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The Water Column Monitoring 2013

Determining the biological effects of two offshore platforms on local fish populations

Norwegian Institute for Water Research
– an institute in the Environmental Research Alliance of Norway

REPORT

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Abstract

The biological effects of two offshore oil platforms on local fish populations were assessed as part of the Water Column Monitoring (WCM) programme for 2013. Wild fish were caught with baited rod and line from within the 500 m safety zone of the Veslefrikk and Oseberg Sør platforms during the summer of 2013. Ling, tusk and saithe were caught and sampled from the Veslefrikk platform, whilst haddock, whiting and saithe were caught from the Oseberg Sør platform. Reference fish were caught on a separate research cruise by trawling from a region of the North Sea less impacted by oil and gas activities (around Egersund bank). Reference tusk were not obtained by trawl but caught by local fishermen from a coastal fjord using long line. 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 inhibition (AChE), ethoxyresorufin *0*-deethylase levels (EROD), lysosomal membrane stability (LMS), histology, PAH metabolites, and PAH body burden. The biomarker data were integrated using the integrated biological response index (IBR/n). Biological responses were observed in fish species from both platforms compared to their respective reference groups with highest responses observed in tusk from the Veslefrikk platform.

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The Water Column Monitoring Programme 2013

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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. However, 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 exposures to drilling muds and sediment held contaminants may be more important in addition to PW chemicals.

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

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Summary

The 2013 water column monitoring programme was designed to investigate the potential biological effects of two offshore oil platforms in the North Sea 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 the impact of produced water (PW) was thought to be less important, whilst impacts from drill cuttings and other sediment sources were likely to be greater. 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 in the sea floor.

Although many of the chemicals that are present in PW effluents and 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 (*Melanogrammus aeglefinus*) collected in areas with extensive oil and gas activity (e.g. Tampen and Oseberg regions). Based on these previous findings, the present investigation focused on PAH exposure in wild fish (and invertebrates) and particularly on possible genotoxic effects. Based on historical chemical data, two different oil fields representing high (Veslefrikk) and moderate (Oseberg Sør) sediment contamination concentrations were used.

Local fish species were caught with baited rod and line from within the 500 m safety zone of the Veslefrikk and Oseberg Sør platforms during the summer of 2013. Ling (*Molva molva*), tusk (*Brosme brosme*) and saithe (*Pollachius virens*) were caught and sampled from the Veslefrikk platform, whilst haddock, whiting (*Merlangius merlangus*) and saithe were caught from the Oseberg Sør platform. Reference fish were caught from a separate research cruise by trawling from a region of the North Sea less impacted by oil and gas activities. Reference tusk were caught by local fishermen from a coastal fjord using long lines. 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), and histology in fish liver; COMET in fish blood; and acetylcholine esterase inhibition (AChE) in fish fillet. Additional biomarker analysis included proteome analysis in fish bile, which was tested on selected fish species for possible application to future WCM programmes. Furthermore, a range of invertebrates were collected from the different locations and analysed for PAH and naphthalene, phenanthrene and dibenzothiophene (NPD) concentrations.

PAH-NPD concentrations were undetected or marginally above the detection limits in fish fillet, stomach content and the invertebrates collected at both Veslefrikk and Oseberg Sør. PFCs in the blood of all fish species were higher in ling than the other fish species although only indicative of background concentrations. PAH metabolites were detected in fish caught near the platforms and were marginally higher than reference fish, although undetected in some fish and only just above the detection limits in others. Overall, chemical data that may indicate exposure to oil related compounds were low in all fish and invertebrates.

Biomarker responses were observed in some fish species from the platform populations, significantly higher levels of DNA adducts were found in saithe at both Veslefrikk and Oseberg Sør compared to the reference group and higher DNA adducts were also found in tusk and whiting at a level above the environmental assessment criteria (EAC). AChE inhibition was also lower in saithe from both Veslefrikk and Oseberg Sør and significantly lower in the former. COMET tails were significantly elevated in ling above reference values, whilst the addition of oxidized base pairs indicated significantly higher DNA damage in tusk and ling from Veslefrikk and whiting from Oseberg Sør.

The biomarker data were also presented using the integrated biological response index (IBR/n). Biological responses were observed in fish species from both platforms compared to their respective reference groups with highest responses observed in tusk from the Veslefrikk platform. Overall, the fish from the Veslefrikk platform were more impacted than those from both the Oseberg Sør and reference groups.

Sammendrag

Vannsøyleovervåkingsprogrammet for 2013 (WCM 2013) ble designet for å undersøke potensielle biologiske effekter på lokale fiskepopulasjoner i nærheten av to offshore oljeplattformer i Nordsjøen. Ulike arter av bunnfisk ble valgt for innsamling ettersom det antas at bunnfisk har lavere sannsynlighet for å migrere bort fra plattformene enn pelagisk fisk. Ved å fokusere på organismer som levere dypere i vannsøylen antas det at påvirkningen av produsert vann vil være mindre viktig mens påvirkning fra borekaks og andre sedimentkilder vil være av større betydning. Både borekaks deponert før utslippsforbudet av oljeholdig borekaks fra 1993 samt nyere utlekking fra deponeringsbrønner er potensielle kilder til oljeeksponering for organismer som spiser og/eller bor i nærheten av disse kildene på havbunnen.

Selv om mange av kjemikaliene som finnes i produsert vann og boreslam har potensiale for å indusere biologiske responser, er det begrensede muligheter for å vurdere potensiale for skadelige effekter på grunn av mangel på tilstrekkelige *in situ* overvåkningsstudier. Tidligere Norske tilstandsovervåkingsprogrammer har påvist eksponering for polyaromatiske hydrokarboner (PAH) og økte nivåer av DNA addukter i hyse (*Melanogrammus aeglefinus*) fra områder med utbredt olje- og gassaktivitet (Tampen- og Oseberg-områdene). På bakgrunn av disse tidligere observasjonene fokuserte WCM 2013 på PAH eksponering i vill fisk (og invertebrater) og spesielt på mulige genotoksiske effekter. Basert på historiske kjemiske data ble det valgt ut to oljefelt som representerte høye (Veslefrikk) og moderate (Oseberg sør) sedimentkonsentrasjoner av miljøgifter.

Lokale fiskearter ble samlet inn med fiskestang og fiskesnøre innenfor sikkerhetssonen på 500 m av Veslefrikk og Oseberg Sør plattformene i løpet av sommeren 2013. Lange (*Molva molva*), brosme (*Brosme brosme*) og sei (*Pollachius virens*) ble samlet fra Veslefrikk plattformen mens hyse, hvitting (*Merlangius merlangus*) og sei ble samlet fra Oseberg Sør plattformen. Referansefisk ble samlet med trål fra et forskningsfartøy fra et område i Nordsjøen som var mindre påvirket av olje- og gassaktivitet. Referansebrosme ble fisket med line av lokale fiskere i en kystnær fjord.

Både den kjemiske belastningen av miljøgifter og en rekke validerte biologiske effekt-endepunkter ble målt i alle fiskegrupper inkludert PAHer i filét og mageinnhold; perfluorinerte forbindelser (PFCer) i blod; PAH metabolitter i galle; DNA addukter, ethoxyresorufin *O*-deethylase (EROD), lysosomal membranstabilitet (LMS), og leverhistologi; COMET i blod; og inhibering av acetylcholin esterase (AChE) i filét. Ytterligere biomarkøranalyser inkluderte proteom-analyser i galle fra utvalgte fiskearter for vurdering av mulig fremtidig bruk i Vannsøyleovervåkingsprogrammer. I tillegg ble det fra de ulike områdene samlet inn en rekke invertebrater som ble analysert for PAH og naftalen, fenantren og dibenzothiophen (NDP).

PAH-NDP konsentrasjoner var enten under eller marginalt over deteksjonsgrensen i filét og mageinnhold fra fisk og i invertebratene samlet ved Veslefrikk og Oseberg Sør. Konsentrasjonene av PFOS i blod fra fisk på nivå med bakgrunnskonsentrasjoner men var høyere i lange enn i de andre artene. PAH metabolitter ble detektert i fisk fanget nær plattformene og var marginalt høyere enn i referansefisken (konsentrasjonene var enten under eller marginalt over deteksjonsgrensen). Kjemiske data som indikerer eksponering til oljerelaterte kjemikalier var generelt sett lave i all fisk og invertebrater.

Biomarkørresponser ble observer i noen fiskearter fra populasjoner rundt plattformene; signifikant høyere nivåer av DNA addukter ble funnet i sei fra både Veslefrikk og Oseberg Sør sammenlignet med fisk fra referanseområdet. Høyere nivå enn miljø vurderingskriterier (EAC) av DNA addukter ble også funnet i brosme og hvitting. AChE-inhibisjon var også lavere i sei fra både Veslefrikk og Oseberg Sør og signifikant lavere i Veslefrikk. Verdien for halelengden fra COMET analysene for lange var signifikant større enn referanseverdiene, mens nivået av COMET og oksiderte basepar var signifikant høyere enn referanseverdier i brosme og lange fra Veslefrikk og hvitting fra Oseberg Sør.

Biomarkørdataene ble også presentert ved bruk av integrert biologisk respons indeks (IBR/n). Biologiske responser ble observert i fiskearter fra begge plattformer sammenlignet med deres respektive referansegrupper. Høyest respons ble observert i brosme fra Veslefrikk-plattformen. Fisk fra Veslefrikk-plattformen var generelt sett mer påvirket enn fisk fra Oseberg Sør og referansegruppene.

1. Introduction

1.1 General purpose of the study

The main purpose of the WCM programme is 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. Within the Norwegian sector of the North Sea, 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. Although approval is required from the Norwegian authorities (Norwegian Environment Agency), the operators together with the Norwegian Environment Agency choose the study area and the design of the programme. In recent years a group of scientific experts, set up by the Norwegian Environment Agency 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 on 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.

In the present campaign, field transplanted animals were not used, instead the monitoring effort was moved towards local fish populations reported to inhabit offshore oil installations in the North Sea. 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 the impact of PW may be less important, whilst impacts from drill cuttings and other sediment sources may be more influential. In the deepest part of the water column close to installations, drilling discharges may be a 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 in the sea floor.

The varying degrees of exposure of drilling muds and/ or PW discharge to fish will be influenced by the depth in the water column that the animals predominantly inhabit. For example, it would be expected for demersal fish species to be mostly influenced by exposure to drilling muds and leakages from disposal wells, whilst pelagic fish would be more influenced to PW whilst occupying the top 50 m. However, seasonal stratification of the water column could potential influence the distribution of the PW leading to mixing with deeper water in the winter and trapping of the PW above the stratified layer in the summer months. The present study was performed in June where stratification of the water column would be expected. This would result in limited mixing of the PW with the deeper sea water. Consequently, the demersal fish and even the deeper dwelling pelagic fish, which occupy waters below the stratified layer, are likely to be mainly influenced by contaminants from the seabed, rather than PW exposure.

Produced water

Produced water (PW) is a complex mixture that varies in chemical composition depending on the formation and the age of the reservoir. However, the main organic chemical components include: benzene, toluene, ethylbenzene and xylene (BTEX); naphthalenes, phenanthrenes and dibenzothiophenes (NPD), polycyclic aromatic hydrocarbons (PAHs), organic acids, and alkylated phenols (APs) (Røe and Johnsen, 1996; Utvik, 1999). Improved cleaning procedures at the platform removes the vast majority of the oil in the PW prior to discharge, with the current discharge limit of oil in water typically maintained below 30 mg/ L. However, large volumes of PW are discharged annually. For example, in 2012 about 130 million cubic meters of PW were discharged within the Norwegian continental Shelf (Norwegian Oil and Gas, 2013).

Drill cuttings and muds

Regulations put in place 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 concentration (Grant and Briggs, 2002). The total hydrocarbon concentration measured in drilling piles collected from the North Sea are in the range 10 000 to 600 000 mg/kg (Bell *et al.*, 2000; Breuer *et al.*, 2004; Park *et al.*, 2001; Westerlund *et al.*, 2001).

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 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 PW effluents and drilling muds 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 (Grøsvik *et al*, 2010; Pampanin *et al.*, 2013). Based on these previous findings, the present investigation will focus on PAH exposure in wild fish (and invertebrates) and particularly on possible genotoxic effects. Two different fields representing high (Veslefrikk) and moderate (Oseberg Sør) contamination will be used. Fish and invertebrates will be collected from around these offshore installations as well as from areas far from oil and gas installations in the North Sea as reference material. The measurement of a suite of biological effects markers and chemical bioaccumulation in these fish species will be used to assess the impact of the offshore installations on the organisms living in the vicinity.

1.3 Objectives

The objective of the work was to perform biological effects monitoring of wild fish populations in the vicinity of two offshore platforms. A suite of biological effects markers and chemical analyses were used to assess the potential impact of these platforms on the local fish populations.

1.4 Background on the biological effects methods selected

The biomarker tools have been selected in most part from the International Council for the Exploration of the Sea (ICES) recommended list of biomarkers for biological effects monitoring in marine organisms (ICES, 2011). Validation data is available for many of these biomarkers in marine fish increasing their reliability as a tool 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 specific genotoxicants. 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 semipermeable membrane that contains numerous hydrolytic enzymes involved in a range of cellular processes including digestion, defence, and reproduction (Viarengo *et al.*, 2007). 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*) (Zorita *et al.*, 2008) and Atlantic cod (*Gadus morhua*) (Holth *et al.*, 2011). It has been suggested that a median destabilization time of less than 10 min would indicate the onset of pathology, 20-30 min indicate compensating stress and values over 40 min reflect an unaffected state (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).

1.4.4 Fish Histology

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 to be used for future water column monitoring purposes.

1.4.5 Acetylcholine esterase inhibition

Acetylcholine esterase (AChE) is an essential enzyme for the neurotransmission because it 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 nervous central 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 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 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.

1.4.8 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, although they have been slowly phased out since their ban in 2007.

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

2. Methods

2.1 Study design

The design of the monitoring programme was to compare the health status of local fish populations living in the vicinity of two offshore platforms with reference fish from the North Sea. The collection of the fish species and the sampling of the fish took place within three separate research cruises. The details of these cruises can be found in the WCM2013 cruise report (Appendix A), although a brief description of the activities related to the collection of the fish is provided below.

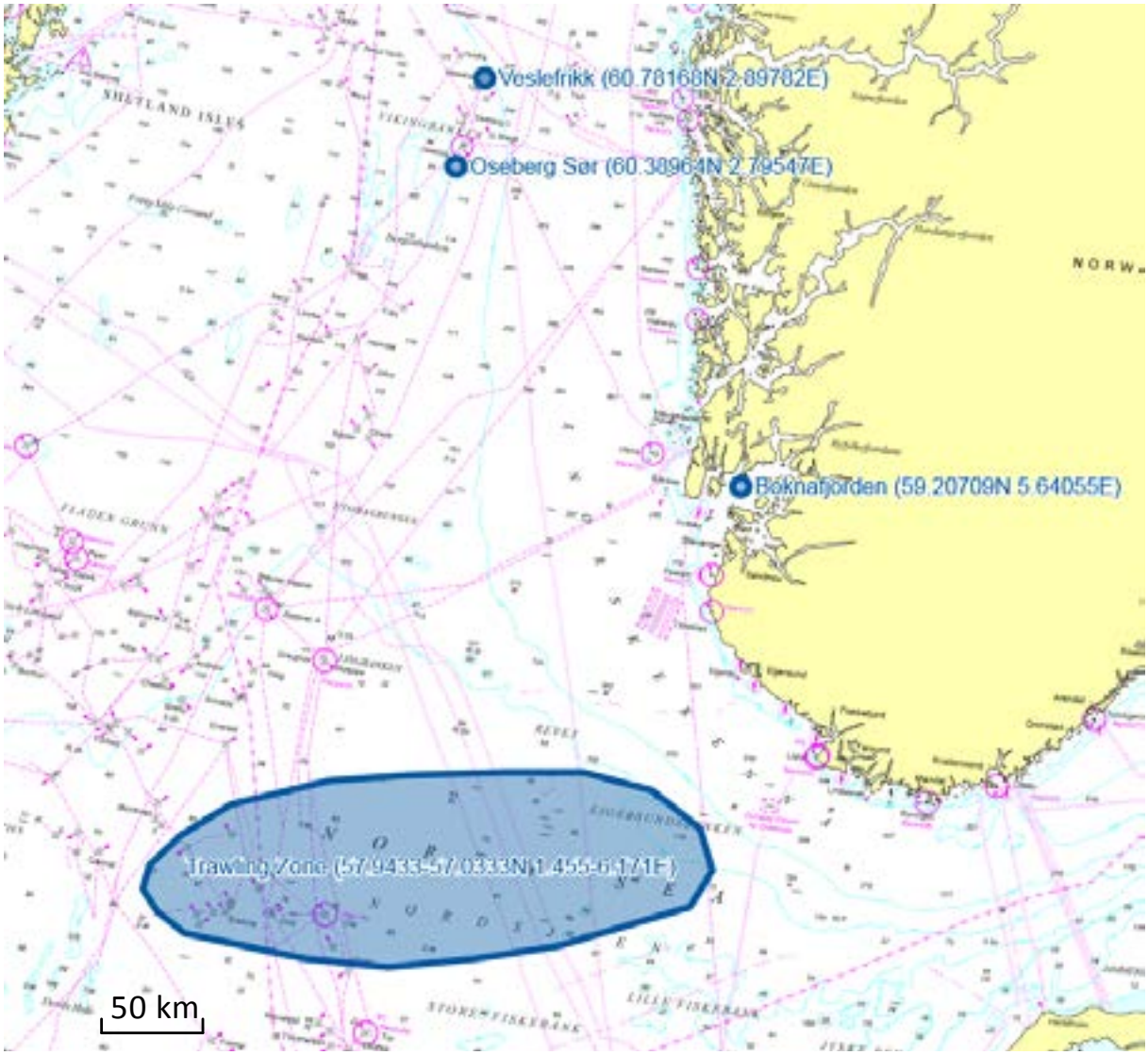

Figure 1. The study area in the North Sea, highlighting approximate positions of the Veslefrikk and Oseberg Sør platforms and the trawling zone for the collection of the reference fish. Co-ordinates in WGS84. (Map taken from www.norgeskart.no).

2.2 Offshore sampling

2.2.1 Collection of fish and invertebrates near the platforms

Fish and invertebrates were collected from a vessel within the 500 m safety zone of two offshore North Sea platforms, Veslefrikk and Oseberg Sør (**Figure 1**). The collection was focused within 100 meters of the platforms. Due to subsea installations near the platforms only "passive" fishing gear could be used. Rod and line fishing using mackerel (*Scomber scombrus)* and herring (*Clupea harengus)* as bait proved extremely effective and a steady stream 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 two platforms are summarised in **Table 1**. A range of samples were taken from each fish, 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. An overview of the samples taken and the analysis performed are shown in **Table 2**.

