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Water Column Monitoring 2011

Gullfaks C platform

Final report

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REPORT

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Abstract

The report presents results from the Water Column Monitoring survey 2011, performed in collaboration by NIVA and IRIS. The objective of the survey was to assess the extent to which produced water (PW) discharged from Gullfaks C platform affects organisms living in the water column. The study was designed to monitor bioaccumulation and biomarker responses in mussels held in cages in the vicinity of the water discharge point, with supporting information from passive sampling devices. Significantly greater bioaccumulation of PAH and NPD compounds was found in mussels from the two stations positioned 500 m from the platform, with concentrations significantly higher in mussels from one of the 500 m stations (i.e. station 2). All other mussel stations positioned 1000 m and 2000 m from the platform had PAH-NPD bioaccumulation typical of offshore background concentrations. There was very good agreement between the biomarker responses and the chemical concentration data. The calculated integrated biological response (IBR/n) was markedly higher in mussels from station 2, indicating poorer health. The IBR/n was also slightly raised in mussels from station 3 (1000 m), which was considered to be due to other chemicals within the PW. Alkyl phenols and naphthenic acids were detected in all POCIS placed at selected mussel stations from 500 to 2000 m, with mussel station 2 (500 m) and 3 (1000 m) showing highest concentrations of these compounds. PAH metabolites were detected in wild caught whiting (*Merlangius merlangus*) and tusk (*Brosme brosme*). The measured PAH metabolites in both fish species were indicative of weathered PW chemicals. Overall chemical bioaccumulation and impaired health to caged mussels was observed in mussels exposed to the PW plume located 500 m downstream from the platform.

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Water Column Monitoring 2011

Gullfaks C platform

Preface

The Water Column Monitoring (WCM) programme performs investigations into the potential biological effects of offshore oil and gas activity on the biota living within the water column of the Norwegian sector of the North Sea. Oil companies in the Norwegian sector with produced water discharges, who funded the project, are obliged by the Norwegian authorities to perform water column monitoring offshore. The work has been performed at various fields within the Norwegian sector over the last 20 years. The methods used are considered to be the best available technology for the assessment of biological effects monitoring, measuring chemical bioaccumulation of oil related compounds in mussels and passive sampling devices as well as a suite of biomarker responses in mussels. Integration of the chemical and biological effects data enables a comprehensive assessment of the effects of the produced water on organism health.

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

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Summary

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Alkyl phenols and naphthenic acids were detected in all passive samplers (POCIS) placed at selected mussel stations from 500 to 2000 m, with mussel station 2 (500 m) and 3 (1000 m) showing highest concentrations of these compounds.

PAH metabolites were detected in wild caught whiting (*Merlangius merlangus*) and tusk (*Brosme brosme*). The measured PAH metabolites in both fish species were indicative of weathered PW chemicals.

Overall chemical bioaccumulation and impaired health to caged mussels was observed in mussels exposed to the PW plume located 500 m downstream from the platform.

List of Symbols

AP	Alkyl phenol
BP	Base pair
Bq	Becquerel
BTEX	Benzene, toluene, etyhyl benzene and xylene
$C_1 - C_9$	referring to the number of carbons in a side chain (e.g. on a PAH or phenol)
CI	Condition index
CYP1A	Cytochrome P450 1A (CYP1A) proteins
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked ImmunoSorbent Assay
FF	Fixed fluorescence
GC-MS	Gas chromatography – Mass Spectrometry
GST	Glutathione-S-Transferase
HPLC	High performance liquid chromatography
IBR	Integrated biological response
IRIS	International Research Institute of Stavanger
LF	Lipofuscin accumulation
LMS	Lysosomal membrane stability
MFO	Mixed function oxidase
MN	Micronucleus/ micronuclei
NA	Naphthenic acid
NIVA	Norwegian Institute for Water Research
NL	Neutral lipid
NRRT	Neutral red retention time
NPD	Naphthalenes, Phenanthrenes and Dibenzothiophenes
OLF	Norwegian Oil Industry Association
PAH	Polycyclic aromatic hydrocarbon
PH	Pyrene hydroxylase
PW	Produced Water
PES	Polyethersulphone
POCIS	Polar organic chemical integrative sampler
RPM	Revolutions per minute
SBE	Seabird electronics
SIM	Selected ion monitoring
SPMD	Semi-permeable membrane device
VTG	Vitellogenin (precursor of egg yolk protein)
WCM	Water Column Monitoring
w.w.	Wet weight
ZRP	Zona radiata protein (egg shell protein)

1. Introduction

1.1 General purpose of the study

The Water Column Monitoring (WCM) programme is an annual study that is designed to use the best available biological effects tools to determine the potential impact of the offshore oil and gas activities on the local marine environment. Within the Norwegian sector of the North Sea, the offshore operators are obliged to carry out environmental monitoring within the water column in the vicinity of the offshore installations. This obligation requires that monitoring of the water column should be carried out in at least one offshore oil and gas field each year. Although approval is required by the Norwegian authorities, the operators can choose the study area and the design of the program. The operators then select contractors that perform the study based on the proposed program. In 2011, the Gullfaks C platform was chosen as the study site. Statoil was the operator at Gullfaks C, whom became the main representative for the OLF group.

1.2 Background on the WCM programme

Organisms living in the water column around offshore oil and gas production facilities are predominantly exposed to chemicals through discharge of production water. Produced water (PW) is the water that is extracted together with oil and gas and usually is a combination of: 1) formation water contained naturally in the reservoir; 2) injected seawater to aid in extraction and 3) treatment chemicals added during the production. However, the amount and composition of PW varies widely from field to field (Røe, 1998).

Typically PW contains dissolved inorganic salts, minerals and heavy metals together with dissolved and dispersed oil components and other organic compounds. The specific chemical composition varies between reservoirs and within a reservoir as production proceeds. A target chemical characterisation of four offshore oil production platforms in the North Sea 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 and Johnsen, 1996; 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. Although the vast majority of oil present in PW is removed before discharge (current discharge limit 30 mg oil/L, actual average discharge from Gullfaks C 14 mg oil/L), significant quantities of the PW compounds are released into the seas due to the large volumes involved. The total discharge of PW in the Norwegian sector of the North Sea in 2010 was approximately 131 million m^3 (Norwegian Petroleum Directorate, 2011).

Some of the organic chemicals found in PW are relatively resistant to biodegradation, have a bioaccumulation potential and may be toxic to organisms in receiving waters (Brendehaug et al., 1992; Tollefsen et al., 1998; Taban and Børseth, 2000; Aas et al., 2000a). This applies in particular to groups of chemicals such as alkyl phenols (APs) and polycyclic aromatic hydrocarbons (PAHs) that are known to produce various toxic effects including reproductive disturbances, mutagenicity and carcinogenicity (Landahl et al., 1990; Bechmann, 1999; Lye, 2000; Meier et al., 2002). Studies from the ICES workshop "Biological effects of contaminants in the pelagic ecosystem (BECPELAG)" indicate that 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 are limited by the lack of sufficient *in situ* monitoring data using biological effects methods with endpoints reflecting long term (ecological) effects.

Biological markers (biomarkers) have been developed to measure the biological response related to an 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, 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-integrating 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 (i.e. population 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. Care must be taken to avoid misuse of biomarker data in trials to extrapolate to ecologically relevant effects (Lam and Gray, 2003). The combination of laboratory and field validation of the different biomarkers 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).

1.3 Objective

The principal objective of the WCM survey 2011 was to assess the extent to which PW discharges from an oil production platform (Gullfaks C) affect organisms living in the water column. To fulfil this objective a combination of chemical concentrations and biological effects measurements in caged mussels, positioned at strategic locations with respect to the PW discharge, were used. Supporting chemical information from passive sampling devices provided additional information to determine the potential biological effects of the PW discharge within the water column.

1.4 Background on the chemical and biological methods used

1.4.1 Mussel PAH bioaccumulation

The organic fraction of PW is dominated by low molecular PAHs, such as NPDs, decalins and their alkyl homologues (Utvik, 1999). High molecular PAHs such as benzopyrene, pyrene and chrysene are also naturally present in effluents of PW from production platforms in the North Sea, although usually at lower concentrations. Many of the low molecular weight PAHs have also been detected in caged organisms deployed downstream from known discharge points (Røe, 1998). This applies in particular to alkyl substituted NPDs, which have been found in higher concentrations than their non-alkylated parent compounds in biological tissues and passive sampling devices (Røe, 1998; Ruus et al., 2006). Measurement of contaminant body burdens in caged animals are commonly used to assess the exposure situation in a specified area (Brooks et al., 2011a,b; Sundt et al., 2011).

1.4.2 ^{226}Radium

Radiation levels in 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. The dominating radionuclides in the PW discharges are ^{226}Ra and ^{228}Ra. At present there is regular monitoring of these radionuclides as well as ^{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 -spectrometry. This method is used for the monitoring of radioactivity in the PW where the activity of the radium isotopes is in the order of Bq/L. A more sensitive analytical technique based on α-spectroscopy was adapted as

part of WCM 2010 (work package 14). This method is suitable for determining the low levels typically present in biota.

1.4.3 *Mytilus* species identification

It has been recently confirmed that a mixed population of *Mytilus* spp. occurs on the Norwegian coastline, with *M. edulis*, *M. trossulus* and *M. galloprovincialis* as well as hybrids between the three species found in a patchy distribution (Väinölä & Strelkov, 2011; Brooks & Farmen, in press). The potential differences in the bioaccumulation of contaminants as well as biological responses to stress either environmental or chemical between the different *Mytilus* spp. has the potential to reduce the effectiveness of biomonitoring programmes. For example, with respect to contaminant exposure, higher metal concentrations were detected in *M. trossulus* compared to *M. edulis* when collected from the same habitat and in the same size range (Lobel et al., 1990). The differences in the bioaccumulation of metals between the two *Mytilus spp*. was suggested to be due to the slower growth rate of *M. trossulus* compared to *M. edulis* rather than to any direct differences between the element metabolisms of the species. Hence, *M. trossulus* of the same size as *M. edulis* would be in fact older and have a longer exposure history resulting in higher contaminant concentrations. Differences in biomarker responses between the different species have not been investigated. However, there are a few cases where differences in general physiology and behaviour of the mussel could affect the overall fitness and biological response, such as reproductive strategy (Hilbish et al. 2002; Dias et al., 2011) and susceptibility to parasitism (Coustau et al., 1991). Consequently, it is highly recommended for *Mytilus* species to be determined for biomonitoring programmes and that only one species should be used.

1.4.4 Pyrene hydroxylase

Pyrene hydroxylase represents an enzymatic activity commonly grouped as mixed function oxidases (MFOs), i.e. cytochrome P450 enzymes. These enzymes metabolise selected PAHs and consequently alter potentially harmful chemicals to non-toxic and readily excretable end products. Pyrene hydroxylase is considered to be a model PAH with a single phase I metabolite i.e. 1-hydroxypyrene. This conjugates to various phase II metabolites that can be enzymatically deconjugated for quantification of total phase I metabolism (Fillman et al., 2004; Jørgensen et al., 2005). Pyrene hydroxylase is thought to be induced by a variety of PAHs and consequently been proposed as a biomarker of exposure to PAH compounds (Fillman et al., 2004).

1.4.5 Micronucleus formation

The frequency of MN is regarded as an important tool for in situ monitoring of genotoxicity. This assay has been used for various organic and inorganic pollutants in laboratory studies as well as in field studies (Bolognesi et al. 1996; Koukouzika and Dimitriadis, 2005; Baršienė et al. 2006) and it has been recommended for offshore biomonitoring (Baršienė et al., 2006; Gorbi et al., 2008). This biomarker shows a continuously increasing trend in animals exposed to increasing concentrations of pollutant and exposure times (Viarengo et al., 2007).

Micronuclei (MN) are chromatin-containing structures that are surrounded by a membrane and have no detectable link to the cell nucleus. As an index of chromosomal damage, the MN assay is based on the enumeration of downstream aberrations after DNA damage and reveals a time-integrated response to complex mixtures of pollutants. Therefore, the MN assay provides the evidence of DNA breakage and spindle dysfunction caused by clastogens and aneuploidogenic poisons (Heddle et al., 1983, 1991; MacGregor, 1991; Seelbach et al., 1993; Kramer, 1998; Zoll-Moreux 1999).

1.4.6 Lysosomal membrane stability

Lysosomes are subcellular organelles surrounded by a semipermeable membrane that contains numerous hydrolytic enzymes involved in a range of cellular processes including digestion, defence,

and reproduction (Viarengo et al., 2007). Lysosome membrane integrity has been found to be affected by a range of stressors, including metals and organic chemicals. One of the most well-established methods to determine changes in membrane integrity is through measurements of the lysosomal membrane stability (LMS) (Lowe et al., 1995).

LMS (assayed as neutral red retention time) is an easy to perform and low cost test to detect impairments of the functional integrity of cells (Livingstone et al., 2000; Moore et al., 2006). The method uses one of a range of available dyes which will accumulate in the lysosomal compartment of cells. A reduction in membrane integrity will cause the dye to leak back into the cytosol, an effect which can then be quantified. The method is used with circulating cells (e.g. haemocytes), but techniques exist to use a similar method on other tissues (Lowe et al., 1995; Viarengo et al., 2007).
The histochemical procedure for LMS determination in frozen samples of mussel digestive gland (Moore et al., 2004) is also an alternative for offshore monitoring programs, where the field conditions such as a moving vessel may be a limiting factor.

Digestive gland lysosomes are cell organelles specialised in digestion of both endogenous and exogenous materials. Impairment of lysosomes and, hence, of food assimilation, can result in severe alterations of cells and whole organisms. Lysosomes of the digestive cell of mussels, apart from their main functions in intracellular digestion of ingested material (Robledo et al., 2006, Izagirre et al., 2008) and autophagic processes (Moore et al., 2007), play an important role in responses to toxic compounds through the sequestration and accumulation of toxic metals and organic xenobiotics. Lysosomal responses to pollutants are widely accepted cellular biomarkers of biological effect, especially in mussels and other bivalve molluscs, whose digestive cells possess a very well developed endo-lysosomal system (UNEP/RAMOGE 1999, ICES 2004).

1.4.7 Lipofuscin accumulation

In mussel, histochemical biomarkers are often analysed in the digestive gland. The digestive gland of molluscs is the main centre for metabolic regulation, participating in the mechanisms of immune defence and homeostatic regulation of the internal medium, as well as in the processes of detoxification and elimination of xenobiotics (Moore and Allen, 2002).

Lipofuscin accumulation represents an oxidative stress biomarker (Viarengo et al., 1990; Regoli, 1992). Elevated lipofuscin accumulation reflects degradation of cellular membrane caused by oxidative damage following the action of different pollutants (Moore, 1988). Lipofuscin granules are constituted by oxidatively modified proteins and lipid degradation products, along with carbohydrates and metals. Although lipofuscin composition may be variable, all lipofuscin pigments are not degradable. Therefore, their accumulation in the lysosome vacuolar system represents an indication of the oxidative stress level in the cells and it is related to the level of membrane lipid peroxidation (Viarengo et al., 2007). During pollutant exposure of mussels, this biomarker typically shows a continuously increasing trend, which reaches a maximum level that it is determined by the rate of secretion of lipofuscin-rich residual bodies into the external fluids.

1.4.8 Neutral lipid accumulation

The effects of pollutants are often associated with unbalanced fatty acid metabolisms and the accumulation of neutral lipids in the lysosomal vacuolar system. The lysosomal storage of neutral lipids in mussel digestive glands has been found to be a useful marker of change in the physiology of the cells (Viarengo et al., 2007). Neutral lipid accumulation appears to be more strictly linked to organic chemical pollution (Lowe and Clarke, 1989, Cajaraville et al., 1992). Lipophilic xenobiotics may alter the metabolism of neutral lipids leading to abnormal accumulation of that lipid class inside lysosomes (Moore, 1988).

1.4.9 Histopathology in selected organs

Histological parameters are commonly used as markers of health status in mussels (*Mytilus* spp.). The identification of pathologies and diseases are increasingly being used as indicators of environmental stress since they provide a definite and ecologically-relevant end-point for chronic/ sub chronic contaminant exposure (Mix, 1988). The application of histological markers in mussels can include measures of reproductive and metabolic condition, and allows for the detection of various pathogens that may affect population mortality. The data generated from this type of analysis in various organs (i.e. gills, gonads, digestive gland) is helpful in providing complementary information to support additional cellular and biochemical based biomarkers techniques such as the ones traditionally used in the WCM programme (Bignell et al., 2011). Furthermore gonad development is an important aspect of research related to the biological effects of contaminants (COMPRENDO, 2006). Histological markers assessing the status of gonads may give an indication of contaminant effect on the reproductive performance of mussels (Aarab et al., 2006, 2011).

1.4.10 Condition Index

Physiological measurements have been frequently used as a means to integrate the possible effects of complex mixture of contaminants (Pampanin et al., 2005). Contaminants can have antagonistic, synergistic, or additive effects on animal health. The identification of easy, practical and low-cost indices capable of recognising physiological stress is required during monitoring activities. Physiological responses are generally nonspecific and are directed at evaluating effects on energy metabolism or influence on growth and reproduction. Their link with effects at higher levels of organisation is very important. In this context, mussel condition index is proposed as an ecophysiological measure of the health status of the organisms, summarizing the physiological activity of mussels (growth, reproduction, secretion, etc.) under given environmental conditions.

1.4.11 POCIS

The principle of passive sampling is the placement of a device in the environment for a fixed period of time, where it is left unattended to accumulate contaminants by diffusive and/or sorptive processes. They offer sensitive, time-averaged sampling without confounding factors which may occur when using biomonitoring organisms. In the present study they are used as a support parameter to indicate exposure to groups of chemicals and not to determine effects, which may only be inferred (see Harman et al., (2011), for a full discussion). The present passive sampling design has been chosen to focus on the alkyl phenols (AP) most abundant in PW (C1-C5) and to screen for the presence of Naphthenic Acids (NA). Biological methods for measuring exposure (not effects) to both these two relevant chemical groups are either lacking or poor. The chosen passive sampling device is the polar organic chemical integrative sampler (POCIS, Alvarez et al., 2004), which has previously been shown to be suitable for measuring PW originating AP (Harman et al., 2009).

1.4.12 Bile metabolites in fish

The high relevance of fish bile metabolites in relation to PW exposure has been observed in previous environmental monitoring campaigns in the North Sea (Brooks et al., 2011a; Hylland et al., 2008), as well as in pilot laboratory studies (Sundt et al., 2009). Fish bile metabolites have been determined using two different methods: fix wavelength fluorescence and gas chromatography with mass spectrometric detection (GC-MS).

- Fixed wavelength fluorescence. Optical excitation wavelength increases with increasing size of PAH molecule. This variability is utilised in a simple detection method for PAH like fixed wavelength fluorescence (FF) detection and synchronous fluorescence spectroscopy (Aas et al., 2000b). However this method is not optimal for standardisation and quantification and should be regarded as a screening method.
- GC-MS. For a more quantitative and qualitative analysis of PAH metabolites, HPLC with fluorescence detection or GC-MS in selected ion monitoring mode (SIM) can be applied. The

selected GC-MS SIM method is the best suited for the detection of PAH compounds containing 2 to 3 ring structures, namely naphthalenes and phenanthrenes (Jonsson et al., 2003 and 2004). Both alkyl substituted and non alkyl substituted compounds are detected.

2. Materials and Methods

2.1 Study design

2.1.1 Source of mussels

Mussels (*Mytilus spp.*) were obtained from a clean location in Trondheimsfjord, the same population used in the WCM programme in 2009, 2008 and 2006. Mussels were transported to IRIS Akvamiljø facility and kept in clean seawater (inlet at 80 m depth) for 6 days prior to pre-exposure sampling and 3 days prior to field deployment. Mussels of the same size were used throughout the study (length approx. 70 mm).

2.1.2 Pre exposure sampling

A sub-sample of the mussels collected from Trondheimsfjord were analysed for the same chemical and biomarker endpoints as measured in the mussels used in the field exposure (**Table 2**). Mussels were sampled after arrival at the IRIS Akvamiljø laboratories in Stavanger.

2.1.3 Cage deployment and retrieval

A detailed description of the field work including pre-exposure sampling, deployment cruise and the sampling cruise can be found in the cruise report (Appendix A). A general outline is provided below. The mussel stations were deployed on the 11th and 12th April 2011 and retrieved 26th to 28th May 2011. Mussels were placed at strategic locations around the Gullfaks C platform at 500 m, 1000 m and 2000 m as shown in figure 1. Approximately 200 mussels were held within the water column at a depth of around 15 m. Twelve mussel stations were used around the platform, with 2 mussel stations located at a reference location approximately 60-80 km north east. The higher number of mussel stations used was to increase the likelihood of some mussels lying in the path of the PW discharge plume, with the intention of analysing mussels from 10 stations.

Table 1. Coordinates of the mussel stations recorded during deployment and retrieval.

Station number	Deployed		Collected		Deviation (m)	Bearing (^{o})
	Latitude (WGS 84)	Longitude (WGS 84)	Latitude (WGS 84)	Longitude (WGS 84)		
1	61°12.730 N	002°16.956 E	61°12.709 N	002°16.952 E	26	190
2	61°12.643 N	002°16.734 E	61°12.648 N	002°16.707 E	30	145
3	61°12.555 N	002°17.471 E	61°12.171 N	002°18.112 E	906	142
4	61°12.470 N	002°15.599 E	61°12.305 N	002°15.812 E	354	147
5	61°13.243 N	002°15.599 E	61°13.212 N	002°15.673 E	83	130
6	61°13.237 N	002°17.142 E	Not found			
7	61°12.380 N	002°18.371 E	61°12.340 N	002°18.363 E	71	186
8	61°12.082 N	002°17.992 E	Not found			
9	61°11.916 N	002°17.535 E	Not found			
10	61°12.078 N	002°14.775 E	Not found			
11	61°13.633 N	002°14.706 E	Not found			
12	61°13.679 N	002°17.925 E	61°13.337 N	002°18.871 E		
13 (Ref 1)	61°39.801 N	002°59.611 E	Not found			
14 (Ref 2)	61°40.545 N	002°58.167 E	Not found			

Current meters were attached to stations 3 and 5 at a depth of 15 ± 2 m to determine the main current direction during the entire exposure duration. In addition, at selected stations, POCIS were placed immediately below the mussels and were used to determine AP and NA concentrations. POCIS were deployed at stations 1, 2, 3, 5, 7, 9 and one reference station.

