

OLF The Norwegian Oil Industry Association

# Water Column monitoring Summary report 2005



DISCHARGES  
RISK ASSESSMENT  
MONITORING

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## PREFACE

Since 1999, environmental monitoring of the water column related to discharges from offshore oil and gas production has been a part of the Norwegian national environmental monitoring programme in the North Sea, as a legislative requirement from the Norwegian authorities. The programme includes regional impact monitoring and environmental condition monitoring on a national scale.

The present report has been written to summarise the activity up to 2005, also including the development phase from 1997-1999, before the official programme was launched. The authors of the report wish to thank scientists from NIVA, Akvamiljø, Battelle and SINTEF for their contribution and comments. We would also like to thank the members of the OLF Discharge to Sea working group for their input to the work.

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## SUMMARY

The present report reviews and summarises the environmental monitoring of the water column for the Norwegian sector of the North Sea in the period 1997-2004. The monitoring program is split in two sections: regional impact monitoring (covering one selected region on an annual basis) and condition monitoring (covering the whole Norwegian Continental Shelf every third year). The programme is subject to dynamic development with respect to new knowledge regarding sampling techniques, chemical parameters and biological responses.

The main objectives of the programme in the early phase (1997-2000) were to establish dilutions patterns and background concentrations of oil originating components and to validate the dilution model for risk assessment. The results from the programme show that the measured and predicted environmental levels of the produced water components are in good agreement.

PAH proves to be the most reliable indicator on produced water dilution. The concentrations are in general low, but for the developed areas elevated levels have been detected as far as 10 km away from the nearest discharge point.

Several of the biological responses confirm the exposure pattern as measured for the chemical parameters. However, due to low exposure, elevated levels for some components in the zero sampling material and exposed reference stations it has been difficult to draw clear conclusions from the results of the biological response.

The results from the condition monitoring shows no evidence of increased levels of oil related components in wild fish, but increased levels of hepatic DNA adducts have been detected.





## 1 INTRODUCTION

### 1.1 PRODUCED WATER

Prior to 1993, the main source of oil discharges from offshore E&P activities in the Norwegian sector of the North Sea was discharges of oil-contaminated cuttings from drilling operations. After these discharges were banned in 1993, and as a result of aging offshore fields discharging higher volumes of produced water, oil originating from produced water has become the most dominating contributor from the E&P industry to hydrocarbon discharges into the marine environment. As a result, produced water discharges have received increased attention over the past decade. The present report summarises the development and execution of the Norwegian environmental monitoring programme of the water column for the period 1997-2004. This programme is primarily designed to assess and follow up the potential environmental impact of produced water discharges.

Produced water originates from oil and gas-producing reservoirs and is basically fossil water, a natural component in such reservoirs. When seawater injection is used for reservoir pressure support, produced water will eventually become a mixture of seawater and formation water. Produced water composition and volume may vary considerably between fields and also throughout the life cycle of a production field. Gas and condensate fields in general produce less water than oil fields, and the produced water from such fields tend to contain relatively higher concentrations of volatile hydrocarbons than oil fields. Oil and oil-related organic compounds are the main contaminants in produced water, but the water also contains a complex mixture of inorganic and organic compounds originating from the reservoir. In addition, produced water contains chemicals added in the production process. A detailed description of produced water composition is given by OGP (2004). An overview of produced water discharges and injection in the Norwegian sector is given in Figure 1-1.

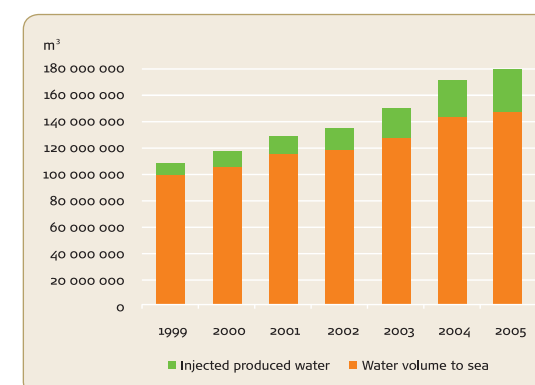


Figure 1-1: Produced water discharges and injection in the Norwegian sector of the North Sea.



1.2 PURPOSE AND OBJECTIVE OF THE WATER COLUMN MONITORING PROGRAMME

The purpose and objectives of the water column monitoring programme are as follows (SFT 2005).

The environmental monitoring offshore includes both monitoring of the sediments and monitoring in the water column. The purpose of the environmental monitoring offshore is to obtain:

- an overview - and control, of pollution from the offshore activity, including environmental impacts
- an overview of the general condition (trends) and development around the various installations and in the surrounding regions

The results from the monitoring will, among other things, be used for:

- early warning of any aggravation of the environmental situation
- forecasts regarding the future environmental condition
- verification of models for calculating the environmental risk as a function of the existing and expected discharges from the offshore industry
- verification of laboratory-based research to increase the knowledge of possible environmental impacts of discharges from the petroleum activity
- evaluation of the risk of environmental damage and ecological effects

1.3 DEVELOPMENT AND EXECUTION OF THE WATER COLUMN MONITORING PROGRAMME

The first field studies in the Norwegian sector of the North Sea addressing the fate and effect of produced water discharges were carried out in the Tampen area in the period 1991-1995. The aim of these early studies was to determine concentrations of produced water compounds in the vicinity of offshore oil fields with large volumes of discharged produced water, and to study the dilution of such compounds in the marine environment. Large water volumes were sampled and extracted or filtered for the purpose of concentrating oil compounds, PAH and alkylated phenols. The results showed that dilution of produced water was higher than expected from existing dilution models (Riksheim and Johnsen 1994). As a result of these observations, further development and improvement of dilution models became important. In the DREAM (Dose related Risk and Effect Assessment Model) project (1997-2000) a three dimensional, time-dependent dilution model was developed (Reed et al, 2001, Johnsen et al., 2000). In 1995 and 1997, two field studies were carried out in the Tampen - Oseberg area where a number of sampling techniques for analysis of produced water compounds in seawater were tested (Røe, 1998). Later on, the Norwegian national regulations for environmental monitoring of the water column (SFT 2005) were developed on the basis of these studies.

The water column monitoring programme is divided into two sections. The Regional Impact monitoring addresses the fate and effect of produced water discharges in selected regions and is carried out on an annual basis. As a consequence of the increased

awareness of potential environmental impact of produced water, concern has been expressed related to contamination of fish in areas with large produced water discharges. To meet this concern, the environmental monitoring programme also includes measurements of selected hydrocarbons in commercially important fish species. Biological exposure and effect parameters (biomarkers) have been used in the past and will be included in the future. This part of the water column monitoring programme is referred to as the Condition monitoring. Sampling and analytical approaches differ between the two areas. While the regional impact monitoring utilises direct water sampling and caged marine organisms (fish and blue mussels) to assess fate and effects of the discharges, the condition monitoring is based on sampling and analysis of feral fish.

The regional impact monitoring is divided into two phases:

- Phase 1 (1997-2000):  
The first official monitoring survey resulting from this requirement was carried out in the Ekofisk region in 1999. This was followed by a survey in the Sleipner region in 2000. Together with the 1997 survey in the Tampen - Oseberg area these field studies have formed Phase 1 of the water column monitoring programme, addressing dilution and the fate of produced water in the marine environment. The results from these surveys have been used to validate and further improve the DREAM dilution model (Durell et al., 2004, Durell et al., 2005). See Chapter 2 for detailed description.
- Phase 2 (2001):  
In parallel with the development of the dilution model and Phase 1 of the monitoring programme, risk assessment methodology for discharges to the water column was developed in the DREAM project (Johnsen at al 2000, Neff et al., 2005). Phase 2 of the water column monitoring programme addresses the potential effect of produced water in the marine environment. The results are also used for the purpose of validation of the environmental risk of produced water, as determined by the DREAM approach. This phase of the programme focuses on measuring biological responses and effects in marine organisms, along with some selected chemical analysis of produced water compounds. See Chapter 3 for a detailed description.

An overview of the field surveys for the regional impact monitoring is given in Table 2 below. The detailed results from this part of the monitoring programme are discussed in Chapters 2 and 3.

Year	Region	Type of monitoring	References
1997	Tampen/Troll/ Oseberg	Chemical analyses, testing of methodology, PEC validation	Durell & Uhler (1997)
1999	Ekofisk	Chemical analyses, PEC validation	Durell (1999)
2000	Sleipner	Chemical analyses, PEC validation	Durell (2000)
2001-02	Tampen	BECPELAG project, biological responses, development and testing of methodology, chemical analyses	Hylland et al. (2002)
2003	Troll	Biological responses, chemical analyses	Børseth et al. (2004)
2004	Tampen	Biological responses, chemical analyses	Hylland et al. (2005)

Table 2-1: Overview of field surveys with regional impact monitoring 1997-2004.

The condition monitoring is in principle carried out every third year. However, due to difficulties related to sampling of sufficient numbers of specimen of the wild fish at some locations, it has been necessary to allow flexibility in the sampling period. This may result in the surveys related to one condition monitoring exercise being extended over a longer period than one calendar year. Further description of the condition monitoring is given in detail in Chapter 4. Figure 2 gives an overview of the actual regions for the two sections of the monitoring programme; regional impact monitoring and condition monitoring.

