WATER COLUMN MONITORING 2014
Determining the biological effects of an offshore platform on local fish populations
Title
Water Column Monitoring 2014: Determining the biological effects of an offshore platform on local fish populations.

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Client(s)
Norwegian Oil and Gas, represented by Statoil ASA

Abstract
The biological effects of an offshore oil platform on local fish populations were assessed as part of the Water Column Monitoring (WCM) programme for 2014. The Njord A platform was chosen as the study location, which was not in operation and had no current discharge of produced water. Demersal fish species were targeted since they were believed to be less likely to migrate away from the platform than pelagic fish. By targeting organisms deeper in the water column and selecting a platform currently not in operation, the impact of drill cuttings and other sediment sources including leakages from well deposits were the main sources of contamination.

Wild fish including ling (Molva molva), tusk (Brosme brosme), redfish (Sebastes sp.) and saithe (Pollachius virens) were caught with baited rod and line from within the 500 m safety zone of the Njord A platform during the summer of 2014. Reference fish were caught on a separate research cruise by trawling from a region of the Norwegian Sea less impacted by oil and gas activities. Limited numbers of reference ling were obtained and the reference data for ling collected from the North Sea as part of the WCM2013 programme were used for comparison of the biomarker responses.

Contaminant body burden and a suite of biological effects endpoints were measured in all fish groups and included DNA adducts, DNA strand breaks by comet assay, acetylcholine esterase (AChE) inhibition, ethoxyresorufin O-deethylase (EROD), vitellogenin (VTG), lysosomal membrane stability (LMS), liver and gill histopathology, PAH metabolites, and PAH body burden. The biomarker data were integrated using the integrated biological response index (IBR/n). Despite low and/or undetected concentrations of PAH and PAH metabolites in fish fillet and bile respectively significant responses in AChE, comet and DNA adducts were found. The biomarker responses indicated exposure to both neurotoxic and genotoxic chemicals in fish inhabiting the lower water column with influence from sediment sources around the Njord A platform. Integration of the biomarker responses (IBR/n) found that all four fish species that were caught from around the platform had markedly higher IBR/n values than their respective reference population. The study shows the advantage of using a suite of biomarkers for assessing the biological effects of low concentrations of complex mixtures with biological effects observed despite low concentrations of PAH measured.

Keywords
1. Oil and gas
2. Offshore monitoring
3. Biological effects
4. Demersal fish
WATER COLUMN MONITORING 2014

Determining the biological effects of an offshore platform on local fish populations
Preface

The Water Column Monitoring (WCM) programme performs investigations into the potential biological effects of offshore oil and gas activity on organisms living within the water column of the Norwegian continental shelf. Oil companies in the Norwegian sector with produced water discharges, are obliged by the Norwegian authorities to perform biological effects monitoring offshore. The work has been performed at various fields within the Norwegian sector over the last 20 years. The methods used are considered to be the best available technology for the assessment of biological effects monitoring. Previous campaigns have investigated the biological effects using field transplanted animals (mussels and fish) at known distances from the PW discharge. In 2013, the biological responses of local fish populations that reside in or around the platforms were used with a particular focus on demersal fish species. Subsequently the focus was changed to the bottom part of the water column where exposure to drilling muds and sediment held contaminants may be more important in addition to PW chemicals. In the present programme of 2014 the biological responses in local demersal fish populations will once again be targeted. The Njord A platform was not in operation during the monitoring period and therefore produced water was not discharged during this time. Consequently, exposure to drilling muds and leaking well deposits were considered to be the main sources of contamination to the sampled fish populations.

The WCM programme has been performed through collaboration between the Norwegian Institute of Water Research (NIVA) and the International Research Institute of Stavanger (IRIS). The work participants from these two laboratories include:


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Additional assistance was provided by the following:

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NorGenoTech AS: Sergey Shaposhnikov, Andrew Collins.

AdnTox, France: Jérémie Le Goff.

Oslo, March 2015

Dr Steven J Brooks
Project Manager
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<th>Definition</th>
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<tr>
<td>AChE</td>
<td>Acetylcholine esterase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>BAC</td>
<td>Background assessment criteria</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CI</td>
<td>Condition index</td>
</tr>
<tr>
<td>CTD</td>
<td>Conductivity, temperature and depth</td>
</tr>
<tr>
<td>CYP P450</td>
<td>Cytochrome P450</td>
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<tr>
<td>CYP1A</td>
<td>Cytochrome 1A</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EAC</td>
<td>Environmental assessment criteria</td>
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<tr>
<td>ELISA</td>
<td>Enzyme linked immune-sorbent assay</td>
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<td>EROD</td>
<td>Ethoxyresorufin 0-deethylase</td>
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<tr>
<td>FF</td>
<td>Fixed fluorescence</td>
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<tr>
<td>FPG</td>
<td>Formamidopyrimidine DNA glycosylase</td>
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<tr>
<td>FTU</td>
<td>Formazin turbidity units</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography mass spectrometry</td>
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<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>GSI</td>
<td>Gonadosomatic index</td>
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<tr>
<td>HES</td>
<td>Haematoxylin, eosin and saffron</td>
</tr>
<tr>
<td>HPLC/F</td>
<td>High performance liquid chromatography with fluorescence detection</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>IBR/n</td>
<td>Integrated biological response (divided by the number of biomarkers)</td>
</tr>
<tr>
<td>ICES</td>
<td>International council for the exploration of the seas</td>
</tr>
<tr>
<td>IMR</td>
<td>Institute of marine research</td>
</tr>
<tr>
<td>IRIS</td>
<td>International research institute of Stavanger</td>
</tr>
<tr>
<td>IU</td>
<td>International unit</td>
</tr>
<tr>
<td>LC-TOF MS</td>
<td>Liquid chromatography – time of flight mass spectrometry</td>
</tr>
<tr>
<td>LDPE</td>
<td>Low density polyethylene</td>
</tr>
<tr>
<td>LMS</td>
<td>Lysosomal membrane stability</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>LP</td>
<td>Labilisation period</td>
</tr>
<tr>
<td>LSI</td>
<td>Liver somatic index</td>
</tr>
<tr>
<td>mDa</td>
<td>Millidaltons</td>
</tr>
<tr>
<td>MEDPOL</td>
<td>Mediterranean pollution programme</td>
</tr>
<tr>
<td>MSD</td>
<td>Mass selective detector</td>
</tr>
<tr>
<td>MZ</td>
<td>Mass charge (number of ions)</td>
</tr>
<tr>
<td>N (n)</td>
<td>Number of replicates (individual measurements) per group</td>
</tr>
<tr>
<td>NEA</td>
<td>Norwegian environment agency</td>
</tr>
<tr>
<td>NIST</td>
<td>National institute of standards and technology</td>
</tr>
<tr>
<td>NIVA</td>
<td>Norwegian institute of water research</td>
</tr>
<tr>
<td>NTH</td>
<td>endonuclease III</td>
</tr>
<tr>
<td>nw-XICs</td>
<td>Narrow mass windows of extracted ion chromatograms</td>
</tr>
<tr>
<td>OSPAR</td>
<td>Oslo Paris convention</td>
</tr>
</tbody>
</table>
Chemicals

6:2FTS 1H,2H-perfluorooctane sulfonate (6:2)
AlkA 3-methyladenine DNA glycosylase II
ATC Acetylthiocholine
Ba Barium
BSTFA N,O-Bis(trimethylsilyl)trifluoroacetamide
CaCl2 Calcium chloride
DCM Dichloromethane
DTNB Dithiobisnitrobenzoate
DTT Dithiothreitol
EDTA Ethylenediaminetetraacetic acid
H2SO4 Sulphuric acid
KCl Potassium chloride
NaCl Sodium chloride
NADPH Nicotinamide adenine dinucleotide phosphate
N-EtFOSA N-ethylperfluoro-1-octanesulfonamide
N-EtFOSE 2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol
N-MeFOSA N-methylperfluoro-1-octanesulfonamide
N-MeFOSE 2-(N-methylperfluoro-1-octanesulfonamido)-ethanol
NPD Naphthalene, Phenanthrene and Dibenzothiophene
OH-PAH Hydroxyl PAH
OPD o-phenylendiamin
PAH Polycyclic aromatic hydrocarbons
PBS Phosphate buffered saline
PCB Polychlorinated biphenyls
PFBS Perfluoro-1-butanesulfonate
PFC Perfluorinated compounds
PFDA Perfluoro-n-decanoic acid
PFDoDA Perfluoro-n-dodecanoic acid
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
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<tbody>
<tr>
<td>PFDoDS</td>
<td>Perfluoro-1-dodecansulfonate</td>
</tr>
<tr>
<td>PFDS</td>
<td>Perfluoro-1-decanesulfonate</td>
</tr>
<tr>
<td>PFHpA</td>
<td>Perfluoro-n-heptanoic acid (PFHpA)</td>
</tr>
<tr>
<td>PFHxA</td>
<td>Perfluoro-n-hexanoic acid</td>
</tr>
<tr>
<td>PFHxDA</td>
<td>Perfluoro-n-hexadecanoic acid</td>
</tr>
<tr>
<td>PFHxS</td>
<td>Perfluoro-1-hexanesulfonate</td>
</tr>
<tr>
<td>PFNA</td>
<td>Perfluoro-n-nonanoic acid</td>
</tr>
<tr>
<td>PFOA</td>
<td>Perfluoro-n-octanoic acid</td>
</tr>
<tr>
<td>PFODA</td>
<td>Perfluoro-n-octadecanoic acid</td>
</tr>
<tr>
<td>PFOS</td>
<td>Perfluoro-1-octanesulfonate</td>
</tr>
<tr>
<td>PFOSA</td>
<td>Perfluoro-1-octanesulfonamide</td>
</tr>
<tr>
<td>PFPA</td>
<td>Perfluoro-n-pentanoic acid</td>
</tr>
<tr>
<td>PFTeDA</td>
<td>Perfluoro-n-tetradecanoic acid</td>
</tr>
<tr>
<td>PFTrDA</td>
<td>Perfluoro-n-tridecanoic acid</td>
</tr>
<tr>
<td>PFUnDA</td>
<td>Perfluoro-n-undecanoic acid</td>
</tr>
<tr>
<td>T4denV</td>
<td>T4 endonuclease V</td>
</tr>
<tr>
<td>TCDD</td>
<td>2,3,7,8-tetrachloro-dibenzo-dioxin</td>
</tr>
<tr>
<td>THC</td>
<td>Total hydrocarbons</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>TNB</td>
<td>5-thio-2-nitrobenzoic acid</td>
</tr>
<tr>
<td>TPBS</td>
<td>Tris phosphate buffered saline</td>
</tr>
</tbody>
</table>
Summary

The 2014 water column monitoring programme was designed to investigate the potential biological effects of an offshore oil platform on local fish populations. Demersal fish species were the main target since they were believed to be less likely to migrate away from the platform than pelagic fish. By targeting organisms deeper in the water column and selecting a platform currently not in operation (i.e. no produce water discharge), the impact of drill cuttings and other sediment sources including leakages from well deposits were the most likely sources of contamination. Both cutting piles deposited before the discharge prohibition of oil containing cuttings in 1993 and recent leakages from disposal wells were potential sources of oil contamination to organisms that feed on and/or live within the sea floor.

Although many of the chemicals that are present in drilling muds have the potential to induce biological responses, the ability to assess the potential for adverse effects is limited by the lack of sufficient in situ monitoring studies. Previous Norwegian condition monitoring programmes have revealed exposure to polycyclic aromatic hydrocarbons (PAH) and elevated levels of DNA adducts in haddock collected in areas with extensive oil and gas activity (e.g. Tampen and Oseberg regions). Furthermore, the WCM2013 programme found elevated levels of DNA adducts and comet tails in fish residing near two offshore installations in the North Sea. Based on these previous findings, the present investigation focused again on PAH exposure in wild fish and particularly on possible genotoxic effects. Based on historical chemical data the Njord A installation was targeted due to high concentrations of oil related chemicals in the sediment and recent leakages from subsea well deposits.

Passive sampling devices (PSDs) were placed at 6 strategic locations within the 500 m safety zone approximately 1 m above the sediment. The PSDs were deployed on the 30th April and retrieved after approximately 3 weeks on the 19th May by a remote operated vehicle (ROV). The PSDs were used to measure PAH-NPD concentrations and to screen for none-target compounds. The PSDs were only able to detect low ng/L concentrations of PAHs in the water column 1 m from the sea floor. However, the non-target analysis was able to detect the presence of other chemicals in the water column from LDPE and POCIS extracts.

Fish were caught with baited rod and line from within the 500 m safety zone of the Njord A platform during 26th-28th May 2014. Sufficient numbers of ling (Molva molva), tusk (Brosme brosme), redfish (Sebastes sp.) and saithe (Pollachius virens) were caught and sampled immediately. Reference fish were caught from a separate research cruise between the 10th and 14th June 2014 by trawling from a region of the Norwegian Sea less impacted by oil and gas activities. Contaminant body burden and a suite of validated biological effect endpoints were measured in all fish groups. These included: PAHs in fish fillet and stomach content; perfluorinated compounds (PFCs) in fish blood; PAH metabolites in fish bile; DNA adducts; ethoxyresorufin 0-deethylase (EROD); lysosomal membrane stability (LMS) in liver; histopathology in fish liver and gill; comet and vitellogenin (VTG) in fish blood; and acetylcholine esterase inhibition (AChE) in fish fillet.

PAH concentrations in fish fillet were low or undetected in all four fish species from both the platform and reference populations. PAH metabolites in fish bile were also low or marginally above the limit of quantification. However, despite the apparent low exposure to PAH compounds significant biomarker responses were observed for AChE, comet and DNA adducts. The biological responses indicate exposure to both neurotoxic and genotoxic chemicals in fish residing in the vicinity of the Njord A platform. Histopathological alterations were observed in both liver and gill tissue of fish from the Njord A platform compared to the reference location, where a species specific response was observed. The sensitivity of the biomarker responses and the wide range of potentially toxic oil and gas related chemicals that could be present in the water column and sediment but were not measured were likely to be why no exposure/effect relationship was established. The biological responses were very similar to the findings from
WCM2013, where neurotoxic and genotoxic responses were also observed in wild fish from the Veslefrikk and Oseberg South platforms.

In all four fish species sampled the calculated IBR/n value was markedly higher in the Njord A platform population then their respective reference groups. The highest IBR/n was calculated in both tusk and redfish from the Njord A platform (IBR/n = 1.8), closely followed by saithe caught near Njord A (IBR/n = 1.6). The higher IBR/n indicates a clear biological response in platform fish compared to their respective reference populations. These results highlight the advantage of using a suite of biomarker tools to assess the potential biological effects of complex mixtures in field scenarios, which contain low individual concentrations of many compounds, which when combined can cause toxicity.
1. Introduction

1.1 General purpose of the study

The main purpose of the study was to ensure that the discharge regulations set by the Norwegian Environment Agency are sufficient for providing adequate environmental protection to organisms living within the water column around offshore oil and gas installations. Within the Norwegian sector of the North and Norwegian Seas, the offshore operators are obliged to perform environmental monitoring within the water column in the vicinity of the offshore installations. This obligation requires that effects monitoring should be performed in at least one offshore oil and gas field each year. Approval is required from the Norwegian authorities (Norwegian Environment Agency, NEA), and the operators together with the NEA agree on the study area and the general design of the programme. In recent years a group of scientific experts, set up by the NEA to evaluate the study, have also been invited to assist with the design of the programme and in the selection of the biological effects methods to be used. The overall aim being to use the best biological effects tools to determine whether offshore oil and gas activities are effecting the health of organisms living within the water column.

1.2 Background to the water column monitoring programme

The water column monitoring (WCM) programme is designed to evaluate the potential biological effects of offshore oil and gas activities on the local marine environment. In recent years the effects of produced water (PW) have been the main concern, investigating the biological effects in field transplanted mussels and/or fish, positioned in the top region of the water column, at known distances from the discharge outlet from an offshore platform (Brooks et al., 2011; Hylland et al., 2008). These investigations have generally found, within a limited distance from the platform (approx. 500 to 1000 m), elevated concentrations of chemical bioaccumulation combined with some low level health effects/biomarker responses. However, in some instances biological effects have been found further away from the point source (e.g. up to 10 km Bilbao et al., 2006b).

In the last two campaigns (WCM2013 and WCM2014) the monitoring effort has been moved towards local fish populations that inhabit offshore oil installations. Since pelagic fish were considered to exhibit more migratory behaviour than demersal fish species, the fishing methods used were targeted towards demersal fish species. This change in approach altered the focus from the upper to the lower part of the water column, where impacts from drill cuttings and other sediment sources are the main source of contamination. In the deepest part of the water column close to installations, drilling discharges and leakages from subsea well deposits are likely to be the main source of hydrocarbon exposure. Both cutting piles deposited before the discharge prohibition in 1993 and more recent leakages from disposal wells are potential sources of oil contamination to organisms that feed on and/or live within the sea floor. In the present study, due to maintenance work being performed at the Njord A platform, there was no PW discharged during or several months prior to the sampling effort. Therefore, the sediment was considered to be the principal source of contamination.

Drill cuttings and muds

Regulations introduced in Norway in 1993 and within the OSPAR region between 1996 and 2000 greatly limited the discharge of oil in cuttings. However, prior to this, oil based drilling muds were used in drilling practices and the legacy of cuttings heavily contaminated with oil based muds remains present today. The toxicity of these oil based cutting piles has been extensively investigated, with the overall toxicity mostly governed by the total hydrocarbon (THC) concentration (Grant and Briggs, 2002). The THC concentration measured in the most contaminated drilling piles collected from the North Sea are in the range of 10 000 to 600 000 mg/kg (Bell et al., 2000; Breuer et al., 2004; Park et al., 2001; Westerlund et al., 2001).
The study site selected was the Njord A installation, which has high concentrations of sediment contamination as well as a recent history of leakages from oil well deposits from the sea floor (DNV, 2013). Within 2000 m of the platform the sediment is characterised as very fine grain where hydrocarbons dominate the contaminants. The main source of contamination was considered to be leakage from an injection well between 2000 and 2006. Differences in the spatial distribution of hydrocarbons in the sediment were mostly attributed to leakages from these wells with highest concentrations found approximately 100 m north-east and south-east of the platform. Highest concentrations of THC (6000 mg/kg d.w.) have been found in sediments at the closest stations to the platform (approx. 250 m). However, comparing historical data from this platform, THC concentrations have markedly reduced from peak concentrations in the years 2000 and 2001 to the lowest values in the most recent year 2012 (350 mg/kg d.w). In contrast, Ba concentrations were highest in 2012 (over 12000 mg/kg d.w.). However, other than Ba, metal concentrations were mostly low mg/kg concentrations.

