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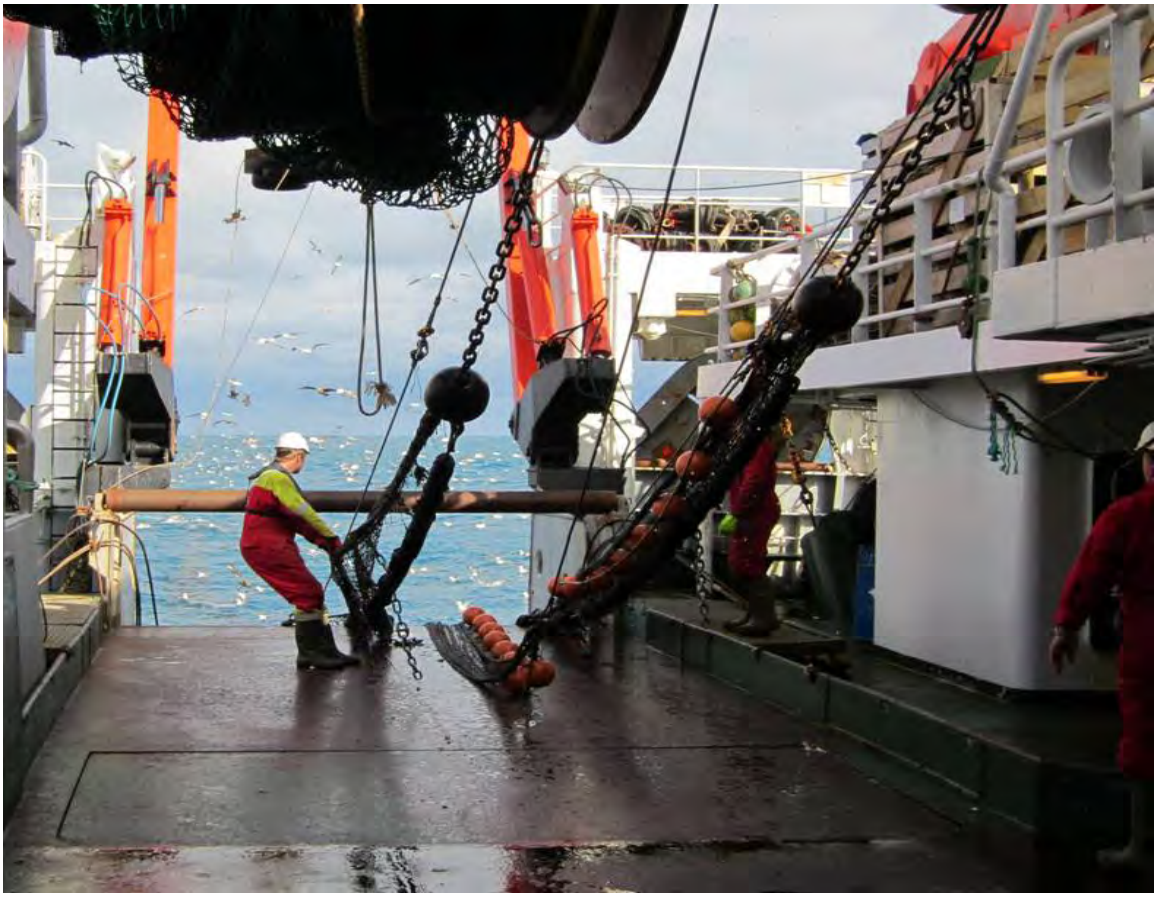

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Condition monitoring in the water column 2011: Oil hydrocarbons in fish from Norwegian waters

Authors:

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The 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 North Sea during summer 2011 by bottom trawl. We sampled cod and haddock from the Egersund Bank (reference area) and from the Tampen region where the installations with largest discharges of produced water are located. In addition haddock were sampled from three sites; Ula area (the Southern North Sea), Bressay Bank (reference area) and the Viking Bank) to obtain better resolution for differences in DNA adduct levels in the North Sea. Differences in DNA adduct levels in haddock from Tampen compared to the Egersund Bank were reported from condition monitoring in 2002, 2005 and 2008.

The following methods were investigated: Biological data and stomach analyses. Lipid class analyses were performed on liver of cod and haddock. Fatty acid profiles were performed on fillet of cod and haddock, algae and zooplankton. Measurements of exposure levels: NPD/PAH in muscle and liver of cod and haddock. NPD/PAH metabolites in bile from cod and haddock. For effect analyses, we measured CYP1A levels in liver of cod and haddock, DNA adducts in haddock liver and parameters of oxidative stress; Vitamin E in cod and haddock fillet and lipid peroxidation in cod and haddock liver.

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. In cod liver, the average levels of NPD ranged from 37±25 ng/g ww (average ± std dev) at the Egersund Bank to 40±20 ng/g ww at Tampen. Sum PAH($_{EPA16}$) varied from 5±10 ng/g ww at Tampen to 10±20 ng/g ww at the Egersund Bank. Sum NPD in liver of haddock were 55±21 ng/g ww at the Egersund Bank and

38±14 ng/g ww and 47±23 ng/g ww at the two stations at Tampen. Levels of sum PAH (EPA_{16}) in haddock liver were 32±16 ng/g ww at the Egersund Bank, 23±14 ng/g ww and 22±11 ng/g ww at the two stations at Tampen.

Low levels of PAH metabolites were measured in haddock bile from the 2011 survey. Sum PAH metabolite level of Haddock south of Tampen (Station H5) were 124±206 ng/g bile, while at H7, between Statfjord and Gullfaks they were 106±74 ng/g bile. At the Egersund Bank, Southern North Sea (Ula area), Bressay Bank and the Viking Bank the levels were 212±297 ng/g bile, 76±64 ng/g bile, 133±78 ng/g bile and 62±49 ng/g bile, respectively. We did not find significantly increased levels in bile metabolites of haddock fished at Tampen compared with fish caught at the Egersund Bank, or for haddock fished at the additional stations from the North Sea. For cod, sum PAH metabolites at Tampen were 37±20 ng/g bile, while at the Egersund Bank they were 81±75 ng/g bile.

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 adducts per 10^9 nucleotides). Two stations had DNA adduct levels defined as high (>6.7 adducts per 10^9 nucleotides). These were one of two stations at Tampen and the station at Viking Bank.

The mean DNA adduct level measured were 1.6 adducts x 10^{-9} nucleotides in station H2 (Ula area, Southern North Sea), around 5.0 adducts x 10^{-9} nucleotides (from 4.3 to 5.5) in stations H1 (Egersund Bank), H4 (Bressay Bank) and H5 (Tampen South of Statfjord), and 7.3 adducts x 10^{-9} nucleotides at station H7 (Tampen between Statfjord and Gullfaks) and 19.5 adducts x 10^{-9} nucleotides at station H6 (Viking Bank). If one individual with very high adduct level at H6 was excluded, mean level at station H6 was reduced to 7.9 adducts x 10^{-9} nucleotides. Station H6 and H7 were significant different from stations H1, H4 and H5 (Wilcoxon/Kruskal-Wallis, $p< 0.05$), independent of the individual with very high DNA adduct level at H6.

The presence of DNA adducts confirms that haddock has been exposed to genotoxic pollutants and the results indicates PAH contamination in the North Sea. The DNA adduct data reported in the condition monitoring of 2002, 2005 and 2008 were performed in another laboratory. 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 (H5) had DNA adduct levels at the same levels as the reference stations Egersund Bank and Bressay Bank. The station at Tampen between Statfjord and Gullfaks (H7) and the station at the Viking Bank (H6) had statistically higher DNA adduct levels, although only 30 and 40 % higher at the stations at Tampen and the Viking Bank, compared with the Egersund Bank, when one extreme individual from the Viking Bank was excluded. Reasons to the increased levels at the Viking Bank should be further investigated. The results demonstrate the importance of higher resolution (i.e. more sampling stations), as earlier studies from the North Sea mainly have focused on the Egersund Bank and Tampen.

The haddock liver somatic index (LSI) was low at all sampling stations and there were no significant difference between Tampen and the other area. LSI levels at Tampen were the same as found in 2008 and 2010. 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 lipids analysis 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.

The present results do not indicate that discharges from oil and gas activities affect food safety aspects as we see no changes in NPD or PAH levels in fillet of liver of the investigated fish species between the Tampen region and the reference areas Egersund Bank and the Bressay Bank. We also did not see changes in PAH metabolite levels in fish bile between Tampen and the Egersund Bank. 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 included the two reference stations and one of the stations at Tampen. The other station at Tampen together with the station at Viking the Bank had levels slightly above 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 present study does 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.

Bjørn Einar Grøsvik
Project leader

Contents

1 Acknowledgements

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2 Abbreviations

BAC - Background levels
CPM - Counts per minute
CTD - Conductivity, temperature and depth
CYP - Cytochrome P450
EAC - Environmental assessment criteria
ELISA - Enhanced liquid immunosorbent assay
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
PE - Phosphatidyl ethanolamine
PI - Phosphatidyl inositol
PS - Phosphatidyl serine
PUFA - Poly unsaturated fatty acids
PW - Produced water
RAL - Relative adduct levels
SD - Standard deviation
SIM - Selected ion monitoring
SPE - solid phase extraction
TBARS- Thiobarbituric acid reactive substances
TLC - Thin-layer chromatography
ww - wet weight

3 Introduction

The Activity regulations require the offshore petroleum industry to perform environmental monitoring of the water column. The condition monitoring shall document if fish from Norwegian ocean areas contain elevated levels of components that originate from discharges from the petroleum activity. The major objective is to document to what extent discharges from the oil and gas installations cause contamination of fish negatively affecting the quality. For both the petroleum industry and the Norwegian fishing industry it is important that safety and quality of Norwegian seafood is documented, as well as environmental health of the marine environment.

Condition monitoring with fish from the Norwegian Continental Shelf are conducted every third year and shall document whether fish from Norwegian Seas are affected by pollution from oil and gas industry activities. The program is decided by the Norwegian State Pollution Control Agency (Klif) (Aktivitetsforskriften, §1.2). Sampling should be performed such that it gives a representative picture of the most important fish species in the region. In this connection knowledge of the species composition and migration pattern in each region is important.

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

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

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

However, the analyses of biomarkers in the 2002 study revealed biological effects in haddock from Tampen and Sleipner compared with fish from the Egersund Bank. In haddock, genotoxicity was reflected in increased levels of hepatic DNA adducts probably due to exposure to NPD/PAH. 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).
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).

Results with condition monitoring from 2002 (Balk et al. 2011), 2005 and 2008 (Grøsvik et al., 2007, Grøsvik et al., 2009) were used as basis for the proposal for monitoring for 2011.

The following methods were investigated: Biological data and stomach analyses. Lipid class analyses were performed on liver of cod and haddock. Fatty acid profiles were performed on fillet of cod and haddock, algae and zooplankton. Measurements of exposure levels: NPD/PAH in muscle and liver of cod and haddock. NPD/PAH metabolites in bile from cod and haddock. For effect analyses, we measured CYP1A levels in liver of cod and haddock, DNA adducts in cod and haddock liver and parameters of oxidative stress; Vitamin E in cod and haddock fillet and lipid peroxidation in cod and haddock liver.

The objectives for this study have been:

1. Measurements of NPD/PAH in muscle and liver of cod and haddock from the Egersund Bank and Tampen.
2. Measurements of metabolites of PAH in bile of cod and haddock from the Egersund Bank and Tampen.
3. Measurements of CYP1A levels in liver of cod and haddock from the Egersund Bank and Tampen.
4. Measurements of oxidative stress parameters in liver (TBARS) and muscle (Vitamin E) in cod and haddock from the Egersund Bank and Tampen.
5. Perform lipid extraction and lipid class separation on cod and haddock liver to analyse ratio of (n-3)/(n-6) poly unsaturated fatty acids. (Results under process)
6. Species characterisation of stomach analyses of cod and haddock.
7. Study possible genototoxic effects in fish from Tampen compared with fish from Egersund Bank and three additional stations by measurements of hepatic DNA adducts.

4 Sampled material and collection sites

Sampling for the Condition monitoring was performed with R/V Johan Hjort 28/6-25/7-2011 during the international bottom trawl survey (IBTS). Bottom trawl was used for collection of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). From each of the regions 25 (±10%) fish of each species were sampled. After killing the fish with a blow to the head, standard IMR procedures were used for collection and storage of muscle, liver, blood and bile samples for the later chemical and biochemical analyses.

Material sampled is listed in Table 1, 2 and 3, while maps of the fish locations are shown in Figures 1-3. Biological data is shown in Table 4 and 5.

Table 1: Overview of haddock sampled from the North Sea, July 2011.

Samples from UK sector were not analysed (n.a.).

Southern North Sea								
Station	**Date**	**Serieno.**	**Depth**	**Latitude**	**Longitude**	**E/W**	**No of haddock**	**Area**
H1	30.06.2011	24311	199	58°08.9	4°42.3	E	5	Egersund Bank
H1	30.06.2011	24312	181	58°06.7	4°49.1	E	4	Egersund Bank
H1	30.06.2011	24313	151	58°03.9	4°55.1	E	9	Egersund Bank
H1	30.06.2011	24314	150	58°03.8	4°54.9	E	7	Egersund Bank
H1	08.07.2011	24335	81	57°24.1	5°40.8	E	10	Egersund Bank
H2	05.07.2011	24323	65	57°06.7	3°10.4	E	12	Ula area, Southern North Sea
H2	07.07.2011	24330	81	57°20.4	2°28.5	E	13	Ula area, Southern North Sea
H3	07.07.2011	24329	91	57°20.6	1°36.7	E	25	Southern North Sea, UK sector, n.a.
	Sum						85	
Northern North Sea								
Station	**Date**	**Serieno.**	**Depth**	**Latitude**	**Longitude**	**E/W**	**No of haddock**	**Area**
H6	29.06.2011	24303	92	60°30.5'	2°35.3'	E	9	Viking Bank
H6	29.06.2011	24304	93	60°29.1	2°34.9	E	11	Viking Bank
H6	29.06.2011	24305	85	60°23.5	2°35.4	E	5	Viking Bank
H4	13.07.2011	24360	136	59°16.8	0°27.4	W	13	Bressay Bank
H4	13.07.2011	24361	133	59°16.9	0°12.5	W	12	Bressay Bank
H5	21.07.2011	24385	161	61°03.0	2°27.6	E	3	Tampen downstream Gullfaks
H5	21.07.2011	24386	162	61°03.4	2°26.9	E	9	Tampen downstream Gullfaks
H5	21.07.2011	24387	162	61°03.9	2°26.2	E	1	Tampen downstream Gullfaks
H5	21.07.2011	24389	166	61°03.9	2°26.8	E	3	Tampen downstream Gullfaks
H5	21.07.2011	24390	177	61°03.9	2°28.7	E	3	Tampen downstream Gullfaks
H5	21.07.2011	24391	131	61°02.1	2°25.7	E	10	Tampen downstream Gullfaks
H7	22.07.2011	24395	192	61°18.0	2°03.7	E	1	Tampen between Statfjord and Gullfaks
H7	22.07.2011	24396	142	61°16.9	1°59.2	E	12	Tampen between Statfjord and Gullfaks
	Sum						92	
Total number of fish							177	

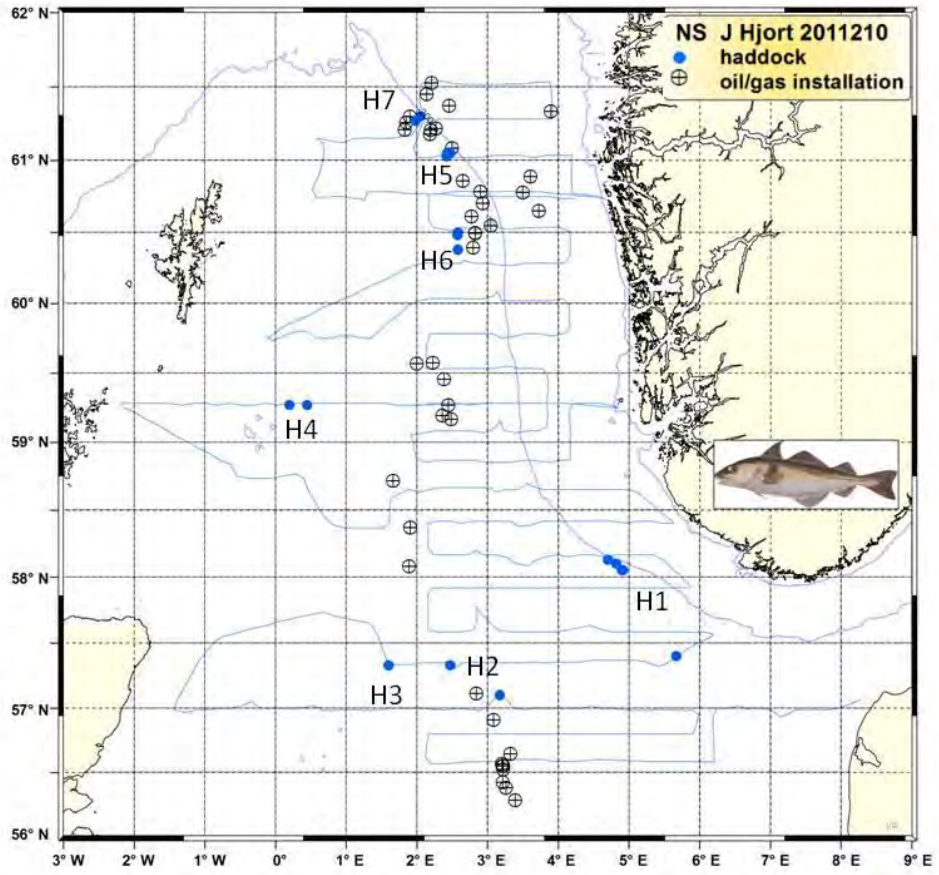


Figure 1. Stations with haddock fished in the North Sea July 2011. H1: Egersund Bank, H2: Ula area, Southern North Sea, H3: Southern North Sea, H4: Bressay Bank, H6: Viking Bank, H5 Tampen South, H7 Tampen between Statfjord and Gullfaks.

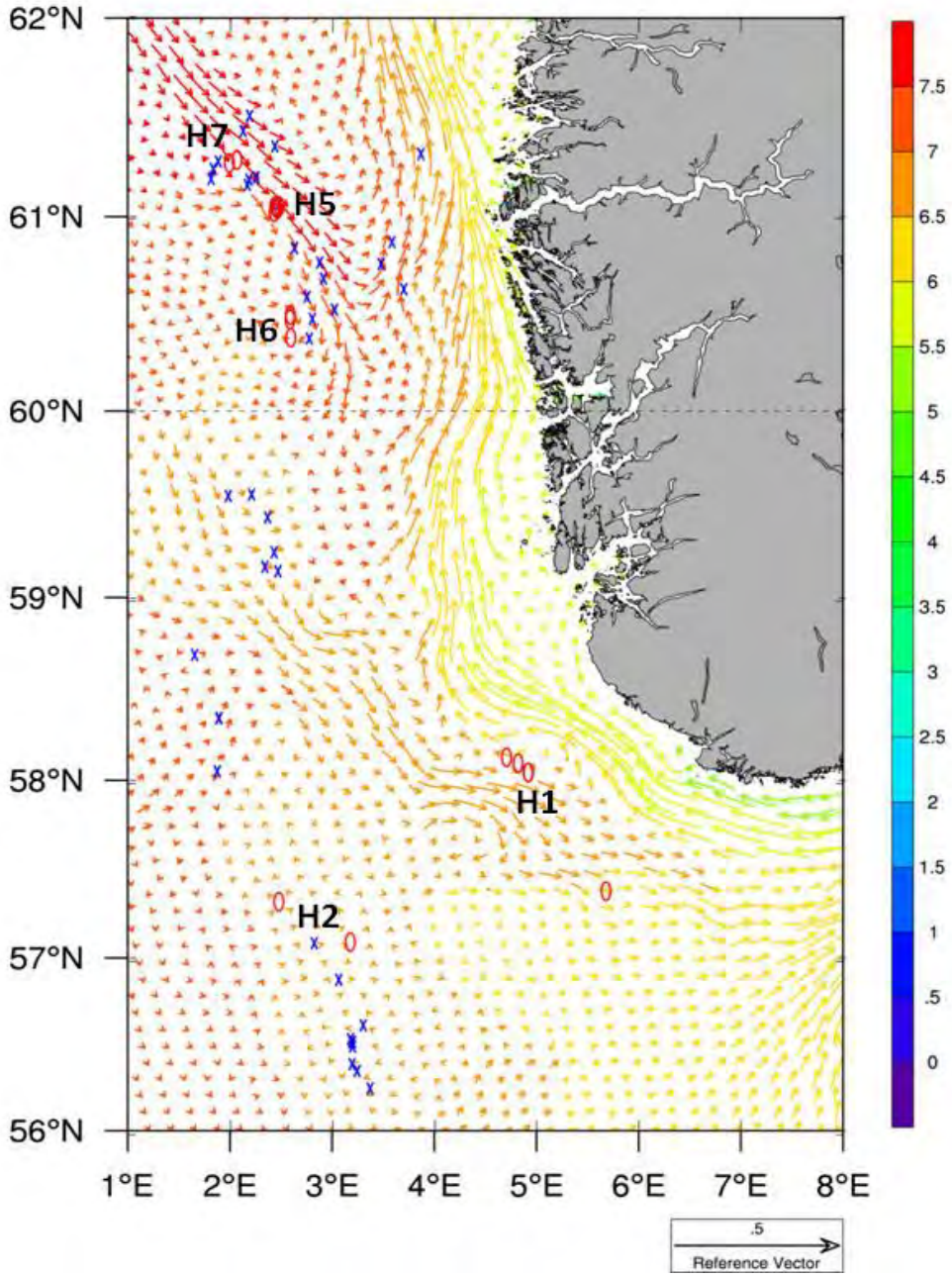


Figure 2. Sampling stations of haddock plotted on a map with weighted currents. The map is based on ROMS model with 4x4 km resolution. Currents averaged on data from April during the period from 1989-2008. Currents given at depth of 20 m. Colours indicate temperature °C. Fish stations: red circle, oil and gas installations: blue cross.

Table 2: Overview of cod, whiting. Plaice, sand eel and European hake sampled from the North Sea, July 2011. Plaice, sand eel and European hake were not in the proposal.

Southern North Sea												
Station	**Date**	**Serie no.**	**Depth**	**Latitude**	**Longitude**	**E/W**	**Cod**	**Whiting**	**Plaice**	**Sand eel**	**European hake**	**Area**
H1	30.06.2011	24311	199	58°08.9	4°42.3	E	4					Egersund Bank
H1	30.06.2011	24312	181	58°06.7	4°49.1	E	1					Egersund Bank
H1	30.06.2011	24313	151	58°03.9	4°55.1	E	20					Egersund Bank
H1	08.07.2011	24336	141	57°34.3	6°12.9	E	9				10	Egersund Bank
H1	01.07.2011	24316	66	57°11.3	6°49.2	E		10				SE of Egersund Bank
H1	01.07.2011	24317	64	57°11.8	7°05.5	E		15				SE of Egersund Bank
H1	08.07.2011	24340	72	57°37.8	4°30.1	E				30		Egersund Bank
H1	09.07.2011	24344	67	57°55.4	3°03.1	E			10			Egersund Bank
H1	09.07.2011	24345	93	57°55.1	4°04.6	E			3			Egersund Bank
H1	10.07.2011	24348	86	58°07.0	3°39.2	E			12			Egersund Bank, North
	Sum						34	25	25	30	10	

Northern North Sea												
Station	**Date**	**Serieno.**	**Depth**	**Latitude**	**Longitude**	**E/W**	**Cod**	**Whiting**	**Plaice**	**Sand eel**	**European hake**	Område
H5	21.07.2011	24386	162	61°03.4	002 26.9	E	5					Tampen downstream Gullfaks
H5	21.07.2011	24387	162	61°03.9	002 26.2	E	3					Tampen downstream Gullfaks
H5	21.07.2011	24389	166	61°03.9	002 26.8	E	2					Tampen downstream Gullfaks
H5	21.07.2011	24391	131	61°02.1	002 25.7	E	9					Tampen downstream Gullfaks
H5	21.07.2011	24392	135	61°02.9	001 56.9	E	4					Tampen downstream Gullfaks
H7	22.07.2011	24395	192	61°18.0	002 03.7	E	4				10	Tampen between Statfjord and Gullfaks
H7	22.07.2011	24396	142	61°16.9	001 59.2	E	5					Tampen between Statfjord and Gullfaks
	24.07.2011	24397	158	61°08.7	000 38.5	E					10	BetweenTampen and Shetland
	Sum						14	0	0	0	20	
Total number of fish							48	25	25	30	30	

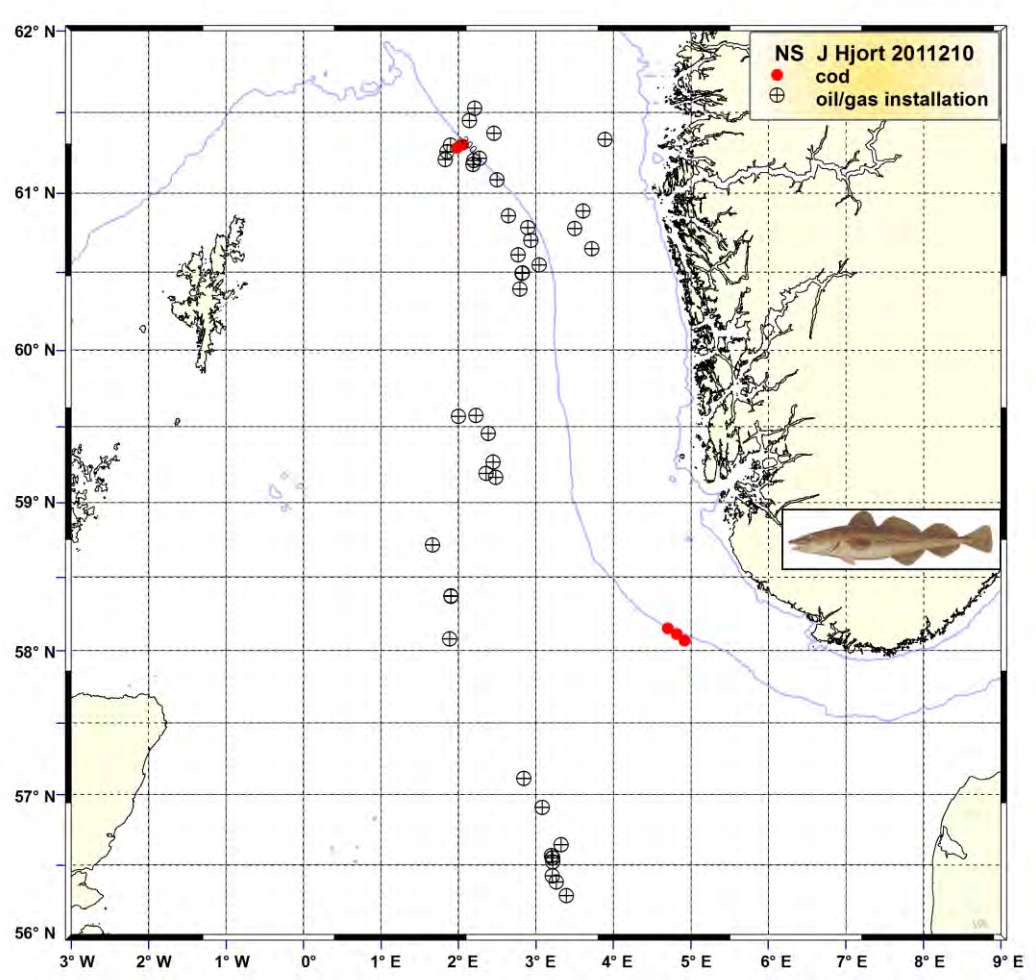


Figure 3. Stations with cod fished in the North Sea July 2011.

Table 3. Sampling of plankton, zooplankton and benthos.

Southern North Sea

Station	Date	Serieno.	St.no.	Depth	Latitude	Longitude	E/W	Plankton	Zooplankton	Benthos	Area	Fishing gear/tool
Station 1	30.06.2011	24311	237	199	58°08.9	4°42.3	E			1	Egersund Bank	Bottom trawl
	30.06.2011	-	463	197	58°07.72	4°47.29	E	2	2		Egersund Bank	CTD, WP-II
	01.07.2011	24315	0054	149	58°03.1	4°58.1	E			1	Egersund Bank	Modified sandeel dredge
	05.07.2011	-	479	65	57°00.05	3°39.74	E		4			MIK, MOC
	05.07.2011	-	480	66	56°59.95	3°23.87	E	2				CTD
	06.07.2011	-	487	91	56°59.70	0°39.96	E		2			MIK
	06.07.2011	-	492	72	56°59.67	1°28.09	E		2			MIK
	08.07.2011	24334	259	64	57°19.9	5°23.8	E			1		Bottom trawl
	08.07.2011	24337	0055	100	57°35.5	5°32.3	E			1		Modified sandeel dredge
	08.07.2011	24338	262	94	57°35.6	5°27.2	E			1		Bottom trawl
	Sum							4	10	5		

Northern North Sea

Station	Date	Serieno.	St.no.	Depth	Latitude	Longitude	E/W	Plankton	Zooplankton	Benthos	Area	Fishing gear/tool
Station 6	29.06.2011	24304	230	93	60°29.1	2°34.9	E			1	Viking Bank	Bottom trawl
	29.06.2011	-	462	87	60°27.25	2°34.83	E	2	2		Viking Bank	CTD, WP-II
	13.07.2011	-	512	110	59°17.06	1°19.12	W	2	2		Utsira - Startpoint	CTD, MIK
	14.07.2011	-	534	280	59°17.05	4°10.92	E		2		Utsira - Startpoint	MIK
	14.07.2011	-	535	119	59°17.0	4°49.88	E	2	2		Utsira - Startpoint	CTD, WP-II
Station 5	21.07.2011	24385		161	61°03.0	2°27.6	E	2	2		Tampen downstream Gullfaks	CTD, WP-II
	21.07.2011	24392		135	61°02.9	1°56.9	E			1	Tampen downstream Gullfaks	Bottom trawl
	22.07.2011	-	555	203	61°16.9	2°06.4	E	2	2		Tampen between Statfjord and Gullfaks	CTD, WP-II
	24.07.2011	-	556	149	61°10.21	0°37.09	E	2			North of Tampen	
	24.07.2011	24397		158	61°08.7	0°38.5	E			1	North of Tampen	
	24.07.2011	-	557	146	60°44.96	0°33.83	E	2				CTD
	Sum							14	12	3		

Examples of catch, upper row: fish and sea snail from bottom trawl, lower row: different species collected from modified sand eel dredge. Photo: BE Grøsvik.

Table 4. Biological data of haddock.

Data given as average ± SD.

Area	Egersund Bank	Ula area Southern North Sea	Bressay Bank	Viking Bank	Tampen South	Tampen between Statfjord and Gullfaks
Station	H1	H2	H4	H6	H5	H7
Females/males	17/18	10/15	10/15	19/6	16/13	8/5
Tot no	35	25	25	25	29	13
Length (cm)	34±5	31±4	34±4	42±9	38±4	35±6
Weight (g)	410±188	302±110	405±172	806±437	562±196	371±74
Liver weight (g)	10.2±4.8	12.8±7.3	12.8±7.5	17±10	15.4±7.0	7.5±2.1
Age	2.9±0.9	2.9±1.3	3.3±1.5	4.3±1.3	2.4±0.8	2.1±0.3
LSI (%)	2.6±0.9	4.3±2.1	3.1±0.7	2.0±0.5	2.7±0.8	2.0±0.5
Fulton	1.01±0.11	0.94±0.10	0.95±0.08	0.96±0.12	1.01±0.06	0.92±0.22

Liver somatic index (LSI) is percentage liver weight per body weight. Fulton index is weight/length^3*100.

Table 5. Biological data of cod

Data given as mean ± SD.

Area	Egersund Bank	Tampen
Station	H1	H5+H7
Females/males	19/15	17/15
Tot. No	34	32
Length (cm)	43±12	58±17
Weight (g)	915±790	2554±2376
Liver weight (g)	19±24	122±202
Age	2.3±0.5	2.7±1.2
LSI (%)	2.0±1.1	3.1±2.4
Fulton	0.96±0.08	1.03±0.07

Liver somatic index (LSI) is percentage liver weight per body weight. Fulton index is weight/length^3*100.

Material for analyses

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 (5 vials), fillet and brain.

Fish > 30 cm were selected for DNA adduct analyses and sent to ADN´tox, Caen, France. 137 fish were sent from the different stations as shown in Table 6.

Chemical analyses of NPD/PAH (Muscle and liver), bile metabolites, CYP1A levels in liver, stomach content and fatty acid analyses were performed at IMR. TBARS of fish liver were analysed by Nifes and α-tocopherol by Vitas, Oslo, Norway. Sampling procedures are described in Appendix 7.1.

Table 6. Number of haddock samples sent for DNA adduct analyses. Fish > 30 cm were selected.

Station	Area	Female	Male
1	Egersund Bank	14	15
2	The Ula area, Southern North Sea	7	10
4	Bressay Bank (on the transect Startpoint – Utsira)	10	14
5	Tampen downstream Gullfaks	13	12
6	Viking Bank	19	6
7	Tampen between Statfjord and Gullfaks	11	6
	Sum	74	63
	Total: 137		

Fish sampling at R/V Johan Hjort. Photo: B.E. Grøsvik

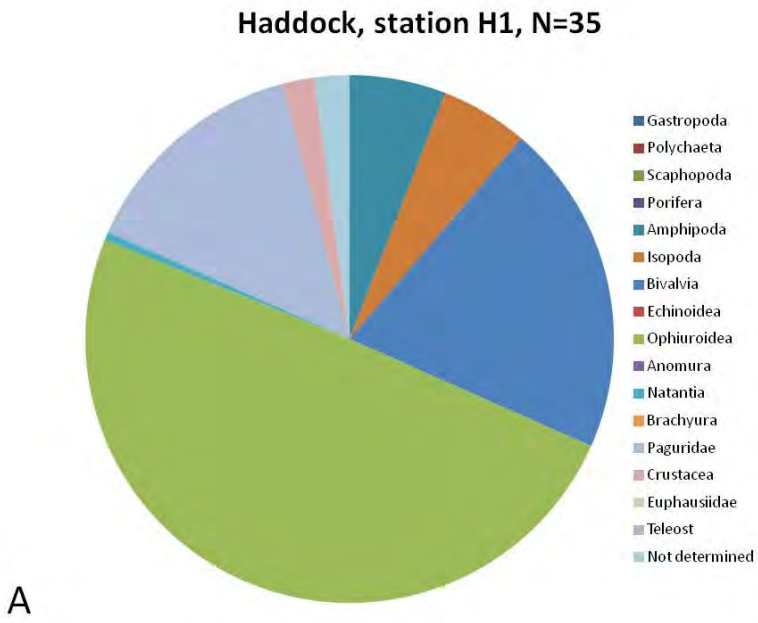
5 Results and Discussion

5.1 Stomach content

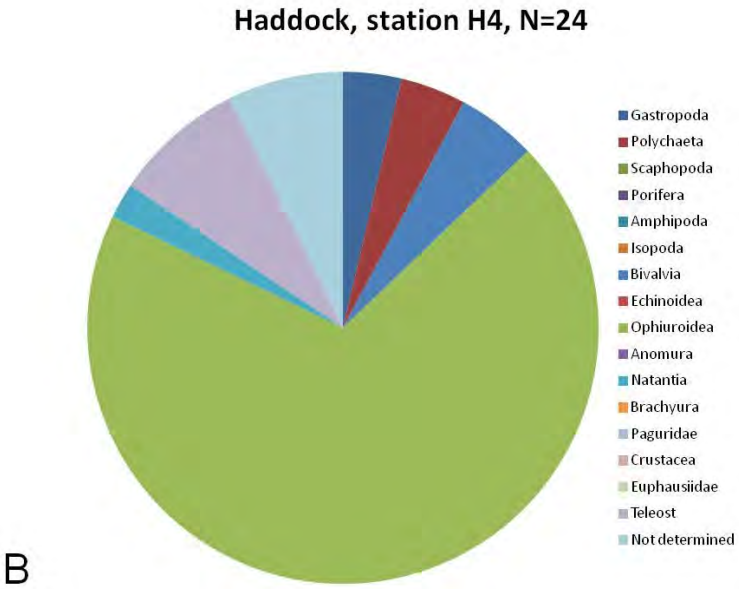
Stomach content were analysed for haddock from 5 stations and cod from 2 stations to see how diet could differ between the different regions in the North Sea. Brittle stars dominated, but bivalves, hermit crabs, isopods and amphipods were also components in the diet of haddock from the Egersund Bank (Station H1) (Table 7 and Figure 4A). At the Bressay Bank (Station H4), brittle stars were the main diet componenet, while at the Viking Bank (Station 6), fish and hermit crabs were the main constituents. At Tampen (Station 5 and 7), the haddock seemed to feed on a varied diet (Table 7 and Figure 4). Stomach content of cod is more dominated of fish compared with haddock (Table 7 and Figure 5), supporting the assumption that haddock is a more benthic feeding species.

Table 7. Sum of stomach content in haddock (H1-H7) and cod per station given in mg. Given as sum of all fish per station. Numer of fish per station were: H1: N=35, H4: N= 24, H6: N= 21, H5: N= 25 and H7: N= 17, cod Egersund Bank: N= 8, cod Tampen: N=22. Not determined = N.D.