There are both pros and cons regarding field sampling of feral fish (Condition monitoring) or caging of organisms (Regional impact monitoring). The field sampling is of high ecological relevance, but at the same time it is difficult to assess source impact in a specific area due to the movement history of the sampled specimens. The natural variation in the sampled material also makes it more difficult to interpret observed

exposure or effect responses. Caging organisms reflects a worst case scenario with respect to local exposure, since the organisms are trapped in the influence area of the discharge plume. On the other hand, organisms with desirable properties such as an advantageous life stage and condition may be selected for this purpose, reducing the variation in the target analyses. An argument against the cage alternative is that this situation reflects a “semi-natural” exposure at one geographical point. The results are limited to the selected species, and the availability of food is also unknown (starvation may affect the results).

Another factor of uncertainty is the obvious lack of real time oceanographic data for the sampling period when using the DREAM model to predict the dilution pattern of the discharges. The cages/sampling sites are selected based on results from the dilution model in DREAM run with data input from a typical situation for the sampling period.

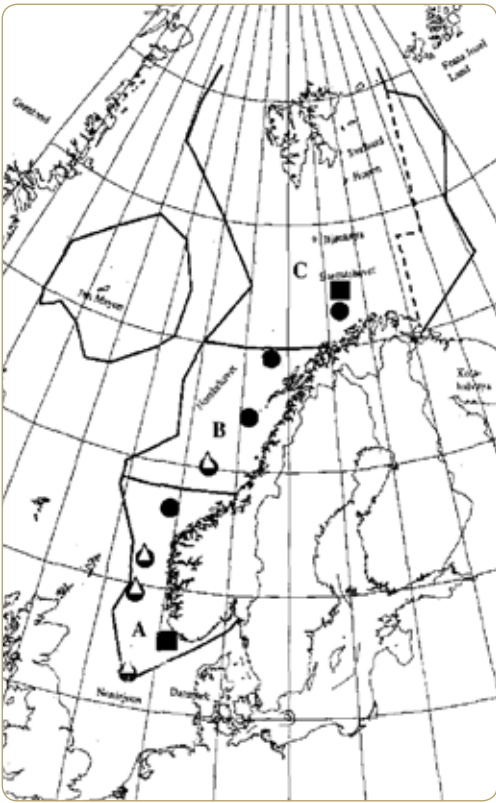


Figure 1-2: The station network for condition monitoring and regional impact monitoring of the water column. The condition monitoring areas are indicated as filled circles (•). The impact monitoring areas are indicated as triangles (▲). The reference areas for condition monitoring are indicated as squares (■).





## 2 THE FATE OF PRODUCED WATER

When produced water is discharged to sea, several chemical, physical and biological mechanisms will affect the concentration of each of the compounds in seawater. The most important of the changes affecting the fate and effects of produced water compounds are dilution, evaporation, adsorption/precipitation, biodegradation, and photo oxidation. Collectively, these processes tend to decrease the concentrations of produced water compounds in the seawater and thereby decrease their potential impact on marine organisms. In addition, both added chemicals and natural components in the discharge may influence the bioavailability of components to marine organisms. Based on evaluation of the fate of the different produced water components, PAHs and alkylphenols as well as metals have been selected for further monitoring studies. The fate of different produced water compounds is described in more detail in the OGP-report "Fate and effects of produced water components", (OGP 2004).

The water column monitoring survey in 1997 focused on development of techniques to quantify the levels of PAHs in seawater. The conclusions from this study formed the basis for detection of produced water compounds in seawater in all later studies (1999-2004). Chapter 2-1 contains an overview of the different methods.

In parallel to the sampling technique development, a dispersion model was developed as a part of the DREAM project. The dispersion model included the most important parameters affecting the concentration of produced water compounds in seawater (dilution, evaporation, adsorption/precipitation and biodegradation). In Chapter 2-2 the dispersion model is further described. Chapter 2-3 gives an overview of monitoring results from 1997-2004 from produced water compounds in seawater, while Chapter 2-4 shows a comparison of modelled and measured data. In Chapter 2-5 the biota exposure concentration in Phase 2 of the water column monitoring is presented.

### 2.1 DEVELOPMENT OF METHODOLOGY FOR MEASURING PRODUCED WATER COMPONENTS IN SEAWATER

Due to the rapid dilution of discharged produced water in the North Sea, sampling methods must be able to detect ultra-trace concentrations of the compounds of concern in the water column. In the following, a brief overview of techniques included in the 1997 field survey is given. For more details about the method development, see Røe Utvik et al., 1999.

#### 2.1.1 DIRECT WATER SAMPLING (BULK SEAWATER)

In the field survey at Statfjord in 1997, direct water sampling was included alongside sampling of plankton at selected locations. Water samples (2 l) were analysed with respect to PAH. Due to low concentration levels, the uncertainty in the results were high. For several compounds the concentrations were below the detection limit (Røe, 1998). It is important to bear in mind that a water sample at a fixed time and location may not be representative of the mean concentration of produced water compounds at that location, due to variations in local current patterns.

#### 2.1.2 SOLID PHASE EXTRACTION (SPE)

This technique concentrates the target compounds on a solid phase by extracting large water volumes. The solid phase is then analysed, and the concentration levels in seawater is back calculated. Several different solid phases can be used, dependent on target compounds. In the 1997 study polystyrene-divinylbenzene disks were used as a solid phase. Water samples were filtrated through the disk in a laboratory on the vessel. This was time-consuming due to a low flow-rate through the disks. The results showed high levels of some PAH compounds in the blank samples. Therefore, the uncertainty in the results was quite high. In later studies, however, this technique has been used with good results (Thomas et al., in press). As for direct water sampling, such a sample is representative only for a fixed time, location and depth. Some studies have also been done with direct SPE extraction of produced water (on the platform).



### 2.1.3 *IN-SITU* LARGE VOLUME WATER SAMPLING

The principle of *in-situ* large volume water sampling is the same as for solid phase extraction, by concentrating target compounds by extraction. The difference from the method described above is that all sampling and extraction is done in the water column. The water sampler used in the 1997 study was a large volume filtration system by McLane which included sampling of particulate and dissolved fractions onto filters and XAD resins. The technique reflected the gradients of PAH components that were expected from dispersion modelling, but the results were depreciated by a breakthrough of the low-molecular weight PAHs such as the naphthalenes. It may be possible to modify the *in-situ* sampler and some of the sampling conditions to improve the retention and efficiency of the system.

### 2.1.4 SEMIPERMEABLE MEMBRANE DEVICES – SPMD

SPMDs concentrate and integrate contaminants over a period of time by binding organic compounds to the lipid phase in the membrane. The lipid phase is then analysed with respect to target compounds. The average concentration of the target compounds in seawater can then be estimated by back calculation from the expected sampling rate of each of the target compounds from the seawater into the lipids (bio concentration factors, BCFs). Such sampling rates are experimentally derived, and the quality of these data has improved considerable over the last 5-10 years. The technique reflected the gradients of PAH components that were expected from dispersion modelling, but for the low-molecular compounds like naphthalene, the sampling time was too long. This means that the sampling rate was no longer in the linear phase, but into the equilibrium phase, which makes the back calculation more uncertain. For more details about the calculation procedure and sampling rate data, see Røe Utvik et al., 1999, Røe Utvik and Gartner 2002, Durell et al., 2004, and Durell et al., 2005. The methodology is, however, well suited for integrating concentration levels at a location over a certain period of time, which probably gives a more correct picture of the exposure concentration for biota at that particular location than a single water sample. The technique has later also been developed for more polar compounds by using other membranes and adsorption materials (POCIS). POCIS was tested as an additional component during the 2004 water column monitoring campaign.

### 2.1.5 BLUE MUSSELS

The principle of deployment of mussels for measuring concentration levels of organic produced water compounds in seawater is almost the same as for SPMDs. The mussels are deployed for a certain period of time at several locations. The concentration levels of target compounds detected in the mussel tissue is then used for back calculation to find the concentration levels in seawater by assuming that a steady state or equilibrium is established for the target compounds. The technique reflected the gradients of PAH components that were expected from dispersion modelling for all components, so the technique appears to be well suited for monitoring purposes. Concentrations of contaminants in blue mussels will reflect both dissolved and particulate contaminant levels in the environment.

### 2.1.6 PLANKTON SAMPLES

Zooplankton was collected with a zooplankton net at a depth of 10 meters. The samples were generally 400-500 ml of thick zooplankton suspension, consisting primarily of *Calanus sp.* Samples were analysed for PAHs. For the near-field stations the organic compounds naphthalene and phenanthrene were detected, while for the far-field and reference stations the concentration levels were below the detection limit. The concentration levels measured at the near-field stations corresponded well with predicted levels calculated from seawater concentration (from mussel data) and experimental bioaccumulation factors from laboratory food-chain studies (Røe, 1998; OLF, 1998). In later studies, analysis of metals in plankton samples was also included.

