Background levels of oil-derived pollution in fish and invertebrates from the coastal zone around the Faroe Islands.

- Biomarker analyses in fish and analyses of PAH and metals in invertebrates

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Master thesis

University of Copenhagen

March 2004

Preface

This project has been carried out under an agreement between the company BP Amoco Exploration and The Food-, Veterinary- and Environmental Agency (FVEA) in the Faroe Islands. At the same time it makes up my Master of Science thesis in biology at the University of Copenhagen.

The project consists of two studies: A **fish study** and an **invertebrate study**. The fish study (chapter 2 and 3) has been funded by FOÍB (Faroese Oil Industry Group) and the results are previously reported in Hoydal, K. "Coastal baseline – exposure and effects of PAH on fish in the coastal zone around the Faroe Islands". The invertebrate study (chapter 4 and 5), along with two additional fish analyses of DNA adducts and PCB and dioxins in cod liver and the completion of the report, has been funded by the BP Amoco Exploration.

I have, partly, participated in the analysis work, by performing the biomarker analyses in the fish study, at NIVA (Norwegian Institute for Water Research) under supervision of the staff at NIVA during a stay in Oslo in the period from the 26th of August to the 21st of September 2002. The chemical analyses in the invertebrate study (including the additional analyses of fish liver) have been bought from qualified laboratories.

Maria Dam from the FVEA has been the project leader, and my supervisors have been Ian Lambert, associate professor at the University of Copenhagen, August Krogh Institute, and professor Ketil Hylland at the Norwegian Institute for Water Research (NIVA).

I wish to thank and acknowledge all the people, who have been involved in different parts of the work of the project:

The project leader Maria Dam from the FVEA, I wish to thank for setting up this project and working to provide the funding and for being helpful during all the work with the project.

My supervisors: Associate professor Ian Lambert at the University of Copenhagen, I wish to thank for good guidance in connection with writing of the report, and professor Ketil Hylland at NIVA, for making it possible for me to participate in the analysis work by performing the biomarker analyses at NIVA, and for discussion of the results.

I also wish to thank the rest of the staff at NIVA: Anders Ruus, Knut-Erik Tollefsen, Åse Kristine Rogne, Åse Bakketun og Harry Efraimsen, from marin department, and Siv-Hege Wang and Erling Bratsberg from chemical department, for the help with the analyses.

Tórvild Østerø and Jóhannis Danielsen, both from the Food and Environmental Agency I wish to thank for assisting me in the field, with sampling and sample preparations, Carlo Petersen for dab fishing, Gunnar Djurhuus and Bjørki Geyti from "Hav-, botn- og streym kanningar" for diving for horse mussels and flounders, Maria G. Hansen, Súni Petersen, Eyð Eidesgaard, Jacob P. Joensen, Kristín Baldvinsdóttur and Rókur Tummasarson for the sampling in Suðuroy.

Summary

In this study the exposure level of PAH and other oil-derived pollutants were analysed in fish and invertebrates from the coastal zone of the Faroe Islands.

Four species of fish: sculpins (*Myoxocephalus scorpius*), dab (*Limanda limanda*, cod (*Gadus morhua*) and flounder (*Plathichthys flesus*) were sampled at two locations (Kaldbak and Kirkjubø) and analysed for the biomarkers: EROD activity, CYP1A protein and DNA adducts in liver, PAH metabolites in bile and vitellogenin in blood. Five invertebrate species: limpets (*Patella vulgata*), blue mussels (*Mytilus edulis*), dogwhelks (*Nucella lapillus*), periwinkles (*Littorina obtusata*) and horse mussels (*Modiolus modiolus*) were sampled at six locations and analysed for PAH and metals. The sampling was performed three times in a year (late winter, spring and summer) to be able to detect seasonal variation.

The fish results generally showed low levels of PAH exposure, although large variations were found within the groups, especially in the EROD results. Also in the concentration of pyrene metabolites in bile and in the the vitellogenin results from July large individual variability was seen. The levels of CYP1A induction were influenced by gonadal development in flounder, dab and sculpin, showing negative correlation between CYP1A induction and gonadal developement. The cods sampled were immature and thus not influenced by spawning. Dab were found to be most sensitive to the biomarkers of PAH exposure. Sculpins showed very low levels of CYP1A activity, probably because the catalytic activity of the CYP1A enzymes had been destroyed. There was not found significant correlation between PAH metabolite content in bile and induction of CYP1A. Vitellogenin was analysed in cod and the results showed high levels compared to other studies from reference areas.

The most suitable fish species to use as an indicator organism for the analysed biomarkers seem to be dab or cod. Since the results of the biomarkers show large variation, probably due to small sample sizes, it is suggested that either cod or dab is subjected to further investigation, by analysing a larger number of individuals for the biomarkers, and minimizing the influence of other parameters by sampling only in one season, not influenced by spawning.

The levels of PAH in invertebrates were found to vary between the stations, probably due to local contamination. For all the species the seasonal pattern seemed to be that the highest PAH content was found in winter (Dec.-Feb.) with lower levels in spring (Mar.-May) and lowest levels in summer (June-Aug.). This pattern was seen for all the stations except in Trongisvágur, where the highest content was found in May, and in Kaldbak where the content in blue mussel was at the same level all three seasons.The highest PAH accumulation was found in blue mussels while horse mussels and the snails had lower levels.

With regard to PAH analysis blue mussel seem to be the species best suited as indicator organism, since it accumulates PAH to the greatest extent. For the metals it is not possible to pick one species which is best suited. Different species accumulate different metals to various extents depending on local factors, where availability of prey may be among the important ones. Thus it is not feasible to base the chemical analyses in future investigations on one species only, but rather a selection of species dependent on the characteristics of the location in question.

Contents

1 Introduction

By opening the Faroese offshore area for hydrocarbon exploration activities, a need for investigations on oil-derived pollution around the Faroe Islands has arisen. The area opened in the 1st licence round in August 2000 is located south east of the Faroe Islands. Although the drilling operations take place far from the coast the possible effects from oil on the environment near the coast of the Faroe Islands need to be elucidated.

Oil exploration activities can be an environmental threat due to the release of oil and other toxic substances into the sea. Oil can be released by larger oil spill accidents at the drilling location, or from tankers, if the oil is brought to land. Oil can also be released to the sea from regular shipping and fishing operations. If an oil spill occurs far from the coast, the oil forms an oil slick on the sea, which can drift with the wind and eventually reach the coast. During the drifting on the sea the oil undergoes weathering and degradational processes, and the oil that reaches the coast will most often be more or less degraded (Børresen, 1993).

Oil is mainly composed of saturated hydrocarbons, but contains variable amounts of polycyclic aromatic hydrocarbons (PAH). Even though the PAHs only constitute a minor fraction of the crude oil, they are very resistant against degradational and weathering processes (Børresen, 1993). This makes them suitable for analysis as indicators of oil-derived pollution, since they can be found in the environment long after visible traces of an oil pollution accident have disappeared.
PAHs are the main toxic components of the oil and several of the PAH compounds, such as benzo(a)pyrene, are known to have carcinogenic properties and PAH has been linked to observations of liver lesions and tumors in fish. PAHs can thus have serious long-term effects on the organisms exposed to them.

The economy of the Faroe Islands is based on fisheries, and around 97% of the export is fish or fish products. The coastal zone, being the habitat for several species of fish, invertebrates and seaweeds, also functions as nursery areas of several fish species, some of which are economically important, such as cod (*Gadus morhua*) and saithe (*Pollachius virens*). Pollution affecting organisms in the coastal zone would, therefore, affect these fish species and by this possibly also the recruitment to the economically important fish stocks and fisheries in the Faroe Islands. Oil-derived pollution in the coastal area can thus have serious ecological and also economical effects to the Faroese society.

To be able to detect a possible effect from an oil exploration industry on the near shore environment, the present level of oil-derived pollutants needs to be determined. This includes both knowledge of the current status of concentrations of chemical substances derived from oil (mainly PAH) or substances used in the oil exploration industry and released with produced water or drilling mud at the drilling locations (e.g. metals and alkylated phenols), and effects of these on coastal organisms.

Invertebrates are able to accumulate PAHs in their tissue, whereas vertebrates metabolize PAHs efficiently. Therefore, chemical analyses of parent PAHs in the tissues of vertebrates is not a suitable parameter. Instead the exposure and effects of PAH can be analysed by using biomarkers by which, the response to the impact of contaminants is measured rather than the concentration of the contaminant (Sandvik, 2002). Analyses of biomarkers are often more biologically relevant and are mostly more cost-efficient than chemical analyses (Aas, 2000).

Purpose of the present investigation:
The aim of this work is to determine baseline levels of oil-derived pollutants in organisms living in the coastal zone on the Faroe Islands, to which levels found in future monitoring investigations can be compared, before a possible establishment of an oil industry beyond the explorative phase.

This is done by analysing biomarkers for PAH exposure in fish, and by measuring the concentrations of PAH and selected metals in invertebrates. The biomarkers are: EROD activity, CYP1A protein, and DNA adducts in liver and PAH metabolites in bile. In addition, vitellogenin is analysed in fish as another biological effect marker of xenoestrogenic exposure, since compounds with xenoestrogenic properties (such as alkylphenols) are used in the oil industry, and released to the sea with produced water.

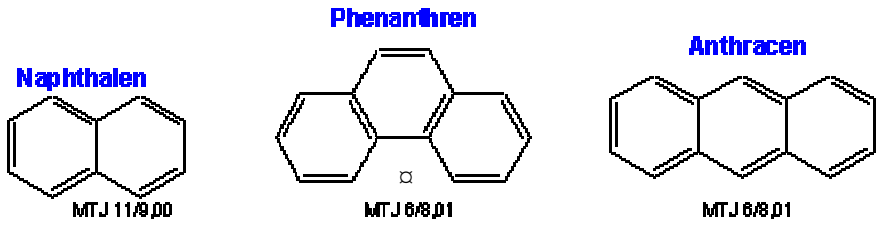
From the results of the analyses, the suitability of the species selected can be evaluated as indicator organisms in future monitoring activities.

The effects of PAH in invertebrates will not be analysed, but the results of the chemical analyses will be used to illustrate background levels of PAH in the waters around the Faroe Islands.

