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Environmental genotoxicity and cytotoxicity studies in fish blood erythrocytes and genotoxicity risk assessment in the Gotland Basin of the Baltic Sea

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VILNIAUS UNIVERSITETAS
GAMTOS TYRIMŲ CENTRAS

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Baltijos jūros Gotlando baseino genotoksiškumo ir citotoksiškumo dėsningumų tyrimai žuvų kraujo eritrocituose bei aplinkos genotoksiškumo rizikos nustatymas

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INTRODUCTION

The Marine Strategy Framework Directive's (MSFD, Directive 2008/56/EC) main goal is to protect the resource base and achieve Good Environmental Status (GES) of EU marine and coastal waters by 2020. The GES of marine waters is defined based on 11 high-level qualitative descriptors laid out in Annex I of the Marine Directive. One of the most critical documents promoting pollution reduction is the HELCOM Baltic Sea Action Plan (BSAP) approved by the Helsinki Commission in 2007. According to the BSAP, the primary purpose is to restore the Baltic marine's good environmental status by 2021 (HELCOM Baltic Sea Action Plan, 2007).

One of the main objectives of the MSFD is to prevent, reduce and control pollution of the marine environment from land-based sources. To meet the GES criteria listed in Descriptor 8, concentrations of contaminants should be at levels not giving rise to pollution effects (Directive 2008/56/EC). Unfortunately, the marine ecosystem contains pollutants that are not part of the MSFD, for example, dumped CW in the sea and the chemicals contained therein. Following MSFD and the International Council for the Exploration of the Sea (ICES), pollution effects ought to be considered at various levels of biological organisation, taking into account effects of the contaminants' interaction with both abiotic and biotic factors (Thain et al., 2008; Lyons et al., 2010; ICES, 2011). Thus, genotoxic methods, such as the micronucleus test, the Comet assay and the DNA adducts test, were introduced to assess pollutants-induced genotoxicity risk to biota (Baršienė et al., 2012c; Martins and Costa, 2015; Bean and Akcha, 2016). Micronucleus (MN) and other nuclear abnormalities test is used as sensitive, fast, minimally invasive and cost-effective measures for determining DNA damage (Bolognesi et al., 2006; Baršienė et al., 2015, 2016, Fenech, 2020). Based on ICES/OSPAR Background Assessment Criteria (BAC), genotoxicity impacts were included in the GES assessment (ICES, 2011; Baršienė et al., 2012c; Hylland et al., 2017).

Up to now, the impact of electromagnetic fields on the marine environment, in particular, on marine organisms, has been receiving insufficient scholarly attention. However, the adoption of MSFD and the relevant Descriptor 11, which deals with the effects of anthropogenic energy forms on the marine environment, has changed this situation (Directive 2008/56/EC). According to MSFD, it is mandatory to assess the effects produced by the anthropogenic energy generation and maintain them at a level that does not affect the marine environment adversely (Directive 2008/56/EC; Otremba et al., 2019).

Based on the European Commission report on the implementation of the Directive 2008/56/EC, the progress in reaching good environmental status has not been fast enough to cover all MSFD descriptors in all EU waters by 2020. The revision of the Directive will therefore aim to implement the European Green Deal, including biodiversity strategy and zero pollution ambition by 2030 (Report from the European Commission to the European Parliament and the Council on the implementation of the Marine Strategy Framework Directive (Directive 2008/56/EC), 2020).

1. LITERATURE REVIEW

1.1. Contaminants in the Gotland Basin of the Baltic Sea

1.1.1. Hazardous substances

Toxic chemicals released into our environment by industries, agriculture, or municipal activities can pose a genetic risk to aquatic organisms (Baršienė et al., 2004, 2006a, 2006b, 2006c, 2012a, 2012b, 2013; Bolognesi and Hayashi, 2011; Marigomez et al., 2013; Turja et al., 2014). The Gotland and Arkona Basins, the northwestern coastal areas of the Bothnian Sea and Kiel Bay, which are among the most polluted open sea areas with the highest ratio of biota contamination (HELCOM, 2017), were assessed. A wide variety and distribution of genotoxic pollutants, such as heavy metals, polycyclic aromatic hydrocarbons, alkylphenols, microplastic and other pollutants (Ricking and Schulz, 2002; Pikkarainen, 2004; Zalewska et. al., 2015; Graca et al., 2016; HELCOM, 2018; Jakubowska et al., 2020; Urban-Malinga et al., 2020) were recorded in the Gotland Basin. Increased concentrations of cadmium, mercury and lead were detected in the liver of *C. harengus membras*, *P. flesus* and *G. morhua callarias* collected from the southeastern part of the Gotland Basin (ICES, 2000). The investigation of potential population health risks of contemporary (post-2000) mercury exposure showed higher risk for bivalves (*Macoma baltica* and *Mytilus edulis*) than fish (Dietz et al., 2021). Moderate–considerable contamination degree in sediment was described for cadmium (Cd), minor–moderate for lead, zinc, copper, low for nickel and chromium in the open Baltic Sea waters (in Lithuanian zone) (Remeikaitė-Nikienė et al., 2018). Increased concentrations of phenanthrene and pyrene were recorded in tissues of bivalves (*M. balthica* and *Astarte borealis*) from the Gotland Basin

(Pikkarainen et al., 2004). In the south-eastern part of the Gotland Deep, the concentration of alkylphenols (4-nonylphenol and 4-tert-octylphenol) in sediments was found to be higher than in sediments of the Bornholm and Gdańsk Deeps of the Baltic Sea (Graca et al., 2016). Due to dioxins (polychlorinated dibenzo-p-dioxins and furans) and polychlorinated biphenyls (PCBs) persistence and lipophilic properties, they are found at relatively high concentrations in fish (Kiviranta et al., 2004). The fatty fish (such as *C. harengus membras*, *Salmo trutta*) often exceeds the EU limits for dioxins and PCBs (Commission regulation, 2006). The highest abundances of anthropogenic microplastic particles were found in the Baltic Proper and the Gotland Basin (Bagaev et al., 2018; Schönlau et al., 2020). The ingestion of plastic has been investigated in the gastrointestinal tract of demersal (*G. morhua*, *Limanda limanda* and *P. flesus*) and pelagic (*C. harengus* and *Scomber scombrus*) species from the North and Baltic Sea. According to the results, 74% of detected plastic particles are microplastic (size < 5 mm). Almost 40% of the particles consist of polyethylene, 22% – polyamide, 13% – polypropylene. Also, significant higher ingestion frequency has been recorded in pelagic fish to compare with demersal fish (Rummel et al., 2016).

Oil spills directly cause PAH pollution and affect aquatic organisms (Honda and Suzuki, 2020; Waszak et al., 2021). The Baltic Sea is an essential route for maritime trade, therefore on average 2 000 ships are at sea every day, including 200 tankers carrying oil or other potentially harmful products (Krek et al., 2018). According to HELCOM (2018), by 2030, the amount of oil shipped in the Baltic Sea would grow by 64% (from about 180 million tonnes to almost 300 million tonnes). With more intensive shipping, the number of accidents will also increase.

1.1.2. Chemical and conventional munition dumpsites

Chemical weapons (CW) were extensively used during World War I, and their production and stockpiling was continued before and during World War II. Chemical weapons sea disposal operations started on a small scale at the end of the war in the Little Belt area. They were performed by the German Army to prevent the capture of the most innovative weapons containing the nerve gas tabun (Knobloch et al., 2013). After the fall of Germany, under the Potsdam agreement, ally forces took control of the German chemical weapons arsenal, which contained roughly 65 000 tons of active agents (Surikov et al., 2014). Large amounts of the captured munitions were dumped in the Skagerrak Strait and the Atlantic Ocean on orders of British and American occupation authorities. In the Baltic Sea, CWs were dumped under the Soviet Military Administration order in Germany (Nawała et al., 2020). In most cases, chemical munitions or containers with chemical warfare agents (CWAs) were thrown overboard. Until the 1970s, dumping was a common global practice, dumping operations being performed by 40 countries. The number of the existing CWA dumpsites is supposed to exceed 300. It is believed that 684 thousand tons of chemical munitions have been disposed of in European waters (Arison, 2013). In recent years, sea-dumped CWAs have been perceived as a worldwide threat (Beck et al., 2018).

After World Wars I and II, the Baltic Sea turned into a dumpsite of about 50 000 tons of CWs (Vanninen et al., 2020), containing 15 000 tons of CWAs, which can exert genotoxic, cytotoxic and carcinogenic effects on humans and marine species (Bolt et al., 2006; Sanderson et al., 2017; Bełdowski et al., 2018; Koske et al., 2019). CWAs were dumped at different sites in the Baltic Sea, mainly near the Island of Bornholm (about 35 000 tons), in the Little Belt (about 5 000 tons), and in the Gotland Basin (2 000 tons) (CHEMSEA Findings, 2014). During the CHEMSEA (Chemical Munitions, Search and Assessment, 2011–2014) project implementation, unofficial CW dumpsites have been detected in the Gdańsk Deep and the Slupsk Furrow.

Due to the growing economy, the marine environment increasingly used, therefore dumped chemical weapon problem raise international concern. Increasingly cases of dumped CWs have been found floating, washed ashore, or fishermen pull them out with their nets (Missiaen and Henriet, 2002; Missiaen et al., 2010). The risks remain high due to the increased use of the Baltic Sea, including the construction of wind farms, pipelines and cables, traffic routes (CHEMSEA findings, 2014; Bełdowski et al., 2016c).

Over time, metallic mantles of munitions and bulk containers rust, their condition deteriorating (CHEMSEA Findings, 2014). There are estimations that CWA-related substances may leak from the containers/canisters during 8–30 years and from artillery projectiles during 100–390 years (Witkiewicz and Popiel, 2005). If only one-sixth of the 50 000 tonnes of munitions leaked, it would destroy a life in the Baltic Sea for 100 years (NATO press release, 2016). According to the latest research, the leakage rates also depend on corroded openings positions on the bomb. If the openings are facing downwards, tabun and several degradation products will leak out and hydrolyse rather quickly. In contrast, if the openings are upwards, the leakage rate is extremely low (Tørnes et al., 2020). The performed inspection of the munitions' condition yielded different results: part of the munitions were found intact. However, in many cases, munition casings were found completely corroded and/or empty (Bełdowski et al., 2016a, 2016b). The concentrations of such CWA-related substances as yperite (Tørnes et al., 2006; Della Torre el al, 2010, 2013), mercury (Della Torre et al., 2010; Bełdowski et al., 2019), arsenic-containing substances (Tørnes et al., 2006; Czub el al., 2018), sulfur mustard and its degradation product 1,4 dithiane and 1,4-thioxane (Amato et al., 2006; Briggs et al., 2016) 2,4,6-trinitrotoluene (TNT) (Appel et al., 2018), hexahydro-1,3,5-trinitro-1,3,5-trazine (RDX) (Ariyarathna et al., 2019) as well as their hydrolysis products detected in sediments from the dumping sites indicate that the leakage of munition compounds has contributed to the environmental contamination. This fact proves that toxic compounds are leaking out from the dumped munitions and are contaminating the environment. Also, increased concentrations of mercury have been detected in the liver of *C. harengus*, *P. flesus* and *G. morhua* collected from the southeast of Gotland (ICES, 2000), suggesting the presence of unexploded munitions containing mercury, i.e. bombs and shells, therein (Della Torre et al., 2010). CWAs contamination is usually local; it may spread, it's outspread depending on various factors. The distribution area of released CWAs and their degradation products may stretch for more than 250 m from contamination sources, in certain cases, the contaminated zone extend to 1 km. The range of contamination depending on bottom currents, topography, and the level of munitions corrosion (CHEMSEA Findings, 2014; Vanninen et al., 2020). Gledhill, with co-authors (2019), have suggested that due to slow release and rapid removal of munition compounds in seawater, CWAs can be only rarely detected in marine zones that are affected by unexploded or discarded munitions. Consequently, it sometimes happens that no CWAs are detected in water or sediment samples from disposal sites. Therefore, the necessity of a highly sensitive method for the detection of munition compounds in the marine environment was reported in their study. The CWAs might leak from corroded munition to the surrounding environment, and their compounds might concentrate in marine biota leading to harmful biological effects (Baršienė et al., 2014).

The chemical warfare agents dumped in the Baltic Sea included sulphur mustard (from 63% (Knobloch et al., 2013) to 80%), α-chloroacetophenone (5%), arsenic-containing compounds such as adamsite (diphenylaminechloroarsine, DM), Clark I (diphenylarsine chloride, DA), Clark II (diphenylarsine cyanide, DC) and arsine oils (is a mixture of phenyldichloroarsine (PDCA; 50%), Clark I (35%), trichloroarsine (TCA;

5%) and triphenylarsine (TPA; 5%) (Missiaen et al., 2010)) (Bełdowski et al., 2017; HELCOM, 2013). In the Gotland Deep, about 608 tons of sulfur mustard was dumped, which turned out to be the most abundant chemical in the dumped stockpile (Knobloch et al., 2013). Such products of sulphur mustard degradation as 1,2,5-trithiepane (Czub et al., 2020) and 1,4,5-oxadithiepane (Chmielińska et al., 2019) are among the most toxic ones. They belong to Chronic Category 2 and are described as “toxic to aquatic life with long-lasting effects”. In water, CWAs usually degrade during hydrolysis, while sulfur and arsenic-containing chemicals by oxidation. The CW agents and their hydrolysis products might concentrate in marine biota and produce chronic or lethal effects (Sanderson et al., 2008, 2010; HELCOM, 2010; Della Torre et al., 2010, 2013; Baršienė et al., 2014, 2016; Bełdowski et al., 2016a; Gledhill et al., 2019). Hydrolysis products of Clark I and Clark II (Francken and Hafez, 2009), nitrogen mustard gas and sulphur mustard gas (Bełdowski et al., 2018) have the same toxicity as their parent compounds and exert long-term effects on marine organisms. It is known that the oxidation product of Clark I, diphenylarsinic acid, and its biotransformation products cause adverse cerebral effects in humans, also encourage liver carcinogenesis in rats (Ishii et al., 2004, 2017; Ochi et al., 2006; Wei et al., 2013). The performed study of acute aquatic toxicity of sulphur mustard and six products of its degradation showed that two of them (1,2,5- trithiepane and 1,4,5-oxadithiepane) are more toxic than the parent compound (Czub et a., 2020). Recent research results show that CWAs and their hydrolysis products can bioaccumulate or exert adverse effects on different aquatic biota levels (Kotwicki et al., 2016; Nawała et al., 2016; Chmielińska et al., 2019; Czub et al., 2020).

Researchers’ interest in the ecological risk posed by CWAs to living marine organisms is steadily growing, it is still poorly understood, with only a few scientific articles being published on the biological or ecological significance of CWAs (Sanderson et al., 2008; 2010; Della Torre et al., 2010, 2013; Baršienė et al., 2014, 2016; Bełdowski et al., 2016b, 2018, 2019; Höher et al., 2019; Strehse et al., 2017, 2020; Valskienė et al., 2018; Koske et al., 2020; Lastumäki et al., 2020; Niemikoski et al., 2017, 2020a, 2020b). Even the lowest concentrations of oxidised forms of Clark I (1.25 μg/L), Adamsite (2.5 μg/L) and chloroacetophenone (5 μg/L) were found to exert adverse effects (cytotoxic, immunotoxic and oxidative stress) on the tissue of mussels (*Mytilus trossulus*), as reported by Höher et al. (2019). Significant cytogenetic, biochemical, histochemical and bioenergetic responses were determined in mussels (*M. trossulus*) caged at the main Bornholm dumping site; however, no direct evidence was gathered to confirm that these responses were elicited by exposure to CWAs, because there were no products (phenarsazinic acid, diphenylarsinic acid and triphenylarsine oxide) of CWAs primary degradation recorded in mussel tissues (Lastumäki et al., 2020). The accumulation of CWAs in living organisms, their intervention in enzyme-controlled reactions and their ability to cause genetic changes are not sufficiently elucidated (Baršienė et al., 2016). However, in recent research, there is evidence that CWA-related phenylarsenic chemicals accumulate into marine biota (Niemikoski et al., 2017). *In vitro* experiments showed that CWA-related phenylarsenic chemicals such as Clark I, Adamsite and phenylarsonic acid (PDCA[ox]) form glutathione (GSH) conjugates and methylated metabolites in cod (*G. morhua*) liver. Triphenylarsine oxide (TPA[ox]) was not found to form GSH conjugates and methylated metabolites, indicating a different biotransformation pathway. Moreover, hydroxylated metabolites were detected for all explored chemical. For detecting CWA-related contamination in fish, methylated and hydroxylated metabolites of phenylarsenic chemicals are more promising targets, because GSH conjugates are difficult to detect in fish samples due to their reactive nature (Niemikoski et al., 2020a). The latest research by Niemikoski et al. (2020b), is the first to yield information on CWAs bioaccumulation and distribution in tissues of *G. morhua* from the Bornholm Basin in the Baltic Sea. The above-mentioned study detected trace amounts of phenylarsenic CWAs in muscle samples of the *G. morhua* caught close to the main dumpsite. Also, significant changes were recorded in some biomarkers in individuals containing trace levels of CWA-related chemicals.

After World War II, conventional and chemical ammunition containing primary (e.g. lead azide, lead styphnate (Lotufo et al., 2017) and secondary (e.g. TNT, RDX and octahydro-1,3,5,7- tetranitro-1,3,5,7-tetrazocine (HMX)) explosives were dumped mainly in the same CW dumpsites. The presence of explosives and products of their decomposition in seabed sediment samples implies the seabed contamination, which certainly affects the marine ecosystem (Nawała et al., 2020). Trinitrotoluene (TNT) is one of the primary organic explosive contaminants (Jenkins et al., 2012). The uptake of such explosive compounds as TNT, 2-amino-4,6-dinitrolouene (2-ADNT) and 4-amino-2,6-dinitrolouene (4-ADNT) by marine organisms has been first identified in blue mussels (*M. edulis*) from the munitions dumpsite (Strehse et al., 2017). The performed study of TNT and its degradation products (2-ADNT and 4-ADNT) showed the extensive toxicity to fish (*Danio rerio*) embryos of munition compounds beyond acute toxicity (Koske et al., 2019). Toxicity studies showed that TNT is more toxic than 2-ADNT, whereas 1,3,5-

trinitrobenzene TNB exposure revealed higher toxicity than TNT to juvenile sheepshead minnows (*Cyprinodon variegatus*) (Lotufo et al., 2010). Toxic explosives and their compounds were identified in 48% of bile samples from dab (*L. limanda*) caught at the munitions dumping site and reference sites in the Baltic Sea. The results show that such explosive compounds as TNT, 4-ADNT, 2-ADNT, HMX, RDX are accumulated by flatfish and may pose a risk to fish health (Koske et al., 2020). These results show that CWA disposal sites require continuous monitoring for environmental genotoxicity and cytotoxicity and CWA-related contaminants.

There are few studies available on cytogenetic effects of CWAs in different ecosystem components, including fish. *In vitro* studies have shown a correlation between the exposure to nitroaromatic compounds (including TNT) and mutagenicity (George et al., 2001), genotoxicity in bacteria (Neuwoehner et al., 2007), mutagenicity in mammalian cells (Kennel et al., 2000), cytotoxicity in human cells (Liao et al., 2017). Environmental genotoxicity effects in the fish caught along the chemical munitions' transportation routes, and in Bornholm dumping areas have been assessed by Baršienė et al. (2014, 2016). Biological effects (biochemical, histochemical, genotoxicity and cytotoxicity, bioenergetic responses) of CWAs were determined using caged mussels (*M. trossulus*) at the Bornholm dumpsite (Lastumäki et al., 2020). TNT and its degradation products (2-ADNT and 4-ADNT) caused DNA damages in zebrafish embryos (*D. rerio*) and poses a potential risk for long-term effects in fish living near munition dumpsites (Koske et al., 2019). Negative effects (e.g. genotoxicity biomarkers, lysosomal membrane stability changes, head kidney pathology) of CWAs on *G. morhua* health were determined at the Bornholm CW dumpsite (Bełdowski et al., 2016a; Lang et al., 2018). The most significantly increased genotoxicity responses were recorded in *C. harengus membras* caught at the stations located along the chemical weapons' transportation routes, close to the Bornholm CW dumping area, in zones with CWAs detected in sediments (Baršienė et al., 2016). Strong genotoxicity and cytotoxicity responses have been determined in fish (*C. harengus membras, P. flesus, G. morhua*) sampled at the chemical and conventional munitions dumping site in the Gdańsk Basin (Valskienė et al., 2018). However, the effects of CWAs genotoxicity and cytotoxicity on fish collected from the chemical munition dumpsites in the Gotland Deep have not been described.

1.1.3. Anthropogenic electromagnetic field

The marine environment may also be affected by an artificial magnetic field (MF) and electromagnetic field (EMF) emissions from wind farms, power transmission cables, hydrokinetic turbines. Depending on the energy transmission technology, submarine cables, which are laid on the seabed, generate electromagnetic or static magnetic fields that may disturb natural geomagnetic physical fields or produce low-frequency electromagnetic fields. There are two types of electricity transmission technologies that may affect the marine environment, i.e. direct current (DC) and alternating current transmission (AC) technologies. DC transmission technologies generate a static MF, while AC transmission technologies a low-frequency EMF (Otremba et al., 2019). High-voltage DC submarine cables are more frequently used for long-distance transmission of electricity, and AC cables are usually used for short-distance (up to 70 km) transmission (Negra et al., 2006). According to literature sources, in terms of mutagenic changes induction, low-frequency EMF is 200 times more hazardous than static MF (Suzuki et al., 2006). Electromagnetic fields have different frequency ranges, e.g. static magnetic and static electric fields have a frequency of 0 Hz, extremely low frequency (ELF) electric and magnetic fields have frequencies within the range of 1 Hz – 300 Hz, intermediate frequency electric and magnetic fields have frequencies ranging from 300 Hz to 100 kHz, and the frequency range of radio frequency electromagnetic fields is 100 kHz – 300 GHz (SCENIHR, 2009). The magnetic field of the Earth ranges from 0.025 mT at the Equator to 0.060 mT at the poles (Poleo et al., 2001). The magnetic field of the Baltic Sea ranges from 0.0501 to 0.0505 mT (Hulot et al., 2010), while AC cables generate EMF with intensity up to 8 mT (Cada et al., 2011a). The environmental effect of the MF depends on cable characteristics, geological properties of the stratum and the conductivity of the water column. Interactions of matter with AC and DC magnetic fields are different. The AC magnetic field ability to penetrate or propagate in saline water is characterised by the skin depth. The penetration depth of 50 Hz MF is about 35 m in Atlantic water, whereas the penetration depth of 1 MHz is only 0.25 m (Öhman et al., 2007).

According to the European Wind Energy Association, new technical uses of marine areas increase in abundance and intensity. By 2050, European offshore wind capacity could expand from the current 230 to 450 GW, and that in the Baltic Sea from 2 to 83 GW. This would make the Baltic Sea the second-largest basin for offshore wind power in Europe after the North Sea.

It is projected that by 2030 there will have been built wind power plants in the Lithuanian EEZ, which will have started generating electricity by that time (European Wind Energy Association, 2019).

Magnetic field induction can be minimised in several ways. Firstly, instead of a single DC cable, two high-voltage cables with bi-polar transmission can be laid in close proximity to each other (Öhman et al., 2007; Otremba and Andrulewicz, 2014). Also, instead of triple single-core AC cables, three-core cables, whose magnetic fields have a shorter extension, can be used for the three-phase current transfer (Otremba et al., 2019). Although there are relevant technical solutions for reducing the disruption of the Earth's magnetic field and the induction of EMF, the environmental effects of these factors (particularly those on the migration and physiology of marine organisms) have not been sufficiently studied yet (Otremba et al., 2019). Marine vertebrates (e.g. fish, sea turtles, sharks, rays, whales), bacteria (Frankel and Blakemore, 1980) and protists (Bazylinski et al., 2000) use electric and magnetic fields for navigation, communication, prey detection, predator avoidance (Kirschvink, 1997; Wiltschko and Wiltschko, 2005; Lohmann et al., 2008). Consequently, artificial EMF exposure can disturb animal behaviour (Gill, 2005; Gill et al., 2014).