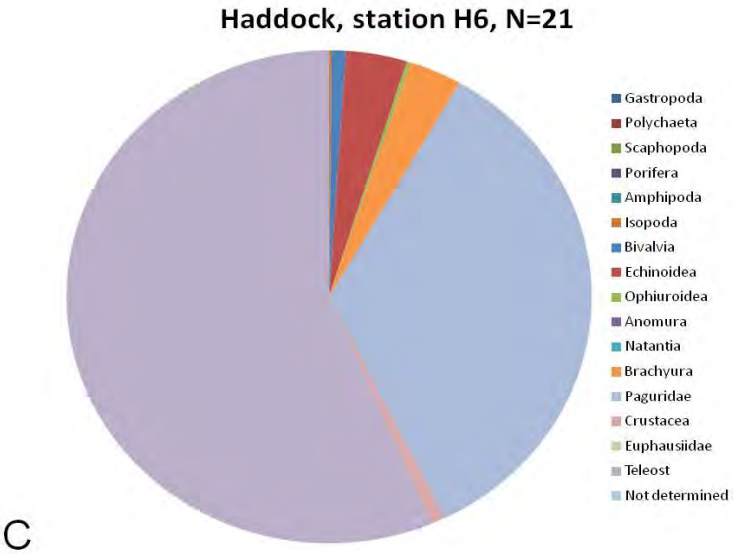
Group	Group	H1	H4	H6	H5	H7	Cod, Tampen	Cod, Egersund Bank
Snails	Gastropoda	0	1599	0	3424	2271	0	2933
Lug worms	Polychaeta	0	1798	0	6102	0	0	0
Tusk shell	Scaphopoda	0	0	0	82	369	0	0
Sponges	Porifera	0	0	0	661	0	0	0
Amphipods	Amphipoda	2485	0	0	6212	0	3520	0
Isopods	Isopoda	2263	0	621	3726	0	405	0
Bivalves	Bivalvia	8580	2182	3417	3363	2939	0	0
Sea urchin	Echinoidea	0	0	14768	6078	5053	0	0
Brittle star	Ophiuroidea	20841	30013	997	4105	6567	0	0
Numida	Anomura	0	0	0	6098	2771	17871	17829
Shrimps	Natantia	207	972	0	2183	0	0	0
Crabs	Brachyura	0	0	12712	3376	11295	48434	33417
Hermit crab	Paguridae	6012	0	135645	7018	0	44376	14990
Crustaceans	Crustacea	813	0	3258	7840	137	0	5300
Krill	Euphausiidae	0	0	0	0	4885	0	0
Fish	Teleost	0	3578	221933	0	0	562936	205608
N.D.		907	3186	0	0	0	0	0


Haddock, station H1, N=35
Gastropoda
Polychaeta
Scaphopoda
Porifera
Amphipoda
Isopoda
Bivalvia
Echinoidea
Ophiuroidea
Anomura
Natantia
Brachyura
Paguridae
Crustacea
Euphausiidae
Teleost
Not determined

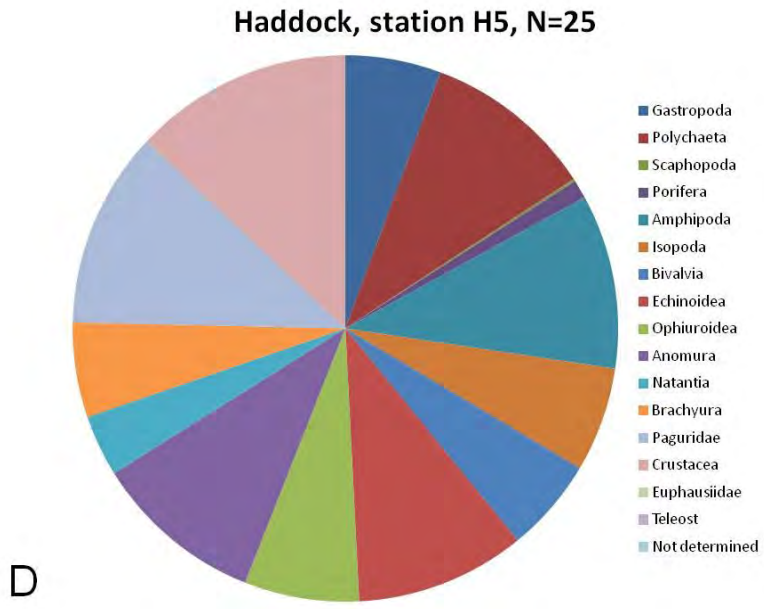
A


Haddock, station H4, N=24
Gastropoda
Polychaeta
Scaphopoda
Porifera
Amphipoda
Isopoda
Bivalvia
Echinoidea
Ophiuroidea
Anomura
Natantia
Brachyura
Paguridae
Crustacea
Euphausiidae
Teleost
Not determined

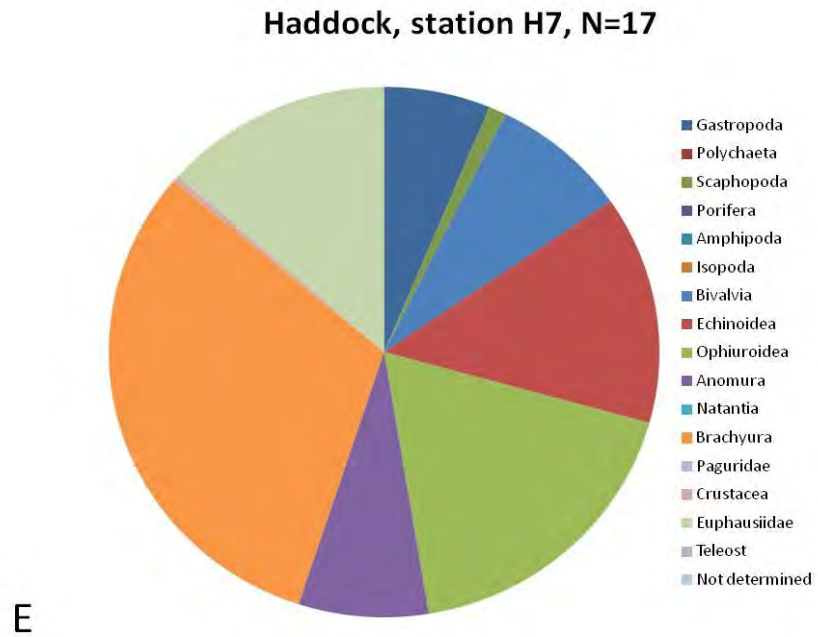
B


Haddock, station H6, N=21
Gastropoda
Polychaeta
Scaphopoda
Porifera
Amphipoda
Isopoda
Bivalvia
Echinoidea
Ophiuroidea
Anomura
Natantia
Brachyura
Paguridae
Crustacea
Euphausiidae
Teleost
Not determined

C

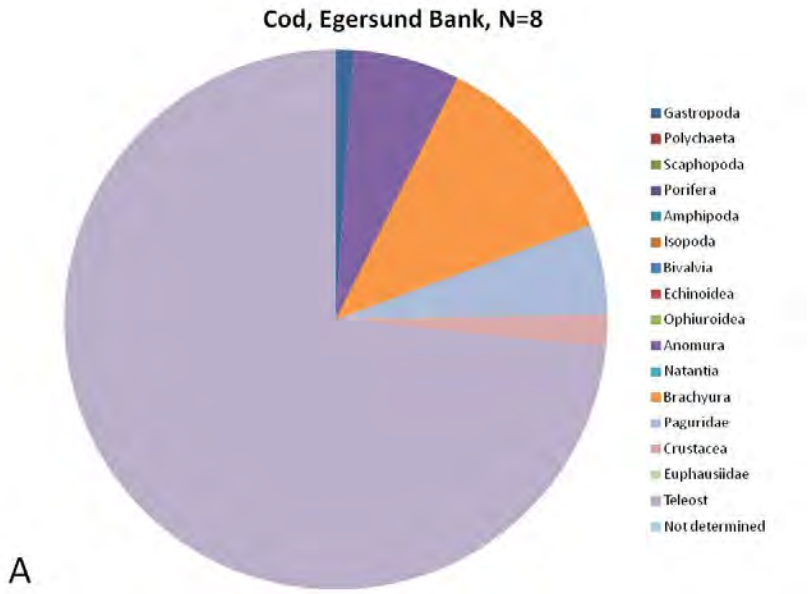


D



E

Figure 4. Distribution of stomach content in haddock per station from sum of all fish per station. Numer of fish per station were: Egersund Bank H1: N=35, Brassey Bank H4: N= 24, Viking Bank H6: N= 21, Tampen South H5: N= 25 and Tampen H7: N= 17.



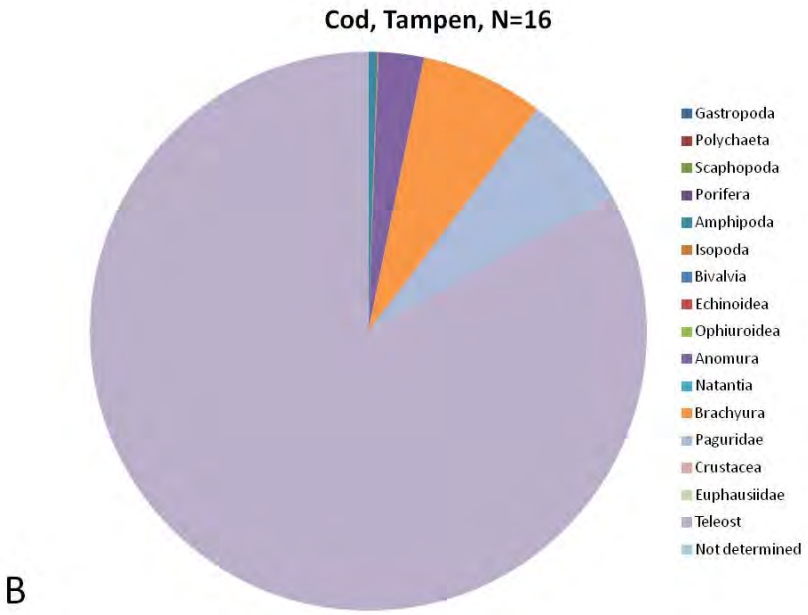


Figure 5. Distribution of stomach content in cod from Egersund Bank and Tampen from sum of all fish per station. Numer of fish per station were: Egersund Bank: N=8, Tampen: N= 16.

5.2 Levels of NPD/PAH in cod and haddock muscle and liver

Analyses of aromatic hydrocarbons (NPD/PAH) were carried out using GC/MS. The compounds included in the analysis are shown in Tables 8-10. NPD is the sum of naphthalene, phenanthrene, dibenzothiophene, and their C_1-C_3 alkylated homologs and 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.

The method is validated to analyse PAH in the concentration range of 0.2 ng/g. For some compounds the detection limit are higher, because of background problems. 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 NPD and PAH in cod and haddock muscle were generally below LOQ (Table 8). In cod liver, the average levels of NPD ranged from 37±25 ng/g at the Egersund Bank to 40±20 ng/g at Tampen (Table 9). In 2008, NPD levels in cod liver at Tampen were 21±10 ng/g (Grøsvik et al., 2008).

Average sum PAH($_{EPA16}$) in cod liver ranged from 5±10 ng/g at Tampen to 10±20 ng/g at the Egersund Bank (Table 9).

Table 8. Levels of NPD/sum PAH compounds in cod and haddock muscle caught at Tampen. Presented as average ± stdev (ng/g wet weight). N= number of fish per station. Compounds included in sum NPD are labelled with [a]. Compounds included in sum PAH (EPA_{16}) are labelled with [b]. Values of naphthalene, dibenzothiophene and phenanthrene are included in sum NPD as well as sum PAH (EPA16).

Compound	Cod muscle Tampen N= 25	Haddock muscle Tampen N=25	LOD	LOQ
Naphthalene [a,b]	< LOQ	< LOQ	0.27	0.58
C1-naphthalene [a]	< LOQ	< LOQ	0.26	0.61
C2-naphthalene [a]	< LOQ	< LOQ	0.12	0.26
C3-naphthalene [a]	< LOQ	0.99±1.81	0.31	0.70
Dibenzothiophene [a,b]	< LOQ	< LOQ	0.02	0.05
C1-dibenzothiophene [a]	< LOQ	< LOQ	0.02	0.06
C2-dibenzothiophene [a]	< LOQ	< LOQ	0.06	0.14
C3-Dibenzothiophene [a]	< LOQ	< LOQ	0.08	0.20
Phenanthrene [a,b]	< LOQ	< LOQ	0.12	0.24
C1-phenanthrene [a]	< LOQ	< LOQ	0.07	0.18
C2-phenanthrene [a]	< LOQ	< LOQ	0.08	0.14
C3-phenanthrene [a]	< LOQ	< LOQ	0.05	0.20
Acenaphthylene [b]	< LOQ	< LOQ	0.02	0.05
Acenaphthene [b]	< LOQ	0.20±0.50	0.03	0.07
Fluorene [b]	< LOQ	0.38±0.83	0.07	0.16

Anthracene [b]	< LOQ	< LOQ	0.03	0.08
Fluoranthene [b]	< LOQ	< LOQ	0.05	0.11
Pyrene [b]	< LOQ	< LOQ	0.04	0.11
Benz(a)anthracene [b]	< LOQ	< LOQ	0.02	0.05
Chrysene [b]	< LOQ	< LOQ	0.04	0.09
Benzo(b)fluoranthene [b]	< LOQ	< LOQ	0.03	0.06
Benzo(k)fluoranthene [b]	< LOQ	< LOQ	0.01	0.04
Benzo(a)pyrene [b]	< LOQ	< LOQ	0.02	0.07
Indeno(1,2,3-cd)pyrene [b]	< LOQ	< LOQ	0.02	0.04
Dibenz(a,h)anthracene [b]	< LOQ	< LOQ	0.03	0.07
Benzo(g,h,i)perylene [b]	< LOQ	< LOQ	0.03	0.07
SUM NPD [a]	< LOQ	**0.99±1.81**		
SUM PAH (EPA_{16}) [b]	< LOQ	**0.58**		

Table 9. Levels of NPD/sum PAH compounds in cod liver.
Presented as average ± stdev (ng/g wet weight). N= number of fish per station. Compounds included in sum NPD are labelled with [a]. Compounds included in sum PAH (EPA_{16}) are labelled with [b]. Values of naphthalene, dibenzothiophene and phenanthrene are included in sum NPD as well as sum PAH (EPA16).

Compound	**Egersund Bank T1 N= 17**	**Tampen T 2 N=32**	**LOD**	**LOQ**
Naphthalene [a,b]	5.00±6.39	2.42±1.78	0.64	0.99
C1-naphthalene [a]	2.80±1.18	2.87±2.00	0.71	1.20
C2-naphthalene [a]	4.04±1.05	5.38±2.93	0.48	0.76
C3-naphthalene [a]	7.33±2.71	8.85±4.73	1.62	2,04
Dibenzothiophene [a,b]	0.18±0.22	0.16±0.10	0.1	0.28
C1-dibenzothiophene [a]	0.75±1.38	1.52±1.34	0.06	0.13
C2-dibenzothiophene [a]	2.02±3.36	4.90±6.43	0.05	0.05
C3-Dibenzothiophene [a]	1.81±2.41	1.63±2.12	0.09	0.09
Phenanthrene [a,b]	2.21±1.73	2.71±1.27	1.35	2.4
C1-phenanthrene [a]	6.11±10.72	3.29±3.40	0.65	1.25
C2-phenanthrene [a]	2.05±2.75	1.43±1.24	0.11	0.11
C3-phenanthrene [a]	3.90±4.16	7.12±6.15	0.24	0.59
Acenaphthylene [b]	0.60±0.62	0.37±0.18	0.17	0.42
Acenaphthene [b]	1.53±1.35	0.82±0.86	0.29	0.5
Fluorene [b]	3.64±1.59	5.18±2.11	1.17	2.12
Anthracene [b]	0.61±1.74	0.17±0.07	0.05	0.09
Fluoranthene [b]	0.81±1.79	0.44±0.17	0.32	0.60
Pyrene [b]	0.62±1.75	0.23+0.08	0.14	0.25
Benz(a)anthracene [b]	0.65±1.29	0.25±0.16	0.04	0.11
Chrysene [b]	0.94±2.88	0.26±0.21	0.05	0.09
Benzo(b)fluoranthene [b]	0.51±1.60	0.11±0.07	0.05	0.09
Benzo(k)fluoranthene [b]	0.41±1.64	0.02±0.03	0.03	0.07
Benzo(a)pyrene [b]	1.06±2.82	0.07±0.06	0.01	0.01
Indeno(1,2,3-cd)pyrene [b]	0.70±2.02	0.06±0.05	0.01	0.01
Dibenz(a,h)anthracene [b]	0.63±1.94	0.13±0.16	0.02	0.02
Benzo(g,h,i)perylene [b]	0.58±1.85	0.06±0.07	0.05	0.12
SUM NPD [a]	**36.5±24.8**	**40.4±19.5**		
SUM PAH (EPA_{16}) [b]	**10.4±20.3**	**4.9±9.5**		

Levels of NPD and PAH in haddock liver were low for all stations. Sum NPD in liver of haddock were 55±21 ng/g at the Egersund Bank and 38±14 ng/g and 47±23 ng/g at the two stations at Tampen (Table 10). NPD levels measured in haddock liver in 2008 ranged from 15.3±7 ng/g at the Egersund Bank, 7.8±5.9 ng/g in the Barents Sea to 10.5±13.3 ng/g at the Halten Bank. Levels found in haddock liver at the Egersund Bank in January 2008 (one month after the Statfjord A discharge) was 31±19 ng/g NPD, while levels found in haddock liver at Tampen 6 days after the discharge were 132±123 ng/g NPD (Grøsvik *et al*., 2008).

Table 10. Levels of NPD/sum PAH compounds in haddock liver.
Presented as average ± stdev (ng/g wet weight). N= number of fish per station. Compounds included in sum NPD are labelled with [a]. Compounds included in sum PAH (EPA_{16}) are labelled with [b]. Values of naphthalene, dibenzothiophene and phenanthrene are included in sum NPD as well as sum PAH (EPA_{16}).

Compound	Egersund Bank H 1 N= 25	Tampen H 5 N=19	Tampen H 7 N=15	LOD	LOQ
Naphthalene [a,b]	3.51±2.27	2.02±1.68	2.43±3.78	0.64	0.99
C1-naphthalene [a]	2.40±0.94	1.80±1.02	1.66±2.21	0.71	1.20
C2-naphthalene [a]	2.90±1.41	3.12±3.57	1.82±0.70	0.48	0.76
C3-naphthalene [a]	8.24±4.62	5.14±1.69	4.69±1.25	1.62	2,04
Dibenzothiophene [a,b]	0.38±0.31	0.56±0.54	0.54±0.47	0.1	0.28
C1-dibenzothiophene [a]	1.04±1.94	1.12±2.51	2.20±1.98	0.06	0.13
C2-dibenzothiophene [a]	2.94±3.83	4.47±3.36	8.39±7.51	0.05	0.05
C3-Dibenzothiophene [a]	2.56±2.51	1.47±1.02	1.86±2.48	0.09	0.09
Phenanthrene [a,b]	12.6±6.5	7.58±5.12	8.52±5.48	1.35	2.4
C1-phenanthrene [a]	4.10±2.17	3.45±1.28	3.57±1.33	0.65	1.25
C2-phenanthrene [a]	7.24±4.39	3.85±2.17	5.40±3.38	0.11	0.11
C3-phenanthrene [a]	9.41±7.42	4.54±3.38	6.13±7.02	0.24	0.59
Acenaphthylene [b]	0.59±0.39	0.36±0.36	0.22±0.09	0.17	0.42
Acenaphthene [b]	1.16±1.25	0.69±0.71	0.41±0.25	0.29	0.5
Fluorene [b]	8.94+5.15	7.12±5.82	5.70±2.47	1.17	2.12
Anthracene [b]	0.50±0.22	0.38±0.11	0.48±0.38	0.05	0.09
Fluoranthene [b]	1.32±0.52	1.32±0.37	1.20±0.38	0.32	0.60
Pyrene [b]	0.56±0.22	0.62±0.20	0.55±0.19	0.14	0.25
Benz(a)anthracene [b]	0.72±0.53	0.71±0.52	0.40±0.27	0.04	0.11
Chrysene [b]	0.31±0.21	0.51±0.26	0.30+0.16	0.05	0.09
Benzo(b)fluoranthene [b]	0.46±0.45	0.45±0.19	0.30±0.22	0.05	0.09
Benzo(k)fluoranthene [b]	0.06±0.06	0.10±0.10	0.08±0.07	0.03	0.07
Benzo(a)pyrene [b]	0.22±0.20	0.23±0.12	0.25±0.17	0.01	0.01
Indeno(1,2,3-cd)pyrene [b]	0.17±0.20	0.27±0.24	0.17±0.19	0.01	0.01
Dibenz(a,h)anthracene [b]	0.11±0.07	0.17±0.15	0.17±0.17	0.02	0.02
Benzo(g,h,i)perylene [b]	0.28±0.31	0.13±0.12	0.10±0.07	0.05	0.12
SUM NPD [a]	**55.4±21.1**	**38.0±14.0**	**47.2±22.5**		
SUM PAH (EPA_{16}) [b]	**31.6±15.5**	**22.9±13.9**	**21.5±11.04**		

Levels of sum PAH (EPA_{16}) in haddock liver were 32±16 ng/g at the Egersund Bank, 23±14 ng/g and 22±11 ng/g at the two stations at Tampen. Similar measurements in haddock liver i 2008 gave levels of sum PAHs at 2.1±3.1 ng/g (Egersund Bank), 2.6±3.5 ng/g (Barents Sea) and 1.5±2.1 ng/g (Halten Bank). Levels of sum PAH in haddock after the Statfjord incident

were 26±16 at the Egersund Bank in January 2008 and 6.3±5.2 at Tampen in December 2008 (Grøsvik *et al*., 2008).

The somewhat lower levels for NPD and PAH reported from 2008 compared to the data from 2011 are probably affected of subtraction of blank for the 2008 data and to a change in extraction method.

5.3 Levels of PAH metabolites by GC MS in cod and haddock bile

The content of PAH metabolites in bile can reflect which compounds are being metabolised in the organism in a small and concentrated volume, and hydroxylated polycyclic aromatic hydrocarbons (PAH) are detected (Aas *et al*., 2000). As PAHs are quickly metabolised by fish, it is more appropriate to monitor the levels of PAH metabolites (hydroxylated PAH) in fish bile than the levels of parent compounds in fish muscle or liver. PAHs are metabolised in fish in two stages, first being oxidised to hydroxylated PAHs and then conjugated into highly water-soluble conjugates of e.g. glucuronic acid. Several methods have been described for analysing PAH metabolites using solid-phase extraction, various types of derivatisation and consequent GC-MS analysis (e.g. Jonsson *et al*., 2003). Based on this, the method for analysing PAH metabolites include deconjugation, derivatisation and pentafluorobenzoyl derivatization, as previously described for alkylphenol analysis (Boitsov *et al*., 2004). This allows low detection limits due to the possibility of using negative chemical ionisation (NCI) mode on GC-MS.

Sum PAH metabolite level of haddock at Tampen South (Station H5) were 124±206 ng/g bile, while at H7, between Statfjord and Gullfaks it was 106±74 ng/g bile. At the Egersund Bank, Southern North Sea and Bressay Bank the levels were 212±297 ng/g bile, 76±64 ng/g bile, 133±78 ng/g bile and 62±49 ng/g bile, respectively, (Table 11). We did not find significantly increased levels in bile metabolites of haddock fished at Tampen compared with fish caught at the Egersund Bank, or for haddock fished at the additional stations from the North Sea. For cod, sum PAH metabolites at Tampen were 37±20 ng/g bile, ng/g bile While at Egersund Bank they were 81±75 ng/g bile (Table 12).

Table 11. PAH metabolites in bile in haddock.
Values given as average ± std dev in ng/g bile. N= number of fish per station.

	H1	**H2**	**H4**	**H6**	**H5**	**H7**	**LOQ**
	N=17	N=6	N=16	N=13	N=25	N=17	
1-Naphthol	2.18±2.81	2.21±1.64	3.81±7.32	0.70±0.60	1.76±2.00	0.77±0.49	2.54
2-Naphthol	2.56±3.36	2.00±1.11	4.65±8.55	1.03±0.66	2.80±5.49	1.30±0.79	0.24
Σ Naphthol	**4.74±6.13**	**4.21±2.74**	**8.47±15.86**	**1.73±1.16**	**4.56±7.11**	**2.07±1.25**	
7-Methyl-1-Naphthol/8-methyl-2-naphthol	0.33±0.84	0.09±0.10	2.25±5.96	0.10±0.25	0.20±0.66	0.11±0.24	0.01
2-Methyl-1-naphthol	6.21±13.31	3.15±2.66	3.22±4.03	1.55±1.68	1.83±3.06	2.76±3.02	1.65
3-Methyl-1-naphthol	1.53±1.92	1.58±0.95	1.88±2.55	0.33±0.30	0.68±0.69	0.98±0.73	0.10
6-Methyl-1-naphthol	1.47 ±3.86	0.69±0.97	1.77±2.40	0.92±1.12	0.84±1.16	0.40±0.48	0.12
3-methyl-2-naphthol	0.68 ±0.79	0.42±0.37	1.19±1.59	0.84±0.79	1.26±1.49	1.57±1.34	0.05
7-methyl-2-naphthol	0.89±1.03	0.58±0.34	2.41±5.59	0.23±0.12	0.99±2.31	0.45±0.31	0.10
6-methyl-2-naphthol	0.64±0.96	0.37±0.35	1.26±2.07	0.18±0.11	0.33±0.50	0.30±0.22	0.06

4-methyl-1-naphthol	3.36±5.73	1.13±1.23	2.25±3.36	0.40±0.31	1.03±1.21	1.43±1.50	0.52
5-methyl-1-naphthol/1-methyl-2-naphthol	0.76±0.87	0.27±0.15	1.31±2.46	0.11±0.06	0.74±1.82	0.33±0.23	0.08
4-methyl-2-naphthol	0.95±1.26	0.65±0.52	0.98±1.08	0.06±0.05	0.38±0.87	0.21±0.14	0.12
5-methyl-2-naphthol	0.36±0.36	0.09±0.07	0.79±1.34	0.05±0.04	0.35±0.52	0.19±0.13	0.03
Σ C1/C2 Naphthol	**17.17±21.38**	**9.03±5.63**	**19.31±22.09**	**4.78±2.28**	**8.63±10.91**	**8.74±6.68**	
2-Hydroxyfluorene	8.42±10.06	4.10±3.03	10.88±15.94	2.36±1.01	4.43±4.87	4.44±2.96	0.67
9-Hydroxyfluorene	81.42±129.65	37.47±56.43	145.33±322.26	21.07±20.63	70.13±147.33	60.70±50.65	10.69
Σ Hydroxyfluorene	89.84±131.13	41.56±57.10	156.21±337.28	23.43±20.92	74.56±151.68	65.13±52.57	
4-Hydroxyphenanthrene	1.31±1.55	0.50±0.23	2.46±4.93	0.25±0.23	1.07±0.80	0.88±0.51	0.14
9-Hydroxyphenanthrene	1.71±1.78	1.01±1.18	3.25±7.37	0.35±0.14	2.04±4.32	1.06±0.76	0.21
3-Hydroxyphenanthrene	3.82±3.92	1.33±0.90	7.03±12.79	1.58±0.71	3.35±4.47	2.34±1.45	0.66
1-Hydroxyphenanthrene	3.57±3.99	2.27±1.98	6.33±12.40	7.86±22.13	2.99±4.82	1.89±1.24	0.82
2-Hydroxyphenanthrene	7.78±8.05	4.70±2.62	17.12±30.69	5.54±6.32	9.09±17.45	6.17±4.90	0.75
Σ Hydroxyphenanthrene	**18.18±17.15**	**9.81±5.42**	**36.19±67.97**	**15.58±22.69**	**18.53±31.19**	**12.34±8.03**	
1-Hydroxychrysense	2.28±3.38	0.70±0.60	10.3±22.21	0.68±0.94	2.70±3.77	2.48±2.59	0.81
1-Hydroxypyrene	79.94±227.91	10.72±8.18	55.35±117.84	16.28±24.52	14.62±8.59	16.16±10.67	8.50
Σ PAH metabolites	**212±297**	**76±64**	**133±78**	**62±49**	**124±206**	**106±74**	

Table 12. PAH metabolites in bile in cod.
Values given as average ± std dev in ng/g bile. N= number of fish per station.

	H1	**H2**	**LOQ**
	N=25	N=28	
1-Naphthol	0.74±2.19	0.11±0.07	2.54
2-Naphthol	1.48±3.46	0.40±0.24	0.24
Σ Naphthol	**2.22±5.65**	**0.51±0.30**	
7-Methyl-1-Naphthol/8-methyl-2-naphthol	0.02±0.04	0.01±0.01	0.01
2-Methyl-1-naphthol	0.56±0.77	0.55±1.44	1.65
3-Methyl-1-naphthol	0.32±0.59	0.11±0.07	0.10
6-Methyl-1-naphthol	0.69 ±0.78	0.30±0.37	0.12
3-methyl-2-naphthol	0.23 ±0.32	0.40±0.52	0.05
7-methyl-2-naphthol	0.55±1.31	0.21±0.12	0.10
6-methyl-2-naphthol	0.16±0.22	0.05±0.03	0.06
4-methyl-1-naphthol	0.24±0.54	0.10±0.15	0.52
5-methyl-1-naphthol/1-methyl-2-naphthol	0.29±0.66	0.12±0.07	0.08
4-methyl-2-naphthol	0.32±0.60	0.10±0.08	0.12
5-methyl-2-naphthol	0.23±0.41	0.09±0.08	0.03
Σ C1/C2 Naphthol	**3.61±5.30**	**2.05±2.18**	
2-Hydroxyfluorene	5.03±4.02	2.85±2.32	0.67
9-Hydroxyfluorene	9.90±18.68	3.38±2.81	10.69
Σ Hydroxyfluorene	14.93±21.21	6.23±3.77	
4-Hydroxyphenanthrene	0.46±0.92	0.28±0.26	0.14
9-Hydroxyphenanthrene	0.57±1.68	0.32±0.36	0.21
3-Hydroxyphenanthrene	2.49±2.00	1.58±1.10	0.66
1-Hydroxyphenanthrene	1.80±2.33	0.75±0.42	0.82

2-Hydroxyphenanthrene	4.53±7.90	2.04±1.39	0.75
Σ Hydroxyphenanthrene	**9.85±14.32**	**4.97±3.09**	
1-Hydroxychrysense	0.95±0.67	0.90±0.71	0.81
1-Hydroxypyrene	49.28±43.55	22.51±16.13	8.50
Σ PAH metabolites	**81±75**	**37±20**	

Since the condition monitoring in 2008, several new PAH metabolite standards have been purchased allowing better resolution of PAH metabolites in bile. For this reason, the sum of PAH metabolites reported are not directly comparable to earlier studies.

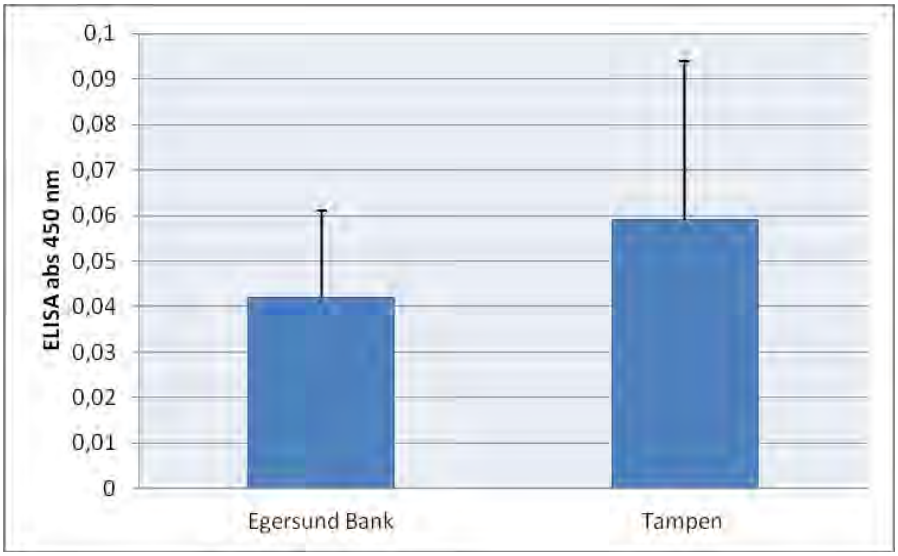
Compared to the PAH metabolite levels reported in bile of haddock from Tampen in 2008 (Grøsvik et al., 2009), much lower levels have been measured in haddock from the 2011 survey. In the 2008 study, sum PAH metabolites of 580 ng/g bile were measured in haddock from Tampen, significantly higher than at the Egersund Bank (231 ng/g bile), The main contributor to sum PAH metabolites was 1-hydroxy phenanthrene. Levels of 1-hydroxy phenanthrene in haddock bile from Tampen were 510±814 ng/g bile, while at the Egersund Bank levels were 133±207 ng/g.

Bile metabolites were performed on cod at Tampen and the Egersund Bank approximately one month after the discharge at Statfjord, December 2007, and levels of PAH metabolites were comparable with levels found in this study, except for those of 1-hydroxy phenanthrene. Mean levels of 1-hydroxy phenanthrene in cod bile in the Statfjord A study were between 6 and 14 ng/g bile (Grøsvik *et al*., 2008).

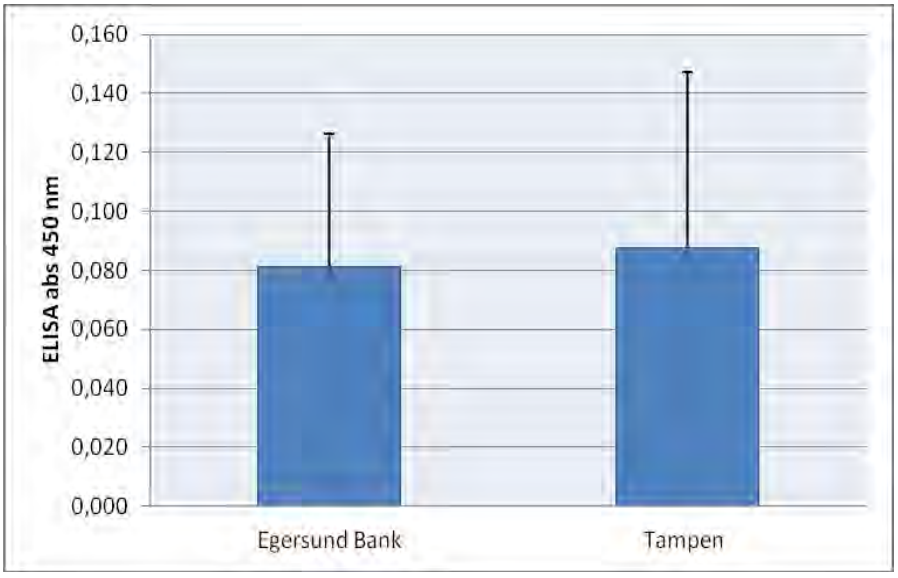
Levels of sum PAH metabolites in bile from two cod kept in cage under the oil slick after the Server accident had levels of 4026 ng/g bile. This level is in the same range as reported in bile from oil exposed cod from laboratory studies (Jonsson *et al*., 2003).

5.4 CYP1A in liver of cod and haddock

ELISA was performed on liver samples of cod and haddock incubated with anti-cod CYP1A. Absorbances were low, from 0.04-0.06, however, an increase in CYP1A levels in cod were observed in cod from Tampen compared with cod from the Egersund Bank, although not statistical significant (Figure 6A). Similar low absorbances (0.035-0.04) were reported at Tampen with cod and anti-CYP1 in 2008 (Grøsvik et al., 2008). No differences were observed in CYP 1A levels for haddock (Figure 6B).



A

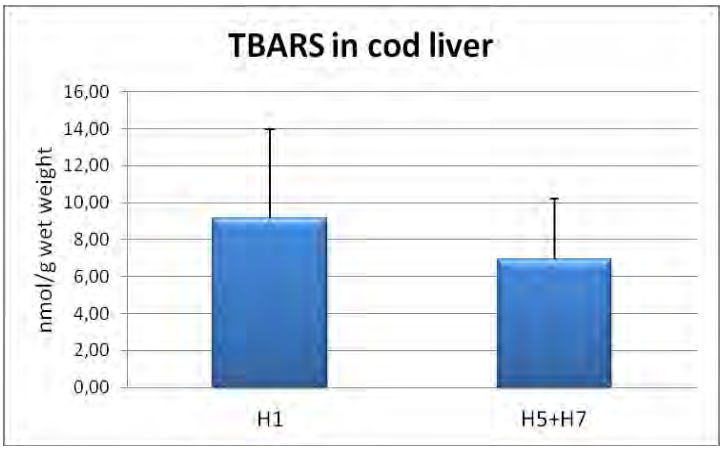


B

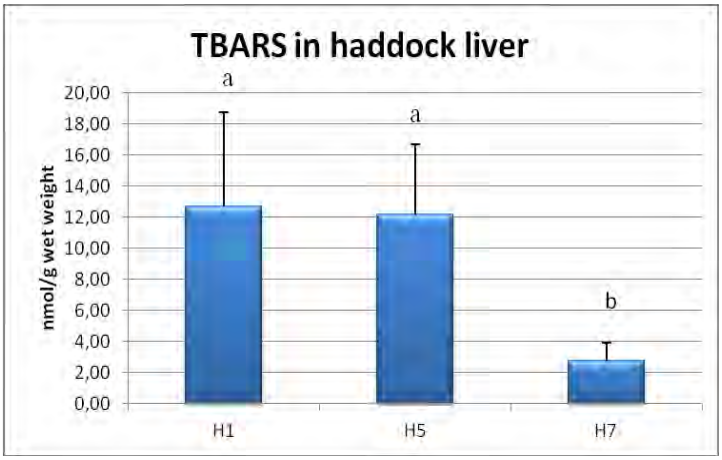
Figure 6. ELISA absorbance of CYP1A in liver of cod (A) and haddock (B). Data presented as average + stdev. A: Monoclonal anti-cod CYP1A (NP-7, Biosense) diluted 1:1000. B: Polyclonal anti-trout CYP1A (CP-226, Biosense) diluted 1:1000.

5.5 Thiobarbituric acid reactive substances (TBARS) in liver

Measurements of thiobarbituric acid reactive substances (TBARS) were included to study possible lipid peroxidation due to oxidative stress. Average levels of TBARS in liver of cod from Egersund Bank were 9.1±4.8 nmol/wet weight, while at Tampen 6.9±3.3 nmol/g wet weight. For haddock, TBARS levels were 12.7±6.1 and 12.1±4.6 at H1 and H5, respectively. At H7, TBARS levels were 2.8±1.2 nmol/g wet weight (Figure 7). TBARS levels < 15 nmol/g wet weight are considered background levels.



A

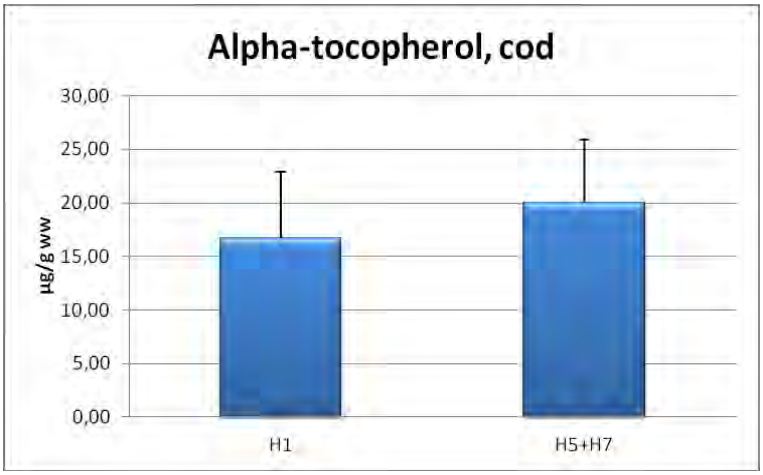


B

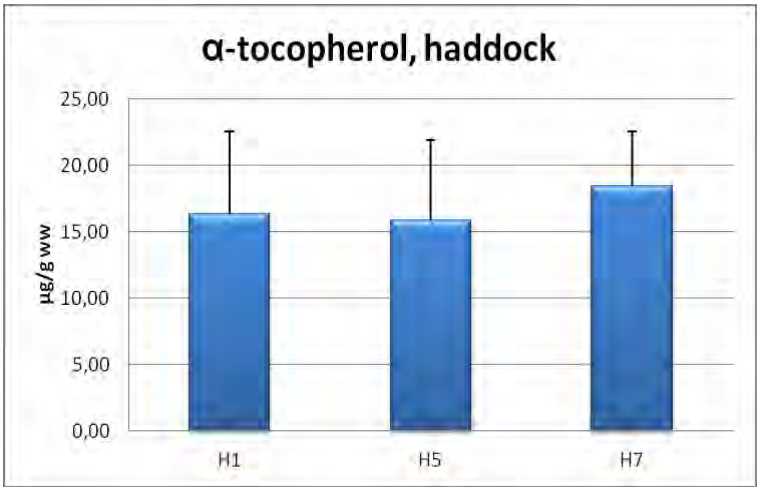
Figure 7. Thiobarbituric acid reactive substances (TBARS) in liver of cod (A) and haddock (B) caught at the Egersund Bank (H1) and Tampen (H5 and H7), given as mean nmol/g wet weight + std dev. For cod, N=25 (HI), N=18 (H5) and N=7 (H7). For haddock, N=25 (H1), N= 8 (H5), N= 17 (H7). Different letters indicate significant changes, $p < 0.05$.

5.6 α-Tocopherol levels in fish muscle

α-Tocopherol were included as it was reported to be significantly reduced at Tampen in the 2002 monitoring (Balk et al., 2011). α-Tocopherol is one of eight lipid soluble compounds included in the term vitamin E. It has antioxidant properties and may be consumed by reactive oxygen species (Burton, 1994). For the 2011 campaign, we did not see similar reduced levels in fish muscle of fish caught at Tampen compared with the Egersund Bank. (Figure 8). Mean levels in cod muscle were 16,7±6.2 µg/g wet weight and 20.0±5.8 µg/g wet weight at Egersund Bank and Tampen, respectively (Figure 8A). For haddock muscle, mean levels were 16.3±4.7, 15.5±4.1 and 18.5±3.8 µg/g wet weight, respectively (Figure 8B).