1.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAH) are compounds consisting of two or more fused benzen rings lying in a single plane. The rings are said to be fused when they are sharing at least two carbon atoms. The compounds can be divided into low-molecular–weight PAHs (LPAHs) possessing two or three rings and high-molecular–weight PAHs (HPAHs) possessing four or more rings (Neff, 1979; Meador et al., 1995) (Figure 1.1).

Low-molecular-weight PAHs (LPAHs):



High-molecular-weight PAHs (HPAHs):

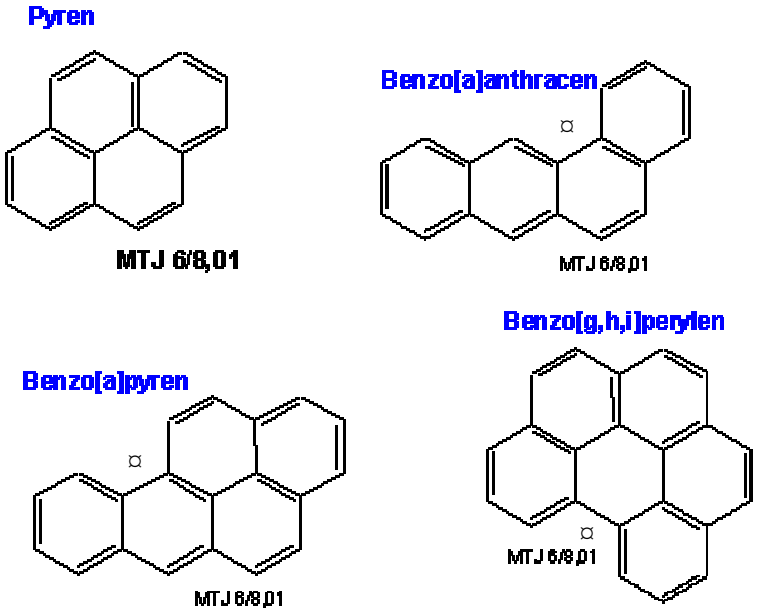


Figure 1.1 Examples of low-molecular-weight and high-molecular-weight PAHs.

The main difference between LPAHs and HPAH is, however, more obvious, when looking at the solubility in water rather than the difference in molecular weight. The solubility tends to decrease as the molecular weight or number of aromatic rings increases, and usually alkylated PAHs have lower solubility than unalkylated compounds, as well as more linear compounds generally are less soluble than angular isomeres (e.g. anthracene is less soluble than phenanthrene, see Figure 1.1 and Table 1.1) (Neff, 1979).

The solubility in water is correlated with the hydrophobicity (or lipofilicity), which can be expressed by the octanol-water partition coefficient (K_{ow})[1]. Generally the hydrophobicity of a PAH compound increases with increasing molecular weight as shown in Table 1.1. The compounds with largest K_{ow} are the most lipophilic and hence have the highest particle affinity. The K_{ow} (generally expressed as log K_{ow}) is thus an important physical parameter when looking at the partitioning behaviour of PAH in the environment (Meador et al., 1995)

Table 1.1 Octanol-water partition coefficients and molecular weights for commonly measured polycyclic aromatic hydrocarbons in environmental samples (modified from Meador et al., 1995).

Chemical	MW	Rings	Solubility at 25°C, µg/l *	Log K_{ow}**
Naphthalene	128,2	2	12500 to 34000	3,34
1-methylnaphthalene	142,2	2		3,88
2-methylnaphthalene	142,2	2		3,91
Biphenyl	153,2	2		3,98
Acenaphthylene	152,2	3	3420	4,08
Acenaphthene	154,2	3		4,08
Fluorene	166,2	2		4,22
2,6-dimethylnaphthalene	156,2	2		4,42
Anthracene	178,2	3	$2,4x10^{-4}$	4,53
Phenanthrene	178,2	3	$6,8x10^{-4}$	4,53
2,3,5-trimethylnaphthalene	170,3	2		4,83
1-methylphenanthrene	192,2	3		5,15
Pyrene	202,3	4	$6,9x10^{-7}$	5,07
Fluoranthene	202,3	4		5,24
Chrysene	228,3	4		5,77
Benz(a)anthracene	228,3	4	$1,1x10^{-7}$	5,90
Benzo(e)pyrene	252,3	5	$5,5x10^{-9}$	6,10
Benzo(a)pyrene	252,3	5	$5,5x10^{-9}$	6,23
Perylene	252,3	5		6,39
Dibenz(a,h)anthracene	278,4	5		6,47
Benzo(b)fluoranthene	252,3	5		6,52
Benzo(k)fluoranthene	252,3	5		6,73
Benzo(ghi)perylene	276,3	6	$1,0x10^{-10}$	7,03
Indeno(1,2,3-cd)pyrene	276,3	6		7,43

*From Nagpal, 1993 (see references therein)
**Mean value of measured or calculated values from several investigations (see Meador et al., 1995 for individual values and references)
MW: Molecular weight in daltons

1.1.1 Sources of PAH

PAHs can be formed by incomplete combustion of organic material at high temperatures (pyrogenic PAH) or by slow transformation of organic matter under pressure (petrogenic PAH). In addition, to a minor extent, PAHs can be formed naturally by biosynthesis in microbes and plants (Neff, 1979, Meador,1995; Aas, 2000). Petrogenic PAH mixtures contain many homologous series of PAH and in each series the concentration of alkyl homologs is greater than the unalkylated parent compound while pyrogenic PAH mixtures are dominated by the unalkylated parent compounds rather than

[1] The octanol-water partition coefficient is the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature. Octanol is an organic solvent that is used as a surrogate for natural organic matter.

alkylated homologs in each homolog series (Neff, 1979; Meador et al, 1995). Pyrogenic PAH consists mostly of HPAH while petrogenic PAH mostly consists of LPAHs (Meador et al. 1995). PAH contamination originating from a petrogenic source show dominance of 2- and 3- ring compounds (LPAHs) while pyrogenic PAH is dominated by 4- and 5- ring compounds (HPAHs) (Meador et al., 1995; Neff, 1979; Aas et al., 2000b). Pyrogenic PAH can be released to the environment naturally from forest fires and volcanic eruptions or from antropogenic sources such as combustion of fossil fuels and industrial waste. Sources of PAH to the marine environment may both be from deposition of airborne particulates from burning of fossil fuels (pyrogenic) and direct discharge of oil to the sea by natural seepage, accidental releases or operational discharges of produced water (petrogenic) (Aas, 2000). Produced water is a by product from oil production, which is separated from the oil and gass and discharged to the sea. is. It is composed of the water that is found naturally in the reservoir, and water that is injected into the reservoir to maintain the pressure, and contains residues of oil along with other chemical substances such as metals alkylated phenols and aromatic hydrocarbons (Meier et al., 2001). PAH from industrial waste generally has a local distribution near the discharge point, while PAH in oil discharged with produced water can be distributed widespread in the ocean (Aas, 2000). The main source of total PAH to the aquatic environment is by petrolium spillage.

1.1.2 Bioavailability

By definition the bioavailable fraction of a compound is the proportion of the total concentration of the compound that is available for uptake by aquatic organisms. The main variable controlling the bioavailable fraction of PAH is organic carbon. In the aquatic environment PAHs are found both dissolved and as particulates in the water column, and the partitioning af PAH into either of these forms is due to their specific water solubility. Thus the distribution of these compounds in adsorbed and in water phase differs for each PAH (Piccardo et al., 2001). The lighter PAHs (LPAHs) seem to remain in the dissolved state to a larger degree than the heavier PAHs (HPAHs), which almost always are bound to particles, due to their low solubility in water and high lipofilicity. The strong particle affinity of heavier PAH can make them more unavailable for metabolism, as they are deposited in the sediments to a greater extent than the lighter PAHs (Næs et al., 1998).

1.1.3 Degradation of PAH

The most important mechanisms of degradation of PAH in the aquatic environment are photooxidation, chemical oxidation and biological transformation by aquatic bacteria, fungi and animals. Some organisms are able to completely oxidize aromatic hydrocarbons to carbon dioxide and water and to use them as a source of energy. Others are not able to carry out the complete oxidation, but are able to partially metabolize aromatic hydrocarbons into various oxygenated metabolites when alternative growth substrate is available (cooxidation). Fungi and aquatic animals possess a cytochrome P450 enzyme system, which is involved in the metabolism of aromatic hydrocarbons. The metabolism of PAH by the cytochrome P450 enzyme system in fish is described in section 1.2.2 and 1.3.

All these mechanisms of degradation require oxygen and hence PAH can be very persistent in anoxic environments, for example when buried in deeper layers of sediments (Neff, 1979).

1.1.4 Acute and long-term toxic properties of PAH

The toxic properties of PAH can be expressed either as acute toxicity or long term damage. Acute toxicity is explained by reversible binding of PAH to lipofilic sites in the cell, such as cellular

membranes and thereby interfering with cellular processes. The LPAHs have significant acute toxicity to aquatic organisms, which HPAHs have not. This can probably be explained by their low solubility in water. Within the LPAHs the toxicity generally increases with increasing molecular weight. Within an aromatic series, the toxicity increases with increasing alkyl substitution on the aromatic nucleus. (Neff, 1979)

Long term damage can be caused by metabolites of PAH, which being more reactive, hydrofilic and electrophilic can bind covalently to macromolecules such as DNA, RNA and proteins in the cell and cause damage such as mutagenis and carcinogenesis. The carcinogenic PAHs are mostly in the four-to-six ring PAH groups. The degree of carcinogenisity of the PAH is related to structure and reactivity of its major metabolites. Highly angular PAHs are more carcinogenic than linear and more condensed forms (Neff, 1979), and the tendency to bind to macromolecules has been found to be connected to the presence of a "bay region" on the PAH molecule (indicated by * on Figure 1.1) (Pahlman & Pelkonen, 1987).

1.2 PAH in aquatic organisms

1.2.1 Uptake

Marine organisms can readily take up PAHs in the marine environment, due to the lipophilic nature of the PAHs. The routes of PAH uptake in pelagic organisms include diffusion from the water through the gills and skin and uptake through the intestine via the diet. For organisms living in or on the sediment an additional route may be diffusion from the sediment through the skin (Meador et al., 1995). The uptake is dependent on several factors such as concentrations of PAH in the environment, the lipofilicity and solubility of the PAH compounds, wether it is dissolved or particle bound along with food preferences and lipid content of the organism (Aas, 2000).