### 2.1.7 RECOMMENDATION

SPMDs and mussels together with *in-situ* sampling of seawater were identified as methodologies capable of measuring levels of produced water compounds in the environment averaged over a certain period of time. This is important when the results are used in comparison with modelled average levels, and also to indicate the exposure levels in the prediction of possible effects from the discharges.

Methodologies for measurements at a fixed time and location, such as direct water sampling and solid phase extraction can be used for near-zone measurements.



2.1.8 OVERVIEW OF SAMPLING TECHNIQUES USED IN THE WATER COLUMN MONITORING SURVEYS 1997-2004

Analysis Parameter	Sampling Matrix					
	SPMD	Mussels	Bulk Seawater	In-situ Seawater	SPE Seawater	Plankton
Tampen Region (1997)						
PAH and alkyl homologues	X	X	X	X	X	
Phenol and C1 through C4 homologues	(X)a	(X)a	X	(X)a	(X)a	
Total extractable organic compounds (TEOC)			X	X	X	
Ekofisk Region (1999)						
PAH and alkyl homologues	X	X	X	X		X
Phenol and C1 through C4 homologues	(X)a	(X)a				(X)a
Total extractable organic compounds (TEOC)			X	X		
Metals		X				X
Sleipner Region (2000)						
PAHs and alkyl homologues	X	X				X
Phenol and C1 through C4 homologues	(X)a	(X)a				(X)a
Metals	X	X				X
Becpelag (2001)						
PAHs and alkyl homologues	X	X		X	X	X
Metals	Xb	X				X
Troll B (2003)						
PAHs and alkyl homologues	X	X				X
Metals		X				
Statfjord (2004)						
PAHs and alkyl homologues		X				X
Phenol and C1 through C4 homologues	Xc	X				
Metals		X				X

Table 2-1: Summary of sampling techniques used in the field surveys 1997-2004.

a Phenolic compound analysis was included with PAH and other semi-volatile aromatics. However, it is recognized that SPMDs, mussels, plankton, and in-situ or SPE seawater do not accumulate these compounds to a significant degree.

b DGT – diffusive gradient in thin films; a passive sampling technique for some metals.

c The sampling matrix was a modified SPMD system called POCIS. The testing of this system was a research activity, not a part of the regular monitoring programme.

2.2 DISPERSION MODELLING

The DREAM model predicts the environmental concentration (PEC) of produced water compounds as input to the risk assessment. This is the routine procedure to determine concentrations of produced water compounds in the water column while the monitoring programme (as stated in Chapter 1) contributes to verify and validate these. The dilution model in DREAM provides three dimensional, time-variable concentration fields of groups of produced water compounds, organized according to chemical properties (Johnsen et al., 2000). Added chemicals may also be included in the model.

The modelling is based on hydrodynamic and meteorological data along with chemical and physical data of the different compounds in the discharge. A comprehensive data set including hydrodynamic and meteorological data for the Norwegian sector south of the Lofoten area serves as the basis for dilution modelling. The data covers the period 1990 - 1994, and the month of May in 1990 has been selected as the representative period for running the model and performing risk assessment. When linked to the monitoring surveys, the model is used to predefine the sampling locations based on predicted dilution of the discharge. After the sampling the model is run with real time hydrodynamic and metrological data for the sampling period to allow direct comparison between modelled and measured concentrations of produced water compounds.

2.3 MONITORING OF PRODUCED WATER COMPOUNDS IN SEAWATER AND BIOTA - RESULTS 1997-2004

There is a large dataset of results from the water column monitoring activities during these years. In the following, a summary is given of:

- Characteristics of a petrogenic PAH profile
- Concentrations of selected organic and inorganic compounds in seawater at various distances from the discharge source
- Concentrations of selected organic and inorganic compounds in mussels and plankton at various distances from the discharge source
- Concentrations of total PAH in the North Sea compared to concentrations observed in other areas

2.3.1 CHARACTERISTICS OF A PETROGENIC PAH PROFILE

To identify the origin of the hydrocarbons detected in seawater, it is important to evaluate the PAH profile. As shown in Figure 2-1, the profile of a sample close to a petrogenic source is characterised by higher concentration levels of the alkylhomologues than the parent compound. The total PAH concentration is dominated by the low-molecular weight compounds such as naphthalenes and phenanthrenes. The decalin group can also be used for identification, even though these compounds are non-aromatic and hence not included in the PAH fraction.





2.3.3 CONCENTRATION LEVELS OF TOTAL PAH IN THE NORTH SEA COMPARED TO LEVELS OBSERVED IN OTHER AREAS

To put the PAH concentrations measured during the monitoring surveys into perspective, Table 2-3 compares these levels with typical PAH concentrations measured in Norwegian coastal waters. As seen from the table, PAH concentrations near the discharge points rapidly dilutes to lower levels than the costal water background. The natural background level of PAH is, as expected, significantly lower in offshore waters than near the coast.

Location	Total PAH (ng/g wet weight)	References
North Sea (<500 m from source)	400 - 1150	OLF 1998
North Sea (<10 km from source)	20 (average Tampen 1997)	OLF 1998
North Sea (background levels)	8 (average 1997-2000)	OLF 1998
Norwegian coast (background)	47	SFT 1995
Norwegian fjords (contaminated)	100 - 4300	Knutzen, & Green (1991) Knutzen (1991 a,b) PMF, Programme on Marine Pollution (1992)

Table 2-3: Comparison of PAH concentrations in the North Sea with Norwegian coastal waters.

2.4 VALIDATION OF THE DISPERSION MODEL

The results described in this chapter focuses on the use of field-deployed blue mussels and SPMDs to collect time-integrated hydrocarbon data, and to estimate concentrations of PAH from produced water in the water column. The PAH concentrations in seawater, estimated from mussels and SPMDs, have been compared to results from dispersion modelling using the Dose-related Risk and Effect Assessment Model (DREAM).

The data are presented according to the major compounds groups; naphthalenes, 2/3-ring PAH, 4/5-ring PAH, and total PAH (the sum of the naphthalenes through 4/5-ring PAH groups).

2.4.1 CONCENTRATION LEVELS IN SEA WATER – PHASE 1

Data from the Tampen and Ekofisk surveys are presented. The Sleipner Survey is not included due to few sampling sites in this region. Figure 2-5 shows the results from modelling of total PAH for the Tampen and Ekofisk regions, and Figure 2-6 shows the comparison of modelled and measured data for the same regions. Figure 2-7 shows concentration of the major compound classes at selected locations in the Ekofisk region.

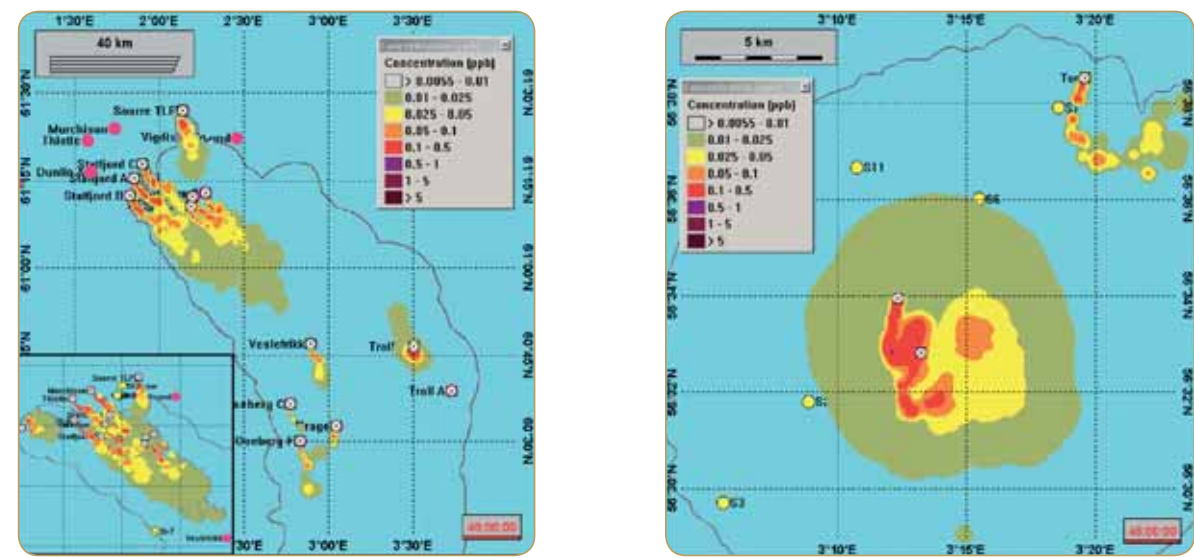


Figure 2-5: Model output map presenting the total PAH (naphthalenes through 5-ring PAH) concentrations in the Tampen (left) and Central Ekofisk (right) region.

