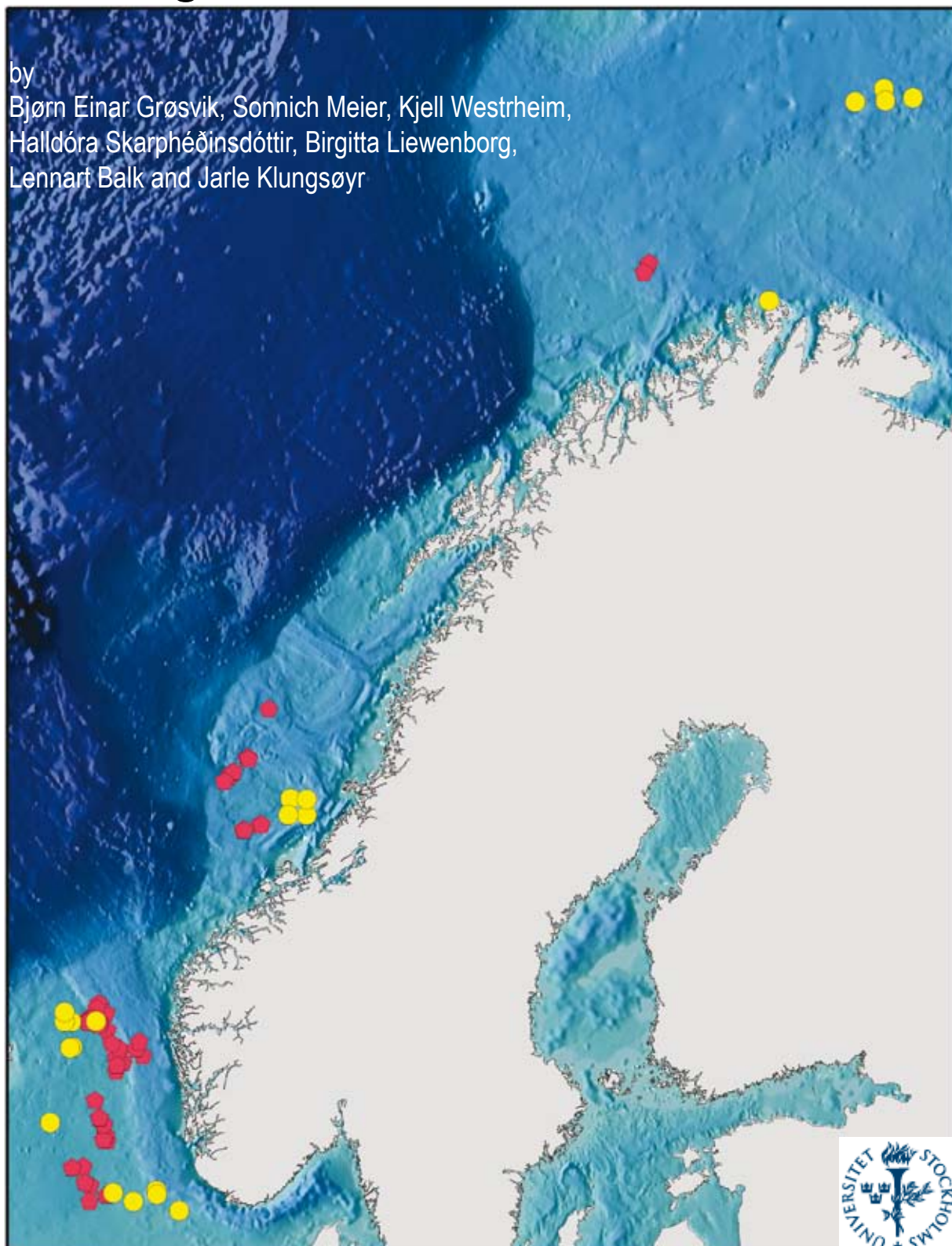Condition monitoring in the water column 2005:

Oil hydrocarbons in fish from Norwegian waters

by
Bjørn Einar Grøsvik, Sonnich Meier, Kjell Westrheim,
Halldóra Skarphéðinsdóttir, Birgitta Liewenborg,
Lennart Balk and Jarle Klungsøyr

INSTITUTE OF MARINE RESEARCH
HAVFORSKNINGSINSTITUTTET

Condition monitoring in the water column 2005: Oil hydrocarbons in fish from Norwegian waters

Authors:

Bjørn Einar Grøsvik[1], Sonnich Meier[1], Kjell Westrheim[1], Halldóra Skarphéðinsdóttir[2], Birgitta Liewenborg[2], Lennart Balk[2] and Jarle Klungsøyr[1]

[1] Institute of Marine Research, Bergen, Norway.
[2] Laboratory for Aquatic Ecotoxicology, Department of Applied Environmental Science, Stockholm University, Sweden.

Project leader:

Jarle Klungsøyr

Contractor:

The operators on the Norwegian Continental Shelf

Summary

This report has been prepared by Institute of Marine Research (IMR) & University of Stockholm (UoS) on behalf on the offshore petroleum industry operators on the Norwegian Continental Shelf as part of the authority requirements in the Health, Safety and Environmental regulation (Activity regulation).

The objectives for this study have been:
1. Determine to what extent fish from the oil installation areas at Tampen and the Halten Bank contain elevated levels of petroleum hydrocarbons compared with fish from reference areas at the Egersund Bank/Ling Bank and in the Barents Sea by measurements of NPD/PAH in fish muscle.
2. Comparison of NPD/PAH bile metabolites in fish from Tampen compared with fish from Egersund Bank/Ling Bank
3. Study possible genotoxic effects in fish from Tampen compared with fish from Egersund Bank/Ling Bank by measurements of hepatic DNA adducts.
4. Document to what extent discharges of alkylphenols in produced water cause estrogenic effects in fish from Tampen by measurements of alkylphenols in muscle and liver, and vitellogenin in plasma.

Di- and polyaromatic hydrocarbons (NPD/PAH) have been analysed in muscle of cod and haddock caught in the North Sea at Ling Bank/Egersund Bank (reference), Tampen, Halten Bank and Barents Sea (reference), autumn 2005 and concentrations found to be below levels of quantification (LOQ) for fish sampled from all regions.
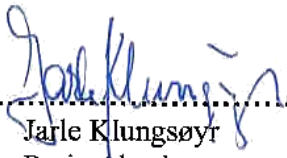
Cod sampled at the Ling Bank/Egersund Bank in the Southern part of the North Sea had the same levels of PAH metabolites in bile as cod sampled from the Tampen region. Haddock demonstrated significantly higher levels of fluorescence for all three wavelength pairs measured, indicating a higher levels of 2-, 3-, 4- and 5-ring PAHs for haddock sampled in the Tampen region compared

with haddock from the Ling Bank/Egersund Bank region. Saithe, on the other hand demonstrated higher levels of 2- and 3-ring compounds at the Ling Bank/Egersund Bank compared with fish sampled at Tampen. Overall, the highest levels of PAH metabolites in bile were measured in haddock.

DNA adducts were analyzed in liver of cod, haddock and saithe at Tampen and from Ling Bank/Egersund Bank (reference site). In both areas the highest levels of DNA adducts were measured in haddock. The percentage of individuals with detectable adducts was also higher in haddock than for the other species. Haddock from Tampen had significant higher DNA adduct levels compared with haddock from Egersund Bank/Ling Bank (Mann-Whitney test), indicative of more PAH exposure in this region. Higher levels of DNA adducts in haddock caught at Tampen compared with the Egersund Bank have earlier been reported by Klungsøyr *et al*. (2003). Significant differences were not found for cod and saithe collected from the same areas.

Analyses of alkylphenols in cod liver, haddock liver and herring muscle from Ling Bank/Egersund Bank and Tampen regions demonstrated levels below limits of detection (LOD) for all stations. The absence of alkylphenols in fish is in line with the results from the 2002 monitoring (Klungsøyr *et al*., 2003), and it supports the risk assessment carried out by Myhre *et al*. (2004) that stated that the risk for estrogenic and reproductive effects in fish after alkylphenols exposure from produced water discharges is very low.

There were no differences in VTG concentration in plasma of cod caught at Tampen compared with Ling Bank/Egersund Bank that could not be explained by differences in size and sexual maturation.

....
Jarle Klungsøyr
Project leader

....
Ole Arve Misund
Research director

2

Contents

Acknowledgements

Introduction

The Activity regulations require the offshore petroleum industry to perform monitoring. The Condition monitoring shall document if fish from Norwegian ocean areas contain elevated values of components that originate from discharges from the petroleum activity. Analyses of di- and polyaromatic hydrocarbons (NPD/PAH) in fish muscle are included in the regular environmental monitoring programme for the offshore petroleum installations in Norwegian areas. The major objective is to document to what extent discharges from the oil and gas installations cause contamination of fish negatively affecting the quality. For both the petroleum industry and the Norwegian fishing industry it is very important that safety and quality of Norwegian seafood is documented, as well as environmental health.

A study reported by Klungsøyr and Johnsen (1997) on cod (*Gadus morhua* L.) and haddock (*Melanogrammus aeglefinus*) concluded that there is no general increase in levels of NPD/PAH in fish caught in the vicinity of oil and gas fields in Norwegian areas compared with remote reference areas.

In the monitoring performed in 2000, haddock were collected from ten regions: Ekofisk, Sleipner, Tampen, Møre, Trøndelag, Nordland, Troms, Finmark, the Barents Sea (reference) and the Egersund Bank (reference). The results from the analyses of 25 muscle samples from each of these regions showed that haddock only contained very low background concentrations of NPD/PAH (Klungsøyr *et al*., 2001).

In 2002, the monitoring was carried out as an integrated part of the project "Contamination of fish in the North Sea by offshore oil and gas industry" (Norwegian Research Council project No. 152231/720). This project had a broader scope than only tracing oil hydrocarbons in fish. The objective was to study to what extent contaminants from offshore petroleum industry bioaccumulate cause effect in fish populations and affect food safety and quality. In this study NPD/PAH were analysed in cod, haddock, saithe and herring from Tampen, Sleipner and the Egersund Bank (reference area). The levels of NPD/PAH in haddock muscle at Sleipner and Tampen were generally very low and at normally occurring background concentrations for fish from the North Sea. Similar results were found for fish liver samples showing that fish from Tampen and Sleipner in general contained very low background concentrations of NPD/PAH. This is in accordance with previous results and can be explained both by low exposure and/or and effective metabolic system in fish resulting in rapid excretion of aromatic hydrocarbons (Klungsøyr *et al*., 2003).

However, the analyses of biomarkers in the 2002 study revealed biological effects in haddock from Tampen and Sleipner compared with fish from the Egersund Bank. In haddock, genotoxicity was reflected in increased levels of hepatic DNA adducts probably due to exposure to NPD/PAH. Anomalies in muscle lipid composition were also detected at the Tampen and Sleipner areas compared to Egersund Bank (Klungsøyr *et al*., 2003).

A new monitoring study to follow up the results from the previous studies was carried out during 2005-2006 and presented here.

The objectives have been:
1. Determine to what extent fish from areas with oil and gas activity; Tampen and the Halten Bank contain elevated levels of petroleum hydrocarbons compared with fish

from reference areas; the Egersund Bank/Ling Bank and the Barents Sea by measurements of NPD/PAH in fish muscle.
   2. Comparison of NPD/PAH bile metabolites in fish from Tampen compared with fish from Egersund Bank/Ling Bank
   3. Study possible genotoxic effects in fish from Tampen compared with fish from Egersund Bank/Ling Bank by measurements of hepatic DNA adducts.
   4. Document to what extent discharges of alkylphenols in produced water cause estrogenic effects in fish from Tampen by measurements of alkylphenols in muscle and liver, and vitellogenin in plasma.

Work packages
   1. Sampling of fish from 4 selected areas: The Egersund Bank/Ling Bank (reference area), Tampen, the Halten Bank and the Barents Sea (reference area).
   2. Chemical analysis of NPD/PAH in muscle of cod and haddock for food quality control purposes.
   3. Study uptake and metabolism of PAH in cod, haddock and saithe from the Egersund Bank/Ling Bank and Tampen area by fluorescence measurements of PAH metabolites in bile.
   4. Study genotoxic effects in cod, haddock and saithe at the Egersund Bank/Ling Bank and Tampen area by analysis of DNA adducts in liver.
   5. Study possible endocrine disrupting effects by analysis of alkylphenols in cod, haddock, saithe and herring from the Egersund Bank/Ling Bank and the Tampen area, and vitellogenin in cod from the same areas.