Some of the organic chemicals found in PW and drilling muds are relatively resistant to biodegradation, have a bioaccumulation potential and may be toxic to organisms in receiving waters (Brendehaug et al., 1992; Tollefsen et al., 1998; Taban and Børseth, 2000; Aas et al., 2000a). This applies in particular to groups of chemicals such as polycyclic aromatic hydrocarbons (PAHs) that are known to produce various toxic effects including reproductive disturbances, mutagenicity and carcinogenicity (Landahl et al., 1990; Bechmann, 1999; Lye, 2000; Meier et al., 2002). Although there is reason to assume that many of the chemicals that are present in drilling muds and sediment deposits may produce biological responses, the ability to assess the potential for adverse effects are limited by the lack of sufficient in situ monitoring data. Previous Norwegian condition monitoring programmes have revealed exposure to PAHs and elevated levels of DNA adducts in haddock collected in areas with extensive oil and gas activity (e.g. Tampen and Oseberg regions). A study conducted in 2011 indicated that the oil based drilling fluids previously discharged may contribute to this exposure and effect (Grosvik et al., 2010; Pampanin et al., 2013). The previous WCM2013 programme also reported elevated levels of genotoxicity, through DNA adducts and comet tails, in fish populations residing near two offshore oil installations in the North Sea compared to reference fish. Based on these previous findings, the present investigation focused on PAH exposure in wild fish and particularly on possible genotoxic effects.

1.3 Objectives

The main objective of the study was to perform biological effects monitoring of wild fish in the vicinity of the Njord A installation in the Norwegian Sea. A suite of biological effects markers and chemical analyses were measured in four fish species in order to assess the potential impact of this installation on the local fish populations. In addition, passive sampling devices (PSDs) positioned at strategic locations within 500 m of the platform and 1 m above the seafloor were used to measure oil and gas related chemicals in the water column considering potential input from high sediment load and leakages from well deposits.

1.4 Background on the selected biological effects methods

The biomarker tools have been selected in most part from the International Council for the Exploration of the Sea’s (ICES) recommended list of biomarkers for biological effects monitoring in marine fish (ICES, 2011). Gill histology was the only other biological effects method that was used, which was not recommended by ICES. Validation data was available for many of the biomarkers in marine fish increasing their reliability as tools for marine biomonitoring and in some cases providing environmental and background assessment criteria (EAC and BAC). A brief description of these biomarkers is provided.

1.4.1 DNA adducts

The presence of DNA adducts in animal cells is considered to be evidence of exposure to genotoxics. A DNA adduct is formed when a non-DNA chemical, e.g. a carcinogenic pollutant chemical, binds covalently to the DNA. Due to the sensitive and consistent responses of hepatic DNA adduct levels to the genotoxic forms of PAH, this parameter is considered to be a reliable biomarker of PAH effect and pro-mutagenic DNA lesions in fish. However, PAHs are not the only groups of chemicals that can form
DNA adducts. The stability of the DNA adduct, i.e. the resistance to DNA repair mechanisms, is an important factor. Carcinogenic PAHs form stable DNA adducts after being bio-activated in the cell, and since PAHs are common pollutants in many aquatic environments, this pollutant class has received much attention. In fish, DNA adducts are commonly measured in the liver, since this is the key organ for biotransformation of xenobiotics. Overall, the frequency of DNA adducts in wild caught fish will provide an indication of long term exposure to genotoxins (Jonsson et al. 2003).

1.4.2 Ethoxyresorufin O-deethylase (EROD)

In the cytochrome P450 (CYP P450) superfamily, CYP1A-subfamily enzymes are one of the most important concerning biotransformation/bioactivation of xenobiotics. These enzymes are induced when the cell is exposed to xenobiotics like 2,3,7,8-tetrachloro-dibenzo-dioxin (TCDD), planar polychlorinated biphenyls (PCBs), or PAHs (Goksøyr and Förlin, 1992). CYP1A are heme-containing proteins, mainly located on the surface of the smooth endoplasmic reticulum within cells. The enzymes are isolated in the so-called microsomal fraction by differential ultracentrifugation following homogenisation of the cell. Most of the enzyme activity is retained using this procedure and CYP1A activity is measured in the microsomal samples in the ethoxyresorufin O-deethylase (EROD) assay. Here, 7-ethoxyresorufin is used as an artificial substrate for CYP1A and fluorescence of the product resorufin is measured as an indication of the CYP1A-activity.

1.4.3 Lysosomal membrane stability (LMS)

Lysosomes are subcellular organelles surrounded by a semi-permeable membrane that contains numerous hydrolytic enzymes involved in a range of cellular processes including digestion, defence, and reproduction (Viarengo et al., 2007; Moore et al., 2008). The measure of the integrity of the lysosome membrane has been found to be sensitive to a range of stressors, including metals and organic chemicals. One of the most well-established methods to determine changes in membrane integrity is through measurements of the lysosomal membrane stability (LMS), which is typically measured in haemocytes and digestive gland cells of mussels (Lowe et al., 1995) and liver hepatocytes of fish (Köhler et al., 2002).

Changes in lysosomal morphology and membrane stability have been frequently used as a biomarker for effects of combined contaminant stress in marine organism such as flounder (Platichthys flesus) (Köhler 1991; Köhler and Pluta, 1995), eel pout (Zoarces viviparus) (Sturve et al., 2005), red mullet (Mullus barbatus) (Viarengo et al., 2007; Zorita et al., 2008), Atlantic cod (Gadus morhua) (Holth et al., 2011), sea bream (Sparus aurata) (Viarengo et al., 2007) and Atlantic stargazer (Uranoscopus scaber) (Viarengo et al., 2007). The LMS test is recommended as a rapid prognostic biomarker for toxicity induced liver injury in monitoring programmes (Köhler et al., 2002). Lysosomal membrane stability has recently been adopted by The United Nations Environment Programme (UNEP) as part of the first tier of techniques for assessing harmful impact in the Mediterranean Pollution programme (MEDPOL Phase IV) (ICES 2007).

1.4.4 Fish histopathology

Liver tissue

Histological parameters are commonly used as markers of health status in marine organisms. The liver of teleosts is the primary organ for the biotransformation of organic xenobiotics. There have been numerous reports of histo-cytopathological changes in the liver of fish exposed to a wide range of organic compounds and heavy metals (Agamy, 2012). Studies on liver histopathology in fish have increasingly been incorporated in marine biological effects monitoring programmes (Lang et al., 2006). According to the ICES guidelines, European flatfish dab (Limanda limanda) and flounder are the main target species for monitoring purposes in the North Sea and adjacent areas, including the Baltic Sea (Feist et al., 2004).

At the same time, few hepatic lesions have been reported from gadoid species such as cod and whiting (Merlangius merlangus). However, it should be noted that these species are not regularly examined for liver pathology in routine monitoring programmes. Consequently, it was suggested that liver pathology information from these fish species should be improved before they are incorporated into biological
effects monitoring programmes (Feist et al., 2004). The aim of this histopathological survey was to provide information about liver alterations in analysed species and to provide support to the suite of biological effects methods used.

**Gill tissue**

Fish gills are the main site of gas exchange, ionic-regulation, acid-base balance, and nitrogenous waste excretion, as well as direct contact with the surrounding water. Therefore, this organ is regarded as a sensitive tissue to analyse for the impacts of environmental contamination (Au, 2004). Gill epithelium may absorb a variety of lipophilic organic compounds, including PAHs (Spies et al., 1996). Furthermore, biotransformation of petroleum fractions may also take place in this tissue (Prasad, 1991).

The histopathological analysis of gill histology is already in use as a useful marker for general environmental contamination (Au, 2004; Dulic et al., 2009; Schwaiger et al., 1997) and does not seem to be significantly influenced by factors such as season, sex or age (Au, 2004). This analysis has also been used for the evaluation of the health status of fish exposed to contaminants, both in laboratory (Biagini et al., 2009; Hoyle et al., 2007; Miron et al., 2008; Monteiro et al., 2008) and in field studies (Fernandes et al., 2008; Khan, 2003; Stentiford et al., 2009; Stentiford et al., 2003).

**1.4.5 Acetylcholine esterase (AChE) inhibition**

Acetylcholine esterase (AChE) is an essential enzyme involved in neurotransmission, which hydrolyses the neurotransmitter acetylcholine at cholinergic synapses. An inhibition of AChE causes an over stimulation of muscarinic and nicotinic receptors, which can cause various effects on the central nervous system (Costa, 2006). Different types of compounds have been described as AChE inhibitors, such as organophosphorus and carbamate pesticides (Assis et al., 2010, Di Tuoro et al., 2011), and PAHs (Kang and Fang, 1997, Kopecka-Pilarczyk and Correia, 2011). The AChE assay has been used to assess the neurotoxicity of environmental samples (e.g. Bocquené et al., 1990; Hildebrandt et al., 2008; Holth and Tollefsen, 2012) and exposure to neurotoxic compounds (Assis et al., 2010; Payne et al., 1996).

**1.4.6 Comet**

The comet assay (single cell gel electrophoresis) is a sensitive and versatile method for detecting DNA damage in eukaryotic cells. Alkaline electrophoresis of agarose-embedded, lysed cells (nucleoids) produces comet-like images, and the intensity of the comet tail (comprising DNA with breaks) relative to the head (unbroken DNA) is a quantitative measure of the strand break frequency. In its basic form, the assay detects single and double strand breaks, and alkali-labile sites. An early modification to the assay was the introduction of digestion of nucleoid DNA with a lesion-specific endonuclease. Formamidopyrimidine DNA glycosylase (FPG) detects 8-oxoguanine and ring-opened formamidopyrimidines; endonuclease III (NTH) cuts DNA at oxidised pyrimidines; AlkA recognises alkylated guanines; T4 endonuclease V (T4denV) is specific for pyrimidine dimers induced by UV light. The increase in comet tail intensity after enzyme digestion indicates the presence of the corresponding DNA lesion.

**1.4.7 Vitellogenin (VTG)**

Vitellogenin (yolk protein) is synthesised in the liver of female fish under the stimulation of endogenous estradiol (Tata and Smith, 1979). In contrast, males and juvenile fish do not produce appreciable quantities of VTG. However, they do have numerous hepatic oestrogen receptors and are capable of producing VTG when exposed to exogenous oestrogens. Induction of this female typical protein in male and juvenile fish has therefore been widely used as a sensitive biomarker for exposure to xenoestrogens (Sumpter and Jobling, 1995).

The use of VTG as a biomarker for xenoestrogens in ecologically relevant fish species has been employed for coastal and freshwater environmental monitoring (Hylland et al., 1998; Hylland et al., 1999) and for monitoring of areas that are effected by discharge from oil production activities (Brooks et al., 2011). Recent studies with freshwater species such as zebrafish and rainbow trout, suggest that induction of
VTG occurs at concentrations of xenoestrogens that also produce alteration in sexual development when exposed during sensitive early life stages of development (Jobling et al., 1996; Örn et al., 2003). Estrogenic-like chemicals have been identified in produced water discharges from offshore oil installations in the North Sea (Boitsov et al., 2007). VTG induction was investigated in wild male fish residing around the Njord A installation to see if estrogenic effects can be seen.

1.4.8 Bile metabolites

The bio-transformation of PAHs by fish reduces the accumulation of these compounds in their tissues. As a result, the concentration of parent PAHs in fish samples does not provide an adequate assessment of PAH exposure (Beyer et al., 2010). Alternatively, the analysis of PAH metabolites in the bile of fish, is considered to be a very sensitive method for the assessment of PAH exposure in laboratory and field studies (Aas et al., 2001; Beyer et al., 2010). Two methods have been employed for the analysis of PAH metabolites.

**Fixed wavelength fluorescence**

The method of fixed wavelength fluorescence works on the principal that the optimal excitation wavelength increases with the size of the PAH molecule. Therefore, smaller PAHs can be detected using a relatively shorter excitation wavelength than larger PAHs (Aas et al., 2000b). However, this direct method is not suitable for standardisation and quantification of the PAH compounds and should be regarded as a screening tool to identify the general groups present. The advantages of this method are that it is relatively cheap with high sample throughput, although different PAH compounds as well as other natural constituents of the bile can interfere with the fluorescence signal, particularly at low PAH metabolite concentrations.

**GC-MS**

For a more quantitative and qualitative analysis of PAH metabolites, high performance liquid chromatography with fluorescence detection (HPLC/F) or gas chromatography with mass spectrometry in single ion mode detection (GC-MS SIM) can be applied. The GC-MS SIM method is the best suited for the detection of PAH compounds containing 2 to 3 ring structures, namely naphthalenes and phenanthrenes (Jonsson et al., 2003; Jonsson et al., 2004). Both alkyl substituted and non-alkyl substituted compounds are detected.

1.5 PFC in fish blood

Perfluorinated compounds (PFCs) have been widely used in manufacturing for several decades due to their ability to make products stain-resistant, water repellent, slippery and long lasting. However, the widespread distribution of PFCs in the environment and the persistent nature of these compounds have led to concern over elevated concentrations in nature and their potential threat to the environment. In addition to their many other applications, PFCs are a known ingredient in Class B firefighting foams, which are designed to target oil based fires. Consequently, these firefighting foams containing PFCs are present on offshore oil and gas installations.

Many PFCs are persistent and bioaccumulative with highest concentrations often found in the blood of animals. In the current study a suite of PFCs were measured in the blood samples of fish caught around the offshore platforms. The data will be compared to PFC concentration in the blood of suitable reference fish, with the aim to determine whether PFCs are elevated in fish that live in the vicinity of offshore platforms.

1.6 Passive sampling devices to detect waterborne concentrations of PAH and non-target chemicals

The principle of passive sampling is the placement of a device in the environment for a fixed period of time, where it is left unattended to accumulate contaminants by diffusive and/or sorptive processes. They
offer sensitive, time-averaged sampling without confounding factors, which may occur when using biomonitoring organisms. In the present study they will be used as a support parameter to indicate waterborne exposure to groups of chemicals originating from historically contaminated sediments. Two passive sampling devices were chosen the polar organic chemical integrative sampler (POCIS) and low-density polyethylene (LDPE). POCIS is suitable for more polar compounds (log $K_{ow} < 3$), whilst LDPE samples more hydrophobic compounds (log $K_{ow} > 3$). LDPE provides very similar results to SPMDs (Allan et al., 2010), but resulting extracts are cleaner due to the lack of the triolein lipid used in SPMDs. This lowers the potential for losing unknown substances during extensive clean-up steps. This is particularly important since it is difficult to determine internal/recovery standards for non-target screening work outlined below. Both SPMDs and POCIS have been used extensively in previous WCM surveys, for measurement of dissolved concentrations of PAH, NPD, alkylated phenols, and naphthenic acids (Harman et al. 2009; 2010; 2011; 2014).

Non-target screening of unknown chemicals in the environment generally involves high resolution mass spectrometric analysis of samples obtained from universal extractions of environmental matrices (Hernández et al., 2011). Highly sensitive accurate-mass measurements over a full spectrum make it possible to screen for a large number of organic contaminants at low concentrations. An advantage of this technique is that the data remains available for subsequent analysis. Then, a suspect target analysis is feasible by obtaining narrow mass windows of extracted ion chromatograms (nw-XICs) at certain abundant ions of the compound in question. The combination of passive sampling and high resolution gas chromatography time-of-flight mass spectrometry has rarely been undertaken (Allan et al., 2013; Gravell et al., 2012). Yet this would allow a suspect analyses (searching for selected compounds) and a non-target (without selection of compounds to be searched) screening, including those compounds which are potentially bioaccumulative and/or bioavailable. Thus, a mixture of target analysis for PAH/NPD compounds in LDPE, and screening in both types of samplers will be carried out, in order to examine concentrations of known and unknown oil originating compounds, respectively.
2. Materials and Methods

2.1 Study design

The design of the monitoring programme was divided into three main activities: 1) to measure the concentration of oil related compounds in the water column around the Njord A platform and approximately 1 m above the seafloor using PSDs; 2) to assess the chemical exposure and health status of local fish populations living in the vicinity of the Njord A installation; and 3) to assess the chemical exposure and health status of fish collected from a reference location in the Norwegian Sea for comparison to those fish from the Njord A platform. Details of the deployment and retrieval of the PSDs around the Njord A platform as well as the collection of the fish species from both the platform and the reference location can be found in the WCM2014 cruise report (Appendix A). However, a brief description of each activity is provided below. The approximate location of the Njord A platform and the reference area are shown in Figure 1.

Figure 1. The approximate position of the Njord A platform and the trawling zone for the collection of the reference fish. Njord A (64.2677° N 7.2311° E); Reference fish trawling zone (63.17931-63.20483° N 6.75251-6.75797° E).

2.2 Concentrations of oil related chemicals at Njord A using PSDs

With the aid of a submersible remote operated vehicle (ROV), PSDs were positioned one meter above the seafloor at six locations within the 500 m safety zone of the Njord A platform. The PSDs used included: low-density polyethylene (LDPE) and polar organic chemical integrated samplers (POCIS), which were exposed to the water column for approximately 3 weeks between 30th April and 19th May 2014. The LDPE extracts were used to estimate water concentrations of PAH-NPDs, whilst non-target and suspect
target screening of oil related compounds was performed on the LDPE and POCIS extracts by liquid chromatography – time of flight mass spectrometry (LC-TOF MS). Sites were chosen based on consultation with the operator, historical analysis and the capabilities of the ROV. The approximate positions of the 6 locations around the Njord A platform are shown in Figure 2.

Figure 2. Seafloor map around the Njord A installation (green square), showing approximate positions of the PSDs (red stars).

2.3 Offshore sampling

2.3.1 Collection of fish near the platform

Fish were collected using a supply vessel (Skandi Stord) within the 500 m safety zone of the Njord A installation (Figure 1). The collection was focused within 100 meters of the platform. Due to subsea installations near the platform only “passive” fishing equipment could be used. Rod and line fishing, using mackerel as bait, proved extremely effective and a steady supply of fish were caught enabling time for processing of the fish on board within a maximum of 1 h and typically within 20 min after capture. The fish species caught at the platform are summarised in Table 1.

Table 1. Number of fish species sampled from near the Njord A installation and the reference location.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Common name</th>
<th>Njord A</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brosme brosme</td>
<td>tusk</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Molva molva</td>
<td>ling</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Sebastes sp.</td>
<td>redfish</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Pollachius virens</td>
<td>saithe</td>
<td>12</td>
<td>20</td>
</tr>
</tbody>
</table>
Samples from individual fish were taken for the suite of biomarkers, chemical analysis and supporting parameters as listed in Table 2. Biological samples were snap frozen in liquid nitrogen and stored at -80°C until analysis. Samples used for chemical analysis were frozen with dry ice and stored below -20°C prior to analysis.