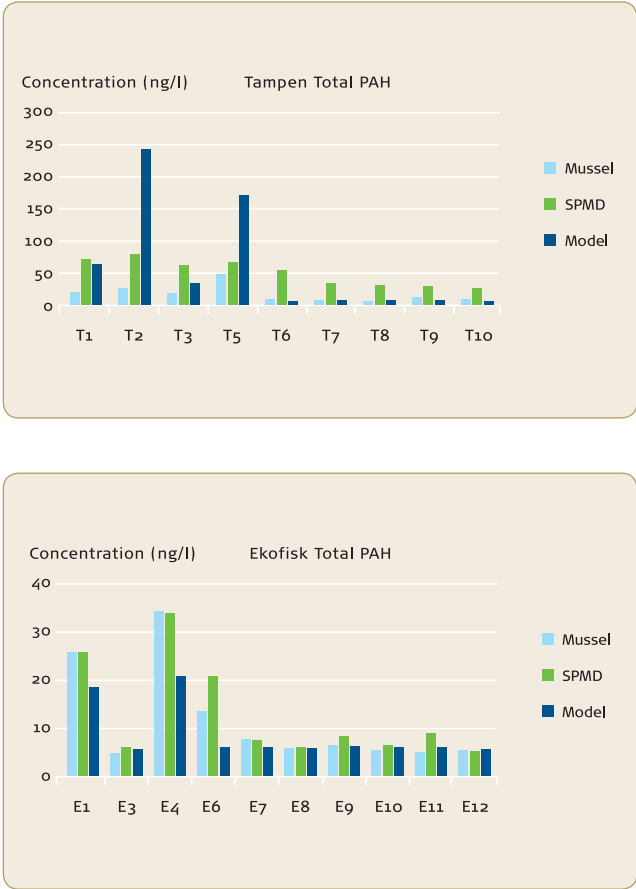


Figure 2-6: Total PAH Concentrations at sampling locations in the Tampen and Ekofisk Regions – Data from mussel and SPMD field measurements and modelled data (ng/l).

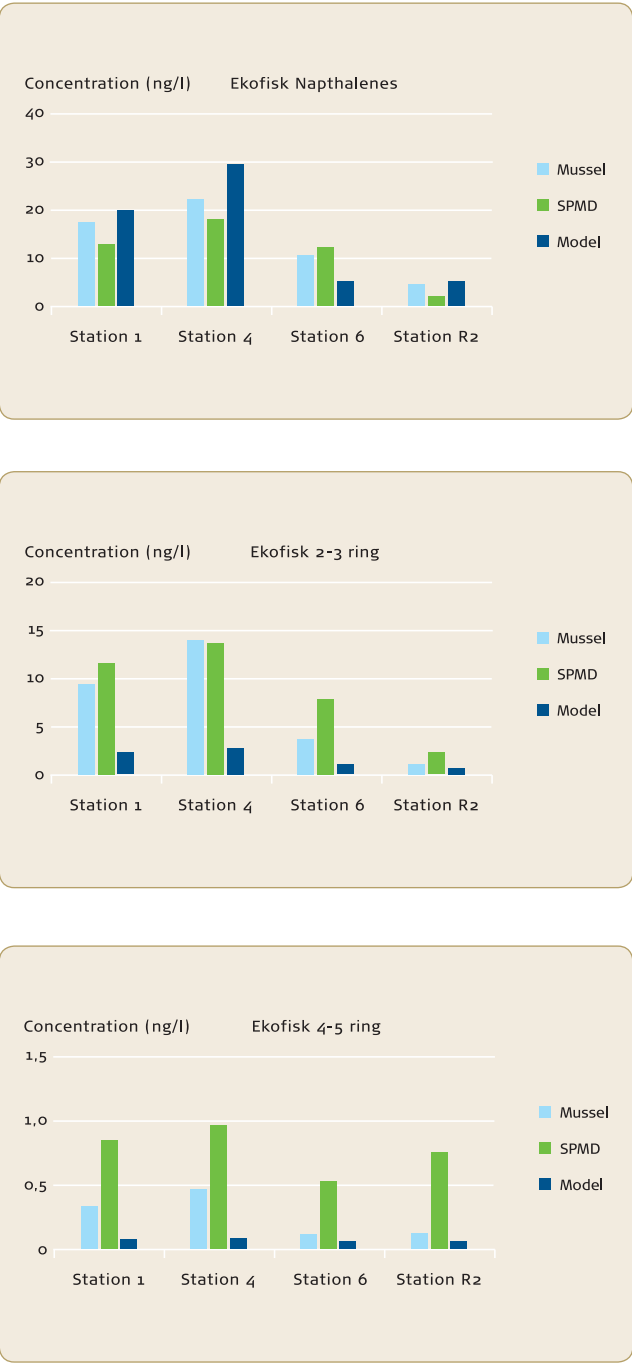


Figure 2-7: Concentrations of the major compound classes at selected locations in the Ekofisk region – Data from mussel and SPMD field measurements and modelled data (ng/l).

### Tampen Data

An important difference between the work in the Tampen and Ekofisk areas was that some of the Tampen area stations were, to a greater degree, influenced by tidal current fluctuations. The mussel, SPMD, and model-based total PAH concentration estimates were less comparable for the stations in the Tampen Region than in the Ekofisk Region. The data indicate that the background/blanks associated with the SPMDs were quite variable and contributed 20-30 ng/l to the SPMD total PAH data (Table 4), making the SPMD data less valueable. The mussel-based and modelled data were more comparable for the

stations away from direct produced water influence (e.g., S6 and S9), but the model predicted considerably higher concentrations than the mussel data for the stations close to produced water discharges (e.g., S2 and S5).

### Ekofisk Data

The mussel, SPMD, and model-based estimated total PAH concentrations in ambient water were highly comparable for the stations in the Ekofisk Region, with the model predicting essentially the same or slightly lower concentrations than were estimated from the field samples. All methods yielded the highest concentrations for the stations closest to the major discharges (stations S1 and S4), and these stations had approximately 4-6 times higher concentrations than the background levels.

### 2.4.2 BIOTA EXPOSURE CONCENTRATION – PHASE 2

The biota exposure concentration is an important parameter, when effect parameter responses are to be evaluated. Unfortunately, the chemical data from analysis of mussel tissue residues from the field studies of 2003 and 2004 were not comparable to data from earlier studies. In 2003 and 2004, as discussed in Chapter 2.3.1, the petrogenic pattern of PAHs was not identified at the near-zone sites and this is most likely caused by the low exposure in 2003 and 2004. Calculations of the oceanic currents in the area during the period of deployment of the cages support this explanation (H. Rye and M.K. Ditlevsen, 2005).





### 3 BIOMARKERS FOR EXPOSURE AND IMPACT MONITORING

Chapter 3 describes the use of biomarkers for environmental monitoring of offshore petroleum activities. Chapter 3.1 describes the development of methodology, while the results and a comparison between the different surveys are given in Chapter 3.2.

#### 3.1 DEVELOPMENT OF METHODOLOGY FOR MEASURING EXPOSURE OF BIOTA – PHASE 2

In 2001/2002 ICES organised a workshop on biological effects in pelagic ecosystems (BECPELAG) with participants from institutions and laboratories all over Europe. During the workshop the availability of about 40 different methods from bacteria to fish were tested in two different areas in the North Sea; the German Bight and the Statfjord (Tampen) area. The participation of the Norwegian offshore industry in this work was defined as the regional impact monitoring offshore both in 2001 and 2002. The results and recommendations from BECPELAG are summarised in Hylland et al. 2002.

The recommendations from BECPELAG were, together with the experiences gained through the existing environmental monitoring (measures of concentrations), used as basis for the survey at Troll in 2003 (Børseth & Tollefsen, 2004). The 2004 survey at Tampen was only slightly modified (Hylland et al., 2005).

##### 3.1.1 METHODOLOGY

The following biomarkers were recommended:

- **EROD:** indicate exposure to planar hydrophobic contaminants among which PAHs. EROD induction represents one of the first steps of planar contaminants metabolism. 2001, 2003, 2004
- **GST:** induction represents one of the second steps of hydrophobic contaminants metabolism. 2001, 2003, 2004
- **DNA adducts:** Indicate exposure to contaminants able to affect DNA integrity. 2001, 2003 (caged), 2004 (feral)
- **Vitellogenin:** Induction of this female typical protein in male and juvenile fish indicates exposure to estrogen-like contaminants (feminisation). 2001, 2003, 2004
- **Histopathology/-chemistry:** Measure structural changes in tissues that may be linked to health impairment as a result of exposure to pollutants. 2001, 2003, 2004
- **PAH metabolites:** Increased levels of the metabolites show increased activity of detoxification of PAHs as a result of increased exposure to these components. 2001, 2003, 2004
- **BaPH activity:** show induction of process to transform PAHs into more water-soluble and excretable end-products (blue mussels). 2001, 2003, 2004



- **Lysosomal stability:** a general health parameter, has shown to respond to PAH and crude oil.  
2001, 2003, 2004
- **Micronuclei:** chromatin-containing structures that are surrounded by a membrane and have no detectable link to the cell nuclei. Breaks in DNA can lead to formation of micronuclei after cell division.  
2004
- **General health criteria:** length, weight, sex and condition are measured. Condition is determined as the ratio between total weight and length.  
2001, 2003, 2004
- **Lipid content:** measure the lipid levels in the analysed tissue. Levels of organic contaminants are dependent of the lipid levels; the more lipids, the more organic components the organisms are capable of accumulating.  
2001, 2003, 2004

### 3.1.2 2003 AND 2004 SURVEYS

For the 2003 survey only caged organisms were selected (Chapter 1). As a result of the measurements from the Condition monitoring in 2002 and the assumption that the exposure time of the caged organisms is too short for development of DNA adducts in fish, the survey in 2004 also included feral fish for measurements of potential findings of DNA adducts (Chapter 4).

