Water column monitoring of offshore produced water discharges. Compilation of previous experience and suggestions for future survey design

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Abbreviations

AP	Alkylated phenol
BCF	Bioconcentration Factor
BECPELAG	Biological Effects of Contaminants in Marine Pelagic Ecosystems (ICES)
CI	Condition Index
CTD	Conductivity, Temperature, Salinity
DREAM	Dose Related Risk and Effects Assessment Model
EIF	Environmental Impact Factor
EDC	Endocrine Disrupting Compound
FA	Fatty Acids
GSI	Gonado Somatic Index
HPG axis	Hypothalamic-Pituitary-Gonadal axis
ICES	International Council for the Exploration of the Sea
IRIS	International Research Institute of Stavanger
Klif	Klima- og forurensningsdirektoratet
LOEC	Lowest Observable Effect Concentration
LSI	Liver Somatic Index
NA	Naphthenic Acids
NIVA	Norsk Institutt for Vannforskning
NL	Neutral Lipids
NPD	Naphthalenes, Phenanthrenes and Dibenzothiophenes
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PL	Phospholipids
POCIS	Polar Organic Chemical Integrative Sampler
PRC	Performance Reference Compounds (passive samplers)
PSD	Passive Sampling Device
PW	Produced Water

SPMD	Semipermeable Membrane Device
TENORM	Technologically Enhanced Naturally Occurring Radioactive Materials
VTG	Vitellogenin
WCM	Water Column Monitoring, in this report used specifically about the mandatory monitoring in Norway
WGBEC (ICES)	Working Group on Biological Effects of Contaminants
ZRP	Zona Radiata Protein

1 Program overview

As an alternative to field monitoring, the WCM 2010 program consisted of desktop and laboratory studies. The scope of the programme was to disseminate and compile previous PW related biomonitoring and effect experience, make available new information and provide suggestions for future survey design. An overview of the different work packages in the program is listed in Table 1.

Table 1. Outline of the work packages (WP) completed within the water column monitoring programme 2010. (Responsible contributors are underlined).

WP	WP short name	Contributors	Task
1	Administration	IRIS, NIVA, IMR	Workshops, coordination
2	PW – cod reproduction	IMR	Publication
3	PW – endocrine disruption	NIVA, IRIS, IMR	Publication
4	PW – effects Atlantic cod	IRIS	Publication
5	PW biomarker validation cod	IRIS, NIVA	Publication
6	PW biomarker validation mussel	IRIS, NIVA	Publication
7	WCM 2006-2009	NIVA, IRIS	Publication
8	Condition monitoring	IMR	Publication
9	Validation of exposure markers	NIVA, IRIS	Publication
10	Assessment criteria	IRIS	Publication
11	Exp levels APs	IRIS, NIVA, IMR	Publication
12	Risk assessment APs	IRIS	Publication
13	Bioavailability	IRIS	Laboratory exposure
14	PW, TENORM	IRIS	Analysis/method development WCM material
15	Gill lesions	IRIS	Additional analysis of WCM material
16	Summary report	IRIS	Summary report with recommendations

2 Introduction

Organisms living in the water column around offshore oil and gas production facilities are potentially exposed to chemicals through discharge of produced water (PW). The composition of PW varies from field to field, but is generally a mixture of: 1) formation water contained naturally in the reservoir; 2) injected water used for the recovery of oil; and 3) treatment chemicals added during production (Røe Utvik, 1999). Typically, PW contains dissolved inorganic salts, minerals and heavy metals together with dissolved and dispersed oil components and other organic compounds. Data from offshore oil production platforms in the North Sea have showed that the major organic components were BTEX (benzene, toluene, ethylbenzene and xylene), NPD (naphthalenes, phenanthrenes and dibenzothiophenes), PAHs (polycyclic aromatic hydrocarbons), organic acids, alkyl phenols (APs) and phenols (Røe Utvik and Johnsen, 1996; Røe Utvik, 1999). As a natural consequence of well exploitation, oil content in the reservoirs will decrease and the need to inject water will increase, thus eventually leading to increase in the discharges of PW. The total discharge of PW in the Norwegian sector of the North Sea in 2009 was 134 million m^3/ year (KLIF, 2010).

Many components of the PW have been found to have detrimental effects on aquatic organisms, including estrogenic, anti-androgenic and anti-estrogenic effects (Thomas et al. 2009; Thomas et al. 2004). This applies in particular to groups of chemicals such as alkylphenols (APs) and polycyclic aromatic hydrocarbons (PAHs) that are also known to produce various toxic effects including reproductive disturbances, mutagenicity and carcinogenicity (Landahl et al., 1990; Bechmann, 1999; Lye, 2000; Meier et al., 2002). Studies from the ICES workshop "Biological effects of contaminants in the pelagic ecosystem (BECPELAG)" indicate that the effects of toxic compounds can be detected several kilometres away from North Sea oil production platforms using *in vitro* bioassays (Thomas et al., 2006; Tollefsen et al., 2006a) and biomarkers (Regoli et al., 2006). Although there is reason to assume that many of the chemicals that are present in PW effluents may produce biological responses, the ability to assess the potential for adverse effects is limited by the lack of sufficient *in situ* monitoring data using biological effects methods with endpoints (e.g. mussel histology and histochemisty) reflecting long term (ecological) effects.

Water Column Monitoring (WCM) is a programme conducted by Oil and Gas operators in Norwegian waters with the objective to determine the potential biological effects of produced water on the local marine environment. This monitoring is mandatory for operators with produced water discharges, and requires that monitoring is carried out in at least one offshore oil and gas field each year, under the so-called Effect Monitoring Programme. Exceptions can be given where field work is replaced by laboratory experiments or desk-top studies. Within the WCM wild fish should be collected every third year for analyses of contaminants and potential effects from these, under the so-called Condition Monitoring Programme.

Table 2. Studies conducted under the Water Column Monitoring Programme in Norwegian waters.

Year	Effect Monitoring
1995	Statfjord C (not caging)
1997	Tampen (not caging)
1999	Ekofisk
2000	Sleipner
2001	Tampen (BECPELAG)
2003	Troll B
2004	Statfjord B
2006	Ekofisk
2007	Laboratory-/ literature study
2008	Ekofisk
2009	Ekofisk
2010	Laboratory-/ literature study

As an alternative to the yearly Water Column Monitoring field investigation, the effort in 2010 was focused on dissemination of unpublished results from previous PW related investigations. The work was divided into 15 work packages as outlined in Table 2. An overview with abstracts of manuscripts produced within the 2010 programme is provided in the Appendix. In addition to the publication processes, three new PW related laboratory studies were performed in 2010, and reports from these are also provided in the Appendix.

The present summary report provides a compilation of aquired results and experiences over the recent years, references to other relevant studies and recommendations for design of future water column monitoring programmes.

3 Water Column Monitoring Norway

The general design of the WCM programme is to measure a suite of biomarkers and chemical exposure concentrations in caged fish and mussels in order to determine the potential biological effects of a PW discharge on the local marine environment. Previous survey designs have used at least 6 exposure stations at increasing distances from the discharge point(s) (i.e. 200-300m to 2 km), with two reference stations approximately 50 km away from the studied platform in a ‘clean’ area. Due to a combination of cost and logistics fish have only been used at 2 exposure stations at Ekofisk and at the reference stations, whilst mussels have been deployed at all stations. The exposure duration of 6 weeks has been used, which was considered to be sufficient time for significant effects in histological endpoints to occur. Chemical measurements are taken in the whole tissue of mussels and from metabolites in fish bile in order to determine exposure to the PW of the exposure animals. Biomarkers measured include specific effect endpoints, genotoxicity and general stress markers.

It is important to ensure that the caged mussels and fish are exposed to the PW plume. Current conditions at Ekofisk used in surveys in 2006, 2008 and 2009 were consistent and reliable and enabled the cages to be positioned within the PW plume. However, this is not likely to be the situation for other platforms, e.g. as experienced in Troll (WCM 2005, 2003). The depth at which the fish and mussels are held within the water column is dependent on the straftification in the area, with animals positioned above the thermocline at between 10 to 15 m.

Pre-exposure sampling of both fish and mussels is performed to determine the chemical concentrations and biomarker levels in animals prior to exposure. One of the main reasons for using fish is the possibility to measure the potential estrogenic effects of the PW discharge, through the measurement of both vitellogenin (VTG) and zona radiata protein (ZRP). In this case blood plasma samples are taken from cod during the pre-exposure sampling, which have been subsequently tagged to provide individual measurements of VTG and ZRP, i.e pre and post exposure. Measurements (pre and post exposure) of individual fish were used to increase the discriminatory power of the estrogenic biomarkers between the different stations. The use of caged fish as opposed to local wild fish has the clear advantage of ensuring that the fish are exposed to the PW and can be positioned at known distances from the platform. This helps to establish the relationship between PW exposure and biological effect. However, additional stress on field transplanted caged fish can occur through food shortage and/ or abiotic factors (e.g. temperature, pH). These additional stressors on caged fish can potentially interfere with the various biomarker responses and care must be taken when interpreting such biomarker data, taking into account supporting parameters that may provide information on the general health of the fish.

3.1 Additional support parameters

Additional parameters are obtained in order to provide supporting information about the physicochemical conditions of the exposure as well as the biological condition of the test animals. To date, the additional supporting parameters used in the Norwegian WCM programme include physicochemical measurements such as temperature, salinity, current direction and strength, as well as biological parameters of the test organisms including Condition Index (CI), Liver Somatic Index (LSI), and Gonad Somatic Index (GSI).

The health status markers (CI, LSI, and GSI) have been shown to be effective in distinguishing between caged and pre exposure fish suggesting starvation within the caged fish with potential effects on biomarker endpoints. These health status markers have proved useful and should be continued when caged fish are included in future monitoring programmes.

Conductivity, temperature and depth (CTD) have been measured from the platform by platform personnel. The frequency of CTD measurements in past programmes has proven difficult due to the difficulty in communications between scientists and platform staff offshore. However, the benefits of these measurements from the platform are not so clear and focus should instead be placed on taking CTD measurements in the surrounding area at locations relevant to the position of the caged mussels and fish.

Recommendations for future monitoring are to increase the number of locations at which CTD measurements are taken from the research vessel. This includes measurements from the research vessel during deployment and retrieval of the cages at 500 m, 1000 m, and 2000 m from the platform. This will provide important information on the statification in the area, which has a strong influence on the height of the discharge plume and exposure to the caged organisms.

The main current direction close to the platform can change on a daily basis. Therefore, confirmation of the current direction in the area is also vital in order to understand the direction of the discharge plume and help to explain the chemical and biomarker data. It is recommended that at least two current meters should be positioned at stations near the platform during the field exposure.

The bioavailability and toxicity of produced water compounds can be influenced by the type and size of particles within the water column. Turbidity should be measured in future monitoring programmes for at least two locations in the vicinity of the platform, to provide supporting information on the exposure and help interprate the chemical data and biomarker responses.

The use of a UV-fluorimeter for the detection of the PW plume and the potential exposure profiles to the caged organisms over the exposure duration should be investigated. UV-fluorimeters have the potential to detect hydrocarbons within the water column. The main benefit of the UV-fluorimeter would be to provide real-time exposure information throughout the entire duration of the field deployment. Positioning the UV-fluorimeter alongside the caged animals could potentially provide important information on the different type of exposure, such as pulses or as a continous stream. However, the sensitivity of the UV-fluorimeters to the hydrocarbons within the PW plume would need to be investigated to determine whether it could detect the plume at varying distances from the platform.

3.2 Duration of exposure

The duration of organism exposure to a contaminant is known to have a strong influence on different biomarkers. For example, relatively fast responses would be expected for biomarkers at the gene and cellular level, whilst longer term exposures would be required before changes in tissue differentiation and damage would occur. This is of particular interest in caged or transplanted organisms that are used to assess biological effects following exposure for a designated duration. Often in monitoring programmes using transplanted organisms a compromise approach is used, where test animals are exposed for long enough to illicit biological responses in the slower acting biomarker endpoints, which can often be too long an exposure for other biomarkers. Single gene expression of EROD and VTG has been found to be significantly upregulated in fish exposed to PW after 1-2 weeks, although this upregulation was significantly reduced after 4 weeks.

The current exposure duration for the WCM programme is 6 weeks. This has been considered an appropriate time to enable changes in physiological responses such as

histology to occur, although there is the possibility that adaption may be experienced in some of the other biomarkers (e.g. EROD).

Bile metabolites of PAHs and APs are often used when measuring exposure to PW compounds. Although an effective method, it should be made clear that metabolites detected are likely to reflect organism exposure within the last 1-2 weeks, rather than for the duration of the exposure.

For passive sampling devices (PSDs) such as POCIS and SPMDs, the ideal exposure duration is mostly dependent on maintaining a linear uptake for all chemicals sampled. Uptake of a compound and the time when equilibrium is reached is dependent on the nature of the chemical and its octanal water partition coefficeint (log K_{OW}), which can differ widely in PW mixtures. However, for most PW compounds, ideal exposure durations have been considered to be between 2 and 4 weeks (Harman et al 2009).

3.3 Season for exposure

The main factors that need to be considered are the following:

1) The condition of the test organisms (e.g. reproductive stage, particularly for mussels). In mussels, biomarker responses and general health status are affected by the reproductive stage. Recently spawned or spawning mussels should therefore be avoided.

2) Stratification of the water column, which can have significant effects on the dispersal and mixing of the discharge plume and thereby affect exposure.

3) Stable weather conditions for working in the vicinity of offshore oil installations.

4) Algal blooms potentially influence the bioavailability and uptake pathways of contaminants in exposure organisms.

5) Comparison of data from previous years.

3.4 Availability of studied species

Mussels (Mytilus spp.)

Mussels are available all year round from the Norwegian coast, and should be collected from locations known to have low levels of contaminant exposure. Collected mussels should be brought back to the laboratory and maintained in clean, filtered seawater at seasonal temperatures for at least 1-2 weeks prior to field deployment. In addition to the reproductive stage, which can alter bioaccumulation and stress marker responses, three different mussel species have been found to exist on the Norwegian coastline (Brooks and Farmen, in prep). The species are *M. edulis*, *M. trossulus* and *M. galloprovincialis* and differences in biomarker responses between species are thought likely. Therefore, it is important that speciation be checked and that only one species of mussel is used in biomonitoring programmes.

Fish (Atlantic Cod, Gadus morhua)

Obtaining a non-contaminanted source of live juvenile cod for biomonitoring purposes has been challenging in WCM programmes. Farmed fish have been recommended by ICES WGBEC, since knowledge of the exposure history is somewhat known. However, elevated biomarker levels have been measured in pre-exposure cod compared to deployed reference fish in the last three WCM programmes (Brooks et al., 2011). Since fish farm facilities are often close to the shore, exposure to unknown anthropogenic sources is likely. Additional sources include the fish feed, although this can be controlled so that fish are fed with a non contaminanted food.

4 Overview of PW related biomonitoring studies

Several PW related biomonitoring studies have been conducted globally. A range of different designs have been applied both with respect to type of exposure and to the selection of biological and chemical markers. The most important areas where PW related field biomonitoring investigations based on wild or caged organisms have been carried out are indicated in Figure 1. General advantages and limitations with different approaches are discussed in the publications.

Figure 1. Circles indicate the main areas where PW related field monitoring based on wild or caged organisms has been carried out.

4.1 Caged organisms

An overview of PW related biomonitoring studies based on caged (transplanted) organisms is given in Table 3. The investigations conducted in the North Sea consisting of the BECPELAG and subsequent WCM programs dominate the picture. Outside the North Sea, caging studies have been conducted in the South of Europe (Adriatic Sea), in North America (California and Gulf of Mexico) and Australia (Australian Northwest shelf). The most commonly used organisms are bivalves (mainly mussel species). Results from only one study outside Norway using caged fish are reported (Stripy Sea Perch, Australia: Zhu et al 2008). Most of the biological parameters that showed effects were correlated with distance from the PW discharge. Bioaccumulation data of PW related compounds dominated the field experiments, but also several effect parameters were evaluated. Most of the biomarkers tested in caged organisms were well known and they were able to identify effects from specific chemicals and different levels of biological effects, and were also able to conclude the absence of anthropogenic impact. The use of organisms in optimal conditions and clean reference station recognition were generally crucial matters. Given limitation of time (both duration of studies and field experiment difficulties) and funding, the selection of parameters to be evaluated and sampling methods are crucial features of the design process. Study designs were similar in these monitoring activities, cage distance from the PW outfall in fact varied between 1 to 1000 m in most cases.

4.2 Wild organisms

An overview of biomonitoring studies based on wild organisms is given in Table 4.

The potential and limitations of biomarkers applied to wild species are similar to those in studies involving caged animals. The evaluation of PW discharge impact in wild organisms has an additional relevance, revealing the risk to wild populations of ecological or environmental significance in the field (Gray, 2002).

Table 3. Overview of published PW biomonitoring studies where caged (transplanted) organisms have been utilized.

Area (Location)	Study/Project	Species	Analysed parameters	Responsive parameters	Reference
North Sea/Ekofisk platform	Water Column Monitoring 2008	Blue mussel (*Mytilus edulis*), Atlantic cod (*Gadus morhua*)	PAH body burden in mussels, alkylphenol (AP) body burden in mussels, PAH metabolites in fish bile, AP metabolites in fish bile	PAH body burden in mussel, PAH metabolites in fish bile, AP metabolites in fish bile	Harman et al., 2011
North Sea/Ekofisk platform	Water Column Monitoring 2006-2009	Blue mussel (*Mytilus edulis*), Atlantic cod (*Gadus morhua*)	Mussel: lysosomal membrane stability (NRRT), DNA damage (micronuclei), PAH body burden. Cod: vitellogenin, zona radiata protein, CYP1A, glutathione S-transferase, DNA adducts, PAH bile metabolites, condition index	Mussel: lysosomal membrane stability (NRRT), DNA damage (micronuclei), PAH body burden. Cod: CYP1A, PAH bile metabolites	Brooks et al., 2011
North Sea/Ekofisk platform	Water Column Monitoring 2006	Blue mussel (*Mytilus edulis*)	Lysosomal membrane stability (NRRT), DNA damage (micronuclei)	Lysosomal membrane stability (NRRT), DNA damage (micronuclei)	Sundt et al., 2011c
North Sea/Ekofisk platform	Water Column Monitoring 2006	Atlantic cod (*Gadus morhua*)	Vitellogenin, CYP1A, DNA adducts, PAH bile metabolites, gonado-somatic, hepato-somatic and condition indices	CYP1A, DNA adducts, PAH and alkylphenol (AP) bile metabolites	Sundt et al., 2011b
North Sea/Ekofisk platform	Water Column Monitoring 2008-2009	Atlantic cod (*Gadus morhua*)	Histopathology of fish gills, PAH bile metabolites	Histopathology of fish gills, PAH bile metabolites	Sundt et al., 2011c
North Sea (Tampen, Statfjord and Troll B	Water Column Monitoring 2001-2004	Blue mussel (*Mytilus edulis*), Atlantic cod (*Gadus morhua*)	Mussel: lysosomal membrane stability (NRRT and histochemical method), DNA damage (micronuclei), PAH body	Mussel: lysosomal membrane stability (NRRT and histochemical method), DNA damage	Hylland et al., 2008

platforms)			burden, histopathology. Cod: PAH bile metabolites, EROD, glutathione S-transferase, DNA adducts, vitellogenin	(micronuclei), PAH body burden, histopathology. Cod: PAH bile metabolites	
North Sea (Statfjord)	ICES workshop-BECPELAG	Blue mussels (*Mytilus edulis*)	PAH bioaccumulation	PAH bioaccumulation	Røe Utvik and Gärtner, 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Blue mussels (*Mytilus edulis*), Atlantic cod (*Gadus morhua*)	PAH, PCB, organotin compound, metal, alkylphenol (AP) bioaccumulation	PAH, PCB, organotin compound, metal bioaccumulation	Ruus et al., 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Atlantic cod (*Gadus morhua*)	Brominated flame-retardant (PBDE, HBCD, TBBP-A) bioaccumulation	No effect	Vethaak et al., 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Blue mussels (*Mytilus edulis*)	Benzo(a)pyrene hydroxylase, acetylcholinesterase (AchE)	Acetylcholinesterase (AchE)	Burgeot et al., 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Blue mussels (*Mytilus edulis*), Atlantic cod (*Gadus morhua*)	Mussel: Lysosomal membrane stability (in digestive gland), lysosomal structural changes (in digestive gland), neutral lipid accumulation, peroxisomal proliferation, AOX, metal accumulation (autometallography), histopathology of liver. Cod: lysosomal structural changes (in digestive gland), metal accumulation (autometallography), AOX	AOX in mussel, peroxisomal proliferation	Bilbao et al., 2006b (chapter 3.4)
North Sea (Statfjord)	ICES workshop-BECPELAG	Blue mussels (*Mytilus edulis*), Atlantic cod (*Gadus morhua*)	Total energy allocation (CEA, total carbohydrates, lipids and protein and ETS)	No effect	Smolders et al., 2006

North Sea (Statfjord)	ICES workshop-BECPELAG	Atlantic cod (*Gadus morhua*)	CYP1A	CYP1A	Förlin and Hylland, 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Atlantic cod (*Gadus morhua*)	PAH metabolites (FF and GC-MS methods), metals in bile	PAH metabolites (FF and GC-MS methods), metals in bile	Aas et al, 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Atlantic cod (*Gadus morhua*)	DNA damage (DNA adducts and alkaline unwinding)	DNA damage (DNA adducts)	Balk et al, 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Atlantic cod (*Gadus morhua*)	AchE and GST	GST	Danischewski, 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Blue mussels (*Mytilus edulis*), Atlantic cod (*Gadus morhua*)	Histological changes	Histological change in cod gonads, histological change in mussel digestive glands and gonads	Feist et al., 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Atlantic cod (*Gadus morhua*)	Vitellogenin	No effect	Scott et al., 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Blue mussels (*Mytilus edulis*)	Antioxidant parameters(catalase, GST, glutathione S-transferase) and TOSC assay	Catalase and TOSC assay	Regoli et al., 2006
North Sea/Tampen and Ekofisk platforms	Norwegian Oil Industry Association (OLF)	Blue mussel (*Mytilus edulis*)	PAH body burben.	PAH body burben	Durell et al., 2006
Mediterranean Sea/Adriatic Sea (Giovanna	Italian Ministry of Environment	Mussel (*Mytilus galloprovincialis*)	Lysosomal membrane stability (NRRT), accumulation of lipofuscin, neutral lipids, malondialdehyde (MDA), DNA	Lysosomal membrane stability (NRRT), DNA damage (micronuclei), peroxisomal	Gorbi et al., 2008

platform)			damage (comet assay and micronuclei), survival in air, metallothioneins, peroxisomal proliferation (AOX), acetylcholinesterase activity, superoxide dismutase, catalase, glutathione S-transferase, glutathione reductase, Se-dependent and Se-independent glutathione peroxidases, total glutathione, TOSC	proliferation (AOX),	
Carpinteria/CA, USA		Mussels (*Mytilus californianus* and *Mytilus edulis*)	Shell growth and condition	No effect	Osenberg et al., 1992
Carpinteria/CA, USA	Mineral Management Service, U.S.	Mussels (*Mytilus californianus* and *Mytilus edulis*), Abalone (*Haliotis rufescens*), Sea urchins (*Strongylocentrotus purpuratus*) Giant kelp (*Macrocystis pyrifera*)	Mussels: shell growth, gonado and somatic tissue mass. Abalone: settlement amd metamorphosis. Sea urchins: gonadal mass, fertilization success, larval early stage development. Giant kelp: sporophyll development.	Mussels: shell growth, gonado and somatic tissue mass. Abalone: settlement amd metamorphosis. Sea urchins: gonadal mass, fertilization success, larval early stage development. Giant kelp: sporophyll development	Canestro et al., 1996
Australian Northwest Shelf (Harriet A platform)		Common rock Oyster (*Saccostrea cuccullata*)	PAH body burden and fatty acid methyl esters (FAMES) accumulation	PAH body burden	Burns et al., 1999

Australian Northwest Shelf (Harriet A platform)	Environmental Toxicology Research Program	Stripey seaperch (*Lutjanus carponotatus*)	CYP1A and CYP2K1/2M1 like proteins	CYP1A	Zhu et al., 2008

Table 4. Overview of published PW bio monitoring studies based on wild organisms.

Area (Location)	Study/Project	Species	Analysed parameters	Responsive parameters	Reference
North Sea (Tampen)	Condition Monitoring in the Water column 2008	Haddock (*Melanogrammus aeglefinus*), Atlantic cod (*Gadus morhua*) and Saithe (*Pollachius virens*)	NPDs and PAH in liver, PAH and AP metabolites, DNA damage (DNA adducts), vitellogenin, histology of gonads, fatty acid and lipid amounts, neutral lipids.	No effects on cod and saithe. Haddock: PAH metabolites, DNA damage (DNA adducts), lipid amount, neutral lipids.	Grøsvik et al., 2009
North Sea (Tampen)	Condition Monitoring in the Water column 2005	Haddock (*M. aeglefinus*), cod (*Gadus morhua*)	AP metabolites, DNA damage (DNA adducts), vitellogenin	DNA damage (DNA adducts in Haddok)	Grøsvik et al., 2007
North Sea (Tampen)	Condition Monitoring in the Water column 2002	Haddock (*M. aeglefinus*), cod (*Gadus morhua*)	AP metabolites, DNA damage (DNA adducts)	DNA damage (DNA adducts in Haddok)	Klungsøyr et al., 2003
North Sea (Statfjord)	ICES workshop-BECPELAG	Mackerel (*Scomber scombrus*), Saithe (*Pollachius virens*), Herring (*Clupea harengus*)	PAH, PCB, organotin compound, metal, alkylphenol (AP) bioaccumulation	PAH, PCB, organotin compound, metal bioaccumulation	Ruus et al., 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Herring (*Clupea harengus*), jellyfish,	Brominated flame-retardant (PBDE, HBCD, TBBP-A)	No effect	Vethaak et al., 2006

		copepods	bioaccumulation		
North Sea (Statfjord)	ICES workshop-BECPELAG	Herring (*Clupea harengus*), Saithe (*Pollachius virens*),	EROD and CYP1A	No effect	McIntosh et al., 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Whiting (*Merlangius merlangius*)	Viral haemorrhagic septicaemia virus and infectious salmon anaemia virus	No effect	Dixon et al., 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Herring (*Clupea harengus*), Saithe (*Pollachius virens*)	Lysosomal membrane stability (in liver), lysosomal structural changes (in liver), neutral lipid accumulation, peroxisomal proliferation, AOX, metal accumulation (autometallography), histopathology of liver	Lysosomal membrane stability (in Saithe liver), lysosomal structural changes (in Saithe liver), neutral lipid accumulation (in Saithe liver), peroxisomal proliferation and AOXin both species	Bilbao et al., 2006 a (chapter 2.4)
North Sea (Statfjord)	ICES workshop-BECPELAG	Herring (*Clupea harengu*)	Hepatic metallothionen	No effect	Chesman et al., 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Zooplancton and fish larvae	Total energy allocation (CEA, total carbohydrates, lipids	No effect	Smolders et al., 2006

			and protein and ETS)		
North Sea (Statfjord)	ICES workshop-BECPELAG	Mackerel (*Scomber scombrus*), Saithe (*Pollachius virens*), Herring (*Clupea harengus*)	PAH metabolites (FF and GC-MS methods), metals in bile	PAH metabolites (FF and GC-MS methods), metals in bile	Aas et al, 2006
North Sea (Statfjord)	ICES workshop-BECPELAG	Saithe (*Pollachius virens*)	Vitellogenin	No effect	Scott et al., 2006
North Sea (Clyde, Fulmar and Auk platforms)		Dab (*Limanda limanda*)	EROD and caynoethoxycoumarin-O-deethylase (cECOD)	EROD and caynoethoxycoumarin-O-deethylase (cECOD)	Stagg et al., 1995
Gulf of Mexico		Torny oysters,, Creole Fish (*Paranthias furcifer*), Yellow Chub (*Kryphosus incisor*)	Bioaccumulation of PAHs and phenols	Bioaccumulation of PAHs	Neff et al., 2009
Gulf of Mexico		Clams (*Chama macerophylla*), oyster (*Crassostrea virginica*)	Bioaccumulation of heavy metals		Trefy et al., 1995
Gulf of Mexico (Pass Fourchon)	U.S. Department of the Interior, Minerals Management Service	Fringed flounder (*Etropus crossotus*), Shoal flounder (*Syacium gunteri*),Bay whiff (*Citharichthys spilopterus*)	EROD	No effect	Reily et al., 1994

Gulf of Mexico (Pass Fourchon)	U.S. Department of the Interior, Minerals Management Service	Fringed flounder, Shoal flounder, Bay whiff, Catfish, Sea robin	Superoxide dismutase (SOD), catalase	Superoxide dismutase (SOD), catalase	Winston et al., 1994a (chapter 4)
Gulf of Mexico (Pass Fourchon)	U.S. Department of the Interior, Minerals Management Service	Fringed flounder, Shoal flounder, Bay whiff, Catfish, Sea robin	NADH, NADPH	No effect	Winston and Dobias, 1994
Gulf of Mexico (Pass Fourchon)	U.S. Department of the Interior, Minerals Management Service	Catfish (*Ictalurus punctatus*)	Genotoxicity (umu mutagencity assay)	No Effect	Winston et al., 1994b (chapter 6B)
Australian Northwest Shelf (Wandoo B, Four Vanguard and Ocean Legend platforms)		Rainbow runner (*Elegatis bipunnulata*), Goldbanded snapper (*Pristipomoides multidens*), Giant trevally (*Caranx ignobilis*)	EROD, PAH bile metabolites, DNA damage (alkaline unwinding), stress proteins (HSP-70), condition factor (CF), liver somatic index (LSI)	EROD, PAH bile metabolites, DNA damage (alkaline unwinding), stress proteins (HSP-70), liver somatic index (LSI)	Gagnon 2010
Australian Northwest Shelf (Harriet A platform)	Pilot study	Gold-spotted trevally (*Carangoides fulvoguttatus*) Bar-cheeked coral trout (*Plectropomus maculates*)	EROD, fluorescent aromatic compounds (FACs) in bile, CYP1A	FACs, CYP1A and to a limited degree EROD	Cod King et al., 2005

Australian Northwest Shelf (Harriet A platform)	Environmental Toxicology Research Program	Gold-spotted trevally (*Carangoides fulvoguttatus*)	CYP1A and CYP2K1/2M1 like proteins	CYP1A	Zhu et al., 2008

4.3 Alternative approaches

Most of the biomonitoring studies have taken into account individual-based parameters, but some activities were performed on population-based parameters, such as density of infaunal organisms and microbial community studies (Canestro et al., 1996).

Accumulation of PAH in zooplankton

Zooplankton can easily be collected, and represent an integrated average over a considerable effective sampling volume (Hylland et al. 2006b). Carls et al. (2006) demonstrated the approach by applying PAH measurements in the copepod *Neocalanus* collected in Port Valdez, Alaska. Areas with strong current advection (transport of water from one region to another) of planktonic organisms through an affected area present an obvious challenge.

Microbial ecology in the water column

PW contains compounds (e.g. organic acids) that may cause organic enrichment increasing the microbial activity in the sea (saprobisation). In a Canadian study influence of PW on the microbial ecology in the water column was investigated. As for the zooplankton approach above, advection of microorganisms through an affected area is an obvious challenge. In previous WCMs, a decreasing gradient of fouling intensity on the cage surfaces with distance to the PW outfall is observed (Sundt pers.obs.) indicating organic enrichment close to the discharge. This is probably a combined effect of PW, sewage from sanitary drains and washing activities.

4.4 Biomarker techniques employed

Biological indicators or markers (biomarkers) have been developed to measure the biological response related to exposure to, or the toxic effect of, an environmental chemical (Peakall, 1992). Some biomarkers are specific in terms of their ability to detect and assess the potential for effects through a specific toxic mechanism (e.g. vitellogenin for estrogenic exposure), whereas others give information about larger groups of chemicals with more diverse mechanisms of action. Common for all of the methods is the capability of performing time-integrated response assessment to complex mixtures over extended periods of time, which is often required in environmental monitoring. Since most of these methods are highly sensitive and responses occur at lower concentrations and/or prior in time to more adverse effects at a higher organisation level, the methods have become convenient early-warning tools for assessing the potential for long term (ecological) effects. The use of biomarkers in sentinel species or specific caging systems with keystone species has consequently facilitated the implementation of such methods in various environmental monitoring programs in freshwater, marine and estuarine areas. In recent years, a combination of laboratory and field validation of the different biomarker and effects-based methods has greatly improved the knowledge of the potential and limitations of these methods and

made it possible to link responses of biomarker signals to the potential for more adverse effects at the ecological level (Collier et al., 1992; Elliot et al., 2003; Bechmann et al., 2000).

For the WCM programme a wide range of biomarker responses combined with chemical exposure concentrations have been measured in transplanted cod (*Gadus morhua*) and mussel (*Mytilus spp.).* A list of the biomarkers used in both species is found in Tables 5 and 6.

4.5 Types of stress monitored

Since PW is a highly complex mixture, monitoring the biological effects can be challenging. The biomarker approach employed has been to use a combination of general health markers together with target specific biomarkers. For example, components of the PW, such as AP and naphthenic acids have been reported to act as potential xenoestrogens. In this case, estrogenic effect biomarkers such as VTG and ZRP have been used in cod. Genotoxicity endpoints thought to be sensitive to components within PW have also been used, such as micronuclei formation in mussels and DNA adducts in cod.