Table 2. The suite of biomarkers, chemical analysis and supporting parameters measured in the various tissues of the wild caught fish species.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomarkers DNA adducts</td>
<td>Liver</td>
</tr>
<tr>
<td>EROD</td>
<td>Liver</td>
</tr>
<tr>
<td>Lysosomal membrane stability</td>
<td>Liver</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Liver and gill</td>
</tr>
<tr>
<td>Comet assay</td>
<td>Blood</td>
</tr>
<tr>
<td>Acetylcholine esterase inhibition</td>
<td>Fillet</td>
</tr>
<tr>
<td>PAH metabolites</td>
<td>Bile</td>
</tr>
<tr>
<td>VTG</td>
<td>Blood plasma</td>
</tr>
<tr>
<td>Chemical analyses</td>
<td></td>
</tr>
<tr>
<td>PFCs</td>
<td>Blood</td>
</tr>
<tr>
<td>PAH-NPD</td>
<td>Fillet</td>
</tr>
<tr>
<td>PAH-NPD</td>
<td>Stomach content</td>
</tr>
<tr>
<td>Supporting parameters</td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>Whole fish</td>
</tr>
<tr>
<td>Weight</td>
<td>Whole fish</td>
</tr>
<tr>
<td>Gonad weight</td>
<td>Gonad</td>
</tr>
<tr>
<td>Liver weight</td>
<td>Liver</td>
</tr>
<tr>
<td>Age</td>
<td>Otoliths</td>
</tr>
</tbody>
</table>

Conductivity, temperature and turbidity profiles were taken at the Njord A platform on two occasions during the fishing effort with the aid of an STD/CTD-model SD204. Vertical profiles down to the seafloor were performed.

2.3.2 Collection of fish at the reference location

Following the collection of the four fish species from the Njord A platform, the same fish species were targeted during a separate research cruise conducted by the Institute of Marine Research (IMR). The collection of the reference fish took place by trawl on 10th June 2014 in the area highlighted in Figure 1. One scientist from NIVA and one from IRIS took part in the reference cruise in order to ensure that the reference fish were sampled in the same manner as those collected from around the platform. The numbers of reference fish sampled during the IMR cruise are listed in Table 1. Since only four reference ling were caught during the reference cruise, the reference values obtained for ling from the WCM2013 programme were also used for comparison.

2.4 Analytical methods

2.4.1 Passive sampler extraction and chemical analysis

LDPE extraction

LDPE samplers were wiped with ultrapure water and paper towels to remove any fouling. Samplers were then extracted in pentane for 24 h after the addition of a deuterated PAH internal standard. The pentane extract was decanted and the 24 h extraction repeated. Pentane extracts were combined and evaporated under nitrogen to approximately 0.5 mL. The extract was divided into 2 aliquots and the first aliquot was cleaned up by Gel Permeation Chromatography (GPC). EnvirogelTM GPC clean-up columns (19 x 150
mm and 19 x 300 mm in series) were used with dichloromethane (DCM) at a flow rate of 5 ml/min collecting the fraction from 14:50 – 20:20 min (see Harman et al., 2008, for further details).

**Targeted analysis**

Cleaned extracts were analysed by gas chromatography – mass selective detector (GC-MSD) for quantitative analysis of PAH and NPD. The GC was fitted with a 30 m × 0.25 mm, 0.25 μm film thickness DB-5MS column with helium carrier gas. Splitless injection at 280°C was used. The initial temperature of 60°C was held for 2 min, followed by an increase of 7°C/ min to 250°C, followed by 15°C/ min to 310°C and held for 15 min. To correct for any possible contamination during procedures, control or ‘blank’ LDPEs were used. These included a field control that was exposed to the air during deployment and retrieval (LDPEs are also efficient air samplers) and a laboratory control that followed the exposure to solvents, glassware etc. during work up (see Allan et al., 2010, for further details).

**Calculation of sampling rates and water concentrations**

Sampling rates and water concentration calculations were only carried out for PAH and NPD compounds in LDPE, as the non-target analysis is not quantitative. An empirical model was used in the calculation of water concentrations from LDPE accumulations. In this model, compound specific or intrinsic effects are adjusted based on the log Kow of the analyte and site-specific or extrinsic factors arising from differences in environmental variables are adjusted by using the performance reference compounds (PRC), which are spiked into samplers prior to deployment. In this way the uptake for each individual compound at each sampling site was established (expressed as a sampling rate, L/ d). This sampling rate is used to calculate water concentrations based on first order uptake kinetics, described previously (e.g. Harman et al., 2008; Allan et al., 2010). Where individual analytes were not detected in LDPEs then the analytical detection limit was used in calculations to provide a maximum theoretical concentration in the water.

**Non-targeted analysis**

The extract aliquots that were not subject to GPC clean-up were analysed by high resolution time of flight mass spectrometry (GC-HRMS). The GC was fitted with a 30 m × 0.25 mm, 0.25 μm film thickness DB-5MS column with helium carrier gas. Splitless injection at 280°C was used. The initial temperature of 60°C was held for 2 min, followed by an increase of 7°C/ min to 250°C, followed by 15°C/ min to 310°C and held for 15 min. The HRMS was operated in full scan positive electron impact mode with a scan range of 50−500 m/ z. Accurate mass spectra to 4 decimal places was used for peak identification with an error threshold of 5 mDa.

The mass spectra were de-convoluted using Micromass software (MassLynx V4.1, ChromaLynx) and compared with reference spectra in the National Institute of Standards and Technology (NIST) mass spectral database (version 2.0 f) for tentative identification. The tentative identification was supported by at least two accurate-mass ions. For identified compounds, the match factor (reverse fit) and probability of correct identification was recorded.

**Suspect target analysis**

Nw XICs of extracted ion chromatograms of 20-50 mDa were used to compare the relative abundance of selected suspect compounds of chemicals (Hernández et al., 2011; Cervera et al., 2012; Hernández et al., 2012) and similar compounds to the ones identified by non-target screening. Extracts were analysed by GC/HR ToF-MS. Mass spectra of detected compounds were compared with mass spectra from the NIST spectral database for tentative identification. Accurate mass spectra were used for peak identification with an error threshold of 5 mDa. In addition, the match factor and probability of correct identification were recorded. The combination of several identification criteria ensured the robustness of the identification process. The abundance of the peaks were normalised to that of the internal standard of deuterated pyrene for the LDPE-samplers. For the POCIS samplers, no internal standards were added, so only the relative areas of the peaks were recorded.
POCIS extraction

POCIS sorbents were removed and rinsed with ultrapure water to remove salts, and dried under vacuum prior to elution with ethyl acetate followed by ethyl acetate (1% ammonium hydroxide). Eluents were combined and evaporated under nitrogen to 200 µL and analysed by GC-HRMS as described above.

2.4.2 PAH-NPD in fish stomach and fish fillet

Samples were defrosted, homogenised and a sub-sample of approximate 5 g was taken. Internal standards were added (naphthalene d8, biphenyl d10, acenaphthene d8, phenanthrene d10, anthracene d10, Pyrene d10, chrysene d12 and perylene d12) before extraction by saponification. Analytes were then extracted twice with 40 mL cyclohexane and dried over sodium sulphate. The extracts were reduced by a gentle stream of nitrogen and cleaned by size exclusion chromatography. Analysis proceeded by gas chromatography with mass spectrometric detection (GC-MS) with the MS detector operating in selected ion monitoring mode (SIM). The GC was equipped with a 30 m column with a stationary phase of 5% phenyl polysiloxane (0.25 mm i.d. and 0.25 µm film thickness), and the injector operated in splitless mode. The initial column temperature was 60°C, which after two minutes was raised stepwise to 310°C. The carrier gas was helium and the column flow rate was 1.2 mL/ min. Quantification of individual components was performed by using the internal standard method. The alkylated homologues were quantified by baseline integration of the established chromatographic pattern and the response factors were assumed equal within each group of homologues.

2.4.3 Bile metabolites

Fixed fluorescence:

Bile samples were diluted 1:1600 in methanol: water (1:1). Slit widths were set at 2.5 nm for both excitation and emission wavelengths, and samples were analysed in a quartz cuvette. All bile samples were analysed by FF at the wavelength pairs 290/335, 341/383 and 380/430 nm, optimised for the detection of 2-3 ring, 4-ring and 5-ring PAH metabolites, respectively. The fluorescence signal was transformed into pyrene fluorescence equivalents (PFE) through a standard curve made by pyrene (Sigma St Louis, USA). Pyrene was measured at the same fluorimeter, with the same cuvette, same solvent, and with the same slit settings as the bile samples. It was, however, measured at the optimal wavelength pair of pyrene, 332/374 nm (excitation/emission). The concentration of PAH metabolites in bile samples was expressed as µg PFE/ mL bile.

GC-MS

Fish bile was prepared for analysis as described by Jonsson et al., (2003; 2004). Briefly, 25–30 µL of bile was weighed accurately into a micro centrifuge vial. Internal standards (2,6-dibromophenol, 3-fluorophenanthrene and 1-fluoropyrene) and β-glucuronidase (3000 units) in sodium acetate buffer (0.4 M, pH = 5) were added and the solution left at 40°C for 2 h. The OH-PAHs were extracted with ethyl acetate (4 times 0.5 mL), the combined extract dried with anhydrous sodium sulphate and concentrated to 0.5 mL. Trimethylsilyl (TMS) ethers of OH-PAHs were prepared by addition of 0.2 mL N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) and heating for 2 h at 60°C. TPA was added as a GC-MS performance standard before transferring the prepared samples to capped vials.

Trimethylsilyl ethers of OH-PAHs (TMS-OH-PAHs) in fish bile samples were analysed by a GC-MS system consisting of a HP5890 series II Gas chromatograph, Shimadzu QP2010 GCMS. Helium was used as carrier gas and the applied column was CP-Sil 8 CB-MS, 50 m x 0.25 mm and 0.25 µm film-thickness (Varian). Samples and calibration standards (1 µL) were injected on a split/ splitless injector with splitless mode on for one minute. The temperatures for the injector, transfer-line and ion source were held at 250°C, 300°C and 240°C, respectively, and the GC oven temperature programme was as follows: 80°C to 120°C at 15°C/ min, 120°C to 300°C at 6°C/ min and held at 300°C for 30 min. Mass spectra were obtained at 70 eV in selected ion mode (SIM). Based on the fragmentation pattern of non-alkylated TMS-O-PAHs (Jonsson et al., 2003); the molecular ions were selected for determination of both alkylated and non-alkylated TMS-O-PAHs.
2.4.4 PFC in fish blood

A suite of 23 PFCs were measured in fish blood based on the method from Verreault et al., (2005). Internal standards were added to 0.5 mL of sample and extracted twice with acetonitrile using an ultrasonic bath. The extract was mixed with ammonium acetate buffer, acetic acid and EnviCarb and then filtered (0.45 µm) before analysed by LC/MS-QToF (ESI negative mode).

The PFCs measured in fish blood included the following: perfluoro-n-pentanoic acid (PFPA); perfluoro-n-hexanoic acid (PFHxA); perfluoro-n-heptanoic acid (PFHpA); perfluoro-n-octanoic acid (PFOA); perfluoro-n-nonanoic acid (PFNA); perfluoro-n-decanoic acid (PFDA); perfluoro-n-undecanoic acid (PFUnDA); perfluoro-n-dodecanoic acid (PFDoDA); perfluoro-n-tridecanoic acid (PFTrDA); perfluoro-n-tetradecanoic acid (PFTeDA); perfluoro-n-hexadecanoic acid (PFHxDA); perfluoro-n-octadecanoic acid (PFODA); perfluoro-1-butanesulfonate (PFBS); perfluoro-1-hexanesulfonate (PFHxS); perfluoro-1-octanesulfonate (PFOS); perfluoro-1-dodecansulfonate (PFDoDS); perfluoro-1-octanesulfonamide (PFOSA); N-methylperfluoro-1-octanesulfonamide (N-MeFOSA); N-ethylperfluoro-1-octanesulfonamide (N-EtFOSA); 2-(N-methylperfluoro-1-octanesulfonamido)-ethanol (N-MeFOSE); 2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (N-EtFOSE); 1H,2H-perfluoro-octane sulfonate (6:2); (6:2FTS).

2.5 Biomarker methods

2.5.1 Supporting parameters- health indices

With the aid of a measuring board and digital fish scale (Berkley® model BTDFS50-1) the length and total weight of each fish was measured on board the survey vessel. The fish were sexed by visual examination of their gonad. A motion compensated balance (Marel M2000 series) was used to measure the total liver and gonad weights on board the vessel.

The condition index of each fish was determined by calculating the ratio between total weight and the cube of the fork length of the fish.

\[
\text{Condition index (CI)} = \left( \frac{\text{weight (g)}}{\text{length (cm}^3)} \right) \times 100
\]

The liver somatic index (LSI) reflects the animals’ nourishment status. The LSI was calculated as:

\[
\text{Liver somatic Index (LSI)} = \frac{[\text{liver weight (g)} \times 100]}{[\text{fish weight (g)}]}
\]

The gonadosomatic index (GSI) reflects the animals’ reproductive status. GSI was calculated as:

\[
\text{Gonadosomatic Index (GSI)} = \frac{[\text{gonad weight (g)} \times 100]}{[\text{fish weight (g)}]}
\]

Age determination

Otolith pairs were removed from the cranium of individual fish and stored dry in labelled paper envelopes. Prior to reading, otoliths were snapped cleanly through the nucleus and soaked in water to reveal the annuli on the transverse surface. The annuli were scored from the transverse surface submerged in water with side light illumination and with the aid of a binocular microscope at 10-32x magnification.

2.5.2 Ethoxyresorufin O-deethylase (EROD)

EROD activity was measured in liver microsomes as described by Burke & Mayer (1974) and modified for the plate reader by Eggens & Galgani (1992). Microsomes were prepared on ice with pre-cooled equipment and solutions. Cryo-preserved liver samples were homogenized in a potassium phosphate buffer (0.1 M, pH 7.8) containing KCl (0.15 M), dithiothreitol (DTT) (1 mM), and glycerol (5% v/v),
using a Potter-Elvehjem Teflon–glass homogenizer. The homogenate was centrifuged (10,000 × g; 30 min, 4°C) before the supernatant was re-centrifuged (50,000 × g; 120 min, 4°C). The microsomal fraction was obtained by resuspending the resulting pellet in potassium phosphate buffer (0.1 M, pH 7.8) containing KCl (0.15 M), DTT (1 mM), EDTA (1 mM), and glycerol (20% v/v). Microsome samples were diluted to ~1 mg/mL in buffer and pipetted (50 μL) in 6 technical replicates onto a 96 well microplate. Prepared resorufin standards (duplicates) were then added to subsequent wells. Reaction mixture (200 μL, containing 0.1 M potassium phosphate buffer, pH 8, and 3 μM 7-ethoxyresorufin) was added to the sample wells, before NADPH solution (2.4 mM in final well volume of 275 μL) was added to initiate the reaction. Transformation of 7-ethoxyresorufin to resorufin was read in 8 steps on the plate reader. Excitation was at 530 nm and fluorescence emission was measured at 590 nm. The EROD activity values were normalised to the protein content in the microsomal fraction and expressed as pmol/ min/ mg microsomal protein. Protein concentrations were determined according to Lowry et al., (1951), adapted to measurement by plate reader. The protein standard used was bovine gamma globulin.

2.5.3 Acetylcholine esterase (AChE) inhibition

The method from Bocquené and Galgani, (1998) to determine the acetylcholine esterase (AChE) activity in biota tissue was followed in the present study to assess AChE in extracts from fish fillet samples. The microsomal fraction was obtained as described above for EROD. The enzyme activity was followed by the production of the yellow coloured 5-thio-2-nitrobenzoic acid (TNB) anion. The production of TNB is based on coupling of these reactions:

\[
\text{Acetylthiocholine (ATC) → thiocholine + acetate}
\]

\[
\text{Thiocholine + dithiobisnitrobenzoate (DTNB) → 5-thio-2-nitrobenzoic acid (TNB)}
\]

The conversion of DTNB to TNB can be used as a measure of the hydrolysis of ATC into thiocholine. ATC is produced from hydrolysisation of the neurotransmitter acetylcholine by AChE. AChE inhibitors will induce a decrease in the production of ATC and therefore a decrease in the production of TNB will be observed.

Experiments were performed in 96-well microplates (Sarstedt, Nürnberg, Germany) and the protocol was automated on a robotic workstation (a Biomek 3000 laboratory automation workstation) to allow high-throughput analysis. Every sample was run in triplicate. For this experiment 340 μL of 0.02 M phosphate buffer (at pH 7) were mixed together with 20 μL of 0.01 M DTNB and 10 μL of supernatant. After 5 minutes of incubation, 10 μL of 0.1 M ATC were added to start the reaction. The enzyme activity was then followed by an absorbance plate reader at 405 nm at room temperature (EMax microplate reader from Molecular Devices, with SoftMax Pro 5 software).

The change in absorbance per minute was used to calculate the AChE activity:

\[
\text{AChE activity (μmol ATC/min/mg protein) = } \frac{[\Delta A \times \text{Volt} \times 1000]}{\varepsilon \times \text{light path} \times \text{Vols} \times [\text{protein}]}
\]

Where \(\Delta A\) = change in absorbance (OD) per minute at 405 nm, corrected for spontaneous hydrolysis, \(\text{Volt} = \text{total assay volume (0.380 mL)}, \varepsilon = \text{extinction coefficient of TNB (M/ cm)}, \text{light path = microplate well depth (1 cm)}, \text{Vols = sample volume (in mL)}, \text{and [protein] = concentration of protein in the enzymatic extract (mg/mL)}.

2.5.4 Lysosomal membrane stability (LMS)

The determination of LMS was based on the time of acid labilisation treatment required to produce the maximum staining intensity after demonstration of naphthol AS-BI phosphate activity in digestive cell lysosomes (Broeg et al., 1999). The latency test for lysosomal enzymes determines the time intervals of weak acid buffer exposure needed to destabilise the lysosomal membrane and, thus, destroy the latency of
lysosomal enzymes. Penetration of the substrate through the lysosomal membrane was assessed as maximum staining intensity of enzyme-substrate inside lysosomes as visualised by azo-coupling with Fast Violet B and quantified by computer assisted image analysis. The longer the acid buffer exposure period needed to destabilise the lysosomal membrane represents high membrane integrity of lysosomes and vice versa.