Mussels are filter-feeding organisms and can directly absorb lower weight PAHs through interstitial filtered water, while heavier molecular weight hydrocarbons (four or more rings) are mainly ingested in particulate form through the digestive system (Piccardo et al., 2001).

Fish can take up PAH from the water through the gills, and to a lesser extent through the skin, or from ingestion through the intestine (Neff, 1979). The uptake routes depend on in which form PAH is found in the water, wether in dissolved state or bound to particulates. PAHs dissolved in the water will more easily be taken up by the gills than the particulate PAH, wheras the particle bound PAH more easily are taken up through the intestine than the dissolved PAH compounds (Connel, 1988).

The uptake from water depends mainly on the diffusion over the body surface of which the gill constitutes most of the body surface area (Randall et al., 1998). The rate of toxicant transfer between the water and organism will depend on the lipofilicity (K_{ow}) of the compound (Randall et al., 1998). Factors that increase oxygen requirement (such as temperature rise or exercise) will also affect the rate of water movement over the gill, and thus the rate of lipophilic chemical uptake, since they are brought into contact with the gills by water flow (Connel, 1988). Hence, the transfer of toxicant between the water and organism will vary with oxygen uptake (Randall et al., 1998; Connel, 1988).

Uptake via the food depends on the feeding rate, the concentration of toxicant in the food, the rate at which food is processed by the gut, and the amount of toxicant absorbed versus the amount excreted. The uptake over the gills is found to be the dominating route, while dietary uptake is found to be limited (Randall et al., 1998; Neff, 1979). Studies of dietary uptake in fish generally indicate low uptake efficiency and that uptake efficiency generally declines with increasing chemical hydrofobicity (Meador et al., 1995). It is suggested that uptake from food can be ignored when estimating toxicant body burden in water breathing animals under natural conditions when the feeding rate is low, and thus uptake from food plays a minor role compared to the uptake from water (Randall et al., 1998). The findings of DNA adducts in gill tissue also indicate that exposure does take place via the passage of water over the gills and not only via the ingestion of food (Ericson et al., 1998).

1.2.2 Elimination of PAH

Elimination of PAH can occur via passive diffusion through body surfaces – mainly the gills – or actively via enzymatic degradation creating metabolites, which are more hydrofilic than the lipofilic parent compound and hence can readily be excreted through the intestine and urine via the liver/gall, kidney or digestive glands.

The elimination through the body surfaces includes mostly unmetabolized PAH and occurs when concentrations in the external environment are lower than internal concentrations (Neff, 1979; Meador et al., 1995; Knutzen, 1989). By the conversion of the PAH to a more hydrofilic metabolite the ability to diffuse through the gill membrane is decreased and the elimination by the excretory route is favoured. The rate of elimination can be affected by environmental factors (temperature and salinity) and physiological factors (reproductive state, age, sex, stress and enzyme induction) in addition to factors such as route of uptake, chemical hydrophobicity and exposure history (Meador et al., 1995).

The enzyme system responsible for transformation of PAHs is the mixed function oxygenase (MFO) system or cytochrome P-450 system, which is an electron transfer system located within the endoplasmatic reticulum (the microsomal fraction) of the cell and responsible for metabolism of many xenobiotics (see section 1.3).
The biotransformation process is occuring in two steps. Phase I, an oxidative step catalysed by the cytochrome P-450 enzyme system, by which an oxygen atom is introduced into the molecule, and phase II, in which a larger endogenous molecule (such as glucoronic acid, sulfate, glutathione etc.) is conjugated to the oxidized compound by the aid of different transeferase enzymes. The created metabolite is generally less toxic and can readily be excreated (Goksøyr & Förlin, 1992; Andersson & Förlin, 1992).

Examples of oxidative reactions involved in the first step are hydroxylation, epoxidation, deethylation, and demethylation as shown in Figure 1.2 (Stegeman,1989; Payne, 1984).

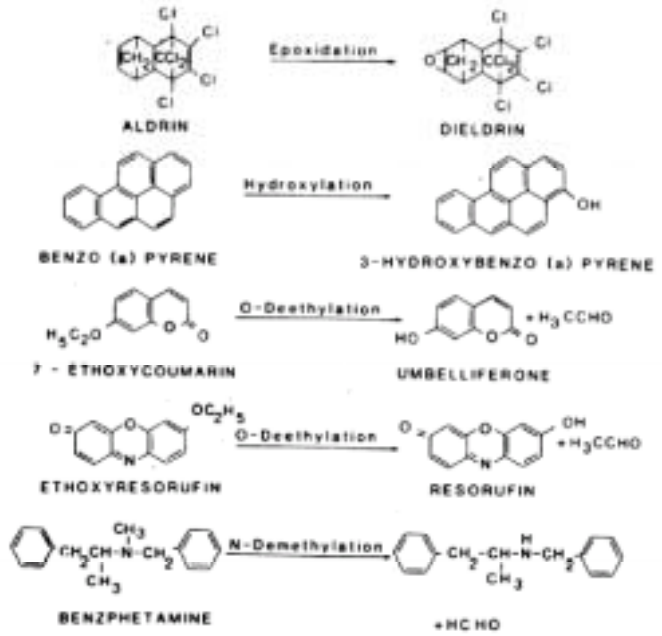


Figure 1.2 Common mixed-function oxydase reactions (Lee, 1981)

Vertebrates can metabolize PAH efficiently by enzymes of the cytocrome P-450 system. The biotransforming processes have shown to be inducible in several fish species, when they are exposed to PAH, by which the excretion capacity for these compounds is enhanced (Aas, 2000). When taken up in fish, PAHs undergo a metabolic transformation, which creates a polar and more water soluble metabolite and thereby enhances excretion. Excretion to the bile is the dominant excretion route of metabolites of especially larger PAH molecules in fish (Meador et al., 1995).

In some cases, however, the biotransformation process can create metabolites that are more toxic than the parental compound. Certain PAHs (e.g. benzo(a)pyrene) can by the fase I metabolism be converted into highly reactive metabolites, such as epoxides, which are able to bind covalently to macromolecules such as DNA. This leads to formation of adducts which eventually can cause mutagenic or carcinogenic effects (Buhler & Williams, 1988; Ericson et al., 1998). Figure 1.3 shows the dominant pathway of benzo(a)pyrene metabolism in fish.

In vertebrates the biotransformational processes mostly take place in the liver, which is the major organ involved in metabolism of organic xenobiotics, but are also found to occur to some extent in other extrahepatic tissues, such as kidney, heart and gills, in analyses of cod and scup, (Husøy et al., 1994; Stegeman et al., 1990). In some organs the induction is found to occur in selected cell types such as endothelial cells in the heart and gills (Stegeman et al., 1990; Husøy et al., 1994). The response of different cells and organs to toxic effects after exposure to xenobiotics is suggested to be determined by the different expression and function of P450 forms in those cells and organs (Husøy et al., 1994).

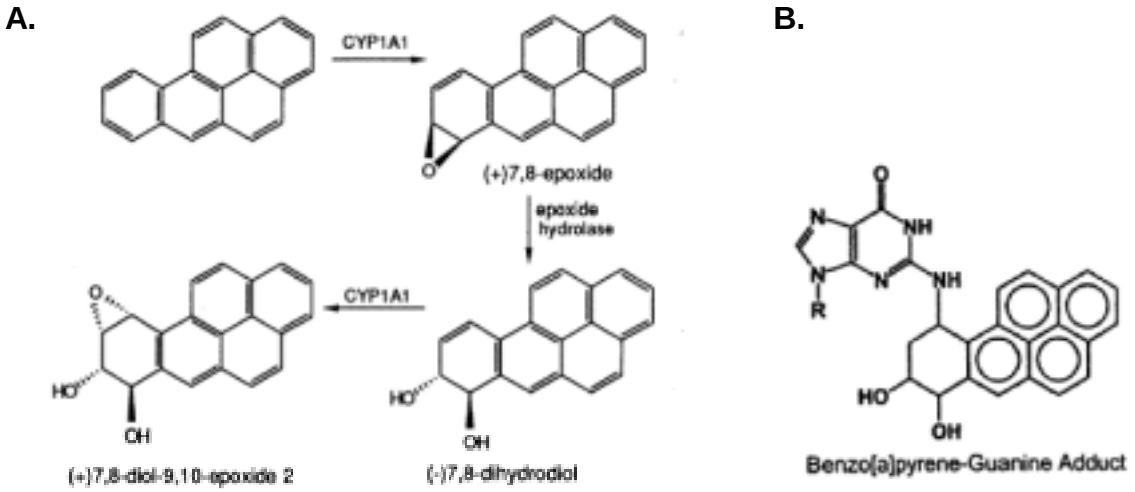


Figure 1.3 A: Metabolic activation of benzo(a)pyrene to dihydrodiol-epoxide metabolites via CYP1A and epoxide hydrolase (Stegeman & Hahn, 1994), **B**: Adduct formation between activated benzo(a)pyrene (7,8-diol-9,10-epoxide) and guanine (Sandvik, 2002).

Although molluscs generally appear to have low cytochrome P-450 monooxygenase activity (Stegeman & Lech, 1991) mussels have been found to have ability to actively metabolize PAH (Gilewicz et al., 1984; Livingstone & Farrar, 1985; Stegeman, 1985). P450 activities measured in molluscan species appear to be concentrated in the endoplasmatic reticulum of the digestive gland (Stegeman & Hahn, 1994; Moore et al.,1989). The activity is, however, low and significant PAH transformation does not take place in mussels (Stegeman & Leech, 1991; Næs et al., 1998). Molluscs are able to accumulate PAH in concentrations above the level of the ambient water (Neff, 1979; Moore et al, 1989), which makes them suitable as indicator organisms for analysis of PAH accumulation

1.2.3 Accumulation of PAH in molluscs

Invertebrates have been found to have different ability to accumulate PAHs. Næs et al. (1995) investigated the accumulation of highly and less soluble and carcinogenic PAHs (KPAHs) in periwinkles, limpets, blue mussels and horse mussels. They found that limpets and periwinkles accumulated relatively higher fractions of the low molecular weight compounds of relatively high solubility and thereby led to underestimates of the KPAH in the ambient environment. Periwinkles appeared to retain the KPAHs to a greater extent than the limpets. Blue mussels have also been found to lead to underestimates of KPAH at some stations, but not at others, while horse mussels were found to accumulate KPAH and other heavy PAHs effectively. Where blue mussels and horse mussels were sampled at the same areas, the horse mussels had higher percentage of KPAH in 20 of 23 cases. Thus, mussels appear to be better indicators than snails, especially when looking at potentially carcinogenic PAH (Næs et al., 1995). The same was found by Næs et al. (1998) where horse mussels were found to have a relative affinity for the heavier PAHs, reflecting the sediment profile, while periwinkle, limpet and blue mussel seemed to have rather similar profiles. Limpets, periwinkle and blue mussels live on rocky shores, while horse mussel is subtidal and generally lives partly buried in sandy or soft sediment substrates. Limpets and periwinkles are grazers while mussels are filter-feeders. The habitat preference of the organism thus seems to be significant for the PAH composition in the tissue, not the feeding behaviour (Næs et al., 1998).