Studies have shown that low-frequency EMF adversely affects the mitotic cycle in embryos of sea urchin *Strongylocentrotus purpuratus* (Levin and Ernst, 1995), activates mitogen-activated protein (MAP) kinases and expression of heat shock proteins in blue mussels *Mytilus galloprovincialis* (Malagoli et al., 2003, 2004), delays the hatching period in zebrafish *D. rerio* (Skauli et al., 2000) and embryos of northern pike *Esox lucius* (Fey et al., 2019a), enhances yolk sac absorption rates in rainbow trout *Oncorhynchus mykiss* (Fey et al., 2019b), increases pineal melatonin level in freshwater brook trout *Salvelinus fontinalis* (Lerchl et al., 1998), stimulates potential effect of bioturbation and ammonia excretion rate reduction in *Hediste diversicolor* (Jakubowska et al., 2019), causes behavioral alterations in different fish species (Bevelhimer et al., 2013), affects physiology and behaviour of crustaceans, bivalves (Aristarkhov et al., 1988; Bochert and Zettler, 2004, 2006; Scott et al., 2018; Hutchison et al., 2020; Albert et al., 2020) and fish at various ontogenetic stages (Formicki et al., 2019). Although some preliminary results are already available, no consistent pattern of EMF-induced genotoxicity and cytotoxicity effects in cells or organisms has been determined yet. Results of some studies show that ELF-EMF exposure does not induce genotoxic or cytotoxic effects in human lymphocytes (Cho and Chung, 2003), mouse embryo fibroblasts (Balb/c 3T3 cells), rat glioma cells

(C6 cells) and human oesophageal squamous carcinoma cells (KYSE150 cells) (An et al., 2015). However, the published findings on *in vitro* and *in vivo* cellular effects of EMF exposure are contradictory (Kocaman et al., 2018).

1.1.4. Micronucleus and other nuclear abnormalities assay and genotoxicity risk assessment

The micronucleus (MN) and other nuclear abnormalities (NAs) assay is described as a simple, reliable, sensitive test system, generating early warnings about environmental quality alterations and providing immediate results (Pollo et al., 2015) not only in the case of hazard identification, but also in that of risk assessment (Hayashi, 2016). The MN and other NAs assay is widely used to identify genomic alterations in the *in vivo, in vitro* (Hayashi, 2016), and the *in situ* assessments (Bolognesi and Hayashi, 2011). Based on ICES/OSPAR Background Assessment Criteria (BAC), genotoxicity hazards were included in the assessment of GES (ICES, 2011; Baršienė et al., 2012c; Hylland et al., 2017).

The morphological features of nuclear abnormalities as biomarkers of environmental genotoxicity (MN, NB, NBf, BNb, BL) and cytotoxicity (DB, 8-shaped, Frag and Apop), which have been detailed by Fenech et al. (2003). The described genotoxicity and cytotoxicity endpoints have been adopted and applied by Baršienė et al. (2004, 2012a) in the analysis of fish and mussel cells. The MN and other NAs assay is a widely used method due to its proven suitability for different fish species (Hussain et al., 2018; Sommer et al., 2020), mussels (Bolognesi and Hayashi, 2011; Baršienė et al., 2014) and other marine (Trifuoggi et al., 2019; Nunes and Costa, 2019; Finlayson et al., 2019) or freshwater (Knapik and Ramsdorf, 2020; Parolini, 2020) organisms. Different tissues such as peripheral blood, cephalic kidney, liver, gill and fins, can be used for genotoxicity and cytotoxicity assessment (Arkhipchuk et al., 2005; Cavas et al., 2005; Bolognesi and Hayashi, 2011; Brinkmann et al., 2014). Genotoxicity and cytotoxicity analysis in peripheral blood erythrocytes seem to be the most appropriate test for animal health and welfare protection. As sample preparation is simple, no harm is done to the health of test organisms.

Following Water Framework Directive requirements, to improve the ecological risk assessment of pollutants, the MN test was used (Hagger et al., 2008). ICES approved methodology has been developed and used by Baršienė et al. (2012c) to assess background responses and genotoxicity risks in different organism species (fish and molluscs) from the Baltic Sea. MN test is recognized by international protection, normalisation and scientific organizations such as International Organization for Standardization (ISO), The Organization for Economic Co-operation and Development (OECD), Atomic Energy Agency (IAEA) (Sommer et al., 2020). The following fish species representing different categories, i.e. *C. harengus membras* (pelagic), *P. flesus* (benthic) and *G. morhua callarias* (demersal) (ICES, 2017), were selected for assessing environmental genotoxicity and cytotoxicity.

The scientific novelty of the thesis

The novelty of this study consists of the following first reported findings:

1. Genotoxicity and cytotoxicity levels were assessed in three most commonly occurring and commercially important fish species (herring (*Clupea harengus membras*), flounder (*Platichtys flesus*) and cod (*Gadus morhua callarias*)) inhabiting the chemical munitions dumping site located in the eastern part of the Gotland Basin of the Baltic Sea.
2. Environmental genotoxicity risk was determined to *C. harengus membras*, *P. flesus* and *G. morhua callarias* caught at the study stations located near or in the chemical munitions dumping zone C in the Gotland Basin of the Baltic Sea.
3. Environmental genotoxicity risk was determined in peripheral blood erythrocytes of *C. harengus membras*, *P. flesus* and *G. morhua*, which were:
    a) sampled from 2010 to 2017 at 52 study stations located in the southern part of the Gotland Basin.
    b) collected from 2011 to 2017 at 47 study stations located in the eastern part of the Gotland Basin.
4. The ecological status of different fish species from the southern part of the Gotland Basin was determined based on the obtained genotoxicity risk results and applying GES assessment criteria.
5. Potential genotoxicity and cytotoxicity of 50 Hz 1 mT electromagnetic field (EMF) exposure were determined to rainbow trout (*Oncorhynchus mykiss*) larvae and Baltic clam (*Limecola balthica*).

Theoretical and practical significance

The theoretical significance of this study lies in the following:

1. In the southern and eastern parts of the Gotland Basin, exceptionally high and high genotoxicity risks were determined to *C. harengus membras* and *P. flesus*.
2. In the southern and eastern parts of the Gotland Basin, responses to environmental genotoxicity and cytotoxicity in fish were found to be species-specific.
3. In the chemical munitions dumping zone, the highest ∑Gentox responses were recorded in *C. harengus membras*, lower ∑Gentox responses being found in the fish caught further away.
4. Genotoxicity levels in *C. harengus membras* and *P. flesus* collected from the Lithuania EEZ were found to decrease over time. In contrast, in fish from the Polish EEZ, no time-related changes in genotoxicity levels were determined.
5. The presence of genotoxic and cytotoxic pressure on fish in the studied areas was confirmed based on the ∑Gentox and ∑Cytox values recorded in the fish sampled at the study stations located relatively close to each other.
6. The potential genotoxicity and cytotoxicity of a 50 Hz 1 mT electromagnetic field to *O. mykiss* larvae and *L. balthica* were determined.
7. Exposure to the EMF of such intensity that is typically generated by submarine cables was first proved to significantly and negatively affect molluscs and the early life stages of salmonids.

The practical significance of this study:

1. The environmental genotoxicity and cytotoxicity effects determined in common fish species of the Baltic Sea will be helpful in conducting the continuous monitoring of chemical munitions dumping sites.
2. Long-term data on cytogenetic damage in the Baltic Sea fish species will prove useful because they will supplement the HELCOM monitoring database and help identify time-dependent changes in genotoxicity and cytotoxicity effects.

3. The obtained results will be helpful in improving the ecological status of the eastern Baltic Sea region as they allow classifying the region according to the GES (Good Environmental Status) criteria.
4. The micronucleus and other nuclear abnormalities assay was confirmed to be a valuable method for investigating the EMF-inflicted cytogenetic damage in aquatic organisms.
5. Based on the study results, *L. balthica* could be proposed as one of the most suitable bio-indicator species for assessing EMF-induced genotoxicity and cytotoxicity.
6. The potential of EMF-inflicted cytogenetic damage determined in aquatic species may encourage the introduction of magnetic field emissions' regulation and standardisation and development of technical solutions for reducing the EMF emission into the marine environment.

The aim and objectives of the thesis

This thesis aims to assess the peculiarities of genotoxicity and cytotoxicity effects in fish species inhabiting the southern and eastern parts of the Gotland Basin of the Baltic Sea.

The objectives of the thesis are as follows:

1. To determine the level of environmental genotoxicity and cytotoxicity in peripheral blood erythrocytes of different fish species.
2. To determine the peculiarities of genotoxic and cytotoxic effects in fish from the Gotland Basin and patterns of their change over time (including the 2010–2017 period in the southern part and the 2011–2017 period in the eastern part of the Gotland Basin).
3. To evaluate the level of environmental genotoxicity and cytotoxicity in native fish species inhabiting the chemical munitions dumping zones located in the eastern part of the Gotland Basin.
4. To assess genotoxicity risks to different fish species at stations located in the Gotland Basin, applying background assessment criteria.
5. To assess genotoxicity and cytotoxicity effects of exposure to a 50 Hz 1 mT electromagnetic field in early development stages of *O. mykiss* and *L. balthica*.

Statements to defend

1. In the Gotland Basin of the Baltic Sea, environmental genotoxicity and cytotoxicity responses in fish are species-specific and sampling location-dependent.
2. In the Gotland Basin, environmental genotoxicity risk to *C. harengus membras* and *P. flesus* is exceptionally high and high, and that to *G. morhua callarias* is increased.
3. The environmental genotoxicity- and cytotoxicity-inflicted cytogenetic damage in fish from the Lithuania EEZ shows a tendency to decrease over time (2011−2016).
4. In the chemical munition dumping zone, the highest ∑Gentox responses were recorded in *C. harengus membras*, while ∑Gentox responses in the fish caught further away from the known sources of pollution with chemical warfare agents were lower.
5. Exposure to a 50 Hz 1 mT EMF was found to produce genotoxic and cytotoxic effects in *O. mykiss* larvae and *L. balthica*.
6. *O. mykiss* larvae and *L. balthica* are suitable bio-indicators for assessing EMF-induced genotoxicity and cytotoxicity effects in freshwater and marine environments. Adult *L. balthica* could be proposed as one of the most suitable bio-indicator species to assess EMF-induced genotoxicity and cytotoxicity effects.
7. The nuclear abnormalities assay was recognised as a suitable and reliable tool for detecting EMF-inflicted cytogenetic damage in aquatic organisms.

2. MATERIALS AND METHODS

2.1. Study design

For analysing genotoxicity and cytotoxicity effects in peripheral blood erythrocytes of adult fish, early development stages of fish and molluscs were used for a micronucleus (MN) and other nuclear abnormalities assay. These research methods and the criteria described by Heddle et al. (1991), Fenech et al. (2003) and Baršienė et al. (2004, 2012a) were applied in this study.

The simplified scheme of the *in situ* and experimental research design is presented in Table 1.

1 Table. A general outline of the investigations conducted within the current thesis as discussed in separate papers.

1 lentelė. Disertacijos metu atliktų tyrimų apžvalga.

The region of the Baltic Sea	Species	Sampling date	Sampling stations (number of specimens)	Stage of development	Paper
The southern part of the Goland Basin	*Clupea harengus membras* (Linnaeus, 1761)	2010-2017	31 (308)	Adult	III
	Platichtys flesus (Linnaeus, 1758)		20 (213)		
	Gadus morhua callarias (Linnaeus, 1758)		24 (237)		
The eastern part of the Gotland Basin	*C. harengus membras*	2011-2017	29 (288)	Adult	IV
	P. flesus		19 (205)		
	G. morhua callarias		12 (112)		
The Gulf of Riga	*C. harengus membras*	2010	6 (89)	Adult	I
	P. flesus		7 (88)		
Experimental design					
Species	Tissue	Exposure duration	Number of specimens	Stage of development	Paper
Oncorhynchus mykiss (Walbaum, 1792)	Peripheral blood erythrocytes	40 days	40	From embryos at eyed-egg stage (244°D) to exogenous feeding larvae	II
Limecola balthica (Linnaeus, 1758)	Gill cells	12 days	40	Adult	II

2.2. Sampling of fish

The southern part of the Gotland Basin. *Clupea harengus membras*, *P. flesu*s and *G. morhua callarias* were collected from 37 study stations located in the Polish EEZ and 13 stations located in the Lithuanian EEZ from November 2010 to June 2017. Peripheral blood samples were collected from 308 *C. harengus membras* (31 stations), 213 *P. flesu*s (20 stations) and 237 *G. morhua callarias* specimens (24 stations) (Table 1). The data from reference stations B09 (December 2003) and BP3 (December 2003) were used to evaluate time-related changes in genotoxicity and cytotoxicity responses of fish.

Samples were collected during the fish research surveys (types BITS and BIAS) carried out mainly by the Polish RV "Baltica" using the standard bottom or pelagic small-meshed trawls. The German RV "Walther Herwig III" monitored 10 (B09, B09/01, B09/17, B09/18, B09/19, B09/19a, B09/20, B09/23, B09, and BP3) stations by bottom trawling using 180 ft trawls. Samples from three (1U, 2U, 3U) stations were collected during the survey carried out by the Lithuanian commercial fishing vessel "Wismar".

The eastern part of the Gotland Basin. Specimens of three fish species were caught from March 2011 to March 2017 at 47 study stations. Sampling for *P. flesu*s was carried out at the reference station 6b (November 2011), for *C. harengus membras* and *G. morhua callarias* at the reference station BP3 (December 2003). Most of the study stations were located near the known Gotland chemical weapons dumpsites. Samples were obtained from the fish research catches carried out mainly by the Polish RV "Baltica" and partly by the German RV "Walther Herwig III".

Peripheral blood samples for genotoxicity and cytotoxicity analysis were collected from 288 *C. harengus membras* (29 stations), 205 *P. flesu*s (19 stations) and 112 *G. morhua callarias* specimens (12 stations) (Table 1).

The Gulf of Riga. Blood samples were taken from *P. flesu*s and *C. harengus membras* specimens collected from seven stations during the German RV "Walther Herwig III" sampling cruise in December 2010. The preparations of slides were taken from 88 *P. flesus* specimens and from 89 *C. harengus* specimens (Table 1).

2.3. Experimental design

The experiment was performed in Poland at the National Marine Fisheries Research Institute, Department of Fisheries Oceanography and Marine Ecology.

The experimental system contains:

- EMF generator, which consists of two identical Helmholtz coils arranged in parallel to one another. The generator is equiped with AC gaussmeter GM-2, which allows measuring and adjusting values of magnetic flux intensity. Helmholtz coils are cooled by circulating water system connected to the water cooling unit Titan 2000. The generator produces EMF with a frequency of 50 Hz with magnetic induction values in the range 0–1mT.
- Two aquaria of the same size (V=25 dm^3, 30×30×28 cm). The experimental aquarium is positioned in the centre of the generator. The reference aquarium is positioned in the natural geomagnetic field.
- Experimental and the reference aquaria connected to a conditioning tank (V=300 dm^3) equipped with a cooling system (Titan 4000) that regulates and maintains temperature of the water at a constant level. The water in the experimental system is continuously aerated and pumped between aquaria and the conditioning tank at the rate of 1100 ml min^{-1}.
- A closedloop pumping system with mechanical and biological filters.

The eyed eggs (244 D⁰) of *Oncorhynchus mykiss* were obtained from the Dąbie Fish Hatchery (Dąbie, Poland). For acclimation, the eggs were kept for 24 h under constant-temperature conditions (T = 9.6ºC).

Early life stages of *O. mykiss* were exposed to a 50 Hz 1 mT EMF for 40 days. The eggs were kept in the dark until the hatching of larvae, after which the day/night cycle was applied. Feeding of larvae was started from the 30^{th} day of the experiment, after significant absorption of yolk-sac volume. After 40 days of exposure to EMF, blood samples were taken from 20 specimens of *O. mykiss* larvae of the treatment (EMF-exposed) group and 20 specimens of the control group.

Limecola balthica specimens were collected from a depth of 70–100 cm at the Kuźnica station located in the inner part of the Puck Bay (the southern part of the Baltic Sea). After catching, *L. balthic*a specimens were placed into tanks equipped with a flow-through water system filled with natural seawater (S=7.2). They were kept at a constant temperature corresponding to field conditions (T=16°C) for one week to acclimate. After 12 days of exposure to EMF, gill arches were sampled from 20 individuals of *L. balthica* of the treatment and control groups. A more detailed description of the experiment is presented in Paper II.

2.4. Blood sample preparation and analysis

Peripheral blood taken directly from the caudal vein of each fish (*C. harengus membras*, *P. flesus* and *G. morhua callarias*) with a drop of blood was directly smeared on a microscopic slide and air-dried. *O. mykiss* larvae heart puncture was performed to collect blood. Small pieces of *L. balthic*a gills were dissected, softly dragged along a clean slide and allowed to dry. Dried smears were fixed in methanol for 10 min and stained with 5% Giemsa solution in phosphate buffer (pH = 6.8) for 8 min.

Blood-stained slides were analysed using a bright-field Olympus BX51 microscope with an immersion objective (1000 x). Four thousand erythrocytes with intact cellular and nuclear membranes per fish, 1000 gill cells per mussel were evaluated using blind scoring. The results were expressed as the mean value (‰) of the sums of the analysed individuals' lesions scored in 1000 cells per organism collected from every study station or study group.

Micronuclei (MN), nuclear buds (NB), nuclear buds on filament (NBf) and bi-nucleated erythrocytes with nucleoplasmic bridges (BNb) and cells with blebbed nuclei (BL) were considered as genotoxicity endpoints. Fragmented (Frag), apoptotic (Apop), bi-nucleated (BN) and 8-shaped nuclei erythrocytes were regarded as cytotoxicity endpoints (Fig. 1). The levels of total genotoxicity (∑Gentox) and total cytotoxicity (∑Cytox) were assessed as the sum of the frequencies of the analysed genotoxicity (MN+NB+NBf+BNb) and cytotoxicity (Frag+Apop+BN+8-shaped) endpoints.

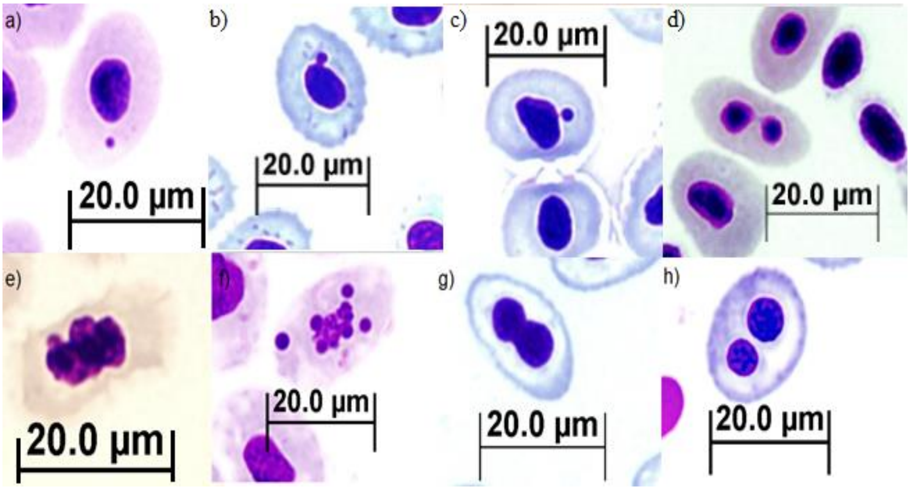

Fig. 1. Nuclear abnormalities in peripheral blood: a) erythrocyte with MN in *P. flesu*s, b) erythrocyte with NB in *C. harengus membras*, c) erythrocyte with nuclear bud on filament (NBf) in *C. harengus membras*, d) a bi-nucleated erythrocyte with nucleoplasmic bridge (BNb) in *P. flesu*s, e) fragmented (Frag) erythrocyte in *C. harengus membras*, f) apoptotic (Apop) erythrocyte in *G. morhua callarias*, g) 8-shaped nuclei erythrocyte in *P. flesu*s, h) bi-nucleated (BN) erythrocyte in *C. harengus membras*.

1 pav. *Branduolio pažaidų analizė žuvų periferinio kraujo eritrocituose. Genotoksinės pažaidos: (a) mikrobranduolys (MB), (b) branduolio pumpuras (BP), (c) branduolio pumpuras su nukleoplazmine jungtimi (BPs), (d) dvibranduolių tiltas (DBt). Citotoksinės pažaidos: (e) branduolio fragmentacija (Fr), (f) apoptozė (Ap), (g) aštuoneto formos branduolys (8), (h) dvibranduolė ląstelė (DB)*.

2.5. Environmental genotoxicity risk assessment in fish

Genotoxicity risk at each of the 99 studied stations in the southern and eastern parts of the Gotland Basin was assessed based on the calculated background ΣGentox levels (BAC) in specimens of *C. harengus membras* (<0.85‰ ΣGentox/1000 erythrocytes), *P. flesus* (<0.40‰ ΣGentox/1000 erythrocytes), and in those of *G. morhua callarias* (<0.55‰ ΣGentox/1000 erythrocytes) (Baršienė et al., 2012c). At seven stations in the Gulf of Riga, genotoxicity risk was assessed based on the determined background MN levels in *C. harengus membras* (0.39‰ MN/1000 erythrocytes) and *P. flesus* (0.23‰ MN/1000 erythrocytes) (Baršienė et al., 2012c). The methodology for BAC calculation and the BAC levels reported for different Baltic Sea organisms are presented in the article by Baršienė et al. (2012c).

Genotoxicity risk at the stations studied was evaluated based on the ∑Gentox level exceeding its BAC value (∑Gentox > BAC). It was rated on a 5-grade scale as low, moderate, increased, high and exceptionally high-risk, which corresponded to ∑Gentox > BAC determined in 0.0−19, 20−39, 40−59, 60−79, 80−100% of the specimens, respectively.

2.6. Statistical analysis

Since genotoxicity and cytotoxicity data did not follow a normal distribution (Kolmogorov-Smirnov test and Shapiro-Wilk test), the non-parametric Mann-Whitney *U* test was used to compare NAs frequencies among the study stations (GraphPad Prism® 5.01 (GraphPad Software Inc., San Diego, CA, USA)). The results were expressed as a mean ± standard error of the mean (SEM). The level of significance was established at $p < 0.05$. The Bonferroni correction was used to adjust the significance level of genotoxicity and cytotoxicity responses in fish from the eastern Gotland Basin of the Baltic Sea (Vickerstaff et al., 2019).

3. RESULTS

3.1. Environmental genotoxicity risk assessment in the Gulf of Riga (Baltic Sea) using *C. harengus membras* and *P. flesus*

Environmental genotoxicity risk (according to MN levels) in the Gulf of Riga was assessed using *C. harengus membras* collected from six study stations and *P. flesus* collected from seven study stations. The obtained environmental genotoxicity (according to MN levels) data showed that genotoxicity (MN) risk to *P. flesus* was exceptionally high at four stations (GOR3/41-S, GOR3/42S at southern coastal area of the Gulf of Riga, GOR2/37-W, GOR2/40-W at western coastal area), high at two stations (GOR1/30-E at eastern coastal area, GOR2/35-W at western coastal area) and increased at one station (GOR1/31-E at eastern coastal area). As for *C. harengus membras*, increased genotoxicity (MN) risk was determined at two stations (GOR1/30-E, GOR3/41-S) and moderate at four stations (GOR2/35-W, GOR2/37-W, GOR2/42-S, GOR1/31-E). A low genotoxicity risk level was not identified for fish (Fig. 2).

The interspecies comparison of environmental genotoxicity at the same study stations revealed that genotoxicity (MN) risk to *P. flesus* was higher than to *C. harengus membras*. Exceptionally high and high genotoxicity (MN) risks to *P. flesus* were found at six out of seven (85.71%) stations surveyed, no risk being determined to *C. harengus membras*. Study stations with exceptionally high and high genotoxicity (MN) risks were mainly located in the Gulf of Riga's southern and western coastal areas (Fig. 2).