After 6 weeks exposure the stations were collected. Unfortunately, due to errors in the rig design only 7 of the 14 stations were retrieved (**Table 1**). However, the location and number of collected stations was deemed sufficient to assess the biological effects of the PW discharge. The collected mussels were sampled on board the research vessel and the different matrices collected for specific chemical and biological endpoints as listed in **Table 2**. Of the stations collected, the main movement was for stations 3 and 12, which were located approximately 1000 m SE from their original position (see cruise report Appendix B). Station 12 mussels were recovered 1 week after the other stations. These mussels were frozen immediately upon collection and stored at -20°C. These samples were analysed for chemical concentrations only. The two reference stations were not found.

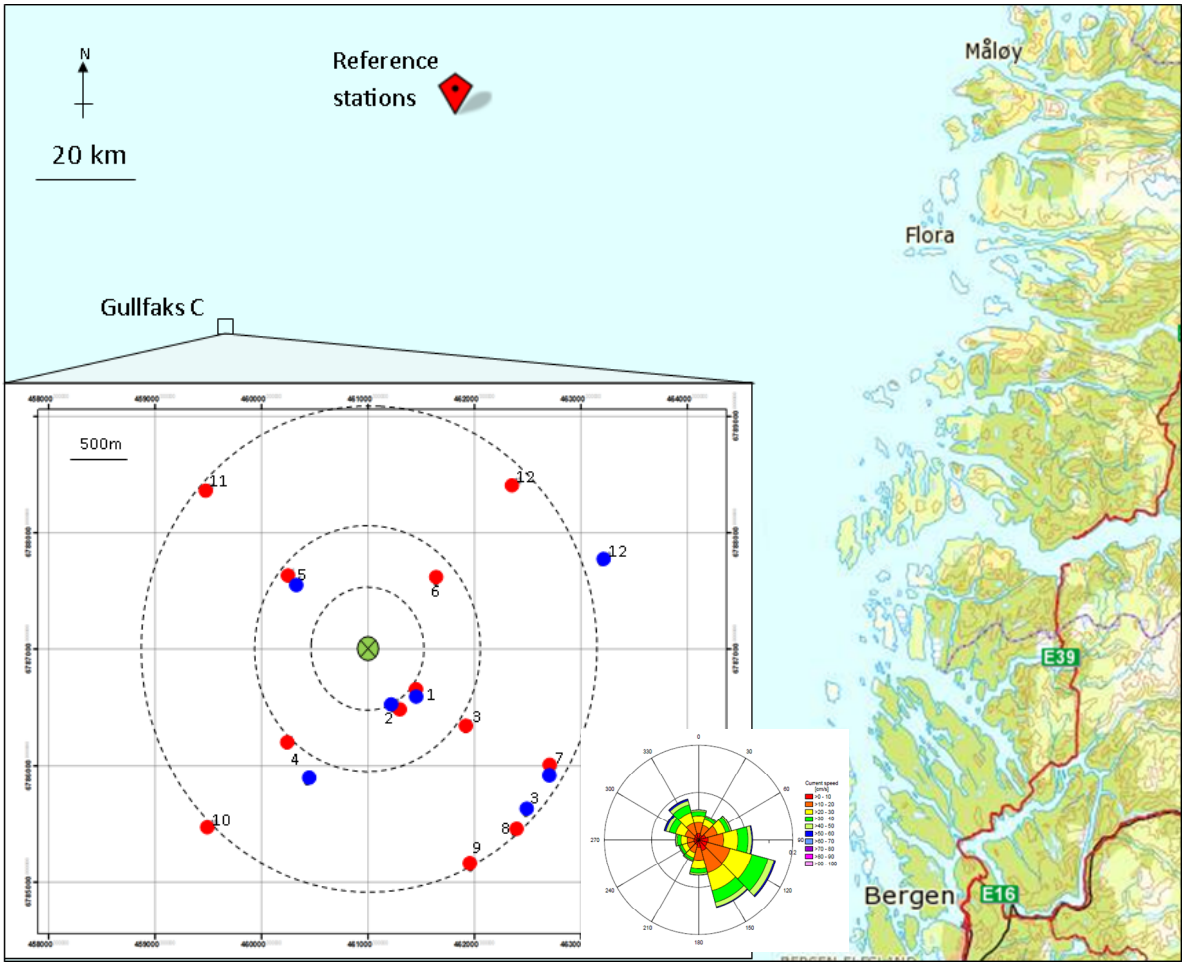


Figure 1. Location of the mussel stations around the Gullfaks C platform (superimposed panel), showing the positions of the deployed stations (red dot) and retrieved stations (blue dot). Current row data showing the average current direction at a depth of 30 m (Statoil report). The reference stations were positioned 60-80 km North East of the Gullfaks field. Green circle with cross denotes the position of Gullfaks C platform. The dotted circles denote 500, 1000 and 2000 m from the platform.

Table 2. Measured chemical and biological endpoints in the mussel

Method	Indication of	Matrix
Species confirmation	*Mytilus* spp. (*edulis, trossulus, galloprovincialis*)	Gill
Pyrene Hydroxylase	Exposure to PAHs with 4 benzene rings	Digestive gland
Lysosomal stability	General stress	Haemocytes, digestive gland
Lipofuscin accumulation	General health	Digestive gland
Neutral lipid accumulation	Peroxidation of lipids	Digestive gland
Histopathology	General stress and pollutant exposure	Digestive gland, gills, gonad
Condition Index	General health	Whole organism
PAH concentration	PAH exposure	Soft tissue
Lipid content	Used for lipid normalization of PAH	Soft tissue
Micronucleus	Genotoxic exposure	Haemocytes
^{226}Radium	Exposure to radiation	Soft tissue

2.1 Temperature depth profiles and current measurements

The SBE "Seabird 901" probe was deployed at 500 m (from station 1, 19:30am, 28.05.11), 1000 m (from station 6, 15:30, 29.05.11) and 2000 m (from station 12, 15:00, 29.05.11) during the deployment and the retrieval cruises. The specifications for the instrument are as follows:

Conductivity: *Inductive cell*, range: 0-70 mS/cm, resolution: 0.01 mS/cm, accuracy: ± 0.02 mS/cm. Salinity: *Calculated from C, T and D*, range: 0-40 ppt, resolution: 0.01 ppt, accuracy: ± 0.02 ppt. Temperature: range: -2 to +40 °C, resolution: 0.001 °C, accuracy: ± 0.01 °C, response time: <0.5 sec. Pressure range 1000 m, resolution: 0.01 % FS, accuracy: ± 0.02 % FS, Sound velocity: *Calculated from C, T and D*, ranges: 1300-1700 m/s, resolution: 5 cm/s, accuracy: ± 10 cm/s

Current meters (RMC9) were positioned at stations 3 and 5 and at one of the reference stations for the duration of the exposure. The current meters from stations 3 and 5 were retrieved successfully. The specifications for the instrument are as follows:

Measuring current velocity (range: 0.5 - 500 cm/s, accuracy: 0.5 cm/s or 1 %), current direction (accuracy/ resolution: 2°/ 0.1°), temperature (range: -4 – 40 °C, accuracy/ resolution: 0.1 °C/ 0.01°C) and depth (range 0-200 m, accuracy/resolution: 0.25 %/ better than 0.005 % of full scale per sample). Accuracy of current velocity is dependent on set-up parameters. During the WCM the accuracy was 0.4 cm/s. The instrument measured current velocity as a 60 seconds average and logged data for every 10 minute, diagnostic data were collected every 12 hours.

2.2 Species determination

Total DNA was extracted from 20-40 mg of gill tissue from frozen mussels using DNAzol reagent (Invitrogen, Madison, Wisconsin, USA) following the manufacturer's recommended protocol. The tissue was homogenised in 1 mL DNAzol using Precellys 24 bead mill (Bertin, Montigny-le-

Bretonneux, France), using ceramic CK14 beads at 5000 rpm for 10 seconds. Cell debris were then removed by centrifugation at 10,000 g for 10 min (4ºC), before DNA was precipitated from the supernatant by addition of 500 µl 100% ethanol. Following two wash steps with 75% ethanol, the DNA was pelleted by centrifugation at 4,000 g for 2 min, air dried and dissolved in 8 mM NaOH. The resulting DNA was quantified and quality controlled on a nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and all samples had OD 260/280 > 1.8 indicative of pure DNA. For species identification, polymerase chain reaction (PCR) were used to amplify a specific 180 base pair (bp) segment for *M. edulis*, 168 bp segment for *M. trossulus* or 126 bp segment for *M. galloprovincialis* as described by Inoue et al. (1995). The 50 µl PCR reactions contained 10 µl of DNA template, 300 µM forward and reverse primers, VWR 2x Taq mastermix (VWR, Radnor, Pennsylvania, USA), and were subjected to a 5 min pre-heating stage at 95ºC followed by 35 cycles of 30 sec at 95ºC, 30 sec at 55ºC, 30 sec at 72ºC, and final extension step of 10 min at 72ºC. One µl of the PCR product was loaded onto a DNA 1000 chip (Agilent technologies, Santa Clara, California, USA) and run in a Bioanalyzer instrument (Agilent technologies, Santa Clara, California, USA) for visualisation of amplicon size.

2.3 Analytical methods in mussels

2.3.1 Chemical body burden in mussels

Approximately 15 whole mussels from each station were excised from their shell and transferred to high temperature treated (560^{o}C) glass containers. The mussels were frozen and transported to NIVA on dry-ice. The samples were stored at -20^{o}C until analysis.

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

2.3.2 ^{226}Radium in mussel soft tissue

For each individual station, mussel soft tissue was dissected and divided into 4 separate pools each containing between 100-200 g (w.w.). All samples were immediately frozen and the 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.

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

2.4 Biomarker methods in mussel

Where possible the biological responses were evaluated on the same individuals. This included: pyrene hydroxylase; LMS in both haemocytes and digestive gland; MN; and lipofuscin and neutral

lipid accumulation. Fifteen mussels were analysed from each station and in the pre exposure group (time zero).

2.4.1 Pyrene hydroxylase

The hydroxylase activity method was adapted from a method described in (Michel et al., 1994). The microsomal fraction (100 µL) was added to sodium phosphate buffer (200 µL, 0.05 M, pH 7.3) containing BSA (2 mg/mL), NADPH (100 µL, 10 mM) and pyrene in acetone (10 µL, 400 µg/mL). The tubes containing the microsomes were incubated on a shaker (room temperature, 30 min.) before the reaction was terminated by adding 500 µL methanol. Internal standard (triphenylamine, 10 µL, 15 µg/ mL) was added to the solution and mixed. The tubes were centrifuged to precipitate protein and the supernatant was injected on an HPLC system for the determination of the metabolite formed.

Determination of metabolites by HPLC
HPLC was performed on a Waters 2695 Separations Module equipped with a 2475 fluorescence detector and fitted with a Waters PAH C18 (4.6 ×250 mm, 5 µm) column. The mobile phase consisted of a gradient from 40:60 acetonitrile: water to 100% acetonitrile at a flow of 1 mL/min, at 35°C. The excitation and emission wavelengths used for detection of pyrene and triphenylamine were 346nm, 384nm and 300nm, 360nm respectively. The injection volume used was 25 µL.

2.4.2 Micronucleus formation

Mussel haemolymph was applied directly on to slides, air-dried and fixed in methanol for 15 min. Slides were stained with 5% Giemsa solution for 10-20 min. Anonymous scoring of MN was performed without knowledge of the exposure status of the samples to eliminate technical variability. The frequency of MN in haemocytes was determined by scoring at 1000× magnification using an Olympus IX71 inverted microscope. A minimum of 200 cells per mussel were counted. Only cells with intact cellular and nuclear membrane were scored. MN were scored when: i) nucleus and MN had a common cytoplasm, ii) colour intensity of MN was the same or lower than the nucleus, iii) the size of the MN was equal or smaller than 1/3 of the nucleus, iv) MN was completely separated from the nucleus, v) cells with multiple MN were not scored.

2.4.3 LMS in haemocytes

Haemolymph 400 µL was taken from each mussel and mixed with filtered sea water at the ratio 1:1. An aliquot (30 µL) of haemolymph/ seawater-mixture was pipetted out on microscope-slides, and incubated in a light-proof box for 15 min before 30 µL neutral red (concentration 0.1 µg/µL) was added. All analyses were performed blind. For a detailed description of the method see Lowe and Pipe, (1994).

Neutral red dye is selectively taken up by haemolymph cells and this adds an extra stress to the membranes. After some time, from 15 to 180 minutes, depending on the health status of the mussels, the integrity of the membrane will weaken and the dye will leak out into the cytosol. This causes the cells to become round in shape. The time from when the dye is added to the cells, until the cells become round and perish is observed visually with a microscope. The cells are observed repeatedly at 15, 30, 60, 90, 120, 150 and 180 minutes of incubation with neutral red. The endpoint of the analysis is when 50% of all cells become round and die. This method is perceived as a general health-parameter, and has been shown to respond to PAH/ oil-exposed mussels.

2.4.4 Histochemical preparation

For histochemical examination, small pieces (5 x 5 x 5 mm) of freshly excised digestive gland tissue were placed in cryovials and snap frozen in liquid nitrogen (-196°C). Prior to sectioning the samples were attached to aluminium chucks. Cryostat sections (10 µm) were cut in a cryostat with the cabinet temperature below -25°C and the knife cooled to - 20°C. The sections were transferred to microscope

slides. The slides were stored in the freezer at -40 °C before use. Cryostat sections were used for analyses of lysosomal membrane stability, lipofuscin and neutral lipid accumulation.

2.4.5 LMS in digestive gland

The determination of LMS was based on the time of acid labilisation treatment required to produce the maximum staining intensity according to UNEP/RAMOGE (1999), after demonstration of hexosaminidase (Hex) activity in digestive cell lysosomes. Slides were put at 4 ºC for 30 min and then 10 min at room temperature prior to staining. Serial cryotome sections (10 µm) were subjected to acid labilisation in intervals of 0, 2, 4, 6, 8, 10, 15, 20, 25, 30 40 and 50 min in 0.1 M citrate buffer (pH 4.5 containing 2.5 % NaCl) in a shaking water bath at 37 ºC, in order to find out the range of pre-treatment time needed to completely labilise the lysosomal membrane. Following this treatment, sections were transferred to the substrate incubation medium for the demonstration of Hex activity. The incubation medium consisted of 20 mg naphthol AS-BI-N-acetyl-β-D glucosaminide (Sigma, N 4006) dissolved in 2.5 ml of 2-methoxyethanol (Merck, 859), and made up to 50 ml with 0.1 M citrate buffer (pH 4.5) containing 2.5 % NaCl and 3.5 g of low viscosity polypeptide (Sigma, P5115) to act as a section stabiliser. Sections were incubated in this medium for 20 min at 37 ºC, rinsed in a saline solution (3.0 % NaCl) at 37 ºC for 2 min and then transferred to 0.1 M phosphate buffer (pH 7.4) containing 1mg/ml of diazonium dye Fast Violet B salt (Sigma, F1631), at RT for 10 min. Slides were then rapidly rinsed in running tap water for 5 min, fixed for 10 min in Baker's formol calcium containing 2.5 % NaCl at 4 ºC and rinsed in distilled water. Finally, slides were mounted in Kaiser's glycerine gelatine and sealed with nail varnish.

The time of acid labilisation treatment required to produce the maximum staining intensity was assessed under the light microscope as the maximal accumulation of reaction product associated with lysosomes (UNEP/RAMOGE 1999). Four determinations were made for each animal by dividing each section in the acid labilisation sequence into 4 approximately equal segments and assessing the labilisation period in each of the corresponding set of segments. The mean value was then derived for each section, corresponding to an individual digestive gland.

2.4.6 Lipofuscin accumulation

The lipofuscin content of lysosomes was determined using the Schmorl reaction. Cryostat sections were fixed in Baker`s calcium-formol for 15 min, rinsed in distilled water and immersed in the reaction medium containing an aqueous solution of 1% ferric chloride and 1% potassium ferrocyanide in a ratio 1:1 (v:v). Sections were stained for 2 min, rinsed in acetic acid (1%) for 2 min, washed in running water for 10 min and rinsed in distilled water before mounting using aqueous mounting medium. Slides were subjected to image analysis and results were expressed as pixel density.

2.4.7 Neutral lipid

For the determination of unsaturated neutral lipids, cryostat sections were fixed in Baker`s calcium-formol for 15 min, rinsed in distilled water and transferred into 60% triethylphosphate (v/v with distilled water) for 1 min. Sections were stained in 1% solution of Oil Red O in 60% triethylphosphate for 15 min. Then they were rinsed in 60% triethylphosphate for 30 s, washed in distilled water and mounted using aqueous mounting medium. Neutral lipid accumulation was assessed by computer assisted image analysis. Results were expressed as pixel density.

2.4.8 Histopathology of selected organs

In order to prevent the appearance of post mortem artefacts, specimens were handled with extreme care. Sampled mussels were kept in ice after collections and the dissection were performed immediately on board. All analysed tissue (gill, gonads and digestive glands) were dissected, putted in pre-labelled histocassette and placed into histological fixative (Baker's calcium-solution: 4% formaldehyde, 1% $CaCl_2$, 2.5% NaCl) for wax sections. Tissue samples were no thicker than 1 cm to

ensure proper fixation, but long and wide enough to represent the different area of a tissue. Samples were then stored at 4˚C until embedding.

A 4 mm cross-section of each mussel, including digestive diverticulum, gills, mantle, kidneys, were dehydrated in alcohols. The tissues were cleared in methyl benzoate ($C_6H_5CO_2CH_3$), rinsed in benzene (C_6H_6) and embedded in paraffin. Histological sections (5 µm) were cut using a microtome HM 355s (Microm, Bergman), mounted on slides, dried at 37 ºC for 24 hours and stained with haematoxylin and eosin. The tissues were examined for health parameters related to reproductive and physiological conditions, inflammatory and non-specific pathologies and those associated with pathogen and parasites infections. The DG tubules atrophy was recorded using a scoring index ranging from 0 to 3 (Brooks et al., 2009). The reproductive status was determined according to (Seed, 1976). The presence of parasites and non-specific inflammation were scored as absent (0) or present (1). All micrographs were captured using an AxioCam MRc5 (Zeiss) digital camera mounted on a *Zeiss Axioplan 2* light microscope (Göttingen, Germany). The slides were analysed blind.

2.4.9 Condition index

Condition index (CI) was calculated on 20 specimens. Mussel shells were cleaned carefully under fresh water flow and then opened to remove all soft tissue. The shells and soft tissue were dried for 48 h at 90˚C and before being weighed. Afterwards, the remained soft tissue was dried for 4 hours at 470˚C to determine the ash weight. CI was calculated according to the following formula (Lucas and Beninger, 1985): CI= ash free dry weight/dry weight of shell

2.4.10 Integrative Biological Response (IBR)

The Integrative Biological Response (IBR) index was developed by Beliaeff and Burgeot (2002) in order to integrate biochemical, genotoxicity and histochemical biomarkers. In the present study neutral lipid, lipofuscin, lysosomal membrane stability, neutral red retention and micronuclei were the biomarkers selected for the IBR calculation. The inverse values of LMS and NRRT were used since a decrease was reflective of adverse impact. The calculation method is based on relative differences between the biomarkers in each given data set. Thus, the IBR index is computed by summing-up triangular star plot areas (a simple multivariate graphic method) for each two neighbouring biomarkers in a given data set, according to the following procedure: (1) calculation of the mean and standard deviation for each sample; (2) standardization of data for each sample: $x_i'=(x_i-x)/s$; where, x_i'=standardized value of the biomarker; xi=mean value of a biomarker from each sample; x=general mean value of x_i calculated from all compared samples (data set); s=standard deviation of x_i calculated from all samples; (3) addition of the standardized value obtained for each sample to the absolute standardized value of the minimum value in the data set ($y_i=x_i'+|x_{min}'|$); (4) calculation of the Star Plot triangular areas by multiplication of the obtained standardized value of each biomarker (y_i) with the value of the next standardized biomarker value (y_{i+1}), dividing each calculation by 2 ($A_i=(y_i*y_{i+1})/2$); and (5) calculation of the IBR index which is the summing-up of all the Star Plot triangular areas ($IBR=\sum A_i$) (Beliaeff and Burgeot, 2002). Since the IBR value is directly dependent on the number of biomarkers in the data set, we divided the obtained IBR value by the number of biomarkers used in each case (n=5) to calculate IBR/n, according to Broeg and Lehtonen (2006).

2.5 Polar chemical integrated sampling devices (POCIS)

2.5.1 Handling, extraction and extract derivitisation

Standard POCIS, with a surface area per mass of sorbent ratio of ca. 180 cm^2 g^{-1} were obtained from ExposMeter (Tavelsjo, Sweden) (Alvarez et al., 2004). The 'pharmaceutical' configuration was used, containing Oasis® HLB sorbent between two discs of polyethersulphone (PES) membrane. POCIS were used at specific stations (1, 2, 3, 5, 7, 9 and one reference) and deployed in commercially available stainless steel canisters (Environmental Sampling Technologies, Saint Joseph, USA,), which

were attached directly to the main rope of each mussel station. After retrieval samplers were frozen until analysis (-20 ^{o}C).

For extraction POCIS were carefully opened and the sorbent washed with water (Option 3, Elga™) into an empty, solvent rinsed solid phase extraction reservoir (International Sorbent Technologies, Hengoed, UK) and dried using nitrogen. The membranes were discarded. Internal standards were added before elution with 20 mL of methanol or acidified methanol. For AP analysis extracts were concentrated to 0.5 mL, derivatised with 10% pentafluorobenzoyl chloride (Sigma-Aldrich, Oslo, Norway) and extracted with 4 mL hexane (Boitsov et al., 2004). NA were derivatised using N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (Sigma-Aldrich, Germany, Thomas et al., 2009).