Table 5 Biomarkers in fish (Atlantic Cod, *Gadus morhua*) used as part of the WCM programme.

Method	Type of mechanism	Suitability	Advantages	Disadvantages
PAH-metabolites in bile by Fixed Fluorescence	Marker of PAH exposure	Responsive in lab / field caging	Cost effective screening tool for PAH exposure	Non-specific, less sensitive than PAH bioaccumulation in mussels
PAH-metabolites by GC/MS	Marker of PAH exposure	Responsive in lab / field caging	Sensitive and capable of detecting individual compounds and substituted compounds	More expensive analysis. less sensitive than PAH bioaccumulation in mussels
AP metabolites	Marker of AP exposure	Responsive in lab / field caging	Sensitive and capable of detecting individual compounds and substituted compounds	Less sensitive to AP exposure than POCIS samplers.
Hepatic GST	Involved in 2^o detoxification mechanisms (Phase 2 reaction enzymes)	Not responsive in field caging studies.	Standardised method for exposure to lipohilic compounds	High baseline levels in reference and pre-exposure fish prevent effects from being seen in exposed fish.
Hepatic Cytochrome 450 1A	Carries out oxidative reactions related to biotransformation of xenobiotics.	Responsive in lab and field caging studies.	Sensitive to planar PAHs found in field exposures.	Elevated levels of CYP1A found in pre-exposure fish.
Vitellogenin (Vtg)	Exposure to xenoestrogens	Responsive in lab, not responsive in the field.	Specific marker to xenoestrogenic exposure that can be measured non-destructively	Not sensitive enough to field exposures of xenoestrogenics in PW. Only measureable in fish.
Zona Radiata Protein	Exposure to xenoestrogens	Responsive in lab, not responsive in the field	Specific marker to xenoestrogenic exposure that can be measured non-destructively	Not sensitive enough to field exposures of xenoestrogenics in PW. Only measureable in fish
DNA adducts	Measure of genetic damage	Responsive in lab, not responsive in field in caged fish	Sensitive genotoxicity marker	Large variablility in response in field exposures. Expensive and labour intensive analysis.
Gill histology	General health marker	Responsive in lab / field caging	Sensitive marker with several different measureable endpoints.	Unsure link between exposure and effect. Whether the effects seen are reversible or perminen t damage
Liver histopathology	General health marker	Not responsive in field caging study with cod, but differences found in wild caught seith.	Tissue level biomarker	Sensitivity needs to be more documented.
Histology internal organs	General marker of animal health status	Responsive in lab and field caging studies.	Measures actual change in tissue composition and structure, physiological change.	Need a long exposure duration for effects to be seen

Table 6 Biomarkers employed within the WCM for mussels (*Mytilus spp.*)

Biomarker/ chemical endpoint	Type of mechanism	Suitability	Advantages	Disadvantages
PAH body burden by GC/MS	Exposure to PAH-NPD compounds	Very effective in PW field investigations. capable of detecting individual compounds and substituted compounds	Best method for the detection of PAH-NPD in environmental monitoring	Analysis is expensive and the number of replicates is often kept low (n= 3)
Benzo(a)pyrene/ pyrene hydroxylase activity	Exposure marker to Benzo(a) pyrene and pyrene	Responsive in lab studies and field caging.	Sensitve and specific to chemicals in PW	Elevated levels of Pyrene Hydroxylase have been found in pre-exposure mussels.
Lysosomal membrane stability	General health marker.	Responsive in lab studies and field caging	Sensitive, fast result and low cost	Subjective assessment of endpoint, need same analyst throught tests.
Micronucleus formation	Genotoxicity	Responsive in lab studies and field caging	Sensitive and non-destructive	
Neutral lipid accumulation	Lipophiliic contaminants such as PAHs can cause increase in neutral lipids	Responsive in lab studies but non-comparable data obtained in previous field studies		
Lipofuscin accumulation	Oxidative damage to cellular membranes	Responsive in lab studies but non-comparable data obtained in previous field studies		
Gill histology	General health marker	Responsive in field caging	Marker with several different measureable endpoints.	Unsure link between exposure and effect. Sensitivity of the markers needs to be checked
Liver histopathology	General health marker	Responsive in lab / field caging	Tissue level biomarker	Sensitivity needs to be more documented.

4.6 Chemical compounds with particular focus

Monitoring programs have confirmed the presence of PW constituents around the offshore installations; however, the studies indicate that the affected areas around each outfall are limited. Processes like dilution, evaporation and biodegradation decrease actual concentrations to marine organisms soon after the PW enters the sea, and uncertainties still exist regarding threshold levels of chronic low dose exposurefor sensitive stages of different organisms.

4.6.1 PAHs

Exposure to PAHs in caged organisms is confirmed by elevated levels of parent compounds in fish liver, metabolites of fish bile, mussel soft tissue and passive samplers (Harman et al 2011, Brooks et al. 2011 respectively).

PAHs related to offshore operational discharges are generally not found in fillet of wild specimens of fish collected in regions with oil and gas activity (Grøsvik et al. 2009). Detectable levels of PAH metabolites have been observed in wild herring and saithe collected in the vicinity of PW outfalls (Aas et al. 2006). For these species the levels detected are generally too low to trigger responses in PAH related biomarkers.

Some species may potentially be more vulnerable to the low PAH levels present in areas with extensive oil and gas production due to their feeding biology, as indicated by the PAHs being detected in bile of wild haddock collected in the Tampen area (Grøsvik et al. 2009). The source of this PAH is presently not known but contribution from PW is not unlikely. Levels of PAHs observed are generally low, but a possible connection to elevated levels of DNA adducts in haddock collected in that particular region is indicated and should be further investigated.

4.6.2 Alkylphenols (APs)

Exposure to APs has been confirmed in caging studies by elevated levels in passive samplers (Harman et al 2011) and in fish bile (Brooks et al 2011). The main toxicological concern has been the oestrogen mimicking potential of some APs. Several laboratory studies have indicated that PW has the potential to cause reproduction related effects in fish when the exposure concentration is high. However, studies indicate that the levels of PW related C_4-C_7 APs are too low to cause any reproductive effects.

Several studies conclude that field realistic concentrations of these compounds are not likely to cause reproductive effects in North Sea fish populations. Sensitive techniques for detection of AP metabolites in biota are available. Results from the condition monitoring confirms that APs are not detected in bile of haddock, saithe and long rough dab in areas with extensive PW discharges (Grøsvik et al. 2007; Grøsvik et al. 2009). Vtg levels in wild caught male cod from Tampen are not observed to be induced compared with fish from reference areas (ibid.).

4.6.3 Other hydrocarbons

In addition to APs and PAHs, PW also contains a large “hump” of unresolved complex mixture (UCM), which is composed of a large number of unknown compounds that also

will contribute to the toxicity (Neff et al. 2000; Rowland et al. 2001; Melbye et al. 2009). Some of these unknown compounds might also have endocrine disruption effects. For example, naphthenic acids present in PW can function as xenoestrogens (Thomas et al. 2009).

4.6.4 Metals

Bioavailability of trace metals has been evaluated in various PW discharge monitoring studies. It is not considered likely that metals from PW can cause biological effects in the North Sea ecosystem. No effects from metals were found in pelagic fish caught in the vicinity of the Statfjord platform (Bilbao et al., 2006; Chesman et al., 2006; Aas et al., 2006), even though a quantifiable amount of cadmium, zinc and copper was found in mackerel liver, and zinc and copper in saithe and herring soft tissues (Ruus et al., 2006). Effect evaluation was made using metallothionein proteins and autometallography (AMG), sensitive biomarkers of exposure, measuring metals effects at the cellular level (Matigomez et al. 2002; Viarengo et al., 1997). Levels of AMG deposits were found at background levels in mussels caged along a gradient in the Statfjord platform area, showing that the metal pollution is not noticeable even in the near zone. All these findings are in agreement with monitoring studies performed in the Adriatic Sea (Gorbi et al., 2008).

4.6.5 Radionucleides

It is considered unlikely that naturally occurring radioactivity associated with PW discharges represents a significant health risk to marine life or humans. E.g. no oxidative stress response were indicated in rag worms (*Hediste diversicolor*) exposed to levels of ^{226}Ra several orders of magnitude higher than levels measured in organisms caged close to a PW discharge (Grung et al 2009b).

4.6.6 Production Chemicals

Even though production chemicals may contribute significantly to the theoretical impact when environmental risk of operational discharges is evaluated by modelling, the compounds have had no particular focus in the Norwegian monitoring programmes. This emphasizes that there should be an increased focus on parameters that could detect such impact for future WCM and condition monitoring. Work is ongoing to increase injection of surfactants to reservoirs to enable a higher oil exploitation. Surfactants are membrane active compounds which could affect oxygen uptake over gills, and possible increased discharges of such compounds from produced water should be risk assessed. Some compounds have significant acute toxic potential in high concentrations, but are generally rapidly degraded and not accumulated. Extensive effort is being put into selection of chemicals with as low environmental impact potential as possible. Some studies have focused on investigating fate and effects of some production chemicals. E.g. levels of diethylene glycol in discharged PW has been investigated (Cappiello et al. 2007), and only low levels of effects from this particular compound have been found in fish (Gorbi et al. 2009).

4.7 Passive samplers

The principle of the passive sampling technique is the placement of a device in the environment for a fixed period of time, where it is left unattended to accumulate contaminants by diffusive and/or sorptive processes. The main advantages of using passive sampling devices (PSDs) over traditional discreet spot water samples are; concentrations are time-integrative during exposure, compensating for fluctuations in discharges etc; lower detection limits are normally achievable as a larger sample has been taken and; that only the freely dissolved and thus more readily bioavailable fraction is measured. Additionally PSDs may offer significant advantages over biomonitoring methods as outlined below in Table 7, most importantly the avoidance of inherent biological variation. A further not insignificant advantage is a reduction in the need for the use of animals in scientific experiments. However, relating PSD accumulations to the overall ecological relevance of contaminants is complicated and effects can only be inferred.

Passive samplers are usually selective to a range of compounds based on some physicochemical property of target analytes. The following discussion is limited to two types of passive sampler, both for organic contaminants, i.e. metals are not considered. These two samplers are the widely used semipermeable membrane device (SPMD), suitable for sampling compounds with log K_{OW} >3-3.5, and the polar organic chemical integrative sampler (POCIS) for compounds with log K_{OW} <3.5.

4.7.1 Passive samplers in field studies

SPMDs have successfully been applied to a wide range of sampling scenarios and target compounds. There is significant literature considering the application of PSDs to oil spill related problems and over 100 studies concerning the general measurement of PAHs in the aquatic environment. Within the framework of monitoring the offshore oil and gas industry discharges several studies are available. The first of these was a comparison of large water volume sampling and accumulations in mussels and SPMDs (Røe Utvik et al., 1999a). The study showed similar estimated water concentrations (within a factor of 2) in mussels and SPMDs at some stations but differences up to a factor of 10 at others. Additionally a gradient in accumulation relative to exposure was shown in both mussels and SPMDs, with some differences in the relative levels attributed to mussels also accumulating the particulate bound fraction and possibly metabolism (Røe Utvik et al., 1999b). Subsequently the same data was compared to dispersion model predictions of PAH concentrations. Very similar results were obtained at stations in the Ekofisk region, with large discrepancies for stations in the Tampen region attributed to inadequate consideration of the variation in direction of the plume. The overall conclusion of the authors was that the techniques are complimentary (Durell et al., 2006). A similar comparison of DREAM model predictions and measured AP concentrations in POCIS is possible and should be considered in order to better understand their environmental fate and thus potential for effects.

In 2008 and 2009 SPMDs were co-deployed at all 8 stations used during the WCM (six exposure and two reference stations). The use of spiked standards (so called performance reference compounds PRCs) which dissipate during deployment to correct for exposure conditions (water flow rates, temperature etc.) were used for the first time

in these surveys. This resulted in more accurate estimations of time-integrated water concentrations. There is far less uncertainty in these estimations compared to estimating exposure water concentrations using literature bioaccumulation/elimination rates, for mussels. All target compounds (PAH 29) were detectable in SPMDs with similar concentrations between stations near to the discharges. Elevated levels of alkylated PAHs were shown (ΣNPD typically tens of ng L^{-1}), compared to the reference sites whereas similar levels were apparent for PAH16 (2-4 ng L^{-1}). Overall results between PAH body burden in mussels and SPMD accumulations showed a similar pattern in exposure relative to discharge for phenanthrenes and dibenzothiophenes, but differences for naphthalenes and some PAHs. Where discrepancies existed these were attributed to differences in the fraction of PAH sampled (mussels may also sample the particulate bound fraction), time required to reach a steady state and due to the least hydrophobic compounds (i.e. naphthalene) not accumulating significantly in mussels. Water concentrations were not estimated from mussels due to the wide range of BCFs available in the literature. Similar to controlled laboratory studies there was a good correlation between PAHs in SPMDs and OH-PAH in fish bile. One of the challenges for trace PAH analysis in SPMDs is contamination from naphthalene and alkylated naphthalenes (Harman et al., 2009b; 2011; Boehm et al., 2005). This may easily be overcome by adding an extra clean-up step at the fabrication stage or by simply switching to other hydrophobic passive samplers such as low density polyethylene (LDPE), reports of which are increasing rapidly in frequency in the primary literature.

Interestingly, measured water concentrations were higher in 2009 than 2008 for the more abundant alkylated PAHs (also for APs in POCIS), whilst the biological effects measured suggested exposure was lower. This change in the biological effects was attributed to the improved performance of the cleaning system installed at the study production area. This cleaning system is most efficient for hydrophobic compounds, i.e. the removal of oil. However, the amount of PW discharged actually increased from 2008 to 2009, resulting in the measured increase of more water soluble compounds, shown in PSDs. This somewhat important observation was not apparently replicated by biota, illustrating the importance of having a biologically independent measure of exposure.

For PW originating polar compounds, the only studies applying passive sampling for measurement, to our knowledge, are those carried out alongside the WCM. The first of these was carried out in 2004, where POCIS extracts were also used for biological testing using small scale bioassays (Harman, et al., 2010). Although the biological results failed to reveal a clear gradient of effect relative to exposure, a gradient in chemical concentrations was apparent for APs in POCIS (and also for PAHs in SPMDs), for the limited number of compounds measured. Another group of compounds analysed for in this study was carbazoles. These were not detected but have been shown to accumulate in SPMDs in laboratory calibrations (Harman et al., 2008), so we can conclude that levels were too low to be measured. Subsequently POCIS was used to measure a larger range of APs, deployed as part of the WCM in both 2008 and 2009 (Harman et al., 2009b; Harman et al., 2011). Nearly 100 individual APs were detected, using a much more sensitive derivitisation method (Boitsov et al., 2004). Although levels were generally low, for most of these compounds this was the first time that they

have been measured in the receiving waters of PW discharges and this measurement would not have been possible using existing water sampling techniques. Additionally, exposure to APs was poorly represented in co-deployed biological samples in either both years (AP in mussels) or in 2009 (AP metabolites in fish).

Challenges remain with using POCIS and other alternative polar passive samplers. These challenges largely revolve around two related issues; the lack of an adequate exposure correction method equivalent to the PRC approach used in hydrophobic passive samplers; and the lack of an uptake model that may predict sampling kinetics based on some physicochemical property of target compounds. Until these issues are resolved in detail, then estimations of time integrated water concentrations from POCIS accumulations are likely to remain more uncertain than those for more hydrophobic samplers such as SPMDs. POCIS is almost certainly applicable to the measurement of naphthenic acids and this possibility should be explored in future studies.

4.7.2 Passive samplers in laboratory studies

PSDs can also be applied to measuring water concentrations in laboratory exposures and their ability to measure only the freely dissolved fraction was of special significance in WP13 of the current WCM. In that study the effect of suspended particles on the availability of PAHs to fish was examined (Appendix WP13). Previously SPMDs have been used to calculate the exposure water concentrations in a year-long experiment exposing Atlantic cod to several levels of a synthetic PW (mixture of PAH and AP). Not only were water concentrations determined but accumulations were also compared to hydroxy-PAH metabolite concentrations in fish bile, with reasonable correlations shown (Harman et al., 2009a). In such laboratory applications care must be taken to ensure that exposure system renewal rates are high enough as to avoid significant depletion of exposure concentrations due to high sampling rates of PSDs. In another recent study the effects of PW from a gas condensate plant were examined in the laboratory. Even with DL in the low pg L^{-1} the target compounds of PAH and alkylated PAH were not detected at elevated levels in the exposure treatments despite a clear profile of biological effects relative to exposure (Brooks et al., 2011). This unexpected result indicates that (in this example) other compounds are likely to have been the cause of the effects measured, illustrating the usefulness of including PSDs in such studies.

A further novel application of passive sampling in laboratory studies is for the dosing of small scale experiments. This technique (passive dosing) involves the spiking of a sampling phase (typically silicone rubber) with target compounds which partition into the exposure system. This offers a much more reliable and predictable method for dosing as concentrations in the exposure system will be held constant even if there is sorption to the exposure vessel and particulates or uptake by study organisms. To the authors knowledge this technique has not yet been applied to PW related studies.

Table 7 Comparison of biomonitoring and passive sampling for measuring exposure to PW originating AP and PAH (from Harman et al., 2011)

	MUSSELS	SPMDs	FISH	POCIS
Suitability for measuring PW AP	Not accumulated	Poor for most relevant AP	Limited	Excellent
Number of compounds detected	<29 PAH	29 PAH	9 OH-PAH, 5 OH-AP	>60 AP
Pattern of exposure represented	Yes PAH	Yes PAH	Limited	Yes AP
Ability to measure exposure to other PW relevant compounds?	Likely for hydrophobic compounds present in significant quantity, e.g. decalins	More or less any compound with log K_{OW}>3.0. e.g. hopanes, carbazoles, decalins.	Complicated method development required	More or less any compound with log K_{OW}>3.0. e.g. organic acids. Configuration easily modified
Inital concentrations of contaminants	Some PAH signal always present	Very low (ng) for most PAH[1]	Some PAH/AP signal always present	Very low (pg) for AP
Ease of deployment	Moderate	Easy	Difficult	Easy
Correction for spatial and temporal differences in deployment conditions?	No	Yes where an appropriate PRC approach is used	No	May be inferred from SPMD PRC results
Typical variation	Moderate (Average RSD 10-41%)	Low (Average RSD 9-17%)	Very high (Average PAH RSD 24-104%, AP 44-182%)	Low-moderate (Average RSD 7-32%)
Sensitivity, as accumulations /LOQ[2]	Good (33,7 for PAH)	Generally very good (15,6 for PAH)[3]	Poor (1,3 for AP, 2,5 for PAH)	Good (14,3, for AP)
Estimation of exposure water concentrations?	Possible for PAH using literature BAFs, but large uncertainties	Excellent, using the PRC approach	Not possible, some evidence that rates of metabolism are not independent of concentrations.	Not as accurate as for SPMDs, but not hampered by any biological processes.
Understanding of uptake process	Complicated, partly dependent on environmental variables	More easily quantifiable then for organisms	Complicated, partly dependent on environmental variables	More easily quantifiable then for organisms
Results in extreme conditions?	No	Yes	No	Yes
Overall Ecological relevance of results	A well accepted measure of bioaccumulation. Mussels not naturally present	Exposure to biota may be inferred	Highly relevant species.	Low, concentrations must be compared to laboratory studies
Ethical issues	No authorised experimental leader required	No animals required	Authorised experimental leader required	No animals required
Direct measurement of biological effects	Yes	No, extracts may be used in bioassay testing.	Yes	No, extracts may be used in bioassay testing.

[1]Alkylated naphthalenes are an exception.[2]Average accumulations for exposure stations 3 and 4. Only compounds measured in both matrices considered. LOQ determined as average blank value + 10 × SD. [3]C3-Alkylated naphthalenes not included due to exception blank value.

5 PW related laboratory studies

In addition to field studies, several PW related laboratory investigations have been carried out on early life stages and reproductive mature fish. The laboratory experiments have been done using both real PW collected from oil platforms and mixes of selected compounds present in PW. Two chemicals groups, alkylphenols (APs) and poly aromatic hydrocarbons (PAHs) have been given special focus in these studies. The APs have been shown to bind to the oestrogen receptor, mimicking the effects of the natural female sex hormone oestrogen and disrupting the endocrine and reproductive systems in fish even in low concentrations (Servos, 1999) and PAHs are known to be genotoxic and highly toxic to early life stages of fish (Barron et al., 2004).

5.1 Approaches

Different approaches have been used to expose fish to PW compounds. There are many practical and environmental difficulties of designing long-term experiments to expose fish via the water when studying large marine fish, like cod or other species present in the North Sea. Most studies have therefore either used relatively short-term water exposure or long-term exposure through food.

The APs found in PW all have moderate lipophilic properties (Log K_{OW} 3.4-4) (Shiu et al., 1994). In fish, the biological uptake of chemicals with log $K_{OW} < 4$ (logarithm of the octanol/water partition coefficient) mainly takes place via the water (theoretically 20 times faster than uptake from food) (Mackay and Fraser, 2000). This means that bioaccumulation of APs in fish probably takes place primarily via uptake through the gills and skin and is not biomagnified through the food chain. However, there are several reports showing a high bioconcentration factor (BCF) for some long chain APs (nonylphenol and octylphenol) in lower trophic levels, so uptake via food cannot be totally ignored as a source of NP in the higher trophic levels, such as juvenile fish (Takahashi et al., 2003; Hecht et al., 2004; Hu et al., 2005; Correa-Reyes et al., 2007).

5.1.1 **Oral exposure**

IMR have studied the effects of APs on cod reproduction using oral exposure. Three experiments have been conducted using a mixture of middle chain length APs (C_4-C_7 APs) and one experiment using PW from Oseberg C (Meier et al., 2007a, Meier et al., 2007b, Meier et al., 2011). It was found that exposure to APs had different effects depending on the developmental stage of the fish. It was observed that juvenile females can be “pushed” into puberty and maturation, while gonad development is delayed in mature females and males. These results suggest that AP-exposure can affect the hypothalamic-pituitary-gonadal (HPG) axis in cod even at extremely low concentrations. In this study the lowest observable effect concentration (LOEC) was found at a nominal dose of 4µg AP /kg body weight for the effects on timing of puberty, and 20 µg AP /kg body weight for the delay effect in mature fish. The real body burden was under the detection limit of the analytical methods (<1 µg/kg) and it is estimated

that the real body burden after oral exposure was less than 10 % of the nominal dose. Importantly, no similar effects were seen in fish exposed to real PW.

It is also important to note, that the endocrine effects of APs on the HPG-axis in cod appear at AP doses that are lower than the doses that induce Vtg in male and juvenile fish, and this suggests that Vtg is not sufficiently sensitive for detection of levels of EDCs that interfere with the HPG-axis in fish (Meier et al., 2011).

5.1.2 Water exposure

5.1.2.1 Surrogate produced water (APs, PAHs etc.)

Several exposure experiments using fish (zebra fish or cod) exposed to mixtures of APs and PAHs at IRIS (Bohne-Kjersem et al 2009; Sundt et al., 2009; Tollefsen et al., 2011); NIVA (Holth et al., 2008; Holth et al., 2009; Holth et al., 2010) and NIFES (Olsvik et al., 2007) have been performed.

Low doses of PW related compounds did not induce Vtg (Tollefsen et al., 2011) or disrupt gonad development in cod (Holth et al., 2010), however, effects on the mRNA levels have been found in many genes from several different biological systems in cod (Holth et al., 2010;) and zebra fish (Olsvik et al., 2007; Holth et al., 2008).

5.1.2.2 Produced water

PW contains numerous toxic compounds of natural origin, such as dispersed oil, metals, APs, PAHs and also a large “hump” of unresolved complex mixture (UCM), which is composed of a large number of unknown compounds (Røe Utvik, 1999; Neff et al., 2000; Neff, 2002; Rowland et al., 2001; Boitsov et al., 2007; Booth et al., 2007; Melbye et al., 2009). In addition, PW also contains different chemicals added during the oil production process (McCormack et al., 2001).

Even through there still are a rather limited number of investigations, some studies have used real PW collected from offshore oil production platforms in laboratory exposure experiments. This approach recreates the most realistic field-exposure regime in which fish will be affected by a wide range of chemicals. The biological effects that are found in this kind of study cannot be assigned to one group of chemicals alone, but are the results of exposures to the complex chemical mixture found in PW.

The early life stages are the most sensitive to harmful effects of PW. Recent studies have shown that exposure to 1% PW prevents the cod yolk sac larvae from beginning to feed on their own. The inability to start-feed could be explained by the increased incidence of lower jaw deformities or by decreased swimming activity caused by narcotic effects from the PW (Meier et al., 2010). Stephens et al., (2000) did not report increases in mortality in turbot (*Scopthalmus maximus*) larvae exposed to PW (different dilutions up to 1%), but they found a reduction in the swimming activity of larvae exposed to 1% PW. Turbot larvae exposed to 0.1% and 1% PW showed changes in the ultrastructure of the cell membranes of the gills which could result in a reduced ability to take up oxygen. In addition, numerous other signs of chronic stress were observed, including increased levels of cortisol and cytochrome P450, and increased activity of

CYP1A and 7-ethoxyresorufin-O-deethylase (EROD) (Stephens et al., 2000). Similarly, an increase in cytochrome P450 activity is also reported in herring larvae exposed to 400-800 times diluted PW (Gamble et al., 1987).

In juvenile and adult cod, high doses of produced water (1:100-1:200 dilution) have been shown to induce Vtg (Meier et al., 2010; Sundt et al., 2012) and CYP1A (Meier et al., 2010), and to interfere with gonad development (Sundt and Björkblom, 2011) and affect the immunosystem in cod (Perez-Casanova et al., 2010).

These data demonstrate that the lowest observable effect concentration (LOEC) in fish larvae and adult fish is between 0.1% and 1% PW (Meier et al., 2010) However, due to the dilution factor this concentration can only be expected to be found very close to oil platforms. After PW is discharged into the sea it is quickly diluted and the bioactive compounds will most likely be diluted to a concentration that does not produce any large scale harmful biological effects (Durell et al., 2006; Neff et al., 2006).

5.2 Biomarker testing and validation

The performance of biomarkers for specific groups of pollutants may be assessed under controlled exposure conditions in laboratory studies. Exposures to PW from the Ekofisk field have been used to assess the performance of chemical and biological markers used for cod and mussels for the WCM (Sundt et al. 2011c; Sundt et al. 2011b). Establishing the relationship between exposure levels (e.g. measured with chemical techniques) and specific effects under controlled conditions may contribute to a more cost efficient field monitoring (see paragraph 9.11.2).

Table 8 and Table 9 report overviews of laboratory studies with produced water exposures and surrogate produced water, respectively.

Table 8 Overview of laboratory studies with produced water exposures.

Reference	Exposure compounds/Source	Administration	Study species	Study topic
Gamble et al., 1987	Produced water Auk and Forties platform, North Sea	water	Cod and herring larvae	development effects and biomarkers
Stephens et al., 2000	produced water AH001 platform, North Sea	water	turbot fry	effects
Olsvik et al., 2007	produced water Oseberg C, North Sea	water	zebrafish	gene expression
Caliani et al., 2009	produced water (PW) from an Italian on-shore oil plant	water	mosquito fish (*Gambusia affinis*)	Genotoxic effects
Sundt et al., 2009 Tollefsen et al., 2011	produced water Oseberg C, North Sea	water	juvenile cod	effects / biomarkers
Sundt et al, 2011c Hannam et al., 2009	produced water Ekofisk, North Sea	water	mussels	effects / biomarkers
Sundt et al, 2012	produced water Ekofisk, North Sea	water	juvenile cod	biomarkers
Sundt and Björkblom 2011	produced water Ekofisk, North Sea	water	mature cod	Reproduction effects
Meier et al. 2010	produced water Oseberg C, North Sea	water	eggs, larvae and juvenile cod	Survival, developmental effects and biomarkers
Bohne-Kjersem et al., 2010	produced water	water	cod larvae	Protein expression,

	Oseberg C, North Sea			biomarker development
Bohne-Kjersem et al. submitted	produced water Oseberg C, North Sea	water	early juvenile cod	Protein expression, biomarker development
Perez-Casanova et al., 2010	produced water Hibernia, Newfoundland	water	juvenile cod	immune response
Lie et al., 2009 Meier et al., 2011	produced water Oseberg C, North Sea	oral	Juvenile and mature cod	development effects Reproduction effects, biomarkers and gene expression.

Table 9. Overview of laboratory studies with surrogate produced water.

Reference	Exposure comp	Administration	Study species	Study topic
Hasselberg et al., 2004a Hasselberg et al., 2004b Meier et al., 2007a Meier et al., 2007b Meier et al., 2007c	AP mix	oral	cod	Reproduction effects, effects and biomarkers

Sundt et al., 2009 Tollefsen et al., 2011	AP mix, PAH mix	water	juvenile cod	effects / biomarkers
Bohne-Kjersem et al., 2009	AP mix, PAH mix	water	juvenile cod	Protein expression/biomarker development
Holth et al., (2008)	AP mix, PAH mix	water	zebrafish	gene expression biomarkers
Grung et al., 2009a Holth et al., 2009 Holth et al., 2010	AP mix, PAH mix	water	Juvenile and mature cod	Reproduction effects, biomarkers, genotoxicity and gene expression.
Lie et al., 2009 Meier et al., 2011	AP mix	oral	Juvenile and mature cod	development effects Reproduction effects, biomarkers and gene expression.

6 Important findings from WCMs

The main aim of the WCM programme is to use the best available biomonitoring tools to assess the biological effects of PW from offshore platforms. For this to occur, the programme needs to continuously evolve, reassessing the choice of individual biomarkers for inclusion in the biomarker suite as well as the choice of chemical measurements. This has resulted in the inclusion and exclusion of biomarkers within the programme over the years with the adoption of new proposed monitoring techniques. The main lessons learnt from the previous three field monitoring programmes are listed below.

1) Increase the number of mussel cages. Cages of mussels are less resource demanding than fish, which allows greater coverage and ensures that some stations are exposed to the plume. This is particularly important at platforms where the direction of the PW discharge plume is less well known.

2) The position of cages is extremely important and great care should be taken to ensure caged organisms are exposed to the PW plume at varying distances. Information on the current direction and strength are often available within the area of the selected offshore platform and this information should be used when designing the survey. Current meters have been and should continue to be used to confirm the current direction and speed during the exposure duration.

3) The timing of the WCM field exposure has been and should continue to be planned to occur in order to avoid significant stratification of the water column and algal blooms, which can potentially influence the dispersal and bioavailability of the PW respectively. For these reasons the WCM programme occurs in early spring.

4) The mussel bioaccumulation of PAHs has proven to be an extremely effective tool for monitoring organism exposure, with a clear distinction with distance from the PW discharge. Alkyl phenols are an important component of the PW and have in previous WCM programmes only been measured as the metabolised form in fish bile. However, it is known that POCIS are currently the best available method for the detection of AP in the water column (Harman et al., 2011). POCIS are therefore highly recommended for future WCM programmes to complement the PAH accumulation in the mussel.

5) The use of Vtg and Zrp biomarkers for the assessment of estrogenic exposure in fish appears to be a duplication of information, and neither has been found to be responsive at Ekofisk. Since the Vtg method is more developed and robust, Vtg alone is recommended for use in future monitoring programmes where fish are used. However, in the absence of fish, it is recommended that PSD extraction combined with *in vitro* bioassays (i.e. YES) are used to measure potential estrogenicity of the PW in the receiving waters. The sensitivity of the *in vitro* assay is suitable for the study of estrogenic effects in PW. In addtion to YES, it is recommended that YAS, anti-YES and anti-YAS are also performed on the PSD extracts, since components of the PW have also been found to have androgenic and anti-androgenic activity (Thomas et al., 2004b; Thomas et al., 2009).

6) GST has been used as a biomarker of exposure to PAH related compounds in previous programmes. However, field data so far has proved disappointing and there is uncertainty about the sensitivity of the GST method. The method measures all GST activity, and since GST has many isomers performing a wide range of biological functions they are thought to be naturally up-regulated within the body. Consequently, a large exposure to a GST stimulating compound is required to elevate GST above background levels. For this reason it appears inappropriate to measure GST to detect exposure to environmental concentrations of PAH related compounds.

7) Obtaining clean and healthy organisms for caging studies is extremely important but often difficult since coastal areas are typically exposed to chronic concentrations of environmental contaminants. For the last several years, the mussels used in the WCM programme have been selected from the same location (Trondheimsfjord) in what has been considered to be a clean location. In general, this has been the case, although a low PAH signature has been observed in these mussels from pre-exposure sampling (WCM report, 2009). Using the same mussel population each year for the WCM was considered advantageous, enabling comparisons to be made from year to year, (e.g. Ekofisk in 2006, 2008 and 2009).

With regard to caged fish, farmed fish have been used in previous programmes. The advantages of using farmed fish as opposed to capturing wild fish for caging are both pragmatic and scientific, some of the key points are listed below.

1) Farmed fish are easier and cheaper to obtain for transplantation studies.

2) Farmed fish are often from a single population and of the same age and size class, reducing variability between fish.

3) The exposure history of farmed fish is known to some extent.

4) Farmed fish are used to being held in captivity so may be more tolerant to being caged.