1.3 The Cytochrome P-450 – Mixed function oxygenase - system

The cytochromes P-450 comprise a superfamily of enzymes which function as mixed function oxygenases (MFOs) involved in the metabolism of both endogenous compounds, such as stereoid hormones, stereoids and fatty acids, and exogenous xenobiotics, such as PAH, polychlorinated biphenyls (PCB) and various drugs (Parke, 1990). Many of them have shown to exhibit substrate induced genomal regulation of their enzyme activity, following exposure of the animal to a specific substrate or chemical (Parke, 1990).
The P450 system consists of several accessory enzymes sitting in the endoplasmatic reticulum together with the P450 isoenzymes. The P450 isoenzymes all consist of a single polypeptide chain with an iron-protoporphyrin IX loosely bound by hydrophobic forces, electrostatic and covalent bonds. Four of the iron ligands are contained in the planar porphyrin ring, the fifth is a thiolate group from a cysteine residue in the polypeptide backbone and the sixth is the site of oxygen binding during the monooxygenase reaction cycle (Goksøyr & Förlin, 1992). Electrons from NADPH (or NADH) are transferred to P450 through a flavoprotein, NADPH-cytochrome P450 reductase (or NADH-cytochrome b_5 reductase) to the P450 enzyme, which then inserts an atom of oxygen into the substrate and reduces the second oxygen atom to form water (see Figure 1.4) (Goksøyr & Förlin, 1992). The overall reaction is:

$$RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O$$

Cytochrome P-450 is the terminal substrate binding component and determines the specificity of the reaction, while NADPH-cytochrome c reductase transfers reducing equivalents from NADPH to cytochrome P450 (Andersson & Förlin, 1992).

The P-450 superfamily can be divided into families and subfamilies according to the percentage of similarity of the amino acid sequences of the proteins produced (Nebert & Gonzales, 1990) (see Table 1.2.). The first four families P-450 1-4 are primarily hepatic, microsomal enzymes involved in the catabolism of foregn compounds (Timbrell, 1991). The substrates and inducers for different P450 gene subfamilies are shown in Table 1.3.

Table 1.2 Recommended nomenclature for the P450 system by Nebert et al. (1991), shown by the P4501A subfamily

superfamily	P450
family	P4501
subfamily	P4501A
gene	*CYP1A1, CYP1A2*
mRNA	CYP1A1, CYP1A2
protein	CYP1A1, CYP1A2 (or P4501A1, P4501A2)

The italicized root symbol *CYP* (denoting cytochrome P-450) is used followed by the designation for the individual P450 form. An Arabic number is denoting the family, a letter the subfamily and an Arabic number the individual gene. At mRNA and protein level the non-italicized form is recommended, but for the protein the P450 1A1 form can also be used (Nebert et al., 1991).

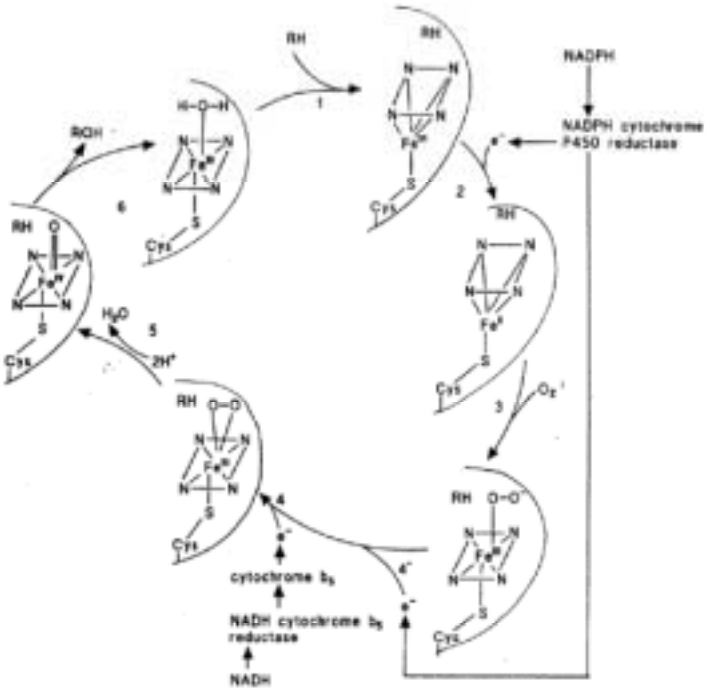


Figure 1.4 The catalytic cycle of the cytochrome P450 monooxygenase (mixed function oxygenase) system. (From Timbrell, 1991). RH: substrate. **Step 1**: Binding of the substrate to cytochrome P450, iron is in the oxydized ferric state. **Step 2**: First electron reduction of the substrate-enzyme complex, the iron atom is reduced from the ferric to the ferrous state, the reducing equivalents are transferred from the NADPH via NADPH cytochrome P450 reductase. **Step 3**: Addition of molecular oxygen and rearrangment of the ternary ferrous oxygenated cytochrome P450-substrate complex. The reduced cytochromes P450-substrate complex binds oxygen and undergoes a rearrangement. **Step 4**: Addition of the second electron from NAPDH via P-450 reductase (alternatively the electron may be donated from NADH via cytochrome b_5 reductase and cytochrom b_5). The complex then rearranges with insertion of one atom of oxygen into the substrate to yield the product. The other oxygen atom is reduced to water, the other product.

Table 1.3 Induction in selected P450 gene subfamilies (redrawn from Stegeman & Hahn, 1994)

Gene family and subfamily	Selected protein members	Prominent substrates[a]	Common inducers[b]	Mechanism
CYP1A	1A1	PAH, planar PCB, 7-ethoxyresorufin	PAH, planar PCB, BNF, chlorinated dioxines (e.g.TCDD) and furans	Mostly transcriptional
	1A2	Acetanilide, estradiol,caffeine	PAH, planar PCB, BNF, chlorinated dioxines (e.g.TCDD) and furans, ISF	Transcriptional, post-transcripptional (protein stabilization)
CYP2B	2B1	Barbiturates, steroids	Barbiturates, non-planar PCBs, DDT	Transcriptional
CYP2E	2E1	Ethanol, alkylnitrosamines	Ethanol, ketones starvation, diabetes	Transcriptional, post-transcripptional (protein stabilization)
CYP3A	3A1	Steroids (6B-hydroxylase)	PCN	Transcriptional
CYP4A	4A1	Lauric acid, arachidonic acid	Clofibrate, phthalates, PCBs	Transcriptional

[a]The substrates listed are common ones for the forms indicated

[b]Abbrevations used: PAH - polynuclear aromatic hydrocarbons; BNF - B-naphthoflavone; DDT - dichlorodiphenyltrichloroethane; ISF - isosafrole; PB - phenobarbital; PCB - polychlorinated biphenyls; PCN - pregnenolone-a-carbonitrile; TCDD - 2,3,7,8-tetrachlorodibenzo-p-dioxin.

The gene family inducible by planar molecules such as PAHs, is the P4501 family (Parke, 1990; Stegeman et al., 1992). Other examples of planar compounds, which are able to induce the P4501 family are some congeners of PCB and polychlorinated dibenzo-*p*-dioxins (PCDD) (Figure 1.5). In mammals, which is the species group most intensively studied, the P4501 family is found to comprise only one subfamily (P4501A) with two genes *CYP1A1* and *CYP1A2* (Nebert & Gonzalez, 1990). Until recently, evidence for only one subfamily has been found in teleost fish species and analyses of DNA sequences have lead to the assumption that this subfamily corresponds to the CYP1A1 family in mammals (Stegeman, 1989) and that the *CYP1A2* gene has evolved by a gene duplication event after the separation of fish and mammalian predecessors (Nebert & Gonzalez, 1990; Goksøyr & Förlin, 1992). Evidence of a second CYP1A gene (*CYP1A3*) has since been found in fish (Berndtson & Chen, 1994). This gene is however not orthologous to the *CYP1A2* gene in mammals, but probably the fish *CYP1A1* precursor gene has undergone its own duplication event separate from the mammalian duplication event (Berndtson & Chen, 1994).

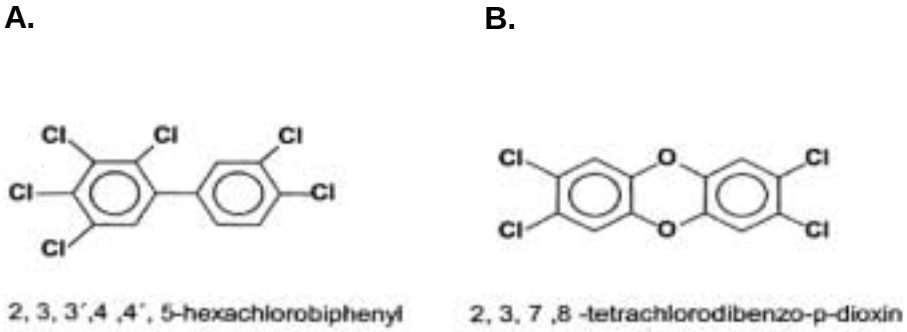


Figure 1.5 Examples of PCB and dioxin, which along with PAHs can induce the P4501A subfamily **A**: PCB 156**, B**: TCDD. (From Sandvik, 2002)

1.3.1 Induction of CYP1A

Inductive response is a process by which a chemical stimulates the rate of gene transcription resulting in increased levels of messenger-RNA and synthesis of the P450 protein (Andersson & Förlin, 1992). The induction of CYP1A involves a cytocolic ligand-activated transcription factor (the Ah receptor), a 90 kDa heatshock protein and a nuclear translocation factor (ARNT) (see Figure 1.6). The chemical agent (inducer) binds as a ligand to the *Ah* receptor, which then undergoes a transformational process involving the dissociation af a 90 kDa heatshock protein and formation of a heterodimer between the Ah receptor-ligand subunit and the ARNT. The heterodimer is then translocated to the nucleus and reacts directly with DNA and initiates transcription of the P4501 genes followed by synthesis of mRNA and proteins (apoproteins; insertions of heme results in active enzyme) (Stegeman et al., 1992; Stegeman & Hahn, 1994; Andersson & Förlin, 1992; Sarasquete & Segner, 2000).

