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Environmental genotoxicity and cytotoxicity studies in mussels before and after an oil spill at the marine oil terminal in the Baltic Sea

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Abstract Environmental genotoxicity and cytotoxicity effects in the gills of mussels *Mytilus edulis*, from the Baltic Sea areas close to the Būtingė oil terminal (Lithuania) before and after accidental oil spill in 31 January 2008 were studied. Mussels from the oil spillage zones were collected in 12 days, in 3 and 6 months after the spill to determine the effects of the spill. Mussels sampled in 2006–2007 were used for the assessment of the background levels of genotoxicity and cytotoxicity in the Būtingė oil terminal area. Comparison of the responses in *M. edulis* before and after the oil spill revealed significant elevation of frequencies of micronuclei (MN), nuclear buds (NB) and fragmented-apoptotic (FA) cells. Environmental genotoxicity and cytotoxicity levels in mussels from the Palanga site before the accident (in June 2007) served as a reference. Six months after the accident, in July 2008, 5.6-fold increase of MN, 2.9-fold elevation of NB, and 8.8-fold elevation of FA cells were observed in mussels from the same site.

Keywords Micronuclei · Nuclear abnormalities · Oil spill · Blue mussel · Baltic Sea

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Introduction

A complex mixture of pollutants enters the marine environment through the discharges of industrial, municipal, and agricultural wastes, those contaminants have increased concern relating to their harmful effects on aquatic organisms. In recent decades, the developments of offshore oil industry as well as shipping activities have increased risk of contamination by petrochemical products from navigation accidents and oil spills. Petroleum products released from the oil industry are composed mainly of non-cyclic and cyclic hydrocarbons, nitrogen–oxygen, sulfur compounds, produced water, alkylphenols and heavy metals (Wake 2005). Polycyclic aromatic hydrocarbons (PAHs), heavy metals, and alkylphenols are of particular concern due to their potential mutagenic and carcinogenic features. Hazardous substances capable of modifying the genetic material of living organisms could occur below the detection limit, but even so act as genotoxins in these low concentrations. Furthermore, contaminants discharged in complex mixtures can result in

interactions between unidentified substances and lead to unpredictability in genotoxic responses to pollution (Jha 2008). Genotoxic compounds can bind to DNA molecules and trigger a damaging chain of biological events, such as an impaired enzyme function, cytotoxicity, immunotoxicity, reproduction disturbances, growth inhibition, or carcinogenesis (Ohe et al. 2004).

Oil spills can result in a wide distribution of petroleum hydrocarbons in the marine environment seriously impacting the DNA of filter-feeding bivalve populations (Hamoutene et al. 2002). Bivalves have a limited ability to metabolize petroleum hydrocarbons and thus there is a comparatively high bioavailability of these compounds (Dyrynda et al. 1997). Positive correlations have been determined between micronuclei incidences and carcinogenic PAHs in the tissues of green-lipped mussels (*Perna viridis*), significant dose- and time-dependent formations of micronuclei was described (Siu et al. 2008). In inter-tidal mussels (*Perna perna*) on the Brazilian coast, chronically contaminated by oil and particularly after an oil spill in 2000, micronuclei (MN) frequencies strongly correlated to PAH concentrations (mainly alkylated homologues). Significant elevations of MN incidences was observed in mussels wherein ΣPAH was above 1,000 μg kg^{-1} d.w. Elevated MN frequencies were observed in specimens were ΣPAH levels were close or above 300 μg kg^{-1} d.w. (Francioni et al. 2007).

Increased frequencies of MN have been found in mussels from zones affected by oil spills (Parry et al. 1997; Harvey et al. 1999; Baršienė et al. 2004, 2006a, b; Bolognesi et al. 2006). Significant elevation of genotoxicity was observed in mussels from marine areas 30 days after oil spill and cytogenetic damage persisted up to 100 days (Parry et al. 1997), 7 months (Baršienė et al. 2006b), and even 5 years after an accident (Baršienė et al. 2008). Statistically significant increases of micronuclei levels have been detected in oysters and fish caged in Haven oil spill zones 10 years after an accident (Bolognesi et al. 2006). In mussels collected from oil terminal and marine port zones in the Baltic Sea, an elevated frequency of cytogenetic damage has been detected (Baršienė and Baršytė-Lovejoy 2000; Baršienė 2002). Significantly increased levels of micronuclei, nuclear buds, and fragmented-apoptotic cells were found in bivalves inhabiting the Baltic Sea after the oil spill in Būtingė oil terminal in November 2001 (Baršienė et al. 2006a) and in the areas close to the Russian oil platform D-6 (Baršienė et al. 2008).