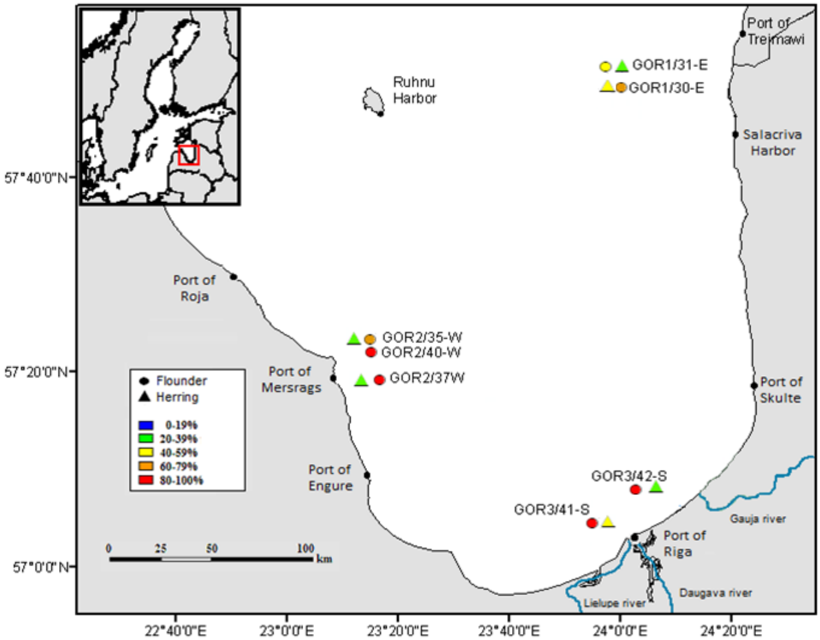

Fig. 2. Environmental genotoxicity risk assessment (according to MN levels) in *C. harengus membras* and *P. flesus* collected from different study stations in the Gulf of Riga (Baltic Sea).

2 pav. *Aplinkos genotototoksiškumo rizikos analizė (remiantis MB dažniu) C. harengus membras ir P. flesus žuvims sužvejotoms tyrimų stotyse Baltijos jūros Rygos įlankoje.*

3.2. Genotoxic and cytotoxic effects of a 50 Hz 1 mT electromagnetic field on larval rainbow trout (*Oncorhynchus mykiss*) and Baltic clam (*Limecola balthica*)

This research represents the first attempts to evaluate the genotoxic and cytotoxic electromagnetic fields (EMF) and their impacts on aquatic organisms *in vivo*. The potential genotoxic and cytotoxic effects of a 50 Hz 1 mT EMF were evaluated in peripheral blood erythrocytes of *O. mykiss* fry after 40 days of exposure and gill cells of *L. balthica* exposed to this factor for 12 days.

3.2.1. Genotoxicity and cytotoxicity responses in *O. mykiss*

As evidenced by the results obtained, EMF exposure increased frequencies of genotoxicity and cytotoxicity endpoints in the analysed cells of the investigated freshwater fish (*O. mykiss*). Exposure of *O. mykiss* to EMF induced the formation of three (MN, NB and BL) out of four analysed genotoxicity endpoints in peripheral blood erythrocytes. The frequencies of MN (0.23‰) and NB (0.17‰) were found significantly elevated in *O. mykiss* larvae (Mann-Whitney *U* test, $p = 0.025$ and $p = 0.027$, for MN and NB, respectively). The frequency of BL (0.08‰) increased 4-fold compared to the control group, but this induction was not statistically significant (Mann-Whitney *U* test, $p > 0.05$). Erythrocytes with NBf were not detected (Fig.3).

Cytotoxicity endpoints (8-shaped nuclei, Frag and Apop erythrocytes) were recorded in the EMF-exposed group. Among the all endpoints analysed, the frequency of 8-shaped (0.58‰) nuclei was the highest. The induced 8-shaped nuclei frequency was statistically significant (Mann-Whitney *U* test, $p = 0.002$). The frequencies of Frag (0.04‰) and Apop (0.02‰) nuclei erythrocytes were low. The induction of BN was not recorded (Fig. 3).

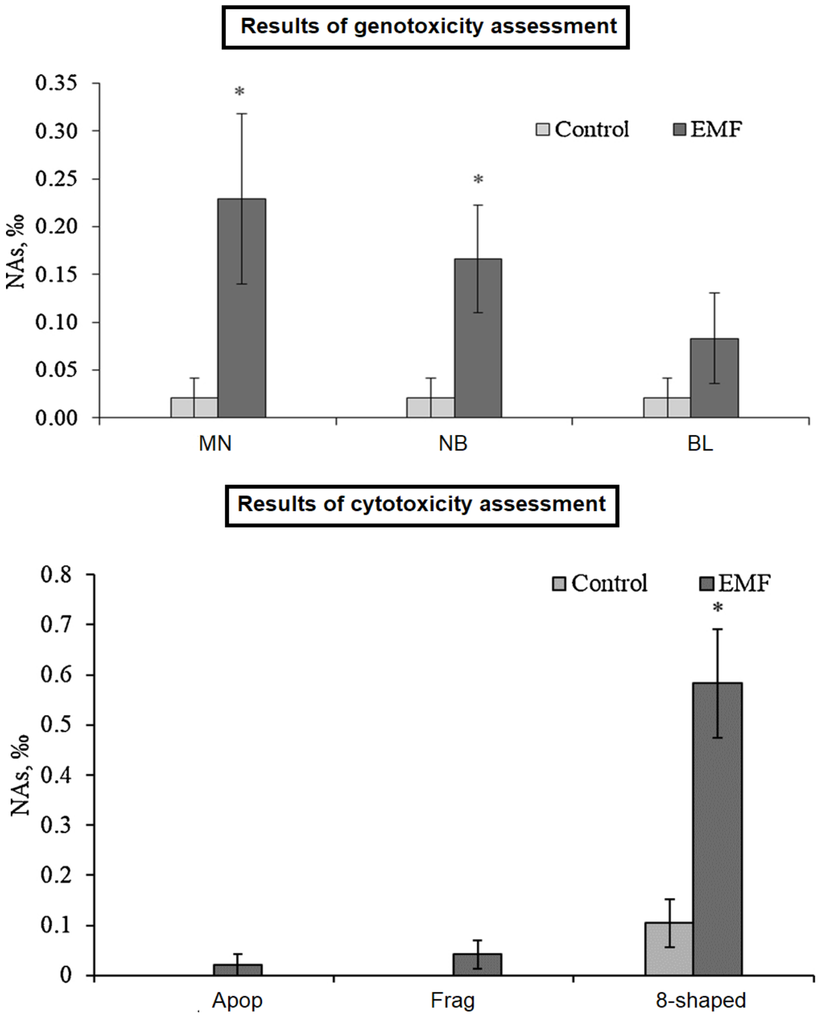

Fig. 3. Genotoxicity and cytotoxicity responses in erythrocytes of *O. mykiss* larvae (22–26 mm TL) after exposure to EMF of 1 mT for 40 days. Data are represented as mean ± SEM. Asterisks (*) denote statistically significant differences from the control group. In each group, N=20.

3 pav. *Genotoksiškumo ir citotoksiškumo atsakas O. mykiss lervų (22–26 mm TL) eritrocituose po 40 parų poveikio 1 mT EML. Žvaigždutės (*) žymi reikšmingus skirtumus tarp poveikio grupių ir kontrolinės grupės. Kiekvienoje grupėje – po 20 individų.*

3.2.2. Genotoxicity and cytotoxicity responses in *L. balthica*

Exposure to 1 mT EMF induced the formation of all analysed genotoxicity endpoints in *L. balthica* gills cells. The frequencies of genotoxicity endpoints NBf (0.5‰), NB (3.55‰), BL (4.1‰) were statistically significant induced in the treatment group compared to the control group (Mann-Whitney *U* test, $p = 0.002$, $p = 0.002$, and $p = 0.003$ for NBf, NB, and BL, respectively). A statistically significant increase of MN (1.25‰) frequency was not observed (Mann-Whitney *U* test, $p > 0.05$) (Fig. 4).

The effect of 1 mT EMF on cytotoxicity endpoints in gills cells of *L. balthica* after a 12-day exposure is presented in Fig. 4. The frequency of Apop, 8-shaped and BN cytotoxicity endpoints were statistically significant in the EMF-exposed group compared to the control group (Mann-Whitney U test, $p = 0.017$, $p = 0.004$, and $p = 0.046$ for Apop, 8-shaped, and BN cells, respectively). The frequency of Frag (1.3‰) nuclei erythrocytes was not statistically significant (Mann-Whitney U test, $p > 0.05$) (Fig.4). The stronger responses to EMF exposure were elicited in *L. balthica* compared to *O. mykiss* larvae.

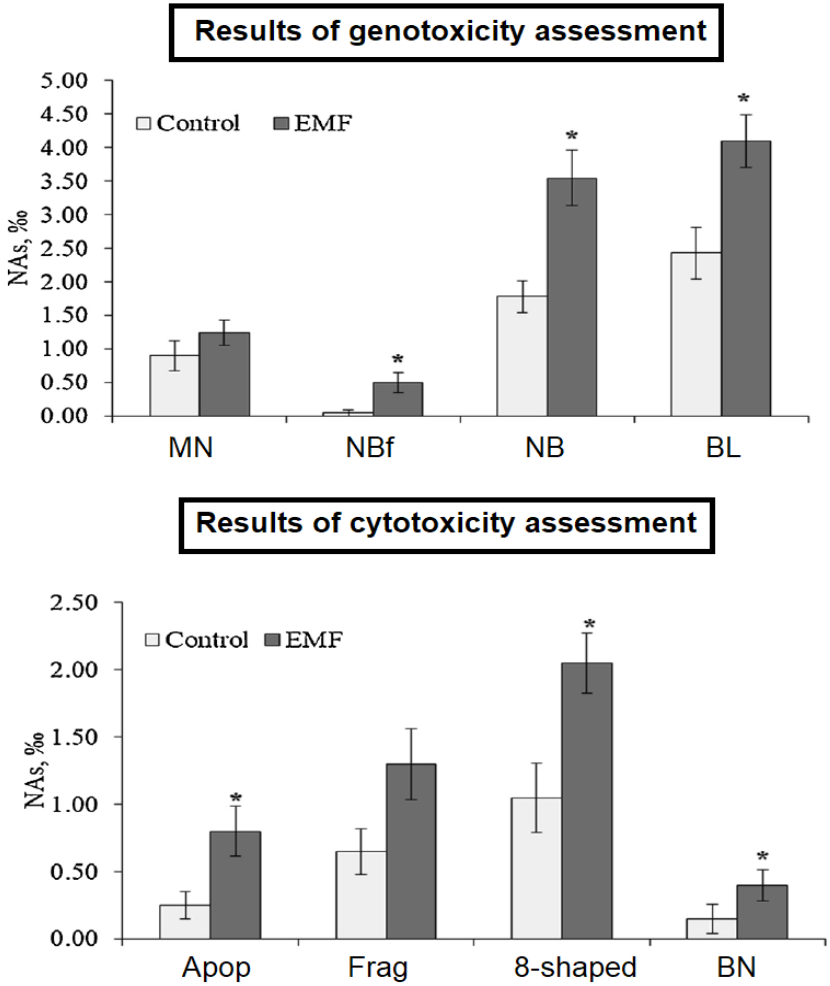

Fig. 4. Genotoxicity and cytotoxicity responses in *L. balthica* gills cells induced by exposure to EMF of 1 mT for 12 days. Data are represented as mean ± SEM. Asterisks (*) denote statistically significant differences from the control group. In each group, N=20.

4 pav. *Genotoksiškumo ir citotoksiškumo atsakas L. balthica žiaunų ląstelėse po 12 parų poveikio 1 mT EML. Žvaigždutės (*) žymi reikšmingus skirtumus tarp poveikio grupių ir kontrolinės grupės. Kiekvienoje grupėje – po 20 individų.*

3.3. Induction of nuclear abnormalities in herring (*Clupea harengus membras*), flounder (*Platichthys flesus*) and Atlantic cod (*Gadus morhua*) collected from the southern part of the Gotland Basin—the Baltic Sea (2010–2017)

This is the first attempt to determine the ecological status of the southern part of the Gotland basin according to GES assessment criteria. The frequencies of genotoxicity and cytotoxicity endpoints were examined in peripheral blood erythrocytes of *C. harengus membras*, *P. flesus* and *G. morhua callarias* collected in 2010–2017 from 37 study stations located in the Polish EEZ and from 13 stations located in the Lithuanian EEZ. Peripheral blood samples were collected from 308 *Clupea harengus membras* (31 stations), 213 *Platichthys flesus* (20 stations) and 237 *Gadus morhua* specimens (24 stations). In the Polish EEZ, study stations were located close to oil and gas platforms, chemical and conventional munitions dumping sites (Fig. 5).

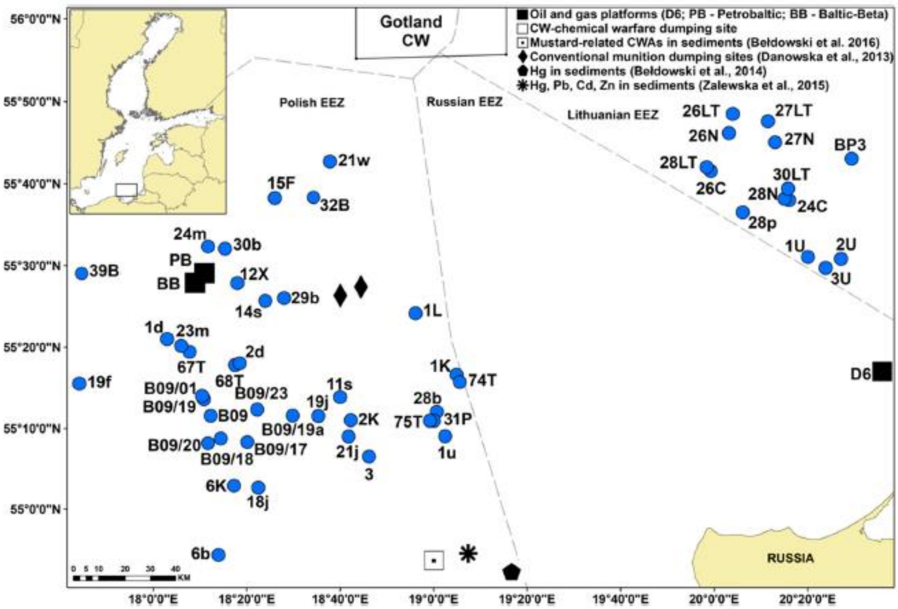

Fig. 5. Location of sampling stations in the Polish and Lithuanian EEZs (Baltic Sea).
5 pav. *Tyrimų stotys Lenkijos ir Lietuvos išskirtinėse ekonominėse zonose (IEZ).*

3.3.1. Environmental genotoxicity and cytotoxicity levels in *C. harengus membras*

In fish from the Polish EEZ, genotoxicity and cytotoxicity frequencies were higher than in fish from the Lithuanian EEZ. Strongly increased frequencies of separate and total ∑Gentox parameters were recorded in *C. harengus membras* at nine stations (Lithuanian EEZ – 30LT, Polish EEZ – 11s, 14s, 21w, 39B, 15F, 1K, 67T, 74T) (Fig. 6). The frequencies of these genotoxicity parameters in *C. harengus membras* specimens that are presented further in this study are without strongly increased frequencies.

Parameter	Stations								
	30LT	11 s	14 s	21w	39B	15F	1K	67T	74T
The values of nuclear abnormalities with strongly increased frequencies									
MN		40.59 ± 39.94	40.98 ± 39.89	6.40 ± 5.96	1.48 ± 0.86	3.65 ± 1.30	1.47 ± 0.72	1.47 ± 0.72	3.55 ± 0.80
NB	6.55 ± 4.83	20.40 ± 19.96	20.55 ± 19.96	20.78 ± 19.91					
NBf		20.50 ± 19.95		4.48 ± 3.95					
BNb	6.53 ± 6.50								
Frag		6.03 ± 6.00	10.15 ± 9.99	2.30 ± 1.97					
∑Gentox	16.63 ± 13.6	81.45 ± 79.84	62.10 ± 59.77	31.65 ± 29.82	2.8 ± 0.95	7.48 ± 1.58	2.36 ± 0.8	2.80 ± 0.90	5.28 ± 0.77
The values of nuclear abnormalities without strongly increased frequencies									
MN	0.81 ± 0.18	0.61 ± 0.21	1.08 ± 0.27	0.44 ± 0.15	0.64 ± 0.22	1.84 ± 0.45	0.78 ± 0.22	0.81 ± 0.29	3.00 ± 0.64
NB	1.72 ± 0.25	0.44 ± 0.10	0.61 ± 0.18	0.86 ± 0.14	1.08 ± 0.18	2.38 ± 0.56	0.81 ± 0.16	1.23 ± 0.23	1.48 ± 0.28
NBf	2.74 ± 2.20	0.56 ± 0.20	0.58 ± 0.21	0.53 ± 0.11	0.25 ± 0.15	1.45 ± 0.41	0.08 ± 0.04	0.10 ± 0.04	0.25 ± 0.10
BNb	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Frag	0.25 ± 0.10	0.03 ± 0.03	0.17 ± 0.06	0.33 ± 0.13	0.20 ± 0.05	0.50 ± 0.09	0.08 ± 0.04	0.30 ± 0.12	0.35 ± 0.09
∑Gentox	3.03 ± 0.48	1.61 ± 0.38	2.33 ± 0.34	1.83 ± 0.19	1.92 ± 0.36	4.64 ± 0.8	0.73 ± 0.09	1.94 ± 0.31	4.90 ± 0.68

Fig. 6. Strongly increased frequencies of MN, NB, NBf, BNb, Frag and ∑Gentox in *C. harengus membras* blood erythrocytes.
6 pav*. Ypač aukšti MB, BP, BPs, DBt, Frag ir ∑Gentox dažnis C. harengus membras periferinio kraujo eritrocituose.*

The frequency of ΣGentox in *C. harengus membras* varied from 0.98‰ at the reference station 6b to 4.90‰ at station 74T (March 2017). The highest ∑Gentox frequencies (exceeding 3‰) were recorded only in *C. harengus membras* caught at stations 29b (3.65‰, November 2011), 19j (3.38‰, November 2012), 15F (4.64‰, September 2015), 31P (3.05‰, November 2016), 74T (4.90‰, March 2017) in the Polish EEZ, and at stations 28LT (4.29‰, March 2011), 30LT (3.03‰, March 2011) in the Lithuanian EEZ. The frequencies of ΣGentox were found to exceed 1‰ in *C. harengus membras* specimens sampled at all study stations, except at station 6b (0.98‰). Significantly increased frequencies of ΣGentox were observed in *C. harengus membras* at 21 out of 31 stations (Polish EEZ – st3, 29b, 1d, 2d, 19f, 19j, 21, 11s, 14s, 21w, 39B, 15F, 1K, 31P, 67T, 74T, Lithuanian EEZ – 26LT, 27LT, 28LT, 30LT, 26C) (Fig.7).

The frequency of ΣCytox in peripheral blood erythrocytes of *C. harengus membras* varied from 0.23‰ at the stations 28N (March 2016) to 1.68‰ at station 15F (September 2015). Significantly increased ∑Cytox frequencies (exceeding 1‰) were recorded in *C. harengus membras* caught at six stations (Polish EEZ – 15F (1.68‰) > 31P (1.15‰) > 74T (1.13) > 67T (1.08‰) > 2d (1.03‰), Lithuanian EEZ – 28p (1.05‰). In blood samples of *C. harengus membras* collected from all study stations located in both marine areas, ∑Gentox frequencies were found to be higher than those of ∑Cytox. The ΣCytox frequencies recorded in *C. harengus membras* at 16 study stations (Polish EEZ – 2d, 19f, 18j, 19j, 21j, 21w, 39B, 15F, 1K, 31P, 67T, 74T, Lithuanian EEZ – 27LT, 28LT, 28p, 26C) significantly differed from those at the reference stations 6b (Polish EEZ) and 28N (Lithuanian EEZ) (Fig. 7).

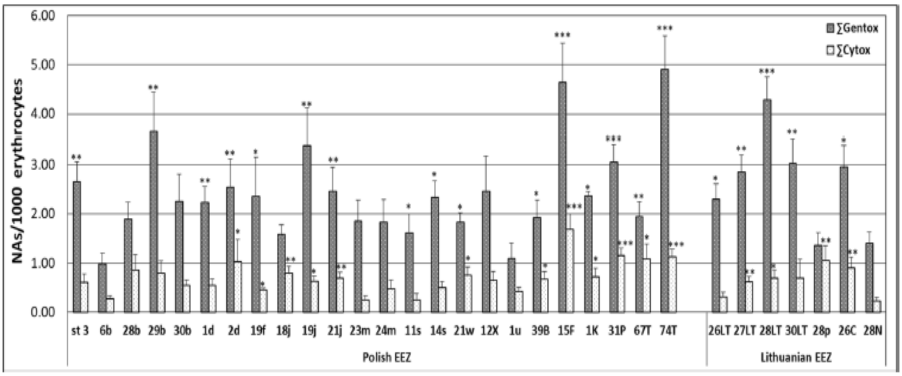

Fig. 7. ΣGentox and ΣCytox levels in *C. harengus membras* collected from the Polish and Lithuanian EEZs in 2010–2017. Data are represented as mean ± SEM. Asterisks indicate ΣGentox and ΣCytox levels recorded at stations in the Polish EEZ that are significantly different from those at the reference station 6b, and significantly different ΣGentox and ΣCytox levels at stations in the Lithuanian EEZ compared to station 28 N; **p* < 0.01, ***p* < 0.001, ****p* < 0.0001 .

7 pav. *∑Gentox ir ∑Cytox lygis C. harengus membras, pagautose 2010–2017 m. Lenkijos ir Lietuvos IEZ. Žvaigždutės žymi statistiškai patikimus skirtumus tarp tyrimų stočių ir kontrolinių stočių 6b (Lenkijos IEZ), 28N (Lietuvos IEZ); *p < 0.01, **p < 0.001, ***p < 0.0001.*

3.3.2. Environmental genotoxicity and cytotoxicity levels in *P. flesus*

In *P. flesus*, the ∑Gentox frequency revealed a variation between 0.48‰ at station B09/01 (December 2012) and 2.73‰ at station 28LT (March 2011). The highest ∑Gentox frequencies (exceeding 2‰) were recorded in *P. flesus* caught at station 3 (2.37‰, November 2010) in the Polish EEZ, and at stations 28LT (2.73‰, March 2011) and 26LT (2.55‰, March 2011). The frequencies of ΣGentox were found to exceed 1‰ in *P. flesus* specimens sampled at nine study stations (Polish EEZ – 75T and 18j (1.43‰) > 6b (1.28‰) > 1d (1.13‰) > 2d (1.08‰) > 12X (1.05‰) > 68T (1.03‰), Lithuanian EEZ – 27LT (1.98‰) > 30LT (1.81‰).

The frequency of ∑Cytox in *P. flesus* varied from 0.13‰ at reference station BP3 (December 2013) to 1.20‰ at station 23m (February 2013). The ΣCytox frequency in *P. flesus* collected from Polish EEZ was higher compared to fish responses from the Lithuanian EEZ. The ΣGentox frequencies higher than ΣCytox frequencies were recorded at most study stations (except three stations 23m (February 2013) and 68T (March 2017) in Polish EEZ, 26C (March 2015) in Lithuania EEZ). Significantly increased frequencies of ∑Gentox and ∑Cytox were recorded at all study stations (Fig. 8).

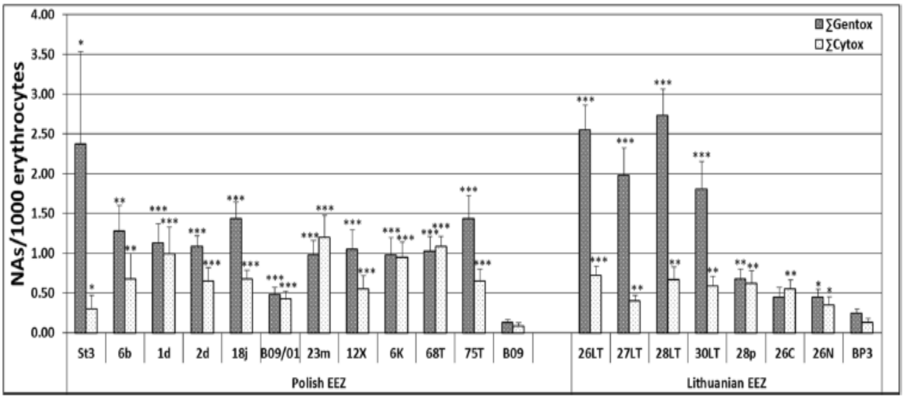

Fig. 8. ΣGentox and ΣCytox levels in *P. flesus* collected from the Polish (reference station B09) and Lithuanian EEZs (reference station BP3) in 2010–2017. Data are represented as mean ± SEM. Asterisks indicate significantly different ΣGentox and ΣCytox levels recorded at the monitored stations compared to reference stations; **p* < 0.01, ***p* < 0.001, ****p* < 0.0001.