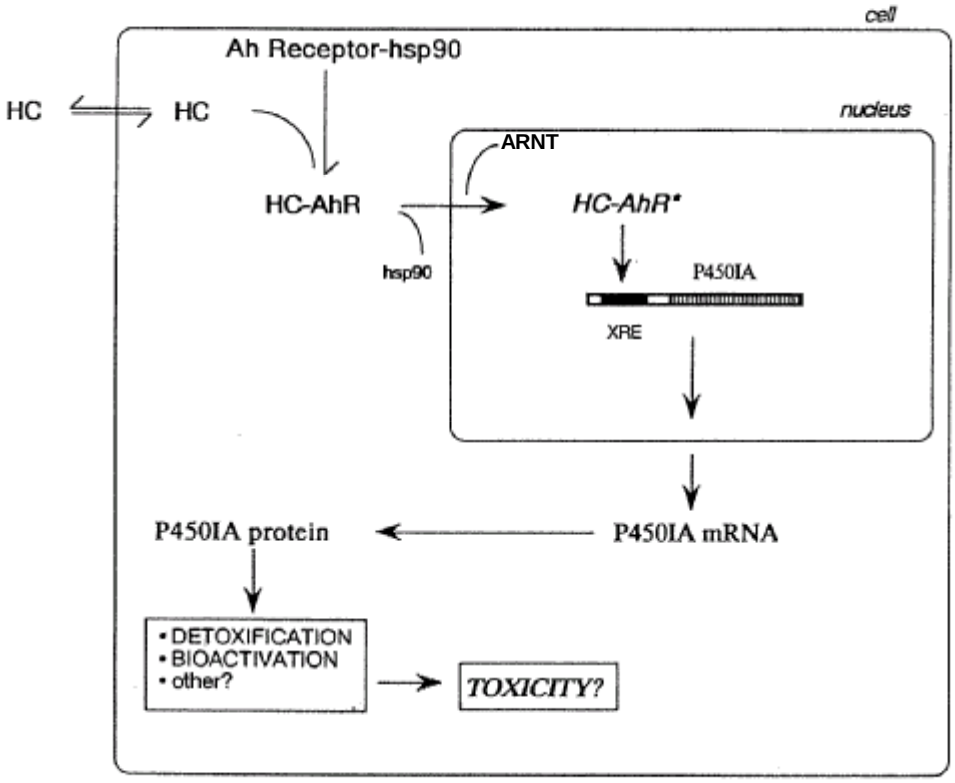


Figure 1.6 The mechanism of CYP1A induction (Stegeman et al., 1992 (modified according to Stegeman & Hahn, 1994). The inducer enters the cell and binds to the Ah receptor (AhR), which is subsequently transformed to its activated form (HC-AhR*) involving the dissociation of a 90-kDa heat shock protein (hsp90) and formation of a heterodimer between the AhR and the Ah receptor nuclear translocator (ARNT) protein. The receptor-inducer complex enters the nucleus and binds to regulatory elements (XRE) in the DNA stimulating the transcription of P4501A mRNA, which can be translated to proteins.

Ligands that induce *Ah* receptor mediated response are restricted to hydrophobic planar molecules which fit into the binding site of the Ah receptor, which is suggested to be rectangular and have a size of approximately 3x10 Å. The most potent CYP1A inducer is 2,3,7,8-tetrachloro-dibenzo-dioxin (TCDD) which fits most closely to this presumed binding site (Figure 1.7) (Landers & Bunce, 1991; Sarasquete & Segner, 2000).

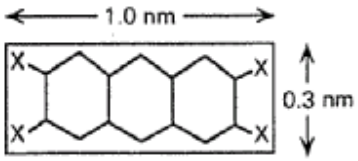


Figure 1.7 Possible dimension of the binding site of the *Ah* receptor, fitting most closely to TCDD which is the most potent inducer of CYP1A (Landers & Bunce, 1991).

1.3.2 Factors affecting the induction of cytochrome P-450

The induction of the cytochrome P450 system in fish can be affected by various factors. Biotic and abiotic factors found to influence the P-450 level and activities in natural fish populations are: Species, sex, reproductive stage, temperature, age, dietary factors, inducing agents and antagonistic agents (Goksøyr & Förlin, 1992).

There can be large variations in the inductive response even between related species. When dab and flounder are sampled at the same place or under same conditions, the MFO activity, measured as 7-ethoxyresorufin-*O*-deethylase (EROD) assay, is three to four times higher in dab than in flounder (Krüner & Westernhagen, 1999). Seasonal- and sex differences have also shown to be less pronounced in flounder than in dab (Westernhagen et al., 1999).

In mammals males generally seem to have higher levels of hepatic P450 than females (Andersson & Förlin, 1992) and this seems also to be the case in winter flounder (*Pleuronectes americanus*) (Vandermeulen & Mossman, 1996; Edwards et al., 1988) and in dab (*Limanda limanda*) at least in some of the stages of the reproduction cycle (Krüner & Westernhagen, 1999; Lange et al., 1999; Goksøyr et al., 1992).

Seasonal variations in MFO activity can be linked to hormonal status, (e.g. levels of steroid hormones), and change in the reproductive state can have influence on the sensitivity to induction of the organism (Payne, 1984). Hence, seasonal effects often reflect to the different reproductive periods (spawning, pre-and post spawning). Spawning is particularly important, as it can be a period of marked biochemical, physiological and histological changes (Payne, 1984). However, temperature can have indirect influence by modulating seasonal changes in physiology and abundance of chemical or natural inducers can also vary seasonally (Payne, 1984).

Seasonal variations in MFO activity are differently expressed in males and females and the EROD activity is found to be low, especially in females, in the spawning period (Krüner & Westernhagen, 1999; Lange et al., 1998; 1999). The differences in MFO response between the sexes is most likely due to the interaction of the MFO system with stereoid hormones in fish (Lange et al., 1999; Edwards et al, 1988; Lindström-Seppä & Stegeman, 1995). Estradiol is a steroid hormone produced by developing ovaries and has been suggested to suppress CYP1A expression in reproductively active females (Stegeman & Hahn, 1994; Förlin et al.,1984; Stegeman & Woodin, 1984). This explanation is supported by the findings of an inverse relationship between EROD activity and Gonadosomatic Index (GSI)[2] (Khan & Payne, 2002; Lange et al., 1998). Sex and seasonality of the reproduction cycle have been found to be the most important variables influencing the MFO activity in winter flounder (Vandermeulen & Mossman, 1996; Edwards et al., 1988).

In dab an inverse relationship has been found between temperature and EROD activity in the post-spawning season and temperature appeared to have stronger influence on regional variability in EROD activity than the organochlorine concentration, which were thought to be the main inducers in this study (Sleiderink et al., 1995; Lange et al., 1998). However, in the spawning season temperature and EROD activity showed positive correlation (Lange et al., 1998) and as the temperature differences not could be explained by qualitative changes such as changes in enzyme affinities and temperature optima, Lange et al. (1998) suggested, that the influence by temperature on EROD activity occurs indirectly via its influence on the duration of the gonadal cycle.

[2] Gonadosomatic Index: (gonad weight/total weight)x100

Temperature would thus have influence on the time of spawning, which is assumed to be coupled with the seasonal variation in EROD activity.

It has been suggested that age and size of the fish are factors to be excluded when selecting species for MFO measurements in monitoring (Edwards et al., 1988). However, Khan & Payne (2002) found that higher activities were found in adult male than in adult female and juvenile winter flounder.

1.4 Biomarkers

Biomarkers have been defined as " Biological responses that can be related to an exposure to, or toxic effect of, an environmental chemical or chemicals" (Peakall, 1994)
The effect of chemical contaminants can occur at different biological levels extending from the molecular or biochemical level to the physiology of the individual organism and ultimately to the level of population and ecosystem (Stegeman et al., 1992). The different levels are:

- ecosystem
- community
- population
- individual
- organ
- tissue
- cell
- organelle
- pathway
- molecules

(redrawn from Stegeman et al.,1992)

Changes at molecular level will underlie the effects at higher level of biological organisation and can be used as an "early warning" signal of the effects of chemical pollution. There is an advantage of measuring changes at the biochemical level, because the biochemical and molecular alterations are usually the first detectable quantifiable responses to environmental change, and can serve as a markers of both exposure and effect (Stegeman et al., 1992).

Certain criteria have been proposed to define biological responses that can serve as a biomarkers (Stegeman et al., 1992):

1) The assay to quantify the biomarker should be sensitive, reliable, and relatively easy;
2) baseline data for the concentration/activity of the biomarker should be known in order to be able to distinguish between natural variability (noise) and contaminant-induced stress (signal);
3) The basic biology/physiology of the test organism should be known so that sources of uncontrolled variation (growth and development, reproduction, food sources) can be minimized;
4) All the factors, intrinsic as well as extrinsic, that affect the biomarker should be known;
5) It should be established whether changes in biomarker concentration are due to physiological acclimation or to genetic adaption;
6) changed levels of the biomarker should be correlated with the "health" or "fitness" of the organism.

1.4.1 Biomarkers of PAH exposure

Figure 1.8 shows the fate of PAH when taken up in fish. PAH is transformed by the CYP1A system into metabolites (Phase I), which can either be conjugated to an endogenous molecule (Phase II) and excreted to the bile, or (if bioactivated during phase I) they can react with DNA creating adducts. Each of these steps can be measured by different biomarker analyses.