On 31 January 2008, an accidental spill of oil products occurred from the tanker "Stena Antarctica" during the pumping of oil from the Būtingė oil terminal. Due to bad weather conditions, contaminants spread widely along the Lithuanian coast near Šventoji. In samples taken the day after the accident, oil products in the water from Šventoji exceeded the Maximum Permissible Concentration (MPC) in surface waters (0.05 mg l^{-1}) by 22 times. The concentration of oil products in the sand from the beach near Šventoji was seven times higher than the level of the most polluted IV category of sediments, more than 1,500 mg kg^{-1} d.w. (CMR 2008).

Among the current environmental genotoxicity tests, the MN test is one of the most frequently used, serving as an index of cytogenetic damage for over 30 years (Fenech et al. 2003). This test is fast and sensitive enough to detect structural and numerical chromosomal alterations induced by clastogenic and aneugenic agents (Heddle et al. 1991). The formation of nuclear buds may reflect the capacity of organisms to expel chromosome fragments without telomeres and centromeres from the nucleus. Besides, nuclear buds can be formed from DNA fragments that have been improperly condensed, amplified, or formed after failed replication (Lindberg et al. 2007). Elimination of cytogenetic damage by the apoptosis and necrosis occurs at different rates in various organisms (Mičic et al. 2002). Despite significant progress in the development of a biomarker approach, there are limited numbers of field and laboratory-controlled studies with environmentally realistic doses of petroleum compounds.

The main objective of this study was to evaluate the level of environmental genotoxicity and cytotoxicity at different sites in the Lithuanian economic zone of the Baltic Sea affected by the oil spill from the tanker "Stena Antarctica". The formation of micronuclei and nuclear buds in gill cells of mussels *Mytilus edulis* was assessed as the endpoint of environmental genotoxicity. The incidence of fragmented-apoptotic and bi-nucleated

gill cells was used as a marker of environmental cytotoxicity.

Materials and methods

Sampling

Data obtained from regular (2001–2008) environmental genotoxicity and cytotoxicity monitoring of Lithuanian coastal areas demonstrates that the site of Palanga in June 2007 could be rated as reference. In order to evaluate the genotoxic and cytotoxic impact of the "Stena Antarctica" oil spill, background levels of studied biomarkers, observed in 2006–2007 before the spill, were compared with the temporal pattern of environmental genotoxicity and cytotoxicity, assessed after the accident (February to August 2008).

Before the accident, in May and August 2006, as well as July and August 2007, blue mussels (*M. edulis*) were sampled close to the Būtingė oil terminal (1B station). In the reference area, located near the small resort town Palanga, 16 mussels were collected in 2007 (close to the second study site; Fig. 1, Table 1).

Twelve days after the accident, which occurred on 31 January 2008, 24 mussels were sampled close to oil spill site in the Būtingė oil terminal area in three study stations (first, 1B, and 2AV).