8 pav. *∑Gentox ir ∑Cytox lygis P. flesus, pagautose 2010–2017 m. Lenkijos ir Lietuvos IEZ. Žvaigždutės rodo statistiškai patikimus skirtumus tarp tyrimų stočių ir kontrolinių stočių B09 (Lenkijos IEZ), BP3 (Lietuvos IEZ); *p < 0.01, **p < 0.001, ***p < 0.0001.*

3.3.3. Environmental genotoxicity and cytotoxicity levels in *G. morhua callarias*

The frequencies of ∑Gentox in *G. morhua callarias* ranged from 0.1‰ at study station B09/19a (September 2013) to 1.85‰ at station 21w (November 2013). Strongly increased frequencies of ∑Gentox were recorded in specimens collected at stations 21w, 1u (1.65‰, November 2013 and September 2014, respectively) and 15F (1.43‰, September 2015) located in the Polish EEZ.

∑Cytox frequencies ranged between 0.13‰ at reference station BP3 (December 2003) to 1.35‰ at station 15F (September 2015). In the Polish EEZ, significantly increased frequencies of ∑Gentox were found at eight stations (21w, 12X, 1u, 32B, 15F, 1L, 31P, 67T) and significantly increased frequencies of ∑Cytox at ten stations (B09/17, 21w, 12X, 1u, 32B, 15F, 1K, 31P, 37T, 74T) out of 18 stations studied compared to reference stations. Frequencies of ∑Gentox and ∑Cytox in *G. morhua callarias* at all (24C, 27N, 1U, 2U, 3U) study stations located in Lithuanian EEZ were significantly higher compared to reference stations (B09/01 and BP3) (Fig. 9).

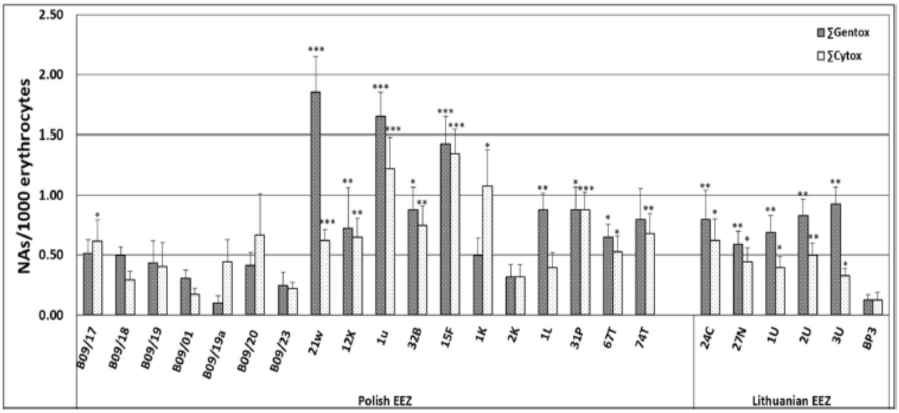

Fig. 9. ΣGentox and ΣCytox levels in *G. morhua callarias* collected from the Polish and Lithuanian EEZs in 2011–2017. The reference station B09/01 is located in the Polish EEZ and the reference station BP3 in the Lithuanian EEZ. Data are represented as mean ± SEM. Asterisks indicate significantly different ΣGentox and ΣCytox levels recorded at study stations compared to reference stations; *p < 0.01, **p < 0.001, ***p < 0.0001.

9 pav. *∑Gentox ir ∑Cytox lygis G. morhua callarias, pagautose 2011–2017 m. Lenkijos ir Lietuvos IEZ. Žvaigždutės rodo statistiškai patikimus skirtumus tarp tyrimų stočių ir kontrolinių stočių B09/01 (Lenkijos IEZ), BP3 (Lietuvos IEZ); *p < 0.01, **p < 0.001, ***p < 0.0001.*

3.3.4. Interspecies comparison of cytogenetic damage

The comparison of environmental genotoxicity and cytotoxicity responses in the fish species caught at the same study stations showed higher ∑Gentox and ∑Cytox responses in *C. harengus membras*, followed by those recorded in *P. flesus* and *G. morhua callarias*. *Clupea harengus membras* and *P. flesus* were collected from thirteen (Polish EEZ - st3, 6b, 1d, 2d, 18j, 23m, 12X, Lithuanian EEZ – 26LT, 27LT, 28LT, 30LT, 28p, 26C) stations, *C. harengus membras* and *G. morhua callarias* from eight (Polish EEZ – 21W, 12X, 1u, 15F, 1K, 31P, 67T, 74T), *P. flesus* and *G. morhua callarias* from one (12X) station. All three fish species were sampled at 12X station. At eleven stations (except stations 6b and 26LT), ∑Gentox values were found to be higher in *C. harengus membras* than in *P. flesus*. In contrast, higher ∑Cytox values in *C. harengus membras* than in *P. flesus* were recorded at nine stations (except stations 6b, 1d, 23m, 26LT). ∑Gentox values were found to be higher in *C. harengus membras* than in *G. morhua callarias* at six stations (except at stations 21w and 1u). In comparison, higher ∑Cytox values in *C. harengus membras* than in *G. morhua callarias* were recorded at six stations (except at stations 1u, 1K). Values of ∑Gentox and ∑Cytox in *P. flesus* were found to be higher than those in *G. morhua callarias*. Comparing ∑Gentox and ∑Cytox responses in all fish species collected from the same station 12X showed higher responses in *C. harengus membras* than in *P. flesus* and *G. morhua callarias* (Figs. 7–9).

3.3.5. Time-related differences in cytogenetic responses in fish from Polish and Lithuanian EEZ

The long-term studies of environmental genotoxicity and cytotoxicity in Polish and Lithuanian waters highlighted the existence of time-related differences in responses of fish from these marine areas. The collected data on environmental genotoxicity and cytotoxicity levels at the stations located close to each other in the Lithuanian EEZ revealed a significant time-related (2011–2016) decrease in genotoxicity endpoints in *C. harengus membras* and *P. flesus* (Fig. 7–9), while genotoxicity and cytotoxicity responses in *G. morhua callarias* remained unchanged over time (2015–2017). Environmental genotoxicity and cytotoxicity studies showed no time-related (2011–2017) changes in cytogenetic responses in all fish species caught at the stations located in the oil-gas platforms area in the Polish EEZ.

3.3.6. Environmental genotoxicity risk assessment in different fish species

The assessment of genotoxicity (according to ∑Gentox levels) risk levels in *C. harengus membras, P. flesus* and *G. morhua callarias* from 52 study stations in the southern part of the Gotland Basin showed exceptionally high and high levels of genotoxicity (∑Gentox) risk to fish species at 76.92% of the stations studied. Exceptionally high and high genotoxicity (∑Gentox) risks to *C. harengus membras* were found at 96%, to *P. flesus* at 92% and *G. morhua callarias* at 39% of the stations surveyed in the Polish EEZ. Exceptionally high and high genotoxicity (∑Gentox) risks to *C. harengus membras* were identified at 100%, to *P. flesus* at 86% and *G. morhua callari*as at 33% of the stations studied in the Lithuanian EEZ. The assessed genotoxicity responses in fish collected from the same stations (Polish EEZ - 1d, 2d, 23m, 18j, 75T, Lithuanian EEZ - 26LT, 27LT, 28LT, 30LT) showed the highest genotoxicity (∑Gentox) risk level for both fish species. According to the results, the highest genotoxicity (∑Gentox) risk exists for fish caught in the zone of oil and gas platforms in the Polish EEZ (marked by a grey circle in Fig. 10). Low genotoxicity risk was indentified for *P. flesus* from the reference stations BP3 (Lithuania EEZ) and B09 (Polish EEZ), for *G. morhua callarias* from 2K, B09/19, B09/19a, B09/20, B09/01, and B09/23 stations (Polish EEZ), for *C. harengus membras* low genotoxicity risk was not determined (Fig. 10). As evidenced by the obtained genotoxicity (∑Gentox) risk results, the Good Environmental Status (GES) level cannot be achieved in the southern part of the Gotland Basin, where high and exceptionally high ΣGentox risks were determined.

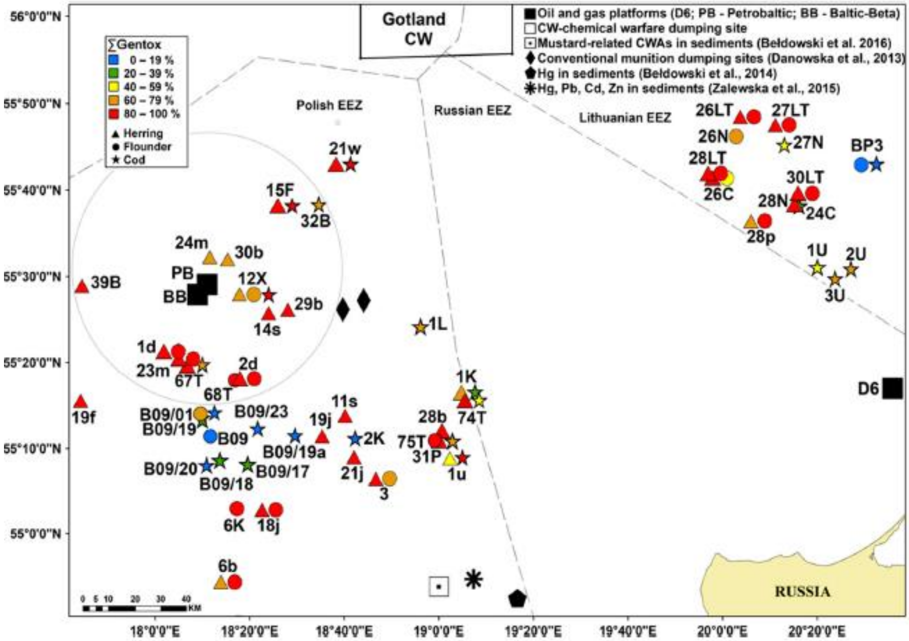

Fig. 10. Results of the environmental genotoxicity assessment based on ΣGentox levels in *C. harengus membras*, *P. flesus* and *G. morhua callarias* collected from 52 study stations in the southern part of the Gotland Basin (2010–2017). The zone of oil and gas platforms in the Polish EEZ is marked with a grey circle.

10 pav. *C. harengus membras, P. flesus, G. morhua callarias aplinkos genotoksiškumo rizikos įvertinimas (remiantis ΣGentox dažniu) pietinėje Gotlando baseino dalyje. Naftos ir dujų platformos esančios Lenkijos IEZ pažymėtos pilku apskritimu.*

3.4. Cytogenetic damage in native Baltic Sea fish species: environmental risks associated with chemical munitions dumping in the Gotland Basin

This study represents the first attempt to evaluate genotoxicity and cytotoxicity responses in *C. harengus membras*, *P. flesus* and *G. morhua callarias* and to assess environmental genotoxicity risk at each of the 47 study stations located in or close to the chemical munitions dumping zone in the eastern part of the Gotland Basin of the Baltic Sea (Fig. 11).

Environmental genotoxicity and cytotoxicity effects were analysed in *C. harengus membras*, *P. flesus* and *G. morhua callarias* caught at 29, 19, and 12 stations, respectively. There were 605 fish specimens analysed (288 specimens of *C. harengus membras*, 205 specimens of *P. flesus* and 112 specimens of *G. morhua callarias*). Most of the study stations were located close to the known Gotland chemical weapons (CW) dumpsite. Study stations 1e, B14/35-37, B14/49, B14/50 were located in the CW dumping area. The group of stations in the eastern Gotland Basin were in the area, where chemical warfare agents (CWA)-related substances were detected in sediment (CHEMSEA Findings, 2014).

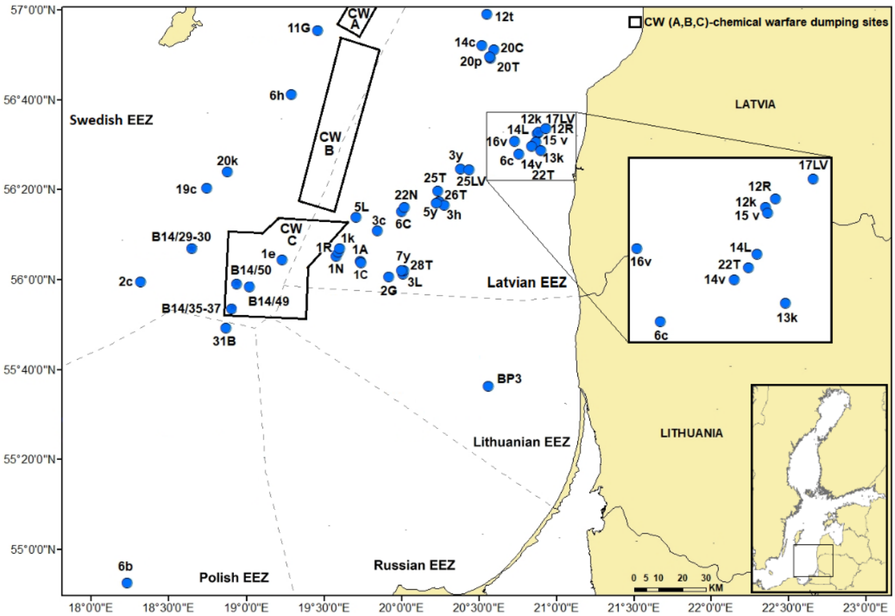

Fig. 11. Location of sampling stations in the eastern part of the Gotland Basin of the Baltic Sea.
11 pav. *Tyrimų stotys rytinėje Baltijos jūros Gotlando baseino dalyje.*

3.4.1. Environmental genotoxicity and cytotoxicity levels in *C. harengus membras*

The ΣGentox frequency in *C. harengus membras* ranged from 0.75‰ at station 15v (December 2013) to 13.13‰ at station 1e (October 2014). The highest ΣGentox frequencies (exceeding 3‰) were found in *C. harengus membras* at station 1e (13.13‰), which is located at the CW dumpsite, and at stations 19c (7.2‰), 1R (3.25‰) that are located near CW zone. High frequencies of ΣGentox (exceeding 1%) were recorded in fish collected from 24 out of 29 stations: 1e (13.13‰) > 19c (7.20‰) > 1R (3.25‰) > 25LV (2.59‰) > 14c (2.35‰) > 20C (2.30‰) > 17LV (2.25‰) > 12k (2.05‰) > 20k (2.03) > 5L (1.93‰) > 6c (1.90‰) > 2c (1.85‰) > 1k = 28T (1.83‰) > 1C (1.75‰) > 20p (1.67‰) > 3c (1.60‰) > 1A (1.30‰) > 5y = 22T (1.28‰) > 6h (1.23‰) > 2G (1.07‰) > 12R = 12t (1.03‰). Significantly higher frequencies of ΣGentox in *C. harengus membras* samples were not recorded (Fig. 12).

The frequency of ΣCytox varied from 0.10‰ at stations 20k (December 2012) to 1.60‰ at station 19c (December 2011). High frequencies of ΣCytox (exceeding 1‰) were recorded in *C. harengus membras* collected from stations 19c (1.60‰, December 2011) and 1e (1.08 ‰, October 2014). In *C. harengus membras* peripheral blood samples from station 1e located in the centre of CW dumpsite, ΣCytox frequency was recorded to be significantly higher compared to reference station 6b. In *C. harengus membras* sampled from all the study stations, the frequency of ΣGentox was found to be higher than that of ΣCytox (Fig. 12).

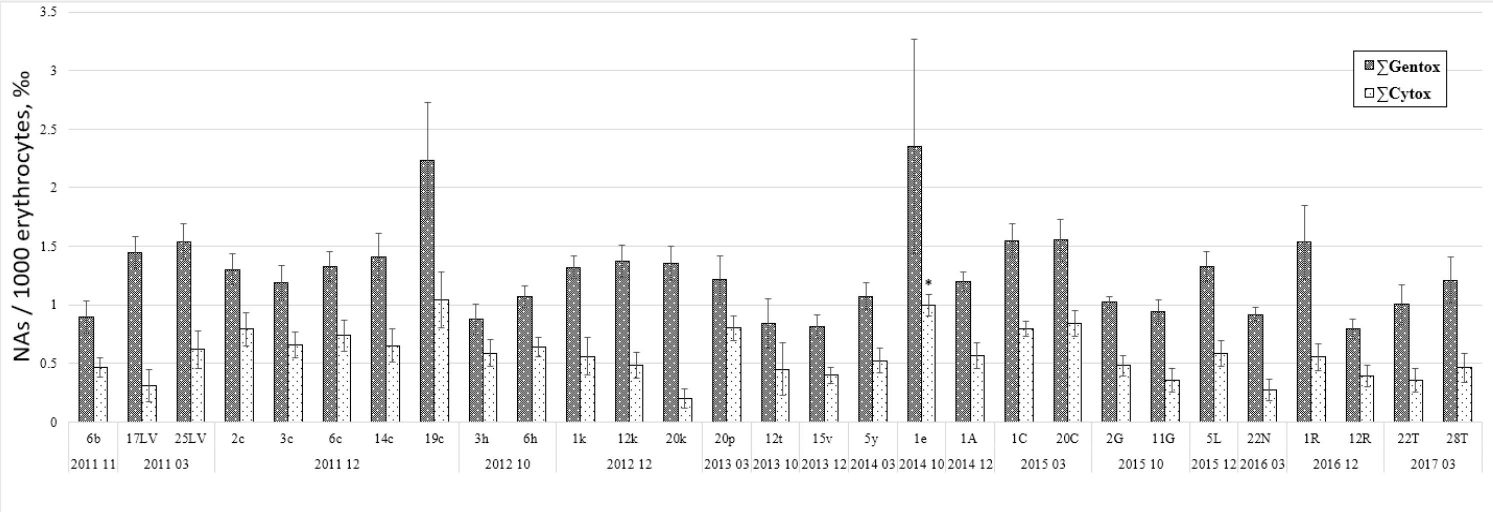

Fig. 12. Levels of ΣGentox and ΣCytox in *C. harengus membras* specimens collected from 28 study stations in 2011-2017 and in those collected from the reference station 6b in November 2011 (axis X). The square root scale was used because of high ΣGentox values recorded in herring at station 1e (13.13‰) and 19c (7.02‰). Data are represented as mean ± SEM. Asterisks (*) indicate statistically significantly different values of ΣGentox and ΣCytox from those recorded at the reference station (Mann-Whitney U test); * $p < 0.002$.

12 pav. *∑Gentox ir ∑Cytox lygis C. harengus membras, pagautų 28 tyrimų stotyse 2011–2017 m. ir 6b (2011 m. lapkritis) kontrolinėje stotyje (vidurkis ± SEM). Dėl aukštų ΣGentox lygių 1e (13,13 ‰) ir 19c (7,02 ‰) stotyse buvo naudojama kvadratinių šaknų skalė. Žvaigždutės rodo statistiškai patikimus skirtumus tarp tyrimų stočių ir kontrolinės stoties;* * $p < 0.002$.

A separate analysis of genotoxicity endpoints in *C. harengus membras* frequencies showed that MN frequency varied between 0.23‰ (at stations 3h (October 2012) and 6b (November 2011) and 12.05‰ at station 1e (October 2014)). Extremely high MN frequencies were found at stations 1e (12.05‰) and 19c (4.08‰). The MN frequency in *C. harengus membras* collected at station 1e was significantly higher compared to the reference stations. The frequencies of nuclear buds (NB+NBf) varied from 0.50‰ at station 22T (March 2017) to 3.05‰ at station 19c (December 2011). The highest frequencies of NB+NBf were determined at stations 1R (2.80‰, December 2016) and 25LV (2.01‰, March 2011). The (NB+NBf) frequencies above 1‰ were recorded in *C. harengus membras* caught at 15 (19c (3.05‰) > 1R (2.80‰) > 25LT (2.01‰) > 17LV (1.82‰) > 14c (1.68‰) > 1C (1.58‰) > 2c = 12k = 20k (1.53‰) > 6c = 1e (1.35‰) > 3c = 1k (1.33‰) > 5L (1.30‰) > 20C (1.03‰) out of 29 stations studied. The fragmented-apoptotic (FA) erythrocytes frequency in *C. harengus membras* revealed a variation between 0.03‰ at stations 2G, 11G (October 2015) and 0.70‰ at station 19c. FA erythrocytes were not detected in specimens collected from study stations 22N (March 2016), 12R (December 2016) and 22T (March 2017) (Fig. 13).

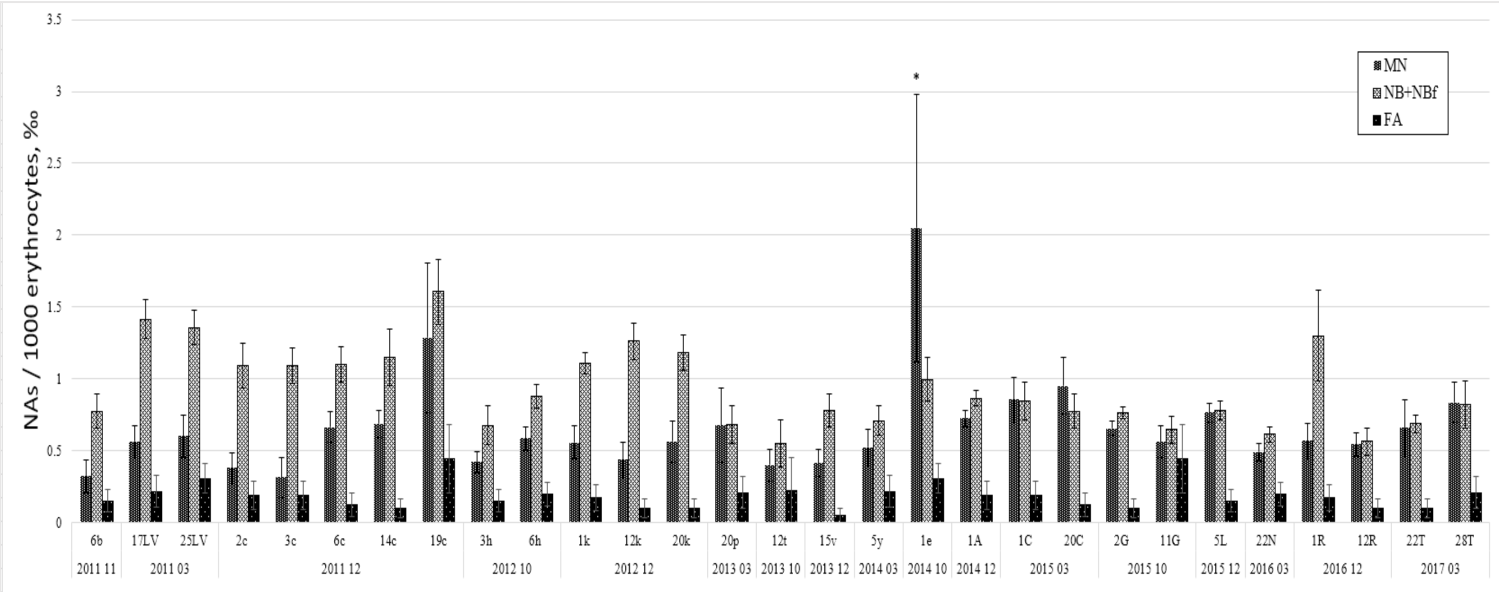

Fig. 13. Micronuclei (MN), nuclear buds (NB + NBf) and fragmented-apoptotic (FA) erythrocytes in herring collected from study stations during the 2011–2017 period (the axis X). Due to high MN values at station 1e (12.05‰) and 19c (4.08‰), were used square root scale. Data are represented as means ± SEM. Asterisks indicate statistically significant differences in MN, NB + NBf and FA frequencies between study stations and the reference station 6b (November 2011); *$p < 0.002$.

13 pav. *Mikrobranduolių (MB), branduolio pumpurų (BP+BPs) ir fragmentuotų-apoptotinių (FA) eritrocitų dažnis C. harengus membras, sužvejotų tyrimų stotyse 2011–2017 m. Žvaigždutės rodo statistiškai patikimus skirtumus tarp tyrimų stočių ir kontrolinės stoties 6b (2011 m. lapkritis); *p < 0.002.*

3.4.2. Environmental genotoxicity and cytotoxicity levels in *P. flesus*

The ΣGentox frequency in peripheral blood erythrocytes of *P. flesus* ranged from 0.07‰ in specimens at reference station BP3 (December 2003) to 2.57‰ at station 17LV (March 2011). The highest ΣGentox frequencies (values above 1‰) were detected in *P. flesus* at stations 10 (25LV (1.81‰, March 2011), 3c and 31B (1.58‰, December 2011 and February 2015, respectively), 13k (1.36‰, December 2012), 1N (1.28‰, March 2016), 1C (1.15‰, March 2015), 12R (1.06‰, December 2016), 6c (1.05‰, December 2011), 3y (1.03‰, March 2014), 19c (1.01‰, December 2011)) out of 19 study stations (Fig. 14).