A



B

Figure 8. Levels of α-tocopherol in muscle of cod (A) and haddock (B) given as µg/g α-tocopherol per wet weight, mean per station + std dev. Egersund Bank (H1), Tampen south (H5), Tampen between Statfjord and Gullfaks (H7). For cod , N=25 (H1), n=17 (H5), n=8 (H7). For haddock, N=25 (H1), N=12 (H5), N=13 (H7).

5.7 DNA adducts in liver of haddock

Studies of DNA adducts in fish coupled with their exposure to certain pollutants represents an important approach in environmental risk assessment since Dawe et al. (1964) claimed that bottom feeding fish were "useful indicators of environmental carcinogens". Measurements of DNA adducts are used as a biomarker of 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 post labelling 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 bullheads sampled from sites in the Buffalo and Detroit Rivers, in association with exposure of fish to high concentrations of polycyclic aromatic hydrocarbons (PAH). Since these early works, a large range of fish species was studied, in a large panel of applications. Numerous published works are focused on the flounder (*Platichthys flesus*). Most of them indicate that adducts are detected in the liver of this fish when exposed to environmental genotoxicants. For example, the prolonged exposure via the diet to a mixture of four PAHs (5 and 50 $mg.kg^{-1}$) lead to the appearance of DNA adducts detected by the ^{32}P postlabelling (Reynolds et al., 2003). Interestingly, the adduct pattern exhibits a major spot probably comparable to the major spot detected in the current study, including the positive control. In a controlled mesocosm system, Harvey et al. (1997) showed the existence of DNA adducts in the liver of flounders associated to exposure to a mixture of PAHs (and PCBs). The concentrations of adducts measured by ^{32}P post-labelling (between 0 and 1 adduct in 10^8 normal nucleotides) were similar to those in the current study. In 1999, Lyons et al. measured hepatic DNA adducts and PAH metabolites in bile of flounders sampled in different stations of the polluted Tyne Estuary (North East England), while other fish were caught in a clean reference site. Finally, a large differences in DNA adduct levels were observed with higher values for contaminated sites, associated to large amounts of PAH metabolites in bile. The combination of two biomarkers provides a better estimate of the bioavailability of certain pollutants and indicated that flounders in Tyne Estuary were actually exposed to subsequent sub-lethal genotoxic effects. More recently, a comparable study was conducted on flounders caught in the Baltic Sea (Malmström et al., 2009). Hepatic DNA adduct levels measured in 10 different sites were low, with generally clean autoradiograms (except for a few detectable spots and rare faint typical radioactive diagonal zone). The authors concluded that in the investigated areas, flounders were not exposed to concentrations of polycyclic hydrocarbons of concentrations leading to increased levels of DNA adducts.

Preparation of DNA solutions

After shipment on dry ice, the samples were stored at -80°C until their handling of DNA extraction. Small pieces of tissue (70 to 90 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 (Appendix 7.1.6). The DNA concentrations were calculated from absorbance at 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 (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 following two weeks.

The absorbance ratios A_{260}/A_{280} and A_{260}/A_{230} obtained on the whole sample set were 1.92 ± 0.06 and 2.20 ± 0.10 respectively. These experimental ratios are considered satisfactory and in accordance with the requirements of the ^{32}P-postlabelling method.

Detection limit and controls

The detailed protocol used by ADn'tox is described in Appendix 7.1.6. Fourteen sets of analysis were necessary in order to analyse the DNA adduct patterns of the overall 137 samples. Two independent adduct measurements have been performed for each DNA sample.

The limit of detection is fixed to half the smallest DNA adduct level (Relative adduct level=RAL) calculated for an observed spot, i.e. ½ x 0.2 = 0.1 adducts per 10^9 nucleotides (RAL x 10^{-9}). For analysis without detectable adducts ("null" results), the concentration in adducts is then defined as $<0.1 \times 10^{-9}$ nucleotides.

In each set of analysis, DNA samples from both positive and negative controls were 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 inter laboratory trials. The DNA damage level was 1107 adducts for 10^9 normal nucleotides (according to F.A. Beland, in Philips and Castegnaro (1999), and Divi et al. (2002) and Zhan et al. (1995) for more details). The negative control was DNA extracted from AG1521 fibroblasts.

The autoradiographic patterns from both positive and negative controls are provided in Appendix 7.1.6. 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 was checked on the base of the direct level of radioactivity (Cerenkov radiation) in the major spot of the positive control, expressed in radiation counts per minute (cpm).

Statistical analysis

When DNA adduct level data were not normal distributed, non-parametric tests (Wilcoxon/Kruskal-Wallis test) were used to test if mean levels were statistical significant different. JMP ver 9.0.2 SAS®software was used for statistical analyses.

Results from DNA adduct measurements

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), Appendix 7.1.6), pattern of adducts and direct level of radioactivity in the major spot (routinely near 22,000 cpm ± 15%). The clean patterns 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).

Individual data are listed in Appendix 7.3. The adduct levels are expressed as nmol adducts per mol normal nucleotides. In general, adducts are associated to patterns with relatively few spots.

Qualitative analysis of DNA adduct patterns

Qualitatively, at least one signal (or spot) attributed to DNA adduct was observed on 214 among the 274 autoradiographic patterns obtained from fish sampled in 2011 (2 patterns per sample, Appendix 7.2, Figures 1 and 2), i.e. 78% of the overall patterns. 109 samples among 137 (80%) present one or more detectable adduct(s) on at least one of the two plates. The proportion of samples without detectable adducts per station is ranged from 4% (1 sample among 25 in H6) and 6% (1 sample among 17 in H7) to 45% (13 samples among 29 in H1). A statistical analysis of DNA adduct patterns in presence/absence of spot(s) for each sample confirms the global difference in the proportion of samples without adducts between stations (Fisher's exact test: p=0.002).

In particular, 15 distinct spots were isolated from their different 2D chromatographic migration on the PEI cellulose sheets (numbered 1 to 15, Figure 9). From the 2011 campaign, one major spot (n°1) was found in 103 samples among 137 (75% of overall samples, 94% of samples with adducts) and two frequent spots (n°2 and n°3, 28% and 19% of overall samples, 36% and 24% of samples with adducts, respectively). Spots n°6, n°10 and n°13 are occasional (9%, 8% and 6% of the 137 samples, respectively). Other spots occur very rarely, two of them are even observed in only one sample (spots n°8 and n°12). It is important to note that the spot n°1 is comparable (i.e. co-chromatographic spots) to the major spot (MS) obtained on all patterns of the positive control.

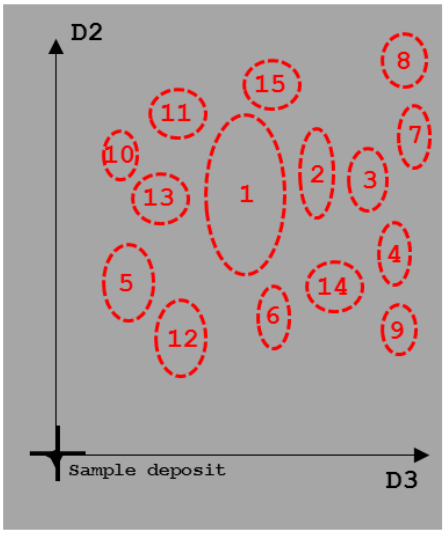


Figure 9: Location template of the different distinct spots attributed to DNA adducts obtained after two-dimensional thin layer chromatography on the overall 300 patterns. D1, D2, D3 and D4 migrations are explained in the Annex.

Interestingly, the spots n°2 and n°3 are frequently detected in only one pattern among both realised for each sample. This to be perhaps attributed to versatility in the 32P labelling efficiency of theses adducts, in association to their chemical structure. In another way, since the two spots are never found together on a same pattern but frequently in one and the other pattern of the same sample, this could mean that it is actually the same adduct.

Concerning the 2011 fish campaign, no spot appears to be limited to a particular site (site-specificity) and/or to only one sex (sex-specificity). However, some interesting variations of the DNA adduct patterns can be noted. The major spot n°1 is detected in more than 80% of the samples in stations H4 (92%), H6 (88%) and H7 (82%). The spot is present in only 3 females among 7 (43%) in station H2, 7 females among 14 in station H1 (50%) while it is encountered in all females in H6 (19 samples), 10 females among 11 (91%) in H7 and 9 females among 10 (90%) in H4. For males, the spot n°1 distribution between stations can be quite different. Only 3 males among 6 (50%) in station H6 and 4 males among 6 (66%) in station H7 exhibit this spot. Finally, the spot n°1 distribution (presence rate in samples) is statistically different from station to station (Fisher exact test on the samples in 2011: p=0.030), but does not depend statistically on the gender (Fisher exact test on samples in 2011: p=1.000).

The presence rate of the spot n°2 is dependent on the station too, with a maximum presence in stations H6 (56% of samples) and H7 (53%) (Fisher exact test on samples in 2011: p=0.001). It is absent from females in station H4 (n=10), males in station H1 (n=15), but present in 8 females among 11 (73%) in station H7. A significant sex effect is observed (presence in 39% of overall females and only 16 % of males) but might be partly associated to the fluctuation of sex ratio between the explored stations (Fisher exact test on samples in 2011: p=0.004).

Spot n°3 is absent from station H2, females in station H4 (n=10) and males in station H5 (n=13), but present in 7 females among 11 (64%) in station H7 and 10 females among 19 (53%) in station H6. As for spots n°1 and n°2, its presence rate in samples is dependent on the station with higher presence in stations H6 (48%) and H7 (47%) (Fisher exact test on samples: p=0.000003), and as for spot n°2, dependent on the sex with the same reservation concerning sex ratio between stations (Fisher exact test on samples in 2011: p=0.05). Interestingly, spot n°2 is associated to spot n°1 in 37 samples, i.e. 95% of samples with spot n°2. The only two samples that exhibit the spot n°2 without n°1 are females from station H2.

The mean number of different adducts (distinct spots) per sample varies significantly from station to station (Anova on samples in 2011: p<0.001). The higher mean spot numbers are described for stations H6 (2.6 *(± 0.2)* spots per sample in average) and H7 (2.3 *(± 0.3)* spots, Table 13). The result is confirmed by a two-by-two comparison of the stations according to Tukey's studentized range test on the spot number per sample. Through this test, the stations H6 and H7 appear significantly different from H1, H2, H4, H5, and H1, H2, H5 respectively. Other paired comparisons were not significant.

Table 13: Richness of the DNA adduct patterns, expressed as number of detected spots per sample for the different fishing stations.

Station	Number of fish/ station	Number of detected spot(s) per sample[1]		
		minimum	maximum	Mean (±*SD*)
H1	29	0	4	1.2 (± 0.2)
H2	17	0	3	1.1 (± 0.2)
H4	24	0	5	1.7 (± 0.2)
H5	25	0	4	1.0 (± 0.2)
H6	25	0	6	2.6 (± 0.2)
H7	17	0	4	2.3 (± 0.3)

[1)] The number is the sum of the different spots observed on both chromatographic pattern associated to each sample.

Quantitative analysis of DNA adduct patterns

The mean relative adduct levels (RAL) per sample ranged from <0.1 (no detectable adducts) to 300 (sample n°11, Viking Bank) adducts per 10^9 normal nucleotides (Appendix 7.3). On the 2011 fish campaign, 47% of the samples (64 among 137) present a mean RAL < 3.0 adduct per 10^9 normal nucleotides, 27 % had adduct levels between 3.0-6.7, and 36 % had adduct levels > 6.7 adduct per 10^9 normal nucleotides.

On the overall results, a large inter individual differences in RAL is observed, with the presence of outliers for females in stations H4 (sample n°22), H5 (sample n°22) and H6 (sample n°11). For males, one outlier is observed in station H5 (sample n°25).

Mean DNA adducts per station for haddock sampled in 2011 are presented in Figure 10. The mean DNA adduct level measured were 1.6±1.1 adducts x 10^{-9} nucleotides in station H2 (Ula

area, the Southern North Sea), around 5.0 adducts x 10^{-9} nucleotides (from 4.3 to 5.5) in stations H1 (Egersund Bank), H4 (Bressay Bank) and H5 (Tampen South of Statfjord), 7.3 adducts x 10^{-9} nucleotides at station H7 (Tampen between Statfjord and Gullfaks) and 19.5 adducts x 10^{-9} nucleotides at station H6 (Viking Bank). If one individual with very high adduct level at H6 was excluded, mean level at station H6 was reduced to 7.9 adducts x 10^{-9} nucleotides. Station H6 and H7 were significant different from stations H1, H4 and H5 (Wilcoxon/Kruskal-Wallis, $p < 0.05$), independent of the individual with very high DNA adduct level at H6.

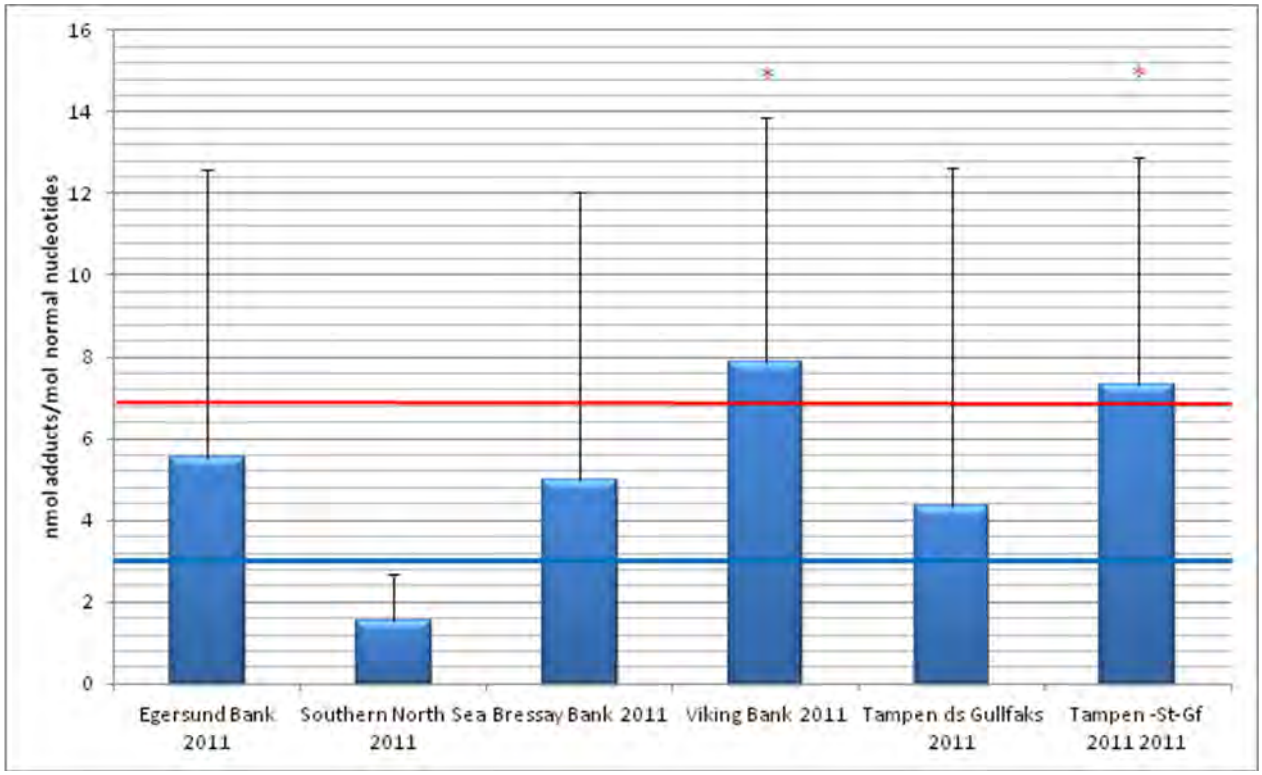


Figure 10. DNA adduct data from 2011.
Data given as mean levels of DNA adducts (nmol adducts/mol normal nucleotides or relative adduct levels (RAL) x 10^{-9}) + standard deviation. Stations: Egersund Bank (H1) (N=29), Ula area, Southern North Sea (H2) (N= 17), Bressay Bank (H4) (N=24), Viking Bank (H6) (N=24), H5: Tampen downstream (ds) Gullfaks (H5) (N=25), Tampen between Statfjord (St) and Gullfaks (Gf) (H7) (N=17). For haddock, background response range is set to ≤ 3.0 RAL x 10^{-9}, Elevated response range > 3.0 RAL x 10^{-9} and levels > 6.7 RAL x 10^{-9} is considered high and cause for concern response (ICES, 2011). * indicates significanct differences from the other stations (Wilcoxon / Kruskal-Wallis non parametric test, $p < 0.05$).

For the 3 stations H1, H4 and H5, the levels of DNA adducts were around 3 to 4 times larger than obtained at station H2. The proportion of samples without detectable adducts was very different between these 3 stations (13/29 (45%) in H1, 8/25 (32%) in H5, 2/24 (8%) in H4). For stations H1 and H5, this proportion is above what is found in the overall samples (23%).

For the two remaining stations H6 and H7, the proportion of samples without detectable adducts was very small, 4% (1/25) in station H6 and 6% (1/17) in station H7 (the 2 samples were males).

The DNA adduct data performed in the condition monitoring of 2002, 2005 and 2008 are compared in Figure 11, although they were performed in another laboratory. 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 Statfjord had DNA adduct levels at the same levels as the reference stations Egersund Bank and Bressay Bank, while the station at Tampen between Statfjord and Gullfaks and the station at the Viking Bank had statistically higher, although only 30 and 40 % higher at the stations at Tampen and the Viking Bank, when one extreme individual was excluded. The results demonstrate the importance of higher resolution from higher number of stations in field monitoring.

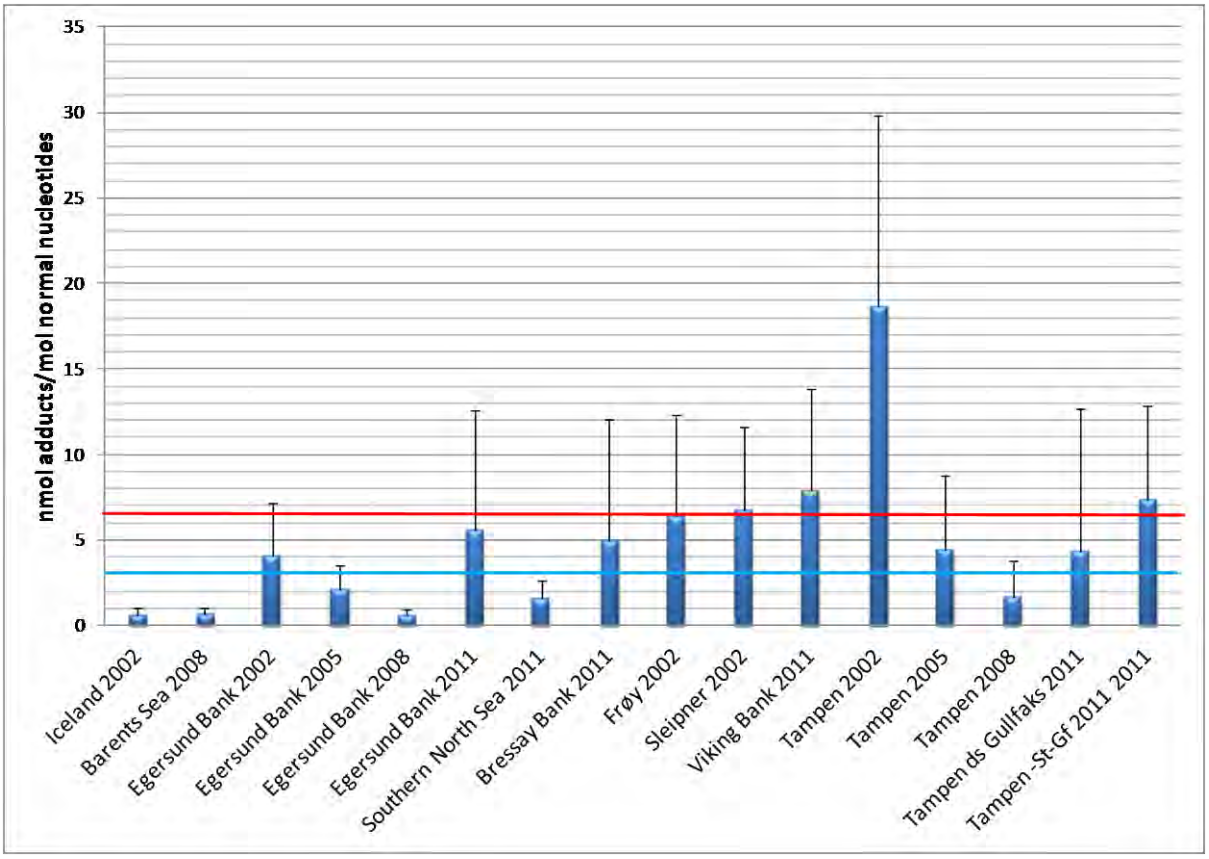


Figure 11. Comparing DNA adduct data from 2002, 2005, 2008 and 2011.
Data given as mean levels of DNA adducts (nmol adducts/mol normal nucleotides or RAL x 10^{-9}) + standard deviation. For haddock, background response range is set to ≤3.0 RAL x10^{-9}, elevated response range > 3.0 RAL x 10^{-9} and levels > 6.7 RAL x 10^{-9} is considered high and cause for concern response (ICES, 2011). Data from monitoring before 2011 are taken from: Balk et al., 2011 (2002 data), Grøsvik et al., 2007 (2005 data), Grøsvik et al., 2009 (2008 data).

Effect of the sex (and station) on DNA adduct levels

On the overall result, the individual mean level in DNA adducts appears about two times higher in females compared to males (mean RAL by sex: 10.2 *(± 37.1)* x 10^{-9} for females; 4.6 *(± 6.5)* x 10^{-9} for males). The difference is of borderline significance (Kruskal-Wallis test: p=0.069; Anova on Log(RAL) in samples with detectable adducts: p=0.044) However, the observed result is probably associated to the station effect, as the male/female ratio varies widely among stations, with a higher proportion of females for stations H6 (19/25 : 76% females) and H7 (11/17 : 65% females) compared to H2 (7/17 : 41% females) and H4 (10/24: 42% females). Further, no statistical difference is observed concerning the proportion of samples with undetectable adducts in males and females on the overall study (Fisher's Exact test: p=0.675). Similarly, no gender difference is observed on the frequency of the more frequent numbered spots (for spot n°1, Fisher's exact test: p= 1.000).

When analysed per station, the mean RAL by sex is higher for males only in the station H1. The mean RAL by sex is similar between males and females in station H4 (and H6 when sample n°11 is discarded), whereas it is higher for females in stations H2, H5, H6 (sample n°11 included) and H7.

In order to define the relative implication of the sampling station and gender in the observed DNA adduct variations, a test on the intersection of the two variables (station x sex) was performed. The gender ratio between stations was not statistically different (Fisher's exact test: p=0.119). The station effect was only observed in samples with detectable adducts: p=0.001 (station); p=0.306 (sex); p=0.446 (station x sex) (Anova on Log(RAL).

Discussion

In the European Union Marine Strategy Framework Directive (MSFD) member states are required to develop a robust set of tools for defining eleven qualitative descriptors of Good Environmental Status (GES), to be able to demonstrate that "Concentrations of contaminants are at levels not giving rise to pollution effects"(GES Descriptor 8). The ICES Study Group for the Integrated Monitoring of Contaminants and Biological Effects (SGIMC) has recommended threshold levels for a set of effect parameters/biomarkers to be able to classify environmental status in a three-colour system where Blue is less than Background Assessment Criteria (BAC), Green is above BAC but less than Environmental Assessment Criteria, and Red is above EAC (ICES, 2011).

For DNA adducts, haddock is included in the recommendation. For adduct levels for haddock, BAC is set to ≤ 3.0 RAL x 10^{-9}, Elevated response range to > 3.0 RAL x 10^{-9} and levels >6.7 RAL x 10^{-9} are set to high and cause for concern response, or environmental assessment criteria (EAC) (ICES, 2011).

32P postlabelling is known to be highly sensitive, but also semi quantitative. The proportion of detectable levels of DNA adducts in tissues of wild fish is of 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 highest levels of DNA adducts were found at Tampen between Statfjord and Gullfaks (H7) and at the Viking Bank (H6). Station H7 and Station H5 (Tampen South) were the two stations with presumed highest potential impact from the O&G industry through produced water discharges and presence of previously discharged oil contaminated drill cuttings.

DNA adduct analyses from fish sampling in the period from 2001 to 2004, revealed significant higher levels of hepatic adducts in haddocks caught in the Tampen region compared to an unpolluted site from southwest of Iceland (Balk et al., 2011). The mean DNA adducts was around 20 nmol adducts per mol normal nucleotides, or 20 adducts per 10^9 nucleotides. In the current study we did not observe the large differences between Tampen and the Egersund Bank as reported in Balk et al., (2011).

The mean adduct levels measured per station in the current study were below the background concentration of 3.0 adducts per 10^9 nucleotides at station H2, around 5.0 adducts per 10^9 nucleotides in stations H1, H4 and H5, and slightly above the EAC threshold of 6.7 adducts per 10^9 nucleotides at stations H6 and H7.

In a qualitative point of view, 15 distinct spots attributed to different adducts are counted on the overall samples. Such qualitative variety in distinct spots can be attributed to a large capability of genotoxicant bioactivation by haddocks and/or the presence of numerous pollutants in fish environment. The richness of DNA adduct pattern per fish appears different from station to station, with higher number of distinct adducts at Tampen and the Viking Bank. One spot is detected in the vast majority of the overall samples, and two others are frequent. The distribution of each of these 3 spots is associated to the fishing station and for two of them, to the gender of haddocks. These spots are mainly observed in stations H6 and H7. Moreover, the most observed adduct in the samples seems to be comparable to the one described in the positive control for the method used, which was exposed to the PAH Benzo[a]pyrene (BaP). According to these 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 stations, in probably very different concentrations. Other explanations 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 sites or the revelation of endogenous DNA adducts (Aas et al., 2003; Swenberg et al., 2011).

Some significant differences in levels of DNA adducts is shown between males and females throughout the study. In all, the measured DNA adduct levels are higher in females. Such differences associated to sex could be explained by variable metabolic capacities and/or different conditions of exposure to pollutants in association with certain behaviours that differ between sexes. However, because of a variable sex ratio between the explored stations, the role of sex in the observed result appears very difficult to assess. This difficulty is mentioned

in most of the environmental studies that have considered this matter, e.g. in Akcha et al., (2004).

The presence of DNA adducts confirms that the fish have been exposed to genotoxic pollutants and indicates PAH contamination in the area. 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). The stations with levels above background levels included the two reference stations and one of the stations at Tampen. The other station at Tampen together with the station at Viking the Bank had levels slightly above 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 present study does not indicate that cod and haddock caught at Tampen are more contaminated with oil related 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.

5.8 Lipid analyses

Detailed lipid class analyses and fatty acids analyses of muscle samples have been conducted from haddock and cod. In addition fatty acid profiles have been analysed for algae and zooplankton. For the lipid class analyses liver lipids were extracted and separated by solid phase extraction (SPE) into neutral lipid (NL) and membrane lipids. Phosphatidylcholine (PC) and phosphatidylethanolamin (PE) eluates together, and phosphatidylserine (PS) and phosphatidylinositiol (PI) are also analysed together. The NL in the liver are totally dominated by triacylglycerids and functions as the most important energy storage in cod fish. The PC and PE are the dominating membrane lipids and contribute approximately 85 % of the membrane lipids and PS/PI contributed approximately 15 %. The fatty acids profile were analysed from the different lipid classes from liver, while total lipids were analysed from muscle, algae and zooplankton samples by gas chromatography.

Balk et al. (2011) found that haddock and cod caught around Tampen had a different fatty acids profiles compared with fish caught more south in the North Sea (Sleipner area and Egersund bank). The relative amount arachidonic acid (20:4 (n-6)) was higher in fish from the Tampen region and this changed the ratio between poly unsaturated n-3 fatty acids (n-3 PUFA, also called ω3 PUFA) and n-6 PUFA. This observed differences raised the question whether "petroleum hydrocarbons may accumulate in the membranes, thereby altering their properties, or interfere directly with the metabolic reactions and/or molecular signalling regulating the fatty acid composition of the membranes" (Balk et al., 2011).

Similar effects have been reported in laboratory studies of cod exposed to alkylphenols. Such exposures resulted in a reduction of the amount of n-3 PUFA and increased the relative amount of n-6 PUFA in the membrane lipids (Meier et al., 2007). However, there are also many natural factors, like temperature and diet that also may affect the lipid composition of fish. There is therefore a need for more investigation to establish if this finding may be correlated to pollution or natural geographic differences.

5.8.1 Lipid analyses of muscle and liver of haddock

IMR has in the condition monitoring and through own funding further investigated lipid composition in haddock. In 2008 haddock were sampled from Tampen, Egersund Bank (North Sea), Halten Bank (Norwegian Sea) and Barents Sea, and in 2010 we sampled analysed from Tampen and Egersund Bank. The present 2011 survey have studied five different areas in the North Sea. We selected only to analyse male fish.

The investigation from 2008 and 2010 confirmed the differences in the fatty acids profiles that were reported by Balk et al. (2011). There were found significantly higher levels of 20:6 (n-6) (and reduced n-3/n-6 ratio) in both the neutral storage lipids (NL) and the membrane lipids (PC/PE) of liver samples of haddock at the Tampen region compared with the other areas (Figure 13). Similar differences were also observed in analysis of muscle lipids of haddock (Figure 14). We also found that haddock caught at the Tampen region in 2008 and

2010 had decreased levels of lipid storage. Haddock from Tampen had smaller livers and reduced lipid content. The amount of liver lipid relative to body weight in haddock at Tampen was only 1/3 of the amount that were found at the other stations (Figure 12).

The present investigation from 2011 did not show the same picture. The LSI (liver somatic index) was at the same low levels at Tampen as reported in 2008 and 2010. However, the LSI was also low at all the other samplings points and there were no significant difference between Tampen and the other areas (Figure 12). A clear difference in the 2011 analysis were that LSI and lipid content of haddock liver were much lower in the references area at Egersund Bank compared with 2008 and 2010.

Also, fatty acid profile measured in 2011 did not show the same differences between Tampen and Egersund Bank as found in 2008 and 2010. There were no significant differences in n-3/n-6 ratio or fatty acids profile between the references area at Egersund Bank and Tampen, nor for liver or muscle analysis (Figure 13 and Figure 14). In 2011 the levels of arachidonic acid (20:4 (n-6)) were higher in the reference area and lower from the Tampen region than reported in 2008 and 2010.

Principal component analysis (PCA) also confirm that there were no clear differences between the fatty acids composition in the different area for haddock liver NL (Figure 17), PC/PE (Figure 18), PS/PI (Figure 19). PCA is a fast and easy method of evaluating if there are different between samples with multiple numbers of variables, like fatty acids profiles. PCA give a graphic presentation where a multivariate space is projected in two dimensions, and samples that are “similar” are located close in the score plot. The loading plot shows how the different variables (fatty acids) are correlated to the samples. If there were large difference in fatty acids profiles between different areas, these samples should clusters in separated groups in the score plot of the PCA.

For haddock muscle there was a trend in the PCA (Figure 21) that the fish from Southern North Sea (Ula area, H2) clustered away from the other areas showing that these fish have different fatty acids profile. Table 21 also shows that the haddock from Ula area (H2) have higher relative levels of the short chain PUFA; 18:3 (n-3) and 18:4 (n-3) in the muscle. Similar trend can also be observed in the NL from the liver (Table 17). It is likely that this differences originates from difference in diet.

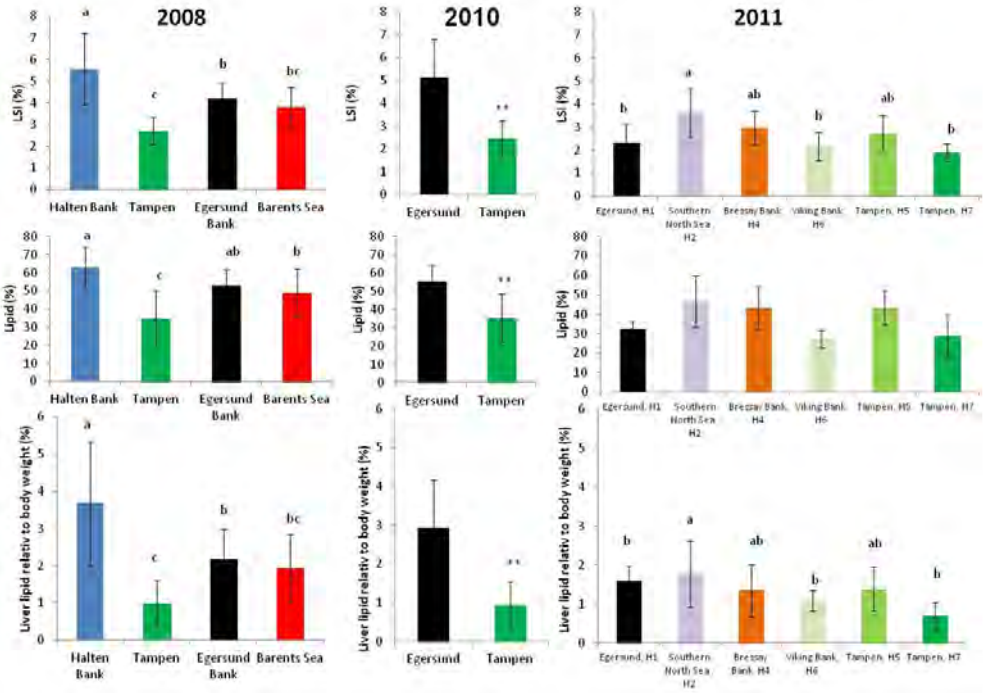


Figure 12. Comparison between different sampling years (2008, 2010 and 2011) and geographic areas for haddock samples. Top figures: liver somatix index (LSI = weight of liver relative to body weight), Middle figures: lipid amount in the liver (% of weight), Bottom figures: the amount of liver lipid relative to body weight. The results are given as mean + std dev. Different letters (or stars for 2010) indicate significant differences, $p < 0.05$.

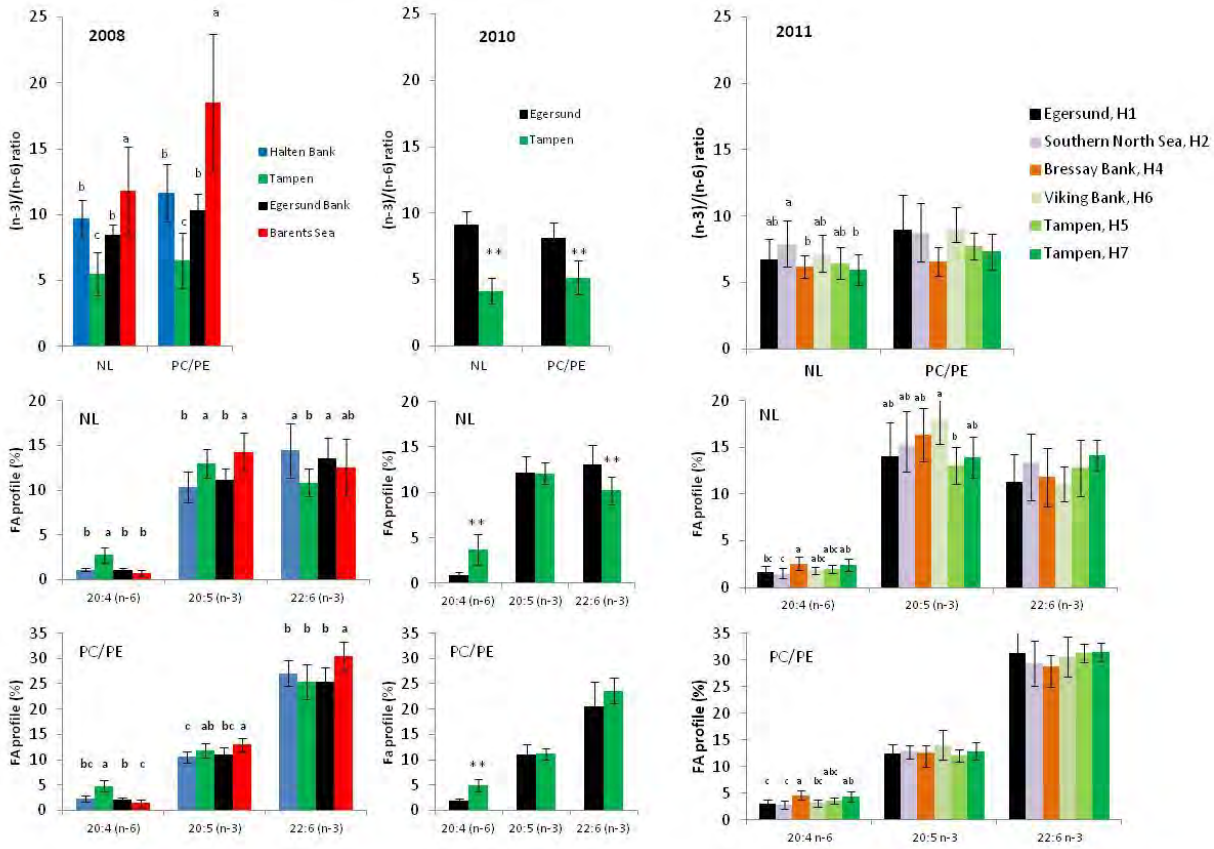


Figure 13. Comparison of sampling years (2008, 2010 and 2011) and geographic area for haddock. Top figures: the ratio between (n-3) and (n-6) fatty acids in the liver neutral lipid (NL) and the dominating phospholipids (PC/PE), Middle figures: the fatty acids profiles of ARA (20:4 (n-6)), EPA (20:5 (n-3)) and DHA (22:6 (n-3)) in NL, Bottom figures: the fatty acids profiles of 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3) in PC/PE. The results are giving as mean per station + std dev. Different letters (or stars for 2010) indicate significant changes, $p < 0.05$.

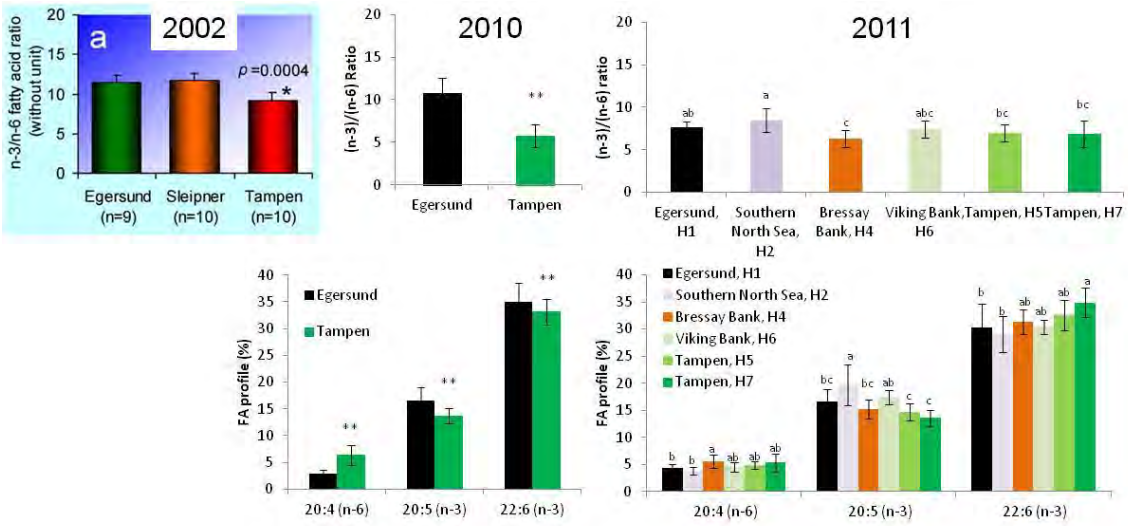


Figure 14. Comparison of results from 2002, 2010 and 2011 with haddock. Data from 2002 are taken from Balk et al. (2011). Top figure: the ratio between (n-3) and (n-6) fatty acids in the total muscle lipids. Bottom figure: the fatty acid profiles of 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3) in muscle lipids from 2010 (left) and 2011 (right). The results are given as mean per station + std dev. Different letters (or stars for 2010) indicate significant differences, $p < 0.05$.