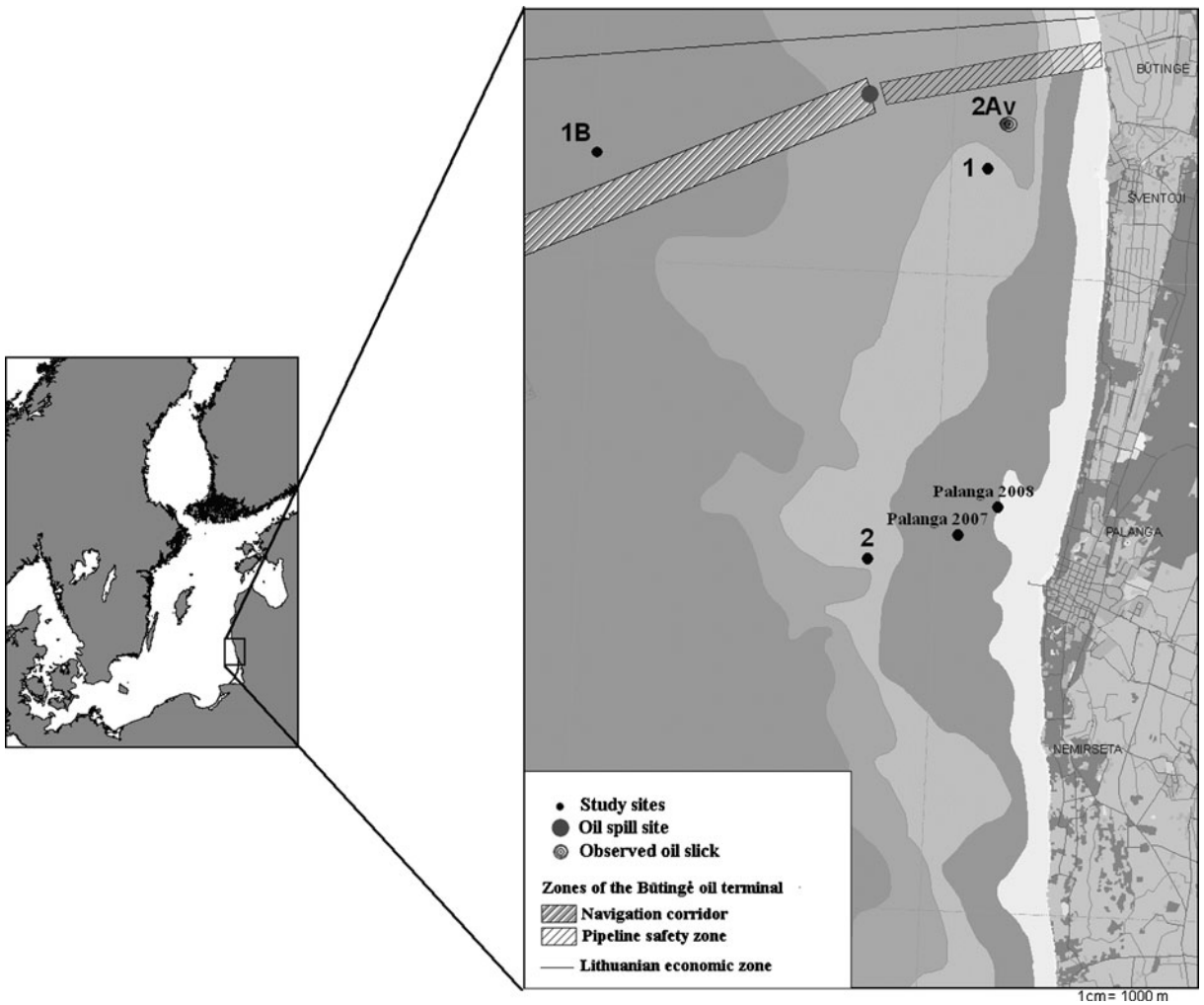

Fig. 1 Sampling locations (Baltic Sea, Lithuanian coast). Definition: black and white drawing

Table 1 Material for micronuclei and other nuclear abnormalities studies in mussels from the Būtingė oil terminal area (Baltic Sea, Lithuanian coast)

Study station	May, August 2006	June–August 2007	February 2008	May–June 2008	August 2008
2AV (Būtingė)			8		
First (Būtingė)			8	8	10
1B (Būtingė)	16	10	8		10
Second (Palanga)		16*		8+16*	10*
Total	16	26	24	32	30

*- close to the second study site

In addition to the annual monitoring sites, mussels were sampled at the 2AV station, the location where the oil spill was first observed (Fig. 1). Three months later, in May 2008, 16 blue mussels were collected from two sites contaminated by the accident, as well as from the reference Palanga site. In August 2008, 30 mussels were sampled from three sites contaminated by the accident. In total, 128 mussel specimens were collected for the study of micronuclei and other nuclear abnormalities (Fig. 1, Table 1).

Sample preparation, criteria, and analysis

The blue mussels were dissected, their gills removed and two gill arches placed in a drop of 3:1 ethanol acetic acid solution on clean microscopic slide and gently nipped with tweezers for 2–3 min (until cells spread within the drop of solution). The cell suspension produced was then carefully smeared on the surface of the slide and air-dried. Dried smears were subsequently fixed in methanol for 10 min and stained with 4% Giemsa solution in phosphate buffer pH = 6.8. The stained slides were analyzed under a light microscope Olympus BX51 at final magnification of × 1,000. Blind scoring of micronuclei and other nuclear abnormalities was performed on coded slides, the origin of samples being unknown.

Micronuclei, nuclear buds, fragmented-apoptotic, and bi-nucleated cells were identified using criteria described by Fenech et al. (2003).