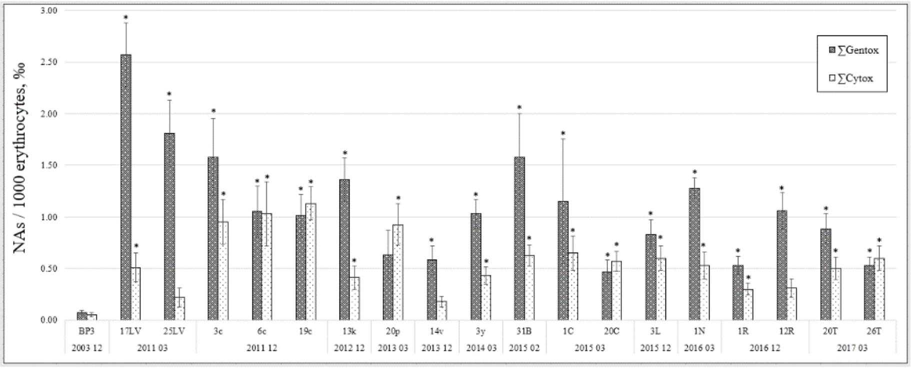

Fig. 14. The levels of ΣGentox and ΣCytox in *P. flesus* specimens collected from 18 study stations in 2011–2017 and from the reference station BP3 in December 2003 (the axis X). Data are represented as mean ± SEM. Asterisks (*) indicate statistically significa*ntly different values of ΣGentox and ΣCytox from those recorded at the reference station; *p < 0.003.*

14 pav. *∑Gentox ir ∑Cytox lygis P. flesus, pagautų 18 tyrimų stočių (2011–2017 m.) ir kontrolinėje stotyje BP3 (2003 m. gruodis) (virdurkis* ± SEM). *Žvaigždutės rodo statistiškai patikimus skirtumus tarp tyrimų stočių ir kontrolinės stoties; *p < 0.003.*

The ΣCytox frequency in *P. flesus* varied from 0.05‰ at reference stations BP3 (December 2003) to 1.13‰ at station 19c (December 2011, located close to CW dumpsite). The ΣCytox frequencies above 1‰ were recorded in *P. flesus* at stations 19c (1.13‰) and 6c (1.03‰). In summary, the research on genotoxicity and cytotoxicity in *P. flesus* indicates that ΣCytox frequencies at all stations sudied (except for the fish collected at stations 19c, 20p (March 2013), 20C (March 2015) and 26T (March 2017) were lower than ΣGentox frequencies. Frequencies of ΣGentox responses in *P. flesus* at 17 studied stations (except station 20p) were significantly higher than those at reference station BP3. At 15 studied stations (except stations 25LV, 14v, 12R), ΣCytox frequencies in *P. flesus* significantly differed from those recorded at the reference station (Fig. 14).

The performed analysis of separate genotoxicity endpoints (MN, NB, NBf and BNb) revealed a low induction of MN in *P. flesus* sampled at the reference station BP3 (0.06‰, December 2003). The highest frequencies of MN were detected in *P. flesus* collected at stations 25LV (1.20‰, March 2011), 3c (0.90‰, December 2011), 31B (0.85‰, February 2015), 1N (0.83‰, March 2016), 17LV (0.74‰, March 2011), 1C (0.70‰, March 2015). A more than ten times higher frequencies of MN were recorded at six out of 19 stations studied, and a 10-fold higher frequency of NB+NBf was recorded at all stations studied. The frequency of MN in *P. flesus* at 13 stations (except for the fish collected at stations 3c, 20p, 14v, 20T and 26T) significantly differed from those at reference station BP3. The frequencies of NB+NB in *P. flesus* from all study stations differed significantly from those at the reference station BP3. The frequency of fragmented-apoptotic (FA) erythrocytes in *P. flesus* varied between 0.02‰ (BP3) and 0.28‰ (20p, March 2013). FA was not recorded at stations 14v and 1R. Significantly increased frequencies of FA were recorded in *P. flesus* at 31B and 20C stations studied (Fig. 15).

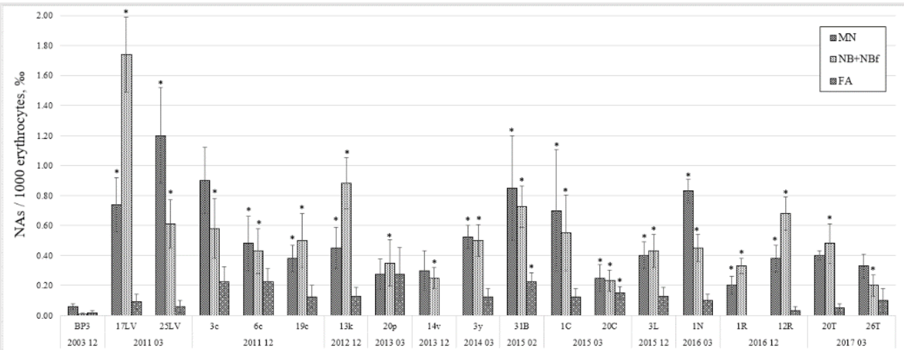

Fig. 15. Micronuclei (MN), nuclear buds (NB + NBf) and fragmented-apoptotic (FA) erythrocytes of *P. flesus* collected from study stations in 2011–2017. Data are represented as mean ± SEM. Asterisks indicate statistically significant differences in MN, *NB + NBf and FA frequencies between study and reference station BP3 (December 2012);*p < 0.003.*

15 pav. *Mikrobranduolių (MB), branduolio pumpurų (BP+BPs) ir fragmentuotų-apoptotinių (FA) eritrocitų dažnis P. flesus sužvejotų tyrimų stotyse 2011–2017 m. Žvaigždutės rodo statistiškai patikimus skirtumus tarp tyrimų stočių ir kontrolinės stoties; *p < 0.003.*

3.4.3. Environmental genotoxicity and cytotoxicity levels in *G. morhua callarias*

The ΣGentox levels in *G. morhua callarias* ranged between 0.13‰ at reference station BP3 (December 2003) and 1.13‰ at station 20T (March 2017). The ΣGentox frequencies higher than 1‰ were found in *G. morhua callarias* at station 20T (1.13 ‰). The ΣGentox frequencies in *G. morhua callarias* from 11 out of 12 stations studied (except stations 16v (December 2013) and 6C (March 2015)) significantly differed from those in the specimens sampled at references station BP3 (Fig. 16).

The frequencies of ΣGentox higher than ΣCytox responses were recorded at most of the study stations monitored, except at B14/29-30, B14/49, B14/50 stations. The frequency of ΣCytox ranged from 0.13‰ at station BP3 to 1.01‰ at station B14/29-30 (May 2012). The ΣCytox frequencies above 1‰ were recorded in *G. morhua callarias* at station B14/29-30 (1.01 ‰, May 2012). The ΣCytox frequencies in *G. morhua callarias* from two (14L (December 2012) and 20T (March 2017)) out of 12 studied ones significantly increased from those in the fish collected at reference station BP3 (Fig. 16).

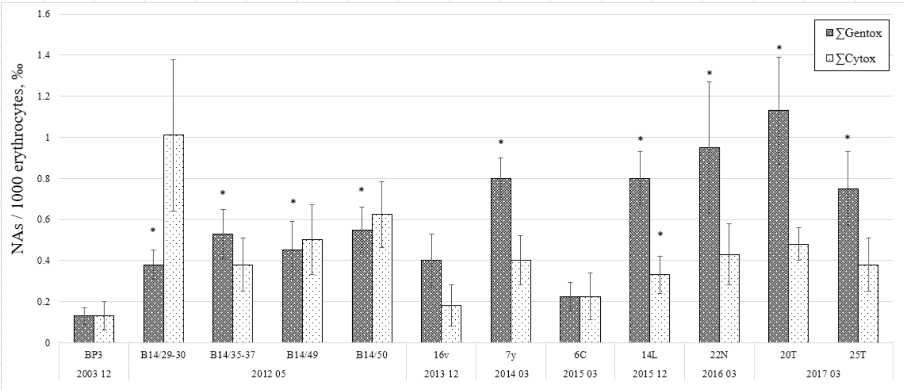

Fig. 16. The levels of ΣGentox and ΣCytox in *G. morhua callarias* specimens collected from 11 study stations in 2012–2017 and from the reference station BP3 in December 2003 (the axis X). Data are represented as mean ± SEM. Asterisks (*) indicate statistically significant differences in ΣGentox and ΣCytox levels from those recorded at the *reference station; *p < 0.004.*

16 pav. *∑Gentox ir ∑Cytox lygiai G. morhua callarias, pagautų 11 tyrimų stočių 2012–2017 m. ir BP3 (2003 m. gruodis) kontrolinėje stotyje (virdurkis* ± SEM). *Žvaigždutės rodo statistiškai patikimus skirtumus tarp tyrimų stočių ir kontrolinės stoties; *p < 0.004.*

The frequencies of MN varied from 0.03‰ at station BP3 to 0.43‰ at stations 7y (March 2014) and 20T (March 2017). Significantly increased frequencies of MN were recorded in *G. morhua callarias* at stations 7y (March 2014), 14L (December 2015), 20T and 25T (March 2017). The highest frequencies of NB+NBf were found at stations 22N (0.85‰, March 2016) > 20T (0.68‰, March 2017) > 25T (0.53‰, March 2017). Significantly higher frequencies of NB+NBf than those at the reference station were found in *G. morhua callarias* from eight (B14/35-37, B14/49 and B14/50 (May 2012), 7y (March 2014), 14L (December 2015), 22N (March 2016), 20T and 25T (March 2017) out of 12 stations. The highest frequencies of FA erythrocytes were recorded at stations B14/29-30 (0.31‰, May 2012). FA did not reveal significantly elevated levels in *G. morhua callarias* (Fig. 17).

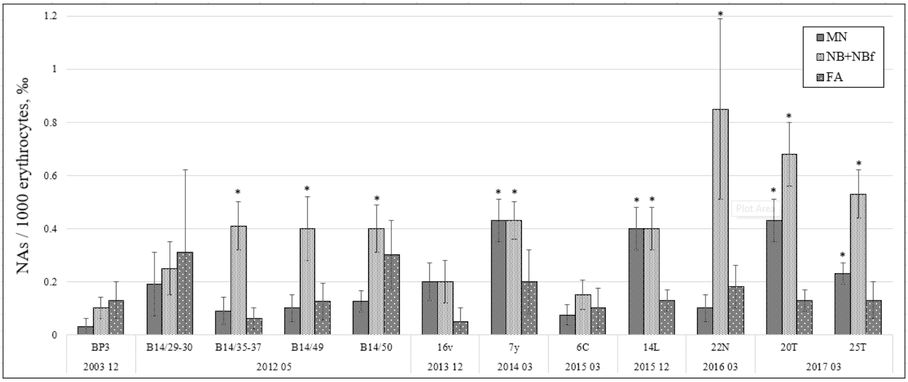

Fig. 17. Micronuclei (MN), nuclear buds (NB + NBf) and fragmented-apoptotic (FA) erythrocytes of *G. morhua callarias* were collected from study stations in 2012–2017. Data are represented as mean ± SEM. Asterisks indicate statistically significant differences in MN, NB + NBf and FA frequencies between study and reference station BP3 (December 2012); **p* < *0.004.*

17 pav. *Mikrobranduolių (MB), branduolio pumpurų (BP+BPs) ir fragmentuotų-apoptotinių (FA) eritrocitų dažnis G. morhua callarias sužvejotų tyrimų stotyse 2012–2017 m. Žvaigždutės rodo statistiškai patikimus skirtumus tarp tyrimų stočių ir kontrolinės stoties; *p < 0.004.*

3.4.4. Comparison of cytogenetic damage among fish species

Analysis of genotoxicity and cytotoxicity endpoints in different fish species caught at the same study stations showed that eight (25LV (March 2011), 3c, 6c and 19c (December 2011), 20p (March 2013), 1C and 20C (March 2015), 1R (December 2016)) out of 12 study stations revealed higher ΣGentox frequencies in *C. harengus membras* than in *P. flesus* or *G. morhua callarias*. Higher ΣCytox responses in *C. harengus membras* than in *P. flesus* or *G. morhua callarias* were at six (25LV, 19c, 20p, 1C, 20C, 1R) out of 12 studied stations (Fig. 18).

High ΣGentox frequencies above 1‰ were found in *C. harengus membras* at all ten study stations, in *P. flesus* at seven stations, in *G. morhua callarias* at one out of two stations studied. High ΣCytox frequencies (values above 1‰) were recorded in *P. flesus* at stations 6c and 19c, in *C. harengus membras* at stations 19c. Statistically significant differences between ΣGentox responses of *C. harengus membras* and *P. flesus* were recorded at four stations (19c (December 2011), 1C and 20C (March 2015) and 1R (December 2016)) (Fig.18).

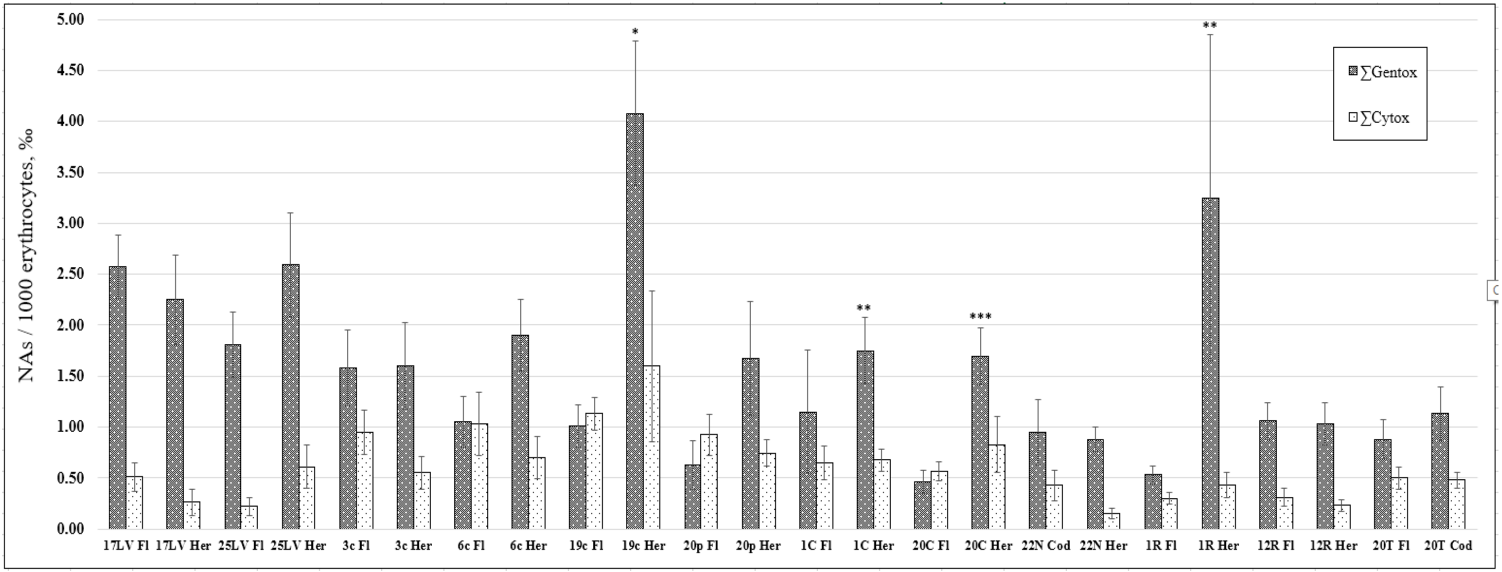

Fig. 18. Frequencies of ΣGentox and ΣCytox in herring (*C. harengus membras*), flounder (*P. flesus*) and cod (*G. morhua callaria*) caught in 2011–2017 at the same study stations (axis X) located in the Gotland Basin of the Baltic Sea. Data are represented as mean ± SEM. Asterisks (*) indicate statistically significant differences in ΣGentox and ΣCytox levels between different fish species.

18 pav. *Tarprūšiniai ΣGentox ir ΣCytox dažnio skirtumai C. harengus membras, P. flesus ir G. morhua callarias, sužvejotų tose pačiose tyrimų stotyse 2011−2017 m. Baltijos jūros Gotlando baseine. Žvaigždutės rodo statistiškai patikimus skirtumus tarp skirtingų žuvų rūšių.*

3.4.5. Environmental genotoxicity risk assessment in fish

The analysis of environmental ∑Gentox frequencies in the *C. harengus membras*, *P. flesus* and *G. morhua callarias* from the study stations in the Gotland Basin revealed that genotoxicity risk levels at 37 (except B14/29-30, B14/49, B14/50, 6C, 3h, 16v, 15v, 12t, 11G, 20C) out of 47 studied stations varied from exceptionally high to high levels. *Clupea harengus membras* was found to be exposed to exceptionally high and high genotoxicity (∑Gentox) risk at 23 stations (79.31%), *P. flesus* at 17 stations (89.47%), *G. morhua callarias* at six (50.00%) stations studied. Increased genotoxicity (∑Gentox) risk to *C. harengus membras* was determined at four stations (3h, 15v, 12t, 11G), to *P. flesus* and *G. morhua callarias* at one station (20C and B14/49, respectively). Low or moderate genotoxicity (∑Gentox) risk to *G. morhua callarias* was determined at five stations (BP3, B14/29-30, B14/50, 16v, 6C), to *C. harengus membras* at two stations (3h, 22N), and *P. flesus* at BP3 (Fig.19).

The analysis of ∑Gentox levels at study stations, from which more than one fish species has been collected, showed that genotoxicity (∑Gentox) risk was uniform and exceptionally high and high at eight (17LV, 25LV, 3c, 6c, 20p, 1C, 20T, 1R) out of 12 study stations. Differentiation between genotoxicity risk to *C. harengus membras* and that to *P. flesus* was revealed at two stations (12R, 20C), to *P. flesus* and *G. morhua callarias* at one station (22N). The long-term assessment of environmental genotoxicity and cytotoxicity to fish in the eastern part of the Gotland basin revealed no changes in cytogenetic responses over time (2011–2017) (Fig.19).

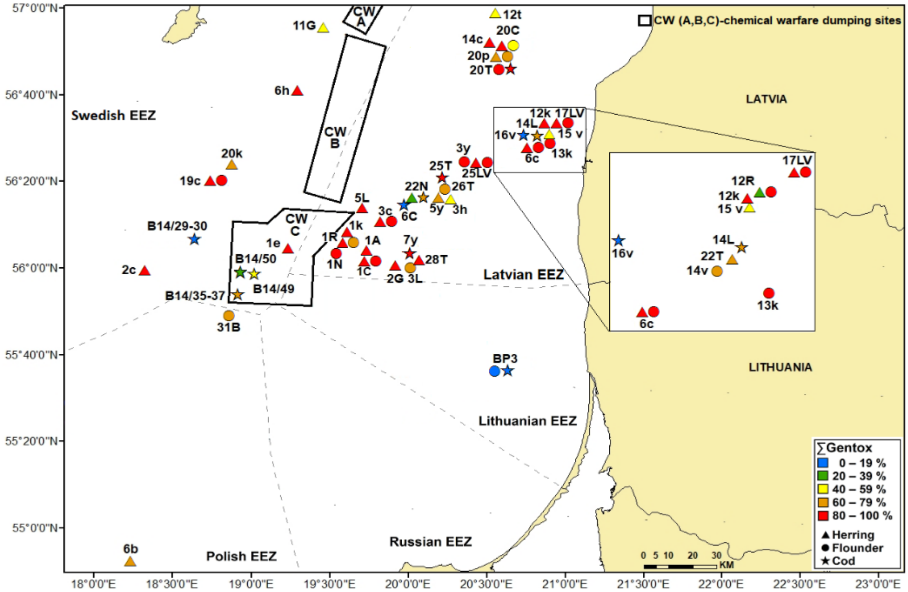

Fig. 19. Environmental genotoxicity (according to ΣGentox levels) risk (2011–2017) to *C. harengus membras*, *P. flesus* and *G. morhua callarias* at 47 study stations in the Gotland Basin of the Baltic Sea.

19 pav. *C. harengus membras, P. flesus, G. morhua callarias aplinkos genotoksiškumo rizikos įvertinimas (remiantis ΣGentox dažniu) 47 tyrimų stotyse rytinėje Gotlando baseino dalyje.*

4. DISCUSSION

Pressure from such contaminants as heavy metals, polycyclic aromatic hydrocarbons (PAH), polybrominated diphenyl ethers (PBDEs) and radionuclides on the marine environment in all parts of the Baltic Sea is known to be high and to pose significant risks to marine ecosystems. Unfortunately, the Baltic Sea also faces a threat from such "new" sources of pollution as chemical warfare agents (CWA) and anthropogenic energy (e.g. magnetic, electromagnetic fields). Therefore, this study aims to provide the first insights into the environmental genotoxicity and cytotoxicity effects as well as genotoxicity risk to native and commercially important fish species (*C. harengus membras, P. flesus, G. morhua callarias*) affected by multiple stressors in the Gotland Basin of the Baltic Sea, including zones of oil and gas platforms, chemical and conventional munitions dumping sites, and port-related water pollution. Moreover, this is the first research to assess the potential genotoxic and cytotoxic effects of 50 Hz 1 mT electromagnetic field (EMF) on marine invertebrates (*L. balthica*) and freshwater fish (*O. mykiss*).

4.1. Genotoxicity and cytotoxicity responses and environmental genotoxicity risk associated with environmental pollution

The assessment of environmental genotoxicity and cytotoxicity responses in the fish (*C. harengus membras, P. flesus, G. morhua callarias*) sampled from the southern part of the Gotland Basin revealed zone- and time-related differences between responses of fish from the Polish and Lithuanian EEZs. Genotoxicity and cytotoxicity responses in fish from the Polish EEZ were higher than those elicited in fish from the Lithuanian EEZ. The high ∑Gentox and ∑Cytox values recorded in fish sampled at the study stations located relatively close to each other indicate increased environmental pollution (Valskienė et al., 2019). The Gotland Basin is one of the most polluted areas in the Baltic Sea. It is polluted with a wide variety of widespread genotoxic pollutants, including microplastics, heavy metals, PAH, PBDE, alkylphenols and other pollutants (Ricking and Schulz, 2002; Pikkarainen, 2004; Zalewska et al., 2015; Graca et al., 2016; HELCOM, 2018; Jakubowska et al., 2020; Urban-Malinga et al., 2020). Statistical analysis of the spatial distribution of oil spills showed that 55% of the total oil spills were detected in the Russian EEZ, 33% in the Polish EEZand 12% in the Lithuanian EEZ in 2004-2015 (Krek et al., 2018). In the Lithuanian territorial waters, the degree of the sediment contamination described as low for nickel and chromium, minor–

moderate for lead, zinc and copper, and moderate–considerable pollution for Cd (Remeikaitė-Nikienė et al., 2018). Investigations on sediment samples showed higher arsenic concentrations near the Gotland CW dumpsite (average 9.7 mg/kg) compared to other Lithuanian coastal areas (2.1 mg/kg) (Garnaga and Stankevičius, 2005).

The results of this thesis show exceptionally high and high genotoxicity risks to three fish species at all study stations in the Polish EEZ zone, where oil and gas platforms are operating intensively. Moreover, at the study stations located outside the oil-gas platforms area, fish also showed very high ∑Gentox and ∑Cytox responses. It should be pointed out that near these stations, increased concentrations of mercury, lead, cadmium and zinc were detected in sediments (Bełdowski et al., 2014; Zalewska et al., 2015).

As evidenced by the results of the long-term environmental genotoxicity and cytotoxicity assessment (2015–2017), a significant time-related decrease in environmental genotoxicity was recorded only in the Lithuanian EEZ, while in the Polish EEZ (2014–2016), no time-related changes in genotoxicity and cytotoxicity were revealed (Valskienė et al., 2019). The time-related stability of genotoxicity and cytotoxicity effects in fish, which was observed in the Polish EEZ, could be the outcome of contaminants dispersion during the implementation of pan-Baltic industrial projects (construction of Nord Stream pipeline and laying of undersea electric cable between St Petersburg and Kaliningrad) and the mine clearance programme in 2008–2011 (Möller, 2011). Good Environmental Status (GES) level cannot be achieved in zones where high and exceptionally high ΣGentox risks were determined.

