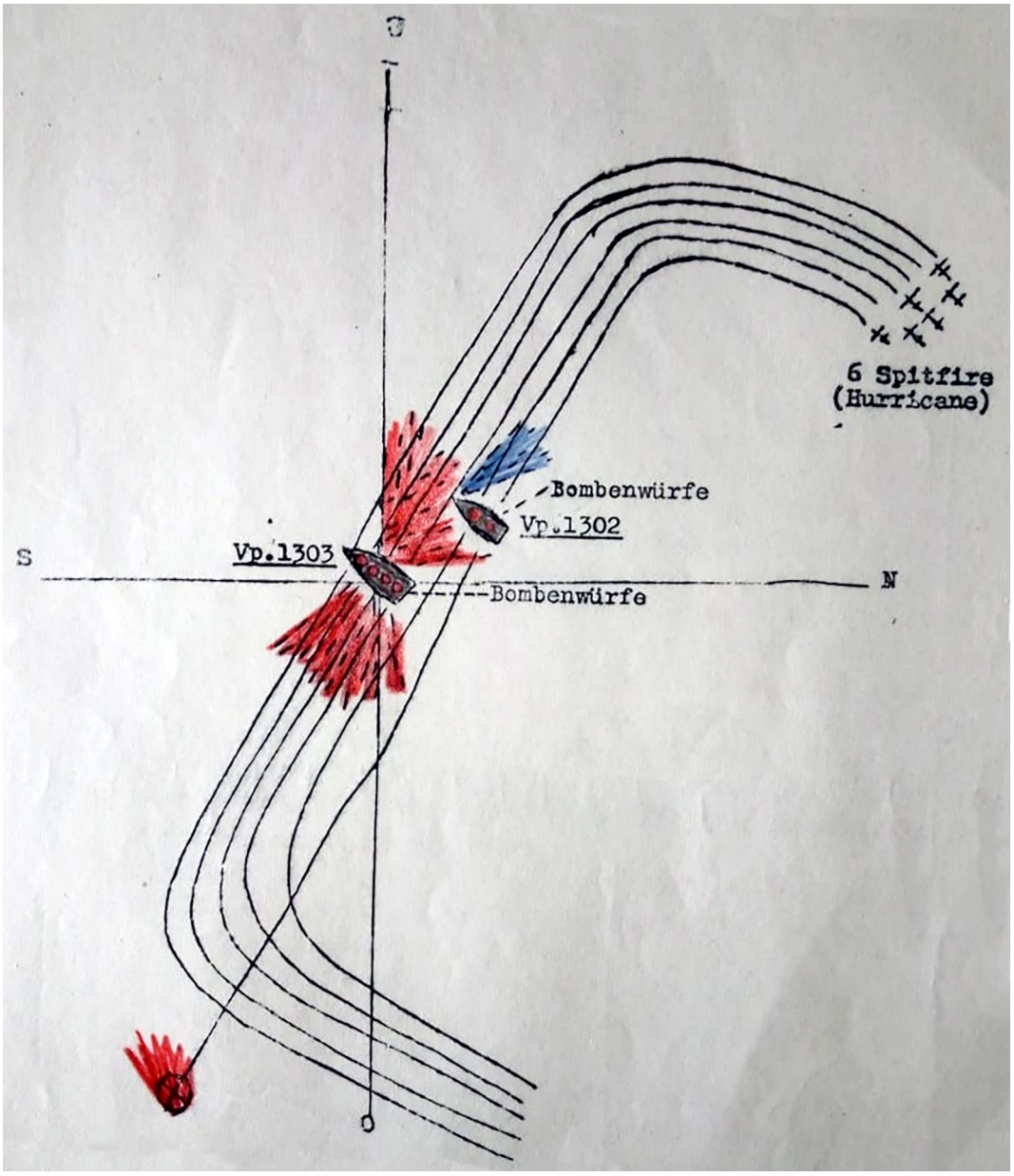

Fig. 2. Military-historical drawing of the battle prior to the sinking of V 1302.
Both V 1303 and V 1302 were attacked by six English Spitfire (Hurricane) aircraft and destroyed by the bombing raids. One aircraft was shot down by V 1302 during the attack. (Source of the document: RM 72-146 Kriegstagebuch 13. VP Flottille).

could actually be interpreted as marginal. The most dominant energetic compound detected in the sediment samples was TNT (up to 12.6 ng/kg), while the concentrations of 4-ADNT and 2-ADNT were generally lower (around or below 5 ng/kg). The level of 4-ADNT was approximately one to twofold higher than 2-ADNT. At all stations, 1,3-DNB and 2,4-DNT were under the LOD. Therefore, the presence of explosive chemicals in all sediment samples obtained from the V 1302 wreck vicinity indicates the leakage of these compounds from munitions onboard the wreck.