Work package 1 – Sampling

Haddock (*Melanogrammus aeglefinus*), cod (*Gadus morhua*) and saithe (*Pollachius virens*) were collected from 4 regions: The Barents Sea, Halten Bank, Tampen, Egersund Bank/Ling Bank. Herring (*Clupea harengus*) was collected from the two North Sea regions only. The sampling took place during the following cruises:

> The Barents Sea: RV G.O. Sars, 5 August - 8 September 2005
> The Halten Bank: RV Johan Hjort, 11 October - 8 November 2005
> Tampen and the Egersund Bank/Ling Bank: RV G.O. Sars, 26 October -16
> December 2005

Bottom trawl was used for collection of cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and saithe (*Pollachius virens*). Pelagic trawl was used for collection of herring (*Clupea harengus*). The fish were kept alive in big tanks onboard the vessels until sampling took place. From each of the regions 25 (±10%) fish of each species were sampled. After killing the fish with a blow to the head, standard IMR procedures were used for collection and storage of muscle, liver, blood and bile samples for the later chemical and biochemical analyses. Figure 1 gives the sampling locations for fish in the North Sea. Table 1 gives details of the sampling from all four areas and biological data is shown in Table 2.
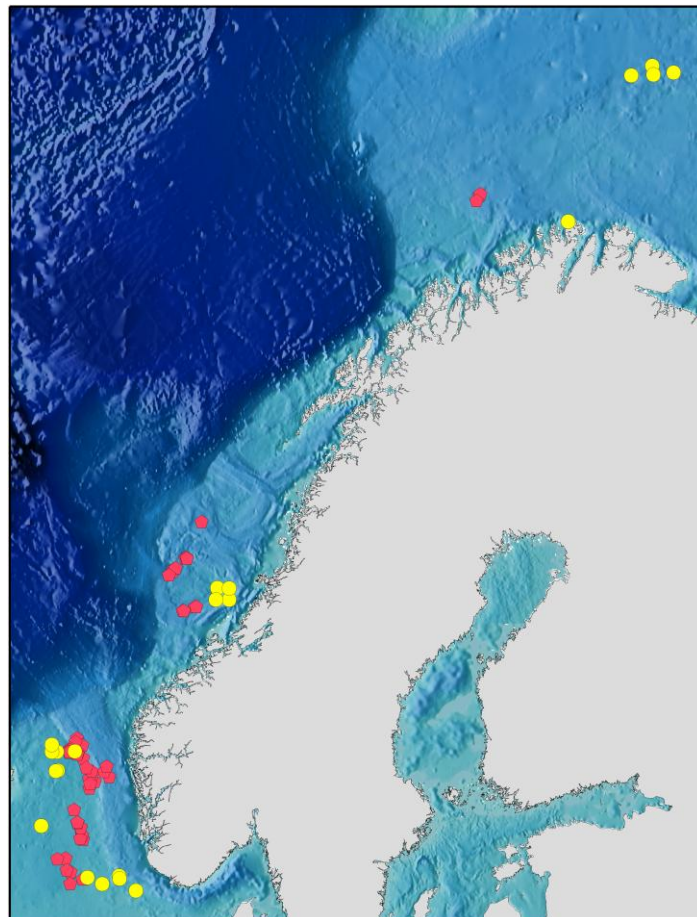

Figure 1. Stations for fish sampling.

Table 1. Sampling data
Abbreviations: G.O. Sars (GOS), longitude (Long.), not measured (nm).

St. no.	Vessel	Date	Species	Latitude	Long.	No. of fish	Depth (m)	Temp. (°C)
Ling Bank/ Egersund Bank								
547	GOS	30.10.05	Cod	N 58 04.6	E 03 23.8	4	87	11.4
549	GOS	30.10.05	Cod	N 58 16.8	E 04 11.8	5	145	11.4
577+	GOS	06.12.05	Cod	N 57 54.4	E 04 59.3	20	100-175	9.5-10.0
546	GOS	30.10.05	Haddock	N 58 14.1	E 02 41.8	4	72	11.5
547	GOS	30.10.05	Haddock	N 58 04.6	E 03 23.8	14	87	11.4
548	GOS	30.10.05	Haddock	N 58 12.7	E 04 13.0	7	136	11.4
549	GOS	30.10.05	Saithe	N 58 16.8	E 04 11.8	10	145	11.4
548	GOS	30.10.05	Saithe	N 58 12.7	E 04 13.0	15	136	11.4
544	GOS	29.10.05	Herring	N 59 30.0	E 00 31.1	25	125	10.7
Tampen								
561/562	GOS	14.11.05	Cod	N 60 47.5	E 01 12.4	1	146	10.8
563	GOS	17.11.05	Cod	N 61 23.4	E 01 01.1	1	167	10.4
565	GOS	17.11.05	Cod	N 61 13.8	E 01 15.5	1	153	10.5
566	GOS	17.11.05	Cod	N 61 14.2	E 02 06.6	1	141	10.5
584+	GOS	07.12.05	Cod	N 60 48.0	E 01 17.8	23	118-180	8.8-9.9
561	GOS	14.11.05	Haddock	N 60 48.0	E 01 17.8	2	146	10.8
562	GOS	14.11.05	Haddock	N 60 47.5	E 01 12.4	13	146	10.8
563	GOS	17.11.05	Haddock	N 61 23.4	E 01 01.1	2	167	10.4
564	GOS	17.11.05	Haddock	N 61 14.0	E 01 01.2	8	165	10.5
561/562	GOS	14.11.05	Saithe	N 60 47.5	E 01 12.4	20	146	10.8
563	GOS	17.11.05	Saithe	N 61 23.4	E 01 01.1	5	167	10.4
563	GOS	17.11.05	Herring	N 61 23.4	E 01 01.1	25	167	10.4
Halten Bank								
732	J. Hjort	01.11.05	Cod	N 64 30.2	E 09 23.8	10	218	9.5-10.0
734	J. Hjort	01.11.05	Cod	N 64 44.1	E 08 50,6		113	9.5-10.0
732	J. Hjort	01.11.05	Haddock	N 64 43.7	E 09 23.8	25	218	9.5-10.0
733	J. Hjort	01.11.05	Haddock	N 64 30.2	E 08 46.2		155	9.5-10.0
732	J. Hjort	01.11.05	Saithe	N 64 43.7	E 09 23.8	25	218	9.5-10.0
733	J. Hjort	01.11.05	Saithe	N 64 30.2	E 08 46.2		155	9.5-10.0
Barents Sea								
595	J. Hjort	30.08.05	Cod	N 73 16.7	E 28 23.9	25	349	8.8-8.9
596	J. Hjort	30.08.05	Cod	N 73 17.2	E 29 26.6		327	8.8-8.9
597	J. Hjort	30.08.05	Cod	N 73 24.4	E 29 23.4		390	8.9
595	J. Hjort	30.08.05	Haddock	N 73 16.7	E 28 23.9	25	349	8.8-8.9
596	J. Hjort	30.08.05	Haddock	N 73 17.2	E 29 26.6		327	8.8-8.9
622	J. Hjort	30.08.05	Haddock	N 73 19.4	E 30 23.8		329	9.1-9.3
640	J. Hjort	30.08.05	Saithe	N 71 11.0	E 25.24.4	25	104	n.m.

Table 2. Biological data

Data given as mean ± stdev.

Station	Species	Length (cm)	Weight (cm)	Males/ Females	Liver weight (g)	Gonad weight (g)	LSI (%)	GSI (%)
Ling Bank/ Egersund B.	Cod	49±14	1657±2088	13/12	71±138	41±106	3.0±1.4	1.0±2.1
Tampen	Cod	76±17	4890±2768	11/14	342±262	125±128	8.2±14.1	3.2±6.9
Halten Bank	Cod	69±14	3685±1808	6/4	n.m.	n.m.	n.m.	n.m.
Barents Sea	Cod	48±7	977±480	16/9	n.m.	n.m.	n.m.	n.m.
Ling bank/ Egersund B.	Haddock	38±5	617±240	16/9	32±17	3.4±4.4	5.4±1.6	0.7±0.8
Tampen	Haddock	42±4	795±248	13/12	45±19	6.1±6.9	5.6±1.35	0.8±0.7
Halten Bank	Haddock	50±7	1408±526	15/10	n.m.	n.m.	n.m.	n.m.
Barents Sea	Haddock	43±12	965±872	21/4	n.m.	n.m.	n.m.	n.m.
Ling bank/ Egersund B.	Saithe	44±5	789±305	12/13	33±28	n.d.	3.9±1.4	n.m.
Tampen	Saithe	51±5	1326±798	18/7	67±36	n.d.	5.1±1.5	n.m.
Ling bank/ Egersund B.	Herring	29±1	173±21	25/0	2.3±0.5	3.3±7	1.3±0.3	2.1±4.8
Tampen	Herring	29±1	208±40	8/17	3.5±4.0	5.8±11.0	1.7±2.0	1.5±3.2

Liver somatic index (LSI) is percentage liver weight per body weight. Gonado somatic index (GSI) is percentage gonade weight per body weight. If not measured, labelled n.m.

Otoliths were sampled and analysed in haddock from Egersund/Ling Bank and Tampen and haddock from these sites were found to be: 5,2±1.5 and 5,8±0.8 years, respectively.

Work package 2 - Chemical analyses of NPD/PAH in fish muscle

The main task in this work package was to document whether discharges from offshore petroleum industry cause oil contamination in cod and haddock muscle negatively affecting the quality for human consumption. Levels of aromatic hydrocarbons (NPD/PAH) will be indicators of exposure, and analyses were carried out using GC/MS. The compounds included in the analysis are shown in Table 3. NPD is the sum of naphthalene, phenanthrene, dibenzothiophene, and their C_1-C_3 alkylated homologs. PAH (EPA list of 16 compounds) is the sum of acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, pyrene.

NPD/PAH analysis of muscle tissue

Wet muscle tissue (ca. 15g) was boiled under reflux with 0.5N alcoholic KOH for 1.5 hours, followed by liquid/liquid extraction with hexane. Extracts were volume reduced and cleaned on silica column prior to injection on a Micromass Autospec Ultima GC/MS in SIM mode (Klungsøyr *et al*., 1988). The GC/MS system was quipped with a HP-6890 GC, a 50m x 0,25mm, 0,25µm Varian Factor Four CC VF-5ms capillary column inserted directly into the ion source. Other conditions were: injector temperature 280ºC; transfer line 275ºC; column temperature, 60ºC for 1 min, 60-100ºC at 15ºC/min, 100-280ºC at 6ºC/min, 9min at final temperature, carrier gas He at 1.5 ml/min. Electron impact ionization at 70eV was used. Samples were injected by auto sampler, 1 µl splitless injection.

The method is validated to analyse PAH in concentration of 0.2 ng/g. For some compounds the detection limit are higher, because of background problems. Levels of detection (LOD) is defined as LOD: $Y = Y_B + 3SD_B$, and levels of quantification (LOQ) is $LOQ = Y = Y_B + 10SD_B$ where Y_B is the response of blank sample signal and SD_B is the standard deviation of the blank samples.

Results

Levels of NPD and PAH in muscle of cod and haddock were low for all regions. All levels measured were below levels of quantification (LOQ) (Table 3 and 4).

Table 3. Mean ± std dev of NPD/PAH compounds in muscle of cod in ng/g w.w.
N=25 per station, except for Halten Bank, where N=10. Abbreviations: Limit of detection (LOD), limit of quantification (LOQ).