2.5.2 Instrumental analysis of AP and NA

Analysis of alkyl phenol pentafluorobenzoate derivatives was carried out using an Agilent Technologies (Santa Clara, USA) 6890 GC linked to a 5973 mass selective detector in selected ion monitoring (SIM) mode. The injection was splitless and a 50m column of 0.25 mm internal diameter and 0.25µm film thickness was used. Temperature at injection was 60°C which was raised stepwise to 290°C. Analytical quantification limits, obtained by the average solvent blank value, (n = 3) + 10 × standard deviation of that average, were below 0.5 ng for all AP, except for phenol (17.0 ng). Further detail concerning the method and the uncertainty etc. are given by Boitsov et al., (2004). POCIS blank values are considered separately. For NA, 1 µL of each sample was injected into an Agilent gas chromatograph fitted with a 30 m × 0.25 mm, 0.25 µm film thickness DB-5MScolumn (J&W Scientific) with helium carrier gas. Splitless injection at 240 °C was used. The initial temperature of 60 °C was held for 2 min, followed by an increase of 5 °C/min to 300 °C, and held at this temperature for 10 min. The high-resolution time-of-flight mass spectrometer (GCT Premier, Waters) was operated in full scan positive electron impact mode with a scan range of 50-1000 m/z. Accurate mass spectra to 4 decimal places was used for peak identification with an error threshold of 4 mDa. See Thomas et al., (2009), for further details. Analysis for AP was carried out by IMR, Bergen, and NA by NIVA, Oslo.

2.6 Wild caught fish

Two long-lines, each containing 200 baited hooks, were placed approximately 1000-2000 m from the platform (start 61^{o} 09.203N 002^{o} 09.656E to end 61^{o} 09.232 N 002^{o} 10.996 E). The long-lines were placed in the water for approximately 5 hours before they were retrieved. The long lines were retrieved from the water and the following fish species were caught: 33 Whiting (*Merlangius merlangus*), 14 Tusk (*Brosme brosme*), 4 Ling (*Molva molva*), 1 Blue Ling (*Molva dypterygia*), 2 Cod (*Gadus morhua*), and 1 Haddock (*Melanogrammus aeglefinus*). The liver and bile were sampled from 25 Whiting and 14 Tusk.

2.6.1 Bile metabolites in Whiting

PAH metabolites were measured in the bile of collected Whiting.

Fix wavelength fluorescence

Bile samples were diluted 1:1600 in methanol: water (1:1). Slit widths were set at 2.5 nm for both excitation and emission wavelengths. And samples were analysed in a quartz cuvette. All bile samples were analysed by FF at the wavelength pairs 290/335, 341/383 and 380/430 nm, optimised for the detection of 2-3 ring, 4-ring and 5-ring PAH metabolites, respectively.

The fluorescence signal was transformed into pyrene fluorescence equivalents through a standard curve made by pyrene (Sigme St Louis, USA). Pyrene was measured at the same fluorimeter, with the same cuvette, same solvent, and with the same slit settings as the bile samples. It was, however, measured at the optimal wavelength pair of pyrene, 332/374 nm (ex/em). The concentration of PAH metabolites in bile samples was expressed as µg pyrene fluorescence equivalents (PFE)/ ml bile.

PAH metabolites in fish bile

Samples of fish bile for PAH metabolite analyses were prepared and analysed as described by Jonsson et al. (2003, 2004). In brief, β-glucuronidase was diluted 1:10 in sodium acetate buffer (0.4 M, pH 5). Internal standards and 25–30 µl of bile were added, followed by incubation at 40˚C for 2 h. The OH-PAH were extracted with ethylacetate (four times 0.5 ml) and the combined extract was dried with anhydrous sodium sulphate and concentrated to 0.5 ml. Trimethylsilyl (TMS) ethers of OH-PAH were prepared by addition of 0.2 ml BSTFA and followed by heating for 2 h at 60˚C and the GC–MS performance standard TPA was added before transferral to capped vials. TMS ethers of OH-PAH in fish bile samples were analysed with a GC–MS system consisting of a HP5890 series II gas chromatograph, Shimadzu QP2010 GCMS. Helium was used as the carrier gas and the applied column was CP-Sil 8 CB-MS (Varian). The temperatures for the injector, transfer line, and ion source were held at 250, 300 and 240˚C, respectively, and the GC oven temperature program was as follows: 80–120˚C at 15˚C/min, 120–300 ˚C at 6˚C/min, and held at 300˚C for30 min. Mass spectra were obtained at 70 eV in selected ion mode (SIM).

2.7 UV fluorimeter (Aquatracka)

During the retrieval cruise, the UV-fluorimeter was towed behind the Esvagt Dee at a distance of 50 m. The Aquatracka was attached to a second rope connected to a surface buoy to ensure that the Aquatracka remained at a depth of 15 m during the tow, the same height as where the mussels were positioned within the water column. The Esvagt Dee circled the Gullfaks C platform at a speed of 2 knots and at a constant distance of 500 m. The starting position of the tow was known (293^{o} from the platform) at 61^{o} 13.372N 002^{o} 15.168E. In addition, four vertical profiles were recorded at 4 locations in the East to South direction, 500 m from the platform: 1) 61^{o} 12.833N 002^{o} 16.995E; 2) 61^{o} 12.755N 002^{o} 16.902E; 3) 61^{o} 12.667N 002^{o} 16.800E; 4) 61^{o} 12.610N 002^{o} 16.682E.

To obtain background measurements the Aquatracka was towed at 2 knots behind the Esvagt Dee at a distance of 50 m and a depth of 15 m between the two reference stations (1 nautical mile). The Aquatracka recorded measurements every second on UNIplot software installed on a laptop computer. The data was transferred to an excel spreadsheet for evaluation.

Laboratory exposures were performed with the Aquatracka, placing the probe in the solutions of varying concentrations of an artificial PW mixture for one minute. The artificial PW mixture contained chemicals typically found in PW and at concentrations typically found from Norwegian platforms in the North Sea (Holth et al., 2008). The concentration series included 1x PW, which was equivalent to the concentration of PW just prior to leaving the discharge outlet, and dilutions of this (i.e. 10x, 100x, 1000x, 10,000x, 100,000x dilution).

2.8 Statistical methods

Biological responses in individuals were subjected to analysis of variance (ANOVA) to clarify whether there were differences between groups (Sokal and Rohlf, 1981). Prior to analyses, homogeneity of variances was checked using the Levene's test. Where necessary variables were log transformed to obtain homogeneity. Where this was not possible, Kruskal-Wallis non-parametric analysis was used. (Sokal and Rohlf, 1981). Where the parametric ANOVA indicated significant differences, groups were compared using Tukey's post-hoc test. The level of significance for rejection of H_0: "no difference between groups" was set to 0.05.

3. Results

3.1 Temperature, salinity and current data

The water currents around Gullfaks C were identified with current meters positioned at station 3 and 5 (**Figure 2**). The results showed a stable current in the South East direction throughout the whole exposure period. The current direction confirmed that the selected station axis were in the path of the plume.

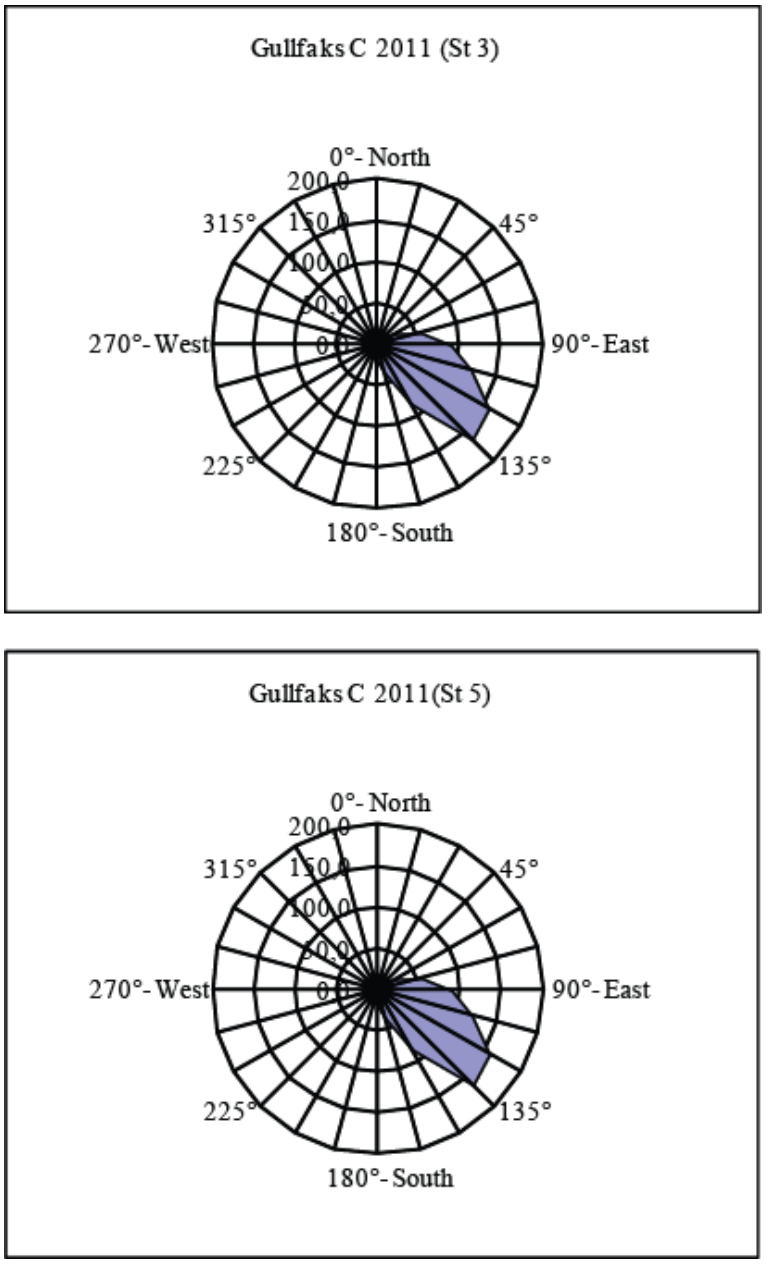


Figure 2. Sum of relative current (in 15^{o} sectors, cm/s).

The temperature and salinity of the water column remained relatively stable at approximately 10 ± 0.5 ^{o}C and 35.3 ± 0.1 psu respectively, with no stratification observed.

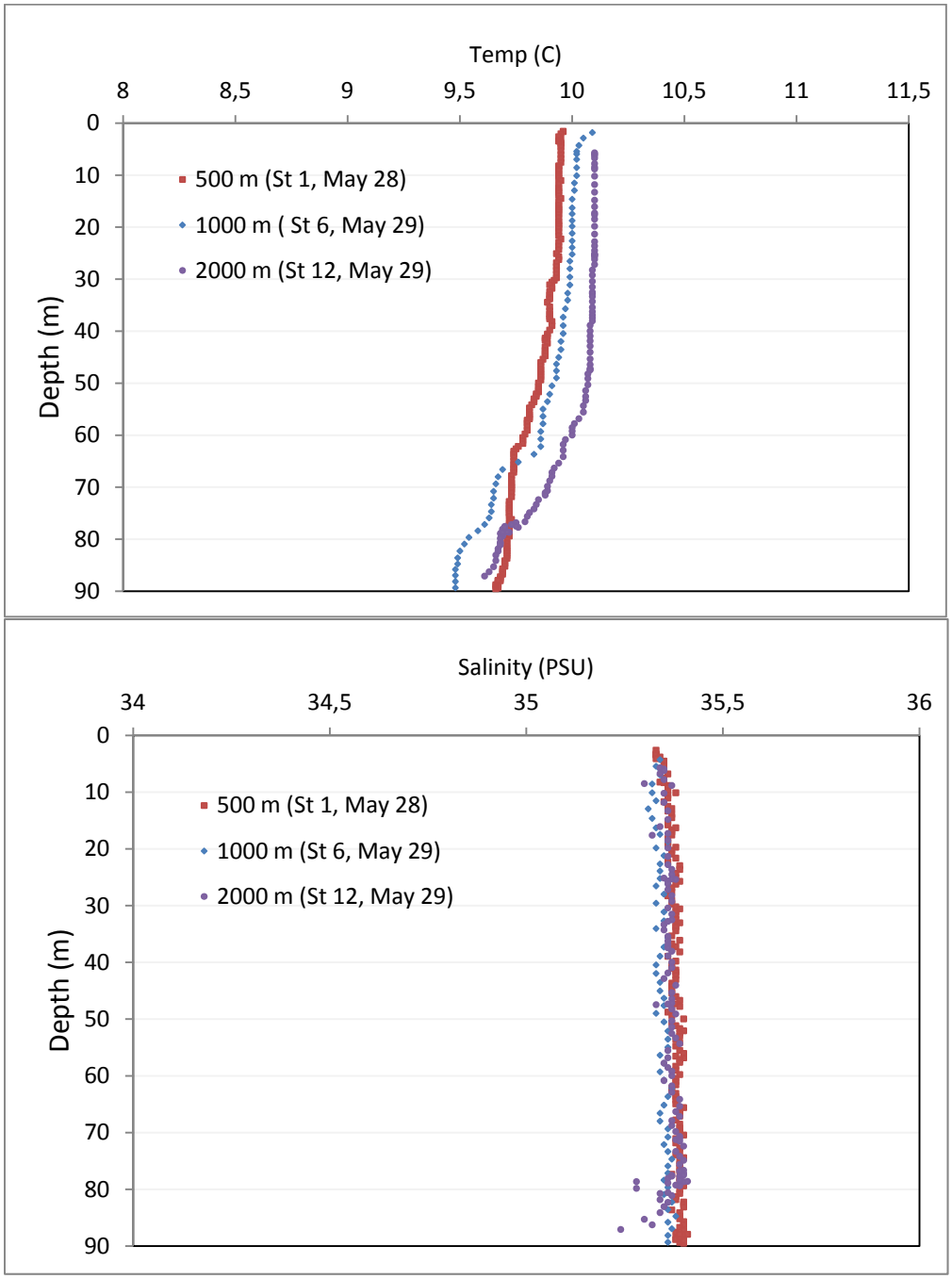


Figure 3. Temperature and salinity profiles taken on board the survey vessel at 500, 1000 and 2000 m from the Gullfaks C platform.

3.2 Identification of *Mytilus* spp.

The percentage distribution of *Mytilus* spp. used in the WCM programme is shown in **Figure 4**. Of the 86 individuals sampled 91% were identified as *Mytilus edulis*. The remaining 9% were hybrids of *M. edulis*, with *M. edulis*/ *M. trossulus* and *M. edulis*/ *M. galloprovincialis* making up 7% and 2% respectively.

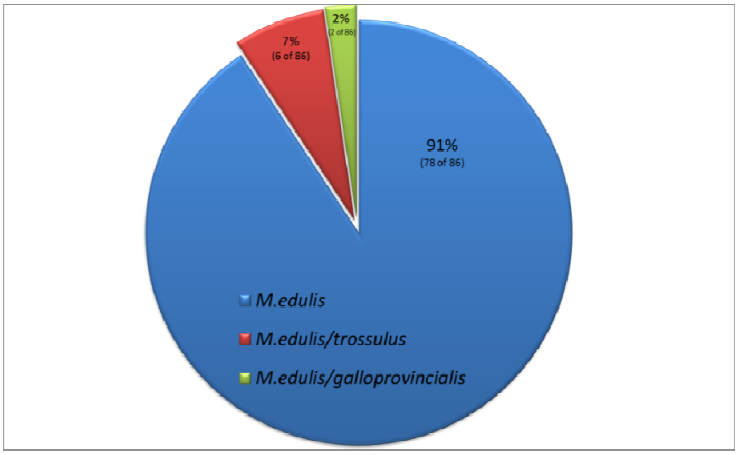


Figure 4. The percentage distribution of *Mytilus* spp. and their hybrids from a sub-sample of the exposure mussels (n = 86).

3.3 Chemical body burden data in mussels

Twenty four pooled mussel samples were analysed for PAHs including NPDs and metals. These included three replicate pooled samples from 7 exposure stations (Station 1, 2, 3, 4, 5, 7 & 12) plus the pre-exposure mussels. Station 3 located 1000 m SE of the platform during deployment was retrieved approximately 2000 m SE of the platform after 6 weeks. Station 12 was retrieved one week after the other mussels approximately 1000 m from its original position. The chemical analysis was carried out by NIVA and the raw data is provided in Appendix B.

With exception to station 12 mussels that had lower lipid content than the other groups (~1%); median lipid content for all groups was between 1.4 and 1.8 % (**Figure 5**). The data for PAHs and NPDs are presented on a wet-weight basis (µg/ kg; **Figure 6** to Figure **9**). Due to the small sample size (n=3 in each group), statistical evaluation was not performed. Groups with no overlapping values can be regarded as different. The results are shown as Sum PAH16, total naphthalenes, total phenanthrenes/anthracenes, and total dibenzothiophenes.

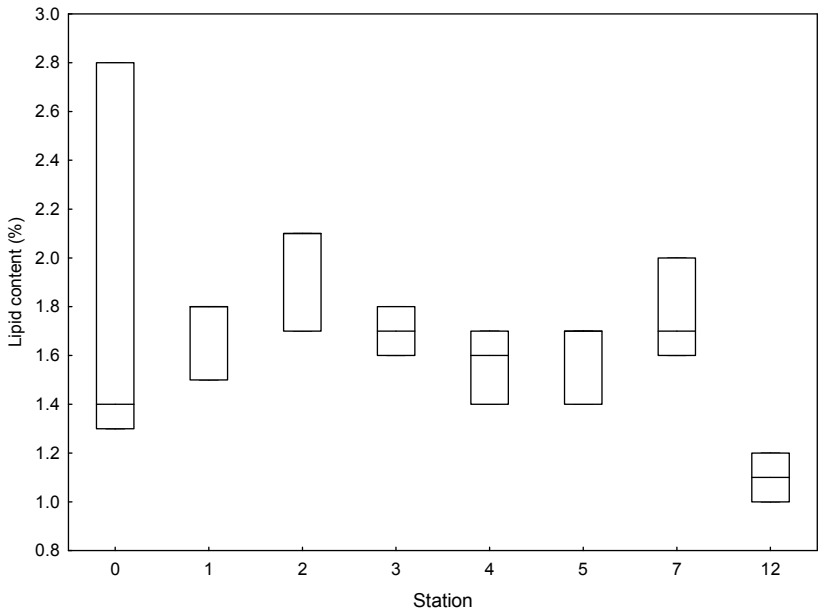


Figure 5. Lipid content of mussels from the groups indicated. The figure shows median and minimum/ maximum values (n=3). Station 0 refers to the pre exposed mussels.

3.3.1 PAH and NPD concentrations in mussels

Due to the small sample size (n=3), statistical evaluation was not performed. Instead, groups with no overlapping values were regarded as different. The sum of PAH16 was significantly higher at station 2, 500 m from the platform, with a median concentration of 30 µg/ kg (w.w) (**Figure 6**). Station 1, also at 500 m from the discharge was almost half the concentration of station 2 at 18 µg/kg (w.w.). PAH16 concentrations were detected at below 10 µg/kg (w.w.) for the pre exposed mussels, whilst all other stations were marginally above or lower than the detection limit, indicating background concentrations for the North Sea.

This pattern of elevated concentrations of PAH16 in the closest stations to the PW discharge at 500 m and background levels at all other exposure stations was consistent for all other PAHs measured including total naphthalenes (**Figure 7**), phenanthrenes & anthracenes (Figu**re 8**), and dibenzothiophenes (Figur**e 9**). Highest median concentrations of total NPD at station 2 were measured at approximately 800, 500 and 300 µg/kg (w.w.) respectively.

Relative concentrations of alkylated PAHs to parent compounds:
It is well established that the PAHs found in coal and petroleum often contain one or more methyl (C1), ethyl (C2), propyl (C3), butyl (C4), or (occasionally) higher alkyl substituents on one or more of the aromatic carbons. These alkyl PAHs are generally more abundant than the parent PAHs in petroleum, but are less abundant than the parent PAHs in pyrogenic PAH mixtures. This has been demonstrated in mussels caged down-stream of PW discharges (Ruus et al., 2006; Hylland et al., 2005). The ratio of alkyl-compounds: parent-compound for the mussels with respect to the NPD compounds are shown in the following graphs (Figur**e 10** to Figur**e 12**). As expected, higher ratios were found in the mussels caged close to the Gullfaks C platform with much lower ratios in the pre-exposure mussels. Therefore, the PAH compounds found in the mussels from the pre-exposure group were likely from pyrogenic sources.

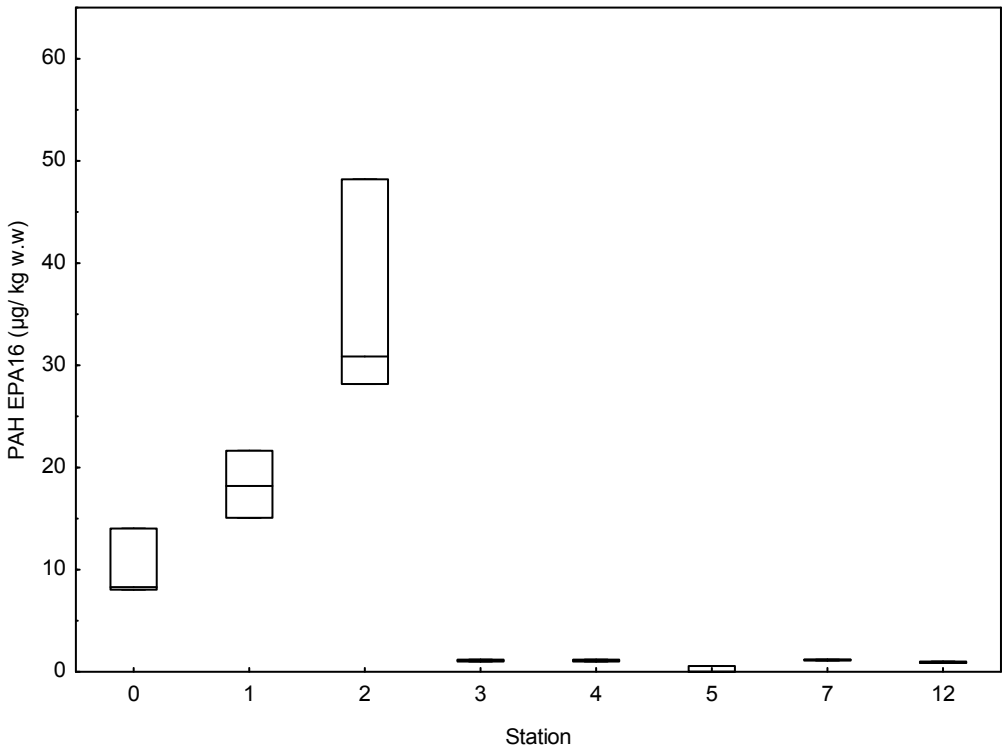


Figure 6. Sum PAH16 concentrations in mussels from the groups indicated. Boxes depict median and minimum/ maximum (n=3). Station 0 refers to the pre-exposed mussels.

