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Biomarker responses in eelpouts from four coastal areas in Sweden, Denmark and Germany

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A B S T R A C T

To increase our understanding of possible chemical impacts on coastal fish populations in the Baltic Sea, Kattegat and Skagerrak, the viviparous eelpout (*Zoarces viviparus*) was used as sentinel species in two major sampling campaigns (spring and autumn) in 16 different coastal sites. Condition factor (CF), liver somatic index (LSI), gonad somatic index (GSI) were measured and the activity of the hepatic enzymes ethoxyresorufin-O-deethylase (EROD), glutathione reductase GR), glutathione S-transferase (GST), catalase (CAT) and muscular activity of acetylcholinesterase (AChE) were assessed. PAH metabolites in bile were also analyzed. The most notable finding in the data set was the low EROD activity in eelpouts collected at the relatively polluted region in Germany compared to the other regions, which could be due to an inhibition of the CYP1A-system or to adaptation to chronic exposure of pollutants in this area. Additionally, low AChE activity was noted in the German region in the autumn campaign and low AChE activity detected in the Danish region in the spring campaign. These differences suggest possible season-specific differences in the use and release of AChE-inhibiting chemicals in the Danish and German regions. Clustering of biomarkers on site level indicated a relationship between CF and GSI and suggested that sites with a high CF contained eelpout that put a larger effort into their larvae development. Clustering of the oxidative stress markers GR, GST and CAT on the individual level reflected a possible coordinated regulation of these enzymes. Overall, the results support the importance of taking into account general regional differences and seasonal variation in biomarker activity when monitoring and assessing the effects of pollution. Despite the expected seasonal variation for most of the measured endpoint, several markers (GSI, EROD and CF) vary similarly between all selected sites in both spring and autumn. This suggests that the differences between sites for these endpoints are independent of season.

1. Introduction

The Baltic Sea area is heavily contaminated by industrial waste, shipping activity, agricultural run-off and other anthropogenic activities. Although the amount of certain classical environmental pollutants, including PCB, DDT and TBT, have decreased in the Baltic Sea since the 1980's, the level of other chemicals such as endocrine disrupters, perfluorinated alkyl acids and brominated flame retardants have increased in concentration (Bignert et al., 2015; HELCOM, 2010). Many of these chemicals are persistent and, can easily bioaccumulate and cause adverse effects individually or as part of complex mixtures of chemical compounds.

Environmental monitoring is an urgent task as it is important to determine the link between the current levels of pollution and the

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effects observed in the field. Biomarkers of effect measure the biological changes that occur as a result of e.g. chemical exposure and are widely used to assess the health status of fish (Hylland et al., 2015; Lehtonen et al., 2014). Recently, several studies in the Baltic Sea area, including large research projects financed by national and international agencies, have focused on developing and improving monitoring measures to access the effect of environmental pollutants. Indices for the assessment of environmental pollution have been developed to measure the overall health status in selected species (Broeg and Lehtonen, 2006). In addition, a multi-biomarker approach has been proposed as an alternative choice in monitoring, especially in combination with assessing the effects on different biological levels (Asker et al., 2015; Lehtonen et al., 2006; Schiedek et al., 2006). Lehtonen and colleagues have developed an integrated monitoring approach to assess the overall health of the ecosystem and have suggested health indices as a tool in environmental management (Lehtonen et al., 2014). In addition, the need for integrated long-term monitoring in marine coastal areas has also been determined (Hanson et al., 2009; Hansson et al., 2006; Ronisz et al., 2005; Sandström et al., 2005).

The fish species eelpout (*Zoarces viviparus*) is widespread throughout most of the Baltic and North Sea regions and has been recommended as sentinel species by the International Council for the Exploration of the Sea (ICES, 2010) and the international commissions HELCOM and OSPAR (HELCOM, 2008; OSPAR, 2007). The stationary behavior and viviparity of the eelpout makes it well suited for environmental monitoring and ecotoxicological research (Hedman et al., 2011; Jacobsson et al., 1986). Eelpouts are included in several research studies and national monitoring programs conducted in the coastal areas of Sweden (Bignert et al., 2015; Ronisz et al., 2005) Denmark (Dahllöf et al., 2010; Strand et al., 2004, 2009), Estonia (Kreitsberg et al., 2012), Poland (Napierska and Podolska, 2006) and Germany (Gercken, 2007; Gercken et al., 2006; Gercken and Sordyl, 2002; Rüdel et al., 2010). Eelpouts are also used in basic research studies, including the identification of masculinized embryos by pulp mill effluents (Larsson and Förlin, 2002; Larsson et al., 2000), hepatic transcriptome sequencing (Kristiansson et al., 2009), gene expression profiling (Asker et al., 2013), oxidative stress assessments (Almroth et al., 2005), and the maternal transfer of pollutants (Brande-Lavridsen et al., 2013; Morthorst et al., 2014; Rasmussen et al., 2002).