Compound	Ling Bank/ Egersund Bank	Tampen	Halten Bank	Barents Sea	LOD	LOQ
Naftalen	0.330±0.069	0.444±0.135	0.298±0.034	0.943±0.371	1.157	1.630
C1-naftalen	0.426±0.057	0.686±0.110	0.435±0.051	1.092±0.366	1.985	3.214
C2-naftalen	0.664±0.465	0.919±0.124	0.739±0.091	1.472±0.302	1.866	3.420
C3-naftalen	0.322±0.348	0.416±0.067	0.315±0.031	0.685±0.233	0.483	0.778
Acenaphtylen	0.081±0.108	0.017±0.003	0.009±0.001	0.031±0.010	0.044	0.081
Acenapthene	0.040±0.011	0.040±0.004	0.038±0.003	0.051±0.019	0.064	0.091
Fluorene	0.166±0.052	0.153±0.025	0.129±0.044	0.247±0.065	0.512	1.102
Anthracene	0.003±0.002	0.005±0.04	0.003±0.004	0.007±0.003	0.009	0.020
Phenantrene	0.073±0.016	0.149±0.072	0.085±0.029	0.200±0.033	0.229	0.386
C1-Phenantrene	0.054±0.020	0.113±0.106	0.052±0.010	0.236±0.267	0.075	0.112
C2-Phenantrene	0.044±0.022	0.090±0.098	0.073±0.125	0.237±0.197	0.062	0.100
C3-Phenantrene	0.043±0.030	0.062±0.090	0.025±0.004	0.213±0.213	0.033	0.066
Dibenzothiophene	0.002±0.002	0.005±0.008	0.003±0.001	0.008±0.008	-	0.2
C1-Dibenzothiophene	0.004±0.001	0.013±0.017	0.005±0.001	0.039±0.039	0.004	0.008
C2-Dibenzothiophene	0.008±0.003	0.037±0.041	0.009±0.003	0.097±0.097	0.012	0.024
C3-Dibenzothiophene	0.013±0.015	0.041±0.045	0.011±0.004	0.138±0.138	0.011	0.031
Fluoranthene	0.022±0.005	0.054±0.08	0.032±0.033	0.056±0.056	0.054	0.098
Pyrene	0.010±0.003	0.027±0.050	0.017±0.022	0.030±0.030	0.021	0.041
Benz(a)anthracene	0.000±0.001	0.002±0.002	0.002±0.003	0.004±0.004	0.004	0.011
Chrysene	0.002±0.002	0.007±0.015	0.004±0.005	0.008±0.006	0.005	0.012
Benzo(b)fluorantene	0.015±0.020	0.016±0.016	0.011±0.008	0.046±0.038	0.015	0.042
Benzo(k)fluorantene	0.013±0.022	0.007±0.07	0.008±0.005	0.027±0.023	0.033	0.095
Benz(e)pyrene	0.013±0.021	0.008±0.09	0.010±0.008	0.018±.021	0.026	0.072
Benz(a)pyrene	0.011±0.02	0.004±0.06	0.007±0.004	0.013±0.020	0.059	0.171
Perylene	0.009±0.015	0.004±0.04	0.005±0.002	0.015±0.020	0.024	0.068
Indeno(1.2.3-cd)pyrene	0.001±0.01	0.002±0.002	0.001±0.002	0.002±0.003	0.004	0.011
Dibenz(a.h)anthracene	0.001±0.02	0.001±0.001	0	0.002±0.001	0.003	0.009
Benzo(g.h.i)perylene	0.001±0.02	0.002±0.002	0.002±0.002	0.003±0.004	0.007	0.048

Table 4. Mean ± std dev of NPD/PAH compounds in muscle of haddock in ng/g w.w. N=25 per station. Abbreviations: Limit of detection (LOD), limit of quantification (LOQ).

Compound	Ling bank/ Egersund Bank	Tampen	Halten Bank	Barents Sea	LOD	LOQ
Naftalen	0.415±0.130	0.281±0.060	0.309±0.037	0.465±0.145	0.440	0.765
C1-naftalen	0.563±0.196	0.416±0.069	0.504±0.059	1.035±0.596	0.757	1.410
C2-naftalen	0.806±0.203	0.716±0.118	0.878±0.101	1.018±0.186	0.931	1.594
C3-naftalen	0.560±0.162	0.286±0.052	0.604±0.105	0.582±0.264	0.691	1.296
Acenaphtylen	0.063±0.032	0.009±0.001	0.031±0.013	0.021±0.019	0.105	0.243
Acenapthene	0.071±0.071	0.037±0.006	0.045±0.027	0.041±0.014	0.068	0.112
Fluorene	0.153±0.028	0.142±0.028	0.152±0.012	0.152±0.029	0.208	0.362
Anthracene	0.005±0.002	0.002±0.001	0.004±0.001	0.004±0.001	0.007	0.016
Phenantrene	0.076±0.013	0.061±0.010	0.077±0.009	0.116±0.023	0.082	0.122
C1-Phenantrene	0.108±0.145	0.043±0.007	0.079±0.014	0.147±0.182	0.099	0.179
C2-Phenantrene	0.076±0.019	0.035±0.006	0.060±0.012	0.093±0.047	0.061	0.114
C3-Phenantrene	0.070±0.019	0.018±0.004	0.060±0.013	0.094±0.069	0.050	0.107
Dibenzothiophene	0.001±0.002	0.001±0.001	0.001±0.001	0.008±0.010	-	0.2
C1-Dibenzothiophene	0.016±0.031	0.003±0.001	0.013±0.003	0.029±0.030	0.007	0.015
C2-Dibenzothiophene	0.016±0.008	0.008±0.002	0.021±0.011	0.065±0.047	0.008	0.021
C3-Dibenzothiophene	0.018±0.009	0.034±0.120	0.025±0.017	0.099±0.097	0.054	0.153
Fluoranthene	0.025±0.007	0.020±0.004	0.022±0.008	0.036±0.008	0.031	0.059
Pyrene	0.014±0.009	0.009±0.002	0.010±0.004	0.017±0.007	0.012	0.022
Benz(a)anthracene	0.002±0.009	0.000±0.000	0.000±0.001	0.003±0.003	0.003	0.007
Chrysene	0.008±0.031	0.002±0.004	0.003±0.004	0.007±0.004	0.004	0.008
Benzo(b)fluorantene	0.018±0.040	0.010±0.015	0.007±0.005	0.018±0.012	0.007	0.019
Benzo(k)fluorantene	0.006±0.008	0.005±0.011	0.002±0.001	0.008±0.010	0.004	0.010
Benz(e)pyrene	0.025±0.098	0.005±0.004	0.003±0.003	0.012±0.010	0.008	0.021
Benz(a)pyrene	0.011±0.024	0.002±0.001	0.003±0.008	0.006±0.008	0.003	0.007
Perylene	0.008±0.030	0.001±0.001	0.002±0.004	0.026±0.030	0.003	0.007
Indeno(1,2,3-cd)pyrene	0.002±0.006	0.000±0.000	0.001±0.001	0.004±0.007	0.004	0.008
Dibenz(a,h)anthracene	0.002±0.003	0.000±0.000	0.001±0.001	0.001±0.001	0.003	0.006
Benzo(g,h,i)perylene	0.029±0.138	0.000±0.000	0.000±0.001	0.003±0.005	0.002	0.004

Work package 3 - Analyses of bile metabolites

The presence of PAH metabolites in fish bile is evidence of exposure, and some of the reactive intermediates can cause genotoxic effects. Presence of PAH metabolites was analysed by a simple fluorescence screening technique. Bile metabolites were analysed in cod, haddock and saithe from the Tampen area and the Ling Bank/Egersund Bank.

The content in bile can reflect which compounds are being metabolised in the organism in a small and concentrated volume. This has shown particularly useful for hydroxylated polycyclic aromatic hydrocarbons (PAH) (Aas *et al*., 2000). Detection of 2- to 3-ring metabolites is read by ex/em 290/335, 4-ring metabolites by ex/em 341/380 and 5-ring metabolites by ex/em 380/430 nm. Small changes were done in ex/em values when adapting to plate reader as producer only could deliver filters for fluorescence for wavelengths in tens.

Material and methods

Fixed wavelength fluorescence method (FF) was adapted to a plate reader, Fluostar Optima, BMG Labtech, and micro plate F96 from NUNC were used for fluorescence measurements. Bile was diluted 200 times in 100 % ethanol. Generally, 50 % ethanol or 50 % methanol are used for fluorometric measurements, but initial analyses demonstrated that 50 % methanol was not optimal for plate reader measurements due to high background fluorescence. 100 % ethanol gave lower background fluorescence than 50 % and was therefore selected. All samples were analysed on the same plate, which allowed maximum 46 samples per plate, 2 parallels per sample. Fluorescence was measured for ex/em 290/330 nm, ex/em 340/380 nm and ex/em 380/430 nm, which is used to detect 2-3 ring, 4 ring and 5 ring PAH structures, respectively. Levels of fluorescence were standardised per mg/ml protein in bile. Protein levels were measured according to Bradford *et al*. (1976), using bovine serum albumin as standard.

Statistical analysis
Student t test was used after testing for normal distribution. The analyses were performed with JMP, ver. 5.0, SAS Institute In., Cary, NC, USA.

Results and discussion

Bile from cod sampled at the Ling bank/Egersund Bank in the Southern part of the North Sea had the same levels of PAH metabolites as cod sampled from the Tampen region (Table 5). Cod deployed in cages in a gradient at Statfjord during the BECPELAG workshop in 2001 demonstrated significant increase in FF measurements in bile closer to the oil field (0.5 to 10 km) compared with a reference station 140 km South East (Aas *et al*., 2006). As seen from Figure 1, most stations in the present study were located further away from oil platforms than 10 km.

Overall, the highest levels of PAH metabolites were measured in haddock. Haddock demonstrated significantly higher FF levels for all three wavelength pairs in the Tampen region compared with haddock from the Ling Bank/Egersund Bank region, which indicate higher levels of PAH contamination. Saithe, on the other hand demonstrated highest levels of 2 and 3 ring compounds at the Ling Bank/Egersund Bank compared with fish sampled at Tampen, while significant differences were not found for 4 and 5 ring type metabolites (Table 5). This warrants further investigations and cannot at present be explained.

Conflicting data has been published on how to normalise bile fluorescence. Van den Hurk (2006) has suggested it to be expressed per protein rather than per biliverdin concentration as mixtures of chemical stress can induce heme oxygenase activity and that this could lead to increased biliverdin expression (Hurk, 2006). In the current study fluorescence data have been standardised to protein concentration.

Table 5. Fixed wavelength fluorescence expressed as fluorescence units standardised per mg/ml protein in bile given as mean ± stdev.

Region	Species (N)	FF ex290/em330	FF ex340/em380	FF ex380/em430
Ling Bank/ Egersund Bank	cod (23)	137561±69575	19578±9647	18124±9211
Tampen	cod (23)	132522±77858	16995±11287	14468±8568
Ling Bank/ Egersund Bank	haddock (22)	381917±255035	64224±40947	42189±27655
Tampen	haddock (17)	659657±285562 (*)	127649±69091 (*)	76654±51353 (*)
Ling Bank/ Egersund Bank	saithe (11)	330499±137951	37848±22591	35700±25762
Tampen	saithe (19)	167346±99476 (*)	25631±11896	30442±16847

(*) indicate significant differences between Tampen and Ling Bank, p< 0.05.

Work package 4 – DNA adducts in liver of cod, haddock and saithe from Tampen and Ling bank/Egersund Bank

Summary

DNA adducts were analyzed in liver of fish sampled in the Tampen area in the North Sea, an area of the North Sea with high oil and gas activity, including several oil fields where there are discharges of produced water. Ling Bank (Ling Bank/Egersund Bank in the case of cod) was used as a reference area. The fish species studied were cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and saithe (*Pollachius virens*), 25 individuals of each species from each area (except for cod from Ling Bank/Egersund were only 21). The results revealed that significant higher DNA adduct levels were found in liver of haddock from Tampen, 4.42 ± 1.80 nmol adducts/mol normal nucleotides (average ± 95% confidence level) compared with haddock from the reference area Ling Bank which had 2.14 ± 0.57 nmol adducts/mol nucleotides (Mann-Whitney test). Cod and saithe from Tampen had 1.21 ± 1.38 and 1.44 ± 0.36 nmol adducts/mol nucleotides respectively, compared to the reference area which had the adduct levels of 0.51 ± 0.19 (cod) and 1.05 ± 0.46 (saithe) nmol adducts/mol nucleotides. All three species had higher number of individuals with detectable DNA adducts in the Tampen area, than in the Ling Bank area, indicating they are more affected by PAH exposure. The observed DNA adduct levels in the liver of fish from Tampen can be considered high when having in mind that the fish is caught in the open North Sea, especially the levels in haddock. The fact that the fish show elevated levels of DNA adducts is an abnormal condition, and confirms that the fish has been exposed to genotoxic pollutants beyond their DNA repair capacity and suggest PAH contamination in the area.

Introduction

In this study, aromatic hydrophobic PAH-DNA adducts were analysed in liver of three different feral fish species; cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and saithe (*Pollachius virens*), sampled in the Tampen area, and in the Ling Bank/Egersund Bank for reference. They were analysed with the ^{32}P post labelling assay, which is the most sensitive and frequently applied technique for detecting PAH-DNA adducts in marine organisms (Reichert *et al*., 1998). PAHs are readily taken up and metabolised by fish, and it is during the metabolic transformation of these compounds, that they are activated to become genotoxic. It is the enzymatic phase I of the biotransformation of PAHs, that leads to the formation of reactive electrophilic metabolites which can undergo attack and bind covalently to nucleophilic centres in large molecules such as lipids, proteins, DNA, and RNA, and form adducts. Factors that affect DNA adduct levels are exposure dose, the degree of bioactivation in phase I into reactive intermediates in relation to the phase II detoxification, DNA repair efficiency, as well as cell turnover. DNA adduct levels are thus a quantifiable measure of the biologically effective dose reaching a critical target site, and they integrate multiple toxicokinetic factors such as uptake, metabolism, detoxification, excretion and covalent binding of reactive metabolites to target tissues (Reichert *et al*., 1998). DNA adducts have shown to be predecessor of both mutagenic and carcinogenic effects, and they have shown to correlate with liver lesions in fish (Baumann, 1998; Reichert *et al*., 1998). They are also widely used as, and considered to be highly relevant biomarker for PAH exposure to fish (Varanasi, 1989).

