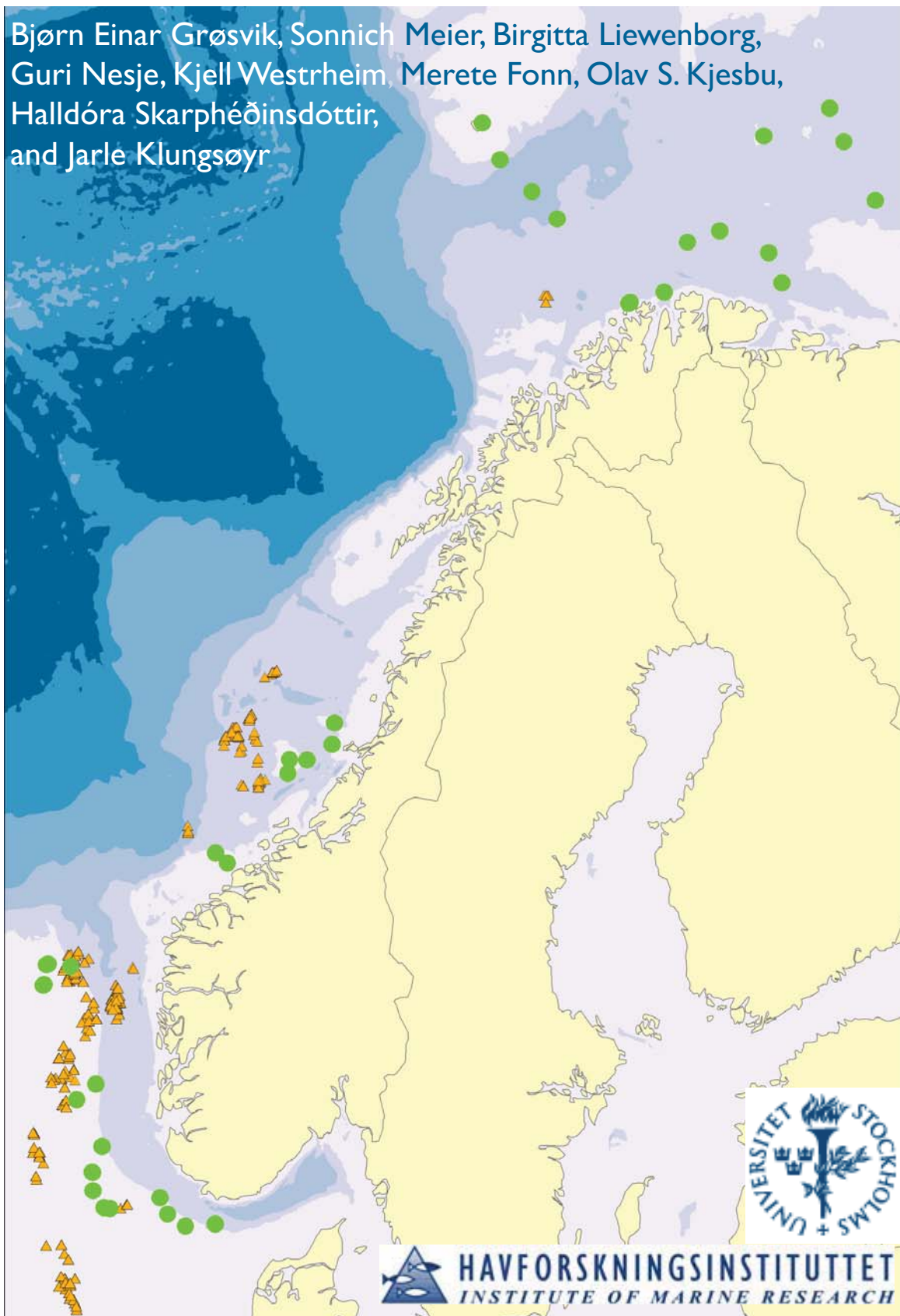


Condition monitoring in the water column 2008: Oil hydrocarbons in fish from Norwegian waters

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Oil hydrocarbons in fish from Norwegian waters

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Summary

This report has been prepared by Institute of Marine Research (IMR) & University of Stockholm (UoS) 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 objectives for this study have been:

1. Measure NPD/PAH in haddock liver from the Egersund Bank, the Halten Bank and the Barents Sea.
2. Determine to what extent fish from the oil installation areas at Tampen and the Halten Bank contain elevated levels of petroleum hydrocarbons compared with fish from reference areas at the Egersund Bank and in the Barents Sea by measurements of metabolites of PAH and alkylphenols in bile of haddock, saithe and long rough dab.
3. Measure vitellogenin levels in blood from male cod from the Egersund Bank, Tampen, the Halten Bank and the Barents Sea.
4. Study possible genotoxic effects in fish from Tampen compared with fish from the Egersund Bank/Ling Bank by measurements of hepatic DNA adducts.
5. Perform gonad histology on haddock and long rough dab to study whether differences can be observed between fish caught at Tampen compared with fish caught at the Egersund Bank and the Barents Sea.

Levels of NPD and PAH in haddock were low for the three regions. Sum NPD in liver of haddock 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.

The highest levels of sum PAH metabolites were measured in haddock from Tampen of 580 ng/g bile. Sum PAH metabolites at the Egersund Bank was 231, at the Halten Bank 199 and in haddock from the Barents Sea 35 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. Levels above LOQ were measured in all of haddock bile from Tampen (N=16). Levels of 1-hydroxy phenanthrene in haddock bile from the Egersund Bank was 133±207 ng/g, while levels from the Halten Bank and the Barents Sea were 43±71 and 19±14 ng/g, respectively. This is the first study on bile metabolites on haddock. Only low levels of PAH metabolites were measured in saithe and long rough dab.

Metabolites of alkylphenols were analysed for a high number of metabolites. Analysed fish included haddock from the Egersund Bank (N=23), Tampen (N=16), the Barents Sea (N=22) and the Halten Bank (N=16), in addition to saithe from the Egersund Bank (N=19) and Tampen (N=19) and long rough dab from the Barents Sea (N=21). In total 143 fish were analysed and most of the alkylphenol metabolites were found to be below LOQ.

The low levels of alkylphenols measured in bile is in accordance with the results from condition monitoring 2005 where levels of alkylphenols measured in cod liver, haddock liver and herring muscle from the Egersund Bank and Tampen regions demonstrated mostly levels below LOD for all stations (Grøsvik et al. 2008), and with the results from the 2002 monitoring (Klungsoyr et al. 2003).

Levels of DNA adducts were significantly higher in liver of haddock from Tampen compared to the Egersund Bank and the Barents Sea, indicative of more PAH exposure in this region. Higher levels of DNA adducts in haddock caught at Tampen compared with the Egersund Bank have earlier been reported by Klungsoyr *et al.* (2003) and by Grøsvik *et al.* (2008). DNA adducts from long rough dab was performed from the Egersund Bank and The Barents Sea. Only background levels were observed.

Levels of Vtg in blood of male cod were generally low from all regions and ranged from 0-5000 ng/ml. The results were in accordance with those obtained from the Egersund Bank/Ling Bank and Tampen in the condition monitoring of 2005 (Grøsvik at al., 2008) and of the work by Scott *et al.* (2006b).

Histological analyses of haddock ovaries showed no differences between the Egersund Bank and Tampen. Prevalence of connective tissue were absent in ovaries from the Barents Sea, while observed in ovaries from Tampen and the Egersund Bank.

Analyses of lipid content and fatty acid profiles in different lipid classes demonstrated significant differences in lipid amount in the livers of haddock from Tampen compared with haddock from the other regions. Haddock from Tampen had small livers with low lipid content (>40 % lipid) and had therefore only approx. 50 % of the energy reserve compared with haddock from the other regions.

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Project leader

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Jarle Klungsoyr
Group leader

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1 Introduction

The Activity regulations require the offshore petroleum industry to perform monitoring. The Condition monitoring shall document if fish from Norwegian ocean areas contain elevated values of components that originate from discharges from the petroleum activity. 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.

Condition monitoring of fish from open seas in the Norwegian areas is conducted every third year and shall document whether fish from these areas are affected by pollution from the oil and gas activities. The programme is decided by SFT (Aktivitetsforskriften, §1.2). Sampling should be performed so 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.

The Barents Sea will be considered as a reference area together with the reference area in the North Sea, the Egersund Bank area, where the Condition monitoring took place also in 2005 and 2002. The reference area in the North Sea is also important to be able to distinguish between differences in the reference levels in the North Sea and the Barents Sea.

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

In the monitoring performed in 2000, haddock were collected from ten regions: Ekofisk, Sleipner, Tampen, Møre, Trøndelag, Nordland, Troms, Finnmark, 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 muscles 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 an effective metabolic system in fish resulting in rapid excretion of aromatic hydrocarbons (Klungsoyr et al. 2003).

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

Main findings from the Condition monitoring 2005 were:

Di- and polyaromatic hydrocarbons (NPD/PAH) were analysed in muscles of cod and haddock caught in the North Sea at the Ling Bank/Egersund Bank (reference), Tampen, the Halten Bank and the Barents Sea (reference), and found to be below levels of quantification (LOQ) for fish sampled from all regions.

Cod sampled at the Ling Bank/Egersund Bank in the southern part of the North Sea, had the same levels of PAH metabolites in bile as cod sampled from the Tampen region. Haddock demonstrated significantly higher levels of fluorescence for all three wavelength pairs measured, indicating 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.

DNA adducts were analyzed in liver of cod, haddock and saithe at Tampen and the Ling Bank/Egersund Bank. 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 the Egersund Bank/Ling Bank, indicating higher exposure of PAH in this region. Significant differences in DNA adduct levels were not found for cod and saithe collected from the same areas.

Analyses of alkylphenols in cod liver, haddock liver, and herring muscle from the Ling Bank/Egersund Bank and Tampen regions demonstrated levels below limits of detection (LOD) for all stations.

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

Results from the Condition monitoring from 2005 (Grøsvik et al. 2007) were used as basis for the proposal for monitoring for 2008.

The objectives for this study have been:

1. Measure NPD/PAH in haddock liver from the Egersund Bank, Halten Bank and the Barents Sea.
2. Determine to what extent fish caught in areas with oil and gas activities at Tampen and the Halten Bank contain elevated levels of petroleum hydrocarbons compared with fish from the reference areas at the Egersund Bank and in the Barents Sea by measurements of metabolites of PAH and alkylphenols in bile of haddock, saithe and long rough dab.
3. Measure vitellogenin levels in blood from male cod from the Egersund Bank, Tampen, the Halten Bank and the Barents Sea.
4. Study possible genotoxic effects in fish from Tampen compared with fish from the Egersund Bank/Ling Bank by measurements of hepatic DNA adducts.
5. Perform gonad histology on haddock and long rough dab to study whether differences can be observed between fish caught at Tampen compared with fish caught at the Egersund Bank and in the Barents Sea.

2 Sampling

Haddock (*Melanogrammus aeglefinus*), cod (*Gadus morhua*) and saithe (*Pollachius virens*) were collected from four regions: The Barents Sea, the Halten Bank, Tampen and the Egersund Bank. Long rough dab (*Hippoglossoides platessoides*) was sampled from the Egersund Bank and the Barents Sea. The sampling took place during the following cruises:

Tampen and the Egersund Bank: RV J Hjort, 1 July – 31 July 2008

The Barents Sea: RV G.O. Sars, 18 August – 1 September 2008

The Halten Bank: RV Johan Hjort, 25 November – 8 December 2008

Bottom trawl was used for collection of cod, haddock, saithe and long rough dab. From each of the regions, 25 ($\pm 10\%$) fish of each species were sampled. After killing the fish with a blow to the head, standard IMR procedures were used for collection and storage of muscle, liver, blood and bile samples for the later chemical and biochemical analyses. Figure 1 gives the sampling locations for fish in the North Sea. Table 1 gives details of the sampling from all four areas, and biological data is shown in Tables 2-5.

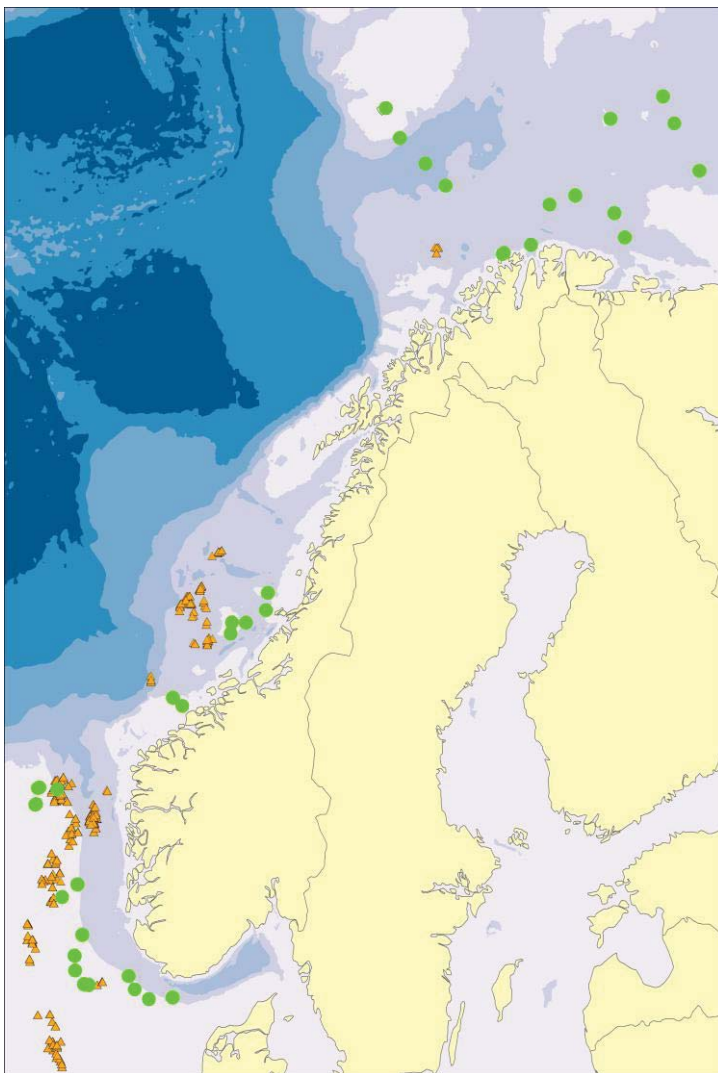


Figure 1. Stations for fish sampling are shown in green circles, oil and gas installations in Norwegian sector in yellow triangles.

Table 1. Planned samples (in parenthesis) vs actually obtained from field sampling.

Parameter	Species	Egersund Bank	Tampen	Barents Sea	Halten Bank
PAH metabolites GCMS, bile	Haddock	(25)	25 (16)	(25) 25	(25) 23
PAH metabolitter GCMS, galle	Long rough dab	(25) 6	25 (0)	(25) 25	(25) 25
PAH metabolitter GCMS, galle	Saithe	(25) 23	25 (19)	25 (11)	25 (21)
DNA addukter, lever	Haddock	(25) 25	(25) 25	(25) 25	
DNA addukter, lever	Long rough dab	(25) 12	(25) 0	25	
Alkylfenol GCMS, galle	Sei	(25) (23)	(25) 19		
Alkylfenol GCMS, galle	Haddock	(25)	(25) 16	(25) 25	(23)
Alkylfenol GCMS, galle	Long rough dab	(25) 6		(25) 25	
Vtg i blod	Cod (M)	(25) 22	25 (8)	25	
Lipidklasse/fettsyre sammensetn.	Haddock	25	25	25	(25)
Histologi, gonade	Haddock	25	25 (12)	25	
Histologi, gonade	Long rough dab	25 (8)	25 (0)	25	
Histologi, gonade	Cod	(13)	(15)	(7)	
PAH/NPD lever	Haddock	(25)		(25)	(25)

Table 2. Biological data of haddock. Data given as mean \pm stdev.

Area	Egersund Bank	Egersund Bank	Tampen	Tampen	Barents Sea	Barents Sea	Halten Bank	Halten Bank
Period	July 08	July 08	July 08	July 08	Aug 08	Aug 08	Dec 08	Decr 08
Sex	Females	Males	Females	Males	Females	Males	Females	Males
Number	23	20	12	13	28	13	13	12
Length (cm)	37 \pm 3	35 \pm 3	39 \pm 4	40 \pm 3	40.0 \pm 7.4	43.4 \pm 7.6	50 \pm 8	47 \pm 8
Weight (g)	537 \pm 141	440 \pm 150	596 \pm 194	623 \pm 194	682 \pm 390	894 \pm 473	1327 \pm 668	1096 \pm 550
Liver weight (g)	25 \pm 9	19 \pm 7	15 \pm 8	19 \pm 9	31.4 \pm 20.8	38.6 \pm 26.8	78 \pm 49	61 \pm 38
Gonade weight (g)	3.3 \pm 1.0	n.m.	3.2 \pm 1.5	1.5 \pm 1.4	5.8 \pm 6.1	n.m.	23 \pm 16	n.m.
Age	3.2 \pm 0.4	3.3 \pm 1.5	2.9 \pm 0.5	3.6 \pm 1.7	3.3 \pm 1.2	4.1 \pm 1.6	4.8 \pm 2.4	4.1 \pm 1.5
LSI (%)	4.7 \pm 1.4	4.2 \pm 0.9	2.4 \pm 0.6	3.0 \pm 0.8	3.9 \pm 1.2	4.0 \pm 1.4	5.8 \pm 2.3	5.3 \pm 1.9
GSI (%)	0.6 \pm 0.2	n.m.	0.53 \pm 0.14	0.22 \pm 0.17	1.3 \pm 4.3	n.m.	1.4 \pm 0.6	n.m.
Fulton	1.02 \pm 0.10	1.04 \pm 0.10	0.97 \pm 0.07	0.96 \pm 0.08	1.0 \pm 0.1	1.0 \pm 0.2	0.99 \pm 0.10	0.98 \pm 0.07

Liver somatic index (LSI) is percentage liver weight per body weight. Gonado somatic index (GSI) is percentage gonade weight per body weight. Fulton index is weight/length³*100. If not measured, labelled n.m.