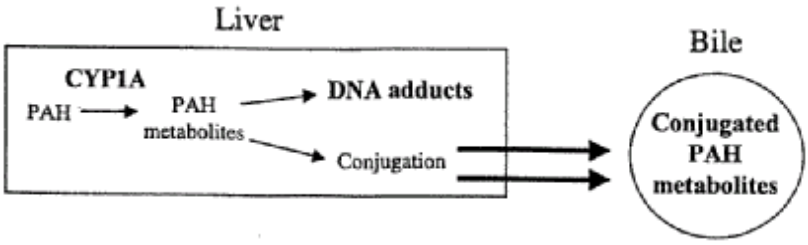


Figure 1.8 Illustration of the fate of PAH after being taken up in an organism, showing the connection between different biomarker analyses (Aas, 2000)

Transformation of PAH into metabolites involves induction of the CYP1A system. Figure 1.9 shows the steps of induction (formation of mRNA, protein and catalytic active enzyme) and each of these steps can be analysed by a suitable assay to measure induction (Goksøyr & Förlin, 1992). The formation of mRNA can be analysed by DNA probes[3], CYP1A protein content by immunodetection analysis (such as ELISA), and the enzyme activity can be analysed by catalytic assays (such as EROD).

Level		Nomenclature	Marker
DNA	→	*CYP1A1*	
▼			
mRNA	→	CYP1A1	DNA probe
▼			
protein	→	P450 1A1	antibody
▼			
enzyme	→	EROD/AHH	catalytic assay

Figure 1.9 Different levels of induction of the cytochrome P450 system and markers which can be used to detect it. (Redrawn from Goksøyr & Förlin, 1992)

EROD

The catalytic activity of the CYP1A enzyme can be analysed by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay. CYP1A catalyses the O-deethylation of 7-ethoxyresorufin, giving the product resorufin which can be measured fluorimetrically. The resorufin formation depends on the presence of enzyme, substrate, oxygen and the cofactor NADPH (see Figure 1.10) (Nilsen et al., 1998).

[3]mRNA analysis for measuring CYP1A expression involves cDNA probes for the CYP1A gene. cDNA are strong cloned copies of otherwise fragile mRNA.

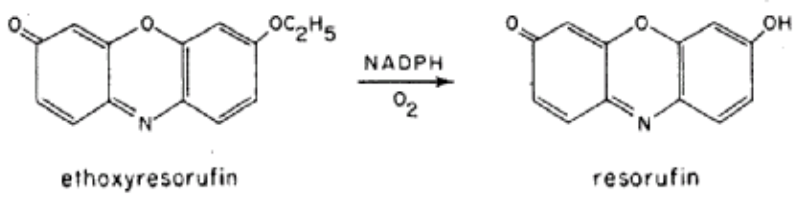


Figure 1.10 The conversion of ethoxyresorufin to resorufin by CYP1A

The catalytic assays EROD and AHH (aryl hydrocarbon hydroxylase[4]) appear to be the most sensitive catalytic probes when determining induction response in fish, showing low or undetectable levels in untreated fish, but are higly induced by exposure to PAH type inducers (Goksøyr & Förlin, 1992). When the two assays (EROD and AHH) are analysed at the same time, the results are highly correlated, and as the EROD assay is highly sensitive and easy and safe to perform, it is most often preferred by researchers when studying PAH induction in fish and mammals (Buhler & Williams, 1989).

CYP1A protein

The measurement of catalytic activity has some disadvantages, because some conditions can lead to underestimation or lack of detection even though induction has occurred. The catalytic activities are sensitive to protein denaturation when conditions during sampling and storage are not optimal (Förlin & Andersson, 1985; Krüner & Westernhagen, 1999). The presence of some compounds, endogenous or exogenous (especially organochlorines, heavy metals, and many xenoestrogens (Sandvik, 2002)), can inhibit the catalytic activity. In addition the sample or tissue may be too small to yield measurable catalytic activity. In such cases measurements of the CYP1A protein content are of crucial importance (Goksøyr & Förlin, 1992). Measurement of CYP1A mRNA can also complement catalytic assays, but as mRNA is also very susceptible to degradation, the information gained by this method in environmental monitoring is limited compared to the immunodetectable analysis of CYP1A protein (Goksøyr & Förlin, 1992).

CYP1A protein content can be measured with an enzyme-linked immunosorbent assay (ELISA), which is a semiquantitative assay giving a relative measure of CYP1A in different samples. In the ELISA procedure the antigen is immobilized on a microtiter plate. A primary antibody specific for the antigen is allowed to bind to the antigen and a secondary antibody, conjugated to the enzyme HRP, is allowed to bind to the primary antibody. The immunocomplex is then detected by addition of a substrate, which is cleaved by the conjugated HRP generating a coloured reaction product, which can be detected spectrofotometrically (Nilsen et al., 1998).

PAH metabolites in bile

As most of the PAH taken up in fish is metabolised, chemical measurements of PAH in fish is not a suitable parameter for analysis of PAH exposition (Aas, 2000). Instead the concentration of metabolites, derived from PAH, can be estimated. When biotransformed by the CYP1A1 system the metabolites generally are hydrofilic and will readily be excreted. Excretion to the bile is the dominant excretion route in fish of metabolites of, especially, larger PAH molecules (Meador et al., 1995). Hence, analyses of PAH metabolites in bile is a suitable matrix for PAH exposure.
Since the PAHs and their metabolites display strong and characteristic fluorescent properties, semi-quantitative measures of such compounds can be obtained by simple fluorescence analyses of bile

[4] BaP-hydroxylase (Buhler & Williams, 1989)

samples. All PAH molecules absorb ultraviolet light followed by an emission of light of longer wavelength. The optimal excitation and emission wavelenghts and signal intensity vary between PAH compounds and are dependent on size, structure and eventual substituents and this variability can be utilized in simple detection methods for PAHs (Aas, 2000.)

The PAH–metabolites in bile are mostly found as conjugated OH-PAH, which means that they are bound to proteins. The protein bound metabolites can be released by treating the material with an enzyme, β-glucuronidase/aryl sulfatase at 37ºC. The proteins are precipitated by the addition of alcohol. Selected metabolites can then be measured by HPLC with fluorescence detection.

The concentration of PAH metabolites in bile is however influenced by differences in bile density (precence of bile proteins) between individuals. The filling and emptying of the gall bladder depends on the feeding status of the individual fish (Aas, 2000). As the fish empties the bile into the gastrointestinal tract during feeding, the gall bladder will be almost empty in the hours after feeding. Shortly afterwards the gall-bladder fills up with water, and the bile first accumulating in the gall bladder after feeding will be rather diluted (Ariese et al., 1997). After long periods of fasting the bile fluid will be more concentrated and the fluorescence signal may be influenced by accumulation of other metabolic products in the bile (Beyer, 1996). The feeding status, thus, influences the bile density and thereby the concentration of metabolites in the bile (Aas, 2000). The variations in bile densities can be minimized by normalizing the result for the bile pigment, biliverdin. This can be done by measuring the absorbance in the bile at 380nm, as biliverdin has one of its two major peaks at this wavelength.

DNA adducts

The traces of exposure can be detected by the formation of DNA adducts which can be quite persistent in fish. Reactive metabolites of carcinogenic PAH (such as benzo(a)pyrene) can bind covalently to DNA and the formation of hepatic DNA adducts has shown to be dose dependent and to increase with time of exposure to PAH contaminated sediment (French et al., 1996; Aas et al., 2000a). Hepatic DNA adducts in contrast to parent PAH reflect cumulative uptake of PAH in fish (Meador et al., 1995).

Hence, the measurement of bile fluorescence and DNA adducts can provide complementary information on short- and long -term exposure to PAH-like contaminants (Meador et al., 1995).

The formation of DNA adducts can be measured by the ^{32}P-postlabeling assay. By this method DNA is hydrolyzed enzymatically to 3'-monophosphates followed by enrichment DNA adducts by the selective removal of normal nucleotides and labelling with [^{32}P]phosphate. The labelled adducts are then separated by two-dimensional, thin-layer chromatography (TLC) on polyethyleneimine (PEI)-modified cellulose sheets. The radiolabeled adducts can then be detected by autoradiography and quantitated by liquid scintillation spectrometry or storage phosphor imaging (Reichert & French, 1994)

1.4.2 Biomarkers for xenoestrogenic exposure

Vitellogenin
Vitellogenin (Vtg) is the egg yolk precursor, and is normally produced in the liver of mature female fish in response to estradiol in the blood. 17β-estradiol (E_2)is produced by developing ovaries. If male or immature female fish are exposed to oestrogenic substances, their livers too will be stimulated to produce Vtg and hence they can be used as a biomarker for environmental oestrogens (Scott & Hylland, 2002). Examples of xenooestrogens are alkylated phenols, which are found in large quantities in produced water from oil exploration activities (Meier, 2001). In addition to induce vitellogenesis xenooestrogens also have an inhibitory effect on P450 expression (Sandvik, 2002).

Vtg can be measured by a competitive ELISA test. The principle in this test is that anti-vitellogenin (antibody) in a polyklonal serum binds to vitellogenin (Vtg) in a solution in competition with a known amount of Vtg, which is bound to the walls of a microtitre plate. An enzyme-conjugated secondary antibody then binds to the primary antibody, which has bound to the Vtg on the walls of the plate. By adding a colour substrate the enzyme conjugate will develop a colour and the the colour end product can be measured by reading the absorbance by a platereader. The concentration of Vtg can then be calculated from a standard curve. The more vitellogenin there is in the sample, the less enzyme-conjugated antibody binds to the walls in the plate and the weaker is the colour development.

1.5 Test organisms

The suitability of species chosen to be monitoring organisms has to be evaluated when performing biomarker analyses. The selected species should be representative for habitat and biota and relative stationary to be sure they are exposed to the actual pollution. Among these species the most sensitive species should be selected (Aas, 2000).