The aim is to get better documentation to what extent discharges from petroleum activities at Tampen cause increased hepatic DNA-adduct levels in fish. This will be a direct follow-up of the 2002 study, which showed that haddock from Tampen contained high amounts of DNA-adducts in liver tissue. In addition to haddock, the study was extended to include cod and saithe. These two species can be seen as complementary to haddock and not so tightly linked to feeding on bottom dwelling organisms as haddock. Cod is an opportunistic feeder that lives on whatever prey organisms it can catch both at the seabed and in the water column, while saithe primarily feed on smaller fish and crustaceans in the water column.

Materials and Methods

Standard DNA (salmon sperm, D-1626), spermidin (S-2626), RNase A (R-4642), micrococcal endonuclease (N-3755) and spleen phosphodiesterase (P-9041) were obtained from Sigma Chemical Company, St. Louis, MO, USA. RNase T1 (109 193), proteinase K (1000144), α-amylase (102814), T4-polynucleotidekinase (3´-phosphatase free, 838 292) and phenol (1814303) were purchased from Roche Diagnostics, Scandinavia AB, Bromma, Sweden. Nuclease P1 (7160) was bought from Yamasa Corporation, Diagnostics Department, Chuo-Ku, Tokyo, Japan, and later Sigma-Aldrich Sweden AB, Stockholm, Sweden. Radiolabelled ATP ([γ-32P]ATP) with specific activity 3000 Ci/mmol (110 TBq/mmol) were obtained from Amersham Biosciences, Uppsala, Sweden. The benzo[a]pyrene standard adduct, 7R, 8S, 9S-trihydroxy, 10R-(N2-deoxyguanosyl-3´-phosphate)-7,8,9,10-tetrahydro-benzo(a)-pyrene (BaPDE-dG-3´p), was obtained from Midwest Research Institute, Kansas City, MO, USA. Cellulose (MN-301) was purchased from Machery-Nagel, Düren, Germany. Vinyl strips (PVC foil, 0.2 mm thickness), used for the groundwork of the polyethyleneimine cellulose sheets were obtained from Andren & Söner, Stockholm, Sweden. Scintillation fluid (Ultima gold) was purchased from CIAB, Lidingö, Sweden. All other solvents and chemicals for DNA purification and adduct analysis were purchased from common commercial sources and were of analytical purity.

DNA adduct analysis

Tissue samples were semi-thawed and the DNA extracted and purified according to Dunn *et al*., 1987; Reichert and French, 1994, slightly modified as described in Ericson and Balk, 2000. DNA adducts were enriched using the Nuclease P1 method, 0.41 μg Nuclease P1/μg DNA, and a 45 min incubation period (Reddy and Randerath, 1986; Beach and Gupta, 1992). The DNA adducts were radiolabelled using 5´-[γ-32P]triphosphate([γ-32P]ATP) and T4 polynucleotide kinase. Separation and cleanup of adducts was performed by a modified multidirectional thin-layer chromatography (TLC) on laboratory produced polyethyleneimine cellulose sheets that serve as anionic exchanger support. After elution, adducts were then located on the sheets and quantified by storage phosphor imaging technology (PhosphorImagerTMSI and ImageQuant 5.0). In addition, several quality control experiments were performed in parallel to the analysis of the various fish tissue samples.

Controls used during the analytical work were: a) Pure salmon sperm as negative control, b) the standard DNA adduct B[a]PDE-dG-3´p, and c) adducted liver tissue from B[a]P exposed perch. These were processed parallel to the samples and served as quality assurance for all the analytical steps in the 32P-postlabeling method. These quality assurance experiments confirm a faultless assay for the DNA adduct measurements performed in this study.

DNA for adduct analysis was quantified on the basis of its absorption at 260 nm in a GeneQuant spectrophotometer from Pharmacia Biotech, Uppsala, Sweden. Liquid scintillation spectroscopy was performed in a Packard Tri-Carb 2100TR liquid scintillation

counter from Packard Instrument Company. A Desaga spreader from Desaga Heidelberg, Germany, was used to prepare the TLC-sheets. The DNA adducts were located and the levels quantified on the TLC sheets with ImageQuant, 5.0 software, Molecular Dynamics, by the storage phosphor imaging technique using a PhosphorImager™ SI instrument (Sunnyvale, CA, USA), essential according to methodology described by Reichert *et al*. (1998).

Results and Discussion

25 individuals of each species from each area were analysed for DNA adduct levels, except for cod. Only 8 cods were caught in the Ling Bank/Egersund Bank area, additional 13 were caught in Egersund, and these individuals taken together were used as a reference group.

The results revealed that significant higher levels of DNA adducts were found in liver of haddock, with 4.42 ± 1.80 nmol adducts/mol normal nucleotides (average $\pm$ 95% confidence level) in fish from Tampen, and 2.14 ± 0.57 nmol adducts/mol normal nucleotides in fish from the reference area Ling Bank (Mann-Whitney test). Haddock caught at Tampen were slightly larger than haddock from Egersund Bank/Ling Bank (Table 2) and analyses of otoliths demonstrated haddock caught at Egersund Bank/Ling Bank to be $5,2\pm1.5$ years and haddock caught at Tampen were $5,8\pm0.8$ years.

Cod and saithe from Tampen had 1.21 ± 1.38 and 1.44 ± 0.36 nmol adducts/mol nucleotides respectively, compared to the reference area which had the adduct levels of 0.51 ± 0.19 and 1.05 ± 0.46 nmol adducts/mol nucleotides in cod and saithe respectively (average $\pm$ 95% confidence level). Average DNA adduct values and 95% confidence levels are presented in Figure 2, individual values in Figure 3, and raw data can be seen in appendix A. That haddock shows higher DNA adduct levels than cod is in agreement with previous investigations from this area (Klungsøyr *et al*., 2003; Balk *et al*., manuscript). Table 6 shows number of individuals of each species that had DNA adducts. It shows that all three species had higher number of individuals with DNA adducts in the Tampen area, than in the Ling Bank/Egersund Bank area, indicating they are more affected by PAH exposure. It also shows that the haddock was more affected than cod or saithe, with as many as 21 individuals (84%) from the Tampen area with DNA adducts, and 19 (76%) from Ling Bank/Egersund Bank. Cod from the Tampen area had 7 individuals (28%) showing DNA adducts compared to 2 (10%) from the Ling bank/Egersund Bank, and saithe 16 (64%) with DNA adducts from Tampen, and 6 (24%) from Ling bank/Egersund Bank. The Ling Bank/Egersund Bank area, however, shows relatively high number of individuals with DNA adducts, especially haddocks, considering it is the reference area. No statistical differences were found between the two areas for cod and saithe. Figure 4 shows representative autoradiograms of the DNA adducts. It shows the typical diagonal radioactive zone (DRZ) pattern of PAH-DNA adducts.

The observed DNA adduct levels in the liver of fish from Tampen can be considered high when having in mind that the fish is caught in the open North Sea, especially the levels in haddock. The fact that the fish show elevated levels of DNA adducts at all is an abnormal condition, and confirms that the fish has been exposed to genotoxic pollutants beyond their DNA repair capacity and strongly suggest PAH contamination in the area. Few studies on DNA adduct levels in fish from the North Sea or neighbouring areas, or even from open seas in general have been published. But for comparison, Aas *et al*. (2003) studied DNA adduct levels in 11 fish species from the open seas of the NE Atlantic. That study showed undetectable levels of DNA adducts in the fish, or levels just above the detection limits. Haddock was not included in that study.

There is somewhat large differences between DNA adduct levels in the different fish species if we assume similar exposure period. Reasons for that could have to do with species specific PAH metabolism, since it is well-known that there are differences between fish species in their responses to PAHs (Aas *et al.*, 2001; Eggens *et al.*, 1998). Additional factors are also different distribution of PAHs in the respectively species habitat and diets.

Unknown DNA adduct in haddock

A distinctive adduct spot on the autoradiograms was found in haddock liver, both from the Tampen oil field, as well as from the reference area Ling bank. This spot is not situated on the typical PAH-adduct diagonal radioactive zone (DRZ) but closer to the right edge of the autoradiograms (shown in Figure 4). This is in accordance with our previous findings from haddock from different areas, including both assumed contaminated and less contaminated areas, which frequently show this corresponding type of spot. This spot represents what is believed at present knowledge, to be an endogenous adduct, and not a PAH-DNA adduct. This spot does not correlate with the levels of other adducts in the same tissue, as is typical for PAH adducts, and is therefore not believed to be caused by anthropogenic PAH exposure. This spot is therefore not included in the calculation of DNA adduct levels. On the pictures showing the autoradiograms, this particular spot is outlined in white. Endogenous DNA adducts, believed to be formed from endogenous compounds such as steroids (Randerath & Randerath 1993), have previously been reported in mammals, but not in teleost fish species. A recent study on DNA adduct levels in 11 fish species (haddock not included) from areas in the northern Atlantic gave no evidence of such natural or endogenous DNA adducts in any of the species studied (Aas *et al.* 2003). This adduct is scientifically interesting, but not of obvious importance here since it is not believed to be caused by anthropogenic PAH exposure.
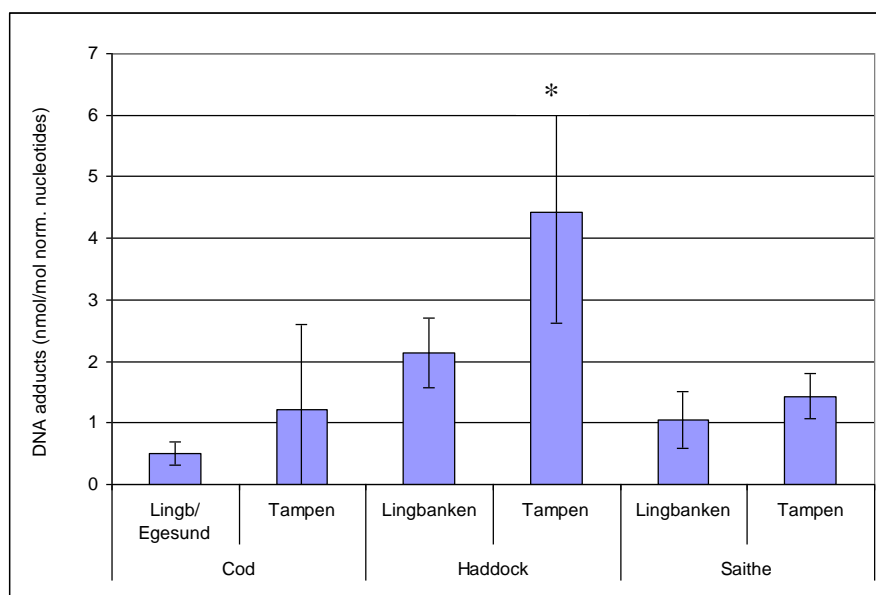

Figure 2. DNA adduct levels (nmol add/mol normal nucleotides) in liver of cod, haddock and saithe from Tampen and the reference area Ling Bank/Egersund Bank. Average $\pm$ 95% confidence level, n= 25 for all staples, except for cod from Ling Bank/Egersund Bank, where n=21. (*) indicates significant changes between Tampen and Ling Bank, $p < 0.05$.

Table 6. Number of individuals with DNA adducts. Others had DNA adducts levels below the detection limits.