Table 3. Biological data of cod. Data given as mean \pm stdev.

Area	Egersund Bank	Egersund Bank	Tampen	Tampen	Barents Sea	Barents Sea	Halten Bank	Halten Bank
Period	July 08	July 08	July 08	July 08	Aug 08	Aug 08	Dec 08	Dec 08
Sex	Females	Males	Females	Males	Females	Males	Females	Males
Number	25	22	15	8	8	24	9	9
Length (cm)	55 \pm 13	55 \pm 16	60 \pm 12	66 \pm 12	62.9 \pm 18.5	63.8 \pm 15.3	66 \pm 12	65 \pm 11
Weight (g)	1875 \pm 1352	2056 \pm 1860	2592 \pm 1563	3165 \pm 1604	2771 \pm 2005	2718 \pm 1753	3268 \pm 1875	3060 \pm 1450
Liver weight (g)	45 \pm 42	69 \pm 87	75 \pm 85	94 \pm 59	159 \pm 165	151 \pm 122	117 \pm 112	113 \pm 94
Gonade weight (g)	8.4 \pm 9.4	10.8 \pm 12.3	8.3 \pm 5.2	3.7 \pm 4.3	18.7 \pm 13.4	n.d.	57 \pm 59	165 \pm 16
Age	3.2 \pm 1.0	3.1 \pm 1.0	3.8 \pm 1.2	4.4 \pm 1.7	4.4 \pm 1.3	5.1 \pm 1.7	3.8 \pm 1.6	3.5 \pm 1.2
LSI (%)	2.2 \pm 0.9	3.2 \pm 1.7	2.4 \pm 1.4	2.8 \pm 1.3	4.4 \pm 2.5	3.9 \pm 2.3	3.1 \pm 1.7	3.1 \pm 1.8
GSI (%)	0.32 \pm 0.11	0.18 \pm 0.17	0.33 \pm 0.12	0.10 \pm 0.06	0.5 \pm 0.2	n.d.	1.0 \pm 0.9	4.2 \pm 1.4
Fulton	0.95 \pm 0.08	1.01 \pm 0.01	1.05 \pm 0.16	0.95 \pm 0.08	0.9 \pm 0.1	0.9 \pm 0.1	1.01 \pm 0.09	1.05 \pm 0.14

Liver somatic index (LSI) is percentage liver weight per body weight. Gonado somatic index (GSI) is percentage gonade weight per body weight. Fulton index is weight/length³*100. If not measured, labelled n.m.

Table 4. Biological data of saithe. Data given as mean \pm stdev.

Area	Egersund Bank	Egersund Bank	Tampen	Tampen	Barents Sea	Barents Sea	Halten Bank	Halten Bank
Period	July 08	July 08	July 08	July 08	Aug 08	Aug 08	Dec 08	Dec 08
Sex	Females	Males	Females	Males	Females	Males	Females	Males
Number	9	16	17	8	2	9	15	11
Length (cm)	55 \pm 4	56 \pm 10	54 \pm 14	52 \pm 10	47 \pm 22	65 \pm 12	55 \pm 8	52 \pm 8
Weight (g)	1657 \pm 372	1591 \pm 555	1743 \pm 1733	1438 \pm 918	1365 \pm 1478	2930 \pm 1736	1522 \pm 623	1344 \pm 739
Liver weight (g)	138 \pm 28	129 \pm 69	138 \pm 221	95 \pm 83	85.5 \pm 98.3	224 \pm 122	82 \pm 53	78 \pm 63
Gonade weight (g)	n.d.	n.d.	10.8 \pm 18.7	2.6 \pm 3.4	n.d.	n.d.	29 \pm 26	n.m.
Age	n.d.	n.d.	4.8 \pm 2.1	4.4 \pm 2.1	n.d.	5.2 \pm 1.3	6.0 \pm 1.8	5.5 \pm 1.8
LSI (%)	8.5 \pm 1.8	7.8 \pm 2.1	6.0 \pm 2.5	5.9 \pm 3.3	5.7 \pm 1.0	6.1 \pm 1.7	5.1 \pm 1.5	4.8 \pm 2.1
GSI (%)	n.d.	n.d.	0.4 \pm 0.2	0.1 \pm 0.1	n.d.	n.d.	1.6 \pm 1.1	n.m.
Fulton	1.00 \pm 0.06	0.95 \pm 0.23	0.88 \pm 0.09	0.91 \pm 0.10	1.0 \pm 0.0	1.0 \pm 0.1	0.85 \pm 0.08	0.92 \pm 0.07

Liver somatic index (LSI) is percentage liver weight per body weight. Gonado somatic index (GSI) is percentage gonade weight per body weight. Fulton index is weight/length³*100. If not measured, labelled n.m.

Table 5. Biological data of long rough dab. Data given as mean \pm stdev.

Area	Barents Sea	Egersund Bank	Egersund Bank
Period	Aug 08	July 08	July 08
Sex	Females	Females	Males
Number	24	8	4
Length (cm)	43.4 \pm 1.7	21 \pm 2	21 \pm 1
Weight (g)	788 \pm 99	68 \pm 16	62 \pm 12
Liver weight (g)	19.0 \pm 4.5	0.9 \pm 0.3	1.1 \pm 0.2
Gonade weight (g)	29.7 \pm 8.6	0.7 \pm 0.2	n.m.
Age	\pm	n.m.	n.m.
LSI (%)	2.4 \pm 0.4	1.4 \pm 0.4	1.8 \pm 0.8
GSI (%)	3.9 \pm 1.0	1.1 \pm 0.3	n.m.
Fulton	1.0 \pm 0.1	0.72 \pm 0.09	0.69 \pm 0.08

Liver somatic index (LSI) is percentage liver weight per body weight. Gonado somatic index (GSI) is percentage gonade weight per body weight. Fulton index is weight/length³*100. If not measured, labelled n.m.

4 Levels of NPD/PAH in haddock liver

In the condition monitoring of 2005, NPD and PAH compounds were only measured in muscle of cod and haddock, and all levels were below levels of quantification (LOQ) in fillet of cod and haddock sampled from the Egersund Bank, Tampen, Halten Bank and the Barents Sea (Grøsvik et al. 2007). NPD and PAH in fish fillet were also measured in several fish species after the oil discharge incident of 4400 m³ crude oil at Statfjord in December 2007. Also in this study levels of NPD and PAH in the fillets were below levels of detection (LOD) for fish sampled 6 days and 1 month after the discharge. However, increased levels of NPD compounds were measured in liver of haddock and pollack sampled 6 days after the discharge (Grøsvik et al. 2008). Therefore, it was decided to include liver of haddock from the Egersund Bank, the Halten Bank and the Barents Sea in this study to learn more about background levels. Levels of liver from haddock from Tampen will be performed in connection with a report on levels after the discharge at Tampen in May 2008.

Analyses of aromatic hydrocarbons (NPD/PAH) were carried out using GC/MS. The compounds included in the analysis are shown in Table 3. NPD is the sum of naphthalene, phenanthrene, dibenzothiophene, and their C₁-C₃ 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 a concentration of 0.2 ng/g. For some compounds the detection limit is higher, because of background problems. Levels of detection (LOD) is defined as $LOD: Y = Y_B + 3SD_B$, and levels of quantification (LOQ) is $LOQ = Y = Y_B + 10SD_B$, where Y_B is the response of blank sample signal and SD_B is the standard deviation of the blank samples.

Levels of NPD and PAH in haddock were low for all regions. Sum NPD in liver of haddock 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 (Table 6). Levels found in haddock 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 at Tampen 6 days after the discharge were 132±123 ng/g NPD (Grøsvik et al. 2008). Levels of sum PAH were low for the three regions. Sum PAHs were 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).

Table 6. Levels of NPD/sumPAH compounds in haddock liver.

Compound	Egersund Bank N= 25	Halten Bank N=26	Barents Sea N=22
Naphthalene ^{a,b}	0.49±2.47	0.27±1.35	0.51±2.41
C1-naphthalene ^a	0	2.26±8.87	0.34±1.60
C2-naphthalene ^a	1.67±3.45	2.65±6.26	0.44±1.42
C3-naphthalene ^a	10.72±3.53	4.75±3.02	4.47±1.79
Dibenzothiofene ^{a,b}	0.11±0.19	0.02±0.04	0.04±0.06
C1-dibenzothiofene ^a	0.06±0.13	0.01±0.07	0
C2-dibenzothiofene ^a	0.13±0.19	0.07±0.20	0.42±0.54
C3-dibenzothiofene ^a	0.34±0.28	0.14±0.50	0.28±0.57
Phenantrene ^{a,b}	0.17±0.60	0.05±0.28	0.30±0.66
C1-phenantrene ^a	0.48±0.55	0	0.09±0.27
C2-phenantrene ^a	0.66±0.39	0.11±0.32	0.46±0.66
C3-phenantrene ^a	0.47±0.38	0.16±0.31	0.48±1.01
Acenaphthylene ^b	0.15±0.15	0.18±0.40	0.06±0.08
Acenaphthene ^b	0.34±0.12	0.69±0.95	0.17±0.12
Fluorene ^b	0	0.17±0.60	0.27±0.58
Anthracene ^b	0.18±0.22	0.13±0.13	0.29±0.64
Fluoranthene ^b	0.48±0.70	0.03±0.16	0.31±0.44
Pyrene ^b	0.08±0.30	0	0.08±0.25
Benz(a)anthracene ^b	0.05±0.07	0.01±0.01	0.07±0.14
Chrysene ^b	0.02±0.11	0	0
Benzo(b,j,k)fluorantene ^b	0	0	0.13±0.25
Benzo(a)pyrene ^b	0.04±0.16	0	0.03±0.10
Indeno(1,2,3-cd)pyrene ^b	0	0	0.01±0.05
Dibenz(a,h)anthracene ^b	0	0	0.05±0.2
Benzo(g,h,i) perylen ^b	0	0	0.11±0.39
SUM NPD ^a	15.29±7.27	10.50±13.27	7.81±5.93
SUM PAH (EPA16) ^b	2.13±3.12	1.53±2.13	2.56±3.51

Presented as mean ± stdev (ng/g wet weight). N= number of fish per station. Abbreviations: Limit of detection (LOD). Compounds included in sum NPD are labelled with ^a. Compounds included in sum PAH (EPA16) are labelled with ^b. Values of naphthalene, dibenzothiophene and phenanthrene are included in sum NPD as well as sum PAH (EPA16).

5 Levels of PAH metabolites and alkyl phenols by GC MS in bile

The content of PAHs and alkyl phenols in bile can reflect which compounds are being metabolised in the organism in a small and concentrated volume. This has shown particularly useful for hydroxylated polycyclic aromatic hydrocarbons (PAH) (Aas et al. 2000). 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 and alkylphenols include deconjugation, derivatisation and pentafluorobenzoyl derivatization, as previously described for alkylphenol analysis (Boitsov et al. 2004). This allows achieving low detection limits due to the possibility of using negative chemical ionisation (NCI) mode on GC-MS.

5.1 Levels of PAH metabolites in bile

Overall, the highest levels of sum PAH metabolites were measured in haddock from Tampen of 580 ng/g bile. Sum PAH metabolites at the Egersund Bank was 231, at the Halten Bank 199, and in haddock from the Barents Sea 35 ng/g bile (Table 7). 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. Levels above LOQ were measured in all haddock bile from Tampen (N=16). Levels of 1-hydroxy phenanthrene in haddock bile from the Egersund Bank was 133 ± 207 ng/g, while levels from the Halten Bank and the Barents Sea were 43 ± 71 and 19 ± 14 ng/g, respectively. This is the first study on bile metabolites on haddock. Bile metabolites were performed on cod at Tampen and the Egersund Bank approximately one month after the discharge at Statfjord December 2007, and the levels of PAH metabolites were comparable with levels found in this study, except for those of 1-hydroxy phenanthrene. The 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 is in the same range as reported in bile from oil exposed cod (Jonsson et al. 2003).

Low levels of PAH metabolites were measured in saithe (Table 8) and long rough dab (Table 9).

Table 7. PAH metabolites in bile in haddock.

Compound	Egersund Bank (N=22)	Tampen (N=16)	Halten Bank (N=23)	Barents Sea (N=22)	LOQ
1-Naphthol	29±18 (N=5)	14±1 (N=10)	21±15 (N=16)	< LOQ	12.8
2-Naphthol	14±9.8 (N=3)	13±6 (n=13)	14±14 (N=15)	6.0±0.2 (N=2)	5.0
2-methyl-1-naphthol	2.7±0.1 (N=2)	7.3±4.6 (N=7)	13±12 (N=8)	< LOQ	2.4
1-methyl-2-naphthol	0.3±0 (N=2)	0.6±0.6 (N=15)	0.7±1.3 (N=20)	< LOQ	0.1
2-Hydroxyfluorene	4.1±2.6 (N=22)	5.4±4.9 (N=16)	16±27 (N=21)	3.0±3.2 (N=15)	0.9
1-Hydroxy phenanthrene	133±207 (N=13)	510±814 (N=16)	43±71(N=21)	19±14 (N=2)	8.9
1-Hydroxypyrene	18±10 (N=10)	13±6 (N=5)	71±136 (N=16)	< LOQ	9.0
1-Hydroxychrysene	30±21 (N=22)	18±14 (N=10)	20±19 (N=19)	7.3±1.5 (N=7)	5.5
Sum PAH metabolites	231	580	199	35	

Values given as mean ± SD in ng/g bile. Total number of bile analysed per station is indicated in the first line, while number of fish with levels of PAH metabolites above LOQ are indicated after mean and SD values.

Table 8. PAH metabolites in bile in saithe.

Compound	Egersund Bank (N=22)	Tampen (N=16)	Halten Bank (N=23)	Barents Sea (N=22)	LOQ
1-Naphthol	29 (N=1)	<LOQ	20±2 (N=3)	15 (N=1)	12.8
2-Naphthol	<LOQ	7±2 (N=11)	<LOQ	10±4 (N=2)	5.0
2-methyl-1-naphthol	6±0 (N=5)	4±1 (N=6)	4 (N=1)	<LOQ	2.4
1-methyl-2-naphthol	<LOQ	<LOQ	<LOQ	0.2 (N=1)	0.1
2-Hydroxyfluorene	4±4 (N=14)	4±3 (N=15)	2±1 (N=9)	10±10 (N=9)	0.9
1-Hydroxyphenanthrene	<LOQ	<LOQ	<LOQ	25±5 (N=3)	8.9
1-Hydroxypyrene	18±7 (N=17)	10±1 (N=4)	16±6 (N=19)	12±2 (N=3)	9.0
1-Hydroxychrysene	12±6 (N=17)	12±6 (N=11)	18±9 (N=19)	10±3 (N=7)	5.5
Sum PAH metabolites	69	36	59	82	

Values given as mean ± SD in ng/g bile. Total number of bile analysed per station is indicated in the first line, while number of fish with levels of PAH metabolites above LOQ are indicated after mean and SD values.

Table 9. PAH metabolites in bile in long rough dab.

Compound	Barents Sea (N=)	LOQ
1-Naphthol	16 (N=1)	12.8
2-Naphthol	7.5±3.6 (N=5)	5.0
2-methyl-1-naphthol	15.4±15.3 (N=2)	2.4
1-methyl-2-naphthol	2.4 (N=1)	0.1
2-Hydroxyfluorene	3.2±4.0 (N=11)	0.9
1-Hydroxyphenanthrene	29±30 (N=3)	8.9
1-Hydroxypyrene	<LOQ	9.0
1-Hydroxychrysene	14 (N=1)	5.5
Sum PAH metabolites	88	

Values given as mean ± SD in ng/g bile. Total number of bile analysed per station is indicated in the first line, while number of fish with levels of PAH metabolites above LOQ are indicated after mean and SD values.

5.2 Levels of alkylphenols in bile

Metabolites of alkylphenols were analysed for a high number of metabolites, as listed in Table 8. Analysed fish included haddock from the Egersund Bank (N=23), Tampen (N=16), the Barents Sea (N=22) and the Halten Bank (N=16), in addition to saithe from the Egersund Bank (N=19), Tampen (N=19), and long rough dab from the Barents Sea (N=21). In total 143 fish were analysed, and most of the alkylphenol metabolites were found to be below LOQ. An overview of the number of fish scoring below and over LOQ is given in Table 10.

The low levels of alkylphenols measured in bile are in accordance with the results from the condition monitoring 2005, where levels of alkylphenols measured in cod liver, haddock liver and herring muscle from the Egersund Bank and Tampen regions demonstrated mostly levels below LOD for all stations (Grøsvik et al. 2008), and with the results from the 2002 monitoring (Klungøy et al. 2003).

Levels of alkylphenol metabolites over LOQ in haddock are shown in Table 11, and in saithe and long rough dab in Table 12. Individuals with no levels over LOQ were cut. One haddock from Tampen had elevated levels of oil related alkyl phenols in the bile. Elevated levels of 4tert-octylphenol were measured in 10 of 22 haddock from the Barents Sea and in 2 of 23 haddock from the Halten Bank, while not in haddock from Tampen and the Egersund Bank.

As this phenomenon was not observed in haddock from the North Sea, it could be due to problems with sample preparation and extraction, as samples were upconcentrated 40 times. 4tert-octylphenol and 4tert-nonylphenol are constituents in many plastic products and is compounds known to cause false positives in extraction analyses.

Table 10. Results from measurements of alkylphenol metabolites in bile.