The current investigation was part of a joint research project called BALCOFISH. This EU Bonus program was supported to investigate possible links between environmental pollution in the Baltic Sea and effects in coastal fish populations. Here, we present the monitoring results from two large sampling campaigns (spring and autumn) for eelpouts, which encompasses 16 different sites at four different coastal regions in the Baltic Sea including Kattegat and part of Skagerrak on the Swedish west coast (Fig. 1). Because the selected regions had each individually been part of previous monitoring efforts, the aim of our investigation was to determine the value of performing an extended monitoring program and thereby provide an overall picture of the impact of environmental pollutants in the selected areas. Measurements included morphometric indices (condition factor, liver somatic index, gonad somatic index) and the analysis of specific biomarkers, including activity measurements of the enzymes ethoxyresorufin-O-deethylase, glutathione reductase, glutathione S-transferase, catalase and acetylcholinesterase. PAH metabolites in bile were also analyzed. The differences in the levels of biomarkers between and within the

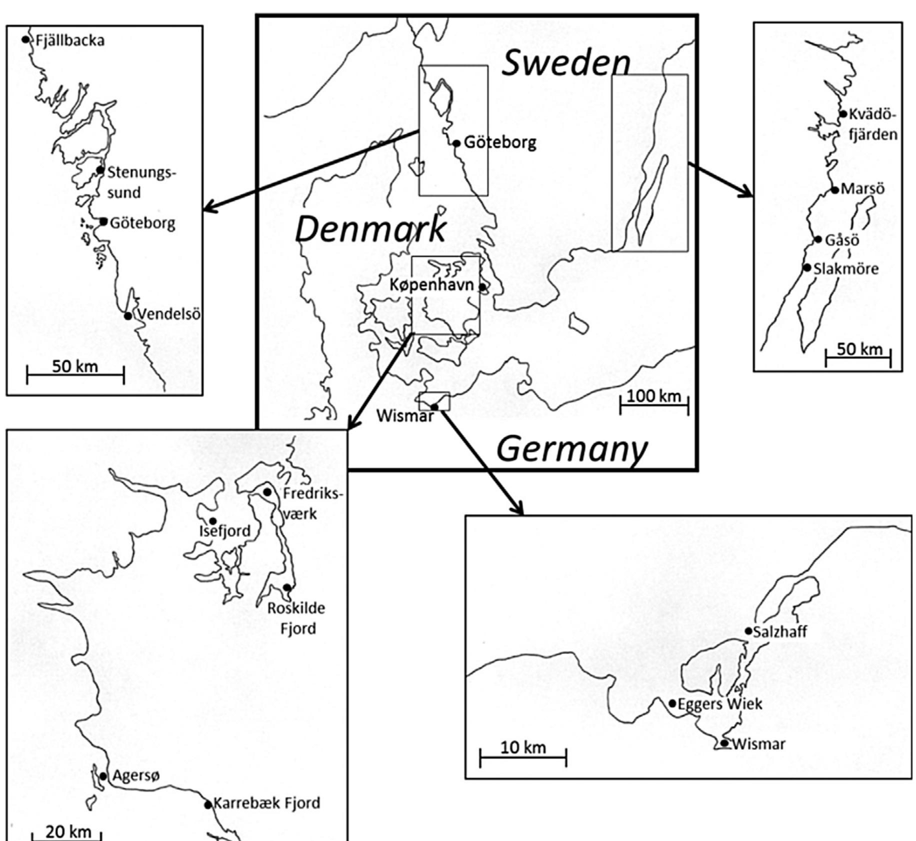

Fig. 1. A map indicating the 16 eelpout sampling sites in the Swedish, German, and Danish coastal areas during the spring and autumn of 2009. Note the scale difference between regions.