In addition one new method, micronuclei and EROD in gills, were included in the 2004 survey. This method was tested on cod with promising results in 2003 as part of the NRF PROOF validation project.

### 3.2 MONITORING BIOLOGICAL RESPONSES OF PRODUCED WATER COMPOUNDS IN BIOTA – RESULTS 2001-2004

In this chapter the different main results are described and compared. In addition, Chapter 3.2.1 discusses the complexity of the results.

In general the environmental surveys show that the condition of the tested organisms (fish and blue mussels) is good. For most of the biomarkers the signals are near background levels.

Results for selected PAHs from BECPELAG (2001/2002), Troll (2003) and Statfjord (2004) surveys are given in Figures 3-1, 3-2 and 3-3. From BECPELAG there were significant differences between the transect stations. The concentrations decreased with increased distance from the discharge point. The measurements showed significantly increased levels of PAH metabolites in caged cod up to 10 km away from Statfjord, compared to the reference station. This is well in line with the results from the chemical analysis in the 1997 survey. On the other hand, results from the 2003 survey show evidence of exposure to PAH at very low levels (not far from background levels). From the 2004 survey the only PAH parameter showing significantly higher levels than the other sampled stations is 4-ring PAH's in wild fish at the 500 m station (Figure 3-4). As discussed in Chapter 2, calculations of the concentration fields in the area for the actual time of deployment of

the cages (August-September 2004) shows that the oceanic current patterns appear not to be directed into the main current direction, were also the cages were deployed (H. Rye and M.K. Ditlevsen, 2005).

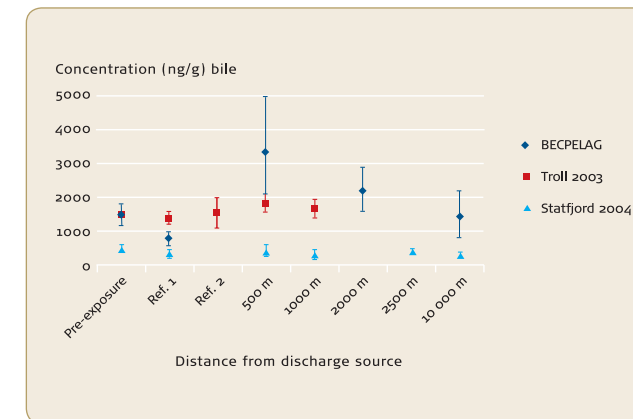


Figure 3-1: Concentration of Co-C1 OH-naphthalenes in bile from caged Atlantic cod (*Gadus morhua* L.) caged at different distances from the discharge source at Statfjord B and Troll B. Mean and standard deviation is given in the figure. Data is compiled from the BECPELAG workshop programme in 2001 (Statfjord B), the Water Column Survey 2003 (Troll B) and the Water Column Survey 2004 (Statfjord B).

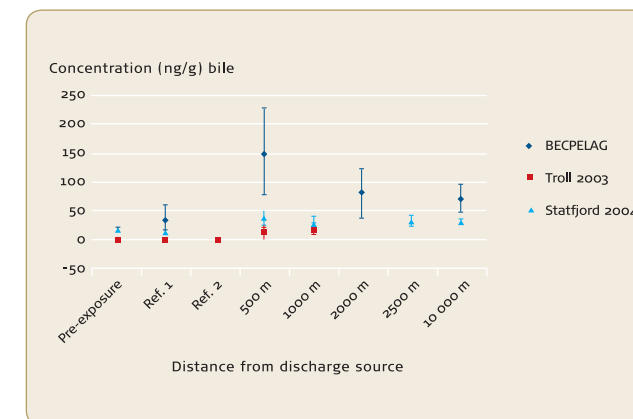


Figure 3-2: Concentration of 2-OH-naphtalenes in bile from caged Atlantic cod (*Gadus morhua* L.) caged at different distances from the discharge source at Statfjord B and Troll B. Mean and standard deviation is given in the figure. Data is compiled from the BECPELAG workshop programme in 2001 (Statfjord B), the Water Column Survey 2003 (Troll B) and the Water Column Survey 2004 (Statfjord B).

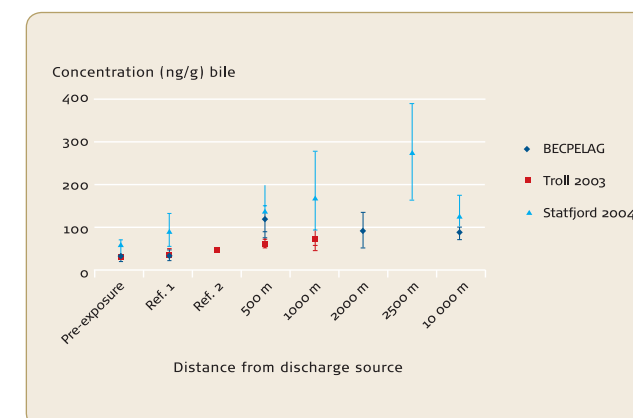


Figure 3-3: Concentration of Co-C2 phenantrene in bile from caged Atlantic cod (*Gadus morhua* L.) caged at different distances from the discharge source at Statfjord B and Troll B. Mean and standard deviation is given in the figure. Data is compiled from the BECPELAG workshop programme in 2001 (Statfjord B), the Water Column Survey 2003 (Troll B) and the Water Column Survey 2004 (Statfjord B).



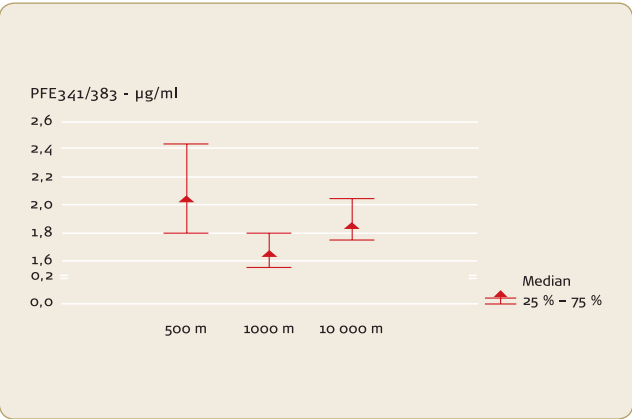


Figure 3-4: 4-ring PAH-metabolites for fixed wave-length (341/383 nm) fluorescence levels in feral saithe (*Pollachius virens*). The figure shows median, quartiles and 10/90-percentiles.

Micronuclei were measured for the first time as part of the biomarker suite for water column monitoring during the 2004 survey. In contrast to the general low measurements of exposure on mussels, the micronuclei from the mussels show significant differences both between the stations and the pre-exposed material (see Figure 3-5).

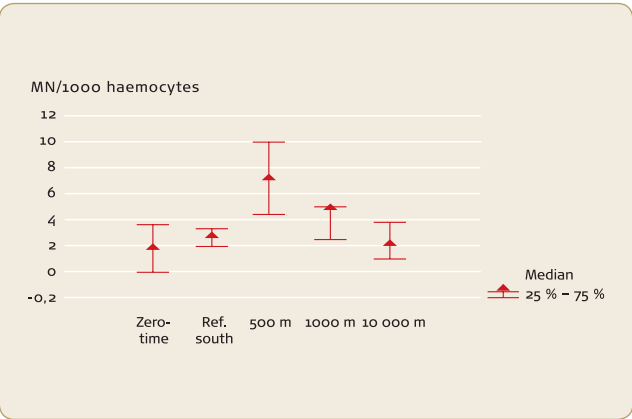


Figure 3-5: Frequency of micronuclei in blue mussels (*Mytilus edulis* L.) from the groups indicated. The figure shows median, quartiles and 10/90-percentiles.

In contrast to the 2003 survey where DNA adduct were measured in caged cod, DNA adducts were measured in feral saithe in 2004. The measurements show no significant differences along the transect, but fish at all the three sites had clearly elevated concentrations of hepatic DNA-adducts, indicating past exposure to genotoxic substances.

In 2003 all stations are higher than the zero-time sample (including the references). Figure 3-6 shows that the transect stations have a tendency to be higher than the references and zero-time samples for the EROD activity induction, but there are no significant differences between the different stations.

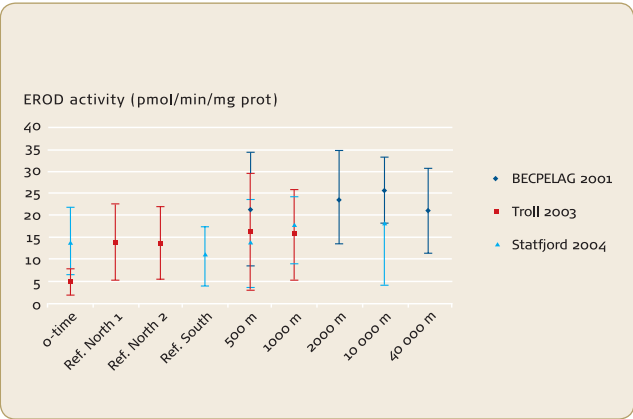


Figure 3-6: EROD activity in liver tissue from caged Atlantic cod (*Gadus morhua* L.) caged at different distances from the discharge source at Statfjord B and Troll B. Median (dot) and standard deviation is given in the figure. Data is compiled from the BECPÉLAG workshop programme in 2001 (Statfjord B), the Water Column Survey 2003 (Troll B) and the Water Column Survey 2004 (Statfjord B).