Using a cryostat chamber (object temperature -18°C and knife -20°C) serial cryotome sections (10 μm) were cut and transferred to glass slides (room temperature). Each slide contained 2 sections from the same specimen. The cryotome sections were subjected to acid labilisation in intervals of 0, 3, 6, 10, 15, 20, 30, 35, 40 and 50 min in 0.1 M citrate buffer (pH 4.5 containing 2.5% NaCl) in a shaking water bath at 37°C, in order to find out the range of pre-treatment time needed to completely labilise the lysosomal membrane. Following this treatment, sections were transferred to the substrate incubation medium for the demonstration of Hex activity. The incubation medium consisted of 20 mg naphthol AS-BI phosphate (Sigma-Aldrich, N2125) dissolved in 2.5 mL of 2-methoxyethanol (Merck, 859), and made up to 50 mL with 0.1 M citrate buffer (pH 4.5) containing 2.5% NaCl and 3.5 g of low viscosity polypeptide (Sigma, P5115) to act as a section stabiliser. Sections were incubated in this medium for 20 min at 37°C, rinsed in a saline solution (3.0% NaCl) at 37°C for 2 min and then transferred to 0.1 M phosphate buffer (pH 7.4) containing 1 mg/ mL of diazonium dye Fast Violet B salt (Sigma, F1631), at room temperature for 10 min. Slides were then rinsed in running tap water for 5 min, fixed for 10 min in Baker's formol calcium containing 2.5% NaCl and 10% CaCl2 at 4°C and rinsed in distilled water. Finally, slides were mounted in aqueous mounting medium Kaiser's glycerine gelatine.

Slides were evaluated under a microscope. Lysosomes stain reddish-purple due to the reactivity of the substrate with Nacetyl-b-hexosaminidase. The average labilisation period (LP) for each digestive gland corresponds to the average incubation time(s) in the acid buffer that produces maximal staining reactivity. Each set of tissue sections are analysed and compared with the staining intensity of the section showing maximal staining (i.e. from which the LP has been derived). A mean value is then derived for each section, corresponding to an individual specimen.

2.5.5 DNA adducts

Preparation of DNA solutions

The fish liver samples were air freighted on dry ice to the AdnTox laboratory, France, for DNA adduct analysis. After receipt, the samples were stored at -80°C until required for DNA extraction. Small pieces of tissue (70 to 90 mg per sample) were taken for the DNA extraction. For each sample, a purified DNA solution was obtained by a method of phenol-chloroform / liquid-liquid extraction, after crushing the liver pieces (tissue-lyser, Qiagen), isolation of cell nuclei (in sucrose 0.32 M) and sample treatment with RNases A, T1 and proteinase K. The DNA concentrations were calculated from the absorbance (optical density) at the wavelength of 260 nm (A260) (Nanodrop Technology, Thermo Scientific ®). The absorbance ratios A260/ A280 and A260/ A230 coupled with the absorbance profile of the samples between 230 nm and 300 nm were used to check the quality of the DNA solutions, particularly with respect to RNA and/ or protein contamination.

32P-postlabelling method

The protocol used by AdnTox was suitable for the detection of so-called "bulky" DNA adducts (Le Goff et al., 2006), which are additional compounds in DNA associated to complex molecules such as PAHs. Each analysis was performed from 5 μg of DNA. The limit of detection was fixed to half the smallest DNA adduct level (relative adduct level=RAL) calculated for an observed spot, i.e. 0.5 x 0.02 = 0.01 adducts per 10⁸ nucleotides (RAL x 10⁻⁸). For analysis without detectable adducts ("null" results), the concentration of adducts was defined as <0.01 x 10⁻⁸ nucleotides.

In each set of analyses, DNA from both positive and negative controls was systematically included. Calf thymus DNA exposed to benzo[a]pyrene dioepoxide (BPDE), kindly provided by F.A Beland (National
Center for Toxicology Research, USA), was used as the positive control. This sample was used as a standard in previous large inter-laboratory trials (Divi et al., 2002; Zhan et al., 1995). Plasmid DNA was used as a negative control.

The autoradiographic patterns from both positive and negative controls assured the smooth technical functioning, by firstly the absence of nonspecific signals (a source of false positives, frequently due to improper disposal of certain reagents/impurities used during handling) and then a correct $^{32}$P labelling on a reference / standard sample. The good labelling efficiency was checked on the base of the direct level of radioactivity (Cerenkov radiation) in the major spot of the positive control, expressed in radioactive counts per minute (cpm).

2.5.6 Comet

**Slide preparation**

The slides were prepared on board the survey vessel with blood samples taken from the caudal vein of the freshly caught fish. A 10 $\mu$L volume of the blood was diluted 1000 fold in ice cold PBS buffer. A 15 $\mu$L volume of this diluted blood solution was added to 85 $\mu$L of warmed low melting point agarose. Then 7 $\mu$L of this agarose/blood solution was placed on an agarose pre-coated slide. Each fish had duplicate spots on 3 pre-coated slides labelled as LYS, FPG and BUF, relating to the treatment of these slides once back in the laboratory. The slides were kept in cool (4°C) lysis buffer, whilst stored on the survey vessel and transported back to the laboratory, Oslo. The slides were processed by NorGenoTech AS, Oslo between 5 and 10 days of being taken.

**Assay**

The comet assay is a simple method for measuring DNA damage at the level of individual cells. A high-throughput method as described in Brunborg et al., (2014) was followed. Briefly, cells embedded in agarose on a microscope slide are lysed, leaving the DNA as a nucleoid, attached to the nuclear matrix. After brief incubation in alkali, gels are electrophoresed at high pH. DNA is attracted to the anode, but moves appreciably only if breaks are present. After neutralisation and staining, the nucleoids (visualised by fluorescence microscopy) resemble comets; the relative intensity of the comet tail reflects the frequency of DNA strand breaks (SBs). Base alterations (e.g. oxidation) are measured by digesting nucleoids with lesion-specific enzymes; formamidopyrimidine DNA glycosylase (FPG) is most often used, to detect 8-oxoguanine and other purine oxidation products.

To increase the number of samples that could be handled simultaneously, the high throughput version of the comet assay with 12 mini gels on one microscope slide coated with polycarbonate film substrate was adopted. The test was performed using three different treatments: 1) Lysis only (to measure SBs); 2) Incubation with FPG buffer after lysis; and 3) Incubation with FPG after lysis (to measure oxidised guanine, oxidised bases). Results are expressed as % DNA in tail (median of, in general, 50 comets per sample). % DNA in tail is linearly related to break frequency over the range of damage levels expected. Net FPG-sensitive sites are calculated as the difference between scores for 3 and 2.

2.5.7 Vitellogenin

A microplate Enzyme Linked Immune-Sorbent Assay (ELISA) was used to semi-quantitatively determine the levels of plasma VTG. The method is based on an ELISA procedure prepared at Biosense Laboratories (Bergen, Norway) and the procedure is described in Goksøyr (1991). This procedure describes an indirect ELISA where the antigen is immobilised on a microtiter plate and a primary antibody specific for the antigen is allowed to bind to the primary antibody. The immuno-complex is detected by the addition of a substrate which is cleaved by the conjugated HRP (horse radish peroxidase) to generate a coloured reaction product that is detected spectrophotometrically. Commercially available antibodies were used: cod antibody (CS-1, prod. no. V01406201) for tusk, ling and saithe and an antibody against sea bream (PO-2, prod. no. V01410201) for redfish. Abnormal levels of the analyte molecule were determined when the signal level in an exposed group significantly differed from the level in a comparable reference group. As positive control for this assay, the analysis was performed on the available female individuals.
Blood samples were taken from the caudal vein of each fish with separate syringes containing heparin (10000 IU/mL, Sigma) and the protease inhibitor aprotinin (5 TIU/mL, Sigma). The blood samples were centrifuged at 2000 g for 5 min in a micro-centrifuge before the supernatant (plasma) was removed and transferred to labelled cryovials and snap-frozen in liquid nitrogen. Plasma samples were stored at -80°C until analysis. Vitellogenin was determined in plasma from fish using a competitive ELISA.

Plasma samples were diluted 1:400 – 1:2000 in coating buffer (carbonate/bicarbonate 50 mM, pH 9.6). The plasma samples were transferred to 96 well microplates, each containing triplicates of the diluted sample and a blank. Individual females were used as positive control. The plates were sealed and incubated at 4°C overnight. The plates were washed three times in PBS buffer. Blocking solution was added in all the wells and incubated for 45 min. Wells were then washed in TPBS. Selected antibody was diluted in 1% BSA in PBS and added. Samples were incubated at 37°C for 1 h. After washing, the secondary antibody was added. The secondary antibody with dilution 1:3000 was added to the wells and incubated for 1 h at 37°C. The plates were washed five times in TPBS buffer and then incubated with the developing solution (0.04% OPD, 0.012% H2O2). The plates were incubated in the dark at room temperature for 30 min. The reaction was stopped with 0.3 M H2SO4 and the absorbance read at 492 nm. To allow comparison of different samples, the total amount of protein added to the microplate wells were the same for each sample. The VTG-concentration was expressed in ng/mL.

2.5.8 Liver histology

In order to prevent the appearance of post mortem artefacts, specimens were handled with care. Sampled fish were dissected on the vessel immediately after capture. Analysed tissues (liver) were dissected and placed in pre-labelled cryovials and snap frozen in liquid nitrogen. The samples were stored at -80°C until further processing.

Inside the cryostat chamber (-20°C) samples were sliced into 4 mm thick specimens to ensure a proper fixation. Samples were put into labelled histological cassettes, placed in histological fixative (Baker's calcium-solution: 4% formaldehyde, 1% CaCl2, 2.5% NaCl) and kept at 4°C. Samples were dehydrated in alcohols and cleared in xylene (C6H4(CH3)2) and embedded in paraffin. Histological sections (3 μm thick) were cut using a microtome HM 355s (Microm, Bergman), mounted on slides, air dried and stained with haematoxylin and eosin.

<p>| Table 3. Categories for the histological liver lesions and scoring system used for their quantification. |
|---------------------------------|-----------------|---------------------|</p>
<table>
<thead>
<tr>
<th>Category</th>
<th>Score range</th>
<th>Score description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis (normal cyclical, non-pathological status of the liver)</td>
<td>0-3</td>
<td>0-absent 1-area affected 2-some areas affected 3-distributed through the entire sampled tissue</td>
</tr>
<tr>
<td>Circulatory disturbances</td>
<td>0-2</td>
<td>0-absent 1-sporadic/small area affected 2-some areas affected</td>
</tr>
<tr>
<td>Inflammatory changes</td>
<td>0-2</td>
<td>0-absent 1-sporadic 2-multiple/widespread</td>
</tr>
<tr>
<td>Melano-macrophage aggregates</td>
<td>0-3</td>
<td>0-absent 1-area affected (1-2 cases) 2-some areas affected/more than 2 in a sample 3-distributed through the entire sampled tissue</td>
</tr>
<tr>
<td>Other pathological changes</td>
<td>0-2</td>
<td>0-absent 1-sporadic 2-multiple/widespread</td>
</tr>
<tr>
<td>Parasites</td>
<td>0-1</td>
<td>0-absent 1-present</td>
</tr>
</tbody>
</table>
The tissues were examined for health parameters related to physiological conditions, inflammatory and non-specific pathologies and those associated with pathogen and parasite infections. All micrographs were captured using an AxioCam MRc5 (Zeiss) digital camera mounted on a Zeiss Axiosplan 2 light microscope (Göttingen, Germany). All slides were analysed blind with no reference to its sample location. Detected histopathological liver lesions were assigned to one of the following groups: steatosis; circulatory disturbance; inflammatory changes; melanomacrophage aggregates; parasites and other pathological changes, according to a developed and adopted scoring system (Bernet et al., 1999; Feist et al., 2004).

Vacuolation condition, macrovesicular and microvesicular steatosis were distinguished based on the size and the pattern of vacuoles present. Circulatory disturbances included various changes in normal structure of blood vessels (congestion, dilation, peliosis). Non-specific lesions were presented as: inflammatory changes (lymphocyte infiltration and granulomatosis); melanoma-macrophage aggregates, parasites, other pathological changes (degenerative – necrosis, proliferative – fibrosis, cirrhotic changes). According to the affected area or prevalence of each disorder within a specimen, all of the parameters were scaled using an established scoring system (Table 3).

2.5.9 Gill histology

One piece of gill arch from each side of the fish was dissected in the field and immediately fixed in Baker’s solution (4% formaldehyde, 1% CaCl₂), and transported to the laboratory for analysis. Tissues were dehydrated in ethanol, rinsed and cleaned in a tissue processor (Shandon Excelsior, Thermo) before embedding in paraffin wax. Histological sections (3 µm), obtained using a microtome (HM 355s, Bergman), were mounted on slides and stained in haematoxylin, eosin and saffron (HES) using an automated staining machine (Tribune Stainer, Surgipath). Finally, sections were evaluated using a microscope (Zeiss Axiosplan 2) and all micrographs were captured with a digital colour camera (AxioCam).

Gills were examined for histopathological alterations related to physiological conditions, inflammatory pathologies and pathogen/parasites infections. Each lesion was scored according to the severity and the frequency of the lesion: 0 = absence of lesion, 1 = ≤ 10 % of the histological section showed the lesion, 2 = between 10% and 50% of the histological section showed the lesion, 3 = between 50% and 70% of the histological section showed the lesion and 4 = between 70% and 100% of the histological section showed the lesion (Benly et al., 2008; Sensini et al., 2008). Slides were analysed blind and to ensure quality, two analysts scored a selection of slides from the first field study.

2.6 Integrative assessment

The Integrative Biological Response (IBR) index was developed to combine biochemical, genotoxic and histochemical biomarkers (Beliaeff and Burgeot, 2002). The method is based on the relative differences between the biomarkers in each given data set. Thus, the IBR index is calculated by summing-up triangular star plot areas (a simple multivariate graphic method) for each two neighbouring biomarkers in a given data set. The procedure is as follows: 1) calculate the mean and standard deviation for each sample group; (2) standardise the data for each sample group: \( x'_i = \frac{x_i - \bar{x}}{s} \); where, \( x'_i \) = standardised value of the biomarker; \( x_i \) = mean value of a biomarker from each sample; \( \bar{x} \) = general mean value of \( x_i \) calculated from all compared sample groups; \( s \) = standard deviation of \( x_i \) calculated from all samples; (3) add the standardised value obtained for each sample group to the absolute standardised value of the minimum value in the data set (i.e. \( y_i = x'_i + |x_{min}'| \)); (4) calculate the Star Plot triangular areas by multiplication of the obtained standardised value of each biomarker (\( y_i \)) with the value of the next standardised biomarker value (\( y_{i+1} \)), dividing each calculation by 2 (\( A_i = y_i \times y_{i+1} / 2 \)); and (5) calculate the IBR index which is the summing-up of all the Star Plot triangular areas (IBR = \( \sum A_i \)) (Beliaeff and Burgeot, 2002). Since the IBR value is directly dependent on the number of biomarkers in the data set, the IBR value was divided by the number of biomarkers used in each case (\( n = 7 \)) to calculate IBR/n, according to Broeg and Lehtonen (2006).
2.7 Statistical treatment

Statistical differences between the groups of biological data were assessed with analysis of variance (ANOVA). Homogeneity of variance for the different groups was checked using the Levene’s test and data were log transformed when necessary to obtain homogeneity. Where homogeneity could not be achieved non-parametric analysis was performed, either as a Kruskal-Wallis test or Mann-Whitney U test. Where homogeneity of variance was achieved a Dunnett’s post-hoc test was performed with the parametric ANOVA to compare significant differences from the reference group. The level of significance was set at p<0.05.
3. Results

3.1 Physicochemical data

Figure 3. Vertical depth profiles for salinity, temperature and turbidity taken within 500 m of the Njord A platform on two occasions during the fish collection.
Salinity, temperature and turbidity profiles were taken at the Njord A platform on two separate occasions during the fishing effort. The profile measurements were taken on the same day within 3 hours of each other and both showed clear stratification of the water column (Figure 3). A halocline was present at around 100 m depth with surface to 100 m salinity changing from 33 to 35 ‰. Salinity remained stable at 35 ‰ at 100 m and below. A thermocline was evident at approximately 50 m with a change in temperature from 9.25°C in the surface waters approaching 6.8°C at around 50 m. A slow gradual decrease in temperature from 8 to 7.5°C was observed between 100 m to 340 m. Turbidity was highest in the surface waters (0.5 to 1 FTU) and was consistently low at around 0.1 FTU between 50 and 340 m depth.

3.2 Chemical concentrations in PSD extracts

3.2.1 Targeted analysis of PAH-NPDs in LDPEs

The seawater concentrations of PAH16 determined from LDPE extracts are shown (Figure 4). The LDPEs were placed 1 m above the seafloor at six stations, all within 300 m of the Njord A platform. Median values were low at all stations ranging between 1.6 and 2.0 ng/ L, which is similar to that assumed to be background (without significant point source) concentrations in previous studies (Harman et al., 2009). Although higher median concentrations were found at stations 2 and 6, there were no marked differences in PAH concentration between the stations. PRC results (not presented), showed that compounds with Log $K_{ow} < 4.5$, were approaching equilibrium after the three week deployment.

![Figure 4](image-url)  
**Figure 4.** Seawater concentrations for the sum PAH16, calculated from LDPE accumulations at the different stations around the Njord A platform. Median, quartiles (box), 10/90 percentiles (outer line), expressed as ng/ L (n=3). The inserted map shows the approximate positions of the stations in relation to the Njord A platform.
The Sum of NPD seawater concentrations measured in LDPE extracts positioned 1 m above the seafloor for 3 weeks are shown in Figure 5. Median concentrations were highest at stations 2 and 6, although large variations were shown at stations 5 and 6 with individual values ranging from 4-42 and 15-45 ng/ L respectively with two out of three replicates showing levels more in line with the other stations. The reason for this variation is unknown, and no-contamination is evident from either laboratory blanks or those taken in the field. Lowest concentrations were found at stations 3 and 4. Based on overlapping values (since n= 3) stations 2 and 3 were considered to be significantly different from stations 1, 2 and 6, with 1 different to 2 and 6. Such differences should be interpreted with caution due to the high variation, and overall concentrations were low.

![Figure 5](image.png)

**Figure 5.** Water concentrations for the sum NPDs, calculated from LDPE accumulations at the different stations around the Njord A platform. Median, quartiles (box), 10/90 percentiles (outer line), expressed as ng/ L, n=3. The inserted map shows the approximate positions of the stations in relation to the Njord A platform.

### 3.2.2 Non-target screening

All sample chromatograms were unresolved complex mixtures (UCM) making non-target analysis using deconvolution software very difficult due to all signals being swamped by the hydrocarbon signals. The number of peaks identified in the POCIS extracts were quite low compared to the LDPE extracts. This was not so surprising, since the nature of compounds accumulating in the POCIS extracts will typically have lower log Kow values, and therefore tend to be less suitable for GCMS identification (and may not be present in sediments in the first instance). However, a few compounds were present in the GCMS identification of the POCIS extracts. In total, 12 and 20 unique peaks that were not present in the field blank samples were recorded by the software for extract 6-1 (i.e. station 6 replicate 1) and 3-1 respectively. These compounds were tentatively identified by the software, and the identifications were checked.
according to the criteria mentioned in materials and methods. For the 20 compounds in extract 6-1, the tentative identification assigned could be confirmed by the combination of fragmentation pattern, accurate mass identification and an evaluation of retention index for 7 of the compounds. An overview of the compounds is given in Table S1, Appendix B along with some of the criteria used for identification purposes. For the rest of the compounds, the manual inspection leaves the tentative inconclusive, and they are not shown in this report.