Table 1. Number of fish species sampled from the two offshore installations

Fish species	Common name	Veslefrikk	Oseberg Sør
Brosme brosme	tusk	20	
Molva molva	ling	21	
Pollachius virens	saithe	17	15
Merlangius merlangus	whiting		20
Melanogrammus aeglefinus	haddock		20

Table 2. Chemical analysis and biological responses measured in different tissue matrices of the wild caught fish populations.

	Analysis	Matrix
Biological response	DNA adducts	Liver
	EROD	Liver
	Lysosomal membrane stability	Liver
	Histology	Liver
	COMET assay	Blood
	Acetylcholine esterase inhibition	Fillet
	Proteome	Bile
	PAH metabolites	Bile
Chemical accumulation and exposure	PFC	Blood
	PAH-NPD	Fillet
	PAH-NPD	Stomach content

Invertebrate samples were collected with baited fish cages deployed overnight at both platforms. The invertebrates caught from the Veslefrikk platform included, four Norway lobster (*Nephrops norvegicus*) and numerous amphipods and isopods; whilst those caught from Oseberg Sør included several hermit crabs (*Pagurus bernhardus*), anemones and whelks. The different invertebrate groups were analysed separately for PAH-NPD concentrations.

2.2.2 Collection of fish at the reference locations

Following the collection of the five fish species from the Veslefrikk and Oseberg Sør platforms, five species were targeted from a separate research cruise conducted by the Institute of Marine Research (IMR). A scientist from NIVA was on board the research vessel to ensure that the reference fish were treated in the same way as those collected from around the platforms. The reference fish were collected by trawling in the area highlighted in **Figure 1**. The reference fish collected from the IMR cruise included: 25 whiting, 24 haddock, 23 saithe and 9 ling. Tusk were not caught with the trawl and a separate activity using local fisherman in Bokna fjord, near Stavanger was used to successfully collect sufficient reference

tusk (location shown in **Figure 1**). The sampling protocol for the collection of the fish samples was identical for all sampling activities.

2.2.3 Oceanographic measurements

Conductivity, temperature and depth as well as turbidity were measured at both Veslefrikk and Oseberg Sør platforms on two occasions during the fishing effort with the aid of an STD/CTD-model SD204. Vertical profiles down to approximately 50 m were performed at each platform.

2.3 Analytical methods

2.3.1 PAH-NPD in fish stomach/ fillet and invertebrates

Samples were defrosted, homogenised and an approximate 5 g sub sample 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.

2.3.2 PFC in fish blood

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 suite of PFCs measured included: perfluorooctanoic acid (PFOA); perfluorooctanesulfonic acid (PFOS); perfluorononanoic acid (PFNA); perfluorobutanesulfonic acid (PFBS); perfluorooctanesulfonamide (PFOSA); perfluorohexanoate (PFHxA); perfluoroheptanoic acid (PFHpA); perfluorohexanesulfonic acid (PFHxS); perfluorodecanoic acid (PFDcA); and perfluoroundecanoic acid (PFUdA).

2.3.3 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 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 pyrene fluorescence equivalents (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 hours. The OH-PAHs were extracted with

ethylacetate (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 BSTFA and heating for two hours 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, Shimudadzu 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^{-1}, 120°C to 300°C at 6°C min^{-1} 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 Biomarker methods

2.4.1 Health Indices

The total weight and length of each fish was measured on board the vessel. The fish were sexed by visual examination of their gonad. A motion compensated balance (Marel M2000 series) was used to measure liver and gonad 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.

$$\text{Condition index} = [\text{Weight (g)}/\text{Length (cm)}^3)]\times 100$$

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

$$\text{LSI} = [\text{Liver weight X } 100] \,/\, \text{fish weight}$$

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

$$\text{GSI} = [\text{Gonad weight X } 100] \,/\, \text{fish weight}$$

2.4.2 EROD

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 recentrifuged (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). EROD activity was assayed fluorimetrically in a plate reader. Briefly, samples of microsomes were diluted to ~2 mg/mL in buffer and pipetted (50 µL) in 6 technical replicates onto a 96 well microplate. Pre-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 normalized 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 was bovine gamma globulin.

2.4.3 Acetylcholine esterase 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:

Acetylthiocholine (ATC) → Thiocholine + Acetate

Thiocholine + Dithiobisnitrobenzoate (DTNB) → 5-thio-2-nitrobenzoic acid (TNB)

The measure of the conversion of DTNB to TNB can be used as a measure of the hydrolysis of ATC into thiocholine. ATC is produced from hydrolysation of the neurotransmitter acetylcholine by the 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, Nürnbecht, 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:

$$\text{AChE activity } (\mu\text{mol ATC/ min/ mg protein}) = \frac{\Delta A \times \text{Volt} \times 1000}{\varepsilon \times \text{lightpath} \times \text{Vols} \times [\text{protein}]}$$

Where ΔA = change in absorbance (OD) per minute at 405 nm, corrected for spontaneous hydrolysis, Volt = total assay volume (0.380 mL), ε = extinction coefficient of TNB (M/ cm), lightpath = microplate well depth (1 cm), Vols = sample volume (in mL), and [protein] = concentration of protein in the enzymatic extract (mg/mL)

2.4.4 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).

Using a cryostat chamber (object temperature -18ºC and knife -20ºC) 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 37ºC, 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 37ºC, rinsed in a saline solution (3.0% NaCl) at 37ºC for 2 min and then transferred to 0.1 M phosphate buffer (pH 7.4) containing 1mg/mL of diazonium dye Fast Violet B salt (Sigma, F1631), at room temperature for 10 min. Slides were then rapidly rinsed in running tap water for 5 min, fixed for 10 min in Baker's formol calcium

containing 2.5% NaCl and 10% $CaCl_2$ at 4ºC and rinsed in distilled water. Finally, slides were mounted in aqueous mounting medium Kaiser's glycerine gelatine.

2.4.5 DNA adducts

Preparation of DNA solutions
The fish liver samples were airfreighted on dry ice to the AdnTox laboratory 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 the crushing of liver pieces (tissue-lyser, Qiagen), isolation of cell nuclei (in sucrose 0.32M) 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, which are additional compounds in DNA associated to complex molecules such as polycyclic aromatic hydrocarbons (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. ½ 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 $<0.01 \times 10^{-8}$ 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 the absence first 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).

2.4.6 COMET

Slide preparation
The slides were prepared on board the research vessel with blood samples taken from the caudal vein of the freshly caught fish. A 10 µL volume of the blood was diluted 1000 fold in ice cold PBS buffer. A 15 µL volume of this diluted blood solution was added to 85 µL of warmed low melting point agarose. Then 7 µL 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 vessel and transported back to the laboratory, Oslo. The slides were processed further by the University of Oslo.

Assay
The comet assay is a simple method for measuring DNA damage at the level of individual cells. 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 or/and 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.

2.4.7 Fish 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 in to 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% $CaCl_2$, 2.5% NaCl) and kept at 4°C. Samples were dehydrated in alcohols and cleared in xylene ($C_6H_4(CH_3)_2$) 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.

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 Axioplan 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); melano-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**).

Table 3. Categories for the histological liver lesions and scoring system used for their quantification.

Steatosis (normal cyclical, non-pathological status of the liver)	0-3	0-absent 1-area affected 2-some areas affected 3-distributed through the hole sampled tissue
Circulatory disturbances	0-2	0-absent 1-sporadic/small area affected 2-some areas affected
Other pathological changes	0-2	0-absent 1-sporadic 2-multiple/widespread
Inflammatory changes	0-2	0-absent 1-sporadic 2-multiple/widespread
Melano-macrophage aggregates	0-3	0-absent 1-area affected (1-2 cases) 2-some areas affected/more than 2 in a sample 3-distributed through the whole sampled tissue
Parasites	0-1	0-absent 1-present

2.5 Additional biomarkers included for testing

Proteome analysis in fish bile samples of selected fish species was included in order to test its suitability for future WCM programmes and was not included in the overall risk assessment in the current study. Complete details of this analysis can be found in the appendices.

2.6 Integrative assessment

The Integrative Biological Response (IBR) index was developed to integrate biochemical, genotoxicity 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: $xi'=(xi-x)/s$; where, xi'=standardised value of the biomarker; xi=mean value of a biomarker from each sample; x=general mean value of xi calculated from all compared sample groups; s=standard deviation of xi 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. $yi=xi'+|xmin'|$); (4) calculate the Star Plot triangular areas by multiplication of the obtained standardised value of each biomarker (yi) with the value of the next standardised biomarker value (yi+1), dividing each calculation by 2 ($Ai=(yi*yi+1)/2$); and (5) calculate the IBR index which is the summing-up of all the Star Plot triangular areas ($IBR=\sum Ai$) (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=6) to calculate IBR/n, according to Broeg and Lehtonen (2006).

In the present study DNA adducts, COMET, AChE, EROD, liver histology and PAH metabolites were the biomarkers selected for the IBR calculation. The inverse values of AChE were used since a decrease was reflective of adverse impact. LMS was not used since this was not performed on all fish. Furthermore, proteome of bile was tested for its suitability for future WCM programmes and was also not included in the IBR/n calculation.

2.7 Statistical analysis

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 if 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

Turbidity, temperature and salinity profiles were taken at both Veslefrikk and Oseberg Sør platforms on two occasions during the fishing effort. At Oseberg Sør stratification of the water column was evident at around 20 m (**Figure 2** and **Figure 3**). Overall, the turbidity was highest in the surface to 10 m depth (ca. 0.6 FTU) and lowest from below 30 m depth (0.1 FTU). From the two temperature profiles a thermocline was present between 15 and 25 m depth, with surface waters of approximately 11°C and approaching 8°C below the thermocline. The halocline appeared to correspond inversely to thermocline with surface salinities of 34.4‰ and become stable at approximately 35.3‰ below 20 m.

At the Veslefrikk platform a similar level of stratification to that described for Oseberg Sør was observed (**Figure 4** and **Figure 5**). Surface turbidity values were slightly higher at Veslefrikk ranging between 0.6 to 1.5 FTU. Temperature profiles showed evidence of a thermocline at around 15-20 m, in addition possible identification of a warmer water plume, possibly indicating the PW discharge at 10 m depth. Surface salinities fluctuated slightly but were stable below 10-20 m depth at 35.3‰.

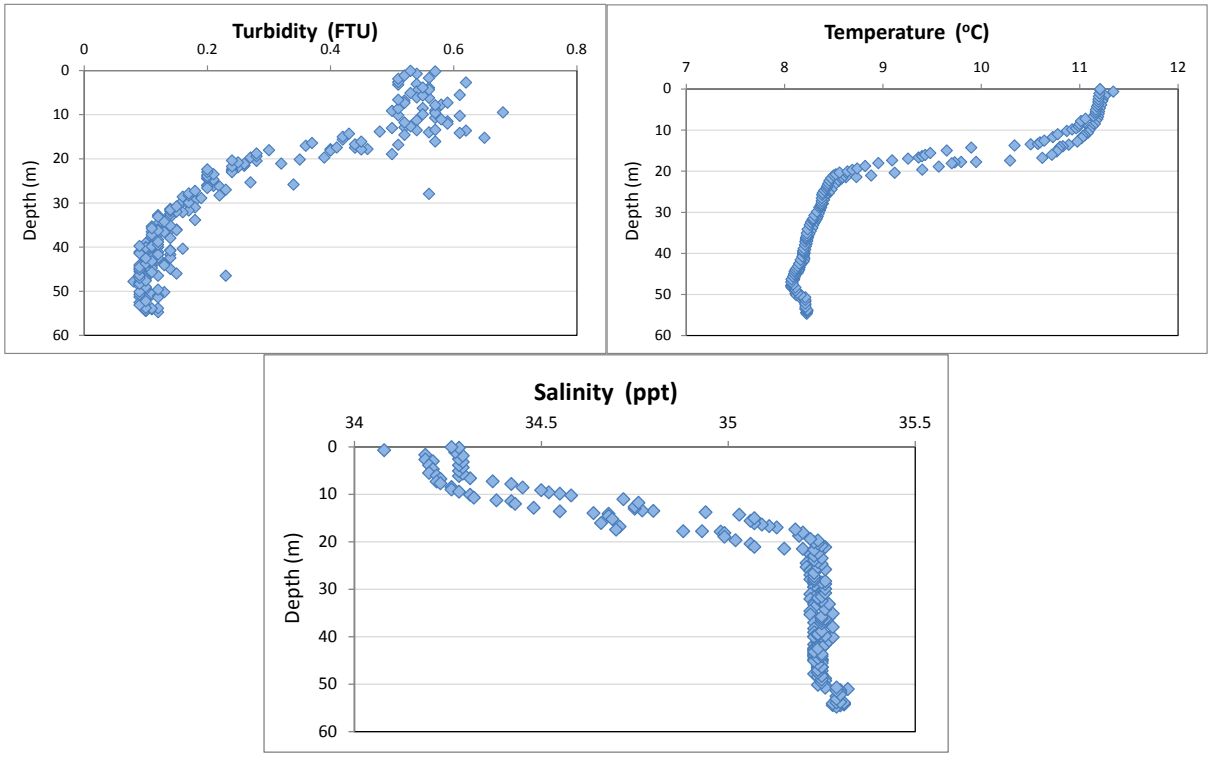

Figure 2. Vertical profiles for turbidity, temperature and salinity taken within 500 m of the Oseberg Sør platform during the fish collection (05.06.12, 13:00).

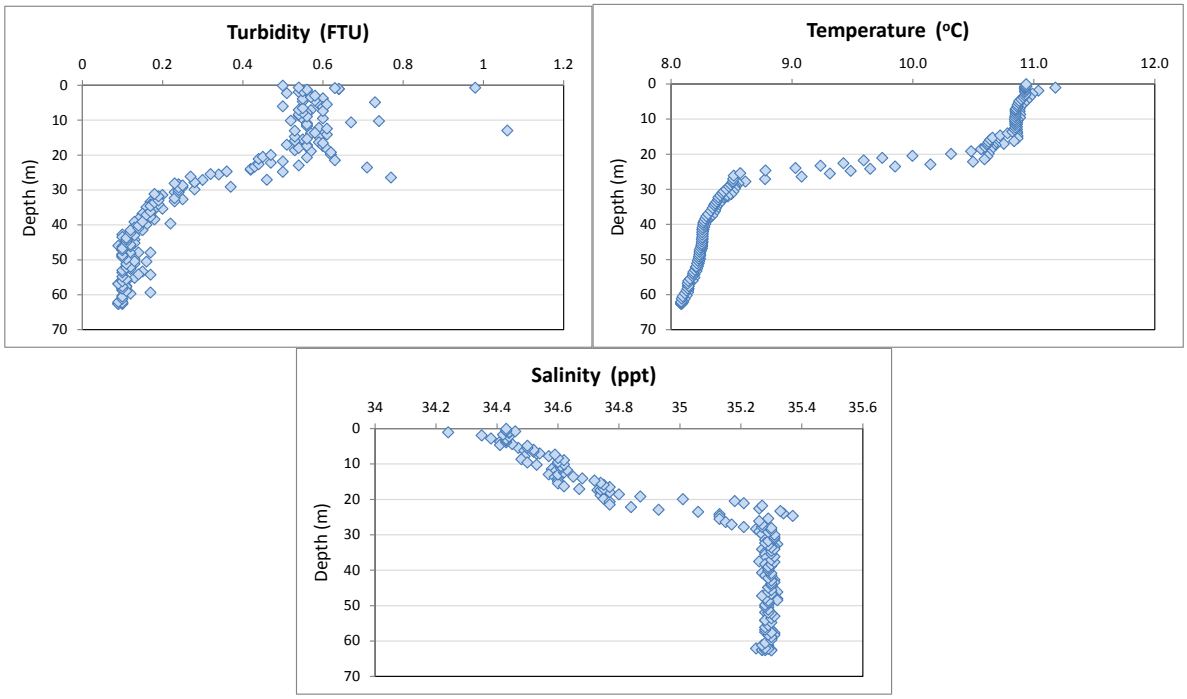

Figure 3. Repeated vertical profiles for turbidity, temperature and salinity taken within 500 m of the Oseberg Sør platform during the fish collection (05.06.12, 19:00).

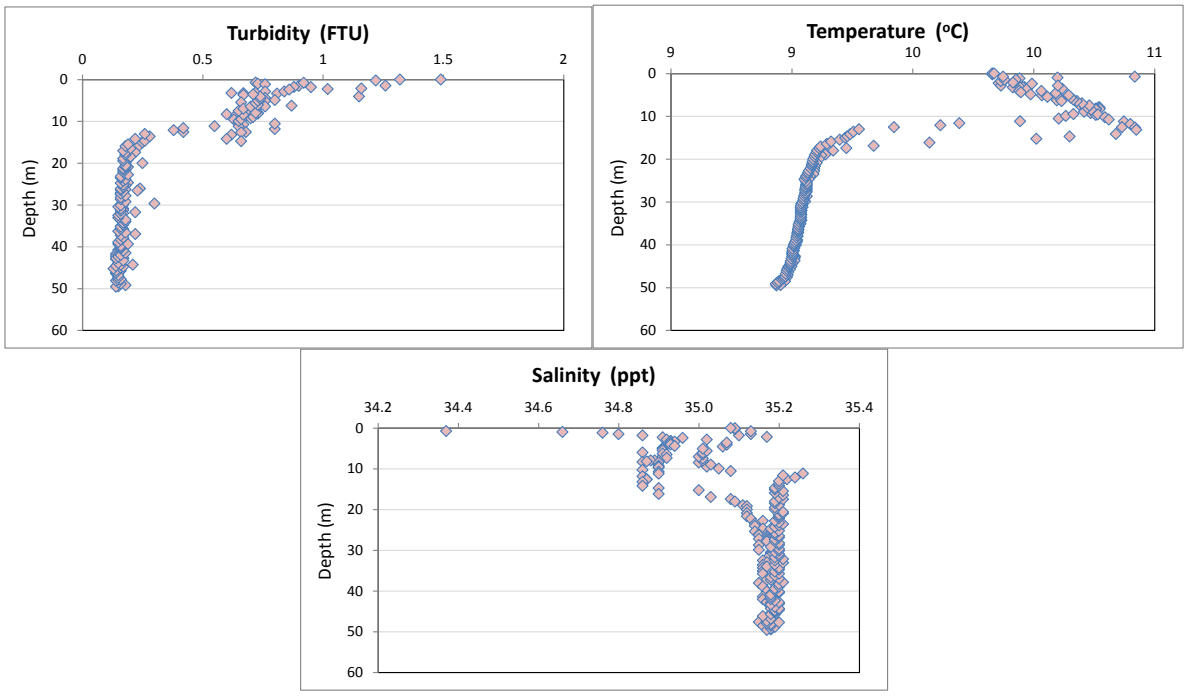

Figure 4. Vertical profiles for turbidity, temperature and salinity taken within 500 m of the Veslefrikk platform during the fish collection (03.06.12, 15:00).