Vitellogenin concentrations are all on the same levels within the same year, but the measured levels were significantly lower in BECPÉLAG than in both 2003 and 2004 (Figure 3-7), which were on the same level. The most probable explanation is that cages were deployed in May/June during BECPÉLAG, whereas cod used during water column monitoring 2003 and 2004 were exposed in August/September. Even juvenile fish have minor fluctuations through the year (Hylland, personal communication). Another explanation may be that the 2001/2002 analyses were carried out by a different laboratory.

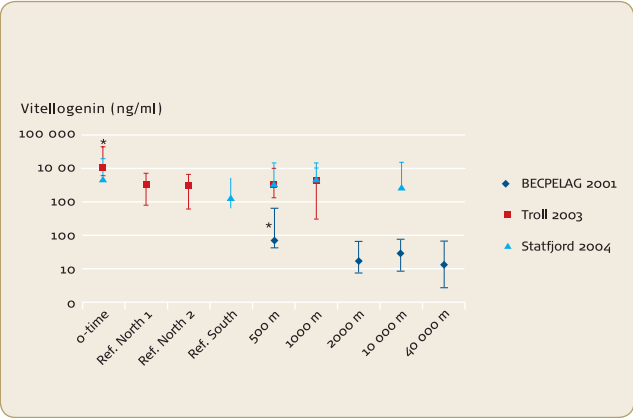


Figure 3-7: Levels of vitellogenin (Vtg) in plasma from male Atlantic cod (*Gadus morhua* L.) caged at different distances from the discharge source at Statfjord B and Troll B. Median (symbol), 25% and 75% quartiles (whiskers) are given in the figure. Data is compiled from the BECPÉLAG workshop programme in 2001 (Statfjord B), the Water Column Survey 2003 (Troll B) and the Water Column Survey 2004 (Statfjord B). Asterisks denote groups different from reference station(s).

The histopathology results from BECPÉLAG showed apparent effects from produced water on tissue (liver) integrity in both wild-caught fish (saithe) and caged cod for the material sampled (Bilbao et al., a, b; in press).

The histopathology results from water column monitoring 2003 and 2004 indicated that the organisms in general were in good condition. However, the histology results from 2004 show differences in gonad indexes (development) at different stations (Figure 3-8). Differences in gonad indexes can affect the spawning period.

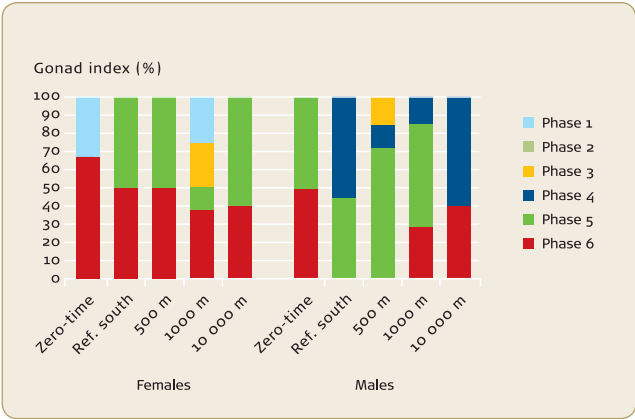


Figure 3-8: Gonad indexes recovered in female (top) and male (bottom) mussels (*Mytilus edulis* L.) from the groups indicated (Phase 1-6).

### 3.2.1 COMPLEXITY OF THE RESULTS

The location of the reference stations and the history and knowledge of the organisms used in the experiments is essential. These issues have been stressed before every survey, but experience shows that these issues have to be highlighted even more. High values found in the pre-exposed/zero-time material and on the reference stations makes it difficult to evaluate and describe the results.

The DREAM model was used to select reference stations that are as optimal as possible, (Figure 2-5). To avoid contaminated reference material, it is important to be careful when choosing what area to take material from and the organisms must also be kept under controlled conditions (i.e. low contaminant levels) before zero-time sampling and deployment.

Another aspect that contributes to the complexity of evaluating the results, is the huge natural variations in biological material. Figure 3-9 is given as an example of the individual variations.

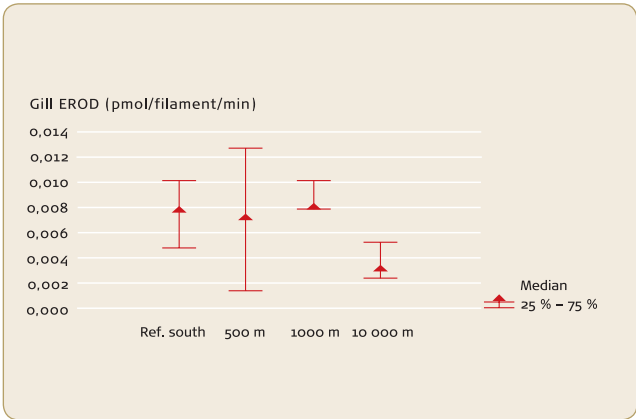
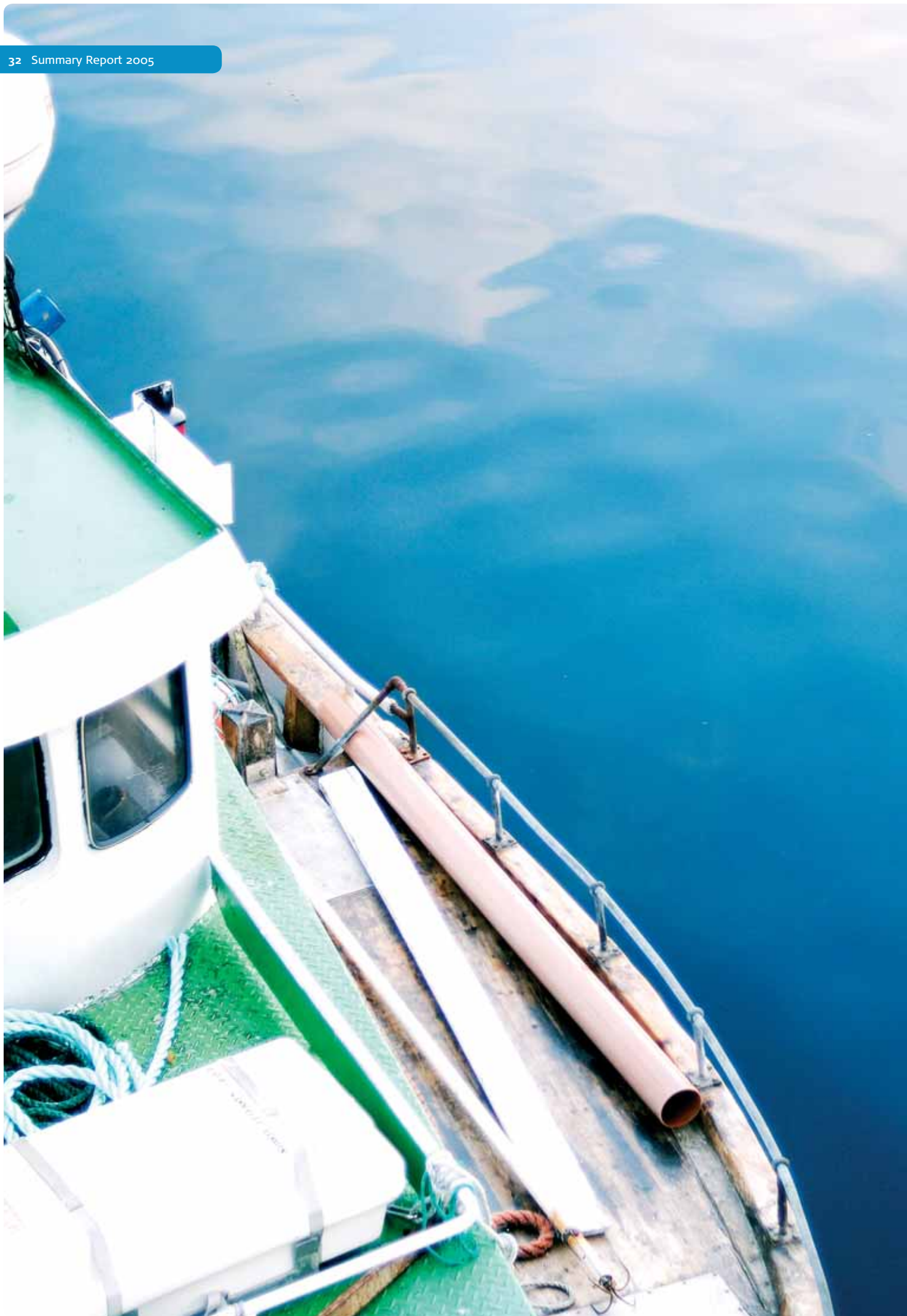


Figure 3-9: EROD activity (pmol/filament/min) in gills from cod (*Gadus morhua* L.) from the groups indicated. The figure shows median, quartiles and 10/90-percentiles. Gill-EROD was a method added to the original programme and zero-time samples were not retrieved.







