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Variability of biological indices, biomarkers, and organochlorine contaminants in flounder (*Platichthys flesus*) in the Gulf of Gdańsk, southern Baltic Sea

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HIGHLIGHTS

- All measured parameters exhibited significant seasonal variations.
- Biological indices were used to interpret biomarker responses.
- EROD correlated positively with DNA SB, both correlated negatively with CF, GSI, HSI.
- Contaminant ww and lw levels varied along the collection times.

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ABSTRACT

Biological indices, biomarkers (EROD activity, DNA SB, 1-OH pyrene), and organochlorine contaminants were investigated in flounder collected in the Gulf of Gdańsk during March–December 2011 to describe their seasonal variability and interrelations. Univariate and multivariate statistics were used to evaluate the relations. The EROD activity positively correlated with DNA SB, both negatively correlated with CF, GSI, and HSI, and there was a moderate positive correlation for EROD and DNA SB with 1-OH pyrene. EROD highest activity corresponded to a resting stage of gonad development. DNA SB, highest during spawning, gradually decreased until late autumn. The PCBs, DDTs, HCB, HCHs, dieldrin, and heptachlor levels in muscle tissue were quantified on a wet and lipid basis. In each case, their levels decreased after spawning, fluctuated over the study period indicating that their accumulation was pronouncedly controlled by chemical-specific properties, their levels in prey, and lipid dynamics.

1. Introduction

Various biological effects tools have been recommended and used for monitoring and assessment of pollutant-induced adverse biological effects to ascertain the quality of marine environments (ICES, 2011; Lehtonen et al., 2014; Lyons et al., 2010). Their application requires however a considerable knowledge of natural variability in their responses related to the influence of intrinsic biological and abiotic factors. Numerous studies have shown that environmental factors such as temperature, salinity and biological factors such as age, size, gender, developmental and reproductive stages can modulate and/or mask the contaminant-induced biological responses (Jimenez et al., 1990; Koenig and Solé, 2012; Lange et al., 1998; Lyons et al., 2011; Nahrgang et al., 2013; Van der Oost et al., 2003). These confounding factors can lead to a misinterpretation of biomarker data. Among the recommended and widely used biomarkers are the activity of ethoxyresorufin-*O*-deethylase (EROD), DNA damage (DNA SB), and biliary 1-OH pyrene in fish. EROD is a member of cytochrome P4501A (CYP1A) family of enzymes involved in the biotransformation of a range of organic contaminants and endogenous substrates (Stegeman et al., 1992). It responds to exposure to a group of organic contaminants that have the capabilities to induce the synthesis of P450 1 A via binding to

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the aryl hydrocarbon receptor (AhR)/ARNT complex e.g., dioxins, furans, dioxin-like PCBs, and some PAHs (Whyte et al., 2000). The induction i.e., increased synthesis of CYP1A protein and related enzymatic activity following exposure, forms the basis for using EROD in environmental monitoring (Collier et al., 1995; Stegeman et al., 1992). DNA SB, measured with the comet assay, reflects changes in the DNA integrity, i.e., DNA strand breaks and other DNA lesions relative to the total DNA content in a single nucleus, induced by gentoxic agents including reactive oxy radicals generated during the metabolism of contaminants (Livingstone, 2001; Regoli et al., 2003). The ecotoxicological applications and significance of comet assay have been well described (Jha, 2008; Martins and Costa, 2015). As the DNA damage can ultimately lead to cell death, carcinogenesis, and mutations, potential effects of DNA SB could lead to both individual and population effects (Depledge, 1998; Devaux et al., 2015; Diekmann et al., 2004). The biliary 1-OH pyrene is a biomarker specific of exposure to PAHs (ICES, 2011; Kammann et al., 2017; Vuorinen et al., 2006). PAHs are among the core hazardous substances monitored in the north-east Atlantic and European seas including the Baltic Sea (HELCOM, 2013; OSPAR, 2010). These contaminants do not accumulate in fish, but are metabolized and excreted via bile fluid, with 1-OH pyrene being the main metabolite (Kammann, 2007; Kammann et al., 2017; Nagel et al., 2012; Ruddock et al., 2002).

The objective of this study was to investigate the EROD activity, DNA SB, and biliary 1-OH pyrene level in flounder (*Platichthys flesus*) in the Gulf of Gdańsk in order to describe their variability in the context of changing biological and environmental factors. The study was focused on flounder, because it is one of the main target species for monitoring European coastal ecosystems, widely distributed from the White Sea in the north to the Mediterranean and the Black Sea in the south (Munroe, 2015; Nielsen, 1986). Flounder have been commonly used as bioindicator species due to their life style and feeding ecology which expose them to sediment-associated contaminants (Beyer et al., 1996; Evrard et al., 2010, 2013; Kirby et al., 2004, 2007). As a demersal species living mostly on the seabed they feed on benthic meio-fauna during the juvenile stage and on macro-fauna thereafter. Their food choice depends on season, prey availability, and habitat characteristics (Aarnio et al., 1996; Florin and Lavados, 2010; Kostrzewska-Szlakowska and Szlakowski, 1990). Adult flounder show a high habitat fidelity which makes them a suitable species for site-specific as well as regional assessment. The biomarkers were investigated in parallel with biological indices i.e., condition factor (CF), hepato- and gonado-somatic indices (HSI and GSI), and the levels of common organochlorine contaminants in muscle tissue, in order to determine their seasonal variability and interrelationships. CF is regarded as a physiological index of fish health, and a good indicator of stock condition and habitat quality. It indicates the magnitude of stored energy reserves which is a factor affecting the status of population through an influence on individual growth, reproductive potential, and probability of survival (ICES, 2017).

