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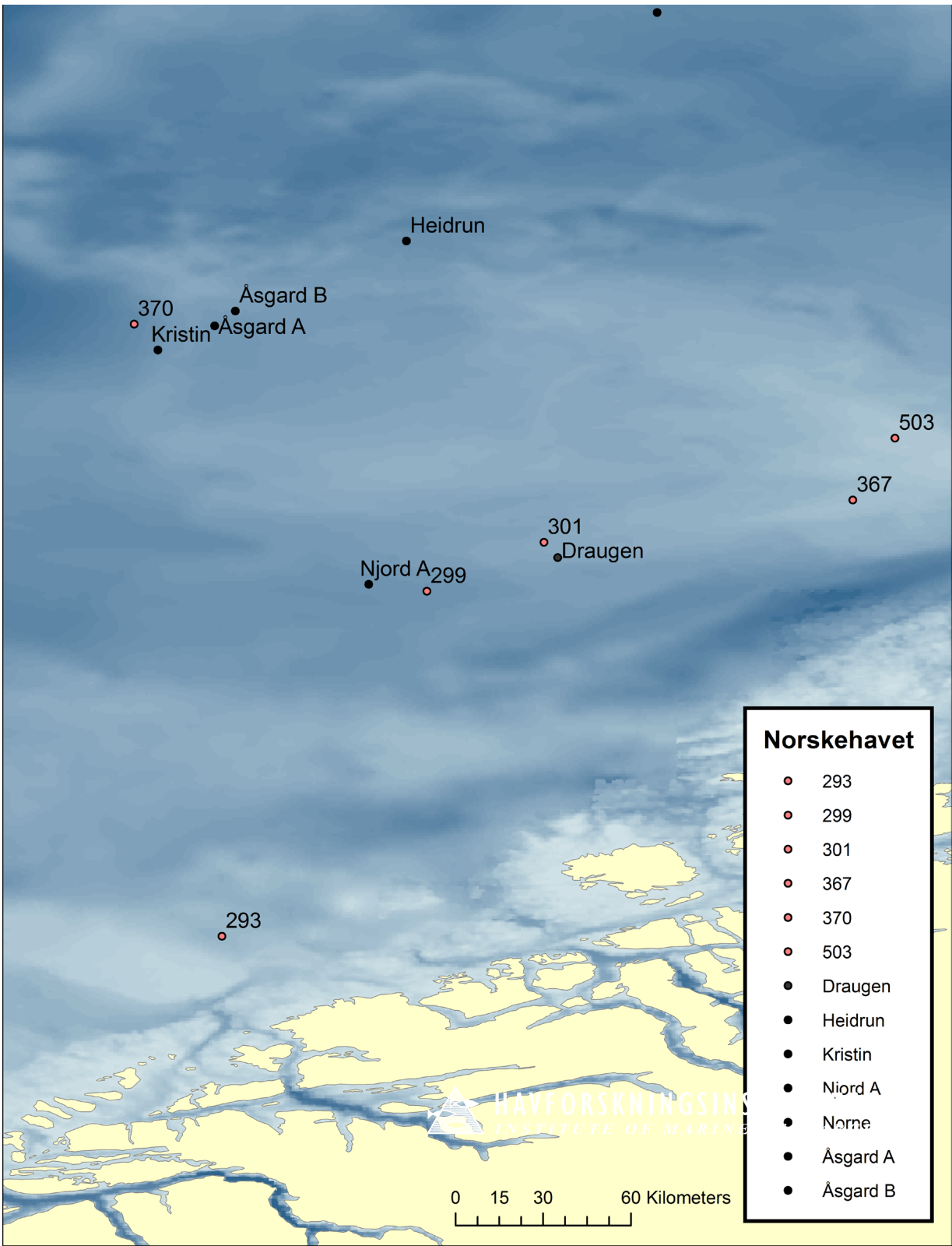

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Condition monitoring 2014
- with focus on the Halten Bank area

Authors:

Bjørn Einar Grøsvik[1], Jérémie Le Goff[2], Pål A. Olsvik[3], Sonnich Meier[1]

1) Institute of Marine Research, Norway
2) ADn´tox, Caen, France
3) NIFES, Bergen, Norway

Project leader:

Bjørn Einar Grøsvik

Contractor:

The Oil and Gas Operators on the Norwegian Continental Shelf

Summary

This report has been prepared by the Institute of Marine Research (IMR) on behalf of 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 condition monitoring shall document if fish from Norwegian ocean areas contain elevated levels of components that originate from discharges from the petroleum activity.

Fish were caught from the Norwegian Sea 10th-14th June 2014 by bottom trawl. For haddock, we compared some of the parameters measured with levels in haddock caught in the North Sea 2013 and with haddock from the Barents Sea in 2008 and 2012. For saithe, parameters were compared with saithe from Masfjorden caught in 2012.

The following methods were investigated: Biological data and stomach analyses. Measurements of exposure levels: PAH in muscle of haddock and saithe. PAH metabolites in bile from haddock. DNA adducts in haddock compared with haddock from the Barents Sea 2012 and the North Sea in 2013. For effect analyses, we measured qPCR levels of gene transcripts of CYP1A, AHRR, GADD45A, GADD45G, P53 and PSTPIP2 in liver of haddock and saithe. Lipid class analyses and fatty acid profiles were performed on phytoplankton and zooplankton.

Levels of PAH in all haddock and saithe muscle measured were below levels of quantification (LOQ). Low levels of PAH metabolites were measured in haddock bile from the Norwegian Sea. Mean levels of sum PAH metabolites in haddock from the Norwegian Sea varied from 85 to 184 ng/g bile, while mean levels from the Barents Sea from 2012 varied from 96 to 113 ng/g bile. Only haddock from the Egersund Bank caught in 2013 had significant higher levels than haddock from the Barents Sea from 2012.

Levels of DNA adducts in liver of haddock from the Norwegian Sea were compared with DNA adduct levels of haddock sampled from one station in the Barents Sea in 2012 and three stations from the North Sea in 2013. Haddock from the Halten Bank region had mean DNA adduct levels of 6.6±7.3 adducts per nmol normal adducts abbreviated to relative adduct level (RAL) and haddock off Kristiansund 5.6±6.1 RAL. Haddock from the Barents Sea had DNA adduct levels of 2.5±3.1 RAL, while haddock from the North Sea from 2013 had RAL levels from 3.3±3.5 (Southern North Sea), 4.8±5.5 (Austbanken) and 8.6±4.0 (Egersund Bank). Haddock from the Egersund Bank 2013 had levels above environmental assessment criteria (EAC) for haddock (> 6.7 RAL) as defined by ICES, and had significantly higher levels of DNA adducts compared with haddock from the Barents Sea 2012.

Haddock caught NW off Kristiansund and at the Halten Bank region had DNA adduct levels comparable with haddock from the North Sea from 2011 and 2013. DNA adduct levels from Halten Bank had levels close to the EAC value of 6.7 RAL and rises concern with regard to PAH exposure of haddock caught in this area. In general, the results with DNA adduct of haddock were higher than expected based on earlier Condition monitoring exercises both for the station at Egersund Bank, the Norwegian Sea and the Barents Sea, and could indicate an increased levels of PAH compounds leading to DNA adducts for all areas since we started these measurements in 2002.

For qPCR analyses in liver of and haddock and saithe, we selected primers to gene transcripts known to be affected by PAH as CYP1A and Ah receptor repressor (AHRR). In addition we wanted to test effects in gene transcripts that are involved in DNA repair processes like growth arrest and DNA damage inducible proteins (GADD) involved in the P53 signaling pathway as GADD45A, GADD45G and p53.

Mean levels of CYP1A and AHRR were not significantly different between haddock from the Halten Bank and the Barents Sea. Levels of GADD45A were increased in haddock from the Halten Bank and NW off Kristiansund compared with haddock from the Barents Sea, and P53 was higher at the Halten Bank compared with haddock from the Barents Sea. Multivariate analyses demonstrated correlations between PAH metabolites and transcript levels of CYP1A and AHRR, but not between DNA adducts and sum PAH metabolites. DNA adducts correlated with GADD45A, and CYP1A levels correlated with sum PAH metabolites, AHRR and GADD45G. The multivariate analyses supported GADD proteins to be interesting proteins to study connected to measurements of DNA adducts and DNA damage repair processes. The results show that it is a challenge to compare transient changes in low levels of PAH metabolites in bile and gene expression levels in liver with DNA adducts which are longer lived covalent bonds.

For saithe we found a reduction in expression of CYP1A in fish caught at the Halten Bank, while GADD45G were upregulated compared to saithe from NW off Kristiansund.

No significant differences in n-3/n-6 ratio or fatty acids profile in phytoplankton or zooplankton were shown for fatty acid profiles.

The present results do not indicate that discharges from oil and gas activities affect food safety aspects as we see no changes in PAH levels in fillet of muscle of haddock and saithe from the investigated fish species in the Norwegian Sea. We also did not see changes in PAH metabolite levels in fish bile from the Norwegian Sea compared with the Barents Sea. Levels of DNA adducts close to EAC for haddock at the Halten Bank raises concern of exposures for PAH compounds related to oil and gas activities.

Bjørn Einar Grøsvik
Project leader

Contents

1. Acknowledgements

The survey for sampling material for the Condition monitoring in the Norwegian Sea was performed with R/V Johan Hjort 10/6-14/6-2014. We acknowledge the crew at Johan Hjort for excellent service and help to make the survey a success. We are grateful to Guri Nesje, Grethe Tveit, Daniela Pampanin (IRIS), Bjørnar André Beylich (Niva), Else Holm and Thomas de Lange Wenneck for help with sample preparation, Anders Fuglevik and Anna Ersland for PAH analyses in muscle and PAH metabolite analyses of bile. We thank Arve Fossen for analyses of fatty acid composition in plankton, Inger Marie Beck for performing analyses of stomach content and Per Arne Horneland for preparing maps.

Crew members on board R/V Johan Hjort in the Norwegian Sea 10th-14th June 2014. Photo: B.E. Grøsvik.

2. Abbreviations

AHRR - Ah receptor repressor
BAC - Background assessment criteria
CPM - Counts per minute
CTD - Conductivity, temperature and depth
CYP - Cytochrome P450
EAC - Environmental assessment criteria
FAME - Fatty acid methyl
GADD - Growth arrest and DNA damage inducible proteins
GC - Gas chromatography
ICES - International Council for Exploration of the Seas
IBTS - International bottom trawl survey
LSI - Liver somatic index
LOD - Levels of detection
LOQ - Levels of quantification
NPD - Naphthalene, phenanthrene, dibenzothiophenene and their alkylated homologues
NL - Neutral lipids
PAH - Polyaromatic hydrocarbons
PC - Phospatidyl choline
PCA - Principal component analysis
PSTPIP2- proline-serine-threonine phosphatase-interacting protein
PUFA - Poly unsaturated fatty acids
PW - Produced water
RAL - Relative adduct levels (nmol adducts per mol normal nucleotides)
SD - Standard deviation
SIM - Selected ion monitoring
SPE - solid phase extraction
TLC - Thin-layer chromatography
ww - wet weight

3. Introduction

Condition monitoring of wild caught fish in the Norwegian Seas has been performed every 3rd year since 2002. The monitoring has been regulated by "Aktivitetsforskriften" and the objective has been to document whether discharges from oil and gas activities are taken up by fish and whether such discharges may impact wild fish populations. Sampling should be representative for the most important fish species in the region.

Objectives are:

- To monitor levels of PAH in fish related to food safety.

- To follow up the results of increased levels of DNA adducts found in the North Sea from the condition monitoring from 2002-2011 by comparing levels from the North Sea to levels in the Norwegian Sea and the Barents Sea.

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 concentration 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 was 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. Significant differences in (n-3)/(n-6) ratio of muscle lipid composition were also detected at the Tampen compared to Egersund Bank (Klungsøyr *et al*.,

2003, Balk et al., 2011). The reported levels in haddock from Tampen in 2002 were surprisingly high, compared to levels in fish from pristine areas (Aas et al, 2003).

In the condition monitoring of 2005, NPD and PAH compounds were only measured in muscle and all levels were below levels of quantification (LOQ) in cod and haddock sampled from the Egersund Bank, Tampen, the Halten Bank and the Barents Sea (Grøsvik *et al*., 2007). Measurements of NPD and PAH in fish fillet were also conducted in several fish species after the oil discharge incident of 4400 m^3 crude oil at Statfjord in December 2007. Also in this study levels of NPD and PAH in fillet were below levels of detection (LOD) for fish sampled 6 days and one month after the discharge. However, increased levels of NPD compounds were measured in liver of haddock and pollock (*Pollachius pollachius*) sampled in the Tampen area 6 days after the discharge (Grøsvik *et al*., 2008).

Other findings from the condition monitoring in 2005 were: 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. Overall, the highest levels of PAH metabolites in bile were measured in haddock (Grøsvik et al., 2007).

DNA adducts were analyzed in liver of cod, haddock and saithe at Tampen and from Ling Bank/Egersund Bank during the condition monitoring in 2005. 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, indicative of more PAH exposure in this region. Significant differences in DNA adduct levels were not found for cod and saithe collected from the same areas (*ibid.*).

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 (*ibid.*).

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 (*ibid*.).

The condition monitoring of 2008 showed similar differences of DNA adduct levels in haddock from Tampen compared to the Egersund Bank as reported from the 2005 monitoring, together with an increase in bile metabolites in haddock from Tampen compared with the Egersund Bank. NPD/PAH levels in haddock liver were at background levels. The ratio of omega-3/omega-6 fatty acids were lower in haddock liver from Tampen compared with the Egersund Bank (Grøsvik et al., 2009).

The condition monitoring of 2011 had focus on haddock and DNA adduct levels in the North Sea. Levels of NPD and PAH in cod and haddock muscle were generally below LOQ. Levels of NPD and PAH in cod and haddock liver were low for all stations. Low levels of PAH metabolites were measured in haddock bile from the 2011 survey. ELISA was performed on liver samples of cod and haddock incubated with anti-cod CYP1A. An increase in CYP1A levels were observed in cod from Tampen compared to Egersund Bank, although not statistical significant. No differences were observed in CYP 1A levels for haddock.

The measured levels of DNA adducts in haddock liver from 6 stations were, apart from the station at the Ula area in the Southern North Sea, above background levels (>3.0 nmol adducts per mol normal nucleotides or relative adduct level (RAL)). Two stations had DNA adduct levels above environmental assessment criteria (EAC) defined to >6.7 RAL (ICES, 2011). These were one of two stations at Tampen and the station at Viking Bank.

The mean DNA adduct level measured were 1.6 RAL at the Ula area, Southern North Sea, around 5.0 RAL at Egersund Bank, Bressay Bank and Tampen South of Statfjord, and 7.3 RAL at Tampen between Statfjord and Gullfaks and 7.9 RAL at the Viking Bank.

DNA adducts in haddock liver were significantly higher at Tampen compared with Egersund Bank in 2005 and 2008, but to a lesser extent, 2-fold in 2005 and 2008 (Grøsvik et al., 2007 and 2008), compared to 5-fold in 2002 (Balk et al., 2011). In the 2011 monitoring, the station south of Tampen had DNA adduct levels at the same levels as the reference stations Egersund Bank and Bressay Bank.

No significant differences in n-3/n-6 ratio or fatty acids profile in haddock or cod between references area at Egersund Bank and Tampen were shown for fatty acid profiles.

The results of the lipid analyses in 2011 compared with earlier monitoring show large natural variation from year to year. We need better understanding of the natural regulation of the lipid homeostasis in wild fish and more experimental studies of how discharges from oil and gas activities effect the lipid metabolism, before we can conclude whether difference in lipid composition between Tampen and other areas as reported in 2002, 2008 and 2010, can be correlated to discharges from the oil and gas activities.

For DNA adduct levels 5 of 6 stations in the North Sea had levels above background levels and two of 6 had levels above environmental assessment criteria (EAC). This raises concern of general increased DNA adduct levels of haddock in the North Sea.

However, due to the low differences between at Tampen and the two reference stations, the Condition monitoring in 2011 did not indicate that cod and haddock caught at Tampen are more contaminated with oil related compounds than fish caught at the reference stations (Egersund Bank and Bressay Bank), although the general PAH pressure in the North Sea Bassin needs more attention.

Results from Condition monitoring in 2002 (Balk et al., 2011), 2005 (Grøsvik et al., 2007), 2008 (Grøsvik et al., 2009), and 2011 (Grøsvik et al., 2012) has been used as a basis for the project proposal for 2014.

We have in this condition monitoring focused on the Halten Bank area in the Norwegian Sea as this area has been less studied due to the need of obtaining more data from the North Sea during the last programmes. The North Sea region has been the region with highest activities with oil and gas production, and 80-85 % of oil discharged with produced water from the Norwegian sector is discharged from the North Sea region (www.miljøstatus.no). The total amount of produced water yearly discharged from Norwegian oil and gas activities has varied around 140 mill m^3 since 2004, while between 30 to 40 mill m^3 has been reinjected to the reservoir (Norsk Olje & Gass, 2015). In 2014, approx 17 mill m^3 produced water were discharged at the Norwegian Sea area, approx. 12 % of the total amount from Norwegian sector. The largest contributors at the Halten Bank area was Draugen contributing with 6.5 mill m^3 produced water. For the field specific water column monitoring, Njord was selected due to problems with leakage of injected slop from drilling and operation from 2000 to 2006. In 2014, the regional and field specific water column monitoring was integrated and fish from IMR's condition monitoring cruise was used as reference material for the field specific water columns monitoring performed by NIVA and IRIS.

As haddock and cod have been the main focus from earlier studies from the North Sea, we suggested these species. However, as we were not able to obtain sufficient number of cod in our field survey, and got good catches of saithe, we included saithe instead of cod for the planned analyses for cod. This was in accordance to prioritise the species that are most representative from the areas in vicinity of the selected oil and gas installations in the Norwegian Sea. Saithe has also been used in water column monitoring from the North Sea, e.g in the BECPELAG workshop (Bilbao et al, 2006).

To obtain more information on possible effects from PAH exposure we included quantitative poly chain reaction qPCR of selected transcripts. Transcripts to CYP1A and Ah receptor repressor (AHRR) were selected because these proteins are shown to be induced by PAHs (Goksøyr & Förlin, 1992; Meyer et al., 2003). In addition we wanted to test effects in gene transcripts that are involved in DNA repair processes and selected transcript from three genes in the P53 signalling pathway (Figure 1). The selected transcripts were p53 and the growth arrest and DNA damage inducible proteins (GADD): GADD45A and GADD45G (Siafakas &Richardson, 2009; Salvador et al., 2013) In addition and proline-serine-threonine phosphatase-interacting protein 2 **(**PSTPIP2), a possible marker for oxidoreductase activity selected because it has been induced in other studies (Olsvik and Grøsvik in prep).

Activation of P53 signaling pathway is induced by a number of stress signals, including DNA damage, oxidative stress and activated oncogenes. The p53 protein is employed as a transcriptional activator of p53-regulated genes. This results in effects on cell cycle arrest, cellular senescence or apoptosis. Other p53-regulated gene functions communicate with adjacent cells, repair the damaged DNA or set up positive and negative feedback loops that

enhance or attenuate the functions of the p53 protein and integrate these stress responses with other signal transduction pathways (http://www.genome.jp/kegg/pathway.html).

Effects on expression levels of genes in the P53 signalling pathway could give information on cellular effects to increased levels of DNA adducts in fish and also information whether such methods could serve as alternative methods to DNA adduct measurements.

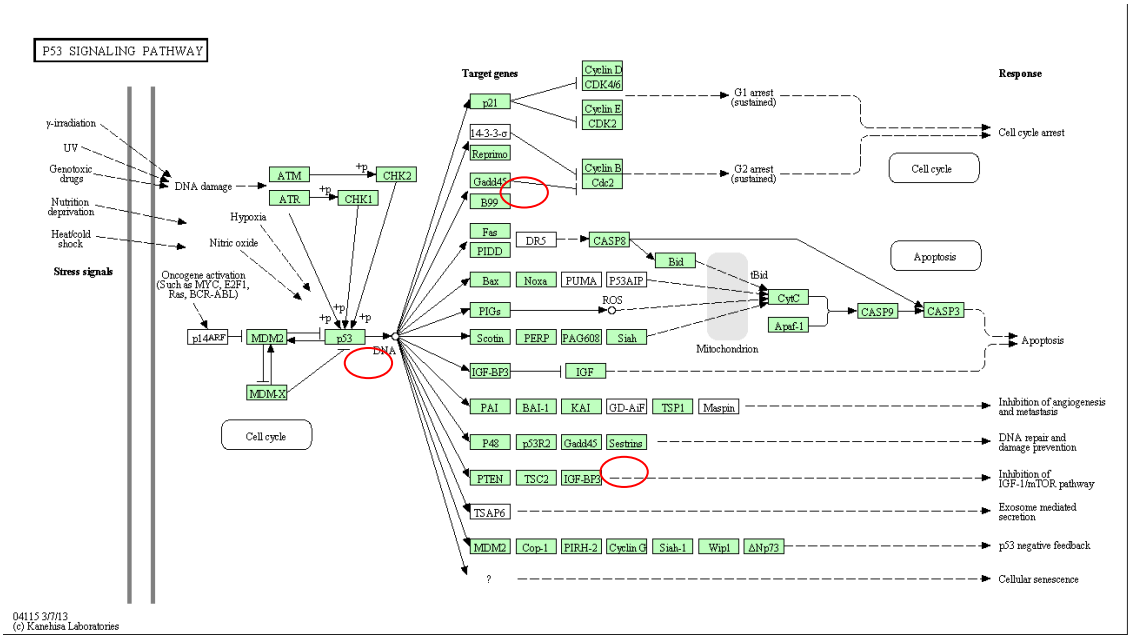


Figure 1. P53 Signaling pathway from zebrafish (*Danio rerio*) taken from (http://www.genome.jp/kegg/pathway.html). Red circles mark p53, and GADD45 proteins.

The objectives for this study have been:

1. Analyses of stomach content of haddock and saithe to get information on diet.
2. Chemical analyses of PAH in muscle of haddock and saithe from the Norwegian Sea to assess exposure and levels related to food safety.
3. Measurements of metabolites of PAH in bile of haddock from the Norwegian Sea and compare with levels in haddock from the North Sea in 2013 and in the Barents Sea in 2012.
4. Study possible genototoxic effects in fish from Tampen compared with fish from Egersund Bank and three additional stations by measurements of hepatic DNA adducts.
5. Measurements of DNA adducts from haddock liver, to compare with levels from the North Sea (2013) and the Barents Sea (2012).
6. Measurements of PAH inducible genes like CYP1A and AHRR and growth arrest and DNA damage inducible genes like GADD45A, GADD45G and P53 levels in liver of haddock and saithe from the Norwegian Sea and compare with levels from the Barents Sea (haddock) and with coastal saithe by qPCR.
7. Fatty acid profiles in phytoplankton and zooplankton to analyse ratio of (n-3)/(n-6) poly unsaturated fatty acids as background information to compare with levels from the North sea.

4. Sampled material and collection sites

Sampling for the Condition monitoring and water column monitoring was performed with R/V Johan Hjort 10/6-14/6-2014. Bottom trawl was used for collection of fish. Soft bottom was sought for trawling. Trawling time was 20 min. From each of the stations we aimed to sample 25 (±10%) fish of each species. 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.

Fish sampled on the cruise with R/V Johan Hjort 10/6-14/6-2014 is listed in Table 1. During dissection of fish, samples were snap frozen in liquid nitrogen and stored in a -80°C freezer. Samples were taken of plasma, bile, liver, muscle and brain.

Chemical analyses of NPD/PAH (Muscle and liver), bile metabolites, levels of CYP1A, AHRR, GADD45A, GADD45G, p53 and PSTPIP2 in liver by qPCR were performed by NIFES, stomach content and fatty acid analyses were performed at IMR. Haddock > 30 cm were selected for DNA adduct analyses and sent to ADN´tox, Caen, France.

Map of stations selected for phytoplankton and zooplankton sampling for analyses of fatty acid profiles are shown in Chapter 6.7.

Table 1- Areas, fish species and number caught.

Species	Latin name	NW off Kristiansund	Halten Bank	Kristin area	Shelf edge	Sum
Haddock	*Melanogrammus aeglefinus*	**21**	**31**			**52**
Cod	*Gadus morhua*	**3**	**1**			**4**
Saithe	*Pollachius virens*	**23**	**25**	**15**		**63**
Ling	*Molva molva*	**4**				**4**
Golden redfish	*Sebastes norvegicus*		**25**	**15**		**40**
Norway redfish	*Sebastes viviparus*		**25**			**25**
Tusk	*Brosme brosme*		**20**			**20**
Greater forkbeard	*Phycis blennoides*		**14**			**14**
Rabbit fish	*Chimaera monstrosa*		**25**			**25**
Atlantic mackerel	*Scomber scombrus*		**25**			**25**
Greenland halibut	*Reinhardtius hippoglossoides*				**25**	**25**

During our sampling cruise, a security zone of 5x 11 nautical miles were closed for other activities due to seismic investigations by Geowave Commander around the Njord platform and we could not obtain samples closer to the Njord platform (Figure 2). We do not know how this activity affected our catch in this area. Maps of stations for haddock and saithe are shown in Figures 3 and 4.

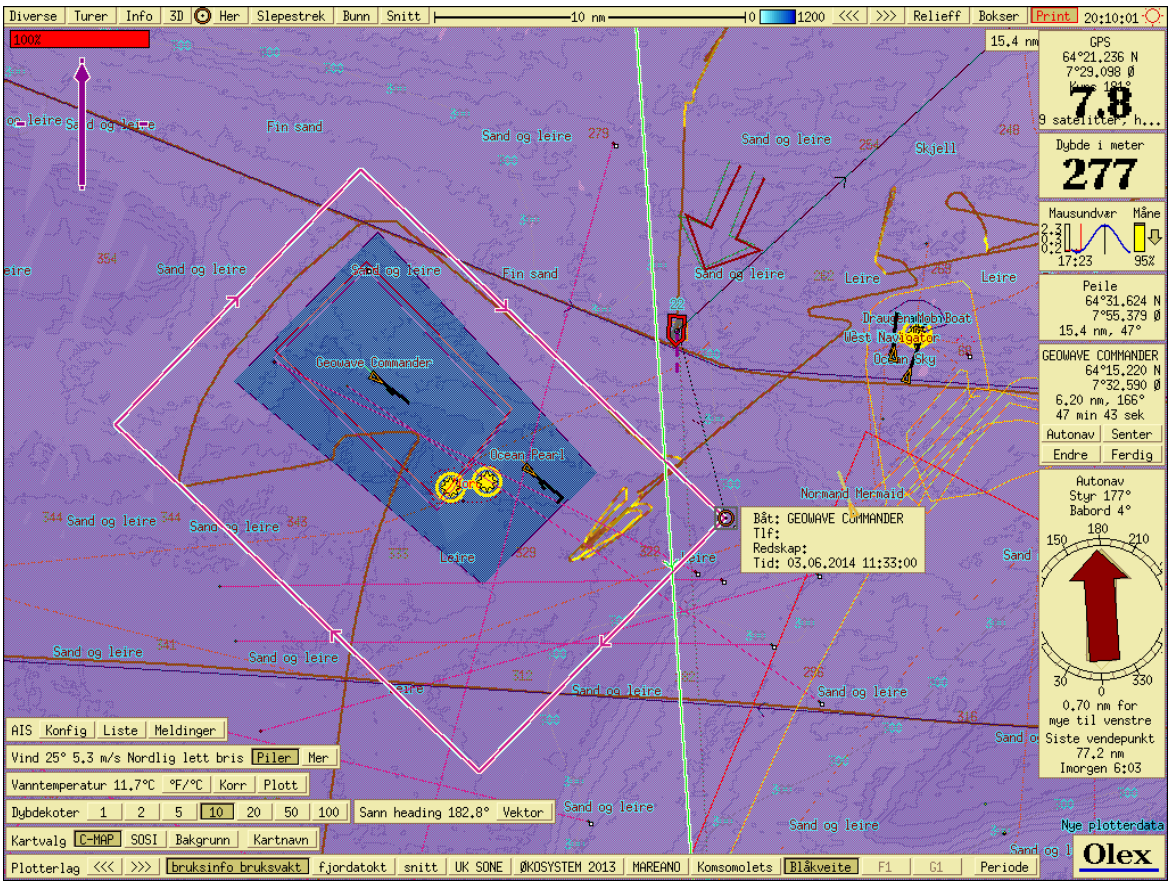


Figure 2. Map of security zone around the Njord platform due to seismic investigations by Geowave Commander during our sampling in this area.

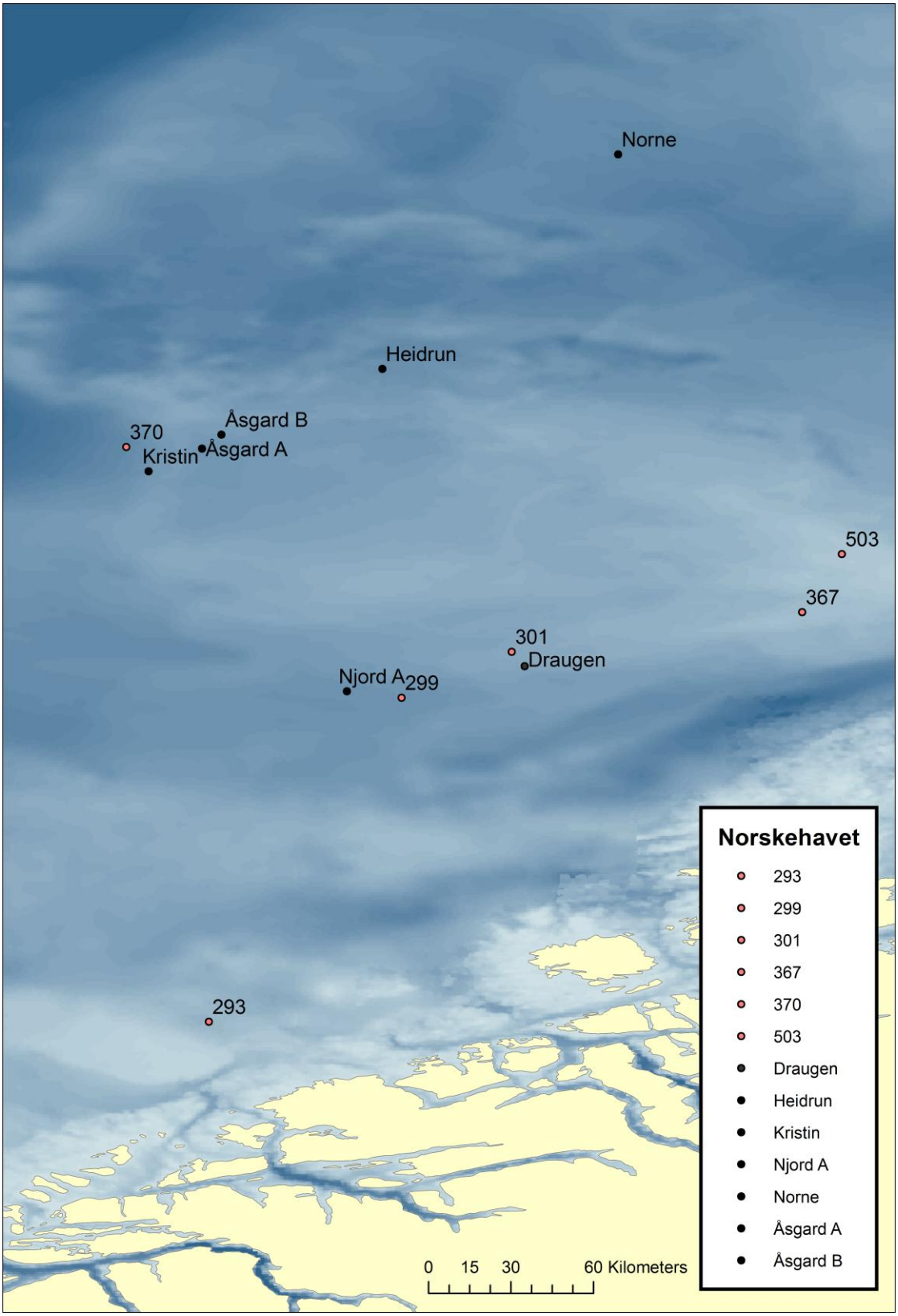


Figure 3. Map over stations where we fished haddock (red circle) and oil and gas installations in the Norwegian Sea (black circles).