The tentative identifications are considered to be quite robust, with in general; high match factors by fragmentation pattern (Abramson, 1975) and accurate mass confirmation with less than 5 mDa deviation of at least two ions. The probability of correct identification according to Stein (1994) was quite low for many of the compounds (Table S1, Appendix B), and in this case it is probably caused by several isomers and similar compounds being possible for the tentatively identified compounds. Many of the compounds identified are natural products that will be present in any environmental extract (fats, fatty acids, steroids). In addition to the natural products, three possible identifications of anthropogenic compounds were made; diphenyl sulfone, a phthalate and possibly a fluorobenzoate. Even though the identifications are quite robust, an injection of the reference standards would have ensured a more unequivocal identification. It is also noteworthy that for most of the compounds, several isomers are possible, and it is difficult to determine which isomer is present in the extract.

The LDPE-extracts have also been subjected to a non-target analysis, revealing that a lot more compounds amenable for GC/MS-analyses were present in these extracts. The number of peaks identified in the extracts was 253; 312 and 20 for samples 3-1, 6-1 and laboratory blank respectively. For station 6-1, 50 of the identifications were checked, and only identifications that were not alkane chains and similar compounds were checked. For 5 of the peaks, the identification could be determined as fairly plausible, three compounds were probably phthalates, one PAH and one organophosphate. This frequency of positive identification is lower than other environmental samples that we have worked with, and is probably caused by an accumulation of long alkane chains, fats, steroids and similar compounds in the LDPE samplers.

3.2.3 Suspect target screening

Non-target analysis is not a sensitive technique and therefore an applied suspect target analysis (Hernández et al., 2012; Hernández et al., 2011) was employed to facilitate a more sensitive comparison. Performing nw-XICs at the exact mass of the three candidates (20-50 mDa window) discussed in the POCIS extract allowed us to evaluate whether the compounds identified were present in other extracts. An example of suspect analysis of the tentatively identified fluorobenzoate in extract 3-1 is shown in Figure 6. As demonstrated, the additional specificity by using the mz resolving power of the ToF instrument means a higher specificity (compare Figure 6b and Figure 6c). This procedure was used for a relative semi-quantification of the compounds in the extract for the same three compounds; the areas indicate the relative abundance of the compounds in the different extracts (Table 4). The semi-quantification allowed a relative comparison without the need of reference standards as reported by Hernández et al., (2011). The response factor for a compound is the same for different extracts, but the response factors will vary from compound to compound. Therefore, the areas reported in Table 4 can be used to compare the relative amount of one compound in several extracts, but not for a comparison of two different compounds in the same extract. In principle, suspect analysis can be performed retrospectively for any compound that is believed to be present in the extract. However, the presence of confirmatory ions based on a known fragmentation pattern, knowledge of the Kovats RI for the compound and co-injection with authentic standards are important for determination of the presence of compounds.
Figure 6. Examples of suspect analysis of extract 3-1 for the compound tentatively identified as fluorbenzoate with mz 173.0795 and confirmatory ion of 155.0662: a) Total ion chromatogram (TIC); b) XIC (extracted ion chromatogram) (mz 173.0795 ± 1 Da); c) nw-XIC (mz 173.0795 ± 0.02 mDa); d) nw-XIC of confirmatory ion (mz 155.0662 ± 0.02 mDa).
Table 4. Comparison of relative area of tentatively identified fluorobenzoate, phthalate and diphenyl sulfone compounds in different extracts. The areas are the response of the signal for mz 173.0795 ± 0.05 mDa, and the presence of the confirmatory ion of mz 155.0662 ± 0.05 mDa.

<table>
<thead>
<tr>
<th>Name</th>
<th>Station- replicate</th>
<th>Fluorobenzoate Rt 19.21</th>
<th>Phthalate Rt 23.13</th>
<th>Diphenyl sulfone</th>
</tr>
</thead>
<tbody>
<tr>
<td>141786_POCIS_5</td>
<td>1-1</td>
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<td>459</td>
<td>50</td>
</tr>
<tr>
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<td>1-2</td>
<td>56.7</td>
<td>572</td>
<td>33</td>
</tr>
<tr>
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<td>2-1</td>
<td>16.9</td>
<td>45.8</td>
<td>28</td>
</tr>
<tr>
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<td>514</td>
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<td>226</td>
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<td>41.9</td>
<td>391</td>
<td>40</td>
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<tr>
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A more suspect analysis was also performed for known geochemical substances. The ions selected were based on those previously published. The data in Table 5 shows the integration of UCMs for each of the masses presented in Table 6. The peak areas are normalised to internal standard but do not take into account individual response factors so compound groups cannot be compared to each other. However, between site comparisons for the same compound group can be performed.

Table 5. Chromatographic peak areas, normalized to internal standard, for selected contaminant groups

<table>
<thead>
<tr>
<th>Station</th>
<th>sampler</th>
<th>14β (H) steranes</th>
<th>Diasteranes /regular steranes</th>
<th>Alkylbenzenes</th>
<th>Substituted methylbenzenes</th>
<th>Trisnorhopanes</th>
<th>Bisnorhopanes</th>
<th>25-norhopanes</th>
<th>Total hopanes</th>
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<td>LDPE 1-2</td>
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<td>Mean</td>
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Table 6. Mass/charge ratio integrated for peak area determination

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<th>Compound group</th>
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<td>14(\beta)(H) steranes</td>
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<tr>
<td>Diasteranes/regular steranes</td>
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<tr>
<td>Alkyl benzenes</td>
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<tr>
<td>Substituted methyl benzenes</td>
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<td>Bisnorhopanes</td>
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<td>177.1643</td>
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<tr>
<td>Total hopanes</td>
<td>191.1878</td>
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</table>

**Figure 7.** Relative chromatographic peak areas for selected contaminant groups from LDPE extracts positioned 1 m above the substrate at the 6 locations around Njord A platform. Data presented as the top and bottom values (box) and median (middle line), n=2. The inserted map shows the approximate positions of the stations in relation to the Njord A platform.

For LDPE sample 6-1, a suspect target analysis for selected PAHs was performed, and a number of these could be detected. Among the compounds that could be detected were trimethyl naphthalene, methyl, dimethyl and trimethyl phenanthrene, and quinolone. For these compounds, a semi-quantification can be performed at a later stage. For some of the suspect compounds we checked, presence could not be confirmed, among them xanthone, acridine and methylquinoline.
3.3 Chemical bioaccumulation and exposure

3.3.1 PAH-NPD in stomach content and fish fillet

PAH-NPD concentrations were measured in homogenised stomach content of fish caught from both the Njord A platform as well as the reference group. For some fish species, particularly ling from the reference group and all fish species from the Njord A platform, the stomachs were regularly empty of food and were therefore not analysed. The PAH-NPD concentrations were low and in most cases undetected in all fish species measured (Table 7). The stomach content from tusk, ling and redfish from the Njord A platform was insufficient in sample volume and resulted in matrix effect interference that prevented quantification. Only the stomach content of the saithe could be adequately measured without interference and in this case PAH-NPDs were below the limit of detection.

Table 7. PAH-NPD concentrations in stomach content of fish collected from the Njord A platform and the reference location. Bold values indicate detected concentrations (µg/ kg w.w.). The following PAHs were below the limit of detection (LOD <0.05 to 0.1 µg/ kg w.w.) in all samples and therefore not presented: acenaphthylene, acenaphthene, fluorene, dibenzoanthiophene, anthracene, pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene; and below LOD (<2 to 4 µg/ kg w.w.) for PAH C1, C2, C3-dibenzothiophenes; C1, C2, C3-phenanthrenes. Note: i, interference caused by insufficient sample volume. Sum measured PAH includes only detected values and not those below their respective LOD.

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<th>C2-Naphthalenes</th>
<th>C3-Naphthalenes</th>
<th>Phenanthrene</th>
<th>Fluoranthene</th>
<th>Chrysene</th>
<th>Sum measured PAH</th>
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<td>&lt;2</td>
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<td>&lt;2</td>
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PAH-NPD concentrations were measured in fillet samples collected from the dorsal surface of the fish (excluding skin) from the Njord A platform and reference group (Table 8). PAH concentrations were undetected in all fish collected from the Njord A platform and reference group. Only C3-naphthalenes and C2-dibenzothiophenes were detected in some of the fish, although when present were only found at low concentrations marginally above the limit of detection.

Table 8. PAH-NPD concentrations in the fillet of fish collected from the Njord A platform and the reference location. Bold values indicate detected concentrations (µg/ kg w.w.). Below limit of detection (LOD <0.05 to 0.1 µg/ kg w.w.) in all samples and therefore not presented for following PAH: acenaphthylene, acenaphthene, fluorene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, indene(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene; and below LOD (<2 to 13 µg/ kg w.w.) for PAH C1, C2-naphthalene, C1, C3-dibenzothiophenes; C1, C2, C3–phenanthrenes. Note: i, interference caused by insufficient sample volume. Sum measured PAH includes only detected values and not those below their respective LOD.

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3.3.2 PAH metabolites in fish bile

**Fixed fluorescence (FF)**

The results of PAH metabolites measured in the bile of the four fish species using the fixed fluorescence (FF) screening method are reported (Figure 8). Fluorescence was measured at the excitation/emission wavelength pairs for the detection of metabolites of naphthalene (2- and 3- rings, 290-334 nm), pyrene (4- rings, 341-383 nm) and benzo[a]pyrene (5- and 6-rings, 380-430 nm). For tusk, a significantly higher fluorescence signal indicating increased concentrations of 2- and 3- ring PAH metabolites were found in fish from the Njord A platform compared to the reference group. No significant differences were found for metabolites of 4-ring or 5-6-ring PAHs.

Due to the small reference sample size for ling (n=4), the platform group were also compared to the reference group measured during the WCM2013 campaign (Ref2013). In these fish significantly lower fluorescence of all three fixed wavelength pairs were found in the WCM2013 reference group compared to both the reference and Njord A platform ling, which would indicate lower metabolite concentrations in WCM2013 reference ling. In redfish, no significant differences were found between reference and platform fish for either of the three PAH metabolite groups. However, saithe from the platform showed significantly lower fluorescence of 2-3-ring PAH metabolites then the reference group. For tusk, ling and saithe, a similar metabolite concentration profile was observed, with higher concentrations of 2-3 ring PAH metabolites followed by 4- ring and then 5-6-ring PAH metabolites in both reference and platform groups.

**Gas Chromatography (GC)**

Results from the FF method were confirmed by GC analysis. The signal level was mostly below the detection limit, indicating a very low presence of PAH metabolites in fish bile. While the FF method is used as a screening tool for the evaluation of PAH metabolites, the GC method provides a more accurate quantification of their presence and is more reliable in the case of strongly coloured fish bile.

For tusk, no significant difference was found between the samples and values were typically below the detection limit. Due to the small reference sample size for ling (n=4), the platform group were also compared to the reference group measured during the WCM2013 campaign (Ref2013). In these fish significantly lower fluorescence of all three fixed wavelength pairs were found in the WCM2013 reference group compared to both the reference and Njord A platform ling, which would indicate lower metabolite concentrations in WCM2013 reference ling. There was also a significant increase in C1-naphthalene in fish around the Njord A platform, compare to both reference samples. In redfish, no significant differences were found between reference and platform fish for either of the three PAH metabolite groups. Finally, saithe collected in the vicinity if Njord A showed significantly higher (and above the LOQ) level of C1- naphthalene and C2-phenanthrene.
Figure 8. Fixed wavelength fluorescence levels in bile from the four fish species from the locations indicated (ref, reference; Ref2013, wcm2013 reference; njord, Njord A platform), expressed as pyrene fluorescence equivalents, PFE µg/mL. The wavelength pair 290/334 nm identifies 2-3 ring structures, 341/383 nm identifies 4 ring structures and 380/430 nm 5-6 ring structures. The figure shows median, quartiles (box) and 10/90-percentiles (bar). The numbers in the parenthesis indicate the specific n value for each group. * indicates significant difference between ref and platform values, whilst † indicates significant difference of Ref2013 from Ref and Njord A values.
Figure 9. PAH metabolites in bile samples measured by gas chromatography (GC) for tusk and ling collected from the reference area and Njord A platform. The figure shows median, quartiles (box) and 10/90 percentiles (bar). Limit of quantification (LOQ) for single compounds (30 ng/g) and groups C1, C2 (200 ng/g) and C3 (500 ng/g). The numbers in the parenthesis indicate the specific n value for each group.
Figure 10. PAH metabolites in bile samples measured by gas chromatography (GC) for redfish and saithe collected from the reference area and Njord A platform. Median, quartiles (box) and 10/90 percentiles (bar). Limit of quantification (LOQ) includes: single compounds (30 ng/g) and groups C1, C2 (200 ng/g) and C3 (500 ng/g). The numbers in the parenthesis indicate the specific n value for each group.

3.3.3 PFCs in fish blood

A total of 23 PFCs were analysed in the whole blood samples of tusk, ling and redfish from the Njord A platform and reference group. From these 23 PFCs, six were measured above the limit of quantification and are shown in Figure 11. Overall, PFOSA was the most abundant PFC found in all three fish sampled,
with highest concentrations found in ling from the reference and platform sites. When comparing between locations, significant differences were only found in tusk, with significantly higher concentrations of PFUnDA, PFHxS and PFOS found in the platform fish compared to the reference group.

**Figure 11.** PFC concentrations in the blood of fish collected from the Njord A platform and reference location. Data expressed as µg/L. Y-axis in logarithmic scale, values presented as median, quartiles (box) 10/90 percentiles (outer line). Perfluoro-n-decanoic acid (PFDA, <0.6), perfluoro-n-undecanoic acid (PFUnDA, <0.6), perfluoro-n-tridecanoic acid (PFTrDA, <0.6), perfluoro-1-hexanesulfonate (PFHxS, <0.1), perfluoro-1-octanesulfonate (PFOS, <0.1), perfluoro-1-octanesulfonamide (PFOSA, <0.1). Values in parenthesis indicate specific n value for each group. * indicates significant difference between ref and platform values.

The 23 PFC that were analysed including individual LOQ values, were as follows: perfluoro-n-pentanoic acid (PFPA, <0.5); perfluoro-n-hexanoic acid (PFHxA, <0.5); perfluoro-n-heptanoic acid (PFHpA, <0.5); perfluoro-n-octanoic acid (PFOA, <0.5); perfluoro-n-nonanoic acid (PFNA, <0.5); perfluoro-n-decanoic acid (PFDA, <0.6); perfluoro-n-undecanoic acid (PFUnDA, <0.6); perfluoro-n-dodecanoic acid (PFDoDA, <0.6); perfluoro-n-tridecanoic acid (PFTrDA, <0.6); perfluoro-n-tetradecanoic acid (PFTeDA, <0.6); perfluoro-n-hexadecanoic acid (PFHxDA, <0.6); perfluoro-n-octadecanoic acid (PFODA, <0.6); perfluoro-1-butanesulfonate (PFBS, <0.1); perfluoro-1-hexanesulfonate (PFHxS, <0.1); perfluoro-1-octanesulfonate (PFOS, <0.1); perfluoro-1-decanesulfonate (PFDS, <0.1); perfluoro-1-octanesulfonamide (PFOSA, <0.1); N-methylperfluoro-1-octanesulfonamide (N-MeFOSA, <0.1); N-ethylperfluoro-1-octanesulfonamide (N-EtFOSA, <0.1); 2-(N-methylperfluoro-1-octanesulfonamido)-ethanol (N-MeFOSE, <2); 2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (N-EtFOSE, <2); 1H,2H-perfluorooctane sulfonate (6:2) (6:2FTS, <0.1).
3.4 Biological supporting parameters

3.4.1 Length, weight and age of fish

A comparison of the length of the four fish species sampled at the platform and for the reference group is shown in Figure 12. Significantly longer tusk and ling and significantly smaller redfish were sampled at the platform compared to their respective reference group(s). The same differences found for length were also observed for weight, with heavier tusk and ling and lighter redfish measured in the platform group compared to their respective references (Figure 13). This pattern was also reflected in the age of the fish with older tusk and ling and younger redfish found at the platform compared to the reference groups (Figure 14). This indicates as expected that all three of these parameters are directly related to each other. Overall, tusk, ling and saithe were generally between 5 and 10 years of age, whilst redfish were much older between 20 and 30 years of age.

Figure 12. Length of fish sampled at the different locations. Median, quartiles (box), 10/90 percentiles (outer line). * significant difference from reference group, † significant difference from ref2013 and reference groups. Values in parenthesis indicate specific number sampled for each group.
Figure 13. Weight of fish sampled at the different locations. Median, quartiles (box), 10/90 percentiles (outer line). * significant difference from reference group, † significant difference from ref2013 and reference groups. Values in parenthesis indicate specific number sampled for each group.
3.4.2 Health indices

Condition index (CI) was obtained from measurements of wet weight and fork length of each fish immediately after capture and is shown in Figure 15. The CI of approximately 1.0 was obtained for tusk and saithe whilst the CI of ling were much lower at approximately 0.5. In redfish, a median CI of 1.6 was calculated in the reference group, which was significantly higher than redfish from the platform. A significantly lower CI was also found in saithe from the platform group compared to their respective reference group.

The liver somatic index (LSI), measured as a ratio between liver weight and fish weight, can provide an indication of the nourishment status of the fish (Figure 16). Significant differences in LSI were found in 3 of the 4 fish species with significant increases in LSI of the platform tusk and saithe, and a significant decrease in LSI for platform redfish compared to their respective reference groups.

The GSI values separated into the individual genders for each fish species are shown in Figure 17. Due to the small sample size, especially male ling, and the absent of male redfish from the platform adequate comparisons for some of the groups could not be made. However, significantly higher GSI values were found only in female tusk and saithe from the platform compared to their respective reference groups.
**Figure 15.** Condition index (CI) of fish sampled at the different locations. Median, quartiles (box), 10/90 percentiles (outer line). * significant difference from reference group. Values in parenthesis indicate specific n value for each group.
Figure 16. Liver somatic index (LSI) of fish sampled at the different locations. Median, quartiles (box), 10/90 percentiles (outer line). * significant difference from reference group. Values in parenthesis indicate specific n value for each group.
Figure 17. Gonadosomatic index (GSI) of fish sampled at the different locations and divided between gender (M-male, F-female). Median, quartiles (box), 10/90 percentiles (outer line). * significant difference from reference group. Values in parenthesis indicate specific n value for each group.
3.5 Biomarkers

3.5.1 Ethoxyresorufin O-deethylase (EROD)

EROD activity in fish liver separated by gender for the four fish species from their different locations are shown in Figure 18. EROD activity was generally low in tusk, ling and redfish from both reference and platform groups. EROD activity of ling from the platform was significantly higher than those from ref2013, although median EROD activity remained low at less than 10 pmol/min/mg protein. In general EROD activity was higher in saithe than the other fish species. Significantly higher EROD activity was measured in male saithe from the reference location compared to their platform group.