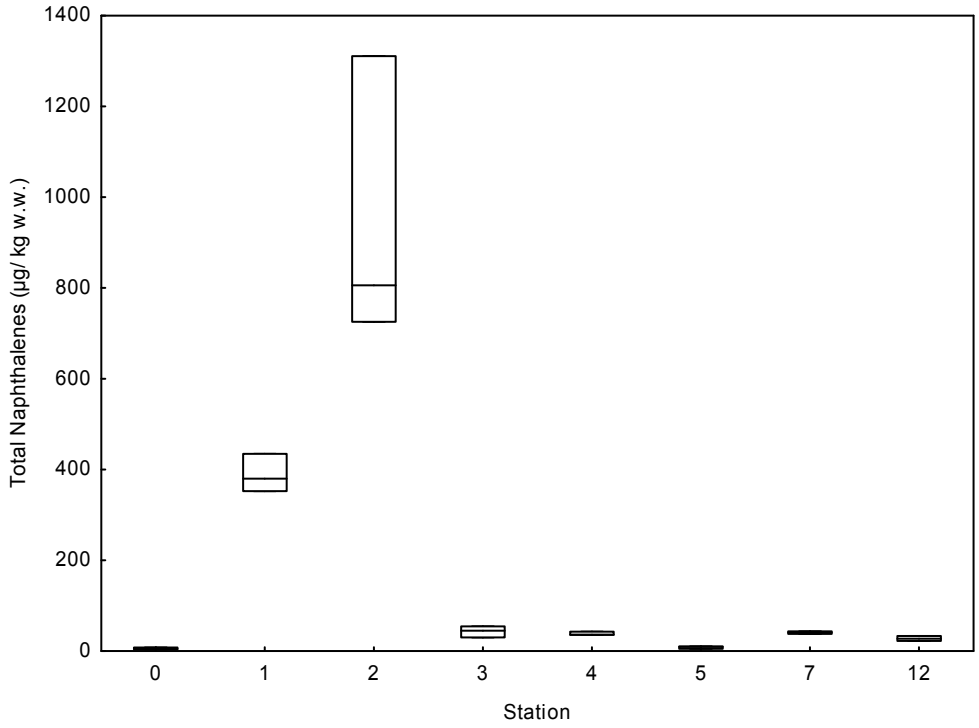
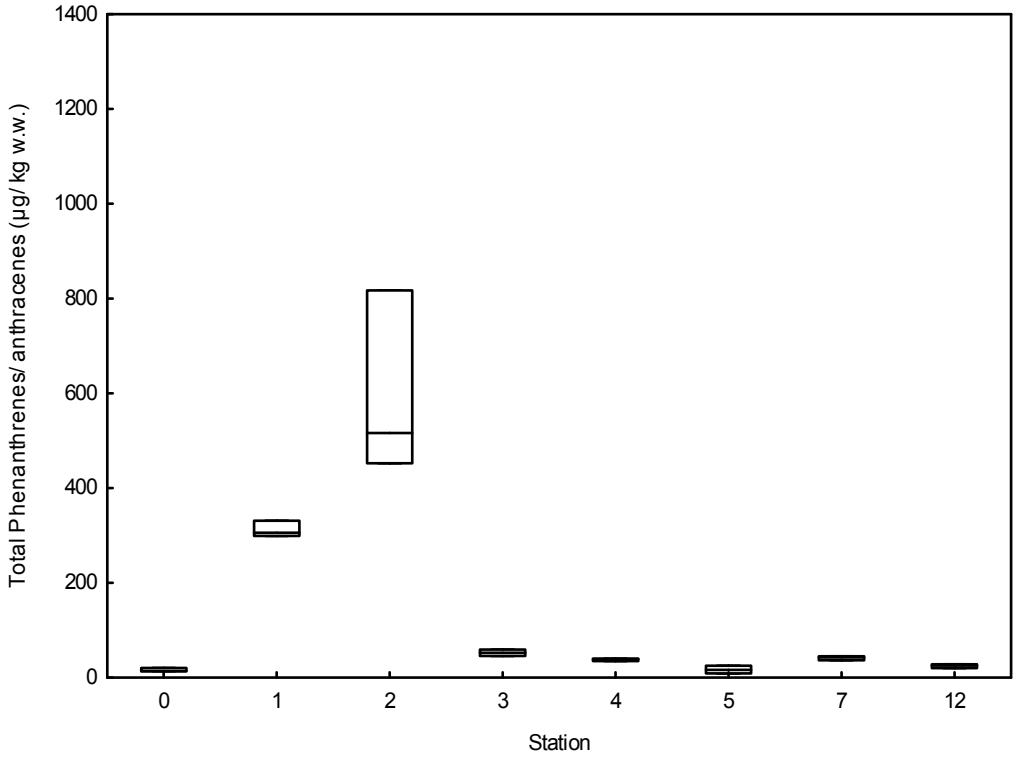
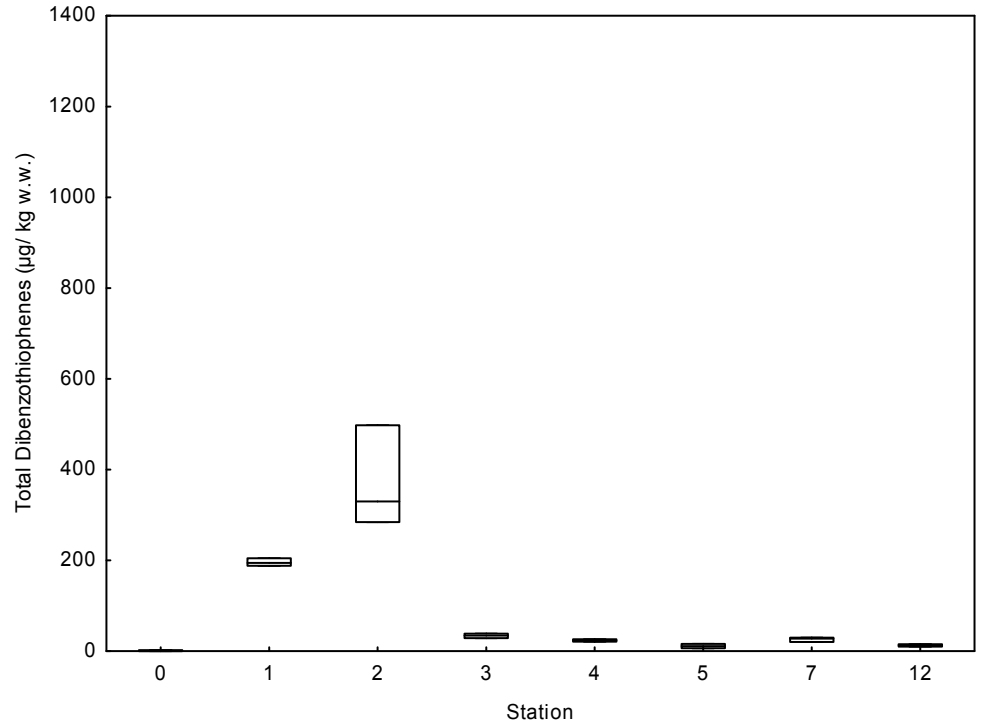


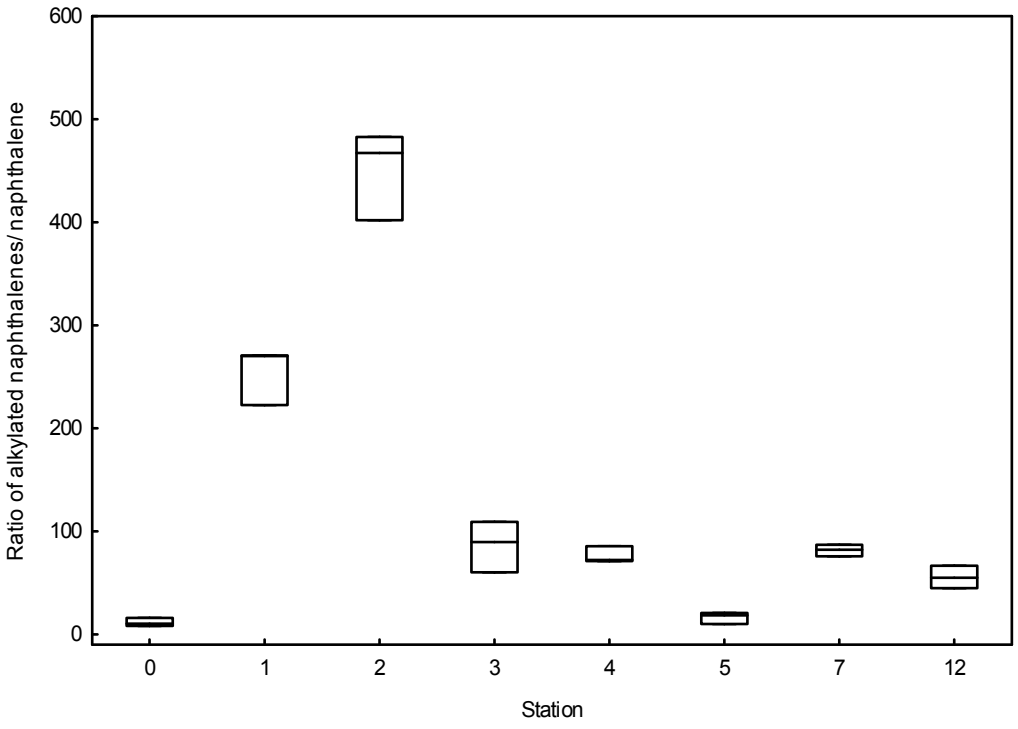
Figure 7. Total naphthalene concentrations shown in mussels from the groups indicated. Boxes depict median and minimum/ maximum values (individual observations since n=3). Data includes the parent compound and alkylated C_1 to C_3. Station 0 refers to the pre-exposed mussels.



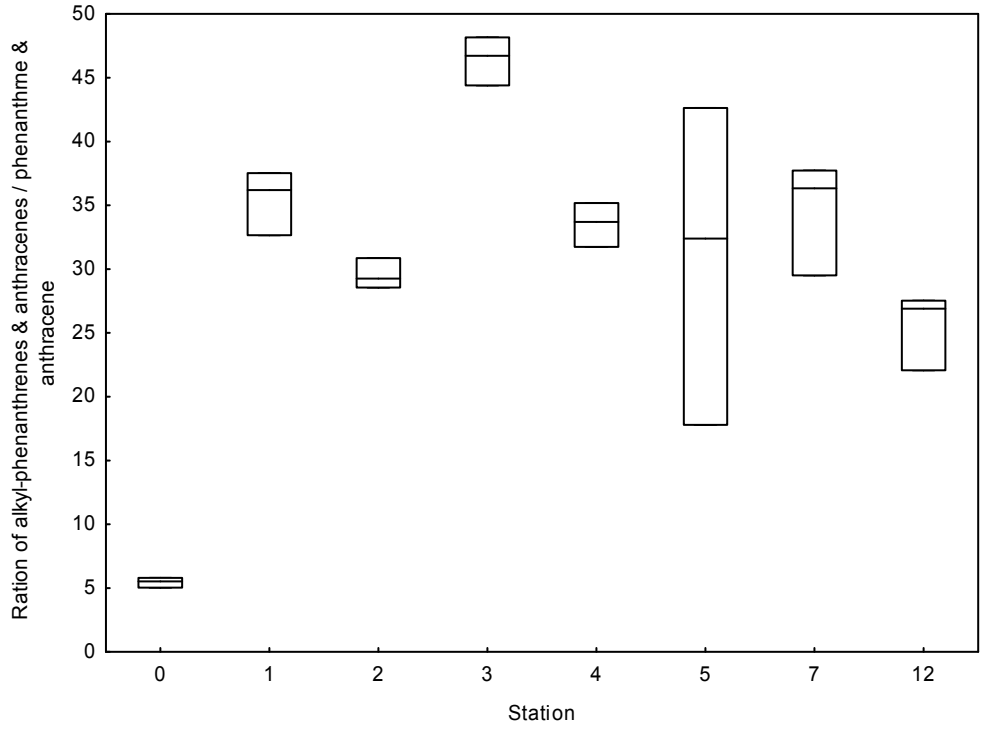
Figu**re 8.** Total phenanthrene/ anthracene concentrations shown in mussels from the groups indicated. Boxes depict median and minimum/ maximum values (individual observations since n=3). Data includes the parent compound and alkylated C_1 to C_3. Station 0 refers to the pre-exposed mussels.



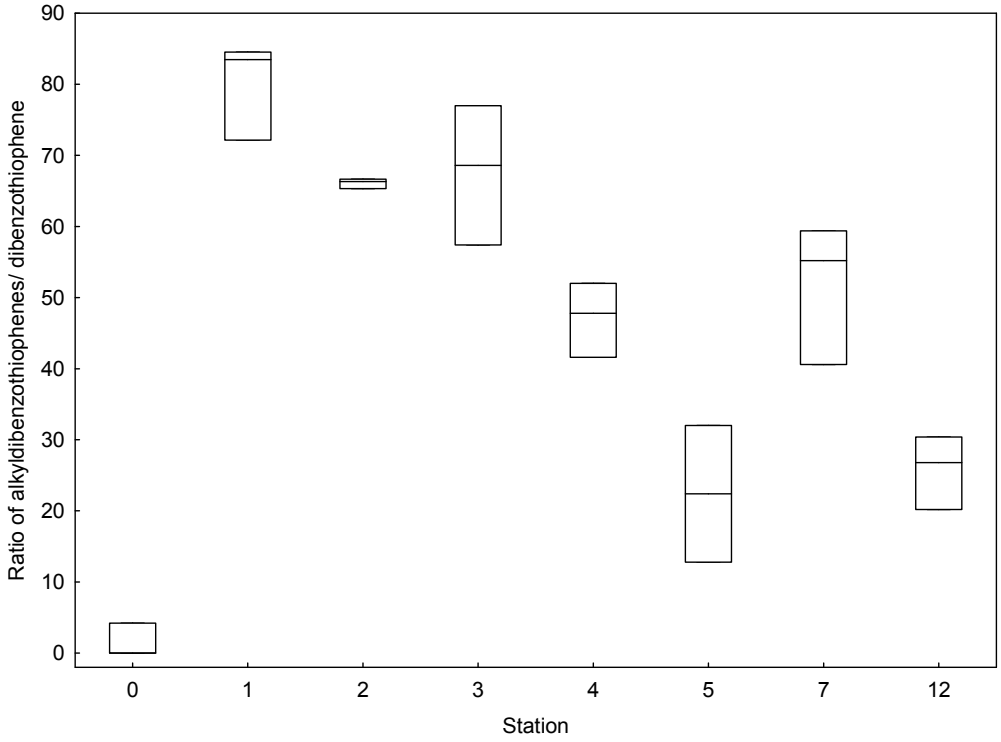
Figur**e 9.** Total dibenzothiophene concentrations shown in mussels from the groups indicated. Boxes depict median and minimum/ maximum values (individual observations since n=3). Data includes the parent compound and alkylated C_1 to C_3. Station 0 refers to the pre-exposed mussels.



Figur**e 10.** Ratio of alkyl-naphthalenes/ naphthalene. Boxes depict median and minimum/ maximum (individual observations, since n=3). Alkylated groups include C_1 to C_3. Station 0 refers to the pre-exposed mussels.



Figur**e 11.** Ratio of alkyl-phenanthrenes & anthracenes/ naphthalene and anthracene. Boxes depict median and minimum/ maximum (individual observations, since n=3). Alkylated groups include C_1 to C_3. Station 0 refers to the pre-exposed mussels.



Figur**e 12.** Ratio of alkyl-dibenzothiophenes/ dibenzothiophene. Boxes depict median and minimum/ maximum (individual observations, since n=3). Alkylated groups include C_1 to C_3. Station 0 refers to the pre-exposed mussels.

3.3.2 Metal concentrations

Table 3. Metal concentrations in the whole tissue homogenates of mussels from the stations indicated. Data expressed as µg/ g w.w. Detection limit for each metal in parentheses)

	Al (0.05)		As (0.05)		Ba (0.002)		Cd (0.001)		Co (0.0005)		Cu (0.03)		Fe (2)		Hg (0.005)		Ni (0.02)		Pb (0.02)		Zn (0.01)	
Station	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Pre-exposed	12.83	4.06	3.87	0.30	0.33	0.13	0.19	0.04	0.06	0.01	1.00	0.13	32.07	6.99	0.02	0.00	0.18	0.03	0.07	0.01	12.23	0.35
1	1.53	0.12	3.00	0.07	0.20	0.04	1.94	0.26	0.10	0.01	1.02	0.05	12.33	0.58	0.01	0.00	0.25	0.02	0.08	0.01	11.40	0.10
2	1.47	0.06	2.85	0.24	0.18	0.05	1.72	0.08	0.09	0.01	1.05	0.02	12.67	0.58	0.01	0.00	0.25	0.04	0.08	0.01	10.23	0.23
3	7.10	8.69	3.10	0.28	0.27	0.17	1.95	0.46	0.12	0.02	1.07	0.14	15.67	1.15	0.02	0.00	0.37	0.06	0.15	0.06	13.10	1.78
4	1.77	0.15	3.04	0.10	0.09	0.01	1.69	0.13	0.10	0.01	0.98	0.11	12.33	1.53	0.02	0.00	0.27	0.02	0.09	0.01	10.82	1.37
5	1.33	0.12	2.76	0.20	0.09	0.01	1.96	0.11	0.10	0.01	0.93	0.05	11.33	0.58	0.01	0.00	0.29	0.02	0.09	0.01	11.43	3.03
7	1.87	0.38	3.88	0.21	0.12	0.01	1.81	0.11	0.11	0.01	1.09	0.08	15.00	1.00	0.02	0.00	0.36	0.05	0.10	0.01	12.77	0.65
12	3.97	3.77	1.37	0.06	0.10	0.08	1.45	0.11	0.06	0.00	0.68	0.08	8.00	1.73	0.01	0.00	0.16	0.01	0.06	0.03	6.71	0.38

A total of 11 metals were measured in the whole mussel homogenates of pre-exposed and caged mussels (**Table 3**). There was no apparent relationship between metal concentration and distance from the platform for any of the metals measured. Higher concentrations of Al and Fe were found in the pre-exposed mussels, whereas Cd concentrations were markedly lower in the pre-exposed group campare to all caged mussels. Overall, metal concentrations typical of North Sea offshore background concentrations were observed.

3.3.3 ^{226}Radium concentrations

Preliminary results indicate that levels of ^{226}Ra in the PW exposed mussels were close to the natural background concentrations, indicating no significant uptake of the radionuclide by the mussels (**Table 4**). This confirms the findings from mussels exposed at Ekofisk in 2009 (Sundt et al. 2010).

Table 4. Concentrations of ^{226}Ra in pooled mussel soft tissue analysed by α-spectrometry (>20 mussels per pool).

Sample	**^{226}Ra** (mBq/kg w.w)		Sample	**^{226}Ra** (mBq/kg w.w)
Lab control A	21 ± 5		Station 4 A	47 ± 8
Lab control B	17 ± 6		Station 4 B	30 ± 7
Lab control C	21 ± 5		Station 4 C	46 ± 11
Lab control D	12 ± 5		Station 4 D	53 ± 12
Station 1 A	27 ± 5		Station 5 A	35 ± 8
Station 1 B	34 ± 6		Station 5 B	44 ± 6
Station 1 C	31 ± 6		Station 5 C	36 ± 6
Station 1 D	33 ± 5		Station 5 D	30 ± 5
Station 2 A	29 ± 5		Station 7 A	22 ± 6
Station 2 B	27 ± 7		Station 7 B	35 ± 7
Station 2 C	25± 6		Station 7 C	32 ± 7
Station 2 D	24 ± 5		Station 7 D	20 ± 5
Station 3 A	43 ± 7			
Station 3 B	31 ± 7			
Station 3 C	35 ± 8			
Station 3 D	38 ± 7			

3.4 Biomarkers in mussels

3.4.1 Pyrene hydroxylase

Pyrene hydroxylase activity in mussels from all exposure stations in Gullfaks C was significantly lower than the activity in the pre-exposure mussels (**Figure 13**). When comparing the pyrene hydroxylase activity in mussels from the different stations at Gullfaks C, no significant difference was found. There was no notable relationship between pyrene hydroxylase activity in mussels and distance from the Gullfaks C platform. In fact mussels from the two closest stations (station 1 and 2, 500 m from the platform) had the lowest median activity levels.

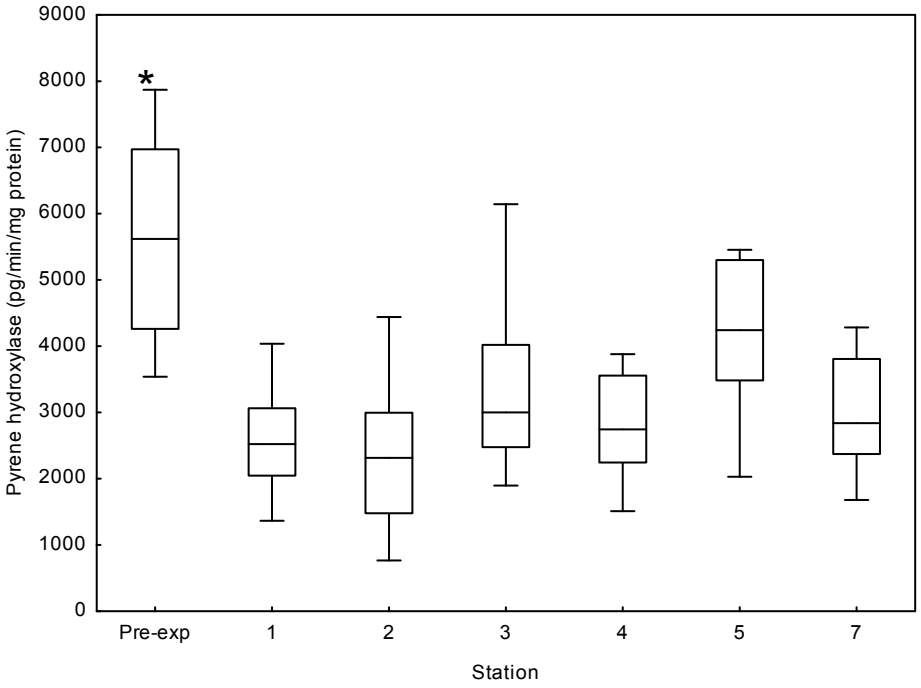


Figure 13. Pyrene hydroxylase activity in the digestive gland of mussels from the stations indicated. Data expressed as median, quartiles and 10/90-percentiles. * Significant difference from all other stations.