Table 14 show the lipid content of liver and muscle in male haddock from the different areas. For all samplings points a few fish had very small livers and low liver lipid content. There were a clear correlation between LSI and the lipid content in the liver (Figure 15). The lipid content in the liver will naturally effect the lipid class composition, lean fish will have less storages lipid (NL) and relative more membrane lipid (Figure 15 and Table 16). To avoid to induce too much "noise" in the data set, we have separated the fish in lean haddock (liver lipid < 15%) and fat haddock (liver lipid > 15 %) (Table 15). There were no significant differences between the different area in LSI or lipid content in liver or muscle from the 2011 survey.

The large variation in liver lipid content from the different sampling points allowed us to test a hypothesis: "Can the large differences in fatty acids content of 20:4 (n-6) found between Tampen and other area be explained by different in lipid content and different mobilization of n-6 and n-3 PUFA between lean and fat fish?"

To test this theory we did correlation analyses between liver lipid (%) and the fatty acids profiles (Table 20 and Figure 16). There were found a small, but significant negative correlation between lipid amount and the relative amount of 20:4 (n-6) (r^2=0.09) in NL, but not in the membrane lipids. For haddock, we did not see any strong correlations between liver lipid content and fatty acids profiles that supported the theory that differences observed between Tampen and other areas could be explained by a simple correlations between lipid amount and fatty acid composition.

Table 14. Lipid content in male haddock liver and amount of fatty acids (FA) in muscle of haddock (mean±std dev).

	(n)	Liver lipid (%)	Muscle FA (%)
Egersund, H1	14	32±18	0.46±0.08
Ula area, H2	10	47±13	0.51±0.08
Bressay Bank, H4	14	43±11	0.48±0.09
Viking Bank, H6	6	27±18	0.46±0.08
Tampen, H5	12	43±15	0.49±0.03
Tampen, H7	6	29±15	0.48±0.07

Table 15. Biological information of samples from lean fish and fat fish.

Lipid content <15%	n	weight (g)	length (cm)	Liver (g)	LSI (%)	Lipid (%)
H1	4	580 ± 224	40,5 ± 3,7	7,75 ± 2,99	1,34 ± 0,15	8,92 ± 4,70
H2	0	-				
H4	0	-				
H6	3	380 ± 92	33,7 ± 3,2	7,00 ± 1,41	1,65 ± 0,49	11,01 ± 0,57
H5	1	260	31,0	3,20	1,23	2,85
H7	1	640	41,0	9,00	1,41	5,06
Lipid content >15%	n	weight (g)	length (cm)	Liver (g)	LSI (%)	Lipid (%)
H1	10	358 ± 39	32,8 ± 1,0	11,5 ± 2,4	3,20 ± 0,52	49,6 ± 3,9
H2	10	357 ± 98	33,5 ± 2,6	13,0 ± 5,0	3,60 ± 1,07	46,6 ± 13,1
H4	14	415 ± 170	34,9 ± 4,2	12,1 ± 5,1	2,97 ± 0,71	43,3 ± 11,1
H6	3	477 ± 202	36,3 ± 4,9	11,3 ± 2,3	2,51 ± 0,47	43,5 ± 4,4
H5	11	588 ± 260	38,4 ± 5,2	16,3 ± 6,7	2,83 ± 0,67	47,1 ± 8,7
H7	5	376 ± 55	37,8 ± 9,1	7,4 ± 0,9	1,99 ± 0,32	33,3 ± 11,1

Table 16. Lipid classes distributed in sample (% of total lipids, mean± std dev).

Lean fish		H1	H2	H4	H6	H5	H7
Lipid content <15%		(n=4)	(n=0)	(n=0)	(n=3)	(n=1)	(n=1)
	NL	62±27			64±5	14	46
	FFA	11±7			11±6	9	17
	PC/PE	23±18			21±8	70	29
	PS/PI	5±4			4±1	6	8
fat fish		H1	H2	H4	H6	H5	H7
Lipid content >15%		(n=10)	(n=10)	(n=14)	(n=3)	(n=11)	(n=5)
	NL	93.5±3.9	95.9±3.7	94.3±3.9	92.6±2.5	92.3±2.3	84.4±7.5
	FFA	1.7±1.6	0.6±0.5	1.0±0.7	2.7±1.4	1.0±0.7	3.9±2.3
	PC/PE	4.1±2.1	3.0±3.0	4.0±2.0	4.0±1.6	5.4±2.1	9.7±6.0
	PS/PI	0.7±0.4	0.5±0.6	0.7±0.5	0.7±0.2	1.0±0.4	2.0±1.2

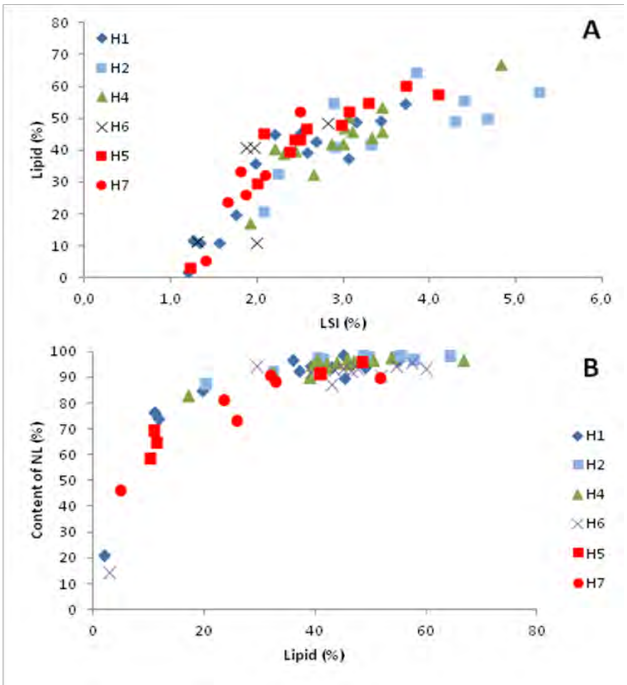


Figure 15. Correlation of LSI and total lipid content (A), and lipid content and amount of neutral lipids (B) in haddock liver.

Table 17. Fatty acids composition (% of total fatty acids) in the neutral lipids (NL) of male haddock liver (mean± std dev). Different letters indicate significant changes, p<0.05.

	Egersund, H1 (n=14)	Southern North Sea, H2 (n=10)	Bressay Bank, H4 (n=14)	Viking Bank, H6 (n=6)	Tampen, H5 (n=12)	Tampen, H7 (n=6)
14:0	3,62 ± 0,90	2,94 ± 0,41	2,89 ± 0,63	3,64 ± 0,84	3,08 ± 0,52	3,56 ± 0,96
i-15:0	0,46 ± 0,15^{ab}	0,40 ± 0,12^{ab}	0,32 ± 0,08^{b}	0,55 ± 0,15^{a}	0,35 ± 0,16^{b}	0,33 ± 0,06^{b}
15:0	0,83 ± 0,15^{a}	0,79 ± 0,11^{ab}	0,82 ± 0,11^{ab}	0,93 ± 0,15^{a}	0,69 ± 0,11^{b}	0,74 ± 0,07^{ab}
16:0	12,46 ± 1,57^{ab}	13,99 ± 1,70^{a}	12,24 ± 1,69^{ab}	11,65 ± 1,30^{b}	13,93 ± 1,03^{a}	12,79 ± 1,53^{ab}
i-17:0	1,24 ± 0,39	1,30 ± 0,28	1,10 ± 0,16	1,41 ± 0,22	1,04 ± 0,38	1,01 ± 0,08
ai-17:0	0,57 ± 0,28	0,64 ± 0,29	0,38 ± 0,12	0,58 ± 0,12	0,46 ± 0,26	0,35 ± 0,06
17:0	0,88 ± 0,21^{abc}	0,76 ± 0,15^{c}	0,88 ± 0,13^{abc}	1,02 ± 0,10^{ab}	0,84 ± 0,16^{bc}	1,11 ± 0,17^{a}
i-18:0	0,27 ± 0,08^{ab}	0,24 ± 0,06^{b}	0,24 ± 0,06^{b}	0,24 ± 0,10^{b}	0,33 ± 0,06^{a}	0,36 ± 0,06^{a}
ai-18:0	0,32 ± 0,16^{ab}	0,25 ± 0,09^{b}	0,28 ± 0,14^{ab}	0,50 ± 0,36^{a}	0,17 ± 0,10^{b}	0,15 ± 0,02^{b}
18:0	4,31 ± 0,86^{b}	4,41 ± 0,55^{b}	4,83 ± 0,70^{ab}	4,89 ± 0,71^{ab}	4,33 ± 0,73^{b}	5,85 ± 0,72^{a}
20:0	0,12 ± 0,03^{b}	0,10 ± 0,03^{b}	0,17 ± 0,04^{a}	0,14 ± 0,02^{ab}	0,14 ± 0,03^{ab}	0,16 ± 0,08^{ab}
∑SFA	**25,09 ± 2,41**	**25,81 ± 2,27**	**24,14 ± 2,30**	**25,55 ± 1,58**	**25,37 ± 1,50**	**26,40 ± 2,20**
16:1 n-11	0,15 ± 0,05^{a}	0,09 ± 0,01^{bc}	0,13 ± 0,05^{abc}	0,08 ± 0,01^{c}	0,14 ± 0,04^{ab}	0,09 ± 0,03^{bc}
16:1 n-9	0,48 ± 0,10	0,49 ± 0,07	0,50 ± 0,12	0,50 ± 0,09	0,48 ± 0,12	0,39 ± 0,05
16:1 n-7	6,91 ± 1,69^{a}	5,78 ± 0,75^{ab}	6,22 ± 1,20^{ab}	7,18 ± 1,28^{a}	5,71 ± 1,06^{ab}	4,53 ± 0,98^{b}
16:1 n-5	0,31 ± 0,07^{a}	0,26 ± 0,08^{ab}	0,20 ± 0,06^{b}	0,26 ± 0,05^{ab}	0,23 ± 0,07^{ab}	0,20 ± 0,02^{b}
17:1 n-x	0,66 ± 0,16	0,77 ± 0,23	0,70 ± 0,14	0,70 ± 0,18	0,79 ± 0,19	0,72 ± 0,12
18:1 n-11	0,73 ± 0,56	0,46 ± 0,21	0,51 ± 0,29	0,94 ± 0,23	0,54 ± 0,22	0,78 ± 0,38
18:1 n-9	11,83 ± 4,13^{ab}	14,21 ± 5,08^{a}	12,53 ± 2,89^{ab}	8,88 ± 1,62^{b}	15,36 ± 2,29^{a}	12,90 ± 1,38^{ab}
18:1 n-7	5,74 ± 1,47^{ab}	4,75 ± 0,99^{b}	5,52 ± 0,91^{ab}	6,43 ± 0,42^{a}	5,58 ± 0,89^{ab}	5,46 ± 0,58^{ab}
18:1 n-5	0,56 ± 0,19	0,56 ± 0,12	0,47 ± 0,09	0,57 ± 0,16	0,53 ± 0,12	0,46 ± 0,05
20:1 n-11	1,98 ± 0,67	1,43 ± 0,74	1,68 ± 0,92	2,43 ± 0,29	1,82 ± 0,28	2,48 ± 1,25
20:1 n-9	3,55 ± 2,51	2,04 ± 0,85	2,53 ± 1,08	1,56 ± 0,33	3,14 ± 1,49	2,35 ± 0,64
20:1 n-7	1,37 ± 0,50^{ab}	0,98 ± 0,38^{b}	1,69 ± 0,50^{a}	2,12 ± 0,81^{a}	1,08 ± 0,56^{b}	0,77 ± 0,40^{b}
22:1 n-11	2,45 ± 2,49	1,27 ± 0,52	1,60 ± 0,86	0,79 ± 0,38	2,16 ± 1,58	1,73 ± 0,82
22:1 n-9	0,38 ± 0,22	0,22 ± 0,05	0,30 ± 0,09	0,32 ± 0,10	0,28 ± 0,17	0,31 ± 0,28
22:1 n-7	0,11 ± 0,05	0,05 ± 0,04	0,12 ± 0,06	0,13 ± 0,04	0,06 ± 0,06	0,11 ± 0,04
24:1 n-9	0,41 ± 0,49	0,22 ± 0,08	0,22 ± 0,07	0,25 ± 0,06	0,34 ± 0,28	0,31 ± 0,23
∑MUFA	**37,62 ± 15,33**	**33,57 ± 10,20**	**34,92 ± 9,31**	**33,13 ± 6,05**	**38,25 ± 9,41**	**33,60 ± 7,25**
16:2 n-4	0,36 ± 0,16^{a}	0,25 ± 0,03^{ab}	0,29 ± 0,11^{ab}	0,39 ± 0,07^{a}	0,22 ± 0,09^{b}	0,17 ± 0,04^{b}
18:2 n-4	0,31 ± 0,08^{abc}	0,28 ± 0,03^{bc}	0,34 ± 0,06^{ab}	0,39 ± 0,06^{a}	0,25 ± 0,05^{c}	0,29 ± 0,04^{bc}
18:4 n-1	0,18 ± 0,09^{ab}	0,12 ± 0,04^{b}	0,15 ± 0,09^{ab}	0,25 ± 0,11^{a}	0,14 ± 0,06^{ab}	0,12 ± 0,03^{ab}
18:2 n-6	1,08 ± 0,23^{ab}	1,24 ± 0,39^{a}	0,87 ± 0,13^{bc}	0,73 ± 0,09^{c}	1,01 ± 0,14^{abc}	1,01 ± 0,19^{abc}
20:2 n-6	0,74 ± 0,18	0,67 ± 0,26	0,74 ± 0,09	0,77 ± 0,09	0,76 ± 0,17	0,81 ± 0,23
20:3 n-6	0,12 ± 0,04^{b}	0,11 ± 0,06^{b}	0,13 ± 0,03^{b}	0,23 ± 0,15^{a}	0,13 ± 0,05^{b}	0,11 ± 0,02^{b}
20:4 n-6	1,68 ± 0,59^{bc}	1,48 ± 0,52^{c}	2,55 ± 0,72^{a}	1,83 ± 0,37^{abc}	1,97 ± 0,46^{abc}	2,46 ± 0,65^{ab}
22:4 n-6	0,90 ± 0,52^{ab}	0,55 ± 0,26^{b}	0,80 ± 0,29^{ab}	1,21 ± 0,45^{a}	0,70 ± 0,18^{ab}	1,01 ± 0,48^{ab}
22:5 n-6	0,39 ± 0,22	0,51 ± 0,24	0,59 ± 0,41	0,33 ± 0,06	0,32 ± 0,04	0,43 ± 0,08
18:3 n-3	0,51 ± 0,16^{b}	1,07 ± 0,62^{a}	0,53 ± 0,19^{b}	0,30 ± 0,03^{b}	0,47 ± 0,07^{b}	0,45 ± 0,09^{b}
18:4 n-3	1,72 ± 0,69^{b}	2,56 ± 1,01^{a}	1,46 ± 0,34^{b}	1,11 ± 0,46^{b}	1,06 ± 0,43^{b}	0,94 ± 0,20^{b}
20:3 n-3	0,32 ± 0,17^{ab}	0,47 ± 0,30^{a}	0,35 ± 0,16^{ab}	0,23 ± 0,05^{b}	0,25 ± 0,03^{b}	0,36 ± 0,03^{ab}
20:4 n-3	0,73 ± 0,22	0,68 ± 0,24	0,69 ± 0,17	0,67 ± 0,15	0,61 ± 0,10	0,82 ± 0,21
20:5 n-3	14,06 ± 3,65^{ab}	15,27 ± 2,81^{ab}	16,37 ± 2,81^{ab}	18,04 ± 2,70^{a}	13,10 ± 1,98^{b}	13,97 ± 2,19^{ab}
21:5 n-3	0,51 ± 0,14^{b}	0,44 ± 0,06^{b}	0,46 ± 0,10^{b}	0,66 ± 0,07^{a}	0,42 ± 0,10^{b}	0,48 ± 0,07^{b}
22:5 n-3	2,42 ± 0,31^{a}	1,49 ± 0,35^{b}	2,80 ± 1,09^{a}	3,08 ± 0,50^{a}	2,16 ± 0,40^{ab}	2,44 ± 0,64^{a}
22:6 n-3	11,26 ± 3,05	13,44 ± 4,13	11,82 ± 3,14	11,09 ± 1,92	12,82 ± 3,01	14,14 ± 1,65
∑PUFA	**37,29 ± 10,49**	**40,62 ± 11,35**	**40,94 ± 9,93**	**41,32 ± 7,33**	**36,39 ± 7,38**	**40,00 ± 6,83**
∑PUFA (n-6)	**4,92 ± 1,78**	**4,55 ± 1,73**	**5,67 ± 1,68**	**5,10 ± 1,21**	**4,89 ± 1,03**	**5,83 ± 1,66**
∑PUFA (n-3)	**31,53 ± 8,38**	**35,42 ± 9,51**	**34,49 ± 8,00**	**35,19 ± 5,87**	**30,89 ± 6,14**	**33,60 ± 5,08**
(n-3)/(n-6)	**6,41 ± 4,71^{ab}**	**7,78 ± 5,49^{a}**	**6,08 ± 4,77^{b}**	**6,90 ± 4,84^{ab}**	**6,31 ± 5,95^{ab}**	**5,76 ± 3,07^{b}**

Table 18. Fatty acids composition (% of total fatty acids) in the membrane lipids (PC/PE) of liver in male haddock (mean± std dev). Different letters indicate significant differences, p< 0.05.

	Egersund, H1 (n=14)	Southern North Sea, H2 (n=10)	Bressay Bank, H4 (n=14)	Viking Bank, H6 (n=6)	Tampen, H5 (n=12)	Tampen, H7 (n=6)
14:0	1,03 ± 0,30	1,05 ± 0,29	0,94 ± 0,21	1,00 ± 0,29	1,04 ± 0,24	1,12 ± 0,36
i-15:0	0,22 ± 0,07	0,25 ± 0,09	0,21 ± 0,08	0,25 ± 0,08	0,20 ± 0,10	0,19 ± 0,05
15:0	0,63 ± 0,13	0,68 ± 0,17	0,66 ± 0,16	0,69 ± 0,12	0,60 ± 0,07	0,60 ± 0,12
16:0	18,40 ± 2,14^{b}	20,76 ± 1,42^{a}	19,35 ± 2,09^{ab}	18,40 ± 1,66^{ab}	19,38 ± 1,06^{ab}	19,05 ± 2,23^{ab}
i-17:0	1,19 ± 0,38^{ab}	1,41 ± 0,24^{a}	1,22 ± 0,15^{ab}	1,28 ± 0,15^{ab}	1,01 ± 0,24^{b}	0,97 ± 0,13^{b}
ai-17:0	0,56 ± 0,25^{ab}	0,67 ± 0,26^{a}	0,48 ± 0,09^{ab}	0,61 ± 0,11^{ab}	0,43 ± 0,16^{ab}	0,38 ± 0,06^{b}
17:0	0,75 ± 0,15	0,68 ± 0,12	0,83 ± 0,12	0,87 ± 0,13	0,69 ± 0,15	0,81 ± 0,15
i-18:0	0,16 ± 0,05	0,13 ± 0,04	0,17 ± 0,04	0,17 ± 0,04	0,17 ± 0,04	0,20 ± 0,09
ai-18:0	0,07 ± 0,02	0,08 ± 0,03	0,08 ± 0,02	0,08 ± 0,02	0,07 ± 0,02	0,07 ± 0,01
18:0	2,41 ± 0,34^{ab}	2,33 ± 0,25^{ab}	2,68 ± 0,54^{a}	2,83 ± 0,33^{a}	2,12 ± 0,39^{b}	2,67 ± 0,56^{ab}
20:0	0,06 ± 0,02^{b}	0,06 ± 0,01^{b}	0,08 ± 0,03^{a}	0,07 ± 0,02^{ab}	0,05 ± 0,01^{b}	0,06 ± 0,02^{ab}
∑SFA	**25,47 ± 2,37^{b}**	**28,08 ± 1,21^{a}**	**26,70 ± 1,71^{ab}**	**26,27 ± 1,51^{ab}**	**25,77 ± 0,99^{b}**	**26,12 ± 1,79^{ab}**
16:1 n-11	0,09 ± 0,07	0,06 ± 0,04	0,06 ± 0,02	0,04 ± 0,01	0,08 ± 0,04	0,05 ± 0,01
16:1 n-9	0,34 ± 0,10	0,35 ± 0,13	0,33 ± 0,08	0,35 ± 0,11	0,34 ± 0,10	0,30 ± 0,05
16:1 n-7	1,51 ± 0,41	1,64 ± 0,25	1,60 ± 0,35	1,72 ± 0,33	1,48 ± 0,24	1,30 ± 0,38
16:1 n-5	0,36 ± 0,13	0,32 ± 0,11	0,33 ± 0,17	0,32 ± 0,06	0,32 ± 0,12	0,27 ± 0,06
17:1 n-x	0,39 ± 0,09	0,48 ± 0,11	0,44 ± 0,06	0,44 ± 0,06	0,47 ± 0,09	0,43 ± 0,07
18:1 n-11	0,39 ± 0,22	0,36 ± 0,15	0,54 ± 0,21	0,63 ± 0,21	0,36 ± 0,10	0,52 ± 0,27
18:1 n-9	6,97 ± 0,98^{ab}	8,49 ± 3,04^{a}	7,13 ± 1,30^{ab}	5,54 ± 0,59^{b}	8,09 ± 0,90^{a}	7,10 ± 0,87^{ab}
18:1 n-7	4,38 ± 1,07	3,81 ± 0,72	4,58 ± 0,53	4,69 ± 0,80	4,34 ± 0,67	3,87 ± 0,61
18:1 n-5	0,84 ± 0,30	0,80 ± 0,17	0,80 ± 0,17	0,76 ± 0,24	0,77 ± 0,17	0,62 ± 0,15
20:1 n-11	0,36 ± 0,09^{ab}	0,26 ± 0,07^{b}	0,35 ± 0,14^{ab}	0,36 ± 0,05^{ab}	0,31 ± 0,14^{ab}	0,46 ± 0,11^{a}
20:1 n-9	1,67 ± 0,93	1,17 ± 0,28	1,32 ± 0,43	0,89 ± 0,14	1,59 ± 0,61	1,15 ± 0,28
20:1 n-7	0,67 ± 0,25^{abc}	0,55 ± 0,11^{bcd}	0,77 ± 0,19^{ab}	0,85 ± 0,26^{a}	0,51 ± 0,17^{cd}	0,36 ± 0,15^{d}
22:1 n-11	0,33 ± 0,21	0,21 ± 0,05	0,19 ± 0,09	0,18 ± 0,06	0,24 ± 0,20	0,23 ± 0,09
22:1 n-9	0,15 ± 0,06^{a}	0,11 ± 0,04^{ab}	0,12 ± 0,03^{ab}	0,15 ± 0,04^{ab}	0,09 ± 0,03^{b}	0,13 ± 0,07^{ab}
22:1 n-7	0,27 ± 0,09^{b}	0,22 ± 0,08^{bc}	0,28 ± 0,05^{b}	0,40 ± 0,08^{a}	0,15 ± 0,08^{c}	0,20 ± 0,05^{bc}
24:1 n-9	3,00 ± 0,88	2,74 ± 0,73	2,66 ± 0,41	2,80 ± 0,63	2,66 ± 0,27	2,85 ± 0,48
∑MUFA	**21,71 ± 0,99**	**21,58 ± 3,20**	**21,50 ± 1,16**	**20,13 ± 2,02**	**21,80 ± 0,77**	**19,83 ± 1,15**
16:2 n-4	0,05 ± 0,02^{a}	0,04 ± 0,02^{ab}	0,04 ± 0,02^{ab}	0,05 ± 0,01^{ab}	0,03 ± 0,01^{b}	0,03 ± 0,01^{b}
18:2 n-4	0,17 ± 0,05	0,15 ± 0,01	0,18 ± 0,05	0,20 ± 0,04	0,15 ± 0,03	0,15 ± 0,02
18:4 n-1	0,04 ± 0,01	0,03 ± 0,01	0,03 ± 0,01	0,04 ± 0,01	0,03 ± 0,01	0,03 ± 0,01
18:2 n-6	0,53 ± 0,13^{ab}	0,65 ± 0,25^{a}	0,48 ± 0,07^{b}	0,38 ± 0,06^{b}	0,54 ± 0,07^{ab}	0,47 ± 0,07^{b}
20:2 n-6	0,56 ± 0,13	0,54 ± 0,18	0,62 ± 0,13	0,55 ± 0,08	0,62 ± 0,10	0,58 ± 0,14
20:3 n-6	0,11 ± 0,03^{abc}	0,08 ± 0,02^{c}	0,09 ± 0,01^{bc}	0,14 ± 0,04ab	0,12 ± 0,03^{ab}	0,10 ± 0,02^{abc}
20:4 n-6	3,11 ± 0,75^{c}	2,79 ± 0,79^{c}	4,50 ± 1,01^{a}	3,18 ± 0,74^{bc}	3,64 ± 0,56^{abc}	4,42 ± 1,00^{ab}
22:4 n-6	0,45 ± 0,21	0,36 ± 0,16	0,43 ± 0,12	0,53 ± 0,14	0,47 ± 0,12	0,45 ± 0,07
22:5 n-6	0,73 ± 0,19^{ab}	0,90 ± 0,28^{a}	0,83 ± 0,17^{ab}	0,65 ± 0,12^{ab}	0,65 ± 0,13^{b}	0,61 ± 0,09^{b}
18:3 n-3	0,15 ± 0,05	0,25 ± 0,20	0,16 ± 0,06	0,11 ± 0,02	0,20 ± 0,06	0,14 ± 0,05
18:4 n-3	0,23 ± 0,15^{ab}	0,28 ± 0,09^{a}	0,13 ± 0,03^{b}	0,13 ± 0,03^{b}	0,16 ± 0,08^{b}	0,12 ± 0,06^{b}
20:3 n-3	0,15 ± 0,09	0,20 ± 0,15	0,15 ± 0,05	0,10 ± 0,03	0,13 ± 0,02	0,15 ± 0,02
20:4 n-3	0,42 ± 0,10	0,40 ± 0,08	0,34 ± 0,06	0,35 ± 0,05	0,39 ± 0,07	0,40 ± 0,08
20:5 n-3	12,54 ± 1,58	12,82 ± 1,24	12,68 ± 1,36	14,07 ± 2,72	12,12 ± 1,13	12,91 ± 1,65
21:5 n-3	0,19 ± 0,06	0,19 ± 0,06	0,16 ± 0,03	0,21 ± 0,03	0,17 ± 0,03	0,16 ± 0,02
22:5 n-3	2,13 ± 0,39	1,30 ± 0,37	2,24 ± 1,39	2,31 ± 0,39	1,77 ± 0,35	1,78 ± 0,57
22:6 n-3	31,24 ± 4,11	29,34 ± 4,19	28,70 ± 2,20	30,56 ± 3,73	31,23 ± 1,72	31,52 ± 1,70
∑PUFA	**52,79 ± 2,69**	**50,32 ± 3,92**	**51,77 ± 2,70**	**53,56 ± 2,01**	**52,41 ± 1,35**	**54,02 ± 2,26**
∑ (n-6)	**5,48 ± 0,94^{b}**	**5,33 ± 1,00^{b}**	**6,95 ± 1,28^{b}**	**5,43 ± 0,88^{b}**	**6,03 ± 0,69^{ab}**	**6,63 ± 1,19^{b}**
∑ (n-3)	**47,05 ± 2,91**	**44,78 ± 4,28**	**44,57 ± 2,24**	**47,84 ± 2,47**	**46,17 ± 1,33**	**47,18 ± 1,89**
(n-3)/n-6)	**8,98 ± 2,61**	**8,75 ± 2,21**	**6,59 ± 1,10**	**9,02 ± 1,64**	**7,76 ± 1,01**	**7,31 ± 1,33**

Table 19. Fatty acids composition (% of total fatty acids) in the PS/PI of male haddock liver (mean± std dev). Different letters indicate significant changes, p< 0.05.

	Egersund, H1 (n=14)	Southern North Sea, H2 (n=10)	Bressay Bank, H4 (n=14)	Viking Bank, H6 (n=6)	Tampen, H5 (n=12)	Tampen, H7 (n=6)
14:0	0,63 ± 0,18^{a}	0,71 ± 0,24^{a}	0,53 ± 0,11^{ab}	0,67 ± 0,16^{a}	0,43 ± 0,16^{b}	0,46 ± 0,05^{ab}
i-15:0	0,15 ± 0,07^{a}	0,15 ± 0,05^{ab}	0,11 ± 0,03^{ab}	0,18 ± 0,07^{a}	0,09 ± 0,03^{b}	0,10 ± 0,02^{ab}
15:0	0,28 ± 0,07^{a}	0,32 ± 0,09^{a}	0,26 ± 0,06^{ab}	0,30 ± 0,04^{a}	0,18 ± 0,03^{c}	0,18 ± 0,02^{bc}
16:0	6,19 ± 1,54^{ab}	7,40 ± 1,95^{a}	6,00 ± 1,43^{ab}	6,04 ± 1,20^{ab}	5,35 ± 0,64^{b}	5,09 ± 1,25^{b}
i-17:0	0,77 ± 0,42	0,89 ± 0,17	0,71 ± 0,15	0,72 ± 0,15	0,67 ± 0,25	0,54 ± 0,15
ai-17:0	0,62 ± 0,41	0,69 ± 0,25	0,47 ± 0,17	0,51 ± 0,10	0,45 ± 0,25	0,35 ± 0,12
17:0	-	-	-	-	-	-
i-18:0	0,26 ± 0,13	0,28 ± 0,10	0,28 ± 0,08	0,18 ± 0,07	0,33 ± 0,13	0,26 ± 0,11
ai-18:0	0,09 ± 0,04^{ab}	0,10 ± 0,04^{a}	0,09 ± 0,02^{ab}	0,09 ± 0,01^{ab}	0,08 ± 0,02^{ab}	0,05 ± 0,02bc
18:0	11,25 ± 2,50	11,30 ± 1,43	11,69 ± 2,81	10,05 ± 3,08	11,38 ± 2,14	11,52 ± 2,56
20:0	0,12 ± 0,03^{b}	0,12 ± 0,03^{b}	0,19 ± 0,05^{a}	0,11 ± 0,02^{b}	0,14 ± 0,04^{b}	0,11 ± 0,03^{b}
∑SFA	**20,36 ± 3,81**	**21,97 ± 2,48**	**20,34 ± 3,02**	**18,85 ± 2,82**	**19,10 ± 2,43**	**18,68 ± 3,77**
16:1 n-11	0,14 ± 0,09^{a}	0,07 ± 0,05^{b}	0,10 ± 0,04^{ab}	0,06 ± 0,02^{b}	0,07 ± 0,03^{b}	0,04 ± 0,01^{b}
16:1 n-9	0,47 ± 0,13^{ab}	0,45 ± 0,18^{ab}	0,46 ± 0,12^{ab}	0,51 ± 0,13^{a}	0,31 ± 0,12^{ab}	0,28 ± 0,07^{b}
16:1 n-7	1,96 ± 0,68^{ab}	1,83 ± 0,32^{abc}	1,83 ± 0,36^{abc}	2,38 ± 0,77^{a}	1,36 ± 0,50^{bc}	1,16 ± 0,26^{c}
16:1 n-5	0,16 ± 0,05^{ab}	0,12 ± 0,03^{bc}	0,13 ± 0,04^{abc}	0,18 ± 0,02^{a}	0,10 ± 0,03^{c}	0,11 ± 0,03^{c}
17:1 n-x	0,37 ± 0,16	0,48 ± 0,13	0,39 ± 0,15	0,43 ± 0,06	0,36 ± 0,06	0,38 ± 0,10
18:1 n-11	0,28 ± 0,22	0,20 ± 0,12	0,23 ± 0,11	0,31 ± 0,12	0,18 ± 0,05	0,19 ± 0,10
18:1 n-9	5,14 ± 1,54^{b}	7,12 ± 2,68^{a}	5,45 ± 1,32^{ab}	3,87 ± 0,42^{b}	6,13 ± 1,00^{ab}	4,78 ± 1,30^{b}
18:1 n-7	4,57 ± 1,12	4,52 ± 0,82	4,59 ± 0,71	4,90 ± 0,61	4,19 ± 0,57	3,82 ± 0,64
18:1 n-5	0,70 ± 0,25	0,73 ± 0,12	0,67 ± 0,16	0,57 ± 0,15	0,73 ± 0,23	0,49 ± 0,16
20:1 n-11	0,54 ± 0,30	0,57 ± 0,32	0,60 ± 0,27	0,61 ± 0,23	0,57 ± 0,10	0,66 ± 0,46
20:1 n-9	2,09 ± 1,32	1,72 ± 0,43	1,73 ± 0,53	1,04 ± 0,11	1,95 ± 0,70	1,39 ± 0,37
20:1 n-7	0,69 ± 0,27^{ab}	0,63 ± 0,15^{ab}	0,87 ± 0,22^{a}	0,86 ± 0,26^{a}	0,64 ± 0,26^{ab}	0,40 ± 0,15^{b}
22:1 n-11	0,39 ± 0,20^{a}	0,36 ± 0,14^{ab}	0,31 ± 0,11^{ab}	0,16 ± 0,05^{b}	0,30 ± 0,21^{ab}	0,22 ± 0,10^{ab}
22:1 n-9	0,06 ± 0,03	0,07 ± 0,06	0,07 ± 0,02	0,06 ± 0,04	0,07 ± 0,10	0,03 ± 0,02
22:1 n-7	0,07 ± 0,04	0,03 ± 0,04	0,07 ± 0,05	0,10 ± 0,07	0,09 ± 0,05	0,07 ± 0,03
24:1 n-9	1,13 ± 0,74	1,27 ± 0,69	1,00 ± 0,53	0,65 ± 0,56	1,48 ± 0,72	0,98 ± 0,76
∑MUFA	18,78 ± 2,74^{ab}	20,16 ± 4,00^{a}	18,49 ± 2,30^{ab}	16,69 ± 1,87^{ab}	18,53 ± 1,65^{ab}	15,01 ± 3,24^{b}
16:2 n-4	0,06 ± 0,03	0,06 ± 0,02	0,05 ± 0,03	0,06 ± 0,05	0,03 ± 0,02	0,03 ± 0,01
18:2 n-4	0,15 ± 0,03^{bc}	0,15 ± 0,02^{bc}	0,16 ± 0,03^{b}	0,22 ± 0,05^{a}	0,12 ± 0,03^{c}	0,13 ± 0,01^{bc}
18:4 n-1	0,06 ± 0,03	0,05 ± 0,03	0,04 ± 0,02	0,07 ± 0,02	0,04 ± 0,02	0,03 ± 0,01
18:2 n-6	1,31 ± 0,35^{ab}	1,54 ± 0,51^{a}	1,24 ± 0,42^{ab}	1,04 ± 0,34^{ab}	0,96 ± 0,36^{b}	1,05 ± 0,57^{ab}
20:2 n-6	1,20 ± 0,25	1,18 ± 0,33	1,27 ± 0,22	1,33 ± 0,18	1,02 ± 0,29	1,13 ± 0,27
20:3 n-6	0,11 ± 0,04^{b}	0,09 ± 0,03^{b}	0,11 ± 0,02^{b}	0,18 ± 0,05^{a}	0,13 ± 0,04^{ab}	0,11 ± 0,02^{b}
20:4 n-6	7,33 ± 1,54^{b}	7,08 ± 1,58^{b}	8,48 ± 1,70^{ab}	6,53 ± 1,57^{b}	9,19 ± 1,57^{a}	9,61 ± 1,29^{a}
22:4 n-6	0,55 ± 0,20^{ab}	0,41 ± 0,18^{b}	0,63 ± 0,25^{ab}	0,84 ± 0,32^{a}	0,71 ± 0,24^{a}	0,66 ± 0,22^{ab}
22:5 n-6	1,91 ± 0,66^{ab}	2,45 ± 0,72^{ab}	2,58 ± 1,09^{a}	1,63 ± 0,52^{ab}	1,56 ± 0,32^{b}	1,86 ± 0,44^{ab}
18:3 n-3	0,23 ± 0,10^{ab}	0,36 ± 0,21^{a}	0,23 ± 0,08^{ab}	0,15 ± 0,03^{b}	0,24 ± 0,08^{ab}	0,15 ± 0,04^{b}
18:4 n-3	0,16 ± 0,06^{b}	0,29 ± 0,10^{a}	0,16 ± 0,06^{b}	0,10 ± 0,03^{b}	0,10 ± 0,03^{b}	0,07 ± 0,02^{b}
20:3 n-3	0,51 ± 0,33	0,62 ± 0,44	0,50 ± 0,18	0,40 ± 0,08	0,33 ± 0,11	0,49 ± 0,14
20:4 n-3	0,45 ± 0,08	0,48 ± 0,06	0,43 ± 0,07	0,47 ± 0,07	0,43 ± 0,10	0,49 ± 0,18
20:5 n-3	8,43 ± 1,79	8,54 ± 2,04	7,91 ± 1,74	9,30 ± 1,49	8,24 ± 1,30	7,46 ± 1,48
21:5 n-3	0,17 ± 0,05	0,14 ± 0,06	0,18 ± 0,07	0,21 ± 0,08	0,19 ± 0,04	0,16 ± 0,04
22:5 n-3	2,47 ± 0,49^{a}	1,52 ± 0,43^{b}	2,74 ± 1,27^{a}	3,07 ± 0,38^{a}	2,37 ± 0,47^{ab}	2,24 ± 0,54^{ab}
22:6 n-3	35,67 ± 6,88	32,81 ± 4,68	34,39 ± 4,97	38,81 ± 3,96	36,62 ± 4,43	40,57 ± 7,40
∑PUFA	**60,78 ± 6,08^{ab}**	**57,75 ± 5,56^{b}**	**61,10 ± 4,53^{ab}**	**64,39 ± 2,89^{ab}**	**62,28 ± 3,65^{ab}**	**66,25 ± 6,59^{a}**
∑ (n-6)	**12,41 ± 1,48^{ab}**	**12,74 ± 1,91^{ab}**	**14,32 ± 1,98^{a}**	**11,54 ± 1,00^{b}**	**13,57 ± 1,13^{ab}**	**14,42 ± 0,97^{a}**
∑ (n-3)	**48,09 ± 6,02^{ab}**	**44,75 ± 5,24^{b}**	**46,53 ± 3,90^{ab}**	**52,51 ± 3,29^{a}**	**48,51 ± 3,97^{ab}**	**51,64 ± 6,74^{ab}**
(n-3)/n-6)	**3,94 ± 0,76^{ab}**	**3,59 ± 0,71^{b}**	**3,31 ± 0,52^{b}**	**4,59 ± 0,64^{a}**	**3,61 ± 0,50^{b}**	**3,60 ± 0,59^{b}**

Table 20. Correlation analysis between liver lipid amount and the fatty acids profile in storages lipid (NL) and the membrane lipids (PC/PE and PS/PI). Given as Pearson correlation and Coefficients of determination (R^2). Significant correlations are marked in bold. Samples from all samplings points are analyzed together (n=62).