5) The stress of capturing wild fish may make them unsuitable for caging studies.

For these reasons farmed fish are recommended for caging studies in future monitoring programmes.

7 Potential effects of PW constituents on the Norwegian North Sea environment

Monitoring programs have confirmed the presence of PW constituents in the vicinity of offshore installations; however the studies indicate that the affected area may be limited when the PW is treated (e.g. Ekofisk). Previous studies have provided information that can be used to estimate the potential for effect from PW constituents in the North Sea ecosystem outside the installation near zones. Processes like dilution, evaporation, biodegradation and photooxidation generally decrease the potential toxicity to marine organisms soon after the PW enters the sea.

7.1 Alkylphenols

The main environmental concern related to APs present in PW discharges has been the potential of reproduction related effects from the weak estrogenic potential of some compounds. However several studies conclude that field realistic concentrations of these compounds are not likely to cause reproductive effects in North Sea fish populations.

Sensitive techniques for detection of AP metabolites in biota are available. Results from the condition monitoring confirm that APs are not detected in bile of haddock, saithe and long rough dab in areas with extensive PW discharges (Grøsvik et al. 2007; Grøsvik et al. 2009).

Relative to reference areas, Vtg levels in male cod from the Tampen area are not elevated (ibid.).

7.2 PAHs

PAHs related to offshore operational discharges are generally not found in muscle of wild specimens of fish collected in regions with oil and gas activity (Grøsvik et al. 2009). Detectable levels of PAH metabolites have been observed in wild herring and saithe collected in the vicinity of the PW outfalls (Aas et al. 2006). For these species the levels detected are generally too low to trigger a response in PAH related biomarkers and the size of the affected areas are limited, hence effects from PAH at the population level are not considered likely.

Some species may potentially be more vulnerable to the low PAH levels present in areas with extensive oil and gas production, probably due to their feeding biology, as indicated by the PAHs detected in bile of wild haddock collected in the Tampen area (Grøsvik et al. 2009). The source of this PAH is presently not known but contribution from PW is not unlikely. Levels of PAH observed are generally low, but a possible connection to elevated levels of DNA adducts in haddock collected in that particular region is indicated and should be further investigated.

7.3 Other hydrocarbons

In addition to APs and PAHs, PW also contains a large “hump” of unresolved complex mixture (UCM), which is composed of a large number of unknown compounds that may contribute to the toxicity (Neff et al. 2000; Rowland et al. 2001; Melbye et al. 2009). Some of these unknown compounds might also have endocrine disruption effects. For example, naphthenic acids present in PW can function as xenoestrogens if concentrations are sufficiently high (Thomas et al. 2009). It is however not considered likely that such effects occur under field realistic conditions.

7.4 Metals

Bioavailability of trace metals has been evaluated using biomarkers, concluding that metals from PW did not cause biological effects in the North Sea ecosystem.

No effects from metals were found in pelagic fish caught in the vicinity of the Statfjord platform (Bilbao et al., 2006; Chesman et al., 2006; Aas et al., 2006), even though a quantifiable amount of cadmium, zinc and copper was found in mackerel liver, and zinc and copper in saithe and herring soft tissues (Ruus et al., 2006). Levels of autometallography deposits were found at background levels in mussels caged along a gradient in the Statfjord platform area, showing that the metal pollution is not noticeable even in the near zone. All these findings are in agreement with monitoring studies performed in the Mediterranean Sea (Gorbi et al., 2008).

7.5 Radionucleides

It is considered unlikely that naturally occurring radioactivity associated with PW discharges represents a significant health risk to marine life or humans. For example no oxidative stress response was indicated in rag worms (*Hediste diversicolor*) exposed to levels of ^{226}Ra several orders of magnitude higher than the levels measured in organisms caged close to a PW discharge (Grung et al 2009b).

7.6 Production Chemicals

Even though production chemicals may contribute significantly to the theoretical impact when environmental risk of operational discharges is evaluated by modelling, the compounds have had no particular focus in the Norwegian monitoring programmes. This emphasizes that there should be an increased focus on parameters that could detect such impact for future WCM and condition monitoring. Some compounds have significant acute toxic potential in high concentrations, but are generally rapidly degraded and not accumulated. Extensive effort is being put into selection of chemicals with as low environmental impact potential as possible.

Some studies have focused on investigating fate and effects of some production chemicals. E.g. levels of diethylene glycol in discharged PW have been investigated (Cappiello et al. 2007), and only low levels of effects from this particular compound have been found in fish (Gorbi et al. 2009). The increased use of surfactants injected to reservoirs to enhance oil recovery may increase discharges of membrane active compounds which could affect the physiology of gills in exposed organisms. The potential risk related to an increased discharge of such compounds should be assessed.

8 Knowledge gaps and emerging monitoring demands

Some of the knowledge gaps that are clearly emerging from previous monitoring studies are reported below. Of course other information is also valuable and will need further attention in the future, e.g. effects on zooplankton, modulation of pelagic processes, sublethal effects on the reproduction, nutrition and general behaviour of fish.

8.1 DNA adducts in Haddock

In samples of haddock collected in the Tampen area, levels of DNA adducts are reported to be elevated relative to haddock collected from areas without extensive oil and gas exploration such as Egersundbanken and the Barents Sea (Hylland et al. 2006b, Balk et al. *submitted*, Grøsvik et al. 2008 and 2009). The exact source of the compounds causing this effect is currently unknown. Compounds causing the effects may come from ongoing PW discharges, previous discharges of cuttings and oil based mud or from other anthropogenic sources (Trans Boundary Pollution). Closely connected to the question of source, is the type of compounds causing the reported effects.

Elevated levels of DNA adducts have not been observed in caged cod exposed to PW (Brooks et al. 2011), but elevated levels of adducts have been observed in cod exposed to high concentrations of PW in laboratory studies (Sundt et al. 2011b). Comparison of autoradiogram profiles from haddock exposed in the laboratory to PW or the mud oil used at the Tampen fields in the past, with autoradiogram profiles from haddock collected in the Tampen area may indicate the source.

For haddock collected in the Tampen area a poor physical condition is reported (Grøsvik et al. 2009). The lipid content in the liver tissue sampled for the DNA adduct analysis may therefore be considerably lower than in specimens from other areas used for comparison. Some previous studies indicate that lipid content in the tissue and levels of biotransformation activity may affect DNA adduct levels (e.g. Acka et al. 2004, Rundle et al. 2007). To what extent physical condition may affect DNA adduct levels or the measurement of adducts in haddock could be investigated by comparing results from exposed groups fed during the exposure with groups starved during the exposure.

8.2 Lesions in cod gills

In the WCM 2010 project (work package 15), the health situation in gills of fish exposed to PW at the Ekofisk field was investigated. Lesions detected by histopathology were observed in the cod material collected in 2008 and 2009.

The lesions observed in 2008 material were confirmed by the 2009 material, however the damages observed were less pronounced in fish material exposed to CTour treated

PW (Sundt et al. 2011a). Monitoring of gill lesions seems to have potential as an indicator of PW exposure.

Gill histology parameters as markers of PW exposure

The study indicated that several of the gill lesions included may have potential as biomarkers of exposure to offshore operational discharges. However as several types of stress may cause the lesions observed, laboratory validation is needed

Types of compounds causing the observed effects

Comparison of lesion prevalence in gills of cod caged at Ekofisk in 2008 and 2009, before and after implementation of new cleaning technology indicated environmental benefit from the new discharge treatment. The fact that the discharged volume at Ekofisk increased from 2008 to 2009 about 26% and that the total discharge of oil compounds discharged was reduced by 24% may indicate that hydrocarbons contributed significantly to the observed effects. Lipophilic compounds are more efficiently removed by the extraction process compared to the often more water soluble production chemicals (e.g. scale and corrosion inhibitors).

Physiological consequences of the observed lesions

Whether wild fish living in the vicinity of PW outfalls may be sufficiently exposed to form gill lesions is presently not known. Knowledge about the organism's potential to repair the type of lesions observed in PW exposed fish will be important when assessing consequences.

8.3 Lipid metabolism in fish

It has been found in 2005, 2008 and 2010 that haddock collected from Tampen had relatively smaller livers with lower lipid content and therefore only had approx. 50 % of the energy reserve compared with haddock from the other regions, Egersundbanken, the Norwegian Sea and the Barents Sea. Fatty acid analysis of different lipid classes showed that fish from Tampen had relatively high levels of arachidonic acid (20:4 (n-6)) compared with haddock from the other areas and the ratio between (n-3)/(n-6) polyunsaturated fatty acids (PUFA) was significantly lower in neutral lipids (NL, storages lipids) and phospholipids (PL, membrane lipids) (Grøsvik et al., 2009; Sonnich Meier, unpublished results). This is an agreement with an earlier investigation that also found that haddock from Tampen had a lower (n-3)/(n-6) ratio compared with haddock from Egersundbanken (Hylland et al., 2006b). High levels of 20:4 (n-6) can be related to increased inflammation and other effects in the immune system (Calder, 2008).

It is not possible however from these data to link the high levels of 20:4 (n-6) directly to oil pollution. High levels of 20:4 (n-6) in the NL were also found, and it is therefore possible that a different diet may be the cause.

It has been found that hydrophobic organic pollution can affect the lipid composition in phospholipids. Crude oil is shown to change the FA profile in bacterial membranes

(Mazzella et al., (2005a and b) and similarly exposure to alkylphenols decreased the amount of (n-3) PUFA in the PL of Atlantic cod (Meier et al., 2007c).

There is a need for future investigation to clarify if the low energy condition of haddock is particularly related to areas in the North Sea with high oil activity and if this low condition can affect the reproductive fitness of the fish in these areas. In addition to field studies IMR also have a NRC project that aims to study mechanisms behind the effect of crude oil and other types of pollution on lipid composition in fish cell membranes: "Study of the effects of oil compounds and persistent organic pollution (POP) on the phospholipid composition and membrane fluidity in Atlantic cod (*Gadus morhua*) with emphasis on the effects on phospholipids synthesis".

Several microarray studies have shown that PW and oil exposure can affect expression of genes involved in lipid metabolism and membrane stability in fish (Olsvik et al., 2007; Holth et al., 2008; Lie et al., 2009; Holth et al., 2010; Olsvik et al., 2011). There is a need for more knowledge on the mechanism behind these effects and the potential biological significance on fish fitness.

8.4 TENORM

The Norwegian Radiation Protection Authority, Petroleum Directorate and the Climate and Pollution Control Authority have emphasized the need for more knowledge regarding radioactive discharges from the oil and gas industry (Anon. 2008) and the environmental authorities have requested monitoring of possible effects on marine organisms.

Radiation levels in produced water (PW) related to Technologically Enhanced Naturally Occurring Radioactive Materials (TENORM) is generally relatively low and the typical seawater dilution for offshore discharges is generally high.

Due to the low levels of radioactivity expected to be present even in organisms caged close to an outfall, isolating biological effects caused by the radioactivity levels alone, from effects caused by other PW constituents like hydrocarbons is expected to be a major analytical challenge. To measure levels of radioactivity in exposed organisms and relate this to background levels is however feasible.

As part of the WCM 2010 project (work package 14), ^{226}Ra in caged organisms from the WCM 2009 was analyzed. The study concluded that α-spectroscopy is suitable for monitoring of ^{226}Ra accumulation and may therefore be included as part of future WCMs. However the study did not indicate any accumulation of ^{226}Ra in cod and mussels caged in the vicinity of the Ekofisk discharge.

9 Suggestions for future monitoring programs

9.1 Evaluation of differences in impact potential of PW from different fields / wells

The last three WCM investigations in 2006, 2008 and 2009 focused on the exposure conditions at the Ekofisk field. Due to differences in chemical profile of PW from different fields and wells (Røe Utvik, 1999, Boitsov et al. 2004) exposure and biological effects may differ considerably among different fields. The need for assessing the range of variation has been expressed by the Klif expert group. Several approaches with a wide range of cost and quality potential can be used to evaluate variability in impact potential among fields. The cost efficiency and scientific potential of the total monitoring approach in future monitoring programmes should be evaluated; it is possible that future investigation design should include a combination of approaches and could be performed in 1 or more PW effluents. The potential for the various approaches is indicated in Table 10. For example, the chemical analyses of PW already performed on an annual basis (9.1.1) could be supplemented by bioassays (9.1.2) providing a screeningfor the toxic potential of various PW types. Morevoer, laboratory exposure studies (9.1.4) will provide direct information of PW effects, especially if performed with "fresh" PW on the platform. In fact changes take place in PW during any processing after release. Finally field collection of organisms is of course a relevant alternative (e.g. recommendations following BECPELAG).

9.1.1 Evaluation based on chemical composition of discharged water

A simple evaluation of effect potential of different PW discharges can be based on the chemical composition of discharges. For such an approach, information about effect levels provided from laboratory studies is needed. Chemical information is already available from the reports to the Climate and Pollution Agency twice a year and could be exploited as a part of the assessment. Compared to other approaches the cost is expected to be low. A limitation with this approach is the relatively limited fraction of the total number of compounds actually present in the discharge that it is feasible to analyse. Such an evaluation will not obtain information about potentially harmful compounds present but not analysed.

9.1.2 *In vitro* techniques / bioassays

As an alternative to *in vivo* studies using caged fish or mussels, chemical extraction methods have mediated the option for *in vitro* exposure of cellular model systems to pre-concentrated environmental water samples. Such bioassays can be used to indicate the presence and potential effects of contaminants and are therefore useful in ecological risk assessment. Advantages of small scale bioassays over *in vivo* studies are related to high time and cost efficiency, as well as possibility for high throughput screening. Chemical extraction techniques compatible with bioassay testing include liquid-liquid extraction (Kuch et al., 2010), solid phase extraction (SPE) (Tollefsen et al., 2007;

Farmen et al., 2010), or passive samplers such as SPMD and POCIS (Harman et al., 2010). As opposed to SPE or liquid-liquid extraction, POCIS and SPMDs are integrative samplers that accumulate organic contaminants typically during a period of some weeks. The bioassays are often based on cell cultures from various model organisms and could be primary cells isolated from fish liver, or genetically modified cellular systems for analyses of specific cellular mechanisms. For instance, a recombinant rat hepatoma cell line known as dioxin responsive (DR)-chemically activated luciferase expression (CALUX) assay, has been used to screen for dioxin equivalents in PW from the UKCS (Hurst et al., 2005). The aryl hydrocarbon receptor (AhR) agonist potency ranged from 1 to 430 ng TCDD TEQ_{CALUX} L^{-1}, reflecting a highly variable composition of PW discharges from the different production fields. In a similar way, recombinant yeast cells in Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS) have been used to measure estrogenic, androgenic and anti-androgenic potential (e.g. binding of organic compounds to the oestrogen and androgen receptors) in PW discharges from several offshore oil production platforms in the British and Norwegian sectors of the North Sea (Thomas et al., 2004a; Thomas et al., 2004b; Tollefsen et al., 2007). Levels of oestrogen equivalents were comparable between the studies and chemical characterisation of the extracts verified the presence of known oestrogen receptor agonists such as short chained alkyl phenols. Moreover, the presence of unidentified anti-androgens in PW was shown for the first time (Tollefsen et al., 2008) indicating that PW discharge represents a significant input into the marine environment of unknown compounds that exert a known biological effect. Effect-directed analyses recently identified naphthenic acids as a group of weak oestrogen receptor agonists responsible for as much as 65 % of the estrogenic activity observed in the bioassays, and that naphthenic acids, as well as certain PAHs and APs contribute to anti-androgenic effects (Thomas et al., 2009).

A bioassay approach has also been applied to discharge from a Chinese oil field, where focus was on identifying genotoxic compounds in PW discharge by a bacterial SOS/umu bioassay for genotoxicity in combination with rat hepatoma Ethoxyresorufin-O-deethylase (EROD) bioassay, (Li et al., 2008). Oilfield produced wastewater was shown to contain substantial quantities of indirect genotoxic substances exclusive of AhR agonists, and it was also shown that neither genotoxic nor AhR agonistic chemicals could be effectively removed by the treatment processes.

Furthermore, primary fish cell cultures have been used in small scale *in vitro* bioassays to assess the potential of PW extracts to cause oxidative stress. In depth analyses of rainbow trout hepatocytes showed dose-response dependent cytotoxicity, increased intracellular formation of reactive oxygen species (ROS), increased levels of the antioxidant glutathione, as well as differential gene expression of important antioxidant enzymes. Finally, an inter-field comparison of PW from 10 offshore oil platforms showed that water soluble organic compounds of PW were major contributors to oxidative stress and cytotoxicity, and that the biological effects were not correlated to the content of total oil in PW (Farmen et al., 2010).

To summarize, chemical pre-concentration of organic contaminants followed by bioassay assessment of potential biological mechanisms of toxicity have shown to be a valuable screening tool. Small scale *in vitro* bioassays have been used to show presence

and biological effects of estrogenic, anti-androgenic, dioxin-like, genotoxic and pro-oxidant contaminants in PW from oil production. Due to the high throughput of these methods, comparisons between different oil fields and continental sectors may be assessed, showing large inter-field variations. Furthermore, the use of such bioassays can be used in effect-directed chemical analyses to identify groups of contaminants causing a specific biological effect.

9.1.3 Caging

The approach currently being employed, involving caging of organisms for effect studies has several advantages. An important factor is that the actual effect potential of the PW is assessed. However due to the costly nature of offshore operations, it will only be realistic to assess a limited number of the discharges present (a single discharge each year). In addition, for some study sites the approach may be considerably challenged by technical limitations. The most obvious limitations are related to design of cage placing where the local plume distribution is challenging and vessel traffic limits the design flexibility. This type of challenging current situation has been experienced at Troll (Hylland et al., 2008); whereas at Ekofisk the circumpolar current pattern increases the likelihood of the plume hitting the cages and consequently designing cage distribution is easier (Brooks et al., 2011).

9.1.4 Laboratory exposure of organisms to PW

Using the same standardized transport and storage conditions and the same dilution for all exposures from all fields, PW from several different fields could be tested simultaneously. In spite of the need for extensive experiment maintenance and relatively extensive laboratory facilities resulting in a high price, the approach may be more cost efficient than field exposures. Previous experience with this type of laboratory exposure could be used as a basis for future design (e.g. Sundt et al., 2009; Sundt et al., 2010). Standardisation of exposure approach is needed and chemical degradation of the PW should be documented, by measurement of selected compounds (e.g. PAHs and APs) in samples collected before and after transport and storage.

Table 10. Various approaches for assessing variability in the effect potential in different PW discharges.

	Chemistry based assessment	*In vitro* studies	Field caging	Laboratory PW exposure
Numbers of discharges realistic to be analyzed	Very high	Very high	Low	High
Number of different effects that can be	Only theoretical evaluation	Limited, (but relevant effects	High	High

assessed		can be selected)		
Amount of PW needed for experiments	Low	Low	(field based)	High
Cost/field	Low	Low	Very high	High

A combination of these four approaches is possibily the most appropriate solution for future evaluation. A chemical based assessment could be followed by laboratory exposure of organisms (i.e. mussels). Experiments should be carried out for each field in established conditions (e.g. fixed dilution rates). The theoretical evaluation of the water volume that each field produces will allow a risk assessment of the environmental effects.

9.2 Coordination of WCM and condition monitoring

Monitoring possible effects of oil and gas activities in open seas should be done with an ecosystem based approach. The water column and sediments should not be considered separate entities, but efforts should be made to integrate results and monitoring strategies. One possibility is wider use of benthic species (fish and invertebrates), as well as planktonic species in the WCM and condition monitoring.

Several of the components in the ecosystem should be included to give a broader view of potential effects of pollutants in the monitoring. The species and methods selected should also aim to study several mechanisms of actions, and should try to cover the most sensitive species and life stages, when this is possible for field studies.

The scope of the WCM is to monitor possible effects from operational discharges, and although the condition monitoring has a more resource related scope, many of the techniques employed are common for the two programs. As the questions that originally brought fish into the WCM caging programme have been answered, it now seems timely to reduce the effort on the relatively demanding caging of fish.

This effort could be shifted to focus on assessing the health situation in wild organisms living in the proximity of the discharges. A significant advantage with a local collection approach is the access to organisms living in the proximity of discharges not available by the trawling approach used in the condition monitoring.

The exposure of wild organisms to operational discharges is expected to be considerably lower than what is seen in organisms caged in the water columns near PW discharges. Relationship between effect levels / corresponding exposure levels established in laboratory studies with exposure levels in field collected organisms should be better established.

Some pollutant stressors act as a general stressor to the organism, while other compounds act more specificly, e.g. endocrine disruptors, neurotoxicants, and

compounds acting directly on nuclear receptors, disruption of metabolic pathways or cell signalling in the organism. As oil and produced water is a complex mixture of a large number of different compounds, both originating from the reservoir but also from chemicals added in the production process, selection of methods should take into consideration several types of mechanisms of action.

9.2.1 Wild organisms for future monitoring

Use of wild organisms for biomonitoring of operational discharges is a frequently used approach. Not being dependent on costly and scientifically challenging caging is a major advantage with such an approach. However the concept of using wild organisms may have biological limitations. The availability of suitable species may vary considerably among different fields reducing the chance of inter field comparisons. A major challenge is the lack of information about geographical distribution of specimens prior to sampling. Factors that should be taken into consideration when selecting wild living species for biomonitoring purposes are listed below. Candidate species in Norwegian waters selected based on the evaluation factors are given in Table 11.

Geographical distribution specimens

Some species may have very specific requirements e.g. bottom substrate. Patchiness in their distribution may therefore be a considerable factor.

Availability for collection

To secure a sufficient number of specimens within the time available, the catch efficiency of the selected gear is of importance. Non-destructive gear suitable for use close to installations and within the platform safety zones is needed.

Movement of specimens in relation to affected locality

Pelagic species like saithe (*Pollachius virens*) and Atlantic mackerel (*Scomber scombrus*) have a considerable migration potential. Lack of information about geographical distribution of specimens prior to sampling makes them unsuitable for biomonitoring studies where local influence of PW is assessed. By selecting species with low self-motion potential the likelihood of a representative local exposure is highest, slow bottom dwelling species will generally be best suited.

Suitability in laboratory studies

In species not previously utilised for biomonitoring, assessment of biomarker performance by controlled laboratory studies may be required. It is therefore an advantage that species are sufficiently robust to be kept under laboratory conditions after collection and transport. Bottom dwelling species are usually far less vulnerable to mechanical influence than pelagic species. For some of the suggested species listed in Table 11, laboratory studies have been carried out and suitability confirmed.

Tissue for sampling

Size of the specimens collected is generally not a limitation. The amount of tissue needed for most chemical and biomarker techniques is low and even small specimens can be exploited.

Previous experience with the species

Selection of species previously exploited for biomonitoring purposes is considered advantageous. Some of the biomarkers routinely used are dependant on the availability of more or less species specific antibodies (e.g. Vtg, Zrp, Cyp1A). Standardisation of species selection also allows comparison of results among different studies.

Table 11. Candidate invertebrate and fish species in Norwegian waters.

Species	Latin name	Norwegian name	Feeding strategy	Catch	Suitability for lab studies
Common whelk	*Buccinum undatum*	Kongsnegl	Scavanger	Pots, Valentinsson et al 1999	Good Siikavuopio et al. 2007
Northern horse mussel	*Modiolus modiolus*	Oskjell	Filter feeder	Diving and dredging	Vingen et al.
Ocean quahog	*Arctica islandica*	Kuskjell	Filter feeder	Diving and dredging	IRIS / Biotaguard, lab tests
Icelandic scallop	*Chlamys islandica*	Haneskjell	Filter feeder	Diving and dredging	IRIS / Biotaguard, lab tests
Common shore crab	*Carcinus maenas*	Strandkrabbe	Scavanger	Pots	IRIS / Biotaguard, lab tests
Great spider crab	*Hyas araneus*	Sandpyntekrabbe	Scavanger	Pots	Sundt et al. 2006
Cold-water coral	*Lophelia pertusa*	Steinkorall	Zooplankton	Scapes, VAG	IRIS/IMR Mortensen et al 2000

Species	Latin name	Norwegian name	Feeding biology	Catch	Suitability for lab studies
Haddock	*Melanogrammus aeglefinus*	Hyse	Bottom/sediment feeder	Trawl, nets, long line, traps	Only prelim. trials
Long rough dab	*Hippoglossoides platessoides*	Gapeflyndre	Bottom feeder	Trawl, nets, long line	Jonsson et al.
Atlantic hagfish	*Myxine glutinosa*	Slimål	Scavanger	Pots	Sundt et al. 2011b
Round ray	*Raja fyllae*	Rundskate	Bottom feeder, (crustaceans)	Trawl, nets, longline	No info. available
Vahl`s eelpout	*Lycodes vahlii*	Vanlig ålebrosme	mostly on endo benthic prey	Traps, trawl	No info. available
Dab	*Limanda limanda*	Sandflyndre	Bottom feeder	Trawl, nets, long line	IRIS

9.2.2 Fishing gear

Trawling, the main approach used for collection of organisms in the Condition Monitoring can not be used close to installations. Simpler non destructive passive gear can be operated from smaller vessels without specialised rigging for fisheries. Live specimens are needed for most analyses used in biomonitoring studies, candidate fishing gear that provides catch with good quality is given in Table 12.

Table 12. Candidate fishing gear that provides catch with the good quality needed for biological sampling.

Gear	General catch quality	Catch efficiency
Baited traps	Excellent	Good for a wide variety of species
Trammel nets	Good	Good for a wide variety of species
Long lines	Good	Good if optimal hooks are used
Sport fishing tackle	Excellent	Can be operated in periods of low activity for sampling crew

9.3 Selection of future WCM sites

The decision of which location(s) to monitor in the coming years should be based on effluent volume and presumed toxicity (as may be determined using bioassays), as well as local oceanographic conditions (depths, stratification, current pattern). Caging locations, timing, etc. then needs to be decided upon according to available information.

9.3.1 Chemical profile and volume of discharge

Effects of APs and PAHs in the PW have had a particular focus in the previous investigations. Results from EIF modelling for different fields often conclude that compounds other than hydrocarbons contribute the most to the theoretical effects (e.g. corrosion inhibitors, H_2S scavenger etc.). Results from available risk modelling evaluations could be more actively exploited when selecting future study sites.

9.3.2 Local plume distribution

One of the most challenging factors in the design of offshore caging studies in a typical offshore environment is to select a geographical distribution of stations within the path of the discharge plume. Results from some previous WCM studies show effect levels that do not appear to be in logical accordance with the distance to the fallout, indicating

this problem (Hylland et al. 2008). Information about the local current situation is crucial for designing a good station grid that could pick up the plume.

The significance of the horizontal plume distributions is obvious. The current speed is also of importance since the shape of the plume is more narrow at high current speed relative to a generally wider (and easier to handle) plume in areas with lower current speed. The WCM was conducted at the Ekofisk field in 2006, 2008 and 2009. Due to tidal influence the current system at that site is circular, resulting in a higher probability for caged organisms to be hit by the discharged PW.

The vertical distribution of the plume is also of importance to the design of the cage distribution. Higher density caused by the high temperature of the PW relative to the surrounding seawater brings the discharge to the surface. After being cooled the plume disperses vertically in the water column, a process potentially significantly affected by density stratification.

9.3.3 Limitation from installations and ship traffic

On several occasions cages have been moved or lost during the WCM exposures due to collision by vessels. In areas with heavy traffic, the rigs could be fitted with acoustic release systems to remove the risk of collisions. Such approaches have been employed for safe operation of cages from technically challenging deep-water environments (Guennegan & Martin 1985; De Broyer et al. 2004; Jones et al. 2003 and Sundt et al. 2009). Where surface buoys are used, positions should be reported to the coastguard central and to the traffic controllers at surrounding installations to avoid possible conflicts.

9.4 Water column effect monitoring as a control of predicted impact

The potential impacts of the different produced water discharges in the North Sea (Norwegian sector) have all been assessed by DREAM/EIF calculations (for instance in the assessments of how to achieve the “zero harmful discharge goal”). It is not a regulatory requirement for the water column effect monitoring to control monitor these impact assessments, and neither did the basis for doing so exist at the time when the DREAM/EIF tool was established and the water column effect monitoring program was initiated.

However, it was expressed by people involved in these processes from operators, authorities and the scientific side that it would have been beneficial to be able to compare (and possibly verify) DREAM/EIF predictions through field effect monitoring. This would strengthen the control aspect of the produced water management. Since that time, knowledge has been gained and tools developed through JIPs, PROOF funded projects and within ICES and OSPAR which better enable this kind of control exercise. Except for an initial attempt in relation to a Master Science study (Nagara, 2009), this has not been done so far.

Elements present in the water column effect monitoring (but also the later condition monitoring program; 2008), can be used in this context, but they do not entirely fulfil

the requirements needed. Different adjustments seem possible in order to fulfil these requirements, but they will be dependant on the overall monitoring design.

The suggestion here is to include this aspect more strongly in the discussion about development of the effect and condition monitoring programs after experience has been gained with the modified design in 2011. A more technically detailed proposal can be given and specifically discussed based on the above mentioned achievements.

It can briefly be mentioned that these achievements include the possibilities to simulate biomarker response fields in DREAM and to make practical monitoring solutions based on single species as well as multiple species monitoring*. This will also be highlighted in the WCM 2010 manuscript regarding assessment criteria (Sanni et al., in prep).

* E.g. PROOF projects: "Validation" (153882/720): Validation of methods and data for Environmental Risk Assessment off-shore; "Biomarker Bridges" (178408/S40): Integration of biomonitoring with risk assessment by construction of biomarker bridges for water column organisms exposed to produced water; Joint Industry Projects: BioSea[a)] and Produced Water[b)] (IRIS et al. for Total E&P Norge[a, b)], ENI Norge[a)], ConocoPhillips Norge[b)]). Recommendations by: ICES WGBEC (Working Group of Biological Effects of Contaminants), and OSPAR/ICES: SGIMC (Study Group of Integrated Monitoring of Contaminants) (ICES 2010; ICES/OSPAR 2009).

9.5 International research network - PW monitoring and effects

Some research groups abroad are currently developing new and improved techniques suitable for biomonitoring of operational discharges from the oil and gas industry.

Exchange of experiences from exposure and analysis design among research groups is considered an important way of improving the scientific quality of the monitoring. A possible way of improving transfer of knowledge suitable in monitoring studies is to support exchange of researchers for short periods of time.

Future monitoring programs may also benefit both in terms of scientific quality and cost efficiency from knowledge transfer through international workshops. Exchange of experiences not available through international publications may be particularly valuable. Workshops could be organised by Norwegian researchers and hosted by KLIF or OLF.

Such networking could also benefit from participation in intercalibration exersizes and method evaluations organized by international working groups that also have advisory roles to European marine areas commissions (i.e. OSPAR, HELCOM, MedPol). The marine environmental regulation is presently in a strong redefinition phase with development of EUs Marine Directive. Several harmonization processes are included in this development, and two-way communication with involved scientists is definitely useful to ensure that the methods that we use on the Norwegian Continental Shelf are within the future harmonized framework.

9.6 Assessment of environmental benefit from new cleaning technology

The oil and gas industry make significant efforts to improve PW treatment approaches. The concept of using biomonitoring before and after implementation of additional cleaning to assess the actual environmental benefit was employed in WCM, WP 7 (Brooks et al. 2011). In this study, a reduction in the mussel bioaccumulation data for all measured PAH NPD compounds was found in mussels from 2009 compared to mussels in 2008 and 2006. This coincided with a reduction in the overall discharge of oil in the PW over the exposure duration, which was thought most likely to have been due to the C-Tour PW treatment system.

The WCM programme at Ekofisk for the three survey years proved to be a very effective assessment of determining the potential environmental benefit of an improved PW cleaning system. The biomonitoring approach, combining biomarker responses with chemical bioaccumulation of exposure measurements, was able to obtain a more comprehensive assessment of the potential environmental impact. This approach has distinct advantages over using chemical analysis alone, where only exposure and not effects can be measured.

9.7 Passive samplers

The addition of passive samplers to future WCMs is recommended, for two main reasons; the removal of inherent biological variability and the ability to isolate accumulations from external environmental or biological factors by modelling the dissipation of spiked standards during exposure using the well-established performance reference compound (PRC) approach. This provides the ability to compare discharges from very different environments spanning large geographical distances which is especially problematic when using biomonitoring. Due to the high variability in the composition of PW both within and between fields, PSDs may be able to link differences in the levels of biological effects measured with differences in concentrations of contaminants. Additionally PSD extracts may be used for studies of toxicological mechanisms using *in vitro* techniques (Harman et al., 2010) and are much more easily screened for additional important target compound groups.

9.7.1 **SPMD**

The SPMD technique is now well established in the scientific community. SPMDs have proved to be capable of detecting very low concentrations (pg L^{-1}) of PW related compounds, in several surveys. The similarity in SPMD and biota accumulations shows the environmental relevance of the technique (for example, see review of mussels and SPMDs by Booij et al., 2006). The ability to correct for differences in exposure conditions between surveys using a PRC based approach means that PSD comparisons are likely to be more reliable for elucidating differences in exposure (not effects) than equivalent mussel surveys. Therefore the independent and reliable measurement of exposure to PW originating hydrophobic organic contamination using SPMDs (or similar PSDs) should be included in future surveys, in order to support the biological effects monitoring. Remaining issues associated with using SPMDs such as

contamination with naphthalene are easily overcome by pre-extraction or by using low density polyethylene, without the triolein fat. Future applications of PSDs for WCM may include bioassays performed on extracts and screening for "new" compounds such as geochemical biomarkers for oil source discrimination (Luellen and Shea, 2003).