In this project species of fish (teleosts) and invertebrates (molluscs) have been sampled as monitoring organisms. Of the fish species shorthorn sculpins (*Myoxocephalus scorpius*), dab (*Limanda limanda*), flounder (*Platichthys flesus*) and a coastal stage of atlantic cod (*Gadus morhua*) called “reyðfiskur” in Faroese (red fish) have been chosen. Dab, flounder and cod are used as indicator organism in international monitoring programmes (JAMP)[5]. Shorthorn sculpin is widespread in the arctic area and due to this and its stationary lifestyle it is used in as indicatorspecies in the AMAP[6] project.

Of the invertebrates species the gastropods: limpets (*Patella vulgata*), periwinkles (*Littorina obtusata*) and dogwhelks (*Nucella lapillus*) and the bivalves: blue mussels (*Mytilus edulis*) and horse mussels (*Modiolus modiolus*) have been chosen.

1.5.1 Fish
Where no other references is given the following text is based upon Joensen & Tåning (1969).

[5] The Joint Assessment and Monitoring Programme under the OSPAR agreement.
[6] Arctic Monitoring and Assessment Programme.

Dab:
Dab (*Limanda limanda*) is common in the fjords, coves and sounds in the Faroes and is found from the tidal zone to a depth of more than 150 m (not taken outside 200 m contour line). On the whole it is a non-migratory fish and does not move any great distances. It leaves the shallow water to spawn, though finds spawning grounds in the fjords and bays.
According to Joensen & Tåning (1969) spawning starts April, reaches a peak around (end of) May and continues to later in the summer. However, a study from 1996-97 (Dam, 2000) shows that spawning occured as early as in March in 1996 and probably even earlier (in January-February) in 1997.
Males reach a length of about 30 cm, females longer (maximum males 38 cm, females 41 cm)
Maturity is reached at a lenght of 18 cm in females and a little less in males.
The diet consists for a large part of mussels (*Abra, Axinus, Cardium* etc.), smaller crustaceans (gammarids, hermit crabs, smaller crabs), and finally bristle worms, echinoderms and smaller fish.
The alga *Ulva* commonly found in the stomach of dab.
Dab is important as foodstuff for predatory fish in the area (Cod, Halibut, Monk-fish).


Photo 1 Dab (*Limanda limanda*).

Flounder:
Flounder (*Platichthys flesus*) is found in probably all Faroese fjords and coves where there are freshwater outfalls. Most of its lifetime it lives in the inner parts of the fjords or coves, where the salinity is low (around fresh water outlets). It can frequently be found at depths as low as 0-3 m but is likely to go to deeper water in the winter. Probably it seeks to more open parts or mouths of the fjords or even the banks during the spawning season. Spawning occurs in winter or early spring (until May).
In the Faroes flounder reaches a length of 38-40 cm (maximum 40-45). It feeds mostly on crustaceans (gammarids, crabs etc.), snails and mussels (young Mytilus and similar forms), larvae of aquatic insects and small fish.


Photo 2 Flounder (*Platichthys flesus*).

Cod:
Cod (*Gadus morhua*) is one of the most common fishes in and around the Faroes. It is mainly found within the 200-300 m contour line, but ranges from depths less than 1m to perhabs 700 m.
Near the Faroes it spawns to the north of the islands (Norðhavið). Spawning takes place at temperature around 6-7ºC. The main spawning season is from early March to May, April being the most important month.
Until the age of 3 years (or more) the cod is rather stationary. When sexually mature the cod migrates to "Norðhavið" (or other locations) to spawn. After spawning they disperse all over the Faroe area and survivors will seek the spawning grounds again the following year.
Many reach sexual maturity at the age of 3 years, but the majority at the age of 4 years, some even later. Cod feeds on nearly everything digestible it comes across. Sand-eel and herring are probably the most favoured food with the addition of crustaceans, brittle star, mussels etc.

Cod, which lives among red algae, acquire an intense red colour and are called "reyðfiskur" (red fish). "The red fish" is stationary until approximately 1 – 1½ years of age, then it migrates to deeper water ultimately to join the spawning population and live with cod on the continental shelf (Steingrund, pers. comm.).


Photo 3 Coastal stage of cod (*Gadus morhua*).

Sculpin:
Sculpin (*Myoxocephalus scorpius*) is very common all around the islands in the stony or sea-weed covered areas of the coastal region. It is often found near piers and similar places. Sculpins are not good swimmers and thus very stationary. It is rare in deep parts of the fjord because of the soft bottom in most of these places. It is found from the low water mark to a depth of ca.200 m, although the number decreases notably from 20-25 m outwards.
The spawning season is from mid-winter until spring.
Sculpins feeds on crustaceans and will indeed take anything. Females reach a size of approximately 32 cm, males less. In Iceland they may reach length of 40 cm.


Photo 4 Sculpin (*Myoxocephalus scorpius*).

1.5.2 Invertebrates

Where no other reference is given the following text is based upon Spärck & Thorson (1969) (limpets, dogwhelks and periwinkles) and Høpner Petersen (1969) (blue- and horse mussels).

Limpets:

Limpets, (*Patella vulgata*), are abundant on almost every rocky coasts of the Faroe Islands and mostly at the places mostly exposed by wave action. They are generally found above low water mark (dry at ebb tide), down to a depth of no more than a few m. The larger specimens mostly have a diameter of 30-40 mm (maximum found 61 mm). Limpets are grazers and feed on microorganisms and algae which they scrape from the rocks with their rasping tongues (Hill, 2000; Næs et al., 1998).

Photo 5 Limpets (*Patella vulgata*).

Dogwhelks:

Dogwhelks, (*Nucella lapillus*), are one of the most common littoral gastropods of the Faroe Islands and are found in almost any place of the coasts in great numbers attached to the rocks above the sea level together with P. vulgata. They have, however, sometimes been found below sea level (down to -20 m). The height of specimens can be around 32-33 mm (maximum 37-39 mm). Dogwhelks are carnivorous and typically feed on barnacles and mussels (Tyler-Walters, 2003).

Photo 6 Dogwhelks (*Nucella lapillus*).

Periwinkles:

Periwinkles, (*Littorina obtusata*), occur all around the northern as well as of the southern Islands of the Faroes in depths of no more than 2-3 m, and not over the high water mark. The Faroese specimens are rather large 10-13 mm (maximum 15,5 mm). Like limpets, periwinkles are grazers and feed by scraping microalgae from the rock with their rasping tongues (Næs et al., 1998).


Photo 7 Periwinkles (*Littorina obtusata*).

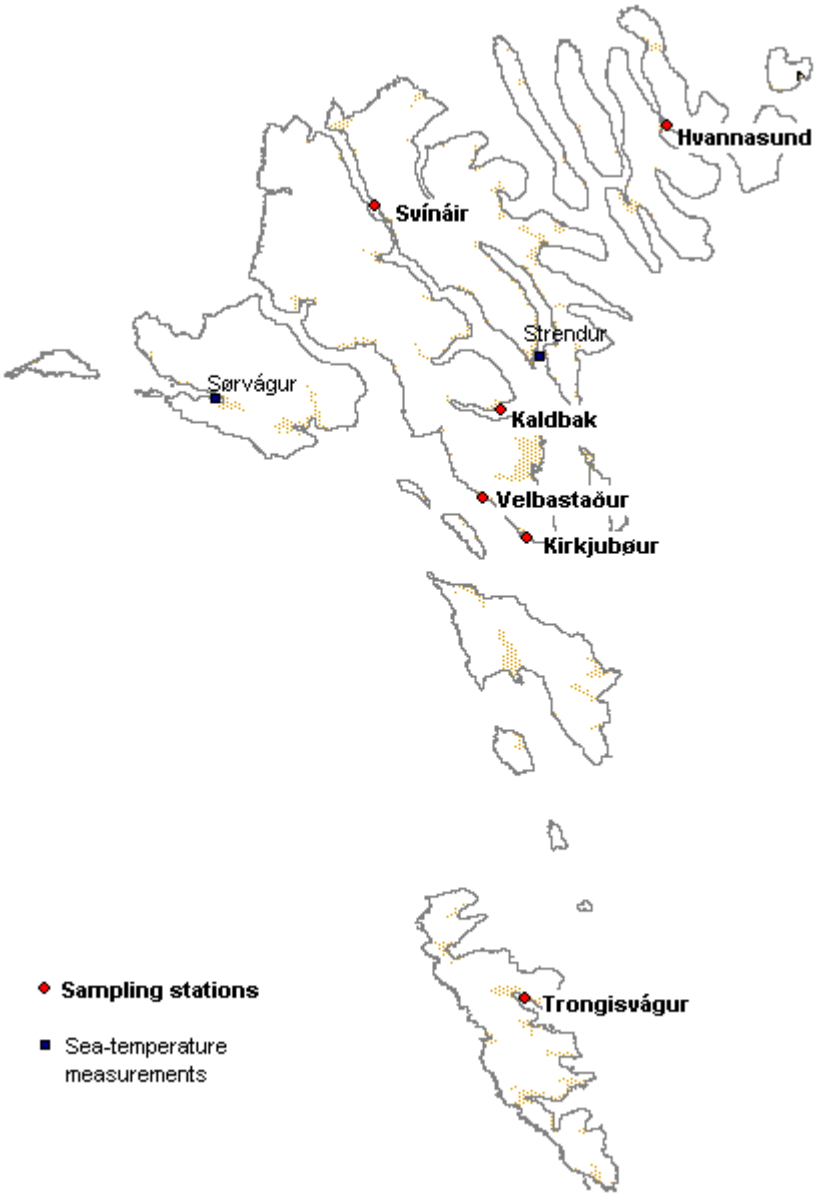
Blue mussels:
Blue mussels (*Mytilus edulis*) are common along the coasts in and below the tidal zone (found down to 50 m). They live attached to suitable substrata by fibrous byssus treads, sometimes occuring as dense masses. Blue mussels are filter-feeders and feed on bacteria, phytoplankton, detritus and dissolved organic matter which they filter from the water (Tyler-Walters, 2002; Næs et al., 1998).


Photo 8 Blue mussels (*Mytilus edulis*).

Horse mussels:
Horse mussels (*Modiolus modiolus*) are commonly found down to a depth of 200 m. They live part buried in soft sediments or coarse grounds or attached to hard substrata often forming extensive beds. Like blue mussels, horse mussels are filter-feeders and feed on organic matter and particles suspended in the water (Tyler-Walters, 2001).


Photo 9 Horse mussel (*Modiolus modiolus*).