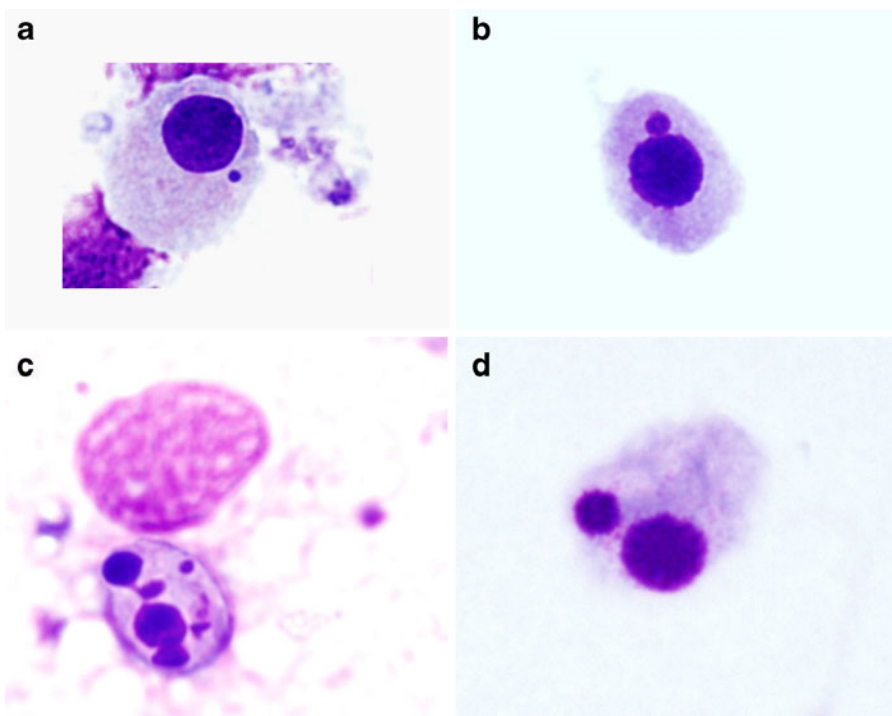

Fig. 2 Nuclear abnormalities in *Mytilus edulis* gills: **a** cell with micronuclei, **b** cell with nuclear bud, **c** fragmented-apoptotic cell and (**d**) bi-nucleated cell. Definition: Photographs

MN were characterized according to the following criteria: (1) round and ovoid-shaped non-refractory particles in the cytoplasm, (2) color and structure similar to chromatin, (3) diameter of 1/3–1/20 of the main nucleus, (4) particles completely separated from the main nucleus (Fig. 2a). Nuclear buds (NB) were characterized as extruded nuclear material that appears like a micronucleus with a narrow or definite nucleoplasmic bridge to the main nucleus (Fig. 2b). Fragmented-apoptotic cells (FA) in early stages were characterized by the presence of chromatin condensation within the nucleus and intact cytoplasmic and nuclear boundaries, late apoptotic cells exhibited nuclear fragmentation (Fig. 2c). The two nuclei of a bi-nucleated (BN) cell are approximately equal in size, the staining pattern and staining intensity have intact nuclear membranes and are situated within the same cytoplasmic boundary. The two nuclei may touch, but ideally should not overlap each other (Fig. 2d).

For each studied specimen of mussel, 2,000 cells with intact cytoplasm were scored. The final results were expressed as the mean value (‰) of sums of the analyzed individual lesions scored in 1,000 cells per mussel collected from every study location (Baršienė et al. 2004).

Statistical analysis was carried out using the PRISM statistical package. The mean and standard error were calculated for each studied group of mussels. The non-parametric Mann–Whitney *U* test was used to compare alteration frequencies in organisms from the site before the oil spill with those contaminated by oil or between time-related groups of mussels collected from the same study location. One-way ANOVA was used to compare results between the studied mussels groups.

Results

In mussels collected on 11–12 February 2008, 12 days after the oil spill in the Būtingė oil terminal, the frequency of micronuclei (MN/1000 cells) varied from 1.99‰ to 2.38‰, nuclear buds (NB/1000 cells) from 1.28‰ to 2.45‰, fragmented-apoptotic cells (FA/1000 cells) from 0.14‰ to 0.49‰, and bi-nucleated cells (BN/1000 cells) from 0.94‰ to 1.20‰. The lowest frequencies of micronuclei, nuclear buds, and fragmented-apoptotic cells were found in mussels from the first location (Fig. 3). The concentration of total oil hydrocarbons in water at the same station was not elevated and did not exceed the MPC (0.05 mg l^{-1}). The highest level of genotoxicity (4.83‰ MN and NB incidences) was registered in mussels from the 2AV station, where an elevated concentration of total oil hydrocarbons (up to 0.11 mg l^{-1}) in water was determined.

The analysis of nuclear abnormalities in gills of mussels was performed in May 2008, 3 months after the oil spill to determine the persistency of the damage. Similar genotoxicity and increased cytotoxicity levels at the first and second stations were comparable to the responses detected in February 2008. Since we have performed annual analysis (2001–2008) of genotoxicity and cytotoxicity in mussels from the Lithuanian coast, it was possible to compare the levels of responses in mussels before and after the oil spill. In the long-term studies, the location close to Palanga has served as a reference site. Comparison of genotoxicity and cytotoxicity levels in the Palanga location in June 2007 and after the accidental spill in May 2008 showed a statistically significant increase of micronuclei ($P = 0.0036$) and fragmented-apoptotic

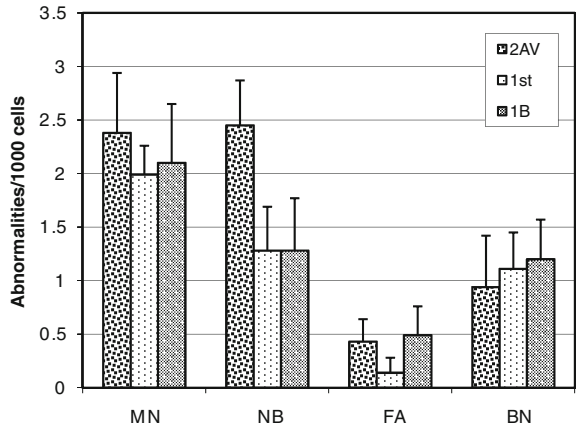

Fig. 3 Frequency of micronuclei (*MN*), nuclear buds (*NB*), fragmented-apoptotic (*FA*) and bi-nucleated (*BN*) cells in gills of mussel collected in February 2008 after the oil spill. Definition: *black* and *white* graphic

cells ($P = 0.0286$) in mussels inhabiting the second (Palanga) station, and nuclear buds ($P = 0.0264$) in mussels from the first station (Fig. 4). One-way ANOVA analysis showed significant differences in MN ($P = 0.0258$, $F = 4.161$), in NB ($P = 0.0125$, F 5.121), and FA ($P = 0.0164$, $F = 4.750$) between the studied mussels groups.