The interspecies comparison of ΣGentox and ∑Cytox responses in fish caught at the same study stations shows *C. harengus membras* to be the most sensitive bioindicator species, followed by *P. flesus* and *G. morhua callarias*. According to the results obtained, ΣGentox levels in *C. harengus membras* were found to be higher than those in *P. flesus* by 81.81% of the study stations and higher than ΣGentox levels in *G. morhua callarias* by 77.78%. The levels of ΣCytox in *C. harengus membras* exceeded those in *P. flesus* by 59%, and those in *G. morhua callarias* by 66,67% of the stations surveyed (Valskienė et al., 2019; Pažusienė et al., 2021). Similar results were reported by Valskienė et al. (2018) in their publication. The interspecies comparison of ΣGentox levels in three fish species revealed higher ΣGentox levels in *C. harengus membras* than in *P. flesus* and *G. morhua callarias* by 65% of the studied stations. According to the results obtained from the thesis research, the percentage of the study stations in the southern and eastern parts of the Gotland Basin, at which exceptionally high and high genotoxicity risks to *P.*

flesus and *C. harengus membras* were determined, is higher than that of the stations with exceptionally high and high genotoxicity risks to *G. morhua callarias* (Valskienė et al., 2019; Pažusienė et al., 2021). The same findings on genotoxicity risks to fish from the Bornholm and Gdańsk Basins were reported in the publications by Baršienė et al. (2012c, 2014) and by Valskienė et al. (2018). Only *P. flesus* from the Gulf of Riga was found to be exposed to exceptionally high and high genotoxicity risks (Butrimavičienė et al., 2018). The exceptionally high and high genotoxicity risks determined to fish at the stations located close to each other indicate an increased environmental genotoxicity pressure on fish in the southern and eastern parts of the Gotland Basin (Valskienė et al., 2019; Pažusienė et al., 2021).

4.2. Genotoxicity and cytotoxicity effects and genotoxicity risk associated with sea-dumped chemical weapons (CWs) containing toxic chemical warfare agents (CWA)

The areas located close to chemical and conventional munition dumpsites are under increased environmental pollution pressure, potentially causing lethal or chronic toxic effects on/in marine organisms (Della Torre et al., 2010, 2013; Baršienė et al., 2014, 2016; Bełdowski et al., 2016a; Koske et al., 2020). The research into environmental genotoxicity and cytotoxicity frequencies near and inside the chemical munitions dumping areas in the eastern part of the Gotland Basin and genotoxicity risks to fish therein, which was performed within the framework of this thesis, revealed that environmental genotoxicity levels in all tested fish species exceeded reference levels. The highest ∑Gentox level was recorded in *C. harengus membras* caught at station 1e (13.13%) located in the centre of the chemical munitions dumping zone C in December 2014, while lower, but statistically significant ∑Gentox levels were recorded in *G. morhua callarias* sampled at station B14/49 (0.53%), B14/35-37 (0.45%), B14/50 (0.38%) located in the same chemical munitions dumping zone C in May 2012. High and statistically significant ∑Cytox levels were determined in *C. harengus membras* collected from station 1e (1.08‰) and in *G. morhua callarias* from station B14/50 (0.63‰) that are also located in this chemical munitions dumping zone. Induction of ∑Gentox and ∑Cytox in the studied fish species signals the contamination of the Gotland CW dumpsites and the surrounding area. It is worth mentioning that the highest ∑Gentox frequencies were detected in *C. harengus membras* collected from station 19c (7.2‰), 1R (3.25‰), and the highest ∑Cytox frequencies were detected in *C. harengus membras* (1.60‰) and *P. flesus* (1.13‰) caught at station 19c, in *G.*

morhua callarias at station B14/29-30 (1.0‰) located close to the CWA dumpsite. Genotoxicity levels were found to be lower at stations located further away from dumping sites. However, these stations were still characterised by exceptionally high and high ∑Gentox risks, which are most likely entailed by the presence of genotoxic CWAs and their effects.

As has been reported by Vanninen et al. (2020), 63% of the sediment samples collected from the relatively large Gotland Deep dumpsite area contain residues of the compounds containing sulfur mustard and phenylarsenic. Such hydrological conditions as weak near seafloor currents and somewhat stable water stratification in the Gotland Basin area are more suitable for the disposal of chemical weapons to avoid threat to the marine ecosystem (MERCW, 2006). The dumped munitions/containers are distributed more randomly in the Gotland Deep than in the Bornholm and Gdańsk Basins (Söderström et al., 2018). The analysis of CWA degradation products in sediments revealed a more varied pattern of local hotspots distribution in the Gotland and Gdańsk Basins and a wide dispersion of contaminants in the Bornholm Deep (Bełdowski et al., 2016a). Although local, CWA contamination may spread, it's outspread depending on various factors. The distribution area of released CWAs and their degradation products may stretch for more than 250 m or even 1 km from sources of contamination, the range of contamination depending on bottom currents, topography and corrosion impact on munitions (CHEMSEA Findings, 2014; Vanninen et al., 2020).

Increased pollution pressure from CW dumpsites produces chronic or/and lethal damages on marine organisms (Sanderson et al., 2008; HELCOM 2010; Della Torre et al., 2010, 2013; Baršienė et al., 2014, 2016; Bełdowski et al., 2016c; Valskienė et al., 2018; Czub et al., 2020, 2021; Ahvo et al., 2020; Straumer et al., 2020). Previous studies have mainly been concerned with the metabolism and toxicity of diphenylarsinic acid (DPA[ox]) and degradation products of chemical warfare agents. DPA[ox] is considered to be the primary degradation product of arsenic-containing warfare chemicals such as diphenylchloroarsine (Clark I) and diphenylcyanoarsine (Clark II) (Kinoshita et al., 2005). *In vitro* research with Chinese hamster V79 cells has shown that DPA[ox] causes cytotoxic effects, chromosome structural aberrations and numerical changes, centrosome abnormalities and spindle organizations in conjunction with the effects of glutathione (GSH) depletion (Ochi et al., 2003, 2004). The presence of GSH strongly affects DPA[ox] toxicity, which is explained by the formation of intermediate conjugates (Ochi et al., 2006). DPA-SG (the GSH adduct of DPA[ox]) was established to be a thousand times more toxic to human HepG2-cells than DPA[ox] itself (Ochi et al., 2006; Kinoshita et al., 2006). Organo-arsenic compounds are degraded to toxic inorganic arsenic (arsenite As (III) and arsenate As (V)) compounds that are associated with acute and chronic health effects (Chaillou et al., 2003; Ellwood and Maher, 2003; Nicholas et al., 2003; Kroening et al., 2009; Franken and Hafez, 2009; Roy et al., 2020). Inorganic arsenic is methylated to dimethylarsinic acid (DMA) and methylarsonic acid (MMA), which are associated with the detoxification mechanism of arsenic-containing CWA (Vahter and Concha et al., 2008). According to other studies (Kumagai and Sumi, 2006; Thomas et al., 2004; Petrick et al., 2000), methylated inorganic arsenic metabolites may pose higher toxicity and reactivity to fish than inorganic arsenic itself. *In vitro* studies have demonstrated that pentavalent dimethylarsinic acid (DMA(V)) cause cytotoxicity (Ochi et al., 1994), genotoxicity (Oya-Ohta et al., 1996), apoptosis (Ochi et al., 1996), abnormalities of mitotic centrosome integrity and spindle organisation effects (Ochi et al., 1999) to mammalian cells. Latest studies show that CWAs and their hydrolysis products can bioaccumulate and/or cause adverse effects in aquatic biota and at multiple levels of biological organization (Kotwicki et al., 2016; Nawała et al., 2016; Chmielińska et al., 2019; Czub et al., 2020, 2021). As has been reported by Höher et al. (2019), blue mussels (*Mytilus trossulus*) bioaccumulate the oxidised forms of CWA such as Clark I, Adamsite (DAox and DMox) and chloroacetophenone into their tissues. Cytotoxic, immunotoxic and oxidative stress effects in *M. trossulus* occurred even at the lowest concentration of different mixtures of CWA (phenylarsenic compounds, Clark I and Adamsite) (Höher et al., 2019). *In vitro* research has shown that CWA-related phenylarsenic chemicals such as Clark I, Adamsite and phenylarsenic acid form glutathione (GSH) conjugates and methylated metabolites in the liver of cod (*G. morhua*). However, was no evidence that triphenylarsine oxide forms GSH conjugates or methylated metabolites, indicating a different metabolic pathway (Niemikoski et al., 2020a). The latest research performed by Niemikoski et al. (2020b) is the first research to provide information on phenylarsenic CWA bioaccumulation, distribution in tissue and adverse effect on *G. morhua* living in the vicinity of Bornholm CWs dumpsite. The above-mentioned research has shown that *G. morhua* muscle samples collected close to the main dumpsite contain trace levels of phenylarsenic CWAs. Moreover, significant changes in some biomarkers are observed in individuals having trace levels of CWA-related chemicals. Sulphur mustard is the most abundant CWAs in the Gotland Deep dumped stockpile (Knobloch et al., 2013). Sulphur mustard degradation products such as 1,2,5-trithiepane (Czub et al., 2020) and 1,4,5- oxadithiepane (Chmielińska et al., 2019) are described as the most toxic ones. Acute aquatic toxicity of sulphur mustard and its six degradation products has been examined using *Daphnia magna*. The results indicate that two sulphur mustard degradation products: 1,2,5- trithiepane (LC50 as low as 224 μg × L−1) and 1,4,5-oxadithiepane LC50 < 10 mg × L−1) (Czub et al., 2020) are highly toxic to *D. magna* (Czub et al., 2020). The latest investigation has shown that CWA (Lewisite, Adamsite, Clark I, phenyldichloroarsine (PDCA)), CWA-related compounds (TPA, arsenic trichloride) and four arsenic-based CWA decomposition products cause highly negative effects to *D. magna* after 48 h exposure. PDCA (LC50 at 0.36 μg × L−1) and Lewisite (EC50 at 3.2 μg × L−1) are very toxic to *D. magna* (Czub et al., 2021).

After World War II, conventional and chemical munitions containing mainly secondary (e.g. trotyl – TNT, hexogen – RDX) and primary (e.g. lead azide – Pb(N3)2) explosives were dumped mainly at the same CW dumpsites (Nawała et al., 2020). The presence of explosives and their degradation products in seabed sediment samples suggest the seabed contamination, which disturb the marine ecosystem (Nawała et al., 2020). DNA damage has been induced in zebrafish (*D. rerio*) embryos after short-term (120 h) exposure to trinitrotoluene (TNT) and its degradation products 2-amino-4,6-dinitrotoluene (2-ADNT) and 4- amino-2,6-dinitrotoluene (4-ADNT). The genotoxicity caused by TNT is about 3-4 times higher than that of 2-ADNT and 4-ADNT. Moreover, TNT and its decomposition products exposure pose a potential risk for long-term effects in fish living close by CW dumpsites (Koske et al., 2019). The uptake of TNT (2-ADNT and 4-ADNT) by marine organisms has been first described in blue mussels (*M. edulis*) from the munitions dumpsite (Strehse et al., 2017). Toxic explosives and their compounds have been identified in 48% of bile samples from dab (*Limanda limanda*) caught at the munitions dumpsite and reference sites in the Baltic Sea. The results show that TNT, 4-ADNT, 2-ADNT, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are accumulated by flatfish and may pose a risk to fish health (Koske et al., 2020). Based on the above-reviewed results showing that marine species (fish, molluscs) sampled from CW dumpsites are contaminated with CWAs, it seems reasonable to assume that the genotoxicity and cytotoxicity effects discussed in the present study could also be produced by CWAs.

The data obtained from long-term research into environmental genotoxicity did not show time-related changes in genotoxicity or cytotoxicity responses in fish from the area of CWs dumping. Exceptionally high and high genotoxicity risks were determined to benthic (*P. flesus*) and to pelagic (*C. harengus membras*) fish collected from all study stations in the chemical munitions dumping zone, except to *C. harengus membras* from stations 11G and 22N. *Clupea harengus membras* and *P. flesus* were found to be exposed to exceptionally high and high ∑Gentox risks at all study stations. The results obtained in the thesis are in agreement with the findings reported by other researchers. Exceptionally high and high genotoxicity risk to *C. harengus membras* and *P. flesu*s has been determined at all the study stations located close to the chemical munitions dumping site in the Gdańsk Basin (Valskienė et al., 2018). The highest genotoxicity levels have been recorded in *C. harengus membras* caught at stations along the CW transportation routes, close to the Bornholm chemical weapons dumping site, in zones with CWAs-related substances in sediments and zones near oil-gas platforms (Baršienė et al., 2016). The highest genotoxicity risk being assessed at or close to the chemical munitions dumpsite, it cannot be excluded that chemical warfare agents are the major causative factors for genotoxicity not only in the primary dumpsite area, but also in the neighbouring areas (Baršienė et al., 2014; Valskienė et al., 2018).

Based on the results of this thesis and those of environmental genotoxicity and cytotoxicity studies and genotoxicity risk assessments at the CW dumping sites in the Baltic Sea performed by other researchers, it can be concluded that the highest genotoxicity risk to fish exists in the Bornholm Basin, those in the Basins of Gotland and Gdańsk being respectively lower (Baršienė et al., 2016; Valskienė et al., 2018; Pažusienė et al., 2021). In conclusion, due to their latent biological hazards with scarcely investigated, but potentially severe environmental consequences, CW-related contaminants and environmental genotoxicity and cytotoxicity at CW disposal sites must be continuously monitored (Pažusienė et al., 2021).

It should be noted that the observed effects may be exerted not only by CWAs, but also by other types of contaminants detected in this area (Ricking and Schulz, 2002; Pikkarainen, 2004; Zalewska et al., 2015; Graca et al., 2016; HELCOM, 2018).

4.3. Genotoxic and cytotoxic effects of electromagnetic field

Research on the effects of anthropogenic activities in the Baltic Sea is generally focused on nutrient loads, inputs of harmful substances, fishing and shipping, far less attention is given to the analysis of the effects produced by the introduction of various forms of anthropogenic energy (high voltage direct current (HVDC) development, construction of numerous wind farm power, etc.) into the marine environment. The number of underwater electric current transmission cables in the sea and freshwater environments is constantly increasing (Fey et al., 2019b). Offshore wind farms, power transmission cables, hydrokinetic turbines emit artificial magnetic (MF) and electromagnetic (EMF) fields in the marine environment (Petersen and Malm, 2006; Cada, 2009; Andrulewicz and Otremba, 2011; Cada and Bevelhimer, 2011; Cada et al., 2011b). As a result, the risk of harmful effects of the magnetic and electromagnetic fields generated in the vicinity of those cables on marine organisms is also growing (Fey et al., 2019b). EMF is one of the most common and fastest-growing environmental influence which may affect living organisms (Kocaman et al., 2018; Hutchison et al., 2020; Albert et al., 2020). In the present study, genotoxic and cytotoxic effects of 50 Hz 1 mT EMF in peripheral blood erythrocytes of *O. mykiss* larvae and in gill cells of *L. balthica* were first determined. Significant inductions of MN, NB and 8-shaped nuclei were detected in *O. mykiss* specimens after exposure to EMF for 40 days. Significantly elevated frequencies of NBf, NB, BL, Apop, 8-shaped nuclei and BN were detected in *L. balthica* after exposure to EMF for 12 days. In the same study were recorded that significant induction of MN and NB was detected in common ragworm (*Hediste diversicolor*) coelomocytes after exposure to EMF for 12 days. Other genotoxicity endpoints, such as NBf and BL increased, but the changes were not statistically significant. Only in *H. diversicolor* coelomocytes EMF exposure did not induce any significant cytotoxic activity. These findings suggest that exposure to the EMF of the intensity typically generated by submarine cables significantly negatively affects common marine invertebrates and early life stages of typical salmonids. Very few *in vivo* genotoxicity and cytotoxicity studies of EMF have been carried out. The genotoxicity effects after *in vivo* or *in vitro* exposure to EMF have previously been investigated in astrocytes of newborn rats (Miyakoshi et al., 2005), in primary astrocytes of rats and C57BL/6 J of male mice (Herrala et al., 2018), in fibroblasts and macrophages of rats (Nakayama et al., 2016), in tracheal cell lines of rats (Lagroye and Poncy, 1997) and human blood cells (Stronati et al., 2004), in neuroblastoma of human and glioma cell lines of rats (Kesari et al., 2016), in internal organs of rats and mice (Soffritti et al., 2016). However, several studies have not detected EMF exposure-induced changes in cytotoxicity response (Mahmoudinasab et al., 2016; Ross et al., 2018). Exposure to 0.25 mT and 0.50 mT EMFs has not been found to bring about cytotoxic effects and morphological changes in MCF-7 cells (Mahmoudinasab et al., 2016). These studies have not recorded the genotoxic effects of exposure to the selected intensities of EMFs, either. Considering that the EMF potential to induce nuclear abnormalities in aquatic animals has not been investigated previously, it is difficult to assess and discuss the results obtained from the current study. According to the study results, *L. balthica* could be proposed as one of the most suitable bio-indicators for assessing EMF-induced genotoxicity and cytotoxicity. Twelve-day exposure to extremely-low-frequency EMF (ELF-EMF) was long enough to detect the elevation of the genotoxicity and cytotoxicity endpoints analysed in *L. baltica* cells, i.e. a significantly elevated frequency of six (NBf, NB, BL, Apop, 8-shaped and BN) nuclear abnormalities out of eight analysed was found.

The findings of EMF-induced genotoxicity and cytotoxicity in the early development stages of teleost fish (*O. mykiss*) suggest that induction and elimination of the analysed endpoints are time-dependent. The elevation of three out of eight genotoxicity and cytotoxicity endpoints in the early development stages of teleost fish was established after 40 days of exposure. The high standard error detected in this study may suggest time-dependent induction, elimination of the analysed genotoxicity and cytotoxicity endpoints as well as variability in fish susceptibility. The study by Mansourian et al. (2016) have revealed a nonlinear time response to EMF exposure and indicated that the maximum EMFexposure effect on apoptosis *in vitro* could be noted between 72 h and five days. The genotoxic and cytotoxic effects of EMF exposure on marine and freshwater organisms, the susceptibility of early life stages of fish to EMF exposure and the life span of genotoxicity and cytotoxicity endpoints are still not known. Therefore, additional attention and more extensive research are required.

In conclusion, these studies provided new information showing that the southern and eastern parts of the Gotland Basin cannot qualify for the Good Environmental Status of European Union marine waters due to exceptionally high and high genotoxicity risks to fish (*C. harengus membras*, *P. flesus*, *G. morhua callarias*). The studies performed within the framework of this thesis revealed the importance of assessing chemical pollution effects on marine organisms and the adverse effects of anthropogenic energy pollution. For the first time, EMF-induced genotoxicity and cytotoxicity effects on marine (*L. balthica*) and freshwater organisms (early life stages of *O. mykiss*) were established. The results presented in the thesis will be helpful in conducting continuous research and monitoring of chemical munitions dumping sites, oil and gas platform areas and the effects of EMF generated by electrical power transmission systems or wind farms.

CONCLUSIONS

1. The highest ∑Gentox level (13.13%) was established in *C. harengus membras* from the chemical munitions dumping zone in the Gotland Basin.

2. In the Lithuanian EEZ, a significant time-related (2011–2016) decrease in genotoxicity (∑Gentox) level was revealed in blood erythrocytes of *C. harengus membras* and *P. flesus*. In the Polish and Latvian EEZs, no changes in cytogenetic responses were observed in fish during the study period.

3. The comparison of genotoxicity and cytotoxicity responses in different fish species collected from the same study stations revealed higher genotoxicity and cytotoxicity levels in *C. harengus membras* than in *P. flesus* and *G. morhua callarias*.

4. Exceptionally high and high genotoxicity risks were determined to *C. harengus membras*, *P. flesus* and *G. morhua callarias* at all the study stations located in the oil and gas platform zone in the southern part of the Gotland Basin.

5. Exceptionally high and high genotoxicity risks to *C. harengus membras* and *P. flesus* were established at all the study stations located in the chemical munitions dumping zone and its vicinity, except to *C. harengus membras* at stations 11G and 22N.

6. Exposure to 50 Hz 1 mT electromagnetic field brings about cytogenetic effects in early development stages of *O. mykiss* and *L. balthica*:

6.1. Exposure to 50 Hz 1 mT electromagnetic field for 40 days induces a significant formation of MN, NB and increased frequencies of 8-shaped nuclei in blood erythrocytes of *O. mykiss* larvae.

6.2. Exposure to a 50 Hz 1 mT electromagnetic field for 12 days induces the formation of all the analysed nuclear abnormalities and significantly elevated frequencies of NB, NBf, BL, Ap, 8-shaped nuclei and BN in *L. balthica* gill cells.

SANTRAUKA

ĮVADAS

Jūrų strategijos pagrindų direktyvos (Direktyva 2008/56/EB, toliau Direktyva), pagrindinis tikslas – apsaugoti jūrų ekosistemą ir užtikrinti tvarią su jūrų aplinka susijusią ekonominę veiklą. Direktyvą sudaro 11 aprašų, kuriuose nustatyti reikalavimai, skirti Europos Sąjungos (toliau ES) šalių strategijoms parengti ir įgyvendinti bendrą tikslą – iki 2020 m. pasiekti arba išlaikyti gerą Europos jūrų ir pakrančių aplinkos būklę. 8 aprašo pagrindinis tikslas – užkirsti kelią taršai patekti į jūros aplinką arba ją sumažinti.

Deja, jūrų ekosistemoje yra teršalų, kurie neaprašyti Direktyvoje, pavyzdžiui, jūrose nuskandintas cheminis ginklas (CG) ir jame esančios cheminės medžiagos. Direktyvoje nurodoma, kad taršos poveikį reikia vertinti skirtingais gyvybės organizacijos lygmenimis bei atsižvelgti į teršalų poveikio priklausomybę nuo abiotinių ir biotinių veiksnių. Remiantis 11 aprašu, nustatant gerą jūrų vandenų aplinkos būklę, privaloma įvertinti antropogeninės energijos poveikį ir išlaikyti tokį lygį, kuris nepakenktų jūrinei ekosistemai (Direktyva 2008/56/EB).

Remiantis Europos komisijos Direktyvos 2008/56/EB įgyvendinimo ataskaitos išvadomis, pažanga, siekiant geros aplinkos būklės, iki 2020 m. nebuvo pakankamai sparti, kad apimtų visus ES vandenis pagal visus aprašus (Europos komisijos ataskaita Europos parlamentui ir tarybai dėl Jūrų strategijos pagrindų direktyvos (Direktyva 2008/56/EB) įgyvendinimo, 2020).

Viena svarbiausių kovos su Baltijos jūros užterštumu priemonių yra 2007 m. patvirtintas Helsinkio komisijos (toliau HELCOM) Baltijos jūros apsaugos veiksmų planas, kurio pagrindinis tikslas – atkurti gerą Baltijos jūros ekologinę būklę iki 2021 m. (HELCOM Baltijos jūros veiksmų planas, 2007).

Mikrobranduolių (MB) ir kitų branduolio pažaidų analizė plačiai taikoma nustatyti aplinkos taršos genotoksiškumo riziką biotai (Theodorakis, 2001, 2012; Baršienė ir kt., 2012; Martins, Costa, 2015; Bean, Akcha, 2016), kuri yra jautri, greita, nesukelianti pavojaus tiriamojo organizmo gyvybei ir sveikatai, ekonomiškai efektyvi priemonė ne tik DNR pažeidimams nustatyti (Bolognesi ir kt., 2006a; Baršienė ir kt., 2015, 2016), bet ir įvertinti aplinkos taršos sukeliamą riziką (Hayashi, 2016). Aplinkos genotoksiškumo rizikos vertinimas jūriniams organizmams pagrįstas ICES/OSPAR patvirtintais foninio lygio vertinimo kriterijais (*angl.* background assessment criteria

(BAC) ir yra vienas iš geros aplinkos būklės vertinimo rodiklių (ICES, 2011; Baršienė ir kt., 2012a; Hylland ir kt., 2017).