	Correlation (Pearson)			Coefficients of determination (R^2)		
Variables	NL	PC/PE	PS/PI	NL	PC/PE	PS/PI
14:0	**-0,307**	-0,234	0,138	**0,094**	0,055	0,019
i-15:0	-0,238	0,030	0,080	0,057	0,001	0,006
15:0	**-0,366**	-0,089	0,243	**0,134**	0,008	0,059
16:0	**0,522**	**0,385**	**0,416**	**0,272**	**0,148**	**0,173**
i-17:0	0,040	**0,356**	**0,446**	0,002	**0,126**	**0,199**
ai-17:0	0,204	**0,379**	**0,454**	0,042	**0,144**	**0,206**
17:0	**-0,326**	-0,173		**0,106**	0,030	
i-18:0	0,073	0,005	0,215	0,005	0,000	0,046
ai-18:0	0,096	**0,252**	**0,354**	0,009	**0,063**	**0,125**
18:0	**-0,283**	-0,185	0,048	**0,080**	0,034	0,002
20:0	-0,160	-0,033	0,241	0,026	0,001	0,058
∑SFA	0,157	**0,396**	**0,344**	0,025	**0,157**	**0,119**
16:1 n-11	0,211	**-0,288**	**-0,266**	0,044	**0,083**	**0,071**
16:1 n-9	-0,085	-0,166	0,070	0,007	0,027	0,005
16:1 n-7	-0,031	0,028	-0,006	0,001	0,001	0,000
16:1 n-5	0,042	0,113	0,042	0,002	0,013	0,002
17:1 n-x	**0,528**	**0,277**	**0,305**	**0,278**	**0,077**	**0,093**
18:1 n-11	-0,036	**0,295**	0,158	0,001	**0,087**	0,025
18:1 n-9	**0,579**	0,207	**0,384**	**0,335**	0,043	**0,147**
18:1 n-7	-0,092	0,066	**0,290**	0,009	0,004	**0,084**
18:1 n-5	**0,418**	**0,264**	**0,306**	**0,175**	**0,070**	**0,093**
20:1 n-11	0,085	0,040	**0,394**	0,007	0,002	**0,155**
20:1 n-9	-0,122	-0,223	-0,085	0,015	0,050	0,007
20:1 n-7	-0,137	0,084	0,247	0,019	0,007	0,061
22:1 n-11	-0,087	**-0,251**	0,071	0,008	**0,063**	0,005
22:1 n-9	**-0,395**	**-0,405**	0,073	**0,156**	**0,164**	0,005
22:1 n-7	**-0,286**	-0,145	0,029	**0,082**	0,021	0,001
24:1 n-9	**-0,428**	-0,162	0,104	**0,183**	0,026	0,011
∑MUFA	**0,322**	0,148	**0,415**	**0,104**	0,022	**0,172**
16:2 n-4	-0,161	0,042	-0,077	0,026	0,002	0,006
18:2 n-4	0,070	0,139	**0,297**	0,005	0,019	**0,088**
18:4 n-1	-0,029	-0,044	0,106	0,001	0,002	0,011
18:2 n-6	-0,033	-0,136	-0,028	0,001	0,018	0,001
20:2 n-6	**-0,376**	**-0,392**	-0,136	**0,141**	**0,154**	0,019
20:3 n-6	-0,051	0,040	0,117	0,003	0,002	0,014
20:4 n-6	**-0,294**	-0,096	-0,083	**0,087**	0,009	0,007
22:4 n-6	**-0,645**	**0,274**	0,184	**0,416**	**0,075**	0,034
22:5 n-6	**-0,262**	0,157	-0,013	**0,069**	0,025	0,000
18:3 n-3	0,137	0,199	0,178	0,019	0,040	0,032
18:4 n-3	0,072	-0,156	**0,307**	0,005	0,024	**0,094**
20:3 n-3	-0,246	**-0,323**	**-0,301**	0,061	**0,104**	**0,091**
20:4 n-3	**-0,414**	-0,250	-0,002	**0,172**	0,062	0,000
20:5 n-3	-0,095	0,141	0,216	0,009	0,020	0,046
21:5 n-3	-0,041	**0,275**	0,226	0,002	**0,076**	0,051
22:5 n-3	**-0,275**	0,121	0,083	**0,076**	0,015	0,007
22:6 n-3	**-0,251**	**-0,368**	**-0,450**	**0,063**	**0,136**	**0,203**
∑PUFA	**-0,325**	**-0,357**	**-0,431**	**0,105**	**0,128**	**0,186**
∑PUFA (n-6)	**-0,524**	-0,081	-0,090	**0,274**	0,007	0,008
∑PUFA (n-3)	-0,239	**-0,316**	**-0,411**	0,057	**0,100**	**0,169**
(n-3)/(n-6)	**0,255**	-0,064	-0,164	**0,065**	0,004	0,027

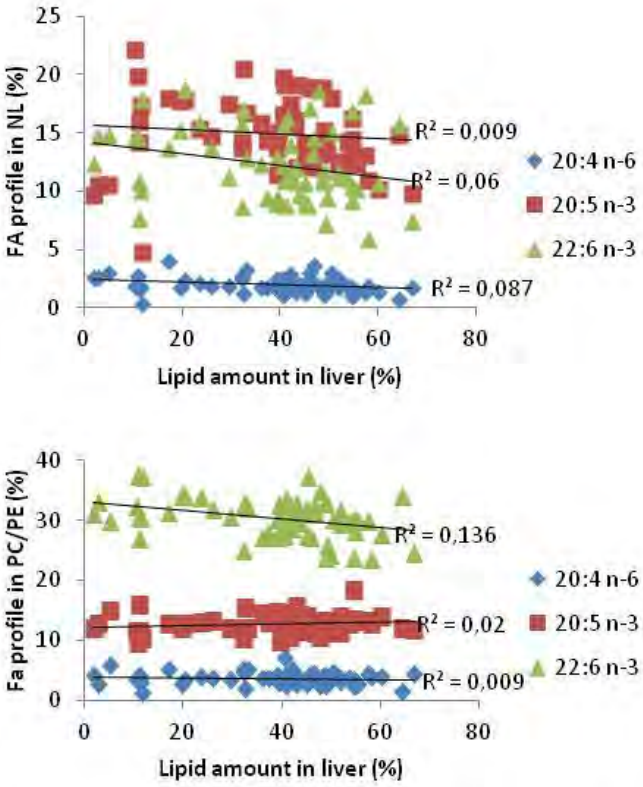


Figure 16. Correlation between lipid amount in haddock liver and fatty acids profile 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3) in neutral lipid (NL) and membrane lipid (PC/PE).

Table 21. Fatty acids composition (% of total fatty acids) in male haddock muscle. Different letters indicate significant changes, p< 0.05.

	Egersund, H1 (n=14)	Southern North Sea, H2 (n=9)	Bressay Bank, H4 (n=13)	Viking Bank, H6 (n=6)	Tampen, H5 (n=12)	Tampen, H7 (n=6)
14:0	1,13 ± 0,34^{b}	1,85 ± 0,46^{a}	1,00 ± 0,13^{b}	0,99 ± 0,21^{b}	0,98 ± 0,16^{b}	0,89 ± 0,13^{b}
Iso 15:0	0,14 ± 0,06^{ab}	0,18 ± 0,05^{a}	0,10 ± 0,02^{b}	0,13 ± 0,03^{ab}	0,11 ± 0,04^{b}	0,09 ± 0,02^{b}
15:0	0,53 ± 0,10^{b}	0,64 ± 0,09^{a}	0,53 ± 0,05^{b}	0,55 ± 0,09^{ab}	0,48 ± 0,06^{b}	0,43 ± 0,03^{b}
16:0	17,41 ± 0,67^{ab}	16,38 ± 1,28^{b}	16,70 ± 1,15^{ab}	17,20 ± 1,23^{ab}	17,68 ± 0,73^{a}	17,46 ± 0,83^{ab}
Iso 17:0	0,69 ± 0,27	0,78 ± 0,20	0,62 ± 0,08	0,72 ± 0,18	0,62 ± 0,20	0,58 ± 0,06
Antiso 17:0	0,26 ± 0,13	0,27 ± 0,11	0,18 ± 0,03	0,25 ± 0,06	0,22 ± 0,09	0,19 ± 0,03
17:0	0,57 ± 0,13	0,51 ± 0,15	0,55 ± 0,09	0,66 ± 0,12	0,61 ± 0,09	0,64 ± 0,07
18:0	5,11 ± 0,23^{a}	4,53 ± 0,77^{b}	5,47 ± 0,41^{a}	5,55 ± 0,56^{a}	5,05 ± 0,34^{ab}	5,59 ± 0,18^{a}
24:0	0,14 ± 0,03^{ab}	0,15 ± 0,02^{a}	0,12 ± 0,01^{b}	0,13 ± 0,02^{ab}	0,12 ± 0,02^{b}	0,12 ± 0,03^{ab}
∑SFA	**25,98 ± 0,66**	**25,29 ± 2,14**	**25,26 ± 0,96**	**26,19 ± 0,99**	**25,86 ± 0,54**	**25,98 ± 0,82**
16:1 (n-11)	0,05 ± 0,02^{ab}	0,06 ± 0,02^{a}	0,05 ± 0,02^{ab}	0,03 ± 0,01^{b}	0,05 ± 0,02^{ab}	0,04 ± 0,01^{ab}
16:1 (n-9)	0,34 ± 0,05^{b}	0,51 ± 0,11^{a}	0,30 ± 0,06^{b}	0,33 ± 0,05^{b}	0,32 ± 0,05^{b}	0,27 ± 0,03^{b}
16:1 (n-7)	1,79 ± 0,71^{b}	3,00 ± 1,09^{a}	1,70 ± 0,20^{b}	1,67 ± 0,28^{b}	1,41 ± 0,23^{b}	1,08 ± 0,15^{b}
16:1 (n-5)	0,39 ± 0,09^{b}	0,51 ± 0,15^{a}	0,30 ± 0,06^{b}	0,40 ± 0,05^{ab}	0,33 ± 0,09^{b}	0,31 ± 0,06^{b}
17:1 (n-9)	0,39 ± 0,08^{ab}	0,47 ± 0,08^{a}	0,38 ± 0,05^{b}	0,42 ± 0,04^{ab}	0,42 ± 0,06^{ab}	0,39 ± 0,02^{ab}
18:1 (n-11)	0,45 ± 0,17	0,40 ± 0,18	0,39 ± 0,19	0,23 ± 0,04	0,46 ± 0,17	0,37 ± 0,08
18:1 (n-9)	6,05 ± 1,02^{ab}	5,67 ± 1,48^{ab}	6,26 ± 0,91^{ab}	5,30 ± 0,59^{b}	6,86 ± 1,01^{a}	6,12 ± 0,24^{ab}
18:1 (n-7)	3,52 ± 0,81	2,80 ± 0,61	3,45 ± 0,54	3,72 ± 0,41	3,33 ± 0,49	3,04 ± 0,33
18:1 (n-5)	0,38 ± 0,11	0,32 ± 0,11	0,30 ± 0,05	0,37 ± 0,07	0,35 ± 0,07	0,28 ± 0,03
20:1 (n-11)	0,53 ± 0,19	0,34 ± 0,25	0,46 ± 0,21	0,53 ± 0,11	0,50 ± 0,07	0,57 ± 0,17
20:1 (n-9)	1,00 ± 0,46	0,77 ± 0,29	0,79 ± 0,24	0,55 ± 0,09	0,83 ± 0,30	0,65 ± 0,09
20:1 (n-7)	0,29 ± 0,11^{a}	0,22 ± 0,07^{ab}	0,28 ± 0,08^{a}	0,36 ± 0,12^{a}	0,22 ± 0,09^{ab}	0,13 ± 0,05^{b}
22:1 (n-11)	0,36 ± 0,12^{ab}	0,54 ± 0,31^{a}	0,28 ± 0,10^{b}	0,21 ± 0,06^{b}	0,33 ± 0,14^{b}	0,30 ± 0,06^{b}
22:1 (n-9)	0,14 ± 0,03^{ab}	0,14 ± 0,03^{ab}	0,16 ± 0,03^{a}	0,11 ± 0,02^{b}	0,12 ± 0,02^{b}	0,10 ± 0,02^{b}
22:1 (n-7)	0,13 ± 0,04^{ab}	0,07 ± 0,08^{c}	0,15 ± 0,03^{ab}	0,18 ± 0,03^{ab}	0,13 ± 0,03^{ab}	0,10 ± 0,03^{bc}
24:1 (n-9)	1,31 ± 0,36^{a}	0,69 ± 0,55^{b}	1,31 ± 0,16^{a}	1,21 ± 0,25^{b}	1,27 ± 0,15^{b}	1,26 ± 0,24^{b}
∑MUFA	**17,14 ± 2,20**	**16,51 ± 2,93**	**16,57 ± 1,55**	**15,64 ± 1,46**	**16,90 ± 1,68**	**15,01 ± 0,89**
16:2 (n-4)	0,10 ± 0,06^{ab}	0,13 ± 0,05^{a}	0,07 ± 0,02^{bc}	0,09 ± 0,02^{abc}	0,06 ± 0,02^{bc}	0,04 ± 0,01^{c}
18:2 (n-4)	0,14 ± 0,05	0,11 ± 0,05	0,13 ± 0,02	0,15 ± 0,02	0,13 ± 0,02	0,13 ± 0,01
18:4 (n-1)	0,07 ± 0,05	0,06 ± 0,03	0,06 ± 0,02	0,08 ± 0,03	0,06 ± 0,02	0,05 ± 0,01
18:2 (n-6)	0,71 ± 0,12^{b}	0,96 ± 0,21^{a}	0,70 ± 0,14^{b}	0,60 ± 0,07^{b}	0,66 ± 0,07^{b}	0,62 ± 0,07^{b}
20:2 (n-6)	0,35 ± 0,07	0,31 ± 0,10	0,37 ± 0,07	0,38 ± 0,06	0,38 ± 0,07	0,34 ± 0,05
20:4 (n-6)	4,28 ± 0,67^{b}	3,86 ± 0,69^{b}	5,50 ± 1,24^{a}	4,49 ± 0,91^{ab}	4,84 ± 0,64^{ab}	5,34 ± 1,68^{ab}
22:4 (n-6)	0,56 ± 0,20^{ab}	0,42 ± 0,19^{b}	0,57 ± 0,12^{ab}	0,76 ± 0,08^{a}	0,63 ± 0,18^{a}	0,64 ± 0,11^{a}
22:5 (n-6)	0,85 ± 0,16^{b}	0,84 ± 0,14^{b}	1,08 ± 0,31^{a}	0,85 ± 0,05^{b}	0,85 ± 0,09^{b}	0,95 ± 0,12^{b}
18:3 (n-3)	0,26 ± 0,09^{b}	0,92 ± 0,56^{a}	0,32 ± 0,12^{b}	0,18 ± 0,04^{b}	0,20 ± 0,03^{b}	0,18 ± 0,02^{b}
18:4 (n-3)	0,48 ± 0,20^{b}	1,34 ± 0,95^{a}	0,43 ± 0,12^{b}	0,32 ± 0,10^{b}	0,32 ± 0,07^{b}	0,26 ± 0,04^{b}
20:3 (n-3)	0,12 ± 0,03^{bc}	0,17 ± 0,02^{a}	0,15 ± 0,06^{ab}	0,09 ± 0,03^{c}	0,10 ± 0,01^{c}	0,11 ± 0,02^{bc}
20:4 (n-3)	0,39 ± 0,06^{ab}	0,46 ± 0,06^{a}	0,39 ± 0,07^{ab}	0,37 ± 0,03^{b}	0,38 ± 0,07^{b}	0,39 ± 0,08^{b}
20:5 (n-3)	16,65 ± 2,23^{bc}	19,62 ± 3,78^{a}	15,16 ± 1,73^{bc}	17,41 ± 1,33^{ab}	14,63 ± 1,62^{c}	13,58 ± 1,46^{c}
21:5 (n-3)	0,24 ± 0,09	0,22 ± 0,04	0,22 ± 0,05	0,26 ± 0,04	0,21 ± 0,04	0,19 ± 0,02
22:5 (n-3)	2,48 ± 0,66	1,62 ± 0,45	2,73 ± 1,51	2,76 ± 0,22	2,31 ± 0,31	2,20 ± 0,19
22:6 (n-3)	30,34 ± 4,31^{b}	29,01 ± 3,30^{b}	31,28 ± 2,31^{ab}	30,38 ± 1,29^{ab}	32,48 ± 2,81^{ab}	34,90 ± 2,74^{a}
∑PUFA	**58,02 ± 2,28**	**60,06 ± 4,31**	**59,16 ± 1,44**	**59,17 ± 1,62**	**58,22 ± 1,63**	**59,90 ± 0,50**
∑n-6	**6,75 ± 0,67^{b}**	**6,39 ± 0,77^{b}**	**8,22 ± 1,38^{a}**	**7,08 ± 0,92^{ab}**	**7,36 ± 0,88^{ab}**	**7,89 ± 1,84^{ab}**
∑n-3	**50,95 ± 2,13**	**53,37 ± 4,75**	**50,68 ± 1,50**	**51,77 ± 1,50**	**50,62 ± 2,06**	**51,80 ± 2,11**
(n-3)/(N-6)	**7,61 ± 0,70^{ab}**	**8,49 ± 1,43^{a}**	**6,32 ± 1,02^{c}**	**7,42 ± 1,01^{abc}**	**6,99 ± 1,02^{bc}**	**6,87 ± 1,58^{bc}**

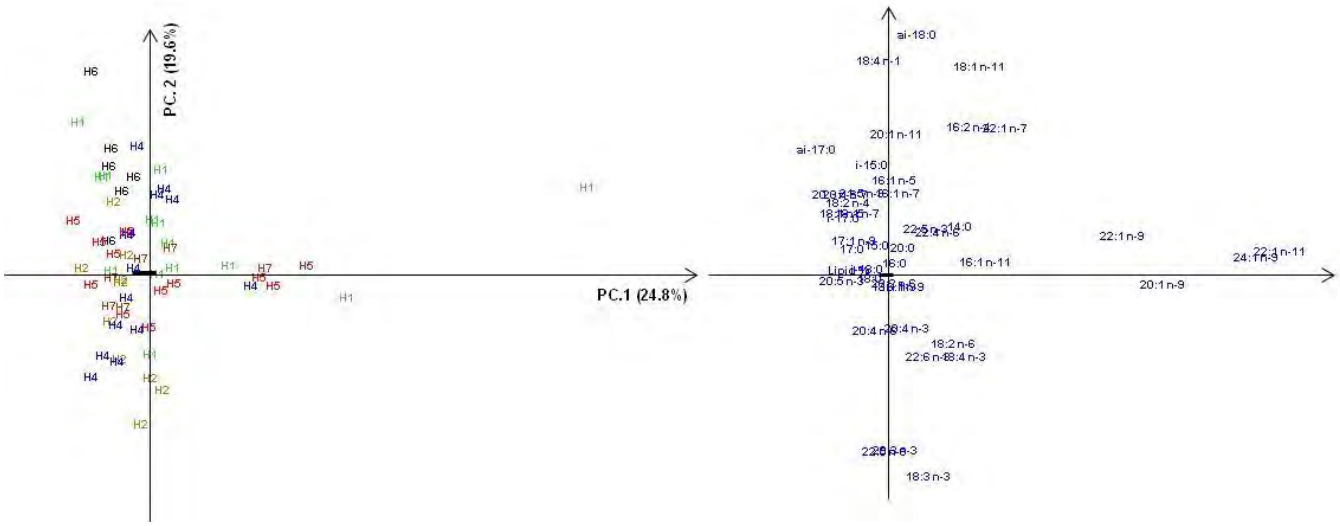


Figure 17. PCA of fatty acid profiles in the neutral lipids of haddock liver. The first plot shows the score values (objects) and the second plot shows the related loading plot with the variable. The model using 2 principal components (PC) explain 44 % of the total variances in the dataset.

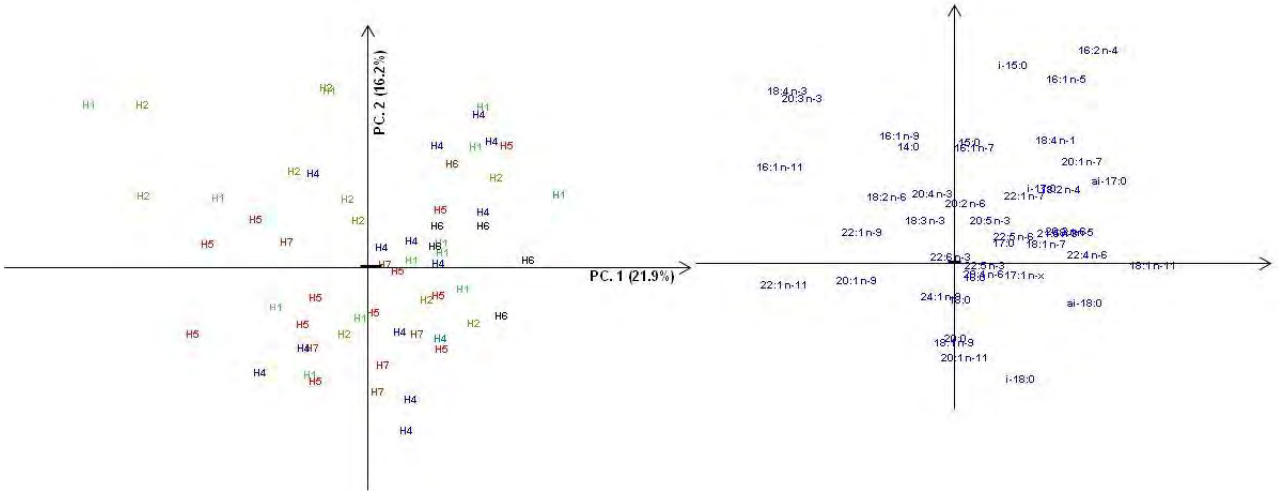


Figure 18. PCA plots of FA profile in PC/PE in Haddock liver. Left: Loading plot, right: Score plot. The model explains 38 % of the total variance in the dataset.

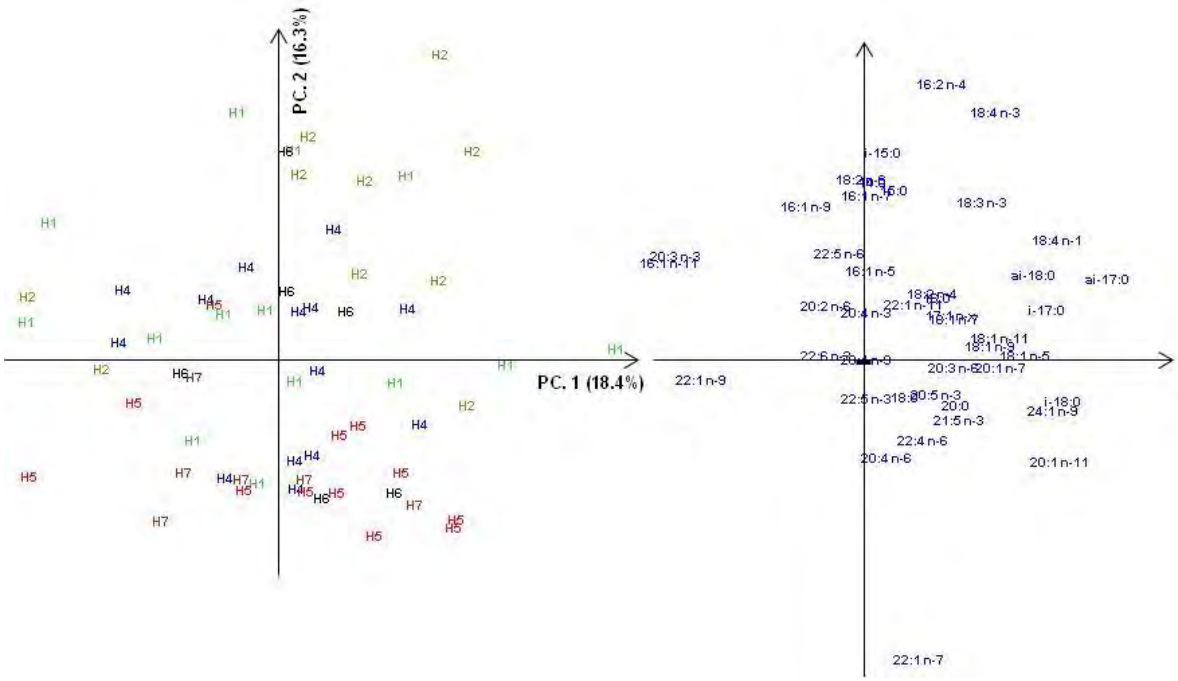


Figure 19. PCA plots of FA profile in PS/PI in Haddock liver. Left: Loading plot, right: Score plot. The model explains 35 % of the total variance in the dataset.

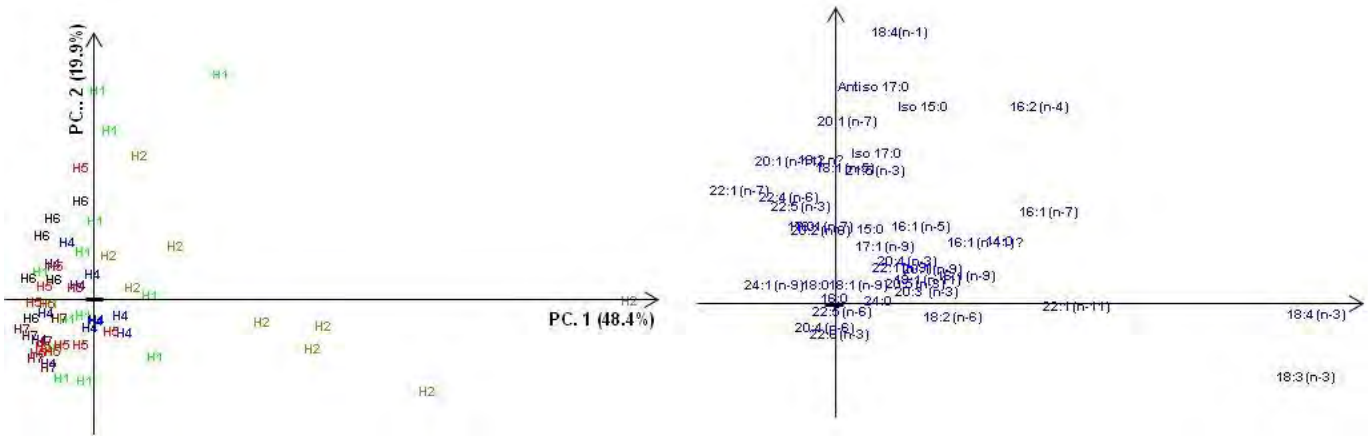


Figure 20. PCA plots of FA profile in total lipid of haddock muscle. Left: Loading plot, right: Score plot. The model explains 68 % of the total variance in the dataset.

5.8.2 Lipid analysis in cod

Lipid analysis were performed of liver (lipid classes; NL, PC/PE and PS/PI) and muscle (total lipid) samples of Altantic cod from Egersund Bank and the Tampen area (Station 5 and 7 are analyzed together). The results of female and male cod were analysed separately.

The lipid analysis of cod shows the same trends as for the haddock: there were no significant differences in the LSI, liver lipid content or fatty acids profiles in the liver lipids (NL, PC/PE and PS/PI). Slightly higher levels of lipid in the muscle of female cod at Egersund Bank were found compared with Tampen.

As for haddock, cod also had large variation in the LSI (0.7-5.3 %) and the lipid content in the liver (4-74%), and there were high correlation between the liver size and lipid content (Figure 21).

PCA shows no group differences in any of the lipid classes of cod liver (Figure 24, 25 and 26). However, lean fish had a different fatty acids profile compared with fat fish. This is also seen in the correlation analysis (Table 24 and Figure 22), as a significant negative correlation were found between liver lipid content and the relative amount of (n-6) PUFA, and a positive correlation observed for (n-3) PUFA, both in the neutral lipid and the membrane lipids. This resulted in a significant positive linear correlation between liver lipid content and the (n-3)/(n-6) ratio (Figure 23). For the neutral lipid (NL), lean fish (liver lipid < 20 %) had (n-3)/(n-6) ratio of 4.6±1.8. For fat fish (liver lipid >20 %), the (n-3)/(n-6) ratio were 10.2±2.6, more than 2 times higher than lean fish. This shows that for cod the energy status is highly important for the fatty acid profile. An explanation to this may be that the fish are constantly mobilizing (n-3) PUFA from the NL to maintain the optimal membrane composition in the body, but when the lipid storages becomes low, the amount of (n-3) PUFA will drop and change the relative ratio between (n-3)/(n-6) PUFA.

The muscle samples show different fatty acids composition between Tampen and Egersund Bank (Figure 27 and Table 28). The loading plot from the PCA shows that the fish at Egersund Bank have relative higher levels of 22:1 (n-11) and other long chain mono unsaturated fatty acid (MUFA) and also contain short chain PUFA, like 18:4 (n-3). This may be explained by differences in the diet. The 22:1 (n-11) and 18:4 (n-3) is typical fatty acids biomarkers for a diet with dominated by *Calanus finmarchicus* or other animal that is eating *Calanus finmarchicus* (Dalsgaard et al. 2003). The zooplankton samples taken at Egersund Bank does also contain high levels of especially 22:1 (n-11) and that support that there are high levels of *Calanus finmarchicus* in this area (Table 30).

The results of the lipid analyses of cod show that one should be careful by using such a general parameter as fatty acids composition as a biomarker for pollution. We need better understanding of the natural regulation of the lipid homeostasis in wild fish and more experimental studies of how oil pollution effect the lipid metabolism, before it will be

possible to conclude if differences in lipid composition between Tampen and other areas (as reported in 2002, 2008 and 2010) can be correlated to discharges from oil and gas activities.

Table 22. Lipid content in cod liver and amount of fatty acids (FA) in muscle of cod.

	(n:L/M)	Liver lipid (%)	Muscle FA (%)
Egersund, female	11/19	42±15	0.55±0.01[a]
Egersund, male	10/14	29±17	0.50±0.07[ab]
Tampen, female	12/14	30±15	0.46±0.06[b]
Tampen, Maile	10/15	33±21	0.46±0.05[b]

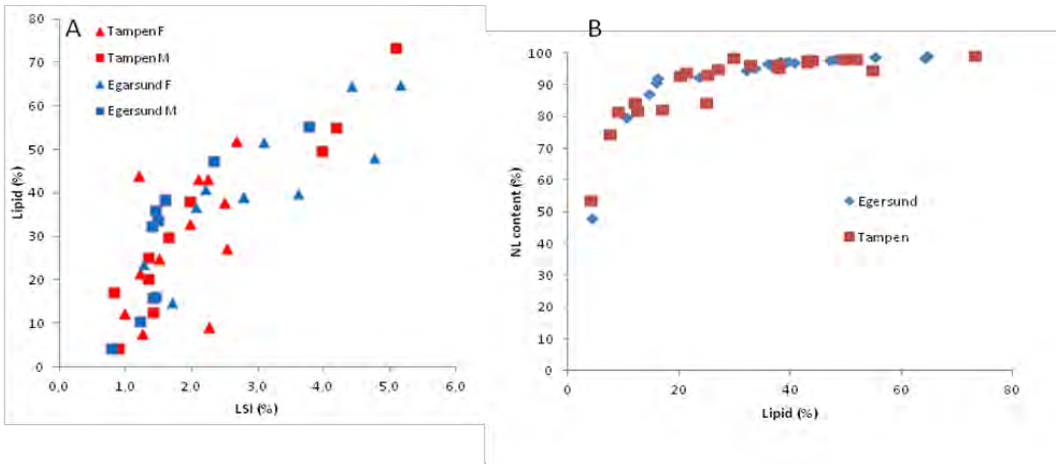


Figure 21. Correlation between LSI and total lipid content (A) and between lipid content and amount of neutral lipid (B) in the liver of cod.

Table 23. lipid classes distributed in sample (wt % of total lipids, mean values ±SD).

Lean fish Lipid content		Egersund (n=5)	Tampen (n=6)
<20%	NL	94.4±4.9	81.5±3.7
	FFA	1.2±1.4	1.5±0.3
	PC/PE	3.9±4.7	15.5±3.6
	PS/PI	0.4±0.4	1.4±0.4
fat fish Lipid content		Egersund (n=16)	Tampen (n=16)
>20%	NL	95.3±4.8	96.4±2.1
	FFA	0.5±0.7	0.3±0.2
	PC/PE	3.8±3.6	3.0±1.8
	PS/PI	0.5±0.6	0.3±0.2

Table 24. Correlation analysis between liver lipid amount and the fatty acids profile in stoages lipid (NL) and the membrane lipids (PC/PE and PS/PI) in cod. Giving as Pearson correlation and Coefficients of determination (R^2). Significant correlations are marked in bold. Samples from all samplings points are analyzed together (n=43).

	Correlation (Pearson)			Coefficients of determination (R^2)		
Variables	NL	PC/PE	PS/PI	NL	PC/PE	PS/PI
14:0	-0,141	0,088	0,229	0,020	0,008	0,052
i-15:0	**-0,521**	**-0,410**	-0,086	**0,272**	**0,168**	0,007
15:0	**-0,519**	**-0,457**	0,073	**0,270**	**0,209**	0,005
16:0	-0,143	**0,434**	**0,326**	0,020	**0,189**	**0,106**
i-17:0	**-0,522**	**-0,397**	**-0,330**	**0,273**	**0,158**	**0,109**
17:0	**-0,668**	**-0,529**	0,057	**0,446**	**0,280**	0,003
ai-18:0	**0,638**	0,100	0,010	**0,407**	0,010	0,000
18:0	-0,168	-0,067	**0,380**	0,028	0,004	**0,144**
ΣSFA	**-0,325**	**0,346**	**0,513**	**0,106**	**0,119**	**0,263**
16:1 n-11	**0,478**	**0,455**	**0,440**	**0,228**	**0,207**	**0,194**
16:1 n-9	**-0,427**	-0,173	-0,094	**0,182**	0,030	0,009
16:1 n-7	**0,325**	0,144	0,299	**0,105**	0,021	0,089
16:1 n-5	0,174	0,194	-0,046	0,030	0,038	0,002
17:1 n-x	-0,283	**-0,404**	-0,081	0,080	**0,163**	0,006
18:1 n-11	0,217	0,141	**0,343**	0,047	0,020	**0,117**
18:1 n-9	0,071	-0,295	0,016	0,005	0,087	0,000
18:1 n-7	-0,214	**-0,508**	-0,219	0,046	**0,258**	0,048
18:1 n-5	**0,314**	0,225	0,162	**0,099**	0,051	0,026
20:1 n-11	-0,188	0,266	0,282	0,035	0,071	0,080
20:1 n-9	**0,446**	0,009	0,273	**0,199**	0,000	0,075
20:1 n-7	**-0,420**	**-0,334**	-0,148	**0,176**	**0,112**	0,022
22:1 n-11	-0,156	**-0,389**	**0,349**	0,024	**0,152**	**0,122**
22:1 n-9	-0,260	-0,125	**0,354**	0,068	0,016	**0,126**
24:1 n-9	**-0,674**	0,125	0,049	**0,455**	0,016	0,002
ΣMUFA	0,045	-0,031	0,117	0,002	0,001	0,014
16:2 n-4	**0,472**	0,270	**0,507**	**0,223**	0,073	**0,257**
18:2 n-4	**0,666**	**0,443**	**0,505**	**0,443**	**0,196**	**0,255**
18:4 n-1	**0,610**	**0,490**	0,263	**0,372**	**0,240**	0,069
18:2 n-6	0,211	-0,092	-0,034	0,045	0,009	0,001
20:2 n-6	**-0,522**	**-0,527**	**-0,541**	**0,272**	**0,277**	**0,293**
20:4 n-6	**-0,552**	**-0,556**	**-0,318**	**0,304**	**0,310**	**0,101**
22:4 n-6	**-0,649**	**-0,464**	**-0,548**	**0,421**	**0,216**	**0,300**
22:5 n-6	**-0,532**	-0,270	0,030	**0,283**	0,073	0,001
18:3 n-3	**0,372**	0,111	0,101	**0,138**	0,012	0,010
18:4 n-3	**0,648**	**0,349**	**0,438**	**0,420**	**0,122**	**0,192**
20:3 n-3	**-0,418**	**-0,551**	**-0,524**	**0,174**	**0,304**	**0,274**
20:4 n-3	0,029	0,203	0,177	0,001	0,041	0,031
20:5 n-3	**0,375**	0,241	0,136	**0,141**	0,058	0,018
21:5 n-3	**0,722**	**0,427**	0,058	**0,521**	**0,182**	0,003
22:5 n-3	-0,071	**-0,458**	-0,085	0,005	**0,210**	0,007
22:6 n-3	-0,039	0,205	**-0,418**	0,002	0,042	**0,175**
ΣPUFA	-0,007	-0,008	**-0,520**	0,000	0,000	**0,271**
ΣPUFA (n-6)	**-0,686**	**-0,598**	**-0,358**	**0,471**	**0,358**	**0,128**
ΣPUFA (n-3)	**0,419**	**0,455**	**-0,384**	**0,175**	**0,207**	**0,148**
n-3/n-6	**0,828**	**0,643**	0,089	**0,686**	**0,414**	0,008

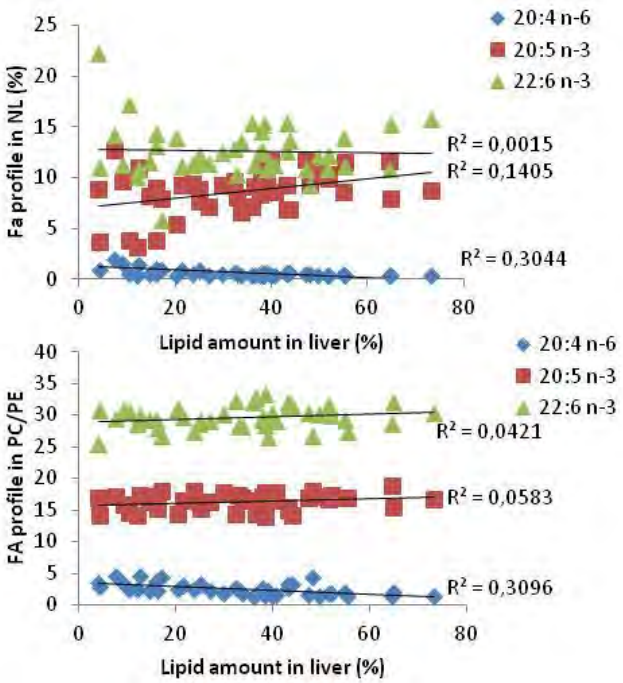


Figure 22. Correlation between lipid amount in cod liver and fatty acids profile 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3) in neutral lipid (NL) and membrane lipid (PC/PE).

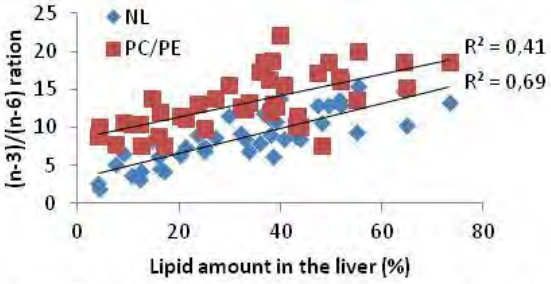


Figure 23. Correlation between lipid amount in cod liver and (n-3)/(n-6) ratio) in neutral lipid (NL) and membrane lipid (PC/PE).

Table 25. Fatty acids composition (% of total fatty acids) in the neutral lipids (NL) of cod liver (mean± std dev). Different letters indicate significant changes, p< 0.05.