3.3. Biomonitoring with blue mussels (Mytilus edulis) and passive sampling systems

The use of mussels as a monitoring system can be regarded as biomonitoring, and it provides the first indication of the entry of munitions chemicals into the marine food chain. The mussels (M) were exposed together with the passive samplers (PS) at four stations (M1/PS1, M2/PS2, M3/PS3, and M4; PS4 was lost) across the entire length of the wreck (Fig. 6A). The presence of energetic compounds in the tissues of the mussels and passive samplers was subsequently examined using GC–MS/MS. Results obtained revealed the presence of explosive chemicals in mussels exposed at the V 1302 wreck, with an average total concentration of approximately two to four ng/g mussel dry weight (d.w.) for all the energetic compounds assessed (Fig. 6A). Furthermore, TNT levels were the highest in all samples; the TNT metabolites 1,3-DNB, 2-ADNT, and 4-ADNT were distinctly lower. 2,4-DNT was not detected (below LOD).

Passive samplers can be regarded as “artificial” mussels. They adsorb chemicals over a period of time (through their membranes) in a passive, rather than active manner (as the name suggests). Three passive samplers PS1, PS2, PS3 at the V 1302 wreck (Fig. 4) were eluted and analyzed for the presence of energetic compounds. Again, data obtained from these samples revealed the presence of explosive chemicals at the V 1302 wreck, with a mean total concentration of approximately 10 to 40 ng/sampler for all the energetic compounds (Fig. 6B). While 1,3-DNB was the most abundant compound in PS2 alone (28.7 ng), TNT levels were noticeably high in all

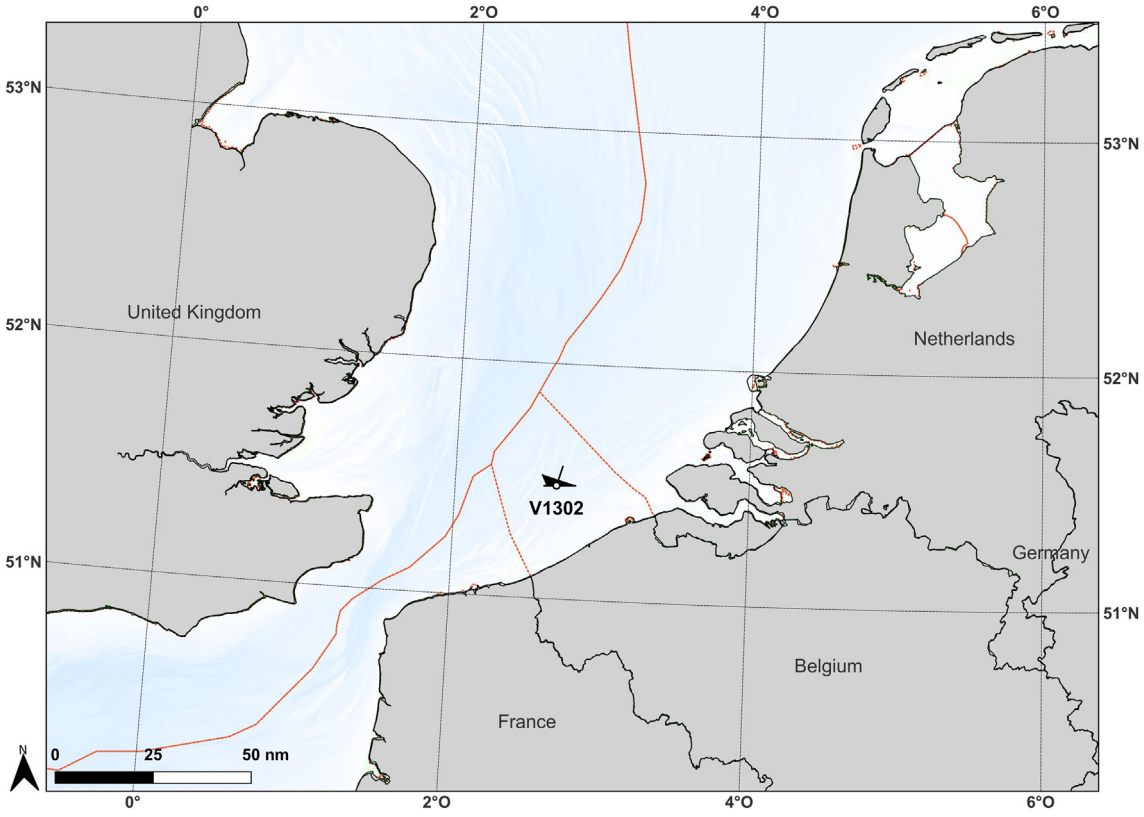

Fig. 3. Location of the V 1302 "JOHN MAHN" wreck in the North Sea
The wreck is located in the Belgian Part of the North Sea (51°28,937′ N; 2°41 E).