9.7.2 **POCIS**

POCIS is a useful tool for screening exposure to polar contaminants. It is the main conclusion from the current WCM (WP 9) that POCIS was the only technique tested which adequately measured exposure to AP in both 2008 and 2009 in the water column. (Harman et al., 2011) and as such should be included in future surveys. Such concentration data can be compared to laboratory studies in order to infer effects on marine species for AP. It is not proposed as a direct measure of effects in itself. However, further work is required in order that POCIS accumulations may be used to estimate water concentrations in a quantitative manner. Other possible applications for POCIS in regard to PW discharges include the testing of extracts using bioassays, the measurement of other polar compounds such as naphthenic acids and production chemicals, and for the verification of dispersion modeling predictions.

9.8 Naphthenic acids

While assessment of the risk posed to the marine environment by the organic constituents of PW has focused on AP and PAH, organic acids are found in discharges are much higher levels. For example in 2009 the total amount released in the Norwegian sector was 27,000 tonnes compared to 310 tonnes of AP (C1-C3) and 51 tonnes of PAHs (EPA16) (OLF, 2010). Whilst the majority of that amount is made up of simple organic acids such as acetic acid, significant quantities of naphthenic acids (NA) are also present. Much of the available risk assessment information concerning NA comes from Canada where these compounds are released in large quantities to freshwater aquatic systems following steam extraction of hydrocarbons from tar sands. NA have consistently been shown to be toxic to a range of aquatic organisms in the freshwater environment, including fish, zooplankton and bacteria (Clemente and Fedorak, 2005).

Recently, NA were reported to represent as much as 65% of the ER agonist potency in North Sea PW, while also disrupting the binding of AR agonists to the AR ligand receptor. The likely overall effect of environmental AR antagonists on exposed male fish is their demasculinization through the inhibition of natural androgenic hormonal signalling. Blocking of the AR during sensitive windows of development would thus serve to disrupt the masculinizing action of androgens and lead to feminization. This may potentially act in concert with the feminization action of ER agonists (Thomas et al., 2009). Further laboratory studies are also required in order in order to assess the potential for effects of these compounds.

To date risk assessment of the feminising effects of PW has particularly focused on AP. It is strongly recommended that future WCM attempt to include measurement of exposure to the complex NA highly abundant in PW. It is also recommended that the feasibility of using PSDs to measure NA be tested, both in the laboratory and in the field. This is likely to represent less of an analytical challenge than measuring exposure

in biological samples, and then these cconcentrations can be used to infer effects based on laboratory studies.

9.9 *Mytilus* spp. And Atlantic cod "omics" approach

The combination of biochemical, histo-pathological and toxicogenomic data has been used as a valuable tool for the assessment of biological risk associated with pollutants within the Tamar River and Estuary (U.K.). The combination of biomarker and 'omics' data is therefore a valuable tool for the assessment of biological risk associated with pollutants in the marine environment. Analysis of gene expression profiles will be carried out using a cDNA microarray. This combination of analysis, i.e. (multivariate) statistics on gene expression profiles and the identification through Gene Ontology (www.geneontology.org) of putative biological processes occurring in mussel, can provide an effective and discriminating approach for assessing the health status of animals; complementing the information generated from the biochemical and histochemical biomarkers.

The microarray developed for mussel and cod is an extremely sensitive tool that could provide important information on the potential biological impact of the PW in the receiving water. The highly complex PW mixture can cause a wide range of biological effects in different target tissues. This method may help to highlight pathways of toxicity and effect that have yet to be considered as well as to confirm those that are being established.

Microarray technology is one possible approach to address "unknown" responses; other possible approaches are "omics", e.g. metabolomics and proteomics. A combination of several assays will probably provide the most useful data for evaluating possible long term effects.

9.10 Speciation of mussels

The mussel *Mytilus spp*. is used widely as a model organism in both coastal and offshore biomonitoring programmes including WCM. On the Norwegian coast it has been assumed for over 20 years that the mussel exist as a single species i.e. *M. edulis* (Gosling et al., 1992). However, recent research has confirmed the presence of three distinct mussel species, *M. edulis, M. galloprovincialis and M. trossulus* along the Norwegian coastline, with hybrid populations between the three species (Brooks and Farmen, manuscript in prep). Differences in the sensitivity of these mussel species to environmental contaminants are considered likely, although to what extent is currently unknown. Species differences in biomarker responses have potentially major impacts on national and international mussel monitoring programmes and work is underway to determine whether species differences do indeed exist. For future mussel biomonitoring programmes it is highly recommended that only one species of mussel is used (i.e. *Mytilus edulis* in the North Sea). To ensure this, mussels should be selected from a location known to contain a single species, which should be confirmed by DNA amplification and gel electrophoresis in a minimum sub-sample of 20 mussels.

9.11 Improved survey design and data interpretation

9.11.1 Flexibility in analysis design, 2-tier approach

In order to focus analysis effort on the most important sample groups, some flexibility in the analysis design is recommended. One way of achieving that could be the use of a 2-tier approach where final biomarker analysis design is determined based on the results of an initial screening. Selected samples from all groups can be analysed by a relevant low cost method capable of indicating general stress (tier-1). Based on the results from the screening, the best possible distribution of higher cost analyses (tier-2) among sample groups can be decided (reviewed by Viarengo et al. 2007). For mussels in the WCM, lysosomal membrane stability is a relevant candidate for screening.

9.11.2 Relation between exposure level and effect measures

The relationship between some effects measured by use of biomarkers and the corresponding exposure levels needed to cause the effects has been established in laboratory studies. In some cases the chemical approaches used to measure exposure to PW provides sensitivity that enables detection of the particular pollutants at far lower levels than what is needed to cause biological effects.

By focusing effort on the most sensitive parameter (often chemistry) better cost efficiency can be achieved. In fish AP metabolites can be quantified at considerably lower levels than what is needed to cause a Vtg response and PAH metabolites can be quantified at significantly lower levels than what is needed to cause Cyp 1a / EROD response. An analysis design focusing effort on the marker technique with the best sensitivity within each target mechanism will improve cost efficiency.

10 References

Aas E, Jonsson G, Sundt RC, Westerlund S, Sanni S (2006) Monitoring PAH metabolites in bile from caged Atlantic cod (*Gadus morhua*) and wild Pelagic fish along expexted contaminant gradients in the North Sea. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K., Vethaak A.D., Lang T. (Eds.) SETAC publications, pp 263-275.

Akcha F, Leday G, Pfohl-Leszkowicz A (2004) Measurement of DNA adducts and strand breaks in dab (*Limanda limanda*) collected in the field: effects of biotic (age, sex) and abiotic (sampling site and period) factors on the extent of DNA damage. Mutation research – Fundamental and molecular mechanisms. 552, 1-2: 197-207

Anon. (2008) Kostnader og nytte for miljø og samfunn ved å stille krav om injeksjon/reinjeksjon av produsert vann, nullutslipp av borekaks og borevæske og inkludere radioaktivitet i nullutslippsmålet. TA-2468/2008 - ISBN 978-82-7655-555-4

Balk L, Liewenborg B, Larsen BK, Aas E, Hylland K, Sanni S (2006) Large Hydrophobic adducts and strand breaks analysed in hepatic DNA from cod (Gadus morhua) caged at the Statfjord oilfield. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K, Vethaak AD, Lang T (Eds.) SETAC publications, pp 277-281.

Barron M G, Carls MG, Heintz R, Rice SD (2004) Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. Toxicological Sciences 78 (1):60-67.

Bechmann RK (1999) Effect of the endocrine disrupter nonylphenol on the marine copepod *Tisbe battagliai*. Science of the Total Environment 233, 1-3: 33-46.

Bechmann RK, Taban IC, Baussant T, Skadsheim A, Sanni S (2000) DREAM – Reproduction effects of a PAH mixture on sheepshead minnow (*Cyprinodon variegates*). Report AM-2000/015.

Beyer J, Tollefsen KE, Meier S, Sundt RC (manuscript) Low risk for alkylphenols in operational discharges of produced water from offshore oil fields to induce xenoestrogenic vitellogenin in feral fish (WCM-2010 WP11).

Bilbao E, Ibane A, Zaldibar B, Soto et al. (2006) Cell and tissue level biomarkers of pollution in Mussels (*Mytilus edulis*) and cod (*Gadus morhua*) caged along a contaminated gradient in Statfjord (North Sea). In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K., Vethaak A.D., Lang T. (Eds.) SETAC publications, pp 215-230.

Bilbao E., Soto M., Cajaraville M.P., Cancio I., Marigomez I. (2006) Cell- and tissue-level biomarkers of pollution in wild pelagic fish, herring (*Clupea harengus*) and saithe (*Pollachius virens*) from the North Sea. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K., Vethaak A.D., Lang T. (Eds.) SETAC publications, pp 121-142.

Boehm PD, Page DS, Brown JS, et al. (2005) Comparison of mussels and semi-permeable membrane devices as intertidal monitors of polycyclic aromatic hydrocarbons at oil spill sites. Marine Pollution Bulletin. 50, 7: 740-750.

Bohne-Kjersem A, Bache N, Meier S, et al. (2010) Biomarker candidate discovery in Atlantic cod (*Gadus morhua*) continuously exposed to North Sea produced water from egg to fry Aquatic Toxicology 96, 4: 280-289.

Bohne-Kjersem A, Skadsheim A, Goksoyr A, et al. (2009) Candidate biomarker discovery in plasma of juvenile cod (*Gadus morhua*) exposed to crude North Sea oil, alkyl phenols and polycyclic aromatic hydrocarbons (PAHs) Marine Environmental Research 68, 5: 268-277

Boitsov S, Mjøs SA, Meier S (2007) Identification of estrogen-like alkylphenols in produced water from offshore oil installations. Marine Environmental Research 64: 651-665.

Booij K, Smedes F, Van Weerlee EM, Honkoop PJC (2006) Environmental monitoring of hydrophobic organic contaminants: The case of mussels versus semipermeable membrane devices. Environ. Sci. Technol. 40, 12: 3898-3900.

Booth AM, Sutton PA, Lewis CA, Lewis AC, Scarlett A, Chau W, Widdows J, Rowland SJ (2007) Unresolved complex mixtures of aromatic hydrocarbons: Thousands of overlooked persistent, bioaccumulative, and toxic contaminants in mussels. Environmental Science & Technology 41: 457-464.

Brooks S, Farmen E (in prep) Distribution of *Mytilus* species on the Norwegian coast.

Brooks SJ, Harman C, Grung M, Farmen E, Ruus A, Vingen S, Godal BF, Baršienė J, Andreikėnaitė L, Skarphéðinsdóttir H, Liewenborg B, Sundt RC (2011) Water column monitoring of the biological effects of produced water from the Ekofisk offshore oil installation from 2006 to 2009. Journal of Toxicology and Environmental Health. 74: 582-604.

Burgeot T., Faucet J., Menard D., Grosjean P., Bocquene G. (2006) Variations of benzo(a)pyrene hydroxylase and acetylcholinesterase activities in mussels caged in the North Sea (German Bight and Statfjord). In “Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K., Vethaak A.D., Lang T. (Eds.) SETAC publications, pp 171-188.

Burns KA, Codi S, Furnas M, et al. (1999) Dispersion and fate of produced formation water constituents in an Australian Northwest Shelf shallow water ecosystem. Marine Pollution Bulletin. 38, 7: 593-603.

Calder PC (2008) The relationship between the fatty acid composition of immune cells and their function. Prostaglandins Leukotrienes and Essential Fatty Acids 79 (3-5): 101-108.

Caliani I, Porcelloni S, Mori G, Frenzilli G, Ferraro M, Marsili L, Casini S, Fossi MC (2009) Genotoxic effects of produced waters in mosquito fish (*Gambusia affinis*). Ecotoxicology 18 (1):75-80.

Canestro D, Raimondi PT, Reed DC, Schrnitt RJ, Holbrook SJ (1996) A study of methods and techniques for detecting ecological impacts. American Academy of Underwater Sciences (AAUS)

Cappiello A, Famiglini G, Palma P, et al. (2007) Application of nano-FIA-Direct-EI-MS to determine diethylene glycol in produced formation water discharges and seawater samples. Chemosphere. 69, 4: 554-560.

Carls MG, Short JW, Payne J (2006) Accumulation of polycyclic aromatic hydrocarbons by *Neocalanus* copepods in Port Valdez, Alaska. Marine Pollution Bulletin 52, 11: 1480-1489.

Chesman B.S., Burt G.R., Langston W.J. (2006) Hepatic metallothionein in pelagic herring (*Clupea harengus*). In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K., Vethaak A.D., Lang T. (Eds.) SETAC publications, pp 143-164.

Clemente JS, Fedorak PM (2005) A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. Chemosphere 60: 585-600.

Collier T, Connor SD, et al. (1992) "Using cytochrome P450 to monitor the aquatic environment: Initial results from regional and national surveys." Marine Environmental Research 34: 195-199.

Correa-Reyes G, Viana MT, Marquez-Rocha FJ, Licea AF, Ponce E, Vazquez-Duhalt R (2007) Nonylphenol algal bioaccumulation and its effect through the trophic chain. Chemosphere 68: 662-670.

Danischewski D, Kammann U, Volbach (2006) Neurotoxicity, genotoxicity and embryotoxicity tests with extracts from oil rig produced water and from SPMDs deployed along two North Sea transects. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K, Vethaak AD, Lang T (Eds.) SETAC publications, pp 377-382.

De Broyer C, Nyssen F, Dauby P (2004) The crustacean scavenger guild in Antarctic shelf, bathyal and abyssal communities. Deep-Sea Research II-Topical Studies in Oceanography 51, 1733–1752.

Dixon P.F., Longshaw C.B., Jones G.J., Stone D.M. (2006) Monitoring of viral haemorrhagic septicaemia virus and infectious salmon anaemia virus at contaminated and reference areas in the North Sea. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K., Vethaak A.D., Lang T. (Eds.) SETAC publications, pp 111-120.

Durell G, Utvik TR, Johnsen S, Frost T, Neff J (2006) Oil well produced water discharges to the North Sea. Part 1: Comparison of deployed mussels (*Mytilus edulis*), semi-permeable membrane devices, and the DREAM model predictions to estimate the dispersion of polycylic aromatic hydrocarbons. Marine Environmental Research 62: 194-223.

Elliot M, Hemmingway KL, et al. Eds (2003) From the individual to the population and community responses to pollution. Effects of pollution on fish. Molecular effects and population responses, Blackwell Publishing.

Farmen E, Harman C, Hylland K, Tollefsen KE (2010). Produced water extracts from North Sea oil production platforms result in cellular oxidative stress in a rainbow trout *in vitro* bioassay. Marine Pollution Bulletin 60: 1092-1098.

Feist SW, Bignell J, Stentiford BG (2006) Histological changes in caged mussels (*Mytilus* sp.) and cod (*Gadus morhua*) at contaminant gradients in the German Bight and the Statfjord offshore oil industry area in the North Sea. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K, Vethaak AD, Lang T (Eds.) SETAC publications, pp 311-323.

Frölin L, Hylland K (2006) Hepatic cytochrome P450 1A concentration and activity in Atlantic cod in two North Sea Pollution gradients. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K, Vethaak AD, Lang T (Eds.) SETAC publications, pp 253-260.

Gagnon MM (2010) Evidence of exposure of fish to PFW at three offshore facilities, North West Shelf, Australia. Petroleum Seminar Series, Curtin University.

Gamble JC, Davies JM, Hay SJ, Dow FK (1987) Mesocosm experiments on the effects of produced water discharges from offshore oil platforms in the northern north-sea. Sarsia 72: 383-386.

Gorbi S, Virno Lamberti C, Notti A, Benedetti M, Fattorini D, Moltedo G, Regoli F (2008) An ecotoxicological protocol with caged mussels, *Mytilus galloprovincialis*, for monitoring the impact of an offshore platform in the Adriatic Sea. Marine Environmental Research 65: 34-49.

Grøsvik BE, Meier S, Liewenborg B et al. (2009) Condition monitoring in the water column 2008: Oil hydrocarbons in fish from Norwegian waters. IMR report Nr.2-2009

Grøsvik BE, Meier S, Westereim K et al. (2007) Condition monitoring in the water column 2008: Oil hydrocarbons in fish from Norwegian waters. IMR report 2-2007

Grung M, Holth TF, Jacobsen MR, et al. (2009a) Polycyclic Aromatic Hydrocarbon (PAH) Metabolites in Atlantic Cod Exposed via Water or Diet to a Synthetic Produced Water Journal of Toxicology and Environmental Health A, 72, 3-4: 254-265.

Grung M, Ruus A, Holth TF, et al. (2009b) Bioaccumulation and lack of oxidative stress response in the rag worm *Hediste diversicolor* following exposure to Ra-226 in sediment. Journal of Environmental Radioactivity 100, 5: 429-434.

Guennegan Y, Martin V (1985) Techniques de pre`le`vements. In Laubier L. and Monniot C. (eds) Peuplements Profonds du Golfe de Gascone. IFREMER, Brest: IFREMER Publications, pp. 571–602.

Hannam M L, Bamber SD, Sundt RC, Galloway TS (2009) Immune modulation in the blue mussel *Mytilus edulis* exposed to North Sea produced water. Environmental Pollution. 157: 1939-1944.

Harman C, Brooks S, Sundt RC, Meier S, Grung M (2011) Field comparison of passive sampling and biological approaches for measuring exposure of PAH and alkylphenols from offshore produced water discharges. Marine Pollution Bulletin 63, 141-148.

Harman C, Farmen E, Tollefsen KE (2010) Monitoring North Sea oil production discharges using passive sampling devices coupled with in vitro bioassay techniques. Journal of Environmental Monitoring. 12, 9: 1699-1708.

Harman C, Holth TF, Hylland K, et al. (2009) Relationship between Polycyclic Aromatic Hydrocarbon (PAH) accumulation in Semipermeable Membrane Devices and PAH Bile Metabolite Levels in Atlantic Cod (*Gadus morhua*) Journal of Toxicology and Environmental Health part A 72, 3-4: 234-243.

Harman C, Tollefsen KE, Boyum O, et al. (2008) Uptake rates of alkylphenols, PAHs and carbazoles in semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (POCIS). Chemosphere. 72, 10: 1510-1516.

Hasselberg L, Meier S, Svardal A (2004a) Effects of alkylphenols on redox status in first spawning Atlantic cod (*Gadus morhua*). Aquatic Toxicology 69: 95-105.

Hasselberg L, Meier S, Svardal A, Hegelund T, Celander MC (2004b) Effects of alkylphenols on CYP1A and CYP3A expression in first spawning Atlantic cod (*Gadus morhua*). Aquatic Toxicology 67, 303-313.

Hecht SA, Gunnarsson JS, Boese BL, Lamberson JO, Schaffner C, Giger W, Jepson PC (2004) Influences of sedimentary organic matter quality on the bioaccumulation of 4-nonylphenol by estuarine amphipods. Environmental Toxicology and Chemistry 23: 865-873.

Holth TF, Beylich BA, Skarphedinsdottir H, et al. (2009) Genotoxicity of Environmentally Relevant Concentrations of Water-Soluble Oil Components in Cod (*Gadus morhua*) Environmental Science and Technology. 43, 9: 3329-3334.

Holth TF, Nourizadeh-Lillabadi R, Blaesbjerg M, Grung M, Holbech H, Petersen GI, Alestrom P, Hylland K (2008) Differential gene expression and biomarkers in zebrafish (*Danio rerio*) following exposure to produced water components. Aquatic toxicology 90: 277-291.

Holth TF, Thorsen A, Olsvik PA, Hylland K (2010) Long-term exposure of Atlantic cod (*Gadus morhua*) to components of produced water: condition, gonad maturation, and gene expression. Canadian Journal of Fisheries and Aquatic Sciences 67: 1685-1698.

Hu JY, Jin F, Wan Y, Yang M, An LH, An W, Tao S (2005) Trophodynamic behavior of 4-nonylphenol and nonylphenol polyethoxylate in a marine aquatic food web from Bohai Bay, North China: Comparison to DDTs. Environmental Science & Technology 39: 4801-4807.

Hurst MR, Chan-Man YL, Balaam J, et al. (2005) The stable aryl hydrocarbon receptor agonist potency of United Kingdom Continental Shelf (UKCS) offshore produced water effluents. Marine Pollution Bulletin 50, 12: 1694-1698

Hylland K, Lang T, Vethaak D (2006b): "Biological Effects of Contaminants in Marine Pelagic Ecosystems" - SETAC Press. 475 pp.

Hylland K, Serigstad B, Thain JE (2006a) *In situ* deployment of organisms and passive samplers during the BECPELAG workshop: Introduction and summary. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K., Vethaak A.D., Lang T. (Eds.) SETAC publications, pp 167-170.

Hylland K, Tollefsen KE, Ruus A, Jonsson G, Sundt RC, Sanni S, Røe Utvik TI, Johnsen S, Nilssen I, Pinturier L, Balk L, Baršienė J, Marigòmez I, Feist SW, Børseth JF (2008) Water column monitoring near oil installations in the North Sea 2001–2004. Marine Pollution Bulletin 56: 414–429.

ICES (2010): Report of the Joint ICES/OSPAR Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC), 25-29 January, Copenhagen, Denmark. ICES CM 2010/ACOM: 30. 211pp.

ICES/OSPAR (2009) Report of the joint ICES/ OSPAR study group on integrated monitoring contaminants and biological effects (SGIMC) ICES CM 2009:30.

Jones EG, Tselepides A, Bagley PM, Collins MA, Priede IG (2003) Bathymetric distribution of some benthic and benthopelagic species attracted to baited cameras and traps in the deep eastern Mediterranean. Marine Ecology Progress Series 251: 75–86.

Jonsson G, Bechmann RK, Bamber SD, Baussant T (2004) Bioconcentration, biotransformation, and elimination of polycyclic aromatic hydrocarbons in sheepshead minnows (*Cyprinodon variegatus*) exposed to contaminated seawater. Environtal Toxicology and Chemistry 23: 1538-1548.

King SC, Johnson JE, Haasch ML, et al. (2005) Summary results from a pilot study conducted around an oil production platform on the Northwest Shelf of Australia. Marine Pollution Bulletin. 50, 11: 1163-1172.

KLIF (2010) Klima- og forurensningsdirektoratets vurdering av måloppnåelse for Nullutslippsarbeidet. Petroleumsvirksomhetens arbeid med nullutslipp. TA2637/ 2010.

Klungsøyr J, Balk L, Berntsen MHG et al (2003) NFR project no. 152231/720. Contamination offish in the North Sea by the offshore oil and gas industry. Summary report to NFR.

Landahl JT, McCain BB, Myeres et al. (1990) Consistent associations between hepatic-lesions in English sole (*Parophrys vetulus*) and PAH in the bottom sediment. Environmental Health Perspectives. 89: 195-203.

Li J, Ma M, Cui Q, Wang Z (2008) Assessing the potential risk of oil-field produced waters using a battery of bioassays/ Biomarkers. Bulletin of Environmental contamination and toxicology. 80: 492-496.

Lie KK, Meier S, Olsvik PA (2009) Effects of environmental relevant doses of pollutants from offshore oil production on Atlantic cod (*Gadus morhua*). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 150: 141-149.

Luellen D, Shea D (2003) Semipermeable membrane devices accumulate conserved ratios of sterane and hopane petroleum biomarkers. Chemosphere 53: 705-713.

Lye CM (2000) Impact of estrogenic substances from oil production at sea. Toxicology Letters. 112: 265-272.

Mackay D, Fraser A (2000) Bioaccumulation of persistent organic chemicals: mechanisms and models. Environmental pollution 110: 375-391.

Mazzella N, Molinet J, Syakti AD, Barriol A, Dodi A, Bertrand JC, Doumenq P (2005a) Effects of pure n-alkanes and crude oil on bacterial phospholipid classes and molecular species determined by electrospray ionization mass spectrometry. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 822 (1-2): 40-53.

Mazzella N, Syakti AD, Molinet J, Gilewicz M, Doumenq P, Artaud J, Bertrand JC (2005b) Effects of crude oil on phospholipid fatty acid compositions of marine hydrocarbon degraders: estimation of the bacterial membrane fluidity. *Environmental Research* 97 (3):300-311.

McCormack P, Jones P, Hetheridge MJ, Rowland SJ (2001) Analysis of oilfield produced waters and production chemicals by electrospray ionisation multi-stage mass spectrometry (ESI-MSn). Water reasarch 35: 3567-3578.

McIntosh AD, Hall CD, Davies IM (2006) Measurements of hydrocarbon concentration in the water column in two areas of the North Sea by continuous fluorescence. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K, Vethaak AD, Lang T (Eds.) SETAC publications, pp 43-50

Meier S, Andersen TC, Lind-Larsen K, Svardal A, Holmsen H (2007c) Effects of alkylphenols on glycerophospholipids and cholesterol in liver and brain from female Atlantic cod (*Gadus morhua*). Comparative Biochemistry and Physiology Part C 145: 420-430.

Meier S, Andersen TE et al. (2002) Hormonal effects of C_4-C_7 alkylated phenols in cod. Bergen, Norway, Institute for Marine Sciences (IMS): 70

Meier S, Andersen TE, Norberg B, Taranger GL, Thorsen A, Kjesbu OS, Klungsøyr J, Svardal A (2007a). Effects of alkylphenols (4-*tert*-butyl-, 4-*n*-pentyl-, 4-*n*-hexyl- and 4-*n*-heptylphenol) on the reproductive system of Atlantic cod (*Gadus morhua*). Manuscript in Meier S (2007) Effects of alkylphenols (C_4-C_7) on the reproductive system of Atlantic cod (*Gadus morhua*). PhD. thesis, University of Bergen, Norway, (https://bora.uib.no/handle/1956/2326)

Meier S, Andersen TE, Norberg B, Thorsen A, Taranger GL, Kjesbu OS, Dahle R, Morton HC, Klungsøyr J, Svardal A (2007b). Effects of alkylphenols on the reproductive system of Atlantic cod (*Gadus morhua*). Aquatic toxicology 81: 207-218.

Meier S, Morton HC, Andersson E, Geffen A, Taranger GL, Larsen M, Pedersen M, Djurhuus R, Klungsøyr J, Svardal A (2011) Low-dose exposure to alkylphenols adversely effects the sexual development of Atlantic cod (*Gadus Morhua*): Acceleration

of the onset of puberty and delayed seasonal gonad development in mature female cod. Aquatic toxicology 105, 136-150.

Meier S, Morton HC, Nyhammer G, et al. (2010) Development of Atlantic cod (*Gadus morhua*) exposed to produced water during early life stages. Effects on embryos, larvae, and juvenile fish Marine Environmental Research 70, 5: 383-394.

Melbye AG, Brakstad OG, Hokstad JN, Gregersen IK, Hansen BH, Booth AM, Rowland SJ, Tollefsen KE (2009) Chemical and toxicological characterization of an unresolved complex mixture-rich biodegraded crude oil. Environmental Toxicology and Chemistry 28: 1815-1824.

Mortensen PB, Roberts JM, Sundt RC (2000) Video-assisted grabbing: a minimally destructive method of sampling live deep-water corals. Journal of the Marine Biological Association 80: 365-366.

Nagara GA (2009) DREAM (Dose-related Risk and Exposure Assessment Model) as a tool to predict biomarker responses in offshore produced water discharge situations - Master's thesis, Faculty of Science and technology, University of Stavanger. 61+8 pp.

Neff JM (2002) Bioaccumulation in marine organisms. Effect of contaminants from oil well produced water. Oxford, Elsevier Science Ltd.

Neff JM (2009) What are harmful levels of produced water discharges in the Arctic? Lecture 3 October 28 2009, Bioaccumulation and Effects of Produced Water Compounds in Marine Organisms.

Neff JM, Johnsen S, Frost TK, Røe Utvik TI, Durell GS (2006) Oil well produced water discharges to the North Sea. Part II: comparison of deployed mussels (*Mytilus edulis*) and the DREAM model to predict risk assessment. Marine Environmental Research 62: 224-246.

Neff JM, Ostazeski S, Gardiner W, Stejskal I (2000). Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel fuel to marine animals. Environmental Toxicology and Chemistry 19: 1809-1821.

OLF (2010) Environmental Report 2009. The Norwegian Oil Industry Association (OLF), Stavanger, Norway, 60 pp.

Olsvik PA, Lie KK, Stavrum AK, Meier S (2007) Gene-expression profiling in gill and liver of zebrafish exposed to produced water. International Journal of Environmental Analytical Chemistry 87: 195-210.

Olsvik PA, Waagbø R, Pedersen, SA, Meier S (2011) Transcriptional effects of dietary exposure of oil-contaminated *Calanus Finmarchicus* in Atlantic herring (*Clupea Harengus*). Journal of Toxicology and Environmental Health 74 (7-9), 508-528.

Osenberg CW, Schmitt RJ, Holbrook SJ, Canestro D (1992) Spatial scale of ecological effects associated with an open coast discharge of produced water. In: Ray JP, Engelhardt FR (Eds.), Produced Water: Technological/Environmental Issues and Solutions. Plenum Press, New York, pp. 387–402.

Peakall DB (1992) A practical basis for using physiological indicators in environmental assessment. Ambio. 21, 6: 437-438.

Perez-Casanova JC, Hamoutene D, Samuelson S, Burt K, King TL, Lee K (2010) The immune response of juvenile Atlantic cod (*Gadus morhua* L.) to chronic exposure to produce water. Marine Environmental Research 70: 26-34.

Regoli F, Frulla S, Aas E, Sanni S (2006) Antioxidant parameters and total oxyrarical scavenging capacity in mussels from the German Bigth and the Statfjord oilfield. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K, Vethaak AD, Lang T (Eds.) SETAC publications, pp 341- 348.

Røe TI, Johnsen S (1996b) Discharges of produced water to the North Sea; Effects in the water column. Produced water 2. Environmental Issues and Mitigation Technologies. S. Johnsen. New York, Plenum Press: 13-25.

Røe Utvik T. And Gärtner (2006) Concentration of polycyclic aromatic hydrocarbons in seawater: comparison of results from dispersion modelling with measured data from blue mussels (*Mytilus edulis*) and SPMD residues. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K, Vethaak AD, Lang T (Eds.) SETAC publications, pp 29-42.

Røe Utvik TI (1999) Chemical characterisation of produced water from four offshore oil production platforms in he North Sea. Chemosphere 39, 15: 2593-2606.

Røe Utvik TI, Durell GS, Johnsen S (1999a) Determining produced water originating polycyclic aromatic hydrocarbons n North Sea waters: comparison of sampling techniques. Marine Pollution Bulletin 38, 11: 977-989.

Rowland S, Donkin P, Smith E, Wraige E (2001) Aromatic hydrocarbon "humps" in the marine environment: Unrecognized toxins? Environmental Science & Technology 35: 2640-2644.

Rundle A, Madsen A, Orjuela M, et al. (2007) The association between benzo[a]pyrene-DNA adducts and body mass index, calorie intake and physical activity. Biomarkers 12, 2: 123-132

Ruus A, Tollefsen KE, Grung M, Klungsøyr J, Hylland K (2006) Accumulation of contaminantions in pelagic organisms, caged blue mussels, caged cod and semi-permeable membrane device (SPMDs). In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K., Vethaak A.D., Lang T. (Eds.) SETAC publications, pp 51-74.

Sanni S, Ravagnan E, Nagara GA, Lyng E, Sundt RC (in prep) Assessment criteria for biomarkers applied to monitoring of oil based discharges. WCM 2010 WP10.

Scott AP, Kristiansen SI, Katsiadaki I, Thain J, Tollefsen KE, Goksøyr A, Barry J (2006) Assessment of oestrogen exposure in cod (*Gadus morhua*) and saithe (Pollachius virens) in relation to their proximity to an oilfield. In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K, Vethaak AD, Lang T. (Eds.) SETAC publications, pp 329-339.

Servos MR (1999) Review of the aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. Water Quality Research Journal of Canada 34: 123-177.

Shiu W Y, Ma KC, Varhanickova D, Mackay D (1994) Chlorophenols and alkylphenols: A rewiew and correlation of environmentally relevant properties and fate in an evaluative environment. Chemosphere 29 (6): 1155-1224.

Smolders R, Cooreman K, Roose P, Blust R, De Coen W (2006) Cellular energy allocation in caged and pelagic free-living organisms along two North Sea Pollution gradients.In "Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K, Vethaak AD, Lang T. (Eds.) SETAC publications, pp 235-247.

Stagg RM, Mcintosh A, Mackie P (1995) Elevation of hetatic monooxygenase activity in the dap (*Limanda Limanda*) in relation to environmental contamination with petroleum-hydrocarbons in the northern North Sea. Aquatic Toxicology. 33, 3-4: 245-264.

Stefania G, Maura B, Claudia VL, et al. (2009) Biological effects of diethylene glycol (DEG) and produced waters (PWs) released from offshore activities: A multi-biomarker approach with the sea bass *Dicentrarchus labrax*. Environmental Pollution. 157, 11: 3166-3173.

Stephens SM, Frankling SC, Stagg RM, Brown JA (2000) Sub-lethal effects of exposure of juvenile turbot to oil produced water. Marine Pollution Bulletin 40: 928-937.