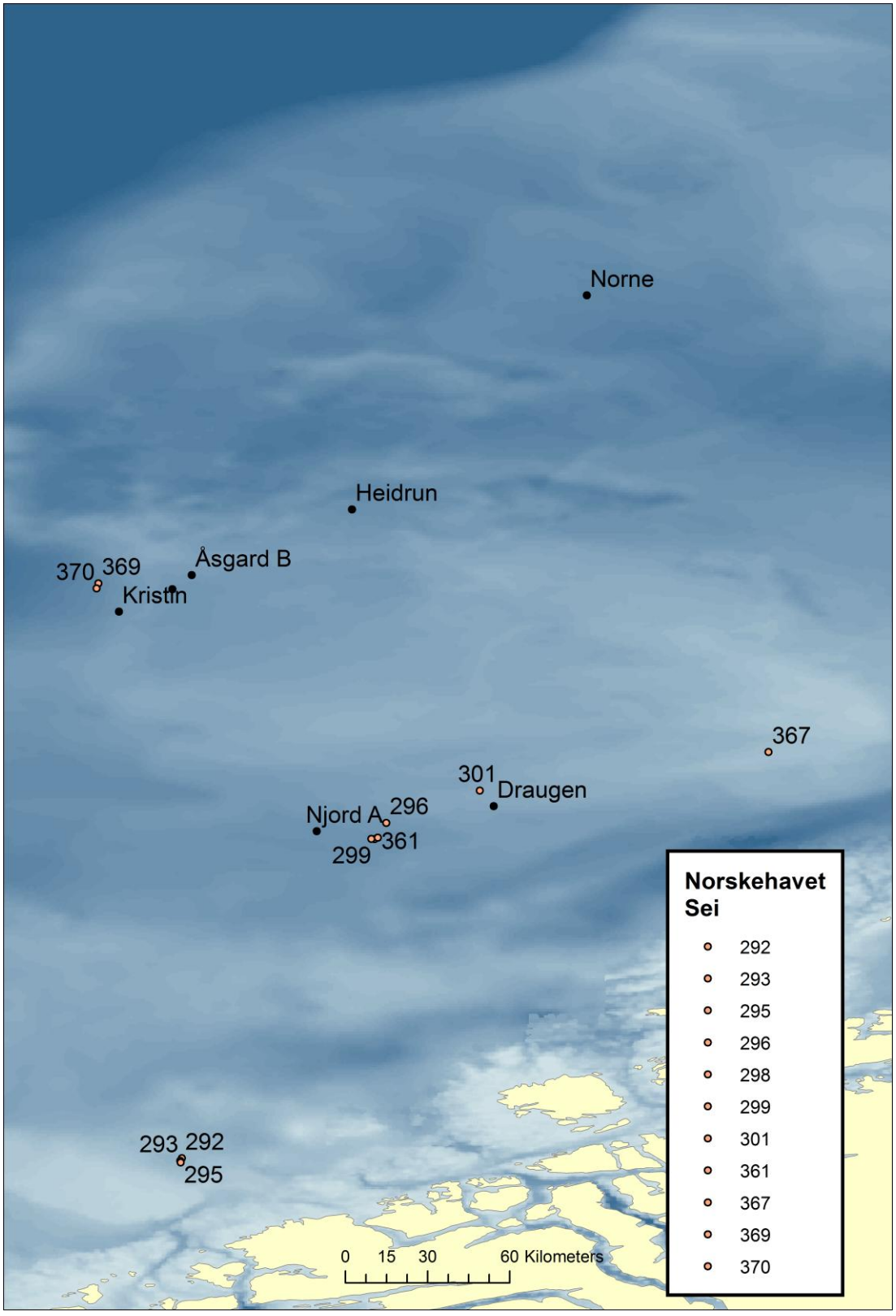


Figure 4. Map over stations where we fished saithe (orange circles) and oil and gas installations in the Norwegian Sea (black circles).

5. Methods

5.1 PAH analysis of muscle tissue

Wet muscle tissue 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 equipped 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 compounds in concentration of 0.2 ng/g. Levels of detection (LOD) are defined as LOD: $Y = YB + 3SD_B$, and levels of quantification (LOQ) is LOQ= $Y = YB + 10SD_B$ where Y_B is the response of blank sample signal and SD_B is the standard deviation of the blank samples.

This method is accredited by Norsk Akkreditering (Appendix 10.4). The method is named O1 and 11 of the 47 reported PAH components reported are included in the yearly certification.
The accredited components are: Phenanthrene, anthracene, 2-methylphenanthrene, 3,6-dimethylphenanthrene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(ghi)perylene. The laboratory participates in ring tests organized by QUASIMEME.

5.2. Analysis of PAH metabolites in fish bile

Bile (100 µl) was diluted in 200 µl sodium acetate buffer (0.01 M, pH 5). 36 µl β-glucuronidase (115600 units/ml) were added, and samples were incubated at 37°C for 2 hours. Surrogate internal standard (SIS) including two deuterated hydroxyl PAH, 1-naphthol-d7 and 1 hydroxypyrene-d9, were added to the solution which was then further diluted with 2 ml acetic acid (0.1 %). The mixture was then loaded onto Oasis (HLB) SPE column (4 cc volume), previously preconditioned with 1 ml methanol and 1 ml acetic acid (0.1 %), successively. The column was rinsed with 3 ml acetic acid (0.1 %) and dried for ½ hour under vacuum. The samples were extracted by 4 ml of methanol. The extract was then evaporated to ca. 0.2 ml under a nitrogen stream (40°C). The eluate was derivatizated with pentafluorobenzoyl chloride as described elsewhere (Boitsov *et al*., 2004) and the samples concentrated to 0.5 ml hexane solution under a nitrogen stream (40°C). All samples were analysed by GC-MS in selected ion monitoring (SIM) mode using negative chemical ionization (NCI).

Conditions for GCMS: We used a 25 m DB-17MS column (J&W Scientific, CA, 0.25 mm i.d., 0.25 µm film thickness). Carrier gas helium, flow rate 1.0 ml/min; injector temperature 300°C; oven temperature gradient from 90°C (2 min) to 160° C at 20°C/min; to 320° C at 4°

C/min, 10 min at 305°C. The following ion masses m/z were scanned for in SIM mode: Naphthol: 338, 1-Naphthol-d7: 345, methylnaphthol: 352, dimethylnaphthol: 366, hydroxyfluorene: 376, hydroxyphenanthrene: 388, hydroxypyrene: 412, 1-Hydroxypyrene-d9: 421, hydroxychrysene: 438.

The method has been quality assured through an inter-laboratory study arranged by WGBEC (Kamman et al., 2013). Masses scanned for in SIM mode are shown in Table 2.

Table 2. Masses scanned for in SIM mode. Methyl-naphthols are coeluating on the GC and are written in italic.

	RT	Quantifier ion (m/z)
1-Naphthol	18,23	338
2-Naphthol	18,80	338
7-Methyl-1-naphthol	*19,55*	352
8-Methyl-2-naphthol	*19,55*	352
2-Methyl-1-naphthol	19,74	352
3-Methyl-1-naphthol	19,82	352
6-Methyl-1-naphthol	20,14	352
3-Methyl-2-naphthol	20,30	352
7-Methyl-2-naphthol	20,73	352
6-Methyl-2-naphthol	20,85	352
4-Methyl-1-naphthol	20,97	352
5-Methyl-1-naphthol	*21,03*	352
1-Methyl-2-naphthol	*21,06*	352
4-Methyl-2-naphthol	21,23	352
5-Methyl-2-naphthol	21,38	352
2-Hydroxyfluorene	24,82	376
9-Hydroxyfluorene	28,32	167
4-Hydroxyphenanthrene	29,51	388
3-Hydroxyphenanthrene	31,79	388
1-Hydroxyphenanthrene	31,85	388
9-Hydroxyphenanthrene	32,27	388
2-Hydroxyphenanthrene	32,66	388
1-Hydroxypyrene	38,61	348
2-Hydroxychrysene	45,58	438
SIS		
1-Naphthol-d7	18,13	345
1-Hydroxypyrene-d9	38,49	356

5.3 Fatty acid profile analyses

Methyl esters of the fatty acids (FAME) from total lipids and the lipids classes were prepared and analyzed on gas chromatography (GC-FID) as described by (Meier *et al*., 2006). The FAME was quantified using Nonadecanoic acid (19:0) as internal standard.

5.4. Analysis of DNA adduct patterns by the ^{32}P postlabelling method

Preparation of DNA

100-120 mg liver samples were cut on ice. 1.5 ml of sucrose (0.32 M) was added and mixed thoroughly to lyse tissue (Tissue lyser, Qiagen: 20 Hz) for 2 minutes. Samples were centrifuged at 800G for 10 minutes, at 4°C. Pellets were dissolved with 1.2 ml of 1 mM EDTA and 20 mM Tris, pH 7.4. 100 µl 10% SDS was added and vortexed for 1 minute.

Vials were incubated for 30 minutes at 37°C with 0.2 mg / ml RNase A and 33.4 U RNase T1. Then incubate for 2.5 hours at 37°C with 0.50 mg/ml proteinase K until complete digestion.

0.5 volume (0.7 ml) of saturated phenol was added and vortexed for 1 minute, before centrifugation for 5 minutes at 5000 rpm. The upper phase (aqueous phase) was removed and transferred to a clean tube. 0.5 volume (0.7 ml) of CIP (phenol + Sevag 1/1) was added and vials vortexed for 1 minute, and centrifuged for 5 minutes at 5000 rpm (+4°C).

The upper phase was removed and transferred to a clean tube. 0.5 volume of Sevag (chloroform + isoamyl alcohol (1/24)) was added and vortexed for 1 minute before centrifugation for 5 minutes at 5000 rpm (+4 ° C). The upper phase was removed.

Precipitation of DNA: 0.1 volumes of a solution of 5 M NaCl and 2 volumes of cold ethanol (stored at -20°C) were added to the aqueous phase. Vials were gently shaked and vortexed. The DNA was air dried before addition of 150 µl ultra pure water.

Spectrophotometric quantification of DNA solutions (Nanodrop, Thermo Scientific): 1 unit of absorbance at 260 nm corresponds to a double-stranded DNA solution concentration equal to 50 µg/ml. Quality criteria selected: 1.85 <A260 / A280 <1.95; A260 / A230> 2.00. Solutions were prepared to be close to 2 µg/µl and kept at -80 °C in 2 ml glass vials.

The ^{32}P-postlabelling method

Each analysis was performed on 5 µg DNA. Ten sets of analyses were necessary in order to analyse the DNA adduct patterns of the overall 100 samples. Two independent adduct measurements have been performed for each DNA sample. For the study, the limit of detection (LOD) is fixed to half the smallest DNA adduct level (Relative adduct level=RAL) calculated for an observed spot in a pattern, i.e. ½ x 0.02 = 0.01 adducts per 10^8 nucleotides (RAL x 10^{-8}). For analysis without detectable adducts ("null" results), the concentration in adducts is then defined as <0.01 x 10^{-8} nucleotides, although

In each set of analysis, DNA from both positive and negative controls was systematically included**.** Positive control was a calf thymus DNA exposed to benzo(a)pyrene dioepoxide (BPDE) kindly provided by F.A Beland (National Center for Toxicology Research, USA). This sample was used as a standard in large interlaboratory trials. The DNA damage level was 110.70 adducts per 10^8 normal nucleotides (according to F.A. Beland, in Philips and

Castegnaro, 1999; see Divi et al., 2002 and Zhan et al., 1995 for more details). The negative control was a plasmid DNA.

The autoradiographic patterns from both positive and negative controls assure technical functioning, by the absence first of nonspecific signals (a source of false positives, frequently due to improper disposal of certain reagents/impurities used during handling) and then a correct ^{32}P labelling on a reference/standard sample. Good labelling efficiency was checked on the basis of the direct level of radioactivity (Cerenkov radiation) in the major spot of the positive control, expressed in radioactive counts per minute (cpm).

As result of the technical variability classically described with the ^{32}P post-labelling method, each sample was analysed twice in two independent runs. Four controls were added to the runs. The two first control samples were one without adducts (cell DNA free of adducts) and the second positive in adducts (DNA rich in adducts of benzo(a)pyrene) with known quantity of adducts according to Philips and Castegnaro, 1999. The third and fourth controls checked 32P-labelling of normal nucleotides (deoxyadenosine 3'phosphate, control of labelling by polynucleotide kinase) and by a small fraction of DNA (1 µg) from the negative control (verification of DNA hydrolysis efficiency).

Hydrolysis
- Prepare 5 µg of DNA/analyse
- Dry sample (Speed Vac SV, 15 minutes)
- Hydrolyse of DNA : MN : 0.7 µg / 5 µg DNA
SPDE : 10 mU / 5 µg DNA } 3.5 hours / 37°C
+ *Buffer solutions*

MN= micrococcal nuclease (Sigma); SPDE: spleen phosphodiesterase (Calbiochem)

Enzymatic enrichment with NP1
- Dry sample (SV) after hydrolysis
- NP1: 5 µg / 5 µg DNA
+ *Buffer solutions* } 30 minutes / 37°C
- Stop incubation with a tris base solution (1.8 µl/sample)

NP1= Nuclease P1 (Sigma)

^{32}P radioactive labelling
-Add to sample:
- PNK : 10U/5µg DNA
- PNK buffer A 1X } 30 minutes / 37°C
- ^{32}P-ATP : 25 µCi / 5 µg DNA

PNK : polynucleotide kinase (+ buffer A 10X ; Fermentas)

Chromatographic separation

Separation of radiolabelled adducts in the previous step was performed by bidirectional thin layer chromatography on polyethyleneimine (PEI) cellulose sheet (12 x 10 cm) (Macherey Nagel), by using D1 to D4 successive migrations (D1 and D4 being "clean-up" migrations). Solvent (mobile phase) composition was provided for each migration.

• D1:

- Mobile phase: Na Phosphate 1 M. pH 6
- Wash sheet in deionized H2O after D1
- Dry sheet
- Cut up PEI Cellulose Sheet (transfer step)

• D2:

- Mobile phase:

Li formate 4.5 M
Urea 8.5 M } pH 3.5

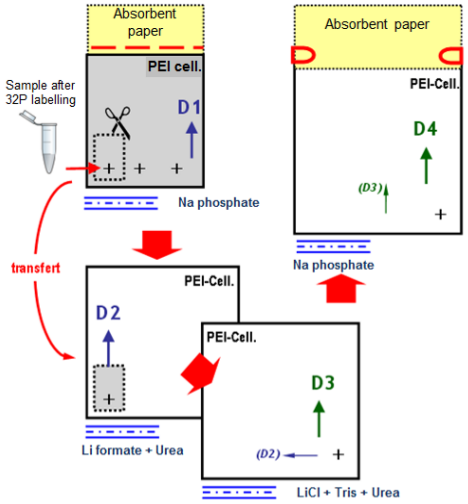
- Wash sheet in deionized H2O
- Dry sheet

• D3:

- Mobile phase:

Li chloride 1.6 M
Tris 0.5 M } pH 8
Urea 8.5 M

- Wash sheet in deionized H2O
- Dry sheet



• D4:

- Mobile phase:

Na Phosphate 1 M. pH 6.8

- Dry sheet

DNA adduct patterns were detected by autoradiography (Kodak X-OMAT® / BIOMAX®). The optimum exposure time is a function of radioactive signal strength (exposure time at -80°C: from 12 to 72 hours).

Quantification / results analysis

Quantification was performed using the scintillation counting of spots cut on chromatographic sheets, by Cerenkov mode, and on the basis of the radioactive signal associated to the labeling of a known quantity of DNA adducts (positive control: 5 µg of a DNA which contained 110.7 adducts for 10^8 normal nucleotides, according to Phillips and Castegnaro, 1999, kindly provided by F.A. Beland, FDA, USA).

5.5. RNA isolation

Liver tissue was thoroughly homogenized before RNA extraction using a Precellys 24 homogenizer and ceramic beads CK28 (Bertin Technologies, Montigny-le-Bretonneux, France). Total RNA was extracted using the BioRobot EZ1 and RNA Tissue Mini Kit (Qiagen, Hilden, Germany), treated with DNase according to the manufacturer's instructions and eluted in 50 µL RNase-free MilliQ H_2O. The RNA was then stored at –80°C before further processing. RNA quality and integrity were assessed with the NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The RNA 6000 Nano LabChip kit (Agilent Technologies, Palo Alto, CA, USA) was used to evaluate the RNA integrity of the liver samples. For haddock, the 260/280 and 260/230 nm ratios of the extracted RNA were 2.10 ± 0.00 and 2.20+ ± 0.00 (n=38), respectively (mean ± SEM). The RNA integrity number (RIN) of the haddock liver samples used for RT-qPCR was 8.7 ± 0.2 (n=9) (mean ± SEM). For saithe, the 260/280 and 260/230 nm ratios of the extracted RNA were 2.10 ± 0.00 and 2.17+ ± 0.00 (n=33), respectively (mean ± SEM). The RNA integrity number (RIN) of the saithe liver samples used for RT-qPCR was 9.2 ± 0.2 (n=16) (mean ± SEM).

5.6. Quantitative real-time RT-qPCR

PCR primers used to quantify the selected genes in haddock and saithe were designed based on genome sequence for the respective species and shown in Table 3. A two-step real-time RT-PCR protocol was used to quantify the transcriptional levels of these genes. The RT reactions were run in duplicate on a 96-well reaction plate with the GeneAmp PCR 9700 machine (Applied Biosystems, Foster City, CA, USA) using TaqMan Reverse Transcription Reagent containing Multiscribe Reverse Transcriptase (50 U/µL) (Applied Biosystems, Foster City, CA, USA). Two-fold serial dilutions of total RNA were made for efficiency calculations. Six serial dilutions (1000–31 ng RNA) in triplicates were analyzed in separate sample wells. Total RNA input was 500 ng in each reaction for all genes. Quality controls “no template controls” (ntc) and “no amplification controls” (nac) were run for quality assessment for each PCR assay.

Reverse transcription was performed at 48°C for 60 min by using oligo dT primers (2.5 µM) for all genes in 50 µL total volume. The final concentration of the other chemicals in each RT reaction was: $MgCl_2$ (5.5 mM), dNTP (500 mM of each), 10X TaqMan RT buffer (1X), RNase inhibitor (0.4 U/µL) and Multiscribe reverse transcriptase (1.67 U/µL) (Applied Biosystems). Twofold diluted cDNA (2.0 µL cDNA in each RT reaction) was transferred to 384-well reaction plates and the qPCR run in 10 µL reactions on the LightCycler 480 Real-Time PCR System (Roche Applied Sciences, Basel, Switzerland). Real-time PCR was performed using Sigma SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich and gene-specific primers (500 nM of each). PCR was achieved with a 5 min activation and denaturizing step at 95°C, followed by 45 cycles of a 10 s denaturing step at 95°C, a 20 s annealing step at 60°C and a 30 s synthesis step at 72°C. Mean normalized expression (MNE) of the target genes was determined using a normalization factor based upon EEF1A and

UBA52 for haddock, and ACTB and UBA52 for saithe (M-values <0.37 for haddock and <0.65 for saithe), as calculated by the *geNorm* software (Vandesompele et al. 2002).

Table 3. PCR primers, contig names, amplicon sizes and PCR efficiencies.

Gene ID	Gene name	Marker for	Contig name	Forward primer	Reverse primer	Amplicon size (bp)	PCR efficiency
Haddock							
CYP1A1	Cytochrome P450, family 1, subfamily A	Detoxification	>Soerhus-mRNA-2-dpf-1_CGATGT_L001_R1_001_(paired)_contig_3768	CTGCGCCACAAAAGACACAT	TTGAAGGTGGACGGTTCCTT	120	2.00
AHRR	Aryl-hydrocarbon receptor repressor	Detoxification	>Soerhus-mRNA-2-dpf-1_CGATGT_L001_R1_001_(paired)_contig_92712	AGCCAGACGCTGAACCTCAT	ATGCCGTGACCCTTGAACTC	122	1.96
p53	Tumor protein p53	DNA damage	>Soerhus-mRNA-2-dpf-1_CGATGT_L001_R1_001_(paired)_contig_2079	CCTGCTGAACTTCATGTGCAA	CCGAGAACATGCCCTTCAGA	102	1.84
GADD45A	growth arrest and DNA-damage-inducible, alpha	DNA damage	>Soerhus-mRNA-2-dpf-1_CGATGT_L001_R1_001_(paired)_contig_13955	ACGGTGTCAAAGGCAATCG	CTGGGTCCGCATTGAGAGAT	103	1.93
GADD45G	growth arrest and DNA-damage-inducible, gamma	DNA damage	>Soerhus-mRNA-2-dpf-1_CGATGT_L001_R1_001_(paired)_contig_5185	GTGCGCGTCAACGATATTGA	AAGGGTCTTTCCATGGGTTTG	121	1.70
PSTPIP2	proline-serine-threonine phosphatase interacting protein 2	Oxidoreductase activity?	>Soerhus-mRNA-2-dpf-1_CGATGT_L001_R1_001_(paired)_contig_85374	TGAGCAAAAGTGCCGAGACA	TGTCTGCTTCGTCAGCGTTT	128	1.81
EEF1A	Eukaryotic translation elongation factor 1 alpha 1	RefGen	>Soerhus-mRNA-2-dpf-1_CGATGT_L001_R1_001_(paired)_contig_221	CACATCGCCTGCAAGTTCAA	GGCTTGCTTGGGATCATGTT	128	1.88
UBA52	Ubiquitin A-52 residue ribosomal protein fusion product 1	RefGen	>Soerhus-mRNA-2-dpf-1_CGATGT_L001_R1_001_(paired)_contig_5918	TGAGGTCGAACCCAGTGACA	CTGCTTGCCAGCGAAGATC	103	1.88
ACTB	Beta actin	RefGen	>Soerhus-mRNA-2-dpf-1_CGATGT_L001_R1_001_(paired)_contig_877	ACAGCCGAGCGTGAGATTGT	TCGGGAAGCTCGTAGCTCTTC	125	1.84
Saithe							
CYP1A1	Cytochrome P450, family 1, subfamily A	Detoxification	>Contig251_Pollock2013	ACATGCCGCAGATCACGTT	CGTCTGCAAGGAGGGTGACT	119	2.08
AHRR	Aryl-hydrocarbon receptor repressor	Detoxification	>Contig105982_Pollock2013	TGTAGCAGCCAGACGCTGAA	CCTTGAACTCGTAGCCGTTGT	118	2.13
p53	Tumor protein p53	DNA damage	>Contig6924_Pollock2013	GCGCTAGTGCATCGTTGATC	CGCCGAAGAGGAGGAGAAG	92	1.85
GADD45A	growth arrest and DNA-damage-inducible, alpha	DNA damage	>Contig41816_Pollock2013	CTGGCTCTCTCCCAGGATCTC	TGCTGCGAAAACGACATCAA	75	1.92
GADD45G	growth arrest and DNA-damage-inducible, gamma	DNA damage	Contig14824_Pollock2013	AGACCTGCTGGTAGCTGCAAA	TCCTCATCGGTGGCAAGAAC	120	2.02
PSTPIP2	proline-serine-threonine phosphatase interacting protein 2	Oxidoreductase activity?	>Contig89427_Pollock2013	GCAGACGAAGCAGACAGGGTAT	GCGTTGGCATGTTCATTCAG	86	2.02
EEF1A	Eukaryotic translation elongation factor 1 alpha 1	RefGen	>Contig44_Pollock2013	CACGCTCTGCCTTCAGTTTG	CCACCGGCCACTTGATCTAC	135	2.03
UBA52	Ubiquitin A-52 residue ribosomal protein fusion product 1	RefGen	>Contig47489_Pollock2013	CAGGTGCAGGGTGGACTCTT	CTGACCAGCAGCGTCTGATC	95	1.75
ACTB	Beta actin	RefGen	>Contig33_Pollock2013	GCCCCACCAGAGCGTAAATA	CCTGCTTGCTGATCCACATCT	91	1.75

5.7. Statistical analyses

Data were tested for normal distribution directly or after being log-transformed (base 10). Data were tested for homogenous variances by ANOVA prior to Dunnett's test to determine which means were significantly different. If not normal distributed, test for statistical significant differences were performed with nonparametric comparisons with control using Steel method. Statistical analyses were performed with JMP ver. 12.0.1, SAS Institute Inc.

For fatty acid analyses, one-way ANOVA and Tukey (HSD) test as a post-hoc test were used. The Principle Component Analysis (PCA) was carried out using Sirius (Version 7.1, Bergen, Norway).

6. Results and discussion

6.1 Biological data

Haddock caught NW off Kristiansund and at the Halten Bank appeared to be in good condition with liver somatic indices of 3.2 and 4.0, respectively. Haddock from the Halten Bank were in average 1 year older than the haddock from NW off Kristiansund. The haddock caught at the Halten Bank also had significantly lower Fulton indices compared with haddock caught NW off Kristiansund (Table 4).

Table 4. Biological data of haddock. Data given as mean ± SD. Values in bold are significant different from haddock caught NW off Kristiansund.

Area	NW off Kristiansund	Halten Bank
Stations	291, 292, 293,295	503, 299, 301,367, 370
Females/males	14/7	8/23
Tot no	21	31
Length (cm)	39±6	41±10
Weight (g)	823±459	729±567
Liver weight (g)	27±20	28±27
Age (year)	2.3±0.7	3.3±1.9
LSI (%)	3.2±0.9	4.0±1.8
Fulton	1.34±0.23	**0.91±0.20**

The saithe from the Norwegian Sea also appeared in good condition, with LSI from mean from 3.7 to 4.5. Saithe caught at the Halten Bank were one year younger and smaller compared to saithe caught NW off Kristiansund. The Fulton index was lower in fish caught at the Halten Bank and at the Kristin area, compared with NW off Kristiansund (Table 5).

Table 5. Biological data of saithe. Data given as mean ± SD. Values in bold are significant different from haddock caught NW off Kristiansund.

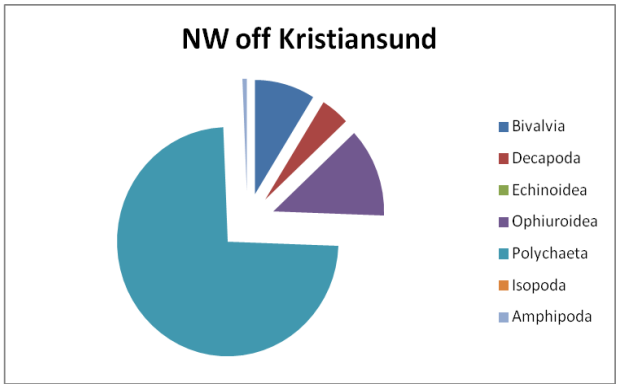
Area	NW off Kristiansund	Halten Bank	Kristin area
Stations	291, 292, 293,295	296, 298, 299, 361, 367	369, 370
Females/males	7/16	10/15	7/8
Tot no	23	24	15
Length (cm)	57±5	**51±9**	55±8
Weight (g)	2044±512	**1387±878**	1562±645
Liver weight (g)	92±30	**58±61**	70±50
Age (year)	6,0±0.9	**5.0±1.5**	6.1±1.4
LSI (%)	4.5±0.9	3.7±2.4	4.1±1.7
Fulton	1.10±0.07	**0.98±0.22**	**0.88±0.06**

6.2 Stomach content of haddock and saithe

Stomach analyses of haddock is given in Table 6 and visualised in Figure 5. Analyses of stomach content in saithe is given in Table 7. The data show haddock to be more benthic species feeding on invertebrates, while saithe is living more pelagic and feed on fish and crustaceans. For all tree stations krill were the most abundant crustaceans in saithe stomachs.

Table 6. Stomach content in sampled haddock from the Norwegian Sea given in g.

	N with content/ N empty	Bivalvia	Decapoda	Echinoidea	Ophiuroidea	Polychaeta	Isopoda	Amphipoda
NW off Kristiansund	13/7	6.1	2.9	0	8.7	51	0	0.5
Halten Bank	4/4	6.1	4.6	9.9	20.2	4.0	0.6	0.5



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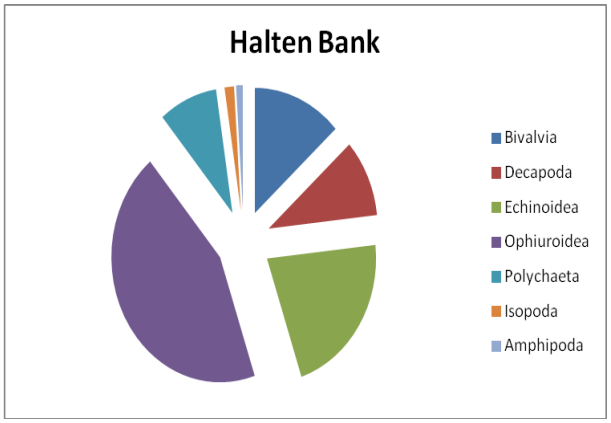


B

Figure 5. Stomach content in haddock visualized as % of sum weight. A: NW off Kristiansund, B: Halten Bank.

Table 7. Stomach content in sampled saithe from the Norwegian Sea given in g.

	N with content/N empty	Fish	Crustacea	Nematoda
NW off Kristiansund	16/6	179	88	1
Halten Bank	7/15	282	4	0
Kristin area	7/7	288	5	0

6.3 Levels of PAH and alkylated homologs in fish muscle

Analyses of PAH compounds in muscle of haddock and saithe were performed to document that oil and gas activities in the Norwegian Sea do not affect sea food quality. Analyses of polyaromatic hydrocarbons (PAH) including alkylated homologs were carried out using GC/MS. The compounds included in the analysis are shown in Table 8. Alkylated homologs are typical petrogenic compounds. PAH (EPA list of 16 compounds) is the sum of acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b,j,k)fluoranthene, benzo(ghi)perylene, chrysene, dibenzo(a,h)anthracene, dibenzothiophene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, pyrene.

Levels of detection (LOD) are defined as LOD: $Y = YB + 3SD_B$, and levels of quantification (LOQ) is LOQ= $Y = YB + 10SD_B$ where Y_B is the response of blank sample signal and SD_B is the standard deviation of the blank samples.

Levels of PAH and alkylated homologs in haddock and saithe muscle were below LOQ for all the components analysed (Table 8), and no contamination effecting sea food quality could be observed. Levels of PAH compounds below LOQ are as expected, as cod, haddock and saithe are lean fish species, and due to documentation from earlier measurements of PAH in muscle of cod and haddock below LOQ in Norwegian Seas (Grøsvik et al., 2012; Grøsvik et al., 2007).

Table 8. Levels of PAH compounds in haddock and saithe muscle caught in the Norwegian Sea. Presented as average ± stdev (ng/g wet weight). N= number of fish per station. Selected alkylated homologs are included in the PAH analyses. Single components in bold are accredited.