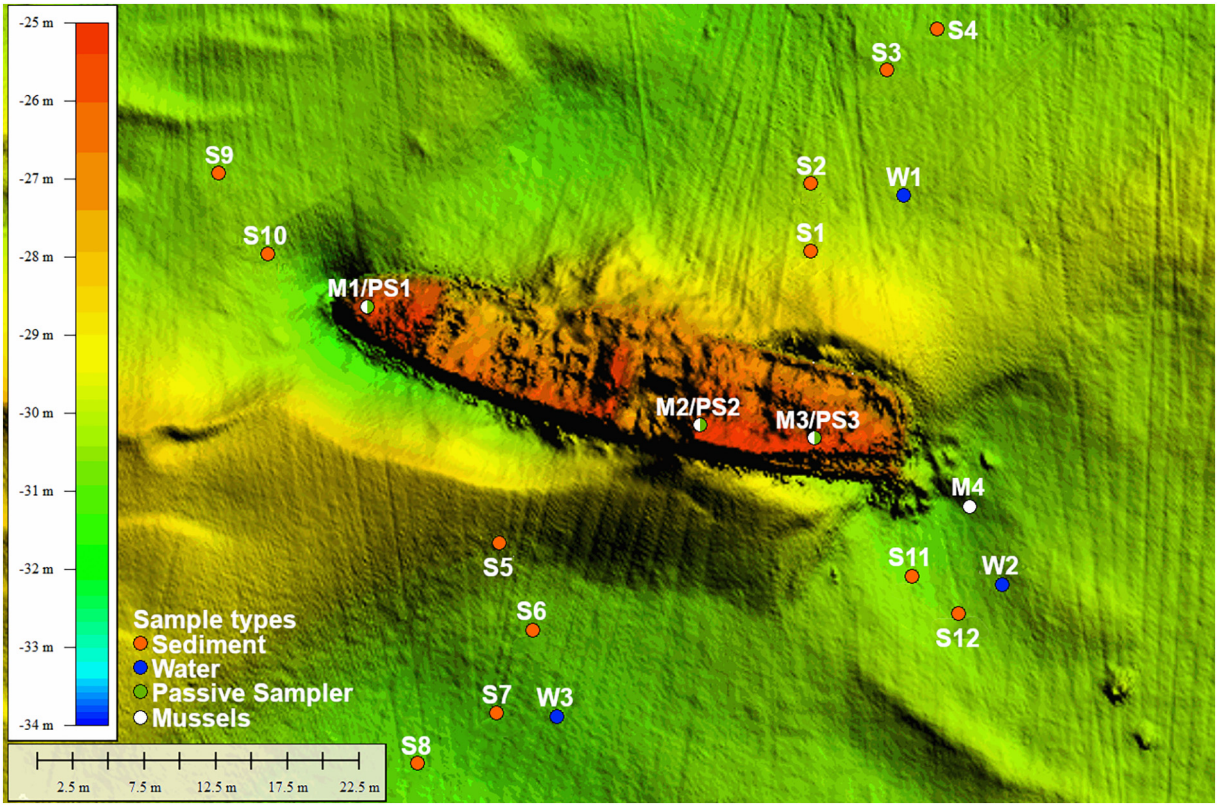

Fig. 4. A multibeam image of the V 1302 wreck indicating sampling stations.
Three water samples (W1 - W3) and 12 sediment samples (S1 - S12, collected in a cross-shaped pattern around the wreck) were obtained from the wreck site. Three nets containing mussels (M) and passive samplers (PS) were placed along a transect of the entire length of the wreck and labelled as M1/PS1 – M3/PS3. The last net M4 was recovered with only the mussels inside, missing the passive sampler.

Table 4
Calculation of the remaining amount of ammunition left on the V 1302 wreck.

Shell type	Rounds (- fired)	Explosive (type)	Explosive (total)
8.8 cm Flak	100	0.776 kg (FP)	77,6 kg
3.7 cm Flak	3000	0.027 kg (FP)	82,2 kg
2.0 cm Flak	20,000 (- 120)	0.006 kg (NP)	124 kg
Depth charges	10	65 kg (Schw. 18)	650 kg
Sunk with the wreck	22,990		933.1 kg

The consumption of ammunition based on distance to the aircrafts and firing rate of the guns allows estimation of the amount of remnants onboard the V 1302 wreck. It is presumed that almost one metric ton of nitroaromatic explosives still remains onboard the V 1302 wreck.

PS samples (PS1:19.1 ng, PS2: 17.36 ng, and PS3: 7,6 ng). The TNT metabolites 2- and 4-ADNT were in the single-digit ng range, whereas 2,4-DNT was not detected.

3.4. Energetic compounds in fish from the V 1302 site

There is considerable interest in the potential absorption of energetic compounds (from munition remnants of the wreck) by fish, and their subsequent accumulation in edible filets. In addition to the filet, the bile (which usually serves as a diagnostic matrix for toxic compounds in an organism) was examined. Interestingly, energetic compounds were detected in the fish (*Trisopterus luscus)* filets. As shown in Fig. 7A, TNT was the most prominent, while 4-ADNT and 2-ADNT were less abundant. The mean total concentrations of all the explosive compounds (TNT and metabolites) were variable, in the single-digit ng/g (d.w.) range. However, two fish specimens contained relatively high TNT concentrations of around 10 and 13 ng/g (d.w.).

Surprisingly, fish bile (which serves as a major excretion route in an organism -from the liver to the intestine) contained only low concentrations of 4-ADNT and 2-ADNT (median was approximately 0.4 ng/mL), while TNT was absent (Fig. 7B). These findings hint at an alternative route for the entry of energetic compounds into fish.