Compared to the background levels detected in mussels before the oil spill, the environmental genotoxicity and cytotoxicity levels remained significantly elevated in mussels collected 6 months after the oil spill (in August 2008). In *M. edulis* from stations contaminated by oil (first, 1B, and Palanga 2008), the frequency of micronuclei varied from 3.74‰ to 6.06‰, nuclear buds from 1.69‰ to 2.65‰, fragmented-apoptotic cells from 0.97‰ to 1.96‰ and bi-nucleated cells from 1.38‰ to 2.36‰ (Fig. 5). In August 2008, significantly higher levels of genotoxicity endpoints were observed in mussels than had been recorded before the accident. Compared to the reference Palanga (June 2007) site, statistically significant elevations of MN ($P = 0.0001$ or <0.0001), NB ($P = 0.0120$ or 0.0020) and FA (P values varied from 0.0013 to 0.0086) were found in mussels from all three contaminated stations.

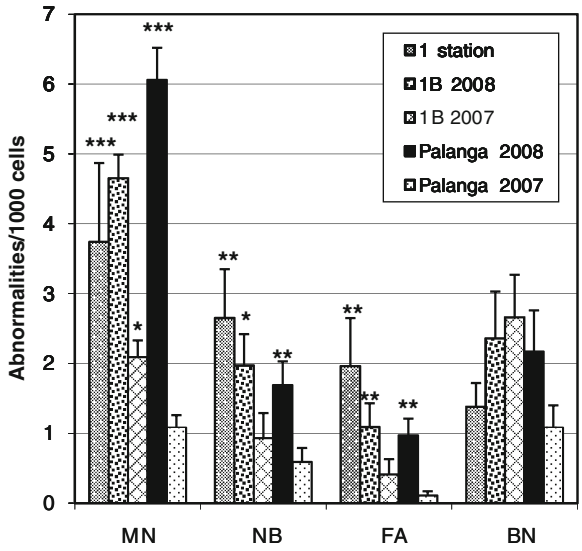

Fig. 5 Frequency of micronuclei (*MN*), nuclear buds (*NB*), fragmented-apoptotic (*FA*) and bi-nucleated (*BN*) cells in gills of mussel collected in August 2008 from the polluted by oil locations (first, 1B, and Palanga 2008 stations) and from the reference (before the oil spill) Palanga 2007 site. Differences between mussels from the reference and contaminated stations shown: *one asterisk* at level $P < 0.05$, *two asterisks* $P < 0.001$, *three asterisks* $P < 0.0001$. Definition: *black* and *white* graphic

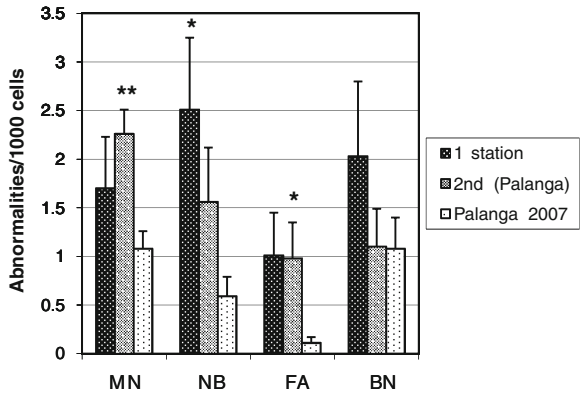

Fig. 4 Frequency of micronuclei (*MN*), nuclear buds (*NB*), fragmented-apoptotic (*FA*) and bi-nucleated (*BN*) cells in gills of mussel collected in May 2008 from the polluted by oil locations (first and second stations) and from the reference (before the oil spill) Palanga site in June 2007. Differences between mussels from the reference and contaminated stations shown: *one asterisk* at level $P < 0.05$, *two asterisks* $P < 0.001$. Definition: *black* and *white* graphic

Investigation of environmental genotoxicity and cytotoxicity in mussels inhabiting 1B station was performed in May and August 2006, in August 2007 and after the oil spill in February and August 2008. The lowest levels of studied abnormalities were in August 2007, the highest after the oil spill in August 2008 (Fig. 6). The increase of MN in August 2008 was significant ($P < 0.0001$) compared to the parameter value in August 2007. One-way ANOVA revealed significant time-related differences only in frequencies of micronuclei ($P = 0.0036$, $F = 4.647$).