Figure 18. EROD activity in liver samples from male and female fish sampled at the different locations. Median, quartiles (box), 10/90 percentiles. * significant difference from reference group, † significant difference from ref2013 group. Values in parenthesis indicate specific n values for each group.
3.5.2 Acetylcholine esterase (AChE) inhibition

AChE activity in the liver of the four fish species from the different locations are shown in Figure 19. Significant inhibition of AChE activity was found in both tusk and saithe from the platform compared to their respective reference groups. No significant differences were found for ling and redfish between the platform and reference groups. Median AChE activity for all fish species measured ranged between 100 and 400 nmol ATC/ min/ mg protein.

![Figure 19](image-url)

**Figure 19.** The inhibition of acetylcholine esterase (AChE) in fish fillet samples from the different locations. Median, quartiles (box), 10/90 percentiles. * significant difference from reference group. Values in parenthesis indicate specific n value for each group.
3.5.3 Lysosomal membrane stability (LMS)

Many of the fish species used in this study have had little application as biomonitoring organisms and have not been widely used for histochemical techniques such as LMS performed on sectioned liver tissue. However, due to the experiences and knowledge obtained from WCM2013, a combination of cooling sprays and special temperature regimes of the chamber, object and knife were used in order to obtain suitable sections for LMS assessment. As a result LMS for all four fish are represented (Figure 20). The median LMS values for all four fish in both platform and reference groups were on or above 20 min. However, significant differences between platform and reference group were reported for tusk and saithe, with significantly lower LMS in their platform groups.

Figure 20. Lysosomal membrane stability (LMS) in fish liver samples from the different locations. Median, quartiles (box), 10/90 percentiles (outer line). * significant difference from reference group. Values in parenthesis indicate specific n value for each group.
3.5.4 DNA adducts

Significant increases in the frequency of DNA adducts were found for both redfish and saithe from the platform population compared to their respective reference group (Figure 21). Elevated adduct frequency was also found in platform ling compared to their reference group, although due to the large intra-variation of the platform ling no significant difference was found. However, the median DNA adducts frequency for the ling from the platform was the same as the reference ling found during WCM2013 programme. Median DNA adducts were highest in redfish from the platform at 6 RAL x10^9 (or nm adducts per mol DNA).

![Graph showing DNA adducts in fish liver samples from the locations indicated.](image)

**Figure 21.** The frequency of DNA adducts in fish liver samples from the locations indicated. Median, quartiles (box), 10/90 percentiles (outer line). * indicates significant difference from the reference group (ANOVA, Dunnett’s, p<0.05. Numbers in parenthesis denote individual n values. Ref, reference; ref2013, reference from WCM2013; platform, Njord A platform; RAL, relative adduct level.)

3.5.5 Comet

The comet assay is used to assess DNA damage in whole blood cells. The main challenge in offshore biomonitoring is to preserve the blood cells so that they can be brought back to the laboratory on land to undergo electrophoresis and further processing. This can be achieved by holding the samples (diluted in PBS and attached to the slide in agarose) in lysis buffer as performed in WCM2013. The quality of the blood cells during the platform cruise were considered suitable in all four fish species sampled for the assessment of DNA damage. However, due to a combination of several potential factors during the reference cruise, a high degree of cellular damage was observed, which prevented the reference samples from being analysed. Consequently, and where possible, the results of the comet assay have been...
compared to the reference fish groups from the WCM2013 cruise (Figure 22 and Figure 23). Since the comet assessment in WCM2013 and WCM2014 was performed by the same laboratory with the same test personnel, the comparison of the data between the two years were considered appropriate.

The percentage DNA damage assessed through the size of the comet like tail showed a significant increase in tusk, ling and saithe collected from Njord A platform compared to their respective reference groups (Figure 22). Median values for DNA damage in these three fish were approximately 60%, although a high variability was observed in tusk and ling. Since redfish were not sampled during the WCM2013 programme, the DNA damage in redfish from the Njord A platform could not be compared to a reference group. However, the % DNA damage in redfish from Njord A was markedly lower than the other fish species sampled there.

The % DNA damage together with the oxidation of base pairs measured through enzyme treatment is shown in Figure 23. When oxidation of base pairs was combined with % DNA damage significantly high values were only found for tusk from Njord A when compared to their respective reference groups. Overall, oxidation of base pairs was responsible for only a small portion of the total DNA damage in all fish sampled.

Figure 22. DNA strand breakage in fish blood samples from the different locations. Median, quartiles (box), 10/90 percentiles. * significant difference from reference group. Values in parenthesis indicate specific n value for each group.
Figure 23. DNA strand breakage and oxidation of base pairs in fish blood samples from the different locations. Median, quartiles (box), 10/90 percentiles. * significant difference from reference group. Values in parenthesis indicate specific n value for each group.
3.5.6 VTG

VTG concentration in male fish from both reference and platform populations are shown in Figure 24. No significant difference in VTG concentration was found between reference and platform groups. Overall the concentration of VTG in the blood of male fish was extremely low.

Figure 24. VTG in blood samples of male fish from the different locations. Median, quartiles (box), 10/90 percentiles. No significant difference from the reference group. Values in parenthesis indicate specific n value for each group.

3.5.7 Liver histology

In fish liver, all the selected histopathological alterations were found in all samples with different levels of distribution (Figure 25). In contrast, the parasites were not common, excluding this parameter from further considerations.

As regards to steatosis, this lesion was found in all the analysed samples. Ling and saithe showed higher values of steatosis in the liver from fish collected around the Njord A platform compared to the reference. There was almost no difference in the % of circulatory disturbance, except for saithe and tusk. Saithe liver showed higher levels of this parameter in fish sampled at the reference site. All fish collected at the platform showed higher levels of melanomacrophage aggregates in the liver compared to the reference, except for saithe where no difference was found. The percentage prevalence of inflammatory changes was higher in liver of fish collected in the vicinity of the Njord A platform only in tusk and saithe. In general a certain amount of other pathology was found in the liver of all species collected at the platform, as well as in ling and redfish from the reference location. Overall, species specific responses were evident.
Figure 25. Fish liver histopathology in the four fish species collected from the Njord A platform and reference group. Data expressed as percentage prevalence. The number of individuals in each group (n) was 15, except for saithe (n=12) and reference ling (ref2013 n=9 and ref =4).

3.5.8 Gill histology

All selected histopathological alterations were found in the four collected species at both locations, showing the natural presence of these lesions (Figure 26). The clearest signal was given by the presence of necrosis and cell degeneration. These lesions were higher in fish collected in the vicinity of Njord A. For three out of four species (excluding redfish), these lesions were present only in individuals collected at the platform site.

Hypertrophy and hyperplasia of gill epithelium showed a species specific response. For tusk and saithe, values were higher in individuals collected at Njord A; whilst for ling and redfish, values were higher in fish sampled at the reference site. As regards fusion and disorganisation of gill lamella, values were higher in fish collected in the vicinity of Njord A for tusk and ling and lower for saithe. The prevalence of necrosis or cell degeneration was higher in all fish species from the Njord A location compared to their respective reference group. In fact necrosis was absent in tusk, ling and saithe from the reference location. With regard to inflammatory changes of the gill epithelium, higher prevalence was observed in tusk, redfish and saithe from the Njord A location compared to their respective reference groups, whilst the opposite was found for ling with 50 % prevalence in reference ling compared to 12% in ling from the Njord A platform.

Overall, as previously described in the liver, the gill histopathology also showed a species specific response to the analysed parameters.
Figure 26. Fish gill histopathology in the four fish species collected from the Njord A platform and reference group. Data expressed as percentage prevalence. The number in the parenthesis indicates the specific n value for each group. The number of individuals in each group (n) was 15, except for saithe (n=12) and reference ling (n=4).

3.6 Integrative assessment

The integrative biological response index (IBR/n) was calculated from star plots of normalised biomarker data from the four fish species. The IBR/n integrates the biomarker data and enables an overall assessment of the relative impact of each biomarker to the specific fish group (Figure 27).

Due to the nature of the assessment only those biomarkers with complete data sets could be used for the analysis, thereby enabling comparisons between fish groups. With this in mind the following biomarkers were used in the IBR/n calculation; DNA adducts, PAH metabolites, EROD, AChE, LMS, gill histopathology and liver histopathology. The inverse values of AChE and LMS were used since a decrease was reflective of an adverse impact and the biomarkers were positioned in relation to function. The mean values of each biomarker for the respective fish species were presented. The histopathology data for both gill and liver were taken as the mean prevalence of all histopathology data (excluding the parasite data for the liver).

In all four fish species sampled the calculated IBR/n value was markedly higher in the Njord A platform population then their respective reference groups. The highest IBR/n was calculated in both tusk and redfish from the Njord A platform (IBR/n =1.8), closely followed by platform saithe (IBR/n = 1.6). For the platform tusk, the contribution to the IBR/n score was equally spread between all biomarkers (excluding EROD) with slightly higher contributions from liver histology and PAH metabolites. The redfish also showed contributions from all biomarkers, with DNA adducts, gill histology and PAH metabolites contributing most to the IBR/n score.
The IBR/n values for the reference fish were relatively low for tusk (0.2), ling (0.3) and saithe (0.56), although slightly elevated in redfish (1.08). However, in all cases the IBR/n of reference fish were below their respective Njord A group.

**Figure 27.** Integrated biological response (IBR/n) calculated from the star plots of mean normalised biomarker data in the fish species from the locations indicated. The biological effects were grouped in relation to function. comet data excluded since reference data was not available. VTG was excluded since insufficient data for all groups.
4. Discussion

4.1 Chemical accumulations in passive sampling devices

Water concentrations of PAH-NPDs as well as non-target and suspect target screening of other organic compounds in the water column approximately one meter above the seafloor were measured with the aid of PSDs. Two different PSDs were used to accumulate both polar (POCIS) and non-polar (LDPE) compounds. The PSDs were used to provide additional information on the potential exposure of the wild fish to waterborne concentrations of oil and gas related chemicals around the platform, which may help to explain some of the biological responses measured.

4.1.1 PAH-NPD concentrations in LDPEs

The PAH16 water concentrations were low at ng/L levels with little difference found between the stations. Four of the stations (1, 2 4 and 6) were positioned above sediments that were considered to be contaminated with oil related chemicals, whilst two of stations (3 and 5) were positioned within a reference area. Here “reference area” refers to an area where bottom sediments are not known to be directly contaminated, however, practical limitations with the platform ROV meant that the reference location was only approximately 300 m from the platform and from potential sources of contamination.

The PAH-NPD concentrations showed larger differences between sites with the highest median values measured at stations 2 and 6 (14 and 17 ng/ L respectively). The highest median values potentially reflect the known contamination at these locations, although the overall concentrations measured were still low at all stations. Large variation in PAH-NPD concentrations were observed at stations 5 and 6. Station 5 was considered a reference station (not directly above contaminated stations) and had one of the highest measurements at 40 ng/ L, although only in one replicate. Proximity to the platform and transfer of contaminants down current of the contaminant source(s) may be responsible.

4.1.2 Non-target and suspect-target screening

A small selection of the POCIS and LDPE extracts were used to see if chemicals associated with oil and gas activities could be detected in the water column 1 m from the seafloor. In the POCIS extracts, seven compounds could be tentatively identified including natural compounds like fats, fatty acids and steroids. Anthropogenic compounds include phthalate, diphenyl sulfone as well as a potential tracer compound (2,6-difluoro-3-methylbenzoic acid). In the LDPE extracts, anthropogenic chemicals detected included three phthalates, a PAH and an organophosphate. Although the actual concentrations were not known, their detection in the seawater near to offshore platforms suggests that the organisms residing there may become exposed to such anthropogenic chemicals and therefore contribute to potential biological effects. Rigorous and quantitative analysis would be required to confirm these preliminary findings. This should include analysis of the POCIS membrane, which may contain substances transferred to the sorbent via water during exposure, but which may not show up in blanks that are dry and thus may not achieve such transfer.

4.2 Chemical concentrations in fish

The concentrations of PAH-NPD were measured in fish fillet and stomach content of the fish collected from around the Njord A platform and the reference area. In all cases the concentrations of PAH-NPD were low or below the limit of detection. This would suggest that living in close proximity to the two offshore platforms did not cause any apparent increase in PAH-NPD body burden concentrations of the species measured. Only C3 naphthalenes were measured above detection limits for both stomach content and fish fillet, although the concentrations measured were much lower than those which may be expected to cause toxicity. It is well established that fish are able to metabolise PAH from their tissues, therefore it is not that surprising that PAH was low or undetected in the fish fillet. PAH metabolites accumulate in the
Concentrations of 23 PFCs were measured in blood samples of native fish. Of the 23 PFCs only 6 (PFDA, PFUnDA, PFTrDA, PFHxS, PFOS, PFOSA) were above the limit of detection. Overall, PFOSA was the most abundant PFC in all three fish species (tusk, ling, redfish), with highest concentrations in ling. However PFOSA concentrations were equal to or even higher in the reference fish compared to those from around the platform. The PFC data were similar to that found in fish during the WCM2013 programme, where PFOSA was also the most abundant PFC in all fish, with highest concentrations found in ling (56 µg/L), and with no difference between platform and reference fish. Previous studies have reported a median concentration of 216 µg/L. PFOSA in the blood of cod collected from the Oslo fjord (Schøyen and Kringstad, 2011). In the present study, highest median concentrations of PFOSA were found in reference ling at approximately 30-40 µg/L, which was similar to the offshore values of ling captured in WCM2013. This indicates a ubiquitous distribution of PFOSA throughout the North Sea, with higher concentrations in fish closer to the coast and anthropogenic sources.

The higher concentrations of PFOSA in the ling compared to the other fish species, was an observation that was also made in WCM2013. The reasons for this are unclear but may be related to differences in diet and habitat niche. However, the concentrations of PFOSA in the blood of fish in our study were markedly lower than that expected to cause toxicity, which is in the region of 10-100 mg/ L concentrations (reviewed in Hekster et al., 2003).

When comparing between reference and platform fish, significant differences were only found in tusk, with significantly higher concentrations of PFOS, PFUnDA and PFHxS in the platform group. This may indicate the platform as a potential source of these PFCs. However the differences observed in tusk were not supported in the other fish species and the overall median concentration of these PFC was low, approaching and below 1 µg/L.

When discussing the contaminant body burden and biomarker data for the different fish species it is important to consider the individual species habitat and feeding habits in order to understanding potential differences in contaminant exposure pathways. Table 9 provides a summary of the habitat and diet for the four adult fish species caught in this study. These factors have been considered with respect to the biomarker responses and have been discussed where relevant in the following sections.

Table 9. Summary information of the habitat and diet of the studied fish species.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Habitat and diet</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tusk</td>
<td>Bottom dwelling species on hard rocky substrates. Feeds mostly on crustaceans and other soft bodied invertebrates and mollusces.</td>
<td>Hare et al., 2012.</td>
</tr>
<tr>
<td>Ling</td>
<td>Bottom dwelling found between rocks and offshore structures (e.g. ship wrecks and offshore installations). Fish feed mostly on small fish (e.g. cod, herring, plaice) as well as starfish and zooplankton.</td>
<td>Wheeler, 1969.</td>
</tr>
<tr>
<td>Redfish</td>
<td>Diurnal vertical migrations linked to their euphausid (small shrimps) prey. Feed mostly at night when they rise off the bottom following the vertical rise of their prey. Diet consists of euphausids, mysids and bathypelagic fish.</td>
<td>Pikanowski et al., 1999.</td>
</tr>
<tr>
<td>Saithe</td>
<td>Exhibit vertical migrations being close to the bottom at night and in midwater during the day. Feed almost entirely on pelagic and demersal fish such as herring, Norway pout, haddock and sandeel, although euphausids and inverts are also consumed</td>
<td>ICES-SCM-Fish map</td>
</tr>
</tbody>
</table>
4.3 Biomarker responses in fish

4.3.1 PAH metabolites

Fish are able to metabolise PAHs from their tissues, which is the main reason why they are found in low or undetectable concentrations in fish fillet samples as described above. The metabolised product is excreted from the cell and ends up in the bile of fish. It is the measurement of bile metabolites therefore that is the preferred matrix for measuring PAH exposure, where if present, can often be found at detectable levels. PAH metabolite values in fish collected from around the platform, as measured by the FF method, were generally low. A significant increase in 2-3 ring PAH metabolites was observed in tusk from the platform population compared to the reference, whilst a significant reduction in 2-3 ring PAH metabolites was found in platform saithe compared to the reference. No other differences in PAH metabolite concentrations were found for the fish species. The results were similar to that observed for PAH metabolites in fish from WCM2013, with an increase in 2-3 ring PAHs in tusk from the Veslefrikk and Oseberg platform groups. The concentration of PAH metabolites were also comparable with previous WCM surveys performed using caged fish that were exposed 500 m from the PW discharge for 6 weeks (Hylland et al., 2008).

The metabolic excretion of PAH in fish is known to be efficient, with most PAH excreted within 2 to 8 days after exposure. Subsequently, PAH metabolites measured in the bile represent exposure over the last few days and at most the last 2 weeks. Therefore, fish that were exposed to PAHs over two weeks ago, although may have triggered a measureable biological response that remains measureable (e.g. DNA adducts), the PAH metabolites would be absent from the bile. As a result, it can often be difficult to establish a dose-response relationship in field monitoring studies.

4.3.2 Supporting biological parameters

For general health parameters including nutritional and reproductive status, length and weight data as well as otolith age were collected from individual fish. These are quick and cheap assessments that are essential when sampling fish and are used as additional parameters to assist in understanding chemical bioaccumulation and biomarker results. In previous WCM campaigns when fish were transplanted into large cages for 6 weeks, quite large differences in condition index (CI) and liver somatic index (LSI) were often observed when compared to pre-exposure fish. This was considered to be due to the shortage of available food for fish held in cages. However, this was not expected to occur in wild caught fish from this study.