Compound	Mean of blank (n=13)	SD	LOQ	Results
Phenol	2.62	1.49	1.75	All samples <LOQ, N=143
o-Cresol	1.29	0.30	0.42	All samples <LOQ, N=143
m-Cresol	0.70	0.22	0.29	All samples <LOQ, N=143
p-Cresol	0.74	0.21	0.29	All samples <LOQ, N=143
2-Etylphenol	10.65	6.91	7.97	All samples <LOQ, N=143
2,6-Dimetylphenol	2.03	1.35	1.55	<LOQ in 142 fish, > LOQ in 1 fish
2,5-Dimetylphenol	13.93	6.99	8.39	<LOQ in 138 fish, > LOQ in 5 fish
2,4-Dimetylphenol	72.44	39.09	46.33	<LOQ in 142 fish, > LOQ in 1 fish
3,5-Dimetylphenol	50.04	25.08	30.08	All samples <LOQ, N=143
4-Etylphenol	163.71	77.52	93.89	<LOQ in 137 fish, > LOQ in 6 fish
2,3-Dimetylphenol	8.68	11.36	12.23	All samples <LOQ, N=143
3,4-Dimetylphenol	12.48	6.17	7.42	<LOQ in 139 fish, > LOQ in 4 fish
2-iso-Propylphenol	146.22	91.93	106.55	All samples <LOQ, N=143
2n-Propylphenol	7.60	3.90	4.66	<LOQ in 142 fish, > LOQ in 1 fish
3-iso-Propylphenol	161.57	97.98	114.14	All samples <LOQ, N=143
2.4.6-Trimetylphenol	19.39	46.36	48.30	All samples <LOQ, N=143
4-iso-Propylphenol	298.10	173.66	203.47	All samples <LOQ, N=143
3-Etyl4-Metylphenol	75.41	45.01	52.55	<LOQ in 142 fish, > LOQ in 1 fish
2.3.6-Trimetylphenol	4.65	4.51	4.98	All samples <LOQ, N=143
2.3.5-Trimetylphenol	128.89	166.71	179.60	All samples <LOQ, N=143
4n-Propylphenol	34.65	25.59	29.06	<LOQ in 142 fish, > LOQ in 1 fish
2-tert-bytul-4-phenol	13.35	4.54	5.88	All samples <LOQ, N=143
3-tert-Bytulphenol	295.73	458.45	488.02	All samples <LOQ, N=143
5-iso-Propyl3-Metylphenol	6.03	3.97	4.58	<LOQ in 142 fish, > LOQ in 1 fish
4-tert-Bytulphenol	919.65	449.33	541.29	All samples <LOQ, N=143
4-sec-Bytulphenol	8.29	7.79	8.62	All samples <LOQ, N=143
4-iso-Propyl3-Methylphenol	2.66	3.49	3.76	<LOQ in 142 fish, > LOQ in 1 fish
4n-Bytulphenol	26.44	23.61	26.26	<LOQ in 142 fish, > LOQ in 1 fish
2-tert-Bytul4-metylphenol	0.26	0.27	0.28	All samples <LOQ, N=143
2-tert-Brytul-6-metylphenol	1.17	0.75	0.84	All samples <LOQ, N=143
4-tert-Butyl2-metylphenol	0.84	0.40	0.51	All samples <LOQ, N=143
4(1,1 Dimetylpropyl)phenol	6.44	3.15	4.02	All samples <LOQ, N=143
4n-Pentylphenol	7.49	6.95	7.66	All samples <LOQ, N=143
2.6-Diisopropylphenol	0.30	0.24	0.27	All samples <LOQ, N=143
2.5-Diisopropylphenol	0.30	0.22	0.24	All samples <LOQ, N=143
2-tert-butyl-4-ethylphenol	1.08	2.55	2.59	All samples <LOQ, N=143
4n-Heksylphenol	0.41	0.20	0.24	<LOQ in 127 fish, > LOQ in 5 fish
Σ c6-phenol				
4-(1-Ethyl-1-methylpropyl)-2-methylphenol	0.24	0.15	0.17	<LOQ in 138 fish, > LOQ in 1 fish
4-n-Heptylphenol	4.45	7.77	7.98	All samples <LOQ, N=143
4tert-Octylphenol	37.22	25.10	28.56	<LOQ in 129 fish, > LOQ in 14 fish
4-n-Octylphenol	0.70	0.44	0.5	<LOQ in 136 fish, > LOQ in 7 fish
2-metyl4-tertOctylphenol	0.98	0.36	0.45	All samples <LOQ, N=143
4n-Nonylphenol	3.82	2.40	2.70	All samples <LOQ, N=143
Σ tert NP	1322.04	964.11	1078	All samples <LOQ, N=143

Mean of 13 blank samples are given in addition to SD and LOQ in ng/g bile.

Table 11. Individual haddock with some of the alkyl phenol metabolites above LOQ. Individuals with all metabolite levels below LOQ

Compound	EH1	EH3	EH11	EH13	EH18	TH2	TH3	TH6	TH8	TH11	TH16	TH19	BH41	BH90	BH91	BH99	BH100	BH101
2,6-Dimethylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	16	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,5-Dimethylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	94	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	91	94	<LOQ	<LOQ
2,4-Dimethylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	501	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4-Etylphenol	<LOQ	<LOQ	<LOQ	1094	1110	1196	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3,4-Dimethylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	83	91	<LOQ	<LOQ
2n-Propylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-Etyl4-Metylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4n-Propylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
5iso-Propyl3-Metylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4iso-Propyl3-Metylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4n-butylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4n-Heksylphenol	3,6	3,3	<LOQ	6,3	9,2	6	5	2	4	6	7	4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4-(1-Ethyl-1-methylpropyl)-2-methylphenol	<LOQ	2,5	2,0	<LOQ	13,5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4tert-Octylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	336	731	972	888	468	58
4-n-Octylphenol	<LOQ	15,1	<LOQ	<LOQ	<LOQ	13	8	9	9	<LOQ	8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Abbreviations: Haddock from Egersund Bank (EH), haddock from Tampen (TH), haddock from the Barents sea (BH) and haddock from the Barents sea (BH)

Table 12. Individual saithe and long rough dab with some of the alkyl phenol metabolites above LOQ.

Compound	ES19	TS10	TS19	TS21	TS23	TS24	TS25	B LRD 72	B LRD78	B LRD85	B LRD89
2,6-Dimethylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,5-Dimethylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	108	118	<LOQ	<LOQ
2,4-Dimethylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4-Etylphenol	<LOQ	1037	<LOQ	<LOQ	<LOQ	<LOQ	1028	<LOQ	996	<LOQ	<LOQ
3,4-Dimethylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	105	<LOQ	<LOQ
2n-Propylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6	<LOQ	<LOQ
3-Etyl4-Metylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	98	<LOQ	<LOQ
4n-Propylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	354	<LOQ	<LOQ
5iso-Propyl3-Metylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	83	<LOQ	<LOQ
4iso-Propyl3-Metylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	208	<LOQ	<LOQ
4n-butylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	346	<LOQ	<LOQ
4n-Heksylphenol	<LOQ	<LOQ	9,6	71,6	18,4	2,5	6,8	<LOQ	<LOQ	<LOQ	<LOQ
4-(1-Ethyl-1-methylpropyl)-2-methylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4tert-Octylphenol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	640	292
4-n-Octylphenol	<LOQ	<LOQ	<LOQ	7,1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Individuals with all metabolite levels below LOQ were cut. Abbreviations: saithe from Egersund Bank (ES), saithe from Tampen (TS) and long rough dab from the Barents sea (B LRD).

6 Measurements of Vtg levels in blood of male cod

Experimental studies in the laboratory have shown that alkylphenols in produced water can cause estrogenic effects in cod, affecting the reproduction. In this study, APs in bile is analysed as well as vitellogenin in blood plasma as a biomarker of estrogenic effects.

Vitellogenin (Vtg) is a glycopospholipoprotein and the main source of yolk proteins and lipids in the growing oocyte. Vtg is synthesized in the liver in response to estrogen. Even though Vtg is a protein specific to female fish, males also possess all of the genetic system needed for VTG protein synthesis. A rise in the level of VTG is commonly used as a biomarker for estrogenic effects in vertebrates. Cod Vtg was analysed in plasma of male cod by a quantitative enzyme-linked immunosorbent assay (ELISA) (Biosense Laboratories, Bergen, Norway).

Levels of Vtg in male cod are plotted versus fish weight both on linear axis and on a logarithmic axis (Figures 2 and 3). A total of 55 fish were analysed, 22 from the Egersund Bank, 7 from Tampen, 6 from the Halten Bank and 20 from the Barents Sea. A larger material should be used to be able to see regional differences. Generally levels of Vtg in feral male cod range from 0 to 5000 ng/ml. Only one cod caught at the Halten Bank had levels of 13400 ng/ml of 5.6 kg weight.

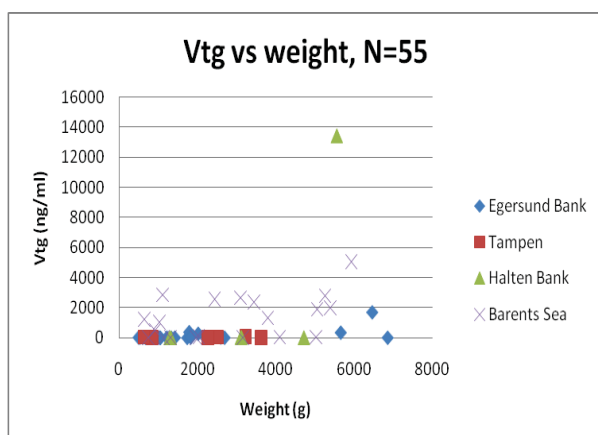


Figure 2. Levels of vitellogenin in plasma of male cod plotted against weight, linear scale.

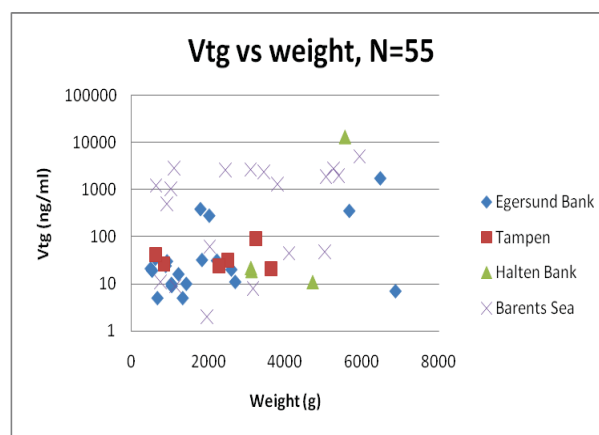


Figure 3. Levels of vitellogenin in plasma of male cod plotted against weight, log scale.

The results were in accordance with those obtained from the Egersund Bank/Ling Bank and Tampen in the condition monitoring of 2005 (Grøsvik et al., 2008) and of the work by Scott et al. (2006b). Scott et al. report Vtg concentrations up to 160 $\mu\text{g/ml}$ in male cod, but from the open sea only in fish over 5 kg. However, in the Oslo Fjord, also many smaller male cod had induced Vtg levels (Scott et al. 2006b). Water column monitoring (in 2001) around oil rigs have found that cod caged closest to the platform (500 m distance) have significant, but marginal elevation of Vtg (Scott et al. 2006a). However, similar studies (2003 and 2004) did not find any differences between cod caged in different distances from oil installations (OLF, 2005).

7 DNA adducts in liver of haddock and long rough dab

7.1 DNA adducts in haddock

DNA adduct levels were significantly highest in haddock from Tampen ($p = 0.005$), with 1.66 ± 0.87 nmol adducts/mol normal nucleotides (average \pm 95% confidence interval) compared to Haddock from the Egersund bank and the Barents Sea with 0.85 ± 0.47 and 0.82 ± 0.38 ¹ nmol adducts/mol normal nucleotides, respectively, $n = 25$ for all sites. Tampen had also the highest number of individuals with detectable DNA adducts or 11 (44%), compared to 7 (28%) and 2 (8%) from the Egersund bank and the Barents Sea, respectively. No obvious relationship between DNA adducts and age was apparent when these variables were plotted against each other. The presence of DNA adducts confirms that the fish has been exposed to genotoxic pollutants beyond their DNA repair capacity and indicates PAH contamination in the area.

7.1.1 Introduction

Aromatic hydrophobic PAH-DNA adducts were analysed in liver of two fish species; haddock (*Melanogrammus aeglefinus*) and long rough dab (*Hippoglossoides platessoides*), sampled in the Tampen oil field area, and for reference in the Egersund bank (North Sea) and from The Barents Sea (NB: DNA adduct results on long rough dab will be reported later). The DNA adducts were analysed with the ³²P postlabelling assay, which is the most sensitive and frequently applied technique for detecting PAH-DNA adducts in marine organisms (Reichert et al. 1998). PAHs are readily taken up and metabolised by fish, and it is during the metabolic transformation of these compounds that they are activated and become genotoxic. The enzymatic phase I of the biotransformation of PAHs leads to the formation of reactive electrophilic metabolites which can undergo attack and bind covalently to nucleophilic centres in large molecules such as lipids, proteins, DNA, and RNA, and form adducts. Factors that affect DNA adduct levels are exposure dose, the degree of bioactivation in phase I into reactive intermediates in relation to the phase II detoxification, DNA repair efficiency, as well as cell turnover. DNA adduct levels are thus a quantifiable measure of the biologically effective dose reaching a critical target site, and they integrate multiple toxicokinetic factors such as uptake, metabolism, detoxification, excretion and covalent binding of reactive metabolites to target tissues (Reichert et al. 1998). DNA adducts have shown to be predecessor of both mutagenic and carcinogenic effects, and they have shown to correlate with liver lesions in fish (Baumann, 1998; Reichert et al. 1998). They are widely used as, and considered to be highly relevant biomarker for PAH exposure to fish.

7.1.2 Results and Discussion

Twenty five individuals from each of the three sampling sites were analysed for DNA adducts. The results revealed that DNA adduct levels were significantly highest in haddock

¹ This average includes one value (4.79 nmol add/mol norm nucleotides) that can possibly be considered an outlier since it is several standard deviations away from the mean. Average value for DNA adducts in fish from the Barents Sea without the outlier is 0.65 ± 0.19 .

from Tampen ($p = 0.005$), with 1.66 ± 0.87 nmol adducts/mol normal nucleotides (average \pm 95% confidence interval) compared to haddock from the Egersund bank and the Barents Sea, with 0.85 ± 0.47 and 0.82 ± 0.38 nmol adducts/mol nucleotides respectively. Tampen had also the highest number of individuals with detectable DNA adducts or 11 (44%), compared to 7 (28%) from the Egersund bank and only 2 (8%) from the Barents Sea. The Barents Sea average includes one value (sample 061: 4.79 nmol add/mol normal nucleotides) that can possibly be considered an outlier since it is several standard deviations away from the mean. Average value for DNA adducts in fish from the Barents Sea without the outlier is 0.65 ± 0.19 . Average DNA adduct values and 95% confidence levels are presented in Table 13 and Figure 4, individual values in Figure 5, and raw data can be seen in the Appendix. Table 13 also shows number of individuals that had detectable DNA adducts. Figure 6 shows pictures of representative autoradiograms of the DNA adducts from each sampling site, and Figure 7 shows autoradiograms of standards that were processed parallel to the samples and served as quality assurance for all analytical steps in the ^{32}P -postlabelling method. DNA adducts from Tampen (the site with highest levels and frequency of adducts) were plotted with age of the fish, but no obvious relationship between adducts and age was apparent, see Figure 8.

Table 13. Average DNA adduct levels and detection limits (nmol add/mol normal nucleotides) \pm 95% confidence interval. (Detection limits are calculated per individual sample and are dependent on the background for each autoradiogram).

	No. of analysed individuals	No. of individuals with DNA adducts	Total average of DNA adduct levels \pm 95% conf. interval	Average of DNA adduct levels in individuals with DNA adducts \pm 95% conf. interval	Average of detection limits for individuals without DNA adducts \pm 95% conf. interval
Tampen	25	11 (44%)	1.66 ± 0.87	3.04 ± 1.75	0.57 ± 0.09
Egersund bank	25	7 (28%)	0.85 ± 0.47	1.81 ± 1.77	0.47 ± 0.06
The Barents Sea*	25	2 (8%)	$0.82 \pm 0.38^*$	$3.53 \pm 16.07^*$	0.58 ± 0.11

*Average includes 1 possible outlier, see text for further explanation (sample 061: 4,79nmol adducts/mol normal nucleotides). See Appendix for mean calculations with and without this outlier.

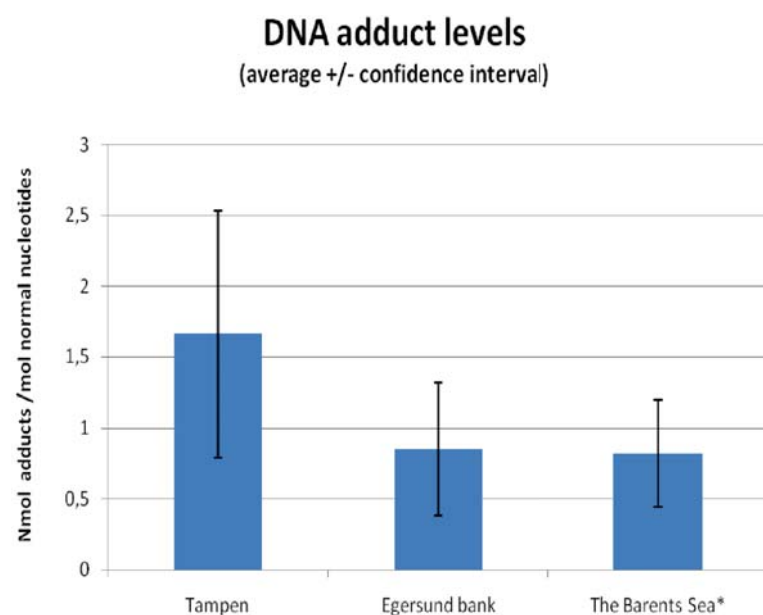


Figure 4. DNA adduct levels (nmol add/mol normal nucleotides) in liver of haddock (*Melanogrammus aeglefinus*) from Tampen and the reference areas Egersund bank and the Barents Sea. The bars represent total average \pm 95% confidence interval, $n = 25$.