Compound	Haddock muscle Norwegian Sea N= 25	Saithe muscle Norwegian Sea N=25	LOQ
Naphthalene	< LOQ	< LOQ	0.2
2-Methylnaphthalene	< LOQ	< LOQ	0.2
1-Methylnaphtalene	< LOQ	< LOQ	0.2
2,6 -Dimethylnaphthalene	< LOQ	< LOQ	0.2
1,3-Dimethylnaphthalene	< LOQ	< LOQ	0.2
1,4 Dimethylnaphthalene	< LOQ	< LOQ	0.2
Acenaphthylene	< LOQ	< LOQ	0.2
Acenaphthene	< LOQ	< LOQ	0.2
1,3,7-Trimethylnaphthalene	< LOQ	< LOQ	0.2
2,3,5-Trimethylnaphtalene	< LOQ	< LOQ	0.2
1,2,3-Trimethylnaphthalene	< LOQ	< LOQ	0.2
1,4,6,7-Tetramethylnaphthalene	< LOQ	< LOQ	0.2
1,2,5,6-Tetramethylnaphthalene	< LOQ	< LOQ	0.2
Fluorene	< LOQ	< LOQ	0.2
Dibenzothiophene	< LOQ	< LOQ	0.2
Phenanthrene	< LOQ	< LOQ	0.2
Anthracene	< LOQ	< LOQ	0.2
4-methyldibenzotiofene	< LOQ	< LOQ	0.2
3-Methylphenanthrene	< LOQ	< LOQ	0.2

2-Methylphenanthrene	< LOQ	< LOQ	0.2
9-Methylphenanthrene	< LOQ	< LOQ	0.2
1-Methylphenanthrene	< LOQ	< LOQ	0.2
4-Ethyldibenzothiophene	< LOQ	< LOQ	0.2
3,6-Dimethylphenantrene	< LOQ	< LOQ	0.2
4-propyldibenzotiophene	< LOQ	< LOQ	0.2
1,7-Dimethylphenantrene	< LOQ	< LOQ	0.2
1,2-Dimethylphenanthrene	< LOQ	< LOQ	0.2
2,6,9-Triimethylphenantrene	< LOQ	< LOQ	0.2
1,2,6-Trimethylphenanthrene	< LOQ	< LOQ	0.2
(1,2,5+1,2,7)-Trimethylphenanthrene	< LOQ	< LOQ	0.2
1,2,6,9-Tetramethylphenantrene	< LOQ	< LOQ	0.2
Fluoranthene	< LOQ	< LOQ	0.2
Pyrene	< LOQ	< LOQ	0.2
Benz(a)anthracene	< LOQ	< LOQ	0.2
Chrysene	< LOQ	< LOQ	0.2
1-Methylchrysene	< LOQ	< LOQ	0.2
6-Ethylchrysene	< LOQ	< LOQ	0.2
6-Propylchrysene	< LOQ	< LOQ	0.2
Benzo(b)fluoranthene	< LOQ	< LOQ	0.2
Benzo(k)fluoranthene	< LOQ	< LOQ	0.2
Benzo(j)fluoranthene	< LOQ	< LOQ	0.2
Benzo(e)pyrene	< LOQ	< LOQ	0.2
Benzo(a)pyrene	< LOQ	< LOQ	0.2
Perylene	< LOQ	< LOQ	0.2
Indeno(1,2,3-cd)pyrene	< LOQ	< LOQ	0.2
Dibenz(a,h)anthracene	< LOQ	< LOQ	0.2
Benzo(g,h,i)perylene	< LOQ	< LOQ	0.2
SUM PAH	< LOQ	< LOQ	

6.4 PAH metabolites in bile of haddock

PAH metabolites in bile of haddock sampled in the Norwegian Sea in 2014 were compared with bile of haddock sampled in the Barents Sea in 2012 and in the North Sea of 2013. Levels of 20 different metabolites are presented in Tables 9 and 10. Levels of sum of the 20 bile metabolites are presented in Figure 6. Sum bile metabolites depend on how many compounds we have standards for, and is therefore a relative number, but used to visualize these levels between the different areas and stations. For sum bile metabolites, only levels in haddock from the Egersund Bank (sampled in 2013) were significantly different from haddock from the Barents Sea (sampled in 2012).

Background assessment criteria of PAH metabolites in bile is set for haddock for 1-hydroxyphenantrene to be 0.8 ng/ml, and 1-hydroxypyrene to be 13 ng/ml (ICES, 2013). We do not have EAC levels for bile metabolites in haddock, but EAC levels in cod for 1-hydroxyphenantrene and 1-hydroxypyrene is given to 528 and 483 ng/g bile, respectively (ICES, 2013). For 1-hydroxyphenathrene, mean levels varied from 1.8 ng/g bile (Halten Bank 2014) to 21 ng/g bile (Viking Bank 2013) (Tables 9 and 10).

For 1-hydroxypyrene, haddock from Austbanken and the Barents Sea-1 had levels below BAC, the other groups had levels from 15 ng/g bile (Barents Sea-3) to 56 ng/g bile (Off Kristiansund (Table 9 and 10).

Levels of 1-hydroxyphenantrene or 1-hydroxypyrene in the haddock analysed were below EAC levels for these metabolites in cod.

Table 9. PAH metabolites in bile of haddock.
Values given as mean ± std dev in ng/g bile. N= number of fish per station.

Station	**2014-Off Kristiansund**	**2014-Halten Bank**	**2012-H1**	**2012-H3**	**LOQ**
Area	**Norwegian Sea**	**Norwegian Sea**	**Barents Sea-1**	**Barents Sea-3**	
Position	**63.19º N 6.75 E**	**64.72 N 8.82º E**	**71.191º N 25.074º E**	**75.69º N 20.82º E**	
N	N=15	N=29	N=21	N=4	
1-Naphthol	4.01±3.55	1.98±3.36	1.03±0.80	1.27±0.98	0.45
2-Naphthol	4.71±2.61	2.57±4.07	2.57±4.07	2.67±1.44	0.23
Σ Naphthol	**8.72±5.58**	**4.55±6.16**	**4.65±4.05**	**3.95±1.17**	
7-Methyl-1-Naphthol/8-methyl-2-naphthol	7.56±7.88	0.74±1.02	4.20±4.98	0.36±0.33	0.10
2-Methyl-1-naphthol/3-methyl-1-naphthol	2.11±1.65	0.82±0.81	2.76±5.64	2.31±0.36	0.05
6-Methyl-1-naphthol	4.16±4.61	0.65±0.82	4.28±5.54	4.21±2.73	0.06
3-methyl-2-naphthol	7.15 ±16.9	1.65±1.79	2.19±2.08	2.33±1.63	0.07
7-methyl-2-naphthol	0.85±0.73	0.87±2.20	1.07±1.34	0.69±0.46	0.11
6-methyl-2-naphthol/4-methyl-1-naphthol	3.70±2.57	1.71±1.78	3.51±3.79	1.91±1.52	0.24
5-methyl-1-naphthol/1-methyl-2-naphthol	0.58±0.44	0.70±1.14	0.34±0.30	0.45±0.60	0.03
4-methyl-2-naphthol	1.25±0.87	1.27±1.84	0.93±0.78	0.60±0.22	0.08
5-methyl-2-naphthol	1.19±1.09	0.95±0.95	0.74±0.91	0.40±0.34	0.06
Σ C1/C2 Naphthol	**28.6±26.1**	**9.4±5.6**	**20.0±19.1**	**13.2±6.9**	

2-Hydroxyfluorene	30.1±21.0	15.3±11.9	23.3±31.7	12.1±5.7	0.20
9-Hydroxyfluorene	41.1±21.6	39.4±28.8	34.2±37.1	38.5±26.3	7.28
Σ Hydroxyfluorene	**71±37**	**55±34**	**58±63**	**51±31**	
4-Hydroxyphenanthrene	3.36±4.81	2.20±7.08	3.31±5.09	0.57±0.29	0.04
9-Hydroxyphenanthrene	3.06±3.36	1.34±1.30	2.16±3.53	4.26±3.65	0.08
1-Hydroxyphenanthrene	3.96±4.67	1.83±2.93	3.37±3.53	3.21±2.94	0.22
3-Hydroxyphenanthrene	1.80±1.50	1.68±1.29	3.91±4.80	1.60±1.26	0.09
2-Hydroxyphenanthrene	7.47±5.21	3.82±2.67	5.57±5.90	3.83±1.98	0.18
Σ Hydroxyphenanthrene	**19.6±12.7**	**10.9±11.2**	**18.3±18.2**	**13.5±7.7**	
1-Hydroxychrysene	0.05±0.06	0.03±0.03	0.05±0.06	0.03±0.04	0.003
1-Hydroxypyrene	55.9±36.0	20±14	12.2±9.9	14.6±10.6	3.11
Σ PAH metabolites	**184±92**	**100±54**	**113±97**	**96±38**	

Table 10. PAH metabolites in bile of haddock.

Values given as average ± std dev in ng/g bile. N= number of fish per station.

Station	**2013-2H**	**2013-3H**	**2013-H3**	**2013-H4**	**LOQ**
Area	**North Sea**	**North Sea**	**North Sea**	**North Sea**	
Area	**Egersund Bank**	**Viking Bank**	**Southern North Sea**	**Austbanken**	
Position	**57.845º N 4.862º E**	**60.372º N 2.602º E**	**57.720º N 0.468º E**	**57.843º N 3.410º E**	
N	N=20	N=6	N=11	N=9	
1-Naphthol	1.98±1.72	1.00±0,45	0.73±1.05	1.34±0.97	0.45
2-Naphthol	6.16±7.40	2.42±1.10	1.97±0.94	2.02±0.89	0.23
Σ Naphthol	**8.14±7.80**	**3.42±1.30**	**2.70±1.83**	**3.36±1.73**	
7-Methyl-1-Naphthol/8-methyl-2-naphthol	4.13±4.43	1.49±1.44	0.75±0.88	0.22±0.12	0.10
2-Methyl-1-naphthol/3-methyl-1-naphthol	2.42±4.42	0.84±0.84	0.74±0.70	0.35±0.24	0.05
6-Methyl-1-naphthol	12.9±13.3	2.60±1.84	3.61±2.15	1.11±1.23	0.06
3-methyl-2-naphthol	5.13 ±5.22	1.84±0.85	1.20±0.90	1.87±1.51	0.07
7-methyl-2-naphthol	2.87±7.02	0.26±0.15	0.58±0.80	0.15±0.18	0.11
6-methyl-2-naphthol/4-methyl-1-naphthol	5.40±6.12	2.48±1.35	1.73±0.90	2.04±0.92	0.24
5-methyl-1-naphthol/1-methyl-2-naphthol	1.88±3.61	0.88±1.49	0.27±0.16	0.22±0.11	0.03
4-methyl-2-naphthol	3.97±9.13	0.85±0.34	0.60±0.32	10.5±14.8	0.08
5-methyl-2-naphthol	2.27±3.71	0.54±0.39	0.43±0.29	0.60±1.20	0.06
Σ C1/C2 Naphthol	**40.9±37.1**	**11.8±5.5**	**9.9±3.2**	**17.1±15.7**	
2-Hydroxyfluorene	55.8±93.2	10.2±6.8	18.9±15.7	37.9±23.9	0.20
9-Hydroxyfluorene	43.6±44.9	29.7±29.1	33.8±66.2	15.8±11.3	7.28
Σ Hydroxyfluorene	**99±133**	**40±34**	**53±78**	**53±28**	
4-Hydroxyphenanthrene	6.45±8.80	0.62±0.37	0.95±1.00	0.89±0.49	0.04
9-Hydroxyphenanthrene	1.58±2.22	2.32±2.38	1.25±1.27	0.70±0.56	0.08
1-Hydroxyphenanthrene	5.13±6.00	20.9±27.6	2.94±2.25	3.29±2.25	0.22
3-Hydroxyphenanthrene	50.6±77.0	1.27±0.94	6.37±10.4	11.2±14.8	0.09
2-Hydroxyphenanthrene	13.8±19.8	5.02±1.86	4.37±2.69	5.89±2.67	0.18

Σ Hydroxyphenanthrene	**78±99**	**30±26**	**16±15**	**22±17**	
1-Hydroxychrysense	0.30±0.78	0.04±0.05	0.06±0.09	0.14±0.11	0.003
1-Hydroxypyrene	35.4±20.9	18.9±13.8	17.7±24.0	7.35±5.30	3.11
Σ PAH metabolites	**262±249**	**104±43**	**99±99**	**104±45**	

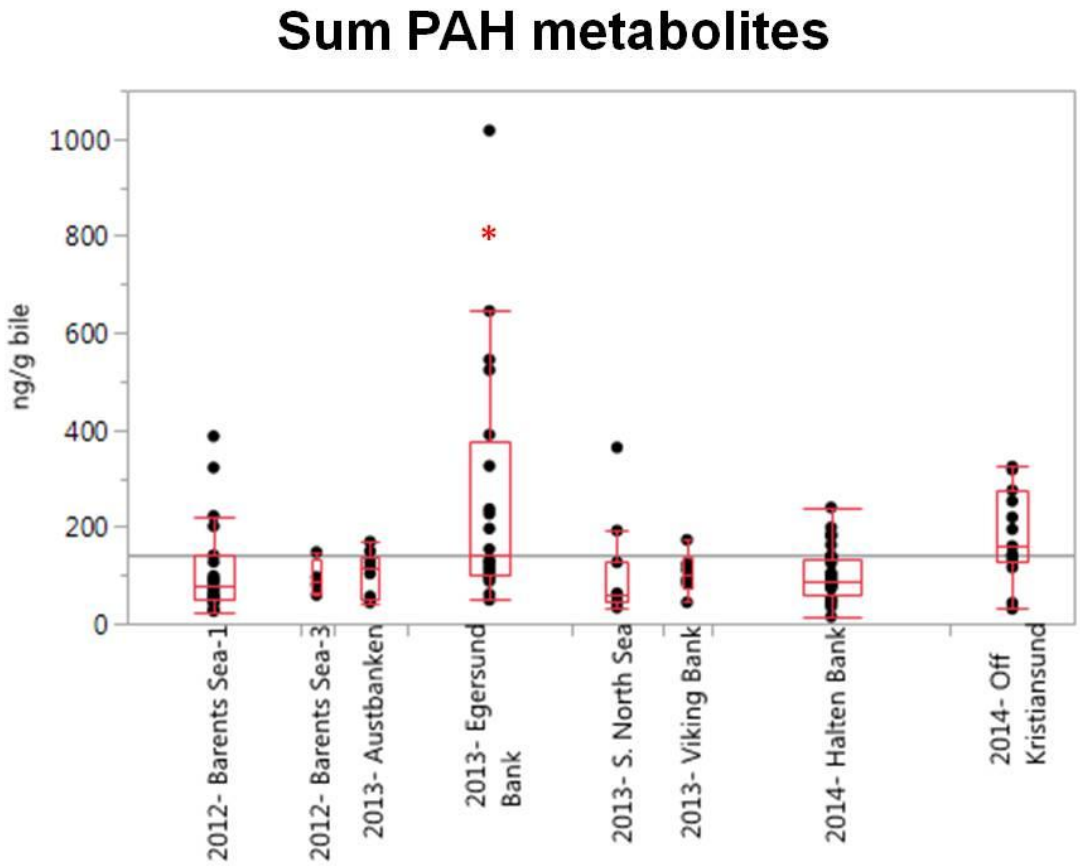


Figure 6. Sum PAH metabolites in bile of haddock from the Norwegian Sea in 2014 compared with bile from haddock from the Barents Sea in 2012 and haddock from the North Sea in 2013 as box plot with median, 10, 25 75 and 90% quantiles. N is given in Tables 9 and 10. Levels in Egersund Bank-2013 were found to be significantly different to levels in Barents Sea-1 from 2012, $p < 0.05$).

6.5 DNA adducts in liver of haddock

The absorbance ratios A_{260}/A_{280} and A_{260}/A_{230} obtained on the whole 100 DNA samples were 1.92 ± *0.16* and 2.10± *0.12*, respectively. These experimental ratios are satisfying with regard to the usual requirements of the ^{32}P-postlabelling method.

Levels of DNA adducts in liver of haddock from the Halten Bank and off Kristiansund were compared with DNA adduct levels of haddock sampled from one station in the Barents Sea in 2012, three stations from the North Sea in 2013 and one station from Tampen from 2011. Haddock from the Halten Bank region had mean DNA adduct levels of 6.6±7.3 adducts per nmol normal adducts or relative adduct level (RAL). Haddock off Kristiansund had DNA adduct levels of 5.6±6.1 RAL. Haddock from the Barents Sea had DNA adduct levels of 2.5±3.1 RAL, while haddock from the North Sea from 2013 had levels from 3.3±3.5 (Southern North Sea) 4.8±5.5 (Austbanken) and 8.6±4.0 (Egersund Bank). DNA adduct levels in haddock from Tampen (between Statfjord and Gullfaks), from the condition monitoring of 2011 is added for comparison. These fish had mean levels of 7.3±5.6 RAL (Grøsvik et al., 2012).

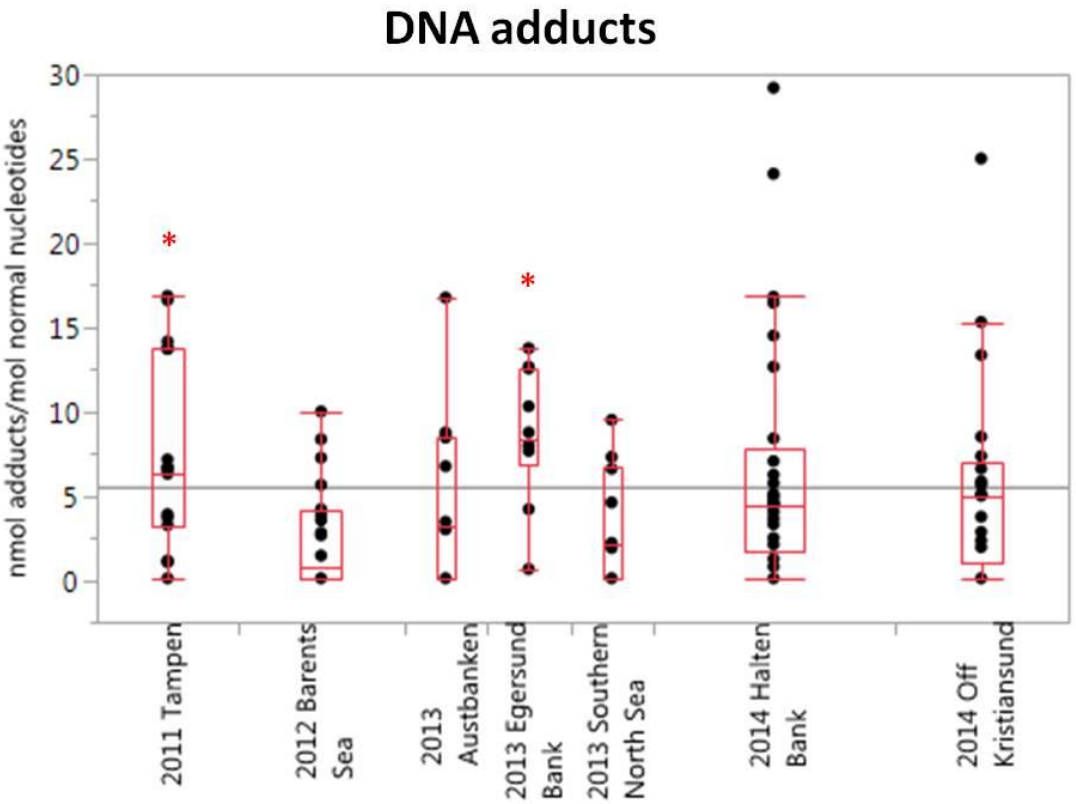


Figure 7. Levels of DNA adducts in liver of haddock from the Halten Bank compared with DNA adduct levels of haddock sampled off Kristiansund, in the Barents Sea in 2012 and from the North Sea in 2013. Shown is box plot with median, 10, 25 75 and 90% quantiles. N=17 (Tampen 2011), N= 20 (Barents Sea 2012), N=10 (Southern North Sea 2013), N=10 (Austbanken 2013), N=21 (Off Kristiansund 2014) and N=29 Halten Bank 2014. (*) indicates significant differences from Barents Sea 2012 (Nonparametric comparisons with control using Steel method, $p < 0.05$).

Haddock from the Egersund Bank 2013 and Tampen 2011 had levels above environmental assessment criteria (EAC) for haddock (> 6.7 RAL) as defined by ICES (ICES, 2011), and had significantly higher levels of DNA adducts compared with haddock from the Barents Sea 2012. Haddock from the Halten Bank 2014 were just below this threshold (Figure 7). The RAL at the Egersund Bank of 2013 were higher compared with RAL at the Egersund Bank of 2011, 8.6±4.0 vs 5.5±7.1, respectively (Grøsvik et al., 2012).

If we compare the RAL levels in haddock from the Barents Sea of 2008 with what we found in 2012, we also see an increase, from 0.8±0.9 to 2.5±3.1, respectively (Grøsvik et al., 2009).

Levels of detection (LOD) for DNA adducts were set to 0.1 RAL, although adducts may be present even if they are not above the detection limit. For the station with highest levels of DNA adducts, Egersund Bank 2013, 100 % of the haddock had DNA adduct. 83 % of haddock from the Halten Bank had adducts, while for haddock from the Barents Sea in 2012, 50 % of the fish had DNA adducts (Table 11).

Table 11. Percentage of haddock livers with DNA adducts. LOD were 0.1 RAL.

Site	Number of samples	% with DNA adducts
Barents Sea 2012	20	50
Southern North Sea 2013	10	60
Austbanken 2013	10	60
Egersund Bank 2013	10	100
NW off Kristiansund 2014	21	76
Halten Bank 2014	29	83

The results indicate higher levels of DNA adducts at the Egersund Bank in 2013 compared with results from the Condition Monitoring in 2005, 2008 and 2011 (Grøsvik et al., 2012). For haddock from the Barents Sea, higher levels were reported in 2012 compared with 2005. DNA adduct levels in haddock from the Halten Bank region and NW off Kristiansund was comparable with levels found in haddock from the North Sea from 2011 and 2013. DNA adduct levels from the Halten Bank were 6.6 RAL which is close to the EAC value of 6.7 RAL. In general, the results with DNA adduct of haddock were higher than expected based on earlier Condition monitorings both for the station at Egersund Bank, the Norwegian Sea and the Barents Sea, and could indicate an increased levels of PAH compounds leading to DNA adducts for all areas since we started these measurements in 2002 (Balk et al., 2011; Grøsvik et al., 2007; Grøsvik et al., 2009 and Grøsvik et al., 2012).

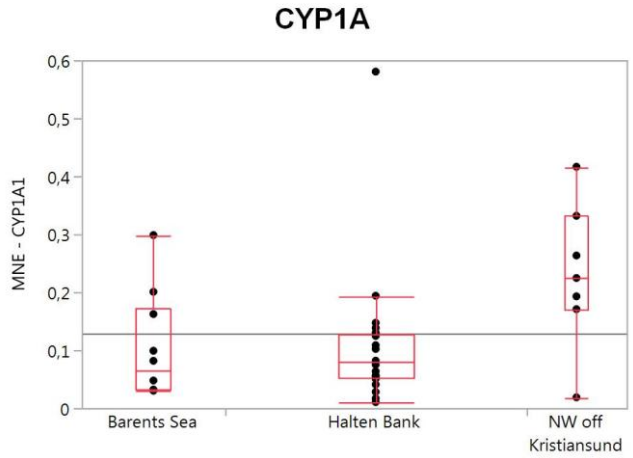
A more detailed discussion of the DNA adducts are given in the analytical report in Appendix 9.3.

6.6 RT-qPCR of selected gene transcripts in haddock and saithe

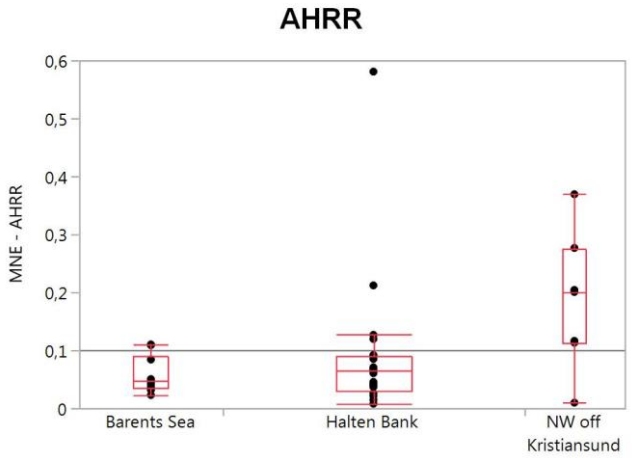
For qPCR analyses liver of and haddock and saithe, we selected species specific primers to gene transcripts known to be affected by PAH as CYP1A and Ah receptor repressor (AHRR) (Meyer et al., 2003). In addition we wanted to test effects in gene transcripts that are involved in DNA repair processes like growth arrest and DNA damage inducible proteins (GADD) like GADD45A and GADD45G, the DNA repair protein p53 (Siafakas &Richardson, 2009) and proline-serine-threonine phosphatase-interacting protein 2 **(**PSTPIP2), a cytoskeletal associated protein which was selected because it has been induced in other studies (Olsvik and Grøsvik in prep).

Haddock

Transcript levels of CYP1A, AHRR and GADD45G were not significantly different between Halten Bank, off Kristiansund and the Barents Sea, while levels of GADD45A were significantly higher at Halten Bank and NW off Kristiansund compared with haddock from the Barents Sea. Transcript levels of P53 were significantly higher in haddock from the Halten Bank compared with haddock from the Barents Sea (Figure 8).



A



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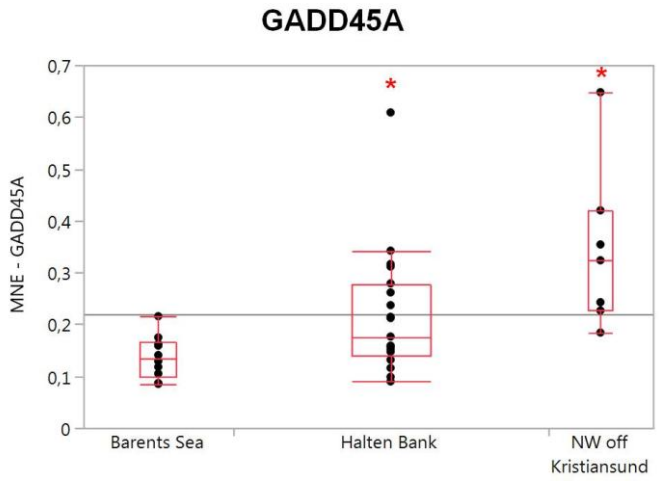

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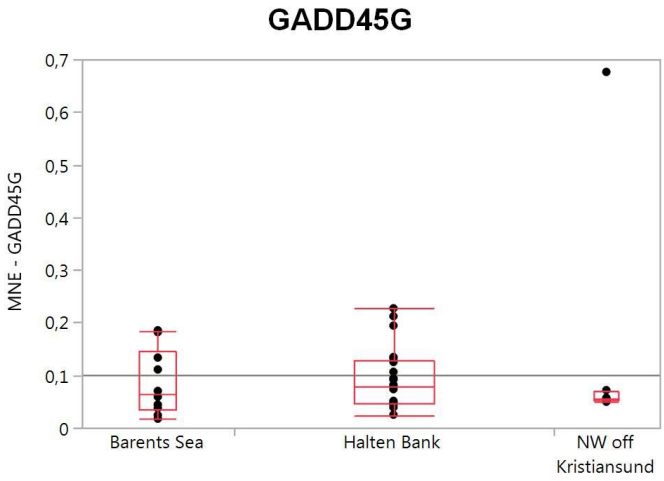

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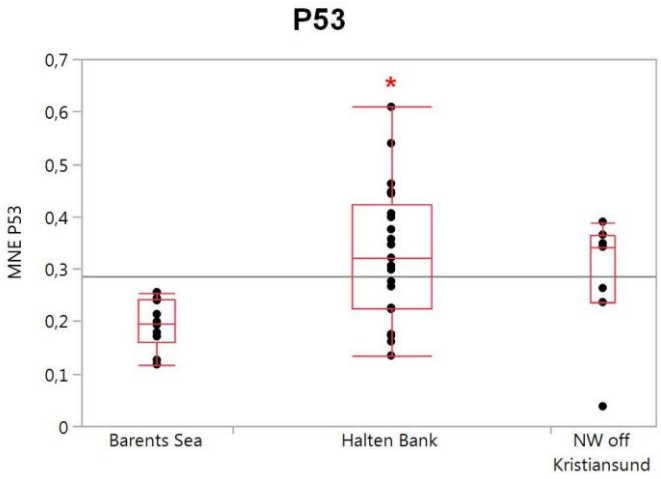


E

Figure 8. Mean normalized expression (MNE) of target genes in liver of haddock. CYP1A (A); AHRR (B); GADD45A (C); GAD45G (D); P53 (E). For off Kristiansund: N= 16 male, Western Halten Bank N= 5 male, Halten Bank: N = 7 male and Barents Sea: N =5 male and 5 female). Shown is box plot with median, 10, 25 75 and 90% quantiles. The haddock from the Barents Sea was sampled in 2008. Different letters indicate significant difference, $p<0.05$.

Multivariate analyses of RT-qPCR levels, DNA adducts and sumPAH metabolites

Multivariate analyses with RT-qPCR transcripts together with data on SumPAH metabolites and DNA adducts demonstrate correlations between bile metabolites and transcript levels of CYP1A and AHRR, but not between DNA adducts and sum PAH metabolites (Table 12). DNA adducts correlated with GADD45A, and CYP1A levels correlated with sum PAH metabolites, AHRR and GADD45G (Table 12). The correlation studies support that GADD proteins are interesting proteins to study connected to measurements DNA adducts and DNA damage repair processes. The results indicate that it is a challenge to compare transient changes in low levels of PAH metabolites in bile and gene expression levels in liver with DNA adducts which are longer lived covalent bonds. This should be further investigated from laboratory exposure for evaluation for future monitoring.

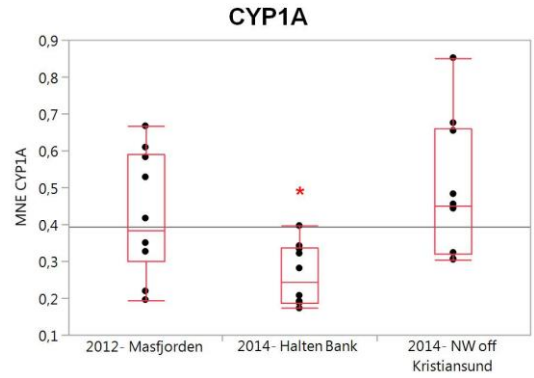
Table 12. Correlations for MNE levels, sum PAH metabolites and DNA adducts for haddock from multivariate analyses. Red and bold numbers correspond to correlations with probability < 0.05.

	Sum PAH metabolites	DNA adducts	CYP1A1	GADD45A	p53	AHRR	PSTPIP2	GADD45G
Sum PAH metabolites	**1,00**	0,03	**0,46**	0,02	0,13	**0,47**	0,24	-0,32
DNA adducts	0,03	**1,00**	-0,03	**0,52**	0,28	0,02	0,24	0,14
CYP1A1	**0,46**	-0,03	**1,00**	0,14	0,08	**0,89**	0,16	**-0,33**
GADD45A	0,02	**0,52**	0,14	**1,00**	0,26	0,22	0,08	**0,44**
p53	0,13	0,28	0,08	0,26	**1,00**	0,23	**0,53**	-0,20
AHRR	**0,47**	0,02	**0,89**	0,22	0,23	**1,00**	0,15	-0,31
PSTPIP2	0,24	0,24	0,16	0,08	**0,53**	0,15	**1,00**	-0,27
GADD45G	-0,32	0,14	**-0,33**	**0,44**	-0,20	-0,31	-0,27	**1,00**

Saithe

Saithe used for qPCR analyses were sampled NW off Kristiansund (Stations 292, 293 and 295) and at the Halten Bank (stations 296, 299 and 361) as indicated in Figure 9. As we did not have any reference material from the Barents Sea we used saithe sampled from a reference site in Masfjorden, Hordaland in 2012 for comparison.

For saithe we found a significant reduction in expression of CYP1A in fish caught at the Halten Bank, while GADD45G were significantly upregulated compared to saithe from NW off Kristiansund. The same pattern was indicated for GADD45A, but the differences were not significant. No significant changes were seen for P53 (Figure 9).