After the oil spill, environmental genotoxicity and cytotoxicity endpoints were repeatedly measured three times in mussels from the first station. Time-related elevations of all studied abnormalities were detected (Fig. 7). Compared to the values of February 2008, highly elevated levels of fragmented-apoptotic cells in August (14-fold) and in May (sevenfold) were recorded. The frequency of micronuclei increased only in

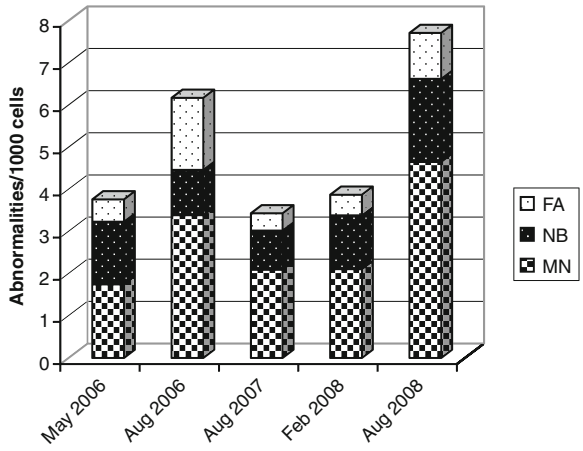

Fig. 6 Incidences of micronuclei (*MN*), nuclear buds (*NB*), and fragmented-apoptotic (*FA*) cells in gills of mussel collected in 2006–2008 from the location 1B near the Būtingė oil terminal area. Definition: *black* and *white* graphic

August (1.9-fold), a twofold increase of nuclear buds was observed in May and in August, and 1.8-fold increase of bi-nucleated cells was found in May. Nevertheless, due to the high variation in individual responses, a time-related statistically significant elevation was detected only for fragmented-apoptotic cells.

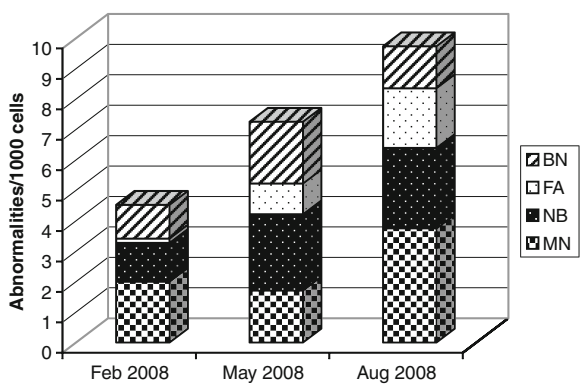

Fig. 7 Incidences of micronuclei (*MN*), nuclear buds (*NB*), fragmented-apoptotic (*FA*) and bi-nucleated (*BN*) cells in gills of mussel collected from the first station in 12 days (February 2008), in 3 months (May 2008) and in 6 months (August 2008) after the oil spill in the Būtingė oil terminal. Definition: *black* and *white* graphic

Discussion

The petroleum industry causes environmental pollution problems worldwide. Various oil compounds have a potential to induce biological consequences to the indigenous biota. Genotoxic effects of certain PAHs could arise due to oxidative biotransformation producing highly DNA-reactive metabolites (Woodhead et al. 1999). The main objective of the current study was to estimate the genotoxicity and cytotoxicity effects induced by the accidental oil spill in the Būtingė oil terminal in 2008 (31 January). A previous accidental oil spill in the Būtingė oil terminal occurred in winter 2001 (23 November). In both cases, oil slicks were driven along the Lithuanian coastline towards the main Lithuanian recreational resorts of Palanga and Šventoji.

After accidents, as soon as oil gets into water it begins to spread on the surface creating a thin film of oil. Short-chain molecules of oil easily evaporates, the other part dissolves, forms emulsions, degrades, oxidizes, aggregates or even penetrates into the sand (Stankevičius 2008). The rate of these processes depends on weather conditions. In case of the accidental oil spill in 31 January 2008, the concentration of total oil hydrocarbons in water 12 days later was still two times higher than the concentrations observed at the same station before the accident (Fig. 8). Further monitoring of PAH in water at Būtingė terminal after the oil spill showed that high concentrations of total oil hydrocarbons were detected mostly in the near-bottom water layer in May and August of 2008 (Mažeikių nafta 2008). Therefore, as demonstrated in the current study, the effects induced by oil contamination in organisms can be observed even in 6 months after an oil spill. The concentration of PAHs (naphthalene, anthracene, fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene) in sediments of the 1B station were analyzed in August of 2006 and 2007 and after the oil spill in August 2008. The cumulative concentrations of PAHs after the oil spill were about five times higher than before the accident (CMR 2008).