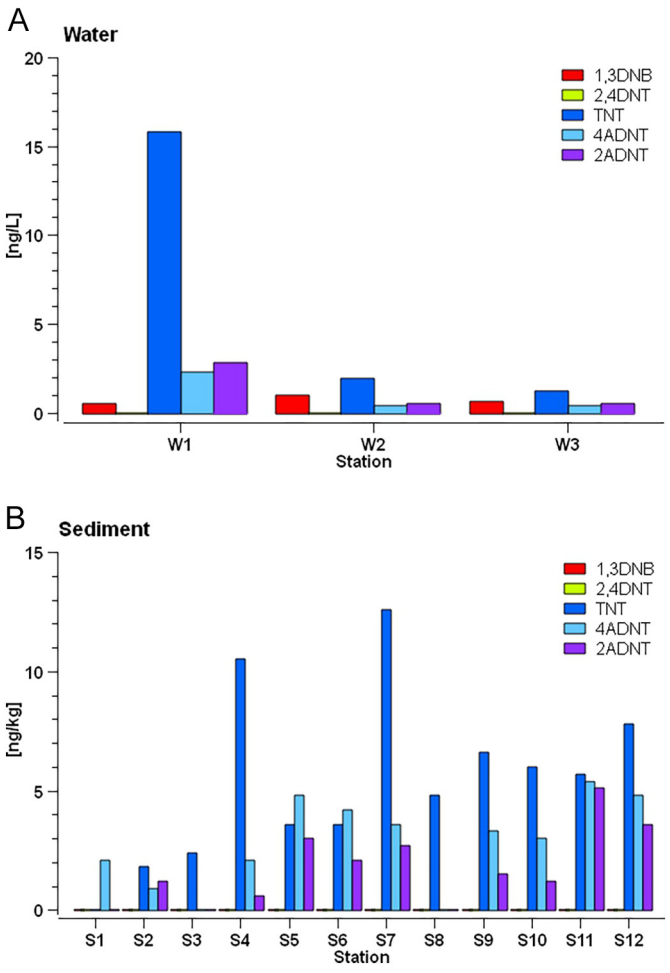

Fig. 5. A. Explosive chemicals in the surrounding water. Analysis of water samples was performed by GC–MS/MS technology. Abbreviations: TNT (2,4,6-trinitrotoluene), 4-ADNT (4-amino-2,6-dinitrotoluene), 2-ADNT (2-amino-4,6-dinitrotoluene), 1,3-DNB (1,3-dinitrobenzene), 2,4-DNT (2,4-dinitrotoluene), and W1 – W3 (sampling stations).
B. Explosive chemicals in the sediment. Analysis of sediment samples was performed by GC–MS/MS technology. Abbreviations: TNT (2,4,6-trinitrotoluene), 4-ADNT (4-amino-2,6-dinitrotoluene), 2-ADNT (2-amino-4,6-dinitrotoluene), 1,3-DNB (1,3-dinitrobenzene), 2,4-DNT (2,4-dinitrotoluene), and S1 – S12 (sampling stations).