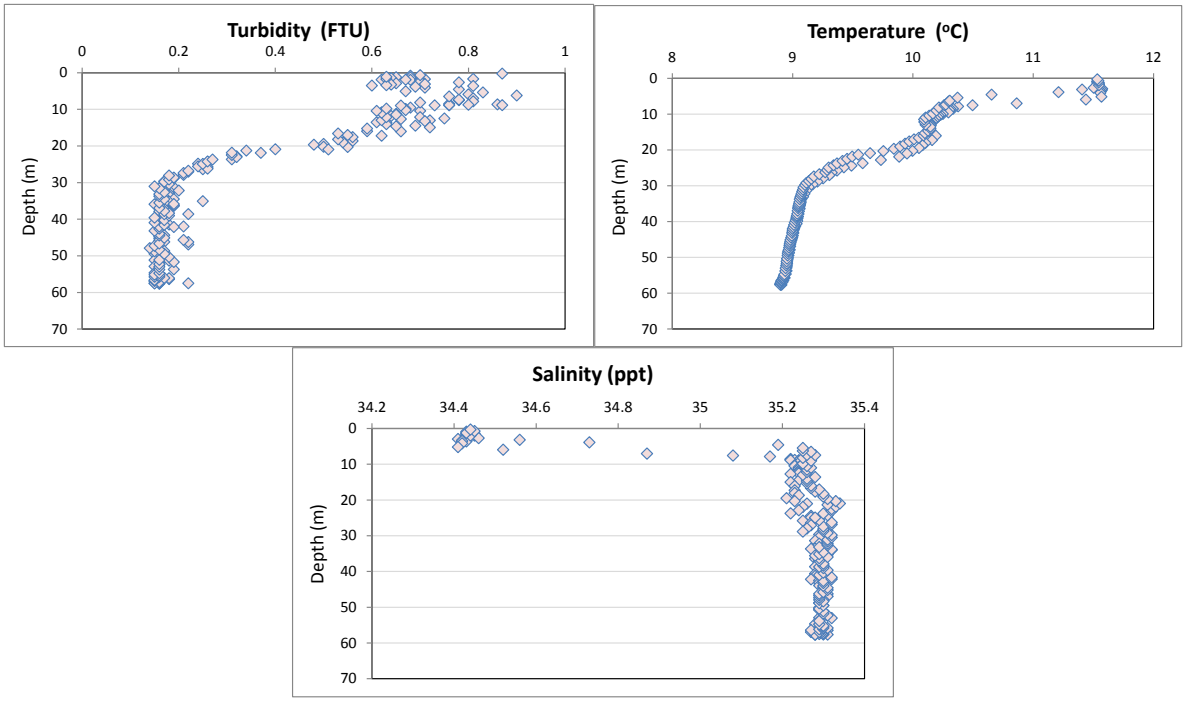

Figure 5. Vertical profiles for turbidity, temperature and salinity taken within 500 m of the Veslefrikk platform during the fish collection (04.06.12, 13:00).

3.2 Chemical bioaccumulation and exposure data

3.2.1 PAH-NPD in stomach and fillet

PAH-NPD concentrations were measured in homogenised stomach content of fish caught from both Veslefrikk and Oseberg Sør platforms as well as the reference source (**Table 4**). For some fish species, particularly ling and tusk from both the reference and Veslefrikk groups, the stomach was regularly empty of food and was therefore not analysed. The PAH-NPD concentrations were low and in most cases undetected in all fish species measured. PAH-NPDs were undetected in the tusk and ling from Veslefrikk and only low concentrations of mostly naphthalenes were detected in whiting, haddock and saithe from Oseberg Sør.

PAH-NPD concentrations were measured in fillet samples collected from the dorsal surface of the fish (excluding skin), from both platforms and reference groups (**Table 5**). PAH-NPD concentrations were undetected in all fish collected from around the two platforms. PAHs were detected in a few reference saithe and haddock but only at low concentrations and not in all individuals.

PAH-NPD concentrations measured in invertebrates collected from the platforms and reference area showed low to undetectable concentrations throughout (**Table 6**).

Table 4. PAH-NPD concentrations in stomach content of fish collected from Oseberg Sør and Veslefrikk platforms and reference fish. Bold values indicate detected concentrations (µg/ kg w.w.). Below limit of detection (LOD, 0.5 µg/ kg w.w.) for following PAH: acenaphthylene, acenaphthene, fluorine, dibenzothiophene, anthracene, benzo(a)anthracene, chrysene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene; and LOD of 2-5 µg/ kg w.w. for NPD: C1-dibenzothiophenes. Note in the final column 'sum measured PAH' zero values are used when below LOD.

Code	Species	Solids	Naphthalene	C1-Naphthalenes	C2-Naphthalenes	C3-Naphthalenes	C2-Dibenzothiophenes	C3-Dibenzothiophenes	Phenanthrene	C1-Phenanthrenes	C2-Phenanthrenes	C3-Phenanthrenes	Fluoranthene	Pyrene	Benzo(b)fluoranthene	Benzo(e)pyrene	Perylene	Indeno(1,2,3-cd)pyrene	Benzo(g,h,i)perylene	Sum measured PAH
Os202	Whiting	**18**	<5	<2	<2	**8.7**	<2	<2	**0.55**	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	**0.8**	<0.5	<0.5	**10.05**
Os204	Whiting	**15**	<5	<2	**2.4**	**9.5**	<2	<2	**0.73**	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**12.63**
Os208	haddock	**33**	**16**	<2	<2	**4.7**	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**20.7**
Os238	haddock	**8.9**	<2	<2	<2	<2	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**0**
Os239	haddock	**11**	<50	<2	**2.6**	**6.3**	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	**1.4**	**0.54**	<0.5	**10.84**
Os240	haddock	**27**	<2	<2	<2	**2.1**	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**2.1**
Os241	haddock	**5.6**	**11**	<2	<2	<2	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**11**
Os216	Saithe	**20**	**5.6**	<2	<2	<2	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**5.6**
V101	Tusk	**17**	<2	<2	<2	<2	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**0**
V104	Ling	**18**	<2	<2	<2	<2	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**0**
V112	Ling	**19**	<5	<2	<2	<4	<2	<2	<0.5	<2	<2	<10	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**0**
R322	Saithe	**11**	**2**	<2	<2	<2	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	**0.94**	<0.5	<0.5	**2.94**
R323	Saithe	**20**	**1.4**	<2	<2	**2.9**	<2	<2	<0.5	<2	<2	<2	<0.5	<0.5	<0.5	<0.5	**0.7**	<0.5	<0.5	**5**
R324	Saithe	**19**	**15**	<2	<2	**5.5**	**13**	**73**	<0.5	**2.3**	**8.3**	**18**	<0.5	<0.5	<0.5	<0.5	**1.1**	<0.5	<0.5	**136.2**
R335	Haddock	**17**	**3.6**	**6.9**	**6**	**17**	<2	<2	**0.99**	<2	**3.4**	<2	<0.5	**0.65**	<0.5	<0.5	<0.5	<0.5	<0.5	**38.54**
R336	Haddock	**20**	<1	<2	<2	**2.2**	<2	<2	<0.5	<2	<2	<2	**0.86**	**0.74**	**1.3**	**0.56**	<0.5	**1.2**	**1.2**	**8.06**
R337	Haddock	**14**	<10	<3	**3.5**	<4	<10	<40	<0.5	<2	<10	<10	**0.72**	**0.61**	<0.5	<0.5	<0.5	<0.5	<0.5	**4.83**
R368	Whiting	**17**	<10	<10	<5	<10	<10	<60	<0.5	<5	<20	<20	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**0**
R369	Whiting	**14**	<10	<3	**4.2**	<4	<10	<40	<0.5	<2	<10	<10	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**4.2**
R370	Whiting	**20**	<10	<3	**3.8**	**4.6**	<10	<40	<0.5	<2	<10	<10	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**8.4**

Table 5. PAH-NPD concentrations in fillet of fish collected from Oseberg Sør and Veslefrikk platforms and reference fish. Bold values indicate detected concentrations (µg/ kg w.w.). Below the LOD of 0.5 µg/ kg w.w. for the following PAH: acenaphthylene, acenaphthene, fluorene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzoIpyrene, benzo(a)pyrene, perylene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene; and NPDs: C1-Naphthalenes(LOD 2-3 µg/kg w.w.), C1-Dibenzothiophenes (LOD 2-5 µg/ kg w.w.). Note in the final column 'sum measured PAH' zero values are used when below LOD.

Code	Species	Solids	Naphthalene	C2-Naphthalenes	C3-Naphthalenes	C2-Dibenzothiophenes	C3-Dibenzothiophenes	C1-Phenanthrenes	C2-Phenanthrenes	C3-Phenanthrenes	Pyrene	Sum measured PAH
Os201	Whiting	**18**	<5	<5	<10	<2	<2	<2	<2	<2	<0.5	**0**
Os202	Whiting	**18**	<6	<10	<20	<2	<2	<2	<2	<2	<0.5	**0**
Os204	Whiting	**19**	<5	<5	<10	<2	<2	<2	<2	<2	<0.5	**0**
Os208	haddock	**18**	<1	<2	<5	<2	<2	<2	<2	<2	<0.5	**0**
Os238	haddock	**19**	<1	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
Os239	haddock	**16**	<5	<2	<5	<2	<2	<2	<2	<2	<0.5	**0**
Os240	haddock	**10**	<1	<2	<5	<2	<2	<2	<2	<2	<0.5	**0**
Os241	haddock	**11**	<5	<2	<5	<2	<2	<2	<2	<2	<0.5	**0**
Os214	Saithe	**19**	<5	<2	<10	<2	<2	<2	<2	<2	<0.5	**0**
Os215	Saithe	**21**	<5	<2	<5	<2	<2	<2	<2	<2	<0.5	**0**
Os222	Saithe	**19**	<5	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
V101	Tusk	**19**	<5	<2	<5	<2	<2	<2	<2	<2	<0.5	**0**
V106	Tusk	**20**	<5	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
V108	Tusk	**21**	<1	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
V102	Ling	**21**	<1	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
V103	Ling	**20**	<1	<2	<5	<2	<2	<2	<2	<2	<0.5	**0**
V104	Ling	**19**	<1	<2	<5	<2	<2	<2	<2	<2	<0.5	**0**
V105	Ling	**20**	<5	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
V112	Ling	**20**	<5	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
V119	Saithe	**20**	<5	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
V127	Saithe	**20**	<0.5	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
V128	Saithe	**21**	<0.5	<2	<2	<2	<2	<2	<2	<2	<0.5	**0**
V129	Saithe	**20**	<0.5	<3	<2	<2	<2	<2	<2	<2	<0.5	**0**
V130	Saithe	**19**	<0.5	<3	<2	<2	<2	<2	<2	<2	<0.5	**0**
R322	Saithe	**20**	**1.2**	<2	<2	<2	<2	<2	<2	<2	<0.5	**1.2**
R323	Saithe	**22**	**12**	<2	**3**	**11**	**65**	**2.8**	**5.7**	**14**	**8.7**	**122.2**
R324	Saithe	**20**	**1.8**	<2	<2	<2	<2	<2	<2	<2	<0.5	**1.8**
R335	Haddock	**21**	<10	**4**	<4	<10	<40	<2	<10	<10	<0.5	**4**
R336	Haddock	**19**	<10	**2.8**	<4	<10	<40	<2	<10	<10	<0.5	**2.8**
R337	Haddock	**20**	<10	**2.8**	<4	<10	<40	<2	<10	<10	<0.5	**2.8**
R368	Whiting	**19**	<10	**3.1**	<4	<10	<40	<2	<10	<10	<0.5	**3.1**
R369	Whiting	**19**	<1	<2	<3	<2	<2	<2	<2	<2	<0.5	**0**
R370	Whiting	**18**	<1	<2	<3	<2	<2	<2	<2	<2	<0.5	**0**
R392	Ling	**19**	<1	<2	<3	<2	<2	<2	<2	<2	<0.5	**0**
R393	Ling	**20**	<1	<2	<3	<2	<2	<2	<2	<2	<0.5	**0**
R394	Ling	**21**	<10	**2.8**	<4	<10	<40	<2	<10	<10	<0.5	**2.8**
R302	Tusk	**21**	<10	<2	<4	<10	<40	<2	<10	<10	<0.5	**0**
R305	Tusk	**20**	<10	<2	<4	<10	<40	<2	<10	<10	<0.5	**0**
R306	Tusk	**18**	<10	<2	<4	<10	<40	<2	<10	<10	<0.5	**0**

3.2.2 PAH-NPD in invertebrates

Table 6. PAH-NPD concentrations in selected invertebrates from the two platforms (Oseberg Sør, Veslefrikk) and reference location. Bold values identify detected concentrations, i indicates interference (µg/ kg w.w.). Below the LOD of 0.5 µg/ kg w.w. for the following PAH: acenaphthylene, acenaphthene, fluorene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene; and NPDs:, C1 and C2-Dibenzothiophenes (LOD 2-10 µg/ kg w.w.), C1-Phenanthrenes (LOD 2 µg/kg w.w.). Note in the final column 'sum measured PAH' zero values are used when below LOD.

Code	Invertebrate group	Solids	Naphthalene	C1-Naphthalenes	C2-Naphthalenes	C3-Naphthalenes	C3-Dibenzothiophenes	C2-Phenanthrenes	C3-Phenanthrenes	Benzo(b)fluoranthene	Perylene	Sum measured PAH
Os1a	Anemones	**33**	**17**	<2	<3	<4	<2	**2**	<10	<3	<0.5	19
Os1b	Anemones	**24**	<10	<2	<3	<4	<2	<2	**2.1**	<3	<0.5	2.1
Os1c	Anemones	**27**	**32**	<2	<3	<4	<2	**2.4**	<10	<3	<0.5	34.4
Os2a	Whelks	**23**	<10	<2	<3	**6.7**	<2	<2	<10	<3	<0.5	6.7
Os2b	Whelks	**24**	<10	<2	<3	<4	<2	<2	<10	<3	<0.5	0
Os2c	Whelks	**23**	**13**	<2	<3	<4	<2	<2	<10	<3	<0.5	13
Os3a	Hermit crabs	**29**	**28**	<2	<3	**7**	<2	<2	<10	<3	<0.5	35
Os3b	Hermit crabs	**30**	<10	<2	<3	**7.3**	<2	**2.6**	<10	<3	<0.5	9.9
Os3c	Hermit crabs	**30**	<10	<2	<3	**6.2**	<2	**2.1**	<10	<3	<0.5	8.3
V1a	Isopods	**24**	<10	<2	<3	<4	**4.2**	**7**	i	<3	<0.5	11.2
V1b	Isopods	**24**	<10	<2	<3	**5.5**	**3.6**	**6.4**	i	<3	<0.5	15.5
V1c	Isopods	**24**	<10	<2	<3	<4	**3.2**	**5.6**	i	<3	<0.5	8.8
V2a	Amphipods	**25**	**11**	**4.7**	<3	<4	<2	**3**	i	<3	<0.5	18.7
V2b	Amphipods	**25**	**25**	**4.5**	<3	**7**	<2	<2	i	<3	**7.8**	44.3
V2c	Amphipods	**25**	<10	**4.7**	<3	<4	<2	**2.3**	i	<3	<0.5	7
V3a	Norway lobster	**20**	<10	<2	<3	<4	<2	<2	<10	<3	<0.5	0
V3b	Norway lobster	**20**	<10	<2	<3	<4	<2	<2	<10	<3	<0.5	0
V3c	Norway lobster	**20**	<10	<2	<3	<4	<2	<2	<10	<3	<0.5	0
R1	Crab	**32**	<10	<3	**4.4**	<4	<40	<10	<10	<0.5	<0.5	4.4
R2	Norway lobster	**23**	<10	<3	**3.5**	<4	<40	<10	<10	<0.5	<0.5	3.5
R3	Anemones	**17**	<10	<3	**6.8**	**84**	<40	<10	<10	**0.64**	<0.5	91.44

3.2.3 PFCs in fish blood

Table 7. Perfluorinated compounds measured in fish blood from the locations indicated (μg/ L). Perfluorooctanoic acid (PFOA); Perfluorooctanesulfonic acid (PFOS); Perfluorononanoic acid (PFNA); Perfluorobutanesulfonic acid (PFBS); Perfluorooctanesulfonamide (PFOSA); Perfluorohexanoate (PFHxA); Perfluoroheptanoic acid (PFHpA); Perfluorohexanesulfonic acid (PFHxS); Perfluorodecanoic acid (PFDcA); perfluoroundecanoic acid (PFUdA), perfluorodecane sulfonate (PFDcS). Below LOD of 0.05 μg/ L for PFBS, and 0.1 μg/L for PFHxA, PFHpA. Note in the final column 'total PFC' zero values are used when below LOD.