Sundt RC, Aarab N, Lyng E, Godal BF, Pampanin DM (2011a) Histopathological alterations in gills of Atlantic cod (*Gadus morhua* L.) exposed to North Sea produced water. IRIS report (WCM-2010, WP 15)

Sundt RC, Baussant T, Beyer J (2009c) Upptake and tissue distribution of C_4-C_7 alkylphenols in Atlantic cod (*Gadus morhua*): relevance for biomonitoring of produced water discharges from oil production. Marine Pollution Bulletin. 58: 72-79

Sundt RC, Beyer J, Vingen S, Sydnes MO (2011b) High matrix interference affecting detection of PAH metabolites in bile of Atlantic hagfish (*Myxine glutinosa*) used for biomonitoring of deep-water oil production.

Sundt RC, Björkblom C (2011) Effects of produced water discharges on the reproductive system of pre-spawning Atlantic cod (*Gadus morhua*) Journal of Toxicology and Environmental Health 74 (7-9): 543-554.

Sundt RC, Børseth JF, Myhre LP (2009a) A simple acoustic release trap system for live sampling of deep-water organisms. Journal of the Marine Biological Association of the UK Marine, Biodiversity Records 2: e65; S1755267209000396; Published online.

Sundt RC, Meier S, Jonsson G, Sanni S, Beyer J (2009b) Development of a laboratory exposure system using marine fish to carry out realistic effect studies with produced water discharged from offshore oil production. Marine Pollution Bulletin 58: 1382-1388.

Sundt RC, Pampanin DM, Grung M, Baršienė J, Ruus A (2011c) PAH body burden and biomarker responses in mussels (*Mytilus edulis*) exposed to Produced Water from a

North Sea oil field: laboratory and field assessments. Marine Pollution Bulletin (WCM 2010, WP6)

Sundt RC, Pampanin DM, Larsen BK, Brede C, Herzke D, Bjørnstad A, Andersen OK (2006) The BEEP Stavanger Workshop: Mesocosm exposures. Aquatic Toxicology 78: 5-12

Sundt RC, Ruus A, Jonsson H, Skarphéðinsdóttir H, Meier S, Grung M, Pampanin DM, Beyer J (2012) Biomarker responses in Atlantic cod (*Gadus morhua*) exposed to Produced Water from a North Sea oil field: laboratory and field assessments. Marine Pollution Bulletin (WCM 2010, WP5)

Takahashi A, Higashitani T, Yakou Y, Saitou M, Tamamoto H, Tanaka H (2003) Evaluating bioaccumulation of suspected endocrine disruptors into periphytons and benthos in the Tama River. Water Science Technology 47: 71-76.

Thomas K, Hurst MR, et al. (2006) *In vitro* bioassay testing of produced and surface water extracts. Pp 357-366. In "ICES workshop on biological effects of contaminants in the pelagic ecosystem (ICES)" Eds: Hylland K, Vethaak AD, Lang T. SETAC special publications.

Thomas KV, Balaam J, Hurst MR, Thain JE (2004b) Identification of *in vitro* estrogen and androgen receptor agonists in North Sea offshore produced water discharges Environ. Toxicol. Chem./SETAC 23: 1156–1163.

Thomas KV, Balaam JB, Hurst MR, Thain JE (2004a) Bio-analytical and chemical characterization of offshore produced water effluents for estrogen receptor (ER) agonists Journal of Environmental Monitoring. 6: 593–598.

Thomas KV, Langford K, Petersen K, Smith AJ, Tollefsen KE (2009) Effect-directed identification of naphthenic acids as important *in vitro* xeno-estrogens and anti-androgens in North Sea offshore produced water discharges. Environmental Science & Technology 43: 8066-8071.

Tollefsen KE, Finne EF, et al. (2006 b). Effluents from oil production activities contain chemicals that interfere with normal function of intra- and extra-cellular estrogen binding proteins. Marine Environmental Research. 62: S191-S194.

Tollefsen KE, Goksøyr A, et al. (2006 a) The use of a fish in vitro bioassay for monitoring of pollutants in the North Sea. In "ICES workshop on biological effects of contaminants in the pelagic ecosystem (ICES)" Eds: Hylland K, Vethaak AD, Lang T. SETAC special publications.

Tollefsen KE, Sundt RC, Beyer J, Meier S, Hylland K (2011) Endocrine modulation in Atlantic cod (*Gadus morhua* L.) exposed to alkylphenols, PAHs, produced water and dispersed oil. Journal of Toxicology and Environmental Health. (in press)

Trefry JH, Naito KL, Trocine RP et al. (1995) Distribution and bioaccumulation of heavy metals from produced water discharges to the Gulf of Mexico. Water Science and Technology 32, 2: 31-36

Vethaak A.D., Brandsma S.H., Kruijt A.W., Leonards P. (2006) Occurence of brominated flame –retardants in zooplankton and pelagic fish in the North Sea. In “Biological effects of contaminants in marine pelagic ecosystems (ICES) Hylland K., Vethaak A.D., Lang T. (Eds.) SETAC publications, pp 75-82.

Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007) The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comparative Biochemistry and Physiology C: Toxicology & Pharmacology 146, 3: 281-300.

Viarengo A, Ponzano E, Dondero F, et al. (1997) A simple spectrophotometric method for metallothionein evaluation in marine organisms: An application to Mediterranean and Antarctic mollusks. Marine Environmental Research. 44, 1: 69-84.

Winston GW, Means JC (1994) Bioavailability and Genotoxicity of Produced Water. Discharges Associated with Offshore Drilling Operations. Afinal report by the Lousiana Universities Marine Consortium for the U.S. Department of Interion, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, Lousiana. OCS Study MMS 95-0020. 124 pp.

Zhu SQ, King SC, Haasch ML (2008) Biomarker induction in tropical fish species on the Northwest Shelf of Australia by produced formation water. Marine Environmental Research 65, 4: 315-324.

11 Appendix

11.1 Publication work WCM 2010 (WP 2-12)

Work package 2-12 in the WCM 2010 were publication processes. Current status on the publication prosesses is given in Table 11. More detailed information (titles, authors, additional dissemination and abstracts) are also listed below.

Table 13. Current status on the publication prosesses (WP 2-12) per April 2011. NETS: Norwegian Environmental Toxicology Symposium 2010; ICES: International Council for the Exploration of the Sea, Annual Science Conference 2010;

WP	WP short name	Responsible contribution	Status	Target journal
2	IMR, PW – cod reproduction	IMR	Submitted	Aquatic Toxicology
3	Endocrine disruption	NIVA, IRIS, IMR	In press	Journal of Toxicology and Environmental Health
4	Effects Atlantic cod	IRIS	In press	Journal of Toxicology and Environmental Health
5	Biomarker validation cod	IRIS, NIVA	Submitted	Marine Pollution Bulletin
6	Biomarker validation mussel	IRIS, NIVA	In press	Marine Pollution Bulletin
7	WCM 2006-2009	NIVA, IRIS	In press	Journal of Toxicology and Environmental Health
8	Condition monitoring	IMR	Manuscript	Marine Pollution Bulletin
9	Val. exp. Markers	NIVA, IRIS	In press	Marine Pollution Bulletin
10	Assessment criteria	IRIS	Manuscript	Marine Pollution Bulletin
11	Exp levels APs	IRIS, NIVA, IMR	Manuscript	Marine Pollution Bulletin
12	Risk assessment APs	IRIS	Manuscript	Marine Environmental Research

WP 2:

Sonnich Meier, H. Craig Morton, Eva Andersson, Audrey Geffen, Geir Lasse Taranger, Marita Larsen, Marianne Pedersen, Rune Djurhuus, Jarle Klungsøyr, Asbjørn Svardal

Low-dose exposure to alkylphenols adversely effects the sexual development of Atlantic cod (Gadus Morhua): Acceleration of the onset of puberty and delaying seasonal gonad development in mature female cod.

Submitted to Aquatic Toxicology

Abstract. Produced water (PW), a by-product of the oil-production process, contains large amount of alkylphenols (APs) and other harmful oil compounds. In the last 20 years, there have been increasing concerns regarding the environmental impact of large increases in the amounts of PW released into the North Sea. We have previously shown that low levels of APs can induce disruption of the endocrine and reproductive systems of Atlantic cod (Gadus morhua). The aims of this follow-up study were to: (i) identify the lowest observable effect concentration of APs; (ii) study the effects of exposure to real PW, obtained from a North Sea oil-production platform; and (iii) study the biological mechanism of endocrine disruption in female cod. Fish were fed with feed paste containing several concentrations of four different APs (4-tert-butylphenol, 4-n-pentylphenol, 4-n-hexylphenol and 4-n-heptylphenol) or real PW for 20 weeks throughout vitellogenisis. AP and PW-exposed fish were compared to unexposed fish and to fish fed paste containing the natural estrogen (17β-estradiol, E2). The cod population in the experiment consisted of both male and female fish. Approximately 60% of the females in the unexposed groups were mature at the end of the experiment, while 40% remained juveniles. 98% of the male fish were mature at the end of the experiment. Our results show that exposure to APs and E2 have different effects depending on the developmental stage of the fish. We observed that juvenile females are “pushed” into puberty and maturation, while gonad development was delayed in mature females and males. The AP-exposed groups contained increased numbers of mature females, and significant differences between the untreated group and the AP-treated groups were seen down to a dose of 4 µg AP/kg body weight. In the high-dose AP and the E2 exposed groups, all females matured and no juveniles were seen. These results suggest that AP-exposure can affect the timing of the onset of puberty in fish even at extremely low concentrations. AP-exposure also impaired oocyte development and reduced estrogen levels in the adult females. Importantly, similar effects were not seen in the fish that were exposed to real PW. Therefore, it is unlikely that the PW discharged into the North Sea has severe endocrine disrupting effects on the wild cod population.

WP 3:

K.-E. Tollefsen, Rolf C. Sundt, Jonny Beyer, Sonnich Meier, Ketil Hylland (2011)

Endocrine modulation in Atlantic cod (*Gadus morhua* L.) exposed to alkylphenols, PAHs, produced water and dispersed oil.

Journal of Toxicology and Environmental Health (*in press*)

Abstract. Effluent from oil production activities contain chemicals that are suspected of causing endocrine disruption in fish. In the present work, Atlantic cod (Gadus morhua L.) were exposed to mixtures of low and medium molecular weight alkylphenols (methyl- to heptylphenol), PAHs, diluted produced water and dispersed oil for 15 days in a flow-through exposure system. Condition index (CI), hepatosomaric index (HSI), gonadosomatic index (GSI), induction of the estrogenic biomarker vitellogenin (Vtg) and modulation of the total sex steroid-binding capacity in plasma were determined to assess whether these mixtures were capable of interfering with endocrine regulated physiological processes in fish. The results document that diluted produced water and low molecular alkylphenols were able to act weakly estrogenic by inducing the estrogenic biomarker vitellogenin in male Atlantic cod. Fish exposed to a mixture of dispersed oil and a mixture of alkylphenols, PAHs and dispersed oil led to up-regulation of the total sex steroid-binding capacity in blood of Atlantic cod. No effects were seen on CI, HSI, and GSI, thus suggesting that the endocrine disrupting potential was too low to elicit effects on general physiological conditions and gonad development in this short test period. The results suggest that complex mixtures of oil-related components are able to act through multiple mechanisms of action to potentially cause endocrine disruption in Atlantic cod.

Additional dissemination: Presentation, NETS 2010 - Norwegian Environmental Toxicology Symposium

WP 4:

Rolf C. Sundt, Carina Björkblom (2011)

Effects of produced water on reproductive parameters in pre-spawning Atlantic cod (*Gadus morhua*)

Journal of Toxicology and Environmental Health (*in press*)

Abstract: Produced water (PW) discharged from offshore oil industry activities contains substances that are known to contribute to a range of mechanisms of toxicity. In the present study selected reproductive biomarkers were studied in pre-spawning Atlantic cod (Gadus morhua) exposed to PW. The fish were exposed for twelve weeks within a continuous flow-through system at realistic environmental near field concentrations.

Concentrations of polyaromatic hydrocarbon (PAH) and alkylphenol (AP) compounds were analysed by gas chromatography with mass spectrometric detection measurement, as were PAH and AP metabolites in fish bile for verification of exposure conditions and presence of the compounds in the PW. A suite of reproductive biomarkers (vitellogenin, zona radiata protein and plasma steroid concentrations) and histological alterations of the gonads were evaluated. The results showed that exposure to sufficiently high levels of PW caused an increase in vitellogenin levels in female fish compared to control fish. Impaired oocyte development and reduced estrogen levels were also observed in PW exposed female fish. In male fish the testicular development was altered, showing an increase in the amount of spermatogonia and primary spermatocytes and a reduction in the amount of mature sperm in the PW exposed fish compared to control fish. The results indicate that sufficiently high levels of PW have the potential to adversely affect the reproductive fitness of cod.

Additional dissemination: Poster, NETS 2010 - Norwegian Environmental Toxicology Symposium

WP 5:

Rolf C. Sundt, Anders Ruus, Henrik Jonsson, Halldóra Skarphéðinsdóttir, Sonnich Meier, Merete Grung, Daniela M. Pampanin

Biomarker responses in Atlantic cod (*Gadus morhua*) exposed to Produced Water from a North Sea oil field: laboratory and field assessments.

Marine Pollution Bulletin, submitted

Abstract. Biological markers of produced water (PW) exposure were studied in Atlantic cod (Gadus morhua) in both laboratory and field experiments, using authentic PW from a North Sea oil field. In the laboratory study, the PW exposure yielded significant elevated levels of metabolites of polycyclic aromatic hydrocarbons (PAHs) and alkylphenols (APs) in bile already at the lowest exposure dose (0.125 % PW). Other biomarkers (hepatic CYP1A induction and DNA adduct formation) responded at 0.25 % and 0.5 % PW concentrations. In the field study, bile metabolite markers and hepatic CYP1A were clearly increased in fish caged close to the PW outfall. Induction of plasma vitellogenin was not found in laboratory or field exposures, suggesting that the levels of estrogen agonists (such as APs) might not have been sufficient to elicit induction, under the present conditions. The applicability of the biomarkers for use in water column biomonitoring programs is indicated.

WP 6:

Rolf C. Sundt, Daniela M. Pampanin, Merete Grung, Janina Baršienė, Anders Ruus

PAH body burden and biomarker responses in mussels (*Mytilus edulis*) exposed to Produced Water from a North Sea oil field: laboratory and field assessments.

Marine Pollution Bulletin, in press

Abstract: In order to study the impact of produced water (PW) from a North Sea oil field on blue mussels (*Mytilus edulis*), chemical and biological markers were selected. A laboratory exposure (0.125%, 0.25% and 0.5% of PW) and a field study (6 stations 0.2–2 km from a PW discharge point) were conducted. In the laboratory study, PAH bioaccumulation increased in mussel soft tissue even at the lowest exposure dose. Micronuclei frequency demonstrated a dose-response pattern, whereas lysosomal membrane stability showed tendency towards a dose-response pattern. The same markers were assessed in the field study, biomarker analyses were consistent with the contamination level, as evaluated by mussel polycyclic aromatic hydrocarbons body burden. Overall, obtained results confirmed the value of an ecotoxicological approach for a scientifically sound characterization of biological effects induced by offshore oilfield operational discharges.

WP 7:

Steven Brooks, Christopher Harman, Merete Grung, Eivind Farmen, Anders Russ, Sjur Vingen, Brit F. Godal, Janina Baršienė, Laura Andreikenaite, Halldóra Skarphéðinsdóttir, Lennart Balk, Birgitta Liewenborg, Rolf C Sundt. (2011)

Monitoring the biological effects of produced water from an offshore oil installation

Journal of Toxicology and Environmental Health (*in press*)

Abstract. The Norwegian water column monitoring programme investigates the biological effects of offshore oil and gas activities in Norwegian waters. In three separate surveys in 2006, 2008 and 2009 bioaccumulation and biomarker responses were measured in mussels and fish held in cages at known distances from the produced water discharge at the Ekofisk oil field. Identical monitoring studies performed in all three years has allowed the biological effects and bioaccumulation data to be compared, and in addition, has enabled the potential environmental benefits of a produced water treatment system (C-Tour), implemented in 2008, to be evaluated. The results of the 2009 survey have shown that caged animals were exposed to low levels of produced

water components with highest tissue concentrations in mussels Mytilus edulis, located closest to the produced water discharge. Mussels located approximately 1-2 km away showed only background concentrations of the organic compounds measured. Concentrations of polycyclic aromatic hydrocarbon (PAH) and alkyl phenol (AP)-metabolites in bile of caged cod Gadus morhua, were slightly elevated suggesting exposure at the Ekofisk stations 200-250 metres from the discharge. There was also a signal of exposure with proximity to the discharge for the biomarkers CYP1A in fish and micronuclei in mussels. All other fish and mussel biomarkers showed no significant exposure effects in 2009. The bioaccumulation data and biomarker responses in 2009 indicated a lower exposure to the produced water effluent than seen previously in 2008 and 2006, resulting in a general improvement in the health of the caged mussels and fish in 2009.

Additional dissemination: Presentation, NETS 2010 - Norwegian Environmental Toxicology Symposium

WP 8:

Bjørn Einar Grøsvik, Sonnich Meier, Birgitta Liewenborg, Guri Nesje, Kjell Westrheim, Merete Fonn, Olav S. Kjesbu, Halldóra Skarphéðinsdóttir and Jarle Klungsøyr

Condition monitoring in the water column 2005 and 2008: Oil hydrocarbons in fish from Norwegian waters and biomarker responses

To be submitted to Marine Pollution Bulletin

Abstract: Fish were caught from open seas from four areas during summer and autumn 2005 and 2008: The Egersund Bank (reference area North Sea), Tampen, the Halten Bank and the Barents Sea (reference area). Haddock, cod and saithe were sampled from all areas, while long rough dab were only sampled from the Barents Sea and the Egersund Bank in 2008. In 2008 more analyses were preformed on haddock, to follow up results obtained from the condition monitoring in 2005. The objectives were to investigate whether fish from Norwegian seas contain elevated levels of components that originate from discharges from the petroleum activity.

Di- and polyaromatic hydrocarbons (NPD/PAH) measured in fish muscle in 2005 were found to be below LOQ for all regions. In 2008 Sum NPD were measured in haddock liver and found to be low for all regions with mean levels of 15.3±7 ng/g at the Egersund Bank, 34±12 ng/g at Tampen, 10.5±13.3 ng/g at the Halten Bank and 7.8±5.9 ng/g in the Barents Sea. Bile metabolites from haddock were measured by GC MS in 2008. The main contributor to sum PAH metabolites at Tampen and at the Egersund Bank was 1-hydroxy phenanthrene with levels of 510±814 and 133±207 ng/g bile,

respectively. Levels of this metabolite in haddock from the Halten Bank and the Barents Sea were 43±71 and 19±14 ng/g bile, respectively. Only low levels of PAH metabolites in bile were measured in saithe and the levels were comparable between the areas. PAH metabolites in long rough dab were only measured in fish from the Barents Sea, and found to be low. Levels of alkylphenols in cod and haddock liver, as well as in herring muscle were found below LOD in 2005. Follow up on 2008 alkylphenol metabolites in bile in 2008 also showed levels below LOQ. Levels of Vtg in blood of male cod were generally low from all regions.

Measurements of DNA adducts in fish liver in 2005 did not show changes for cod and saithe, while a significant increase were observed in haddock from Tampen compared to haddock from the Egersund Bank. Such significantly increase in DNA adducts in liver of haddock from Tampen were also measured in 2008, compared with haddock from the Egersund Bank and the Barents.

Analyses of lipid content in the liver showed also significantly reduction in haddock from Tampen. Fatty acid profiles showed that haddock from Tampen had relatively high levels of arachidonic acid (20:4 (n-6)), and the ratio between omega-3 and omega-6 (n-3)/(n-6) poly unsaturated fatty acids were significantly lower in neutral lipids, free fatty acids and phosphotidylcholine/phosphotidylethanolamine, but not phosphotidylserine/ phosphotidylinositol compared with haddock from the other regions.

For cod and saithe, we did not see significant effects of oil compound discharges, but haddock from the Tampen region seemed to be affected by such exposures, although more work is needed to establish whether the reduced (n-3)/(n-6) ratio at Tampen is influenced by diet or oil pollutants.

Additional dissemination:

- NETS 2010 - Norwegian Environmental Toxicology Symposium, Presentation.

- ICES Annual Science Conference, 20-24 September 2010, Nantes, France. Session F: Monitoring biological effects and contaminants in the marine environment: where do we go from here?

WP 9

Christopher Harman, Steven Brooks, Rolf C Sundt, Sonnich Meier, and Merete Grung (2011)

Field comparison of passive sampling and biological approaches for measuring exposure of PAH and alkylphenols from offshore produced water discharges.

Marine Pollution Bulletin (*in press*)

Abstract: Organic constituents such as polycyclic aromatic hydrocarbons (PAH) and alkylphenols (AP) that are present in large and increasing routine discharges of produced water (PW) from the offshore oil and gas industry in the North Sea continue to cause concern. An analysis of the suitability of biological and chemical based passive sampling devices (PSDs) to determine exposure to these compounds was carried out in situ. PSDs, Atlantic cod (*Gadus morhua*) and blue mussels (*Mytilus edulis*) were deployed in cages around an oil installation and at reference sites in the North Sea, for a period of six weeks. The most relevant PAH and AP were analysed either as parent compounds in passive samplers and biota or as metabolites in fish bile. Generally speaking the pattern of exposure relative to discharge and compared to the reference sites was represented by mussels, SPMDs and fish for PAH. Fish and SPMDs showed good correlation in accumulations, whereas some differences were apparent between mussels and SPMDs. Whilst metabolites in bile indicated that fish had been exposed to AP, the polar organic chemical integrative sampler (POCIS) was the only technique tested that could adequately describe exposure to those most abundant in PW. In a subsequent survey an apparent increase in water concentrations of selected compounds based on PSD accumulations, appeared to be poorly represented in biota. Further advantages in also having a biologically independent measure of exposure means that passive sampling techniques in general are recommended for inclusion in similar discharge monitoring studies.

Additional dissemination: Presentation, 15th International Symposium on Toxicity Assessment Hong Kong

WP 10:

Steinar Sanni, Elisa Ravagnan, Genia Atma Nagara, Emily Lyng, Rolf C. Sundt (manuscript)

Assessment criteria for biomarkers applied to monitoring of oil based discharges.

Abstract. Biomarkers of contamination are increasingly used in monitoring of biological effects of contaminants in the marine environment, for instance in the OSPAR, HELCOM and MED POL European marine management regions. On the Norwegian

Continental Shelf (NCS), biomarkers have been in mandatory use for monitoring effects of produced water discharges. The selected biomarker techniques for the monitoring programmes in the mentioned regions have been tested under controlled laboratory conditions and validated for field use, however criteria for assessment based on these biomarkers have so far been a shortage. Recently a study group initiated by OSPAR and ICES (SGIMC) have developed assessment criteria for the most important biomarkers in use in the North East Atlantic region.

The SGIMC group established two different kinds of assessment criteria – one called Background Assessment Criteria (BAC) and the other called Environmental Assessment Criteria (EAC). The BAC is based solely on field data and consists of threshold values that correspond to a certain deviation of biomarker signals from background levels. The BAC thresholds do not necessarily correspond to thresholds for adverse biological effects, and therefore the EAC has also been defined, based on toxicological data from laboratory experiments. In an evaluation of the SGIMC work in 2010 it is recommended to establish a more extensive set of EAC thresholds. The amount of published data available to establish EAC threshold values have been scarce, although a number of experiments including several fish and invertebrate species have been conducted recently for the oil and gas industry operating on the NCS (i.e. the projects “BioSea I & II JIPs”, “Biomarker Bridges” and “PW JIP”). The results of these experiments are currently in the process of being published, and in this paper the EAC threshold values that can be calculated from these data have been processed and compiled.

It is required by the oil and gas producers on the NCS to conduct risk based managements of their discharges, and large efforts have been made to conduct impact and risk assessment as bases for the operators plans to avoid harmful discharge effects. It is therefore of interest to be able to link the results of their bomonitoring program to these impact and risk assessments. On the basis of the mentioned experimental results the paper discusses how the EAC values can be used in such an integrated risk and biomonitoring approach. The potential problem of representative species and species differences is highlighted, and a solution based on further extension of the EAC type of assessment criteria to a range of species, which at the same time links the biomonitoring results to the risk predictions, is also discussed.

Additional dissemination: Presentation at BIOMARKERS – effects of hazardous substances in aquatic ecosystems- seminar February 1, 2011 Finnish Environment Institute SYKE

WP 11:

Jonny Beyer, Knut Erik Tollefsen, Sonnich Meier, Rolf C. Sundt

Low risk for alkylphenols in operational discharges of produced water from offshore oil fields to impact health and reproduction in fish

To be submitted to Marine Pollution Bulletin

Abstract: What is the chance that alkylphenols (APs) released to sea in offshore oil industry produced water (PW) discharges will cause adverse effects, such as endocrine disruption, in feral fish? This question was raised because APs that are common constituents in offshore PW can possibly act as xenoestrogens, i.e. they are able to induce abnormal feminine responses in male and juvenile fish, such as Vtg induction. In this study, data from controlled PW exposures of fish in laboratory and comparable data from field studies with caged fish at the Ekofisk oil production field was utilised to decide (a) the exposure concentration that is required for PW to induce Vtg synthesis in fish and (b) whether fish at an offshore location close to a PW discharge will possibly be exposed to this concentration. We did observe Vtg induction in PW exposed fish but the LOEC (lowest observable effect concentration) value was in the region one order of magnitude higher than the exposure concentration observed in fish caged in close proximity (<200 m) of the PW outfall at Ekofisk. These findings are in accordance with two previous studies using a model approach and an *in vitro* approach, to investigate the same issue. Thus, we conclude that xenoestrogenicity in local offshore fish populations is unlikely to occur as a consequence of upstream offshore PW discharges.

WP 12

Jonny Beyer , Lars Petter Myhre, Rolf C. Sundt, Thierry Baussant, Steinar Sanni, Sonnich Meier, Rune Vabø, Jarle Klungsøyr

Effects of alkylphenols in offshore oil industry produced water on reproduction in North Sea fish stocks: an environmental risk assessment.

PRIMO16 Conference, Pollutant Responses in Marine Organisms

Abstract: Questions have been raised on whether alkylphenols (APs) in offshore produced water (PW) discharges represent a hazard to fish stock reproduction in the North Sea. An environmental risk assessment (ERA) was done to investigate this issue in three economically important fish populations, the North Sea Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*) and haddock (*Melanogrammus aeglefinus*). The ERA inputs included PW discharge data, fate information of PW plumes, fish distribution information, and environmental hazard information of PW APs (both toxicokinetic and toxicodynamic data). The exposure and risk regimes of fish were simulated using the DREAM (Dose related Risk and Effect Assessment Model) software in two steps. First the DREAM-EIF software as a screening step to pinpoint the areas with potential risk. Then the full capabilities of the DREAM model were used taking into account fish movement and uptake/elimination rates for the areas with a potential risk. The model results show that the environmental exposure of fish to APs from PW is to low to cause any significant effect on the reproduction in North Sea fish stocks.

11.2 Report: WP 13

Project report WCM 2010 work package 13

Impact of suspended particles on bioavailability of petrogenic PAH in cod (*Gadus morhua*), mussels (*Mytilus edulis*) and passive samplers exposed to Produced Water

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Abstract

In environmental monitoring of operational discharges from offshore oil production, comparison of exposure situations over time may be required. Sorption of Produced Water (PW) related hydrocarbons to particles in the sea water may influence the bioavailability of these compounds. Temporal variations of microalgae density may affect exposure measurements in caged organisms and passive samplers. In order to investigate the consequence of differences in biological particle density on bioavailability of petrogenic polycyclic aromatic hydrocarbons (PAHs), a screening study was conducted in blue mussel (*Mytilus edulis*), Atlantic cod (*Gadus morhua*) and passive samplers exposed to PW. Except for a reduction in bioavailability of the highest molecular weight PAHs in mussels from water with a high particle density, only minor influences of particles on bioavailability of PW related PAHs were measured for both species and passive samplers. Implications for the design of offshore bio-monitoring studies and consequences for interpretation of exposure results are discussed.

1. Introduction

As a consequence of production of oil and gas, large quantities of produced water (PW) are released into the marine environment (OLF, 2010). Since the water cut (ratio between water and oil) increases with the age of the well, the volume discharged increases through its life span. Levels of polycyclic aromatic hydrocarbons (PAHs) in semipermeable membrane devices (SPMDs) or biological matrixes like mussel soft tissue or fish bile are routinely used as measurements of PW exposure in monitoring studies (Hylland et al., 2008; Harman et al., 2009, Brooks et al. 2011). Comparison of exposure situations over time may be required, e.g. if there is a need to validate environmental benefit of new cleaning technology (Brooks et al. 2011, Sundt et al. 2011). In addition to treatment efficiency, other factors may also affect exposure of passive samplers and caged organisms during an experiment. Temporal changes in the composition of the raw PW and variations in local oceanographic conditions should be documented and taken into consideration when the results are interpreted. However, knowledge about the possible influence of particle density on the bioavailability of petrogenic organic compounds is sparse (Schrap, 1991).

Considerable spatial and temporal variation in marine micro-algae density is observed, particularly under spring bloom conditions (Bresnan et al. 2009). Organic hydrophobic compounds tend to adsorb to particles and in particular on biological surfaces, therefore an algae mix was selected to study the variation in PAH bio-availability due to suspended particles (Schrap and Opperhuizen, 1990). Presence of particles may theoretically increase bioavailability of compounds for filter feeding organisms like mussels or grazing zooplankton. On the other hand bioavailability for fish and passive samplers may decrease and these factors could affect estimation of PAH presence at oil fields. Information about influence of particles is also important when biomarker approaches are validated through laboratory and field investigations. If bioavailability is significantly modified by presence of particles, bio-concentration data estimated from exposures in laboratory studies may not reflect the real values in field and the resulting bio-concentration factors used in risk assessment modelling may be inaccurate.

The aim of this study was to investigate the importance of biological particles as a modifier of bioavailability of petrogenic PAHs in mussel (*Mytilus edulis*), Atlantic cod (*Gadus morhua*) and SPMDs. This was done by uptake comparison in groups of organisms and passive samplers exposed to diluted PW combined with various densities of microalgae. The following parameters were analysed: PAHs in water, PAH concentrations in SPMD and mussel soft tissues, and PAH metabolites in fish. Due to the important role of gills for absorption of waterborne PAHs and previous findings of PW induced lesions in this tissue (Sundt et al. 2011), a histophatological investigation of fish gills was conducted as a support parameter.

2. Material and methods

2.1 Studied organisms and passive samplers

Farmed juvenile Atlantic cod (weight 72 ± 15 g) were purchased from Atlantic Cod Farms AS and blue mussels (length 56 ± 5 mm) were collected at a clean site in SW Norway (WGS 84: N 58° 55`25 E 5° 58`172). Cod and mussels were transferred to the IRIS research facility in Stavanger and divided into five groups of up to 20 individuals. Three SPMDs per exposure group (91.4 × 2.5 cm LDPE tubing, containing 1 mL triolein) were spiked with five deuterated PAHs as performance reference compounds for exposure adjustment (Booij et al., 1998; Huckins et al., 2002). SPMDs were obtained from ExposMeter (Tavelsjo, Sweden). Solvents were purchased from Rathburn (Walkerburn, Scotland) except for cyclohexane (J.T. Baker, Deventer, Holland) and were HPLC grade. All glassware was baked in a muffle furnace at 560 ºC before use.

2.2 Produced Water and algae mix

PW was tapped downstream from the low pressure separator and hydrocyclone at the North Sea oil field Ekofisk (platform *2/4 J*) and transported to the laboratory facility by ship. Upon arrival the water was sieved through a 30 μm mesh to remove the largest particles and distributed into 5L polyethylene cans before being frozen at -20°C to reduce degradation of PW compounds. Composition of selected PAHs in the PW used for the exposure is reported in Table 1.

As introduced biological particles, Shellfish Diet 1800® (Reed Mariculture Inc. California, USA) containing 30% *Isochrysis*, 20% *Pavlova*, 20% *Tetraselmis* and 30% *Thalassiosira weissflogii* was used. Nominal algae concentrations were 6000 cells/mL (low concentration), 20000 cells/mL (medium concentration) and 60000 cells/mL (high concentration).

Table 1. Content of selected Polycyclic Aromatic Hydrocarbons (PAHs) in samples of the PW stock used for the exposure (collected at day 4, 9 and 15). Limit of quantification (LOQ) was 0,005 µg/ L for single compounds (n=3).