	Cod		Haddock		Saithe	
	No. of analysed individuals	No.of individuals with DNA adducts	No. of analysed individuals	No.of individuals with DNA adducts	No. of analysed individuals	No.of individuals with DNA adducts
Tampen	25	7	25	21	25	16
Ling Bank/ Egersund Bank	21	2	25	19	25	6

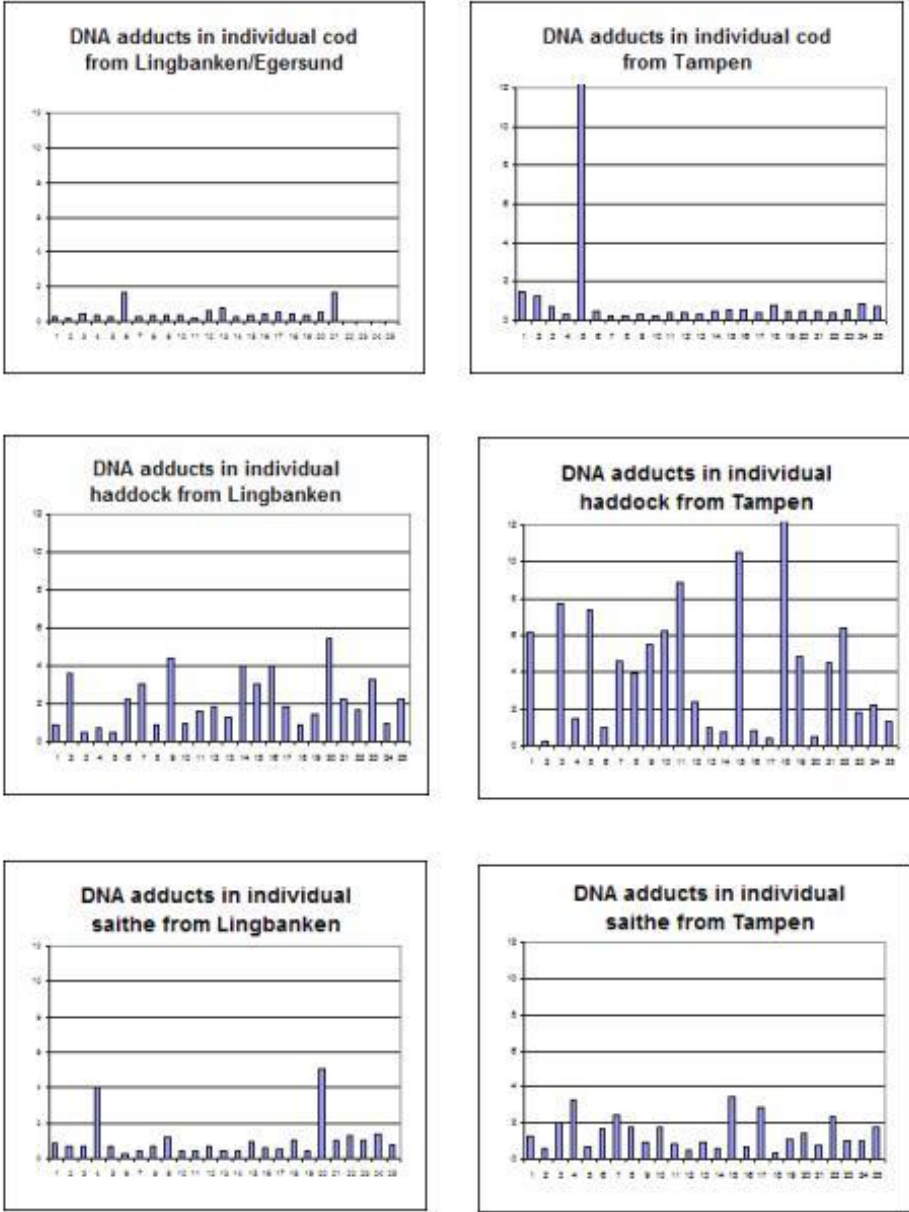**Figure 3.** DNA adducts (nmol/mol normal nucleotides) in liver of individual fish from Tampen and Ling Bank/ Egersund Bank.

Cod

Ling bank

Egersund Bank

Egersund Bank

3   ≤ 0.388 nmol

18   ≤ 0.405 nmol

21   ≤ 1.06 nmol

Tampen
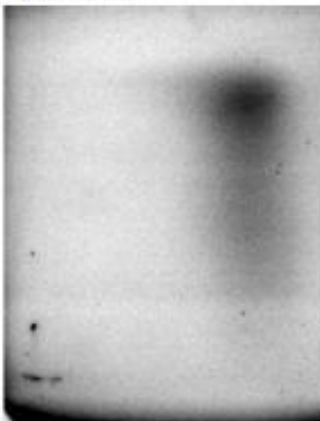
Tampen

Tampen

6   21.7 nmol (runned twice)

12   ≤ 0.853 nmol

14   ≤ 0.607 nmol

Saithe

Ling Bank
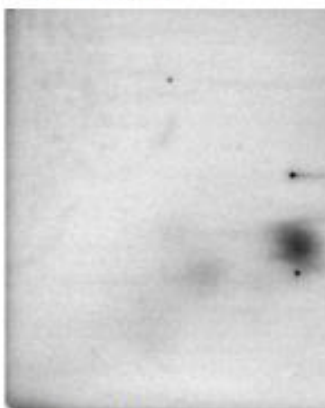
Ling Bank

Ling Bank

4   4.02 nmol
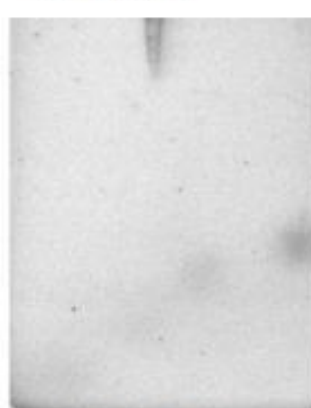
5   0.705 nmol
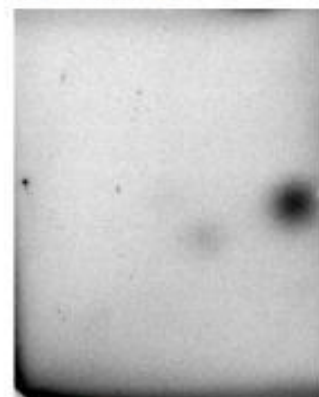
20   5.08 nmol

Saithe cont.

Tampen   Tampen   Tampen
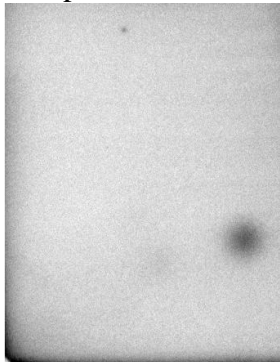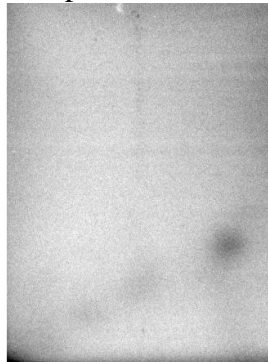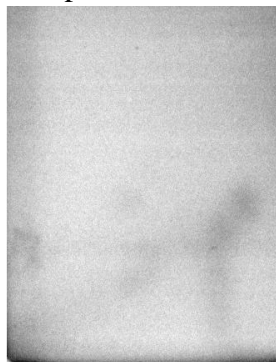
3 1.98 nmol   8 1.73 nmol   17 2.85 nmol

Haddock

Ling Bank/Egersund Bank Ling Bank/Egersund Bank Ling Bank/Egersund Bank
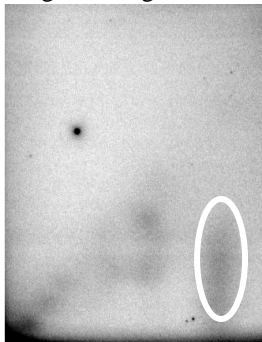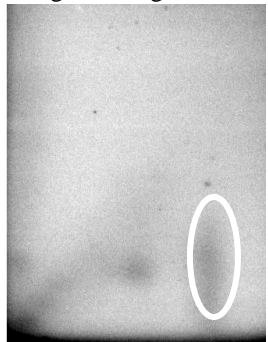
2 3.61 nmol (2.90)   7 3.06 nmol (2.21)   21 2.26 nmol
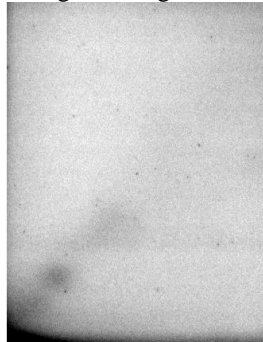
Tampen   Tampen   Tampen

3 7.76nmol   (4.24)   7 4.57nmol   (2.86)   11 8.85nmol (2.18)

Tampen   Tampen   Tampen

15 10.5nmol   (1.70) 18 19.8nmol   (2.00) 19 4.87nmol
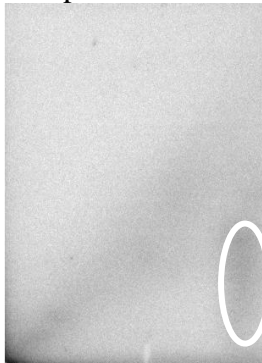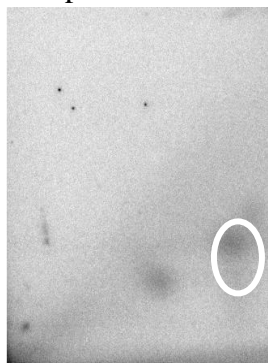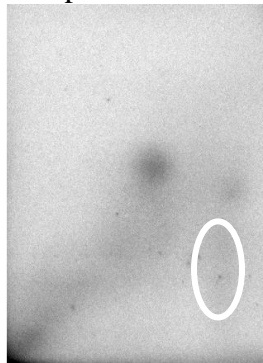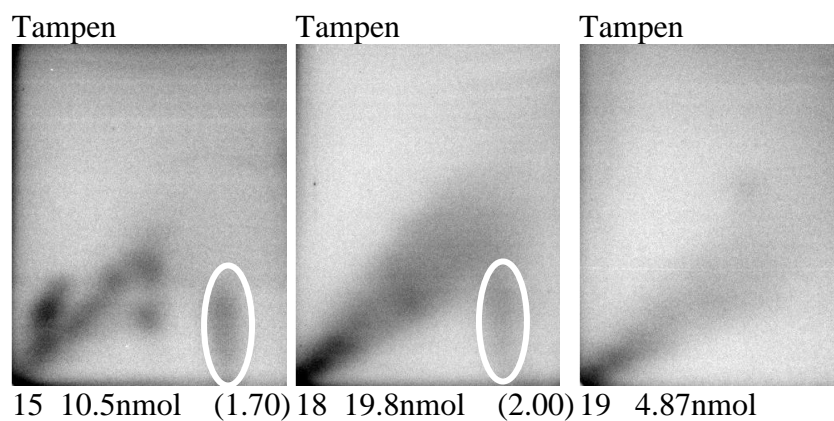
Figure 4. Representative autoradiogram and DNA adduct levels in liver samples of cod, saithe and haddock from Tampen area and the reference area Ling bank/Egersund Bank. Numbers under the autoradiograms represent sample number (fish), DNA adducts (nmol add/mol normal nucleotides), and for haddock: numbers within parenthesis represent what is believed at present knowledge, to be an endogen adduct. That is, an adduct that could *not* be associated with anthropogenic release of PAHs. White circles on the autoradiograms indicate that particular DNA adduct.

Work package 5 - Estrogenic effects in fish due to exposure to alkylphenols.

Experimental studies in the laboratory have shown that alkylphenols in produced water can cause estrogenic effects in cod affecting reproduction. There is still very limited knowledge of alkylphenols (AP) in fish from the North Sea, but modelling of a probable accumulation in pelagic fish living near oil production platforms suggests that levels are very low (Myhre *et al*., 2004). However, it is important to try to verify the results from such risk assessments. In this study APs in liver tissue is analysed as well as vitellogenin in blood plasma as a biomarker of estrogenic effects in fish from Tampen and the Ling Bank.

Vitellogenin (VTG) is a glycophospholipoprotein and the main source of yolk proteins and lipids in the growing oocyte. VTG is synthesized in the liver in response to estrogen. Even though VTG is a protein specific to female fish, males also possess all of the genetic system needed for VTG protein synthesis. A rise in the level of VTG is commonly used as a biomarker for estrogenic effects in vertebrates.

Material and methods
Alkylphenols in cod and haddock liver and herring muscle were analysed by gas chromatography-mass spectrometry as pentafluorobenzoate derivatives (Meier *et al*., 2005). 90 samples were analyzed for 47 individually alkylphenols form phenol to nonylphenol (C9), together with a cluster analysis of "technical" nonylphenol (19 isomers).

There were problems with background contamination of APs, especially from "technical" nonylphenol in the procedure blanks. It seems that some APs are widely spread in most indoor environments (Rudel *et al*., 2003) and phenol and some *para*-substituted APs are intensively used in plastics industry (Cascaval *et al*., 1996). However, despite a significant effort to avoid these problems, we still detected small amounts of some APs in the blank samples. It is therefore important to have a good and intensive control of procedural blanks. The trace amounts of APs in blank samples increase the risk of false positive results and the levels of contaminants are determinate the detection limit. We have in this investigation also analyzed 21 procedural blanks. Levels of detection (LOD) is defined as LOD: $Y = Y_B + 3SD_B$, where Y_B is the response of blank sample signal and SD_B is the standard deviation of the blank samples.

Cod VTG was analysed in plasma of female and male cod by a quantitative enzyme-linked immunosorbent assay (ELISA) (Biosense Laboratories, Bergen, Norway) according to Scott *et al*. (2006a).