3.4.2 Micronucleus assay

Highest MN frequency was found in haemocytes of mussels from station 2 (8 ± 2 per 1000 cells) located 500 m SE of the discharge (**Figure 14**). MN frequencies between 4 and 6 (per 1000 cells) were found in mussels caged at stations 1, 3, 4 and 7, whilst the lowest MN frequency in exposed mussels was observed at Station 5 (2.9 ± 1.0/ 1000 cells). Overall, the lowest MN frequency was found in pre exposed mussels (2.0 ± 1.1). However, no statistically significant differences were observed between the mussel stations.

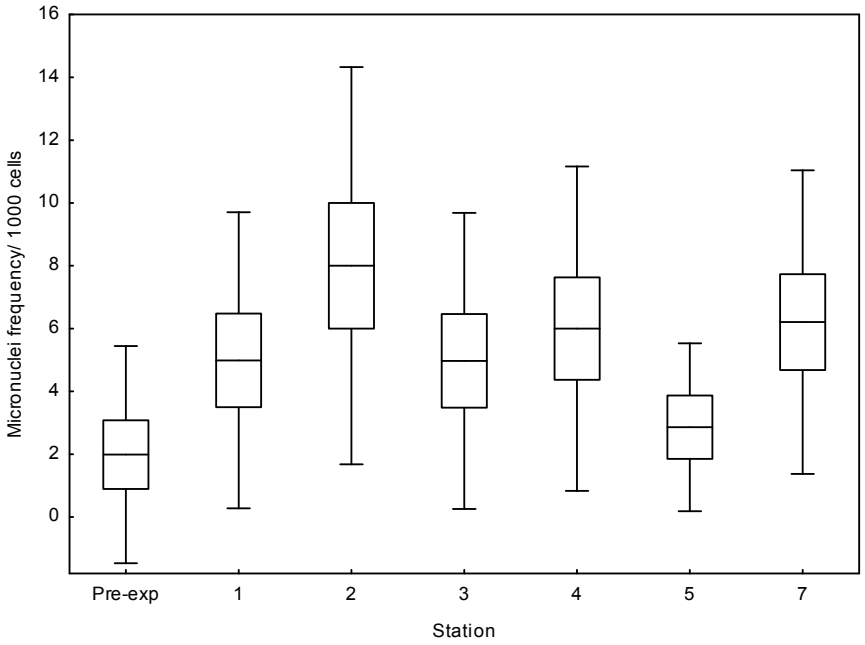


Figure 14. Frequency of micronuclei in haemocytes of mussels from the stations indicated. Data expressed as mean, standard error and standard deviation.

3.4.3 Lysosomal membrane stability in haemocytes

Lysosomal membrane stability was measured in mussel haemocytes as NRRT (min) and ranged from 90 to 180 min (end of the test time) (**Figure 15**). Most of the individual showed values equal or above 120 min that is considered as the threshold level for healthy mussels. The only statistical difference was found between mussel caged at station 2 (120 min) compared to the ones caged at station 4 (180 min) and the pre-exposure group (180 min).

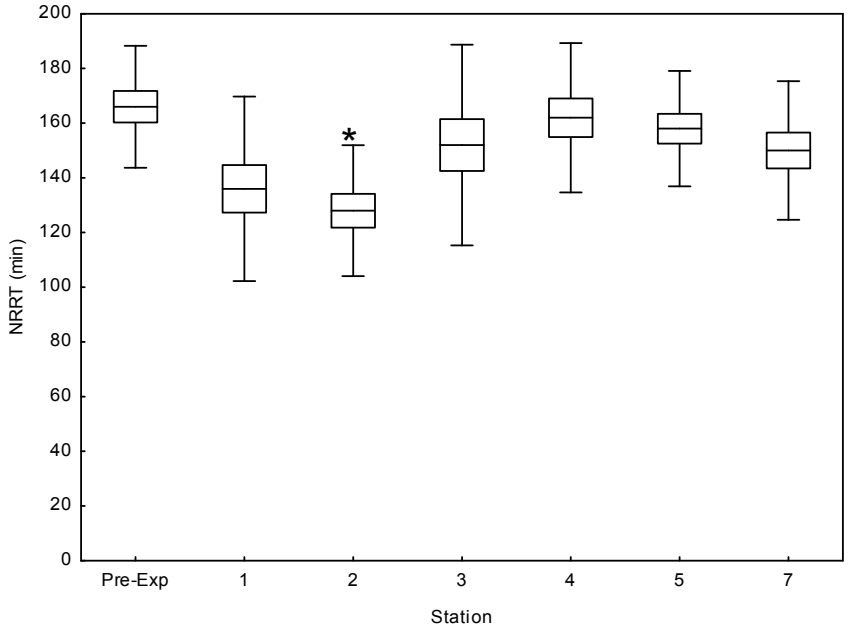


Figure 15. LMS measured as NRRT in haemocytes of mussels from the stations indicated. Data expressed as mean, standard error and standard deviation. * significant difference from station 4 and Pre-exposure.

A new approach for the assessment LMS by NRRT was proposed in this years WCM programme consisted of analysing mussel haemocytes with slide images (Dondero et al., 2006). The retention time of neutral red dye within the lysosomes of haemocytes was monitored after 1 h incubation, instead of 3 h (as normally used in the NRRT assay "live" on board). Due to the poor weather the quality of the slide images was not suitable for quantification of the lysosomal neutral red leakage; therefore the pictures were analysed for comparison of the lysosomal neutral red leakage expressed as % of labilised cells at 60 min. Moreover, due to time constraints, it was not possible to take pictures of all mussel groups (**Figure 16**). Mussels from station 1 and 2 had NRRT values significantly higher compared to mussels from the pre-exposure group. Furthermore, NRRT values in mussels from station 5 and 7 were not significantly different from the pre-exposure ones.

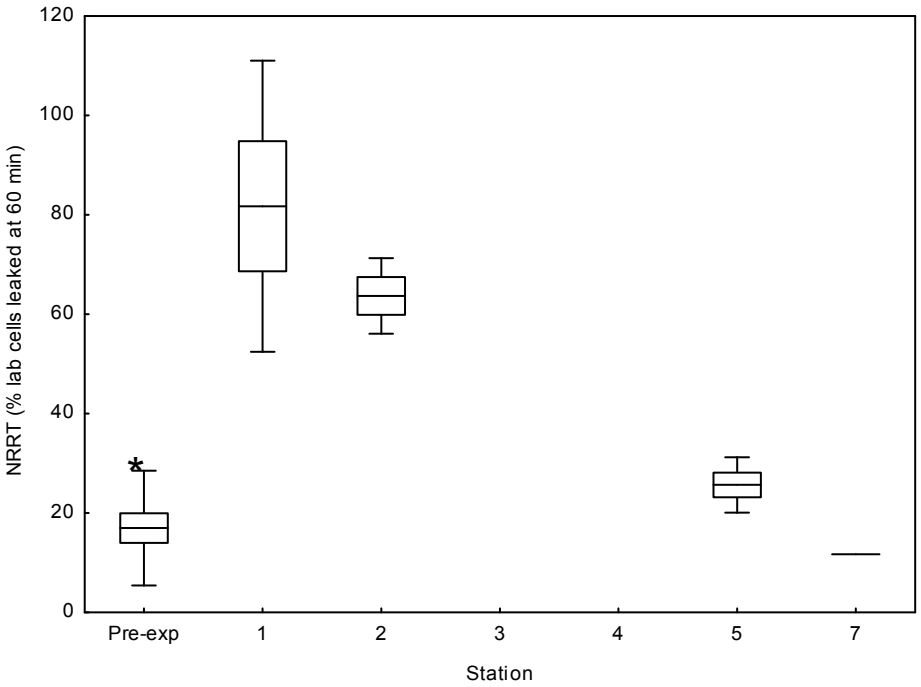


Figure 16. Photographic method for the measurement of LMS by determing NRRT in haemocytes of mussels from the stations indicated. Data expressed as the percentage of cells found to leak the neutral red dye after 60 min (mean, standard error and standard deviation). * Significant difference from stations 1 and 2.

3.4.4 Lysosomal membrane stability in digestive gland

LMS values were lower in digestive glands of mussels caged around the platform area compared to the values reported in the pre-exposed group (**Figure 17**). Moreover, values recorded in mussels from station 3 and 4 were significantly lower compared to the ones from station 1, 5 and 7 and the pre-exposure group.

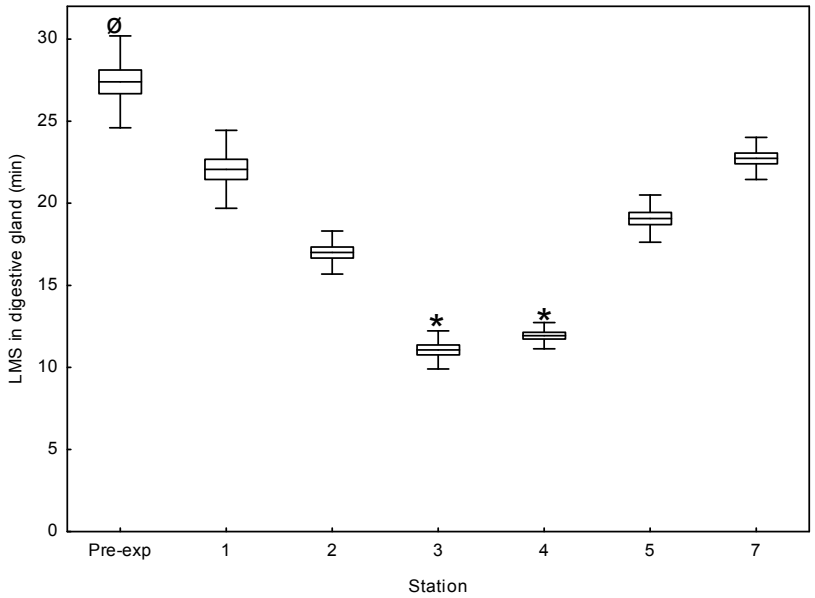


Figure 17. LMS measured in digestive glands of mussels from the stations indicated. Data expressed mean, standard error and standard deviation. * Significant difference from pre exp and stations 1, 5, and 7; ø significant difference from stations 2, 3, 4 and 5.

3.4.5 Lipofuscin accumulation

Lipofuscin accumulation is regarded as an end product of lipid peroxidation, which occurs when polyunsaturated fatty acids are damaged by free radical/ reactive oxygen species. Digestive glands from mussels caged at station 2 had significantly higher lipofuscin accumulation compared to mussels from the pre-exposure group and from station 7 (**Figure 18**). Moreover, mussels for the pre-exposure group showed the lowest level of lipofuscin accumulation in their digestive glands and were significantly different from all mussels caged at the platform area. In general, mussels from station 1, 2, 3 and 4 recorded the higher values.

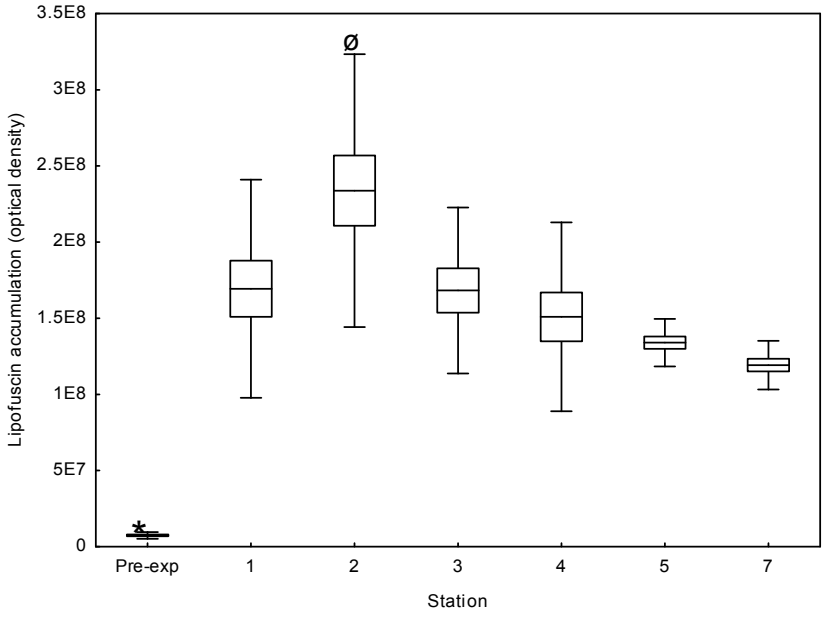


Figure 18. Lipofuscin accumulation in digestive glands of mussels from the stations indicated. Data expressed mean, standard error and standard deviation. * Significant difference from all other stations; ø significant difference from pre-exp and stations 7.

3.4.6 Neutral lipid accumulation

Digestive glands of mussels caged at station 3 showed the highest levels of neutral lipid significantly different from the values registered in mussels caged at station 1, 4, 5 and 7 and in pre-exposure mussels (**Figure 19**). Moreover, mussels caged at station 2 had significantly higher values compared to the ones caged at station 1 and 7 and in the pre-exposure mussels.

In general, all mussels caged around the platform had high level of neutral lipid accumulation in digestive glands compared to the pre-exposure group (even if the values were not always statistically different, probably due to the high standard deviation of the pre-exposure group).

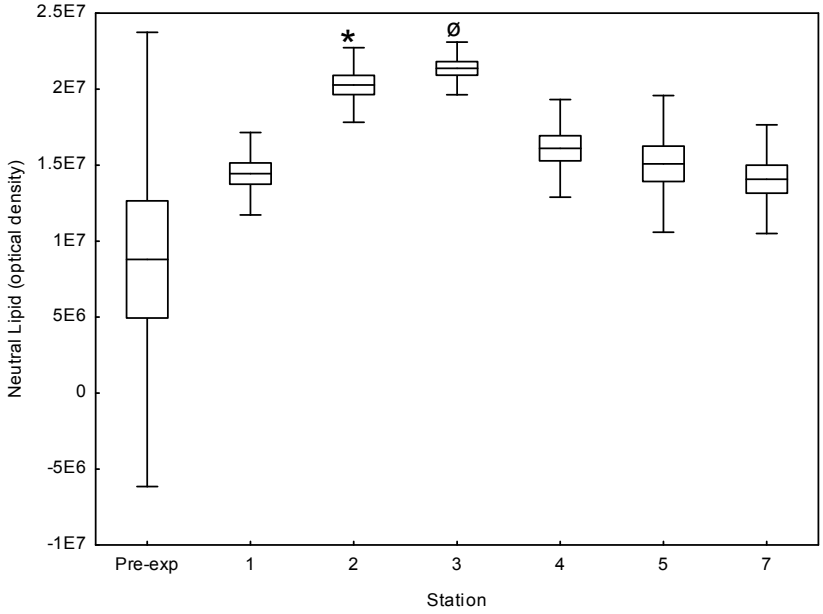


Figure 19. Neutral lipid accumulation in digestive glands of mussels from the stations indicated. Data expressed mean, standard error and standard deviation. * Significant difference from pre-exp and stations 1 and 7; ø significant difference from pre-exp and stations 1, 4, 5 and 7.

3.4.7 Histopathology in selected tissues

Numerous histological parameters were used as markers of health status in mussel caged at the Gullfaks platform. Twenty-three histological parameters were assessed in gonad, digestive gland and gill tissues of mussels including presence of pathogens, inflammatory lesions, non-specific pathologies and reproductive conditions. Obtained results are shown in **Figure 20** and **Figure 21**.

Several of the examined mussels showed histopathological alterations in digestive gland, gonads and gills. These alterations have been scored for each individual mussel. For gill lesions (i.e. epithelial lifting, cuboidal cells, haemolymph vessel, hyperplasia, presence of parasites, fusion of lamella and haemocyte infiltration), no differences were found between mussel groups, either caged at the platform or pre-exposure (**Figure 21**A&B).

As regards the digestive gland and gonad analysis, mussels from the different stations produced quite different results. By using the statistical analysis software JMP, a more objective analysis of the score values is presented. The overall histopathology results indicated that mussels caged at station 1, 2, 3, 4 and 5 were statistically different from the one caged station 7 and the pre-exposure group (**Figure 21**C). Results are presented as quantiles box plots in order to visualise the uneven distribution of data in an appropriate manner. Diagrams show sum of all lesions that have been found in different tissue as box plots (median value, 0%, 50%, 75% and 100% quantiles) at each station. The horizontal line in the middle of the plot indicates the grand mean. A visualisation of statistical analyses is shown at the right

part of each diagram. All red coloured circles mean that there are no significant differences. When a circle appears in a grey tone it indicates a significant different group of data. The size of the circle indicates the power of the data with regard to number of individual samples, e.g. small circle means high power.

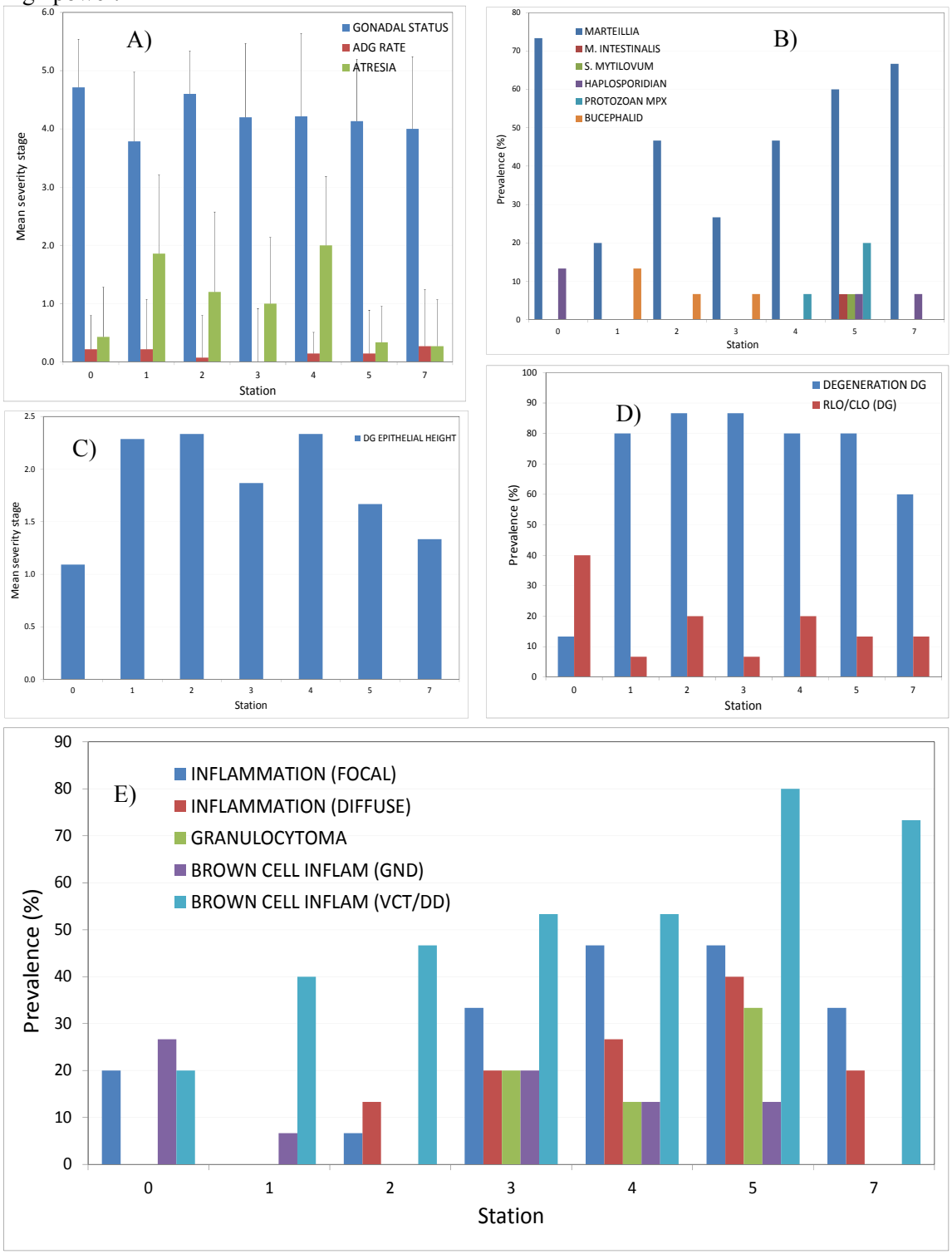


Figure 20. Histopathology markers recorded in mussels collected from the stations indicated. Station 0 indicates the pre-exposure groups. A) reproductive markers, B) parasitic infections, C-D) digestive gland pathology, E) inflammatory lesions.