Dėl hidrobiontuose susikaupusios didelės teršalų koncentracijos Gotlando baseinas vertinamas kaip vienas labiausiai užterštų atviros Baltijos jūros regionų (HELCOM, 2017). Gotlando baseine nustatoma padidėjusi sunkiųjų metalų, policiklinių aromatinių angliavandenilių, polibromintų difenilo eterių, radionuklidų, alkilfenolių ir kitų teršalų koncentracija (Schulz, 2002; Pikkarainen, 2004; Zalewska ir kt., 2015; Graca ir kt., 2016; HELCOM, 2018). Baltijos jūros ekosistemos būklei vis didesnį pavojų kelia po I ir II pasaulinių karų apie 50 000 tonų nuskandintos cheminės ir konvencinės ginkluotės (Vanninen ir kt., 2020), kurią sudaro apie 15 000 tonų cheminio ginklo (toliau CG) medžiagų, turinčių genotoksinį, citotoksinį, kancerogeninį poveikį tiek žmonėms, tiek jūriniams organizmams (Bolt ir kt., 2006; Sanderson ir kt., 2017; Bełdowski ir kt., 2018; Koske ir kt., 2019). Daugiausiai šios ginkluotės rasta netoli Bornholmo salos (apie 35000 tonos), Mažajame Belte (apie 5000 tonų), Gotlando baseine (apie 2000 tonų). Neoficialios CG laidojimo vietos nustatytos Gdansko gilumoje ir Slupsko vagoje 2011–2014 m. projekto „Cheminio ginklo paieška ir vertinimas“ metu (CHEMSEA findings, 2014).

Laikui bėgant, dėl korozijos metaliniai cheminės ir konvencinės ginkluotės konteinerių korpusai yra, dėl to į ekosistemą patenka garstyčių (iprito), chloracetofenono dujų, fenilarseno junginių (arseno aliejų, difenilchloroarsino (Klarkas I), difenilcanarsino (Klarkas II), liuizito, adamsito ir kt.) (Sanderson, Fauser, 2008; HELCOM, 2013). CG medžiagos, jų hidrolizės ir oksidacijos produktai kaupiasi jūriniuose organizmuose, sukeldami lėtinius ir / ar mirtinus susirgimus (Sanderson ir kt., 2008, 2010; HELCOM 2010; Della Torre ir kt., 2010, 2013; Baršienė ir kt., 2014, 2016; Bełdowski ir kt., 2016a; Gledhill ir kt., 2019). Nustatyta, kad Klarkas I, Klarkas II (Francken, Hafez, 2009), azoto iprito ir sieros iprito (Bełdowski ir kt., 2018) hidrolizės ir oksidacijos produktų toksiškumas ir ilgalaikis poveikis jūriniams organizmams toks pats kaip ir jų pirminių junginių.

Iki šiol CG nuskandinimo zonų biologinio poveikio jūriniams organizmams tyrimai atlikti Bornholmo ir Gdansko baseinuose. Bornholmo baseino CG nuskandinimo zonoje sugautoms strimelėms (*C. harengus membras*) nustatyta genotoksinių pažaidų, lizosomų membranos stabilumo pokyčių, inkstų patologija (Bełdowski ir kt., 2016a; Lang ir kt., 2018). Reikšmingų citogenetinių, biocheminių, histocheminių ir bioenergetinių atsakų nustatyta midijoms (*Mytilus trossulus*), kurios buvo patalpintos varžose ir panardintos pagrindinėje nuskandinto CG zonoje netoli Bornholmo salos (Lastumäki ir kt., 2020).

Naujausi tyrimai atskleidė, kad CG medžiagos bioakumuliuoja menkių (*G. morhua*) audiniuose. Tyrimų metu nustatyta fenilarseno pėdsakų menkių raumenų mėginiuose, surinktuose netoli pagrindinio CG sąvartyno, esančio Baltijos jūros Bornholmo baseino zonoje (Niemikoski ir kt., 2020b). Minėtame baseine nustatytas aukštas genotoksinių pažaidų lygis strimelėse (*C. harengus membras*), sugautose tyrimų stotyse, esančiose CG gabenimo trasoje, CG nuskandinimo zonoje, vietose, kuriose nustatytos CG medžiagų koncentracijos nuosėdose, naftos ir dujų platformų zonose (Baršienė ir kt., 2016). Aukštas genotoksiškumo ir citotoksiškumo atsakas nustatytas žuvų (*C. harengus membras*, *P. flesus*, *G. morhua*), sugautų cheminės ir konvencinės ginkluotės nuskandinimo zonoje Gdansko baseine, kraujo eritrocituose (Valskienė ir kt., 2018). Gotlando baseino CG nuskandinimo zonoje aplinkos genotoksiškumo ir citotoksiškumo tyrimai iki šiol nebuvo vykdyti.

Vandens ekosistema yra veikiama ne tik cheminės taršos, bet ir antropogeninės fizikinės taršos – magnetinio (toliau ML) ir elektromagnetinio laukų (toliau EML), kuriuos skleidžia vėjo jėgainės, elektros perdavimo kabeliai, hidrokinetinės turbinos (Otremba ir kt., 2019). Natūralus Žemės magnetinis laukas svyruoja nuo 0.025 mT iki 0.06 mT (Poleo ir kt., 2001), Baltijos jūros – nuo 0.0501 iki 0.0505 mT (Hulot ir kt., 2010), tuo tarpu kintamosios srovės elektros perdavimo kabeliai gali sukurti 8 mT intensyvumo EML (Cada ir kt., 2011). Remiantis Europos vėjo energetikos asociacijos duomenimis, iki 2050 m. visoje Baltijos jūroje turėtų būti instaliuota 83 GW (šiuo metu 2 GW) galios vėjo jėgainių. Tokiu atveju Baltijos jūra taptų antru pagal dydį (po Šiaurės jūros) jūros vėjo elektrinių parku Europoje (Europos vėjo energetikos asociacijos ataskaita, 2019). Didėjant povandeninių kabelių, perduodančių elektros srovę, tinklui, lygiagrečiai didėja ir neigiamo poveikio rizika jūriniams organizmams (Fey ir kt., 2019b).

Nepaisant egzistuojančių techninių sprendimų, galinčių sumažinti EML intensyvumo sklaidą, EML poveikis vandens organizmams mažai ištirtas (Otremba ir kt., 2019). Paskelbti kelių mokslinių tyrimų rezultatai, kurių rezultatai rodo dirbtinio EML neigiamą poveikį jūros ežių (*Strongylocentrotus purpuratus*) embrionų mitoziniam ciklui (Levin, Ernst, 1995), mitogenų aktyvinamų proteinkinazių aktyvacijai, šiluminio šoko baltymų ekspresijai mėlynosiose midijose (*Mytilus galloprovincialis*) (Ottaviani ir kt., 2002; Malagoli ir kt., 2003, 2004), zebrinės danijos (*Danio rerio*) (Skauli ir kt.,

2000) ir šiaurinių lydekų (*Esox lucius*) embrionams (Fey ir kt., 2019a), perėjimo laikotarpio trukmei, sukelia trynio maišo absorbcijos laipsnio padidėjimą vaivorykštiniuose upėtakiuose (*O. mykiss*) (Fey ir kt., 2019b), melatonino lygio padidėjimą kankorėžinėse upėtakių (*Salvelinus fontinalis*) liaukose (Lerchl ir kt., 1998), veikia skirtingų žuvų rūšių elgseną (Bevelhimer ir kt., 2013), vėžiagyvių ir dvigeldžių moliuskų fiziologiją, elgseną (Aristarkhov ir kt., 1988 ; Bochert, Zettler, 2004, 2006; Scott ir kt., 2018). Kai kurių tyrimų duomenimis (Cho, Chung, 2003; An ir kt., 2015) žemo dažnio EML nesukėlė genotoksinio ar citotoksinio poveikio žmogaus limfocitams (Cho ir Chung, 2003), pelių, žiurkių, žmonių ląstelių kultūroms (An ir kt., 2015). Iki šiol EML genotoksinis ir citotoksinis poveikis vandens organizmams nebuvo tirtas.

Darbo naujumas

Šiame darbe pirmą kartą:

1. Įvertintas trijų vietinių ir komerciškai svarbių žuvų rūšių: strimelių (*Clupea harengus membras),* plekšnių (*Platichtys flesus*) ir menkių (*Gadus morhua callarias*), sugautų tyrimo stotyse, esančiose cheminės ginkluotės nuskandinimo zonoje rytinėje Gotlando baseino dalyje, genotoksiškumo ir citotoksiškumo lygis periferinio kraujo eritrocituose.

2. Aplinkos genotoksiškumo rizika nustatyta *C. harengus membras, P. flesus* ir *G. morhua callarias,* sužvejotoms tyrimų stotyse, esančiose nuskandintos cheminės ginkluotės zonoje arba netoli jos.

3. Nustatyta aplinkos genotoksiškumo rizika *C. harengus membras, P. flesus* ir *G. morhua callarias*:

a) sugautoms 52 tyrimų stotyse, esančiose pietinėje Gotlando baseino zonoje, 2010–2017 m.

b) sugautoms 47 tyrimų stotyse, esančiose rytinėje Gotlando baseino zonoje, 2011–2017 m.

4. Pagal geros ekologinės būklės reikalavimus, remiantis skirtingų žuvų rūšių genotoksiškumo rizikos rezultatais, įvertinta pietinės Gotlando baseino dalies ekologinė būklė.

5. Nustatytas genotoksinis ir citotoksinis 50 Hz 1 mT elektromagnetinio lauko poveikis vaivorykštinio upėtakio (*Oncorhynchus mykiss*) lervoms bei baltijinei makomai (*Limecola balthica).*

Mokslinė ir praktinė darbo reikšmė

Mokslinė reikšmė:

1. Aplinkos genotoksiškumo įvertinimas pietinėje ir rytinėje Gotlando baseino dalyse atskleidė aukštą ir labai aukštą genotoksiškumo riziką *C. harengus membras* ir *P. flesus*.

2. Pietinėje ir rytinėje Gotlando baseino dalyse genotoksiškumo ir citotoksiškumo atsakas priklauso nuo žuvies rūšies.

3. Nustatytas didžiausias genotoksinių (∑Gentox) pažaidų lygis *C. harengus membras,* sugautų nuskandintos cheminės ginkluotės zonoje. Tolstant, ∑Gentox lygis žuvų kraujo eritrocituose mažėja.

4. Nustatytas aplinkos genotoksinio poveikio mažėjimas per laiką *C. harengus membras* ir *P. flesus,* sugautoms Lietuvos išskirtinėje ekonominėje zonoje (IEZ). Lenkijos IEZ sugautoms žuvims genotoksiškumo lygio pokyčių per laiką nenustatyta.

5. Aplinkos taršą tyrimo zonoje įrodo aukštas ∑Gentox ir ∑Cytox pažaidų lygis žuvų, sugautų mažai viena nuo kitos nutolusiose tyrimų stotyse.

6. Įvertintas genotoksinis ir citotoksinis 50 Hz 1 mT elektromagnetinio lauko potencialas *O. mykiss* lervoms ir *L. balthica*. Pateikti pirmieji rezultatai, įrodantys galimą povandeninių elektros perdavimo kabelių sukuriamo elektromagnetinio lauko reikšmingą neigiamą citogenetinį poveikį moliuskams ir lašišinėms žuvims ankstyvose jų vystymosi stadijose.

Praktinė reikšmė:

1. Vietinių Baltijos jūros žuvų rūšių genotoksiškumo ir citotoksiškumo tyrimų rezultatai yra naudingi paskandintos cheminės ginkluotės poveikio stebėsenai.

2. Baltijos jūros žuvų ilgalaikių citogenetinių tyrimų rezultatai naudingi pildant HELCOM monitoringo duomenų bazę ir stebint genotoksiškumo ir citotoksiškumo pokyčius per laiką.

3. Rezultatai naudingi siekiant pagerinti rytinio Baltijos jūros regiono ekologinę būklę bei suskirstyti regioną pagal integruotą geros ekologinės būklės vertinimo klasifikacijos skalę.

4. Tyrimų metu pasitvirtino mikrobranduolių ir kitų branduolio pažaidų analizės tinkamumas ir naudingumas tiriant elektromagnetinio (EML) lauko poveikį ir citogenetinį atsaką vandens organizmuose.

5. *L. balthica* nustatyta kaip optimali bioindikatorinė rūšis vertinti EML genotoksiškumą ir citotoksiškumą.

6. EML citogenetinio poveikio vandens organizmams rezultatai suteikia informacijos, galinčios paskatinti pirminį povandeninių elektros perdavimo kabelių generuojamo EML intensyvumo standartizacijos procesą bei priimti techninius sprendimus, mažinančius EML indukciją.

Darbo tikslas ir uždaviniai

Tikslas - įvertinti aplinkos taršos sukelto genotoksinio ir citotoksinio poveikio žuvims ypatumus Baltijos jūros Gotlando baseino pietinėje ir rytinėje dalyse.

Uždaviniai:

1. Nustatyti genotoksinių ir citotoksinių pažaidų lygį skirtingų žuvų rūšių periferinio kraujo eritrocituose.
2. Išanalizuoti Gotlando baseino genotoksinio ir citotoksinio poveikio žuvims ypatumus ir nustatyti pokyčių (nuo 2010 iki 2017 m. pietinėje baseino dalyje ir nuo 2011 iki 2017 m. rytinėje baseino dalyje) dėsningumus per laiką.
3. Įvertinti vietinių žuvų rūšių, sugautų cheminės ginkluotės nuskandinimo zonoje rytinėje Gotlando baseino dalyje, genotoksinį ir citotoksinį lygį periferinio kraujo eritrocituose.
4. Įvertinti Gotlando baseino aplinkos genotoksiškumo riziką skirtingų rūšių žuvims.
5. Įvertinti 50 Hz 1 mT elektromagnetinio lauko genotoksinį ir citotoksinį poveikį *Oncorhynchus mykiss* ankstyvoje vystymosi stadijoje ir *Limecola balthica*.

Ginamieji teiginiai

1. Baltijos jūros Gotlando baseino aplinkos genotoksinis ir citotoksinis poveikis skiriasi priklausomai nuo žuvų rūšies bei tyrimo zonos.
2. Gotlando baseine būdinga aukšta ir labai aukšta aplinkos genotoksiškumo rizika *C. harengus membras* ir *P. flesus*, padidėjusi rizika – *G. morhua callarias.*

3. Aplinkos genotoksinio ir citotoksinio poveikio mažėjimo per laiką (2011–2016 m.) tendencija nustatyta žuvims, sugautoms Lietuvos IEZ.

4. Cheminės ginkluotės nuskandinimo zonai būdingas išskirtinai aukštas ∑Gentox atsakas *C. harengus membras*, tolstant nuo zonos, ∑Gentox lygis mažėja.

5. 50 Hz 1 mT EML turi genotoksinį ir citotoksinį poveikį *O. mykiss* lervoms ir *L. balthica* moliuskams.

6. *O. mykiss* lervos ir *L. Balthica* – tinkami biologiniai indikatoriai vertinti EML sukeliamą genotoksinį ir citotoksinį poveikį jūrinėje ir gėlavandenėje ekosistemose. *L. balthica* yra jautresnė ir tinkamesnė rūšis nustatant EML genotoksinį ir citotoksinį potencialą.

7. Mikrobranduolių ir kitų branduolio pažaidų analizė yra tinkamas metodas EML citogenetinių poveikio tyrimams vandens organizmuose.

Tyrimų medžiagos surinkimas

1. Baltijos jūros Gotlando baseino *C. harengus membras, P. flesus, G. morhua callarias* periferinio kraujo mėginiai surinkti:

• pietinės dalies – 52 tyrimų stotyse 2010–2017 m. laikotarpiu. Žuvų kraujo mėginiai surinkti mokslinių ekspedicijų metu Lenkijos „Baltica“ ir Vokietijos „Walther Herwig III“ mokslinių tyrimų laivais, Lietuvos verslinės žvejybos laivu „Wismar“, naudojant standartinius žvejybinius tralus. Pietinės Gotlando baseino dalies 31 tyrimų stotyje sužvejoti 308 *C. harengus membras* individai, 20 stočių – *213 P. flesus,* 24 stotyse – 237 *G. morhua callarias* (1 lentelė, žr. disertacijos 23 psl.).

• rytinės dalies – 47 tyrimų stotyse 2011–2017 m. laikotarpiu. Mėginiai surinkti mokslinių ekspedicijų metu Lenkijos „Baltica“ ir Vokietijos „Walther Herwig III“ mokslinių tyrimų laivais, naudojant standartinius žvejybinius tralus. Rytinės Gotlando baseino dalies 29 tyrimų stotyse sužvejoti 288 *C. harengus membras* individai, 19 stočių – *205 P. flesus*, 12 stočių – 112 *G. morhua callarias* (1 lentelė, žr. disertacijos 23 psl.).

2. Rygos įlankoje 2010 m. gruodį 7 tyrimų stotyse *C. harengus membras, P. flesus* periferinio kraujo mėginiai surinkti Vokietijos „Walther Herwig III“ mokslinių tyrimų laivais, naudojant standartinius žvejybinius tralus. Rygos įlankoje 6 tyrimų stotyse sugauti 89 *C. harengus membras* individai, 7 stotyse – 88 *P. flesus* individai (1 lentelė, žr. disertacijos 23 psl.).

Eksperimentinis tyrimas

Genotoksinis ir citotoksinis 50 Hz 1 mT elektromagnetinio lauko potencialas buvo vertinamas žuvims ankstyvose jų vystymosi stadijose ir moliuskams. *O. mykiss* lervos buvo veikiamos 40 parų, *L. balthica* – 12 parų.

O. mykiss ikrai (244 D^{o}) gauti iš „Dąbie“ žuvininkystės ūkio (Dąbie, Lenkija). Aklimacija – 24 valandos pastovios temperatūros sąlygomis (T = 9,6 ^{o}C). Nuo 30-tos eksperimento paros *O. mykiss* lervos pradėtos maitinti.

L.balthica surinkti 70–100 cm gylyje Kuźnica tyrimų stotyje (Pucko įlanka, Baltijos jūra). Aklimacija – 7 paros 16 °C temperatūros pratekančio jūrinio (druskingumas = 7,2) vandens rezervuare. Natūraliame jūros vandenyje ir nuosėdose gausu organinių medžiagų, todėl moliuskai papildomai nemaitinti.

Eksperimentinis tyrimas atliktas Nacionaliniame Jūrų žuvininkystės tyrimų institute (Gdynė, Lenkija).

Mėginių analizė

Aplinkos (tyrimai *in situ*) ir elektromagnetinio lauko (eksperimentinis tyrimas) poveikio tyrimai atlikti vertinant genotoksinių (mikrobranduolių (toliau MB), branduolio pumpurų (toliau BP), branduolio pumpurų su nukleoplazmine jungtimi (toliau BPs), dvibranduolių tiltų su nukleoplazmine jungtimi (toliau DBt) ir citotoksinių (fragmentuotų (toliau Frag), apoptotinių (toliau Ap), aštuoneto formos branduolių (toliau 8-formos) ir dvibranduolių eritrocitų (toliau DB) branduolio pažaidų indukciją žuvų periferinio kraujo eritrocituose ir moliuskų žiaunų ląstelėse (1 pav., žr. disertacijos 26 psl.). Vertinimas atliktas remiantis Carassco ir kt. (1990), Heddle ir kt. (1991), Fenech ir kt. (2003), Baršienė ir kt. (2006a; 2014) aprašytais kriterijais.

Žuvų genotoksinės ir citotoksinės pažaidos vertinamos 4000 eritrocitų, moliuskų – 1000 ląstelių. Bendras genotoksiškumo (∑Gentox) lygis buvo vertinamas susumavus atskirų genotoksinių parametrų dažnį, tuo tarpu bendras citotoksiškumo (∑Cytox) lygis – susumavus citotoksinių parametrų dažnį bei išreiškus promilėmis (branduolio pažaida / 1000 eritrocitų).

Aplinkos genotoksiškumo rizikos žuvims vertinimas

Aplinkos genotoksiškumo rizika įvertinta remiantis Baršienė ir kt. (2012a, 2014) publikacijose pateiktomis foninio lygio reikšmėmis, kurias Tarptautinė jūrų tyrinėjimo taryba (*angl.* International council for the exploration of the sea, ICES) patvirtino kaip tinkamas aplinkos genotoksiškumo rizikos jūriniams organizmams vertinti (Davies, Vethaak, 2012). Rytinėje ir pietinėje Gotlando baseino dalyse esančiose stotyse genotoksiškumo rizika įvertinta remiantis ∑Gentox dažniu, Rygos įlankoje – remiantis MB dažniu žuvų kraujo eritrocituose. Kiekviena tyrimų stotis vertinama pagal 5 lygių genotoksiškumo rizikos skalę: žema rizika žuvims, kai 0.0–19 % tirtų individų genotoksinių pažaidų lygis didesnis už ikislenkstinę (*angl.* Bacground Assessment Criteria (BAC) reikšmę, vidutinė – 20–39 %, padidėjusi – 40–59 %, aukšta – 60–79 %, labai aukšta – 80–100 % tirtų individų.

Statistinė duomenų analizė

Genotokiškumo ir citotoksiškumo analizės duomenys neatitiko duomenų pasiskirstymo normalingumo ir homogeniškumo (Kolmogorov‘o-Smirnov’o ir Shapir‘o-Wilk’o testai). Todėl naudotas neparametrinis Mann’o-Whitney U testas palyginti tyrimo ir kontrolinių stočių žuvų branduolio pažaidų lygį. Kiekvienoje tyrimų stotyje nustatytas pažaidų vidurkis ± standartinė paklaida (toliau SE). Reikšmingumo lygis – $p < 0,05$. Taikant Bonferroni’o korekciją, koreguotos *p* vertės reikšmės (Vickerstaff ir kt., 2019; Pažusienė et al., 2021).

Statistiniams skaičiavimams naudoti GraphPad Prism® 5.01 (GraphPad Software Inc., San Diego, CA, USA) ir STATISTICA 7.0 (StatSoft Inc., Tulsa, Oklahoma, USA) programiniai paketai.

REZULTATAI IR JŲ APTARIMAS

Pietinės Gotlando baseino dalies genotoksinių ir citotoksinių pažaidų analizė žuvų periferinio kraujo eritrocituose

Gotlando baseino pietinėje dalyje (2010−2017 m.) įvertinti genotoksinių ir citotoksnių pažaidų lygiai, genotoksiškumo rizika *C. harengus membras, P. flesus* ir *G. morhua callarias* 52 tyrimų stotyse (5 pav., žr. disertacijos 34 psl.). Lenkijos išskirtinėje ekonominėje zonoje (IEZ) sugautų žuvų kraujyje nustatytas aukštesnis genotoksiškumo ir citotoksiškumo pažaidų atsakas nei Lietuvos IEZ. Ypač aukštas ∑Gentox lygis (daugiau nei 3‰) nustatytas *C. harengus membras*, sužvejotų penkiose stotyse (15F, 29b, 19j, 31P, 74T) Lenkijos IEZ, dviejose (28LT ir 30LT) Lietuvos IEZ. ∑Gentox lygis aukštesnis nei 1‰ nustatytas *C. harengus membras* visose tyrimų stotyse (išskyrus 6b Lenkijos IEZ), *P. flesus* − 12 iš 20 tyrimų stočių (Lenkijos IEZ − st3, 6b, 1d, 2d, 18j, 12X, 68T, 75T, Lietuvos IEZ – 26LT, 27LT, 28LT, 30LT), *G. morhua callarias* − 3 iš 24 stočių (Lenkijos IEZ − 21w, 1u, 15F). Aukštas ∑Cytox lygis (daugiau nei 1‰) nustatytas *C. harengus membras,* sugautų šešiose tyrimų stotyse (Lenkijose IEZ − 15F, 2d, 31P, 67T, 74T, Lietuvos IEZ − 28p), *P. flesus* − trijose (Lenkijos IEZ − 1d, 23m, 68T), *G. morhua callarias* − trijose (Lenkijos IEZ − 1u, 15F, 1K) stotyse (7−9 pav., žr. disertacijos 36−38 psl.). Palyginus ∑Gentox lygio skirtumus skirtingų žuvų rūšių periferinio kraujo eritrocituose, aukštesnis pažaidų atsakas nustatytas *C. harengus membras* nei *P. flesus* ir *G. morhua callarias*.