	Egersund, Female NL (n=11)	Egersund, male NL (n=10)	Tampen, Female NL (n=12)	Tampen, Male NL (n=10)
14:0	5,14 ± 0,76	5,32 ± 0,93	5,07 ± 1,11	4,76 ± 1,19
i-15:0	0,18 ± 0.03[b]	0,27 ± 0.09[a]	0,23 ± 0.07[a,b]	0,22 ± 0.07[a,b]
15:0	0,34 ± 0,06	0,43 ± 0,08	0,44 ± 0,13	0,42 ± 0,12
16:0	11,66 ± 0,74	11,47 ± 1,69	12,55 ± 1,65	12,47 ± 1,32
i-17:0	0,27 ± 0,05	0,40 ± 0,13	0,39 ± 0,14	0,39 ± 0,14
17:0	0,22 ± 0,05	0,30 ± 0,17	0,35 ± 0,19	0,34 ± 0,12
ai-18:0	0,54 ± 0.11[a]	0,35 ± 0.20[b]	0,34 ± 0.18[b]	0,33 ± 0.144[b]
18:0	2,32 ± 0,37	2,22 ± 0,67	2,79 ± 0,95	3,01 ± 0,66
ΣSFA	**20,67 ± 1,16**	**20,75 ± 1,92**	**22,15 ± 1,99**	**21,94 ± 2,12**
16:1 n-11	0,16 ± 0.04[a]	0,15 ± 0.05[a,b]	0,11 ± 0.04[b]	0,13 ± 0.03[a,b]
16:1 n-9	0,25 ± 0,08	0,31 ± 0,08	0,30 ± 0,13	0,32 ± 0,08
16:1 n-7	6,56 ± 0,34	5,97 ± 1,47	6,02 ± 0,64	5,73 ± 1,31
16:1 n-5	0,18 ± 0.02[a,b]	0,21 ± 0.05[a]	0,16 ± 0.03[b]	0,16 ± 0.02[b]
17:1 n-x	0,29 ± 0,07	0,32 ± 0,07	0,39 ± 0,14	0,40 ± 0,13
18:1 n-11	2,70 ± 0,48	2,43 ± 0,66	2,38 ± 0,51	2,15 ± 0,88
18:1 n-9	8,70 ± 2,04	7,79 ± 1,74	9,99 ± 3,32	9,12 ± 3,03
18:1 n-7	2,97 ± 0,24	2,84 ± 0,59	3,35 ± 1,10	3,36 ± 0,68
18:1 n-5	0,24 ± 0,04	0,22 ± 0,09	0,21 ± 0,06	0,22 ± 0,04
20:1 n-11	2,28 ± 0,35	2,47 ± 0,67	2,69 ± 0,44	2,69 ± 0,83
20:1 n-9	12,08 ± 1.15[a]	10,68 ± 1.43[a,b]	9,04 ± 2.60[b]	8,64 ± 2.23[b]
20:1 n-7	0,24 ± 0,04	0,26 ± 0,10	0,23 ± 0,10	0,23 ± 0,09
22:1 n-11	10,42 ± 1,02	11,58 ± 3,66	11,16 ± 3,73	10,34 ± 2,13
22:1 n-9	0,71 ± 0,06	0,75 ± 0,13	0,66 ± 0,18	0,62 ± 0,11
24:1 n-9	0,44 ± 0.07[b]	0,80 ± 0.45[a,b]	0,74 ± 0.24[a,b]	0,89 ± 0.54[a]
ΣMUFA	**52,41 ± 10,38**	**51,33 ± 10,26**	**51,53 ± 10,66**	**50,62 ± 13,70**
16:2 n-4	0,69 ± 0,10	0,54 ± 0,14	0,51 ± 0,23	0,51 ± 0,19
18:2 n-4	0,24 ± 0,05	0,19 ± 0,08	0,18 ± 0,05	0,20 ± 0,06
18:4 n-1	0,22 ± 0,05	0,16 ± 0,07	0,15 ± 0,07	0,16 ± 0,06
18:2 n-6	1,15 ± 0,14	1,34 ± 0,43	1,15 ± 0,11	1,21 ± 0,16
20:2 n-6	0,28 ± 0,04	0,33 ± 0,09	0,40 ± 0,19	0,40 ± 0,16
20:4 n-6	0,44 ± 0,08	0,55 ± 0,26	0,77 ± 0,51	0,95 ± 0,95
22:4 n-6	0,52 ± 0,38	2,29 ± 2,50	1,03 ± 0,98	1,71 ± 2,22
22:5 n-6	0,12 ± 0.02[b]	0,19 ± 0.06[a]	0,19 ± 0.04[a]	0,21 ± 0.06[a]
18:3 n-3	0,66 ± 0,13	0,75 ± 0,31	0,68 ± 0,10	0,67 ± 0,17
18:4 n-3	2,18 ± 0,28	1,68 ± 0,76	1,72 ± 0,71	1,74 ± 0,80
20:3 n-3	0,16 ± 0,04	0,18 ± 0,07	0,22 ± 0,09	0,20 ± 0,07
20:4 n-3	0,66 ± 0,10	0,80 ± 0,55	0,62 ± 0,14	0,66 ± 0,14
20:5 n-3	9,73 ± 1,27	7,64 ± 3,12	8,36 ± 2,29	8,76 ± 1,58
21:5 n-3	0,62 ± 0,10	0,44 ± 0,21	0,45 ± 0,16	0,47 ± 0,17
22:5 n-3	1,93 ± 0,56	1,92 ± 0,52	1,74 ± 0,33	1,88 ± 0,27
22:6 n-3	11,50 ± 1,48	13,46 ± 2,14	12,25 ± 1,57	13,33 ± 4,14
ΣPUFA	**31,11 ± 1,48**	**32,47 ± 1,33**	**30,42 ± 3,33**	**33,06 ± 6,20**
ΣPUFA (n-6)	**2,50 ± 0,43**	**4,71 ± 2,56**	**3,54 ± 1,26**	**4,47 ± 3,22**
ΣPUFA (n-3)	**27,45 ± 1,62**	**26,87 ± 2,81**	**26,03 ± 3,29**	**27,71 ± 4,46**
(n-3)/(n-6)	**11,33 ± 2.27[a]**	**7,43 ± 4.09[b]**	**8,12 ± 2.61[a,b]**	**8,02 ± 3.74[a,b]**

Table 26. Fatty acids composition (% of total fatty acids) in the membrane lipids (PC/PE) of cod liver (mean± std dev). Different letters indicate significant changes, p< 0.05.

	Egersund, Female PC/PE (n=11)	Egersund, male PC/PE (n=10)	Tampen, Female PC/PE (n=12)	Tampen, Male PC/PE (n=10)
14:0	3,13 ± 0,53	3,07 ± 0,63	2,91 ± 0,53	2,85 ± 0,58
i-15:0	0,15 ± 0,03	0,17 ± 0,04	0,18 ± 0,06	0,17 ± 0,04
15:0	0,44 ± 0,07	0,49 ± 0,07	0,52 ± 0,15	0,48 ± 0,10
16:0	19,69 ± 1,26	18,78 ± 1,82	19,58 ± 1,50	19,11 ± 1,24
i-17:0	0,39 ± 0,09	0,44 ± 0,10	0,44 ± 0,14	0,46 ± 0,12
17:0	0,27 ± 0,09	0,30 ± 0,09	0,36 ± 0,17	0,35 ± 0,14
ai-18:0	0,05 ± 0,01	0,04 ± 0,01	0,04 ± 0,01	0,07 ± 0,08
18:0	2,04 ± 0,23	1,99 ± 0,30	2,14 ± 0,24	2,28 ± 0,28
ΣSFA	**26,15 ± 1,27**	**25,27 ± 1,75**	**26,18 ± 1,34**	**25,77 ± 1,29**
16:1 n-11	0,24 ± 0,09	0,23 ± 0,07	0,16 ± 0,05	0,16 ± 0,07
16:1 n-9	0,38 ± 0,08	0,42 ± 0,10	0,42 ± 0,11	0,38 ± 0,07
16:1 n-7	2,19 ± 0,13	2,16 ± 0,52	2,08 ± 0,18	2,03 ± 0,20
16:1 n-5	0,28 ± 0.04^{ab}	0,32 ± 0.06^{a}	0,26 ± 0.04^{b}	0,24 ± 0.04^{b}
17:1 n-x	0,23 ± 0,07	0,25 ± 0,05	0,29 ± 0,09	0,27 ± 0,10
18:1 n-11	1,40 ± 0,30	1,33 ± 0,33	1,24 ± 0,30	1,20 ± 0,36
18:1 n-9	6,19 ± 0.59^{b}	6,22 ± 0.34^{b}	6,85 ± 0.92^{ab}	7,19 ± 0.95^{a}
18:1 n-7	2,36 ± 0,42	2,48 ± 0,43	2,62 ± 0,49	2,73 ± 0,62
18:1 n-5	0,29 ± 0,04	0,29 ± 0,06	0,26 ± 0,04	0,27 ± 0,04
20:1 n-11	0,64 ± 0,10	0,65 ± 0,20	0,67 ± 0,20	0,88 ± 0,68
20:1 n-9	3,17 ± 0.43^{a}	3,28 ± 0.64^{a}	2,35 ± 0.96^{b}	2,15 ± 0.74^{b}
20:1 n-7	0,06 ± 0,01	0,08 ± 0,05	0,05 ± 0,02	0,07 ± 0,07
22:1 n-11	0,79 ± 0,14	1,11 ± 0,65	0,80 ± 0,65	0,84 ± 0,44
22:1 n-9	0,10 ± 0.02^{ab}	0,11 ± 0.02^{a}	0,08 ± 0.02^{b}	0,09 ± 0.02^{ab}
24:1 n-9	2,13 ± 0,31	2,19 ± 0,29	2,07 ± 0,36	2,18 ± 0,30
ΣMUFA	**25,34 ± 11,21**	**26,46 ± 11,46**	**24,90 ± 11,15**	**26,11 ± 11,07**
16:2 n-4	0,09 ± 0,02	0,08 ± 0,03	0,07 ± 0,04	0,07 ± 0,03
18:2 n-4	0,18 ± 0,03	0,14 ± 0,05	0,17 ± 0,13	0,15 ± 0,04
18:4 n-1	0,10 ± 0,03	0,07 ± 0,03	0,07 ± 0,03	0,07 ± 0,02
18:2 n-6	0,58 ± 0.05^{b}	0,75 ± 0.21^{a}	0,60 ± 0.11^{b}	0,62 ± 0.07^{ab}
20:2 n-6	0,26 ± 0,11	0,32 ± 0,09	0,32 ± 0,12	0,33 ± 0,15
20:4 n-6	2,04 ± 0,85	2,16 ± 0,73	2,81 ± 0,79	2,77 ± 1,13
22:4 n-6	0,25 ± 0,14	0,37 ± 0,25	0,31 ± 0,14	0,29 ± 0,13
22:5 n-6	0,28 ± 0.06^{b}	0,30 ± 0.06^{ab}	0,36 ± 0.04^{a}	0,35 ± 0.04^{a}
18:3 n-3	0,25 ± 0,04	0,32 ± 0,11	0,25 ± 0,07	0,24 ± 0,06
18:4 n-3	0,60 ± 0,10	0,62 ± 0,22	0,48 ± 0,22	0,47 ± 0,16
20:3 n-3	0,11 ± 0,02	0,13 ± 0,04	0,12 ± 0,04	0,12 ± 0,05
20:4 n-3	0,44 ± 0.06^{ab}	0,48 ± 0.06^{a}	0,39 ± 0.10^{b}	0,43 ± 0.07^{ab}
20:5 n-3	17,02 ± 1,31	15,66 ± 1,32	16,06 ± 1,14	16,61 ± 1,13
21:5 n-3	0,31 ± 0,05	0,26 ± 0,06	0,27 ± 0,12	0,25 ± 0,05
22:5 n-3	1,60 ± 0,22	1,76 ± 0,47	1,54 ± 0,44	1,63 ± 0,30
22:6 n-3	29,30 ± 2,17	30,18 ± 1,99	29,81 ± 1,22	29,14 ± 1,71
ΣPUFA	**53,40 ± 1,05**	**53,61 ± 1,81**	**53,62 ± 1,14**	**53,54 ± 1,49**
ΣPUFA (n-6)	**3,41 ± 1,11**	**3,90 ± 0,99**	**4,39 ± 0,91**	**4,36 ± 1,38**
ΣPUFA (n-3)	**49,63 ± 1,25**	**49,41 ± 1,64**	**48,92 ± 1,01**	**48,90 ± 1,51**
(n-3)/(n-6)	**15,63 ± 3.92^{a}**	**13,45 ± 3.62^{ab}**	**11,61 ± 2.59^{b}**	**12,35 ± 4.14^{ab}**

Table 27. Fatty acids composition (% of total fatty acids) in the PS/PI of cod liver (mean± std dev). Different letters indicate significant changes, p< 0.05.

	Egarsund, Female PS/PI (n=11)	Egarsund, male PS/PI (n=10)	Tampen, Female PS/PI (n=12)	Tampen, Male PS/PI (n=10)
14:0	0,88 ± 0,37	0,93 ± 0,25	1,15 ± 0,68	0,90 ± 0,44
i-15:0	0,08 ± 0,02	0,10 ± 0,03	0,12 ± 0,07	0,10 ± 0,03
15:0	0,13 ± 0,04	0,14 ± 0,04	0,15 ± 0,08	0,13 ± 0,04
16:0	5,98 ± 1,19	6,42 ± 3,38	5,70 ± 1,13	5,03 ± 1,09
i-17:0	0,18 ± 0,04	0,22 ± 0,05	0,23 ± 0,08	0,23 ± 0,07
17:0	0,80 ± 0,40	0,87 ± 0,76	1,05 ± 0,50	1,01 ± 0,68
ai-18:0	0,06 ± 0,04	0,06 ± 0,02	0,06 ± 0,03	0,06 ± 0,02
18:0	13,16 ± 2,68	12,02 ± 1,74	11,66 ± 1,80	12,92 ± 1,74
ΣSFA	**21,28 ± 2,91**	**20,75 ± 4,37**	**20,11 ± 1,66**	**20,36 ± 2,88**
16:1 n-11	0,16 ± 0.03^{ab}	0,19 ± 0.05^{a}	0,12 ± 0.03^{b}	0,12 ± 0.04^{b}
16:1 n-9	0,35 ± 0,16	0,41 ± 0,11	0,36 ± 0,16	0,37 ± 0,08
16:1 n-7	2,59 ± 0,32	2,58 ± 0,50	2,83 ± 0,58	2,63 ± 0,24
16:1 n-5	0,08 ± 0,03	0,16 ± 0,22	0,11 ± 0,14	0,06 ± 0,01
17:1 n-x	0,23 ± 0,09	0,22 ± 0,06	0,28 ± 0,17	0,25 ± 0,05
18:1 n-11	1,32 ± 0,32	1,30 ± 0,50	1,14 ± 0,25	1,10 ± 0,33
18:1 n-9	4,33 ± 0,56	4,17 ± 0,75	4,90 ± 0,71	4,70 ± 0,56
18:1 n-7	3,02 ± 0,31	3,12 ± 0,39	3,17 ± 0,44	3,08 ± 0,35
18:1 n-5	0,28 ± 0,04	0,30 ± 0,08	0,37 ± 0,35	0,26 ± 0,03
20:1 n-11	1,04 ± 1,85	0,44 ± 0,10	0,55 ± 0,15	0,50 ± 0,10
20:1 n-9	4,53 ± 1,88	4,60 ± 0,87	3,73 ± 1,39	3,54 ± 1,11
20:1 n-7	0,12 ± 0,04	0,14 ± 0,05	0,10 ± 0,04	0,09 ± 0,02
22:1 n-11	0,94 ± 0,50	0,79 ± 0,31	0,84 ± 0,32	0,78 ± 0,41
22:1 n-9	0,07 ± 0,03	0,06 ± 0,03	0,06 ± 0,02	0,06 ± 0,03
24:1 n-9	1,38 ± 0,57	1,21 ± 0,54	1,03 ± 0,58	1,48 ± 0,49
ΣMUFA	**25,80 ± 13,03**	**26,21 ± 14,67**	**25,36 ± 14,27**	**24,46 ± 10,49**
16:2 n-4	0,12 ± 0,02	0,09 ± 0,03	0,11 ± 0,06	0,10 ± 0,04
18:2 n-4	0,15 ± 0,04	0,14 ± 0,06	0,13 ± 0,04	0,14 ± 0,03
18:4 n-1	0,09 ± 0,05	0,06 ± 0,03	0,06 ± 0,02	0,06 ± 0,01
18:2 n-6	0,92 ± 0.16^{b}	1,14 ± 0.26^{a}	1,03 ± 0.15^{ab}	1,12 ± 0.11^{ab}
20:2 n-6	0,53 ± 0,08	0,61 ± 0,12	0,59 ± 0,10	0,58 ± 0,13
20:4 n-6	11,09 ± 2,88	10,40 ± 1,66	11,67 ± 2,08	12,30 ± 2,08
22:4 n-6	0,11 ± 0,08	0,13 ± 0,10	0,18 ± 0,10	0,15 ± 0,09
22:5 n-6	0,66 ± 0,14	0,68 ± 0,16	0,79 ± 0,13	0,80 ± 0,13
18:3 n-3	0,33 ± 0.06^{b}	0,43 ± 0.13^{a}	0,36 ± 0.06^{ab}	0,36 ± 0.06^{ab}
18:4 n-3	0,25 ± 0,06	0,24 ± 0,10	0,21 ± 0,09	0,21 ± 0,06
20:3 n-3	0,25 ± 0,04	0,30 ± 0,11	0,28 ± 0,08	0,28 ± 0,08
20:4 n-3	0,47 ± 0,05	0,48 ± 0,09	0,48 ± 0,08	0,52 ± 0,06
20:5 n-3	8,22 ± 1.92^{a}	7,64 ± 1.39^{ab}	6,43 ± 1.13^{b}	6,38 ± 0.88^{b}
21:5 n-3	0,17 ± 0,13	0,11 ± 0,02	0,13 ± 0,06	0,11 ± 0,02
22:5 n-3	2,10 ± 0,54	1,81 ± 0,33	1,90 ± 0,34	1,98 ± 0,32
22:6 n-3	32,83 ± 5,32	35,28 ± 6,07	35,96 ± 3,25	35,53 ± 3,82
ΣPUFA	**58,29 ± 5,13**	**59,55 ± 6,90**	**60,32 ± 2,88**	**60,60 ± 4,58**
ΣPUFA (n-6)	**13,32 ± 2,90**	**12,96 ± 1,66**	**14,26 ± 2,07**	**14,96 ± 2,23**
ΣPUFA (n-3)	**44,61 ± 5,32**	**46,29 ± 6,23**	**45,75 ± 3,29**	**45,35 ± 3,68**
(n-3)/(n-6)	**3,51 ± 0,97**	**3,60 ± 0,55**	**3,29 ± 0,61**	**3,09 ± 0,51**

Table 28. Fatty acids composition (% of total fatty acids) in cod muscle. Different letters indicate significant changes, p< 0.05.

	Egersund female (n=19)	Egersund male (n=14)	Tampen, Female (n=14)	Tampen, Female (n=15)
14:0	1,26 ± 0,31	1,25 ± 0,38	1,07 ± 0,24	1,10 ± 0,29
Iso 15:0	0,06 ± 0,01^{ab}	0,06 ± 0,01^{a}	0,05 ± 0,01^{b}	0,05 ± 0,01^{b}
15:0	0,28 ± 0,03	0,29 ± 0,05	0,27 ± 0,04	0,27 ± 0,03
16:0	17,66 ± 1,05^{c}	17,79 ± 1,04^{bc}	18,74 ± 0,89^{ab}	19,05 ± 1,05^{a}
Iso 17:0	0,30 ± 0,04	0,33 ± 0,07	0,30 ± 0,06	0,29 ± 0,05
Antiso 17:0	0,11 ± 0,03	0,11 ± 0,04	0,08 ± 0,02	0,08 ± 0,03
17:0	0,30 ± 0,05	0,31 ± 0,08	0,28 ± 0,07	0,26 ± 0,07
18:0	4,26 ± 0,33	4,48 ± 0,36	4,34 ± 0,22	4,48 ± 0,38
24:0	0,17 ± 0,04	0,16 ± 0,02	0,16 ± 0,02	0,15 ± 0,03
∑SFA	**24,39 ± 1,02^{b}**	**24,78 ± 0,99^{ab}**	**25,30 ± 0,88^{a}**	**25,73 ± 1,23^{a}**
16:1 (n-11)	0,10 ± 0,03^{a}	0,09 ± 0,04^{ab}	0,08 ± 0,02^{b}	0,08 ± 0,02^{b}
16:1 (n-9)	0,38 ± 0,05	0,37 ± 0,08	0,33 ± 0,03	0,35 ± 0,03
16:1 (n-7)	1,50 ± 0,37^{a}	1,51 ± 0,43^{a}	1,09 ± 0,18^{b}	1,08 ± 0,19^{b}
16:1 (n-5)	0,22 ± 0,02	0,23 ± 0,03	0,22 ± 0,04	0,22 ± 0,03
17:1 (n-9)	0,28 ± 0,05	0,28 ± 0,07	0,25 ± 0,04	0,25 ± 0,05
18:1 (n-11)	1,11 ± 0,30	0,96 ± 0,29	1,02 ± 0,24	1,08 ± 0,32
18:1 (n-9)	6,82 ± 1,02	6,71 ± 1,81	6,56 ± 0,66	6,26 ± 0,90
18:1 (n-7)	2,33 ± 0,34^{a}	2,19 ± 0,38^{ab}	1,97 ± 0,27^{b}	1,94 ± 0,35^{b}
18:1 (n-5)	0,21 ± 0,03^{a}	0,21 ± 0,04^{a}	0,16 ± 0,02^{b}	0,16 ± 0,03^{b}
20:1 (n-11)	0,51 ± 0,10^{a}	0,50 ± 0,11^{ab}	0,41 ± 0,09^{b}	0,41 ± 0,11^{b}
20:1 (n-9)	2,62 ± 0,90^{a}	2,43 ± 0,97^{a}	1,50 ± 0,39^{b}	1,50 ± 0,42^{b}
20:1 (n-7)	0,09 ± 0,02^{a}	0,10 ± 0,04^{b}	0,05 ± 0,01^{b}	0,05 ± 0,03^{b}
22:1 (n-11)	1,43 ± 0,75^{a}	1,46 ± 0,90^{a}	0,86 ± 0,32a^{b}	0,81 ± 0,26^{b}
22:1 (n-9)	0,20 ± 0,06^{a}	0,22 ± 0,11	0,12 ± 0,03^{b}	0,12 ± 0,03^{b}
22:1 (n-7)	0,10 ± 0,04^{a}	0,10 ± 0,04	0,06 ± 0,02^{b}	0,05 ± 0,01^{b}
24:1 (n-9)	1,33 ± 0,32	1,50 ± 0,84	1,26 ± 0,08	1,26 ± 0,18
∑MUFA	**19,23 ± 2,62^{a}**	**18,89 ± 4,08^{a}**	**15,95 ± 1,47^{b}**	**15,62 ± 1,65^{b}**
16:2 (n-4)	0,12 ± 0,05^{a}	0,11 ± 0,06^{ab}	0,07 ± 0,03^{b}	0,08 ± 0,03^{b}
18:2 (n-4)	0,10 ± 0,03	0,09 ± 0,03	0,08 ± 0,02	0,08 ± 0,02
18:4 (n-1)	0,08 ± 0,04^{a}	0,06 ± 0,03^{ab}	0,05 ± 0,03^{b}	0,05 ± 0,02^{b}
18:2 (n-6)	0,58 ± 0,07	0,62 ± 0,09	0,56 ± 0,09	0,61 ± 0,11
20:2 (n-6)	0,20 ± 0,04^{a}	0,20 ± 0,05^{a}	0,17 ± 0,04^{ab}	0,15 ± 0,04^{b}
20:4 (n-6)	2,60 ± 0,54	2,43 ± 0,65	2,88 ± 0,64	2,79 ± 0,82
22:4 (n-6)	0,21 ± 0,08	0,21 ± 0,13	0,20 ± 0,08	0,18 ± 0,09
22:5 (n-6)	0,53 ± 0,06	0,57 ± 0,10	0,58 ± 0,05	0,57 ± 0,06
18:3 (n-3)	0,23 ± 0,05	0,24 ± 0,05	0,20 ± 0,04	0,22 ± 0,05
18:4 (n-3)	0,60 ± 0,19^{a}	0,56 ± 0,19^{a}	0,44 ± 0,12^{b}	0,44 ± 0,14^{b}
20:3 (n-3)	0,09 ± 0,02^{a}	0,08 ± 0,03^{ab}	0,07 ± 0,02^{bc}	0,06 ± 0,02^{c}
20:4 (n-3)	0,37 ± 0,06	0,37 ± 0,04	0,35 ± 0,05	0,36 ± 0,05
20:5 (n-3)	12,93 ± 1,37	11,80 ± 1,72	12,31 ± 1,20	12,61 ± 1,55
21:5 (n-3)	0,26 ± 0,07^{a}	0,22 ± 0,06^{ab}	0,19 ± 0,04^{b}	0,19 ± 0,03^{b}
22:5 (n-3)	1,91 ± 0,53	1,73 ± 0,36	1,74 ± 0,23	1,71 ± 0,20
22:6 (n-3)	36,82 ± 3,04^{b}	38,31 ± 3,87^{ab}	39,92 ± 2,05^{a}	39,65 ± 3,25^{a}
∑PUFA	**57,64 ± 2,01^{b}**	**57,58 ± 3,48^{b}**	**59,83 ± 1,06^{a}**	**59,75 ± 1,83^{a}**
∑n-6	**4,13 ± 0,65**	**4,03 ± 0,89**	**4,39 ± 0,75**	**4,30 ± 0,90**
∑n-3	**53,21 ± 1,88^{b}**	**53,31 ± 3,23^{ab}**	**55,23 ± 1,18^{a}**	**55,23 ± 2,08^{a}**
(n-3)/(N-6)	**13,19 ± 2,05**	**13,79 ± 2,82**	**12,98 ± 2,61**	**13,46 ± 3,33**

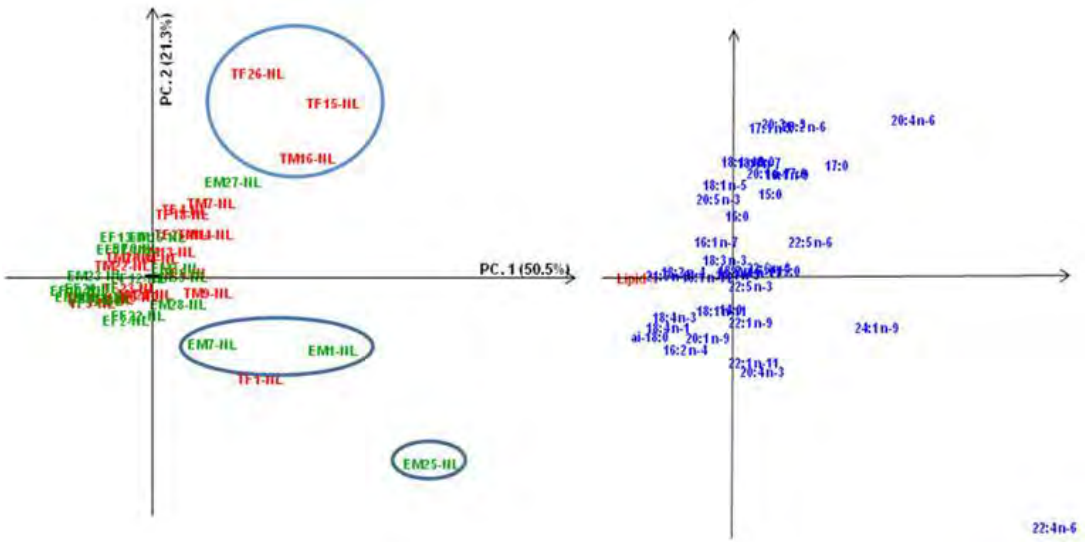


Figure 24. PCA plots of neutral lipids of cod liver. Left: Loading plot, right: Score plot. Red samples are from Egersund Bank and green are from Tampen. The model explains 72 % of the total variance in the dataset. The circle indicate fish with lower liver lipids.

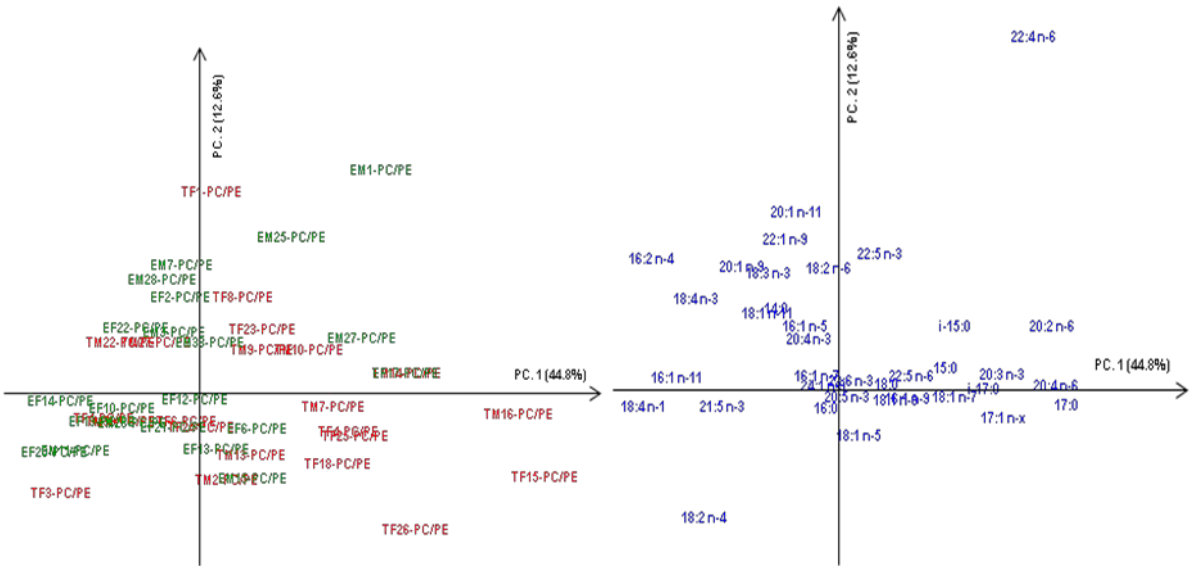


Figure 25. PCA plots of PC/PE of cod liver. Left: Loading plot, right: Score plot. The model explains 58 % of the total variance in the dataset.

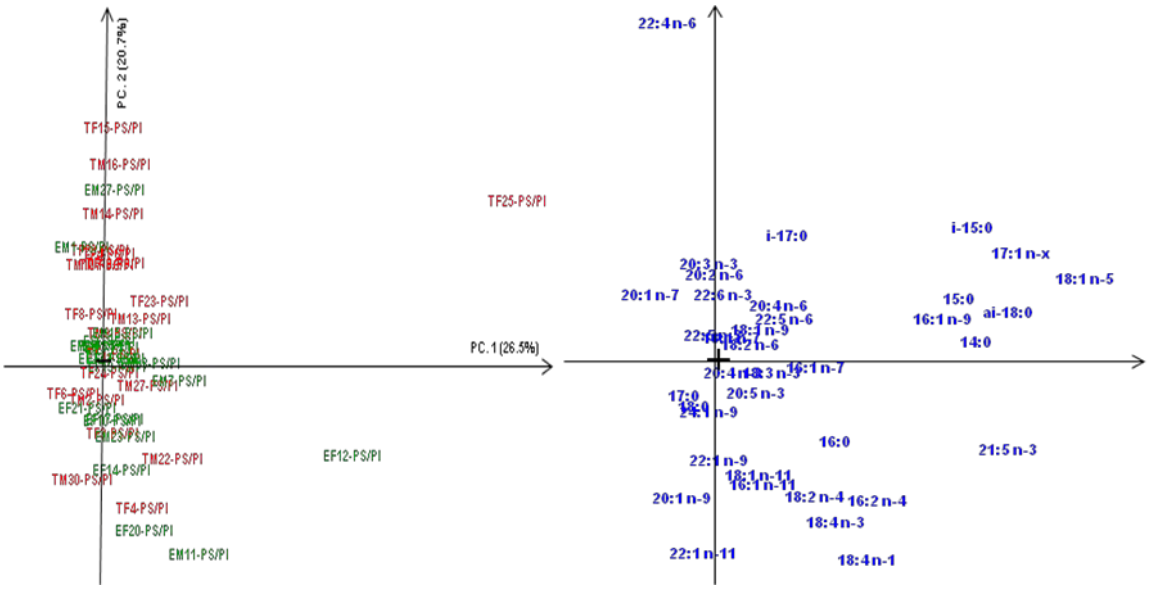


Figure 26. PCA plots of PS/PI of cod liver. Left: Loading plot, right: Score plot. The model explains 47 % of the total variance in the dataset.

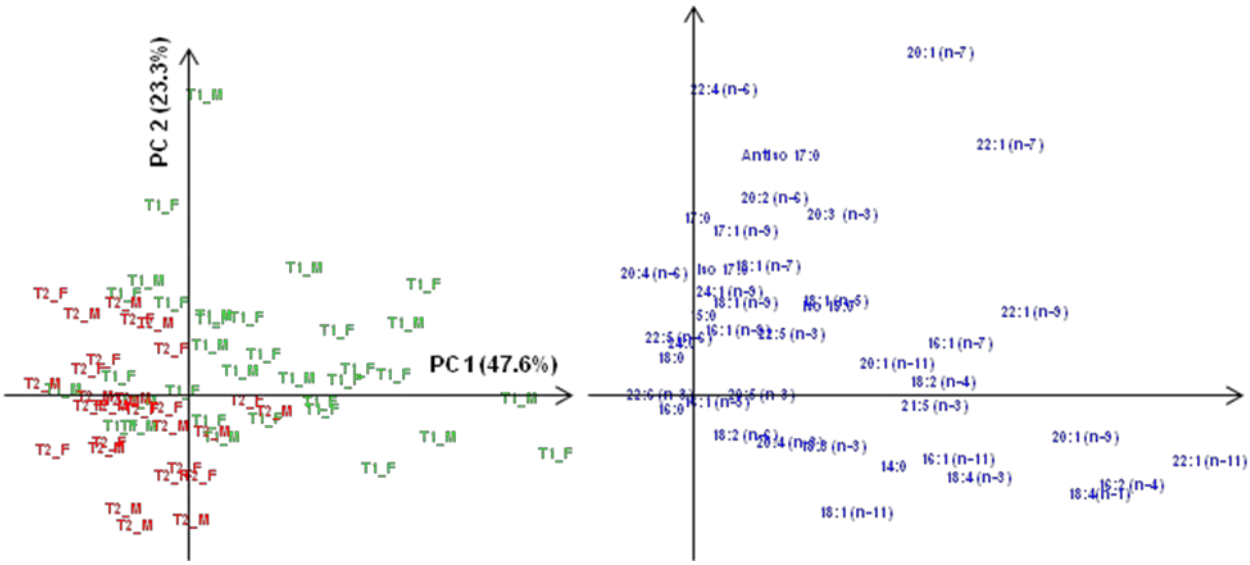


Figure 27. PCA plots of fatty acids in cod muscle. Left: Loading plot, right: Score plot. The model explains 71 % of the total variance in the dataset.

5.8.3 Fatty acid composition in algae

Water samples collected together with CTD measurement were filtered on to micro filters and the fatty acid composition of the algae were analysed by GC-FID. Two samples were taken per station, one at 10 m and one at 20 m. The results are given as mean values of both samples (Table 29). PCA plot of FA profiles from algae is shown in Figure 28.

Table 29. Fatty acids composition (% of total fatty acids) in algae.