*Barents Sea average includes 1 possible outlier: sample 061: 4.79 nmol add/mol normal nucleotides

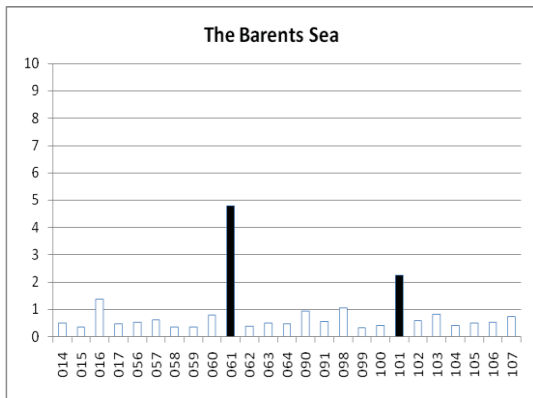
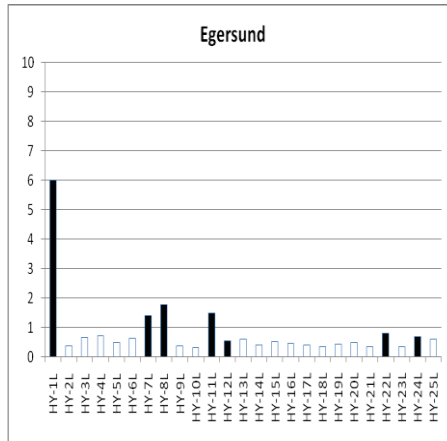
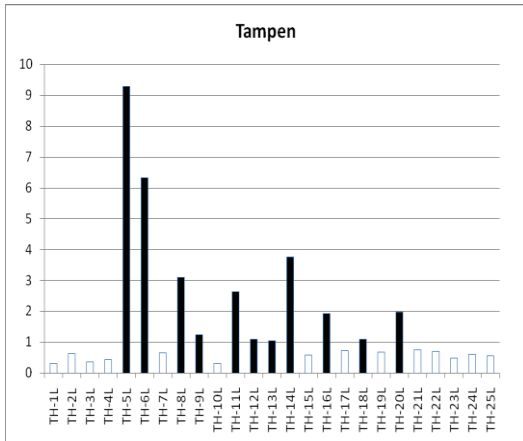
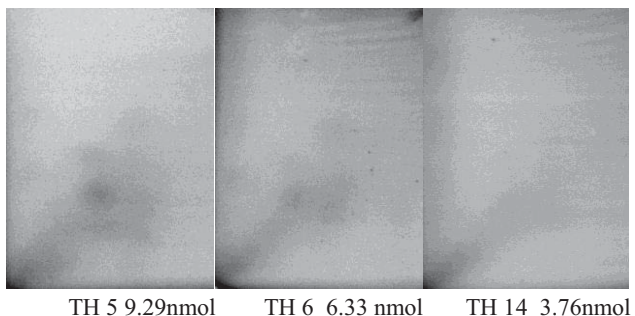
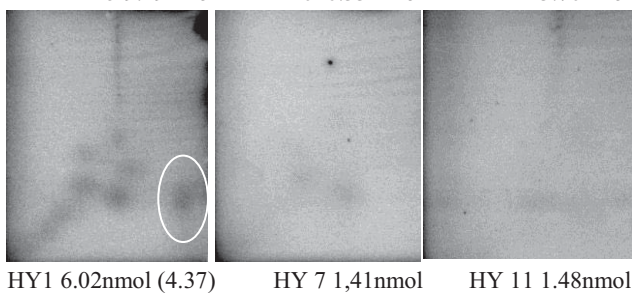


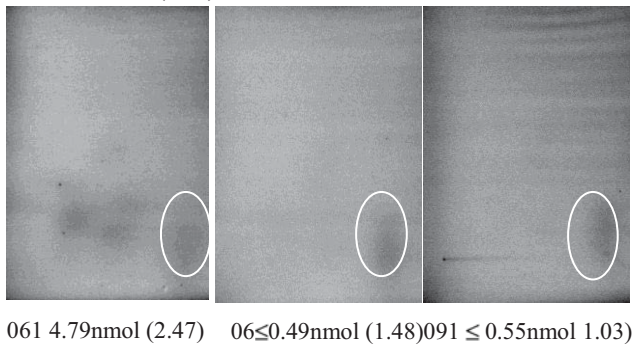
Figure 5. DNA adduct levels (nmol add/mol norm. nucleotides) in individual haddock from different sampling sites. Black bars indicate individuals with adduct levels above the detection limits. White bars are levels below the detection limits. (If any adducts are present in the sample, their value is below the background value of the autoradiogram, and could range from zero up to the background value. Therefore an average of zero and the background is taken. For every value below detection limits in order to be able to calculate averages for groups).



Tampen



Egersund Bank



Barents Sea

Figure 6. Representative autoradiogram and DNA adduct levels in liver samples of haddock from Tampen, the Egersund Bank and the Barents Sea. Numbers under the autoradiograms represent sample number (fish), DNA adducts (nmol add/mol normal nucleotides), and numbers within parenthesis show endogen adduct value. That adduct is not associated with anthropogenic release of PAHs. White circles on the autoradiograms indicate that particular DNA adduct.

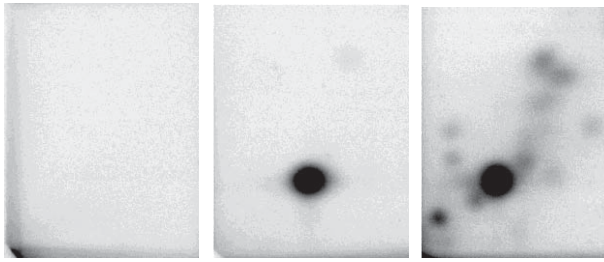


Figure 7. Controls used during the analytical work were: a) Pure salmon sperm as negative control, b) the standard DNA adduct B[a]PDE-dG-3'p, and c) adducted liver tissue from benzo[a]pyrene exposed perch (*Perca fluviatilis*). The standards were processed parallel to the samples and served as quality assurance for all the analytical steps in the ^{32}P -postlabelling method for analysing DNA adducts.

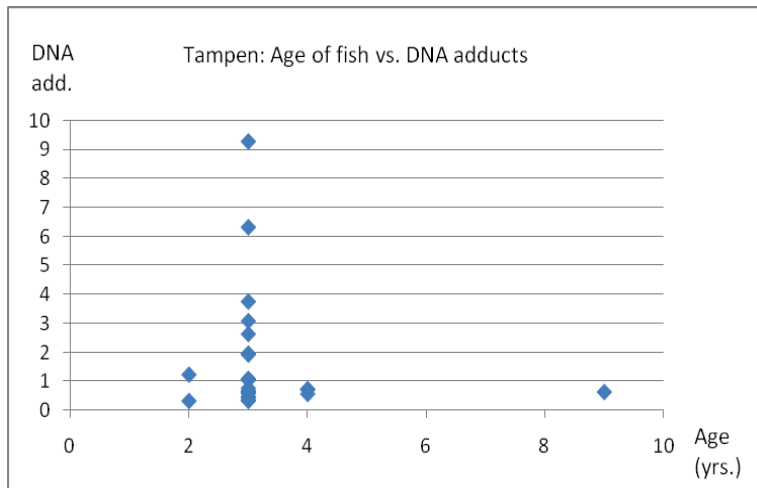


Figure 8. Age of fish from Tampen plotted against DNA adduct levels (nmol add/mol norm nucleotides).

The observed DNA adduct levels in the liver of haddock from Tampen can be considered high when having in mind that the fish is caught in the open North Sea. The fact that the fish show elevated levels of DNA adducts at all is an abnormal condition, and confirms that the fish has been exposed to genotoxic pollutants beyond their DNA repair capacity, and suggest PAH contamination in the area. Few studies on DNA adduct levels in fish from the North Sea or neighboring areas, or even from open seas in general, have been published. But for comparison, Aas et al. (2003) studied DNA adduct levels in 11 fish species from the open seas of the NE Atlantic. That study showed undetectable levels of DNA adducts in the fish, or levels just above the detection limits.

7.1.3 Unknown DNA adduct in haddock

A distinctive adduct spot on the autoradiograms was found in some of the haddock livers. This spot is not situated on the typical PAH-adduct diagonal radioactive zone (DRZ), but closer to the right edge of the autoradiograms. This is in accordance with our previous findings from haddock from different areas, including both assumed contaminated and less contaminated areas, which frequently show this corresponding type of spot. The spot represents what is believed to be an endogenous adduct, and not a PAH-DNA adduct. This spot does not correlate with the levels of other adducts in the same tissue, as is typical for PAH adducts, and is therefore not believed to be caused by anthropogenic PAH exposure and is not included in the calculation of DNA adduct levels. In the pictures showing the autoradiograms, this particular spot is outlined in white. Endogenous DNA adducts, believed to be formed from endogenous compounds such as steroids (Randerath et al. 1993), have previously been reported in mammals, but not in teleost fish species.

7.2 DNA adducts in liver of long rough dab

DNA adducts were analysed in liver of 12 long rough dabs from The North Sea, and 25 from the The Barents Sea. See earlier presented report for method description. The results revealed that DNA adduct levels were below the detections limits in all individuals, except in one from The North Sea area. Average DNA adduct levels were 0.37 ± 0.26 nmol adducts/mol normal nucleotides in fish from The North Sea and 0.29 ± 0.04 in fish from The Barents Sea (average \pm 95% confidence interval). Average DNA adduct values and 95% confidence intervals are presented in Table 14 and Figure 9, individual values in Figure 10, and raw data can be seen in appendix b. Figure 11 shows pictures of representative autoradiograms from each sampling site.

Table 14. Average DNA adduct levels and detection limits (nmol adducts/mol normal nucleotides) in liver of long rough dab \pm 95% confidence interval. Detection limits are calculated per individual sample and are dependent on the background for each autoradiogram.

	No. of analysed individuals	No. of individuals with DNA adducts	Total average of DNA adduct levels \pm 95% conf. interval	Average of DNA adduct levels in individuals with DNA adducts \pm 95% conf. interval	Average of detection limits for individuals without DNA adducts \pm 95% conf. interval
The North Sea	12	1 (8%)	0.37 ± 0.26	1,67	0.25 ± 0.05
The Barents Sea	25	0	0.29 ± 0.04	0	0.29 ± 0.04

(About levels below detections limits: If any adducts are present in the sample, their value is below the background value of the autoradiogram, and could range from zero up to the background value. Therefore an average of zero and the background is taken for every value below detection limits in order to be able to calculate averages for groups).

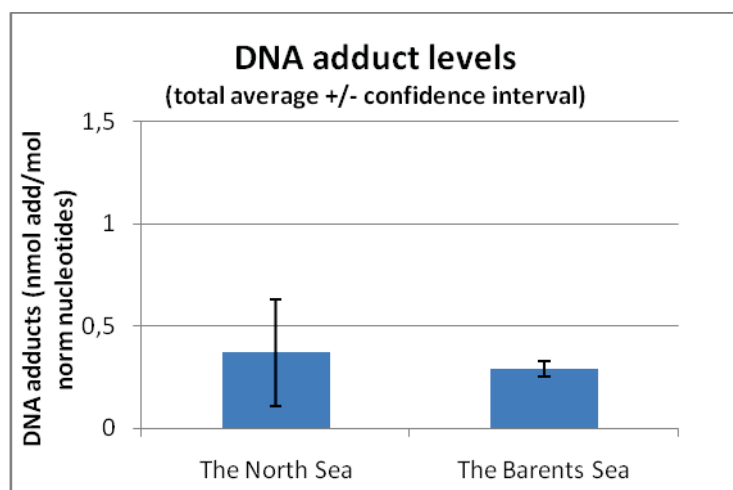


Figure 9. DNA adduct levels (nmol add/mol normal nucleotides) in liver of long rough dab from The North Sea and The Barents Sea. The bars represent total average \pm 95% confidence interval, n= 12 for The North Sea and 25 for The Barents Sea.

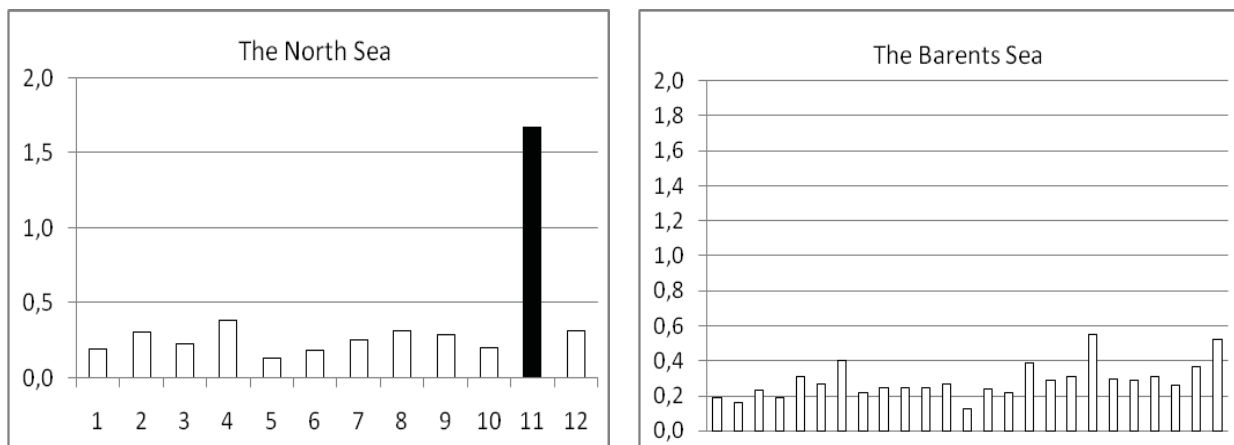


Figure 10. DNA adduct levels (nmol add/mol norm. nucleotides) in individual long rough dabs from the two sampling sites. The black bar indicates individual with adduct levels above the detection limits. White bars are levels below the detection limits.

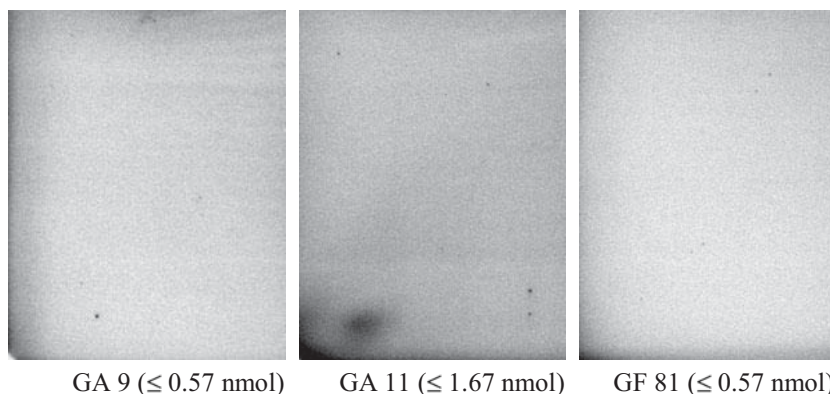


Figure 11. Autoradiograms of liver samples of long rough dab from The North Sea (GA 9, GA 11) and the Barents Sea (GF 81). Numbers under the autoradiograms represent sample number and DNA adduct levels within parenthesis (nmol adducts/mol normal nucleotides).

8 Histology of ovaries of haddock

Samples of haddock ovaries from the three areas of the study, the Barents Sea (N = 24), the Egersundbank (N = 23) and Tampen (N = 11), were processed histologically using conventional protocols, i.e. using resin (Technovit®) as embedding medium and 2% toluidine blue and 1% sodium tetraborate as stain. A series of these plastic sections (4 µm) appearing in different colors of blue and lilac was produced from each ovary for tests on representativeness.

8.1 Classification of various structures in the haddock ovary

The histological sections were evaluated with a high-performance light microscope at different magnifications depending on structures evaluated. These were of four types:

1. Post-ovulatory follicles (POFs), which are formed and left in the ovary when the eggs are spawned. Thus, a POF is the remains of the ovarian follicle. More specifically, it consists of the previous supporting follicle cells covering the follicle proper. As a consequence, the presence of a POF was presently used as a spawning marker: showed that the female had been through at least one spawning season (POFs are known to last for many months in gadoids).
2. The presence of atretic eggs (oocytes) in the ovary. Atresia (Atr) refers to that the developing oocyte is resorbed prior to egg release.
3. Encapsulated eggs (Enc.) are eggs ready to be spawned, i.e. fully developed, but where the actual egg release was not executed. Enc structures may remain in the ovary for a very long time (due to the robust egg shell (chorion)).
4. Stroma (Tissue) is connective tissue supporting the internal ovarian organization. A high amount of stroma is indicative of previous spawning, but as for point 2-3 this can also be indicative of some sort of reproductive performance problems.

More details on these topics can be found in Witthames et al. (2009).

The data were grouped into presence or absence of a given structure. Thus, we used prevalence instead of actual estimation of the level of intensity.

8.2 Classification of various types of oocytes in the long rough dab

Oocytes refer to female germ (sex) cells. Presently, only the first stages of developing oocytes were discovered in the samples, obviously due to sampling well outside the spawning season (July 2008). These stages were:

- CA: the cortical alveoli (CA) stage, i.e. small vesicles are formed in the cytoplasm showing that the oocyte has started to develop for the next spawning season
- CA/YG: the interface between CA and the first, initial uptake of yolk represented by yolk granules (YG)
- YG early: yolk granules are easily detected in the cytoplasm but are still low in numbers

8.3 Histological structures across study areas

The light micrographs showed evidently different haddock ovarian morphology in the Barents Sea (Figure 12), Egersund Bank (Figure 13) and Tampen (Figure 14), with the Egersund Bank showing a more disorganized ovarian structure followed by Tampen. This related mainly to significantly more ovarian tissue in the first case. All defined ovarian structures could be easily detected: POFs (Figure 15); Atresia (Figure 16); Enc. (Figure 17); Tissue (Figure 16). As expected, females showing spawning makers such as POFs were longer in total length than those without these structures within the same geographical area (Figure 18a). These data also indicated that length-at-sexual maturity increased northwards, although the difference amounted to a few centimeters only. Atresia (Figure 18b) and Enc. (Figure 18c) were detected in all three areas while there was no extraordinary amount of tissue in the Barents Sea (Figure 18d).

