

# 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)

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#### Abstract

Eight nuclear abnormalities of geno-cytotoxicity were studied in peripheral blood erythrocytes of herring (Clupea harengus membras), flounder (Platichthys flesus) and Atlantic cod (Gadus morhua) sampled (2010-2017) from the Polish and the Lithuanian Exclusive Economic Zones (EEZs) in the Baltic Sea. At all study stations, total genotoxicity ( $\Sigma$ Gentox) were found to be higher than total cytotoxicity ( $\Sigma$ Cytox). A significant time-related decrease in genotoxicity was detected in the Lithuanian EEZ (2015–2017), while in the Polish EEZ (2014–2016), the opposite tendency was revealed. The highest  $\Sigma$ Gentox and  $\sum$ Cytox values recorded in the fish sampled at the study stations located relatively close to each other clearly indicate an increased environmental genocytotoxicity pressure for fish in these areas. Exceptionally high and high level genotoxicity risks to herring followed by those to flounder and cod were determined at a higher percentage of the stations studied.

**Keywords:** Genotoxicity; Cytotoxicity; Oil extraction; Risk assessment; Marine fish

# 1. Introduction

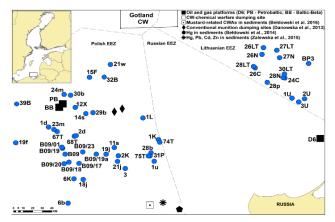
The eastern part of the Gotland basin is one of the most contaminated areas in the Baltic Sea. Chemical and conventional munition dump-sites as well as areas along CW transportation routes are under increased environmental pollution pressure, potentially causing lethal or chronic toxic effects on marine organisms such as fish and mussels or entire food webs (Della Torre et al. 2013). In the southern part of the Gotland Basin, in the Polish EEZ, there are two intensively operating oil and gas platforms. The development of offshore oil industry can increase the risk of contamination from oil extraction processes or due to accidental events.

#### 2. Materials and Methods

#### 2.1. Sampling of fish

Herring, flounder and cod were collected from November 2010 to June 2017, from 37 study stations located in the Polish EEZ and 13 stations located in the Lithuanian EEZ

(Figure 1). For the evaluation of time-related changes in geno-cytotoxicity responses of fish, we additionally used relevant data from reference stations B09 (located in the Polish EEZ, December 2003) and BP3 (located in the Lithuanian EEZ, December 2003).



**Figure. 1.** Location of sampling stations in the Polish and the Lithuanian EEZs (Baltic Sea), where fish were sampled in the period of 2010–2017 (Valskiene et al. 2019).

#### 2.2.Sample preparation and analysis

The frequencies of eight nuclear abnormalities were examined as markers of environmental geno-cytotoxicity in fish blood erythrocytes following the methodology presented in Valskienė et al. (2019). Induction of micronuclei (MN), nuclear buds (NB), nuclear buds on filament (NBf) and bi-nucleated erythrocytes with nucleoplasmic bridges (BNb) were assessed as genotoxicity endpoints, and induction of fragmented (Frag), apoptotic (Apop), bi-nucleated (BN) and 8-shaped nuclei erythrocytes as cytotoxicity endpoints (Fenech et al. 2003).

# 2.3. Environmental genotoxicity risk assessment

Environmental genotoxicity risk at each of the 52 stations studied was assessed based on the established BAC (Background Assessment Criteria) as the sum  $\sum$ Gentox of the analysed biomarker responses such as MN, NB, NBf and BNb in studied fish species. The BAC value for

 $\Sigma$ Gentox in herring is 0.85‰; in flounder – 0.40‰; in cod – 0.55‰ (MN+NB+NBf+BNb/1000 erythrocytes) (Baršienė et al. 2012b).

## 3. Results

# 3.1. Environmental genotoxicity and cytotoxicity levels in herring (C. harengus membras)

The analysis of genotoxicity levels in herring revealed a strongly increased frequency of MN, NB, NBf, BNb or Frag in fish specimens caught at 9 out of 31 investigated stations. Total cytotoxicity levels ( $\Sigma$ Cytox) varied between 0.23-0.25‰ (stations 6N, 6b, 23m) and 1.68‰ (station 15F). The  $\Sigma$ Cytox responses higher than 1.0% were found in gradient in fish at stations 15F > 28p > 31P> 67T > 74T > 2d. The research on genotoxicity and cytotoxicity in herring caught in the Polish and the Lithuanian EEZs indicates that  $\sum$ Cytox levels at all the stations studied were lower than  $\sum$ Gentox levels. The long-term data obtained during the sampling campaigns performed in March of 2011, 2013, 2015 and 2016 reveal the tendency for genotoxicity in Lithuanian EEZ to decrease. A time-related herring response tendency was not observed in the Polish EEZ, where oil platforms PB and BB are operating.