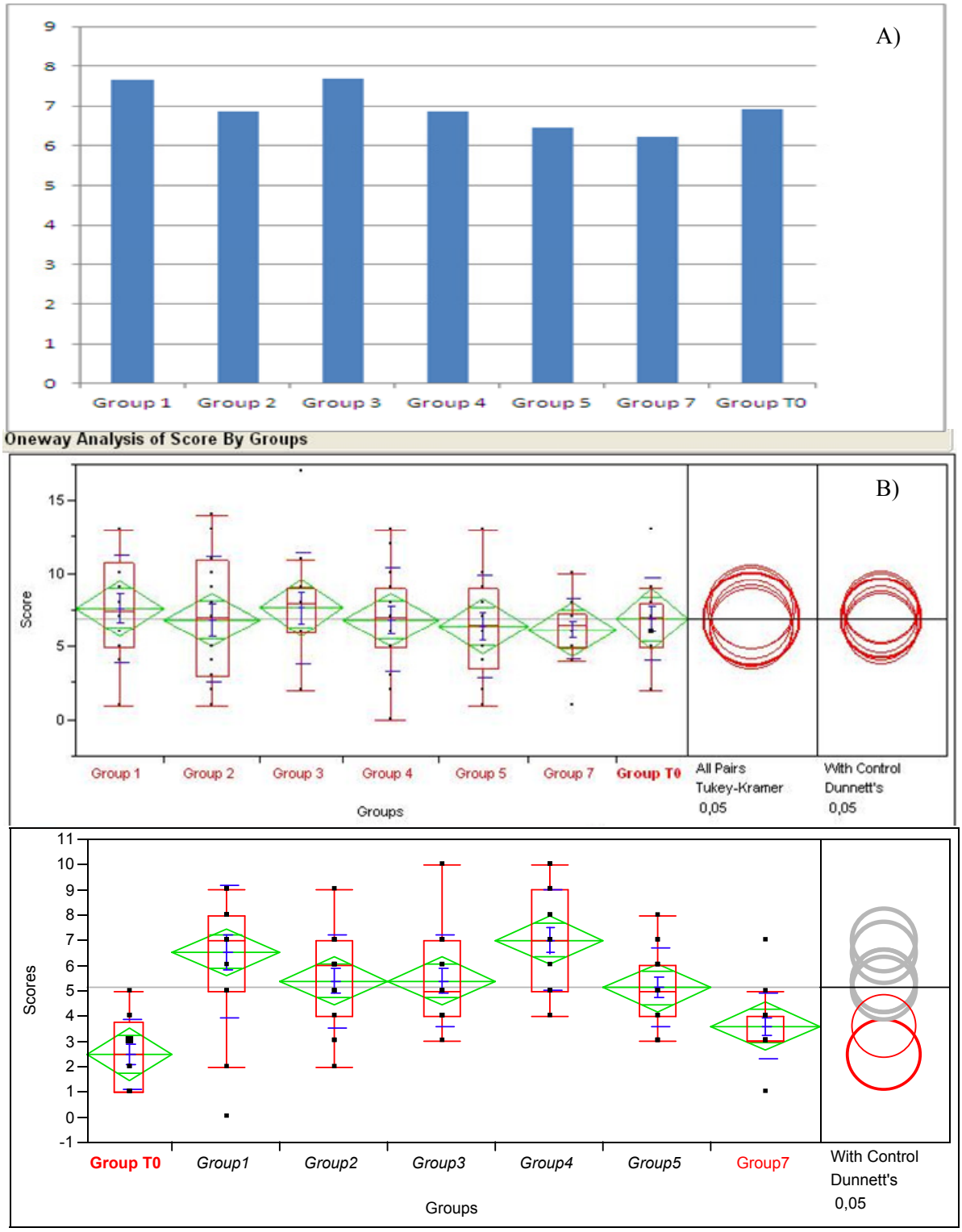


Figure 21. A) Scores of gill lesion in all stations (denoted as group), B) statistical comparison using *JMP version 5.1* by *SAS Institute Inc.* C) Statistical comparison (using one-way ANOVA) of the different mussel stations according to their abnormalities in digestive gland and gonad and parasite analysis. Statistical analyses have been carried out using *JMP version 5.1* by *SAS Institute Inc.*

3.4.8 Condition Index

This physiological measurement of the health status of organisms summarises the overall condition of the animals. It is a physiological response that describes the disruptive effects of pollutants on "normal" functioning and is directed at evaluating effects on energy metabolism or influence on growth and reproduction. In general, CI values were similar in mussels from all stations. The highest values were recorded in mussel caged at station 2 (value statistically different from station 3, 4 and 5) (**Figure 22**).

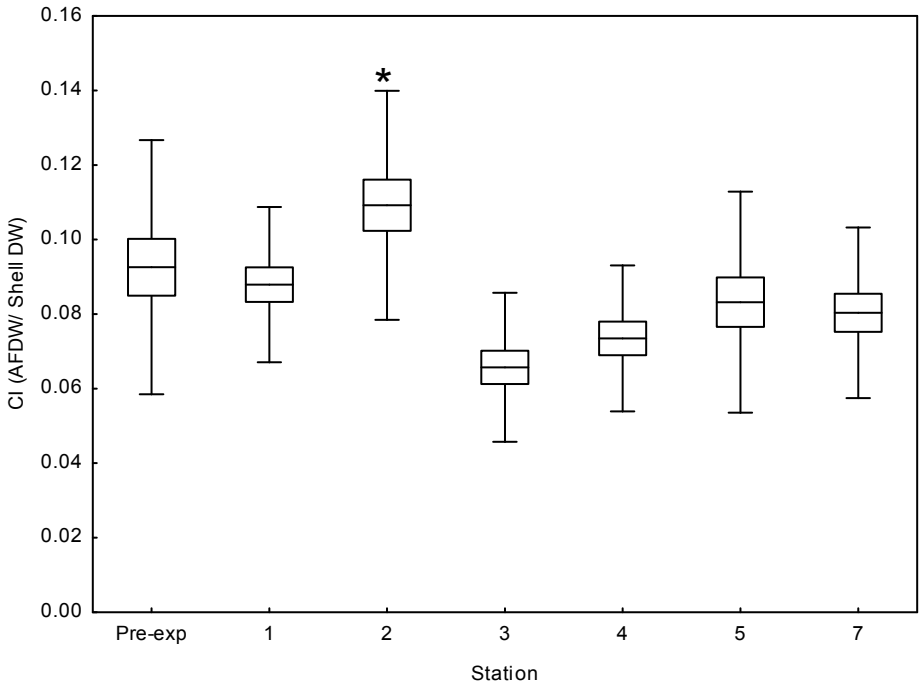


Figure 22. Condition index of mussels from the stations indicated. Data expressed mean, standard error and standard deviation. * Significant difference from stations 3, 4 and 7.

3.4.9 Integrated Biological Response (IBR)

The IBR/n index was calculated from star plots of normalised biomarker data from, NL, LF, LMS, NRRT and MN (**Figure 23**). The IBR/n was zero in mussels from the pre-exposure group. Highest IBR/n of 3.29 was found in mussels from station 2, located 500 m from the discharge. The biomarkers NRRT, MN and NL contributed mostly to the IBR/n score of mussels from station 2. Mussels from station 3 had the next largest IBR/n, although this was almost half of the values shown by station 2. Station 1, had one of the lowest IBR/n values despite being one of the closest stations at 500 m from the discharge.

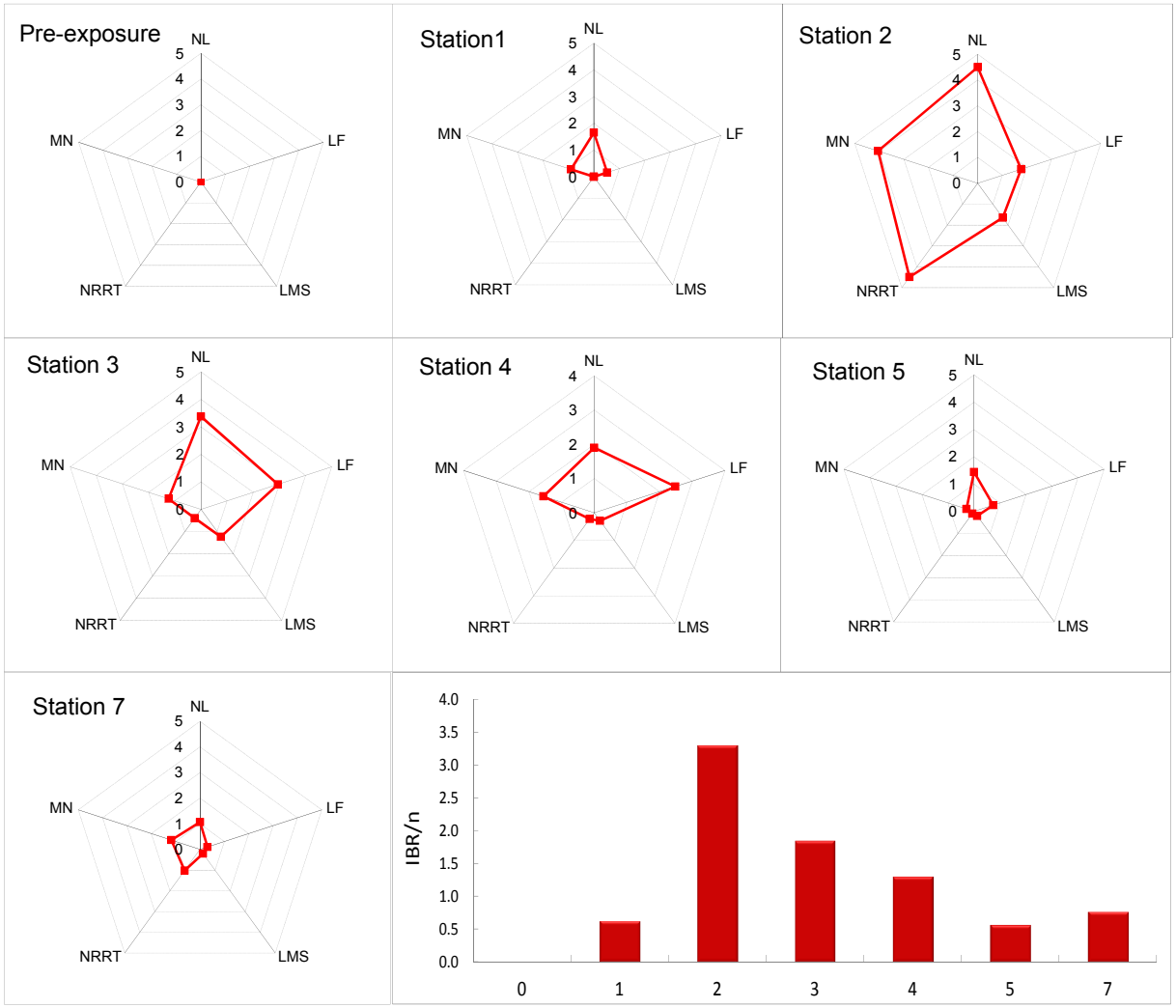


Figure 23. Integrated biological response (IBR) in mussels from the stations indicated and a histogram of the IBR/n calculated from areas connecting biomarker responsiveness in the star plots.

3.5 POCIS extracts

3.5.1 Alkyl phenol concentrations

Alkyl phenols were detected in all POCIS samples from all stations where they were retrieved (**Figure 24**). The highest concentrations of AP were measured in POCIS at stations 2 and 3 located 500 m and 1000 m SE of the platform respectively. C2 phenols were the most abundant group followed by cresol, and then decreasing in concentration from C3 to C9 phenols. The profile of the phenol groups was the same at all stations.

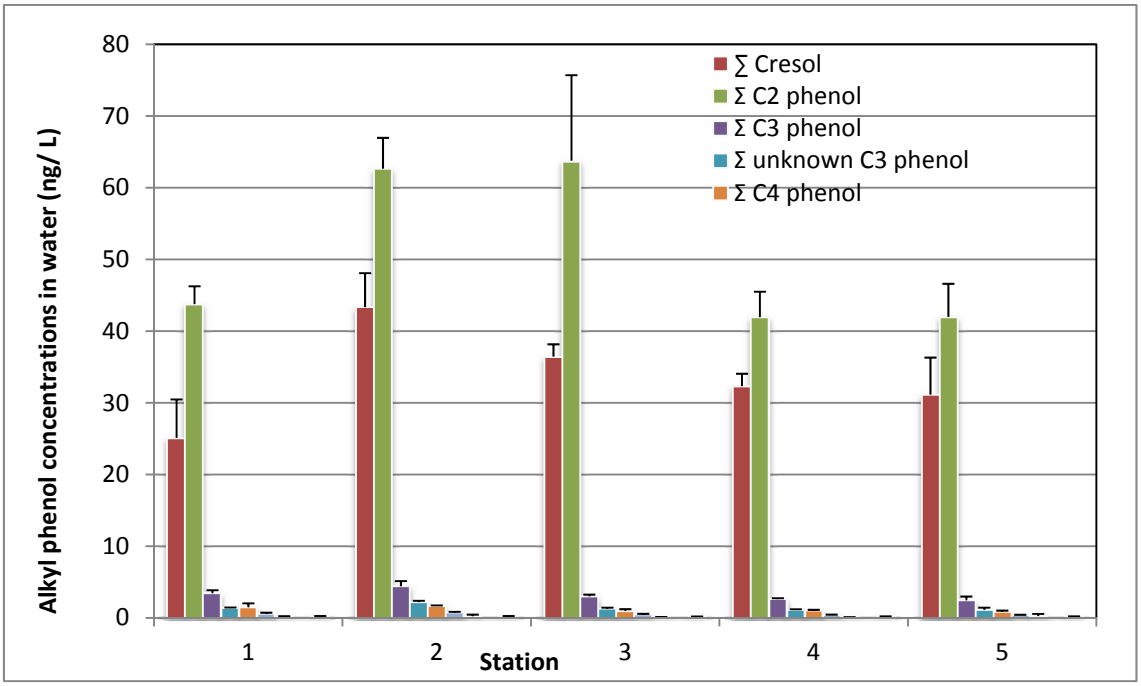


Figure 24. Alkyl phenol concentrations measured in POCIS extracts placed at the stations indicated for 6 weeks. Values are calculated concentrations in water based on the uptake rate coefficients determined from SPMDs (mean ± SD).

3.5.2 Naphthenic acid screening

Screening of NA in POCIS extracts at selected stations allowed for the detection of 10 groups of naphthenic isomers (**Figure 25**). Relative concentrations of these NA have been presented since a quantitative method is not currently available. In all cases, highest NA concentrations (i.e. peak area) were found at the closest station 500 m from the discharge point at station 2. At station 2, the most abundant isomer groups were bicyclic C8, C9 and C10. NA were detected at station 3 with approximately half the intensity of that shown at station 2. The other station positioned 500 m from the discharge (Station 1) had much lower relative amounts of NA than station 2, and even station 3. Station 7, 2000 m from the discharge in the SE direction, and station 5 1000 m NW of the platform appeared to have relatively lower levels of NA.

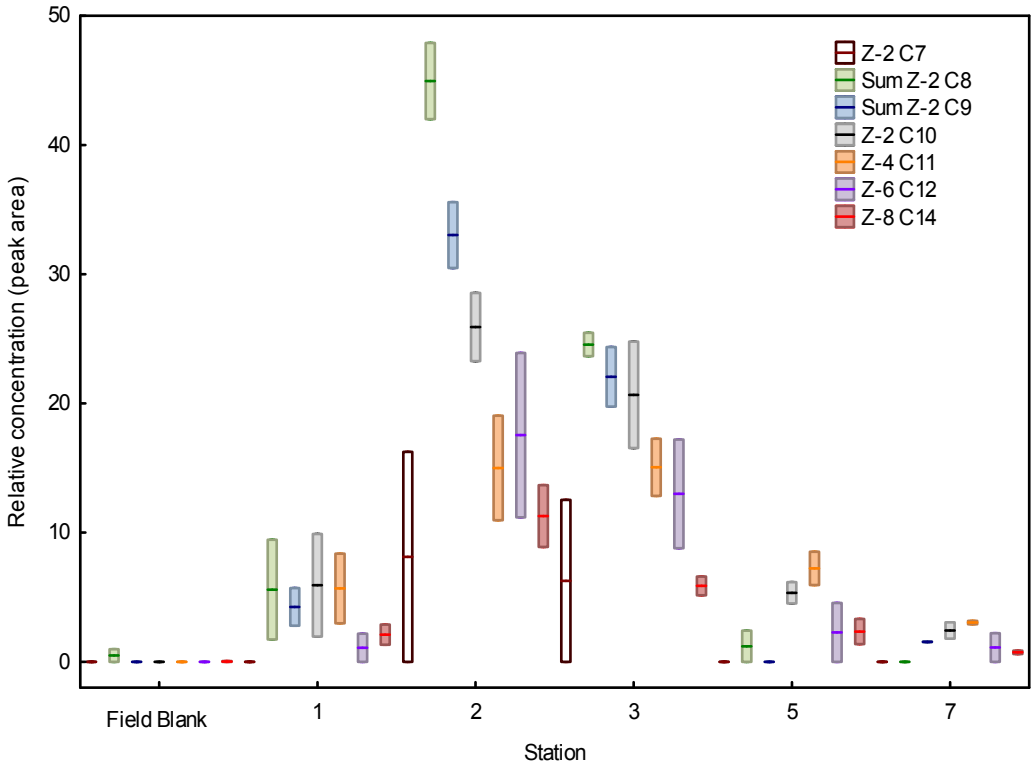


Figure **25.** Naphthenic acids detected in POCIS extracts from the stations indicated. Z= -2 (monocyclic), -4 (bicyclic), -6 (tricyclic), -8 (tetracyclic). C is the number of carbons. Sum Z-2 C8 = sum of 3 compounds, Sum Z-2 C9 = sum of 2 compounds

3.6 Wild caught fish

3.6.1 PAH metabolites

PAH metabolites measured as fixed wavelength fluorescence where detected in both whiting (*Merlangius merlangus*) and tusk (*Brosme brosme*) (**Table 5**). Highest concentrations for both fish species were found in the wavelength pair $PFE_{290/334}$ identifying 2-3 ring structures. There was no apparent difference in the concentration of PAH metabolites between the two fish species.

Table 5. Bile fluorescence in wild fish by Fixed Wavelength Fluorescence.

Species	PFE $_{290/334}$ µg/ml	PFE $_{341/383}$ µg/ml	PFE $_{380/430}$ µg/ml
Whiting	8.4 ± 2.5	1.9 ± 0.7	1.2 ± 0.6
Tusk	8.0 ± 3.2	1.6 ± 1.0	0.9 ± 0.4

PAH metabolites in wild fish by GCMS

Low but quantifiable concentrations of C_1-OH-Naphthalene, C_3-OH-Naphthalene and C_2-OH-Phenanthrene were found in whiting (*M. merlangus*) and tusk (*B. brosme*) caught in the vicinity of the Gullfaks C platform (**Table 6**, **Table 7**).

Table 6. Concentrations of PAH metabolites in tusk bile. LOQ: Limit of Quantification, n.d.: not detected (n=13).

Compounds	ng/g (mean±SD)	Range ng/g	LOQ ng/g
1-OH-Naphthalene	n.d.		30
2-OH-Naphthalene	n.d.		30
C_1-OH-Naphthalene	253±33	218-325	200
C_2-OH-Naphthalene	n.d.		200
C_3-OH-Naphthalene	61±219	<LOQ-791	500
1-OH-Phenanthrene	n.d.		30
C_1-OH-Phenanthrene	n.d.		200
C_2-OH-Phenanthrene	676±955	<LOQ-3601	200
1-OH-Pyrene	n.d.		30

Table 7. Concentrations of PAH metabolites in whiting bile. LOQ: Limit of Quantification, n.d.: not detected (n=12).

Compounds	ng/g (mean±SD)	Range ng/g	LOQ ng/g
1-OH-Naphthalene	n.d.		30
2-OH-Naphthalene	n.d.		30
C_1-OH-Naphthalene	243±86	<LOQ-343	200
C_2-OH-Naphthalene	n.d.		200
C_3-OH-Naphthalene	136±303	<LOQ-759	500
1-OH-Phenanthrene	n.d.		30
C_1-OH-Phenanthrene	n.d.		200
C_2-OH-Phenanthrene	218±175	<LOQ-439	200
1-OH-Pyrene	n.d.		30

3.7 UV fluorimeter data

3.7.1 Field trial

The UV fluorimeter, known as the Aquatracka was towed 50 m behind the research vessel at a speed of 2 knots in a radius of 500 m from the Gullfaks C platform. The Aquatracka was held at a depth of 15 m and relative hydrocarbons were measured every second at a wavelength of 360 nm (**Figure 26**). The relative hydrocarbon values range from 1.4 to 1.6 between 293^o and 220^o from the platform and again from approximately 110^o to 0^o. Relative hydrocarbons were slightly elevated above the background noise to a maximum of 1.7 between 150^o and 130^o in the SE direction.

As a control, the Aquatracka was towed 50 m behind the research vessel at the same speed and depth for 1 nautical mile between the two reference stations (**Figure 27**). The data showed a fairly consistent band for the duration of the tow with a relative hydrocarbon range between 1.4 and 1.65.

The depth profiles taken 500 m from the platform at 90^o (E), 112^o(ESE) 135^o (SE) and 157^o (SSE) from the platform are shown in **Figure 28**. The first three profiles show very little difference in relative hydrocarbons with depth with mean relative hydrocarbons values between 1.4 and 1.5. However, for profile 4, SSE of the platform, a significant increase in relative hydrocarbons was observed at a depth of 10 m (1.78 ± 0.34), suggesting the detection of the PW plume.