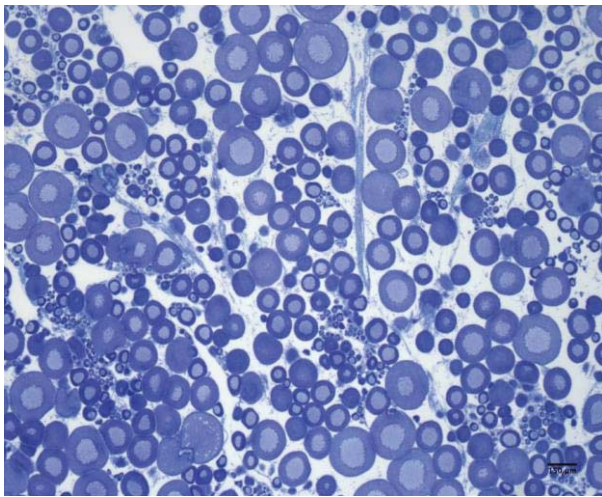


Figure 12. Representative histological section of ovary of haddock from the Barents Sea.

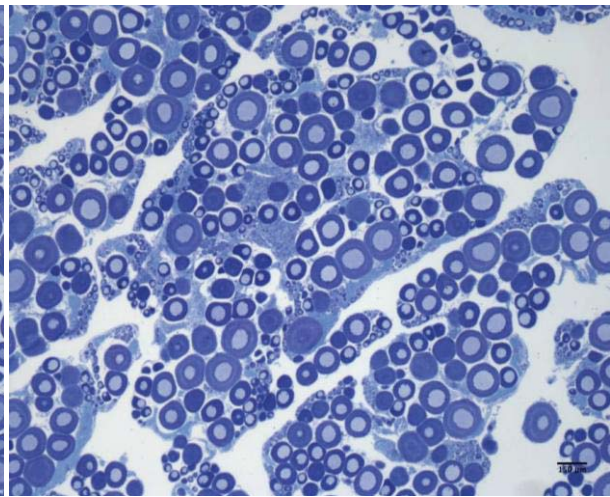


Figure 13. Representative histological section of ovary of haddock from the Egersund Bank.

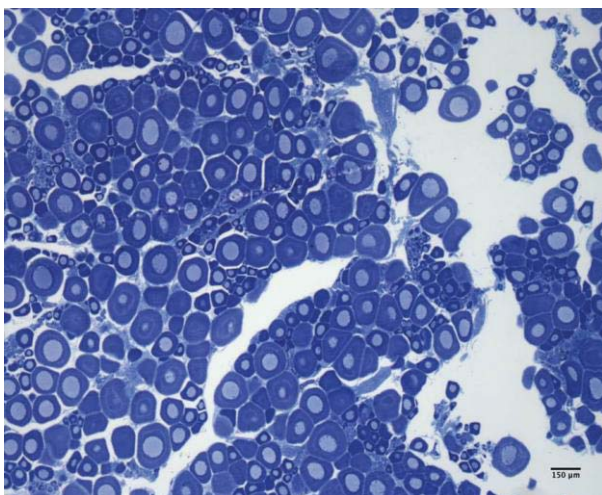


Figure 14. Representative histological section of ovary of haddock from the Tampen.

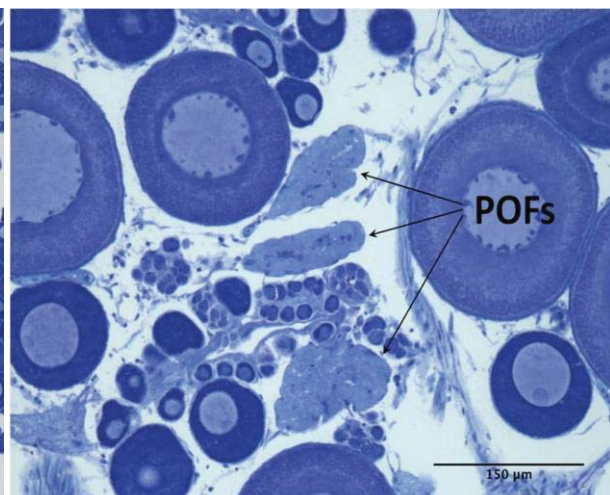


Figure 15. Post-ovulatory follicles (POFs), which are formed and left in the ovary when the eggs are spawned. Thus, a POF is the remains of the ovarian follicle. More specifically, it consists of the previous supporting follicle cells covering the follicle proper.

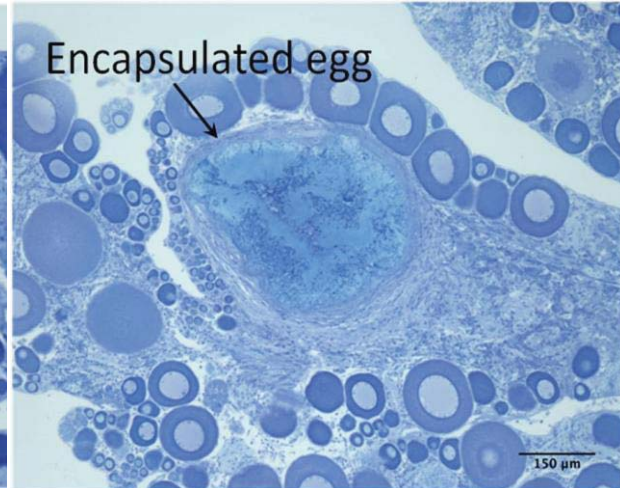
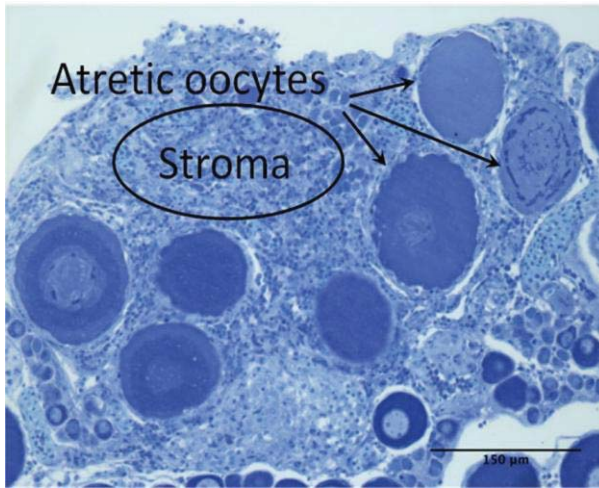


Figure 16. The presence of atretic eggs (oocytes) in the ovary. Atresia (Atr) refers to that the developing oocyte is resorbed prior to egg release. Stroma (tissue) is connective tissue supporting the internal ovarian organization.

Figure 17. Encapsulated eggs (Enc.) are eggs that were ready to be spawned, i.e. were fully developed, but where the actual egg release was not executed. Enc. structures may remain in the ovary for a very long time (due to the robust egg shell (chorion)).

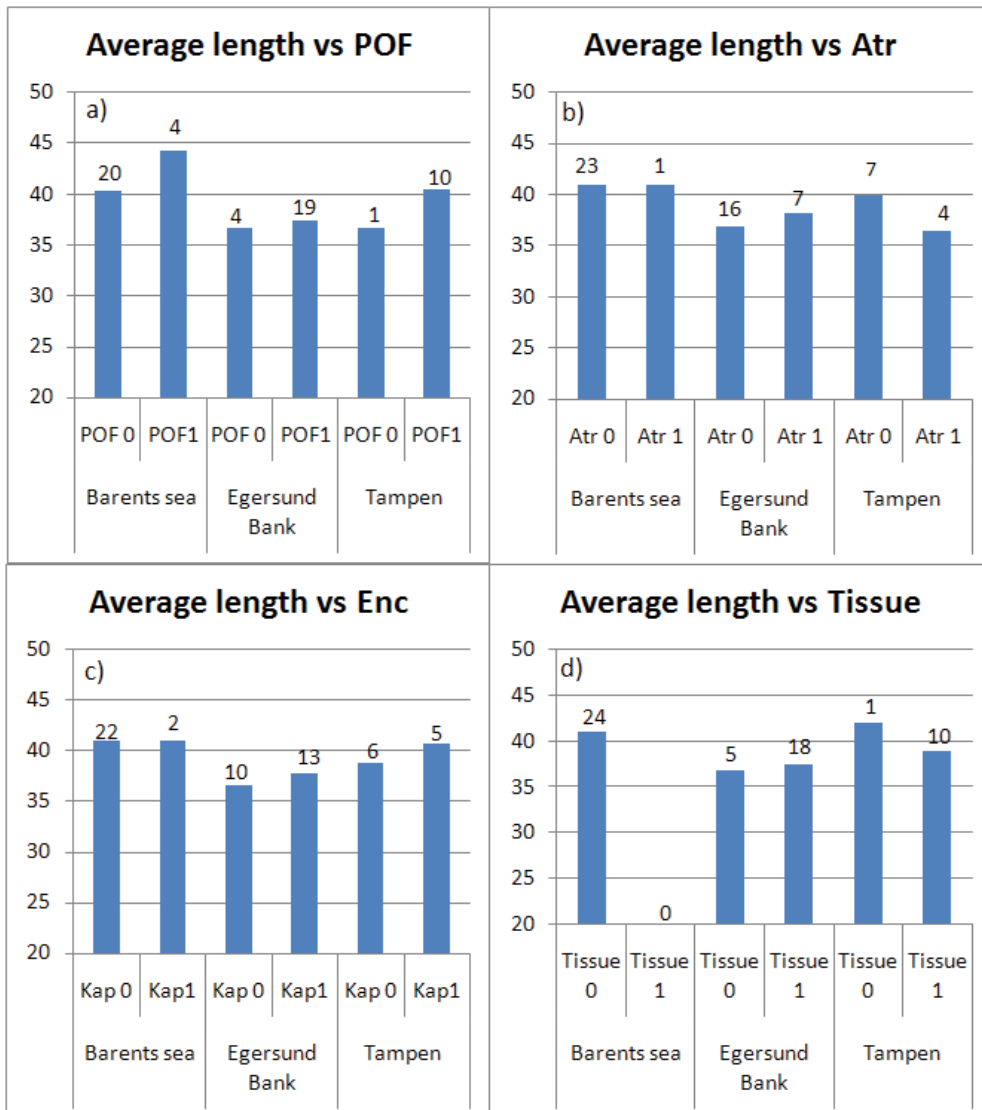


Figure 18. Frequency of (A) average length vs POF, (B) average length vs Atr, (C) average length vs Enc and (D) average length vs tissue in haddock.

In relative terms (prevalence), it appeared that a high fraction of the sampled haddock in the Egersund Bank and Tampen area had spawned before (POFs: 80%), but very few of those from the Barents Sea (POFs: 20%) (Figure 19a). Prevalence of encapsulated eggs (Enc.) (Figure 19b) and atresia (Atr) (Figure 19c) was also significantly higher in the first two areas. The most noticeable difference was seen in terms of prevalence of tissue (Figure 19d). Despite that 20% of the Barents Sea haddock had spawned before, there was no extraordinary amount of tissue at all in any histological section. For the other structures examined there were no any striking differences when taking into account the relative difference in number of sexually immature and mature individuals (Figure 18). Thus, the previous spawning history of the fish apparently very much influenced the presence or absence of the analysed structures.

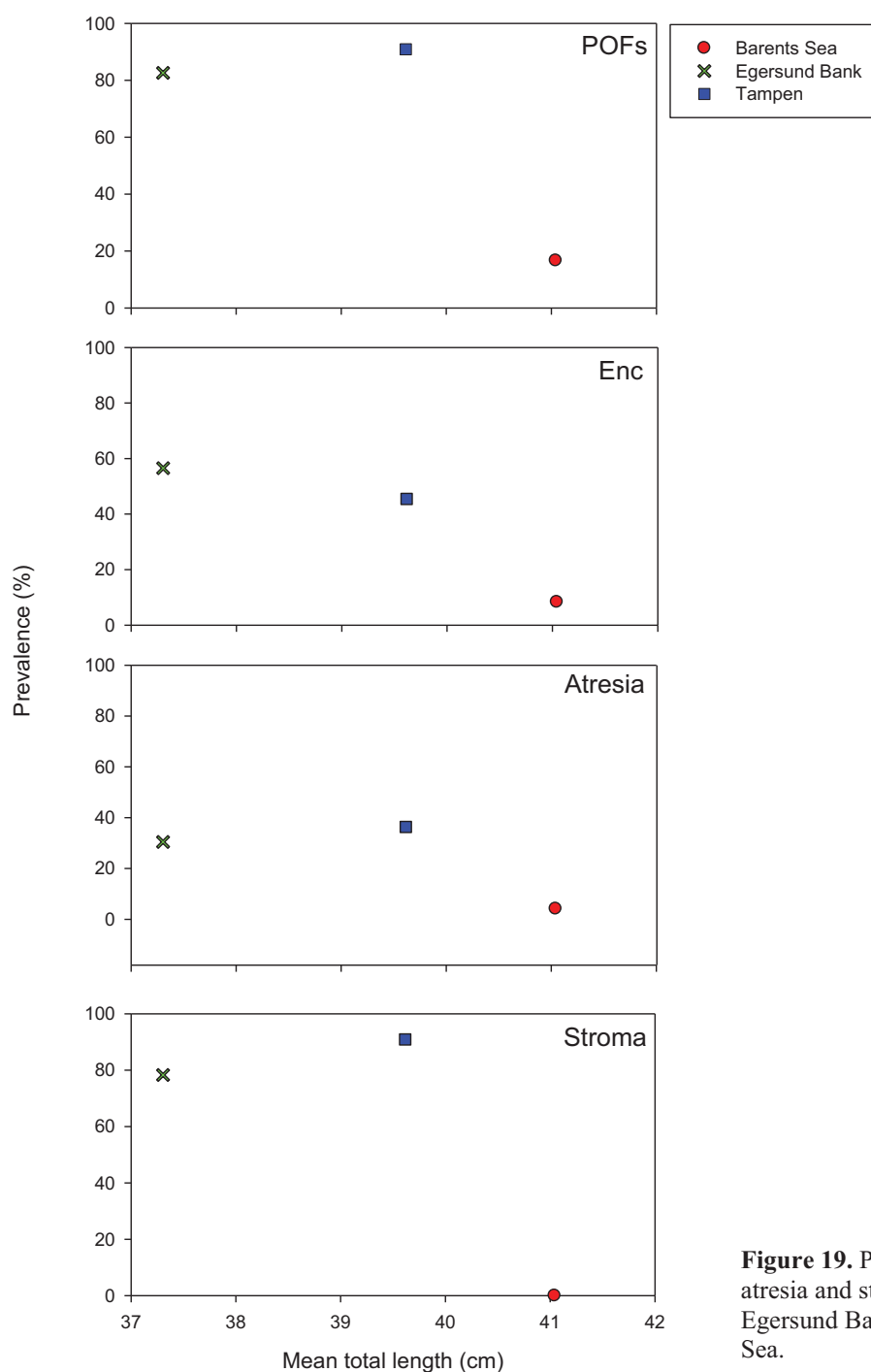


Figure 19. Prevalence (%) of POFs, Enc, atresia and stroma of haddock from the Egersund Bank, Tampen and the Barents Sea.

The histological analyses were complicated by that female haddock spawned earlier in the Egersund Bank and Tampen than in the Barents Sea. Thus, the sample of the last category was dominated by immature fish while the samples of the two first categories were dominated by sexually mature fish. This difference in previous history obviously influenced the ovarian internal morphology. The total absence of extraordinary tissue, as defined, in the Barents Sea haddock ovary points, however, to that there might be some underlying differences. However, as seen from Figs 1-3, all three ovarian samples show a high number of healthy oocytes, indicating an overall successful production of these germ cells in the three areas of study.

9 Lipid extraction and lipid class separation.

Haddock have, like others gadidaes, big, fatty livers. These lean fish store most of its energy as fat in the liver. Analyses of lipid content and fatty acid profiles in different lipid classes demonstrated significant differences in lipid amount in the livers of haddock from Tampen compared with haddock from the other regions (Fig 20). Haddock from the Halten Bank were fished in beginning of December, and had the largest livers with the highest lipid content (<60 % lipid). Haddock from the Egersund Bank and Tampen were fished in July, and haddock from the Barents Sea in August. Generally, haddock from Tampen were in poorer condition than haddock from the other regions.

Haddock from Tampen had small livers with low lipid content (>40 % lipid). The relationship between the relative size of the liver and the amount of lipids for the different areas, are shown in Figure 21. Fish with reduced LSI also had reduced levels of lipid % in the liver. This means that haddock from Tampen had approx. only 50 % of the energy reserve compared with haddock from the other regions (Figure 22).