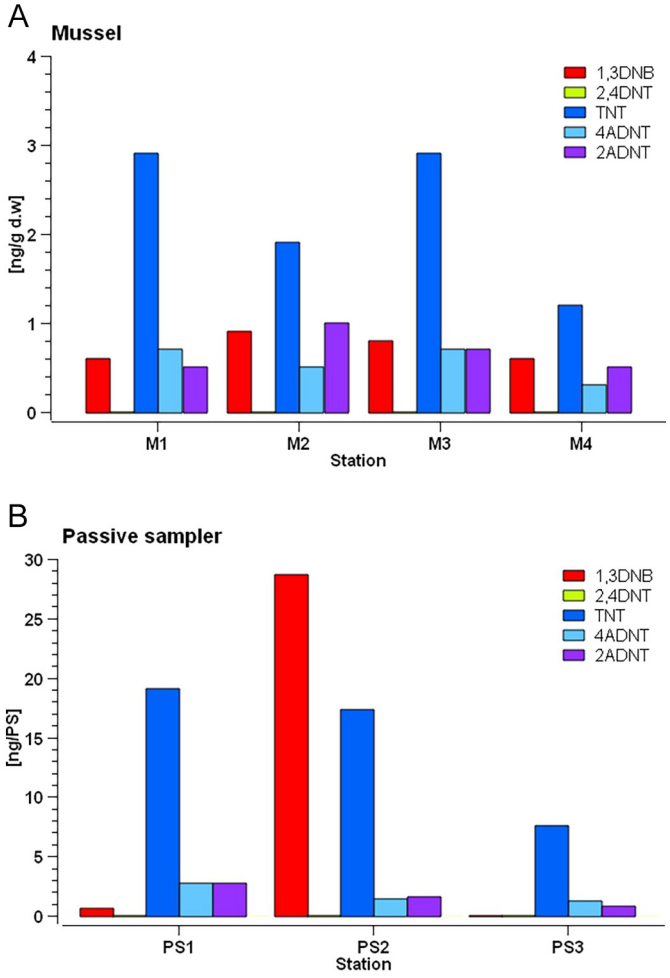

Fig. 6. A. Explosive chemicals in the mussels. Analysis of mussel samples was performed by GC–MS/MS technology. Abbreviations: TNT (2,4,6-trinitrotoluene), 4-ADNT (4-amino-2,6-dinitrotoluene), 2-ADNT (2-amino-4,6-dinitrotoluene), 1,3-DNB (1,3-dinitrobenzene), 2,4-DNT (2,4-dinitrotoluene), and M1 – M4 (monitoring stations).
B. Explosive chemicals in the passive samplers. Analysis of passive samplers was performed by GC–MS/MS technology. Abbreviations: TNT (2,4,6-trinitrotoluene), 4-ADNT (4-amino-2,6-dinitrotoluene), 2-ADNT (2-amino-4,6-dinitrotoluene), 1,3-DNB (1,3-dinitrobenzene), 2,4-DNT (2,4-dinitrotoluene), and PS1 – PS3 (monitoring stations).

4. Discussion

Until recently, sunken ship wrecks have only been regarded as hazardous to sea traffic, cable routes and wind farms. However, emerging perspectives

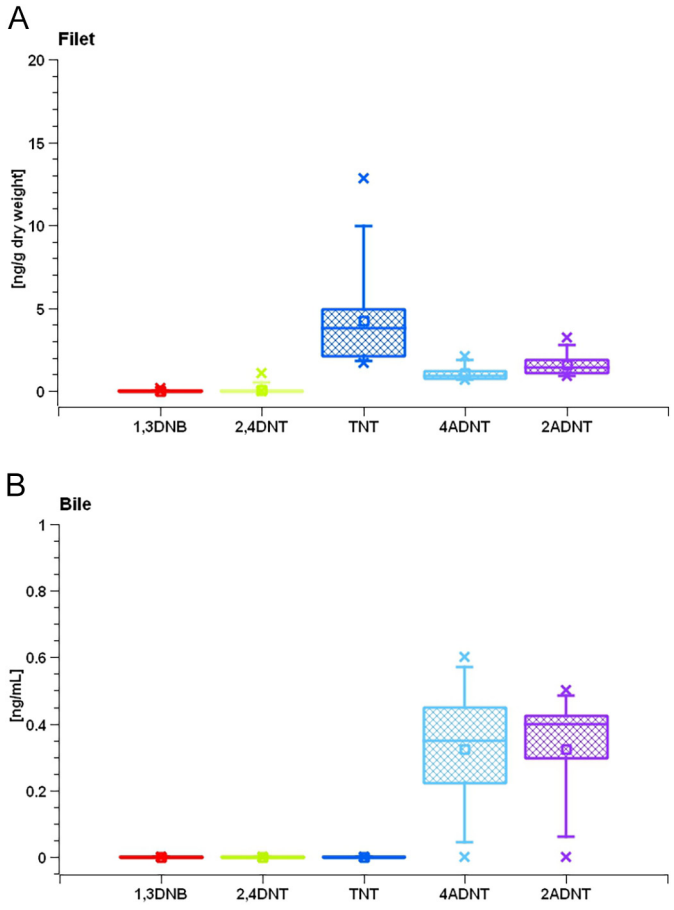

Fig. 7. A. Explosive chemicals in the fish filet. Analysis of fish filets was performed by GC–MS/MS technology. Abbreviations: TNT (2,4,6-trinitrotoluene), 4-ADNT (4-amino-2,6-dinitrotoluene), 2-ADNT (2-amino-4,6-dinitrotoluene), 1,3-DNB (1,3-dinitrobenzene), and 2,4-DNT (2,4-dinitrotoluene).
B. Explosive chemicals in the fish bile. Analysis of fish bile was performed by GC–MS/MS technology. Abbreviations: TNT (2,4,6-trinitrotoluene), 4-ADNT (4-amino-2,6-dinitrotoluene), 2-ADNT (2-amino-4,6-dinitrotoluene), 1,3-DNB (1,3-dinitrobenzene), and 2,4-DNT (2,4-dinitrotoluene).

on wrecks have raised concerns about the contents of sunken ships (such as munitions, fuel, and dangerous cargo). In order to collate all the available information, determine the hazards, and perform a tailored risk assessment of the wrecks (with respect to munitions), the North Sea Wrecks (NSW) project (funded by the Interreg North Sea Region Program) was launched in 2018. The risks of sea-dumped munitions to both the marine environment and the human seafood consumer has previously considered by projects from the Baltic Sea region (project UDEMM: https://udemm.geomar.de/de and project DAIMON: https://interreg-baltic.eu/project/daimon/), (Beck et al., 2022; Maser and Strehse, 2021; Schuster et al., 2021; Maser and Strehse, 2020; Strehse et al., 2020; Koske et al., 2020; Appel et al., 2018; Strehse et al., 2017).

In the current study, research of historical data in the Federal Archives-Military Archives (BArch-MA) in Freiburg (Breisgau, Germany) led to the identification of sunken ship wrecks that could be used for comprehensive investigations by the NSW project. Following an assessment of the availability of historical information and the sinking scenario at site, the accessibility of the wreck to divers, and the suspected amount of munitions onboard at the time of sinking, the wreck of "Vorpostenboot 1302 (V 1302)" was selected for this study. According to the historical documents regarding the armament and ammunition load prior to the battle, as well as the calculation of the ammunition fired from the war diary, about one metric ton of remnant nitroaromatic explosives on the V1302 wreck could be expected at the time of sinking. More than half of this (650 kg) would come from depth charges, the rest from anti-aircraft ammunition (Table 4).