	Egersund St 463 (n=2)	Utsira St 512 (n=2)	North for Tampen St 556 (n=2)	East for Tampen St 557 (n=2)	Viking Bank St 462 (n=2)	Tampen, st5 st 553 (n=2)	Tampen, st7 St 555 (n=2)
14:0	16,56 ± 2,98	9,01 ± 0,48	8,79 ± 0,85	8,54 ± 1,01	8,71 ± 1,14	10,68 ± 2,22	9,10 ± 0,75
Iso 15:0	1,34 ± 0,13	0,78 ± 0,15	0,93 ± 0,04	0,96 ± 0,08	0,78 ± 0,15	0,95 ± 0,09	1,13 ± 0,09
Antiso 15:0	0,28 ± 0,04	0,22 ± 0,03	0,53 ± 0,09	0,54 ± 0,09	0,30 ± 0,02	0,27 ± 0,12	0,89 ± 0,10
15:0	0,65 ± 0,28	0,67 ± 0,04	1,57 ± 0,19	1,53 ± 0,24	0,72 ± 0,01	0,48 ± 0,18	2,03 ± 0,34
Iso 16:0	0,00 ± 0,00	1,86 ± 0,47	1,15 ± 0,23	1,58 ± 0,34	0,07 ± 0,10	1,02 ± 1,41	0,87 ± 0,40
16:0	24,10 ± 3,84	17,45 ± 3,29	21,24 ± 0,66	19,13 ± 1,67	22,65 ± 3,17	15,73 ± 2,86	20,64 ± 0,11
Iso 17:0	0,35 ± 0,09	0,47 ± 0,02	0,58 ± 0,12	0,69 ± 0,02	0,80 ± 0,05	0,46 ± 0,29	0,37 ± 0,53
Antiso 17:0	0,15 ± 0,05	0,19 ± 0,04	0,36 ± 0,02	0,36 ± 0,01	0,45 ± 0,11	0,16 ± 0,08	0,48 ± 0,06
17:0	0,56 ± 0,10	0,45 ± 0,03	0,87 ± 0,05	0,84 ± 0,03	0,77 ± 0,10	0,40 ± 0,00	0,96 ± 0,10
18:0	2,58 ± 0,61	3,65 ± 0,95	6,00 ± 0,57	5,10 ± 0,55	4,96 ± 0,73	2,33 ± 0,39	4,72 ± 0,78
20:0	7,36 ± 3,15	2,82 ± 0,74	6,53 ± 0,30	4,52 ± 0,15	2,60 ± 0,24	6,02 ± 1,09	4,81 ± 0,09
22:0	0,44 ± 0,06	0,33 ± 0,07	0,57 ± 0,06	0,58 ± 0,04	0,69 ± 0,07	0,27 ± 0,05	0,71 ± 0,07
24:0	0,43 ± 0,07	0,39 ± 0,04	0,94 ± 0,03	0,93 ± 0,02	0,71 ± 0,09	0,14 ± 0,20	1,01 ± 0,05
∑SFA	**54,79 ± 4,98**	**38,30 ± 4,26**	**50,06 ± 0,86**	**45,32 ± 3,52**	**44,21 ± 4,83**	**38,91 ± 3,39**	**47,72 ± 0,74**
14:1 (n-7)	0,11 ± 0,03	0,09 ± 0,01	0,48 ± 0,14	0,54 ± 0,11	0,16 ± 0,02	0,09 ± 0,06	0,00 ± 0,00
14:1 (n-5)	0,24 ± 0,06	0,30 ± 0,11	0,58 ± 0,01	0,59 ± 0,09	0,20 ± 0,20	0,34 ± 0,01	0,67 ± 0,09
16:1 (n-9)	0,00 ± 0,00	0,63 ± 0,12	3,34 ± 1,00	4,21 ± 1,37	1,61 ± 0,40	0,00 ± 0,00	5,82 ± 1,06
16:1 (n-7)	6,25 ± 0,03	4,62 ± 0,00	3,42 ± 0,52	4,34 ± 0,24	5,17 ± 0,56	8,67 ± 0,78	5,26 ± 0,73
16:1 (n-5)	0,39 ± 0,03	0,19 ± 0,21	0,69 ± 0,00	0,62 ± 0,06	0,52 ± 0,04	0,55 ± 0,12	0,81 ± 0,07
17:1 X	0,24 ± 0,34	0,58 ± 0,11	1,30 ± 0,00	1,07 ± 0,02	0,79 ± 0,23	0,00 ± 0,00	1,72 ± 0,08
18:1 (n-11)	1,52 ± 0,10	0,46 ± 0,23	1,33 ± 0,10	0,91 ± 0,17	1,27 ± 0,40	1,32 ± 0,01	5,67 ± 0,27
18:1 (n-9)	4,19 ± 0,10	9,59 ± 1,82	8,74 ± 1,03	8,26 ± 0,09	3,88 ± 0,12	3,34 ± 0,29	3,74 ± 0,33
18:1 (n-7)	2,31 ± 0,21	1,66 ± 0,36	1,77 ± 0,05	1,92 ± 0,15	1,91 ± 0,27	2,21 ± 0,53	1,81 ± 0,02
20:1 (n-11)	0,04 ± 0,06	0,19 ± 0,16	0,12 ± 0,11	0,22 ± 0,02	0,25 ± 0,13	0,00 ± 0,00	0,26 ± 0,24
20:1 (n-9)	0,01 ± 0,02	0,80 ± 0,56	0,39 ± 0,09	0,27 ± 0,03	0,24 ± 0,12	0,62 ± 0,43	0,43 ± 0,09
20:1 (n-7)	0,02 ± 0,01	0,10 ± 0,03	0,10 ± 0,02	0,14 ± 0,02	0,43 ± 0,05	0,03 ± 0,01	0,04 ± 0,06
22:1 (n-11)	0,00 ± 0,00	0,83 ± 1,11	0,10 ± 0,00	0,21 ± 0,03	0,00 ± 0,00	0,44 ± 0,57	0,02 ± 0,03
22:1 (n-9)	0,13 ± 0,03	0,55 ± 0,02	0,33 ± 0,03	0,41 ± 0,04	0,33 ± 0,26	0,24 ± 0,10	1,27 ± 0,37
∑MUFA	**15,45 ± 0,65**	**20,58 ± 0,49**	**22,69 ± 1,93**	**23,69 ± 2,08**	**16,77 ± 0,05**	**17,87 ± 0,68**	**27,52 ± 0,56**
16:2 (n-4)	0,00 ± 0,00	0,00 ± 0,00	0,02 ± 0,00	0,03 ± 0,01	0,01 ± 0,01	0,00 ± 0,00	0,04 ± 0,02
16:3 (n-4)	0,08 ± 0,00	0,09 ± 0,06	0,08 ± 0,02	0,06 ± 0,01	0,07 ± 0,04	0,18 ± 0,12	0,25 ± 0,11
18:2 (n-4)	0,13 ± 0,14	0,10 ± 0,01	0,11 ± 0,01	0,14 ± 0,00	0,02 ± 0,01	0,24 ± 0,03	0,02 ± 0,00
16:4 (n-1)	1,62 ± 1,24	7,19 ± 1,93	1,55 ± 0,22	3,58 ± 2,32	0,78 ± 0,37	0,00 ± 0,00	0,00 ± 0,00
18:4 (n-1)	0,19 ± 0,09	0,56 ± 0,53	0,22 ± 0,03	0,14 ± 0,06	0,09 ± 0,02	0,60 ± 0,46	0,11 ± 0,12
16:2 (n-7)	0,00 ± 0,00	0,03 ± 0,03	0,26 ± 0,08	0,28 ± 0,06	0,12 ± 0,09	0,00 ± 0,00	0,50 ± 0,05
18.2 (n-7)	0,08 ± 0,02	0,12 ± 0,00	0,13 ± 0,09	0,18 ± 0,02	0,12 ± 0,00	0,12 ± 0,14	0,17 ± 0,11
16:2 (n-6)	0,20 ± 0,06	0,47 ± 0,19	0,33 ± 0,18	0,38 ± 0,01	0,09 ± 0,13	0,49 ± 0,04	0,24 ± 0,18
18:2 (n-6)	3,19 ± 0,16	3,38 ± 0,98	3,47 ± 0,29	2,87 ± 0,45	2,47 ± 0,15	3,11 ± 0,37	2,29 ± 0,38
18:3 (n-6)	0,00 ± 0,00	0,15 ± 0,07	0,09 ± 0,02	0,04 ± 0,06	0,35 ± 0,45	0,22 ± 0,05	0,07 ± 0,10
20:2 (n-6)	0,58 ± 0,02	0,20 ± 0,03	0,10 ± 0,05	0,15 ± 0,07	0,67 ± 0,30	0,23 ± 0,09	0,04 ± 0,06
20:3 (n-6)	0,01 ± 0,01	0,07 ± 0,03	0,03 ± 0,02	0,03 ± 0,01	0,05 ± 0,07	0,05 ± 0,03	0,01 ± 0,01
20:4 (n-6)	0,22 ± 0,06	0,39 ± 0,09	0,23 ± 0,00	0,32 ± 0,07	1,16 ± 0,09	0,30 ± 0,04	0,16 ± 0,02
22:4 (n-6)	0,05 ± 0,00	0,09 ± 0,03	0,08 ± 0,01	0,11 ± 0,00	0,72 ± 0,63	0,11 ± 0,15	0,04 ± 0,00
22:5(n-6)	0,43 ± 0,15	0,16 ± 0,02	0,18 ± 0,01	0,18 ± 0,02	0,47 ± 0,14	0,21 ± 0,05	0,27 ± 0,04
16:4 (n-3)	0,17 ± 0,02	0,03 ± 0,02	0,09 ± 0,01	0,08 ± 0,01	0,16 ± 0,01	0,03 ± 0,01	0,10 ± 0,03
18:3 (n-3)	2,95 ± 0,46	2,10 ± 0,63	1,89 ± 0,27	1,64 ± 0,01	1,36 ± 0,27	3,61 ± 0,27	2,40 ± 0,28
18:4 (n-3)	5,47 ± 1,86	4,00 ± 0,54	4,64 ± 0,38	4,02 ± 0,51	3,83 ± 0,39	6,65 ± 1,99	4,99 ± 0,49
20:3 (n-3)	0,31 ± 0,05	0,18 ± 0,01	0,16 ± 0,02	0,20 ± 0,06	0,17 ± 0,01	0,29 ± 0,20	0,09 ± 0,06
20:4 (n-3)	0,98 ± 1,09	0,71 ± 0,11	0,54 ± 0,05	0,83 ± 0,25	2,06 ± 2,37	0,54 ± 0,23	0,57 ± 0,05
20:5 (n-3)	3,82 ± 0,13	8,32 ± 1,60	4,54 ± 0,67	5,39 ± 0,81	9,08 ± 0,67	8,76 ± 0,57	5,44 ± 0,05
21:5 (n-3)	0,39 ± 0,46	0,10 ± 0,01	0,24 ± 0,02	0,24 ± 0,02	1,05 ± 1,24	0,38 ± 0,39	0,33 ± 0,01
22:4 (n-3)	0,00 ± 0,00	3,83 ± 5,18	0,34 ± 0,13	0,32 ± 0,16	0,01 ± 0,01	1,53 ± 2,02	0,64 ± 0,03
22:5 (n-3)	0,34 ± 0,02	0,60 ± 0,17	0,42 ± 0,03	0,54 ± 0,07	1,70 ± 0,53	0,50 ± 0,00	0,00 ± 0,00
22:6 (n-3)	8,56 ± 1,31	8,27 ± 2,79	7,50 ± 1,84	9,26 ± 2,03	12,42 ± 0,71	15,09 ± 1,38	5,98 ± 1,34
∑PUFA	**29,76 ± 4,33**	**41,13 ± 4,75**	**27,25 ± 2,79**	**30,99 ± 5,60**	**39,01 ± 4,78**	**43,22 ± 4,07**	**24,76 ± 1,30**
∑n-6	**4,67 ± 0,15**	**4,90 ± 0,90**	**4,51 ± 0,02**	**4,07 ± 0,20**	**5,98 ± 0,96**	**4,71 ± 0,00**	**3,12 ± 0,36**
∑n-3	**22,99 ± 5,35**	**28,13 ± 3,10**	**20,36 ± 3,22**	**22,51 ± 3,56**	**31,83 ± 3,39**	**37,37 ± 3,37**	**20,54 ± 1,14**
(n-3)/(N-6)	**4,91 ± 0,99**	**5,89 ± 1,71**	**4,52 ± 0,74**	**5,56 ± 1,15**	**5,35 ± 0,29**	**7,94 ± 0,71**	**6,60 ± 0,39**
FA (µg/l)	20,30 ± 0,83	70,73 ± 44,89	38,09 ± 5,75	31,73 ± 1,09	25,17 ± 7,02	23,68 ± 2,26	24,92 ± 1,19

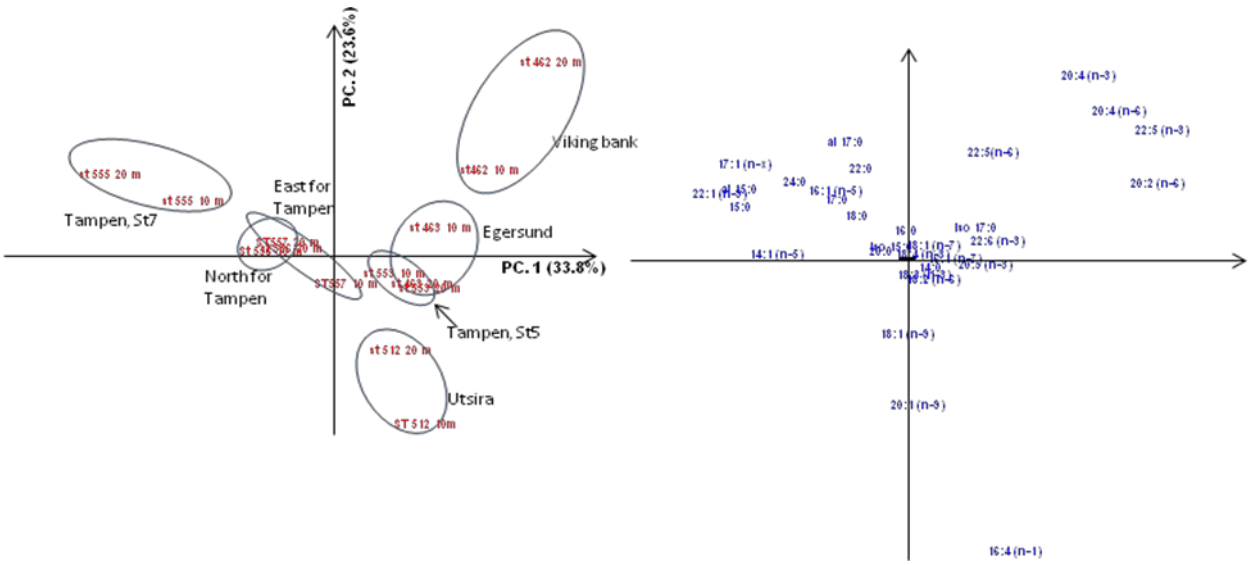


Figure 28. PCA plot of FA profiles from algae. Left: Loading plot, right: Score plot.

5.8.4 Fatty acid composition in zooplankton

Zooplankton was captured by dragging a 180 µm net from the bottom to the surface. The samples were separated into a 1000 µm fraction and a 180 µm fraction. The results are given as mean values of both fractions. Zooplankton (*Calanus*) are rich in wax esters. We have analyzed both the fatty acids (Table 30) and the fatty alcohols (Table 31). PCA plot of FA and FA-OH profiles from zooplankton is shown in Figure 29.

Table 30. Fatty acid composition (wt. % of total fatty acids) in zooplankton.

	Egersund St 463 (n=2)	Utsira-Statpoint St 535 (n=2)	Utsira-Statpoint St 534 (n=2)	Viking Bank St 462 (n=2)	Tampen, st7 St 555 (n=2)
14:0	17,31 ± 1,69	12,32 ± 1,08	15,03 ± 0,73	7,66 ± 0,07	18,08 ± 0,10
Iso 15:0	0,75 ± 0,06	0,53 ± 0,04	0,81 ± 0,04	0,47 ± 0,01	0,69 ± 0,11
Antiso 15:0	0,20 ± 0,02	0,18 ± 0,01	0,25 ± 0,00	0,11 ± 0,00	0,19 ± 0,06
15:0	1,24 ± 0,09	0,84 ± 0,09	1,26 ± 0,06	0,72 ± 0,01	1,05 ± 0,15
Iso 16:0	0,12 ± 0,00	0,10 ± 0,03	0,13 ± 0,01	0,12 ± 0,03	0,12 ± 0,02
16:0	12,61 ± 0,51	13,85 ± 1,64	13,91 ± 0,08	16,43 ± 0,27	13,00 ± 0,94
Iso 17:0	0,35 ± 0,03	0,31 ± 0,03	0,36 ± 0,04	0,37 ± 0,01	0,28 ± 0,04
Antiso 17:0	0,07 ± 0,01	0,07 ± 0,03	0,10 ± 0,00	0,05 ± 0,02	0,06 ± 0,03
17:0	0,40 ± 0,11	0,58 ± 0,05	0,49 ± 0,06	0,71 ± 0,03	0,39 ± 0,05
iso 18:0	0,28 ± 0,02	0,17 ± 0,03	0,25 ± 0,01	0,31 ± 0,05	0,23 ± 0,01
18:0	0,98 ± 0,22	1,71 ± 0,35	1,37 ± 0,28	2,72 ± 0,52	0,97 ± 0,15
20:0	0,62 ± 0,03	0,90 ± 0,37	0,43 ± 0,01	0,63 ± 0,09	0,40 ± 0,05
∑SFA	**34,93 ± 0,99**	**31,54 ± 1,53**	**34,38 ± 0,45**	**30,31 ± 1,07**	**35,46 ± 1,02**
16:1 (n-9)	0,36 ± 0,02	0,36 ± 0,06	0,41 ± 0,01	0,45 ± 0,04	0,42 ± 0,05
16:1 (n-7)	4,14 ± 0,19	5,14 ± 0,70	4,74 ± 0,20	2,98 ± 0,41	6,66 ± 1,39
16:1 (n-5)	0,42 ± 0,01	0,54 ± 0,19	0,54 ± 0,03	0,53 ± 0,06	0,67 ± 0,10
17:1 (n-9)	0,45 ± 0,01	0,49 ± 0,07	0,56 ± 0,02	0,44 ± 0,08	0,52 ± 0,09
18:1 (n-9)	4,58 ± 0,81	7,20 ± 1,56	4,37 ± 0,35	3,99 ± 1,13	4,60 ± 0,27
18:1 (n-7)	0,44 ± 0,07	0,70 ± 0,22	0,52 ± 0,09	0,97 ± 0,12	0,46 ± 0,17
18:1 (n-5)	0,36 ± 0,03	0,38 ± 0,09	0,40 ± 0,04	0,59 ± 0,02	0,44 ± 0,04
20:1 (n-11)	0,80 ± 0,02	0,47 ± 0,06	0,54 ± 0,11	0,30 ± 0,04	0,75 ± 0,12
20:1 (n-9)	3,15 ± 0,08	1,20 ± 0,13	1,58 ± 0,41	1,36 ± 0,08	2,66 ± 0,77
20:1 (n-7)	0,09 ± 0,01	0,08 ± 0,03	0,06 ± 0,00	0,16 ± 0,02	0,07 ± 0,02
22:1 (n-11)	8,78 ± 0,37	2,75 ± 0,18	4,98 ± 1,22	2,84 ± 0,29	4,65 ± 2,58
22:1 (n-9)	0,49 ± 0,00	0,20 ± 0,02	0,28 ± 0,06	0,30 ± 0,04	0,41 ± 0,07
22:1 (n-7)	0,09 ± 0,00	0,06 ± 0,01	0,07 ± 0,01	0,14 ± 0,03	0,09 ± 0,03
24:1 (n-9)	1,34 ± 0,35	1,49 ± 0,01	1,73 ± 0,25	2,29 ± 0,08	1,50 ± 0,04
∑MUFA	**25,48 ± 0,57**	**21,07 ± 1,01**	**20,78 ± 2,14**	**17,34 ± 0,80**	**23,90 ± 0,01**
16:4 (n-1)	0,52 ± 0,07	0,53 ± 0,28	0,58 ± 0,03	0,60 ± 0,17	0,43 ± 0,09
18:4 (n-1)	0,55 ± 0,02	0,29 ± 0,06	0,41 ± 0,10	0,10 ± 0,07	0,62 ± 0,02
16:2 nr 1	0,08 ± 0,01	0,12 ± 0,03	0,11 ± 0,01	0,06 ± 0,00	0,15 ± 0,01
18.2 (n-7)	0,05 ± 0,01	0,05 ± 0,01	0,07 ± 0,01	0,07 ± 0,02	0,05 ± 0,01
18:2 (n-6)	1,40 ± 0,00	1,62 ± 0,01	1,31 ± 0,05	1,41 ± 0,03	1,38 ± 0,12
18:3 (n-6)	0,19 ± 0,01	0,24 ± 0,07	0,19 ± 0,00	0,13 ± 0,02	0,27 ± 0,05
20:2 (n-6)	0,16 ± 0,02	0,19 ± 0,03	0,15 ± 0,01	0,35 ± 0,08	0,14 ± 0,01
20:3 (n-6)	0,05 ± 0,01	0,06 ± 0,01	0,05 ± 0,01	0,03 ± 0,01	0,08 ± 0,02
20:4 (n-6)	0,37 ± 0,00	0,44 ± 0,03	0,38 ± 0,03	0,45 ± 0,05	0,52 ± 0,08
22:5(n-6)	0,23 ± 0,03	0,31 ± 0,07	0,23 ± 0,03	0,29 ± 0,01	0,23 ± 0,08
18:3 (n-3)	1,93 ± 0,05	2,15 ± 0,11	1,80 ± 0,07	1,03 ± 0,11	1,80 ± 0,42
18:4 (n-3)	10,61 ± 1,39	8,75 ± 1,40	9,63 ± 0,32	4,93 ± 0,44	9,70 ± 0,45
20:3 (n-3)	0,17 ± 0,02	0,23 ± 0,02	0,15 ± 0,02	0,14 ± 0,00	0,13 ± 0,00
20:4 (n-3)	1,37 ± 0,03	1,28 ± 0,31	1,20 ± 0,10	1,21 ± 0,03	1,37 ± 0,01
20:5 (n-3)	10,25 ± 0,05	12,70 ± 0,54	12,64 ± 0,40	15,65 ± 0,63	12,39 ± 0,41
22:5 (n-3)	0,65 ± 0,01	0,61 ± 0,03	0,61 ± 0,00	0,59 ± 0,18	0,71 ± 0,04
22:6 (n-3)	11,01 ± 1,95	17,83 ± 0,44	15,32 ± 2,75	25,29 ± 0,01	10,67 ± 1,48
∑PUFA	**39,59 ± 0,42**	**47,39 ± 0,51**	**44,84 ± 2,59**	**52,34 ± 0,26**	**40,64 ± 1,03**
∑n-6	**2,40 ± 0,03**	**2,85 ± 0,15**	**2,31 ± 0,05**	**2,67 ± 0,04**	**2,62 ± 0,11**
∑n-3	**35,98 ± 0,44**	**43,54 ± 0,61**	**41,36 ± 2,69**	**48,84 ± 0,09**	**36,77 ± 1,05**
(n-3)/(N-6)	**14,98 ± 0,01**	**15,28 ± 0,58**	**17,93 ± 1,54**	**18,27 ± 0,32**	**14,05 ± 0,17**

Table 31. Fatty alcohols composition (% of total fatty alcohols) in zooplankton.

	Egersund St 463 (n=2)	Utsira-Statpoint St 535 (n=2)	Utsira-Statpoint St 534 (n=2)	Viking Bank St 462 (n=2)	Tampen, st7 St 555 (n=2)
14:0 ALK	1,52 ± 0,60	6,68 ± 1,52	2,12 ± 1,02	5,54 ± 2,35	1,52 ± 0,16
16:0 ALK	9,69 ± 1,20	26,03 ± 0,85	13,96 ± 2,52	19,56 ± 3,52	11,39 ± 0,20
18:0 ALK	0,83 ± 0,10	1,90 ± 0,08	1,41 ± 0,18	2,39 ± 0,28	1,12 ± 0,22
20:0 ALK	0,34 ± 0,02	0,36 ± 0,04	0,40 ± 0,05	1,28 ± 0,82	0,36 ± 0,10
16:1 ALK	1,51 ± 0,07	3,39 ± 1,30	2,91 ± 1,14	1,85 ± 0,11	2,47 ± 0,14
18:1 (n-9) ALK	3,01 ± 0,21	3,98 ± 0,39	4,84 ± 0,79	2,73 ± 0,30	3,92 ± 0,41
18:1 (n-7) ALK	1,29 ± 0,08	2,01 ± 0,65	1,95 ± 0,26	2,88 ± 1,04	1,63 ± 0,29
18:1 (n-5) ALK	0,34 ± 0,03	0,60 ± 0,07	0,42 ± 0,04	0,32 ± 0,03	0,39 ± 0,00
18:2 (n-6) ALK	1,84 ± 0,16	2,41 ± 0,25	2,99 ± 0,69	2,01 ± 0,28	2,12 ± 0,02
18:3 (n-3) ALK	1,93 ± 0,13	2,35 ± 0,57	3,10 ± 1,00	3,03 ± 0,81	1,97 ± 0,11
20:1 (n-11) ALK	0,87 ± 0,50	0,64 ± 0,15	0,79 ± 0,12	0,35 ± 0,06	0,65 ± 0,06
20:1 (n-9) ALK	24,24 ± 0,35	17,99 ± 0,56	19,73 ± 2,54	20,76 ± 0,73	23,70 ± 1,95
22:1 (n-11) ALK	48,54 ± 1,83	25,69 ± 6,81	39,78 ± 5,19	31,01 ± 0,30	44,34 ± 0,71
22:1 (n-9) ALK	2,88 ± 0,59	4,43 ± 1,05	4,49 ± 0,02	4,52 ± 1,12	3,27 ± 1,72
22:1 (n-7) ALK	1,17 ± 0,16	1,54 ± 0,78	1,12 ± 0,07	1,76 ± 0,26	1,13 ± 0,44

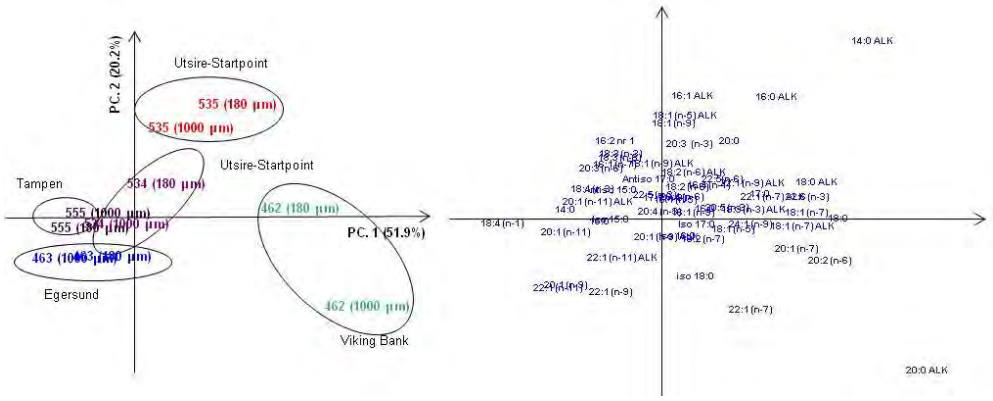


Figure 29. PCA plot of FA and FA-OH profiles in zooplankton. Left: Loading plot, right: Score plot.

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7 Appendix

7.1 Methods

7.1.1 NPD/PAH analysis of liver tissue

Wet liver 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 in concentration of 0.2 ng/g. For some compounds the detection limit are higher, because of background problems. 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.

7.1.2 Analysis of NPD/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 analytes 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). The following masses were scanned for in SIM mode (methyl-naphthols in cursive are coeluating on the GC):

	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

7.1.3 ELISA analyses of CYP1A content in liver

Buffer for homogenising

0,1 M sodiumphosphate ($NaH_2PO4 \cdot H_2O$), 0,15 M potassiumchloride, 1 mM EDTA, 1 mM DTT, 10% v/v glycerol, pH 7,4.

Homogenising of liver and preparation of postmitochondrial supernatant (PMS)

Approx. 0,5 g liver vas added homogenising buffer (2 ml pr 0.5 g liver) and homogenised with use of Potter Elvehjem homogeniser (7 strokes). The homogenate was transferred to Eppendorf vials and centrifuged for 20 min at 12.000xg, 4˚C. Samples were stored at -80˚C.

Measurements of protein content

Performed according to Bradford (1976). PMS-fraction of fish liver was diluted 1:1000 in dH2O. 50 µl of sample (in triplicate) was added ELISA-plate (Nunc 96 wells, flat bottom). 300 µl Coomassie G-250 / 17% phosphoric acid (1:1) was added the samples and incubated for 5 min. Absorbance was measured at 595 nm by plate reader (Tecan SPECTRA Fluor). Protein concentration determined by standard curve with bovine serum albumin.

ELISA

Performed as described in Nilsen et al. (1998). 1 µg total protein added per well, 4 parallels per sample, divided on two plates. For measurements of CYP1A1 in cod liver we used monoclonal mouse anti-cod CYP1A (NP-7, Biosense, Norway), diluted 1:1000. For CYP1A measurements in haddock, we used polyclonal rabbit anti-trout CYP1A (CP-226, Biosense, Norway), diluted 1:1000. For secondary antibodies we used polyclonal goat anti-mouse/rabbit from DacoCytomation, Denmark, diluted 1:2000. Plates were incubated with TMB substrate for 22.5 minutes before addition of 0.5 M H_2SO_4 and absorbance read at 450 nm.

7.1.4 Thiobarbituric acid reactive substances (TBARS) in liver

Lipid peroxidation was measured colourimetrically as thiobarbituric acid reactive substances using a modification of the method of Schmedes and Hölmer (1989).

After lipid extraction of 0.3 g frozen (-80°C) tissue sample in 4 ml of a solution containing 66% (v/v) chloroform: methanol (2:1), 0.005% butylated hydroxytoluene, and 1mM EDTA (in an N_2 atmosphere), 2 ml aliquots of the water extract were added to 2 ml TBA reagent (5% TCA, 1% thiobabituric acid in 0.25 N HCl). The absorbance of the sample was compared to that of a malondialdehyde standard curve at 532 nm with a spectophotometer (Shimadzu, Graplicord, UV 240, Japan). The standard curve was prepared by dissolving 50 µl 1,1,-3,3,-tetraethoxyporpon in 50 ml 0.1 M HCl (0.1 mM malondialdehyde stock solution) which was diluted in TBA reagent to give a 0, 2.5, 5 7.5, 10, and 12.5 mM malondialdehyde standard solution.

7.1.5 Muscle α-tocopherol

Vitas AM-171 – Determination of Tocopherol in fish filet by HPLC- Fluorescence detection (FLD).

Fish filet, thawed in fridge over night, are cut with scissors, added distilled water and homogenized with Ultra Turrax. Samples are weighed into vials and protein is precipitated and tocopherols extracted with isopropanol added internal standard (tocol). After thorough mixing and subsequent centrifugation, an aliquot of the isopropanol phase is injected into the HPLC-FLD.

Analysis is performed on a 1100-series HPLC with a G1314A fluoroscence detector (ex:295, em:330)(Agilent Technologies, Palo Alto, CA). Separations is performed on a Zorbax SB-C18 (50 mm × 4.6 mm i.d. × 1.8 µm film thickness) column from Agilent.

The concentration of α-tocopherol in the muscle was expressed as µg α-tocopherol per g tissue (wet weight).

7.1.6 DNA adduct analyses

DNA extraction

The procedure is to extract purified DNA after isolation of the cell nuclei in the samples. It is applicable to any type of biological sample containing DNA, from 50 to 100 mg of tissue (such as "liver") or any cell pellet.

● Process for tissues treatment
- On the ice, finely cut tissue (take 70 to 80 mg)
- Add 1.5 ml of sucrose 0.32 M and mix thoroughly to lyse tissue (Tissue lyser, Qiagen: 20 Hz, 2 minutes)
- Centrifuge at 800G for 10 Minutes, at +4 °C

● Dissolve the pellet with 1.2 ml of EDTA / Tris (1 / 20 mM. pH 7.4)
Add 100 µl of 10% SDS solution and vortex for 1 minute.

● Incubate 30 minutes at 37 °C with:
0.2 mg / ml RNase A
33.4 U RNase T1

● Incubate 2.5 hours at 37 °C with 0.50 mg / ml proteinase K
(Until complete digestion of samples)

● Add 0.5 volume (0.7 ml) of saturated phenol and vortex 1 minute
Centrifuge 5 minutes at 5000 rpm.

● Remove the upper phase (aqueous phase) and transfer it to a clean tube
Add 0.5 volume (0.7 ml) of CIP (phenol + Sevag 1 / 1) and vortex 1 minute
Centrifuge 5 minutes at 5000 rpm (+4 ° C)

● Remove the upper phase and transfer it to a clean tube
Add 0.5 volume of Sevag (chloroform + isoamyl alcohol (1 / 24)) and vortex 1 minute
Centrifuge 5 minutes at 5000 rpm (+4 ° C)

● Remove the upper phase

● **Precipitation of DNA:**

Add to the aqueous phase 0.1 volumes of a solution of NaCl 5 M and 2 volumes of cold ethanol (stored at -20 ° C)
Shake and vortex lightly manually

● Allow to air dry the DNA. Add 150 µl of ultra pure water.

● **Spectrophotometric quantification of DNA solutions** (Nanodrop, Thermo Scientific)

- Spectrophotometric assay:
Principle: 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

- Prepare solutions close to 2 µg / µl
- Keep these solutions at -80 °C in glass vials (type 2 ml)

Procedure for DNA adduct detection

2.1 Biological material

In order to allow a search of DNA adducts by the described ^{32}P post-labelling protocol, biological material supplied must meet requirements in both quantitative (2 x 5 mg DNA about 15 to 25 mg tissue and / or 5x10^6 to 10^7 cells) and qualitative aspects (cell richness of the tissue samples)

2.2 Procedure for ^{32}P post-labelling

As result of the technical variability classically described with the ^{32}P post-labelling method, each sample was analysed twice in two independent manipulations (runs). Four controls are systematically added to the manipulations to check the successful completion of the manipulation. The two first control samples are one negative in 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 are realised by 32P-labelling of 1) normal nucleotides (deoxyadenosine 3'phosphate, control of labelling by polynucleotide kinase) and 2) a small fraction of DNA (1 µg) coming from the negative control (verification of DNA hydrolysis efficiency).

2.2.1 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
  + Buffer solutions
  } 3.5 hours / 37°C

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

2.2.2 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)

2.2.3 ^{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)

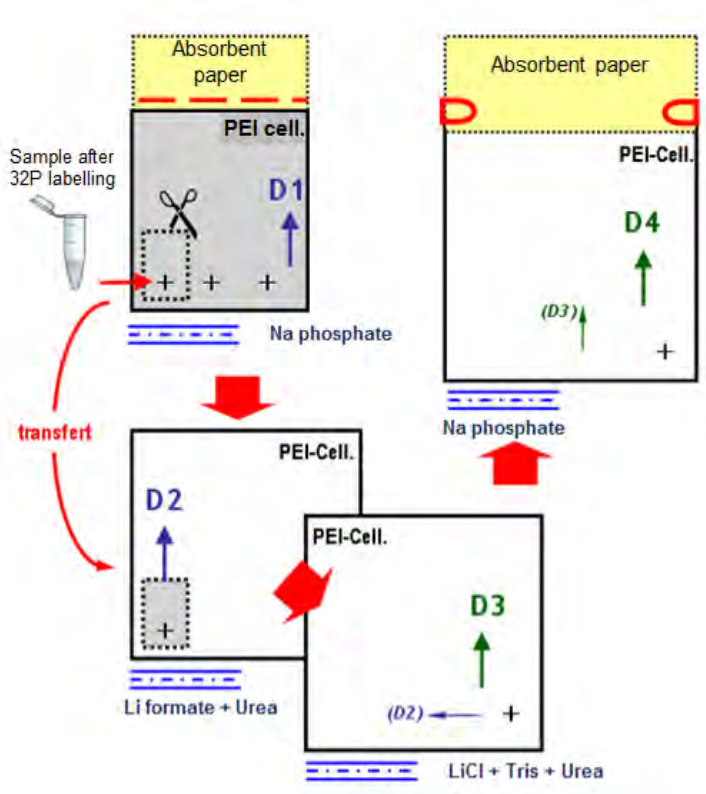
2.2.4 Chromatographic separation

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

PEI-cellulose sheet (Macherey-Nagel)

• 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

Revealing of DNA adducts
DNA adduct patterns are revealed 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
The quantification is 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 contains 110.7 adducts for 10^8 normal nucleotides, according to Phillips and Castegnaro, 1999, kindly provided by F.A. Beland, FDA, USA).

The results are given in two complementary approaches:

• Quantitative Approach:
- Results in relative levels of adducts (= RAL)
- By interest: Results per spot or per sample.
- Statistical Analysis

• Qualitative approach:
- Analysis of spots of interest in potential patterns
- Statistical analysis (presence / absence of a spot under the experimental conditions ...)
The exploitation of the results is made on the basis of two analysis per sample in two different manipulations.

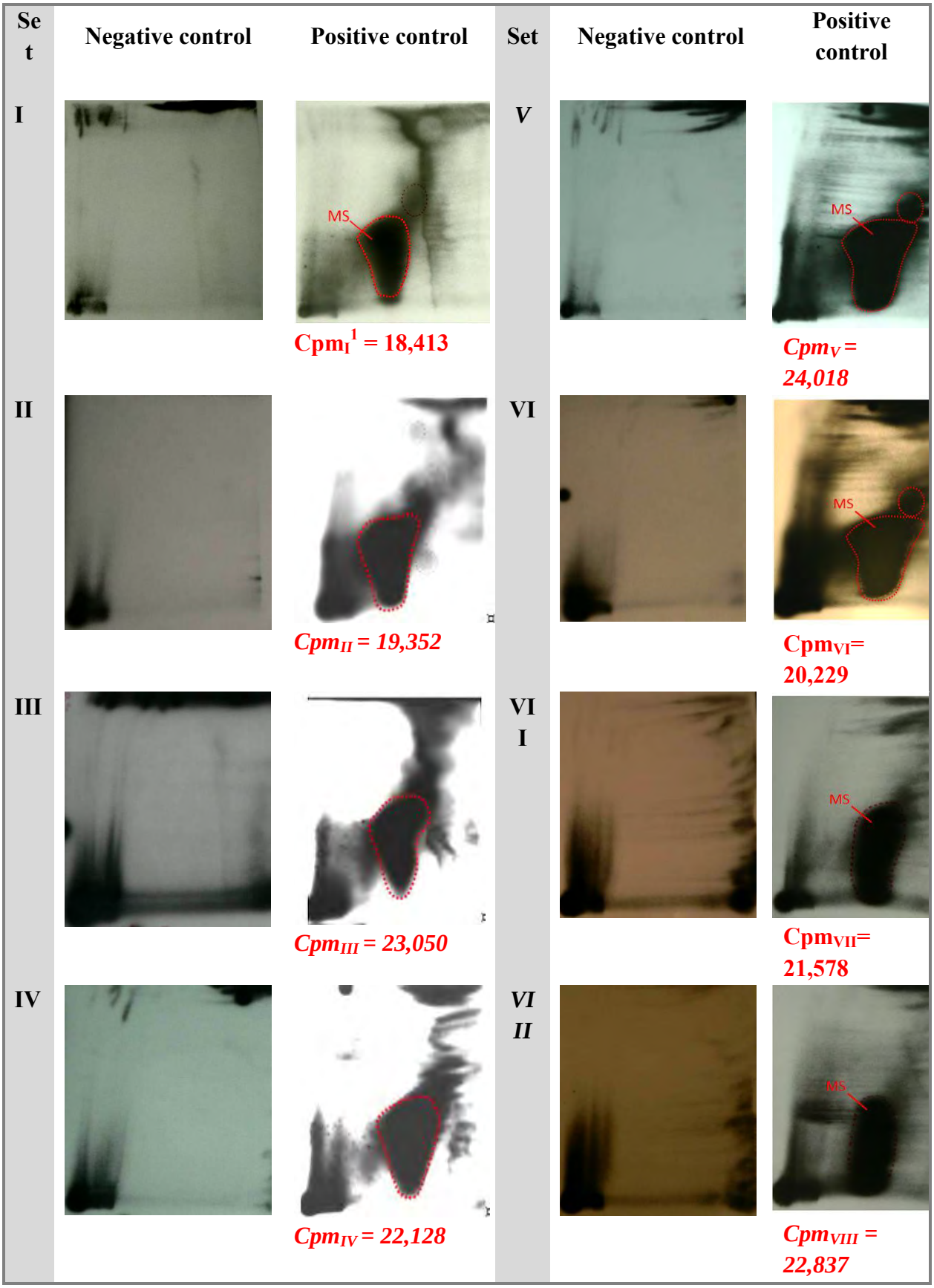


Figure 1-Appendix. Autoradiographic patterns of the negative and positive controls included in each set of 32P-postlabelling (sets I to XVI).

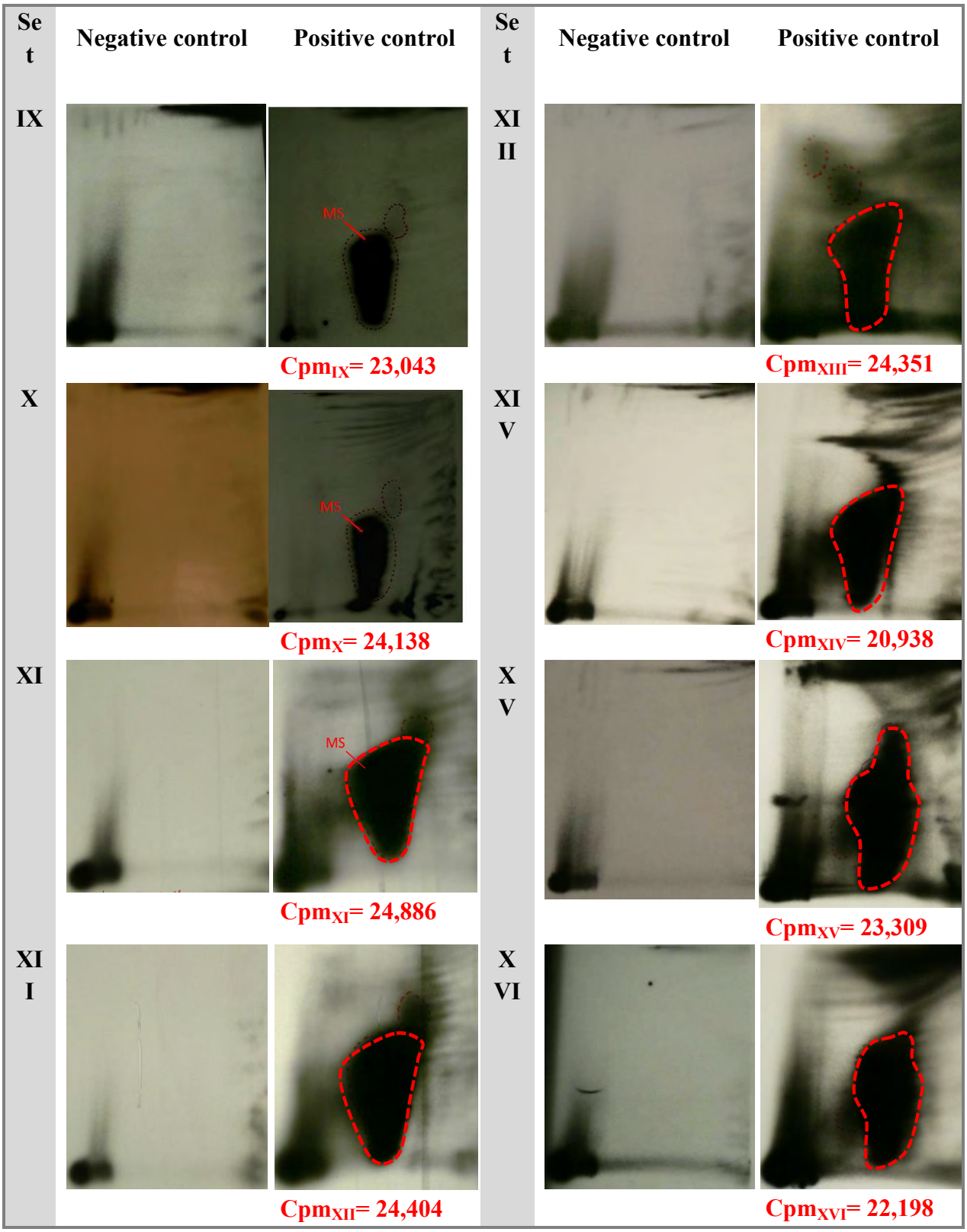


Figure 1-Appendix (Continued). Autoradiographic patterns of the negative and positive controls included in each set of 32P-postlabelling (sets I to XVI).