Location	code	Species	PFOA	PFNA	PFOS	PFOSA	PFHxS	PFDcA	PFUdA	PFDcS	Total PFC
Veslefrikk	V101	Tusk	**0.2**	**0.72**	**1.3**	**0.54**	**0.3**	**0.56**	**3.4**	<0.02	7.02
Veslefrikk	V106	Tusk	<0.2	**0.67**	**1.1**	**3.1**	**0.2**	**0.56**	**3.1**	<0.02	8.73
Veslefrikk	V108	Tusk	<0.2	**0.4**	**1.9**	**7**	<0.02	**0.77**	**4.4**	**0.04**	14.51
Veslefrikk	V102	Ling	<0.2	**0.3**	**1.1**	**12**	**0.04**	**0.37**	**1.9**	<0.02	15.71
Veslefrikk	V103	Ling	<0.2	**0.28**	**1.4**	**17**	**0.03**	**0.41**	**2.4**	**0.03**	21.55
Veslefrikk	V104	Ling	<0.2	**0.42**	**1.3**	**35**	**0.05**	**0.62**	**2.7**	<0.02	40.09
Veslefrikk	V119	Saithe	<0.2	**0.34**	**1.3**	**5.8**	**0.07**	**0.56**	**1.7**	<0.02	9.77
Veslefrikk	V127	Saithe	<0.2	<0.2	**0.96**	**2.4**	<0.02	**0.42**	**2.2**	**0.03**	6.01
Oseberg Sør	OS201	Whiting	<0.2	**1**	**1.4**	**18**	**0.06**	**0.52**	**1.6**	<0.02	22.58
Oseberg Sør	OS202	Whiting	<0.2	**0.71**	**0.88**	**13**	**0.03**	**0.4**	**1.4**	<0.02	16.42
Oseberg Sør	OS204	Whiting	<0.2	**0.45**	**0.76**	**13**	<0.02	<0.3	**0.67**	<0.02	14.88
Oseberg Sør	OS203	Haddock	**0.74**	**3.7**	**7.1**	**1.6**	**1.2**	**0.75**	**1.6**	**0.04**	16.73
Oseberg Sør	OS205	Haddock	<0.2	**0.55**	**1.2**	**1.8**	**0.09**	**0.84**	**1.4**	<0.04	5.88
Oseberg Sør	OS208	Haddock	**0.89**	**4.8**	**2.3**	**4.3**	**0.27**	**0.75**	**1.4**	**0.02**	14.73
Oseberg Sør	OS214	Saithe	<0.2	**0.37**	**1.9**	**12**	**0.05**	**0.38**	**2**	**0.02**	16.72
Oseberg Sør	OS215	Saithe	<0.2	**0.41**	**1.7**	**11**	**0.06**	**0.49**	**2.4**	<0.02	16.06
Oseberg Sør	OS216	Saithe	<0.2	**0.58**	**1.5**	**5.7**	**0.07**	**0.47**	**2.3**	<0.02	10.62
Reference	R301	Tusk	**0.33**	**0.81**	**1.4**	**1.8**	**0.1**	**0.93**	**2.9**	<0.02	8.27
Reference	R302	Tusk	**0.41**	**0.59**	**1.1**	**4.8**	<0.05	**0.68**	**2.8**	<0.03	10.38
Reference	R303	Tusk	<0.2	<0.2	**0.51**	**6.8**	**0.03**	**0.32**	**1.4**	<0.02	9.06
Reference	R322	Saithe	<0.2	<0.2	**1.6**	**17**	**0.05**	<0.3	**1.6**	<0.02	20.25
Reference	R323	Saithe	<0.2	**0.29**	**1.1**	**7.2**	<0.02	**0.62**	**4.2**	0.02	13.43
Reference	R324	Saithe	**0.22**	**0.58**	**1.6**	**5.1**	**0.04**	**0.67**	**2.5**	<0.02	10.71
Reference	R335	Haddock	<0.2	**1.3**	**0.89**	**1.8**	<0.02	**0.71**	**1.3**	<0.02	6
Reference	R336	Haddock	<0.2	**0.63**	**0.77**	**0.95**	<0.02	**0.55**	**0.86**	<0.02	3.76
Reference	R337	Haddock	<0.2	**0.67**	**0.64**	**1.8**	<0.02	**0.52**	**1**	<0.02	4.63
Reference	R369	Whiting	<0.2	<0.2	**0.39**	**17**	<0.02	<0.3	**0.98**	<0.02	18.37
Reference	R373	Whiting	<0.2	**0.37**	**0.42**	**20**	<0.02	<0.3	**0.85**	<0.02	21.64
Reference	R378	Whiting	<0.2	**0.27**	**0.65**	**24**	**0.03**	**0.4**	**1.3**	<0.02	26.65
Reference	R392	Ling	<0.2	**0.55**	**1.5**	**56**	**0.05**	**0.85**	**4.6**	<0.02	63.55
Reference	R393	Ling	<0.2	<0.2	**1.3**	**23**	**0.03**	**0.35**	**1.5**	<0.02	26.18
Reference	R394	Ling	<0.2	**0.36**	**1.5**	**17**	**0.04**	**0.39**	**1.8**	<0.02	21.09

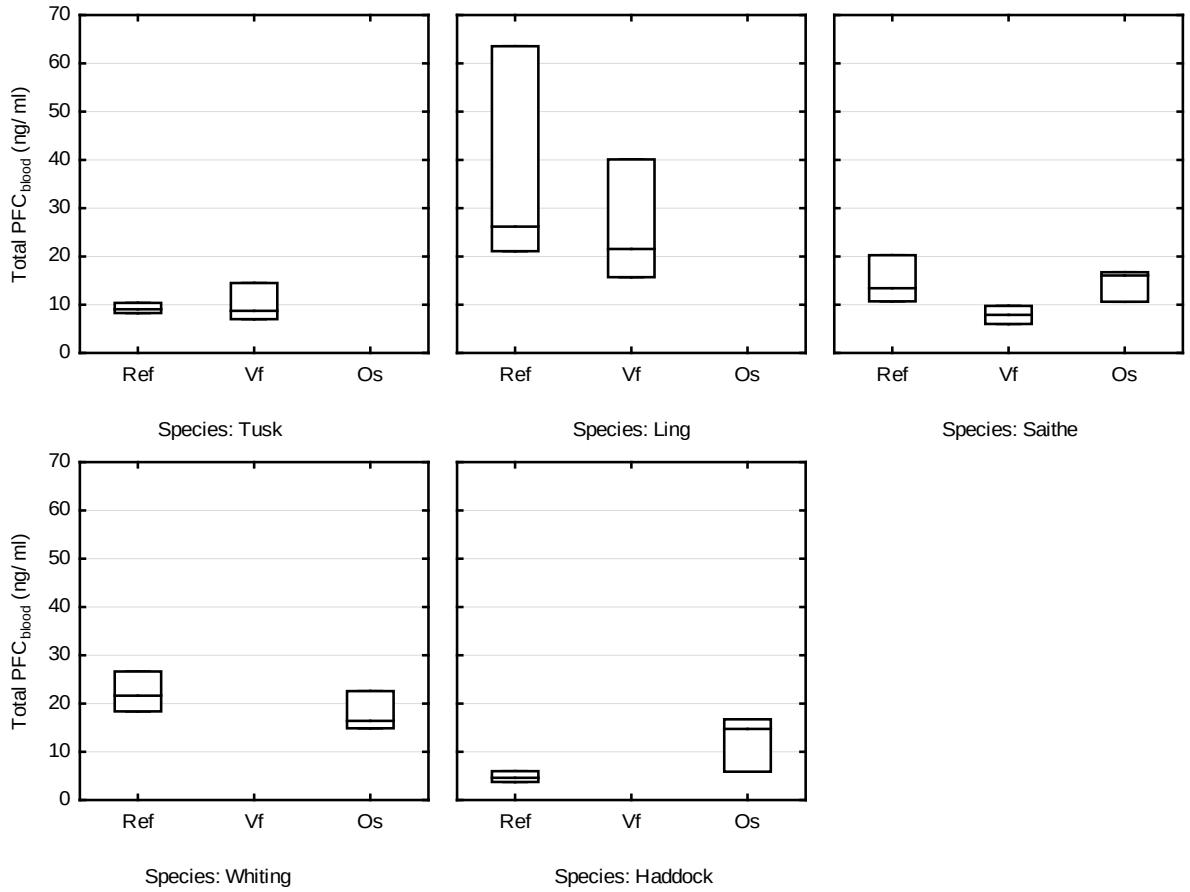

Figure 6. PFC concentrations in blood samples collected from the five fish species (ng/mL w.w.). Vf, Veslefrikk; Os, Oseberg Sør; Ref, Reference. Sum of 11 PFC compounds: Perfluorooctanoic acid (PFOA); Perfluorooctanesulfonic acid (PFOS); Perfluorononanoic acid (PFNA); Perfluorobutanesulfonic acid (PFBS); Perfluorooctanesulfonamide (PFOSA); Perfluorohexanoate (PFHxA); Perfluoroheptanoic acid (PFHpA); Perfluorohexanesulfonic acid (PFHxS); Perfluorodecanoic acid (PFDcA); perfluoroundecanoic acid (PFUdA). Median, quartiles (box), 10/90-percentiles (bar), n=3 (Vf saithe n=2). No significant difference between locations for each species (ANOVA, Dunnett's, $p<0.05$).

Of the eleven PFCs measured in the blood of the fish, PFOSA was overall the most abundant followed by PFUdA, PFOS, PFNA and PFDcA (**Table 7**). The highest concentration was 56 ng PFSOA/mL in ling collected from the reference area. Highest total PFC blood concentrations, based on 11 PFCs measured, were found in ling, being slightly higher in the reference than those collected from Veslefrikk (**Figure 6**). Since only three replicates were taken no statistics could be performed, although overlapping values should be treated as not different from each other. In general, PFCs were not higher in fish blood collected closer to the platform.

3.2.4 Bile metabolites

Fixed fluorescence (FF)

The results of PAH metabolites measured in the fish bile of the five species using the FF screening method are reported (**Figure 7**). Fluorescence was measured at the excitation/emission wavelength pairs for the detection of metabolites of naphthalene (2- and 3- rings), pyrene (4-rings) and benzo[*a*]pyrene (5- and 6-rings).

Higher fluorescence signals for indicating increased concentrations of 2-3 ring PAH metabolites were measured in all fish species, although the concentrations in all groups were very low. No significant differences were found in all fish species between platform and reference groups.

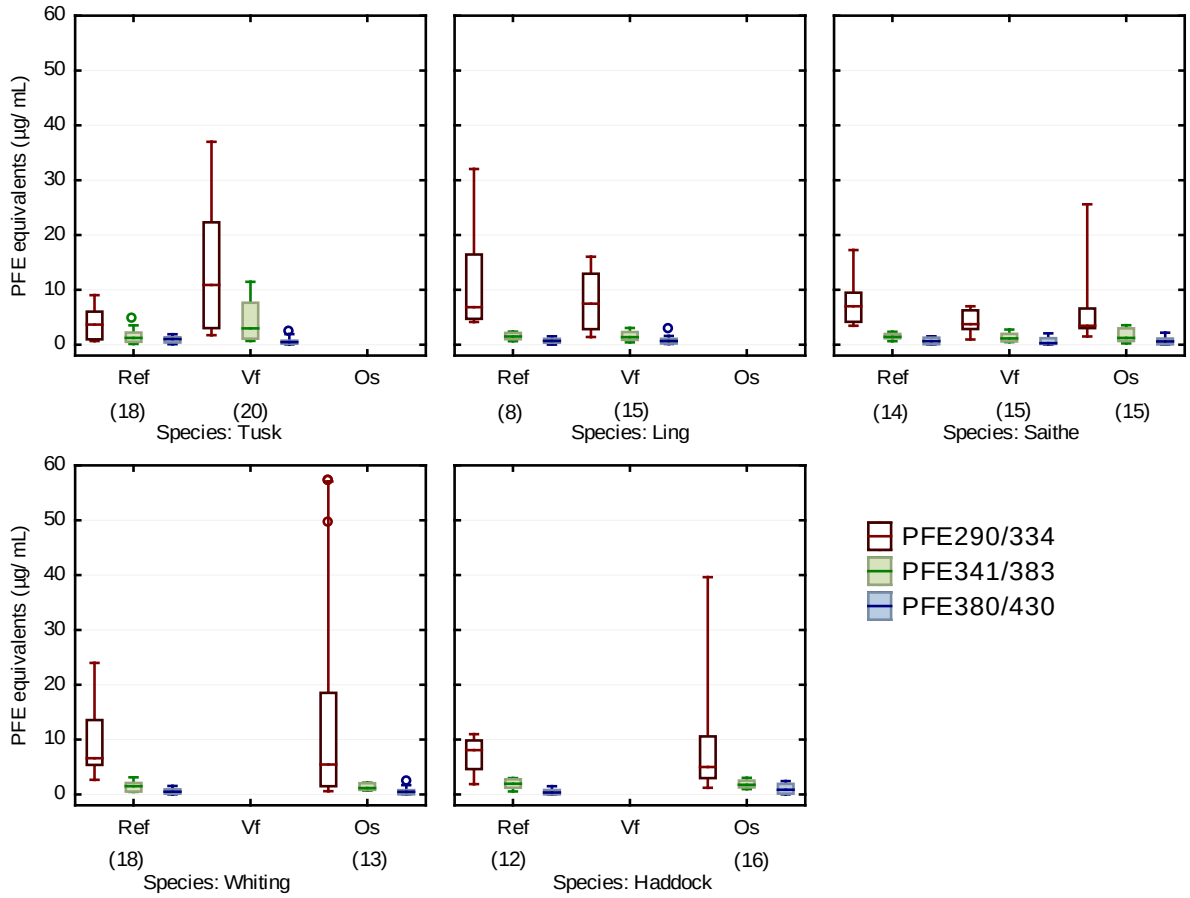

Figure 7. Fixed wavelength fluorescence levels in bile from the five fish species from the locations indicated (Ref, Reference; Vf, Veslefrikk; Os, Oseberg Sør), expressed as pyrene fluorescence equivalents, PFE µg/mL. The wavelength pair 290/334 nm identifies 2-3 ring structures, 341/383 nm identifies 4 ring structures and 380/430 nm 5-6 ring structures. The figure shows median, quartiles (box) and 10/90-percentiles (bar). o outliers, the numbers in the parenthesis indicates the specific n value for each group.

Gas chromatography (GC)

PAH metabolite profiles were found to differ between the fish species (**Figure 8**). With regard to tusk, the PAH metabolites were in general below the detection limits, with few exceptions. Higher values for C_1-OH-phenanthrene were found in few tusk collected in the vicinity of the platform compare to the reference group. For ling, naphthalene metabolite concentrations were all below the detection limits while phenanthrene and pyrene were at detectable levels although low and comparable between platform and reference groups. Detectable concentrations of phenanthrene and pyrene were found in saithe, values were higher in fish collected in the vicinity of the Veslefrikk platform compare to Oseberg Sør or reference groups. Higher values of all PAH metabolites were detected in haddock caught in the vicinity of the Oseberg Sør platform compared to the reference group. Finally for whiting, values were higher in fish collected in the vicinity of the Oseberg Sør platform compared to the reference location, although several individuals had concentrations below the quantification limits.

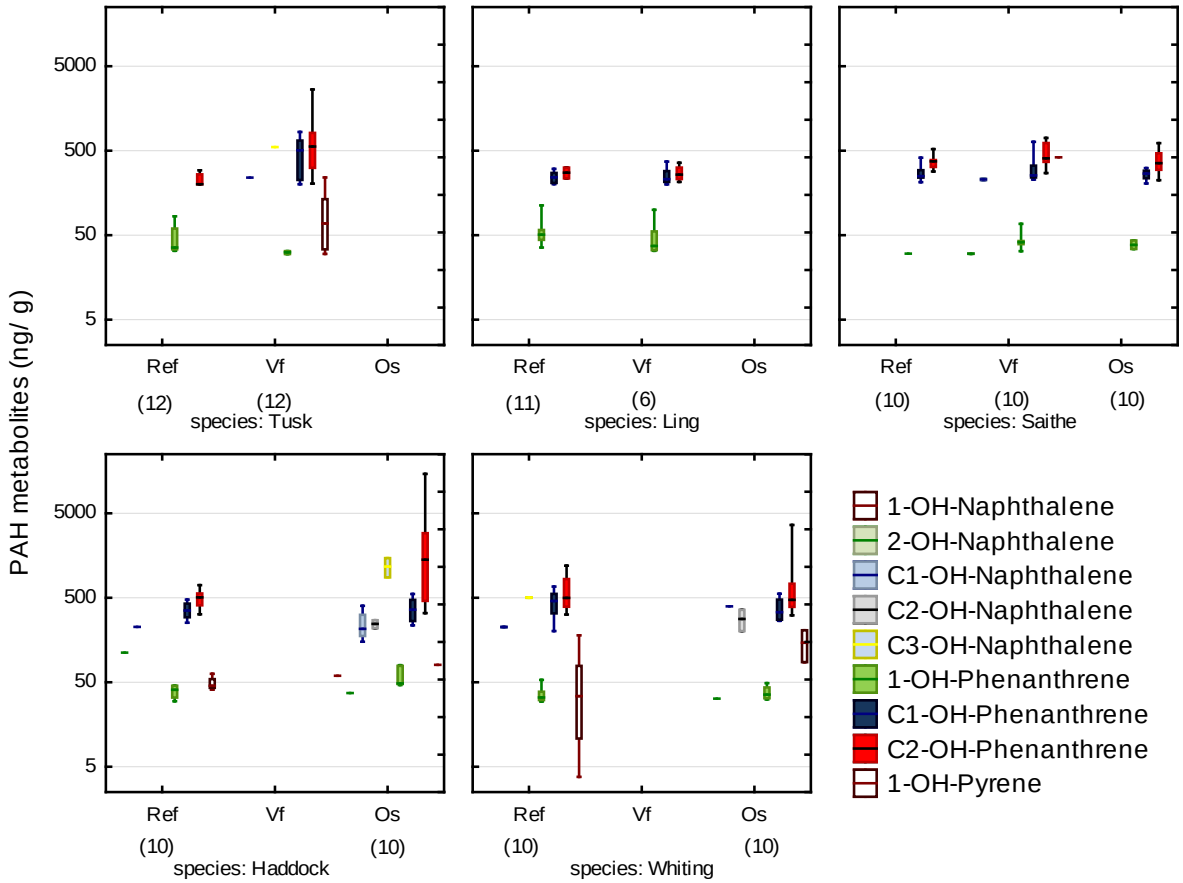

Figure 8. PAH metabolite concentrations of bile samples measured by gas chromatography (GC) for the five fish species at either reference or platform. The figure shows median, quartiles (box) and 10/90-percentiles (bar). Limits of quantification: Single compounds, 30ng/g; C1 and C2-OH-naphthalene/ phenanthrene, 200 ng/g; C3-OH-naphthalene 500 ng/g. The numbers in the parenthesis indicates the specific n value for each group.

3.3 Biomarkers

The summary of the size distribution of each fish species caught at the different locations is shown in the table below (**Table 8**). For all fish species, the reference population was overall smaller than those caught around the platform(s). The largest difference in fork length between the reference and platform groups was observed for ling and saithe.

Table 8. Summary of fork length data measured in all fish species from both the reference location and next to the platform(s). Mean (χ), standard deviation (SD), number of observations (n).

Fish	Parameter	Reference			Oseberg			Veslefrikk		
		χ	SD	n	χ	SD	n	χ	SD	n
Tusk	length (cm)	72	9	20				80	7	20
	weight (g)	4151	1594					5466	1669	
Ling	length (cm)	87	24	9				112	14	21
	weight (g)	4590	3677					7314 *	6733	
Saithe	length (cm)	50	13	23	72	21	15	65	9	17
	weight (g)	1458	1939		4404	3453		2315	784	
Whiting	length (cm)	34	6	24	39	18	20			
	weight (g)	344	204		1106	3271				
Haddock	length (cm)	40	2	25	55	9	20			
	weight (g)	672	114		2002	604				

* 14 ling were not weighed that were in excess of 5.5 kg.