Differences in the length, weight and otolith age of the different fish groups for three of the four species were observed. Tusk and ling had significantly longer, heavier and older individuals from the platform than their respective reference populations. Inversely redfish sampled at the platform were smaller, lighter and younger than their reference group. Since these three parameters (length, weight and age) are somewhat related it is not surprising that they respond in unison. The reasons for the differences are likely to be as a result of randomised subsampling from wild populations, compounded by the different fishing techniques that were employed at the different locations. For instance, due to the combination of subsea obstructions around the platform and the lower density of fish in the open sea, the methods of fishing at the platform and at the reference site required different approaches, which could inadvertently lead to sample bias. For example, the passive approach of rod and line fishing, performed at the platform, required activity on the part of the fish to search for food, which may lead to fewer weak or diseased fish sampled. In contrast, trawling at the reference location would tend to be less discriminatory, selecting all fish irrespective of activity and may therefore be more representative of the population. The same fishing approach was taken for WCM2013, where, as was observed in the present study, larger tusk and ling were collected from the platform (Veslefrikk) compared to the reference group. Whether fishing method was responsible for the differences in the size and age of the fish selected from the different groups is not certain, although the differences observed should be taken into account when evaluating the chemical bioaccumulation and biomarker responses from the fish groups.
The CI, which can provide a general assessment of individual fitness, did show differences between species, with lower CIs in ling, typically half the value of the other fish species. The CI in ling did not show any difference between reference and platform and perhaps was an indication of differences in body shape (i.e. ling being long and thinner than the other fish species), rather than an indication of poor condition. The same observation was made for ling collected in WCM2013, and appears to be a natural condition due to body shape. For both the redfish and saithe, significantly lower CIs were found in the platform populations compared to their respective reference group, which may suggest some impairment in general fitness of the platform fish. This may be an interesting point since food availability is likely to be higher in the platform fish, which is often linked with a higher CI. However, the median CI values for redfish and saithe at the platform were still above or around 1, which would typically denote a fish in good condition.

A significantly lower LSI in redfish from the Njord A platform compared to the reference population may suggest a toxicity response to contaminant exposure. However, LSI can also indicate fish malnourishment, which may explain the observed differences in this fish species. In contrast, the LSI in tusk and in saithe was significantly higher in the platform groups. These observed differences in LSI may reflect the differences in diet and malnourishment between the locations with more food available at one location than another. The differences in diet of the fish species (Table 9) may provide some insight.

The GSI values, which provide a measurement of reproductive status, were presented in relation to gender for each fish species due to the obvious influence of gender on gonad development. However, due to the limited number of females for ling (1) and redfish (0) caught at the platform some comparisons in GSI were not possible. For females, no significant differences in GSI were observed between populations of tusk and saithe. A small but significant increase in GSI was recorded for male tusk and saithe compared to their reference groups. This may suggest the platform fish were at a more advanced reproductive developmental stage than their respective reference population. Since reproductive status can potentially influence certain biomarker responses (e.g. EROD), care should be taken when interpreting the respective biomarker data.

### 4.3.3 EROD

The measurement of EROD activity in fish is a well-established and sensitive biomarker of exposure to PAHs and structurally similar compounds (reviewed in Whyte et al., 2000). EROD induction has been performed in over 150 fish species from laboratory and field investigations. However, the relationship between EROD and biological effects at higher levels of biological organisation is still uncertain, although it has been linked with apoptosis and embryonic mortality. One of the main challenges when using EROD as a biomarker in biomonitoring studies is that it can be influenced by a wide range of biotic and abiotic factors including: fish species; age/ size; reproductive stage as well as temperature and pH. In addition, a variety of chemicals and chemical mixtures have been known to inhibit the induction of EROD in fish, thereby making the measurement of EROD activity in field biomonitoring studies difficult to interpret. Despite considering many of these confounding factors (e.g. species, gender, season, temperature, fish size), which can influence EROD activity it was very difficult to control all variables in such a field based study.

EROD activity showed no discernible increase in fish located near the platforms compared to those from the reference areas in either males or females. In fact, significantly higher EROD activities were observed in the reference saithe and reference ling compared to those from around the platform. In order to determine if some confounding factors such as age, size, general fitness and reproductive stage were influencing EROD activity, correlation analysis was performed between EROD activity and fish length, weight, age, CI, LSI, gonad weight and GSI for each gender of each species. A significant positive correlation between EROD activity and CI was found for male saithe indicating that EROD increased as CI increased. However the opposite (significant negative correlation) was found for female ling with respect to CI and EROD. The only other relationship found was a significant positive correlation between
EROD and LSI for male redfish ($r^2$ 0.51, df6, p<0.05). However, due to the limited data and contradictory findings no discernible pattern in the correlations could be seen.

It is recognized that baseline/background response levels have an important role in integrating biological effect parameters into environmental impact assessments of the marine environment. In general, an elevated level of a particular biomarker, when compared with a background response, indicates that a hazardous substance has caused an unintended or unacceptable level of biological effect. Background assessment criteria (BAC) and environmental assessment criteria (EAC) have subsequently been developed for certain biomarkers where validation data exist for the different species (ICES, 2011).

With respect to assessment criteria, a range of BACs for EROD have been developed for a variety of fish species, separated by both gender and the purity of the homogenate (i.e. S9 or microsomal fraction). Within the microsomal fraction of the liver, as measured in the current study, the BACs are available for dab, *Limanda limanda* (680 pmol/min/mg protein); cod, *Gadus morhua* (145 pmol/min/mg protein); plaice, *Pleuronectes platessa* (255 pmol/min/mg protein); dragonet, *Synchirus sp.* (202 pmol/min/mg protein); and the four spotted megrim, *Lepidorhombus boscii* (13 pmol/min/mg protein).

With the lack of specific BACs for the 4 fish species sampled in the present study, cod could be regarded as the best available estimate. All EROD measurements from fish collected from around the platform as well as at the reference location were well below the BAC suggested for cod. The field EROD data collected during this study for the five species should be used to help develop specific assessment criteria in wild fish, particularly since these species are not often used in biomonitoring programmes. Furthermore, since they typically reside within communities around offshore installations, they are likely to develop into important biomonitoring species for offshore oil and gas activities.

4.3.4 AChE

The AChE inhibition test has been used as a sensitive biomarker of neurotoxic exposure in environmental monitoring programmes and is an ICES recommended biomarker for biological effects monitoring (ICES, 2011). Environmental contaminants including pesticides, organophosphate and carbamate (Galjani and Bocquene, 1990), heavy metals and PAHs (Kang and Fang, 1997) have been found to inhibit AChE activity. Assessment criteria have been developed for a few marine fish including dab, *Platichthys flesus*; flounder (*Platichthys flesus*) and eelpout (*Zoarces viviparus*) with BACs ranging from 235 to 124, and EACs ranging from 165 to 87 nmol/min/mg protein in fish muscle tissue. It is important to remember that since the test measures the inhibition of the AChE enzyme, the EACs are lower than the respective BACs. Overall the median AChE activity values for the four fish species ranged between 100 and 400 nmol/min/mg protein, which would suggest that the assessment criteria maybe similar to those already established. However, species specific assessment criteria will need to be developed before they can be used effectively in risk assessment. The data generated from this study as well as from WCM2013 can be used to help establish such criteria in field populations.

For the fish species analysed in the present study a significant reduction in AChE activity was observed in tusk and in saithe from the Njord A platform compared to their respective reference group. This indicates that both tusk and saithe were exposed to neurotoxic chemicals due to proximity to the offshore platform. In WCM2013, significant AChE inhibition was observed in saithe from both the Veslefrikk and Oseberg platforms, which may indicate the sensitivity of this fish species for detecting exposure to neurotoxic chemicals. However, more information on the AChE response of the fish species used in this study through controlled laboratory exposures to oil and gas related compounds would help to further understand their responses in field scenarios. Controlled laboratory studies have shown that heavier molecular weight PAHs (i.e. 3 rings or more) were responsible for inhibiting AChE activity in the electric eel (Kang and Fang, 1997), whilst smaller PAHs did not. The concentration of PAHs and PAH metabolites in the fish fillet and bile respectively were either below or around the limit of detection and therefore no obvious relationship could be made between exposure and effect. However, the lack of
exposure effect relationship is more likely due to the lack of sensitive chemical methods for the detection of PAHs as well as the limited range of chemicals measured that the fish may be exposed to.

Correlation analysis was performed to understand if there were possible relationships between the biological parameters (age, length, weight, CI, LSI and GSI) of the different fish species and AChE activity. For tusk, significant negative correlations were found between AChE and three biological parameters age, length and weight ($r^2$ values 0.40, 0.36, 0.24 respectively; df 27; $p<0.01$), indicating that AChE activity decreases with older and larger fish. Since significantly older and larger tusk were found around the platform it raises the question as to whether the inhibition in AChE activity observed in tusk at Njord A is related to proximity to the platform or merely as a result of older and larger fish being sampled there or a combination of both. However, significant correlations between AChE and age ($r^2$ 0.265, df13, $p<0.05$), length ($r^2$ 0.365, df13, $p<0.01$) and weight ($r^2$ 0.352, df13, $p<0.01$) were calculated in reference tusk, but not for tusk collected from near the platform, which may suggest that age and size of the fish are influencing AChE independent of contaminant exposure.

For redfish, significant negative correlations were found between AChE activity and length ($r^2$ 0.27, 28df, $p<0.01$) and weight ($r^2$ 0.27, 28df, $p<0.01$), with lower AChE activity in larger fish. However, in contrast to tusk, significantly smaller and younger redfish were found at Njord A platform compared to the reference location. In addition, redfish from the platform showed a significant correlation between AChE and fish weight ($r^2$ 0.274, 13df, $p<0.05$). It appears therefore that fish size may be influencing AChE activity in addition to contaminant exposure. Further significant correlations were found between AChE activity and CI ($r^2$ 0.34, 28df, $p<0.01$) and LSI ($r^2$ 0.1, 28df, $p<0.05$), which suggests that redfish health and nutritional status is influencing AChE. In these cases significant positive correlations were calculated with healthier fish showing higher AChE activities. When analysing only platform redfish, CI and AChE were significantly correlated ($r^2$ 0.347, 13df, $p<0.01$).

In saithe, a significant correlation was calculated between AChE and CI ($r^2$ 0.17, 21df, $p<0.05$), although in this case healthier saithe had a tendency to lower AChE activity, which was the inverse of that observed for redfish. Further significant correlations were found in platform saithe with regards to age ($r^2$ 0.291, 10df, $p<0.05$) and LSI ($r^2$ 0.263, 10df, $p<0.05$), suggesting age and nutritional status may be linked with AChE activity. There were no significant differences between the size and health/nutritional status of saithe from the platform and reference location, and the inhibition of AChE reported in saithe from the platform is likely to be due to exposure to neurotoxic compounds.

AChE activity has been measured in a range of tissue but mostly in the brain or fillet (muscle) of fish where activity levels would be expected to be higher than other tissues. There are limited data on the effects of oil related compounds on AChE activity particularly in tusk, ling redfish and saithe that are rarely used as biomonitoring species. Based on in vitro experiments with a commercially available purified AChE from the electric organ of Japanese eel, _Electrophorus electricus_, a combination of AChE inhibiting compounds and compounds stimulating AChE enzymatic activity from PW exposure were reported (Holth and Tollefsen, 2012). The AChE inhibition was considered by the authors to be unidentified aromatic compounds in the oil/particulate fraction of PW, whereas polar compounds in both the water soluble and oil/particulate fraction of PW caused an apparent stimulation of AChE activity. Therefore, exposure of the native fish to a mixture of oil related compounds containing both AChE inhibiting and AChE stimulating compounds may lead to unusual outcomes or a cancelling out of effects. Whether this was seen in the fish sampled around the platform is unsure but differences in responses by the fish species were observed, probably due to differences in exposure.

4.3.5 LMS

Lysosomal membrane stability was measured in cryostat sections of fish liver. In WCM2013, due to the high fat content of some of the fish livers sectioning the liver of tusk and ling was not achieved despite applying a range of different sectioning techniques. However, from the lessons learnt during WCM2013,
the liver of tusk and ling was treated in such a way that enabled liver sections to be obtained. As a result, the LMS assessment was performed on all four fish species.

As reported previously for AChE activity, significant reductions in LMS were found in tusk and saithe from the platform compared to their reference group. However, despite the significant reductions, the median LMS for tusk and saithe from the platform was 20 min. Although specific assessment criteria are not available for tusk, ling, redfish or saithe, there are general BAC and EAC values of 20 min and 10 min that are available (ICES, 2011). Therefore, the median value of tusk and saithe from the platform was on the BAC and indicative of a background response signifying a healthy condition. In comparison, mean LMS values measured in the hepatocytes of saithe collected at 0.2, 5 and 10 km from the Statfjord platform were 8, 11 and 10 min respectively indicating a stress response compared to the reference group of 30 min (Bilbao et al., 2006b).

Overall the median values of all fish from both platform and reference were on or above the BAC value of 20 min indicating a healthy condition. However, further development of LMS in these fish species is required to determine whether the baseline levels of LMS and the assessment criteria described are suitable.

4.3.6 DNA adducts

Significantly higher DNA adducts were found in both saithe and redfish from the Njord A platform compared to their respective reference groups. In WCM2013, significantly elevated DNA adducts were also found in saithe from the Veslefrikk and Oseberg South installations, which suggests that the saithe are either particularly sensitive to or have more exposure to genotoxic compounds. Differences in food source and habitat niche may be factors responsible. For instance, saithe are active predators that feed in both deep and mid-water. Diet is mostly composed of herring, Norway pout and sand eel. Bioaccumulation of chemicals through the food web as a predator is likely to increase the exposure to genotoxic compounds. Adult saithe are believed to display diel vertical migrations, coming up in to the mid water column during the night and moving close to the bottom during the day, therefore they can be described as benthopelagic and not bound to a demersal life style (Bergstad, 1991). Consequently, they may be expected to show more extensive geographical migrations then the demersal fish species such as ling and tusk. Previous tagging experiments have found recaptures of saithe over a wide range with some population exchange between the Norwegian coast, Faroe Islands and Iceland (Reinsch, 1976). Therefore, the genotoxic response shown in saithe may not be totally attributed to the proximity to the Njord A platform only, but from other regions within the North Sea.

Redfish are also considered to be benthopelagic, found close to the bottom in the day and moving up in the water column during the night in response to their prey. Redfish are semi-pelagic and are more likely to exhibit geographical migration away from the platform for a limited time. As a result the DNA adducts shown for the redfish in this study may reflect exposure to genotoxic compounds in other areas of the North Sea and not solely due to proximity to the Njord A installation.

The diet of redfish consists mostly of planktonic crustaceans (i.e. amphipods, copepods, calanoida and euphausids), as well as small fish (Pálsson, 1983). This differs to that described for saithe, although exposure to genotoxic compounds via the food web is a likely source. Redfish are known to be slow growing and long-lived, the fish sampled in our study were mostly between 20 and 30 years with a narrow length range of 40 to 50 cm. The older fish are more likely to acquire contaminants in their tissues with a longer exposure duration and increased likelihood to bioaccumulate. The higher DNA adducts in Njord A redfish may reflect the longer exposure duration. However, when considering the age of the fish, the redfish from the Njord A platform were significantly younger than those from the reference area, despite significantly higher DNA adducts in the platform group. Therefore, the genotoxic response exhibited by redfish was unlikely to be an artefact of age.
ICES assessment criteria for DNA adducts have been developed for a handful of fish species including dab, flounder, cod and haddock (ICES, 2011). With the frequency of BACs for DNA adducts ranging from 1 (dab, flounder), 1.6 (cod) and 3 (haddock) nm adducts per mol DNA. For the four fish species in our study, the two demersal fish species tusk and ling, median DNA adduct levels of were <1 and 2 respectively. However, saithe and particularly redfish had median DNA adduct levels above the highest BAC available at 3.4 and 6 nm adduct per mol DNA. The redfish value was comparable to the established EAC in fish and indicates a genotoxic response that could result in detrimental effects on the individual.

The lack of response in DNA adduct formation in the demersal fish (tusk and ling) was surprising, particularly since they inhabit and feed off the contaminated sediments below the Njord A platform. In WCM2013, DNA adducts in tusk were 31 nmoles adduct/ mole DNA, which was 5 times the EAC value and indicative of extremely high genotoxic response. The same method and laboratory was used in these two studies.

The suitability of the available assessment criteria is somewhat uncertain due to the absence of species specific assessment criteria for the fish sampled in our study. Establishment of species specific BACs and EACs would greatly improve the assessment of environmental harm and should be given priority in future research activities. Despite the lack of species specific assessment criteria, the high median DNA adducts exhibited by redfish were likely to be above any proposed EAC for this species.

DNA adducts have a few advantages over other biomarkers one of these is that they are persistent for several months once formed (Stein et al., 1993). Therefore, they provide an assessment of chronic exposure accumulated over many weeks. This is in contrast to other biomarkers such as EROD or the presence of PAH metabolites, and is particularly useful when measuring responses in wild fish as opposed to field transplanted fish, where the exposure period is increased. Furthermore, DNA adducts are not thought to be influenced by factors such as gender, season or nutrition, thereby increasing the reliability of the biological response.

4.3.7 Comet

Due to the challenges in performing the comet assessment offshore, the prepared slides containing the attached blood sample in agarose were stored in a buffer solution for transportation back to the laboratory. For the platform fish, the quality of the blood cells was suitable for comet tail assessment; however, those from the reference cruise were particularly damaged and could not be scored. The poor quality of the blood cells was thought to be an artefact of the sample preparation and the extended period at which the prepared slides were kept in the buffer solution before being brought back to the laboratory. For example, the approximate time from when the samples were collected and finally processed was 3-5 days for the platform fish and up to 10 days for the reference fish. This was mostly due to logistical differences and the longer duration of the reference cruise. As a result, reference fish from WCM2013 were used for comparison of the comet data. However, in 2013, redfish were not sampled and therefore only comparisons were available for tusk, ling and saithe. The comet analysis in WCM2013 and WCM2014 was performed by the same laboratory using identical protocols and assessment. Therefore, it was considered acceptable for the reference values from WCM2013 to be used for comparison. With the exception of ling, the reference fish from WCM2013 were of similar size to those fish from Njord A. Ling collected from the platforms in both years were significantly larger than their reference fish and therefore may have had an impact on the higher DNA strand breaks observed in ling at the platform.

The frequency of DNA strand breaks were measured with the comet assay. Data were presented as percentage comet tail as well as enzyme treatment, which provides a measure of the oxidation of DNA base pairs. For the percentage comet tail, indicating DNA damage, significantly higher % DNA damage was found for tusk, ling and saithe from the platform compared to their respective reference groups from WCM2013. As stated above, reference fish for comparison were not available for redfish, although the level of DNA damage exhibited by the redfish from the platform were comparable to that of reference tusk and saithe. Although large intra-variation was observed for tusk and ling, median % DNA damage
was approximately 60% for tusk ling and saithe compared to the reference group (<20%). The median DNA damage from the comet assay in tusk, ling and saithe from the Njord A platform was markedly higher than that found for the same fish species at the Veslefrikk (ling 10%, tusk 5%) and Oseberg South platforms (saithe 20%). The significantly higher DNA damage detected in Njord A fish may be compounded by methodical challenges with performing the assay offshore as stated earlier, leading to higher levels of DNA damage. However, the extent at which such problems with the method have influenced the level of DNA damage is uncertain since such studies of this nature are not regularly performed and effects of sample storage are not fully known.