## 4 CONDITION MONITORING

In 1994, a comprehensive project aim clarify whether commercially important fish species in Norwegian waters were contaminated of oil compounds or not. Samples of cod and haddock were collected from different regions in the Norwegian sector (Figure 1-1) and analysed for content of THC (Total Hydrocarbon Content), PAH and decalines. No increased levels of any of the target compounds were observed when comparing fish caught in the vicinity of production fields with fish from regions regarded as background areas. However, multivariate data analysis proved that the pattern (fingerprint) of decalines were different in fish from the Tampen and Ekofisk regions compared to the background areas. This indicated that fish in these regions are exposed to a different oil source than fish in the other regions (Johnsen et al., 1998).

After the initial study, the first regular condition monitoring surveys was carried out in 2000-2001 (Klungsøyr et al., 2001). This study included analysis of selected oil-derived hydrocarbons (PAH and decalines) in haddock from 10 sampling regions in the Norwegian sector. The study concluded that no increased hydrocarbon levels were detected in fish from regions exposed to produced water compared to background areas.

In 2002 a limited survey with respect to sampling locations was carried out, including the Tampen, Sleipner and Egersundbanken areas. Saithe, cod and haddock were included in the sampling. In addition to the required chemical analyses haddock livers were analysed for content of DNA adducts. Again, the study concluded that there was no significant increase in hydrocarbon concentrations in the fillet or liver of fish from the regions exposed to produced water compared to the reference region (Klungsøyr et al., 2003).

This study detected increased levels of DNA adducts in haddock from the Tampen and Sleipner area compared to Egersundbanken.

As described in Chapter 3, the measurements on feral saithe showed no significant differences along the transect, but fish at all three sites had clearly elevated concentrations of hepatic DNA-adducts, indicating past exposure to genotoxic substances.

In conclusion, the three condition monitoring surveys showed that fish caught in regions exposed to produced water do not contain increased levels of produced water originating hydrocarbons compared to fish from non exposed background areas. The initial study (Johnsen et al., 1998) however showed that decalines in fish from exposed areas differed from patterns found in fish from background areas, indicating a local exposure source. The finding of increased levels of DNA adducts in haddock from the Tampen and Sleipner regions have raised concern about the potential implications of this. It is presently unclear whether these levels are alarming with respect to potential effects on the fish, or whether they reflect an increased exposure of insignificant harm. Also, since haddock is a species living on the seabed, the nature of the exposure is uncertain. Oil-contaminated sediments may be a more probable source than produced water. OLF has initiated research studies to clarify these questions.





## 5 DISCUSSION

### METHODOLOGY

For both practical and scientific reasons, a limited number of sampling techniques and determinants must be selected to describe the dilution and potential exposure to the complex mixture of chemical compounds discharged to the marine environment from offshore oil and gas production. For the regional impact monitoring, focusing on the water column, both direct and indirect sampling were considered in order to measure the water concentrations of the target compounds. Mainly due to challenges related to sensitivity (ultra-low concentrations) and risk of sample contamination, the use of indirect sampling methods (SPMD and blue mussels) has been recommended (Chapter 2).

As representatives of produced water discharges, total hydrocarbon content, PAH, alkylated phenols and a limited number of heavy metals were initially selected. However, the results from the three first monitoring surveys clearly showed that the PAHs were the best representatives among these. Alkylated phenols have not been detected in the recipient, probably due to lack of sensitive analytical methods, while the selected heavy metals rarely appear at higher levels than the natural background (OGP, 2004). Total hydrocarbon content may be detected at elevated levels in the vicinity of discharge points, but is less sensitive and provides no additional information compared to PAH.

In addition to serving the specific purpose of regional impact monitoring (Chapter 1), the 1997, 1999 and 2000 surveys were used for development, testing and selection of methodology for determining environmental concentrations of produced water compounds. In addition, these early surveys provided valuable information for validation and improvement of the dilution model in DREAM. The results and recommendations of these surveys, as listed in Chapter 2, have been summed up by Durell et al., 2005 and Neff et al., 2005. The recommended methods presently form the basis for sampling and analysis of produced water compounds in the regional impact monitoring, and are regarded as sufficient, together with the DREAM model, to give an acceptable image of the fate of produced water compounds in the marine environment.

The aim of the BECELAG workshop (2001-2002) was to test, qualify, identify and select biological response indicators (biomarkers) for the regional impact monitoring. The ambition when introducing such indicators was to establish a link between exposure and effect of different chemical compounds discharged to the sea for the purposes described in Chapter 1-2. As a result of the recommendation from the Steering Committee for BECELAG (Hylland et al., 2002) a number of biomarkers were included in the following monitoring surveys (Chapter 3). The experience and results from these surveys show that further development and testing is needed, both with respect to a better understanding of the link between biomarkers and biological effects, and in order to select optimal indicators for the regional impact monitoring of the discharges from the offshore petroleum activities.



## EXPOSURE OF PRODUCED WATER COMPOUNDS

From the results presented in Chapter 2-2 and 3-2 it is evident that the water column in the vicinity of produced water discharge sources contains elevated concentrations of PAH compared to natural background levels. The area/distance from the source where elevated levels are detected, varies between the different regions included in the monitoring programme. Tampen is by far the region with the highest environmental abundance of such elevated levels, with oil-derived PAH observed at concentrations higher than the natural background up to 10 km from the nearest discharge point. Ekofisk and Sleipner only show increased levels of PAH closer than 5 km and 1 km from the nearest discharge point, respectively. The difference between the regions is explained by the difference in amounts of produced water discharged in each area. Tampen has the highest discharge volumes in the Norwegian sector and may also be influenced by discharges in the UK sector due to the direction of the prevailing currents. Ekofisk also has relatively high discharge volumes while the discharges in the Sleipner region are relatively low. Comparing PAH concentrations from the offshore areas to concentrations in Norwegian coastal waters (Table 2.4) shows that the levels rapidly dilutes down to lower than coastal background (>500m). PAH concentrations at the 500 m locations offshore are comparable to levels in a typical industrial contaminated fjord area.

Comparing measured and modelled results demonstrate that the model is able to give an acceptable image of the fate and abundance of PAH, given the fact that monitoring results have been used to improve the model. Based on this, it is assumed that the model also gives acceptable dilution images for the chemical compounds in produced water that can not be measured in the monitoring programme. Validation of this assumption is desirable, but in practice very difficult due to lack of analytical methods. Recent experience from the water column monitoring surveys have highlighted the need for use of real time data when modelling dilution, both because of limitations in their vertical resolution, stratification variations, and because modelling must necessarily be done with previous current and wind data, which may not reflect the situation when cages are deployed.

Due to difficulties in drawing a direct connection between exposure and biological effects, it is difficult to assess whether an exposure will lead to an effect. There are different definitions of the term effect, but anyway a biomarker response is an early warning of an environmental impact.

Biomarker responses, can in principle provide information on both exposure and effects. However, the exact line between these two endpoints is difficult to draw. For the regional impact monitoring the aim of introducing biomarkers as indicators is to obtain an early warning regarding discharges from our activities, in addition to information about possible effects of the discharges and information to validate the risk assessment.

The results from the BECPELAG workshop (Chapter 3-2) show that the observed biological responses in the Tampen region are well in line with the exposure information gained from the chemical analyses. For the following two surveys (2003 and 2004) the biomarker responses are lower. This is probably due to lower exposure concentrations during the two later surveys, but because of e.g. high levels of some of the analysed

components in the zero samples, affected reference stations, insecurity related to the thermocline and location of discharge plume it is difficult to rely on the results that show slightly increased levels of exposure.

As described above, the numbers and levels of exposure signals are general low, except for some measured result from the BECPELAG where the PAH metabolites and GST show responses up to 10 km away from the discharge point. The decrease in responses for several of the measured biomarkers from BECPELAG to the 2004 survey, even though the input was almost the same, may be many. The main reason is most likely, as discussed in Chapter 3-2, that the cages only were limited exposed to the produced water (H. Rye and M.K. Ditlevsen, 2005).

## EFFECTS OF PRODUCED WATER COMPOUNDS

Even though elevated concentrations of chemical compounds discharged with produced water are present in areas around the discharge points (based on measured and modelled results), this does not necessarily imply a direct impact on the ecosystem quality. In addition to the presence of these compounds, the exposure dose also represents a critical parameter for an effect to occur, but it is an early warning which should be eliminated or reduced as much as possible. PAH concentrations, representing produced water compounds, observed at distances greater than 500 m from the discharge points are low both compared to established toxic threshold levels (Frost, 2002) and to levels in coastal regions (Chapter 2-3). For areas close to the discharge point the observed levels are higher, and for some samples even within a range where toxic effects may occur. However, these levels are rapidly diluted as the water is transported away with ocean and tidal currents and the marine organisms will most probably, unless they are permanently located in the exposed area, be unable to accumulate so high levels of the contaminants that an effect will occur.