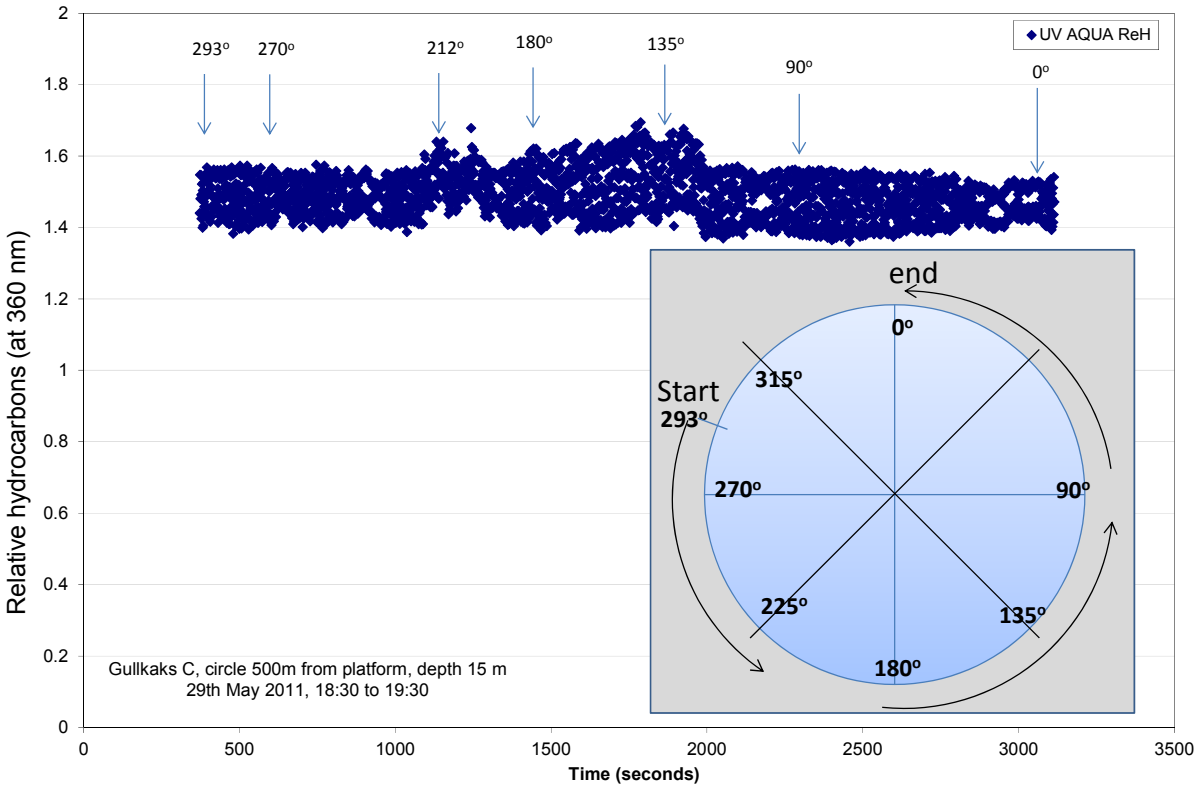


Figure 26. The detection of hydrocarbons in the water in a 500 m circle around the Gullfaks C platform using the Aquatracka UV fluorimeter. Values shown are relative hydrocarbons measured at a wavelength of 360 nm. The inserted graphic describes the starting position of the Aquatracka, which was towed at 50 m behind the research vessel at a depth of 15 m.

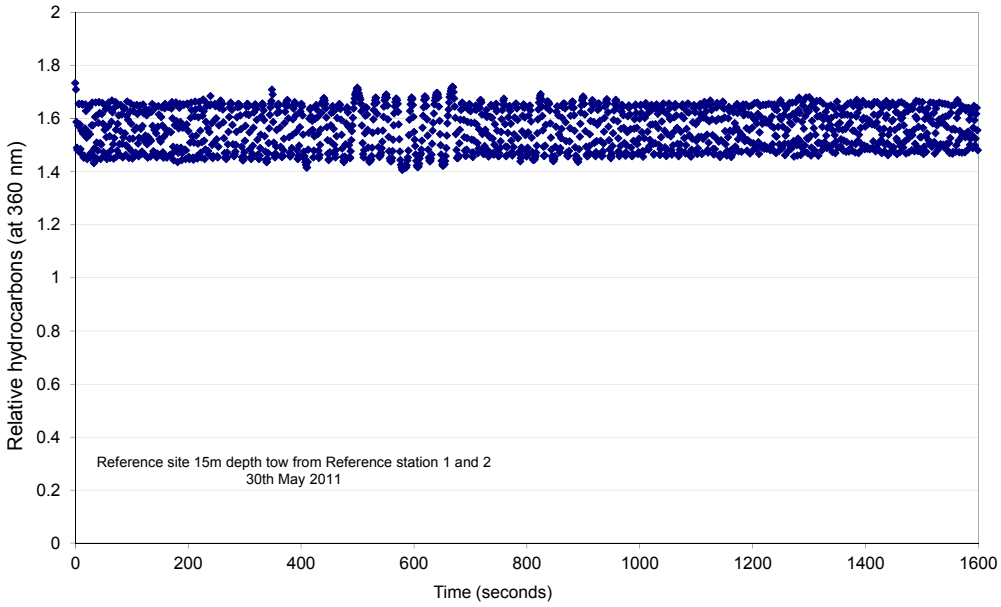


Figure 27. The detection of hydrocarbons in the water using the Aquatracka UV fluorimeter towed 50 m behind the research vessel at a depth of 15 m between reference stations 1 and 2 (approx. 1 nautical mile). Values shown are relative hydrocarbons measured at a wavelength of 360 nm.

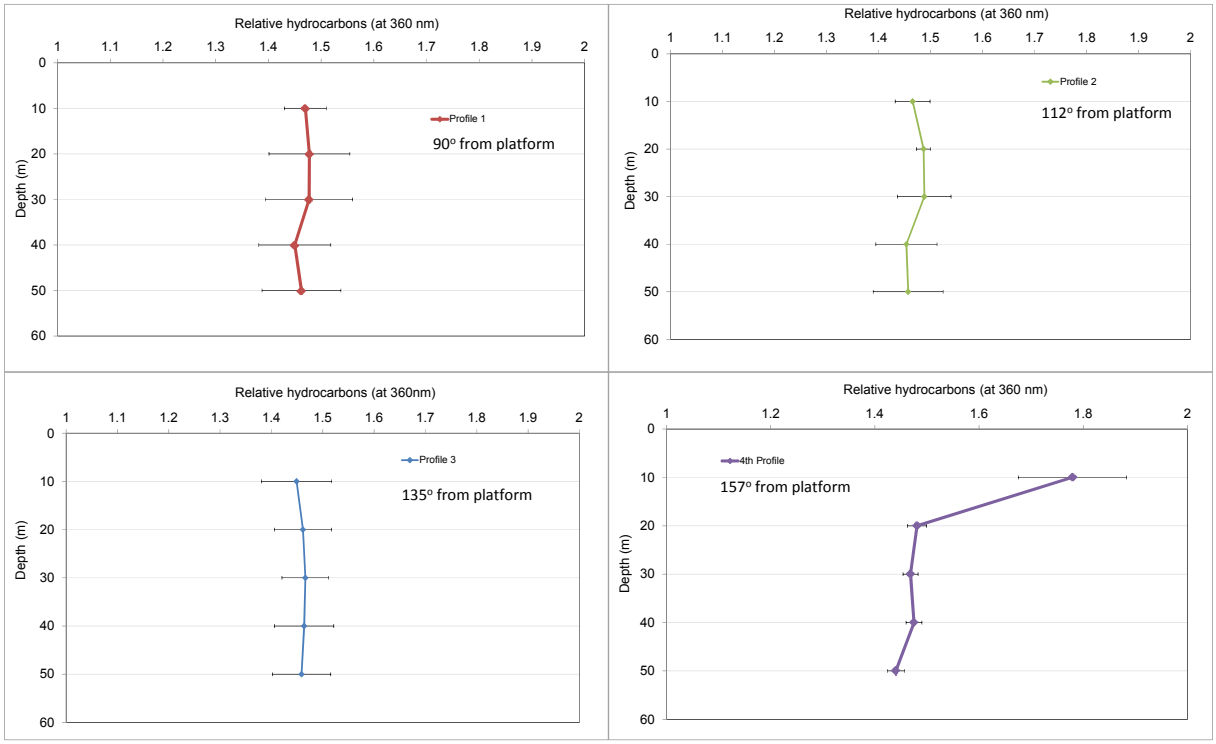
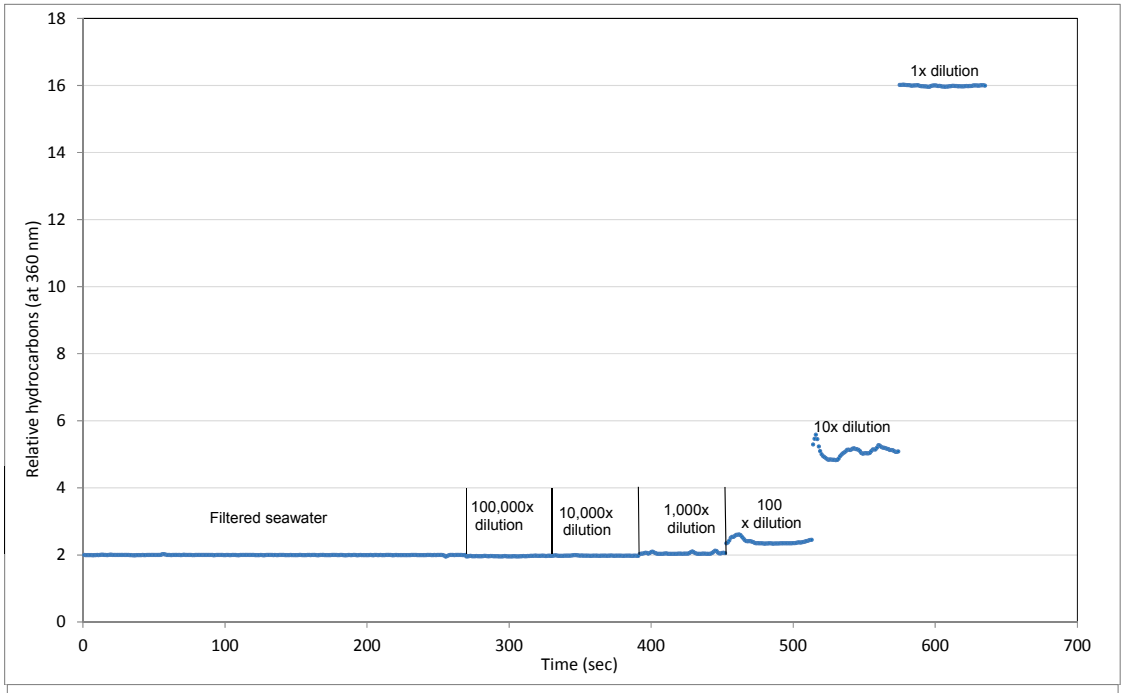


Figure 28. Hydrocarbons detected in four vertical profiles approximately 500 m from the platform using the Aquatracka (absorbance at 360 nm). The Aquatracka was held for 10 seconds at each depth and the relative hydrocarbon measurement recorded. Measurements were taken at 10 m increments to a maximum depth of 50 m.

3.7.2 Laboratory trial

The relative hydrocarbon data following exposure of the Aquatracka to varying concentrations of the artificial PW mixture are shown in **Figure 29**. A clear difference in signal from the filtered seawater can be seen when exposed to 1x, 10x, 100x and perhaps even 1,000 dilution of the artificial PW mixture. However, concentrations of 10,000 dilution and below were not elevated above reference seawater values.



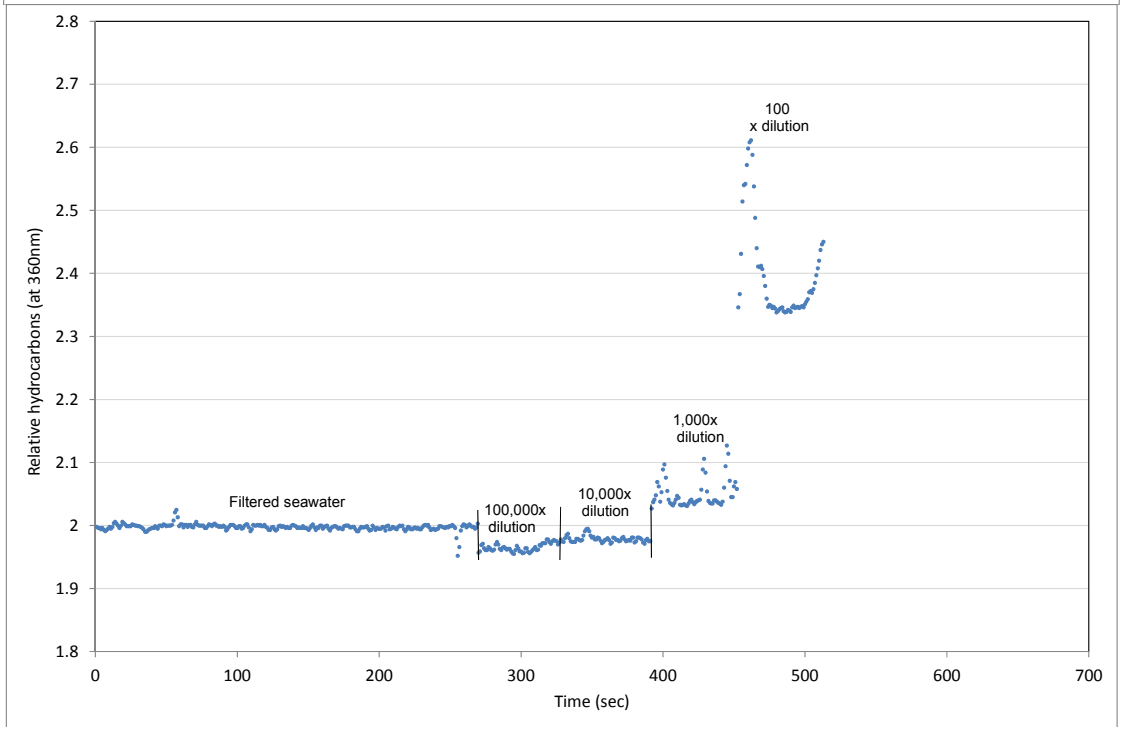


Figure 29. The relative hydrocarbon measurement of the Aquatracka following exposure to varying concentrations of an artificial PW mixture. Graphs present the same data but with different y-axis scaling. 1x dilution refers to the mean concentration of PW chemicals typically found in the Norwegian sector of the North Sea prior to dilution with the receiving water (based on Holth et al., 2008), with subsequent 10-fold dilutions of this with filtered seawater.

4. Discussion

The physicochemical data revealed no stratification of the water column with respect to salinity and temperature that could affect the distribution and direction of the PW discharge. The mussels were held within the water column at a depth of 15 m, whilst the depth of the discharge outlet at Gullfaks C was approximately 20 m. The depth at which the mussels were held during the exposure was deemed suitable for exposure to the PW plume.

The current meter data confirmed that the direction of the plume was in the SE direction, therefore the recovered mussel stations were in the optimal position for an assessment of the effects of the PW discharge from Gullfaks C. Unlike the situation previously seen at Ekofisk, were the tidal current was considered to expose and re-expose the mussels to PW as the tide oscillates; at Gullfaks C, the strong current entirely in the SE direction would ensure that the PW would be transported away from the platform and diluted in the receiving waters with the mussels exposed only for the first instance.

4.1 PAH concentrations in caged mussels

The results of the WCM 2011 survey have shown that mussels caged 500 m from the PW discharge outlet were exposed to elevated concentrations of PAH and NPDs compared to mussels at 1000 m and beyond. The PAH16 concentration at station 2, located 500 m from the platform, where above 30 µg/kg (w.w.). This was significantly higher than the other mussel station at 500 m (Station 1), indicating that station 2 mussels were exposed more to the discharge plume during the 6 week exposure. Despite station 1 and 2 having very different exposures, they were only approximately 200-400 m away from each other, which may suggest that the PW discharge plume from Gullfaks C was rather narrow. Therefore, this highlights the importance of using multiple stations even when the average current direction and speed is previously known.

The maximum PAH16 concentration measured at Gullfaks C was higher than the maximum concentration measured at Ekofisk during the 2006, 2008 and 2009 surveys following similar exposure durations (Brooks et al., 2011a). In addition, PAH16 concentrations measured at Gullfaks C were above those previously measured in mussel tissue after a 6 week exposure in the vicinity of the Troll and Statfjord B platforms (Utvik, 1999 and Hylland et al., 2008 respectively). However, the maximum PAH16 concentration at Gullfaks C was still within the lower end of the range of the concentrations found in mussels from coastal waters of the Nordic countries, where background concentrations have been found to range from 10 to 111 µg/ kg w.w. (Granby & Spliid, 1995).

The mussels positioned at 1000 m and 2000 m contained PAH EPA 16 concentrations typical of offshore background levels of the North Sea, with concentrations measured barely above detection limits at < 2 µg/ kg (w.w.). The pre-exposure mussels contained PAHs from pyrogenic sources, whilst it was clear that the mussels held in cages in and around the platform bioaccumulated PAHs from petroleum.

The NPD concentrations showed the same pattern in concentration with respect to mussel station as that described for PAH EPA16. Concentrations of NPD where significantly higher in mussels from station 2, elevated slightly at station 1, and just above detection limit at all other exposure stations. Overall naphthalene was measured at the highest concentrations in mussel tissues followed by phenanthrene and dibenzothiophenes. The highest median naphthalene concentrations in station 2 mussels (800 µg/kg w.w.) were markedly higher than that previously measured at Ekofisk in 2006, 2008 and 2009 (300 µg/ kg w.w. Brooks et al., 2011a). This was also the same for phenanthrene and dibenzothiophene with maximum median concentrations of 500 and 320 µg/ kg w.w. respectively, compared to 375 and 110 µg/kg w.w. at Ekofisk (Brooks et al., 2011a).

4.2 Biological responses in caged mussels

Bioaccumulation data have indicated that only mussels caged at 500 m from the Gullfaks C platform were exposed to PAHs, while the biomarker results showed a more complex picture of the area. These effects may be in response to exposure to other chemicals in the PW such as AP and NA that were measured from these outer stations using POCIS, or other compounds that were not measured. Single biomarker information will be discussed separately and an overview of the results will be shown through integration of the biological responses.

Since mussels were not retrieved from the planned reference stations, results will be discussed using the pre-exposure group of mussels as a reference. Moreover, animals caged far from the PW discharge, station 7 (2000 m from the platform) and 5 (1000 m from the platform and on the other direction of the PW plume) will be regarded as potential references for the biomarker result discussion.

4.2.1 Pyrene hydroxylase

PH is an MFO enzyme involved in PAH metabolism within the mussel digestive gland. Its activity has been therefore previously proposed as a biomarker of exposure to PAHs (Fillman et al., 2004, Bebianno and Barreira 2009). This enzymatic activity has been extensively studied as an exposure marker of organic xenobiotics in mussels and clams (Michel et al., 1994, Peters et al., 1999, Nasci et al., 2000, Porte et al., 2001). In this study, PH activity was significantly lower in all the animals caged at the Gullfaks platform compared to the pre-exposure one. However, the PH activity showed no relationship with either distance from the platform or with the PAH bioaccumulation in the mussel tissues, with no statistical difference found between stations. The relatively high PH activity in the pre-exposure group may suggest exposure to pyrene or pyrene like compounds either in the mussels natural habitat or whilst held in the holding tanks of flowing seawater at the IRIS research facility prior to day 0 sampling.

Comparison of the PH activity at Gullfaks with that of Ekofisk in 2008 and 2009 (Brooks et al., 2011a) was not possible due to the refinement in the method between Gullfaks and Ekofisk samples. Improvements in the method has led to an increase in the sensitivity resulting in Gullfaks mussels measuring an order of magnitude higher than that measured in mussels at Ekofisk.

4.2.2 Micronucleus assay

The frequency of MN is regarded as an important tool for *in situ* monitoring of genotoxicity. This marker has been extensively used and recommended for off-shore biomonitoring (Barsiene et al., 2006, Gorbi et al., 2008, Sundt et al., 2011). This biomarker shows a continuously increasing trend in animals exposed to increasing concentration of pollutants and exposure time (Viarengo et al., 2007). In this study, the MN frequency is in the same range as previous WCM data from Ekofisk. In general, no statistical differences were found between groups, suggesting no genotoxicity. However, suggested assessment criteria for MN has been established (ICES WGBEC 2010) using data available on studies of molluscs from the North Sea, Northern Atlantic and the Mediterranean, calculating the empirical 90% quantile as background/threshold level of micronuclei incidences. From this report, based on over 600 data points, background levels in *M. edulis* held in cages for 4 to 6 weeks were 4.06 MN per 1000 cells, with MN incidence above 4.06 considered as an effect response (i.e. genotoxicity). From the MN incidence in the Gullfaks mussels, only the mussels from the pre-exposed and station 5 group had MN lower than 4.06 MN /1000 cells, with possible genotoxic responses observed in stations 1,2,3,4 and 7. The chemicals causing these apparent genotoxic responses are likely to be PAH compounds in the closest stations, although at the stations further from the platform, PAH-NPDs were almost absent. However, AP and NA were measured in many of these outer stations and may be at least partly contribute to the observed biological response.

4.2.3 Lysosomal membrane stability

LMS is one of the most used biomarker in monitoring activities and laboratory studies and has been proposed as a screening test for field surveys, in particular for PW monitoring (Viarengo et al., 2007, Sundt et al., 2011). Biomarker based risk assessment is often limited by a lack of connection between marker levels and effect data. However, for LMS (as NRRT assay) threshold levels have been defined (ICES/OSPAR, 2009). Mussels are considered to be healthy if the NRRT value is above 120 min, stressed but capable of compensating it if the value is between 120 min and 50 min and severely stressed and probably exhibiting pathology if the value is below 50 min. LMS, evaluated through the usual NRRT assay, did not show any statistical difference between the groups with only one exception (station 2). However, despite this significant difference in station 2 mussels, all mean values were equal or above 120 min indicating healthy mussels.

A new approach for the LMS evaluation was adopted in this years WCM programme, using image analysis (Dondero et al., 2006). Due to poor weather offshore, non-optimal equipment and time constraints, it was not possible to fully compare the two approaches. Nevertheless, the image analysis approach indicated a statistical difference between mussels caged at station 1 and 2 and the pre-exposure group. This is contrary to the standard NRRT data where no stress response was observed in all mussels sampled. However, supplementary information is needed before a full assessment can made, although the technique does show promise particularly for offshore operations.

LMS in the digestive gland of mussels indicated relatively poor conditions in mussel caged around the platform area. These results agreed well with the NRRT Image analysis method. Assessment criteria have not yet been clearly established for LMS in caged mussels. However, in native shore mussels LP values over 20 min indicate a healthy condition; <20 and >10 min indicate a minor stress, whereas LP values lower than 10 min indicate a severe stress situation (Viarengo et al., 2000). Based on these assessment criteria, pre-exposed mussels and those from stations 1 and 7 were indicative of a healthy mussel, whilst all other mussels exhibited minor stress.

4.2.4 Lipofuscin accumulation

Enhanced lipofuscin accumulation has been consistently observed in digestive gland of mussels exposed to PAHs in both laboratory and field studies (Au, 2004). Significantly higher level of lipofuscin deposits were found in mussels caged at all stations compared to the pre-exposure group, with highest values at the closest station (station 2) corresponding with the highest PAH-NPD concentrations at this station. The high lipofuscin accumulation was indicative of oxidative effects in the mussel digestive glands. Assessment criteria are currently not available for lipofuscin accumulation in field mussels.