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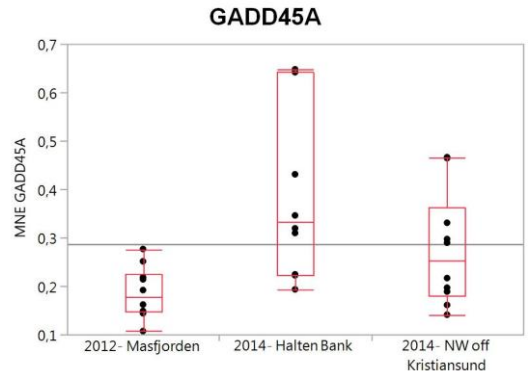

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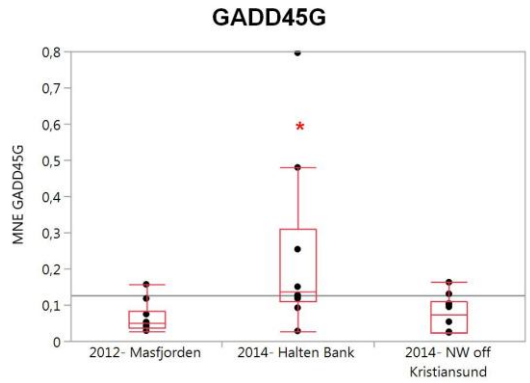

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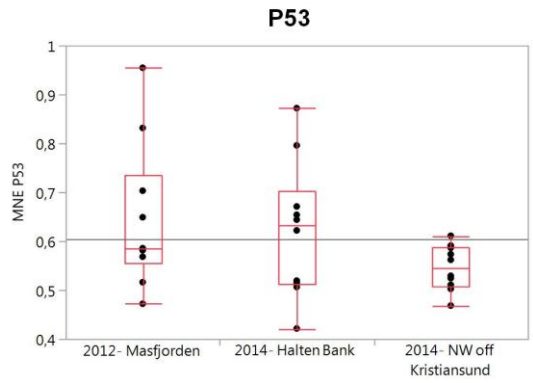

D

Figure 9. Mean normalized expression (MNE) of target genes in liver of male saithe. CYP1A (A); GADD45A (B); GADD45A (C); P53 (D). N=10 for all stations. Shown is box plot with median, 10, 25 75 and 90% quantiles. (*) indicate significant different from NW off Kristiansund, $p < 0.05$.

Correlations of RT-qPCR levels in saithe

For saithe we also could see correlations between expression patterns of GADD45A, GADD45G and p53 (Table 13). For this material, we did not have data on PAH metabolites and DNA adducts.

Table 13. Correlations for qPCR data for saithe multivariate analyses.
Red and bold numbers correspond to correlations with probability < 0.05.

	CYP1A1	GADD45A	p53	AHRR	PSTPIP2	GADD45G
CYP1A1	**1,00**	-0,25	-0,09	0,35	0,16	-0,34
GADD45A	-0,25	**1,00**	**0,36**	-0,03	-0,28	**0,35**
p53	-0,09	**0,36**	**1,00**	-0,11	-0,17	-0,17
AHRR	0,35	-0,03	-0,11	**1,00**	0,07	-0,13
PSTPIP2	0,16	-0,28	-0,17	0,07	**1,00**	-0,24
GADD45G	-0,34	**0,35**	-0,17	-0,13	-0,24	**1,00**

6.7 Fatty acid profiles in phytoplankton and zooplankton

Phytoplankton

Water samples collected together with CTD measurement (529 ml) were filtered on to micro filters and the fatty acid composition of the phytoplankton was analyzed by GC-FID. Three samples were taken at 5, 10 and 20 m per station. Locations for sampling are shown in Figure 10. The fatty acid profiles are shown in Table 16. Figure 11 shows a PCA of the fatty acids. The ratio of (n-3)/(n-6) poly unsaturated fatty acids (PUFA) are shown in Figure 12. The ratio varied with different sampling depth for some of the stations. The ratio varied from 7.4 to 23.6 (Figure 12), no significant differences (ANOVA) were found between the different stations.

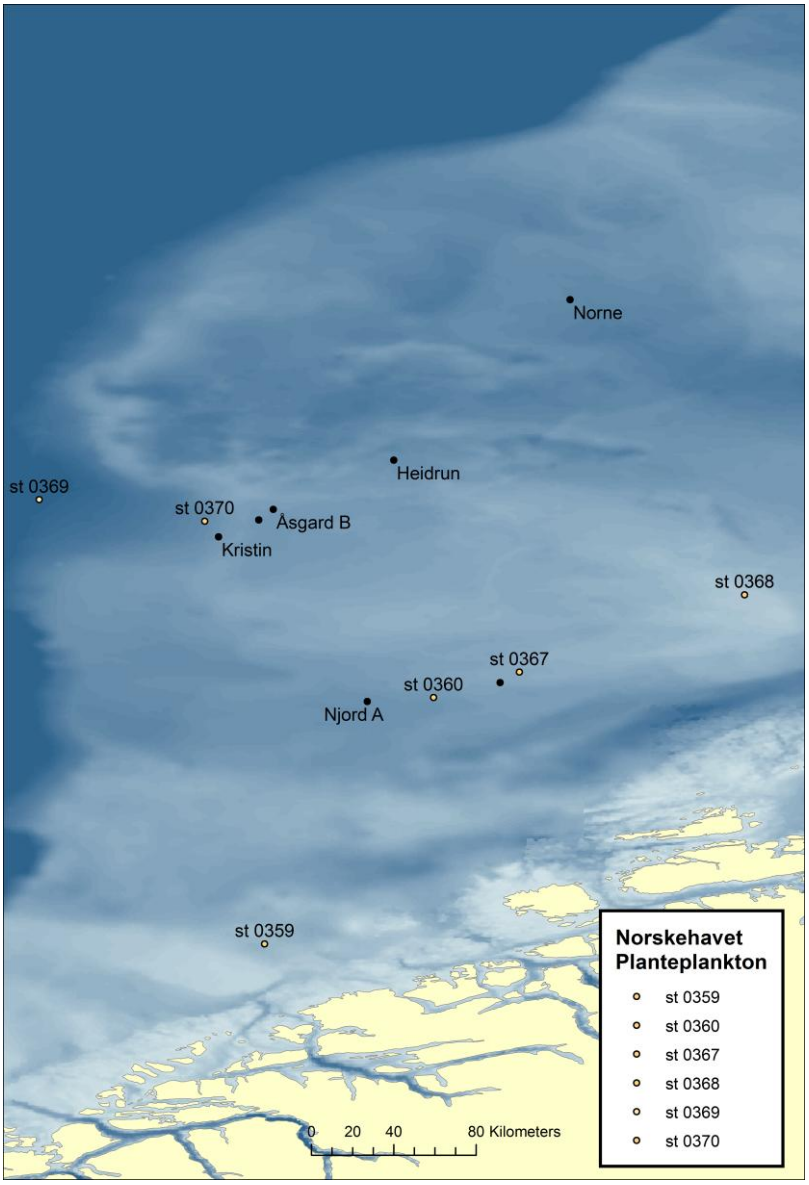

Figure 9. Map of station for phytoplankton sampling (yellow circles) and oil and gas installations in the Norwegian Sea (black circles).

Table 16. Fatty acids profile (% of total FA) sum saturated fatty acids (SFA), sum monounsaturated fatty acids (MUFA), sum polyunsaturated fatty acids (PUFA), sum (n-6) PUFA, sum (n-3) PUFA, the ratio of

(n-3)/(n-6) and FA in µg/L in phytoplankton collected at 10 m. A table of individual fatty acid measurements for phytoplankton collected at 5, 10 and 20 m is shown in Appendix 8.2.

	ST359 10 M	ST360 10 M	ST367 10 M	ST368 10 M	ST369 10 M	ST370 10 M
∑SFA	51,3	43,5	29,9	42,6	49,8	40,2
∑MUFA	15,7	19,7	16,4	16,9	21,0	24,1
∑PUFA	33,8	38,2	54,0	41,7	30,3	37,1
∑(n-6)PUFA	3,0	3,9	2,2	3,8	2,0	3,6
∑(n-3)PUFA	28,1	32,2	50,9	34,6	24,6	32,3
(n-3)/(n-6)	9,4	8,3	23,6	9,0	12,2	9,1
FA (µg/L)	20,2	18,0	126,6	23,4	34,7	24,9

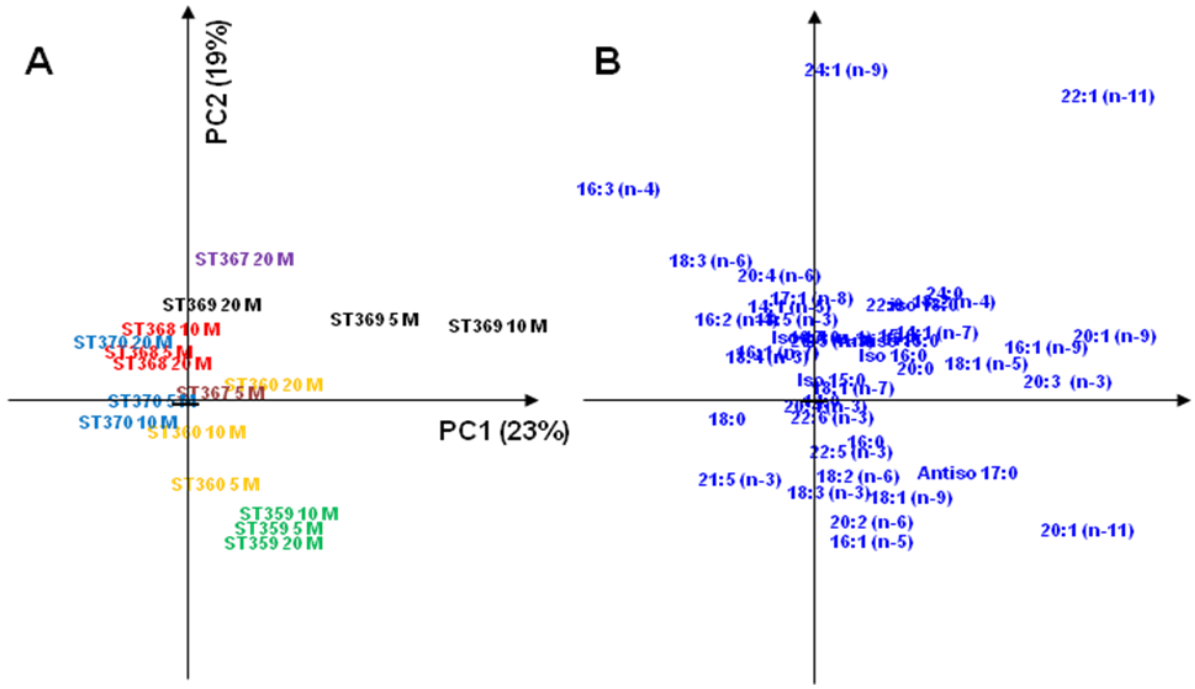

Figure 10. Principal component analyses of the fatty acid profiles in phytoplankton. A: Score plot, B: Loading plot. Water sampled at 5, 10 and 20 m depth (529 ml) and filtered. Phytoplankton sampled at different depth from each station have same colour.

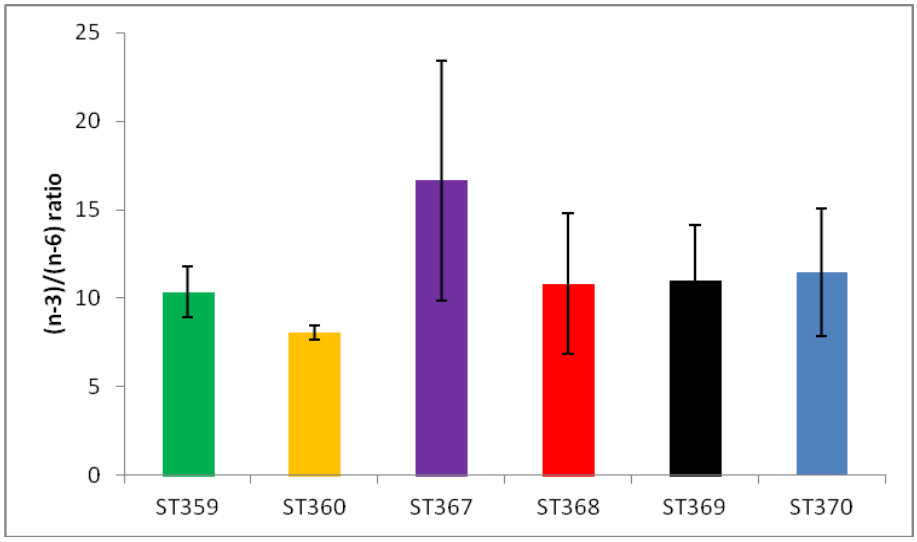

Figure 11. Ratio of (n-3)/(n-6) PUFA in phytoplankton from the Norwegian Sea as average for 3 different depths ± SD. Colour similar to PC analyses in Figure 10.

Zooplankton

Fatty acid composition of the zooplankton were analysed by GC-FID. Zooplankton was sampled by WP2 trawl from 80m to surface from locations as shown in Figure 12. Samples were filtered at 180 and 1000 µm and sum fatty acid profiles of the different fractions are shown in Table 17. A table of individual fatty acid measurements for zooplankton and a table of fatty fatty alcohols profile (% of total FA-ALK) in zooplankton are shown in Appendix 8.2.

A PCA of the fatty acids profile are shown in figure 13 and the ratio of (n-3)/(n-6) PUFA are shown in Figure 14. No significant differences (ANOVA) in the (n-3)/(n-6) ratio were found between the different stations. Similar measurements of zooplankton from the North Sea in 2011 had (n-3)/(n-6) ratio from 14 to 18 (Grøsvik et al., 2012).

Table 17. Fatty acids profile (% of total FA) sum saturated fatty acids (SFA), sum monounsaturated fatty acids (MUFA), sum polyunsaturated fatty acids (PUFA), sum (n-6) PUFA, sum (n-3) PUFA, the ratio of (n-3)/(n-6) and FA in µg/L in zooplankton filtered at 180 and 1000 µm.

	St359 180µm	St359 1000µm	St360 180µm	St360 1000µm	St368 180µm	St368 1000µm	St370 180µm	St370 1000µm
∑SFA	30,0	32,9	35,9	33,3	33,5	32,8	33,5	32,1
∑MUFA	22,4	22,9	29,8	18,1	25,0	24,7	32,6	18,6
∑PUFA	47,6	44,2	34,3	48,6	41,5	42,5	34,0	49,3
∑(n-6)PUFA	3,1	2,4	4,7	2,5	2,9	2,2	5,3	2,6
∑(n-3)PUFA	43,1	39,7	27,7	44,3	36,7	36,4	27,4	44,7
(n-3)/(n-6)	14,0	16,5	5,8	17,5	12,8	16,8	5,1	16,9
FA (mg/100 mg sample)	2,0	1,4	2,0	2,5	3,5	4,6	2,1	1,2

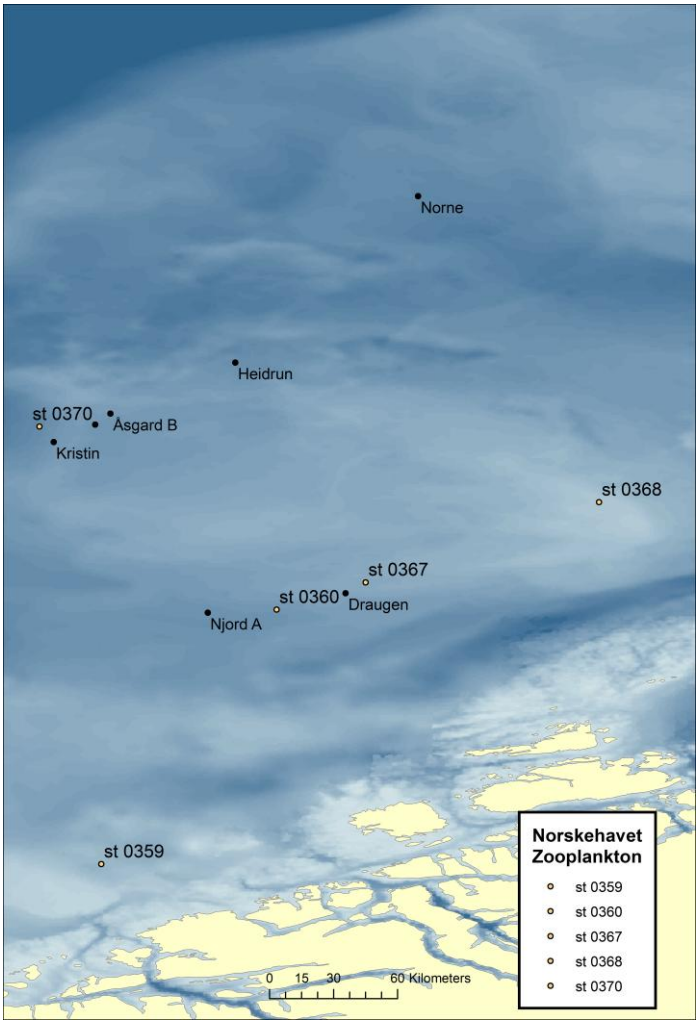

Figure 12. Map of station for zooplankton sampling (yellow circles) and oil and gas installations in the Norwegian Sea (black circles).

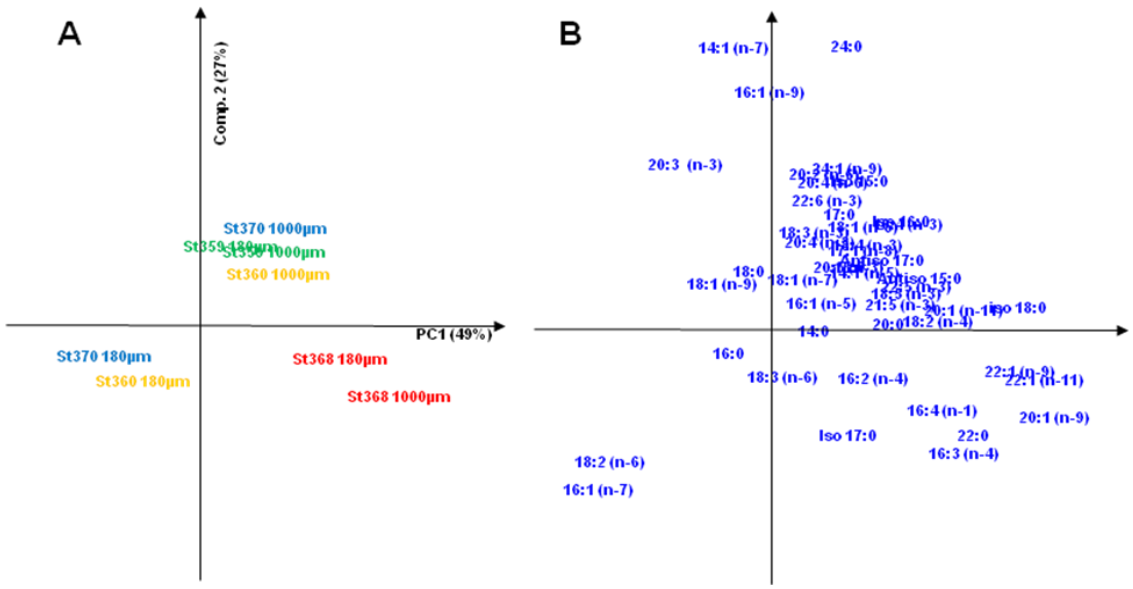

Figure 13. Principal component analyses (PCA) of fatty acid profiles in zooplankton. A: Score plot, B: Loading plot, Zooplankton were filtered at 180 and 1000 µm.

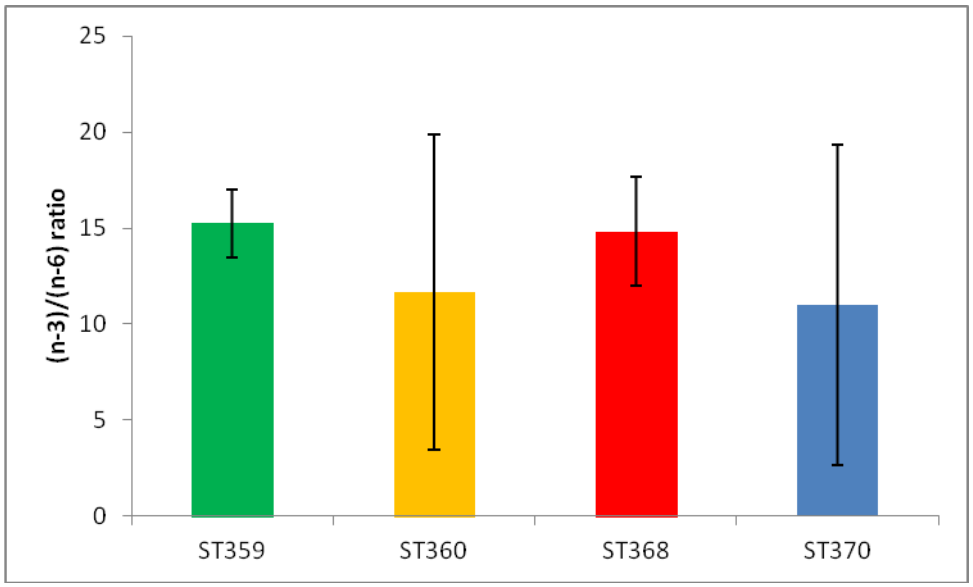

Figure 14. Ratio of (n-3)/(n-6) PUFA in zooplankton from the Norwegian Sea. Zooplankton was filtered at 180 and 1000 µm. Colours similar to group in PC analyses in Figure 13.

The fatty acid analyses of phytoplankton and zooplankton are conducted to look for differences in (n-3)/(n-6) PUFA ratio. There have been several reports that have found that fish caught in the North Sea with high oil and gas activities and discharges of produced water had high levels of (n-6) PUFA and lower levels of (n-3) PUFA (Balk et al, 2011; Grøsvik et al., 2012). In the present study no fish samples were analyzed due to limited budget.

In this study, we did not find any significant differences in the FA profiles of phyto- and zooplankton between the different stations.

7. Conclusion

Levels of PAH in all haddock and saithe muscle measured were below levels of quantification (LOQ). Low levels of PAH metabolites were measured in haddock bile from the Norwegian Sea. Only haddock from the Egersund Bank caught in 2013 had significant higher levels than haddock from the Barents Sea from 2012. No significant differences in n-3/n-6 ratio or fatty acids profile in phytoplankton or zooplankton were shown for fatty acid profiles.

Levels of DNA adducts in liver of haddock from the Norwegian Sea were compared with DNA adduct levels of haddock sampled from one station in the Barents Sea in 2012 and three stations from the North Sea in 2013. Haddock from the Egersund Bank 2013 had levels above environmental assessment criteria (EAC) for haddock (> 6.7 RAL) as defined by ICES, and had significantly higher levels of DNA adducts compared with haddock from the Barents Sea 2012.

Haddock caught NW off Kristiansund and at the Halten Bank region had DNA adduct levels comparable with haddock from the North Sea from 2011 and 2013. DNA adduct levels from the Halten Bank had levels close to the EAC value of 6.7 RAL and rises concern with regard to PAH exposure of haddock caught in this area. In general, the results with DNA adducts of haddock were higher than expected based on earlier Condition monitoring exercises both for the station at Egersund Bank, the Norwegian Sea and the Barents Sea, and could indicate an increased levels of PAH compounds leading to DNA adducts for all areas since we started these measurements in 2002.

Mean levels of CYP1A were not significantly different between haddock from the Halten Bank and the Barents Sea. Multivariate analyses demonstrated correlations between PAH metabolites and transcript levels of CYP1A and AHRR, but not between DNA adducts and sum PAH metabolites. DNA adducts correlated with GADD45A, and CYP1A levels correlated with sum PAH metabolites, AHRR and GADD45G. The multivariate analyses supported GADD proteins to be interesting proteins to study connected to measurements DNA adducts and DNA damage repair processes. The results show that it is a challenge to compare transient changes in low levels of PAH metabolites in bile and gene expression levels in liver with DNA adducts which are longer lived covalent bonds.

The present results do not indicate that discharges from oil and gas activities affect food safety aspects as we see no changes in PAH levels in fillet of muscle of haddock and saithe from the investigated fish species in the Norwegian Sea. We also did not see changes in PAH metabolite levels in fish bile from the Norwegian Sea compared with the Barents Sea. Levels of DNA adducts close to EAC for haddock at the Halten Bank raises concern of exposures for PAH compounds related to oil and gas activities.

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9. Appendix

9.1 Biological data

Table 10.1: Biological data of haddock sampled from the Norwegian Sea 2014.
For sex: 1= female, 2 = male.

Date	Serie	St.nr.	Position		Label	Area	Fish no	Weight (g)	Lengh (cm)	Liver (g)	Gon (g)	Sex	Otolith	LSI (%)	Fulton
10.06.2014	55501	291	63,15	6,72	D-1	NW off Kristiansund	1	490	37	10	2	1/1	2	2,04	0,97
10.06.2014	55503	293	63,19	6,75	D-2	NW off Kristiansund	2	1010	42	39	5	1/1	3	3,86	1,36
10.06.2014	55503	293	63,19	6,75	D-3	NW off Kristiansund	3	970	41	38	1	1/1	2	3,92	1,41
10.06.2014	55503	293	63,19	6,75	D-4	NW off Kristiansund	4	760	38	24	2	1/1	2	3,16	1,39
10.06.2014	55503	293	63,19	6,75	D-5	NW off Kristiansund	5	710	37	27	0	2/1	2	3,80	1,40
10.06.2014	55503	293	63,19	6,75	D-6	NW off Kristiansund	6	660	42	4	7	2/1	4	0,61	0,89
10.06.2014	55503	293	63,19	6,75	D-7	NW off Kristiansund	7	610	35	26	2	1/1	2	4,26	1,42
10.06.2014	55503	293	63,19	6,75	D-8	NW off Kristiansund	8	820	39	28	2	1/1	2	3,41	1,38
10.06.2014	55503	293	63,19	6,75	D-9	NW off Kristiansund	9	780	39	20	3	1/1	2	2,56	1,31
10.06.2014	55503	293	63,19	6,75	D-10	NW off Kristiansund	10	900	40	35	3	1/1	2	3,89	1,41
10.06.2014	55503	293	63,19	6,75	D-12	NW off Kristiansund	11	560	35	17	2	1/1	2	3,04	1,31
10.06.2014	55503	293	63,19	6,75	D-13	NW off Kristiansund	12	500	34	19	2	1/1	2	3,80	1,27
10.06.2014	55503	293	63,19	6,75	D-14	NW off Kristiansund	13	600	36	29	1	2/1	2	4,83	1,29
10.06.2014	55505	295	63,19	6,75	D-15	NW off Kristiansund	14	2430	57	101	12	1/1	4	4,16	1,31
10.06.2014	55505	295	63,19	6,75	D-16	NW off Kristiansund	15	1717	51	43	10	1/1	4	2,50	1,29
10.06.2014	55505	295	63,19	6,75	D-17	NW off Kristiansund	16	910	35	19	1	2/1	2	2,09	2,12
10.06.2014	55505	295	63,19	6,75	D-18	NW off Kristiansund	17	670	35	18	1	2/1	2	2,69	1,56
10.06.2014	55505	295	63,19	6,75	D-19	NW off Kristiansund	18	510	35	18	1	2/1	2	3,53	1,19
10.06.2014	55505	295	63,19	6,75	D-20	NW off Kristiansund	19	550	35	18	2	1/1	2	3,27	1,28
10.06.2014	55505	295	63,19	6,75	D-21	NW off Kristiansund	20	630	36	17	1	1/1	2	2,70	1,35
10.06.2014	55505	295	63,19	6,75	D-22	NW off Kristiansund	21	490	34	14	1	2/1	2	2,86	1,25
11.06.2014	55509	299	64,25	7,38	H-1	Halten Bank	1	1495	54	18	10	1/4	4	1,20	0,95
12.06.2014	55511	301	64,40	7,74	H-2	Halten Bank	2	340	32	22	1	2/1	2	6,47	1,04
12.06.2014	55512	367	64,53	8,69	H-3	Halten Bank	3	1615	55	60	3	2/1	5	3,72	0,97
12.06.2014	55512	367	64,53	8,69	H-4	Halten Bank	4	965	48	28	3	2/1	5	2,90	0,87
12.06.2014	55512	367	64,53	8,69	H-5	Halten Bank	5	820	42	49	1	2/1	3	5,98	1,11
12.06.2014	55512	367	64,53	8,69	H-6	Halten Bank	6	294	31	6	1	1/1	2	2,04	0,99
12.06.2014	55512	367	64,53	8,69	H-7	Halten Bank	7	193	27	4	1	1/1	2	2,07	0,98
13.06.2014	55518	370	65,07	6,48	H-8	Halten Bank	8	1150	48	20	24	2/3		1,74	1,04
12.06.2014	55513	503	64,72	8,82	L-1	Halten Bank	1	1390	52	57	9	1/1	5	4,10	0,99
12.06.2014	55513	503	64,72	8,82	L-2	Halten Bank	2	625	41	10	1	2/1	4	1,60	0,91
12.06.2014	55513	503	64,72	8,82	L-3	Halten Bank	3	970	46	67	4	1/1	3	6,91	1,00
12.06.2014	55513	503	64,72	8,82	L-4	Halten Bank	4	2255	59	127	27	1/4	5	5,63	1,10
12.06.2014	55513	503	64,72	8,82	L-5	Halten Bank	5	1170	49	55	11	1/1	7	4,70	0,99
12.06.2014	55513	503	64,72	8,82	L-6	Halten Bank	6	1755	52	34	12	2/1	9	1,94	1,25
12.06.2014	55513	503	64,72	8,82	L-7	Halten Bank	7	1455	57	17	13	2/1	8	1,17	0,79
12.06.2014	55513	503	64,72	8,82	L-8	Halten Bank	8	535	40	32	32	2/1	3	5,98	0,84
12.06.2014	55513	503	64,72	8,82	L-9	Halten Bank	9	1220	49	71	7	2/1	3	5,82	1,04
12.06.2014	55513	503	64,72	8,82	L-10	Halten Bank	10	265	34	14	2	2/1	2	5,28	0,67
12.06.2014	55513	503	64,72	8,82	L-11	Halten Bank	11	325	36	23	1	2/1	2	7,08	0,70
12.06.2014	55513	503	64,72	8,82	L-12	Halten Bank	12	270	34	14	1	2/1	2	5,19	0,69
12.06.2014	55513	503	64,72	8,82	L-13	Halten Bank	13	450	36	27	3	2/1	2	6,00	0,96
12.06.2014	55513	503	64,72	8,82	L-14	Halten Bank	14	395	33	19	3	2/1	2	4,81	1,10
12.06.2014	55513	503	64,72	8,82	L-15	Halten Bank	15	195	29	8	1	2/1	2	4,10	0,80
12.06.2014	55513	503	64,72	8,82	L-16	Halten Bank	16	340	35	17	2	2/1	2	5,00	0,79
12.06.2014	55513	503	64,72	8,82	L-17	Halten Bank	17	250	32	3	2	2/1	2	1,20	0,76
12.06.2014	55513	503	64,72	8,82	L-18	Halten Bank	18	325	31	17	1	1/2	2	5,23	1,09
12.06.2014	55513	503	64,72	8,82	L-19	Halten Bank	19	300	51	16	2	2/1	2	5,33	0,23
12.06.2014	55513	503	64,72	8,82	L-20	Halten Bank	20	305	32	9	2	2/1	2	2,95	0,93
12.06.2014	55513	503	64,72	8,82	L-21	Halten Bank	21	360	34	13	1	2/1	2	3,61	0,92
12.06.2014	55513	503	64,72	8,82	L-22	Halten Bank	22	260	36	7	1	2/1	2	2,69	0,56
12.06.2014	55513	503	64,72	8,82	L-23	Halten Bank	23	305	31	9	2	2/2	2	2,95	1,02

Table 9.2: Biological data of saithe sampled from the Norwegian Sea 2014.

For sex: 1= female, 2 = male.