Results and Discussion
Analyses of alkylphenol of cod liver, haddock liver and herring muscle from Ling Bank and Tampen regions demonstrated mostly levels below levels of detection (LOD) for all stations (Table 6). The absence of alkylphenol in fish is in line with the results from the 2002 monitoring (Klungsøyr *et al*., 2003).

Table 6. Limits of detection (LOD) and results with alkyl phenol analyses of cod liver, and haddock and herring muscle from Ling Bank and Tampen. Results presented as mean in µg/kg.

	Liver LOD (µg/kg)	Cod liver Ling bank (n=15)	Tampen (n=15)	Haddock liver Ling bank (n=15)	Tampen (n=15)	Muscle LOD (µg/kg)	Herring muscle Ling bank (n=17)	Tampen (n=15)
Phenol	1056	332 ± 225	239 ± 193	505 ± 74	479 ± 75	528	31 ± 10	34 ± 10
C1-phenol								
o-Cresol	45	37 ± 10	38 ± 11	36 ± 15	39 ± 11	22	9 ± 1	9 ± 2
m-Cresol	15	11 ± 3	9 ± 3	12 ± 4	11 ± 3	7	2 ± 0	2 ± 0
p-Cresol	16	21 ± 7	18 ± 6	20 ± 9	19 ± 6	8	4 ± 0	4 ± 1
C2-phenol								
2-Etylphenol	0	0,1 ± 0,1	0,1 ± 0,1	0,1 ± 0,0	0,1 ± 0,1	0	0,1 ± 0,1	0,1 ± 0,1
2,6-dimetylphenol	0	0,1 ± 0,3	0,0 ± 0,0	0,1 ± 0,1	0,0 ± 0,0	0	0,2 ± 0,3	0,1 ± 0,1
2,5-Dimetylphenol	0	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,1	0,1 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
2,4-Dimetylphenol	0	0,2 ± 0,0	0,2 ± 0,0	0,1 ± 0,0	0,2 ± 0,0	0	0,0 ± 0,0	0,1 ± 0,0
3-Ethylphenol	0	0,2 ± 0,1	0,1 ± 0,0	0,2 ± 0,0	0,1 ± 0,0	0	0,1 ± 0,0	0,1 ± 0,0
3,5-Dimetylphenol	2	1,0 ± 0,8	0,7 ± 0,3	1,0 ± 0,4	0,8 ± 0,2	1	0,1 ± 0,0	0,2 ± 0,1
4-Etylphenol	0	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,1	0,1 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
2,3-Dimetylphenol	0	0,1 ± 0,1	0,1 ± 0,1	0,2 ± 0,2	0,1 ± 0,1	0	0,1 ± 0,0	0,1 ± 0,0
3,4-Dimetylphenol	0	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
C3-phenol								
2-iso-Propylphenol	1	0,5 ± 0,2	0,5 ± 0,1	0,6 ± 0,2	0,5 ± 0,1	1	0,2 ± 0,1	0,2 ± 0,1
2-n-Propylphenol	0	0,1 ± 0,2	0,0 ± 0,0	0,1 ± 0,1	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
3-iso-Propylphenol	1	0,4 ± 0,2	0,4 ± 0,1	0,5 ± 0,1	0,4 ± 0,1	0	0,2 ± 0,1	0,2 ± 0,1
2,4,6-Trimetylphenol	0	0,0 ± 0,1	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
4-iso-Propylphenol	1	0,9 ± 0,3	0,8 ± 0,2	1,2 ± 0,2	0,9 ± 0,3	1	0,3 ± 0,1	0,4 ± 0,2
3-n-Propylphenol	0	0,2 ± 0,2	0,2 ± 0,1	0,1 ± 0,1	0,1 ± 0,1	0	0,1 ± 0,0	0,1 ± 0,0
3-Etyl-4-Metylphenol	0	0,2 ± 0,2	0,2 ± 0,1	0,1 ± 0,1	0,1 ± 0,1	0	0,0 ± 0,0	0,1 ± 0,0
2,3,6-Trimetylphenol	0	0,1 ± 0,1	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,0	0	0,1 ± 0,1	0,0 ± 0,1
2,3,5 Trimetylphenol	0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
4-n-Propylphenol	0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
C4-phenol								
2-Tert-butylphenol	0	0,1 ± 0,1	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,0	0	0,1 ± 0,1	0,0 ± 0,0
5-Methyl-4-isopropylphenol	6	0,2 ± 0,1	0,2 ± 0,2	0,1 ± 0,2	0,2 ± 0,2	3	0,5 ± 0,5	0,4 ± 0,4
3-Tert-Butylphenol	0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
5-iso-Propyl-3-Metylphenol	0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
4-Tert-butylphenol	2	1,0 ± 0,4	0,6 ± 0,1	0,9 ± 0,2	0,5 ± 0,1	1	0,6 ± 0,5	0,5 ± 0,2
4-Sec-butylphenol	0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
4-Iso-propyl-3-Metylphenol	0	0,1 ± 0,1	0,1 ± 0,0	0,1 ± 0,1	0,0 ± 0,1	0	0,0 ± 0,0	0,0 ± 0,0
4-n-Butylphenol	0	0,2 ± 0,1	0,2 ± 0,0	0,3 ± 0,1	0,2 ± 0,1	0	0,1 ± 0,0	0,1 ± 0,0
2,3,5,6-Tetramethylphenol	0	0,1 ± 0,0	0,0 ± 0,0	0,1 ± 0,1	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
C5-phenol								
2-tert-Butyl-4-metylphenol	50	28 ± 29	8 ± 4	15 ± 10	10 ± 4	25	7 ± 10	5 ± 12
2-tert-Butyl-5-metylphenol	64	36 ± 37	11 ± 6	18 ± 13	12 ± 6	32	9 ± 13	6 ± 15
2-tert-Butyl-6-methylphenol	3	0,3 ± 0,7	0,1 ± 0,1	0,1 ± 0,1	0,1 ± 0,1	1	0,1 ± 0,2	0,3 ± 1,2
4-(1,1-Dimetylpropyl)phenol	0	0,1 ± 0,2	0,0 ± 0,0	0,0 ± 0,1	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
4-n-Pentylphenol	0	0,2 ± 0,3	0,1 ± 0,1	0,3 ± 0,1	0,3 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
C6-phenol								
2,6-Diisopropylphenol	0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
4-(1,1-Dimethylbutyl)phenol	247	57 ± 39	88 ± 138	49 ± 15	45 ± 12	123	23 ± 22	28 ± 16
4-(1-Methyl-2,2-dimethylpropyl)Phenol	30	8 ± 7	6 ± 4	17 ± 13	7 ± 6	15	23 ± 39	20 ± 20
4-n-Heksylphenol	10	3 ± 2	2 ± 1	4 ± 2	3 ± 1	5	4 ± 6	5 ± 5
C7-phenol								
4-(1,1-Dimethylpentyl)phenol	0	0,1 ± 0,3	0,2 ± 0,4	0,0 ± 0,0	0,2 ± 0,5	0	0,0 ± 0,1	0,0 ± 0,0
4-n-Heptylphenol	0	0,2 ± 0,1	0,1 ± 0,1	0,1 ± 0,1	0,2 ± 0,1	0	0,0 ± 0,0	0,0 ± 0,0
C8-phenol								
4-tert-Octylphenol	16	8 ± 9	7 ± 7	14 ± 8	5 ± 11	8	1 ± 4	1 ± 4
4-n-Octylphenol	129	29 ± 21	30 ± 18	34 ± 33	37 ± 27	65	42 ± 9	36 ± 11
C9-phenol								
Tec. NP (sum av 19 isomerer)	78	67 ± 33	56 ± 8	80 ± 19	61 ± 14	39	25 ± 20	26 ± 20
2-Metyl4-tert-Octylphenol	0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0	0,0 ± 0,0	0,0 ± 0,0
4-n-Nonylphenol	3	1,9 ± 2,3	2,9 ± 3,2	1,9 ± 3,4	1,2 ± 2,4	2	0,3 ± 0,6	0,3 ± 0,3

There were large difference in body size and sexual maturation between the cod from Ling Bank and Tampen. The fish from Tampen were generally larger and more mature (Table 2). This has great influence on the VTG data from the female fish, VTG is a yolk protein and the plasma concentrations therefore followed the gonad development closely (Figure 5). There were no differences in VTG concentration between the two stations that could not be explained through the differences in size and sexually maturation (Table 7).

The male cod showed the same difference in body size and maturation between the two stations as the females (Table 8). There were one male fish from Ling bank that clearly had induced VTG levels, with 266 µg VTG /ml plasma is this close to what one finds in maturing female cod. In addition to this did 3 male cod from ling bank and 3 male cod from Tampen have lightly induced VTG levels (0.5-5 µg/ml). This limited dataset shows that about 30 % of the male cod have induced VTG concentrations, and a correlation between VTG concentration and body size. However, we did not find any difference between VTG levels in male cod from Tampen compared with Ling Bank (Figure 6).

The results agree well with a recently publish article that also have found a size dependent induction of VTG in male cod from the North Sea (Scott *et al.*, 2006b). The authors find VTG concentration up to 160 µg/ml in male cod, but from the open sea only in fish over 5 kg. However, in the Oslo Fjord, also many smaller male cod had induced VTG levels (Scott *et al.*, 2006b). Water column monitoring (in 2001) around oil rigs have found that cod caged closest to the platform (500 m distance) have significant, but marginal elevation of VTG (Scott, *et al.*, 2006a). However, similar studies (2003 and 2004) did not find any differences between cod caged in differences distance from oil installations (OLF, 2005).

Table 7. Body weight, gonad weight, gonado somatic index (GSI) and plasma VTG levels in female cod from Ling Bank and Tampen.

	Weight (g)	Gonad (g)	GSI	VTG (ng/ml)		Weight (g)	Gonad (g)	GSI	VTG (ng/ml)
Ling bank	615	2	0,3	18	Tampen	1330	3	0,20	8
Ling bank	785	3	0,4	4	Tampen	422	1	0,24	12
Ling bank	1160	4	0,3	3	Tampen	2720	7	0,24	14
Ling bank	304	1	0,2	7	Tampen	2400	8	0,33	56
Ling bank	682	2	0,2	19	Tampen	3780	28	0,75	4246
Ling bank	1160	4	0,3	17	Tampen	5060	45	0,90	101907
Ling bank	1390	4	0,3	7	Tampen	4160	42	1,02	241635
Ling bank	1200	5	0,4	45	Tampen	8200	137	1,70	250000
Ling bank	815	4	0,4	25	Tampen	5760	109	1,93	205996
Ling bank	1420	6	0,5	11	Tampen	7260	141	1,98	1449085
Ling bank	3300	17	0,5	34	Tampen	8720	173	2,02	1677220
Ling bank	733	4	0,6	16	Tampen	9640	275	2,94	4251501
Ling bank	8882	433	5,1	7078455	Tampen	5480	174	3,28	8135425
-					Tampen	7340	306	4,35	5801759
-					Tampen	9060	392	4,52	5306095
-					Tampen	6560	417	6,79	5651401
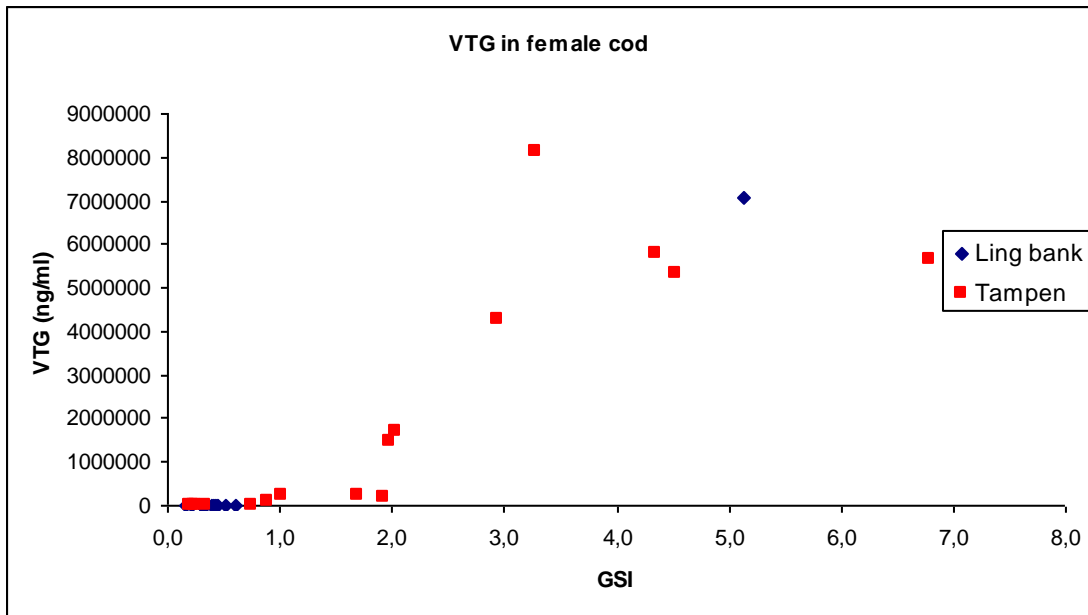
Figure 5. Levels of vitellogenin in plasma of female cod plotted against gonado somatic index (GSI).