Ilgalaikių (2010−2017 m.) tyrimų duomenys atskleidė genotoksinių ir citotoksinių pažaidų lygio mažėjimo tendenciją per laiką. *C. harengus membras ir P. flesus,* sugautose 2015−2017 m. Lietuvos IEZ, Lenkijos IEZ tiriamuoju laikotarpiu, pažaidų lygio pokyčių per laiką nenustatyta. Aukštesnius genotoksiškumo ir citotoksiškumo pažaidų lygio rezultatus žuvų, sugautų Lenkijos IEZ, galėjo lemti naftos ir dujų platformų tarša. Netoli šios zonos nustatytas aukštas ∑Gentox ir ∑Cytox atsakas žuvų kraujyje iš st3, 19j, 11s, 1u, 1K, 31P ir 74T stočių. Greta minėtų stočių, dugno nuosėdose, nustatyta padidėjusi Hg, Pb, Cd ir Zn koncentracija (Bełdowski ir kt., 2014; Zalewska ir kt., 2015). Taip pat žuvų branduolio pažaidų lygio pastovumą galėjo sąlygoti nuolatinė tarša vykdant pramoninius (Nord Stream dujotiekio ir kabelio Sankt Peterbugas−Kaliningradas tiesimo darbai) projektus ir išminavimo programas 2008−2011 m. (Moller, 2011).

Rytinės Gotlando baseino dalies genotoksinių ir citotoksinių pažaidų analizė žuvų periferinio kraujo eritrocituose

Gotlando baseino rytinėje dalyje (2011−2017 m.) pirmą kartą įvertinti genotoksinių ir citotoksnių pažaidų lygiai, genotoksiškumo rizika strimelėms (*C. harengus membras*), plekšnėms (*P. flesus*) ir menkėms (*G. morhua callarias*) 47-iose tyrimų stotyse, esančiose nuskandinto cheminio ginklo (CG) zonoje ir netoli jos (11 pav., žr. disertacijos 42 psl.). Tyrimo rezultatai atskleidė, kad ∑Gentox ir ∑Cytox pažaidų lygis viršijo kontrolinį lygį visose tirtose žuvų rūšyse, išskyrus ∑Gentox lygį *C. harengus membras* iš 3h, 15v, 22N stočių, ∑Cytox lygį *C. harengus membras* iš 17LV, 20k, 15v, 11G, 22N, 12R, 22T stočių. Aukščiausias ∑Gentox lygis periferinio kraujo eritrocituose nustatytas 2014 m. gruodį *C. harengus membras,* sugautų 1e (13.13‰) stotyje, esančioje nuskandinto CG zonos centre. Šioje stotyje nustatytas aukštas ir statistiškai reikšmingas ∑Cytox (1.08‰) lygis. Ypač aukštas ∑Gentox lygis (daugiau nei 3‰) nustatytas *C. harengus membras,* sugautų 1e, 19c, 1R stotyse, esančiose CG zonoje. Aukštas ∑Gentox pažaidų lygis (daugiau nei 1‰) nustatytas *C. harengus membras* 24 iš 29 tyrimo stočių, *P. flesus* – 10 iš 19, *G. morhua callarias* − 1 iš 12 stočių. Aukštas ∑Cytox atsako dažnis (daugiau nei 1‰) nustatytas *C. harengus membras* 19c, 1e stotyse, *P. flesus* − 19c, 6c, *G. morhua callarias* − B14/29-30 stotyje. Nuskandinto CG zonoje 2012 m. gegužę nustatytas padidėjęs ∑Gentox lygis *G. morhua callarias* iš B14/49 (0.53‰), B14/35-37 (0.45‰), B14/50 (0.38‰) tyrimų stočių, ∑Cytox lygis − B14/50 (0.63‰) (12, 14, 16 pav., žr. disertacijos 44−49 psl.). Vertinant skirtingų žuvų rūšių, sugautų tose pačiose tyrimų stotyse, ∑Gentox ir ∑Cytox atsakus, nustatyta reikšmingų ∑Gentox pažaidų skirtumų, lyginant *C. harengus membras* ir *P. flesus* atsakus 19c, 1C, 20C, 1R tyrimų stotyse. Ilgalaikių tyrimų (2011−2017 m.) rezultatai neatskleidė genotoksinių ir citotoksinių pažaidų lygio sumažėjimo.

Aukštas tiriamų žuvų genotoksinių ir citotoksinių pažaidų dažnis rodo padidėjusį aplinkos taršos poveikį. Gotlando baseino CG zonoje 63 % nuosėdų mėginių nustatyta sieros garstyčių ir fenilarseno koncentarcija (Vanninen ir kt., 2020). Žuvų genotoksinių ir citotoksinių pažaidų lygio svyravimą galėjo lemti atsitiktinis nuskandintos ginkluotės išsisklaidymas Gotlando CG zonoje, lyginant su Bornholmo ir Gdansko CG zonomis (Söderström ir kt., 2018; Vanninen ir kt., 2020). Įvertinus CG medžiagų degradacijos produktų koncentraciją nuosėdose, nustatyta, kad Gotlando ir Gdansko baseinams būdinga lokali tarša, Bornholmo baseine tarša išplitusi plačiai (Bełdowski ir kt., 2016; Soderstrom ir kt., 2018). Nors užterštumas CG medžiagomis daugiausia lokalus, tačiau priklausomai nuo taršos šaltinio dydžio, užterštumo diapazono, dugno srovių, topografijos ir korozijos poveikio amunicijai gali išplisti toliau nei 250 m nuo pagrindinio taršos šaltinio (CHEMSEA findings, 2014; Vanninen ir kt., 2020). Remiantis kitų mokslininkų tyrimų rezultatais, Baltijos jūros Bornholmo baseino CG transportavimo trasoje, netoli Bornholmo CG nuskandinimo zonos, vietose, kuriose nuosėdos užterštos CG medžiagomis bei naftos ir dujų platformų zonoje nustatytas aukštas genotoksinių pažaidų lygis *C. harengus membras* (Baršienė ir kt., 2016). Gdansko baseino cheminės ir konvencinės ginkluotės zonoje nustatytas aukštas genotoksinių ir citotoksinių pažaidų atsakas žuvų kraujo eritrocituose (Valskienė ir kt., 2018). Vykdant laboratorinius tyrimus, po poveikio mažomis oksiduotų formų Klarko I (1.25 μg/L), adamsito (2.5 μg/L) ir chloroacetofenono (5 μg/L) koncentracijomis midijoms (*Mytilus trossulus*) nustatytas citotoksinis, imunotoksinis poveikis ir oksidacinis stresas (Höher ir kt., 2019).

Gotlando baseine paskandinta apie 608 tonos sieros garstyčių (Knobloch ir kt., 2013). Naujausi sieros garstyčių ir šešių skilimo produktų ūmaus toksiškumo tyrimai atskleidė, kad skilimo produktai (1,2,5-tritiepanas ir 1,4,5-oksaditiepanas) yra toksiškesni dafnijoms (*Daphnia magna*) nei pirminis junginys (Czub ir kt., 2020). Taip pat nustatyta, kad CG medžiagos (liuizitas, adamsitas, Klarkas I, fenildichloroarsinas), susiję junginiai (trifenilarsinas, arseno trichloridas) ir keturi arseno pagrindu pagaminti skilimo produktai po 48 val. sukėlė neigiamą poveikį *D. magna* (Czub ir kt., 2021). Sprogstamosios medžiagos: trinitrotoluenas (TNT), 4-aminodinitrotoluenas (4-ADNT), 2-aminodinitrotoluenas (2-ADNT), ciklotetrametilentetranitraminas (HMX) kaupiasi *Limanda limanda* audiniuose ir gali sukelti neigiamą poveikį (Koske ir kt., 2020).

Aplinkos genotoksiškumo rizika žuvims pietinėje, rytinėje Gotlando baseino dalyse ir Rygos įlankoje

Pietinėje Gotlando baseino dalyje, Lenkijos IEZ sugautoms žuvims nustatyta aukštesnė genotoksiškumo rizika nei žuvims iš Lietuvos IEZ. Naftos ir dujų platformų zonoje (Lenkijos IEZ) visose tyrimo stotyse visoms sugautoms žuvims būdinga aukšta ir labai aukšta genotoksiškumo rizika. Lenkijos IEZ aukšta ir labai aukšta ∑Gentox rizika nustatyta *C. harengus membras*, sugautoms 96 % tyrimo stočių, *P. flesus* – 92 %, *G. morhua* *callarias* – 39 %. Lietuvos IEZ aukšta ir labai aukšta ∑Gentox rizika nustatyta *C. harengus membras*, sugautoms 100 % tyrimo stočių, *P. flesus* – 86 %, *G. morhua callarias* – 33 % (10 pav., žr. disertacijos 41 psl.). Aukšta ∑Gentox rizika bentosinėms (*P. flesus*) ir pelaginėms (*C. harengus membras*) žuvims rodo genotoksinių medžiagų poveikį visoje vandens storymėje. Remiantis aplinkos ∑Gentox rizikos rezultatais, pietinė Gotlando baseino dalis neatitinka geros aplinkos būklės savybių.

Rytinėje Gotlando baseino dalyje aukšta ir labai aukšta ∑Gentox rizika *P. flesus* nustatyta 89.47 % tyrimo stočių, *C. harengus membras* − 79.31 %, *G. morhua callarias* – 50 % (19 pav., žr. disertacijos 54 psl.). Nuskandintos cheminės ginkluotės zonoje ir šalia jos sugautoms *C. harengus membras* ir *P. flesus* nustatyta aukšta ir labai aukšta genotoksiškumo rizika, *G. morhua callarias* – padidėjusi rizika (Pažusienė ir kt., 2019). Remiantis Valskienės ir kitų mokslininkų (2018) duomenimis, Gdansko baseino cheminės ir konvencinės ginkluotės palaidojimo zonoje taip pat nustatyta aukšta genotoksiškumo rizika *C. harengus membras* ir *P. flesus*. Aukščiausia genotoksiškumo rizika žuvims nustatyta CG palaidojimo zonoje, todėl negalima atmesti prielaidos, kad CG medžiagos yra pagrindiniai genotoksinių pažaidų sukėlėjai (Baršienė ir kt., 2014, Valskienė ir kt., 2018), neatmetant ir kitų teršalų poveikio (Ricking, Schulz, 2002; Pikkarainen, 2004; Zalewska ir kt., 2015; Graca ir kt., 2016; HELCOM, 2018).

Apibendrinant genotoksiškumo tyrimus cheminės ginkluotės nuskandinimo vietose, didžiausias genotoksiškumo pavojus žuvims nustatytas Bornholmo baseine, mažesnis − Gotlando ir Gdansko baseinuose (Baršienė ir kt., 2016; Valskienė ir kt., 2018; Pažusienė ir kt., 2019). Todėl reikalinga nuolatinė palaidotos cheminės ginkluotės būklės ir jos medžiagų poveikio vandens organizmams stebėsena, įskaitant genotoksinių ir citoksinių pažaidų lygio pokyčius.

Rygos įlankoje aukšta ir labai aukšta genotoksiškumo rizika nustatyta tik *P. flesus* (2 pav., žr. disertacijos 29 psl.).

Tarprūšinis palyginimas

Skirtingų žuvų rūšių, sugautų tose pačiose tyrimų stotyse, ∑Gentox ir ∑Cytox atsako palyginimas atskleidė, kad *C. harengus membras* yra tinkamesnė bioindikatorinė žuvis lyginant su *P. flesus ir G. morhua callarias*. Remiantis gautais rezultatais, aukštesnis ΣGentox lygis *C. harengus membras* nei *P. flesus* nustatytas 81,81 % tyrimo stočių, nei *G. morhua callarias* − 77,78

%. Aukštesnis ∑Cytox lygis didesnis *C. harengus membras* nei *P. flesus* nustatytas 59 % tyrimo stočių, nei *G. morhua callarias* − 66,67 %. Panašūs rezultatai buvo pateikti Valskienės ir kt. (2018) publikacijoje, kurioje tarprūšinis žuvų palyginimas parodė aukštesnį ΣGentox lygį *C. harengus membras* nei *P. flesus* ir *G. morhua callarias* 65 % tyrimo stočių.

Disertacijoje nurodyta, kad tyrimo stotyse, esančiose pietinėje ir rytinėje Gotlando baseino dalyse, nustatyta aukšta ir labai aukšta genotoksiškumo rizika *P. flesus* ir *C. harengus membras*. Tokie patys rezultatai buvo gauti tiriant žuvis, sugautas tyrimų stotyse, esančiose Bornholmo ir Gdansko baseinuose.

Elektromagnetinio lauko poveikis

Disertacijos tyrimų metu pirmą kartą nustatytas 50 Hz 1 mT EML genotoksinis ir citotoksinis potencialas *O. mykiss* (ankstyvoje vystymosi stadijoje) periferinio kraujo eritrocituose ir *L. balthica* žiaunų ląstelėse. Po 40 parų EML poveikio *O. mykiss* lervų eritrocituose nustatytas MB, BP, AT, Apop, Frag ir 8-formos branduolių indukcija, reikšmingai padidėjo MB, BP ir 8-formos branduolių dažnis (3 pav., žr. disertacijos 31 psl.). Po 12 parų EML poveikio *L. balthica* žiaunų ląstelėse nustatytos visos genotoksiškumo (MB, BP, BPs, AT) ir citotoksiškumo (Apop, Frag, DB, 8-formos branduolių) pažaidos, reikšmingai padidėjo šešių (BPs, BP, AT, Apop, 8-formos branduolių ir DB) branduolio pažaidų dažnis iš aštuonių tirtų (4 pav., žr. disertacijos 33 psl.). 12 parų trukmės EML poveikio pakako sukelti reikšmingą genotoksinių ir citotoksinių pažaidų dažnio padidėjimą *L. baltica* ląstelėse, todėl *L. balthica* gali būti pasiūlyta kaip vienas tinkamiausių bioindikatorių EML sukeliamiems citogenetinių efektų tyrimams.

Remiantis rezultatais, galima daryti išvadą, kad EML vertė (50 Hz 1 mT), kurią paprastai sukuria povandeniniai kabeliai, reikšmingai neigiamai paveikė lašišines žuvis ankstyvoje vystymosi stadijoje ir moliuskus. Iki šiol EML genotoksiškumo ir citotoksiškumo *in vivo* tyrimų atlikta nedaug, dauguma jų atlikta su žinduoliais. MB susidarymas *in vivo* ir *in vitro* po EML poveikio tirtas žiurkių jauniklių astrocituose (Miyakoshi ir kt., 2005), žiurkių pirminiuose astrocituose, pelių patinų C57BL/6J (Herrala ir kt., 2018), žiurkių trachėjos ląstelių kultūrose (Lagroye ir Poncy, 1997), žmogaus kraujo ląstelėse (Stronati ir kt., 2004). Dalyje ankstesnių EML poveikio organizmams tyrimų metu nebuvo nustatyta citotoksiškumo (Mahmoudinasab ir kt., 2016; Ross ir kt., 2018). EML (0,25 mT ir 0,50 mT) nesukėlė citotoksinio poveikio ir morfologinių pokyčių pelės ląstelėse (Mahmoudinasab ir kt., 2016). Atsižvelgiant į tai, kad EML potencialas sukelti genotoksines ir citotoksines pažaidas vandens organizmuose nebuvo tirtas anksčiau, sunku palyginti ir aptarti disertacijos metu atlikto tyrimo rezultatus.

Įvertinus EML genotoksiškumą ir citotoksiškumą žuvų (*O. mykiss*) lervose, galima daryti išvadą, kad analizuojamų pažaidų indukcija ir eliminacija priklauso nuo laiko. Po 40 dienų poveikio buvo nustatytas 6 iš 8 analizuotų genotoksiškumo ir citotoksiškumo pažaidų padidėjimas ankstyvoje žuvų vystymosi stadijoje. Šiame tyrime nustatytos aukštos analizuotų pažaidų vidurkio standartinės paklaidos, tai gali reikšti genotoksiškumo ir citotoksiškumo pažaidų indukcijos ir eliminacijos priklausomybę nuo laiko, taip pat – žuvų individualų jautrumą. Mansourian ir kitų mokslininkų (2016) tyrimas atskleidė, kad maksimalų EML poveikį apoptozei *in vitro* galima pastebėti nuo 72 h iki 5 dienų. EML genotoksinis ir citotoksinis poveikis įvairioms jūrinėms ir gėlavandenėms organizmų rūšims, jautrumas pagal skirtingas jų vystymosi stadijas, genotoksinių ir citotoksinių pažaidų cirkuliavimo trukmė vis dar nežinomi, todėl reikalingi išsamesni tyrimai.

Disertacijoje pristatyti *in situ* ir eksperimentinių tyrimų rezultatai paskatins atkreipti dėmesį į antropogeninės veiklos poveikį vandens ekosistemų organizmams ne tik dėl cheminės, bet ir dėl fizikinės taršos. Tyrimų rezultatai atskleidė ne tik nuskandinto CG taršos poveikio jūrų organizmams įvertinimo ir stebėjimo svarbą, bet ir antropogeninės fizikinės taršos neigiamą poveikį. Disertacijoje pateikti rezultatai svarbūs CG nuskandinimo, naftos ir dujų platformų zonų bei EML poveikio stebėsenai.

Tyrimų duomenys suteikė naujos informacijos apie rytinės ir pietinės Gotlando baseino dalių ekologinę būklę. Remiantis aukštos ir labai aukštos aplinkos genotoksiškumo rizikos žuvims (*C. harengus membras*, *P. flesus*, *G. morhua callarias*) rezultatais, rytinė ir pietinė Gotlando baseino dalys apibūdintos kaip neatitinkančios geros jūros aplinkos būklės savybių.

Pirmą kartą EML genotoksiškumo ir citotoksiškumo potencialas nustatytas tiek jūriniams dvigeldžiams (*L. balthica*), tiek gėlavandenėms žuvims (*O. mykiss*) ankstyvose vystymosi stadijose.

IŠVADOS

1. Aukščiausias genotoksinis (∑Gentox) lygis (13.13 %) nustatytas *C. harengus membras,* sugautų cheminės ginkluotės nuskandinimo zonoje Baltijos jūros Gotlando baseino rytinėje dalyje.

2. Lietuvos IEZ nustatytas genotoksinių (∑Gentox) pažaidų *Clupea harengus membras* ir *Platychtys flesus* kraujo eritrocituose lygio mažėjimas 2011–2016 m. Lenkijos ir Latvijos IEZ žuvų pažaidų lygio pokyčių tiriamuoju laikotarpiu nenustatyta.

3. Skirtingų žuvų rūšių, sugautų tose pačiose Gotlando baseino stotyse, citogenetinio atsako palyginimas atskleidė didesnį genotoksiškumo ir citotoksiškumo lygį *C. harengus membras* lyginant su *P. flesus* ir *G. morhua callarias*.

4. Aukšta ir labai aukšta genotoksiškumo rizika nustatyta *C. harengus membras, P. flesus* ir *G. morhua callarias* visose tyrimų stotyse, esančiose naftos ir dujų platformų zonoje pietinėje Gotlando baseino dalyje.

5. Aukšta ir labai aukšta genotoksiškumo rizika nustatyta *C. harengus membras* ir *P. flesus* visose tyrimų stotyse, esančiose nuskandintos cheminės ginkluotės zonoje ir šalia jos, išskyrus *C. harengus membras* iš 11G ir 22N stočių.

6. 50 Hz 1 mT elektromagnetinis laukas sukelia citogenetinius pokyčius *Oncorhynchus mykiss* ankstyvoje vystymosi stadijoje ir *Limecola balthica*:

   6.1. Eksperimentiškai nustatyta, kad 50 Hz 1 mT elektromagnetinis laukas po 40 parų poveikio sukelia reikšmingą mikrobranduolių, branduolio pumpurų ir 8-formos branduolio pažaidų dažnio padidėjimą *O. mykiss* lervos kraujo eritrocituose.

   6.2. Eksperimentiškai nustatyta, kad 50 Hz 1 mT elektromagnetinis laukas po 12 parų poveikio sukėlė visų tiriamų branduolio pažaidų dažnio padidėjimą *L. balthica* žiaunų ląstelėse. Nustatytas reikšmingai padidėjęs branduolio pumpurų su nukleoplazmine jungtimi, branduolio pumpurų, ataugų, 8-formos branduolio, dvibranduolių ir apoptozinių ląstelių dažnis palyginus su kontroline grupe.

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LIST OF PUBLICATIONS AND AUTHOR‘S CONTRIBUTION

Publications with an impact factor on the Clarivate Analytics Web of Science (WoS) database:

I. Butrimavičienė L, Baršienė J, **Greiciūnaitė J***, Stankevičiūtė M, Valskienė R (2018) Environmental genotoxicity and risk assessment in the Gulf of Riga (Baltic Sea) using fish, bivalves and crustaceans. *Environmental Science and Pollution Research* 25(25): 24818–24828, (Q2).

II. Stankevičiūtė M, Jakubowska M, **Pažusienė J**, Makaras T, Otremba Z, Urban-Malinga B, Fey DP, Greszkiewicz M, Sauliutė G, Baršienė J, Andrulewicz E (2019) Genotoxic and cytotoxic effects of 50 Hz 1 mT electromagnetic field on larval rainbow trout *(Oncorhynchus mykiss*), Baltic clam (*Limecola balthica*) and common ragworm (*Hediste diversicolor*). *Aquatic toxicology* 208: 109–117, (Q1).

III. Valskienė R, Baršienė J, Butrimavičienė L, **Pažusienė J**, Grygiel W, Stankevičiūtė M, Rybakovas A (2019) Induction of nuclear abnormalities in herring (*Clupea harengus membras*), flounder (*Platichthys flesus*), and Atlantic cod (*Gadus morhua*) collected from the southern part of the Gotland Basin—the Baltic Sea (2010–2017). *Environmental Science and Pollution Research* 26(13): 13366–13380, (Q2).

IV. **Pažusienė J**, Valskienė R, Grygiel W, Stankevičiūtė M, Butrimavičienė L, Baršienė J (2020) Cytogenetic damage in native Baltic Sea fish species: environmental risks associated with chemical munitions dumping in the Gotland Basin. *Environmental Science and Pollution Research. After the second revision.*

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[1]Asterisk (*) indicates the change of surname from Greiciūnaitė to Pažusienė.

[2] Declaration of contribution. Janina Pažusienė designed the study with Janina Baršienė and Milda Stankevičiūtė. J. Pažusienė initiated and planned Paper IV. She was responsible for genotoxicity and cytotoxicity analysis, genotoxicity risk assessment and statistical analysis. J. Pažusienė was responsible for the part of genotoxicity and cytotoxicity analysis of certain parts in Papers II-III. J. Pažusienė was responsible for the genotoxicity risk assessment and graphical data analysis in Paper I.

APPROBATION OF THE RESULTS

I. **Pažusienė J**, Baršienė J, Valskienė R, Stankevičiūtė M. Research on genotoxicity impacts of CWAs on marine organisms. The permanent representations of the Republic of Lithuania and the Republic of Poland to the Organization for Prohibition of Chemical Weapons (OPCW). Side event in the framework of the 24th CWC Conference of State Parties. Sea-dumped chemical weapons: research and international co-operation – current state of play. 26th November 2019, The Hague, The Netherlands.

II. **Pažusienė J**, Valskienė R, Stankevičiūtė M, Butrimavičienė L, Baršienė J. Environmental genotoxicity and risk assessment in herring (*Clupea harengus*), Atlantic cod (*Gadus morhua*) and flounder (*Platichthys flesus*) caught in the Gotland Basins from the Baltic Sea (2010–2017). The 16th International Conference on Environmental Science and Technology, 4th-7th September 2019, Rhodes, Greece.

III. **Pažusienė J**, Butrimavičienė L, Baršienė J, Stankevičiūtė M, Valskienė R. Environmental genotoxicity and risk assessment in the Gulf of Riga (Baltic Sea) using fish, bivalves, and crustaceans. 62nd international conference for students of physics and natural sciences "Open Readings 2019", 19th-22nd March 2019, Vilnius, Lithuania.

IV. **Pažusienė J**, Stankevičiūtė M, Valskienė R, Butrimavičienė L, Baršienė J. Environmental genotoxicity and risk assessment in herring (*Clupea harengus*) blood erythrocytes collected in the Bornholm and the Gotland Basins from the Baltic Sea (2009–2017). 8th Young Environmental Scientists „Yes 2019" meeting, 05th-10th February 2019, Ghent University, Belgium.

V. **Greiciūnaitė* J**, Valskienė R, Butrimavičienė L, Baršienė J. Genotoxicity studies in blood cells of fish collected in the Gotland Basin (the Baltic sea). 60th international conference for students of physics and natural sciences „Open Readings 2017", 14th-17th March 2017, Vilnius, Lithuania.

Academic achievements

I. Research council of Lithuania. Scholarship for study results (2020).

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