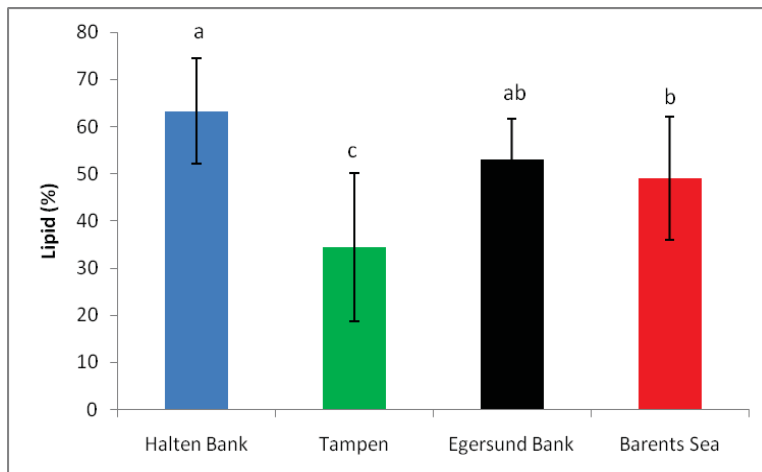


Figure 20. Lipid content in the liver (% of wet weight). Different letters indicate statistical difference $p < 0.05$.

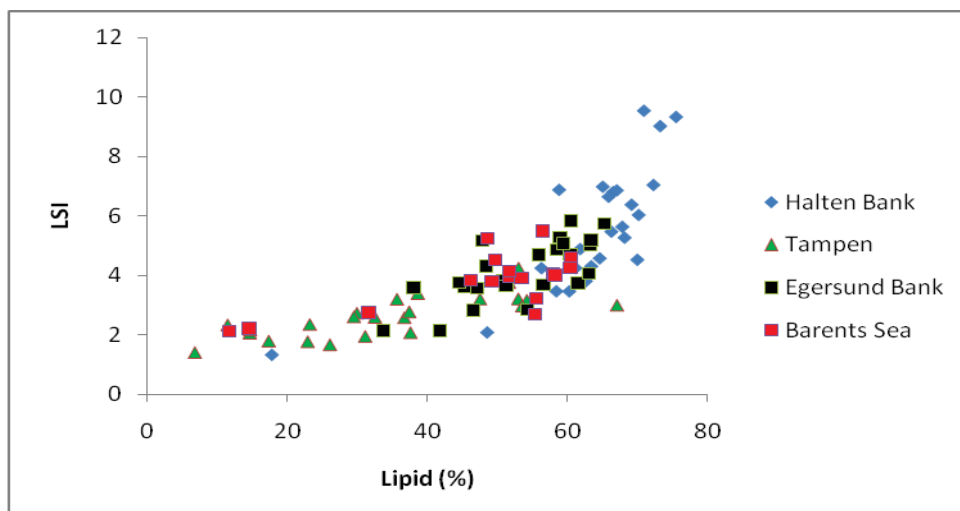


Figure 21. Liver somatic index (LSI) vs lipid content (%) in haddock from the studied areas.

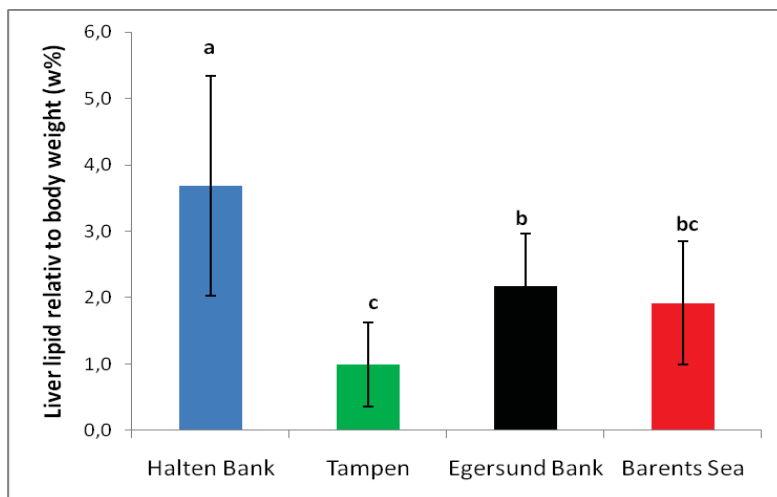


Figure 22. Amount of liver lipids relative to body weight (Total amount of lipid (g)/Body weight (g) x 100) in haddock from the different regions. Different letters indicate statistical difference $p < 0.05$.

Table 15 shows the lipid classes composition calculated as distribution of fatty acids (% of total fatty acids). The neutral lipids (NL) is the energy storages of haddock and does mainly consist of triacylglycerols (TAG). The NL dominated the liver lipids and constituted more than 94 % of the total amount of fatty acids. 2-4 % of the total lipids are polar lipids (PL). The PL are phospholipids, found as membrane lipids. The PL is analyzed as Σ of phosphatidylcholin (PC)/phosphatidylethanolamine (PE) and Σ of phosphatidylserine (PS)/ phosphatidylinositol (PI). PC and PE are the dominating phospholipids and contribute to more than 80 % of the PL, and PS/PI stands for the rest. The free fatty acids (FFA) contribute to around 1 % of the total fatty acids in the lipids.

Table 15. Lipid classes composition (amount of FA/totally FA x 100).

	NL	FFA	PC/PE	PS/PI
Halten Bank	97.1 ± 1.9	1.3 ± 0.7	1.4 ± 1.4	0.3 ± 0.4
Tampen	94.6 ± 3.4	1.6 ± 0.9	3.4 ± 2.5	0.4 ± 0.4
Egersund Bank	96.3 ± 1.0	1.6 ± 0.4	1.7 ± 0.6	0.4 ± 0.2
Barents Sea	95.8 ± 4.4	0.8 ± 0.3	2.8 ± 3.1	0.7 ± 1.0

We found no large differences in lipid class distribution in haddock liver caught from the four regions. In haddock with a low amount of lipid (>20 %), the PL contributed up to 13 % of the total lipids.

The fatty acids profile from haddock are given in Appendix 3.

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11 Appendices

11.1 Methods

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 (Klungsoyr et al. 1988). The GC/MS system was equipped with a HP-6890 GC, a 50 m x 0,25 mm, 0.25 μm Varian Factor Four CC VF-5ms capillary column inserted directly into the ion source. Other conditions were: injector temperature 280° C; transfer line 275° C; column temperature, 60° C for 1 min, 60-100° C at 15° C/min, 100-280° C at 6° C /min, 9 min at final temperature, carrier gas He at 1.5 ml/min. Electron impact ionization at 70 eV was used. Samples were injected by auto sampler, 1 μl splitless injection.

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

Analysis of NPD/PAH and alkyl phenols in fish bile

Bile (100 μl) was diluted in 100 μl sodium acetate buffer (0.01 M, pH 5). 18 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 derivatized with pentafluorobenzoyl chloride as described elsewhere (Boitsov et al. 2007), and the samples concentrated to 0,3 ml hexane solution under a nitrogen stream (40° C). All samples were added 100 μl relative internal standard (RIS, 360 ng/ml) and analysed by GC-MS in selected ion monitoring (SIM) mode using negative chemical ionisation (NCI). The following masses were scanned for in SIM mode:

Compound	Molecular mass / Quantifier ion
2-Naphthol	338
1-Naphthol	338
1-methyl-2-Naphthol	352
2-methyl-1-Naphthol	352
2-hydroxyfluorene	376
1-hydroxyphenanthrene	388
1-hydroxypyrene	412
1-hydroxychrysene	438
1-Naphthol-d7	345
1-Hydroxypyrene-d9	421

DNA adducts

Chemicals

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

DNA adduct analysis

Tissue samples were semi-thawed and the DNA extracted and purified according to Dunn et al. 1987 and Reichert and French 1994, slightly modified as described in Ericson and Balk, 2000. DNA adducts were enriched and normal nucleotides hydrolyzed to nucleosides by the nuclease P₁ method, using 0.41 μg Nuclease P₁/ μg DNA and a 45 min incubation period at 37 °C (Reddy and Randerath 1986; Beach and Gupta 1992). The DNA adducts were radiolabelled using 5'- $[\gamma\text{-}^{32}\text{P}]\text{triphosphate}$ ($[\gamma\text{-}^{32}\text{P}]\text{ATP}$) and T₄ polynucleotide kinase. Separation and cleanup of adducts was performed by a modified, multidirectional, thin-layer chromatography (TLC) on laboratory-produced polyethyleneimine cellulose sheets that serve as anionic exchanger support. After elution, adducts were then located on the sheets and

quantified by storage phosphor imaging technology (PhosphorImagerTMSI and ImageQuant 5.0). In addition, several quality control experiments were performed in parallel to the analysis of the various fish tissue samples.

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

DNA for adduct analysis was quantified on the basis of its absorption at 260 nm in a GeneQuant spectrophotometer from Pharmacia Biotech, Uppsala, Sweden. Liquid scintillation spectroscopy was performed in a Packard Tri-Carb 2100TR liquid scintillation counter from Packard Instrument Company. A Desaga spreader from Desaga Heidelberg, Germany, was used to prepare the TLC-sheets. The DNA adducts were located and the levels quantified on the TLC sheets with ImageQuant, 5.0 software, Molecular Dynamics, by the storage phosphor imaging technique using a PhosphorImagerTM SI instrument (Sunnyvale, CA, USA), essential according to methodology described by Reichert et al. 1998.

DNA adduct levels at different sites were compared statistically with one-way ANOVA.

For every value that is below the detection limits, an average of zero and the background is taken in order to be able to calculate average values for groups. Because, if any adducts are present in the sample, their value is below the background value of the autoradiogram, and could range from zero up to the background value.

Lipid extraction and lipid classes separation

Material and methods

Total lipid were extracted from haddock samples (0.5 g) by a modified Folch method (Folch et al. 1957) with chloroform/methanol (2:1 v/v). 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 phosphatidylserine (PS)/phosphatidylinositol (PI).

The lipid classes separation were done using the same columns (500 mg aminopropyl SPE, Supelco) and same solvent regime as described in Perez-Palacios *et al.* (2007), but it was found that the amount of solvent had to be modified for these marine samples. The column was loaded with 0.5 ml of lipid extraction (10 mg/ml): NL were eluted with 4 ml chloroform; FFA were eluted with Diethylether:Acetic acid (98:2 v/v); PC/PE were eluted with with 10 ml of methanol; PS/PI were eluted with 17 ml methanol/3N HCL in MeOH (9:1, v/v).

The purity of the lipid classes fractionations were tested using thin layer chromatography (TLC) (Olsen et al. 1989). All lipid classes fraction were evaporated to dryness with nitrogen (g) at 40° C.

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 after tests for normal variation.

11.2 Biological data on fish sampled

Table 1. Biological data on haddock from the Egersund Bank.

Females										
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length (cm)	Weighth (g)	Liver weight (g)	Gonad weight (g)	Sex, stage
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	6	34	418	24	3	1,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	7	35	446	16	3	1,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	13	33	466	10	3	1,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	15	30	320	15	2	1,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	21	38	497	25	3,1	1,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	24	41	638	18	5	1,1
14.07.2008	24418-258	58°14.3	03°27.3	Haddock	26b	36	460	18	2,3	1,1
14.07.2008	24419-259	58°14.6	03°26.4	Haddock	34	35	391	12	2,1	1,1
14.07.2008	24419-259	58°14.6	03°26.4	Haddock	35	35	459	30	4,3	1,1
14.07.2008	24419-259	58°14.6	03°26.4	Haddock	36	36	475	31	2,7	1,1
14.07.2008	24419-259	58°14.6	03°26.4	Haddock	37	36	493	25	3,7	1,1
14.07.2008	24419-259	58°14.6	03°26.4	Haddock	38	37	446	12	4	1,1
14.07.2008	24419-259	58°14.6	03°26.4	Haddock	39	40	649	29	4,6	1,1
14.07.2008	24419-259	58°14.6	03°26.4	Haddock	40	42	794	27	4,5	1,1
14.07.2008	24419-259	58°14.6	03°26.4	Haddock	41	39	631	38	3	1,1
14.07.2008	24419-259	58°14.6	03°26.4	Haddock	42	37	552	38	2,1	1,1
14.07.2008	24418-258	58°14.3	03°27.3	Haddock	27	44	960	40	6	1,4
14.07.2008	24418-258	58°14.3	03°27.3	Haddock	28	40	650	35	3,2	1,4
14.07.2008	24418-258	58°14.3	03°27.3	Haddock	29	40	580	20	3,2	1,4
14.07.2008	24418-258	58°14.3	03°27.3	Haddock	30	38	524	32	3	1,4
14.07.2008	24418-258	58°14.3	03°27.3	Haddock	31	37	440	24	3	1,4
14.07.2008	24418-258	58°14.3	03°27.3	Haddock	32	36	450	30	2,6	1,4
14.07.2008	24418-258	58°14.3	03°27.3	Haddock	33	39	622	28	2	1,4
				23	Mean	37	537	25,09	3,28	

Table 1. Continued.

Males										
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length (cm)	Weigth (g)	Liver weight (g)	Gonad weight (g)	Sex, stage
12.07.2008	24407-249	57°44.2	04°03.4	Haddock	1	37	499	18	n.m.	2,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	2	37	514	25	n.m.	2,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	3	33	367	19	n.m.	2,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	4	31	320	12	n.m.	2,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	5	32	347	13	n.m.	2,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	8	30	313	12	n.m.	2,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	9	34	387	11	n.m.	2,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	10	31	275	10	n.m.	2,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	11	31	325	14	n.m.	2,1
12.07.2008	24409-249	57°44.2	04°03.4	Haddock	12	34	424	20	n.m.	2,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	14	34	520	27	n.m.	2,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	18	34	418	17	n.m.	2,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	19	37	492	26	n.m.	2,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	20	35	446	26	n.m.	2,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	22	35	374	8	n.m.	2,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	23	36	462	17	n.m.	2,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	25	33	398	20	n.m.	2,1
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	16	37	493	25	n.m.	2,2
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	17	45	979	35	n.m.	2,4
12.07.2008	24410-250	57°44.4	03°53.0	Haddock	26a	34	n.m.	15	n.m.	n.m.
				20	Mean	35	440	18,50		
					Stdev	3	150	7,02		

Table 2. Biological data on cod from Egersund Bank.

Females										
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length (cm)	Weighth (g)	Liver weight (g)	Gonad weight (g)	Sex, stage
09.07.2008	24401-241	N 57°42.5	E 05°53.9	Cod	1	61	2215	50	n.m.	1,1
09.07.2008	24401-241	N 57°42.5	E 05°53.9	Cod	2	51	1316	16	n.m.	1,1
09.07.2008	24401-241	N 57°42.5	E 05°53.9	Cod	3	50	1263	20	n.m.	1,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	5	56	1642	50	n.m.	1,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	8	44	860	33	n.m.	1,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	12	62	2562	95	n.m.	1,1
12.07.2008	24407-248	N 57°43.4	E 05°40.2	Cod	16	49	1006	18	n.m.	1,1
12.07.2008	24407-248	N 57°43.4	E 05°40.2	Cod	20	54	1529	18	n.m.	1,1
12.07.2008	24407-249	N 57°44.2	E 04°03.4	Cod	22	39	637	17	n.m.	1,1
12.07.2008	24407-249	N 57°44.2	E 04°03.4	Cod	23	31	280	5	n.m.	1,1
12.07.2008	24407-248	N 57°43.4	E 05°40.2	Cod	15	54	1500	22	n.m.	1,1
13.07.2008	24415-255	N 57°57.6	E 05°25.0	Cod	31	44	612	10	1,6	1,1
14.07.2008	24418-258	N 58°14.3	E 03°27.3	Cod	33	49	1117	10	3,2	1,1
14.07.2008	24418-258	N 58°14.3	E 03°27.3	Cod	34	53	1246	12	4,4	1,1
14.07.2008	24418-258	N 58°14.3	E 03°27.3	Cod	35	56	1720	41	4	1,1
19.07.2008	24438-278	N 59°18.1	E 02°45.7	Cod	4	56	1795	74,5	4,6	1,1
19.07.2008	24438-278	N 59°18.1	E 02°45.7	Cod	6	53	1385	42,7	4,1	1,1
22.07.2008	24447-287	N 59°34.1	E 03°15.6	Cod	7	67	2784	70	5,2	1,1
13.07.2008	24415-255	N 57°57.6	E 05°25.0	Cod	26	70	3216	75	15	1,4
13.07.2008	24415-255	N 57°57.6	E 05°25.0	Cod	27	50	1124	12	3,8	1,4
13.07.2008	24415-255	N 57°57.6	E 05°25.0	Cod	28	69	2958	65	8,7	1,4
13.07.2008	24415-255	N 57°57.6	E 05°25.0	Cod	29	86	5800	140	36	1,4
13.07.2008	24415-255	N 57°57.6	E 05°25.0	Cod	32	37	480	7	1,3	1,4
19.07.2008	24428-268	N 58°38.8	E 03°38.4	Cod	39	80	5245	174	19,4	1,4
				24	Mean	55,04	1846	44,88	8,56	
					Stdev	12,83	1373	43,28	9,79	

Table 2. Continued.

Males										
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weighth	Liver	Gonad	Sex,
						(cm)	(g)	weight (g)	weight (g)	stage
13.07.2008	24415-255	N 57°57.6	E 05°25.0	Cod	30	48	1223	30	n.m.	2
09.07.2008	24401-242	N 57°37.3	E 06°60.4	Cod	4	47	1048	22	n.m.	2,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	6	58	1744	66	n.m.	2,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	7	44	892	46	n.m.	2,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	9	41	676	36	n.m.	2,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	10	64	2700	80	n.m.	2,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	11	40	623	30	n.m.	2,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	13	35	538	22	n.m.	2,1
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Cod	14	36	501	30	n.m.	2,1
12.07.2008	24407-248	N 57°43.4	E 05°40.2	Cod	17	48	1062	15	n.m.	2,1
12.07.2008	24407-248	N 57°43.4	E 05°40.2	Cod	18	53	1334	22	n.m.	2,1
12.07.2008	24407-248	N 57°43.4	E 05°40.2	Cod	19	51	1427	26	n.m.	2,1
12.07.2008	24407-248	N 57°43.4	E 05°40.2	Cod	21	48	1040	16	n.m.	2,1
12.07.2008	24407-250	N 57°44.4	E 03°53.0	Cod	24	43	927	30	n.m.	2,1
14.07.2008	24418-258	N 58°14.3	E 03°27.3	Cod	36	56	1795	20	n.m.	2,1
14.07.2008	24418-258	N 58°14.3	E 03°27.3	Cod	38	57	1835	31	n.m.	2,1
12.07.2008	24407-250	N 57°44.4	E 03°53.0	Cod	25	88	6475	198	n.m.	2,4
14.07.2008	24418-258	N 58°14.3	E 03°27.3	Cod	37	63	2222	28	n.m.	2,4
19.07.2008	24438-278	N 59°18.1	E 02°45.7	Cod	1	89	6870	346	27,5	2,4
19.07.2008	24438-278	N 59°18.1	E 02°45.7	Cod	3	86	5670	250	13,1	2,4
22.07.2008	24447-287	N 59°34.1	E 03°15.6	Cod	8	57	2025	129	1,2	2,4
19.07.2008	24438-278	N 59°18.1	E 02°45.7	Cod	5	63	2600	51	1,2	2,5
				22	Mean	55,23	2056	69,26	10,75	
					Stdev	15,60	1860	86,61	12,50	

Table 3. Biological data on saithe from Egersund Bank.