3.3.1 Health indices

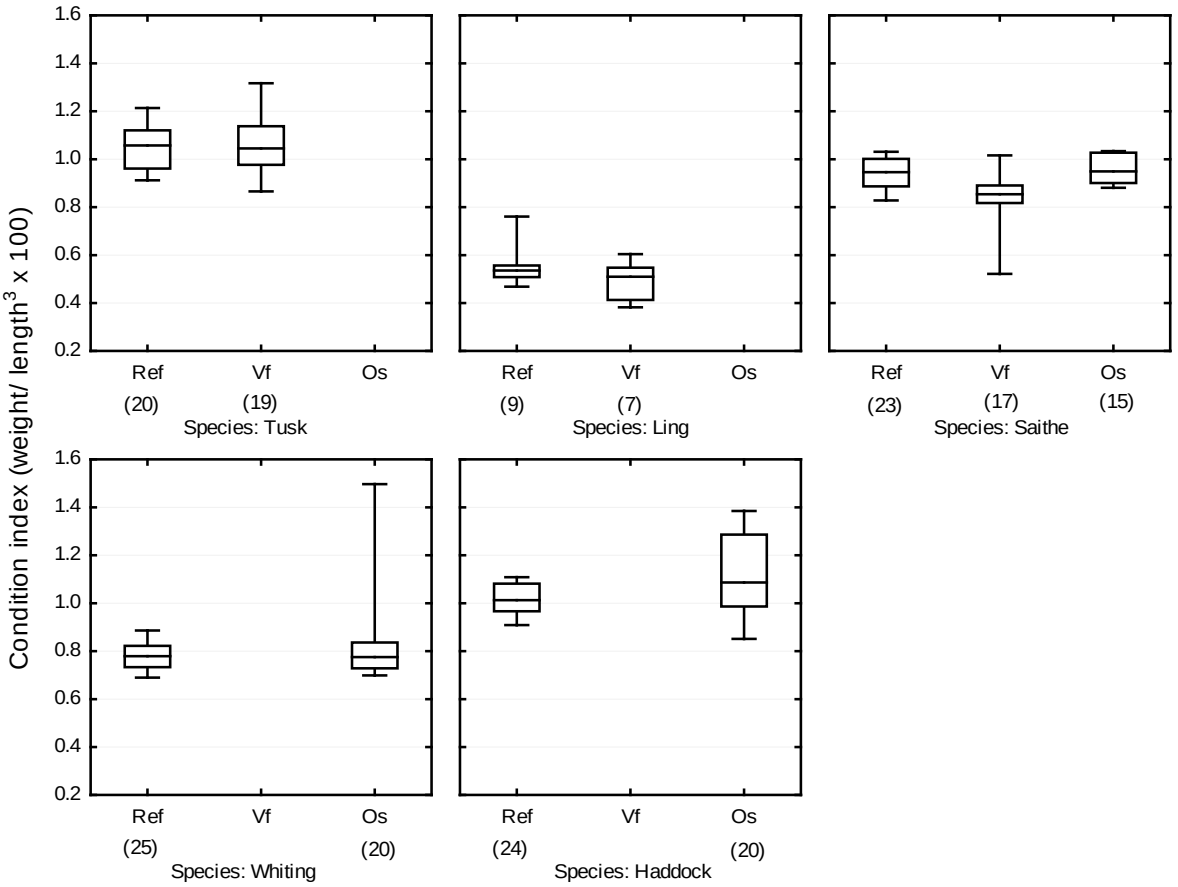

Figure 9. Condition index (CI) of the five fish species caught in the vicinity of the offshore platforms and at the reference locations. Vf, Veslefrikk; Os, Oseberg Sør; Ref, Reference. The figure shows median, quartiles (box) and 10/90-percentiles (bar). * significantly different from reference group (ANOVA, Dunnett's test, $p<0.05$). The numbers in the parenthesis indicates the specific n value for each group.

Condition index (CI) was obtained from measurements of wet weight and fork length of each fish immediately after capture and are shown in **Figure 9**. The CI of approximately 1.0 was obtained for saithe, haddock and tusk, while whiting were approximately 0.8. In contrast, the CI of ling was much lower at approximately 0.5. However, despite the differences between the fish species there were no marked differences between the reference group and the platform populations within species.

The liver somatic index (LSI), measured as a ratio between liver weight and fish weight, can provide an indication of nourishment status of the fish (**Figure 10**). No significant differences in LSI were found for saithe, whiting, ling or tusk between reference and their respective platform(s). The LSI for haddock did show a significant difference with a lower LSI in haddock from the Oseberg Sør platform compared to the reference group (ANOVA, Dunnett's, $p<0.05$). In addition, the LSI of saithe from Veslefrikk was significantly lower than the reference group (ANOVA, Dunnett's, $p<0.05$).

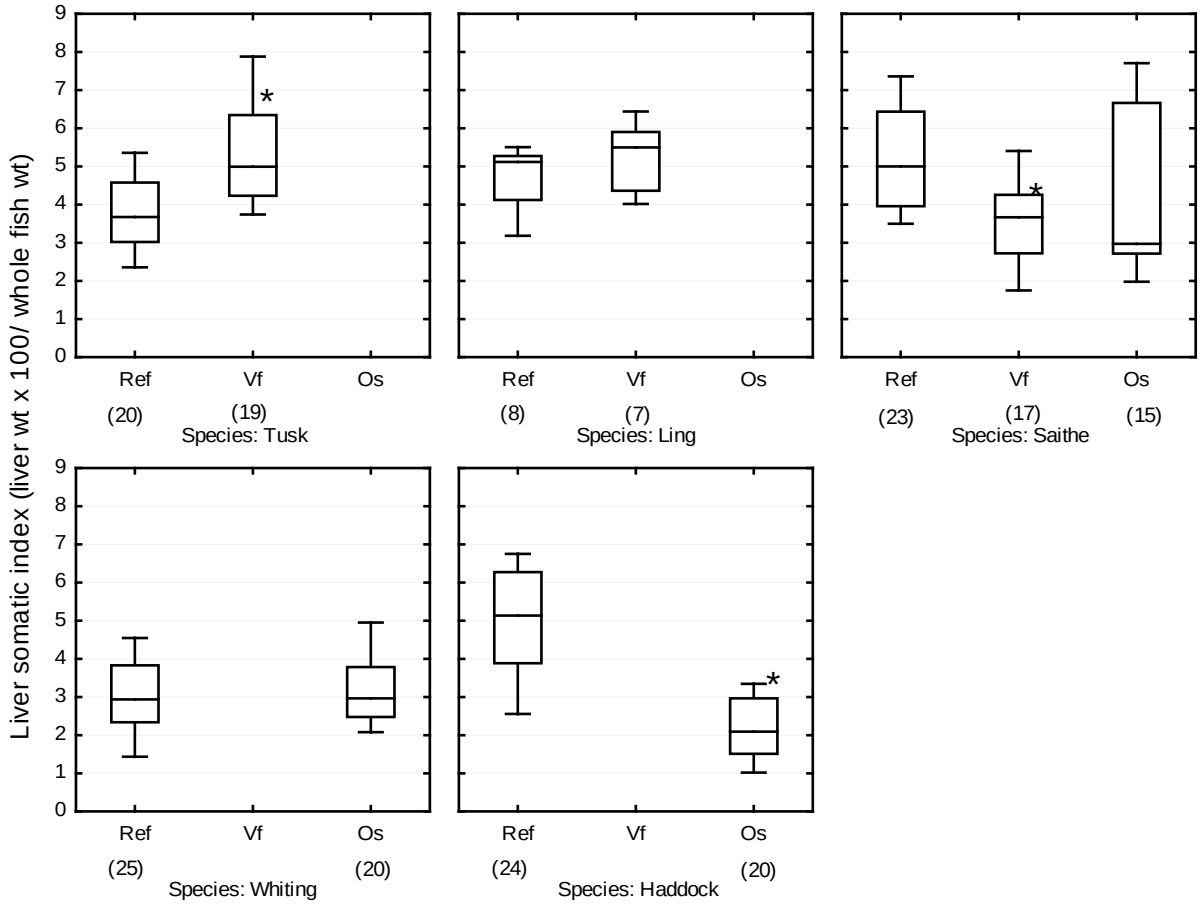

Figure 10. Liver somatic index (LSI) of the five fish species caught in the vicinity of the offshore platforms and at the reference locations. Vf, Veslefrikk; Os, Oseberg Sør; Ref, Reference. * significant difference from reference (ANOVA, Dunnett's, $p<0.05$). The numbers in the parenthesis indicates the specific n value for each group.

The GSI values separated into the individual genders for each fish species are shown in **Figure 11**. In male fish, saithe, haddock, ling and tusk had a GSI below 1.0, whilst male whiting were approaching 1.0 and above. In female fish, whiting and saithe were found to have significantly higher GSI values in fish collected from Oseberg Sør platform compared to their reference groups (ANOVA, Dunnett's, $p<0.05$).

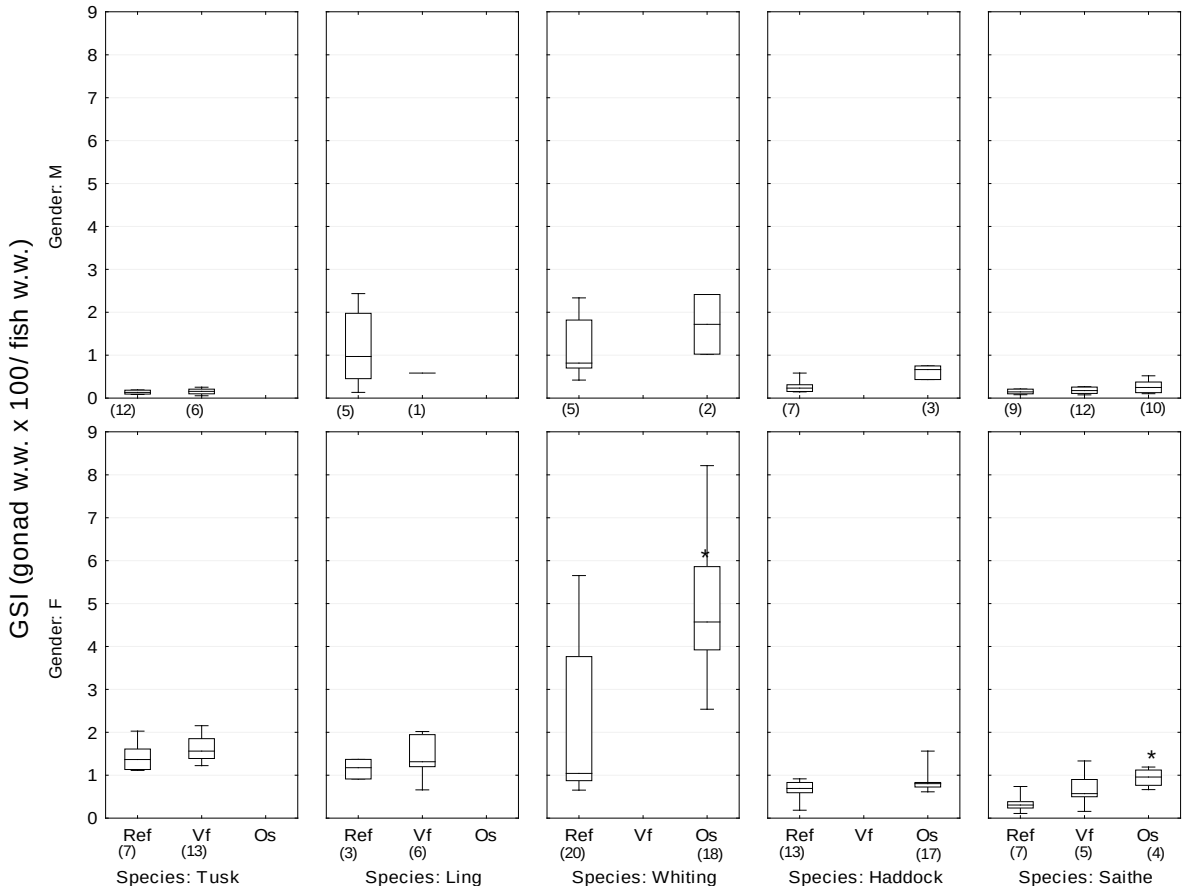

Figure 11. Gonadosomatic index (GSI) of the five fish species caught in the vicinity of the offshore platforms and at the reference locations. The figures have been further divided to represent male and female for each species separately. Vf, Veslefrikk; Os, Oseberg Sør; Ref, Reference. Median, quartile (box), 10/90 percentile (bar). * significant difference from reference (ANOVA, Dunnets, $p<0.05$). The numbers in the parenthesis indicates the specific n value for each group.

3.3.2 EROD

EROD activity in fish liver separated by gender for the five fish species from their different locations are shown in **Figure 12**. No significant differences were found for each of the individual species between the reference and platform(s). There were also no apparent differences between the sexes for the five fish species, although the number of males for some fish species prevented a full comparison to be made.

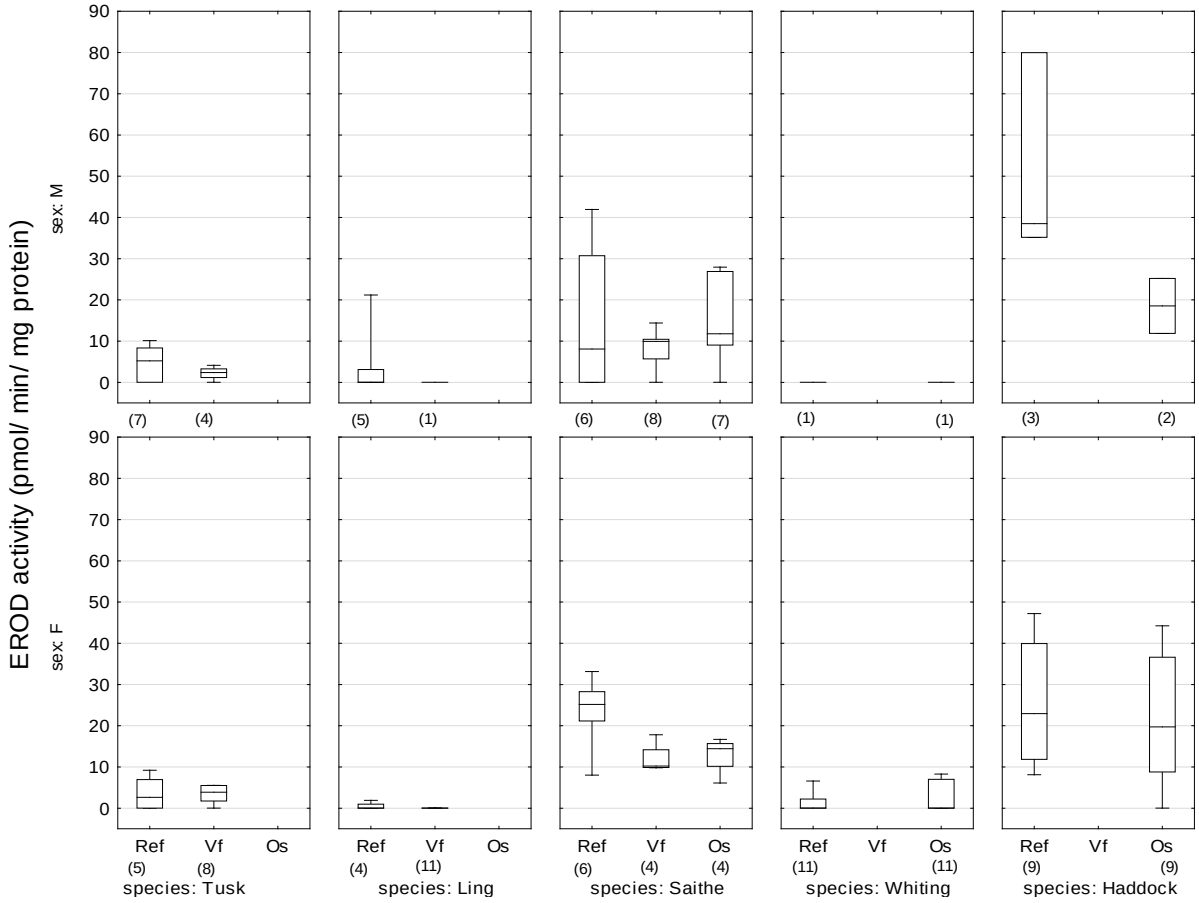

Figure 12. EROD activity in liver samples of the five fish species from the locations indicated. Data are divided by gender (male [top row], females [bottom row]). Ref, Reference; Vf, Veslefrikk; Os, Oseberg Sør. No significant differences found (ANOVA, Dunnett's $p>0.05$). Data expresses as median (line), quartiles (box) and 10/90 percentiles (bar). Numbers in parenthesis denote individual n values.

3.3.3 Acetylcholine esterase inhibition

AChE activity in the liver of the five fish species from the different locations are shown in **Figure 13**. No significant differences in AChE activity were found in haddock, whiting, tusk or ling when comparing the platform population to the reference group. However, for saithe significantly lower activities of AChE were seen in saithe from both Oseberg Sør and Veslefrikk platforms compared to the reference group (ANOVA, Dunnett's, $p<0.05$).

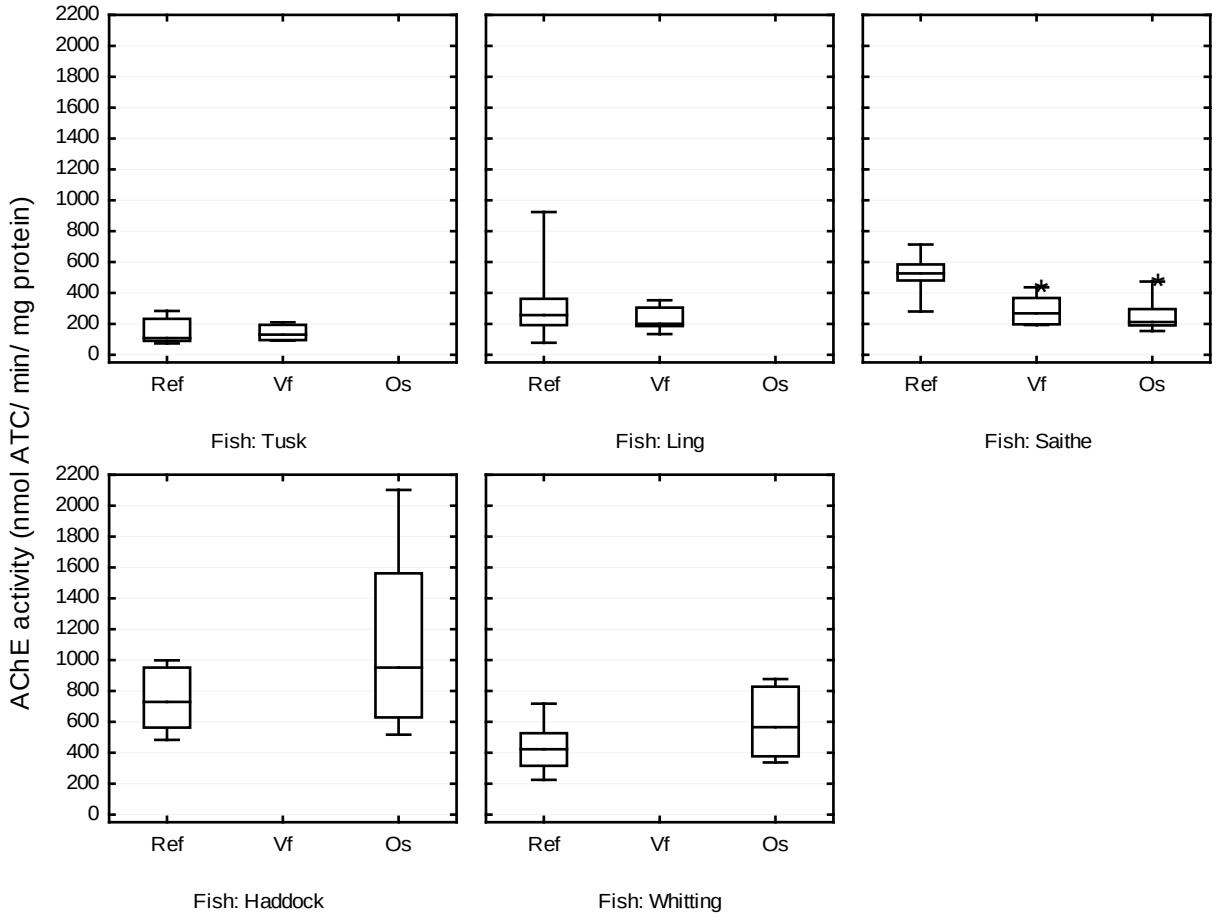

Figure 13. The inhibition of acetylcholine esterase (AChE) in fish fillet samples from the locations indicated. Ref, Reference; Vf, Veslefrikk; Os, Oseberg Sør. Data expresses as median (line), quartiles (box) and 10/90 percentiles (bar). * significant difference from reference groups (ANOVA, Dunnett's, $p<0.05$, n=12, except ref ling n=9).