Table 8. Body weight, gonad weight, gonado somatic index (GSI) and plasma VTG levels in male cod from Ling Bank and Tampen.

	Weight (g)	Gonad (g)	GSI	VTG (ng/ml)		Weight (g)	Gonad (g)	GSI	VTG (ng/ml)
Ling bank	357	<0,5	-	3	Tampen	1200	1	0,08	8
Ling bank	400	<0,5	-	5	Tampen	1550	2	0,10	8
Ling bank	509	1	0,20	19	Tampen	2000	1	0,05	46
Ling bank	736	4	0,55	5	Tampen	2140	1	0,05	26
Ling bank	1005	1	0,05	6	Tampen	4880	275	5,97	24
Ling bank	1085	<0,5	-	546	Tampen	4900	74	1,53	99
Ling bank	1270	16	1,28	9	Tampen	5260	61	1,17	1684
Ling bank	1420	1	0,04	21	Tampen	6000	190	3,27	575
Ling bank	1430	2	0,14	20	Tampen	6920	51	0,74	6
Ling bank	1460	1	0,03	7	Tampen	8280	192	2,37	4732
Ling bank	1600	1	0,03	8	-				
Ling bank	1710	1	0,06	1221	-				
Ling bank	2700	22	0,82	3971	-				
Ling bank	8740	243	2,86	266334	-				
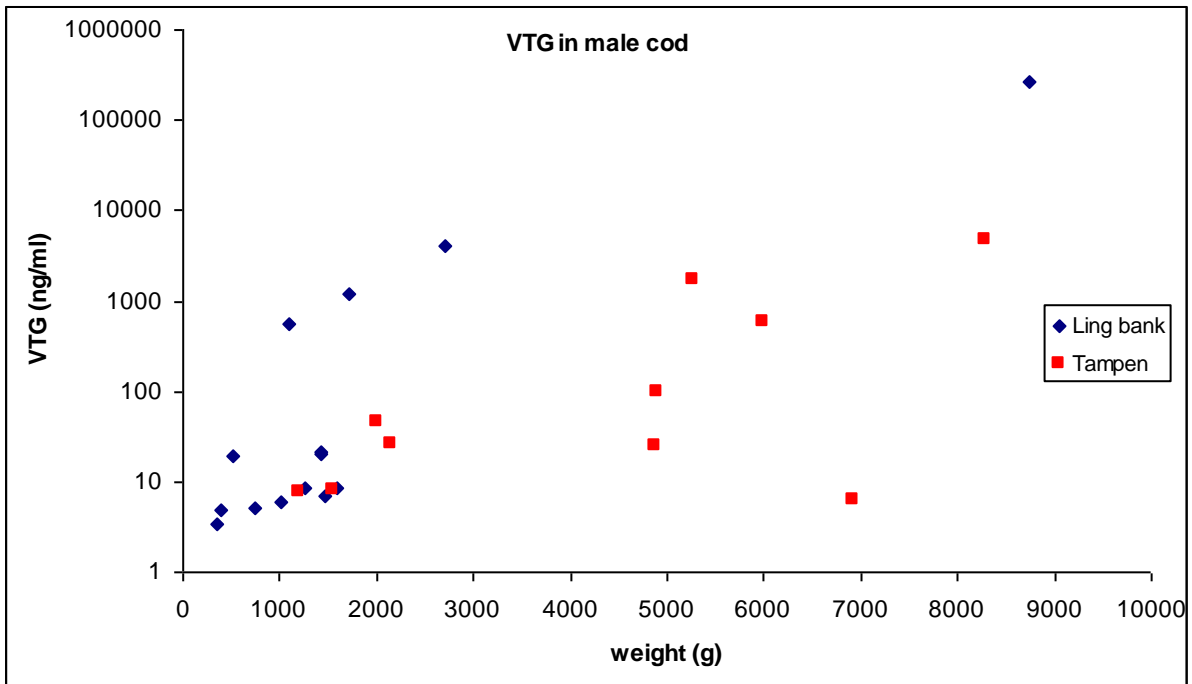
Figure 6. Concentration of vitellogenin in plasma of male cod plotted against body weight (logarithmic scale).

References

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

Aas E, J Beyer, G Jonsson, WL Reichert, OK Andersen. 2001. Evidence of uptake, biotransformation and DNA binding of polyaromatic hydrocarbons in Atlantic cod and corkwing wrasse caught in the vicinity of an aluminium works. Mar. Env. Res. 52:213-229.

Aas E, G Jonsson, RC Sundt, S Westerlund, S Sanni. 2006. Monitoring of PAH metabolites and metals in bile from caged cod Gadus morhua) and wild pelagic fish along expected contaminant gradients in the North Sea. In: Biological effects of contaminants in pelagic ecosystems. K Hylland, AD Vethaak, T Lang (Eds). SETAC Books. p 263-276.

Aas E, B Liewenborg, BE Grøsvik, L Camus, G Jonsson, JF Børseth, L Balk. 2003. DNA adduct levels in fish from pristine areas are not detectable or low when analysed using the nuclease P1 version of the P^{32}-postlabelling technique. Biomarkers 8(6): 445-460.

Baumann PC. 1998. Epizootics of cancer in fish associated with genotoxins in sediment and water. Mutat. Res. 411:277-233.

Beach AC, RC Gupta. 1992. Human biomonitoring and the ^{32}P postlabeling assay. Carcinogenesis 13:1053-1074.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Biochem. 72: 248-254.

Cascaval C N, Rosu D, Agherghinei I. 1996. The thermal behaviour of some epoxy-acrylic polymers based on phenol and para-alkyl substituted phenols. Polymer Degradation and Stability. 52:253-257.

Dunn PB, JJ Black, A Maccubbin. 1987. ^{32}P-Postlabelling analysis of aromatic DNA adducts in fish from polluted areas. Cancer Res. 47:6543-6548.

Eggens ML, AD Vethaak, MJ Leaver, GJMJ Horbach, JP Boon, W Seinen. 1996. Differences in CYP1A response between flounder (Platichthys flesus) and plaice (Pleuronectes platessa) after long-term exposure to harbour dredged spoil in a mesocosm study. Chemosphere. 32: 1357-1380.

Ericson G, L Balk. 2000. DNA adduct formation in northern pike (*Esox lucius*) exposed to a mixture of benzo[a]pyrene. benzo[k]fluoranthene and 7H-dibenzo[c.g]carbazole: time-course and dose response studies. Mutat. Res. 454:11-20.

Hurk PVD. 2006. Bile fluorescence, heme oxygenase induction, and increased biliverdin excretion by mixtures of environmental toxicants. Aquatic Toxicology 77:202-209.

Klungsøyr J, L Balk, MHG Berntssen, J Beyer, AG Melbye, K Hylland. 2003 NFR project No. 152231/720 – Contamination of fish in the North Sea by the offshore oil and gas industry. Summary report to NFR. 30 pp.

Klungsøyr J, S Johnsen. 1997. Oil hydrocarbons in fish from Norwegian waters 1993-95. Fisken og Havet No. 17 – 1997.

Klungsøyr J, G Tveit, K Westrheim. 2001. Tilstandsovervåkning 2000-2001: oljehydrokarboner i hyse (Melanogrammus aeglefinus). Technical Report. Institute of Marine Research. Bergen. Norway.

Klungsøyr J, S Wilhelmsen, K Westrheim, E Sætvedt and KH Palmork (1988). The GEEP Workshop: Organic chemical analyses. Mar. Ecol. Progr. Series 46: 19-26.

Meier S, J Klungsoyr, S Boitsov, T Eide, A Svardal 2005. Gas chromatography-mass spectrometry analysis of alkylphenols in cod (Gadus morhua) tissues as pentafluorobenzoate derivatives. Journal of Chromatography A. 1062:255-268.

Myhre LP, T Baussant, RC Sundt, S Sanni, R. Vabø, HR Skjoldal, J Klungsøyr. 2004. Risk assessment of reproductive effects of alkyl phenols in produced water in the North Sea. Report AM-2004/018. pp 76.

OLF The Norwegian Oil Industry Association. 2005. Water column monitoring, Summary report 2005. pp 47.

Randerath E, K Randerath. 1993. Monitoring tobacco smoke-induced DNA damage by ^{32}P-postlabelling. In: Postlabelling Methods for Detection of DNA adducts. IARC Scientific Publication No. 24. International Agency for Research on Cancer. (eds. DH Phillips, M Castegnaro & H Bartsch) Lyon. France. pp. 305-314.

Reddy MV, K Randerath. 1986. Nuclease P1-mediated enhancement of sensitivity of ^{32}P-postlabeling test for structurally diverse DNA adducts. Carcinogenesis 7:1543-1551.

Reichert WL, B French. The ^{32}P-postlabeling protocols for assaying levels of hydrophobic DNA adducts in fish. 1994. NOAA Tech. Memo. NMFS-NWFSC-14. National Technical Information Service. Springfield. VA.

Reichert WL, MS Myers, K Peck-Miller, BF French, BF Anulacion. 1998. Molecular epizootiology of genotoxic events in marine fish: Linking contaminant exposure. DNA damage and tissue level alterations. Mutat. Res. 411:215-225.

Rudel R A, Camann D E, Spengler J D, Korn L R, Brody J G. 2003. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Env. Sci & Technol. 37:4543-4553.

Scott AP, SI Kristiansen, I Katsiadaki, J Thain, KE Tollefsen, A Goksøyr, J Barry. (2006a). Assessment of Oestrogen Exposure in Cod (*Gadus morhua*) and Saithe (*Pollachius Virens*) in Relation to their Proximity to an Oilfield. In: Biological effects of contaminants in pelagic ecosystems. K Hylland, AD Vethaak, T Lang (Eds). SETAC Books. p. 329-339.

Scott AP, I Katsiadaki, PR Witthames, K Hylland, IM Davies, AD McIntosh, J. Thain. (2006b) Vitellogenin in the blood plasma of male cod (Gadus morhua): A sign of oestrogenic endocrine disruption in the open sea? Mar. Env. Res. 61:149-170.

Varanasi U. 1989. Metabolism of polycyclic aromatic hycrocarbons in the aquatic environment. CRC press. Boca Raton. Florida. USA. 341 pp.

Fish from Ling Bank and Egersund Bank, 2005
DNA adducts (nmol adducts/mol normal nucleotides)

Cod

	sample no	Cod	add	add+0,5*
Ling Bank	2	06326	≤0,594	0,297
	3	06327	≤0,388	0,194
	4	06328	≤0,820	0,410
	5	06329	≤0,742	0,371
	6	06330	≤0,550	0,275
	7	06331	1,63	1,63
	8	06332	≤0,571	0,286
	9	06333	≤0,723	0,362
Average				0,478
Stdev				0,470
Confidence level(95,0%)				0,393
number				8
Egersund	10	06359	≤0,648	0,324
	12	06360	≤0,681	0,341
	15	06361	≤0,418	0,209
	16	06362	0,630	0,630
	17	06363	≤1,66	0,830
	18	06364	≤0,450	0,225
	19	06365	≤0,680	0,340
	20	06366	≤0,829	0,415
	21	06367	≤1,06	0,530
	22	06368	≤0,816	0,408
	23	06369	≤0,761	0,381
	24	06370	≤1,02	0,510
	25	06371	≤3,33	1,67
Average				0,524
Stdev				0,383
Confidence level(95,0%)				0,231
number				13
Average				0,507
Stdev				0,407
Confidence level(95,0%)				0,185
number				21

Haddock

sample no	Haddock	add	add+0,5*	endogen adduct**
1	06279	0,91	0,910	
2	06280	3,61	3,61	2,90
3	06281	≤0,926	0,463	
4	06282	≤1,37	0,685	
5	06283	≤0,909	0,455	
6	06284	2,21	2,21	
7	06285	3,06	3,06	2,21
8	06286	0,853	0,853	
9	06287	4,42	4,42	
10	06288	0,980	0,980	
11	06289	1,59	1,59	1,61
12	06290	1,88	1,88	2,01
13	06291	≤2,58	1,29	
14	06292	4	4,00	0,960
15	06293	3,02	3,02	
16	06294	3,97	3,97	
17	06295	1,88	1,88	
18	06296	≤1,81	0,905	
19	06297	1,41	1,41	
20	06298	5,48	5,48	
21	06299	2,26	2,26	
22	06300	1,69	1,69	
23	06301	3,27	3,27	
24	06302	≤1,99	0,995	
25	06303	2,20	2,20	
Average			2,14	
Stdev			1,37	
Confidence level(95,0%)			0,567	
number			25	