Females											
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weigth	Liver	Gonad	Sex,	G
						(cm)	(g)	weight (g)	weight (g)	stage	(%)
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	1	52	1422	126	n.m.	1,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	4	57	1777	128	n.m.	1,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	5	54	1457	140	n.m.	1,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	7	49	1130	110	n.m.	1,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	11	53	1637	178	n.m.	1,1	n.n.
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Saithe	23	63	2280	105	n.m.	1,1	n.n.
12.07.2008	24407-247	N 57°34.2	E 06°11.4	Saithe	25	51	1423	115	n.m.	1,1	n.n.
09.07.2008	24401-241	N 57 °42.5	E 05°53.9	Saithe	2	60	2180	182	n.m.	1,4	n.n.
09.07.2008	24401-241	N 57 °42.5	E 05°53.9	Saithe	14	54	1609	154	n.m.	1,5	n.n.
				9	Mean	55	1657	138			
					Stdev	4	372	28			
Males											
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weigth	Liver	Gonad	Sex,	G
						(cm)	(g)	weight (g)	weight (g)	stage	(%)
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	3	61	2160	163	n.m.	2,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	6	49	1208	120	n.m.	2,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	9	47	963	57	n.m.	2,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	10	51	1420	108	n.m.	2,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	12	58	1863	104	n.m.	2,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	13	46	1117	108	n.m.	2,1	n.n.
12.07.2008	24407-247	N 57°34.2	N 06°11.4	Saithe	16	49	1112	64	n.m.	2,1	n.n.
12.07.2008	24407-247	N 57°34.2	N 06°11.4	Saithe	17	87	1130	60	n.m.	2,1	n.n.
12.07.2008	24407-247	N 57°34.2	N 06°11.4	Saithe	20	47	1150	83	n.m.	2,1	n.n.
12.07.2008	24407-247	N 57°34.2	N 06°11.4	Saithe	21	50	1190	90	n.m.	2,1	n.n.
12.07.2008	24407-247	N 57°34.2	N 06°11.4	Saithe	22	51	1440	118	n.m.	2,1	n.n.
12.07.2008	24407-247	N 57°34.2	N 06°11.4	Saithe	24	53	1414	66	n.m.	2,1	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	8	59	1884	230	n.m.	2,4	n.n.
09.07.2008	24401-241	N 57 °42.5	N 05°53.9	Saithe	15	62	2778	255	n.m.	2,4	n.n.
12.07.2008	24407-247	N 57°34.2	N 06°11.4	Saithe	18	62	2200	178	n.m.	2,4	n.n.
12.07.2008	24407-247	N 57°34.2	N 06°11.4	Saithe	19	66	2433	262	n.m.	2,4	n.n.
				16	Mean	56	1591	129			
					Stdev	10	555	69			

Table 4. Biological data on Long rough dab from Egersund Bank.

Females										
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weigth	Liver	Gonad	Sex,
						(cm)	(g)	weight (g)	weight (g)	stage
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	1	23	88	1,1	1	1,2
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	2	22	73	1,3	1	1,2
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	4	20	72	1,2	0,8	1,2
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	6	20	61	0,8	0,3	1,1
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	7	20	59	0,7	0,5	1,2?
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	8	20	51	0,7	0,6	1,1
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	9	20	50	0,8	0,7	1,2
14.07.2008	259	58°14.6	03°26.5	Long rough dab	11	24	92	0,6	0,9	1,1
				8	Mean	21	68,3	0,9	0,7	1,2
					Stdev	2	15,9	0,3	0,2	0,1
Males										
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weigth	Liver	Gonad	Sex,
						(cm)	(g)	weight (g)	weight (g)	stage
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	3	22	70	0,8	n.m.	2,1
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	5	21	73	1	n.m.	2,1
13.07.2008	24413-253	57°58.0	03°31.4	Long rough dab	10	20	48	1,4	n.m.	2,1
14.07.2008	259	58.146305	3.264891	Long rough dab	12	20	57	1,1	n.m.	2,1
				4	Mean	21	62,0	1,1		2,1
					Stdev	1	11,6	0,2		0,0

Table 5. Biological data on Haddock from Tampen.

Females										
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weighth	Liver	Gonad	Sex,
						(cm)	(g)	weight (g)	weight (g)	stage
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Hyse	1	35	410	7,4	2,2	1,1
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Hyse	4	42	615	16	3,6	1,1
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Hyse	9	34	348	7,2	1,9	1,1
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Hyse	10	35	431	11,8	2,2	1,1
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	14	35	432	9,4	1,8	1,1
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Hyse	5	38	582	18,7	0,7	1,4
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Hyse	6	41	641	9,1	3,7	1,4
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Hyse	7	44	835	19,7	5,5	1,4
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Hyse	8	40	617	11	3,4	1,4
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	18	35	452	10,6	3,1	1,4
27.07.2008	24471-311	N 61°15.8	E 01°19.0	Hyse	21	44	833	26,8	4,6	1,4
27.07.2008	24471-311	N 61°15.8	E 01°19.0	Hyse	22	45	950	30,1	5,3	1,4
				12	Mean	39	596	14,8	3,2	
					Stdev	4	194	7,6	1,5	
Males										
Date	Serie/st.no.	Latitude	Longitude	Species	Lable	Length	Weighth	Liver	Gonad	Sex,
						(cm)	(g)	weight (g)	weight (g)	stage
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	11	39	585	15,3	1,1	2,1
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	12	39	566	11,1	0,8	2,1
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	13	37	491	12,7	0,6	2,1
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	16	37	451	9,4	0,4	2,1
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	17	38	495	21	0,8	2,1
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Hyse	2	48	1070	31,9	2	2,4
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Hyse	3	38	499	8,4	0,5	2,4
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	15	42	855	36,3	1,5	2,4
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	19	36	431	12	0,6	2,4
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Hyse	20	38	499	15	1,6	2,4
27.07.2008	24471-311	N 61°15.8	E 01°19.0	Hyse	23	40	590	18,9	0,7	2,5
27.07.2008	24471-311	N 61°15.8	E 01°19.0	Hyse	24	40	709	24,1	4,4	2,5
27.07.2008	24471-311	N 61°15.8	E 01°19.0	Hyse	25	45	857	32,3	4,4	2,5
				13	Mean	40	623	19,1	1,5	
					Stdev	3	194	9,4	1,4	

Table 6. Biological data on cod from Tampen.

Females										
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weigth	Liver	Gonad	Sex,
						(cm)	(g)	weight (g)	weight (g)	stage
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Torsk	1	60	3030	130,4	8	1,1
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Torsk	2	47	1055	18	2,5	1,1
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Torsk	3	71	4175	81,8	10,4	1,1
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Torsk	6	55	1610	28,9	4,4	1,1
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Torsk	12	54	1910	73,3	4,5	1,1
27.07.2008	24469-309	E 60°56.8	E 01°17.3	Torsk	15	41	714	13,4	2,3	1,1
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Torsk	16	39	614	17	1,9	1,1
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Torsk	21	64	2110	27,4	5,5	1,1
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Torsk	5	69	2615	39,6	10,5	1,4
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Torsk	7	80	5385	273,1	17,2	1,4
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Torsk	11	53	1300	11,1	9,6	1,4
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Torsk	18	61	2415	34,2	8,9	1,4
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Torsk	4	78	5660	261,7	20,2	1,5
27.07.2008	24471-311	N 61°15.8	E 01°19.0	Torsk	22	70	3655	79,7	10,4	1,5
27.07.2008	24471-311	N 61°15.8	E 01°19.0	Torsk	23	62	2625	38,3	7,9	1,5
				15	Mean	60	2592	75,2	8,3	
					Stdev	12	1563	84,7	5,2	
Males										
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weigth	Liver	Gonad	Sex,
						(cm)	(g)	weight (g)	weight (g)	stage
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Torsk	14	43	849	14,7	0,5	2,1
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Torsk	8	74	3635	56,5	6	2,4
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Torsk	9	62	2500	57,8	1,7	2,4
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Torsk	10	62	2515	66,3	2	2,4
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Torsk	13	70	3235	170,6	2,2	2,4
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Torsk	17	85	6350	151,9	13,6	2,4
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Torsk	19	63	2275	-	2,5	2,4
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Torsk	20	72	3960	137,5	1,4	2,4
				8	Mean	66	3165	93,6	3,7	
					Stdev	12	1605	59,0	4,3	

Table 7. Biological data on saithe from Tampen.

Females												
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weigth	Liver	Gonad	Sex,	GSI	LSI
						(cm)	(g)	weight (g)	weight (g)	stage	(%)	(%)
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	6	49	1139	74,2	3,9	1,1	0,34	6,51
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	9	46	904	58,4	3	1,1	0,33	6,46
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	10	48	858	30,2	2,7	1,1	0,32	3,52
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	11	52	1435	111,2	5,1	1,1	0,36	7,75
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	12	39	471	13,6	1,4	1,1	0,30	2,89
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	14	53	1090	75,3	4,5	1,1	0,41	6,91
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	15	49	983	59,1	3,8	1,1	0,39	6,01
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	16	45	696	25,7	1,4	1,1	0,20	3,69
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Sei	17	40	530	14	1,3	1,1	0,25	2,64
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Sei	24	47	892	30,8	2,8	1,1	0,31	3,45
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	4	59	2140	141,8	11,6	1,4	0,54	6,63
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Sei	20	71	3370	270,3	32,8	1,4	0,97	8,02
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Sei	21	94	7640	946	76,8	1,4	1,01	12,38
27.07.2008	24469-309	N 60°56.8	E 01°17.3	Sei	23	67	2570	159,3	13,1	1,4	0,51	6,20
27.07.2008	24470-310	N 61°15.2	E 01°16.6	Sei	25	66	2745	228,3	12,8	1,4	0,47	8,32
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	7	53	1276	71,9	3,6	1,5	0,28	5,63
27.07.2008	24468-308	N 60°55.9	E 01°15.8	Sei	18	46	899	41,1	3	1,5	0,33	4,57
				17	Mean	54	1743	138,3	10,8		0,43	5,98
					Stdev	14	1733	220,9	18,7		0,23	2,45
Males												
Date	Serie/st.no.	Latitude	Longitude	Species	Label	Length	Weigth	Liver	Gonad	Sex,	GSI	LSI
						(cm)	(g)	weight (g)	weight (g)	stage	(%)	(%)
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	1	48	1046	63,2	0,7	2,1	0,07	6,04
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	2	42	655	30,5	0,4	2,1	0,06	4,66
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	3	42	600	22,4	0,3	2,1	0,05	3,73
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	5	51	1165	55,9	0,7	2,1	0,06	4,80
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	13	47	869	16,4	2,9	2,1	0,33	1,89
27.07.2008	24467-307	N 61°16.8	E 02°02.2	Sei	8	68	3055	230,9	10,1	2,4	0,33	7,56
27.07.2008	24468-308	N 60°55.9	N 01°15.8	Sei	19	68	2630	142,3	4,8	2,4	0,18	5,41
27.07.2008	24469-309	N 60°56.8	N 01°17.3	Sei	22	51	1485	194,4	0,9	2,4	0,06	13,09
				8	Mean	52	1438	94,5	2,6		0,14	5,90
					Stdev	10	918	83,4	3,4		0,12	3,34

Table 8. Biological data on Haddock from the Barents Sea.

Females													
Date	Station	Serie no	Latitude	Longitude	Label	Species	Length (cm)	Weight (g)	Liver weight (g)	Gonad weight (g)	Sex, Stage	Age (Years)	LS
24.08.2008	144	2666	N 73 50.6	E 20 05.8	5	Haddock	38	520	11	2,1	1,1	3	2,1
24.08.2008	144	2666	N 73 50.6	E 20 05.8	6	Haddock	54	1395	73	11,2	1,4	6	5,2
25.08.2008	148	2670	N 72 44.8	E 22 31.1	13	Haddock	28	265	n.m.	n.m.	1,1	2	n.r
25.08.2008	148	2670	N 72 44.8	E 22 31.1	14	Haddock	31	335	17	n.m.	1,1	2	5,0
25.08.2008	148	2670	N 72 44.8	E 22 31.1	16	Haddock	41	665	35	n.m.	1,1	3	5,2
26.08.2008	155	2677	N 71 10.9	E 25 02.2	41	Haddock	49	1150	53	n.m.	1,5	5	4,6
26.08.2008	155	2677	N 71 10.9	E 25 02.2	42	Haddock	31	340	4	1	1,1	2	1,1
26.08.2008	155	2677	N 71 10.9	E 25 02.2	43	Haddock	58	2080	91	20	1,4	5	4,3
26.08.2008	155	2677	N 71 10.9	E 25 02.2	45	Haddock	24	140	n.m.	26	1,1	2	n.r
26.08.2008	156	2678	N 71 12.5	E 26 50.3	48	Haddock	40	650	42	3,5	1,1	3	6,4
27.08.2008	163	2685	N 71 50.6	E 30 25.0	56	Haddock	37	485	n.m.	3,3	1,1	3	n.r
27.08.2008	163	2685	N 71 50.6	E 30 25.0	58	Haddock	39	570	n.m.	2,6	1,1	3	n.r
27.08.2008	163	2685	N 71 50.6	E 30 25.0	59	Haddock	37	495	n.m.	2	1,1	3	n.r
28.08.2008	171	2693	N 72 57.7	E 34 25.9	60	Haddock	38	530	21	2,9	1,1	3	3,9
28.08.2008	171	2693	N 72 57.7	E 34 25.9	61	Haddock	31	270	6	1,1	1,1	2	2,2
28.08.2008	171	2693	N 72 57.7	E 34 25.9	62	Haddock	40	705	27	4	1,1	3	3,8
30.08.2008	183	2705	N 71 16.2	E 38 04.7	90	Haddock	45	820	32	8,1	1,1	4	3,9
30.08.2008	183	2705	N 71 16.2	E 38 04.7	91	Haddock	40	620	34	4	1,1	3	5,4
30.08.2008	183	2705	N 71 16.2	E 38 04.7	98	Haddock	40,5	595	24,6	3	1,1	n.m.	4,1
30.08.2008	183	2705	N 71 16.2	E 38 04.7	99	Haddock	43	705	32,3	3	1,1	n.m.	4,5
30.08.2008	186	2708	N 71 14.5	E 32 17.8	100	Haddock	41	670	18,1	5,6	1,1	5	2,7
30.08.2008	186	2708	N 71 14.5	E 32 17.8	101	Haddock	39	535	17,2	3,0	1,1	3	3,2
30.08.2008	186	2708	N 71 14.5	E 32 17.8	102	Haddock	44	780	34,9	4,8	1,1	n.m.	4,4
30.08.2008	186	2708	N 71 14.5	E 32 17.8	103	Haddock	41	630	23,7	3,3	1,1	n.m.	3,7
30.08.2008	187	2709	N 70 43.2	E 32 18.4	104	Haddock	49	1165	50,9	10,8	1,1	5	4,3
30.08.2008	187	2709	N 70 43.2	E 32 18.4	105	Haddock	44	820	25,1	5,2	1,1	4	3,0
30.08.2008	187	2709	N 70 43.2	E 32 18.4	106	Haddock	34	390	18	2,2	1,1	3	4,6
30.08.2008	187	2709	N 70 43.2	E 32 18.4	107	Haddock	43,5	760	n.m.	n.m.	1,1	n.m.	n.r
						Mean	40,0	681,6	31,4	5,8		3,3	4,
						stdav	7,4	389,8	20,8	6,1		1,2	1,

Table 8. Continued.

Males												
Date	Station	Serie no	Latitude	Longitude	Label	Species	Length (cm)	Weight (g)	Liver weight (g)	Gonad weight (g)	Sex, Stage	Age (Years)
25.08.2008	147	2669	N 73 15.2	E 21 30.4	7	Haddock	37	442	18	n.m.	2,1	3
25.08.2008	148	2670	N 72 44.8	E 22 31.1	10	Haddock	55	1645	70	n.m.	2,2	7
25.08.2008	148	2670	N 72 44.8	E 22 31.1	11	Haddock	36	550	22	n.m.	2,1	3
25.08.2008	148	2670	N 72 44.8	E 22 31.1	12	Haddock	47	980	27	n.m.	2,2	5
25.08.2008	148	2670	N 72 44.8	E 22 31.1	15	Haddock	50	1380	n.m.	n.m.	2,2	6
26.08.2008	154	2676	N 71 18	E 25 08.1	17	Haddock	32	315	14	n.m.	2,1	2
26.08.2008	155	2677	N 71 10.9	E 25 02.2	39	Haddock	52	1560	85	n.m.	2,2	4
26.08.2008	155	2677	N 71 10.9	E 25 02.2	40	Haddock	38	500	13	n.m.	2,1	3
26.08.2008	155	2677	N 71 10.9	E 25 02.2	44	Haddock	41	720	12	n.m.	2,1	3
26.08.2008	156	2678	N 71 12.5	E 26 50.3	46	Haddock	51	1315	63	n.m.	2,4	4
26.08.2008	156	2678	N 71 12.5	E 26 50.3	47	Haddock	49	1215	64	n.m.	2,4	6
27.08.2008	159	2681	N 71 12.5	E 26 50.3	49	Haddock	41	510	37	n.m.	2,1	n.m.
27.08.2008	163	2685	N 71 50.6	E 30 25.0	57	Haddock	35	485	n.m.	n.m.	2,5	3
						Mean	43,4	893,6	38,6			4,1
						stdav	7,6	473,4	26,8			1,6

Table 9. Biological data on cod from the Barents Sea.