This wreck was first surveyed using a multibeam echosounder to verify the condition of the wreck and assess the chance of finding any remaining munitions. After visual inspection by scientific divers, water and sediment samples (collected from the wreck site) were analyzed for the presence of energetic chemicals such as TNT and derivatives thereof, using sensitive GC–MS/MS technology (Bünning et al., 2021). According to our results, the sediment values are not distributed in a logical manner, since amidships the two most distant positions (S4 and S7) are higher than those amidships closer to the wreck (S1-S3 and S5-S6), while the bow (S9-S10) and stern (S11-S12) sediment samples show intermediate values. With regard to explosives in the water, the W1 (starboard) position is particularly prominent with approx. 5-fold higher concentrations for total nitroaromatics compared to the W2 (stern) and W3 (port) positions. In addition, the water values of W1 do not match S1 to S3, but they do match S4. One explanation for this could be that local conditions (e.g. current speed, temperature, salinity) are constantly changing on various time scales, going from a single tidal regime to the seasons. This could lead to very intense rearrangements in the sediment and spread of the nitroaromatic explosives around the wreck. Nevertheless, our investigations with the exposed mussels and passive samplers directly on the wreck (M1/PS1 - M1PS3) show comparable values (except for 1.3 DNP at passive sampler PS2), so that flow-related factors may actually have played a role in the sediment and water samples farther away from the wreck. As has been discussed in a comprehensive review by Lotufo et al. (2021) relatively little has been done to assess the porewater - sediment equilibrium, which comprises release from sediment to porewaters following remobilisation and redistribution of energetic compounds. In our case this is even more complicated, since the North Sea is a very turbulent body of water with rapidly changing conditions. However, the results obtained from this study confirm the leakage of energetic compounds such as TNT and its derivates from the munitions onboard the wreck.

Interestingly, we did not only find the nitroaromatic explosives in significant concentrations in the mussels that we had exposed, but also in fish living around the wreck. This again reveals that these substances are leaking from corroding munitions in or on the wreck. Moreover, this finding indicates that the explosives enter the marine biota and may bioconcentrate in the marine food chain. However, the concentrations measured so far in the mussels and fish are not alarming as far as the human seafood consumer is concerned (Maser and Strehse, 2021). A future step will be to compare the levels of munition compounds in the water and sediment around V 1302 with respect to their toxicity in animals and humans (in particular) which will enable an assessment of their impact on marine life and food safety at the wreck site. Considering the mutagenic and carcinogenic (as well as toxic) properties of these munitions compounds, such assessments are quite challenging (Maser and Strehse, 2021; Koske et al., 2019; Bolt et al., 2006).

What further complicates is the fact that energetic chemicals do not only enter the water and sediment, they are also distributed by currents, natural disturbances due to regular tidal motions and episodic storm events, or even by human activities such as fishing. The environmental fate of energetic compounds is determined by physio-chemical properties such as their solubility and octanol-water partitioning coefficients, or by the temperature, pH, salinity, oxygen dissolution, UV light levels, and the ionic strength of seawater (Beck et al., 2018; Juhasz and Naidu, 2007). Depending on these factors, the substances disperse in the seawater leading to lower concentrations at wreck site. Moreover, degradation by microbial communities are also factors that influence the occurrence of munition compounds at site (Lotufo et al., 2021). However, the concentrations measured in water and sediment from the vicinity of the V 1302 wreck were in the ng/L (water) or ng/kg (sediment) range. That means, first of all, that there is currently no danger of any acute toxic or lethal effects to marine organisms outside the wreck.

The concentrations of munition compounds detected in the mussel tissues were in the low single-digit ng/g range, and are therefore not comparable to laboratory studies in which a battery of biomarkers (including catalase, superoxide dismutase, and lipid peroxidation) were investigated at TNT concentrations above 0.31 mg/L (Schuster et al., 2021). Interestingly, TNT (median levels of approximately 4 ng/g) was the most prominent energetic compound in fish filets, whereas 2-ADNT and 4-ADNT were less abundant (Fig. 7A). On the contrary, concentrations of explosive chemicals in bile from the same specimens were lower by a factor of ten (median of approximately 0.4 ng/mL); TNT was completely absent (Fig. 7B). Usually, the parent compound TNT undergoes metabolism to 2-ADNT and 4-ADNT when ingested via predatory diet (Beck et al., 2022; Schuster et al., 2021; Strehse et al., 2017). However, the fact that the filet contained higher concentrations of TNT than the bile hints at an alternative route of absorbance in the fish. Possibly, the parent compound TNT (which leaks from the corroding munitions and occurs in higher abundance, compared to 2-ADNT and 4-ADNT, in water and sediment, respectively) enters the fish upon respiration (through the gills), and is then directly transported via the blood to the muscle (filet) tissues. This kinetic behavior of TNT has recently been shown in the Atlantic salmon (Mariussen et al., 2018).