Compounds (µg/L)	**Mean ± standard deviation**
Naphthalene	20.36 ± 10.71
C_1-Naphthalene	20.33 ± 7.34
C_2-Naphthalene	23.07 ± 4.58
C_3-Naphthalene	27.05 ± 2.48
Acenaphthylene	<LOQ
Acenaphthene	1.01 0.35
Fluorene	0.71 ± 0.12
Phenanthrene	1.74 ± 0.16
Anthracene	<LOQ
C_1-Phen/Anthr	6.46 ± 0.59
C_2-Phen/Anthr	15.58 ± 1.65
Dibenzothiophene	<LOQ
C_1-Dibenzothiophene	1.61 ± 0.28
C_2-Dibenzothiophene	2.84 ± 0.30
Fluoranthene	0.49 ± 0.05
Pyrene	0.19 ± 0.32
Benzo(a)anthracene	0.61 ± 0.10
Chrysene/Triphenylene	0.55 ± 0.11
C_1-Chrysene	1.16 ± 0.16
C_2-Chrysene	1.68 ± 0.42
Benzo(b, j,k)fluoranthene	<LOQ
Benzo(a)pyrene	<LOQ
Indeno(1,2,3-cd)pyrene	<LOQ
Benzo(g,h,i)perylene	<LOQ
Dibenzo(a,h)anthracene	<LOQ

2.3 Experimental set-up

Study organisms and SPMDs were kept in 600 L glass fiber tanks supplied with sand filtered seawater. Water temperature was 8±1ºC and water salinity was 34‰ throughout the experiment. Study organisms were acclimated to these conditions for two weeks

before the start of the exposure. Fish were fed with dry fish feed (Dana feed marine, 14% fat) during the acclimatization period. In order to reduce particles from feed and faeces and to secure enough bile for the PAH metabolite analyses, the fish were not fed during the exposure. The algae supply was stopped 24 hours prior to sampling in order to evacuate mussels' guts.
Organisms and SPMDs were exposed for 17 days to diluted PW in a continuous flow-through system previously described by Sundt et al. (2009). The dilution was set at 1:1000, from day 6 until day 10 the flow was set to 1:500 to mimic real field conditions with varying PW concentration over time. The sea water flow rate was set to 3L/minute for each tank. The exposure set up included: a negative control with sea water only, a positive control with diluted PW only and three tanks containing diluted PW and algae mix at low, medium and high concentrations (Fig. 1).

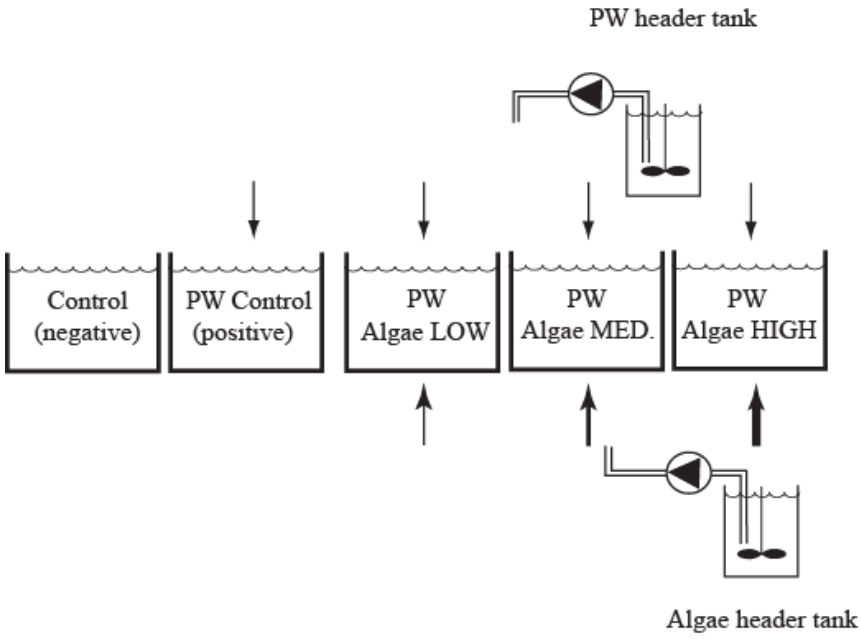

Figure 1. Experimental set up: each treatment group contained 20 cod specimens, 20 mussels and 3 SPMDs. Control = negative control containing only seawater, PW Control = positive control containing diluted produced water only, PW Algae LOW=diluted produced water containing low concentration of algae, PW Algae MED=diluted produced water containing medium concentration of algae, PW Algae HIGH=diluted produced water containing high concentration of algae.

2.4 PAH analysis in water

Water samples for PAH analysis in the exposure tanks were siphoned into 1 L glass flasks at day 2, 7 and 13. PAHs were immediately captured by liquid-liquid extraction. The concentration of selected PAH compounds in PW and in the exposure water (PW diluted in sea water) were measured by means of gas chromatography-mass spectrometry (GC-MS) (Jonsson et al., 2004). The analyses included 16 PAHs

recommended by the EPA and alkylated congeners of naphthalene, phenanthrene, chrysene and dibenzothiphene. Limit of quantification (LOQ) for single components was 0.005 µg/L. Data were expressed as µg/L (n = 3).

2.5 Algae particles

Concentration of algae particles in the range 2-12 µm was measured daily during the exposure by means of a Multisizer™ 3 coulter counter® (Beckman coulter) and the results were treated with the Win-situ® 5 software. For each sample the average of three individual measurements was used.

2.6 PAH in passive samplers

SPMDs were extracted as described by Huckins et al. (1990) Briefly, SPMDs were removed from their steel cans, rinsed in distilled water, dried with paper towel and mounting loops were cut off before being placed in a glass jar with 150 mL hexane. Solvent dialysis proceeded for 24 hrs at 18ºC with appropriate internal standards added and was repeated once. Extracts were combined and reduced to 2 mL under a stream of nitrogen and cleaned up using gel permeation chromatography as previously described by Harman et al. (2008). PAH concentration in SPMDs was determined by GC-MS. An Agilent Technologies (Santa Clara, USA) 6890 GC linked to a 5973 mass selective detector was used in selected ion monitoring (SIM) mode. The injection was pulsed splitless and the GC was equipped with a 30 m column with a stationary phase of 5% phenyl polysiloxane (0.25 mm internal diameter and 0.1 µm film thickness). The initial column temperature was 40ºC, which was raised stepwise to 310ºC over 34 minutes. Data were expressed as ng/L (n = 3 for each group).

2.7 PAH metabolites in fish bile

All fish were treated and sacrificed according to Norwegian national legislation for laboratory animals. After 17 days of exposure fish were anaesthetized with tricaine (MS-222, Sigma-Aldrich) and measured for total length and weight. A condition factor (CF) was calculated as: weight/$(\text{length})^3$ x 100. Bile was sampled and stored at -80 °C until analysis. Biliverdin and semi-quantitative PAH metabolite levels in fish bile were determined by the fixed fluorescence method (FF) (Aas et al., 2000). Data were expressed as Pyrene Fluorescence Equivalents (PFE, n =10-20). Quantitative measurements of PAH metabolites in bile were conducted with a GC-MS method as described by Jonsson et al., (2004) and LOQ was 0.03 µg/L. Data were expressed as ng/g bile (n = 5).

2.8 PAH body burden in mussels

Mussels were sampled as pools of three individuals where whole soft tissue was dissected, excess water was wiped off and tissue was transferred into a pre heated glass vial with Teflon seal. Samples were stored at -20 °C until analysis. PAH analyses in biota were performed by means of GC-MS (Baussant et al., 2009). LOQ ranged from 1 to 2 µg/kg for single compounds. Data were expressed as µg PAHs/g of lipid (n = 3).

2.9 Lipid content in mussel soft tissues

Lipid content in mussel soft tissue was determined gravimetrically based on the method of Blight and Dyer (1959). This measurement was used to normalize mussel body burden data.

2.10 Histopathology in fish gills

A piece of gill arch from each side of the fish was dissected and immediately fixed in Baker's solution (4% formaldehyde, 1% $CaCl_2$). The fixed gill tissue was dehydrated through a series of graded ethanol solutions (50-99%) and cleared in toluene using a tissue processor (*Shandon Excelsior, Thermo*) before embedding in paraffin wax. Histological sections (3 µm) were obtained using a microtome (*HM 355s, Bergman*), mounted on slides and stained in haematoxylin, eosin and saffron (HES) in an automated staining machine (*Tribune Stainer, Surgipath*). The sections were evaluated using a microscope (*Zeiss Axioplan 2*) and all micrographs were captured using a digital colour camera (*AxioCam*). Analyses of histological changes were performed by visual scoring of coded slides (blind reading). The following lesions were scored: aneurisms, epithelial lifting, epithelial hyperplasia, necrosis, leukocyte infiltration, chloride cell degeneration, lamellar fusion, lamellar clubbing, epithelial proliferation and excess mucus. For each fish, four different gill sections were analysed (two from each opercula gill). Each histopatohlogical lesion was scored according to generally accepted classification criteria (Benly et al., 2008; Sensini et al., 2008): 0 = absence of lesion; 1 = ≤ 10 % of the histological section had the lesion, 2 = between 10% and 50% of the histological section had the lesion, 3 = between 50% and 70% of the histological section had the lesion and 4 = between 70% and 100% of the histological section had the lesion. Slides were analyzed blind. To ensure quality of the data, two analysts scored a selection of slides.

2.11 Statistical treatment

Results were presented as mean ± standard deviation. Treated and control groups were compared using a one-way analysis of variance and Fischer LSD pair wise multiple comparison test. Alternative: exposure groups were analyzed for statistical differences to the corresponding control group by means of Dunnett´s test. Values were log-transformed when needed for homogeneity of variance. The non-parametric Kruskal-Wallis test was used when normality or homogeneity tests failed. All calculations were made with the PASW version 18.0 program package (SPSS Inc. Chicago).

3. Results

3.1 Studied organisms

The sex ratio in fish was 65% females and 35% males, individuals were equally distributed in the treated groups. There were no statistical differences regarding fish length, weight or condition factor between groups (Table 2).

Table 2. Morphometric parameters: length, weight and condition factor (CF) of cod (n=12) and size and lipid content of mussels (n=20) in the different groups. PW=diluted produced water; PW Low=diluted produced water with low concentration of algae; PW Medium=diluted produced water with medium concentration of algae; PW High=diluted produced water with high concentration of algae. Results are reported as mean ± standard deviation.

Treatment	Cod length (cm)	Cod weight (g)	Cod CF	Mussel size (mm)	Mussel lipid content (%)
Negative control	21.1±1.5	75.7±20.0	0.79±0.07	57.4±4.0	0.98±0.29
Positive control	20.6±1.0	68.7±11.7	0.78±0.05	56.2±4.9	0.91±0.20
PW Low	21.4±1.2	82.3±15.3	0.83±0.07	53.6±4.9	1.19±0.38
PW Medium	20.5±0.9	67.3±10.4	0.78±0.05	58.7±3.7	1.06±0.09
PW High	20.4±1.6	68.0±16.2	0.79±0.11	54.6±4.8	1.16±0.13

3.2 PAH analysis in water

Exposure concentrations were estimated from 26 quantified PAH groups and single compounds in water samples (Fig. 2). Naphthalenes constituted 70-80 % of total amount of PAHs in all tanks receiving PW, and PAHs with more than three aromatic rings were not detected in any of the exposure treatments. PAH composition was similar and stable in all the tanks receiving PW. Measurements of PAH in mussel soft tissue, cod bile and passive samplers confirmed uptake of PAH in all groups exposed to PW.

3.3 Algae particles

The size of cells from the algae mix (4 types of algae) were mainly between 5 and 10 µm, according to results from the Multisizer coulter counter analysis. The background concentration of particles in the sand filtered sea water used for the experiment was approximately 1200 particles/mL and PW contributed an additional 1200 particles/mL. Results for all groups are given in Table 3.

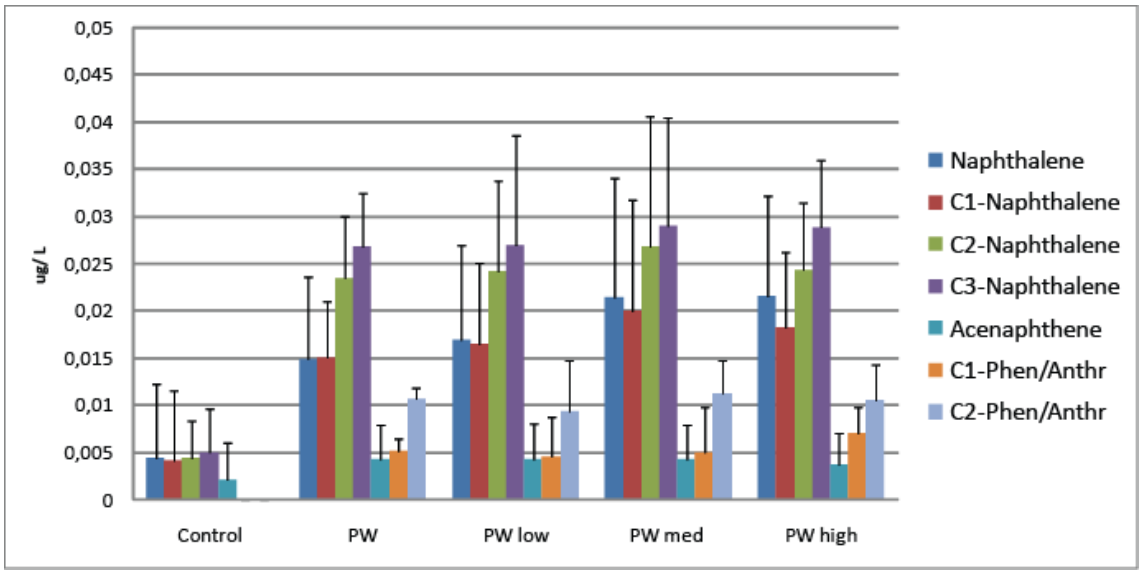

Figure 2. PAH in water samples from the exposure tanks collected at day 2, 7 and 13 (n= 3). Control = only seawater; PW = diluted produced water; PW low = diluted produced water with low concentration of algae; PW med = diluted produced water with medium concentration of algae; PW high = diluted produced water with high concentration of algae.

Table 3. Results of particle density, sum of 26 PAHs in samples of exposure water, sum of 9 PAH metabolite groups in cod bile samples, sum of 6 PAH metabolite groups in mussel soft tissues, sum of 8 PAH groups in passive samplers (SPMDs).
Control = negative control containing only seawater, PW Control = positive control containing diluted produced water only, PW Algae Low = diluted produced water containing low concentration of algae, PW Algae Medium = diluted produced water containing medium concentration of algae, PW Algae High = diluted produced water containing high concentration of algae.

Treatment	Particle density (cells/mL, n=42)	Sum water PAHs (µg/L, n=3)	Sum PAH metabolites in cod bile (µg/g, n=5)	Sum PAH in mussels (µg/g lipid, n=3)	Sum PAH in SPMDs (ng/L triolein, n=3)
Control (negative)	1237±532	0.048 ±0,02	0.701± 0.23	1.036±0.37	0.96±0.11
PW control	2476±765	0.103 ± 0,03	5.385 ± 0.47	65.990±18.83	271.95±54.81
PW Algae Low	6684±1345	0.112 ± 0,05	6.576± 1.10	79.244±19.37	193.06±25.34
PW Algae Medium	17161±2863	0.133 ± 0,07	8.975± 3.14	53.743±21.98	310.90±111.33
PW Algae High	49909±8490	0,118 ± 0,04	6.081 ± 0.99	29.684±4.84	198.33±26.81

3.4 PAH in semipermeable membrane devices

Significantly lower levels of C_1 Naphthalenes, C_2 Phenanthrenes and C_3 Dibenzothiophenes were observed in SPMDs exposed to diluted PW containing low concentration of algae (Fig. 3). Significantly lower levels of C_2 Phenantrenes and C_3 Dibenzothiophenes were also observed in fish exposed to diluted PW containing high concentration of algae. In all groups of fish exposed to PW, C_3 Naphthalenes were the dominating PAH group. Overview of the total amount of PAHs measured in the different organisms and in the SPMDs is reported in Table 3.

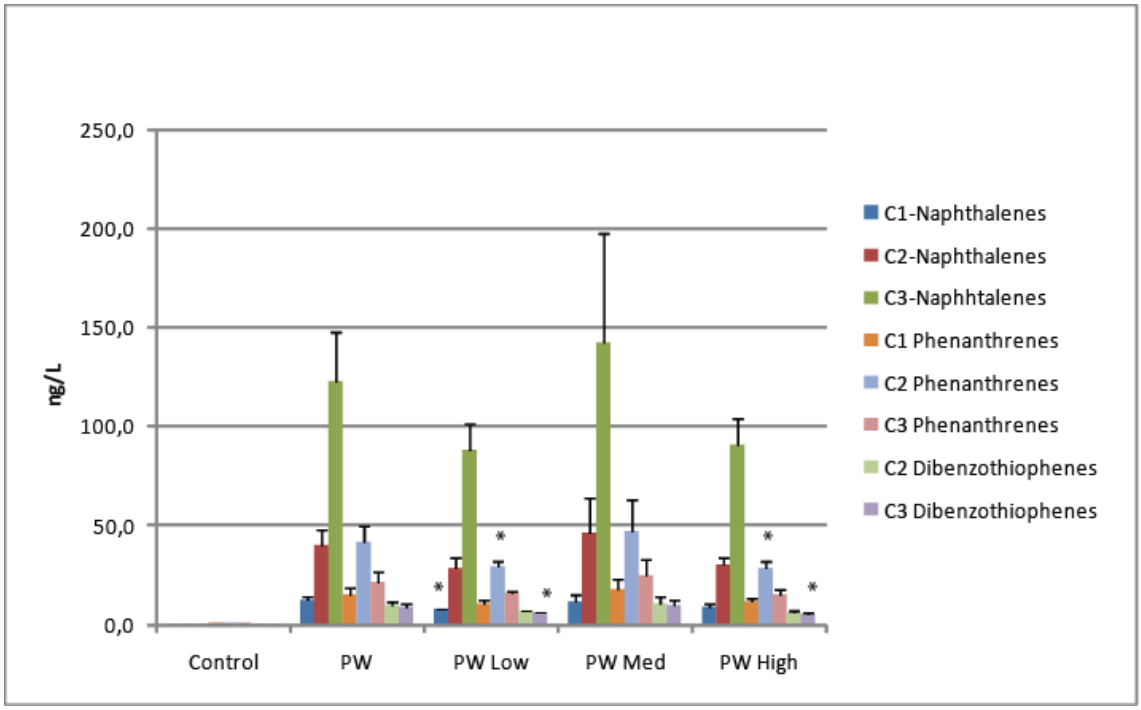

Figure 3. Polycyclic aromatic hydrocarbons (PAHs) levels in SPMDs (mean and standard deviation, Wilcoxon test, * $p<0.05$, n = 3). Control = negative control containing only seawater l, PW = positive control containing diluted produced water, PW low=diluted produced water containing low concentration of algae; PW med = diluted produced water containing medium concentration of algae, PW high=diluted produced water containing high concentration of algae.

3.5 PAH metabolites in fish bile

Analyses of PAH metabolites in fish collected before the experiment (pre-exposure) indicated only low background levels (Fig. 4 and 5). Results obtained with both methods (FF and GC-MS) indicated higher uptake of PAHs in fish from all PW exposed groups compared to the control. PAH metabolite levels in bile of fish exposed to PW and to PW containing algae were similar when analyzed by the FF method (Fig. 4). However, results from the GC-MS analysis indicated significantly increased levels of C_1, C_2 and C_3 OH-Naphthalenes in addition to C_1 OH-Phenanthrenes in bile of fish exposed to the PW containing medium concentration of algae (Fig. 5). The majority of PAH compounds detected in bile were 2- and 3 ring structures.

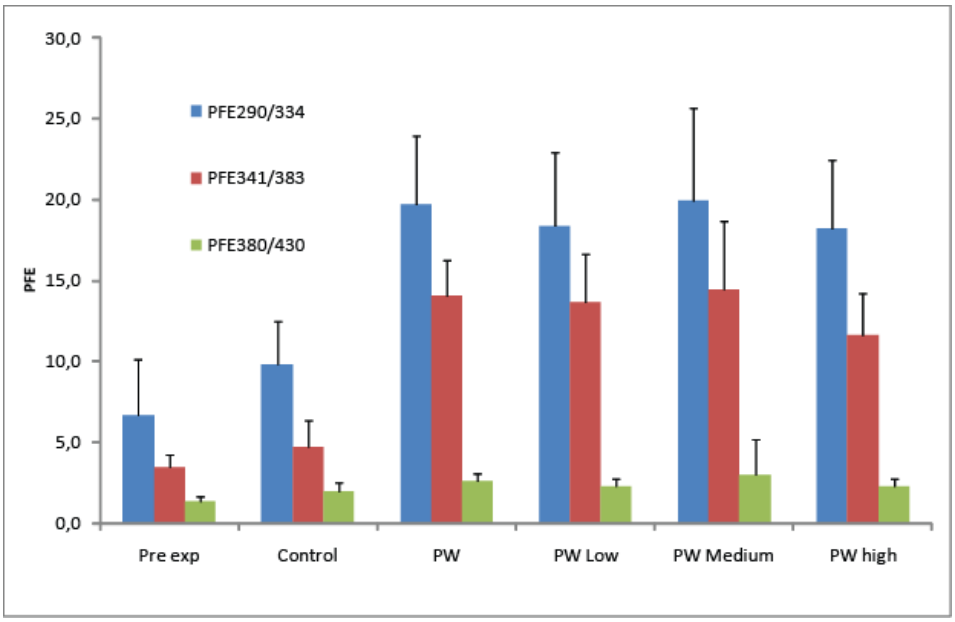

Figure 4. Semi quantitative levels of bile naphthalene/phenanthrene-, pyrene- and B[a]P-type metabolites analysed by fixed wavelength fluorescence (FF) screening method (mean ± standard deviation, n= 10-20). Pre exp = pre-exposure, Control = negative control containing only seawater, PW = positive control containing diluted produced water, PW Low=diluted produced water containing low concentration of algae; PW Medium = diluted produced water containing medium concentration of algae, PW High=diluted produced water containing high concentration of algae. PFE=pyrene fluorescence equivalents

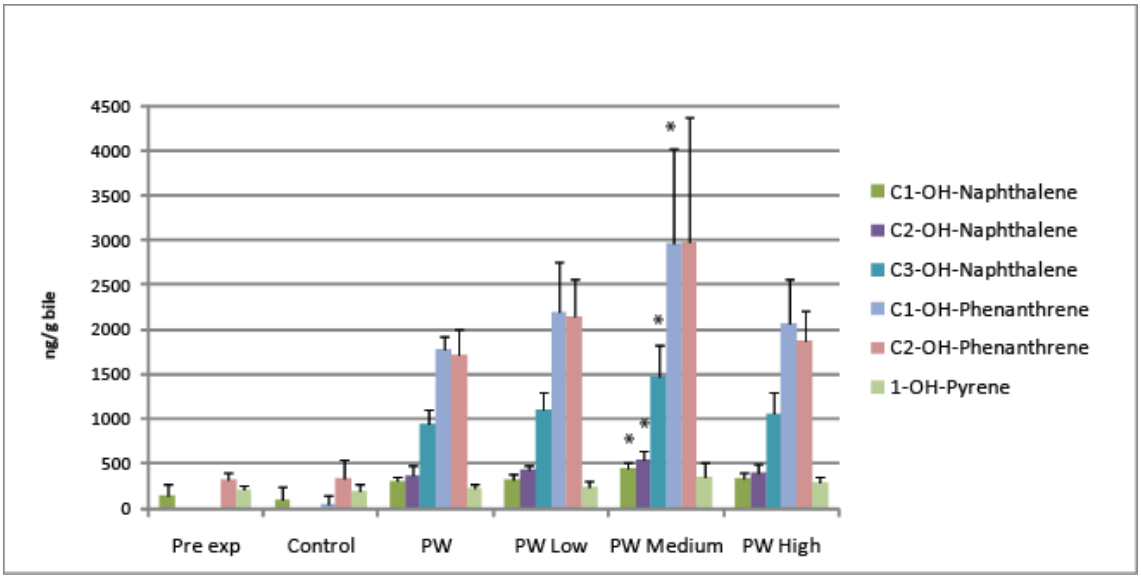

Figure 5. Levels of polycyclic aromatic hydrocarbons (PAHs) metabolites in cod bile (ng/ g bile, mean ± standard deviation, n = 5, Wilcoxon test, * $p<0.05$). Pre exp = pre-exposure, Control = negative control containing only seawater, PW = positive control containing diluted produced water, PW Low=diluted produced water containing low concentration of algae; PW Medium = diluted produced water containing medium concentration of algae, PW High=diluted produced water containing high concentration of algae.

3.6 PAHs in mussel soft tissues

Analyses of PAHs in mussel soft tissues collected before the experiment (pre-exposure) indicated only low background levels (Fig. 6). Increased levels of PAHs were found in all groups of mussels exposed to PW in comparison to the control ones, confirming the hydrocarbon exposure. Significantly increased levels of C_1 and C_2 Naphtalenes were observed in mussels exposed to PW containing low concentration of algae compared to the control ones. Significantly lower levels of all analysed PAHs (except naphthalenes) were also observed in mussels exposed to PW containing high concentration of algae in comparison to the control group (Fig. 6).

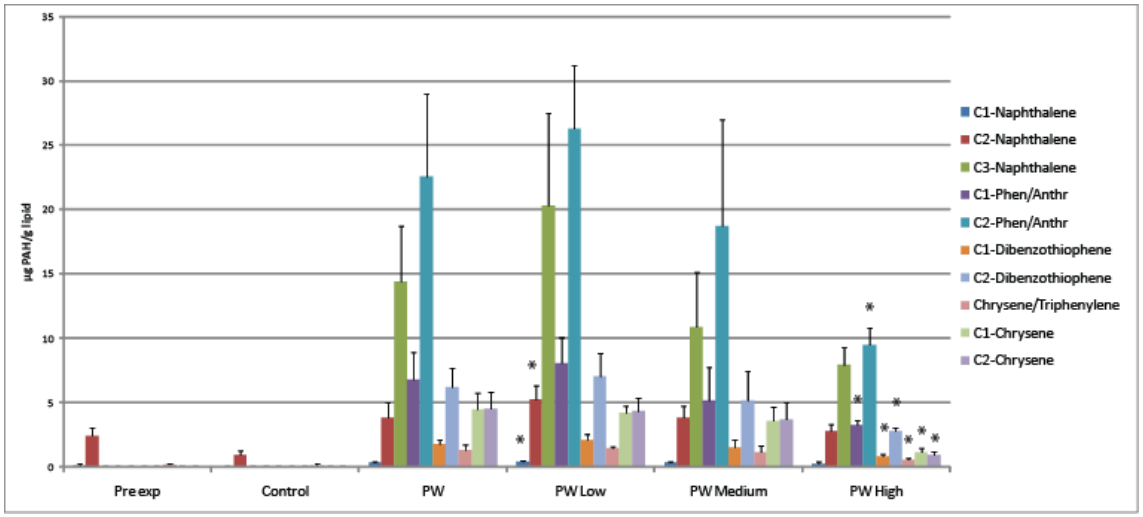

Figure 6. PAH body burden results in mussel soft tissues, data were normalised for the lipid content (mean ± standard deviation, Wilcoxon test, * $p<0.05$, n= 3). Pre exp = pre-exposure, Control = negative control containing only seawater, PW = positive control containing diluted produced water, PW Low = diluted produced water containing low concentration of algae; PW Medium = diluted produced water containing medium concentration of algae, PW High = diluted produced water containing high concentration of algae.

3.7 Lipid content

In order to normalize PAH body burden results in mussels, levels of lipid content in soft tissues from the different experimental groups were measured. The lipid content ranged from 0.91% to 1.19 %, details are given in Table 2.

3.8 Histopathology in fish gills

The following lesions were scored: aneurisms, epithelial lifting, epithelial hyperplasia, lamellar fusion, lamellar clubbing, excess mucus secretion, necrosis and proliferation of epithelial cells. Results from the histological investigation of cod gills are given in Table 4. Even though mild responses for several parameters were observed in some individuals from all groups, no indication of PW or particle induced lesions were found.

Table 4. Comparison of histological lesion parameters in cod gills indicating those considered as chronic (I) or acute (II) responses to environmental conditions (Poppe, 1999). Each histopathological lesion was scored according to generally accepted classification criteria (Benly et al., 2008; Sensini et al., 2008): 0 = absence of lesion; 1 = ≤ 10 % of the histological section had the lesion, 2 = between 10% and 50% of the histological section had the lesion, 3 = between 50% and 70% of the histological section had the lesion and 4 = between 70% and 100% of the histological section had the lesion.

Lesion	Aneurisms					Epithelial lifting [I]					Epithelial hyperplasia [II]					Lamellar fusion [II]					Lamellar clubbing [II]					Excess mucus secretion					Necrosis					Proliferation of epithelial cells				
Scores / Treatment	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Pre exposure (n=12)	12	0	0	0	0	11	1	0	0	0	5	7	0	0	0	12	0	0	0	0	11	1	0	0	0	5	6	1	0	0	11	1	0	0	0	0	10	2	0	0
Negative control (n=16)	14	2	0	0	0	15	1	0	0	0	5	10	1	0	0	14	2	0	0	0	16	0	0	0	0	5	7	4	0	0	12	4	0	0	0	0	12	4	0	0
Positive control (n=17)	17	0	0	0	0	17	0	0	0	0	5	11	1	0	0	17	0	0	0	0	17	0	0	0	0	9	5	3	0	0	16	1	0	0	0	0	14	3	0	0
Algae low (n=17)	17	0	0	0	0	17	0	0	0	0	9	6	2	0	0	16	1	0	0	0	17	0	0	0	0	11	6	0	0	0	15	2	0	0	0	0	16	6	0	0
Algae medium (n=20)	20	0	0	0	0	20	0	0	0	0	3	14	3	0	0	19	1	0	0	0	20	0	0	0	0	11	8	1	0	0	18	2	0	0	0	0	8	10	2	0
Algae high (n=20)	18	2	0	0	0	19	1	0	0	0	2	15	3	0	0	19	1	0	0	0	20	0	0	0	0	11	8	1	0	0	19	1	0	0	0	0	17	3	0	0

4. Discussion

Blue mussels (*Mytilus edulis*), Atlantic cod (*Gadus morhua*) and SPMDs are routinely used for oil production related environmental monitoring programs (Hylland et al., 2008). The main aim of this study was to assess the role of suspended biological particles as modifiers of bioavailability of petrogenic PAHs in these matrixes. Due to spatial and temporal differences in density of marine microalgae, such knowledge is required to support interpretation of results when measurements obtained in different time periods are compared (Brooks et al., 2011).
The chemical composition of Ekofisk PW used in the experiment is representative of North Sea discharges (Røe Utvik, 1999, Boitsov et al., 2007) and the dilution concentrations (0.05 – 0.1%) used in the exposure are representative of offshore near field conditions. PAH levels in mussel soft tissue and fish bile from specimens collected before the experiment (pre-exposure) indicated only low background levels of PAHs, confirming the suitability of the employed organisms for the experiment.
The uptake of a compound is determined by both its concentration in the environment and the compounds bio concentration factor (BCF). The PAH profile in PW is dominated by naphthalenes but the BCF of these compounds is relatively low (BCF in fish is 170 – 3550) compared to PAHs with higher molecular weight (e.g. BCF for benzo(a)pyrene in fish is 23500, Neff, 2002). In addition to uptake, excretion potential is also an important factor. Various organisms will give different responses according to their capability to metabolize contaminants. Nevertheless, since different compounds may have different uptake and excretion dynamics in organisms and no biological excretion occurs in passive samplers, SPMDs provide the most representative picture of PAH profile in the PW discharge (Harman et al., 2009).

4.1 PAH analysis in water

Chemical analysis of water samples confirmed equal PAH concentrations in all tanks with diluted PW. In theory, since binding of hydrocarbons to particles may affect the efficiency of the extraction process, an underestimation of PAH levels in water with a high concentration of algae may be expected. However even levels of all measured PAHs among PW exposed groups were observed, there was therefore no indication of such an effect.

4.2 Algae particles

Mineral particles like diatomaceous earth or river sediment have previously been used to investigate similar issues (van den Heuvel and van Noort, 2003; Mittal and Rockne 2009). The microalgae mix employed in the present study was considered to be more suitable to mimic the particles likely to be present in an offshore water column environment distant from river runoff. The size distribution of particles in the exposure tanks were dominated by algae particles in the size range 5-10 µm, comparable to marine microalgae dominating offshore North Sea blooms. A concentration of more than 10000 cells per ml is considered a blooming and the concentrations used in the experiment were within the range often seen in North Sea spring blooms.
Measured particle densities were acceptably close to the intended concentrations; they were 11.7% higher in the “low algae concentration”, 15% lower in the “medium algae concentration” and 16.7% lower in the “high algae concentration” groups.

Measurements of particle density showed an average background level of 1237 particles/mL in the seawater and an additional contribution of 1239 particles/mL in the PW groups. Daily measurements of particle density showed that the exposure system rendered a stable particle density in all treatments throughout the experiment. Since sedimentation of particles in exposure tanks may affect the particle density in the water, agitation and flow through set up design with continuous flows of 3 L/min (theoretical retention time 3.3 hours), excluded this effect and no sedimentation was observed during the exposure.

4.3 PAH in passive samplers

For SPMDs, significantly lower levels of C_1 Naphthalenes, C_2 Phenanthrenes and C_3 Dibenzothiophenes were observed in the group exposed to PW containing low concentration of algae in comparison to the group exposed to PW only. And significantly lower levels of C_2 Phenanthrenes and C_3 Dibenzothiophenes were observed in the group exposed to PW containing high concentration of algae compared to the group exposed to PW only. These results indicated only a minor effect of the particles in the concentration of PAHs in the passive samplers.