3.3.4 LMS

Many of the fish species used in this study have had little application as biomonitoring organisms and have not been widely used for such histochemical techniques as LMS performed on sectioned liver tissue. Consequently some challenges appeared during investigations. One issue was that not all the fish were successfully sectioned with the aid of the cryostat. The liver of some fish, particularly tusk and ling, had a high lipid content, which prevented the liver from freezing adequately enough prior to cutting resulting in poor quality sections. Various methods were used to obtain sufficient adequate sections suitable for further analyzing, including cooling sprays, thicker sections, special temperature regimes of chamber, block and knife blade (Peters, 2010). Unfortunately, the attempts failed to enable all fish from being analysed. The method of touch imprint cytology was also attempted but proved ineffective due to an unclear hepatocellular impression and noise presence in sections. As a result only three fish species were used for analysing LMS including whiting, saithe, and haddock, although for haddock only the platform group was possible and the reference group is not reported (**Figure 14**). The observed lysosomal responses in fish liver cryo-sections from all the stations were within the normal range of destabilization times. No significant differences were found between the groups.

Fatty liver is known amongst histologists as being difficult to section and problems with sectioning have been previously report in the Atlantic cod (Holth *et al.*, 2011). In this case the head kidney instead of the

liver was successfully used, which may be a suggestion for future WCM programmes when fish are targeted.

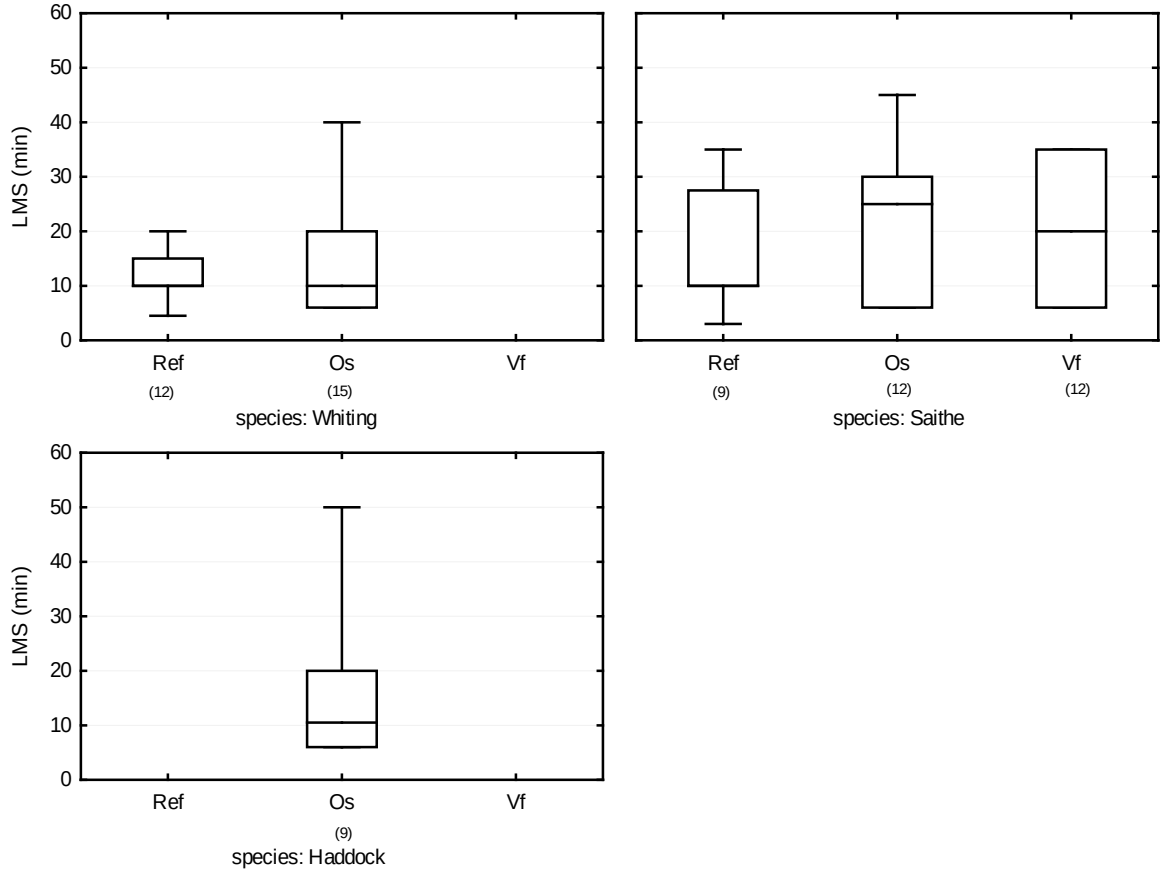

Figure 14. Lysosomal membrane stability (LMS) in fish liver samples from the locations indicated. Ref, Reference; Vf ,Veslefrikk; Os, Oseberg Sør. No significant difference from reference group. Numbers in parenthesis denote individual n values.

3.3.5 DNA adducts

Significant increases in the frequency of DNA adducts were found in populations of saithe from both the Oseberg Sør and Veslefrikk platforms compared to its reference group (ANOVA, Dunnett's, $p<0.05$) (**Figure 15**). Elevated levels of DNA adducts above reference values were also observed in tusk, ling, haddock and whiting although not statistically significant. This was particularly so for tusk with a median value of 3.1. However, due to large variations observed, the p value was slightly above the 0.05 significance level. The DNA adduct frequency was almost zero in all reference fish. With the exception of tusk all median values were below 2.

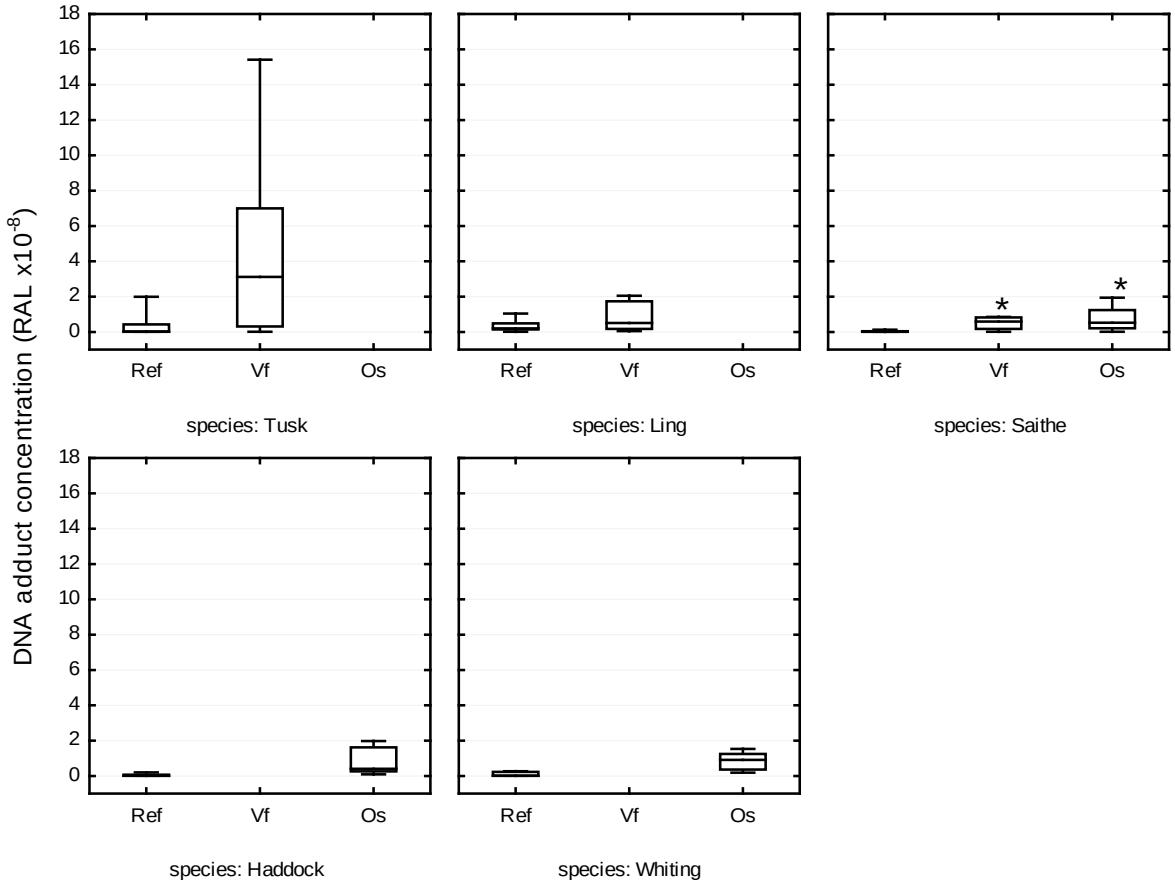

Figure 15. The frequency of DNA adducts in fish liver samples from the locations indicated. Ref, Reference; Vf, Veslefrikk; Os, Oseberg Sør. * indicating significant difference from reference group (ANOVA, Dunnett's, $p<0.05$, n=12 except ref ling n=9).

3.3.6 COMET

The percentage DNA damage assessed through the size of the comet like tail showed a significant increase in ling collected from Veslefrikk compared to its reference group (**Figure 16)**. No significant differences between groups were observed in the other fish species. High variability in DNA damage was observed in saithe, particularly from the saithe reference group. The oxidation of base pairs measured through enzyme treatment revealed further differences between the groups for two of the demersal fish species (**Figure 17**). When oxidation of base pairs was combined with % DNA damage significantly high values were found in tusk and ling from the Veslefrikk platform and whiting from the Oseberg Sør platform compared to their respective reference groups (ANOVA, Dunnett's, $p<0.05$). In contrast, reference saithe showed higher DNA damage and base pair oxidation than saithe from Oseberg Sør.

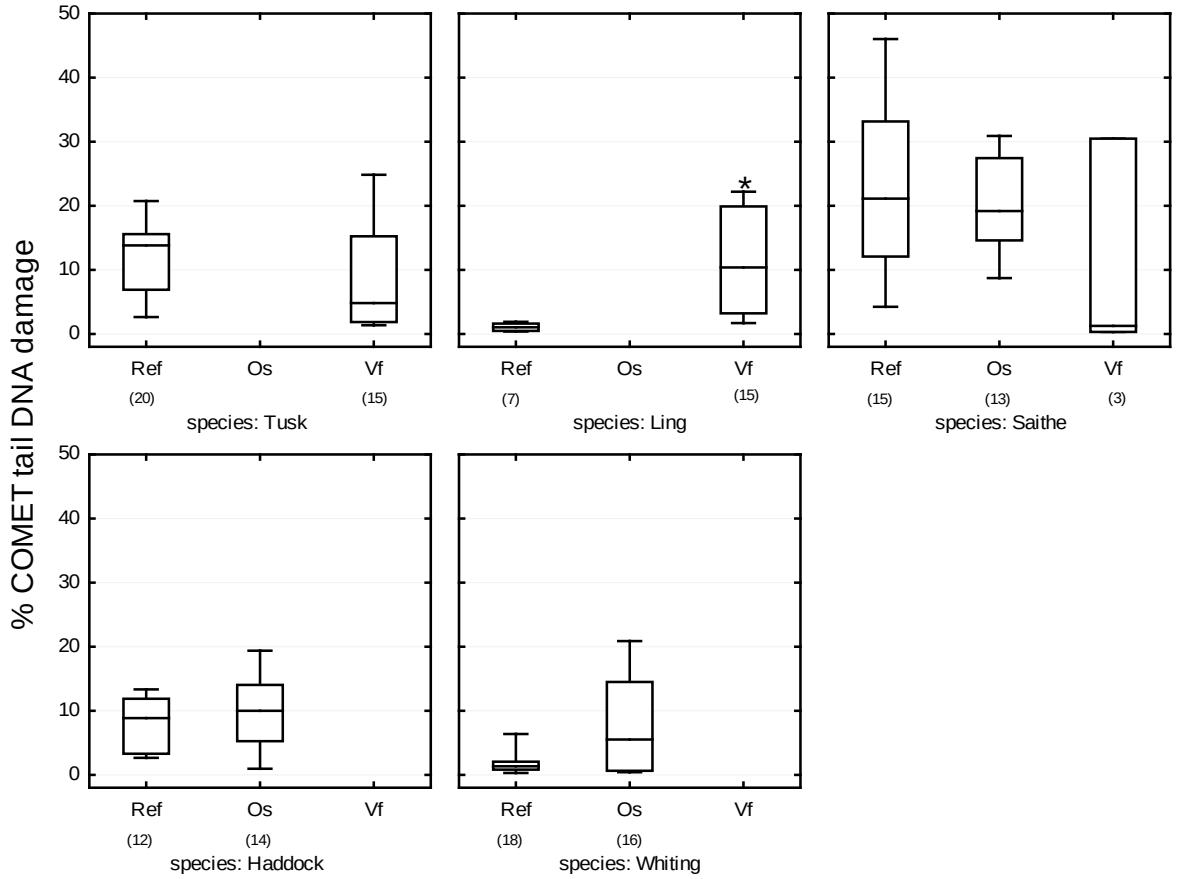

Figure 16. DNA strand breakage in fish blood samples from the locations indicated. Ref, Reference; Vf, Veslefrikk; Os, Oseberg Sør. * significant difference from the reference group (Mann-Whitney, $p<0.05$). Numbers in parenthesis denote individual n values.

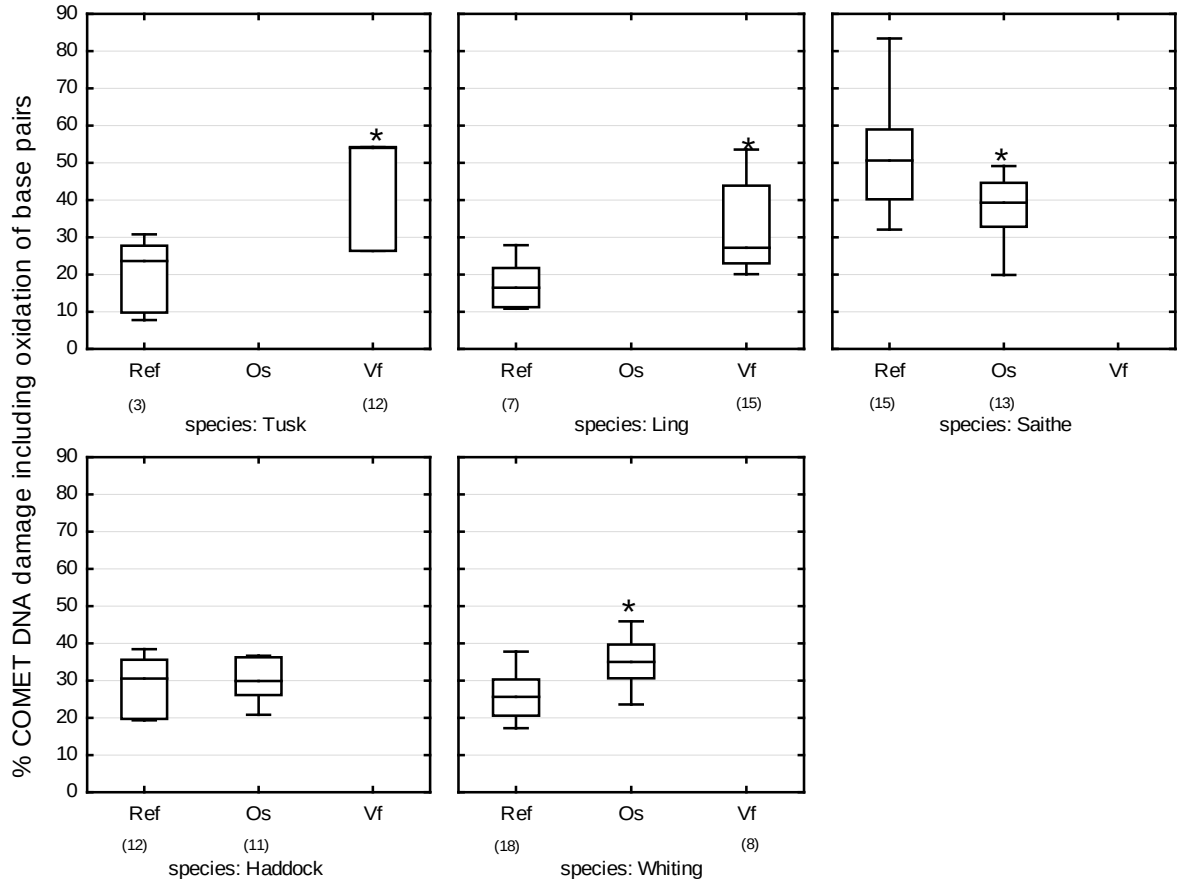

Figure 17. DNA strand breakage including oxidation of DNA base pairs in fish blood samples from the locations indicated. Ref, Reference; Vf, Veslefrikk; Os, Oseberg Sør. (median, quartiles (box) 10/90 percentiles (bar)). * significant difference from the reference group (ANOVA, Dunnett's, $p<0.05$). Numbers in parenthesis denote individual n values.

3.3.7 Fish Histology

In total, liver sections of 109 sampled fish were analysed for the presence of histopathological lesions. By using the scoring system, 22.9% of all specimens were categorized as being non-affected. The degree of hepatocyte vacuolation is a measure that was not used as pathological lesion. It is species-specific and highly dependent on the stage in the reproductive cycle and the availability of an adequate food supply as was shown for European flatfish dab (*Limanda limanda* L.), flounder (*Platichthys fleus* L.) (Feist *et al.* 2004) and Baltic eel pout (*Zoarces viviparus*) (Frick *et al.* 2012). Analysed species showed various degree of hepatocellular vacuolation: normal with the uniform vacuolation, low, medium and high degree of vacuolation (**Figure 18**).

Steatosis lesions noted almost for all specimens were suggested to be part of the normal cyclical (and non-pathological) status of the liver. This conclusion was made according to fatty content of the liver of studied species. Such, total liver lipid contents of Atlantic cod (*G. morhua*) and haddock (*M. aeglefinus*) is high: 36.9% and 67.2% respectively (Zeng *et al.* 2010). According to that fact that all the analysed species belong to the same order Gadiformes, and are cold- and deep-water fish, it can be assumed that all of them have high liver lipid content. During histological processing normal liver vacuolar structure can be disrupted, which can led to formation of enlarged vacuoles characteristic for macrovesicular steatosis (**Figure 19**). For the same reason it was difficult to distinguish between degrees of hepatocellular vacuolation.