Nevertheless, as recently estimated, energetic compounds in mussels and fish do not pose any risk to the human seafood consumer, provided that the sum of all explosive chemicals in the edible part is in the single-digit ng/g range (Maser and Strehse, 2021). In contrast, due to the carcinogenicity of TNT and based on daily and life-long consumption of seafood, there could be a threat to human health if concentrations of these compounds exceed 300 ng/g in the edible part of mussels or fish. Fortunately, the concentrations measured in the current study do not pose any risk to human seafood consumers.

With regard to the toxicity of energetic compounds in aquatic organisms, there are a number of laboratory studies available in which TNT-spiked water was used to derive species-specific toxicity endpoints. In most cases, the exposure concentrations were significantly higher than those expected in the environment. This implies that the resulting effects represent acute rather than chronic adverse effects (Schuster et al., 2021; Lotufo et al., 2013; Nipper et al., 2009; Juhasz and Naidu, 2007; Talmage et al., 1999). The toxicity of nitroaromatic explosive chemicals has been reported in freshwater invertebrates, including amphipods, midges, rotifers, cladocerans, and oligochaetes (summarized in Talmage et al. (1999); Nipper et al. (2009); Lotufo et al. (2013)). Fewer studies have also addressed their effects in marine invertebrates, such as sea urchins, mysid shrimps (Nipper et al., 2001), bivalves (Schuster et al., 2021; Strehse et al., 2020; Rosen and Lotufo, 2007b; Goodfellow et al., 1983; Won et al., 1976), copepods (Ek et al., 2008; Ek et al., 2007; Ek et al., 2006; Dave, 2003; Dave et al., 2000; Won et al., 1976), and polychaetes (Nipper et al., 2001). TNT caused toxicity in all invertebrate species investigated, with LC_{50} values in the low mg/L range (reviewed in Lotufo et al. (2017)). Likewise, adverse biological effects and lethal toxicity of explosive chemicals (in the low mg/L range) have been reported for freshwater and marine fish (Lotufo et al., 2013; Lotufo et al., 2010b; Lotufo et al., 2010a; Lotufo and Blackburn, 2010; Nipper et al., 2001). Liver histopathological studies in Atlantic hagfish (*Myxine glutinosa*, a sediment-dwelling chordate) indicated pre-neoplastic and neoplastic liver lesions (Straumer et al., 2020). In another study, exposure to dumped CWA (at a wreck site) led to significant differences in the activities of glutathione reductase, superoxide dismutase, and glutathione S-transferase in hagfish; catalase and acetylcholinesterase activities were unaltered (Ahvo et al., 2020).

The mutagenicity and carcinogenicity of nitroaromatic compounds have also been shown in laboratory studies with zebrafish embryos (*Danio rerio*) (Koske et al., 2019). Exposure to TNT as well as 2- or 4-ADNT revealed a high proportion of chorda deformations at concentrations in the mg/L range in the surviving embryos. In addition, genotoxicity of the nitroaromatic compounds was shown by a comet assay, with cells isolated from whole embryos after 48 h exposure in vivo. Significant genotoxicity was induced by all the three tested compounds at 0.1 mg/L and 1.0 mg/L, in comparison to the corresponding controls. In contrast to laboratory studies, concentrations of TNT and its derivatives measured in the present study (in water and sediment outside the V 1302 wreck) were in the low ng/L (water) or ng/kg (sediment) range. These concentrations are at least several thousandfold lower than exposure levels reported in marine species in laboratory studies. Thus, acute toxic or lethal effects in organisms outside of the wreck may be rather improbable.

An important but yet unresolved question remains on the effect of long-term exposure to low concentrations of TNT and its metabolites on marine organisms. This threat is currently poorly understood, although few studies are available. In their study on potential bioaccumulation of phenylarsenic CWAs and their negative effects in fish living in the vicinity of a major dumpsite in the Bornholm Basin (Baltic Sea), Niemikoski et al. reported trace levels of phenylarsenic compounds in 14 % of cod (*Gadus morhua*) muscle samples; and significant changes of acetylcholinesterase, glutathione *S*-transferase, catalase, glutathione peroxidase, and superoxide dismutase were observed (Niemikoski et al., 2020). Caged mussels (*Mytilus trossulus*) deployed at depths of 35 m and 65 m above a 90 m deep CWA-dumping hot spot (25 m vertical distance from the bottom) also presented with deteriorated health (in mussels at the hotspot) compared to unburdened reference sites (Lastumäki et al., 2020). TNT-induced oxidative stress in blue mussels (*Mytilus* spp.), observed via upregulation of the carbonyl reductase gene expression, has also been shown in both laboratory and field studies - near a munitions dumping site (Strehse et al., 2020). This finding is currently being developed as a new promising biomarker for explosive-containing sea-dumped war relicts (Strehse et al., 2020).

On the other hand, and importantly enough, sunken shipwrecks provide an ideal ecological niche for all kinds of marine organisms. Sánchez-Caballero et al. (2021) have shown that they are a refuge to commercially-important fish species as well as threatened species. The wrecks may even represent a good strategy for reef restoration, enhanced fishing grounds and fishing management (Sánchez-Caballero et al., 2021). Wrecks may also function as islands of biodiversity for microorganisms, creating a patchwork of habitats with influence radiating out onto the seabed (Hamdan et al., 2021). However, if these niches become polluted, the marine organisms may directly be exposed to harmful concentrations of the toxicants onboard, including munition compounds and heavy metals.