4.4 PAH metabolites in fish bile

PAH metabolites in fish bile were measured by two different techniques, semi-quantitative screening (Fixed Wavelength Fluorescence) and a GC-MS based technique providing quantifiable levels. Differences in metabolite levels in fish from the different PW exposed groups were not sufficiently high enough to be detected by the screening technique. However, results from the GC-MS analysis indicated increased levels of the least lipophilic compounds, C_1, C_2 and C_3 OH-Naphthalenes in addition to C_1 OH-Phenanthrenes only in the bile of fish exposed to PW containing medium concentration of algae. Since this is the only significant difference, and no dose response was seen for these compounds, it is difficult to draw any conclusion from this.

4.5 PAH body burden in mussels

Significantly increased levels of C_1 and C_2 Naphthalenes were observed in mussels from the tank containing diluted PW and low concentration of algae relative to the one containing only diluted PW. And significantly lower levels of all PAH groups except naphthalenes were observed in the mussels exposed to PW containing high concentration of algae, indicating an effect on bioaccumulation of PAHs in mussel soft tissues. Since mussel gonads contain a significant portion of the total lipids in the organism, a possible source of error could therefore be loss of lipid bound PAHs from the organisms by spawning during the exposure. However, large amounts of spawning products would most likely be detected by the daily particle measurements (sperm size is approximately 5µm) but no such event was recorded. The analyzed lipid content in the mussels was within the range normally seen in this species.

4.6 Histopathology in fish gills

Gill lesions have previously been observed in fish caged close to a PW discharge (Sundt et al. 2011). Such lesions may cause a reduction of the available gill surface potentially affecting the uptake of waterborne organic contaminants. This may be caused either by

reduced diffusion surface or from changes in respiration rate due to less efficient O_2 uptake (Au, 2004). Histological investigation of cod gills was therefore included as a supporting parameter. At what level of influence such biological changes start being a significant factor in the animal physiology is not easy to establish. In the present study, histological investigation of 8 different lesions did not show differences among groups, indicating that neither exposure of PW or to PW containing algae caused fish gill damages.

4.7 Bioavailability of PAH compounds in the presence of algae particles

For cod, mussels and SPMDs generally small differences in PAH content were found between groups exposed to PW only and to PW containing algae. This only indicates minor impact on the bioavailability from the particles. However interpretation of these results is challenging due to the complexity of physical, chemical and biological factors affecting the availability of organic pollutants.

Information about sorption of low molecular weight PAHs typically present in crude oil related discharges like PW is sparse. The minor reduction of bioavailability in fish is in accordance with the findings of McCarty and Jimenez (1985) who concluded that dissolved humic material had little effect on uptake of [C^{14}] naphthalene in fish. Several other studies have indicated that adsorption of high molecular weight PAHs into particulates has the potential to be a major controlling factor for their bioavailability. Black and McCarthy (1988) found a decrease in BaP extraction efficiency in rainbow trout with an increase in concentration of suspended organic matter. The same study suggested that only the freely dissolved chemicals are available for uptake by fish gills. The uptake situation is believed to be similar for passive samplers but not for bivalves and other filter feeders that can accumulate the pollutants through several routes of exposure. Moreover, Kukkonen et al. (1989) showed that increased humus concentration decreased bioavailability of BaP in *Daphnia magna*.

Since fish, mussels and passive samplers were exposed in the same tanks, exposure conditions within each group were identical. The results of the PAH profiles therefore could indicate which matrix (organisms and/or SPMDs) is more sensitive as bioindicator for PW exposure. In SPMDs where no metabolism occurs, C_3 Naphthalenes was the dominant PAH group. Fish generally are known to have extensive capacity for metabolising low molecular weight PAHs and in fact metabolites of C_1 and C_2 Phenanthrenes dominated in the fish bile. Compared to fish, mussels are known to have lower metabolic capacity; in this species C_3 Naphthalenes and C_2 Phenanthrenes were the predominant groups.

5. Conclusions

- As previously demonstrated, the bioavailability of organic pollutants is a complex issue.
- This screening study indicates that presence of realistic densities of organic particles have only minor impact on the bioavailability of low molecular weight PAHs in fish, mussels and passive samplers.
- Bioavailability of 3-ring PAHs was only significantly reduced in mussels when algae particle density was high (~50000 cells/ml).

- The low sorption observed in the present study is also interesting from an environmental fate point of view. If sorption of PW related PAHs is low, particles will only to a low extent alter the fate and transport of the compounds to bottom sediment and a more widespread distribution can be expected.
- Histopathological analysis of gills was used as supporting parameter, the exposure time was probably not sufficient to produce effects on fish gills.

Acknowledgements
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References

Au, D.W.T., 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Marine Pollution Bulletin 48, 817-834.

Aas, E., Beyer, J., Goksøyr, A., 2000 Fixed wavelength fluorescence (FF) of bile as a monitoring tool for polyaromatic hydrocarbon exposure in fish: an evaluation of compound specificity, inner filter effect and signal interpretation. Biomarkers 5 (1), 9-23.

Baussant, T., Bechmann, R.K., Taban, I.C., Larsen, B.K., Tandberg, A.H., Bjornstad, A., Torgrimsen, S., Naevdal, A., Oysaed, K.B., Jonsson, G., Sanni, S., 2009. Enzymatic and cellular responses in relation to body burden of PAHs in bivalve molluscs: A case study with chronic levels of North Sea and Barents Sea dispersed oil. Marine Pollution Bulletin 58, 1796-1807.

Benly, A.Ç.K., Köksal, G., Özkul, A., 2008. Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus* L.): effects on gill, liver and kidney histology. Chemosphere 72, 1355-1358.

Black, M.C., McCarthy, J.F., 1988. Dissolved organic macromolecules reduce the uptake of hydrophobic organic contaminants by the gills of rainbow-trout (*Salmo gairdneri*). Environmental Toxicology and Chemistry. Canadian Journal of Biochemistry and Physiology 7, 593-600.

Blight E.G., Dyer W.J. 1959 A rapid method of total lipid extraction and purification. 37(8), 911-917.

Boitsov S., Mjos S.A., Meier S. 2007. Identification of estrogen-like alkylphenols in produced water from offshore oil installations. Marine Environmental Research 64(5), 651-665.

Booij, K., Sleiderink, H., Smedes, F., 1998. Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. Environmental Toxicology and Chemistry 17, 1236-1245.

Bresnan, E., Haya S., Hughes S.L., Fraser S., Rasmussen J., Webster L., Slesser G., Dunn J., Heath M.R. 2009. Seasonal and interannual variation in the phytoplankton community in the north east of Scotland. Journal of Sea Research. 61, 1-2: 17-25

Brooks, S.J., C.Harman, M.Grung, Farmen, E., Ruus, A., Vingen, S., Godal, B.F., Baršienė, J., Andreikėnaitė, L., H.Skarphéðinsdóttir, Liewenborg, B., Sundt, R.C., 2011. Water column monitoring of the biological effects of produced water from the Ekofisk offshore oil installation from 2006 to 2009. Journal of Toxicology and Environmental Health, in press.

Harman, C., Thomas, K.V., Tollefsen, K.E., Meier, S., Boyum, O., Grung, M., 2009. Monitoring the freely dissolved concentrations of polycyclic aromatic hydrocarbons (PAH) and alkylphenols (AP) around a Norwegian oil platform by holistic passive sampling. Marine Pollution Bulletin 58 (11), 1671-1679.

Harman, C., Tollefsen, K.E., Boyum, O., Thomas, K.V., Grung, M., 2008. Uptake rates of alkylphenols, PAHs and carbazoles in semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (POCIS). Chemosphere 72, 1510-1516.

Hylland, K., Tollefsen, K.E., Ruus, A., Jonsson, G., Sundt, R.C., Sanni, S., Utvik, T.I.R., Johnsen, S., Nilssen, I., Pinturier, L., Balk, L., Barsiene, J., Marigòmez, I., Feist, S.W., Børseth, J.F., 2008. Water column monitoring near oil installations in the North Sea 2001-2004. Marine Pollution Bulletin 56 (3), 414-429.

Huckins, J. et al., 2002. Development of the permeability/performance reference compound approach for in situ calibration of semipermeable membrane devices. Environmental Science and Techmology 36, 85-91.

Huckins, J., Tubergen, M.W., Manuweera, G.K., 1990. Semipermeable membrane devices containing model lipid – a new approach to monitoring the bioavailability of lipophillic contaminants ans estimating their bioconcentration potential. Chemosphere 20, 533-552.

Jonsson, G., Bechmann, R.K., Bamber, S. and Baussant, T., 2004. Bioconcentration, biotransformation, and elimination of polycyclic aromatic hydrocarbons in sheepshead minnows (Cyprinodon variegatus) exposed to contaminated seawater. Environmental Toxicology and Chemistry 23, 1538-1548.

Kukkonen, J., Oikari, A., Johnsen, S., Gjessing, E., 1989. Effects of humus concentrations on benzo[a]pyrene accumulation from water to Daphnia Magna: Comparison of natural-waters and standard preparations. Science of the Total Environment 79, 197-207.

McCarthy, J. F., and B. D. Jimenez. 1985. Interaction between polycyclic aromatic hydrocarbons and dissolved humic material: binding and dissociation. Environmental Toxicolology and Chemistry 19:1072-1076.

Mittal M., Rockne, K.J. 2009. Naphthalene and phenanthrene sorption to very low organic content diatomaceous earth: Modeling implications for microbial bioavailability Chemosphere 74, 8: 1134-1144

Neff J.M. 2002. Bioaccumulation in marine organisms: effect of contaminants from oil well produced water. Elsevier. ISBN 0-08-04-3716-8.

OLF, 2010. Environmental Report 2009. The Norwegian Oil Industry Association, pp 60.

RøeUtvik, T.I.R., 1999. Chemical characterisation of produced water from four offshore oil production platforms in the North Sea. Chemosphere 39 (15), 2593-2606.

Schrap S.M. (1991) Bioavailability of organic chemicals in the aquatic environment. Comp. Biochem. Physiol. 100C, 1-2: 13-16.

Schrap S.M., Opperhuizen A. 1990 Relationship between bioavailability and hydrophobicity - reduction of the uptake of organic- chemicals by fish due to the sorption of particles. Environmental Toxicology and Chemistry 9(6),715-724.

Sensini, C., Della Torr C., Corsi, I., Focardi S., 2008. First observations of histopathological effects of 2,4,6-trinitrotoluene (TNT) in gills of European eel Anguilla anguilla (Linnaeus, 1758). Cellular Biology and Toxicology, 24, 621-628.

Sundt R.C., Meier, S., Jonsson, G., Sanni, S., Beyer, J., 2009. Development of a laboratory exposure system using marine fish to carry out realistic effect studies with produced water discharged from offshore oil production. Marine Pollution Bulletin 58 (9), 1382-1388.

Sundt R.C., Aarab N., Lyng E., Godal B.F., D.M. Pampanin 2011. Histopathological alterations in gills of Atlantic cod (*Gadus morhua* L.) exposed to North Sea Produced Water: a comparison of two field studies. Project report WCM-2010, WP15.

van den Heuvel, H., van Noort, P.C.M., 2003. Competition for adsorption between added phenanthrene and in situ PAHs in two sediments. Chemosphere 53, 1097-1103.

11.3 Report: WP 14

Project report WCM-2010, WP14

Monitoring of ^{226}Ra accumulation in caged Atlantic cod (*Gadus morhua*) and blue mussels (*Mytilus edulis*) exposed to an offshore produced water discharge

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Abstract

The scope of the present study was to assess the suitability of caged organisms for monitoring accumulation of a TENORM from oil field operational discharges. Muscle and gill tissue from Atlantic cod (*Gadus morhua*) and soft tissue from mussels (*Mytilus edulis*) caged close to a discharge of produced water (PW) was analysed. Exposure to PW was confirmed by elevated levels of petrogenic PAHs in the biota. Activity levels were measured by means of gamma-spectrometry, liquid scintillation and α-spectrometry. Due to high content of calcium in the gills, analysis of this tissue was not successful with the applied method. The analytical approach provided sufficient sensitivity for comparison of ^{226}Ra activity by α-spectrometry in PW exposed mussels and fish relative to non exposed reference; however accumulation of ^{226}Ra from the current discharge was not indicated.

1. Introduction

Radiation levels in produced water (PW) related to Technologically Enhanced Naturally Occurring Radioactive Materials (TENORM) is generally relatively low and the typical seawater dilution for offshore discharges is generally high. However, the Norwegian Radiation Protection Authority, Petroleum Directorate and the Pollution Control Authority have emphasized the need for more knowledge regarding radioactive discharges from the oil and gas industry (Anon. 2008) and the environmental authorities have requested monitoring of possible effects on marine organisms.

Industrial cleaning processes for purifying the produced water discharge for these components are not available and the use of scale inhibitors reduces the precipitation of the compounds which therefore may reduce sedimentation and therefore lead to a more widespread contamination. KLIF is at present evaluating the cost benefit in including the TENORM discharge in the zero discharge concept (Anon. 2008).

The dominating radionuclides in the produced water discharges are ^{226}Ra and ^{228}Ra. At present there is regularly monitoring of these radionuclides and ^{210}Pb on a monthly or quarterly basis, depending on the volume of the discharged PW. The simplest method for measuring these radionuclides is to use γ-spectrometry. This method is used for the monitoring of radioactivity in the produced water where the activity of the radium isotopes is in the order of Bq/l. Some limited information about the presence of these radionuclides in the sediment around the Ekofisk centre is also available. When measuring radionuclides in sediment, γ-spectrometry is normally used. This method is fairly simple for measuring all gamma emitting radionuclides in one simple run with very limited sample preparation. The activity in background sediment in the North Sea is in the Bq/kg order.

Very few data on these radionuclides in biological material is available, however the activity of ^{226}Ra in biota in the North Sea not exposed for any point source is expected to be at the level 0.1 Bq/ kg or lower (Hosseini et al. 2010). For such levels γ-spectrometry is not sensitive enough and there is need for more sensitive methods, e.g. chemical separation followed by α-spectroscopy. The drawback with using α-spectroscopy is that it is not possible to measure all relevant radionuclides simultaneously (^{226}Ra, ^{228}Ra, ^{210}Pb and ^{228}Th). To be able to measure both ^{226}Ra and ^{228}Ra, an ingrowth period of approximately 6 months is necessary to allow for build-up of ^{228}Th, a daughter nuclide of ^{228}Ra. In this study we decided to only measure ^{226}Ra which is the radionuclide with the highest activity in the produced water discharge at Ekofisk (Table 1).

In the present study we wanted to assess if monitoring of ^{226}Ra accumulation could be included in the annual effect monitoring by exploiting tissue samples from caged cod and mussels exposed to PW. Material for such an assessment was available from the routine monitoring program “Water Column Monitoring 2009” commenced at the Ekofisk field. A major advantage with the current biological material was the extensive amount of chemical and biomarker data available, documenting exposure to the PW plume at the study site.

Activity from the radionuclides, ^{226}Ra ^{228}Ra and ^{210}Pb in the Ekofisk PW seams to be on a rather stable level the last years (Table 1). The main contributor to the radioactivity is the ^{226}Ra isotope with an average activity of 1.5 Bq/L. The activity for ^{226}Ra in the Ekofisk PW is approximately 1000 times higher than the natural background in the North Sea. Due to the considerable dilution typical to offshore discharges, the activity level is expected to be reduced to background level (~2 mBq/L) once the plume has reached a few hundred meters from the outfall. Considerable bio magnification of the radionuclides is therefore necessary, if radiation levels in exposed organisms should reach a level causing biological effects detectable with biomarker techniques presently available. Due to the low levels of radioactivity expected to be present even in organisms caged close to an outfall, isolating biological effects caused by the radioactivity levels alone, from effects caused by other PW constituents like hydrocarbons is expected to be a major analytical challenge. To measure levels of radioactivity in exposed organisms and relate this to background levels is however feasible.

Gills of cod were selected based on the possibility of Ra to precipitate on the large surface of the respiratory tissue. In an experiment where cod and mussels were exposed to drilling mud particles it was concluded that barium (Ba) accumulated particularly in gills (Beckmann et al. 2006). As ^{226}Ra has similar properties as Ba it is likely that the particle bound ^{226}Ra will be trapped on gills of organisms caged close to a produced water discharge. Cod muscle was of interest as it was the only tissue available being used for human consumption. Soft tissue of mussels was considered relevant as ^{226}Ra may be present as particles expected to be efficiently captured from the water by filtering organisms.

Table 1. Radioactivity discharge data from the Ekofisk J platform field. (ConocoPhillips Ekofisk Discharge reports 2007, 2008 and 2009)

Year	**Produced water**	**^{226}Ra**	**^{228}Ra**	**^{210}Pb**
	m^3	Bq/l (yearly average concentration)		
2007	8 507 462	1.81	0.61	0.57
2008	9 400 000	1.53	0.52	0.63
2009	10 700 000	1.53	0.49	0.54

2. Material and methods

2.1 Field exposure

Field caging was commenced at the Ekofisk field in the North Sea as part of environmental monitoring annually imposed by Norwegian authorities. Mussels and cod originating from local farms were transported to the study site by a fish carrier (IMO 9264269). Rigs with mussels were deployed along the dominating current axis, from

approximately 0.2 to 1 km from the outfall and a rig placed at clean locations NE of Ekofisk were regarded as reference (Figure 1). On reference, station 3 and station 4 also cod (*Gadus morhua*) were deployed. All cages were deployed for 6 weeks (4^{th}-5^{th}. April to 21^{st}-22^{nd} May 2009).

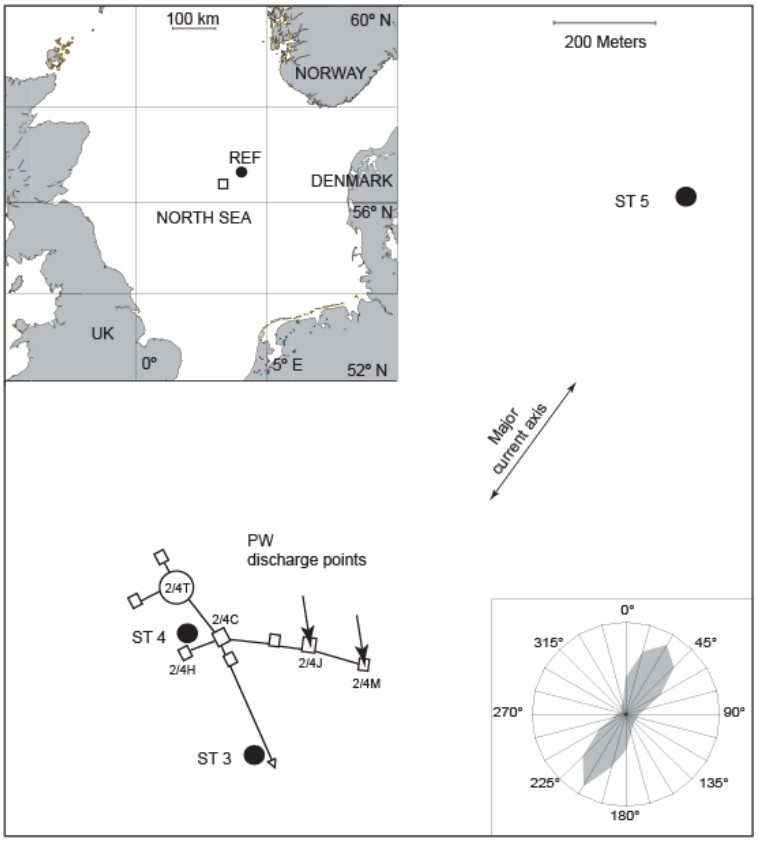

Figure 1. Sketch showing location of caging stations (ST. 3, 4 and 5) in relation to the PW discharge at the Ekofisk installations. Superimposed map show location of field in the North Sea and location of the reference station (REF, upper left), the predominant current axis is indicated by current measurements during the exposure (lower right).

2.2 Collection of tissue samples

Tissue samples of cod (gills and muscle) and mussels (soft tissue) were used for the study; rationale for selection is given in the introduction. After dissection the different tissues was divided into 3 samples containing between 100-200 g wet tissue. All samples were immediately frozen and the tissue stored at - 20°C until analysis. Due to the ^{226}Ra half life of 1602 years, degradation by storage for some months prior to analyses is considered insignificant.

2.3 Documentation of PW exposure

The main purpose of the caging experiment with mussels and cod was to monitor the discharge of produced water using biomarkers and body burden analysis of selected chemical compounds. A full report from that investigation is reported elsewhere (Brooks et al. 2011).

Increased levels of PAH in the soft tissue of mussels confirmed that the organisms that were caged close to the PW discharge was indeed exposed to PW. For the cod the best indicator for PW exposure is bile metabolites which followed the same pattern as the mussels with higher levels closer to the discharge (Table 2).

Table 2. Levels of PAH in mussel soft tissue and cod bile (Sum all PAH measured, mean ±SD) confirming exposure of PW to the caged organisms. Data from Brooks et al. (2011).

Station	Species	Analysis matrix	Distance to discharge	n	∑ measured PAH metabolites	Distance to discharge
Reference	Cod	Bile	86 km	n=16	933±187 ng/g bile	86 km
Station 3	Cod	Bile	200 m	n=16	3531±1791 ng/g bile	0.2 km
Station 4	Cod	Bile	200 m	n=16	2957±1029 ng/g bile	0.2 km
Reference	Mussel	Soft tissue	86 km	n=3 (pools)	29±1 ng/g wet weight	86 km
Station 5	Mussel	Soft tissue	1100 m	n=3 (pools)	226±182 ng/g wet weight	1.1 km
Station 3	Mussel	Soft tissue	200 m	n=3 (pools)	214±129 ng/g wet weight	0.2 km

2.4. Analyses of radioactivity levels in biota samples

Three different approaches for analysing the samples were utilized.

2.1.1 Gammaspectrometry

All samples were dried and ashed at 450 °C. The ash was then packed in closed containers. After approximately one month, the activity of ^{226}Ra was estimated by gammaspectrometry. The detection limit by using this method was too high for the results to be useful in this study,

2.4.2 Liquid scintilation

Another approach was therefore to dissolve the ashed samples in aqua regia after adding ^{133}Ba (for determination of chemical yield), followed by chemical separation of ^{226}Ra by lead- and barium sulphate precipitations. After approximately one month, the activity of ^{226}Ra was determined using liquid scintillation (Quantulus, Liquid Scintillation Spectrometer, PerkinElmer).

Due to problems with a high degree of quenching (loss of counts due to sample or cocktail characteristics) in the samples, no results were obtained using this method.

2.4.3 Analyses by α-spectroscopy

Finally the remaining ash samples were ashed again at 600 °C, spiked with ^{133}Ba for recovery estimation and subsequently treated with 8 M HNO_3. Radium was then separated by $PbSO_4$ and $BaSO_4$ precipitation before activity of ^{226}Ra was determined by α-spectroscopy.

Due to high levels of calcium in the gill tissue, this method was unsuccessful for these samples. An attempt to separate radium from calcium using manganese dioxide precipitation was attempted, but unfortunately this did not solve the problem, and no results were obtained for these samples.

The reported uncertainty is an expanded uncertainty with a coverage factor of 2 (approx. 95 % confidence level).

3. Results and discussion

We had originally planned to analyse 3 replicates of each tissue in each location. In the first test all samples was measured by γ-spectrometry. As expected the radiation levels was low and all samples ended up below detection limit. This test confirmed that the activity in these samples was low and an extensive sample pre-treatment is needed to be able to document the activity in this material. Moving into α-spectrometry was considerably more demanding as the matrix is critical. During this workup procedure some samples was lost due to lack of material.

Table 3. Levels of ^{226}Ra in individual samples of pooled organisms caged off a PW discharge (for location of stations see Figure 1).

Station	Sample type	Tissue	Sample code	^{226}Ra (mBq kg^{-1} wet weight)
Reference	Cod	Muscle	WCM09-M7	7 ± 5
Reference	Cod	Muscle	WCM09-M8	≤ 10
Reference	Cod	Muscle	WCM09-M9	17 ± 7
Station 4	Cod	Muscle	WCM09-M4	≤ 27
Station 4	Cod	Muscle	WCM09-M5	≤ 26
Station 4	Cod	Muscle	WCM09-M6	9 ± 6
Station 3	Cod	Muscle	WCM09-M1	14± 8
Station 3	Cod	Muscle	WCM09-M2	12± 8
Station 3	Cod	Muscle	WCM09-M3	17± 8
Reference	Blue mussel	Soft tissue	WCM09-BM7	≤ 90
Reference	Blue mussel	Soft tissue	WCM09-BM8	31 ± 19
Reference	Blue mussel	Soft tissue	WCM09-BM9	45 ± 19
Station 5	Blue mussel	Soft tissue	WCM09-BM4	46 ± 20
Station 5	Blue mussel	Soft tissue	WCM09-BM5	50 ± 18
Station 5	Blue mussel	Soft tissue	WCM09-BM6	30 ± 14
Station 3	Blue mussel	Soft tissue	WCM09-BM3	≤ 22

Results from the α-spectroscopy measurements are reported in Table 2. The uncertainty is individually calculated for each sample based on counting statistics and chemical yield.

The concentration of ^{226}Ra in Norwegian PW discharges ranges from below the detection limit (0.5-1 Bq/L) to 16 Bq/L (average 3.3 Bq/L, Gäfvert and Færevik 2005). Ekofisk represents fields with total radium discharge below the North Sea average.

The radiation measured in this experiment shows low levels and within the range expected for background North Sea (Hosseini et al 2010, Strålberg et al. 2003). We do have measurable activity for ^{226}Ra for both mussels and Cod tissue. From these data there is no difference between the exposed location and the reference either for cod or mussel. It was clearly documented that the cod was exposed for the produced water; however the concentration of ^{226}Ra in the PW is not sufficiently high for any measurable accumulation to take place after 6 weeks of exposure. To conduct a proper statistic treatment of the results a larger data set is needed, particularly with techniques that generate individual variance. However the main purpose for this work was to make a screening of the situation in order to evaluate if such measurements was suitable when evaluating if radiation may cause effects in biota exposed to PW discharges.

Bio magnification in the hard shell part of mussels have been reported, however with the limited shell growth expected during the standard WCM exposure duration (approximately 6 weeks) the hard shell is not considered relevant.

5. Conclusion

The scope of the present study was to investigate whether ^{226}Ra would accumulate in fish and filter feeders caged near a PW discharge and evaluate if this is a suitable approach for monitoring of PW related TENORM in the water column.

The applied analytical methods have sufficiently sensitivity to be applied on material from caged fish and mussels; however no indication of ^{226}Ra accumulation in biota caged at Ekofisk was found.

We suggest that the present study is followed up by performing α-spectrometry analyses on biota samples from organisms caged in the vicinity of PW discharge with a higher discharge of TENORM.

Acknowledgement

This study was funded by the oil companies having PW discharges to the Norwegian Continental Shelf (Project: Water Column Monitoring 2010).

References

Anon. (2008) Kostnader og nytte for miljø og samfunn ved å stille krav om injeksjon/reinjeksjon av produsert vann, nullutslipp av borekaks og borevæske og inkludere radioaktivitet i nullutslippsmålet. TA-2468/2008 - ISBN 978-82-7655-555-4

Beckman RK, Westerlund S, Baussant T, Taban IC, Pampanin DM, Smith M, Lowe D. 2008 Impacts of drilling mud discharges on water column organisms and filter feeding bivalves. Report IRIS 2006/038

Brooks, S., Harman, C., Grung, M., Finne, E.F., Ruus, A., Vingen, S., Godal, B.F., Baršienė, J., Andreikenaite, L., Skarphéðinsdóttir, H., Liewenborg, B., Sundt, R.C. 2011. Monitoring the biological effects of produced water from an offshore oil installation. Journal of Toxicology and Environmental Health (*in press*).

ConocoPhillips Ekofisk Discharge reports 2007, 2008 and 2009

Gäfvert, T., Færevik, I., 2004. Natural radioactivity in produced water from the Norwegian oil and gas industry in 2003. NRPA (Norwegian Radiation Protection Authority) report number 2005:2.

Hosseini A, Beresford N A, Brown J E, Jones D G, Phaneuf M, Thørring H and Yankovich T 2010. Background dose-rates to reference animals and plants arising from exposure to naturally occurring radionuclides in aquatic environments. Journal of Radiological Protection 30 (2010) 235–264

Strålberg, E., Varskog, A.T.S., Raaum, A., Varskog, P., 2003. Naturally Occurring Radionuclides in the Marine Environment – An Overview of Current Knowledge with Emphasis on the North Sea Area. ND/E-17/03. Report to the Norwegian Research Council. Norwegian Research Council, Norse Decom, ISBN 82-92538-01-1.

11.4 Report: WP 15

Project report WCM 2010 work package 15

Histopathological alterations in gills of Atlantic cod (*Gadus morhua* L.) exposed to North Sea Produced Water: a comparison of two field studies.

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Abstract

Atlantic cod (*Gadus morhua* L.) were caged close to an offshore Produced Water (PW) discharge for six weeks in two different field campaigns in 2008 and 2009, before and after implementation of a new treatment technology. The effects of PW exposure on gill tissue were assessed. In fish caged in 2008, the presence of various histopathological alterations (lamella fusion, hypertrophy of pavement cells, excess of mucus secretion and proliferation of epithelial cells) were significantly different relative to the reference group, supporting the use of the histopathological analysis of gills as markers of biological effects for PW monitoring. In fish caged in 2009, only epithelial hyperplasia and proliferation of epithelial cells were significantly different in prevalence relative to the reference fish. The decrease of gill lesions in samples analysed in 2009 indicated an environmental benefit from the new treatment technology.

Introduction

Produced water (PW) discharged during petroleum extraction, contains polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), decalins, organic acids and other chemical compounds (Røe Utvik, 1999). In order to monitor possible biological effects caused by PW discharges, the Norwegian environmental authorities impose biomonitoring programs annually since 1996 (Water Column Monitoring, WCM, Hylland et al. 2008; Brooks et al. 2011). Due to the relatively low toxicity and high dilution typical for offshore PW discharges, sensitive markers of exposure and effects are needed to detect effects on sentinel organisms. In this context, various biomarkers have been assessed in both laboratory and field experiments (Sundt et al. submitted a and b). In the present study Atlantic cod (*Gadus morhua* L.) was chosen since it is a relevant species for both fisheries and aquaculture and it is commonly used for exposure experiments in both laboratory and field studies (Perez-Canova et al. 2010).

Biomarkers at physiological and tissue level include histopathology of various organs. The gill is the dominant site of gas exchange, ionic-regulation, acid-base balance, and nitrogenous waste excretion. Direct contact with the surrounding water makes this organ particularly sensitive to the presence of environmental pollutants (Au 2004). Since gill epithelium may absorb a variety of lipophilic organic compounds, including PAHs (Spies et al., 1996) and is able to biotransform/metabolise them (Prasad, 1991), histopatholgical analyses of this tissue are in use as biomarkers for environmental contamination (Arellano et al., 1999; Au, 2004; Dulic et al., 2009; Oliva et al., 2009; Schwaiger et al., 1997). Moreover, these parameters seem to be less affected by physiological factors such as age, gender and gonad development status (Au, 2004). Analyses of gill lesions have been previously used as biomarkers to evaluate the health status of fish exposed to contaminants, both in laboratory (Biagini et al., 2009; Hoyle et al., 2007; Miron et al., 2008; Monteiro et al., 2008; Thophon et al., 2003) and in field studies (Fernandes et al., 2008; Khan, 2003; Stentiford et al., 2003 and 2009).

Histopathological symptoms may decrease individual fitness and are therefore markers of high ecological relevance (Woodward et al., 1983). Such pathological changes in gills are considered responsive but not always specific to pollutant exposure. Au (2004) defined such parameters to be: 1) of high ecological relevance, 2) of high sensitivity, 3) of low specificity, 4) generally not affected by confounding factors such as age, sex, temperature and 5) of medium cost effectiveness.

The aim of the present work was to assess the potential of gill lesions as biomarkers of exposure to PW compounds in Atlantic cod. The following lesions were scored: aneurisms, epithelial lifting, epithelial hyperplasia, necrosis, leukocyte infiltration, chloride cell degeneration, lamellar fusion, lamellar clubbing, epithelial proliferation, hypertrophy of pavement cells and excess mucus. Two field experiments were carried out in 2008 and 2009 at the Ekofisk oil field. In 2008 the PW discharge was treated by mechanical processes involving hydrocyclones, while the following year an additional cleaning process (CTour) was added. This technology involves extraction of lipophilic compounds by means of pressurised liquid natural gas. A detailed description of this

process and its cleaning performance is reported by Voldum et al. (2008). The comparison between the two field surveys was used to evaluate the effect of the additional cleaning treatment technology to reduce effects on biota measured as gill damages.

2. Material and methods

2.1 Field studies

As part of WCM 2008 and 2009, farmed Atlantic cod (weight: 370-870 g, length 34-44 cm) were purchased from Rygjabø aquaculture school (Finnøy, South Western Norway) and transported to the oil field for the experiments. Fish were caged at the Ekofisk field in the North Sea (Position N 56º 32' 58" E 03º 12' 45" - ED 50) for 6 weeks. Two caging rigs (station 3 and 4) were deployed less than 200 meters from the PW discharge point and a third rig was placed at a clean location northeast of the discharge (reference station, Figure 1). A detailed description of the cage design is reported in Hylland et al. (2006). Pre-exposure samples were also collected before both caging experiments.