Date	Serie no	St.nr.	Position		Label	Area	Fish no	Weight (g)	Lengh (cm)	Liver (g)	Gon (g)	Sex	Otolith	LSI (%)	Fulton
10.06.2014	55501	291	63,15	6,72	B-1	NW of Kristiansund	1	1590	54	68	1	2	5	4,28	1,01
10.06.2014	55502	292	63,2	6,76	B-2	NW of Kristiansund	2	2070	55	98	4	2	6	4,73	1,24
10.06.2014	55502	292	63,2	6,76	B-3	NW of Kristiansund	3	2250	58	105		2	7	4,67	1,15
10.06.2014	55502	292	63,2	6,76	B-4	NW of Kristiansund	4	2990	64	143	2	2	7	4,78	1,14
10.06.2014	55502	292	63,2	6,76	B-5	NW of Kristiansund	5	2520	61	106	11	1	7	4,21	1,11
10.06.2014	55502	292	63,2	6,76	B-6	NW of Kristiansund	6	2060	58	131	8	1	7	6,36	1,06
10.06.2014	55502	292	63,2	6,76	B-7	NW of Kristiansund	7	3830	73	169	6	2	6	4,41	0,98
10.06.2014	55502	292	63,2	6,76	B-8	NW of Kristiansund	8	1630	52	52	6,5	2	5	3,19	1,16
10.06.2014	55502	292	63,2	6,76	B-9	NW of Kristiansund	9	2070	59	110	7	1	6	5,31	1,01
10.06.2014	55502	292	63,2	6,76	B-10	NW of Kristiansund	10	1840	55	103	10	1	6	5,60	1,11
10.06.2014	55502	292	63,2	6,76	B-11	NW of Kristiansund	11	1750	55	82	3	2	7	4,69	1,05
10.06.2014	55503	293	63,19	6,75	B-13	NW of Kristiansund	12	1660	52	46	7	1	5	2,77	1,18
10.06.2014	55503	293	63,19	6,75	B-14	NW of Kristiansund	13	2160	60	73	12	1	7	3,38	1,00
10.06.2014	55503	293	63,19	6,75	B-15	NW of Kristiansund	14	2040	56	74	7	1	6	3,63	1,16
10.06.2014	55503	293	63,19	6,75	B-16	NW of Kristiansund	15	1940	56	98	2	2	6	5,05	1,10
10.06.2014	55503	293	63,19	6,75	B-17	NW of Kristiansund	16	2010	57	107	1	2	7	5,32	1,09
10.06.2014	55505	295	63,19	6,75	B-18	NW of Kristiansund	17	1920	55	54	5	2	6	2,81	1,15
10.06.2014	55505	295	63,19	6,75	B-19	NW of Kristiansund	18	1940	56	95	3	2	7	4,90	1,10
10.06.2014	55505	295	63,19	6,75	B-20	NW of Kristiansund	19	1680	52	73	1	2	5	4,35	1,19
10.06.2014	55505	295	63,19	6,75	B-21	NW of Kristiansund	20	1920	57	95	10	2	6	4,95	1,04
10.06.2014	55505	295	63,19	6,75	B-22	NW of Kristiansund	21	2080	57	106	1	2	5	5,10	1,12
10.06.2014	55505	295	63,19	6,75	B-23	NW of Kristiansund	22	1640	53	67	1	2	4	4,09	1,10
10.06.2014	55505	295	63,19	6,75	B-24	NW of Kristiansund	23	1420	50	67	1	2	6	4,72	1,14
11.06.2014	55506	296	64,3	7,43	C-1	Halten Bank	1	1390	53	58	3	2	5	4,17	0,93
11.06.2014	55506	296	64,3	7,43	C-2	Halten Bank	2	1390	53	38	6	1	6	2,73	0,93
11.06.2014	55506	296	64,3	7,43	C-3	Halten Bank	3	860	44	23	3	1	5	2,67	1,01
11.06.2014	55508	298	64,25	7,39	C-4	Halten Bank	4	2460	59	132	14	1	7	5,37	1,20
11.06.2014	55508	298	64,25	7,39	C-5	Halten Bank	5	2200	56	235	1	2	4	10,68	1,25
11.06.2014	55508	298	64,25	7,39	C-6	Halten Bank	6	1150	46	28	4	1	4	2,43	1,18
11.06.2014	55508	298	64,25	7,39	C-7	Halten Bank	7	900	43	34	1	2	3	3,78	1,13
11.06.2014	55508	298	64,25	7,39	C-8	Halten Bank	8	1690	53	77	1	2	5	4,56	1,14
11.06.2014	55508	298	64,25	7,39	C-9	Halten Bank	9	2270	59	99	15	1	7	4,36	1,11
11.06.2014	55508	298	64,25	7,39	C-10	Halten Bank	10	800	41	22	1	2	4	2,75	1,16
11.06.2014	55508	298	64,25	7,39	C-11	Halten Bank	11	850	40	31	1	2	4	3,65	1,33
11.06.2014	55509	299	64,25	7,38	C-12	Halten Bank	12	570	57	61	3	2	7	10,70	0,31
11.06.2014	55509	299	64,25	7,38	C-13	Halten Bank	13	920	48	20	4	1	4	2,17	0,83
11.06.2014	55510	361	64,25	7,4	C-14	Halten Bank	14	4255	76	201	15	2	9	4,72	0,97
11.06.2014	55510	361	64,25	7,4	C-15	Halten Bank	15	595	46	11	3	1	4	1,85	0,61
11.06.2014	55510	361	64,25	7,4	C-16	Halten Bank	16	1030	47	8	1	2	5	0,78	0,99
11.06.2014	55510	361	64,25	7,4	C-17	Halten Bank	17	1050	44	18	1	2	4	1,71	1,23
11.06.2014	55511	301	64,4	7,74	C-18	Halten Bank	18	3120	67	158	17	1	8	5,06	1,04
11.06.2014	55511	301	64,4	7,74	C-19	Halten Bank	19	1220	50	32	1	1	5	2,62	0,98
11.06.2014	55511	301	64,4	7,74	C-20	Halten Bank	20	1050	51	18	2	2	4	1,71	0,79
11.06.2014	55511	301	64,4	7,74	C-21	Halten Bank	21	650	42	17	1	2	4	2,62	0,88
11.06.2014	55511	301	64,4	7,74	C-22	Halten Bank	22	880	46	17	1	2	4	1,93	0,90
12.06.2014	55512	367	64,53	8,69	C-23	Halten Bank	23	860	45	32	1	2	4	3,72	0,94
12.06.2014	55512	367	64,53	8,69	C-24	Halten Bank	24	1695	60	28	11	1	6	1,65	0,78
12.06.2014	55512	367	64,53	8,69	C-25	Halten Bank	25	820	45	43	1	2	4	5,24	0,90
13.06.2014	55517	369	65,08	6,48	G-2	Kristin area	1	2150	62	91	13	1	7	4,23	0,90
13.06.2014	55517	369	65,08	6,48	G-3	Kristin area	2	1710	58	55	16	1	7	3,22	0,88
13.06.2014	55517	369	65,08	6,48	G-4	Kristin area	3	710	43	13	3	1	4	1,83	0,89
13.06.2014	55517	369	65,08	6,48	G-5	Kristin area	4	2550	69	127	3	2	7	4,98	0,78
13.06.2014	55517	369	65,08	6,48	G-6	Kristin area	5	1550	55	76	3	2	7	4,90	0,93
13.06.2014	55517	369	65,08	6,48	G-7	Kristin area	6	1930	59	93	3	2	7	4,82	0,94
13.06.2014	55518	370	65,07	6,48	G13	Kristin area	7	1390	56	28	7	1	7	2,01	0,79
13.06.2014	55518	370	65,07	6,48	G14	Kristin area	8	1450	55	68	2	2	7	4,69	0,87
13.06.2014	55518	370	65,07	6,48	G15	Kristin area	9	810	45	51	1	2	4	6,30	0,89
13.06.2014	55518	370	65,07	6,48	G16	Kristin area	10	1600	57	105	7	1	5	6,56	0,86
13.06.2014	55518	370	65,07	6,48	G17	Kristin area	11	3000	68	205	5	2	7	6,83	0,95
13.06.2014	55518	370	65,07	6,48	G18	Kristin area	12	1250	54	51	2	2	7	4,08	0,79
13.06.2014	55518	370	65,07	6,48	G19	Kristin area	13	940	46	13	4	1	4	1,38	0,97
13.06.2014	55518	370	65,07	6,48	G20	Kristin area	14	1510	57	49	10	1	7	3,25	0,82
13.06.2014	55518	370	65,07	6,48	G21	Kristin area	15	886	46	25	1	2	4	2,82	0,91

9.2. Fatty acid profiles in phyto- and zooplankton

Table 9.3. Fatty acids profile (% of total FA) in phytoplankton.

	ST359 5 M	ST359 10 M	ST359 20 M	ST360 5 M	ST360 10 M	ST360 20 M	ST367 5 M	ST367 10 M	ST367 20 M	ST368 5 M	ST368 10 M	ST368 20 M	ST369 5 M	ST369 10 M	ST369 20 M	ST370 5 M	ST370 10 M	ST370 20 M
14:0	10.12	11.73	9.69	13.54	12.84	12.26	10.80	14.14	10.74	12.64	11.99	12.36	11.21	8.45	8.57	11.55	7.69	8.44
Iso 15:0	0.74	0.70	0.59	0.76	0.79	0.81	1.08	0.54	0.76	1.04	0.85	0.84	0.51	0.71	0.61	0.70	0.47	0.61
Antiso 15:0	0.43	0.42	0.31	0.50	0.52	0.85	0.77	0.32	0.43	0.71	0.55	0.65	0.53	0.77	0.49	0.63	0.34	0.47
15:0	1.27	1.47	0.90	1.53	1.54	3.44	2.29	0.90	1.22	1.73	1.50	2.05	1.76	2.76	1.70	1.80	0.75	1.53
Iso 16:0	1.25	1.24	0.79	0.78	0.85	2.32	0.73	0.11	0.74	0.76	1.58	1.46	1.06	1.94	1.18	1.10	1.10	1.57
16:0	22.73	26.45	21.03	21.62	19.98	26.96	25.39	11.13	14.93	15.62	16.42	17.58	18.91	22.35	15.02	21.61	16.79	18.23
Iso 17:0	0.21	0.27	0.28	0.37	0.41	0.26	0.38	0.17	0.39	0.37	0.47	0.43	0.24	0.28	0.40	0.32	0.16	0.35
Antiso 17:0	0.77	0.57	0.43	0.32	0.19	0.63	0.42	0.09	0.14	0.19	0.30	0.34	0.27	0.52	0.69	0.30	0.12	0.19
17:0	0.60	0.71	0.57	0.51	0.62	1.45	0.96	0.36	0.62	0.59	0.81	0.81	0.63	1.15	1.00	0.58	0.37	0.52
iso 18:0	1.68	1.06	0.99	1.10	0.68	2.67	0.21	0.39	1.89	0.74	2.87	1.37	0.56	2.70	2.53	1.29	1.12	0.37
18:0	5.33	5.42	6.07	3.26	3.51	6.51	5.89	1.23	2.68	2.73	3.45	3.61	3.09	5.39	3.79	16.93	10.41	15.91
20:0	0.36	0.40	0.42	0.31	0.39	0.51	0.49	0.18	0.32	0.40	0.36	0.38	0.49	0.70	0.38	0.41	0.26	0.34
22:0	0.27	0.29	0.35	0.31	0.41	0.43	0.31	0.15	0.33	0.65	0.63	0.52	0.57	0.63	0.43	0.29	0.19	0.26
24:0	0.60	0.57	0.66	0.61	0.72	0.92	0.55	0.21	1.16	0.94	0.82	0.83	1.17	1.46	0.62	0.52	0.39	0.50
∑SFA	**46.35**	**51.29**	**43.06**	**45.51**	**43.45**	**60.02**	**50.28**	**29.91**	**36.36**	**39.13**	**42.60**	**43.23**	**40.98**	**49.81**	**37.40**	**58.03**	**40.17**	**49.30**
14:1 (n-5)	0.26	0.19	0.14	0.31	0.45	0.44	0.32	0.15	0.33	0.51	0.55	0.68	0.26	0.33	0.36	0.41	0.25	0.34
14:1 (n-7)	0.30	0.11	0.12	0.44	0.41	0.55	0.17	0.12	0.29	0.22	0.25	0.31	0.42	0.44	0.35	0.17	0.18	0.19
16:1 (n-9)	1.53	1.06	0.98	2.23	1.89	2.48	0.83	0.54	0.78	0.86	1.32	1.58	2.96	3.49	1.20	0.60	0.78	0.73
16:1 (n-7)	3.46	3.52	4.24	11.59	7.51	4.90	6.41	3.46	7.60	8.14	7.47	5.97	9.33	3.22	8.02	10.74	10.67	5.51
16:1 (n-5)	0.54	0.81	0.54	0.60	0.49	0.19	0.41	0.52	0.52	0.45	0.20	0.15	0.16	0.18	0.32	0.30	0.09	0.19
17:1 (n-8)	0.36	0.30	0.60	0.28	0.31	0.47	0.72	0.29	0.62	0.37	1.22	0.91	0.20	0.51	0.83	0.33	0.41	0.34
18:1 (n-9)	6.92	6.82	5.58	5.57	5.52	4.27	4.00	2.71	3.40	3.30	3.05	3.23	5.08	5.42	2.90	1.78	9.34	3.55
18:1 (n-7)	1.83	1.86	1.98	2.13	2.40	1.76	2.18	0.72	1.91	2.25	2.11	1.62	1.53	1.82	2.61	2.07	1.15	1.34
18:1 (n-5)	0.14	0.05	0.13	0.21	0.17	0.14	0.18	0.33	0.13	0.01	0.01	0.15	0.39	0.32	0.03	0.15	0.26	0.23
20:1 (n-11)	0.17	0.16	0.33	0.09	0.08	0.00	0.06	0.49	0.09	0.03	0.09	0.06	0.16	0.27	0.05	0.00	0.12	0.07
20:1 (n-9)	0.34	0.38	0.56	0.11	0.17	0.12	0.30	2.31	0.40	0.08	0.16	0.12	0.72	0.73	0.46	0.11	0.15	0.18
22:1 (n-11)	0.06	0.24	0.00	0.00	0.12	0.00	0.18	3.94	0.55	0.15	0.23	0.19	0.69	0.71	0.37	0.20	0.11	0.19
22:1 (n-9)	0.11	0.16	0.07	0.07	0.09	0.00	0.16	0.19	0.05	0.09	0.09	0.06	6.20	3.34	9.21	1.01	0.16	0.30
24:1 (n-9)	0.03	0.01	0.03	0.02	0.04	0.18	0.39	0.65	0.77	0.23	0.18	0.03	0.41	0.21	0.28	0.01	0.38	0.49
∑MUFA	**16.06**	**15.68**	**15.31**	**23.66**	**19.66**	**15.51**	**16.32**	**16.42**	**17.44**	**16.67**	**16.93**	**15.06**	**28.51**	**21.00**	**26.98**	**17.89**	**24.06**	**13.65**
16:2 (n-4)	0.21	0.17	0.22	0.26	0.40	0.20	0.21	0.21	0.31	0.45	0.47	0.52	0.24	0.16	0.39	0.36	0.37	0.41
16:3 (n-4)	0.00	0.00	0.05	0.03	0.06	0.00	0.05	0.07	0.09	0.06	0.10	0.15	0.04	0.00	0.19	0.08	0.11	0.17
18:2 (n-4)	1.71	2.30	2.74	0.88	1.48	3.42	1.91	0.39	4.29	5.22	2.30	1.68	2.79	3.21	1.35	0.90	0.47	0.46
16:4 (n-1)	0.24	0.23	0.35	0.19	0.22	0.16	0.15	0.31	0.23	0.23	0.37	0.28	0.28	0.33	0.49	0.25	0.33	0.51
18:2 (n-6)	2.61	2.33	2.30	2.85	2.58	1.40	1.91	1.16	1.23	2.19	1.83	1.43	2.70	1.38	1.41	1.57	2.53	1.38
18:3 (n-6)	0.15	0.18	0.24	0.25	0.63	0.40	0.16	0.42	0.50	1.13	1.12	0.64	0.27	0.21	0.37	0.37	0.44	0.35
20:2 (n-6)	0.42	0.33	0.31	0.18	0.12	0.15	0.20	0.14	0.19	0.23	0.32	0.17	0.13	0.10	0.12	0.01	0.16	0.10
20:4 (n-6)	0.15	0.13	0.15	0.34	0.55	0.45	0.63	0.44	0.52	0.80	0.58	0.25	0.27	0.33	0.41	0.29	0.42	0.39
16:4 (n-3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18:3 (n-3)	3.10	2.91	3.42	2.97	3.14	1.42	1.98	1.69	1.75	2.00	2.04	2.14	1.78	1.39	1.76	1.53	2.41	2.69
18:4 (n-3)	4.39	3.97	6.76	4.82	6.35	3.80	3.93	10.43	7.09	5.97	7.55	8.64	3.24	3.52	6.93	4.66	7.63	6.73
18:5 (n-3)	4.26	3.54	4.43	3.48	4.60	2.55	3.63	1.74	6.83	5.25	5.46	7.80	3.80	2.95	6.53	4.30	2.70	5.69
20:3 (n-3)	0.82	0.84	1.00	0.65	0.52	0.61	0.92	0.26	0.69	0.68	0.77	0.67	0.56	2.53	0.91	0.52	0.34	0.30
20:4 (n-3)	0.45	0.38	0.52	0.43	0.43	0.22	0.51	1.03	0.45	0.46	0.42	0.46	0.42	0.58	0.38	0.00	1.39	0.50
20:5 (n-3)	6.21	6.05	8.09	5.81	7.00	6.01	7.44	21.87	11.12	10.51	8.55	7.42	6.67	6.90	6.40	4.90	7.52	8.59
21:5 (n-3)	0.12	0.09	0.20	0.09	0.08	0.00	0.12	0.36	0.10	0.12	0.10	0.11	0.06	0.05	0.07	0.05	0.34	0.12
22:5 (n-3)	0.61	0.49	0.61	0.48	0.48	0.20	0.45	1.21	0.52	0.45	0.44	0.42	0.43	0.29	0.27	0.26	0.42	0.38
22:6 (n-3)	12.14	9.83	10.90	8.71	9.57	5.18	10.18	12.29	11.40	9.79	9.28	10.51	7.91	6.34	7.57	5.53	9.52	9.53
∑PUFA	**37.59**	**33.76**	**42.29**	**32.40**	**38.21**	**26.17**	**34.37**	**54.02**	**47.33**	**45.52**	**41.69**	**43.28**	**31.58**	**30.29**	**35.56**	**25.57**	**37.10**	**38.30**
∑(n-6)PUFA	**3.33**	**2.98**	**2.99**	**3.62**	**3.88**	**2.40**	**2.90**	**2.16**	**2.45**	**4.34**	**3.85**	**2.48**	**3.37**	**2.02**	**2.31**	**2.24**	**3.55**	**2.21**
∑(n-3)PUFA	**32.10**	**28.10**	**35.92**	**27.44**	**32.18**	**19.99**	**29.16**	**50.88**	**39.96**	**35.23**	**34.60**	**38.17**	**24.87**	**24.57**	**30.82**	**21.74**	**32.27**	**34.54**
(n-3)/(n-6)	**9.63**	**9.44**	**12.00**	**7.59**	**8.30**	**8.32**	**10.05**	**23.56**	**16.32**	**8.11**	**8.99**	**15.39**	**7.39**	**12.19**	**13.34**	**9.71**	**9.09**	**15.63**
FA (µg/L)	21.23	20.22	27.04	38.63	18.00	15.15	47.68	126.62	27.83	39.61	23.36	20.07	50.57	34.70	27.22	25.99	24.93	40.10

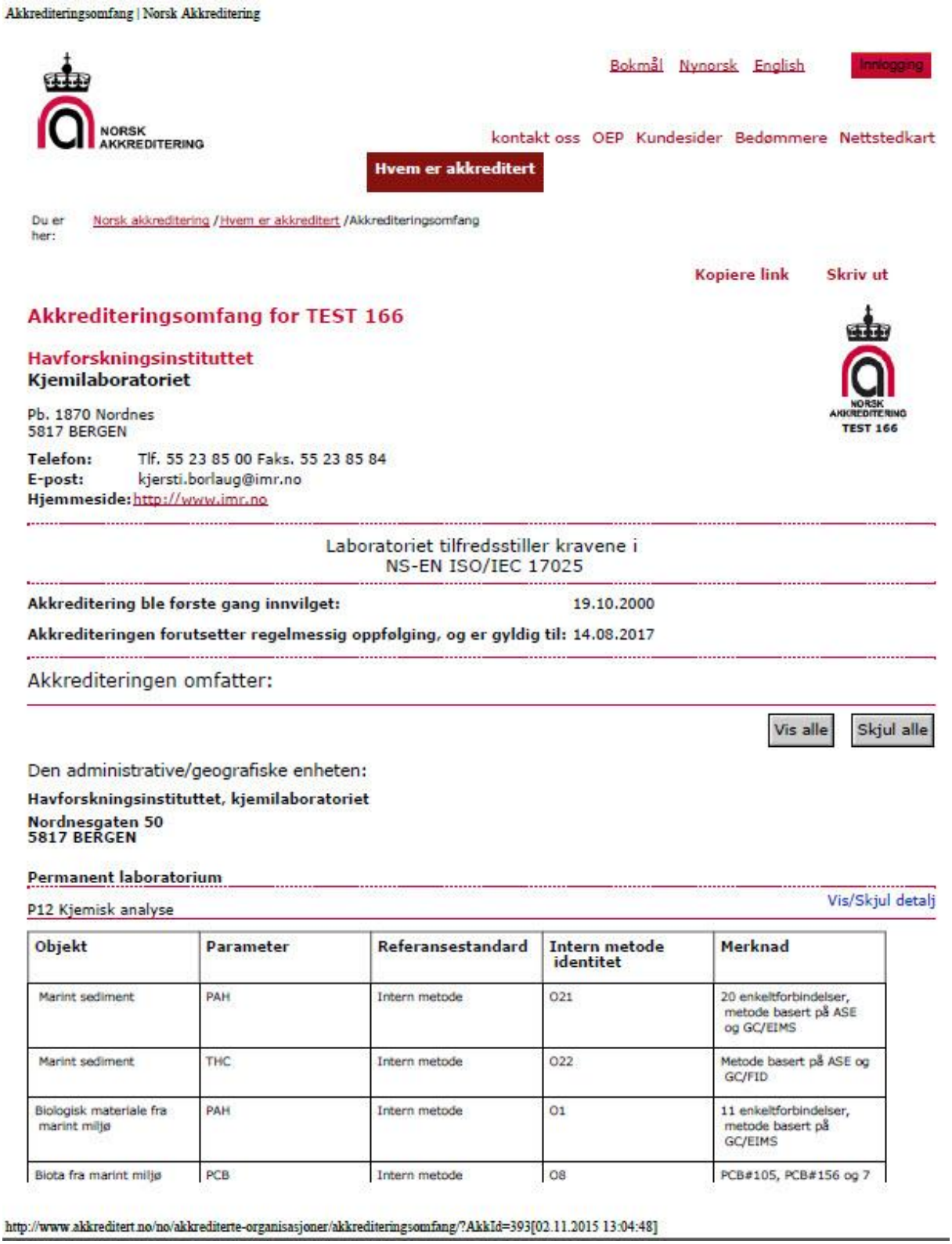
Table 9.4. Fatty acids profile (% of total FA) in zooplankton.

	St359 180µm	St359 1000µm	St360 180µm	St360 1000µm	St368 180µm	St368 1000µm	St370 180µm	St370 1000µm
14:0	14,76	17,92	18,50	19,79	18,76	19,52	15,18	17,99
Iso 15:0	0,40	0,45	0,25	0,48	0,34	0,30	0,17	0,48
Antiso 15:0	0,13	0,14	0,09	0,18	0,19	0,16	0,07	0,16
15:0	0,78	0,88	0,61	0,82	0,87	0,76	0,44	0,80
Iso 16:0	0,06	0,06	0,03	0,06	0,05	0,06	0,02	0,06
16:0	12,17	12,02	15,23	10,65	11,80	10,71	16,17	11,02
Iso 17:0	0,12	0,08	0,12	0,10	0,13	0,17	0,10	0,06
Antiso 17:0	0,05	0,04	0,02	0,04	0,05	0,04	0,03	0,04
17:0	0,30	0,24	0,14	0,25	0,27	0,20	0,16	0,30
iso 18:0	0,03	0,03	0,01	0,02	0,04	0,04	0,00	0,02
18:0	1,07	0,86	0,80	0,72	0,81	0,65	1,03	0,99
20:0	0,09	0,10	0,06	0,06	0,11	0,13	0,06	0,10
22:0	0,03	0,01	0,03	0,04	0,06	0,07	0,01	0,04
24:0	0,02	0,04	0,01	0,06	0,02	0,02	0,02	0,08
∑SFA	**29,98**	**32,87**	**35,89**	**33,28**	**33,49**	**32,82**	**33,46**	**32,13**
14:1 (n-5)	0,10	0,11	0,09	0,15	0,12	0,11	0,07	0,12
14:1 (n-7)	0,06	0,08	0,04	0,02	0,01	0,01	0,03	0,07
16:1 (n-9)	0,43	0,42	0,27	0,50	0,04	0,16	0,20	0,40
16:1 (n-7)	7,90	8,33	21,91	6,52	8,06	7,00	21,94	5,84
16:1 (n-5)	0,44	0,48	0,46	0,58	0,45	0,46	0,40	0,44
17:1 (n-8)	0,29	0,25	0,16	0,27	0,27	0,23	0,16	0,25
18:1 (n-9)	6,56	3,88	3,59	3,49	3,55	3,01	6,70	3,70
18:1 (n-7)	0,61	0,46	0,32	0,34	0,42	0,42	0,48	0,40
18:1 (n-5)	0,32	0,29	0,14	0,30	0,26	0,28	0,21	0,33
20:1 (n-11)	0,49	0,58	0,22	0,45	0,63	0,78	0,23	0,49
20:1 (n-9)	1,41	2,40	0,74	1,60	4,22	4,34	0,57	1,82
22:1 (n-11)	2,19	4,04	1,23	2,68	5,60	6,64	0,85	3,29
22:1 (n-9)	0,19	0,32	0,09	0,18	0,42	0,47	0,09	0,22
24:1 (n-9)	1,43	1,26	0,55	1,03	0,92	0,79	0,64	1,24
∑MUFA	**22,43**	**22,90**	**29,82**	**18,10**	**24,96**	**24,70**	**32,57**	**18,60**
16:2 (n-4)	0,41	0,50	0,49	0,50	0,42	0,80	0,39	0,43
16:3 (n-4)	0,29	0,42	0,38	0,36	0,40	0,96	0,21	0,36
18:2 (n-4)	0,12	0,15	0,08	0,14	0,21	0,20	0,08	0,16
16:4 (n-1)	0,55	1,06	0,86	0,82	0,94	1,93	0,55	0,96
18:2 (n-6)	1,96	1,42	3,79	1,23	1,86	1,25	4,41	1,31
18:3 (n-6)	0,30	0,30	0,52	0,45	0,44	0,40	0,46	0,41
20:2 (n-6)	0,21	0,14	0,02	0,10	0,12	0,10	0,14	0,14
20:4 (n-6)	0,61	0,54	0,41	0,75	0,44	0,41	0,35	0,78
16:4 (n-3)	0,34	0,45	0,19	0,36	0,31	0,33	0,10	0,27
18:3 (n-3)	3,05	2,28	1,63	2,47	1,85	1,79	1,77	1,96
18:4 (n-3)	11,72	12,39	8,73	15,53	12,40	11,55	7,19	14,08
18:5 (n-3)	0,25	0,22	0,15	0,26	0,36	0,31	0,17	0,33
20:3 (n-3)	0,23	0,19	0,11	0,02	0,12	0,01	0,18	0,15
20:4 (n-3)	1,69	1,33	0,91	1,51	1,22	1,13	1,18	1,33
20:5 (n-3)	11,73	11,81	9,54	13,04	10,10	12,39	8,20	13,31
21:5 (n-3)	0,43	0,43	0,26	0,45	0,52	0,57	0,30	0,46
22:5 (n-3)	0,60	0,67	0,34	0,64	0,67	0,80	0,33	0,61
22:6 (n-3)	13,10	9,93	5,86	10,01	9,14	7,54	7,97	12,21
∑PUFA	**47,60**	**44,23**	**34,29**	**48,62**	**41,55**	**42,48**	**33,97**	**49,27**
∑(n-6)PUFA	**3,09**	**2,41**	**4,75**	**2,53**	**2,87**	**2,16**	**5,34**	**2,64**
∑(n-3)PUFA	**43,14**	**39,69**	**27,72**	**44,27**	**36,71**	**36,43**	**27,39**	**44,71**
(n-3)/(n-6)	**13,98**	**16,50**	**5,84**	**17,46**	**12,81**	**16,85**	**5,12**	**16,91**
FA (mg/100 mg sample)	1,97	1,36	2,02	2,53	3,51	4,58	2,07	1,16

Table 9.5. Fatty alcohols profile (% of total FA-ALK) in zooplankton.

	St359 180 µm	St359 1000 µm	St359 2000 µm	St360 180 µm	St360 1000 µm	St360 2000 µm	St368 180 µm	St368 1000 µm	St368 2000 µm	St370 180 µm	St370 1000 µm
14:0 ALK	8,71	2,67	2,42	6,96	2,28	4,50	3,33	2,17	2,13	22,94	3,52
15:0 Alk	0,75	0,48	0,48	0,56	0,39	0,86	0,42	0,26	0,28	0,84	0,52
16:0 ALK	22,37	16,77	16,93	20,45	15,03	20,46	12,11	10,42	10,29	31,07	16,17
18:0 ALK	1,16	0,90	0,95	1,18	0,87	1,30	0,73	0,66	0,70	1,35	1,00
20:0 ALK	0,16	0,16	0,17	0,16	0,18	0,13	0,15	0,15	0,02	0,11	0,15
24:0 Alk	0,30	0,23	0,23	0,25	0,36	0,00	0,20	0,23	0,17	0,12	0,00
16:1 ALK	3,94	5,55	4,58	4,03	3,96	7,41	2,81	3,48	3,50	1,73	4,53
16:1 Alk 2	0,36	0,26	0,37	0,36	0,44	0,37	0,21	0,25	0,19	0,17	0,35
18:1 (n-9) ALK	4,88	5,18	5,42	4,87	4,46	7,65	2,65	2,36	2,49	2,85	4,86
18:1 (n-7) ALK	2,17	2,20	2,21	2,06	1,88	2,03	1,31	1,42	1,45	1,33	1,75
18:1 (n-5) ALK	0,40	0,32	0,35	0,44	0,52	0,24	0,31	0,30	0,29	0,44	0,31
20:1 (n-9) ALK	17,61	22,35	19,91	20,14	22,44	19,86	28,29	25,57	29,55	12,49	22,73
20:1 (n-7) ALK	2,22	3,33	2,95	3,26	4,57	1,71	6,16	8,13	5,64	1,39	2,68
22:1 (n-11) ALK	21,03	25,52	26,97	21,55	23,27	19,92	26,19	30,33	29,58	15,27	26,79
22:1 (n-9) ALK	2,73	3,21	3,53	2,94	6,24	2,17	5,77	4,07	5,49	1,92	3,31
22:1 (n-7) ALK	1,99	1,44	3,01	2,23	4,21	0,28	4,50	6,20	4,05	1,14	2,49
18:2 (n-6) ALK	3,15	3,68	3,57	3,15	3,41	4,14	2,05	1,69	1,79	1,59	3,47
18:3 (n-3) ALK	4,67	4,62	4,68	4,04	4,28	5,93	1,92	1,62	1,64	2,41	4,43
20:2 (n-6) Alk	0,43	0,43	0,44	0,40	0,50	0,27	0,25	0,33	0,20	0,25	0,33
20:3 (n-6) Alk	0,95	0,72	0,82	0,96	0,72	0,77	0,63	0,33	0,55	0,59	0,59
Amount (mg/100 mg)	0,75	0,59	0,71	0,64	1,65	0,13	2,05	3,62	1,40	0,39	0,42

9.3. Certificate of Accreditation by Norsk akkreditering



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Kjemilaboratoriet

Pb. 1870 Nordnes
5817 BERGEN

Telefon: Tlf. 55 23 85 00 Faks. 55 23 85 84
E-post: kjersti.borlaug@imr.no
Hjemmeside: http://www.imr.no

Laboratoriet tilfredsstiller kravene i NS-EN ISO/IEC 17025

Akkreditering ble første gang innvilget: 19.10.2000

Akkrediteringen forutsetter regelmessig oppfølging, og er gyldig til: 14.08.2017

Akkrediteringen omfatter:

Vis alle Skjul alle

Den administrative/geografiske enheten:

Havforskningsinstituttet, kjemilaboratoriet
Nordnesgaten 50
5817 BERGEN

Permanent laboratorium

P12 Kjemisk analyse Vis/Skjul detalj

Objekt	Parameter	Referansestandard	Intern metode identitet	Merknad
Marint sediment	PAH	Intern metode	O21	20 enkeltforbindelser, metode basert på ASE og GC/EIMS
Marint sediment	THC	Intern metode	O22	Metode basert på ASE og GC/FID
Biologisk materiale fra marint miljø	PAH	Intern metode	O1	11 enkeltforbindelser, metode basert på GC/EIMS
Biota fra marint miljø	PCB	Intern metode	O8	PCB#105, PCB#156 og 7

http://www.akkreditert.no/no/akkrediterte-organisasjoner/akkrediteringsomfang/?AkkId=393[02.11.2015 13:04:48]

				-Dutch, metode basert på GC-ECD
Biota fra marint miljø	Klorerte pesticider	Intern metode	O8	Metode basert på ekstraksjon og GC-ECD. 8 enkeltforbindelser
Sjøvann	Nitrat	Intern metode	U3-1	Metode basert på UV-fotometri
Sjøvann	Fosfat	Intern metode	U3-2	Metode basert på UV-fotometri
Sjøvann	Nitritt	Intern metode	U3-3	Metode basert på UV-fotometri
Sjøvann	Silikat	Intern metode	U3-4	Metode basert på UV-fotometri

P22 Ioniserende stråling og radioaktivitet

Vis/Skjul detalj

Norsk akkreditering

Skedsmogata 5
2000 Lillestrøm

Postboks 155
Lillestrøm bedriftssenter
2001 Lillestrøm

E-post: akkreditert@akkreditert.no

Telefon: (+47) 64 84 86 00

Organisasjonsnummer: 986 028 307

Fakturaadresse: EHF 986 028 307

9.4. DNA adduct analyses- Analytical report from Adn`tox

EXPERT IN GENOTOXICOLOGY

ANALYTICAL REPORT

Study reference:	IMR_2014
Purpose of report:	Final report
Date:	26 November 2014

Title of report:

ANALYSIS OF BULKY DNA ADDUCT PATTERNS IN THE LIVER OF FISH (HADDOCK SPECIES) HARVESTED IN REFERENCE SITES IN THE NORTH SEA IN 2014, 2013 and 2012

Dr Jérémie LE GOFF,
Head of ADn'tox
Chief Scientist

ADn'tox
Bâtiment GRECAN
Centre François BACLESSE
3, Avenue du Général Harris
14076 CAEN cedex 5 / France
+33.2.31.45.52.18 / +33.2.31.45.50.70
j.legoff@adntox.com

Purpose of the study

This final report consists of the analysis of the bulky DNA adduct patterns obtained in the liver of 100 fish from Haddock species (and one pool of Haddock larvae), caught in several sites of the North Sea (Reference sites / Oil Platforms) in 2012, 2013, 2014. The detection of the DNA adducts is realised by a nuclease P1 version of the ^{32}P post-labelling method which detailed protocol is provided in annexe.