# 3.2. Environmental genotoxicity and cytotoxicity levels in flounder (P. flesus)

In flounder,  $\Sigma$ Gentox values varied from 0.33‰ at station 12X (February 2014) to 2.73‰ at station 28LT (March 2011). During the 2010–2017 period, ∑Gentox values in flounder caught in the Polish EEZ remained approximately at the similar level, whereas in the Lithuanian EEZ, during the 2011-2016 period, ∑Gentox values were found to be following a downward trend. The measurements of  $\sum$ Cytox values in flounder revealed a variation between 0.13‰ at station BP3 and 1.20‰ at station 23m. In general, cytotoxicity levels in fish collected from the Polish EEZ were higher compared to that in fish from the Lithuanian EEZ. At all the study stations located in the area of oil platforms PB and BB (the Polish EEZ), frequencies of nuclear buds (NB+NBf) in flounder were found to be 6-19-fold higher compared to those in fish caught at reference station B09 (2003).

# *3.3. Environmental genotoxicity and cytotoxicity levels in cod (G. morhua)*

 $\Sigma$ Gentox values higher than  $\Sigma$ Cytox values were recorded at most of the stations monitored, except four stations (B09/17, B09/19a, B09/20 and 1K). Levels of  $\Sigma$ Gentox and  $\Sigma$ Cytox in cod at all the stations located in the Lithuanian EEZ significantly differed from those at reference stations (B09/01 and BP3). In the Polish EEZ, significantly higher levels of  $\Sigma$ Gentox were recorded at eight stations and significantly higher levels of  $\Sigma$ Cytox – at 10 stations out of the 18 stations studied compared with the reference station B09/01. Frequencies of MN, NB and NBf in blood erythrocytes of cod from the stations located near oil and gas platforms PB and BB in the Polish EEZ were found to be significantly increased compared to those in fish from the reference station B09 (2003). In the Polish and the Lithuanian zones studied, more than 2-times higher level of NB+NBf was found in fish from 91% of the stations studied (in 21 out of the 23 studied stations in 2010–2017).

## 3.4. Environmental genotoxicity risk assessment

The performed assessment of environmental  $\sum$ Gentox in fish from 39 study stations (including the reference station B09) located in the Polish EEZ revealed that genotoxicity risk levels at most of them varied from exceptionally high to high levels. The study stations at which genotoxicity risk to herring (at 18 out of the 24 studied stations) and flounder (at 6 out of the 7 study stations) was found to be exceptionally high were distributed throughout the monitored Polish areas. The highest genotoxicity risk exists for fish caught in the zone of oil and gas platforms.

The performed analysis of  $\sum$ Gentox responses in fish from the Lithuanian EEZ showed low genotoxicity risk for fish only at the reference station BP3. The assessed genotoxicity responses in herring and flounder collected from all the stations (26LT, 27LT, 28LT and 30LT) in March 2011 showed exceptionally high genotoxicity risk levels. The assessment of genotoxicity risk for cod carried out in this area in 2015–2017 revealed lower risk compared to that for herring and flounder at the same station or at the same time of sampling.

### 4. Conclusions

Exceptionally high and high level of genotoxicity risk to fish was determined almost at all the stations sampled in the southern part of the Gotland Basin. Therefore, these marine zones cannot qualify for the Good Environmental Status.

#### References

- Baršienė J., Rybakovas A., Lang T., Grygiel W., Andreikėnaitė L., Michailovas A. (2012b), Risk of environmental genotoxicity in the Baltic Sea over the period of 2009– 2011 assessed by micronuclei frequencies in blood erythrocytes of flounder (*Platichthys flesus*), herring (*Clupea harengus*) and eelpout (*Zoarces viviparus*), *Marine Environmental Research*, **77**, 35-42.
- Della Torre C., Petochi T., Farchi C., Corsi I., Dinardo Sammarini V., Alcaro L., Mechelli L., Focardi S., Tursi A., Marino G., Amato E. (2013), Environmental hazard of yperite released at sea: sublethal toxic effects on fish. Journal of hazardous materials, 248-249, 246-253.
- Fenech M., Chang W.P., Kirsch-Volders M., Holland N., Bonassi S., Zeiger E. (2003), HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures, *Mutation Research*, **534**, 65-75.
- Valskienė R, Baršienė J., Butrimavičienė L., Pažusienė J., Grugiel 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*. DOI: 10.1007/s11356-019-04687-0