Statistically significant differences of the frequencies of micronuclei and other nuclear

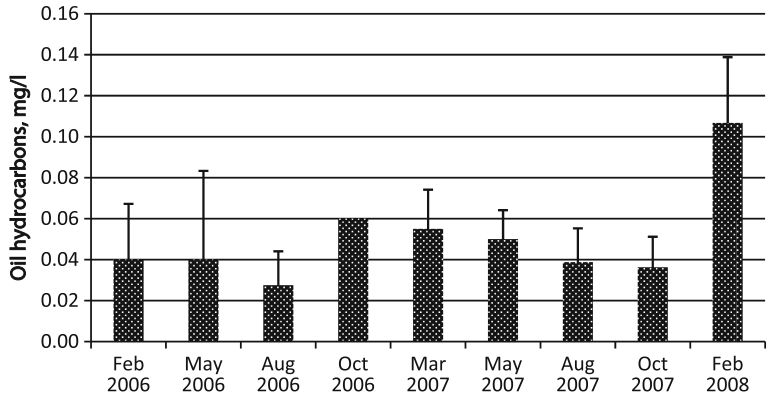

Fig. 8 Average concentrations of total oil hydrocarbons in water at 1B station in 2006–2008 (CMR data). Definition: *black* and *white* graphic

abnormalities were detected in mussels, collected 1 to 2 years before and after the accident, from station 1B, which is located near the Būtingė oil terminal. Furthermore, extremely high levels of environmental genotoxicity and cytotoxicity effects were observed in mussels from the Palanga location in August 2008, 6 months after the oil spill. Our previous studies on environmental genotoxicity in different sites of the Baltic and North Seas revealed a MN baseline in mussels consisting of 1–1.5 MN/1000 cells (Baršienė et al. 2004, 2006a). The frequency of MN after the discussed oil spill was six times higher than the baseline.

The micronuclei level in molluscs reflects the action of clastogenic and aneuploidogenic substances and it was suggested to use as marker of cytogenetic damage in marine monitoring programs (Brunetti et al. 1992; Bolognesi et al. 1996, 2006; Baršienė et al. 2006a; Siu et al. 2008). Environmental genotoxicity and cytotoxicity measurements in mussels and different fish species from the Lithuanian coastal and offshore areas started in September 2001 and thus long-term data regarding changes in these parameters has now been collected. In Lithuanian coastal areas, the MN frequency in mussels ranged from 1.08‰ (MN/1000 cells) in the Palanga location in June 2007 to 6.06‰ in the same location in August 2008. The level which is close to the reference (1.2‰) in Palanga site was established in June 2001. After an accidental oil spill in Būtingė oil terminal in November 2001, the Palanga location was contaminated by oil and the genotoxicity levels increased up to 3 MN/1000 cells (in 2002–2003) (Baršienė et al. 2004) and remained elevated until 2005. The eventual recovery of mussels was observed only in June 2007. In January 2008, a second oil spill occurred with a similar trend of spilled oil distribution, resulting in genotoxicity elevation. As a result, the frequency of micronuclei in mussels from the Palanga site increased to 6.06‰, reaching the highest level observed during the entire observation period (2001 to 2008) in all study locations on the Lithuanian coastal and offshore zones.

It is worth stressing that, in comparison to the level before the spill, a two- to fourfold elevation of nuclear buds, the other endpoint of the environmental genotoxicity, was found after the oil spill in 2008. The level of bi-nucleated cells in the gills of mussels was elevated as much as twofold; the induction of fragmented-apoptotic cells increased up to nine times. Such induction of aberrations in mussels appears evidently as a result of the action of genotoxic and cytotoxic compounds. Therefore, the formation of other nuclear abnormalities, such as nuclear buds, fragmented-apoptotic and bi-nucleated cells also serve as sensitive and informative biomarker in assessment of oil spill damage. Analysis of nuclear buds, fragmented-apoptotic, bi-nucleated cells, and some other nuclear abnormalities in mussels have been successfully applied in environmental studies aiming to assess pollutant effects (Dolcetti and Venier 2002; Izquierdo et al. 2003; Carvalho Pinto-Silva et al. 2005; Venier

and Zampieron 2005; Baršienė et al. 2006a, b, c, d, 2008; Baršienė and Rybakovas 2006; Baršienė and Andreikėnaitė 2007; Koukouzika and Dimitriadis 2008).

Genotoxic and cytotoxic effects in mussels *M. edulis* were induced by oil or its components after 1, 2, 4 and 8 days (Baršienė et al., 2010). Increase genotoxicity and cytotoxicity was also defined after a 3-week treatment with 0.5 ppm crude oil processed from the Statfjord B platform in the North Sea. Moreover, the co-exposure to 0.5 ppm of oil spiked with a mixture of alkylphenols ($\Sigma =$ 0.1 ppm) and 0.1 ppm of PAHs induced a 2.8-fold increase of MN and fourfold increase of nuclear buds and fragmented-apoptotic cells via control levels (Baršienė and Andreikėnaitė 2007).