Saithe

sample no	Saithe	add	add+0,5*
1	06334	≤1,64	0,820
2	06335	≤1,39	0,695
3	06336	≤1,35	0,675
4	06337	4,02	4,02
5	06338	0,705	0,705
6	06339	≤0,519	0,260
7	06340	≤0,883	0,442
8	06341	≤1,43	0,715
9	06342	1,23	1,23
10	06343	0,427	0,427
11	06344	≤0,858	0,429
12	06345	0,687	0,687
13	06346	≤0,862	0,431
14	06347	≤0,874	0,437
15	06348	≤1,89	0,945
16	06349	≤1,23	0,615
17	06350	≤0,976	0,488
18	06351	≤2,07	1,04
19	06352	≤0,900	0,450
20	06353	5,08	5,08
21	06354	≤2,08	1,04
22	06355	≤2,60	1,30
23	06356	≤2,08	1,04
24	06357	≤2,73	1,37
25	06358	≤1,60	0,800
Average			1,05
Stdev			1,11
Confidence level(95,0%)			0,457
number			25

Fish from Tampen, 2005
DNA adducts (nmol adducts/mol normal nucleotides)

sample no.	Cod	add	add+0,5*
2	OLF	1,49	1,49
3	OLF	1,21	1,21
4	OLFxtra	0,730	0,730
5	06220	≤0,608	0,304
6	06221	12,5 / 21,7	**17,2**
7	06222	0,457	0,457
8	OLF	≤0,470	0,235
9	OLF	≤0,538	0,269
10	OLF	≤0,555	0,278
11	OLF	0,266	0,266
12	06223	≤0,853	0,427
13	06224	≤0,828	0,414
14	06225	≤0,607	0,304
15	06226	0,451	0,451
16	06227	≤1,11	0,555
17	06228	≤1,05	0,525
18	06229	≤0,809	0,405
19	06230	≤1,60	0,800
20	06231	≤1,01	0,505
21	06232	≤0,961	0,481
22	06233	≤0,891	0,446
23	06234	≤0,842	0,421
24	06235	≤1,11	0,555
25	06236	≤1,67	0,835
26	06237	≤1,47	0,735
Average		**1,21**	
Stdev		**3,34**	
Confidence level(95,0%)		**1,38**	
number		**25**	

sample no.	Haddock	add	add+0,5*	endogen adduct**
1	OLF	6,18	6,18	1,37
2	06238	≤0,568	0,284	
3	OLF	7,76	7,76	4,24
4	06239	≤2,92	1,46	
5	OLFxtra	7,41	7,41	2,41
6	06240	≤1,98	0,99	
7	OLF	4,57	4,57	2,87
8	OLFxtra	3,91	3,91	
9	OLF	5,52	5,52	1,61
10	OLF	6,23	6,23	0,900
11	OLF	8,85	8,85	2,15
12	OLFxtra	2,42	2,42	
13	06241	≤1,91	0,955	
14	06242	0,770	0,770	
15	06243	10,5	10,5	1,7
16	06244	0,852	0,852	
17	06245	0,743	0,372	
18	06246	19,8	19,8	2,00
19	06247	4,87	4,87	
20	06248	0,530	0,530	
21	06249	4,55	4,55	
22	06250	6,38	6,38	
23	06251	1,77	1,77	
24	06252	2,18	2,18	
25	06253	1,33	1,33	
Average		**4,42**		
Stdev		**4,37**		
Confidence level(95,0%)		**1,80**		
number		**25**		

sample no.	Saithe	add	add+0,5*
1	06254	1,27	1,27
2	06255	≤1,11	0,555
3	06256	1,98	1,98
4	06257	3,25	3,25
5	06258	0,679	0,679
6	06259	1,65	1,65
7	06260	2,45	2,45
8	06261	1,73	1,73
9	06262	0,938	0,938
10	06263	1,80	1,80
11	06264	0,824	0,824
12	06265	≤1,06	0,530
13	06266	≤1,90	0,950
14	06267	≤1,22	0,610
15	06268	3,41	3,41
16	06269	0,661	0,661
17	06270	2,85	2,85
18	06271	≤0,456 / ≤0,827	0,321
19	06272	≤1,98 / ≤2,48	1,12
20	06273	≤2,91	1,46
21	06274	≤1,57	0,785
22	06275	2,35	2,35
23	06276	≤2,00	1,00
24	06277	0,966	0,966
25	06278	1,75	1,75
Average		**1,44**	
Stdev		**0,871**	
Confidence level(95,0%)		**0,360**	
number		**25**	

* This column was created for calculations.

When a number is < (less than), no adducts were detected, and the value given is the background value of the autoradiogram.

If any adducts should be present in that sample, their value is below the background value of the aturadiogram, and could range from zero up to the background value.
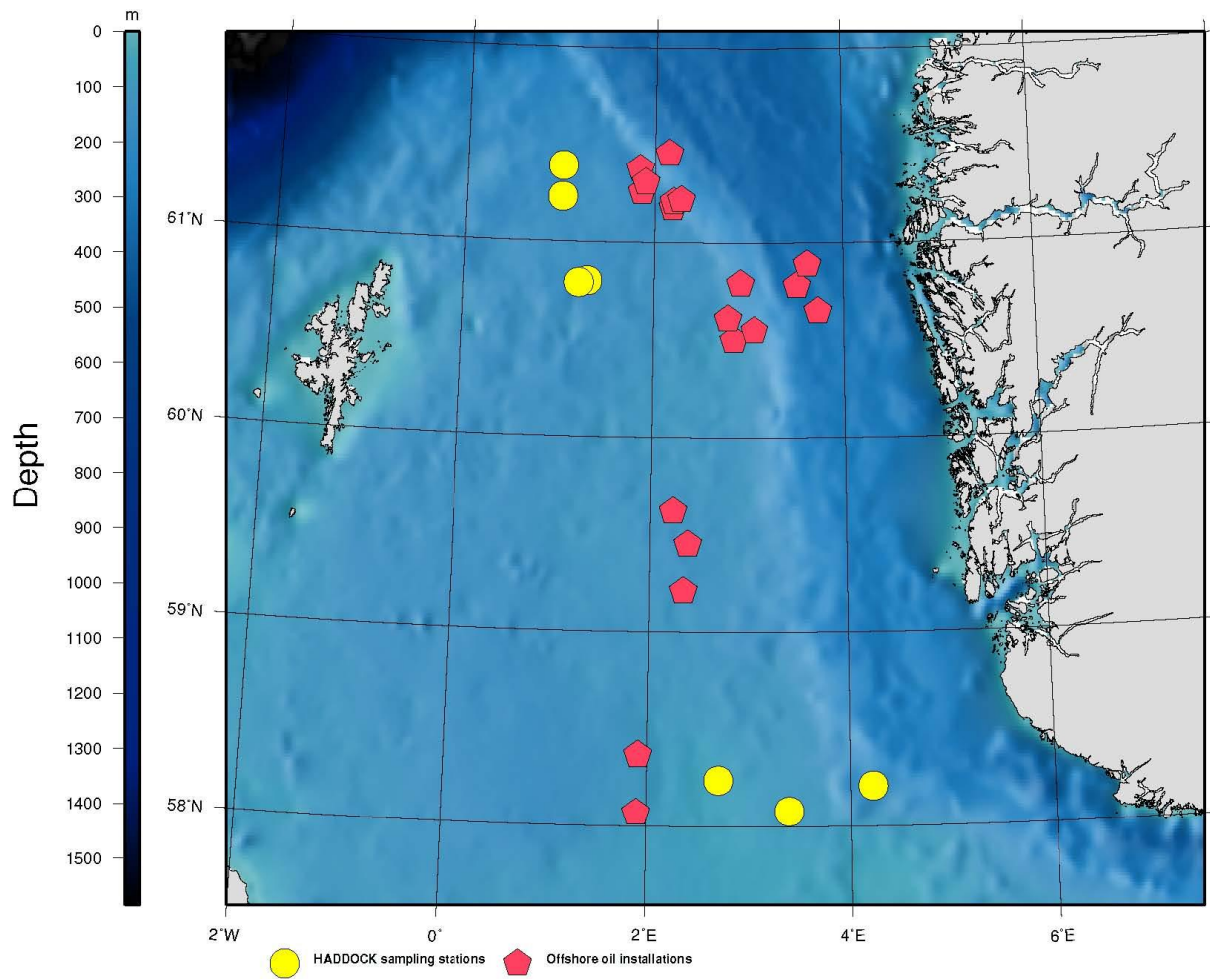
Therefore an average of zero and the background is taken, in order to be able to calculate average values for the groups.

** Explained in text

Appendix B. Map for sampling of cod from the North Sea
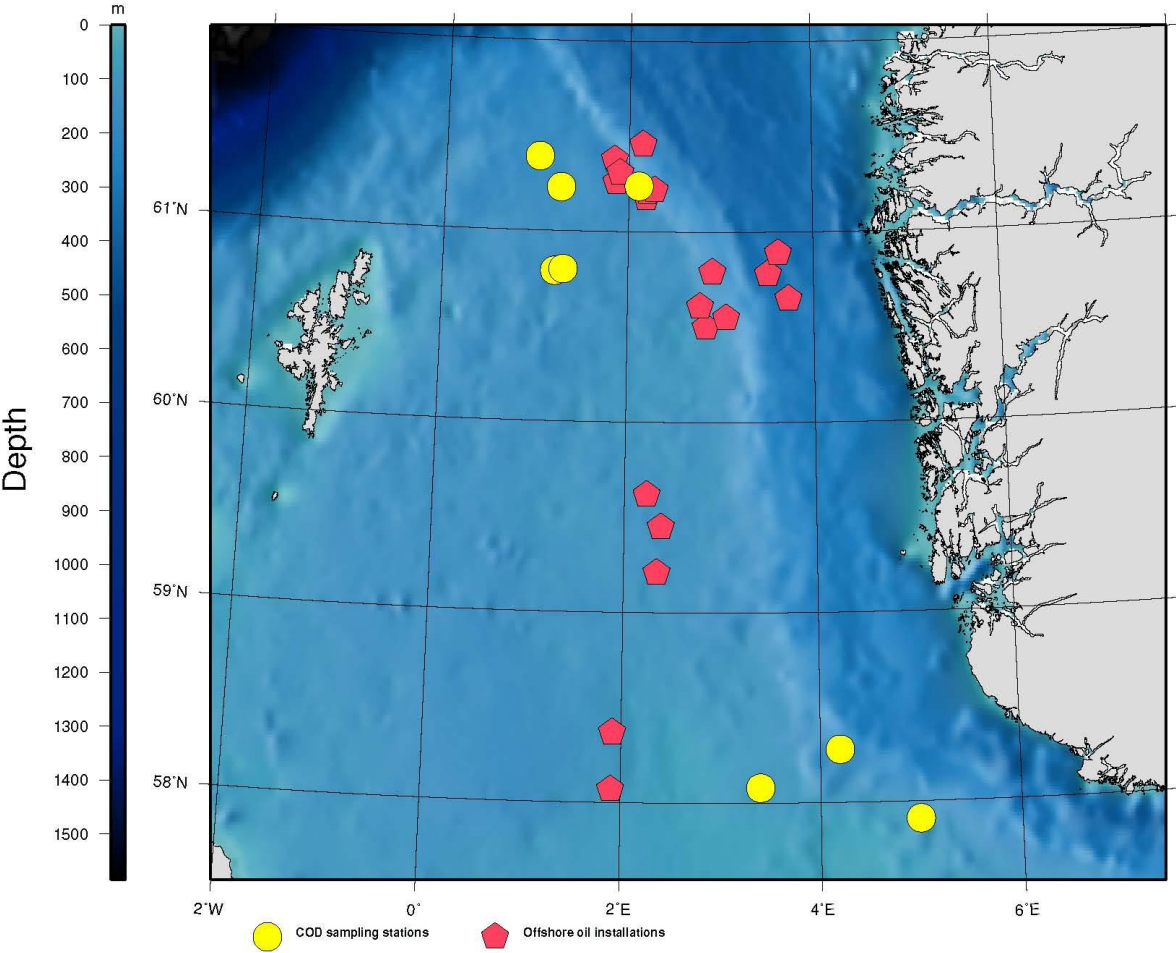
Appendix C. Map for sampling of haddock from the North Sea

Appendix D. Map for sampling of saithe from the North Sea