The document contains

1) The raw data from the 100 samples (all quantitative results and autoradiographic patterns of interest),
2) A statistical analysis based on the available information associated to the 100 samples (species and site).
3) A first discussion of the results in the light of some published studies in the field.

The analysis of the overall samples was spread over a four month period (July 2014-October 2014).

Preparation of the DNA solutions

After receipt, the samples were stored at -80°C until their handling of DNA extraction. Small pieces of tissue (100 to 120 mg per sample) were taken for the DNA extraction.

For each sample, a purified DNA solution was obtained by a method of phenol-chloroform / liquid-liquid extraction, after the crushing of liver pieces (tissue-lyser, Qiagen ®), isolation of cell nuclei (in sucrose 0.32M) and sample treatment with RNases A, T1 and proteinase K (**Annexe 2a**).

The DNA concentrations were deduced from the absorbance (optical density) at the wavelength of 260 nm (A_{260}) (Nanodrop Technology, Thermo Scientific ®). The absorbance ratios A_{260}/A_{280} and A_{260}/A_{230} coupled with the absorbance profile of the samples between 230 nm and 300 nm were used to check the quality of the DNA solutions (more especially the absence of contamination by RNA and/or proteins).

In order to always work on material freshly extracted, the extraction of DNA was separated in time. The extracted samples were systematically analysed in ^{32}P post-labelling in the next two weeks.

The absorbance ratios A_{260}/A_{280} and A_{260}/A_{230} obtained on the whole 100 DNA samples are of 1.92 *± 0.16* and 2.10*± 0.12* respectively. These experimental ratios are satisfying enough in regards to the usual requirements of the ^{32}P-postlabelling method.

Analysis of DNA adduct patterns by the ^{32}P postlabelling method

Materials and methods

The ^{32}P-postlabelling method

The detailed protocol used by ADn'tox is described in the **Annexe 2b**. It is suitable for the research of so-called "bulky" DNA adducts, some additional compounds in DNA that are associated to numerous complex molecules such as certain polycyclic aromatic hydrocarbons (PAHs). Each analysis is realised from 5 micrograms DNA.

Ten manipulations (sets of analysis) were necessary in order to analyse the DNA adduct patterns of the overall **100 samples**. Two independent adduct measurements have been realised for each DNA sample. For the study, the limit of detection (LOD) is fixed to half the smallest DNA adduct level (Relative adduct level=RAL) calculated for an observed spot in a pattern, i.e. ½ x 0.02 = 0.01 adducts per 10^8 nucleotides (RAL x 10^{-8}). For analysis without detectable adducts ("null" results), the concentration in adducts is then defined as <0.01 x 10^{-8} nucleotides (**Table 1**).

In each set of analysis, DNA from both positive and negative controls are systematically included. Positive control is a calf thymus DNA exposed to benzo[a]pyrene dioepoxide (BPDE) kindly provided by F.A Beland (National Center for Toxicology Research, USA). This sample was used as a standard in large interlaboratory trials. The DNA damage level was 110.70 adducts per 10^8 normal nucleotides (according to F.A. Beland, in Philips and Castegnaro, 1999; see Divi et al., 2002 and Zhan et al.,1995 for more details). The negative control was a plasmid DNA.

The autoradiographic patterns from both positive and negative controls are provided in **the Annexe 1**. These results assure the smooth technical functioning, by the absence first of nonspecific signals (a source of false positives, frequently due to improper disposal of certain reagents/impurities used during handling) and then a correct ^{32}P labelling on a reference / standard sample. The good labelling efficiency is checked on the base of the direct level of radioactivity (Cerenkov radiation) in the major spot of the positive control, expressed in radioactive counts per minute (cpm).

Statistical analysis associated to DNA adduct patterns

Usually, the DNA adduct levels measured in the overall samples of a study (expressed as Relative Adduct level per 10^8 normal nucleotides (RAL x 10^{-8})) do not respect the classical normal distribution (Gaussian distribution), due mainly to the large proportion of samples without detectable adducts. This distribution leads to the inability to properly use the parametric tests for statistical analysis of the DNA adduct data, especially the very common analysis of variance (ANOVA). Then, all results are initially treated by nonparametric statistics.
The logarithmic with base 10 transformation (results in Log10) tends to standardise the data distribution for nonzero results (and outliers, i.e. sample values with extreme deviations from the mean) and authorizes the use of parametric statistics on these truncated data.

An analysis is made taking into account the presence or absence of adducts for each sample (qualitative approach of DNA adduct patterns). This approach is of interest given the semi-quantitative aspect of technique, especially in the context of measuring low levels of adducts.
The statistical analysis presented in the report is based on the use of SAS® software by Mr. Didier Pottier, engineer biostatistician at the University of Caen (EA 4651 ABTE-TOXEMAC, France).

Results from DNA adduct measurements on the 100 samples

The samples in the current study were analysed in duplicate in two separate manipulations (sets), including a total of ten ^{32}P postlabelling manipulations (sets) and 202 analyses. One positive control (benzo[*a*]pyrene diol epoxide (BPDE) + calf thymus DNA) and one negative control (plasmid DNA without detectable adducts) were added in each manipulation.

The proper conduct of each independent manipulation is validated according to the qualitative and quantitative results in the positive control (DNA rich in adducts of benzo[*a*]pyrene diol epoxide (BPDE), **Annexe 1**): pattern of adducts and direct level of radioactivity in the major spot (routinely near 17,000 cpm ± 15%). The reproducible clean pattern of the negative control (DNA without detectable adducts) confirm the absence of unwanted interfering signals that could be misattributed to adducts (prevention of false positive).

The **Table 1** and **Figure 1** present the DNA adduct results per site for the 100 fish sampled in 2012, 2013 and 2014. It is worth noting that in the absence of specific information, the fish caught in different years are considered as belonging to different sites.

Overall, apart from a few exceptions, the measured signals attributed to DNA adducts are quantitatively low and associated to patterns with relatively few spots, whatever the site. The adduct levels or concentrations (expressed as a relative adduct level, i.e. the number of detected adducts per 10^8 normal nucleotides (RAL x 10^{-8})) are consistent with published data from environmental studies (see discussion & conclusion).
Predictably, the DNA adduct levels measured in the overall samples, considered by site, do not respect the classical Normal distribution, even after the logarithmic with base 10 transformation (Shapiro-Wilk test, results not shown). Therefore, all of the following statistical analyses are above all based on some non-parametric tests. Parametric tests are used in order to complete (or reinforce) the statistical results.

Qualitative analysis of the DNA adduct patterns

Proportion of samples without detectable DNA adducts

In this field study based on Haddock only, **the proportion of samples without any detectable DNA adducts is 28%** (28 out of 100), together all the seven investigated sites. The value is in accordance with previous results (see reports of earlier studies). Interestingly, this proportion varies significantly from site to site (p=0.02, Fisher's Exact Test), with highest proportions of samples without adducts at site 7 (10 samples out of 20, 50% of samples), site 6 (4 samples out of 10, 40% of samples) and site 5 (4 samples out of 10, 40% of samples). Lower proportions of samples without any detectable adducts are observed at sites 1 and 3 (for both sites: 5 samples out of 21, 24% of samples). The overall samples collected at sites 2 and 4 are characterized by the presence of at least one detectable DNA adduct.

Proportion of samples with high concentrations of DNA adducts

The samples with mean DNA adduct concentrations greater than or equal to 0.4 adduct per 10^8 normal nucleotides (RAL $\geq 0.4 \times 10^{-8}$) are of particular interest (see chapter Discussion). On the overall study, these high concentrations have been measured in about half of the samples (51 samples out of the 100, i.e. 51% of all samples). Interestingly, the proportion of samples with elevated concentrations in DNA adducts varies significantly from site to site (p=0.008, Fisher's Exact Test): 20% of samples (5 samples among 20) at site 7, 40% of samples (4 samples among 10) at sites 5 and 6, 52% of samples (11 samples among 21) at sites 1 and 3, 87% of samples (7 samples among 8) at site 2 and 90% of samples (9 samples among 10) at site 4. It is to note that this proportion is especially high at Sites 2 and 4. These results must be considered in the light of the absence of samples without detectable adducts at both sites.

Qualitative analysis of DNA adduct patterns by the individual number of spots

The mean number of spots (DNA adducts) per sample is statically different from one site to another (p=0.007, Anova). This value is ranged from 1.0 spot per sample at Site 7 to 2.9 spots per sample at Site 2. The value is over 2.0 for Site 2 (2.9 adducts per sample), Site 4 (2.4 adducts per sample) and Site 1 (2.4 adducts per sample) and between 1.0 and 2.0 for Site 3 (1.6 adducts per sample), Site 6 (1.3 adducts per sample), Site 5 (1.2 adducts per sample) and Site 7 (1.0 adducts per sample). In a direct side-by-side comparison, the difference of mean number of spots per sample is only significant between extreme Sites 2 and 7 ($p<0.05$, Tukey Studentized Range Test).

Qualitative analysis by spots

On the overall study, 12 distinct spots (and one specific area called DRZ for Diagonale Radioactive Zone) were isolated from their different 2D chromatographic migration on the PEI cellulose sheets (numbered 1 to 12 and DRZ, according to the chronological order, **Figure 5**). Overall, four spots were present in at least one quarter (25%) of the samples: spots n°1 (48% of the samples, i.e. 48 samples among 100 analysed), n°2 (36%), n°3 (43%), and n°4 (25%). For these four frequent spots, a statistical analysis of their intersite distribution in the samples has been realised (see below). All other spots (except spot n°8 and n°9 (7% and 6% of the samples respectively)) were detected in less than 5% of the samples. The DRZ was occasionally observed for only 2 samples (2% of the samples).

No spot among the more frequent (≥ 25 % of samples) appears to be strictly limited to a particular site (site-specificity). However, some interesting variations of the DNA adduct patterns can be noted, especially for the three frequent spots n°1, n°2 and n°3 (see **table 2**). The proportion of sample with spot 2 is different from site to site, at the limit of statistical significance (p=0.06, Fisher's Exact Test). Highest proportions are encountered at sites 1 (62% of samples) and 2 (50% of samples). The proportion of sample with spots 1 is significantly different from site to site (p=0.02, Fisher's Exact Test). Highest proportions are encountered at sites 1 (67% of samples), 2 (75% of samples) and 4 (70% of samples). It is to note that this frequent spot (48% of the overall samples) is present in only 20% of samples at site 5. The proportion of sample with spot 3 is highly different from site to site ($p<0.001$, Fisher's Exact Test). For this spot, highest proportions are encountered at sites 4 (90% of samples) and 2 (87% of samples).

Quantitative analysis of the DNA adduct patterns

The overall results indicate that the DNA adduct concentrations (expressed in relative adduct level per 10^8 normal nucleotides (RALx10^{-8}) are low for the most part, combined with large interindividual differences, even

within groups of supposed similar conditions of exposure to (potential) genotoxicants (same site of the field study).

Quantitatively, the mean relative adduct levels (RAL) per sample (mean of two independent measurements) were measured between <0.01 (no detectable adducts) and 2.92 adducts per 10^8 normal nucleotides. It is to note that only three individual means (3% of the samples) exceed 2 adducts per 10^8 nucleotides (One sample at site 1 (IMR2014_13) and two samples at site 2 (IMR2014_23 and IMR2014_28)). 14 individual means (14% of the samples) are over 1 adduct per 10^8 nucleotides. 51 individual means (51% of the samples) are over 0.4 adduct per 10^8 nucleotides, an interesting cut-off value (see discussion).

The mean DNA adduct concentration per site varies from 0.25 *± 0.31* x10^{-8} adducts at site n°7 to 1.28 *±14.61* x10^{-8} adducts at site n°2. **(Figure 1).** The intersites difference of DNA adduct concentrations is statistically significant (p=0.001, Kruskal-Wallis Test; p=0.0002, anova). By a pair-wise comparison of the sites, significant differences of the mean RAL levels per site are observed between the Site 2 and each of the other sites except site 4 (p<0.05, Tukey's Studentized Range (HDS) Test). Another significant difference is notified between Site 4 and site 7 (p<0.05, Tukey's Studentized Range (HDS) Test).

Comparison of sites 1 and 3 after rearranging of samples

A rearrangement of samples is proposed in the light of a more detailed analysis of the sampling areas of different fish during the 2014 field campaign. This rearrangement is to switch samples n°24, 25, 26, 27 and 28 from Site 2 to Site 3, motivated by the fact that these samples were collected near the site 3, in order to compare Site 1 and Site 3. Rearranged as mentioned, the mean DNA adduct level at site 3 is 0.55 *± 0.59* x10^{-8} adducts (n=26 samples). This value is very close to that calculated on the site 1 (0.56 *± 0.61* x10^{-8} adducts; n=21 samples). The difference between both sites is not significant, as expected (p=0.881, Wilcoxon test). No significant difference was also observed on qualitative data, i.e. the proportion of samples without detectable DNA adducts (5 samples/21 (24%) at site 1 Vs 5 samples/26 (19%) at site 3, p=0.734; Fisher's Exact Test), the proportion of samples with high concentrations of DNA adducts (11 samples/21 (52%) at site 1 Vs 15 samples/26 (58%) at site 3, p=0.774; Fisher's Exact Test), the qualitative analysis of DNA adduct patterns by the individual number of spots (2.38 x10^{-8} adducts per sample at site 1 in average, Vs 1,88 x10^{-8} adducts per sample at site 1 in average, p=0.293; Student test), the qualitative analysis by spots (*spot 1*: 14 samples/21 (67%) at site 1 Vs 16 samples/26 (61%) at site 3, p=0.768; Fisher's Exact Test; *spot 2*: 13 samples/21 (62%) at site 1 Vs 11 samples/26 (42%) at site 3, p=0.244; Fisher's Exact Test; *spot 3:* 11 samples/21 (52%) at site 1 Vs 11 samples/26 (42%) at site 3, p=0.564; Fisher's Exact Test; *spot 4:* 4 samples/21 (19%) at site 1 Vs 6 samples/26 (23%) at site 3, p=1.000; Fisher's Exact Test).

Sample identification		Species	Site	Relative Adduct Level (RAL) x 10^{-8}		Mean RAL per sample (x10^{-8})	Mean RAL*(± SD)* per condition (x10^{-8})
ADN'TOX	IMR			Run 1 [1]	Run 2		
IMR2014_01	D1-L1	Haddock	SITE 1	0.35	0.22	**0.29**	0.56 ± *0.61*
IMR2014_02	D2-L1	Haddock		0.44	0.58	**0.51**	
IMR2014_03	D3-L1	Haddock		0.65	0.52	**0.59**	
IMR2014_04	D4-L1	Haddock		0.54	0.78	**0.66**	
IMR2014_05	D5-L1	Haddock		0.62	0.38	**0.50**	
IMR2014_06	D6-L1	Haddock		1.39	1.67	**1.53**	
IMR2014_07	D7-L1	Haddock		< *0.01* [2]	< *0.01*	***< 0.01***	
IMR2014_08	D8-L1	Haddock		0.61	0.86	**0.74**	
IMR2014_09	D9-L1	Haddock		1.60	1.07	**1.34**	
IMR2014_10	D10-L1	Haddock		0.46	0.29	**0.38**	
IMR2014_11	D11-L1	Haddock		< *0.01*	< *0.01*	***< 0.01***	
IMR2014_12	D13-L1	Haddock		0.66	0.51	**0.59**	
IMR2014_13	D14-L1	Haddock		2.66	2.34	**2.50**	
IMR2014_14	D15-L1	Haddock		0.25	0.22	**0.24**	
IMR2014_15	D16-L1	Haddock		0.19	0.20	**0.20**	
IMR2014_16	D17-L1	Haddock		<*0.01*	<*0.01*	***< 0.01***	
IMR2014_17	D18-L1	Haddock		0.21	0.17	**0.19**	
IMR2014_18	D19-L1	Haddock		<*0.01*	<*0.01*	***< 0.01***	
IMR2014_19	D20-L1	Haddock		<*0.01*	<*0.01*	***< 0.01***	
IMR2014_20	D21-L1	Haddock		0.96	0.74	**0.85**	
IMR2014_21	D22-L1	Haddock		0.72	0.41	**0.57**	

[1] Run 1 and Run 2: two independent measurements of DNA adducts per sample.
[2] Analysis without any detectable spot/adduct

Table 1: Concentrations in bulky DNA adducts (expressed in Relative Adduct Level per 10^8 normal nucleotides (RAL x 10^{-8})) measured on the 100 fish included in the field study, classified according the sampling conditions (Site and year of sampling).

Sample identification		Species	Site	Relative Adduct Level (RAL) x 10^{-8}		Mean RAL per sample (x10^{-8})	Mean RAL*(± SD)* per condition (x10^{-8})
ADN'TOX	IMR			Run 1[1]	Run 2		
IMR2014_22	**H1-L1**	Haddock	SITE 2	0.48	0.33	**0.41**	1.28 ± *0.95*
IMR2014_23	**H2-L1**	Haddock		2.85	2.99	**2.92**	
IMR2014_24	**H3-L1**	Haddock		0.52	0.73	**0.63**	
IMR2014_25	**H4-L1**	Haddock		1.07	*0.61*	**0.84**	
IMR2014_26	**H5-L1**	Haddock		1.20	1.33	**1.27**	
IMR2014_27	**H6-L1**	Haddock		0.24	0.42	**0.33**	
IMR2014_28	**H7-L1**	Haddock		2.84	1.98	**2.41**	
IMR2014_29	**H8-L1**	Haddock		1.61	1.29	**1.45**	
IMR2014_30	**E24-L1** [3]	?		*<0.01* [2]	_	***<0.01***	

[1] Run 1 and Run 2: two independent measurements of DNA adducts per sample.
[2] Analysis without any detectable spot/adduct
[3] IMR2014_30: sample finally not included in the study

Table 1 (continued): Concentrations in bulky DNA adducts (expressed in Relative Adduct Level per 10^8 normal nucleotides (RAL x 10^{-8})) measured on the 100 fish included in the field study, classified according the sampling conditions (Site and year of sampling).

Sample identification		Species	Site	Relative Adduct Level (RAL) x 10^{-8}		Mean RAL per sample (x10^{-8})	Mean RAL *(± SD)* per condition (x10^{-8})
ADN'TOX	IMR			Run 1[1]	Run 2		
IMR2014_31	L2-L1	Haddock	SITE 3	2.01	1.35	**1.68**	0.42 ± *0.47*
IMR2014_32	L3-L1	Haddock		0.55	0.35	**0.45**	
IMR2014_33	L4-L1	Haddock		0.53	0.45	**0.49**	
IMR2014_34	L5-L1	Haddock		0.80	0.61	**0.71**	
IMR2014_35	L7-L1	Haddock		0.30	0.20	**0.25**	
IMR2014_36	L8-L1	Haddock		0.15	0.10	**0.13**	
IMR2014_37	L9-L1	Haddock		0.50	0.42	**0.46**	
IMR2014_38	L10-L1	Haddock		0.76	0.39	**0.58**	
IMR2014_39	L11-L1	Haddock		0.27	0.15	**0.21**	
IMR2014_40	L12-L1	Haddock		*<0.01* [2]	*<0.01*	***<0.01***	
IMR2014_41	L13-L1	Haddock		2.03	1.26	**1.65**	
IMR2014_42	L14-L1	Haddock		*<0.01*	*<0.01*	**<0.01**	
IMR2014_43	L15-L1	Haddock		0.57	0.45	**0.51**	
IMR2014_44	L16-L1	Haddock		0.54	0.35	**0.45**	
IMR2014_45	L17-L1	Haddock		*<0.01*	*<0.01*	**<0.01**	
IMR2014_46	L18-L1	Haddock		0.43	0.30	**0.37**	
IMR2014_47	L19-L1	Haddock		0.49	0.31	**0.40**	
IMR2014_48	L20-L1	Haddock		0.56	0.32	**0.44**	
IMR2014_49	L21-L1	Haddock		0.15	*<0.01*	**0.08**	
IMR2014_50	L22-L1	Haddock		*<0.01*	*<0.01*	**<0.01**	
IMR2014_51	L23-L1	Haddock		*<0.01*	*<0.01*	**<0.01**	

[1] Run 1 and Run 2: two independent measurements of DNA adducts per sample.
[2] Analysis without any detectable spot/adduct

Table 1 (continued): Concentrations in bulky DNA adducts (expressed in Relative Adduct Level per 10^8 normal nucleotides (RAL x 10^{-8})) measured on the 100 fish included in the field study, classified according the sampling conditions (Site and year of sampling).

Sample identification		Species	Site	Relative Adduct Level (RAL) x 10^{-8}		Mean RAL per sample (x10^{-8})	Mean RAL *(± SD)* per condition (x10^{-8})
ADN'TOX	IMR			Run 1[1]	Run 2		
IMR2014_52	**2013-2HL1**	Haddock	**SITE 4**	0.12	*< 0.01* [2]	**0.07**	0.86 ± *0.40*
IMR2014_53	**2013-2HL2**	Haddock		0.77	0.83	**0.80**	
IMR2014_54	**2013-2HL3**	Haddock		1.41	1.34	**1.38**	
IMR2014_55	**2013-2HL4**	Haddock		1.20	1.31	**1.26**	
IMR2014_56	**2013-2HL5**	Haddock		0.74	0.79	**0.77**	
IMR2014_57	**2013-2HL6**	Haddock		0.94	1.12	**1.03**	
IMR2014_58	**2013-2HL8**	Haddock		1.40	1.13	**1.27**	
IMR2014_59	**2013-2HL9**	Haddock		1.02	0.73	**0.88**	
IMR2014_60	**2013-2HL11**	Haddock		0.82	0.76	**0.79**	
IMR2014_61	**2013-2HL12**	Haddock		0.36	0.48	**0.42**	

[1] Run 1 and Run 2: two independent measurements of DNA adducts per sample.
[2] Analysis without any detectable spot/adduct

Table 1 (continued): Concentrations in bulky DNA adducts (expressed in Relative Adduct Level per 10^8 normal nucleotides (RAL x 10^{-8})) measured on the 100 fish included in the field study, classified according the sampling conditions (Site and year of sampling).

Sample identification		Species	Site	Relative Adduct Level (RAL) x 10^{-8}		Mean RAL per sample (x10^{-8})	Mean RAL(± SD) per condition (x10^{-8})
ADN'TOX	IMR			Run 1[1]	Run 2		
IMR2014_62	2013-H3L1	Haddock	SITE 5	0.27	0.17	**0.22**	0.33 ± *0.35*
IMR2014_63	2013-H3L2	Haddock		0.86	0.60	**0.73**	
IMR2014_64	2013-H3L3	Haddock		0.50	0.82	**0.66**	
IMR2014_65	2013-H3L4	Haddock		*< 0.01* [2]	*< 0.01*	**< 0.01**	
IMR2014_66	2013-H3L5	Haddock		0.37	*< 0.01*	**0.19**	
IMR2014_67	2013-H3L6	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_68	2013-H3L7	Haddock		0.51	0.41	**0.46**	
IMR2014_69	2013-H3L8	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_70	2013-H3L9	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_71	2013-H3L10	Haddock		1.07	0.83	**0.95**	

[1] Run 1 and Run 2: two independent measurements of DNA adducts per sample.
[2] Analysis without any detectable spot/adduct

Sample identification		Species	Site	Relative Adduct Level (RAL) x 10^{-8}		Mean RAL per sample (x10^{-8})	Mean RAL(± SD) per condition (x10^{-8})
ADN'TOX	IMR			Run 1[1]	Run 2		
IMR2014_72	2013-H4L1	Haddock	SITE 6	0.57	0.78	**0.68**	0.48 ± *0.55*
IMR2014_73	2013-H4L2	Haddock		1.70	1.65	**1.68**	
IMR2014_74	2013-H4L3	Haddock		0.95	0.74	**0.85**	
IMR2014_75	2013-H4L4	Haddock		*< 0.01* [2]	*< 0.01*	**< 0.01**	
IMR2014_76	2013-H4L5	Haddock		0.71	1.04	**0.88**	
IMR2014_77	2013-H4L6	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_78	2013-H4L7	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_79	2013-H4L8	Haddock		0.36	0.24	**0.30**	
IMR2014_80	2013-H4L9	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_81	2013-H4L10	Haddock		0.29	0.40	**0.35**	

[1] Run 1 and Run 2: two independent measurements of DNA adducts per sample.
[2] Analysis without any detectable spot/adduct

Table 1 (continued): Concentrations in bulky DNA adducts (expressed in Relative Adduct Level per 10^8 normal nucleotides (RAL x 10^{-8})) measured on the 100 fish included in the field study, classified according the sampling conditions (Site and year of sampling).

Sample identification		Species	Site	Relative Adduct Level (RAL) x 10^{-8}		Mean RAL per sample (x10^{-8})	Mean RAL *(± SD)* per condition (x10^{-8})
ADN'TOX	IMR			Run 1[1]	Run 2		
IMR2014_82	2012-H3L1	Haddock	SITE 7	*< 0.01* [2]	0.28	**0.15**	0.25 *± 0.31*
IMR2014_83	2012-H3L2	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_84	2012-H3L3	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_85	2012-H3L4	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_86	2012-H3L5	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_87	2012-H3L6	Haddock		0.81	0.64	**0.73**	
IMR2014_88	2012-H3L7	Haddock		0.46	0.31	**0.39**	
IMR2014_89	2012-H3L8	Haddock		0.37	0.47	**0.42**	
IMR2014_90	2012-H3L9	Haddock		0.35	0.36	**0.36**	
IMR2014_91	2012-H3L10	Haddock		1.05	0.95	**1.00**	
IMR2014_92	2012-H3L11	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_93	2012-H3L12	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_94	2012-H3L13	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_95	2012-H3L14	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_96	2012-H3L15	Haddock		1.07	0.60	**0.84**	
IMR2014_97	2012-H3L16	Haddock		0.68	0.45	**0.57**	
IMR2014_98	2012-H3L17	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_99	2012-H3L18	Haddock		0.25	0.31	**0.28**	
IMR2014_100	2012-H3L19	Haddock		0.31	0.22	**0.27**	
IMR2014_101	2012-H3L20	Haddock		*< 0.01*	*< 0.01*	**< 0.01**	
IMR2014_102	H1-11/4	Haddock -Larvae		3.46	3.22	**3.34**	

[1] Run 1 and Run 2: two independent measurements of DNA adducts per sample.
[2] Analysis without any detectable spot/adduct

Table 1 (continued): Concentrations in bulky DNA adducts (expressed in Relative Adduct Level per 10^8 normal nucleotides (RAL x 10^{-8})) measured on the 100 fish included in the field study, classified according the sampling conditions (Site and year of sampling).

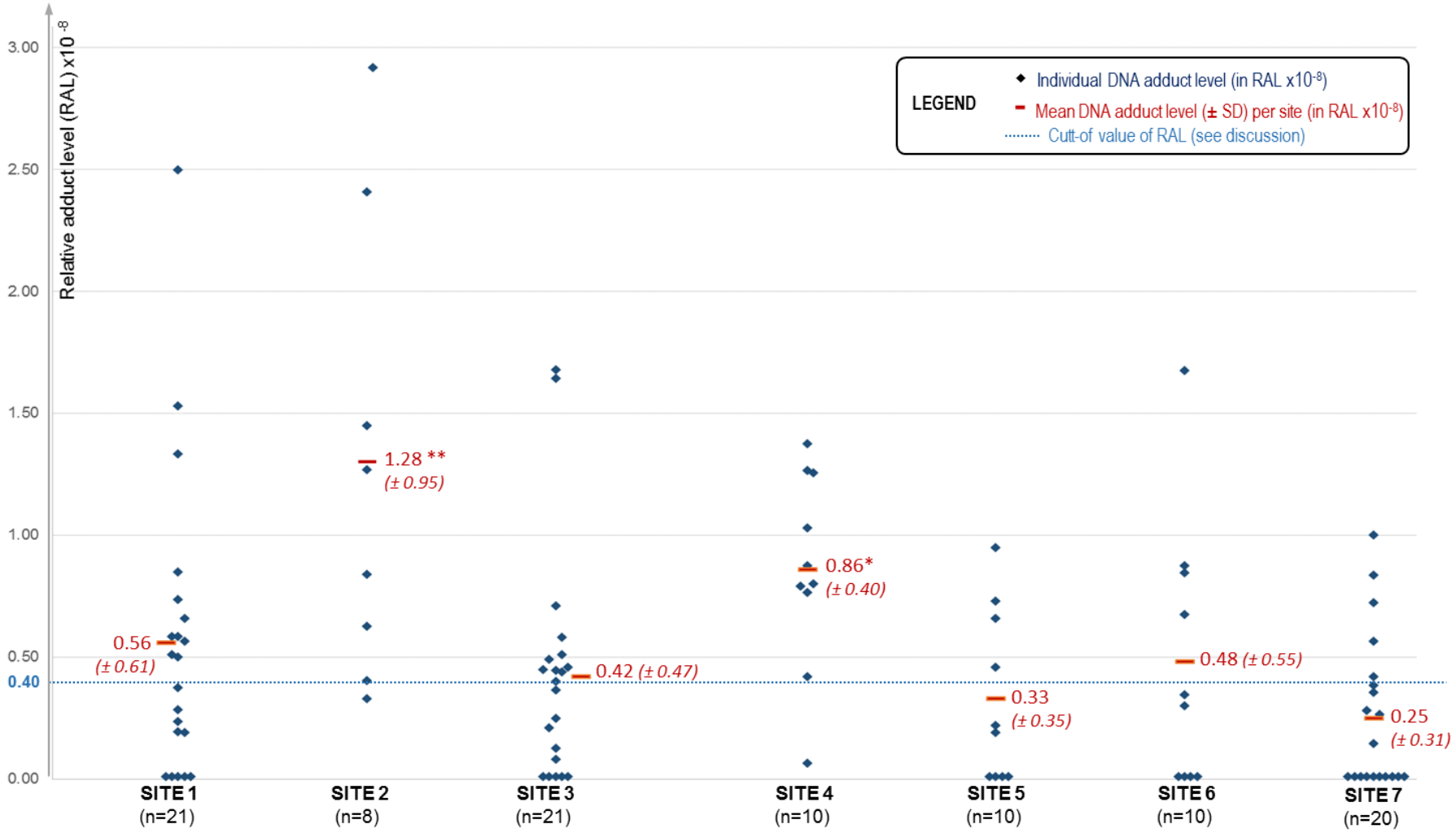


Figure 1: Mean concentrations of DNA adducts (in RAL x 10^{-8}) measured in the liver of 100 fish (Haddock species), classed by sampling site.