Crude oil is composed from different components, such as various hydrocarbons, heavy metals and nitrogen–oxygen compounds. Data on the genotoxicity of ten polycyclic aromatic hydrocarbons (anthracene, benz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene, dibenz[*ah*]anthracene, dibenz[*ac*]anthracene, 3-methylcholanthrene, benzo[*a*]pyrene, benzo[*e*]pyrene, chrysene and pyrene) in mice skin cells has been reported. The genotoxicity of these compounds correlated with their carcinogenicity (Nishikawa et al. 2005). Genotoxic effects of benzo[*a*]pyrene (B[*a*]P) and dimethylbenz[*a*]antracene in the gills and hemolymph of marine molluscs have been observed earlier (Burgeot et al. 1995; Bolognesi et al. 1996; Venier et al. 1997; Siu et al. 2004). Comet and MN assays demonstrate clear dose- and time-dependent responses to B[*a*]P exposure in *Mytilidae* bivalve *P. viridis* (Siu et al. 2004). The level of DNA strand breaks in scallops *Chlamys farreri* estimated after treatment from 1–6 days with 0.5 and 3 $\mu g\, l^{-1}$ B[*a*]P B[*a*]P was significantly different to the control. Later, after a 20-day exposure, a gradual decrease of DNA damage was assessed (Pan et al. 2008). An increased frequency of MN was observed in mussels after 15-day exposure to 0.1 $\mu g\ l^{-1}$ of phenanthrene (Koukouzika and Dimitriadis 2008). In zebra mussel *Dreissena polymorpha*, 2-, 3-, and 4-day treatment with different concentrations (2 and 10 $\mu g\, l^{-1}$) of B[*a*]P (Binelli et al. 2008) induced MN formation. In juvenile fish, *Dicentrarchus labrax*, significant induction of MN and other nuclear abnormalities was observed at 4 h treatment with 0.3, 0.9, and 2.7 μM, at 6 h exposure to 0.9 and 2.7 μM and at 8 h exposure to 0.3, 0.9, and 2.7 μM of naphthalene. Significantly increased levels of nuclear abnormalities have been also described in erythrocytes of *D. labrax* after a 2-h exposure to 0.1, 0.9, and 2.7 μM (B[*a*]P), and after 4 h exposure to 0.1 and 0.9 μM (Gravato and Santos 2002). In eels, *Anguilla anguilla*, treatment with 0.3, 0.9, and 2.7 μM of naphthalene, resulted in the induction of micronuclei and other nuclear abnormalities (Teles et al. 2003). Our recent monitoring studies of environmental genotoxicity within the water column near the Statfjord B oil platform revealed a clear gradient increase in the frequency of MN in hemocytes of mussels and liver erythrocytes of Atlantic cod, caged for 6 week in the area of the platform. In comparison to the reference site, significantly increased levels of micronuclei were also detected in mussels and cod deployed 500 m from the platform, (Hylland et al. 2008). Among the biomarkers (EROD, DNA adducts, PAH metabolites, etc.) used to monitor impacts, to the caged mussels affected by the contaminants in studied area of the North Sea, lysosomal destabilization in mussel hepatopancreas and MN induction in hemocytes were the only methods that demonstrated the impact. Micronuclei formation was suggested as a sensitive biomarker that should be used to monitor low levels of petroleum contaminants (Hylland et al. 2008). Significant elevations of MN incidences have been described after 8-, 12-, 16-, and 30-day caging of green-lipped mussels in different coastal areas polluted by PAHs zones in Hong Kong. In some study locations, strong relationships ($r > 0.9$) between concentrations of carcinogenic PAHs and MN have been detected in mussels caged up to 16 days (Siu et al. 2008).

Among the parameters used to serve as an early warning signal of pollution-induced genetic damage in wildlife species, MN test as well as morphological alterations of the cell nuclei including nuclear buds and fragmented-apoptotic could be successfully applied in the future monitoring of genotoxins in zones affected by the oil industry. Taking into consideration species-specific

responses to contaminants and pollution patterns, ecologically relevant information about oil industry areas could be obtained by the assessment of genotoxic effects in indigenous species (fish and mussels) both *in situ* and in caged organisms from wild populations. Considering the danger of oil spill in oil installations, further elaboration of laboratory-controlled studies using environmentally realistic doses of genotoxic compounds should help to describe in detail the harm to resident species inhabiting areas of chronic contamination. Laboratory-controlled experiments, active monitoring approaches, and *in situ* assessment of DNA damage in various tissues of target species will help to archive a substantial progress in assessment of early responses as well as short- or long-term adaptations to chronic pollution originating from petroleum installations.

Conclusion

The results of the present study pointed to the comparatively quick formation of oil spill induced genotoxicity and cytotoxicity in winter at low temperature and the need to highlight harmful effects after an oil spillage in the marine environment. Since the temperature-dependent gradient February < May < August of studied effects was defined, further monitoring should be performed considering time-related effects in mussels.

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