Currently, neither the concentrations of explosive chemicals within the shipwreck (V 1302) nor the conditions (for example, the corrosion status of the munitions) is known. In the worst case, the munitions could be without metal shells or housings so that the solid explosives could dissolve in the water, reaching concentrations in the mg/L range. These mg/L concentrations have been found to be toxic in aquarium experiments using several marine animal species (see references above). Even if the concentrations are neither lethal nor acutely toxic, chronic exposure to low concentrations of munition compounds may also impact marine life. A future task will also be the comparison between our measurement data and oceanographic parameters. From this, flow models could be generated to better understand and interpret the distribution of explosives on site.

Nevertheless, caution is advised regarding the risk assessment of ship wrecks: Firstly, the metal casings of the munitions within and outside the wreck will continue to corrode and release more munitions chemicals into the surrounding environment. Secondly, this trend will also be intensified by the forthcoming climate change and ocean warming. Higher temperatures enhance the water solubility of pollutants (especially energetic compounds) and this could increase the uptake of pollutants by marine fauna, to a higher extent. Thirdly, the presumably weaker current inside the wreck could mean higher concentrations of energetic compounds in wrecks. Finally, in addition to the acute effects (days to weeks), chronic effects must also be considered, especially when organisms with a long lifespan are exposed to munition compounds throughout their whole lives. Not only would they be severely affected, but the energetic compounds would also possibly accumulate, for instance, in the local food web or along the trophic chain inside or outside the wreck. Clearly, uptake

by aquatic fauna may not only occur from direct exposure to water and/or sediment (e.g., dermal contact and via gills) but also through a predatory lifestyle (when contaminated biota are ingested).

Over all, sunken shipwrecks fully armed with all kind of munitions are sources of munitions chemicals and could ultimately endanger the human seafood consumer through neighboring aquaculture facilities or fishing activities, where these toxic substances may enter the marine food chain. Moreover, it should always be considered that explosives are not only toxic but also mutagenic and carcinogenic.

5. Conclusion

The extent to which munition compounds impact marine species and ecosystems as well as the human seafood consumer is of great interest. Unfortunately, knowledge on the long-term effects of energetic substances such as TNT and its metabolites on the environment is still limited; thus more research is required. Recent research indicates that marine organisms take up energetic compounds, and this might also pose a risk to human seafood consumers. In the case of sunken shipwrecks, current risk assessments are centred on the dangers to critical infrastructure such as offshore installations, harbors, pipelines, cables and tourist regions. However, risk assessments such as the potential impact of a particular wreck on the marine environment (due to its armament and fuel) are missing. Wrecks are a source of several pollutants. Therefore, in view of the long-term ecological effects from chronic exposure, the idea of considering diffusion of pollutants in the ocean and a dilution approach as solutions to pollution must also be rejected here. The V 1302 (JOHN MAHN) is representative of several wrecks (in the waters of Belgian, Dutch, German, Danish, and Norwegian consortium partners) that are still being sampled for risk assessments.

With our investigations we could provide evidence that sunken warship wrecks emerge as a point source of contamination with nitroaromatic energetic compounds leaking from corroding munitions cargo still on board. In the V 1302 case we found these explosive substances in water and sediment samples around the wreck. Moreover, caged mussels being exposed for some weeks as well as wild fish living at the wreck site were shown to take up the compounds touching the problem of seafood safety. The concentrations found in mussel flesh and fish filet, so far, are fortunately only in the one-digit ng per gram range thus indicating no current concern for the human seafood consumer. However, the situation may worsen as the corrosion continues, such that alarming concentrations of energetic compounds are leaking from the wrecks and affecting the marine environment and biota living at these sites. From this study, it is proposed that wrecks should not only be ranked according to critical infrastructure and human activities at sea, but also to the threats they pose to the environment and the human seafood consumer. A tailored monitoring approach (for the individual wrecks) could then be developed and the remediation options discussed.

CRediT authorship contribution statement

E.M. supervised and coordinated the study and wrote the manuscript.
T.H.B. performed the chemical analyses.
M.B. coordinated and performed the sampling.
S.V.H. coordinated and performed the sampling.
M.D.R. coordinated and performed the sampling.
P.M. provided historic data and designed the models.
U.W. provided historic data and designed the models.
J.S.S. coordinated the study.

Declaration animal experiments

All animal experiments comply with the ARRIVE guidelines and have been carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Research Council's Guide for the Care and Use of Laboratory Animals.

Declaration of data derived

I, as the corresponding author, declare that the work described here has not been published previously nor is under consideration for publication elsewhere. The publication is approved by all of the coauthors.

Data availability

Data will be made available on request.

Declaration of competing interest

I, as the corresponding author, declare on behalf of all the authors of the submission, that there is not any financial interest or personal relationship with other people or organizations that could inappropriately influence this work.

Acknowledgement

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