1: 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 1107.0 adducts for 10^9 normal nucleotides (according to F.A. Beland, in Philips and Castegnaro, 1999)
Negative control: DNA extracted from AG1521 fibroblasts.

7.1.7 Lipid extraction and lipid class separation.

A new extraction methods combined two step extraction, using hexane/methanol to extract neutral lipids and chloroform/methanol to extract polar lipids, has been developed and validation. Four times methanol wash were done to hexane/methanol extract and merged with chloroform/methanol extract to ensure optimized lipid classes distribution between two solvent systems. The glass aminopropyl bonded column was used to fractionate the lipid classes into: neutral lipids (NL), free fatty acids (FFA), phosphatidylcholine + phosphatidylethanolamine (PC/PE) and phosphatidylserine + phosphatidylinositol (PS/PI). The results show that 99% of neutral lipids were extracted into hexane while the chloroform/methanol remained 95 % of PC/PE and 88% of PS/PI. According to verification of thin-layer chromatography (TLC) and gas chromatography (GC) results, the solid phase extraction (SPE) separated the lipid classes effectively with good recoveries. The fatty acids profiles were compared with Folch extraction.

Total lipid were extracted from haddock and cod samples (0.5 g) by a combined two step extraction, using hexane/methanol to extract neutral lipids and chloroform/methanol to extract polar lipids in a modified Folch method (Folch *et al*., 1957). An aliquot of the sample was separated into four different lipid classes: neutral lipids (NL: triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG), cholesterol and cholesterol esters); free fatty acids (FFA); phosphatidylcholin (PC) + phosphatidylethanolamine (PE) and phosphotydylserine (PS) + phosphatidylinisitol (PI).

Solid Phase Extraction (SPE) Procedure

The SPE procedure was adapted from the research result of Perez-Palacios *et al.* (2007) using aminopropyl bonded phase columns to separate lipid mixtures into individual classes.
Briefly, 0.5 ml of each extract (approximately 8 mg lipid) was loaded in a 500 mg aminopropyl modified silica minicolumn (Macherey-Nagel GMBH & Co. Germany), which had been previously activated with 4 ml of hexane. Neural lipid (NL), free fatty acid (FFA), and phosphatidylcholine + phosphatidylethanolamine (PC/PE) were sequentially eluted with 7 ml of chloroform/isopropanol (2:1, v/v), 5 ml of 2% acetic acid in diethyl ether, and 10 ml of methanol. The eluates were saved in 15 ml thick-walled glass tubes with teflon lined screw caps, which contained nonadecanoic acid (19:0) as internal standard. The phosphatidylserine + phosphatidylinositol (PS/PI) fraction was collected by methylating the stationary phase of column directly. Blank column eluates were collected periodically without loading samples. All the eluates were evaporated to dry by nitrogen gas and stand by for the thin-layer chromatography (TLC) and gas chromatography (GC) analysis.

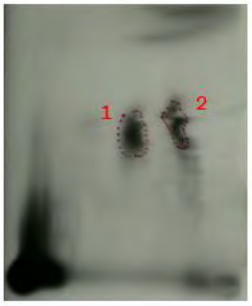
Fatty acids analysis

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

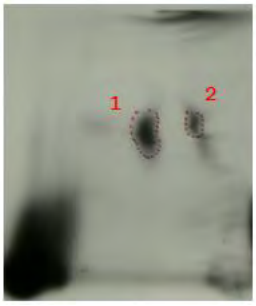
Statistical analyses

One-way ANOVA and Tukey (HSD) test as a post-hoc test. The Principle Component Analysis (PCA) was carried out using Sirius (Version 7.1, Bergen, Norway).

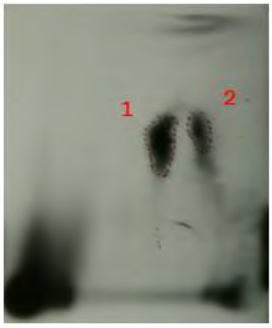
7.2 Representative DNA adduct patterns



Sample n°63

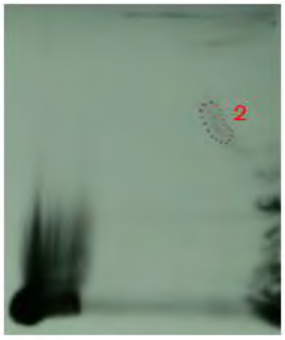


Sample n°64



Sample n°65

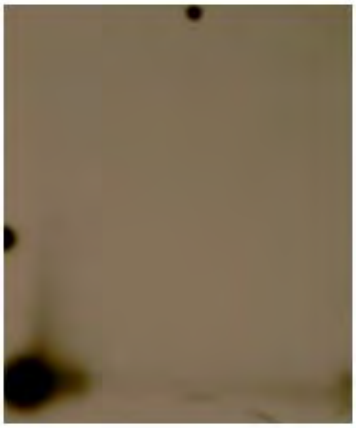
Sample n°71



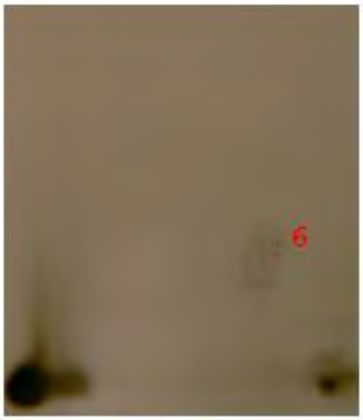
Sample n°72

Figure 1-Appendix 7.2. Representative DNA adduct patterns (Station H1, females).
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 9). Time of autoradiographic exposure (ToE): up to 72 h.

Sample n°44



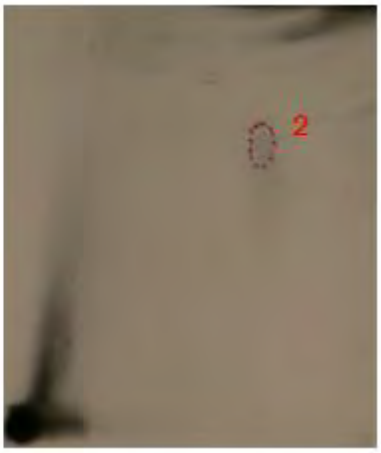
Sample n°45



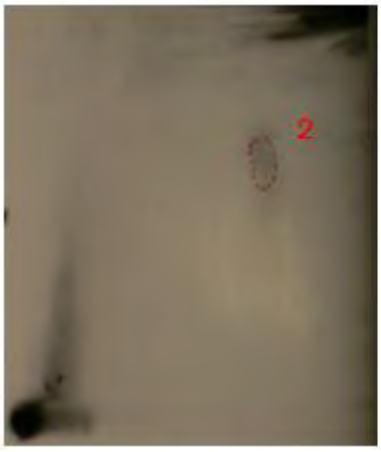
Sample n°49

Figure 1-Appendix 7.2. (continued): Representative DNA adduct patterns (Station H2, females). 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 in Figure 9). Time of autoradiographic exposure (ToE): up to 72 h.

Sample n°54



Sample n°55



Sample n°56

Sample n°58

Sample n°59

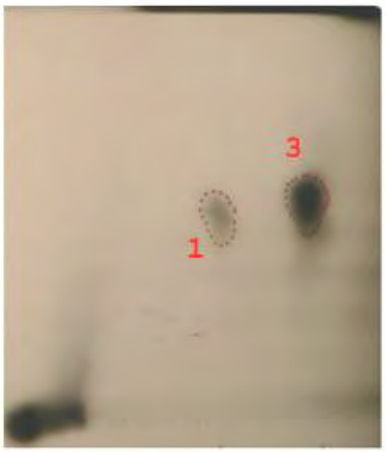
Figure 1-Appendix 7.2. (continued). Representative DNA adduct patterns (Station H4, females). For represented samples, autoradiographic pattern is one among both realised (two analyses per sample). Spots are numbered according to their location on PEI-cellulose plates (see template at Figure 9). Time of autoradiographic exposure (ToE): up to 72 h.

Sample n°32

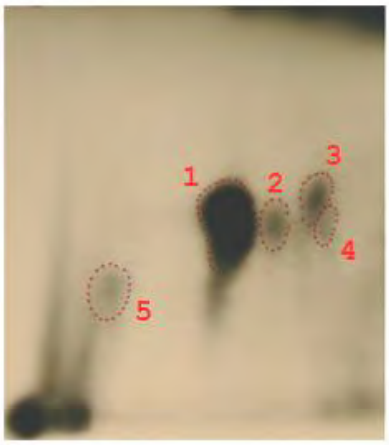
Sample n°33

Sample n°34

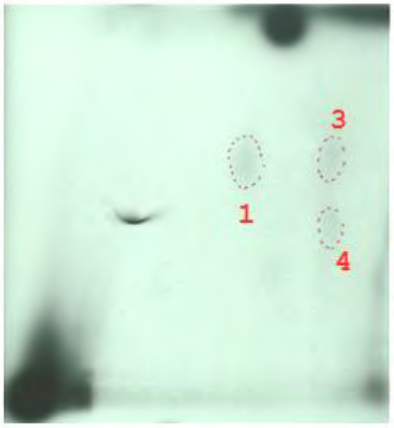
Figure 1-Appendix 7.2. (continued). Representative DNA adduct patterns (Station H5, females). For represented samples, autoradiographic pattern is one among both realised (Two analyses per sample). Spots are numbered according to their location on PEI-cellulose plates (see template at Figure 9). Time of autoradiographic exposure (ToE): up to 72 h.



Sample n°12

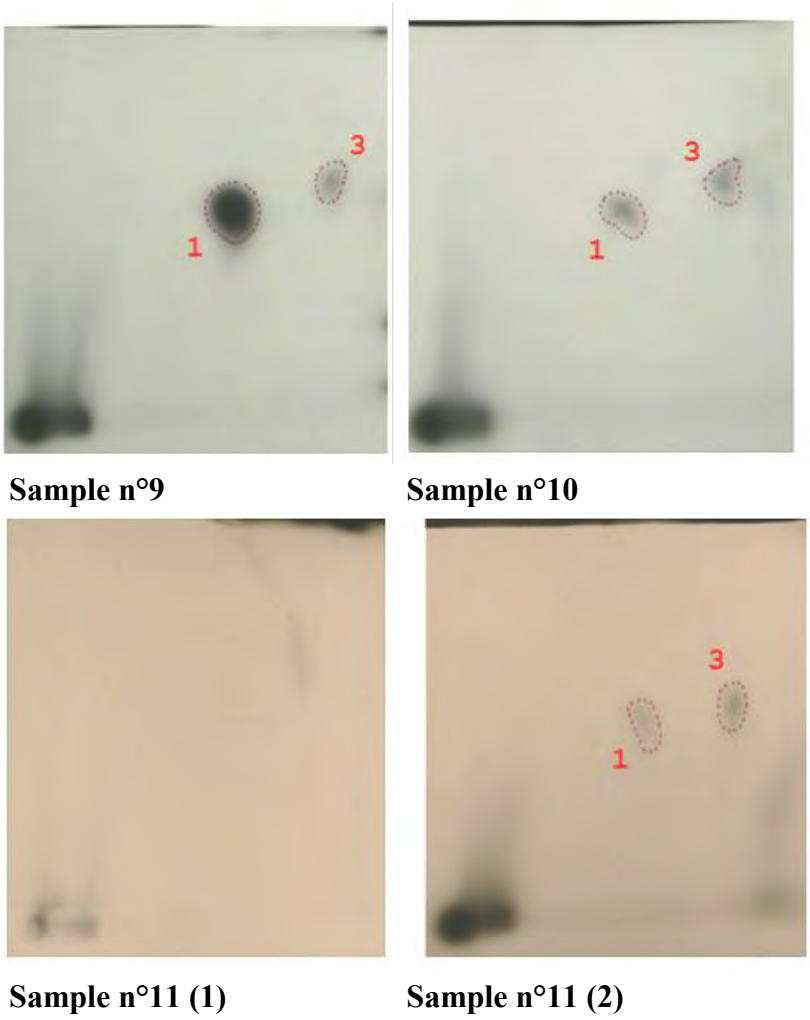


Sample n°17



Sample n°23

Figure 1-Appendix 7.2. (Continued). Representative DNA adduct patterns (Station H6, females). 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 9). Time of autoradiographic exposure (ToE): up to 72 h.



Sample n°9 **Sample n°10**

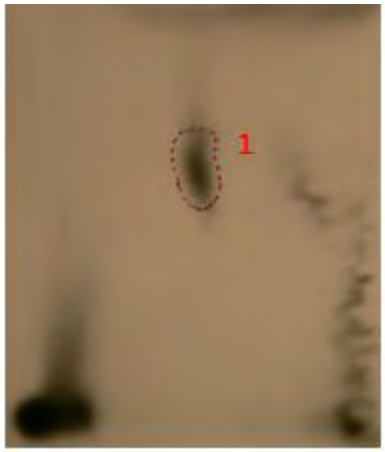
Sample n°11 (1) **Sample n°11 (2)**

Figure 1-Appendix 7.2. (continued). Representative DNA adduct patterns (Station H7, females). 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 9). Time of autoradiographic exposure (ToE): up to 72 h.

Sample n°105

Sample n°112

Sample n°113



Sample n°114

Sample n°115

Figure 2-Appendix 7.2. Representative DNA adduct patterns (Station H1, males). 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 9). Time of autoradiographic exposure (ToE): up to 72 h.

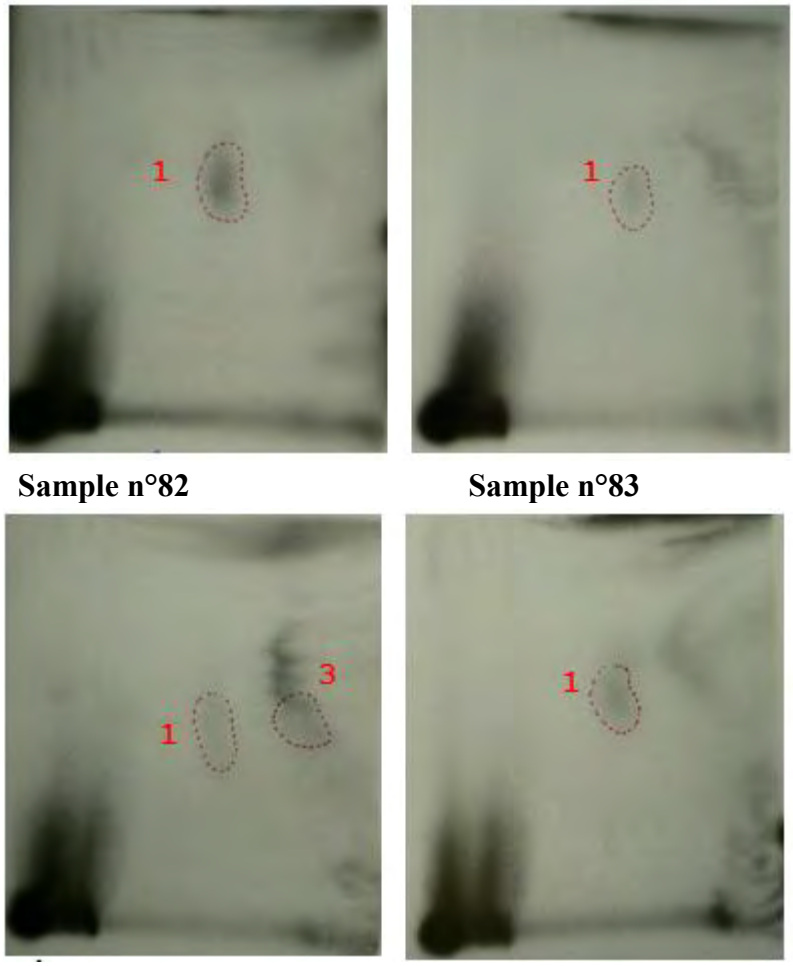


Figure 2-Appendix 7.2. (continued).
Representative DNA adduct patterns (Station H2, males).
For represented samples, autoradiographic pattern is one among both realised (Two analyses per sample). Spots are numbered according to their location on PEI-cellulose plates (see template at Figure 9). Time of autoradiographic exposure (ToE): up to 72 h.

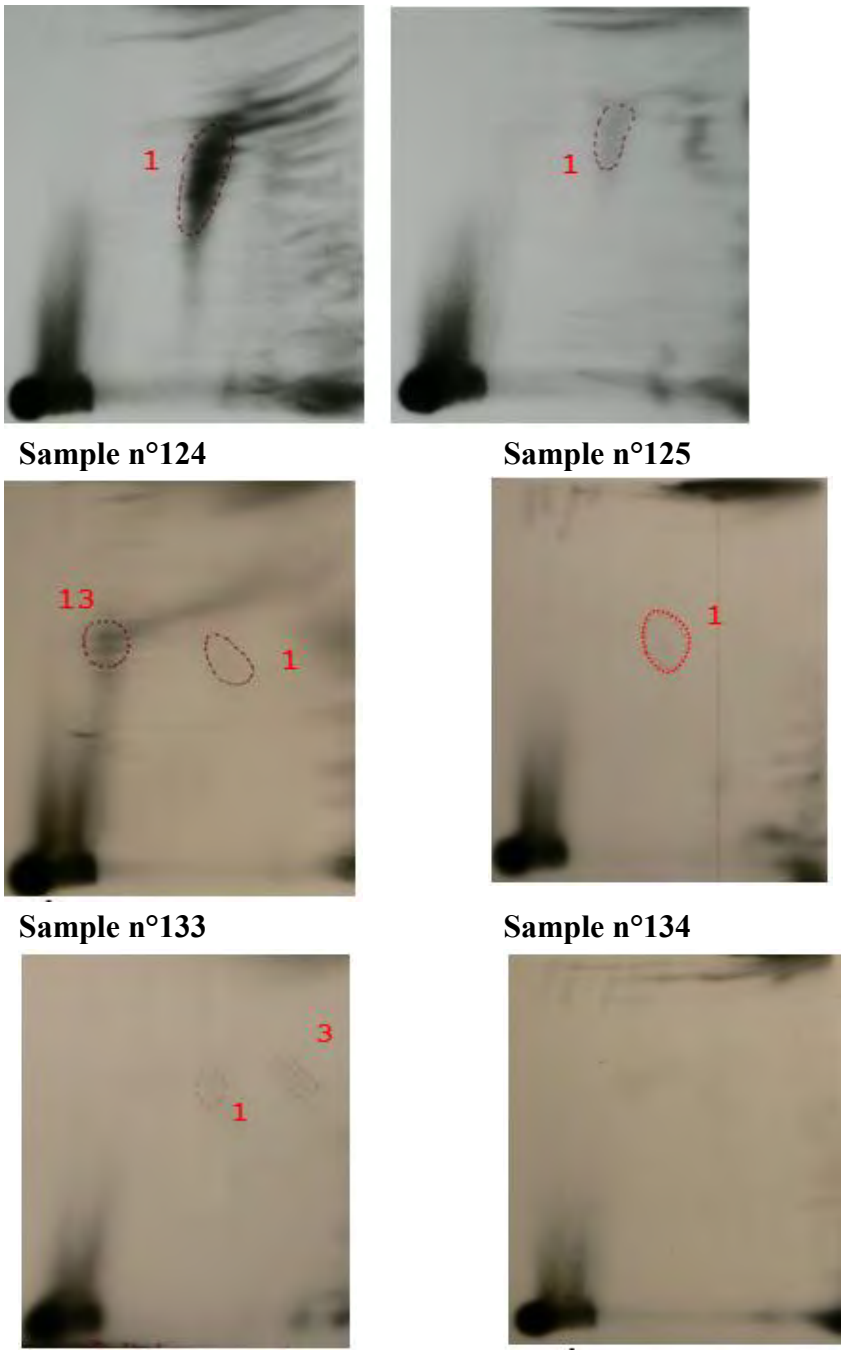
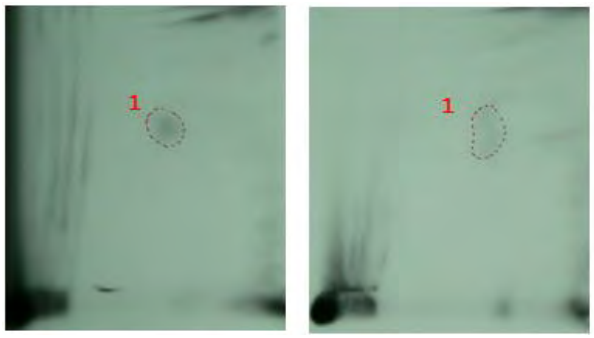
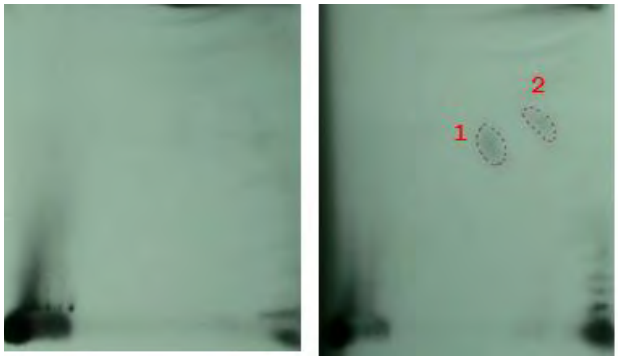


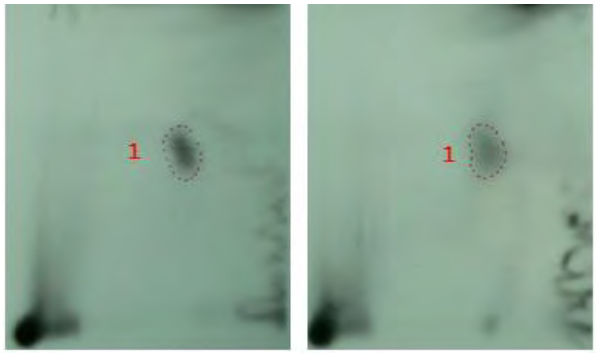
Figure 2-Appendix 7.2. (continued). Representative DNA adduct patterns (Station H4, males). For represented samples, autoradiographic pattern is one among both realised (Two analyses per sample). Spots are numbered according to their location on PEI-cellulose plates (see template at Figure 9). Time of autoradiographic exposure (ToE): up to 72 h.



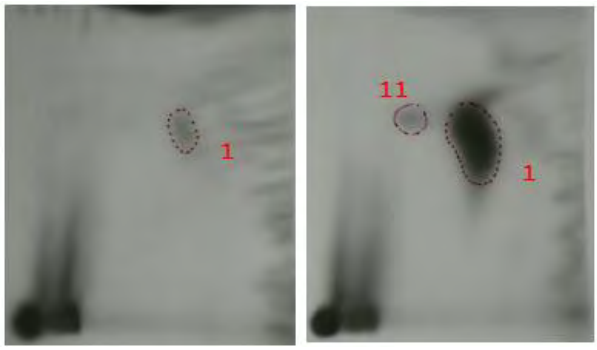
Sample n°93 **Sample n°94**



Sample n°95 **Sample n°97**



Sample n°98 **Sample n°99**



Sample n°101 **Sample n°102**

Figure 2-Appendix 7.2. (continued). Representative DNA adduct patterns (Station H5, males). 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 9). Time of autoradiographic exposure (ToE): up to 72 h.

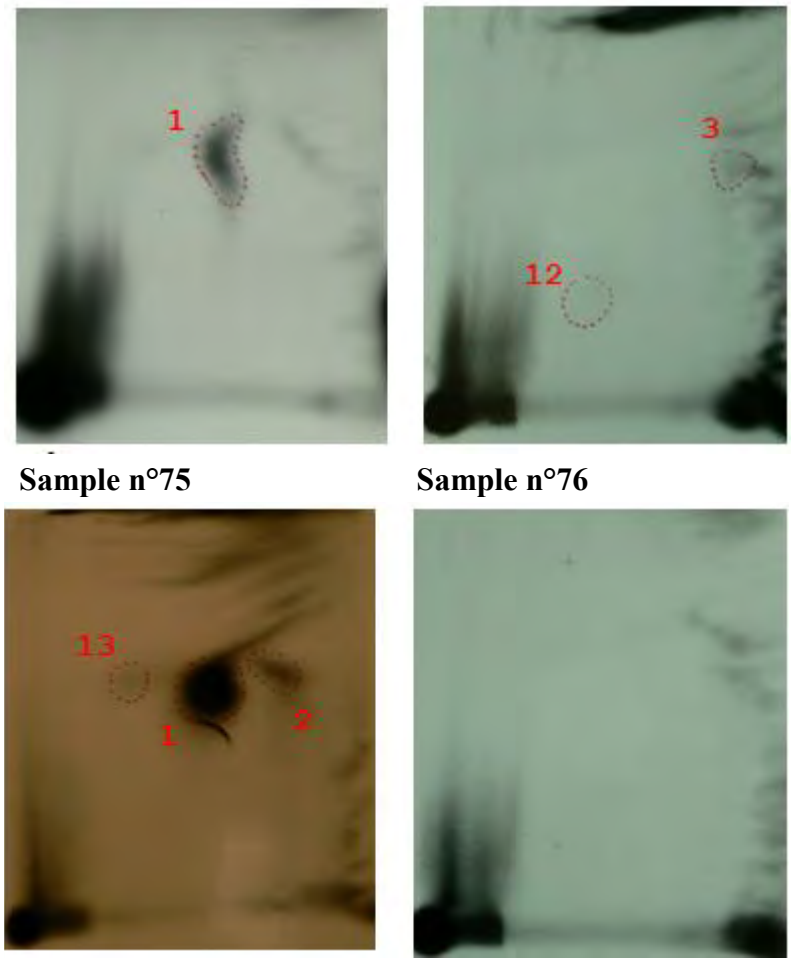
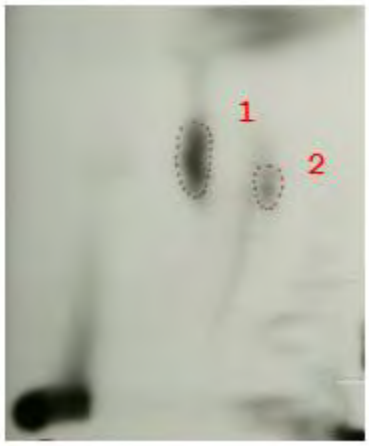


Figure 2-Appendix 7.2 (continued). Representative DNA adduct patterns (Station H6, males). For represented samples, autoradiographic pattern is one among both realised (two analyses per sample). Spots are numbered according to their location on PEI-cellulose plates (see template at Figure 9). Time of autoradiographic exposure (ToE): up to 72 h.



Sample n°118 **Sample n°119**

Sample n°120

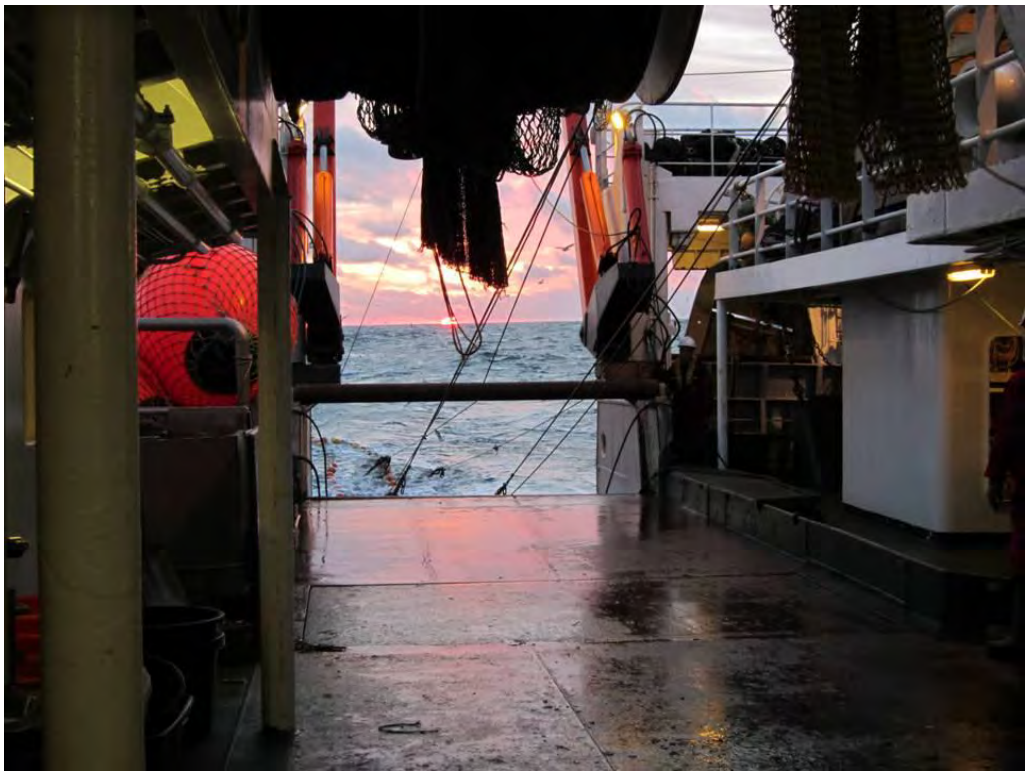
Figure 2-Appendix 7.2. (continued). Representative DNA adduct patterns (Station H7, males). For represented samples, autoradiographic pattern is one among both realised (Two analyses per sample). Spots are numbered according to their location on PEI-cellulose plates (see template at Figure 9). Time of autoradiographic exposure (ToE): up to 72 h.

7.3 DNA adduct levels in liver of haddock.

Station	Area	Fish no	Sex (F, M)	1 run	2 run	Mean RAL
1	Egersund Bank	2	F	<0.1	<0.1	<0.1
1	Egersund Bank	3	F	7.6	7.8	7.7
1	Egersund Bank	6	F	12.9	18.4	15.65
1	Egersund Bank	9	F	9.0	13.8	11.4
1	Egersund Bank	11	F	12.2	22.2	17.2
1	Egersund Bank	14	F	3.5	4.0	3.75
1	Egersund Bank	15	F	<0.1	< 0.1	< 0.1
1	Egersund Bank	18	F	6.7	8.3	7.5
1	Egersund Bank	20	F	<0.1	<0.1	<0.1
1	Egersund Bank	21	F	<0.1	<0.1	<0.1
1	Egersund Bank	22	F	<0.1	<0.1	<0.1
1	Egersund Bank	23	F	4.0	8.1	6.05
1	Egersund Bank	27	F	<0.1	<0.1	<0.1
1	Egersund Bank	30	F	<0.1	<0.1	<0.1
1	Egersund Bank	1	M	<0.1	<0.1	<0.1
1	Egersund Bank	5	M	21.3	12.3	16.8
1	Egersund Bank	8	M	<0.1	<0.1	<0.1
1	Egersund Bank	10	M	<0.1	<0.1	<0.1
1	Egersund Bank	12	M	5.4	8.8	7.1
1	Egersund Bank	13	M	2.5	5.7	4.1
1	Egersund Bank	17	M	0.5	1.6	1.05
1	Egersund Bank	19	M	4.7	2.9	3.8
1	Egersund Bank	24	M	<0.1	<0.1	<0.1
1	Egersund Bank	26	M	<0.1	<0.1	<0.1
1	Egersund Bank	28	M	<0.1	<0.1	<0.1
1	Egersund Bank	29	M	8.7	5.4	7.05
1	Egersund Bank	32	M	15.9	9.6	12.75
1	Egersund Bank	34	M	27.9	28.1	28.0
1	Egersund Bank	35	M	6.6	11.8	9.2
2	Southern North Sea	8	M	<0.1	<0.1	<0.1
2	Southern North Sea	12	M	1.6	1.2	1.4
2	Southern North Sea	13	M	0.8	<0.1	0.45
2	Southern North Sea	15	M	1.5	0.9	1.2
2	Southern North Sea	18	M	2.5	1.1	1.8
2	Southern North Sea	20	M	3.3	3.3	3.3
2	Southern North Sea	21	M	2.1	1.1	1.6
2	Southern North Sea	22	M	1.0	0.6	0.8
2	Southern North Sea	23	M	0.8	1.4	1.1
2	Southern North Sea	24	M	<0.1	<0.1	<0.1
2	Southern North Sea	1	F	3.3	2.0	2.65
2	Southern North Sea	4	F	<0.1	<0.1	<0.1
2	Southern North Sea	14	F	3.6	2.0	2.8
2	Southern North Sea	16	F	1.7	1.0	1.35
2	Southern North Sea	17	F	1.9	1.2	1.55
2	Southern North Sea	19	F	4.7	2.0	3.35
2	Southern North Sea	25	F	3.3	2.6	2.95
4	Bressay Bank	2	F	6.2	3.4	4.8
4	Bressay Bank	6	F	2.0	1.0	1.5
4	Bressay Bank	9	F	3.0	0.9	1.95
4	Bressay Bank	12	F	<0.1	<0.1	<0.1
4	Bressay Bank	16	F	1.1	<0.1	0.6
4	Bressay Bank	18	F	2.0	<0.1	1.05
4	Bressay Bank	20	F	1.9	<0.1	1.0

4	Bressay Bank	22	F	33.4	23.6	28.5
4	Bressay Bank	23	F	6.0	3.6	4.8
4	Bressay Bank	25	F	5.1	3.2	4.15
4	Bressay Bank	1	M	25.3	22.5	23.9
4	Bressay Bank	2	M	2.1	4.9	3.5
4	Bressay Bank	4	M	3.0	1.1	2.05
4	Bressay Bank	7	M	3.4	4.4	3.9
4	Bressay Bank	8	M	1.5	0.5	1.0
4	Bressay Bank	10	M	2.9	4.7	3.8
4	Bressay Bank	11	M	4.6	6.5	5.55
4	Bressay Bank	13	M	5.9	2.2	4.05
4	Bressay Bank	14	M	15.5	9.0	12.25
4	Bressay Bank	15	M	4.5	2.9	3.7
4	Bressay Bank	17	M	1.0	1.4	1.2
4	Bressay Bank	19	M	1.4	1.2	1.3
4	Bressay Bank	21	M	5.0	3.9	4.45
4	Bressay Bank	24	M	<0.1	<0.1	<0.1
5	Tampen Down stream Gullfaks	2	M	<0.1	<0.1	<0.1
5	Tampen Down stream Gullfaks	3	M	2.5	3.2	2.85
5	Tampen Down stream Gullfaks	4	M	0.5	1.0	0.75
5	Tampen Down stream Gullfaks	12	M	1.6	0.7	1.15
5	Tampen Down stream Gullfaks	14	M	<0.1	<0.1	<0.1
5	Tampen Down stream Gullfaks	15	M	3.0	1.0	2.0
5	Tampen Down stream Gullfaks	17	M	2.5	2.4	2.45
5	Tampen Down stream Gullfaks	19	M	9.1	3.8	6.45
5	Tampen Down stream Gullfaks	20	M	6.6	2.6	4.6
5	Tampen Down stream Gullfaks	23	M	<0.1	<0.1	<0.1
5	Tampen Down stream Gullfaks	24	M	2.0	1.7	1.85
5	Tampen Down stream Gullfaks	25	M	36.3	12.9	24.6
5	Tampen Down stream Gullfaks	1	F	<0.1	<0.1	<0.1
5	Tampen Down stream Gullfaks	5	F	1.0	1.4	1.2
5	Tampen Down stream Gullfaks	6	F	4.9	7.1	6.0
5	Tampen Down stream Gullfaks	7	F	<0.1	<0.1	<0.1
5	Tampen Down stream Gullfaks	8	F	<0.1	<0.1	<0.1
5	Tampen Down stream Gullfaks	9	F	2.3	5.2	3.75
5	Tampen Down stream Gullfaks	10	F	2.7	2.6	2.65
5	Tampen Down stream Gullfaks	11	F	<0.1	2.3	1.2
5	Tampen Down stream Gullfaks	13	F	<0.1	<0.1	<,0.1
5	Tampen Down stream Gullfaks	16	F	<0.1	<0.1	<0.1
5	Tampen Down stream Gullfaks	18	F	3.2	4.9	4.05
5	Tampen Down stream Gullfaks	21	F	8.2	5.5	6.85
5	Tampen Down stream Gullfaks	22	F	35.9	34.6	35.25
6	Viking Bank	1	F	13.0	13.9	13.45
6	Viking Bank	2	F	4.3	3.0	3.65
6	Viking Bank	3	F	3.5	9.8	6.65
6	Viking Bank	6	F	12.7	17.4	15.05
6	Viking Bank	10	F	18.5	19.1	18.8
6	Viking Bank	11	F	355.9	243.7	299.8
6	Viking Bank	12	F	18.1	11.2	14.65
6	Viking Bank	13	F	6.1	9.0	7.55
6	Viking Bank	14	F	3.7	5.4	4.55
6	Viking Bank	15	F	5.6	2.4	4.0
6	Viking Bank	16	F	12.7	1.3	7.0
6	Viking Bank	18	F	2.2	0.5	1.35
6	Viking Bank	19	F	8.5	3.1	5.8
6	Viking Bank	20	F	4.6	3.2	3.9
6	Viking Bank	21	F	4.1	4.3	4.2
6	Viking Bank	22	F	9.2	8.0	8.6

6	Viking Bank	23	F	7.6	3.4	5.5
6	Viking Bank	24	F	5.4	4.5	4.95
6	Viking Bank	25	F	14.0	8.0	11.0
6	Viking Bank	4	M	18.2	29.5	23.85
6	Viking Bank	5	M	5.7	10.6	8.15
6	Viking Bank	7	M	12.6	14.3	13.45
6	Viking Bank	8	M	0.9	2.2	1.55
6	Viking Bank	9	M	0.8	1.2	1.0
6	Viking Bank	17	M	<0.1	<0.1	<0.1
7	Tampen Between Statfjord and Gullfaks	1	F	8.9	5.4	7.15
7	Tampen Between Statfjord and Gullfaks	3	F	8.8	19.5	14.15
7	Tampen Between Statfjord and Gullfaks	4	F	10.7	16.7	13.7
7	Tampen Between Statfjord and Gullfaks	6	F	4.6	3.2	3.9
7	Tampen Between Statfjord and Gullfaks	7	F	<0.1	2.2	1.15
7	Tampen Between Statfjord and Gullfaks	9	F	15.3	12.2	13.75
7	Tampen Between Statfjord and Gullfaks	10	F	15.7	17.5	16.6
7	Tampen Between Statfjord and Gullfaks	11	F	7.5	5.9	6.7
7	Tampen Between Statfjord and Gullfaks	12	F	18.2	15.5	16.85
7	Tampen Between Statfjord and Gullfaks	14	F	8.3	4.9	6.6
7	Tampen Between Statfjord and Gullfaks	17	F	<0.1	2.0	1.05
7	Tampen Between Statfjord and Gullfaks	2	M	4.2	8.3	6.25
7	Tampen Between Statfjord and Gullfaks	5	M	<0.1	<0.1	<0.1
7	Tampen Between Statfjord and Gullfaks	8	M	4.2	2.2	3.2
7	Tampen Between Statfjord and Gullfaks	13	M	3.8	3.6	3.7
7	Tampen Between Statfjord and Gullfaks	15	M	7.3	5.3	6.3
7	Tampen Between Statfjord and Gullfaks	16	M	4.2	2.3	3.25



Sunset from R/V Johan Hjort. Photo BE Grøsvik.