Each point of the graph corresponds to the individual mean RAL (mean of two independent adduct measurements)

The dashed line in blue indicates the cut-off value of 0.40 adducts per 10^8 normal nucleotides (see discussion)

Statistical analysis on the site effect in DNA adduct levels (Kruskal Wallis test, anova and Tukey's studentized range test): Global site effect with in particular ** Site 2 different from all other sites except site 4 ($p<0.05$); * Site 4 different from Site 7 ($p< 0.05$).

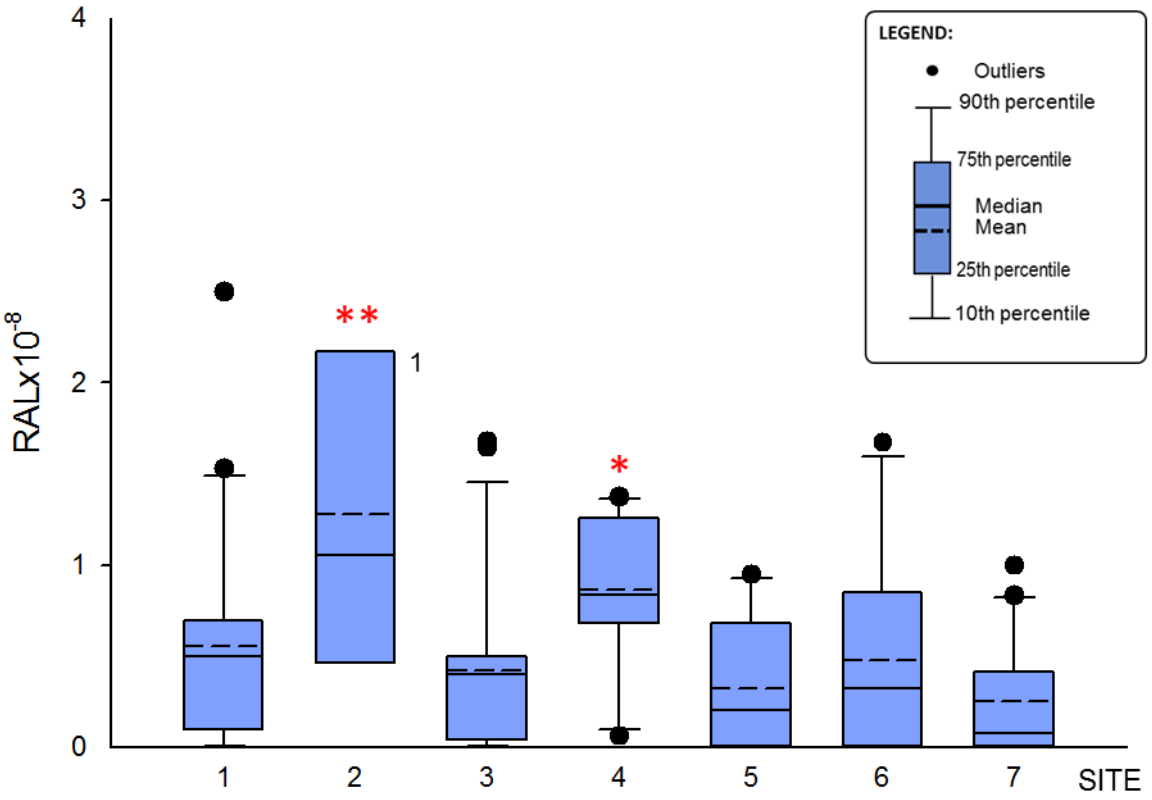
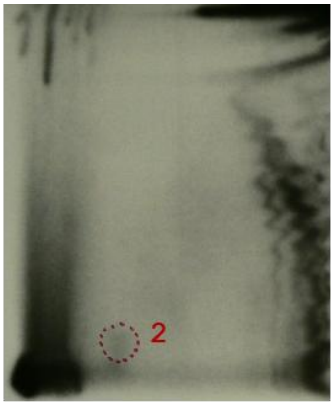


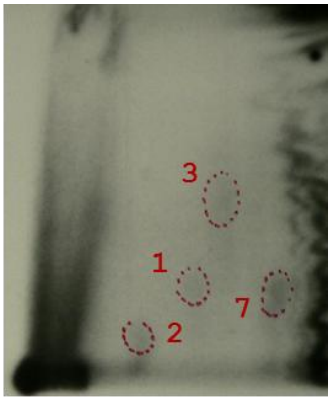
Figure 2: Mean concentrations in DNA adducts (in RAL x 10^{-8}) measured in the 100 haddock, classed by sampling site.
Representation in box plot (see legend)
[1] At least nine points are required to compute the 5th, 10th, 90th and 95th percentiles (SigmaPlot®)
Statistical analysis on the site effect in DNA adduct levels (Kruskal Wallis test, anova and Tukey's studentized range test): Global site effect with in particular ** Site 2 different from all other sites except site 4 ($p<0.05$); * Site 4 different from Site 7 ($p< 0.05$)

Figure 3: Representative DNA adduct patterns at **Site 1**

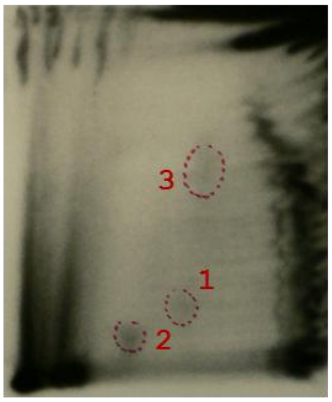
For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).



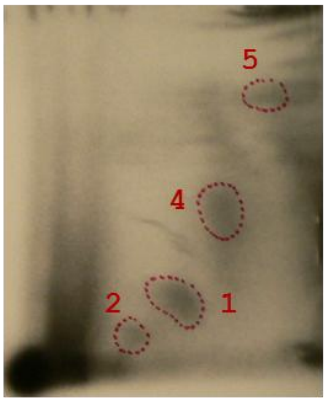
Sample n° 1 (D1-L1)



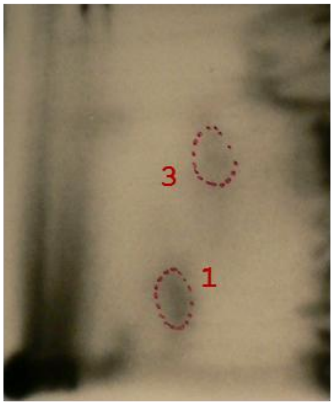
Sample n° 2 (D2-L1)



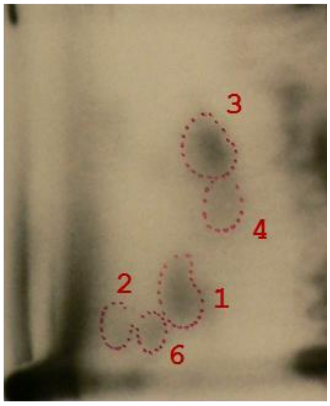
Sample n° 4 (D4-L1)



Sample n° 6 (D6-L1)



Sample n° 8 (D8-L1)

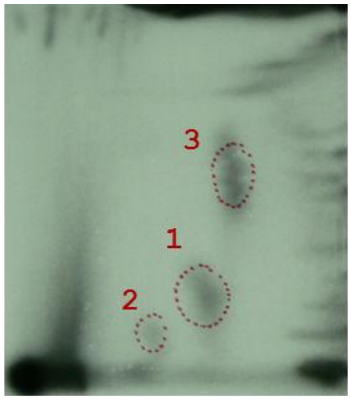


Sample n° 9 (D9-L1)

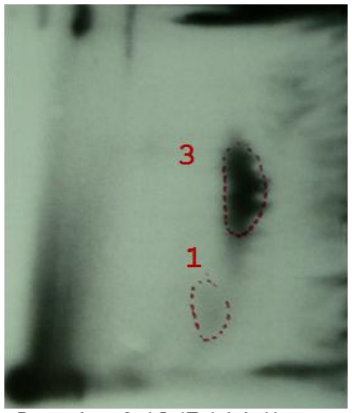
Figure 3: Representative DNA adduct patterns at **Site 1 (continued)**

For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).

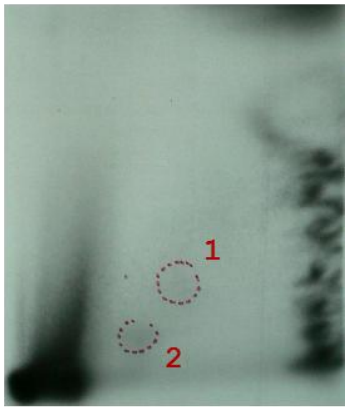
Sample n° 11 (D11-L1)



Sample n° 12 (D13-L1)



Sample n° 13 (D14-L1)



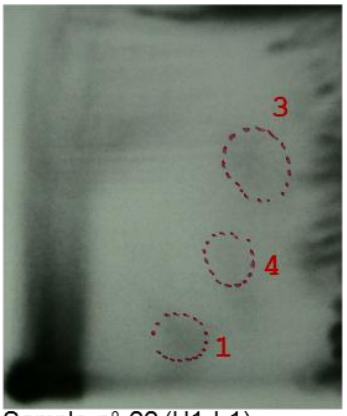
Sample n° 15 (D16-L1)

Sample n° 18 (D19-L1)

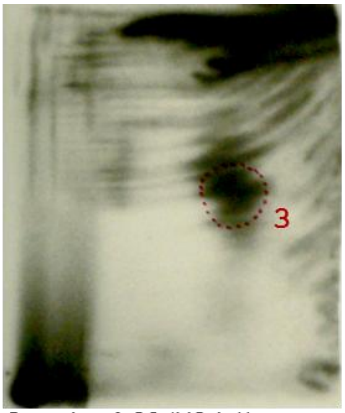
Sample n° 19 (D20-L1)

Figure 3: Representative DNA adduct patterns at **Site 2**

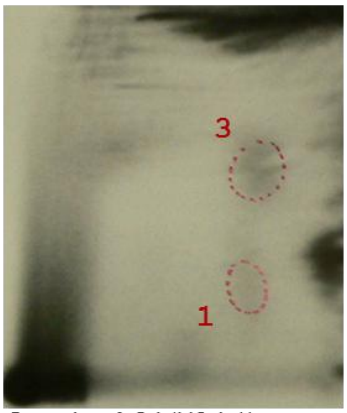
For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).



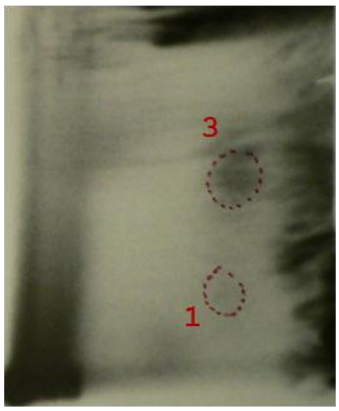
Sample n° 22 (H1-L1)



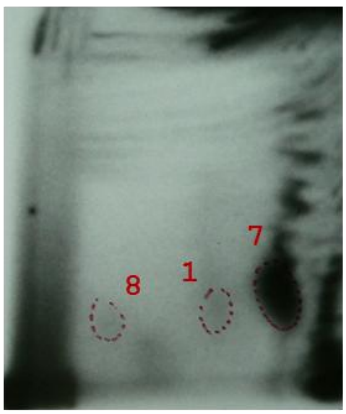
Sample n° 23 (H2-L1)



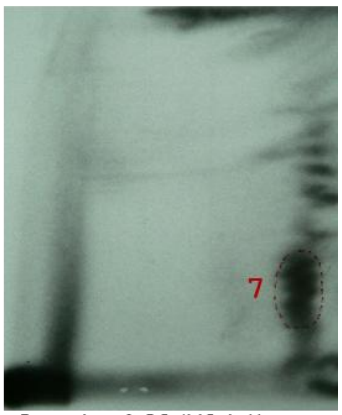
Sample n° 24 (H3-L1)



Sample n° 25 (H4-L1)



Sample n° 28 (H7-L1)



Sample n° 29 (H8-L1)

Figure 3: Representative DNA adduct patterns at **Site 3**

For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).

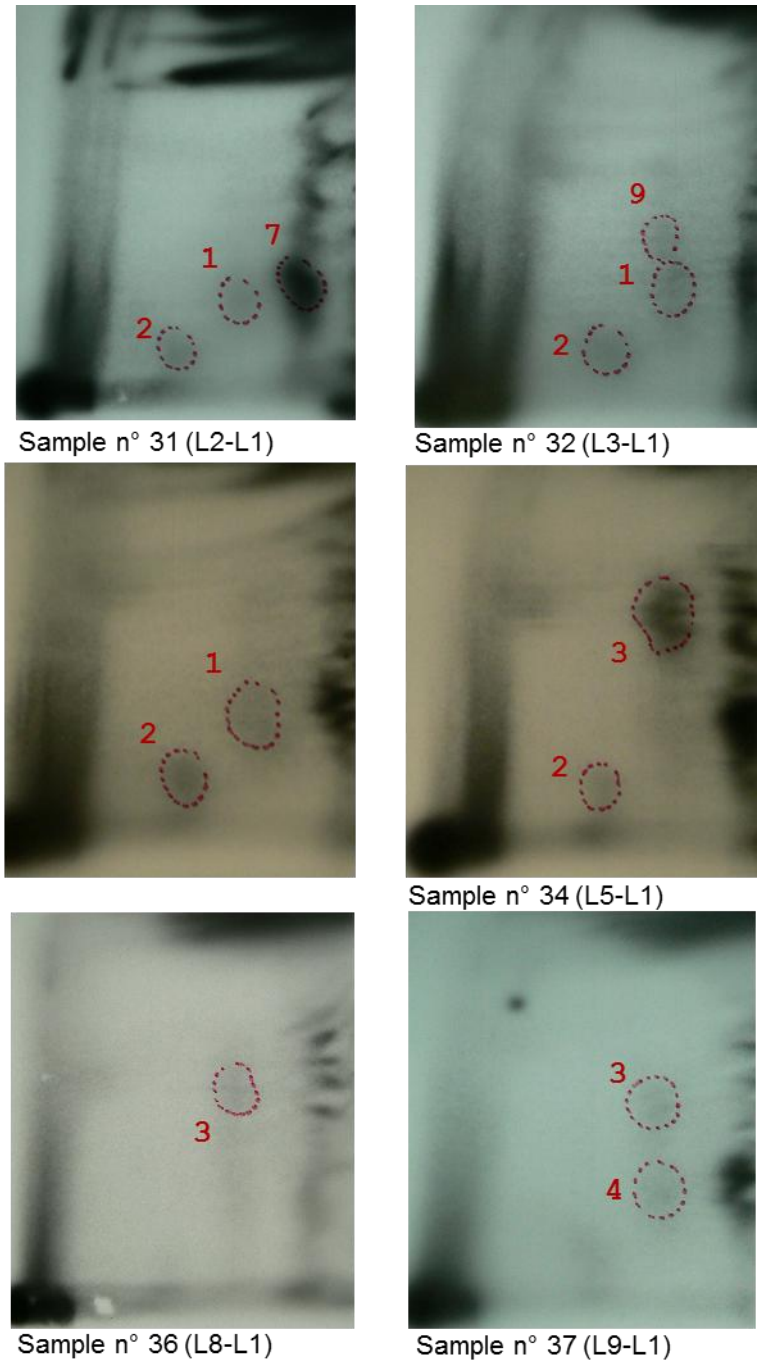


Figure 3: Representative DNA adduct patterns at **Site 3 (continued)**

For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).

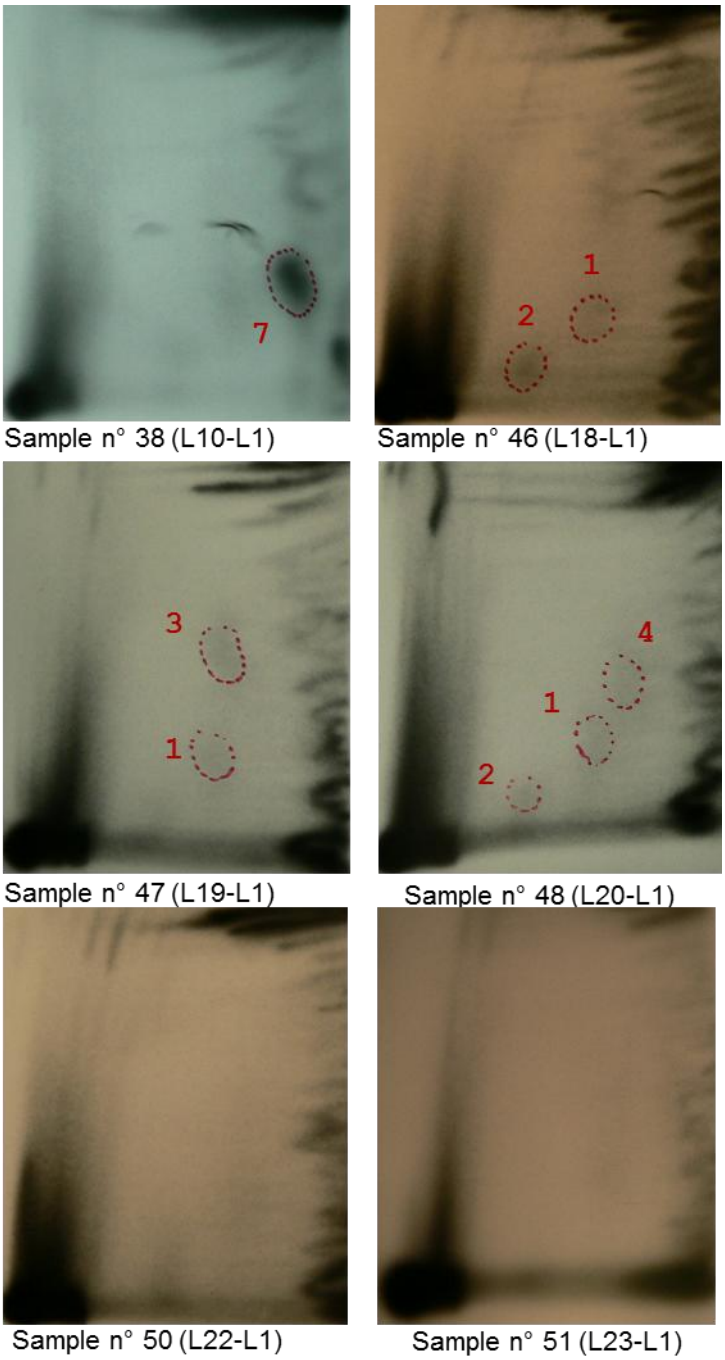


Figure 3: Representative DNA adduct patterns at **Site 4**

For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).

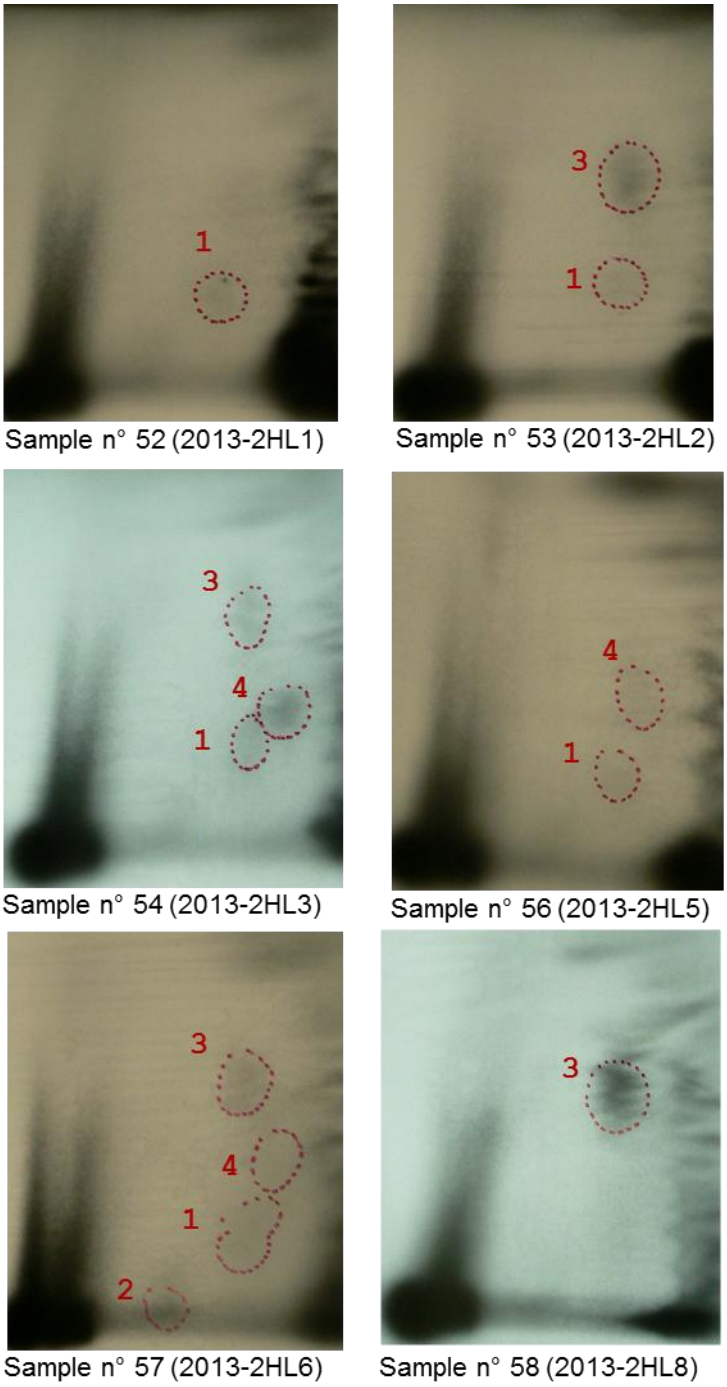


Figure 3: Representative DNA adduct patterns at **Site 5**

For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).

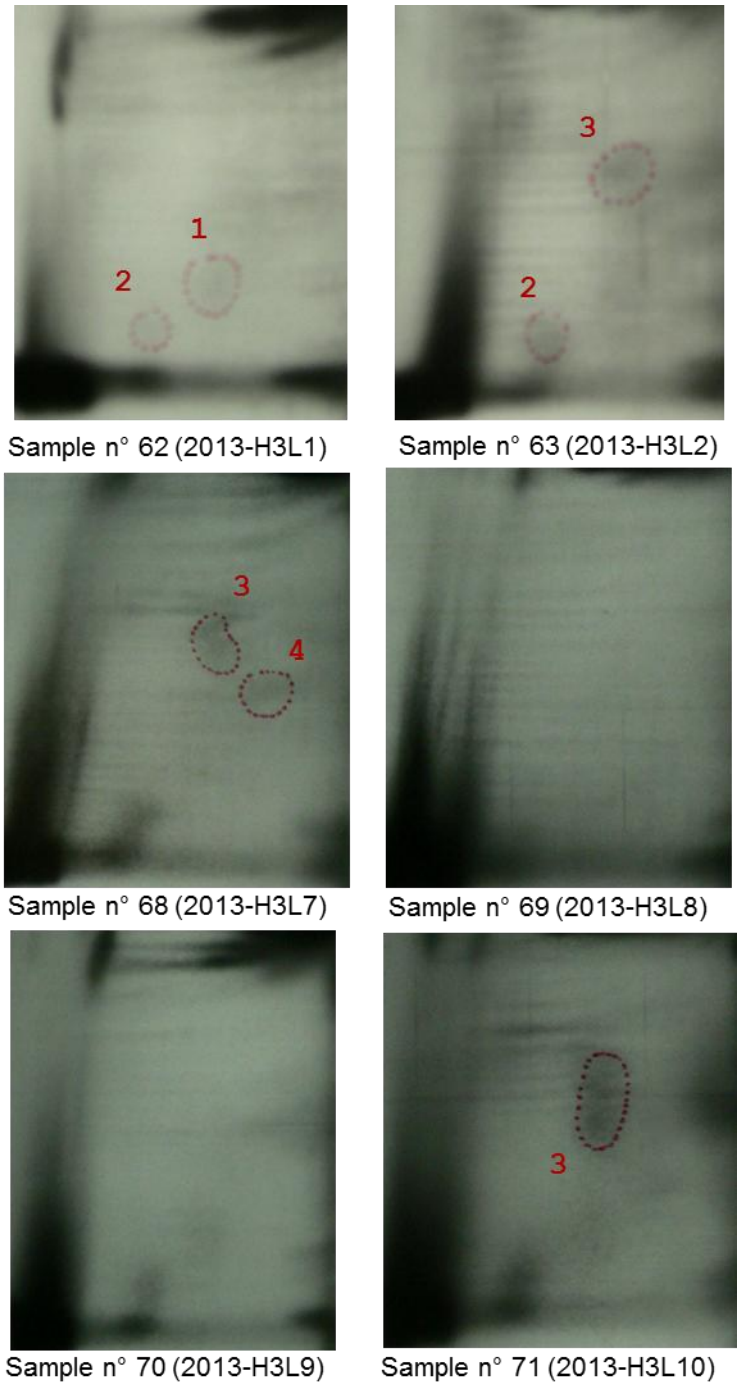
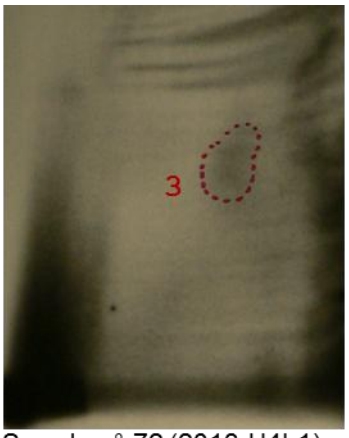
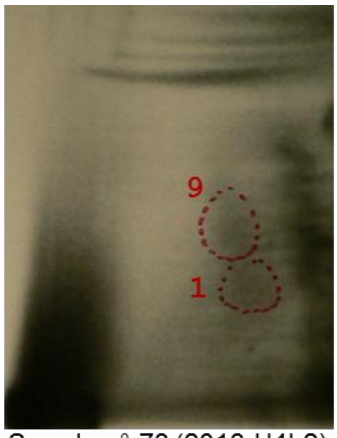


Figure 3: Representative DNA adduct patterns at **Site 6**

For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).

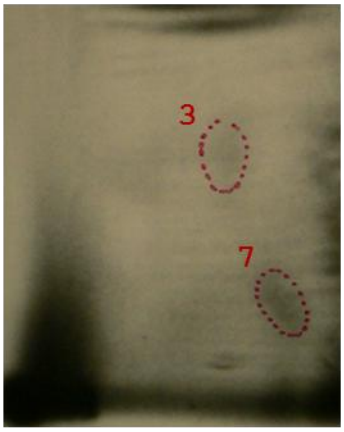


Sample n° 72 (2013-H4L1)



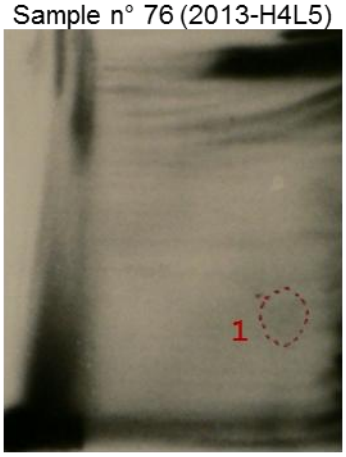
Sample n° 73 (2013-H4L2)

Sample n° 75 (2013-H4L4)



Sample n° 76 (2013-H4L5)

Sample n° 78 (2013-H4L7)



Sample n° 79 (2013-H4L8)

Figure 3: Representative DNA adduct patterns at **Site 7**

For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).