Tusk and ling are true demersal fish species living on the sea floor, these fish are more likely to be exposed to sediment dwelling contaminants from historic drilling muds and leakages from disposal wells that are thought to occur around the Njord A platform. However, similarly high levels of DNA damage were also found for saithe, which is not a demersal fish species but prefers to occupy the lower part of the water column, contaminant exposure via the diet may be a more likely exposure pathway for this species.

Assessment criteria for the comet assay have not yet been established for the fish species in our study and currently only BACs are available for dab and cod, both of which stand at 5% DNA tail (ICES, 2011). Considering this value for our data would clearly show that in all cases, except for reference ling, the median value was above this BAC, including many of the reference groups. This was thought to reflect the sub-optimal conditions under which the gels were prepared, rather than a true indication of DNA damage in the fish from these areas. However, since species specific baseline data are not available in wild fish populations, it is difficult to make the assumption. Assessment criteria need to be established for wild populations of the fish species in our study in order to improve the current risk assessment. Consideration of the assessment criteria should be made with respect to offshore sampling, since sample processing needs to be compromised due to the difficulty in performing the electrophoresis on board a research vessel.

High background levels of DNA damage have been found to derive from variations in methodology. Performing the assessment under either mild alkaline (pH 12.1) or alkaline conditions (pH>13) can have varying results, and the mild alkaline version of the assay should be used for fish blood cells to maintain low background levels of DNA damage (Moretti et al., 1998; Wirzinger et al., 2007). In the current study mild alkaline conditions were employed and were therefore not responsible for the higher than expected background concentrations.

Enzyme treatment provided an assessment of the frequency of oxidation of the DNA base pairs. This assessment showed that significant increases were found only for tusk from the platform compared to the reference group. High variability in ling from the platform group prevented a significant difference from being calculated. For example, the median value was approaching 80% DNA damage including oxidation of base pairs in ling from the platform, compared to <20% in the ling reference group.

The measurement of base oxidation is considered to be less influenced by the sub-optimal conditions under which the gels were prepared and therefore should be considered as a more reliable effect endpoint in this study. In environmental monitoring, the additional step that enables base oxidation to be assessed is not always performed and so data and assessment criteria are not available for comparison.

Mostly due to the difficulty in performing the comet assay offshore, there is little data on comet from marine fish. With respect to PAH exposure, eelpout (Zoarces viviparus) were used to monitor the effects of a bunker oil spill in Gothenburg harbour, Sweden, where DNA damage was correlated with the presence of PAH metabolites in the bile (Frenzilli et al., 2004). The levels of DNA damage ranged from approximately 10% DNA tail in reference eelpout to 20 and 30% DNA tail in more exposed areas. These values, approaching 30% DNA tail, were below the DNA damage measured in tusk, ling and saithe, although comparable to redfish from around the platform.
Offshore monitoring studies with dab have shown that both sex and age of the fish can have significant effects on the presence of DNA strand breaks (Akcha et al., 2003; 2004). In the present study the comet data were compared to the reference fish from WCM2013. Significantly larger and assumingly older ling and saithe were sampled from the Njord A platform than the respective WCM2013 reference group. Therefore age may have contributed to the higher % of DNA damage in the Njord A ling and saithe.

With respect to gender differences, adult dab have been reported to have higher levels of DNA strand breaks in males then in females (Akcha et al., 2004). The only marked differences in gender ratios were found in ling from the Njord A platform where 19 of the 20 ling were female. The high degree of DNA strand breaks in this group of females is contrary to Akcha et al. (2004) and is unlike to act as a confounding factor. Akcha et al., (2004), also found a significant interaction between age and sex that may suggest reproductive status could interfere with the extent of % DNA strand breaks. However, simple correlations were performed on comet strand breaks in relation to GSI (data not shown) with no significant relationship observed.

4.3.8 Histopathology of liver and gill

The histopathological examination of the liver in fish provides important information concerning fish health status at tissue/organ level. All of the four analysed species from all groups (platform and reference) showed various percentage/occurrence of different histological abnormalities and pathologies. There was an increased prevalence of melanomacrophage aggregates in fish from the Njord A platform compared to the reference group, which may suggest a response to contaminant exposure. Melanomacrophage centres are known to vary in size and colour according to the variety of pigments and the cellular content (Agius and Roberts, 2003; Fricke et al., 2012). Their increase in size and presence is associated with environmental stress conditions (Wester et al., 1994; Bilbao et al., 2006). In particular, as regard saithe, previous studies also showed a higher presence in hepatic melanomacrophage aggregates in fish collected closer to the Statfjord platform (Bilbao et al., 2006). It has been proposed as a reliable biomarker for water quality in term of both deoxygenation and chemical pollution (Agius and Roberts, 2003). However, some findings suggest that the morphological features of melanomacrophage aggregates may be weight or age related. Significant differences in the size and age of some fish species were found between locations. For instance, ling and tusk were older and heavy, whilst redfish were lighter and younger at the platform than their reference group. However, no obvious pattern between melanomacrophage aggregates and age for the different fish species were observed, since younger redfish and older tusk and ling were found to have increased occurrence of melanomacrophage aggregates at the platform.

Steatosis lesions were found in all the analysed samples. They are suggested to be part of the normal cyclic (and non-pathological) status of liver. Similar occurrence of steatosis lesions were found in WCM2013. There was almost no difference in the presence of circulatory disturbance, except for the saithe. In general, other minor pathologies were found in liver of all species collected at Njord A. As regards parasite presence, this parameter did not show liver parasitic invasion. Furthermore, this parameter is less likely to be influenced by exposure to contaminant stress.

Since the presented species are not used routinely in monitoring programmes and thus not regularly examined for liver pathology, improved information on liver pathology in these fish species are required before they could be better incorporated into biological effects monitoring programmes. The obtained results from the analysed species will be useful for further investigation of normal liver histology as well as establishing new biomarkers in fish pathology (e.g. melanomacrophage centres), which are widely used in histopathology of other species. All these improvements together with obtained results will strengthen use and inclusion of histopathology in future monitoring programmes.

Since the gills of fish are in close contact with the surrounding water, the tissue structure is particularly vulnerable to pollutants. Gill pathologies are common symptoms of toxic effects on fish from a wide range of aquatic pollutants including PAHs (Au, 2004). Previous histopathological studies of effects from

Epithelial hyperplasia and lamellar fusion are believed to represent chronic responses to environmental effects (Poppe, 1999), where epithelial hyperplasia is an early stage of lamellar fusion (Cerqueira and Fernandes, 2002; Oliva et al., 2009; Poppe, 1999). Hypertrrophy and hyperplasia of gill epithelium showed a species specific response. For tusk and saithe, values were higher in individuals collected at Njord A, whilst for ling and redfish, values were higher in fish sampled at the reference site. As regards fusion and disorganisation of gill lamella, values were higher in fish collected in the vicinity of Njord A for tusk and ling. The clearest signal was given by the presence of necrosis and cell degeneration. These lesions were higher in fish collected in the vicinity of Njord A. For three out of four species (excluding redfish), these lesion were present only in individuals collected at the platform site.

4.3.9 Integrative assessment

The integrated biological response index (IBR/n) was applied to the data in order to determine the overall impact of the Njord A platform on the local fish populations. Based on sediment contaminant and leakage from disposal wells the Njord A platform was considered to represent a contaminated site. The IBR/n scores were able to differentiate between the reference and the platform populations, with markedly higher IBR/n values in all platform fish compared to their respective reference group. The two demersal fish species (tusk and ling) that occupy and feed from the seafloor were expected to be exposed to contaminants in the sediment and drill cuttings. However, although a marked response in IBR/n was observed for tusk, ling only showed a slight increase in IBR/n compared to the reference group. Instead redfish and saithe were found to respond together with tusk and were clearly able to differentiate between the platform and the reference groups.

When examining the biomarkers that contributed to the IBR/n, it was interesting to note that EROD contributed very little to the overall score in all fish from the platform. Although the main contributor to the IBR/n differed between the species, overall histology (liver and gill) and DNA adducts appeared to contribute most. These endpoints are retained for a longer time by the fish compared to enzyme responses. Therefore, this may suggest that these fish were not necessarily experiencing effects from recent contaminant exposure but exposure weeks or months prior to sampling. AChE inhibition also contributed to the IBR/n score of tusk, saithe and redfish. However, from the individual AChE data, significant inhibition of AChE activity was only found in tusk and saithe indicating exposure of these fish to neurotoxic compounds at the Njord A platform. It was unfortunate that the results of the comet assay could not be added to the IBR/n calculation, due to unsuitable reference samples, since significant levels of DNA strand breaks were recorded in tusk, ling and saithe, and would have supported the genotoxicity effects found from the DNA adduct data.

It should be mentioned that the difference in fishing methods between the platform and reference group may have led to sample bias. For instance, using passive fishing methods of rod and line fishing requires activity on the part of the fish, which could potentially lead to selecting the most active and fittest individuals, whilst trawling may be more representative of the population. However, if this was the case and the fish caught from the platform were fitter individuals of the population, the biological effects observed would represent a conservative evaluation of fish health status.

4.4 Considerations for future WCM programmes

The suite of biomarkers selected provided effect measurements at different levels of biological complexity, from subcellular (DNA adducts, comet), enzymatic (AChE, EROD), cellular (LMS) and tissue (histopathology) responses. Of these biomarkers the sub-cellular endpoints measuring DNA damage
appeared to be the most responsive with significant increases in the frequency of DNA adducts and % comet tails in fish species living in the vicinity of the offshore platform. These two biomarkers were found to be the most sensitive for monitoring the apparent low exposure environment and should be included in future WCM programmes where fish are utilised. DNA adducts and comet were also the most responsive biomarkers in WCM2013 and confirms the advantages of using these methods in future WCM programmes. However, difficulties with the comet assay offshore were evident in the present study, with poor quality of the reference slides preventing comet analyses being performed. A review of the best methods for the collection and storage of the comet slides needs to be performed in order to standardise the quality of the samples for future offshore activities.

AChE activity also showed responses with significant reductions in saithe and redfish from the Njord A platform compared to the reference group and showed potential for measuring biological responses from oil related compounds. AChE inhibition was also responsive in WCM2013, where saithe again showed significant inhibition in their platform populations (Veslefrikk and Oseberg South). In contrast, EROD showed no response in any of the wild fish living around the Njord A platform or around the Veslefrikk and Oseberg South platforms in WCM2013, despite its known relationship to organic chemical exposure (Goksøyr and Förlin, 1992). Its effectiveness in biomonitoring programmes relies on the control of the many confounding factors that are known to influence EROD activity. The lack of response in field studies may be related to one or more of these cofactors. Despite this EROD continues to be a sensitive and well validated biomarker in fish and should remain as a biomarker measure in future WCM programmes.

Due to difficulties in sectioning the fatty liver, LMS was only performed fully in two of the five species in WCM2013. However, mostly due to the lessons learnt during the previous year, in the present campaign high quality sections of the fish liver were obtained. The LMS was not able to differentiate between the groups and all fish were considered to be in good health. LMS is a reliable biomarker of fish health and should be used in future programmes. Further work on the species specific assessment criteria for the four species should be performed to confirm if the present values are appropriate.

Liver histopathology is often considered to be a sensitive biomarker of longer term exposure and more appropriate for wild fish species compared to field transplanted studies due to the increased length of exposure. Histopathological responses would be expected to remain in the exposed tissues of the fish for longer, in contrast to enzymatic measures, which may vary from day to day. Histopathological responses were observed in fish from the Njord A platform compared to the reference groups, which may be related to the genotoxic responses observed at the subcellular level in these fish. However, the challenge of performing histopathology on new species makes interpretation difficult, and more validation data for these species is required from both controlled laboratory exposures and field investigations.
5. Conclusions

- Passive samplers positioned 1 m above the substrate detected low ng/ L concentrations of PAH and NPD compounds in seawater. Non-target and suspect target analysis detected a range of organic compounds in LDPE and POCIS extracts, with LDPEs accumulating many more measureable compounds than POCIS.

- In the POCIS extracts, several natural products and anthropogenic compounds were tentatively identified, while a few anthropogenic compounds could also be identified in the LDPE extracts.

- PAH-NPD concentrations in fish fillet and fish stomach samples from tusk, ling, redfish and saithe were either undetected or marginally above the detection limits at both the Njord A platform and the reference location.

- Only 6 of 23 PFCs were detected in the blood of tusk, ling and redfish. The 6 PFCs included PFDA, PFUnDA, PFTrDA, PFHxS, PFOS and PFOSA, of which PFOSA was the most abundant. Highest concentrations of PFOSA were found in reference ling at 40 µg/L.

- Significant biological responses in AChE, comet, DNA adducts and histopathology were observed for fish collected at the Njord A platform compared to the reference group, indicating exposure to both neurotoxic and genotoxic chemicals.

- The absence of a VTG response in exposed fish suggests that the level of exposure to estrogenic compounds was below VTG threshold concentrations.

- In all four fish species sampled the calculated IBR/n value was markedly higher in the Njord A platform population then their respective reference groups. The highest IBR/n was calculated in tusk (1.8), redfish (1.8) and saithe (1.6) from the Njord A platform.

- The lack of cause effect relationship between measured chemical exposure and observed biological response can be explained by both the increased sensitivity of the biomarkers as well as the wide range of oil and gas related chemicals that were not measured but were likely to be present.

- These results highlight the advantage of using a suite of biomarker tools to assess the potential biological effects of complex mixtures in field scenarios, which contain low individual concentrations of many compounds, which when combined can cause toxicity.
6. References


Agamy E. 2012. Histopathological liver alterations in juvenile rabbit fish (Siganus canaliculatus) exposed to light Arabian crude oil, dispersed oil and dispersant. Ecotoxicology and Environmental Safety. 75:171-179.


Assis CRD, Castro PF, Amaral IPG, Carvalho EVMM, Carvalho LB, Bezerra RS. 2010. Characterization of acetylcholinesterase from the brain of the amazonian tambaqui (Colossoma macropomum) and in vitro effect of organophosphorus and carbamate pesticides. Environmental Toxicology and Chemistry. 29:2243-2248.


Bocquené G; Galgani F; Truquet P. 1990. Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. Marine Environmental Research. 30:75-89.


Burke MD, Mayer RT. 1974. Ethoxyresorufin – direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inductible by 3-methylcholanthrene. Drug Metabolism and Disposition 2:583-588.


Di Tuoro D; Portaccio M; Lepore M; Ardunii F; Moscone D; Bencivenega U; Mita DG. 2011. An acetylcholinesterase biosensor for determination of low concentrations of paraoxon and dichlorvos. New Biotechnology. 29:132-138.


Feist SW, Lang T, Stentiford GD, Koehler A. 2004. Use of liver pathology of the European flatfish dab
(Limanda limanda L.) and flounder (Platichthys flesus L.) for monitoring. ICES Techniques in Marine
Environmental Sciences 38, ICES, Copenhagen.

and liver of Liza saliens, from the Esmoriz-Paramos coastal lagoon, Portugal. Arch. Environ. Contam.
Toxicol. 55, 262-269.

Frenzilli G, Scarelchi V, Barga I del, Negro M, Forlin L, Bolognesi C, Sturve J. 2004. DNA damage in

Fricke NF, Stentiford GD, Feist SW, Lang T. 2012. Liver histopathology in Baltic eelpout (Zoarces
viviparus)—a baseline study for use in marine environmental monitoring. Mar Environ Res 82:1–14

organophosphates and carbamates. Bulletin of Environmental Contamination and Toxicology. 45:243–
249.

Goksøyr A, Förlin L. 1992. The cytochrome P-450 system in fish, aquatic toxicology and environmental
monitoring. Aquatic Toxicology. 22:287-311.

Goksøyr A. 1991. A semi-quantitative cytochrome P450IA ELISA: a simple method for studying the
monooxygenase induction response in environmental monitoring and ecotoxicological testing of fish.
Science of the total environment 101 (3), 255-262.

Grant A, Briggs AD. 2002. Toxicity of sediments from around a North Sea oil platform: are metals or

Gravell A, Mills GA, Civil W. 2012. Screening of Pollutants in Water Samples and Extracts from Passive

Grosvik BE, Meier S, Lievenborg B, Nesje G, Westreheim K, Fonn M, Kjesbu OS, Skarpheðinsdóttir H,
Klungsøyr J. 2010. PAH and biomarker measurements in fish from condition monitoring in
Paper ICES CM 2010/F:06.

Hare JA, Manderson JP, Nye JA, Alexander MA, Auster PJ, Borggaard DL, Capotondi AM, Damon-
Randall KB, Heupel E, Mateo I, O’Brien L, Richardson DE, Stock CA, and Biege ST. 2012. Cusk
(Brosme brosme) and climate change: assessing the threat to a candidate marine fish species under the US

PAHs and carbazoles in semipermeable membrane devices (SPMMDs) and polar organic chemical
integrative samplers (POCIS). Chemosphere 72, 1510-1516.

Harman, C., Thomas, K.V., Tollefsen, K-E., Meier, S., Boyum, O., Grung, M. 2009. Monitoring the freely
dissolved concentrations of polycyclic aromatic hydrocarbons (PAH) and alkylphenols (AP) around a
Norwegian oil platform by holistic passive sampling. Marine Pollution Bulletin 58, 1671-1679.

passive sampling devices coupled with in vitro bioassay techniques. Journal of Environmental
Monitoring 12, 1699 – 1708.

Harman, C., Brooks, S., Sundt, R.C., Meier, S., Grung, M. 2011. Field comparison of passive sampling and
biological approaches for measuring exposure of PAH and alkylphenols from offshore produced water

receiving waters around an offshore oil platform by passive sampling. Environmental Toxicology and
Chemistry 33, 1946-1949.

substances. Review of Environmental Contamination and Toxicology. 179:99-121.

Hernández F, Portolés T, Pitarch E, López FJ. 2011. Gas chromatography coupled to high-resolution
time-of-flight mass spectrometry to analyze trace-level organic compounds in the environment, food


Holth TF, and Tollefsen KE. 2012. Acetylcholine esterase inhibitors in effluents from oil production platforms in the North Sea. Aquatic Toxicology. 112:92-98.


ICES Conference on Fish Map web link: http://www.ices.dk/explore-us/projects/EU-RFP/EU%20Repository/ICES%20FishMap/ICES%20FishMap%20species%20factsheet-Saithe.pdf


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