Females											
Date	Station	Serie no	Latitude	Longitude	Label	Species	Length (cm)	Weight (g)	Liver weight (g)	Gonad weight (g)	Sex Stage
26.08.2008	155	2677	N 71°10.9	E 25°02.2	18	Cod	77	4230	231	27	1-3
26.08.2008	155	2677	N 71°10.9	E 25°02.2	21	Cod	50	1045	17	4,6	1-3
26.08.2008	155	2677	N 71°10.9	E 25°02.2	22	Cod	50	1040	32	4,9	1-3
26.08.2008	155	2677	N 71°10.9	E 25°02.2	23	Cod	32	215	4	n.m.	1-3
26.08.2008	155	2677	N 71°10.9	E 25°02.2	28	Cod	80	5000	462	39,2	1-4
26.08.2008	155	2677	N 71°10.9	E 25°02.2	33	Cod	80	5110	312	25,1	1-4
27.08.2008	162	2684	N 71°50.2	E 28°42.5	53	Cod	79	4000	152	23,3	1-4
27.08.2008	162	2684	N 71°50.2	E 28°42.5	55	Cod	55	1530	63	7	1-3
					8 females	Mean	63	2771	159,1	18,7	
						Stdev	18	2005	164,7	13,4	

Table 9. Continued.

Males											
Date	Station	Serie no	Latitude	Longitude	Label	Species	Length (cm)	Weight (g)	Liver weight (g)	Gonad weight (g)	Sex Stage
24.08.2008	141	2663	N 74°28.0	N 19°26.9	1	Cod	50	1110	56	n.m.	2-1
24.08.2008	141	2663	N 74°28.0	N 19°26.9	2	Cod	73	3170	106	n.m.	2-2
24.08.2008	141	2663	N 74°28.0	N 19°26.9	3	Cod	41	645	28	n.m.	2-1
24.08.2008	141	2663	N 74°28.0	N 19°26.9	4	Cod	61	1950	33	n.m.	2-1
26.08.2008	155	2677	N 71°10.9	E 25°02.2	19	Cod	45	920	22	n.m.	2-1
26.08.2008	155	2677	N 71°10.9	E 25°02.2	20	Cod	81	5400	385	n.m.	2-2
26.08.2008	155	2677	N 71°10.9	E 25°02.2	24	Cod	53	1320	39	n.m.	2-1
26.08.2008	155	2677	N 71°10.9	E 25°02.2	25	Cod	79	5030	317	n.m.	2-4
26.08.2008	155	2677	N 71°10.9	E 25°02.2	26	Cod	61	2445	171	n.m.	2-1
26.08.2008	155	2677	N 71°10.9	E 25°02.2	27	Cod	86	5065	287	n.m.	2-5
26.08.2008	155	2677	N 71°10.9	E 25°02.2	29	Cod	46	1145	26	n.m.	2-1
26.08.2008	155	2677	N 71°10.9	E 25°02.2	30	Cod	68	3445	158	n.m.	2-2
26.08.2008	155	2677	N 71°10.9	E 25°02.2	31	Cod	49	1030	20	n.m.	2-1
26.08.2008	155	2677	N 71°10.9	E 25°02.2	32	Cod	75	3920	296	n.m.	2-2
26.08.2008	155	2677	N 71°10.9	E 25°02.2	34	Cod	82	5265	314	n.m.	2-2
27.08.2008	162	2684	N 71°50.2	E 28°42.5	51	Cod	82	4110	142	n.m.	2-2
27.08.2008	162	2684	N 71°50.2	E 28°42.5	52	Cod	87	5940	272	n.m.	2-2
27.08.2008	162	2684	N 71°50.2	E 28°42.5	54	Cod	73	3800	199	n.m.	2-2
30.08.2008	183	2705	N 71°16.2	E 38°04.7	92	Cod	45	750	29	n.m.	2-1
30.08.2008	183	2705	N 71°16.2	E 38°04.7	93	Cod	72	3100	125	n.m.	2-4
30.08.2008	183	2705	N 71°16.2	E 38°04.7	94	Cod	65	1970	n.m.	n.m.	2-4
30.08.2008	183	2705	N 71°16.2	E 38°04.7	95	Cod	39	445	n.m.	n.m.	2-1
30.08.2008	183	2705	N 71°16.2	E 38°04.7	96	Cod	64	2040	n.m.	n.m.	2-4
30.08.2008	183	2705	N 71°16.2	E 38°04.7	97	Cod	54	1220	n.m.	n.m.	2-1
					24 males	Mean	63,8	2718	151		
						Stdev	15,3	1753	122		

Table 10. Biological data on saithe from the Barents Sea.

Females											
Date	Station	Serie no	Latitude	Longitude	Label	Species	Length (cm)	Weight (g)	Liver weight (g)	Gonad weight (g)	St
01.09.2008	190	2712	70 04,86	30 31,89	109	Saithe	31	320	16	n.m.	1
17.09.2008	305	2827	71057	23 209	111	Saithe	62	2410	155	n.m.	1
						Mean	47	1365	85,5		
						Stdev	22	1478	98,3		
Males											
Date	Station	Serie no	Latitude	Longitude	Label	Species	Length (cm)	Weight (g)	Liver weight (g)	Gonad weight (g)	St
Dato, kl	Stasjon	Serie/St nr	Breiddegrad	Lengdegrad	Fisk nr	Art	Lengde	Vekt	Levervekt	gonade	(1=H
26.08.2008	155	2677	71109	25022	35	Saithe	72	3300	n.m.	n.m.	2
26.08.2008	155	2677	71109	25022	36	Saithe	51	1220	n.m.	n.m.	2
26.08.2008	155	2677	71109	25022	37	Saithe	52	1350	n.m.	n.m.	2
26.08.2008	155	2677	71109	25022	38	Saithe	61	2210	n.m.	n.m.	2
01.09.2008	190	2712	70 04,86	30 31,89	108	Saithe	64	2850	n.m.	n.m.	2
17.09.2008	305	2827	71057	23 209	110	Saithe	70	3050	135	n.m.	2
17.09.2008	305	2827	71057	23 209	112	Saithe	53	1490	105	n.m.	2
17.09.2008	305	2827	71057	23 209	113	Saithe	86	6720	327	n.m.	2
17.09.2008	305	2827	71057	23 209	114	Saithe	76	4180	334	n.m.	2
					9 males	Mean	65	2930	225,3		
						Stdev	12	1736	122,2		

Table 11. Biological data on long rough from the Barents Sea.

Females											
Date	Station	Serie no.	Latitude	Longitude	Label	Species	Length (cm)	Weight (g)	Liver weight (g)	Gonad weight (g)	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	65	Long rough dab	42,5	791	21,8	29,3	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	66	Long rough dab	42,5	703	10,6	10,6	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	67	Long rough dab	41	642	15,2	26,3	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	68	Long rough dab	44	695	12,7	21,6	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	69	Long rough dab	43	757	21,1	24,7	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	70	Long rough dab	43	853	19,4	44,5	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	71	Long rough dab	41	672	18,2	30,4	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	72	Long rough dab	44	875	22,8	35,1	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	73	Long rough dab	41,5	688	16,5	25,2	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	74	Long rough dab	44	775	22,4	29,4	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	75	Long rough dab	40	577	9,2	23,4	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	76	Long rough dab	44	869	24,3	40	
29.08.2008	175	2697	N 72°53.6	E 38°15.4	77	Long rough dab	46	892	27,9	36,5	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	78	Long rough dab	43	795	nd	nd	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	79	Long rough dab	43,5	860	18,7	18,5	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	80	Long rough dab	43,5	760	18,9	35,8	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	81	Long rough dab	46	950	24,7	43,6	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	82	Long rough dab	40,5	710	13,8	24,4	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	83	Long rough dab	45	840	21,8	29,5	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	84	Long rough dab	44,5	885	22,8	33	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	85	Long rough dab	44,5	880	18,6	46,2	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	86	Long rough dab	44,5	800	17,7	23,5	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	87	Long rough dab	45	780	18,2	29,3	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	88	Long rough dab	42,5	685	19,7	30,1	
29.08.2008	178	2700	N 72°19.0	N 38°04.7	89	Long rough dab	46	955	19,7	22,7	
					24 females	Mean	43,4	788	19,0	29,7	
						Stdev	1,7	99	4,5	8,6	

Table 12. Biological data of Haddock from Halten Bank.

Females										
Date	St.nr,serienr	Posisjon	Label	Species	Length (cm)	Vekt(g) (g)	Lever (g)	Gonad (g)	Sex, stage	Age (year)
30.11.2008	552.55112	64°42.19` 09°25.54`	H3	Haddock	64	2818	120	51	1--2	7
30.11.2008	552.55112	64°42.19` 09°25.54`	H4	Haddock	60	2084	142	43	1--2	6
30.11.2008	552.55112	64°42.19` 09°25.54`	H7	Haddock	53	1562	104	28	1--2	4
30.11.2008	552.55112	64°42.19` 09°25.54`	H8	Haddock	52	1422	98	22	1--2	4
30.11.2008	552.55112	64°42.19` 09°25.54`	H9	Haddock	60	1643	22	21	1--4	11
30.11.2008	553.55113	64°29.80` 08°45.08`	H11	Haddock	48	942	36	13	1--2	4
30.11.2008	553.55113	64°29.80` 08°45.08`	H13	Haddock	50	1162	82	19	1--2	6
30.11.2008	553.55113	64°29.80` 08°45.08`	H14	Haddock	45	903	51		1--1	3
30.11.2008	553.55113	64°29.80` 08°45.08`	H16	Haddock	44	856	80	10	1--2	3
01.12.2008	554.55114	64°42.36` 08°48.30`	H17	Haddock	55	1903	172	47	1--2	6
01.12.2008	554.55114	64°42.36` 08°48.30`	H18	Haddock	38	518	18	4	1--2	2
01.12.2008	554.55114	64°42.36` 08°48.30`	H20	Haddock	44	931	64	14	1--2	3
01.12.2008	554.55114	64°42.36` 08°48.30`	H23	Haddock	37	509	22	5	1--2	3
				Mean	50,0	1327	77,8	23,1		4,8
				stdev	8,4	668	48,6	16,1		2,4
Males										
30.11.2008	551.55111	64°56.58` 10°20.08`	H1	Haddock	55	1796	88		2--2	6
30.11.2008	551.55111	64°56.58` 10°20.08`	H2	Haddock	47	977	34		2--2	3
30.11.2008	552.55112	64°42.19` 09°25.54`	H5	Haddock	45	921	88		2--2	7
30.11.2008	552.55112	64°42.19` 09°25.54`	H6	Haddock	51	1258	88		2--2	4
30.11.2008	552.55112	64°42.19` 09°25.54`	H10	Haddock	37	470	20		2--2	3
30.11.2008	553.55113	64°29.80` 08°45.08`	H12	Haddock	57	1654	100		2--2	4
30.11.2008	553.55113	64°29.80` 08°45.08`	H15	Haddock	54	1674	107		2--2	4
01.12.2008	554.55114	64°42.36` 08°48.30`	H19	Haddock	38	549	29		2--2	4
01.12.2008	554.55114	64°42.36` 08°48.30`	H21	Haddock	36	430	9		2--1	2
01.12.2008	554.55114	64°42.36` 08°48.30`	H22	Haddock	43	872	40		2--2	3
01.12.2008	554.55114	64°42.36` 08°48.30`	H24	Haddock	60	1933	106		2--2	6
01.12.2008	554.55114	64°42.36` 08°48.30`	H25	Haddock	39	617	28		2--2	3
				Mean	46,8	1095,9	61,4			4,1
				stdev	8,4	549,5	37,6			1,5

Table 13. Biological data of cod from Halten Bank.

Females											
Date	St.no.-	Position	Label	Species	Length	Weight	Liver	Gonade	Sex,	Age	GSI
	Serie no.				(cm)	(g)	(g)	(g)	Stage	(Year)	(%)
29.11.2008	549-55109	16,29` 10°25,	T2	Cod	60	2048	46		1,1	3	0,00
29.11.2008	549-55109	16,29` 10°25,	T3	Cod	66	2885	137	23	1,1	4	0,80
01.12.2008	554-55114	42,36` 08°48,	T8	Cod	52	1579	30	4	1,1	2	0,25
02.12.2008	558-55118	04,88` 06°47,	T13	Cod	66	2423	52	26	1,2	4	1,08
02.12.2008	558-55118	04,88` 06°47,	T15	Cod	68	3067	45	9	1,1	3	0,29
02.12.2008	558-55118	04,88` 06°47,	T16	Cod	78	5217	342	113	1,2	4	2,21
04.12.2008	566-55126	54,32` 06°11,	T18	Cod	74	4233	139	67	1,4	5	1,61
04.12.2008	566-55126	54,32` 06°11,	T19	Cod	85	6909	247	160	1,4	7	2,37
04.12.2008	567-55127	53,62` 05°33,	T20	Cod	48	1055	19		1,1	2	0,00
			9	Mean	66,3	3268	117,4	57,4		3,8	1,0
				stdev	11,9	1875	111,8	59,3		1,6	0,9
Males											
Date	St.no.-	Position	Label	Species	Length	Weight	Liver	Gonade	Sex-	Age	GSI
	Serie no.				(cm)	(g)	(g)	(g)	Stage	(Year)	(%)
29.11.2008	549-55109	16,29` 10°25,	T1	Cod	68	3128	113		2,2	4	
30.11.2008	552-55112	42,19` 09°25,	T4	Cod	82	5570	270	176	2,2	5	3,26
30.11.2008	552-55112	42,19` 09°25,	T5	Cod	66	3111	85	153	2,2	5	5,17
01.12.2008	554-55114	42,36` 08°48,	T6	Cod	56	1907	35		2,1	2	
01.12.2008	554-55114	42,36` 08°48,	T7	Cod	47	1128	18		2,1	2	
01.12.2008	554-55114	42,36` 08°48,	T9	Cod	69	3988	230		2,2	4	
01.12.2008	554-55114	42,36` 08°48,	T10	Cod	73	4351	235		2,2	4	
01.12.2008	554-55114	42,36` 08°48,	T11	Cod	61	2747	61		2,2	4	
02.12.2008	558-55118	04,88` 06°47,	T12	Cod	56	1318	10		2,1	2	
02.12.2008	558-55118	04,88` 06°47,	T14	Cod	51	1286	12		2,1	2	
03.12.2008	560-55120	13,61` 06°22,	T17	Cod	80	4726	106		2,2	5	
04.12.2008	568-55128	58,91` 05°08,	T21	Cod	66	3461	178		2,2	3	
			12	Mean	64,6	3060	112,8	164,5		3,5	4,2
				stdev	10,9	1450	93,9	16,3		1,2	1,4

Table 14. Biological data of saithe from Halten Bank.

Females												
Date	St.no.- Serie no.	Position	Label	Species	Length (cm)	Weight (g)	Liver (g)	Gonade (g)	Sex, Stage	Age Year	GSI (%)	LSI (%)
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S2	Saithe	45	833	28	6	1,2	5	0,73	3,36
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S3	Saithe	48	865	39	3	1,1	4	0,35	4,51
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S5	Saithe	49	962	26	7	1,2	6	0,73	2,70
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S7	Saithe	55	1723	98	40	1,2	6	2,38	5,69
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S9	Saithe	61	1870	88	27	1,2	6	1,47	4,71
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S11	Saithe	56	1491	89	23	1,2	6	1,57	5,97
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S14	Saithe	56	1248	33	9	1,1	5	0,73	2,64
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S15	Saithe	51	1185	58	7	1,1	4	0,59	4,89
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S17	Saithe	63	2071	86	38	1,2	7	1,87	4,15
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S18	Saithe	67	2787	218	86	1,2	9	3,18	7,82
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S20	Saithe	63	2022	123	41	1,2	7	2,07	6,08
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S21	Saithe	63	2004	119	71	1,2	9	3,67	5,94
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S23	Saithe	60	1740	108	46	1,2	7	2,72	6,21
30.11.2008	552-55112	N 64°42.19' E 09°25.54'	S25	Saithe	38	502	31	2	1,1	3	0,40	6,18
			15	Mean	55,4	1521,6	81,7	29,0		6,0	1,60	5,1
				stdev	8,3	622,8	52,7	26,3		1,8	1,08	1,5

Table 14. Continued.

Date	St.no.- Serie no.	Position	Label	Species	Length (cm)	Weight (g)	Liver (g)	Gonade (g)	Sex, Stage	Age Year	GSI (%)
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S1	Saithe	51	1255	52		2,2	6	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S4	Saithe	49	1070	37		2,2	6	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S6	Saithe	47	942			2,2	5	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S8	Saithe	57	1722	100		2,2	6	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S10	Saithe	51	1249	52		2,2	5	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S12	Saithe	50	1061	52		2,2	5	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S13	Saithe	43	849	64		2,2	4	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S16	Saithe	54	1275	57		2,2	5	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S19	Saithe	55	1455	93		2,2	5	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S22	Saithe	71	3363	245		2,2	10	
30.11.2008	552-55112	N 64° 42.19` E 09° 25.54`	S24	Saithe	39	540	24		2,2	3	
			11	Mean	51,5	1344	77,6			5,5	
				stdev	8,3	739	63,1			1,8	