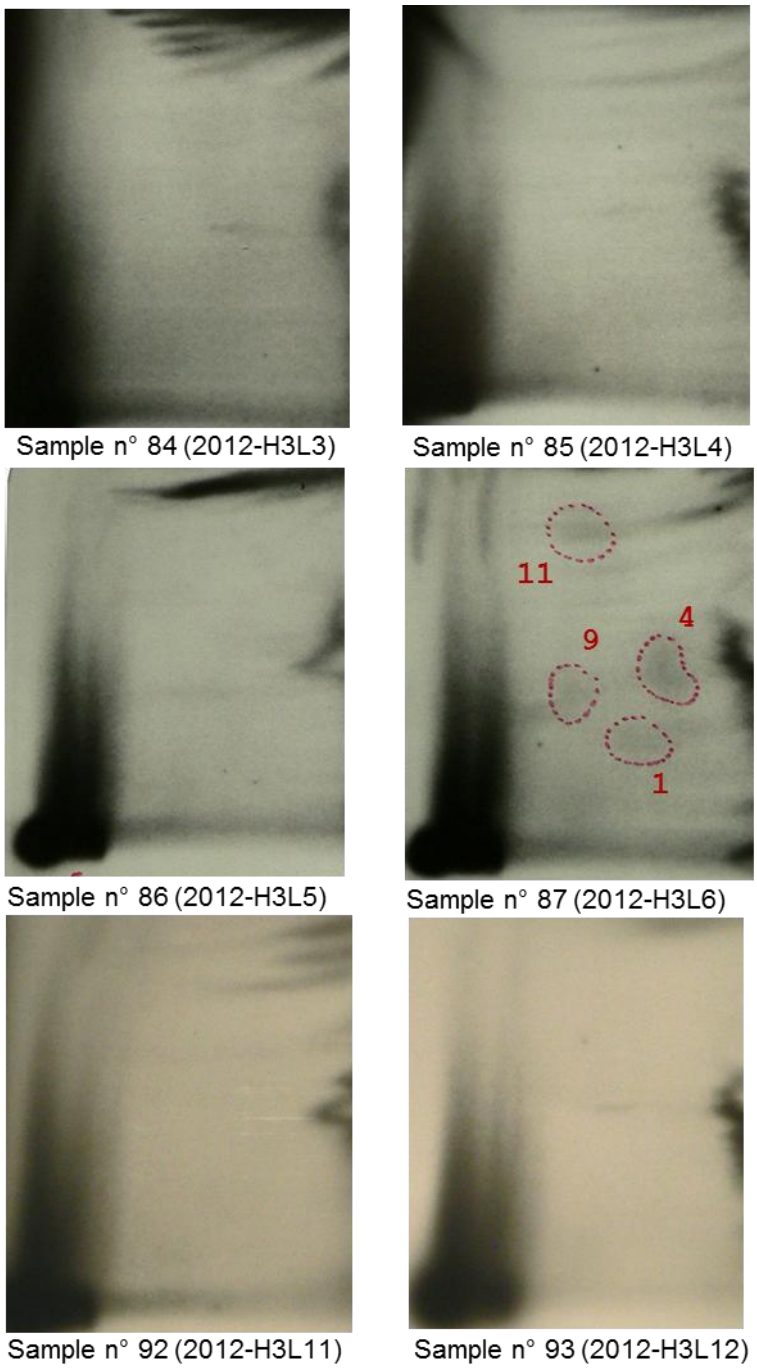
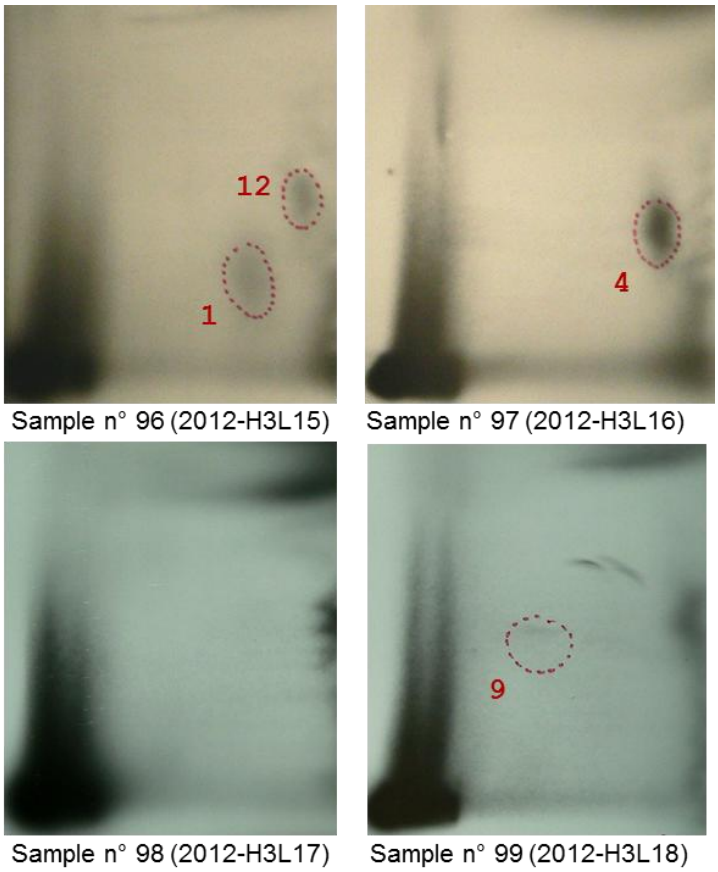


Figure 3: Representative DNA adduct patterns at **Site 7 (continued)**

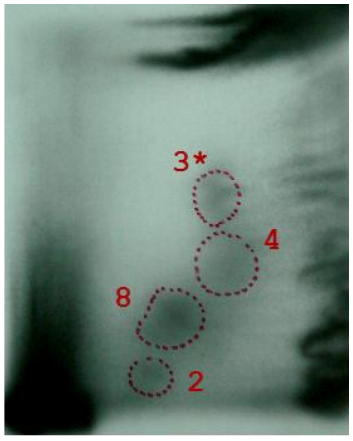
For represented samples, autoradiographic pattern is one among both realised (two analyses per sample).
Spots are numbered according to their location on PEI-cellulose plates (see template at **Figure 4**)
Exposure conditions: Amersham Hyperfilm MP (GE Healthcare), exposure time: 72 hours (-80°C).



Sample n° 96 (2012-H3L15) Sample n° 97 (2012-H3L16)

Sample n° 98 (2012-H3L17) Sample n° 99 (2012-H3L18)

Figure 3: DNA adduct patterns associated to a pool of **haddock larvae**



Sample n° 102 (H1-11/4)

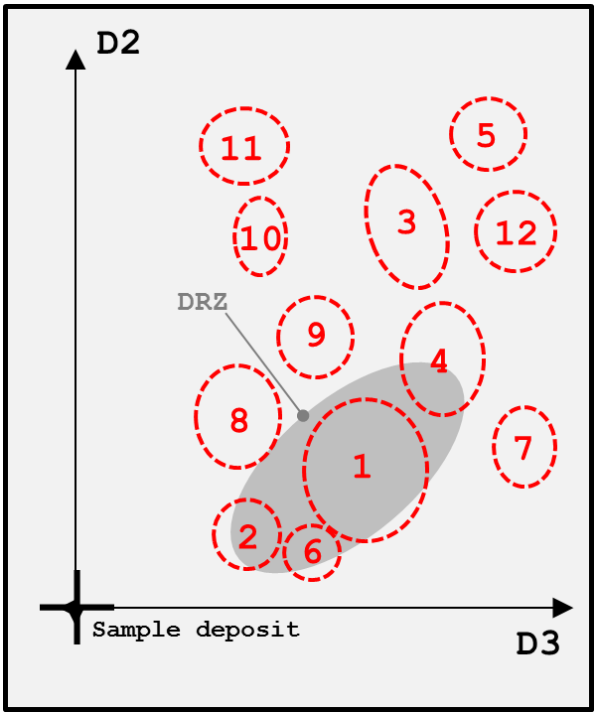
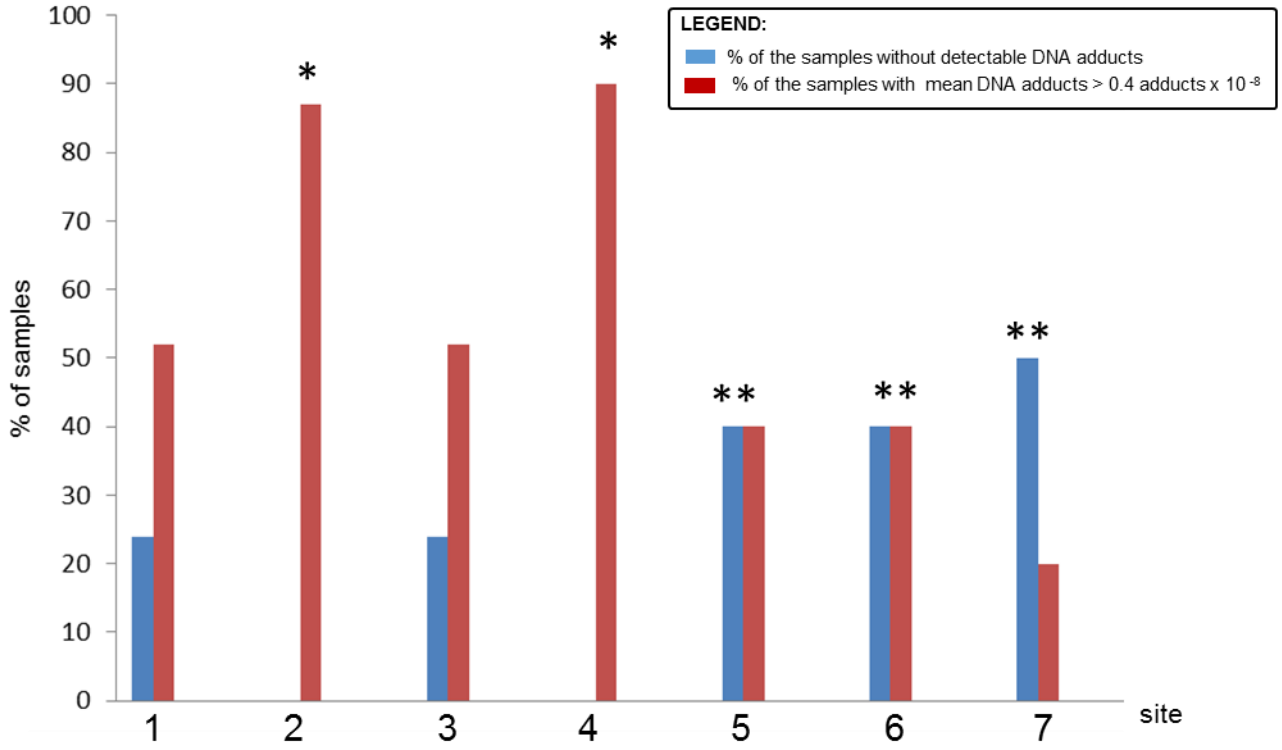


Figure 4: Location template of the different distinct spots attributed to DNA adducts obtained after two-dimensional Thin Layer Chromatography on the overall 200 patterns (100 samples).

D1, D2, D3 and D4 migrations are detailed in annexe 2b.



* Statistically significant global effect of the site in the proportion of samples with elevated DNA adduct levels (p=0.008, Fisher's Exact Test), with higher proportions for sites n°2 and 4.
** Statistically significant global effect of the site in the proportion of sample without detectable DNA adducts (p=0.02, Fisher's Exact Test), with higher proportions for sites n°5, 6 and 7.
and proportion of samples with elevated DNA adduct levels): *** p<0.001; ** p< 0.01; * p<0.05

Figure 5: Occurrence frequency of the samples without detectable DNA adducts and samples with higher concentrations in DNA adducts (>0.4 adducts per 10^8 nucleotides) classed by site.

Site	number of samples	number of samples with defined spot *(% of samples with defined spot at the site)*			
		Spot 1**	Spot 2*	Spot 3***	Spot 4
1	21	14 *(67%)*	13 *(62%)*	11 *(52%)*	4 *(19%)*
2	8	6 *(75%)*	4 *(50%)*	7 *(87%)*	2 *(25%)*
3	21	11 *(52%)*	8 *(38%)*	7 *(33%)*	5 *(24%)*
4	10	7 *(70%)*	3 *(30%)*	9 *(90%)*	3 *(30%)*
5	10	2 *(20%)*	3 *(20%)*	2 *(20%)*	2 *(20%)*
6	10	3 *(30%)*	3 *(30%)*	3 *(30%)*	3 *(30%)*
7	20	5 *(25%)*	5 *(25%)*	5 *(25%)*	5 *(25%)*
Total	*100*	*48 (48%)*	*36 (36%)*	*43 (43%)*	*25 (25%)*

* The proportion of sample with spot 2 is different from site to site, at the limit of statistical significance (p=0.06, Fisher's Exact Test); ** the proportion of sample with spot 1 is different from site to site (p=0.02, Fisher's Exact Test); *** the proportion of sample with spot 3 is highly different from site to site (p<0.001, Fisher's Exact Test)

Table 2: Occurrence frequency of the samples that present the four major spots n°1, n°2, n°3 and n°9, classed by site.

Discussion and Conclusion

The present field study consists in the analysis of the DNA adduct patterns in the liver of 100 individual fish of the same species, haddock (*Melanogrammus aeglefinus*), sampled in different collection areas of the North Sea (Norwegian waters), in the different years 2012, 2013 and 2014. The purpose of the study is to contribute to the evaluation of the genotoxic impact of the offshore oil and gas activities on the marine environment of the North Sea.

Historically, the analysis of DNA adduct profiles in fish exposed to environmental pollutants represents an important approach in environmental risk assessment since Dawe et al. claimed in 1964 that bottom feeding fish were “useful indicators of environnemental carcinogens”. DNA adducts are now considered as a crucial biomarker of exposure, especially for there early emergence after a genotoxic exposure, which may play a key role in establishing a mode of action for cancer (Pottenger et al., 2009). Because of its high sensibility and versatility, the method of ^{32}P postlabelling has been applied to environmental fish studies as early as 1980s, few years after the first publication of the method (1981). Thus, in 1987, Dunn et al. measured significant DNA adduct levels in livers of wild Brown bulheads sampled from sites in the Buffalo and Detroit Rivers, in association with exposure of fish to high concentrations of polycyclic aromatic hydrocarbons. Since these early works, a large range of fish species was studied, in a large panel of applications (laboratory and field studies).

In the marine environment, numerous published works are focused on the flounder (*Platichthys flesus*). Most of them indicate that adducts are detected in the liver when fish are exposed to environmental genotoxicants. Data are available in relation with controlled laboratory exposures and environmental field studies (Harvey et al. 1997, Reynolds et al., 2003, Malmström et al., 2009). The published data on DNA adduct measurement in other fish like haddock seem to be less abundant.

In the present study, the measured DNA adduct concentrations remain relatively low and in general below the value of 1 adduct per 10^8 normal nucleotides. These concentrations are in accordance with literature associated to field studies, whatever locations and fish species.

On the 2014 part of the field study including 3 sampling sites (sites 1, 2 and 3), the mean concentration of DNA adducts in the liver of haddock at site 2 is between 2 or 3 times those of the other two sites. Interestingly, fish associated to site 2 have been caught in the (large) area of Njord and Draugen platforms, two potential sources of PAH (and other pollutants). It should be noted that the mean concentrations of

DNA adducts measured in fish livers are around the value of 0.4 adducts per 10^8 nucleotides for both sites 1 and 3 (see below for the interest of this cut-off value).

In 2011, a comparable study to the current one was conducted on haddock (*Melanogrammus aeglefinus*) and atlantic cod (*Gadus morhua*) caught in two areas of the North Sea with extensive oil production: Tampen and Sleipner (Balk et al., 2011). From 2001 to 2004 fish campaigns, Balk et al. revealed significant higher levels of hepatic adducts in haddock from the Tampen area compared to a control site located in southwest Norway (Egersund bank). Similarly, such quantitative differences in DNA adduct levels between reference sites and oil platform areas were observed in previous study conducted in our laboratory from fish caught in the same areas (IMR report, 2011). But, surprisingly, if DNA adduct patterns seem to be conserved at the Tampen site between 2011 and 2013 (similar DNA adduct level in average and comparable individual distribution of DNA adduct concentrations) the intersite differences were no longer observed in the 2012-2013 part of the present study (sites 4 to 7). In particular, the more elevated concentrations in DNA adduct were observed at site 4, a sampling site associated to Egersund Bank area explored in 2013, with mean DNA adduct level 2 to 3 times those observed at the other sites: Viking bank area in 2013 and 2012 (sites 5 and 7) and Tampen in 2013 (site 6). Finally, the difference is only significant with Viking bank in 2012. Different hypotheses can be formulated to explain these fluctuating results (see later in the discussion).

To go into more detail, it is to note that the low concentrations in DNA adducts globally observed are associated with a large proportion of samples without detectable DNA adducts (nearly between one third and one quarter of all the samples, with large intersite variations), or more precisely below the calculated detection limit of 0.01 adducts for 10^8 normal nucleotides. The measured concentrations are most often about a few adducts for 10^9 normal nucleotides, approaching the limits of detection / quantification accepted for the method. These low values probably explain the technical variability observed between both independent DNA adduct measurements applied on each sample, and could be partly associated to the observed variability of the results from one study to another, and for a given site, to one sampling campaign to the next. The method is known to be particularly sensitive and polyvalent, but certainly semi quantitative. The large proportion of undetectable and very low levels of DNA adducts in tissues of wild fish is of frequent concern. From 98 samples (11 species) caught in presumably pristine areas of the northern Atlantic, DNA adduct levels in liver were below the detection limit of the ^{32}P-postlabelling method in three quarters of cases and just above in the remaining quarter (Aas et al., 2003). The sensitivity of the methods used in the environmental studies focused on genotoxicity is a crucial analytical parameter.

In the present study, the proportion of samples without detectable DNA adducts is varying from one site to another, with highest proportion at site 7 (Vikingbank in 2012, 50% of samples). In contrast, all the samples at Site 2 and 4 revealed DNA adducts, in accordance with the highest mean DNA adduct concentrations per site. Once again, if this observation seems to be expected for the site 2 (area close to 2 oil platforms), it is more surprising concerning the site 4 (Egersund Bank). Moreover, if the relatively elevated proportion of samples with undetectable DNA adducts at site 6 (Tampen, 2013: 40% of samples (6/10)) is unexpected at first sight, this result is nevertheless fairly close to the previous results of the 2011 campaign (Tampen 2011 (site H5): 32% of samples (8/25) with undetectable adducts).

In a Qualitative point of view, 12 distinct spots assigned to different DNA adducts and a diagonal radioactive zone (DRZ) are counted on the overall study. Such qualitative variety can be attributed to the large capability of haddock to realize enzymatic bioactivation of xenobiotics and/or the presence of numerous genotoxic pollutants in fish environment. Thus, the richness of DNA adduct pattern per fish is statistically different from one site to another, with higher distinguished spots per sample at sites 2, 4 and 1 (more than 2 spots per sample in average). Concerning the 3 sites of the 2014 campaign, the site number 2 shows as expected the largest number of distinct spots per individual. In the rest of the study (2012-2013 campaigns) the largest number of spots per individual is more surprisingly observed at site 4 (Egersund Bank). In particular, values at Viking bank in 2012 and 2013 (sites 5 and 7) are the lowest observed, in contrast to the previous study realised in 2011 (highest number of distinct spots were observed at site H6, area near Viking Bank). The less diversified DNA adduct profiles are concerning 3 among the 4 sites belonging to 2012 or 2013 sampling campaign, thus leading to consider a potential effect associated to sampling campaign and/or technical artefact (see later in the discussion).

No spot appears to be specific of one or more sites. Among the 4 major spots (detected in more than one quarter of the overall samples), the 3 more frequently occurring spots (spots n°1, 2 and 3) are differently distributed according to the sampling site. By assuming that the higher is the exposure of a population of fish to a defined genotoxic pollutant or mixture, the more frequent is the associated damage in sampled individuals, it is possible to argue that genotoxicants that led to DNA adducts of the spots 1, 2 and 3 can be probably more present in the environment at sites 2 and 4 by comparison with other sites. Interinsting, the most frequent spot n°1 seems to be comparable to the one described in the positive control for the method used (both spots had the same chromatographic profiles and overlaps). So, it can be hypothesise that this adduct is directly related to benzo[a]pyrene diol epoxide (BPDE), a major metabolite of Benzo[a]pyrene (BaP). On this basis, fish that have been caught in sites n°1, 2 (Njord and Draugen platforms) and 4 (Egersund Bank in 2013) may globally have been more exposed to baP than fish of other sites.

According to the qualitative results, the absence of real specificity of spots in presumably contaminated areas could be attributed to the relative presence of certain genotoxic pollutants in the overall sites, in probably very different concentrations. The high proportion of samples without detectable adducts in some stations could reflect the presence of genotoxic pollutants in very low levels, under an undefined level that contributes to a detectable DNA adduct formation in haddock liver. Other hypothesis for the non-specificity of spots and the presence of detectable DNA adducts in supposed unpolluted areas are the possible migration of fish from other contaminated site or the revelation of endogenous DNA adducts (Aas et al., 2003; Swenberg et al., 2011). Endogenous DNA adducts are lesions of the DNA that can occur outside of exposure to xenobiotics.

In contrast, individuals with higher DNA adduct levels (>0.40 adducts per 10^8 normal nucleotides) are thought to be exposed to higher levels of genotoxicants and/or are especially sensitive to the genotoxicity of certain pollutants because of the genetic susceptibility combined with environmental exposures during vulnerable periods of development.

The mean adduct level measured in each site is below or somewhat above the value of 0.40 adducts per 10^8 nucleotides for four (or five) sites and well above this value at two others, one site in the vicinity of Njord and Draugen offshore oil platforms. Interestingly, in previous comparable field studies, this cut-off value of 0.40 adduct per 10^8 nucleotides has been proposed by our laboratory as a possible threshold value for the detection of a significant genotoxic effect attributed to environmental pollutants (unpublished data). It is to note that this hypothesised value is equal to the mean DNA adduct concentration measured by Balk et al (2011) in haddock at the control site Egersund bank. In the way of a better environmental risk assessment, the determination of reliable threshold values for biomarkers is now a crucial issue. The question is under discussion, as shown in a report of the study Group on integrated monitoring of contaminants and biological effects dated 14-18 march 2011 (ICES, 2011). The proposed BAC (Background assessment concentrations) and EAC (Environmental Assessment criteria) values for DNA adducts are in accordance with the results obtained in our laboratory since 2009. Surprisingly, from the present study, the mean DNA adducts concentration in the liver of haddock caught in 2013 was twice this reference value at Egersund Bank (Site 4) and just below at Tampen (Site 6).

The fluctuation of the DNA adduct concentrations between different sampling campaigns for a same fish species at a same site, as well as the inconsistent intersite differences, could be partly explained by (apart from technical aspects) 1) The interindividual variability in the response to pollutant exposure and sampling inhomogeneity, 2) a quantitative and qualitative fluctuation in exposure to the complex cocktails of pollutants over time, 3) the variation of environmental parameters associated to fish's living conditions at

each site 4) the migration of fish from site to another (for example during spawning season). These different points are not exhaustive and have to be considered with regard to the response of integrative biomarkers of exposure like DNA adducts. To illustrate the potential role of the cocktails of environmental pollutants on biomarker response, our research team observed in previous field study a very significant (and unexpected) more elevated level of bulky DNA adducts in blue mussels collected at a reference site (lightly contaminated by PAHs) compared to mussels collected at more polluted sites (Rocher et al., 2006). In response to this surprising situation associated to a well know sentinel organism, two major hypotheses were advanced: the exposure of mussels to unknown genotoxicants and/or an acquired defect of repair of DNA damage like DNA adducts. In particular, Arsenic was measured in soft tissue of mussels at the reference site with the highest values. Interestingly, this toxicant is considered as capable of enhancing the B[a]P genotoxicity by inhibiting repair of DNA damage in rodents.

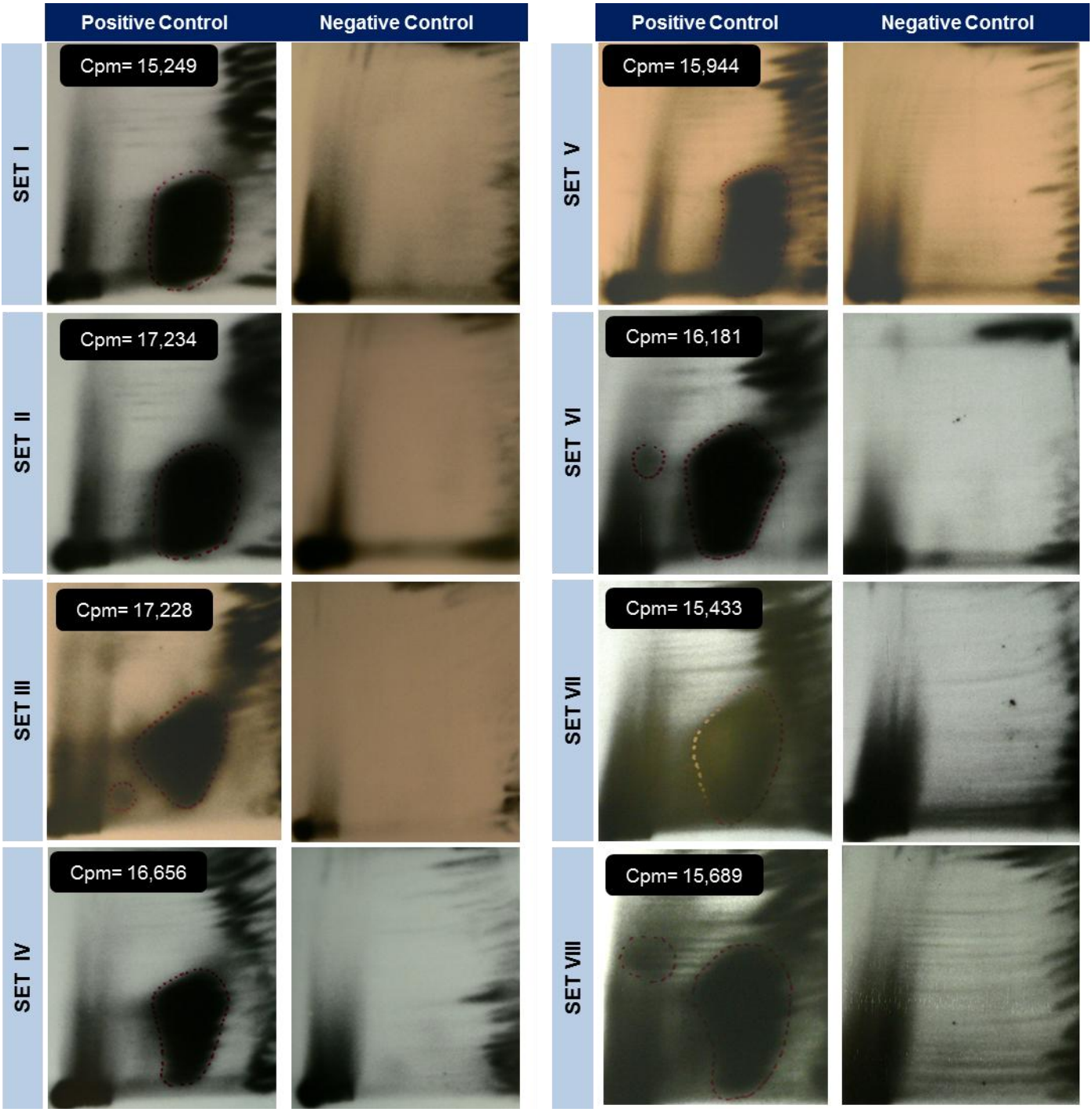
In our previous report (IMR 2011), variation of DNA adduct in haddock caught at different years in the area of Tampen was already described. In 2008, the mean DNA adduct concentration in a group of 5 haddock was only 0.18 $x10^{-8}$ adducts. In 2010, this value reached 0.62 $x10^{-8}$ adducts, with high proportion of samples without detectable adducts (4 samples among 8, i.e. 50% of the samples). Unfortunately, the groups were smaller to conclude definitively on a sampling year effect.

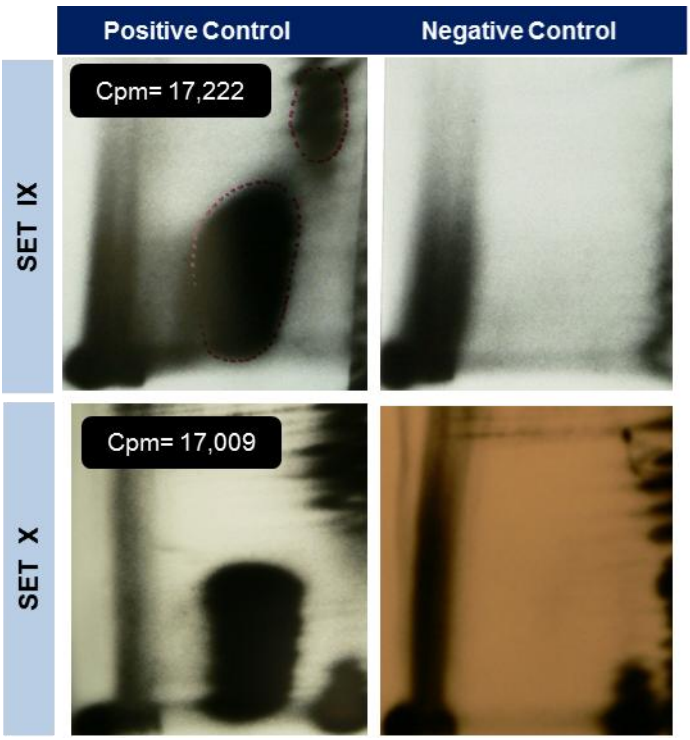
In a more technical point of view, the hypothesis of a potential effect of the storage period of the samples on the DNA adduct profiles, and a possible loss of DNA adducts during this steep, cannot be excluded. The question of the stability of DNA adducts, in tissue or isolated DNA, is not clearly elucidated, especially after an extended period of storage. According to Gupta, one of the greatest expert in the ^{32}P postlabeling method, DNA adducts associated to PAH exposure are thought to be stable at -80°C in both tissue or purified DNA for several weeks to months (in “Technologies for detection of DNA Damage and mutation”, Pfeifeir, 1996) . Other DNA adducts like aromatic-derived ones are probably more labile and must be explored “without excessive storage”. In stored tissue, and especially in liver where enzymatic activities are elevated, it cannot be excluded a biologic degradation of DNA adducts, even at -80°C. In 1996, Binkova et al. measured benzo(a)pyrene DNA adducts in different tissues of rats exposed to 100 mg/kg BaP for 24h, after a long-term storage at -20°C and -80°C. No particular effect was detected up to 10 months storage. Unfortunately, the trial did not include a longer follow-up period. In fact, the most critical point seems to be associated to sample preparation before freezing. A significant decrease in benzo(a)pyrene DNA adduct levels in rat liver was observed in association with a delay in freezing organs (especially for organs that were kept for several hours to several days at + 20 ° C + 37 !) (Izzotti, 1993).
Further to the technical point, prolonged storage of samples may alter DNA too, and consequently DNA adduct contents. However, the NOAA technical Memorandum 14 (1994) indicates that “DNA appears to be

stable in whole tissue for at least 2 years". Our spectrophotometric results associated to the DNA extraction from samples collected in 2012 and 2013 seems to confirm this suggestion, and leads to the reasonable exclusion of this technical artefact.

ANNEXES

ANNEXE 1: Autoradiographic patterns of the negative and positive controls included in each set of 32P-postlabelling (sets I to XI).





Cpm= count per minute= direct radioactivity measured in the major spot (MS) in the positive control (after subtraction of background noise), for each set of analyses.

Autoradiography is realised after the specific ^{32}P labelling of DNA adducts and 2D-chromatographic separation on PEI-cellulose sheet. Time of exposure is to 72 hours.
Spot radioactivity is measured on PEI cellulose sheet with a scintillation counter (Cerenkov mode).
Positive control: calf thymus DNA treated by benzo[a]pyrene dioepoxide (BPDE) with a final concentration of 110.70 adducts for 10^8 normal nucleotides (according to F.A. Beland, in Philips and Castegnaro, 1999)
Negative control: plasmid DNA.

ANNEXE 3: Qualitative raw data.

Qualitative results on the 100 samples according to the presence/absence of spots.

When spot (n°1 to 15 + DRZ) is present for only one of both analyses, the number 1 is indicated

When spot (n°1 to 15 + DRZ) is present for both analyses, the number 2 is indicated

Sample Number	Site	Spot number															
		1	2	3	4	5	6	7	8	9	1	11	12	13	14	15	DRZ
1	site1	1	1														
2	site1	2	2	1				1									
3	site1	1	1	1													1
4	site1	2	1	2													
5	site1	2	1	1													
6	site1	1	2		2	2			1								
7	site1																
8	site1	1	1	1	1					1							
9	site1	2	2	1	2		1			1							
10	site1	2	2														
11	site1																
12	site1	1	2	1													
13	site1	1		1													1
14	site1	1	2														
15	site1	2	1														
16	site1																
17	site1	2	1	1													
18	site1																
19	site1																
20	site1			2	1												
21	site1			2													
22	site2	1		1	2												
23	site2		1	2							1						
24	site2	2		2													
25	site2	1	1	1													
26	site2	1	1	1	1												
27	site2	2															
28	site2	1	1	1				1	1								
29	site2			1				1									
31	site3	1	2					1									
32	site3	2	2							2							
33	site3	2	2														
34	site3	1	1	1	1												
35	site3	2															
36	site3			2													
37	site3			2	2												
38	site3							2									
39	site3		2														
40	site3																
41	site3			1	1												
42	site3																
43	site3	1	2	2													
44	site3	1		2													
45	site3																
46	site3	1	2														
47	site3	1		1	1												
48	site3	1	2		1												
49	site3	1															
50	site3																
51	site3																

Sample Number	Site	Spot number															
		1	2	3	4	5	6	7	8	9	1	11	12	13	14	15	DRZ
52	site4	1															
53	site4	1		2													
54	site4	1	1	2	1												
55	site4	1		2							1	1					
56	site4	1		1	1												
57	site4	2	2	1	2												
58	site4			2													
59	site4			2													
60	site4	1	1	2													
61	site4			2													
62	site5	1	1	1													
63	site5		1	2													
64	site5	1			1												
65	site5																
66	site5		1														
67	site5																
68	site5			2	1												
69	site5																
70	site5																
71	site5			2				1									
72	site6			2													
73	site6	2	1		1					1							
74	site6		1	2													
75	site6																
76	site6	1		2				1									
77	site6																
78	site6																
79	site6	1			1												
80	site6																
81	site6			2													
82	site7	1															
83	site7																
84	site7																
85	site7																
86	site7																
87	site7	1			2					2		1					
88	site7			1	1								1				
89	site7	1	1		2												
90	site7		2		2												
91	site7				2												
92	site7																
93	site7																
94	site7																
95	site7																
96	site7	2											1				
97	site7		1		1												
98	site7																
99	site7									2							
100	site7	1			1												
101	site7																

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