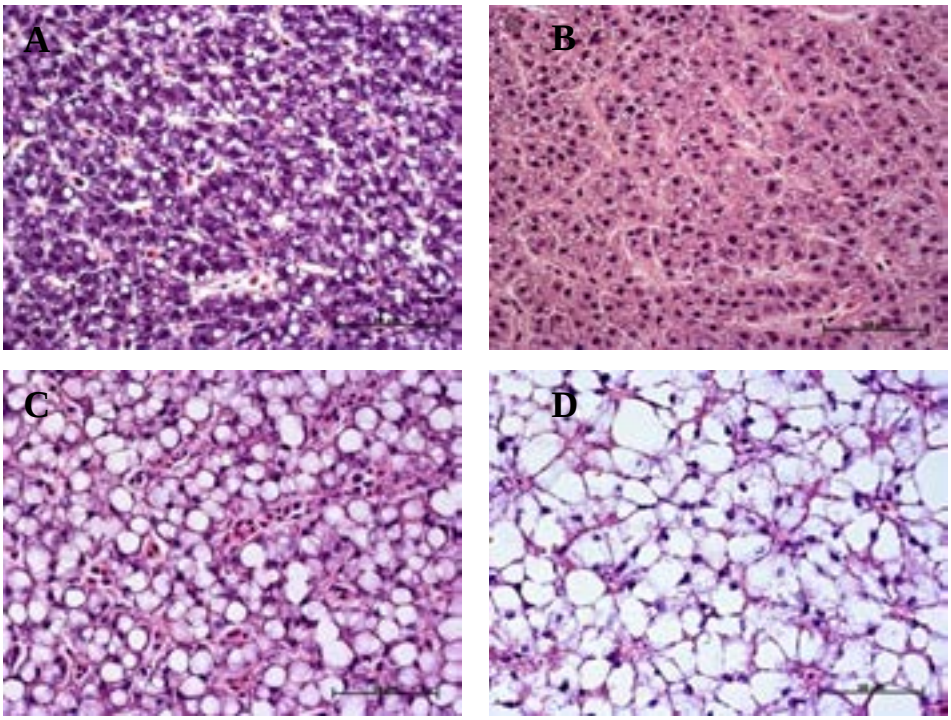

Figure 18. Normal liver sections with varying degree of hepatocellular vacuolation: a) normal, uniform vacuolation, whiting, b) low degree of vacuolation, saithe, c) medium degree of vacuolation, saithe, d) high degree of vacuolation, common ling.

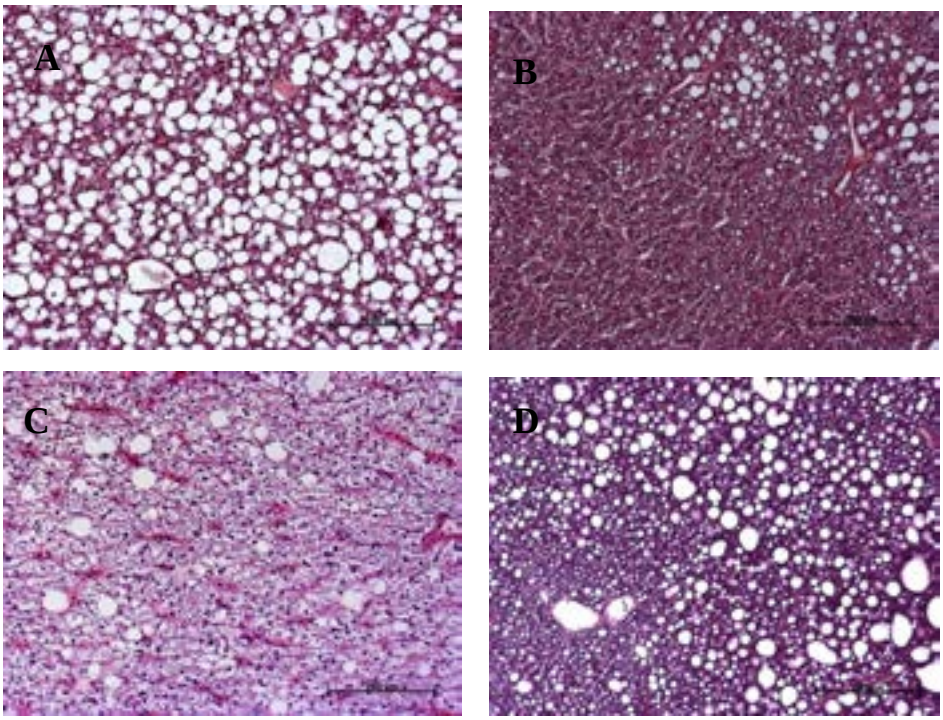

Figure 19. Liver sections with varying degree of steatosis: a) tusk; b) haddock, note the varying hepatocellular vacuolation; c) haddock, note granular appearance of hepatocytes (microsteatosis); d) whiting, note the varying hepatocellular vacuolation.

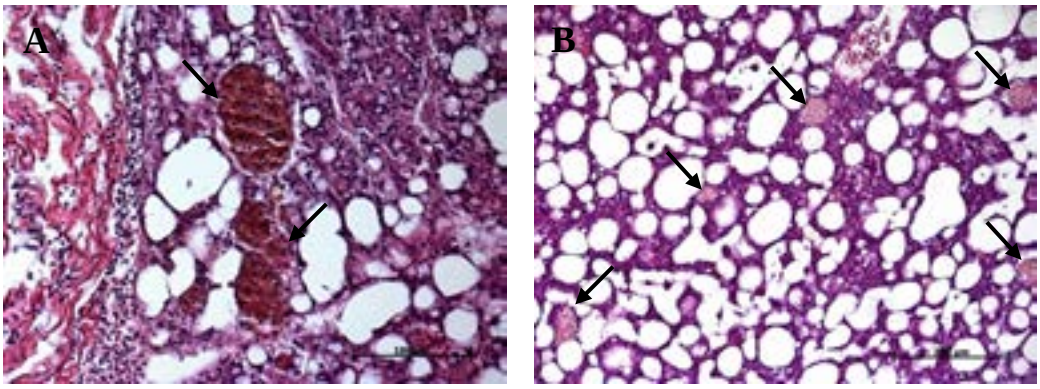

Figure 20. Melano-macrophage centres: a) haddock and b) tusk.

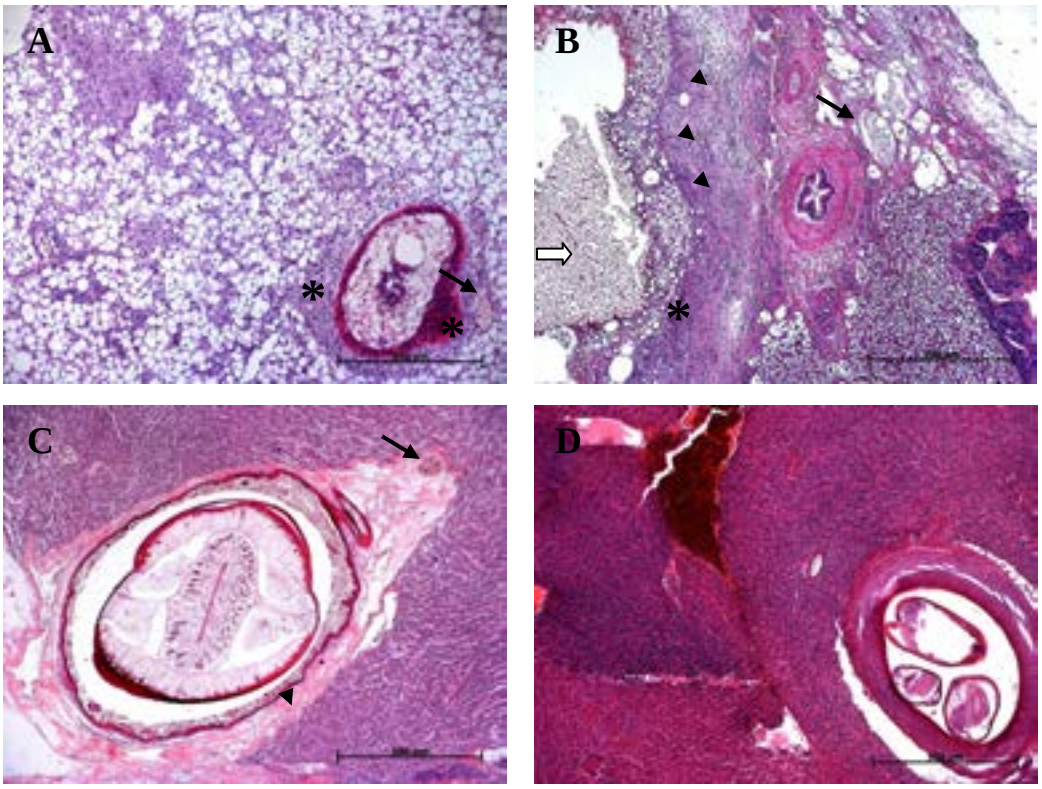

Figure 21. Pathological alterations: a) common ling, granulomatous formation (left corner), inflammatory reaction around pathological changes (right corner); b) cirrhotic changes in saithe – hepatocytes nodule (open arrow) surrounded by wall of fibrous/connective tissue; c) parasite invasion in haddock; d) peliosis hepatis in saithe, note the presence of large "blood lake" containing intact erythrocytes and eosinophilic serous material (left corner), parasite invasion (right corner).
Arrow – MMC, * - inflammatory reaction, infiltrated lymphocytes, arrow head - fibrous/connective tissue

Melano-macrophage centers (macrophage aggregates, MMC) were present in analysed liver to varying degrees, singly or large in numbers. As in other species they varied in size and in colour (from yellow or pink to golden brown and black) according to the variety of pigments and the cellular content (Agius and Roberts, 2003; Fricke *et al.* 2012) (**Figure 20**; **Figure 21**b, c). Melano-macrophage centers increase in size or frequency in conditions of environmental stress and have been suggested as reliable biomarkers for water quality in terms of both deoxygenation and chemical pollution (Agius and Roberts, 2003). Some findings suggest that the morphological features of MMCs are weight or age related. For better understanding of MMC role in monitoring programmes it is advisable to use them as a separate criterion

and in relation with other parameters (diseases, parasites presence, age-weight, etc.). Parasite invasions reflect only organisms that were found in analysed specimen. This parameter does not contain information from gross observation of liver parasitic invasion status.

Circulatory disturbances, inflammatory changes and other pathological changes are present with different occurrence in analysed species (**Figure 22**). The prevalence of inflammatory changes was markedly elevated above reference values in tusk, ling and saithe from Veslefrikk, as well as saithe from Oseberg Sør.

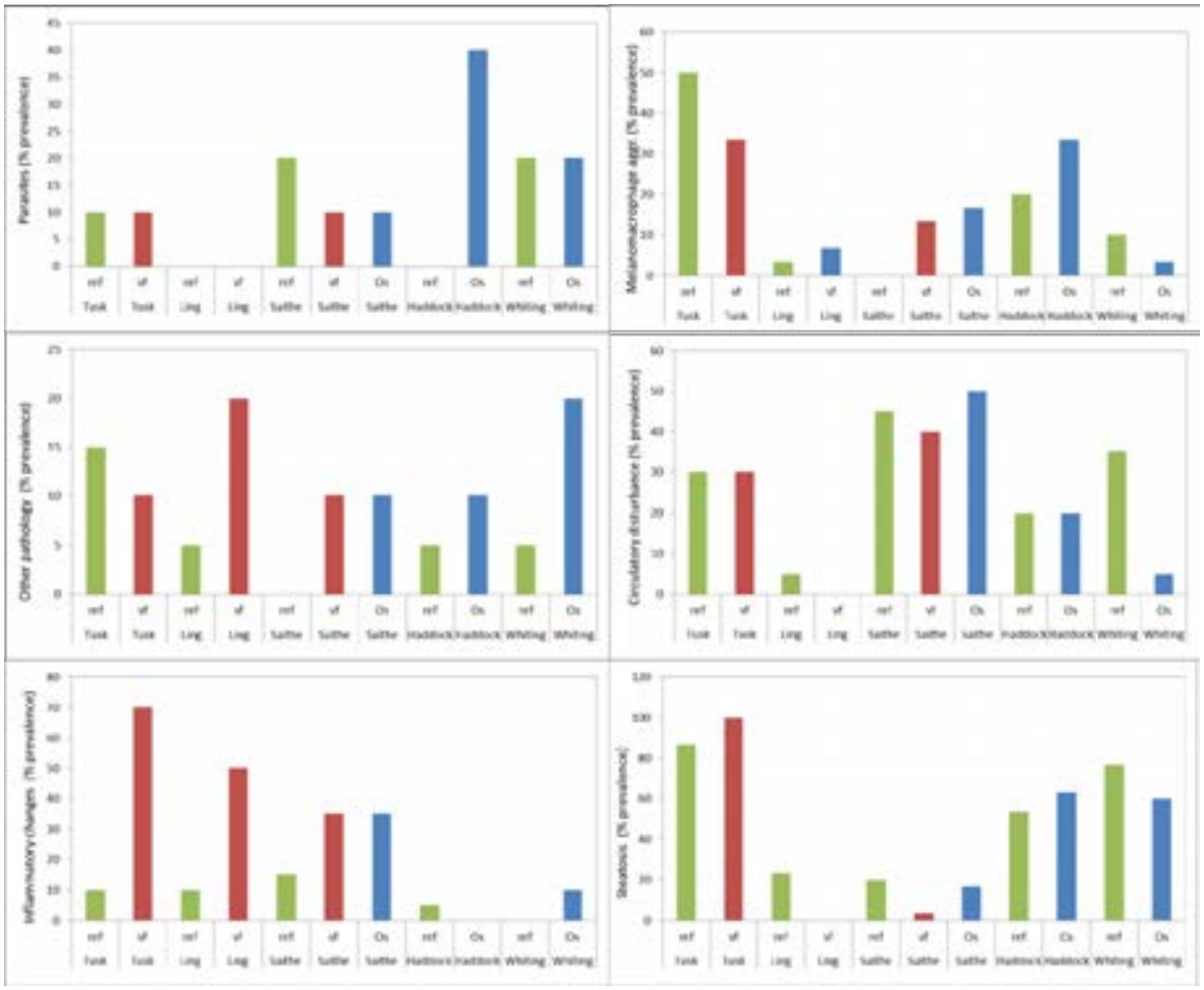

Figure 22. Fish histopathology in the five fish species collected from the platforms and reference groups. Data expressed as percentage prevalence.

3.4 Integrative assessment

The integrative biological response index (IBR/n) was calculated from star plots of normalised biomarker data from the five 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 23**). The biomarkers were positioned in relation to function and include DNA adducts, COMET, AChE, EROD, histology and PAH metabolites. The mean values of each biomarker for the respective fish species were presented. The histopathology data were taken as the mean prevalence of all histopathology data excluding the parasite data, whilst the PAH metabolite data was taken as the mean of all detected PAH metabolites from 10 fish per group.

The highest IBR/n was calculated in tusk from the Veslefrikk platform (IBR/n =1.5), with largest contributions coming from DNA adducts, histopathology and AChE. The IBR/n from reference tusk was approximately half of the Veslefrikk tusk value. However, the IBR/n of the reference tusk was higher than all other reference values. The IBR/n values for ling at the Veslefrikk platform were higher than the reference group, although the IBR/n score was much lower than that obtained for tusk. Of the fish from the Oseberg platform, haddock had the higher IBR/n value, with contributions from PAHmet, EROD and histology. Higher IBR/n values were also found in saithe from both platforms compared to the reference group, although slightly higher in the Veslefrikk population. For whiting, very similar IBR/n scores were found at both reference and the Oseberg Sør platform. Overall, IBR/n scores were slightly higher in fish from the Veslefrikk platform and lower in the reference groups.

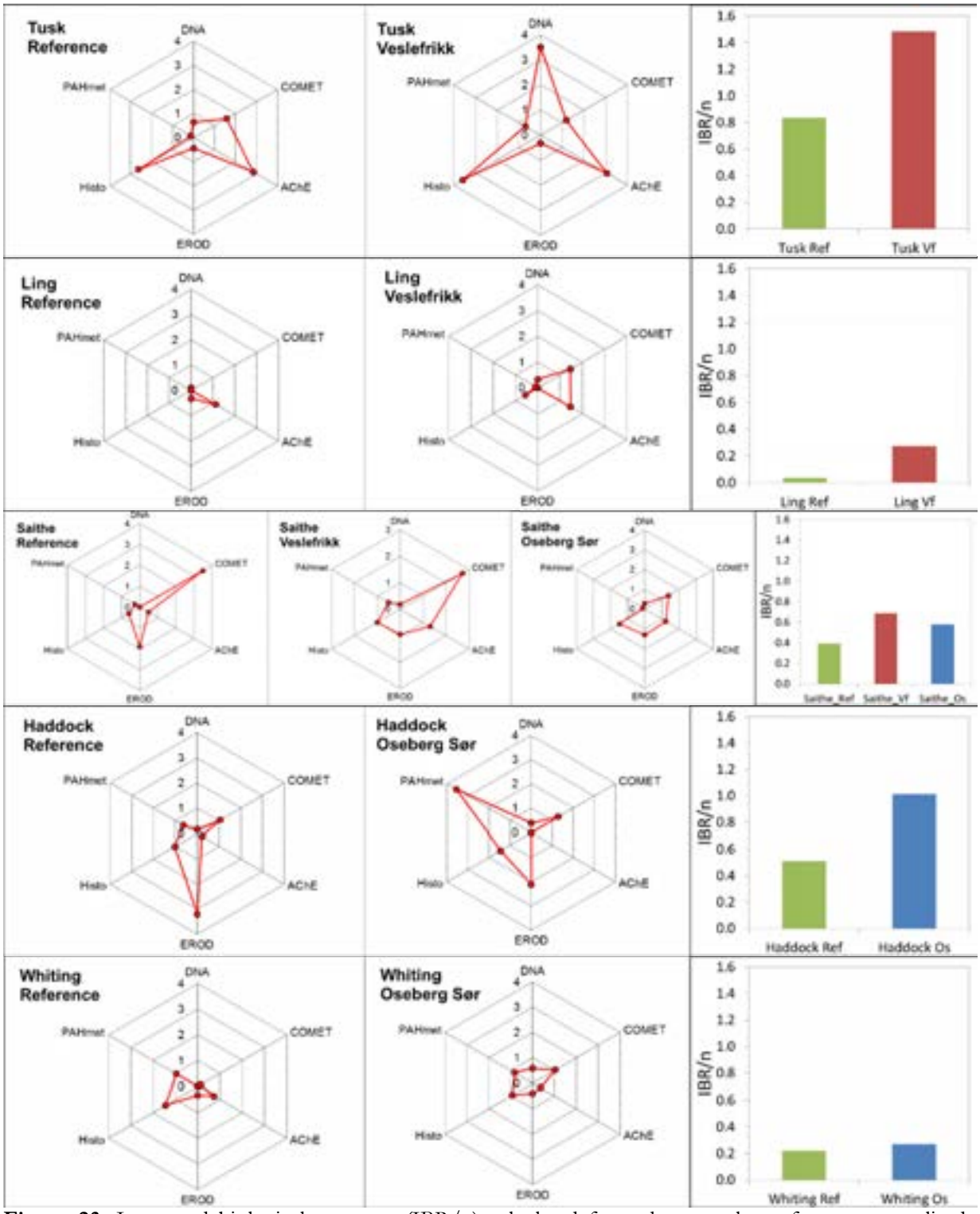

Figure 23. Integrated biological response (IBR/n) calculated from the star plots of mean normalised biomarker data in the fish species from the locations indicated. The biological effects were grouped in relation to function. Ref, Reference; Vf, Veslefrikk platform; Os, Oseberg Sør platform.