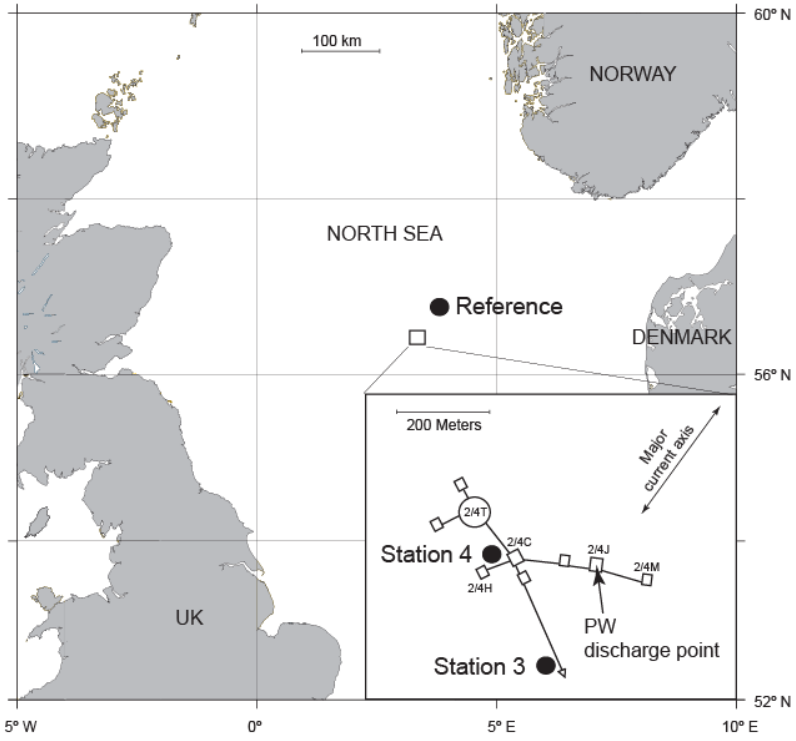

Figure 1. Location of exposed field sites (station 3 and station 4) and the unexposed reference site (Reference) in relation to the produced water (PW) fallout. The predominant current axis indicates the oscillating, tidal movement of water in the area, being mainly SW or NE. (Discharge position N 56º 32' 58" E 03º 12' 45" – ED50).

2.2 Exposure documentation

In order to document actual exposure to PW, metabolites of PAHs were analysed in fish bile. Fish bile samples were prepared and analysed by gas chromatography-mass spectrometry (GC-MS) as described by Jonsson et al. (2003; 2004). Detailed results are reported and discussed in Brooks et al. (2011).

2.3 Sampling and analysis

After 6 weeks of exposure, fish were collected from cages (22-26 fish per group) and kept for less than two hours in a tank with continuous seawater supply until sampling. Prior to dissection, fish were anesthetised with Metomidate (*Hypnodil* ^{TM}) in order to prevent histopathological alterations in the gill epithelium caused by rough handling. One piece of a random gill arch from each side of the fish was dissected. Subsequently, fish were killed by a blow to the head and macroscopically examined for fin erosion, skeletal malformation and epidermal hyperplasia. Gill samples were immediately fixed in Baker's solution (4% formaldehyde, 1% $CaCl_2$). Tissues were dehydrated in ethanol, then rinsed and cleared in a tissue processor (*Shandon Excelsior, Thermo*) before embedding in paraffin wax. Histological sections (3 µm thickness) were obtained using a microtome (*HM 355s, Bergman*), mounted on slides and stained in haematoxylin, eosin and saffron (HES) in an automated staining machine (*Tribune Stainer, Surgipath*). Sections were evaluated using a microscope (*Zeiss Axioplan 2*) and all micrographs were captured using a digital colour camera (*AxioCam*).

2.4 Scoring of histopathological lesions in gills

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

2.5 Statistical analysis

Since obtained data were not normally distributed, a non-parametric test (Mann-Whitney U test) was used to compare groups (Dytham, 2003) A probability level of <0.05 was considered significant (SPSS, version 18).

3. Results

3.1 Exposure documentation

Measured levels of PAH metabolites in fish bile are reported in Table 1. Levels were higher in fish caged in the vicinity of the PW outfall relative to the levels recorded in fish caged at the reference station in both field studies.

Table 1. Levels of PAH metabolites (ng/g) in cod bile. Quantification limits (LOQ) were 0.005 ng/g for single compound; nd: not detected, n= 16, data from Brooks et al. 2011)

Compounds	**2008**			**2009**		
	Reference	**Station 3**	**Station 4**	**Reference**	**Station 3**	**Station 4**
1-OH-Naphthalene	nd	<LOQ	<LOQ	nd	<LOQ	nd
2-OH-Naphthalene	<LOQ	41±42	27±15	nd	26±27	<LOQ
C_1-OH-Naphthalene	266±60	415±145	340±86	204±36	392±145	344±78
C_2-OH-Naphthalene	<LOQ	554±286	359±163	nd	557±436	370±169
C_3-OH-Naphthalene	<LOQ	1194±285	962±269	<LOQ	933±459	<LOQ
1-OH-Phenanthrene	<LOQ	31±32	39±27	<LOQ	<LOQ	<LOQ
C_1-OH-Phenanthrene	214±52	634±287	586±225	<LOQ	868±473	762±351
C_2-OH-Phenanthrene	<LOQ	767±219	784±212	<LOQ	725±316	679±224
1-OH-Pyrene	<LOQ	21±15	21±10	<LOQ	22±23	17±17

3.2 Sampling

Pathological symptoms such as fin erosion, skeletal malformation and epidermal hyperplasia were checked in sampled fish. Only cod without major visible external damages were used in the present study.

3.3 Histopathological lesions in gills

Eleven gill histopathological lesions were identified in fish caged at the Ekofisk field in 2008 (Table 2). Proliferation of epithelial cells, epithelial hyperplasia and epithelial lifting represented the more common lesions in fish caged close to the PW discharge. These lesions were also recorded in a few fish from the reference station. Prevalence of hypertrophy of pavement cells, excess of mucus secretion and proliferation of epithelial celle were significantly higher in gills of fish caged at station 3 compared to the reference station. Levels of lamellar fusion and proliferation of epithelial cells were significantly higher in gills from fish caged at station 4 relative to the reference group. Moreover, chloride cell degeneration, excess mucus secretion, hypertrophy of pavement cells and aneurisms were recorded only in fish caged at station 3 and 4 and not in fish caged in the reference station.

Only nine lesions were detected in gills of fish caged at the Ekofisk oil field in 2009, as hypertrophy of pavement cells and chloride cell degeneration were not recorded in any fish. Prevalence of epithelial hyperplasia and proliferation of epithelial cells was

significantly higher in gills of fish caged close to the PW outfall (station 3 and 4) relative to the prevalence recorded in gills of fish caged at the reference station. The total amount of the other lesions were comparable in fish caged at station 3 and 4 and at the reference station. With only one exception, no aneurism lesions were scored.

Statistical comparison of data from pre exposure and reference station fish is reported in Table 2b. In 2008, the two exposed groups were similar for all the recorded gill lesions (with the exception of lamellar clubbing). In 2009, the two exposed groups were comparable according to seven of the lesions scored.

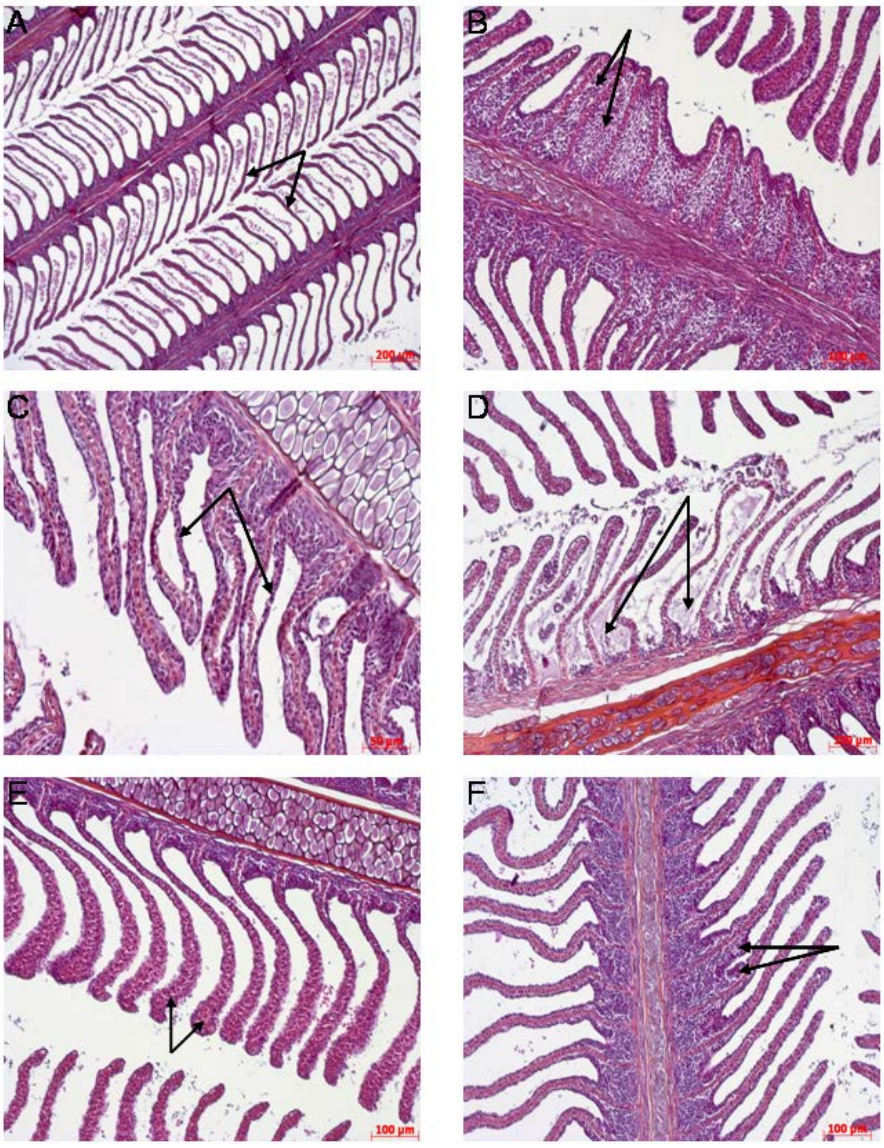

Figure 2. Histological sections (3µm) of gills from Atlantic cod (*Gadus morhua* L.) stained with haematoxylin, eosin and saffron (HES). A: gill from the reference station, arrows indicate secondary lamellas. B-F: representative sections of gills from station 3 and 4, arrows indicate the following lesions: B, fusion of secondary lamellae; C, epithelial lifting, D: necrotic epithelium, E: epithelial proliferation; F, epithelial hyperplasia. Scale bars are given in lower right corner of each panel.

Table 2. All histopathological lesions scored in gill samples from both field studies. Results are reported as sum of all lesions of fish per station (a) and as statistical comparison (p-values from Mann-Whitney U test, significantly differences are reported in colour) between all groups and the reference station (b) (nd = not determined) (n of analysed fish = 21-22).

a		Aneurisms	Epithelial lifting	Epithelial hyperplasia	Lamellar fusion	Lamellar clubbing	Hypertrophy pavement cells	Chloride cells damage	Leukocyte infiltration	Excess mucus secretion	Necrosis	Proliferation of epithelial cells
2008	Pre-exposure	1	1	9	10	0	0	0	0	2	0	17
	Reference	0	4	13	10	4	0	0	0	0	2	19
	Station 3	4	23	26	8	1	10	12	0	11	11	42
	Station 4	9	11	26	34	6	6	14	7	6	2	52
2009	Pre-exposure	0	4	23	4	1	0	0	1	18	3	37
	Reference	0	12	29	9	0	0	0	3	39	4	34
	Station 3	0	9	48	15	0	0	0	6	43	5	57
	Station 4	1	14	47	9	1	0	0	2	34	7	53

b		Aneurisms	Epithelial lifting	Epithelial hyperplasia	Lamellar fusion	Lamellar clubbing	Hypertrophy pavement cells	Chloride cells damage	Leukocyte infiltration	Excess mucus secretion	Necrosis	Proliferation of epithelial cells
2008	Reference											
	Pre exp	nd	0,172	0,644	0,937	0,041	1,000	1,000	1,000	0,211	0,100	0,396
	Stat 3	nd	0,070	0,068	0,858	0,229	0,025	0,092	1,000	0,048	0,654	0,013
	Stat 4	nd	0,234	0,209	0,005	0,635	0,359	0,188	1,000	0,102	0,453	0,000
2009	Reference											
	Pre exp	1,000	0,047	0,427	0,542	0,292	nd	nd	0,595	0,005	0,794	0,416
	Stat 3	1,000	0,196	0,004	0,368	1,000	nd	nd	0,273	0,332	1,000	0,003
	Stat 4	0,278	0,797	0,006	0,738	0,278	nd	nd	0,629	0,775	0,270	0,044

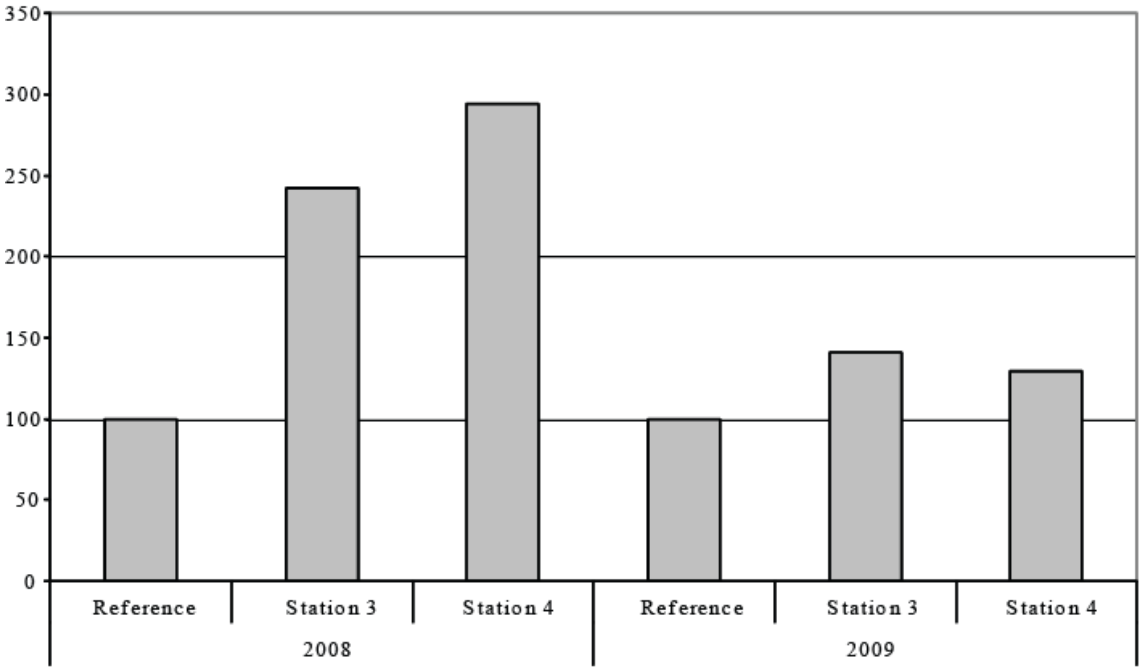

Figure 3. Comparison between the 2008 and 2009 field studies. Sum of histological lesions, normalised to the mean of the reference values.

To compare levels of gill lesions scored in caged fish in the two different campaigns (2008 and 2009), data were normalised to the mean of the reference station values (Figure 3). A decrease in the total amount of lesions in exposed fish from the 2009 campaign relative to the exposed fish from the 2008 campaign was evident.

Some individuals collected from both field studies were infected by intracellular bacteria, possibly epitheliocystis, in the gills (7% of the fish caged in 2008 and 9.5% of the fish caged in 2009). Exclusion of infected individuals did not change conclusions from the statistical analysis.

4. Discussion

4.1 Exposure documentation

The high relevance of fish bile metabolites in relation to PW exposure has been observed in previous environmental monitoring campaigns in the North Sea (Hylland et al., 2008, Brooks et al 2011, Sundt et al. submitted b), as well as in laboratory studies (Sundt et al, 2009). PAH metabolite levels in bile were higher in fish caged close to the PW outfall relative to the levels recorded in fish caged at the reference station in both campaigns, confirming exposure of the fish to PW.

Discharged volume of PW, concentration of oil in water, estimated concentration of PAHs in the water and the total amount of oil discharged were different in 2008 and

2009 (Table 3), expectedly due to the effect of the new cleaning technology implemented.

Table 3. The volume and components (concentration of Oil in Water and PAH, and Total oil discharged) of the PW discharged during the individual monitoring surveys at Ekofisk, comparing 2008 with 2009. The percentage difference from 2008 to 2009 is indicated in brackets. *: estimated concentrations provided by ConocoPhillips based on a correlation between concentration of Oil in Water and PAH concentration.

Study	**2008**	**2009**	**Discharge development from 2008 to 2009**
Discharged volume (x1000L)	935	1.180	+26%
Measured concentration of Oil in the water (mg/ml)	11.8	7.1	-40%
Estimated concentration of PAHs (µg/L) *	341	278	-19%
Total oil discharged (kg)	11.024	8.392	-24%

4.2 Histopathological lesions in gills

Since the gills of fish are in close contact with the surrounding water the tissue structure is vulnerable to pollutants. Gill pathologies are common symptoms of toxic effects on fish of a wide range of aquatic pollutants including PAHs, organophosphate compounds and heavy metals (Mallatt, 1985; Au, 2004). Previous histopathological studies of effects from different exposure sources (including PW, oil refinery discharges, water-soluble fractions of diesel and crude oil, oil sands process-affected water and mining-associated waters, seep-related hydrocarbons and refined oil) reported the occurrence of several of the same lesions observed in the present study (Khan, 1998, Simonato et al., 2008, Khan, 2003, Prasad, 1991, Spies et al., 1996, Woodward et al., 1983, Van den Heuvel et al., 2000, Stephens et al., 2000, Brand et al., 2001, Khan and Kiceniuk, 1988, Nero et al., 2006).

Lifting of the gill epithelium is considered to be an acute response to environmental conditions (Au, 2004, Sensini et al., 2008). The presence of this lesion was high only in fish exposed to PW in 2008, indicating an improvement of the environmental condition.

Epithelial hyperplasia, lamellar fusion and clubbing are believed to represent chronic responses to environmental effects (Poppe, 1999), where epithelial hyperplasia is an early stage of lamellar fusion (Cerqueira and Fernandes, 2002; Oliva et al., 2009; Poppe, 1999). Values of the more severe lamellar fusion lesion were significantly higher in fish caged close to the PW discharge in the 2008 field survey. Only in 2009, where the scores of epithelial hyperplasia were significantly higher in fish caged close

to the Ekofisk outfall relative to the reference group. Since epithelial hyperplasia is considered as a low level damage, as it represents an initial stage of lamellar fusion, these results indicated an improvement of the water quality.

Excess of mucus secretion was significantly higher in fish caged close to the outfall only in the 2008 campaign. Some of the observed histological changes indicated a classic defence response to stressors. Epithelial lifting and excess of mucus secretion increase the distance through which the toxicant has to travel to reach the blood stream (Ortiz et al., 2003), while lamellar fusion diminishes the amount of vulnerable gill surface area (Mallatt, 1985).

Even though fish had gills lesions expected to be caused by PW compounds in both 2008 and 2009 campaigns, the comparison between results from the two years showed a general decrease in gill lesions in fish caged in 2009, indicating an improvement of the environmental condition that may be associated to adding additional treatment technology, as the *CTour*, to the one already in place. A parallel study applying other biomarkers showed similar results (Brooks et al. 2011). PW is a complex mixture of organic compounds from oil in the reservoir and chemicals added to facilitate production. In order to optimize cleaning strategy, knowledge about what types of compounds cause the different effects is important. The fact that the discharged volume increased from 2008 to 2009 by about 26% and that the exposure of most PAH compounds was reduced by 19%, may indicate that hydrocarbons contributed significantly to the observed effects. However Srephens et al. (2000) indicated that lesions observed in PW exposed turbot may be caused by production chemicals. Lipophilic compounds are more efficiently removed by the extraction in the cleaning process compared to water soluble production chemicals (e.g. scale and corrosion inhibitors) which are expected to be less efficiently captured by the process.

4.3 Physiological consequences of gill lesions

The present study showed that Ekofisk PW contains compounds with potential to cause detectable lesions in gills of caged fish, given a sufficiently high exposure concentration and long duration. Histopathology can reflect the disturbance in physiological and/or biochemical function (Hinton et al., 1992). The observed gill lesions may disturb both gas exchange and ionic regulation of fish, possibly affecting fish health. For example, potential physiological effects were seen in cutthroat trout (*Oncorhynchus clarkii*) exposed to refined oil. Significantly reduced swimming performance has been reported in fish affected by hyperplasia, lamellar fusion and necrotic tissue (Woodward et al., 1983).

Due to the significant dilution typical for offshore discharges and the fact that wild fish are capable of escaping unfavourable conditions close to the discharge point, it is likely that wild fish are less affected if affected at all.

4.4 Suitability of histological lesions in gills as biomarkers of exposure to PW

To be useful in practical and routine monitoring, selected biomarkers should be ecologically relevant, sensitive and responsive to environmentally realistic

concentrations, and preferably exhibit a good dose response relationship to levels of pollutants (Au, 2004).

As previously mentioned, several of the alterations scored in the present study have previously been observed in fish exposed to other types of hydrocarbons. The present study showed that several lesions (epithelial hyperplasia, lamellar fusion, hypertrophy of pavement cells, excess of mucus secretion and proliferation of epithelial cells) may be suitable as sensitive biological markers for biomonitoring of PW discharges. Epithelial hyperplasia and proliferation of epithelial cells were the most sensitive parameters, being capable of discriminating both caging stations placed close to the PW outfall in relation to the reference site in both field surveys.

Laboratory exposure to PW, including a recovery period, should be conducted to better assess the potential of these biomarkers and to find possible dose response relationships. NOEC (No Observable Effect Concentration) values for the different histological alterations could be established for risk assessment evaluations.

5. Conclusions

- Exposure to North Sea PW can cause detectable lesions in gills of Atlantic cod if the exposure is sufficiently extensive in terms of concentration and duration.
- The present study indicated that several of the scored gill lesions may be suitable as biomarkers of exposure to PW.
- The comparison of results from two different field exposures indicated reduction in gill lesions after installation of additional cleaning technology.
- Whether wild fish living in the vicinity of PW outfalls may be sufficiently exposed to form gill lesions should be investigated.

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7. References

Arellano, J.M., Storch, V., Sarasquete, C., 1999. Histological changes and copper accumulation in liver and gills of the senegales sole, *Solea senegalensis*. Ecotox. Environ. Safe. 44, 62-72.

Au, D.W.T., 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Marine Pollution Bulletin 48, 817-834.

Benly, A.Ç.K., Köksal, G., Özkul, A., 2008. Sublethal ammonia exposure of Nile tilapia (Oreochromis niloticus L.): effects on gill, liver and kidney histology. Chemosphere 72, 1355-1358.

Biagini, F.R., David, J.A.D., Fontanetti, C.S., 2009. The use of histological, histochemical and ultramorphological techniques to detect gill alterations in *Oreochromis niloticus* reared in treated polluted waters. Micron 40, 839-844.

Brand, D.G., Fink, R., Bengeyfield, W., Birtwell, I.K., McAllister, C.D., 2001. Salt water-acclimated pink salmon fry (*Oncorhynchus gorbuscha*) develop stress-related visceral lesions after 10-day exposure to sublethal concentrations of the water-soluble fraction of north slope crude oil. Toxicol. Pathol. 29, 574-584.

Brooks, S.J., Harman, C., Grung, M., Farmen, E., Ruus, A., Vingen, S., Godal, B.F., Baršienė, J., Andreikėnaitė, L., Skarphéðinsdóttir, H., Liewenborg, B., Sundt, R.C.,2011. Water column monitoring of the biological effects of produced water from the Ekofisk offshore oil installation from 2006 to 2009. Journal of Toxicology and Environmental Health, in press.

Cerqueira, C.C.C., Fernandes, M.N., 2002. Gill Tissue Recovery after Copper Exposure and Blood Parameter Responses in the Tropical Fish *Prochilodus scrofa*. Ecotox. Environ. Safe. 52, 83-91.

Dytham C., 2003. Choosing and using statistics. a biologist's guide. blackwell publishing, pp 248.

Dulic, Z., Poleksic, V., Raskovic, B., Lakic, N., Markovic, Z., Zivic, I., Stankovic, M., 2009. Assessment of the water quality of aquatic resources using biological methods. Desalin. Water Treat. 11, 264-274.

Fernandes, C., Fontainhas-Fernandes, A., Ferreira, M., Salgado, M.A., 2008. Oxidative stress response in gill and liver of *Liza saliens*, from the Esmoriz-Paramos coastal lagoon, Portugal. Arch. Environ. Contam. Toxicol. 55, 262-269.

Jonsson G., Beyer J., Wells D., Ariese F. 2003. The application of HPLC-F and GC-MS to the analysis of selected hydroxy polycyclic hydrocarbons in two certified fish bile reference materials. Journal of Environmental Monitoring 5(3): 513-520

Jonsson, M.E., Brunström, B., Ingebrigtsen, K., Brandt, I., 2004. Cell-specific CYP1A expression and benzo[a]pyrene adduct formation in gills of rainbow trout (Oncorhynchus mykiss) following CYP1A induction in the laboratory and in the field. Environmental Toxicology and Chemistry 23 (4), 874-882.

Hinton, D.E., Baumann, P., Gardner, G., Hawkins, W., Hendricks, J., Murchelano, R., Okihiro, M., 1992. Histopathologic biomarkers, in: Huggett RJ, K.R., Mehrle PM, Bergman HL. (Ed.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Boca Raton, FL. , pp. 155-210.

Hoyle, I., Shaw, B.J., Handy, R.D., 2007. Dietary copper exposure in the African walking catfish, *Clarias gariepinus*: Transient osmoregulatory disturbances and oxidative stress. Aquat. Toxicol. 83, 62-72.

Hylland, K., Serigstad, B., Thain, J.E., 2006. *In situ* deployment of organisms and passive samplers during BECPELAG workshop: introduction and summary. In "Biological effects of contaminants in marine pelagic ecosystem" (ICES), Hylland K., Vethaak A.D., Lang T. (eds.) SETAC publications, pp 167-170.

Hylland, K., Tollefsen, K.-E., Ruus, A., Jonsson, G., Sundt, R.C., Sanni, S., Røe Utvik, T.I., Johnsen, S., Nilssen, I., Pinturier, L., Balk, L., Barsiene, J., Marigòmez, I., Feist, S.W., Børseth, J.F., 2008. Water column monitoring near oil installations in the North Sea 2001-2004. Marine Pollution Bulletin 56, 414-429.

Khan, R.A., 1998. Influence of petroleum at a refinery terminal on feral winter flounder, *Pleuronectes americanus*. Bull. Environ. Contam. Toxicol. 61, 770-777.

Khan, R.A., 2003. Health of flatfish from localities in Placentia Bay, Newfoundland, contaminated with petroleum and PCBs. Arch. Environ. Contam. Toxicol. 44, 485-492.

Khan, R.A., Kiceniuk, J.W., 1988. Effect of Petroleum Aromatic-Hydrocarbons on Monogeneids Parasitizing Atlantic Cod, *Gadus-Morhua* L. Bull. Environ. Contam. Toxicol. 41, 94-100.

Mallatt, J., 1985. Fish Gill Structural Changes Induced by Toxicants and Other Irritants: A Statistical Review. Canadian Journal of Fisheries and Aquatic Sciences 42, 630-648.

Miron, D.D., Moraes, B., Becker, A.G., Crestani, M., Spanevello, R., Loro, V.L., Baldisserotto, B., 2008. Ammonia and pH effects on some metabolic parameters and gill histology of silver catfish, *Rhamdia quelen* (Heptapteridae). Aquaculture 277, 192-196.

Monteiro, S.M., Rocha, E., Fontainhas-Fernandes, A., Sousa, M., 2008. Quantitative histopathology of *Oreochromis niloticus* gills after copper exposure. J. Fish Biol. 73, 1376-1392.

Nero, V., Farwell, A., Lister, A., Van der Kraak, G., Lee, L.E.J., Van Meer, T., MacKinnon, M.D., Dixon, D.G., 2006. Gill and liver histopathological changes in yellow perch (*Perca flavescens*) and goldfish (*Carassius auratus*) exposed to oil sands process-affected water. Ecotox. Environ. Safe. 63, 365-377.

Oliva, M., Garrido, M.C., Marquez, D.S., de Canales, M.L.G., 2009. Sublethal and lethal toxicity in juvenile Senegal sole (*Solea senegalensis*) exposed to copper: A preliminary toxicity range-finding test. Exp. Toxicol. Pathol. 61, 113-121.

Ortiz, J.B., Gonzales De Canales, L., Sarasquete, C., 2003. Histopathological changes induced by lindane (γ-HCH) in varipus organs of fishes. Scientia Marina, 67, 53-61.

Perez-Casanova, J.C., Hamoutene, D., Samuelson, S., Burt, K.. King, T.L., Lee. K., 2010. The immune response of juvenile Atlantic cod (Gadus morhua L.) to chronic exposure to produce water. Mar. Environ. Res. 70, 26-34.

Poppe, T., 1999. Systematisk patologi og patofysiologi, in: Poppe, T. (Ed.), Fiskehelse og fiskesykdommer. Universitetsforlaget, Oslo, pp. 12-36.

Prasad, M.S., 1991. Sem Study on the effects of crude-oil on the gills and air breathing organs of Climbing perch *Anabas Testudineus* Bull. Environ. Contam. Toxicol. 47, 882-889.

Røe Utvik, T.I.R., 1999. Chemical characterisation of produced water from four offshore oil production platforms in the North Sea. Chemosphere 39, 2593-2606.

Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W., Triebskorn, R., 1997. The use of histopathological indicators to evaluate contaminat-related stress in fish. Journal of Aquatic Ecosystem Stress and Recovery 6, 75-86.

Sensini, C., Della Torr C., Corsi, I., Focardi S., 2008. First observations of histopathological effects of 2,4,6-trinitrotoluene (TNT) in gills of European eel Anguilla anguilla (Linnaeus, 1758). Cellular Biology and Toxicology, 24, 621-628.

Simonato, J.D., Guedes, C.L.B., Martinez, C.B.R., 2008. Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. Ecotox. Environ. Safe. 69, 112-120.

Spies, R.B., Stegeman, J.J., Hinton, D.E., Woodin, B., Smolowitz, R., Okihiro, M., Shea, D., 1996. Biomarkers of hydrocarbon exposure and sublethal effects in embiotocid fishes from a natural petroleum seep in the Santa Barbara Channel. Aquat. Toxicol. 34, 195-219.

Stentiford, G.D., Bignell, J.P., Lyons, B.P., Feist, S.W., 2009. Site-specific disease profiles in fish and their use in environmental monitoring. Mar. Ecol.-Prog. Ser. 381, 1-15.

Stentiford, G.D., Longshaw, M., Lyons, B.P., Jones, G., Green, M., Feist, S.W., 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar. Environ. Res. 55, 137-159.

Stephens, S.M., Frankling, S.C., Stagg, R.M., Brown, J.A., 2000. Sub-lethal effects of exposure of juvenile turbot to oil produced water. Marine Pollution Bulletin 40, 928-937.

Sundt, R.C., Meier, S., Jonsson, G., Sanni, S., Beyer, J., 2009. Development of a laboratory exposure system using marine fish to carry out realistic effect studies with produced water discharged from offshore oil production. Marine Pollution Bulletin 58 (9), 1382-1388.

Sundt R.C., Pampanin D.M., Grung M., Baršienė. J., Ruus A., submitted a. Bioaccumulation and biomarker responses in mussels (Mytilus edulis) exposed to produced water from a North Sea oil field: laboratory and field validations.

Sundt, R.C., Ruus, A., Jonsson, H., Skarphéðinsdóttirc, H., Meier, S., Grung, M., Pampanin, D.M., submitted b. Biomarker responses in Atlantic cod (Gadus morhua) exposed to Produced Water from a North Sea oil field: laboratory and field assessments.

Thophon, S., Kruatrachue, M., Upathan, E.S., Pokethitiyook, P., Sahaphong, S., Jarikhuan., S., 2003. Histopathological alterations of white seabass, Lates calcarifer in acute and subchronic cadmium exposure. Environmental Pollution, 307-320.

Van den Heuvel, M.R., Power, M., Richards, J., MacKinnon, M., Dixon, D.G., 2000. Disease and gill lesions in yellow perch (*Perca flavescens*) exposed to oil sands mining-associated waters. Ecotox. Environ. Safe. 46, 334-341.

Voldum, K., Garpestad, E., Anderssen, N.O., Henriksen, I.B., 2008. The CTour Process, An Option to Comply with Zero Harmful Discharge Legislation in Norwegian Waters - Experience of CTour Installation on Ekofisk After Start up 4th Quarter 2007, Abu Dhabi International Petroleum Exhibition and Conference, 3-6 November 2008 Abu Dhabi, UAE.

Woodward, D.F., Riley, R.G., Smith, C.E., 1983. Accumulation, Sublethal Effects, and Safe Concentration of Refined Oil as Evaluated with Cutthroat Trout. Arch. Environ. Contam. Toxicol. 12, 455-464.