2. Materials and methods

2.1. The study area and sampling of fish

The Gulf of Gdańsk (GoG) is a southeastern bay of the Baltic Sea, enclosed by a large curve of the Hel peninsula of Poland and the Sambia peninsula of Russia. It is a relatively shallow water basin (average depth 50 m, maximal 118 m) of mostly sandy bottoms, with an estimated surface area of 4296 km^2 and a coastline of 491 km. The gulf has a mixture of brackish and marine waters typical for this type of basin, with freshwater coming from the Polish and Kaliningrad (Russia) regions. The GoG area is influenced by surrounding urban conglomerates and industrial developments including two dynamic international sea ports. Elevated levels of chemical contaminants in sediment and fish inhabiting GoG have been reported among which the dominant were legacy persistent organic pollutants and some metals (Dabrowska et al., 2013; Lubecki and Kowalewska, 2010; Strandberg et al., 1998; Waszak et al., 2014), although the so called newer contaminants i.e., polyfluorinated and organotin compounds, are also present (Falandysz et al., 2006; Filipkowska et al., 2014).

Adult female flounder (*Platichthys flesus*) were collected from the Gulf of Gdańsk (GoG; E18°54′-19°10'; N54°25′-54°30' Fig. S1, Supplementary data) eight times in the period from March 2011 to December 2011 by a fisherman using a bottom trawl (105–120 mm mesh width). There was no sampling in January, February, August and September due to technical reasons. Although an investigation of both genders would be desirable, yet considering the overall work load and existing data (Akcha et al., 2004; Beyer et al., 1996; Janssen et al., 1995), only females were selected for the study. Flounder of 25–30 cm in length were targeted for sampling. On each sampling occasion an excess of fish was caught, yet larger individuals were sometimes included to provide a sufficient number of specimens. Upon collection, the fish were transferred to 120 L tanks filled with aerated site-collected water and transported to the NMFRI laboratory. There, they were anesthetized, blood samples were taken, weight and total length was recorded. They were then sacrificed and dissected, the weight of liver, gonads, and soma (gutted body weight i.e., carcass without internal organs) was recorded, and samples of liver and bile, and both muscle fillets (skin removed) were collected. The liver and bile samples were frozen in liquid nitrogen and stored at −80 °C for measurement of EROD and PAH metabolites, respectively. Both muscle fillets were grounded, freeze-dried, and stored at −20 °C for chemical analyses. The gonad maturity stage was assessed visually according to the scale of Maier (1908). This scale has been used for fish in the Baltic Sea until recently (ICES, 2012). Condition factor (CF), hepato-somatic (HSI) and gonado-somatic (GSI) indexes were calculated according to the following formulas (ICES, 2011): CF = [[BW (g)/(total length)^3] *100], HSI = [HW/(BW-HW)*100], and GSI = [GW/(BW-GW)*100], where HW – liver weight (g), GW – gonad weight (g), BW – body weight (g). In addition, these indexes were calculated based on the weight of soma, and were marked as CF $_{gutted}$, GSI $_{gutted}$ and HSI $_{gutted}$, respectively.

2.2. Biomarker analyses

DNA SB was measured with the comet assay as follows: a 50 μl aliquot of blood cells diluted with 0.01 M phosphate buffer mixed with 0.5% low-melting point agarose was placed onto glass slides pre-coated with 1% normal-melting point agarose. After solidifying the agarose (10 min, 4 °C), the slides were immersed in a chilled lysis solution (1 h, 4 °C), then rinsed with deionized water and placed in an electrophoresis buffer (30 min, 4 °C) to allow the DNA to unwind. Electrophoresis was conducted at 25 V and 300 mA for 30 min. Thereafter the slides were neutralized in a 0.4 M Tris buffer (pH 7.5). Cells were stained with ethidium bromide and examined under a fluorescent microscope (Nikon *Eclipse 80i*) with excitation set to 440 nm and emission 520 nm. DNA SB was quantified as tail DNA (the percent of DNA in the comet tail) in 100 cells (50 cells in two replicate slides) per one specimen using the image analysis software Lucia (Laboratory Imaging, Prague, Czech Republic). All blood samples, prior to be assayed, were checked for cell viability using a trypan blue exclusion test (Strober, 2001). A viability greater than 95% was taken as satisfactory.

EROD activity was measured by a kinetic fluorimetric assay as described by Stagg and McIntosh (1998) and used by Kopecka and