Both the chemical fingerprinting and the observation of DNA adducts in haddock (benthic feeder) and saithe (pelagic feeder) from the Tampen region in 2002 and 2004 respectively, demonstrate that exposure to oil-derived compounds is evident for this area. The source of this exposure is however not clear, but taking into account historical discharges, there will most probably be several sources

## 6 CONCLUSIONS

The following conclusions can be drawn from the environmental monitoring surveys of the water column 1997-2004:

- The programmes are dynamic and developed from one year to another based on experience from previous surveys and new knowledge.
- The methodology for sampling and analysis of chemical tracers (PAH) for the purpose of water column monitoring are well defined and of sufficient quality.
- Methodology for sampling and analysis of biological response indicators is still in need of development. A reliable set of biological response indicators has still not been finally established, partly due to low exposure, unfortunate circumstances related to high levels of measured components in pre-exposed samples and reference stations with elevated levels.
- The dilution model gives a sufficient reflection of potential environmental concentrations of the tracer compounds (PAH), and can be trusted for risk assessment purposes. This is likely to be case also for other chemical compounds discharged with produced water.
- Elevated concentrations of oil-derived PAHs can be found in the recipient in areas with produced water discharges. The abundance and distribution varies, depending on the volume of produced water discharges and the density of discharge sources. In the Tampen area elevated concentrations of 2-3 ring PAH have been detected as far as 10 km from the nearest discharge source, while in other areas, like Sleipner and Ekofisk, elevated levels are only observed in the vicinity of the discharge sources (1 and 5 km respectively).
- The concentrations of PAH outside the near zone (500 m) are generally low compared to expected effect threshold levels (PNEC) and concentrations observed in costal areas.
- Biological responses showing exposure to and to produced water compounds (PAH) have been observed at similar distance as the elevated PAH concentrations for the Tampen area 10 km, BECPELAG workshop 2001-2002.
- The 2003 and 2004 surveys show lower exposures in terms of both chemical and biological indicators than the previous surveys. This may be explained by limited exposure of the organisms, time of year (when variability in biological responses) surveys were conducted, and the influence of the natural pycnocline on the dilution factors of the discharge plume.
- The condition monitoring shows no evidence of increased levels of oil related components in wild fish, but increased levels of DNA adducts have been detected.

## 7 THE WAY FORWARD

The results from the water column monitoring surveys so far has demonstrated that measuring both exposure and effect parameters in the field is very challenging. There is still a need to develop monitoring methodology, both related to analysis of chemical compounds discharged into the environment and the biological responses to these pollutants may cause. The further work of the monitoring programme will thus have a considerable focus on the development and testing of such methods.

Another important experience from the monitoring programme is the continuous need continuums needed to reduce the uncertainty. A number of factors contribute to the uncertainty of the measured results and these have to be minimised in order to secure the quality of the core results in the monitoring studies. Such factors may be related to oceanographic data (modelled vs. measured), background levels of chemical compounds, natural variation in biomarker activity, and more. Uncertainty in the interpretation of field data may be lowered by conducting controlled lab exposures simultaneously to the field surveys using the same discharge water. Efforts to minimise these uncertainty factors are a high priority.

The water monitoring programme will in the period 2006-2007 focus on identifying the effect of the zero discharge work related to implementation of discharge-reducing measures at the Ekofisk field. This is important for the evaluation of the zero discharge work and evaluation of the need for further discharge-reducing measures.

Today's monitoring programme distinguishes between the regional impact monitoring and the condition monitoring, both with respect to frequency and content. Over the past years a need for overlapping methodology between these two programmes has become evident. The potential of a closer link, even a merger between the two programmes should be considered in the future.

Work is also needed to develop links between biomarker monitoring and risk assessment, i.e. what does certain biomarker levels mean with regard to risk of real impact.



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## APPENDIX

- **Cytochrome P4501A activity (EROD) and concentration (CYP1A) in liver; EROD in gills on a testing basis in 2004**

- 2001 (EROD and CYP1A in all samples)**

- Saithe and herring (natural populations):

- no significant differences in EROD response between locations; significant differences in CYP1A concentration in saithe between locations, but not related to gradient

- Atlantic cod (caged):

- no differences between locations in either EROD activity or CYP1A concentration

- 2003 (EROD only)**

- Atlantic cod (caged):

- low EROD at all field locations; significantly lower in cod prior to deployment (from fish farm) compared to field deployed fish

- 2004 (EROD only)**

- Atlantic cod (caged):

- no significant differences between locations for either hepatic (liver) EROD or gill EROD

- **GST (Atlantic cod)**

- 2001:**

- significantly elevated GST at innermost location (500 m) compared to reference location

- 2003:**

- significant differences between locations, but not related to gradient

- 2004:**

- no significant differences between locations

- **DNA adducts (fish)**

- 2001 (caged Atlantic cod):**

- higher concentration at reference location than at location closer to platform

- 2003 (caged Atlantic cod):**

- low concentrations of DNA adducts, somewhat elevated in cod prior to exposure (fish farm)

- 2004 (natural population of saithe):**

- no significant differences, but individual fish at all three sites had clearly elevated concentrations of hepatic DNA-adducts indicating past exposure to genotoxic substances

- **Vitellogenin (Atlantic cod)**

- 2001:**

- Histopathology generally show trends towards higher concentration closer to platform, but no significant differences between locations

- 2003:**

- low concentrations of vitellogenin in fish held in cages; significantly higher in fish sampled prior to exposure (at fish farm)

- 2004:**

- no significant differences between locations (within each sex)

- **Histopathology and histochemistry**

- 2001**

- Saithe (natural population):

- significant differences in the tissue integrity of the liver and peroxisomal proliferation (AOX activity and density of peroxisomes) between locations close to the platform and reference location; lower prevalence of melanomacrophage centers in fish from reference location compared to fish in gradient

- Atlantic cod (caged):

- no significant differences between locations in the parameters measured

- Blue mussel (caged):

- significant differences in peroxisomal proliferation (AOX activity) between locations closest to platform (500 m and 2000 m) and reference; non-significant trend in relative density of cell types in hepatopancreas

- 2003**

- Atlantic cod (caged):

- individual fish with lesions, not contaminant-related

- Blue mussel (caged):

- higher prevalence of melanised lysosomes at locations close to platform compared to reference

- 2004**

- Saithe (natural population):

- no significant differences, although individuals collected closest to the discharge point had higher prevalence of inflammations than fish from other locations

- Atlantic cod (caged):

- not included

- Blue mussel (caged):

- mussels were stressed prior to deployment (presumably due to proximity to spawning period), but no effects of the gradient

- **PAH metabolites (fixed fluorescence; fish)**

- 2001**

- Saithe and herring (natural populations):

- no significant differences between locations

- Atlantic cod (caged):

- significant differences between locations; gradient in 2-3 ring PAHs with highest levels close to platform

- 2003 (caged Atlantic cod):**

- not included

**2004**

Saithe (natural population):

no significant differences between locations

Atlantic cod (caged):

no gradient-related differences between locations, highest for fish prior to deployment (from fish farm)

- **PAH metabolites (GC/MS; fish)**

**2001**

Saithe and herring (natural populations):

no significant differences between locations

Atlantic cod (caged):

significant differences between locations; gradient in naphthalene metabolites with highest levels close to platform

**2003 (caged Atlantic cod):**

significant differences between locations; gradient in one naphthalene metabolite (2-OH-naphthalene) with highest levels closest to platform

**2004**

Saithe (natural population):

no differences between locations that could be related to gradient

Atlantic cod (caged):

no differences between locations that could be related to distance to platform

- **BaPH activity (blue mussel)**

**2001:**

not measured due to loss of samples

**2003:**

significant differences between locations, but not related to distance from platform

**2004:**

no significant differences between stations

- **Lysosomal stability by histochemical method (fish)**

**2001 (natural population of saithe):**

significant effects (decreased membrane stability) related to gradient from platform

**2003:**

not included

**2004:**

not included

- **Lysosomal stability by histochemical method (blue mussel - hepatopancreas)**

**2001:**

significant effects related to gradient from platform

**2003:**

all field stations apparently lower lysosomal stability (i.e. affected) compared to pre-exposure mussels, but only mussels kept at reference site significantly different from pre-exposure mussels

**2004:**

not included

- **Lysosomal stability by neutral red retention (blue mussel - hemocytes)**

**2001:**

no results reported

**2003:**

significant differences between locations, but not related to gradient from platform (lowest retention at reference location)

**2004:**

no significant differences between locations

- **Micronucleus formation**

**2001:**

not included

**2003:**

not reported

**2004**

Atlantic cod (caged):

non-significant trend of increased formation of micronuclei in kidney cells of cod caged closer to platform

Blue mussel hemocytes (caged):

significant differences between the locations with higher levels closer to platform and between locations and the zero-time sample

- **General health criteria**

Organisms deployed in cages (blue mussels, Atlantic cod) have generally been in good health after deployment (all years).

- **Lipid content (blue mussels)**

**2001:**

not reported

**2003:**

no significant differences between locations

**2004:**

no significant differences between locations



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