4. Discussion

4.1 Chemical concentrations in fish and invertebrates

PAH-NPD concentrations were measured in fish fillet and stomach content of the fish collected from around the two platforms and the reference area. In all cases the concentrations of PAH-NPD were low or below detection limits. The same suite of PAH-NPD chemicals were also measured in selected invertebrates from the three locations with similarly low or undetected concentrations. 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.

Concentrations of eleven PFCs were measured in blood samples of native fish. PFOSA, PFUdA, PFOS, PFNA and PFDcA were detected in the blood of all fish although the concentrations were relatively low. In the literature PFCs have been measured in a wide range of marine organisms and in various tissues from whole homogenates, liver and blood (Hekster *et al.*, 2003). However, data available for fish is mostly from the liver where PFOS is usually most abundant. However, of the limited data available in fish blood, PFOSA has been found to be the most abundant PFC. The median PFSOA concentration measured in the blood of 25 cod from the inner Oslo fjord was 216 µg/ L. This was approximately 6 times higher than that found for PFOS in the blood of the same fish (Schøyen and Kringstad, 2011). Incidentally, PFOS is typically more abundant than PFOA in fish liver samples (Green *et al*, 2010). From the fish sampled in the North Sea, ling showed markedly higher concentrations of PFOSA than any other fish species with maximum blood PFOSA concentrations of 56 µg/ L. The reasons for this are unclear but possibly related to differences in diet and habitat niche. The concentrations of PFOSA in the blood of fish in our study were markedly lower than those collected from the Oslo fjord and much lower than expected to cause toxicity, which are in the region of low to mid mg/ L concentrations (reviewed in Hekster *et al.*, 2003).

4.2 Biomarker responses in local fish

4.2.1 PAH metabolites

PAH metabolite values in fish collected from around the platforms, measured by the FF method, were generally low. No apparent differences were found for the fish species except for tusk, where a small increase in 2-3 ring PAH metabolites was observed in the platform population. The concentrations were 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).

PAH metabolite data were confirmed by GC-MS. Most of the reported values were below the limit of quantification. Nevertheless, small differences were found in concentrations measured in haddock and whiting caught at the Oseberg Sør platform compared to the reference groups. Although these differences were small, they could be related to differences in fish behaviour (e.g. living area, feeding habits, etc.). However, there was no relationship found between fish behaviour and PAH metabolites

4.2.2 Health indices, CI, LSI and GSI

For general health parameters including nutritional and reproductive status, length and weight data were collected from individual fish. These are quick and cheap assessments that should be taken when sampling fish and can be useful as an additional parameter for explanation of 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 seen compared to pre-exposure fish due to the availability of food in caged fish. However, this was not expected to occur in wild caught fish from this study. Fish length was slightly different between groups with reference fish being smaller than those caught near the platforms. This was particularly the case for ling and saithe. Although all fish measured were considered adult, the smaller reference fish would indicate relatively younger fish than those from the platforms.

Increases in both chemical bioaccumulation and certain biomarker responses may have been expected in the older (larger) fish, although no obvious pattern between size and response was observed.

The CI 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 species), rather than an indication of poor condition. The CI did not differ between locations for all the fish species measured and suggests that the biomarker data is suitable for comparison between the platform(s) and reference groups.

A significantly lower LSI in haddock and tusk from the Oseberg and Veslefrikk platforms respectively compared to their reference population may suggest a toxicity response to contaminant exposure. However, LSI can also indicate fish malnourishment, which may explain the observed differences in these two demersal fish species. In contrast, the LSI in saithe from the Veslefrikk platform was significantly higher than the reference group. Saithe are more pelagic than haddock and tusk and feed higher in the water column. Differences in malnourishment between the fish species and locations may reflect the differences in habitat and diet.

The calculated GSI values were similar in all species with exception to whiting and saithe, which both had a significantly higher GSI in females from the Oseberg Sør platform compared to their female reference group. This suggests that the female whiting and saithe populations from the platform where at a different reproductive stage than there reference population. Since reproductive status can influence certain biomarker responses (e.g. EROD), care should be taken when interpreting the respective biomarker data.

4.2.3 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 with 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; fish 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. Large variations in EROD activity were found, which prevented any statistical differences from being made, although in many cases higher EROD activities were observed in the reference fish compared to those from around the platforms. In order to determine if some confounding factors such as age, general fitness and reproductive stage were influencing EROD activity, correlation analysis between EROD activity and fish length, CI, LSI, gonad weight and GSI were performed (data not shown). However, no significant correlations for any of the fish species were found.

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 suggested for a variety of different fish species, which are influenced 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 suggested BACs are included for dab, *Limanda limanda* (680 pmol/min/mg protein); cod (145 pmol/min/mg protein); plaice (255 pmol/min/mg protein); dragonet (202 pmol/min/mg protein); and the four spotted megrim (13 pmol/min/mg protein). With the lack of a specific BAC, cod could be the best available estimate for the fish species in the current study. All EROD measurements from fish collected around the platform as well as at the reference location were well within 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.

4.2.4 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 (Galgani and Bocquene, 1990), heavy metals and PAHs (Kang and Fang, 1997) have been found to inhibit AChE activity. Assessment criteria are available for a few marine fish including dab, flounder, red mullet and eelpout with BACs ranging from 235 to 124, and EACs ranging from 165 to 87 nmol/min/mg protein in fish muscle tissue. The median values for the five fish species measured in the present study were within these values and above ranging from 100 to 900 nmol/min/mg protein. However, species specific assessment criteria are required before they can be effectively used to indicate potential biological effects.

For the fish species analysed in this study a significant reduction in AChE activity was observed in saithe from both the Oseberg Sør and Veslefrikk platforms compared to the reference group. A previous study based on a controlled laboratory exposure suggested that PAHs of 3 rings or move were required to inhibit AChE activity in the electric eel, whilst small molecular weight PAHs (below 3 rings) failed to inhibit the enzyme (Kang and Fang, 1997). The chemical data from fish fillet and bile metabolites in the saithe did not show any major influence of PAH exposure that could attribute an effect, although the AChE response from the saithe would suggest that PAH exposure did occur to a certain extent.

AChE activity has been performed in various tissues 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 ling, tusk, whiting 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, 2011). 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.

The findings demonstrated that saithe were exposed to potentially neurotoxic compounds with a reduction in AChE activity, although more information on the AChE response of the fish species used through controlled laboratory studies would prove useful in understanding their responses in field scenarios.

4.2.5 LMS

Lysosomal membrane stability was measured in cryostat sections of fish liver. However, due to the high fat content of some of the fish livers, making the tissue difficult to section, not all fish species were successfully analysed. This problem could be solved by choosing an alternative organ (e.g. head kidney) for cryo-sectioning and analysis in future studies with these fish. Despite the challenges in sample preparation, lysosomal responses where measured in two of the fish species (i.e. whiting and saithe). Although assessment criteria are not specifically available for whiting and ling, there are general BAC and EAC values of 20 min and 10 min that are available (ICES, 2011). Median values for whiting from both the reference and platform had a labilisation time of 10 min, which was below the background level and equal to the EAC. Reference saithe also had a labilisation time of 10 min, whilst the median values of the Oseberg Sør and Veslefrikk were 20 and 25 min respectively. However, due to the large variation in the data no significant differences were found between the groups. 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.

4.2.6 DNA adducts

Although only significantly higher DNA adducts were found in saithe from both Veslefrikk and Oseberg Sør platforms, all fish species collected from around the platforms had higher concentrations of DNA adducts than their respective reference groups. The ICES assessment criteria for DNA adducts have been developed for a handful of fish species including dab, flounder, cod and haddock. With the frequency of BACs for DNA adducts ranging from 1 (dab, flounder), 1.6 (cod) and 3 (haddock) nm adducts per mol DNA. The DNA adduct data in the current study was expressed as RAL x 10^8, therefore multiplying the values by 10 enables the data to be directly compared with assessment criteria.

The median DNA adduct values for both tusk and whiting were above the EAC of 6 indicating exposure of these fish to genotoxic compounds. The median tusk value of 31 nmoles adduct/ mole DNA was more than 5 times above the EAC. The median DNA adducts for haddock from Oseberg Sør were above the species specific BAC (3) and below the EAC (6), suggesting some level of genotoxicity. Ling and saithe from Veslefrikk and Oseberg Sør respectively showed median DNA adducts marginally below the EAC value. However, the suitability of these assessment criteria is somewhat uncertain due to the absence of species specific assessment criteria for the fish sampled in our study (except for haddock). Despite the lack of species specific assessment criteria, the high median DNA adducts exhibited by tusk were likely to be well above any proposed EAC for this species and therefore should be treated as a substantial genotoxic response.

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. This is particularly useful when measuring responses in wild fish as opposed to field transplanted fish, with the period of monitoring markedly 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.

4.2.7 COMET

The results from the COMET analysis showed DNA strand breaks as percentage COMET tail as well as enzyme treated, which indicates the oxidation of base pairs. For the percentage COMET tail indicating DNA damage, a significant difference was only found for ling having significantly higher DNA damage in the fish from the Veslefrikk platform compared to the reference group. The ling, being a true demersal fish species, would be expected to live on the sea floor and may be subject to contaminants from historic drilling muds known to occur at Veslefrikk. However, the other demersal fish (tusk) found at Veslefrikk did not show elevated levels of DNA damage compared to its reference group using this method. From the Oseberg Sør platform haddock and saithe showed no increase in DNA damage close to the platform,

although for whiting despite not being a demersal fish did exhibit elevated levels compared to the reference group, although not statistically significant.

Assessment criteria for the COMET assay has not been established for the fish species in our study and currently only BACs are available for dab (*Limanda limanda*) and cod (*Gadus morhua*), both of which stand at 5% DNA tail (ICES, 2011). Considering this value for our data would clearly show that in most cases the median value was above this BAC, including many of the reference groups. Higher than expected DNA damage was observed in many of the fish from the reference groups (tusk, saithe and haddock). 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 baseline data are not available for these species it is difficult to make the assumption. 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 for both demersal fish species (tusk and ling) from the Veslefrikk platform as well as whiting from the Oseberg Sør platform. In contrast, saithe from Oseberg showed significantly lower oxidation of the DNA base pairs (FPG treated) than the reference group, although this more likely reflects the higher damage in the reference saithe rather than the lower values in the platform fish. 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 above the DNA damage measured in the fish (except saithe) from around the platforms.

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). However, there were no within species gender differences with respect to % COMET tail for the fish species measured in the current study. Furthermore, there were no correlations found between size (length, weight) and % COMET tail.

4.2.8 Fish liver histology

The histopathological examination of the liver in fish provides important information concerning fish health status at tissue/organ level. All of the five analysed species from all five groups (platform and reference) showed various percentage/occurrence of different histological abnormalities, pathologies, and no one species or group appeared to be more or less affected than another. However, there was an increased prevalence of inflammatory changes in fish from the Veslefrikk platform and to a lesser extent from the Oseberg Sør platform compared to the reference group, which may suggest a response to contaminant exposure. These changes at tissue level may reflect the subcellular effects observed in these fish species from Veslefrikk, particularly with regard to the genotoxic responses.

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 incorporated into biological effects monitoring programmes. The obtained results from the analysed species can 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 in other species. All these improvements together with obtained results will strengthen use and inclusions of histopathology in future monitoring programmes.

Overall, biological responses were observed in fish from around the platforms despite the low or undetected concentrations of PAH-NPD concentrations measured in the fish fillet or PAH metabolites in the fish bile. This 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 benefit of using biomarker tools in order to assess the potential biological effects of complex mixtures, which contain low individual concentrations of many compounds, although when combined can cause toxicity.

4.3 Integration of the biological effects data

The integrated biological response index was applied to the data in order to determine the overall impact of the two offshore platforms on the local fish populations. Based on historical sediment contaminant data the Veslefrikk platform was considered to be the more contaminated of the two. Although the IBR/n scores were not particularly elevated, they were able to differentiate between the reference and the platform populations. This was most obvious for the Veslefrikk platform, which exhibited a higher IBR/n for tusk in particular and to a lesser extent in saithe compared to their reference groups. However, an elevated IBR/n was also calculated for haddock from Oseberg compared to the reference group.

The two demersal fish species (tusk and haddock) that occupy and feed from the seafloor were likely to be exposed to contaminants in the sediment and drill cuttings. For tusk, the main contributions were DNA adducts and histopathology, 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, as well as ling and saithe indicating that neurotoxic compounds were present from the Veslefrikk area. For haddock, PAH metabolites and COMET contributed most alongside EROD and histology, which may suggest a link between PAH exposure and genotoxicity.

The IBR/n of the reference tusk, although markedly lower than the tusk from Veslefrikk, was higher than all but one of the other fish groups. Since trawling offshore was not possible to catch tusk, due to their tendency to inhabit rocky substrates, tusk were caught by local fisherman from a coastal fjord. Proximity to the shore and to anthropogenic sources of contaminants cannot be excluded and perhaps were responsible for the slightly higher biological responses in the reference group than expected.

Saithe were the only fish species that were caught at both platforms, enabling a comparison of effect between the platforms to be considered. Although small differences were found, the biological responses found in saithe from the Veslefrikk platform were the more pronounced. This may correspond to the higher historical contamination at Veslefrikk compared to Oseberg Sør.

4.4 Confounding factors

With all environmental monitoring programmes there is inevitably a series of confounding factors that have the potential to inadvertently influence the outcome of the data. A well-designed monitoring programme could minimise such factors although often logistical issues, particularly with offshore monitoring, make it difficult to remove completely. For instance, due to the combination of subsea

obstructions around the platform and the lower density of fish in the open sea, the selected methods of fishing at the platform and at the reference site where different. These differences in fishing methods can potentially lead to sample bias. For example, the passive approach of rod and line fishing, performed at the platforms, requires 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 different sampling methods may also lead to differences in the time taken to process individual fish from the time of capture. With rod and line fishing, the fish are caught individually and processed immediately upon capture. This is in contrast to trawled fish, where the trawl takes time to be brought onto deck, and following sorting of the catch, larger volumes of fish are available for processing. In this case fish are kept alive in water tanks on the deck to wait processing and the fish are sampled as quickly as possible. The effect of the different fishing methods on the integrity of the biological samples is kept to a minimum by using organised sampling routines on board the vessel. An experienced sampling crew on board the Johan Hjort that was well operated in its sampling routines was able to maintain a good integrity of the biological samples during the present study. However, differences in sample integrity between the methods of fishing cannot be completely excluded.

Since insufficient numbers of tusk were collected by trawl from the reference area, reference tusk were collected with long lines from the Bokna fjord with the help of local fishermen. The fishing method of long line reflects that used at the platforms, potentially selecting the more active and healthier fish. The deepest part of the Bokna fjord is approximately 600 m. The location selected for the reference tusk was in the outer part of the Bokna fjord close to Kvitøy (N59 05 10.8 E5 26 11.2, WGS 84) with depths between 150-200 m. This area is open with direct water communication with the North Sea and was considered to be a suitable location for reference fish for the North Sea. However, care must be taken when using coastal populations as reference populations since exposure to anthropogenic contaminants from land run off and local discharges cannot be completely prevented. Proximity to land will likely increase the chance of exposure to anthropogenic sources of contaminants, which could potentially lead to effects on the biological responses.

4.5 Considerations for future WCM programmes

The 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 two offshore platforms. 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. AChE activity also showed responses with significant reductions in saithe from both platforms compared to the reference group and showed potential for measuring biological responses from oil related compounds. In contrast, EROD showed no response in fish living around the two platforms 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. 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.

Although LMS was only performed fully in two species there was no response observed in hepatocytes of those two fish living close to the offshore platforms. The use of the head kidney rather than the liver should be considered for LMS measurements in future WCM programmes, particularly in fish with lipid rich livers (e.g. tusk, ling). However, suitable validation data will be required before head kidney is fully incorporated as a valid alternative.

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 Veslefrikk platform compared to the reference groups, which may be related to the genotoxic responses observed at the subcellular level in these fish species (e.g. tusk). The challenge of performing histopathology on new species makes interpretation difficult, and more validation data for these species is required.

The fish species used in the current study were a combination of pelagic and demersal fish that were likely to have different exposure profiles based on their modes of life. In addition, migratory behaviour, although considered to be minimal, cannot be totally excluded and caution must be taken when explaining the data. For instance, in most part it was assumed that the fish caught in the vicinity of the platform(s) were living within the local area and were not merely passing through. Therefore, the biological responses measured were considered to be as a result of living in close proximity to the offshore platforms. In this study it did appear that overall the demersal fish species showed the greater biological effects compared to the pelagic fish. It may be assumed that exposure to oil related contaminants in the sediment or consumption of sediment dwelling organisms were responsible for these differences, although the PAH metabolite data failed to link the exposure with the effect. However, only PAH metabolites were measured and although these are considered to be the most abundant oil related compounds, others such as alkylphenol, naphthenic acids and other organic acids and metals are likely to have contributed to the contaminant exposure and perhaps should be considered for analysis in future studies.

5. Conclusions

- PAH-NPD concentrations in fish fillet, fish stomach content and invertebrate samples were undetected or marginally above the detection limits at Veslefrikk and Oseberg Sør platforms as well as the reference group.
- PFCs in fish blood samples were detected with PFOSA the most abundant. However, the concentration of PFCs were relatively low, six times lower than PFCs measured in the inner Oslo fjord and up to six orders of magnitude below concentrations expected to cause environmental toxicity. No differences were observed between the two oil fields and the reference location
- Significant biological responses in DNA adducts, COMET, liver histology and AChE were observed in fish collected from around the Veslefrikk and Oseberg Sør platforms as compared to the reference location.
- 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 biomarker tools in order to assess the potential biological effects of complex mixtures, which contain low individual concentrations of many compounds, although when combined can cause toxicity.
- Integration of the biological effects data revealed that tusk from the Veslefrikk platform showed the highest integrative response.
- Overall the fish from the Veslefrikk platform appeared to be more impacted than those from Oseberg Sør and reference groups.

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Appendices

Appendix	Description
A	WCM2013 cruise report
B	Raw data: NIVA analysis
C	Raw data: IRIS analysis
D	DNA adduct report from AdnTox
E	DNA adduct appendix from AdnTox
F	Additional techniques: proteome analysis of fish bile
G	DNA adduct literature study

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