4.2.5 Neutral lipid accumulation

Neutral lipid accumulation in digestive cells may be considered indicative of exposure to organic chemicals of different physicochemical properties (Brooks et al., 2011a). In our study, higher levels of neutral lipid accumulation were recorded in all mussels caged at the platform area compared to the pre-exposure group, even if only mussels from station 2 and 3 resulted statistically different, which may have been due to the high standard deviation in the pre-exposure group. Currently no assessment criteria are available for lipofuscin accumulation in field mussels.

4.2.6 Histopathology

Histopathological examination of the mussel tissues can assist in the interpretation of biomarker results (e.g. responses may be influenced by gender, gamete developmental cycle, presence of parasites or lesions that imply cell loss/hypertrophy/migration/etc.) and provides sensitive, useful and potential indications for the screening of the mussel health status (Brooks et al., 2009). Therefore, the

generated data aimed to provide complementary information to support cellular and biochemical based biomarkers such as those commonly used in monitoring programme. Compared to the pre exposure mussels, caged mussels around the Gullfaks platform showed a loss of histological integrity in digestive gland tissue and other alterations, which was typical of a minor stress environment (Lowe et al., 1981; Cajaraville et al., 1990, 1992; Marigómez et al., 2006; Wedderburn et al., 2000; Usheva et al., 2006; Kim et al., 2008; Aarab et al., 2008). For example in particular, the presence of atrophy in digestive tubules, present in some individuals, may be explained by the presence of PAH compunds (Aarab et al., 2008 and 2011). The study of the gonads aimed to reveal possible disturbance in the mussel reproduction in mussel caged close to the platform. In this study, various abnormalities have been recorded. To summarise the histopathological data, the scored parameters from both digestive gland and gonad were merged in a matrix and analysed statistically for differences. The overall results indicated that mussels caged at station 1, 2, 3, 4 and 5 were statistically different from the one caged station 7 and the pre-exposure group. As regards, the gill tissue, there was no difference in the frequency of gill lesions detected between the stations and these measuements were not included in the final overall analysis.

Finally, International Council of Exploration of the Seas (ICES) guidelines for conducting histopathology on mussels have been initiated through the ICES Working Group on the Biological Effects of Contaminants (i.e. both NIVA and IRIS are participating). The development of grading criteria for histological health parameters, together with adequate SOP and QA controls, will strength their use and inclusion in future monitoring programmes.

4.2.7 Condition Index

Physiological markers are aiming to summarising the general status of the analysed organisms, providing an overview of the animal conditions and an indication of the level of damage caused by pollutant exposure (Pampanin et al., 2005). For the Gullfaks mussels, no differences were found between mussels caged near the platform and the pre-exposure group, indicating general good health conditions of the animals.

4.2.8 Integrative Biological Response (IBR/n)

Integrative Biological Responses (IBR/n) has been previously applied to fishes and mussels including different suites of biomarkers (Baussant et al., 2009; Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006; Brooks et al., 2011b; Damiens et al., 2007; Pytharopoulou et al., 2008; Raftopoulou and Dimitriadis, 2010.). Five different biomarkers (NRRT, MN, neutral lipid accumulation, lipofuscin accumulation, LMS in digestive gland) were used to calculate the IBR index developed by Belaieff and Burgeot (2002), and they were orderly represented in the five axes of star plots according to their biological complexity level. IBR/n discriminated successfully between the different stations within the platform areas. In fact, the IBR/n values were moderately higher in stations 2, 3 and 4 compared to the pre-exposure group and station 7 (suggested as references from the chemical analysis results). Station 5 resulted similar to station 7 and the pre-exposure group. The highest IBR/n of 3.29 at station 2 was indicative of low to moderate effects and was comparable to that found in caged mussels from other studies investigating the effects of PW discharge in the receiving waters (Brooks et al., in press).

Overall, the results are in good agreement with the location of the stations in relation to both the PW discharge distance and the PW plume direction (as revealed by the current data). The biomarker responses were not severe (as supported by the physiological and histopathology markers) indicating a relatively low effect of the PW discharge in the platform area that decrease with increasing distance from the discharge point.

4.3 Alkyl phenols and naphthenics in POCIS

Exposure to both AP and NA in the vicinity of the discharge was clearly shown by POCIS extracts. Levels of AP were similar to those measured at similar distances (ca. 500 m) from offshore PW discharges in previous studies, where levels of < 55 ng/ L in 2008 and < 200 ng/ L in 2009 were shown at Ekofisk and < 70 ng/ L in 2004 at Statfjord B (Harman et al., 2009; 2010; 2011). It should be remembered that chemical uptake by POCIS cannot currently be corrected for differences in exposure conditions between laboratory calibrations and field exposures in contrast to hydrophobic passive samplers such as SPMDs. Calibration data for alkyl phenols and POCIS are available and hydrophobic samplers have been used as surrogates for the correction of exposure conditions (Harman et al, 2008; 2009). This means that water concentrations of AP derived from POCIS should be considered as semi-quantitative, providing information about the order of the average concentrations during the exposure period. The ecological consequences of such accumulations may only be inferred, but based on laboratory studies the estimated concentrations appear several orders of magnitude lower than those shown to illicit both acute and sub lethal effects.

There is a reasonable agreement between the AP POCIS results and the biological results, however the gradient of exposure is far less pronounced from the POCIS results than the biological data. The rather flat concentrations between stations for AP may indicate that these compounds are at or nearing equilibrium. The data are dominated by cresols and C1 AP, and this may indicate that such compounds are more widely distributed around the installation than that which would be expected for PAH, for example. Although many AP have been reported to have some degree of estrogenic potency, the estrogenicity is markedly higher in the C8 and C9 AP (Tollefsen et al., 2008). From the POCIS extracts, C8 and C9 AP were below detection limits in the POCIS. This would suggest that the estrogenic risk from exposure to chemicals in the PW, 500 m downstream from the discharge, was low.

For NA there is excellent agreement between the chemical measurements and the overall IBR, (Figs. 24 and 22, respectively). The NA analysis method is purely qualitative, indicating only that these compounds are present in the receiving waters. Even though NA have been shown to be acutely toxic in several studies (reviewed by Clemente and Fedorak, 2005) much work is required to elucidate both the modes of action, potential chronic effects and chemical characterisation as to which compounds are responsible.

Overall levels of AP (and NA) appear low even taking the uncertainty of the measurements into account. In the absence of adequate biological measures of exposure, the POCIS results confirm that mussels have been exposed to both groups of compounds during the deployment.

4.4 PAH metabolites in wild caught fish

Concentrations of C_1-OH-Naphthalene, C_3-OH-Naphthalene and C_2-OH-Phenanthrene were found in both wild caught whiting (*Merlangius merlangus*) and tusk (*Brosme brosme*). These data support the previous findings from the Norwegian condition monitoring programme where the presence of 1-OH-phenanthrene in the bile of haddock collected in the Tampen area was indicated. When comparing the PAH metabolite data from Gullfaks C with those measured in cage fish exposed to PW at Ekofisk in 2006, 2008 and 2009 (Brooks et al., 2011a) a striking difference can be seen. The wild fish from Gullfaks did not contain C_2-OH-Naphthalene or C_1-OH-Phenanthrene unlike the Ekofisk fish, which would suggest that the source of PAH exposure to the Gullfaks wild fish was not from fresh unweathered PW.

4.5 Aquatracka UV fluorimeter

The Aquatracka UV fluorimeter was tested within the WCM 2011 programme to assess its suitability for the detection of hydrocarbons within the PW discharge plume. The Aquatracka was designed to measure hydrocarbons at a fixed wavelength of 360 nm, which may be more suitable for 4- ring PAHs and less suitable for 2-3 ring PAHs.

The circle tow at 500 m around the platform at a depth of 15 m did show a very weak signal compared to the background when positioned SE of the platform. This would support the current meter data as the expected direction of the PW discharge plume. However, this signal was extremely weak and from this data alone the Aquatracka would appear not sensitive enough to detect the plume at 500 m from the platform. However, a much clearer signal was found at a depth of 10 m during the 4th vertical profile when the Aquatracka was lowered in the water whilst positioned 500 m SSE of the platform.

The laboratory exposure to the mixture of PW related compounds suggests that dilution of the PW to 1000x and below would make it difficult for the Aquatracka to distinguish the signal from background noise. At 500 m, dilution of the PW may be expected to be diluted in the region of 1000 times to 2500 times its original concentration (Durrell et al., 2006). Although it is realised that the artificial mixture of PW related compounds made within the laboratory would be very different to the original PW discharged offshore, and that dilutions may be specific to individual platforms, it does provide some indication that the Aquatracka may not be able to achieve the required sensitivity at 500 m away from the platform.

The main benefit and original purpose for the inclusion of the Aquatracka in the WCM programme was to provide real-time exposure information throughout the entire 6 week field deployment. By exposing the Aquatracka alongside the mussels, the Aquatracka could potential provide important information on the different type of exposure to the PW plume experienced by the mussels, such as either in pulses or as a continuous stream. However, since a battery powered Aquatracka was not commercially available, the Aquatracka could not be left at station for the duration of the exposure, but was instead only used from the survey vessel. This significantly reduces its application and usefulness to the WCM2011 and unless a battery powered UV fluorimeter can be obtained for future WCM2011 the advantages of the Aquatracka to the current programme are limited.
In its current form, the benefit of the Aquatracka may only be to assist in the location of the PW plume prior to deployment of mussel stations. However, for this to be effective the Aquatracka would need to be used closer than 500 m as tested in this programme.

5. Conclusions

- Mussels from station 2, located 500 m SE of the platform showed significantly higher concentrations of PAH and NPD concentrations then all other mussel groups.
- There was good agreement between the biological effects data and the chemical bioaccumulation data with the IBR/n index and PAH, NPD concentrations higher in mussels from station 2.
- The biological responses of the mussels from the closest station (station 2), 500 m from the discharge were indicative of low to moderate effects resulting in partially impaired health.
- Mussel stations located 1000 m and 2000 m from the Gullfaks platform showed PAH and NPD concentrations typical of offshore background concentrations.
- Alkyl phenols and naphthenic acids were detected in POCIS extracts from all the stations that were analysed, with the highest concentrations detected at stations 2 and 3.

- The Aquatracka was only able to detect a weak hydrocarbon signal, marginally above background concentrations when SE of the platform in the assumed direction of the PW plume.

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7. Appendices

Appendix	Description
A	Characterisation of discharges from Gullfaks C in 2011
B	Quality assurance
C	Cruise report (on disk)
D	Data report, NIVA (on disk)
E	Data report, IRIS (on disk)

Appendix A. Characterisation of discharges from Gullfaks C in 2011

Cleaning system for produced water

There is a produced water (PW) discharge from Gullfaks C. Prior to discharge the PW it is cleaned by means of oil-water separation in hydrocyclones and flotation cells as described in principle in the figure below. Ballast water that may contain oil is cleaned by separation in dedicated storage and sedimentation tanks prior to discharge to sea.

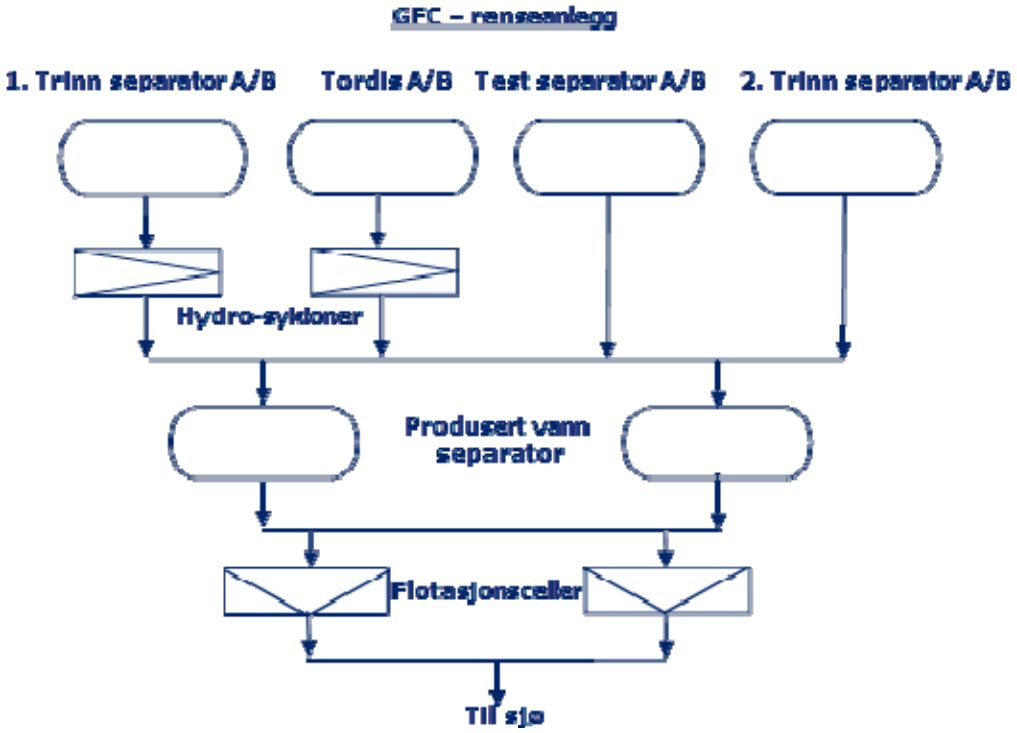

Figure A.1 Principle of the cleaning process of PW at the Gullfaks C platform.

Volumes of PW discharges and oil in water concentrations

Discharges of PW in the deployment period April and May 2011 at Gullfaks C were representative for the yearly average discharges from this platform (slightly above).

Table A.1. Discharges of PW during the last 12 months from Gullfaks C, as per December 2011.

Oil in PW (kg)	PW discharge (m3x1000)	Oil in PW (mg/l)	Target (mg/l
102.868	7.411	13,9	15

Table A.2. Concentration of oil in PW and PW discharge volumes in the last 12 months at Gullfaks C, as per December 2011.

	2010		2011									
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Oil in water (mg/l)	15,8	13,0	14,7	10,7	7,5	16,0	16,8	12,7	22,9	11,4	8,4	8,0
PW (m3x1000)	943	715	713	485	552	764	559	527	717	667	647	122

DREAM/EIF modelling

DREAM/ Environmental Impact Factor (EIF) modelling is carried out regularly for all fields with produced water discharges on the Norwegian shelf. The results are used for relative comparisons over time for installations/fields, between installations and to assess environmental impact between different technical strategies for produced water management; cleaning, reinjection etc. The model calculates a volume of water where there could be a potential negative impact on organisms around the discharge point. This is where the predicted Environmental Concentrations (PEC) is higher than predicted no effect concentrations (PNEC) of organisms. This volume is proportional to the EIF value which is calculated per installation/ field.

It should be stated that the modelling leading to this calculated volume of water or area of impact is associated with high uncertainties, and that results should be treated with this in mind. The modelling of discharges from Gullfaks C indicate an area of potential impact (PEC > PNEC) that stretches about 1 km from the platform in a southerly direction (Figure x.2). The drift model shows a south- and south-eastwards drift of the plume, which coincide with the observed stable current direction in the area. (Current data from May 1990 is being used in this modelling).

Natural components in produced water are thought to contribute with about 60% of the total environmental risk in Gullfaks C, while added chemicals is making up the remaing 40 %. The main contributors to these 40% are an H2S scavenger and a corrosion inhibitor. These results are taken from modelling carried out using 2010 discharge data.

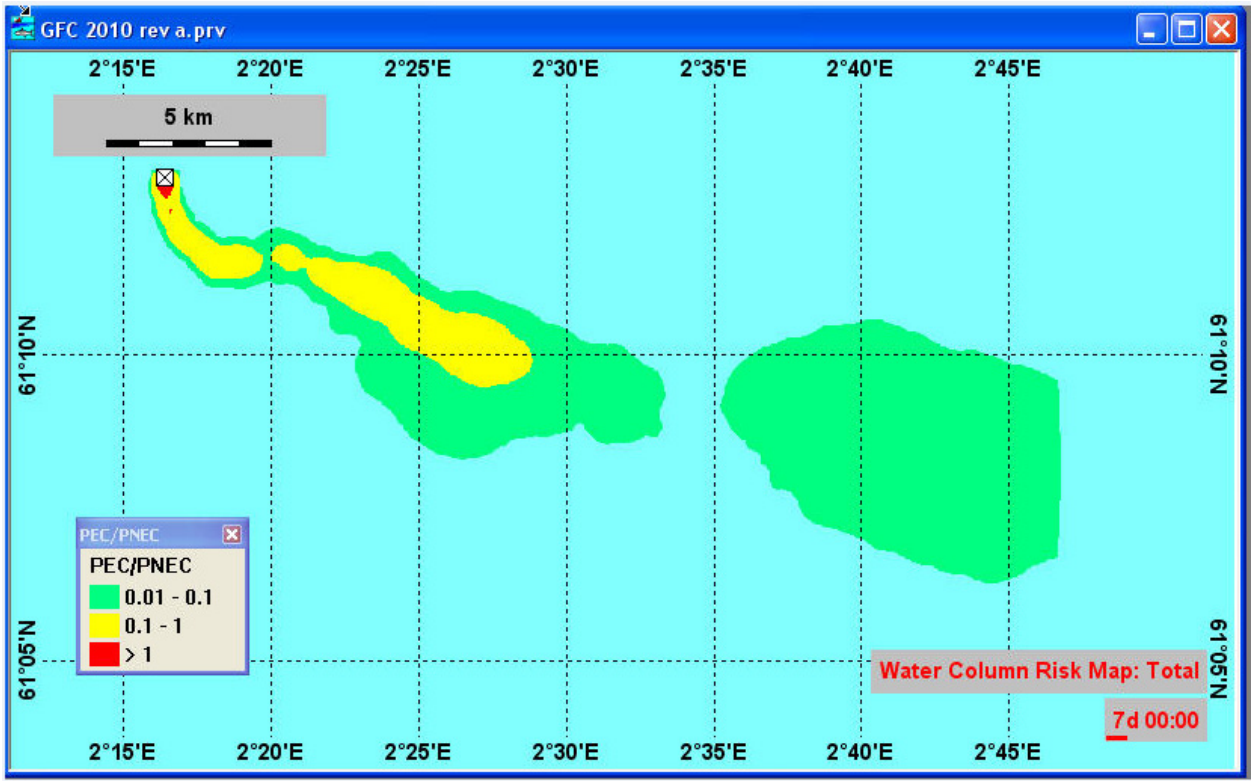

Figure A.2 Modelling of produced water discharges from Gullfaks C. DREAM/ Environmental Impact Factor (EIF) model.

Appendix B. Quality assurance

The following is a description of the quality assurance measures that were taken for each procedure. For all laboratory analysis, standard operating procedures of the analysing laboratory were adhered to. For the chemical analysis, accredited procedures were used.

Quality assurance measures taken

Procedure	**Quality measure**
Sampling protocols	All samples were collected by trained scientific personnel. All samples were clearly marked in pre-labelled vials with individual labels and stored in the appropriate conditions prior to analysis. All biological data was recorded and stored in a project folder.
PAH body burden	Accredited method complying with the requirements of NS-EN ISO/IEC 17025.
Species Identification	Established methods based on Inoue et al., (1985). Mussels selected at random.
Pyrene hydroxylase	The samples were randomised before analysis took place and analysed blind. The samples were analysed within 24 hour of work-up. When determining hydroxy pyrene by HPLC in the samples, two procedural blanks were run for each series of samples. These include 1) a sample without pyrene, and 2) a sample containing pyrene but without microsomes. The median of the blank 2 was subtracted from each sample. A series contained not more than 30 samples. When analysing a series, a standard was run every 10-20 samples, to check that the levels of hydroxy pyrene were correct.
LMS (NRRT)	IRIS SOP NRRT (Lysosomal Membrane Stability) Haemolymph cells . The method is described in Lowe et al., (1995). One batch of neutral red stock solution was used for all individuals. The light level was kept to a minimal tolerable level and maintained throughout the subsequent analysis. Examination time for each slide was kept less than a minute.
Neutral lipid	IRIS 2.2-421 SOP - Neutral lipid accumulation Rev 02 A piece of digestive gland of mussels were immediately put in a cryovial and frozen in liquid nitrogen after dissection. Neutral lipids were detected in cryostat sections (10 µm) by the Oil Red O technique according to Bayliss, (1984). All slides were stained using one batch of the Oil Red O stock solution. Two pictures of each individual were taken using an objective lens of 400 x magnifications and the image analysis program AxioVision (Zeiss). For every picture taken similar settings were used for both microscope and image analysis program. A scale bar was added in each picture. The measurements of neutral lipids were carried out in each picture using an automatic measurement program (AxioVision). Only the secondary tubules in the digestive gland were measured.
Lipofuscin accumulation	IRIS 2.2-423 SOP - Lipofuscin accumulation Rev 02 A piece of digestive gland of mussels were immediately put in a cryovial and frozen in liquid nitrogen after dissection. Demonstration of lipofuscin was performed histochemically in cryostat sections (10 µm) using Schmorl's method (Pearse, 1985). All slides were stained using one batch of the stock solution. Two pictures of each individual were taken using an objective lens of 400 x magnifications and the image analysis program AxioVision (Zeiss). For every picture taken similar settings were used for both microscope and image analysis program. A scale bar was added in each picture. The measurements of lipofuscin were carried out in each picture using an automatic measurement program (AxioVision). Only the secondary tubules in the digestive gland

	were measured.
Micronuclei formation	All samples were randomised and analysed blind. Only experienced scientists that were trained in the standard operating procedures were allowed to carry out the assessment. Co-ordinates of the micronuclei were recorded to enable assessment checks to be carried out at intervals.
Alkyl phenols in POCIS	Analytical limits of detection/quantification set to 3 and 10 times the standard deviation of four analysed solvent blanks plus the average. POCIS blanks (n=3) are reported alongside exposure POCIS for comparison as it is incorrect to subtract these (depends on stage of equilibrium). Water concentrations provided are semi-quantitative, due to the lack of an exposure correction method. Samples analysed blind.
Naphthenic acids in POCIS	This is a qualitative method, to screen for the presence of NA only. Solvent blanks analysed as per instrument protocol, with no target compounds detected. POCIS blanks are reported alongside exposure POCIS for comparison (n=3). Samples analysed blind.
Data archive	All raw data were stored electronically in the appropriate project folder on the secured NIVA and/or IRIS hard drives. Data will be stored for a minimum of five years.