Fish study



Map 1 Map of the Faroe Islands showing the sampling stations for fish and inverebrates, and the stations where temperatures have been measured by the Faroese Office of Public Works.

2 Methods

2.1 Sampling of fish

Fish were sampled in Kaldbak and Kirkjubøur (see Map 1 on page 32). The plan was to collect sculpins in Kaldbak and dab in Kirkjubøur, but as there was a not negligible by-catch of other species, these were taken as samples as well. The additional sampling included a coastal stage of Atlantic cod, flounder and dab in Kaldbak, and flounder in Kirkjubøur. The only flounder samples that were analysed were those from Kirkjubøur in February.

In Kaldbak the samples in the first sampling period were secured by fishing with fishing rods at the quay, but as it turned out to be quite difficult to get enough samples and because of the close vicinity to a fish processing plant, the sampling method was changed to fish-traps placed more distantly from land. The traps normally were soaked for 24 hours, before they were taken up, but sometimes 2-4 days elapsed.

In Kirkjubøur the sampling in the first sampling period was done by diving near the quay. In the second and third sample period the fish were caught in fishing nets, which were set in Brandansvík and were deployed for about two hours. Some of the fish was also caught by fishing rod from a boat.

The fish were transported alive in containers with seawater to the laboratory, where the sample preparation was done. Table 2.1 gives a summary of the fish catches.

Table 2.1 Fish samples from Kaldbak and Kirkjubøur

	Sampling period	**Short-horn sculpin** (*Myoxocephalus scorpius)*	**Cod –** coastal variety (*Gadus morhua*)	**Flounder** (*Platichthys flesus*)	**Dab** (*Limanda limanda)*
Kaldbak	Jan-Feb ’02	6	-	-	-
	Apr-May ’02	12	12	8	-
	July ’02	10	11	1	9
Kirkjubøur	Jan-feb ’02			6	-
	Apr-May ’02			8	7
	July ’02			-	13

Grey shadow: Not analysed

Prior to dissection the weight and length of the fish were measured. Then the fish was killed with a blow to the head. A blood sample of 1-5 ml was taken with a syringe and a BD vacutainer containing heparin (LH 143 I.U.). The blood sample was kept on ice until centrifugation (5 min. at 3000 rpm). The plasma was transferred into a cryo tube with a pasteur pipette and frozen in liquid nitrogen.

The liver and gall bladder were dissected. The gall bladder was transferred whole into a cryo tube and frozen in liquid nitrogen. When the gall bladder was too big to fit in to a cryo tube, it was pierced and the content carefully poured into the tube. The weight of the liver was recorded and

three samples of liver, approximately 1 g each, were taken into cryo tubes from each fish (if there was sufficient liver tissue) and frozen in liquid nitrogen.

Sex was determined and the gonads were weighed. The gonads and the rest of the liver and the stomach, with contents, were stored in -20ºC and registered in the Environmental Specimen Bank for use in later studies.

Water temperature was not measured in connection with sampling, but the Faroese Office of Public Works makes regular measurements of sea-water temperatures (one meter above the sea-floor) at selected stations. The stations which are nearest to Kaldbak and Kirkjubø are "Strendur" and "Sørvágur" (see Map 1 on page 32). The mean sea-temperature for each month during 2002 is shown in attachment 1. The mean temperature in Strendur and Sørvágur respectively was 5,7-6,5ºC in January, 6,2ºC in April (only measured in Strendur) and 10,7-10,2ºC in July.

2.2 Analysis of fish samples

The analyses of the **fish samples** were performed at NIVA (Norwegian Institute for Water Reseach) in the period from the 26th of August to the 21st of September by Katrin Hoydal, under supervision of the staff at NIVA, except for additional analysis of EROD activity in sculpin by HPLC method, which were performed by the National Veterinary Institute in Norway, and the analysis for CYP1A protein which were performed by NIVA.

The **liver** samples from the fish were analysed for EROD activity, CYP1A protein content and total microsomal protein content.
Preparation of the samples was done by homogenisation to attain cell lysis without affecting proteins and enzymes. The cytosol and organelles were separated with centrifugation as described in section 2.2.1 .
The protein content in the microsomal fraction of the liver was measured as described in section 2.2.3
The EROD activity was measured fluorimetrically as described in section 2.2.4.
The CYP1A protein content was analysed by an enzyme-linked immunosorbent assay (ELISA) (section 2.2.5).

The **bile** samples were analysed for PAH-metabolites using HPLC (High Performance Liquid Chromatography) with fluorescence detection. The preparation and analysis of bile samples are described in section 2.2.6 and 2.2.7 respectively.

The **blood** samples were analysed for vitellogenin content using an enzyme-linked immunosorbent assay (ELISA) as described in section 2.2.8.

2.2.1 Chemicals

7-ethoxyresorufin, resorufin, NADPH, Trizma base, Trizma Hydrochloride, Tween-20 (Polyoxyethylene sorbitan), and BSA (Bovine serum albumin) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Bovine gamma globulin (protein standard), protein assay reagent A (alkaline copper tartrate solution) and B (diluted Folin reagent) were from BioRad Laboratories (Hercules, CA, USA). Primary antibodies: Rabbit anti-cod Vtg, Polyclonal antibody, CS-1, Rabbit anti-

wolffish Vtg, Polyclonal antibody, CS-3, Rabbit anti-Arctic char Vtg, Polyclonal antibody, PO-1, Rabbit anti-sea bream Vtg, Polyclonal antibody, PO-2, anti-fisk CYP1A antibody (CP226) were from Biosense laboratories (Bergen, Norway) and Secondary antibody: Goat anti-rabbit IgG Horseraddish Peroxidase Conjugate (GAR-HRP) was from AmDEX (Jyllinge, Denmark).

2.2.2 Preparation of liver samples

The liver samples were thawed on ice and approximately 1 g of liver was weighed and put in a glass homogenising tube. A 0,1 M K-phosphate homogenising buffer was added until 5 ml of solution was obtained. The solution was homogenised using a motorised homogeniser (Potter-Elvehjem type) with at least ten passes with a teflon pestle. The homogenate was transferred to centrifugation tubes and centrifuged at 10000 *x* g at 4ºC for 30 min. The supernatant (PMS[7] fraction) was collected with a pipette, carefully avoiding the pellet and the floating lipid layer, transferred to new centrifugation tubes and centrifuged at 48000 *x* g at 4ºC for 120 min. The supernatant (cytosol fraction) was removed and frozen at -80ºC and not analysed in this context. The pellet (microsomal fraction) was resuspended in 1,5 ml resuspending buffer and homogenised using a glas-teflon homogeniser. The homogenate was transferred to eppendorf tubes and frozen at -80ºC until analysis for microsomal protein content (2.2.3), EROD activity (2.2.4) and CYP1A protein content (2.2.5).

2.2.3 Protein

The microsomal protein content was analysed quantitatively by a modified method of Lowry et al. (1951). The analysis is based on the two-step reaction of protein with an alkaline copper tartrate solution and Folin reagent. The blue colored end product can be measured at 750 nm. A standard curve was made using dilutions of Bovine gamma globulin protein standard in Tris buffer (0,125 – 1,00 mg/ml).
10 µl of diluted sample (the resuspended pellet obtained by the preparation of the liver samples) or standard were added to a microtiter plate. Then 25 µl of alkaline copper tatrate solution and 200 µl of diluted Folin reagent was added and the plate was mixed by the mixing function in the plate reader. After incubation for 15 minutes the absorbance was read in the plate reader at 750 nm. The protein concentrations in the samples could then be determined by comparison to the linear standard curve.

Microtiter plate for the protein assay:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Std 2	s 1	s 3	s 6	s 9	s 11	s 14	s 17	s 19	s 22	ref. G.m
B	Blank	Std 2	s 1	s 4	s 6	s 9	s 12	s 14	s 17	s 20	s 22	ref. G.m
C	Blank	Std 3	s 1	s 4	s 7	s 9	s 12	s 15	s 17	s 20	s 23	ref. G.m
D	Blank	Std 3	s 2	s 4	s 7	s 10	s 12	s 15	s 18	s 20	s 23	ref. G.m
E	Std 1	Std 3	s 2	s 5	s 7	s 10	s 13	s 15	s 18	s 21	s 23	ref. L.l.
F	Std 1	Std 4	s 2	s 5	s 8	s 10	s 13	s 16	s 18	s 21	s 24	ref. L.l.
G	Std 1	Std 4	s 3	s 5	s 8	s 11	s 13	s 16	s 19	s 21	s 24	ref. L.l.
H	Std 2	Std 4	s 3	s 6	s 8	s 11	s 14	s 16	s 19	s 22	s 24	ref. L.l.

s1-24: sample 1-sample 24
ref. G.m. and ref. L.l.: reference material of cod and dab respectively

[7] Postmitochondrial supernatant

2.2.4 EROD analysis

The EROD (7-ethoxyresorufin-*O*-deethylase) analysis was performed according to Eggens & Galgani (1992), which is a modified version of the method described by Burke and Mayer (1974), using a plate-reader. The reaction solution was made by diluting a stock solution of 0,2 mM 7-ethoxyresorufin in dimethylsulfoxide (DMSO) in 0,1 M K-phosphate buffer and the absorbance at 450 nm was measured. A standard curve of 8 standard solutions (0 – 0,64 µM) was made of a 1 mM stock solution of resorufin in DMSO diluted in K-phosphate buffer. The starting concentration (highest standard concentration) of 0,64 µM was obtained by adding 50 µl of 10 µM resorufin in DMSO to 5,2 ml K-phosphate buffer and the absorbance was read at 572 nm (exact concentration was calculated using the extinction coefficient 73,2 $mM^{-1}cm^{-1}$). The other standard solutions were then made by dilution of this solution in K-phosphate buffer. A 2,4 mM NADPH stock solution was made of 20 mg NADPH in 10 ml K-phosphate buffer. As ethoxyresorufin and resorufin are sensitive to light, the EROD analysis was performed protected from direct light.

If necessary the samples were diluted in K-phosphate buffer. 275 µl of standard was added to 16 of the wells (A1-B8) in a microtiter plate (dublicates of each standard). To the rest of the wells 50 µl of buffer and 50 µl of sample (6 replicates) were added. To half of the wells containing sample (three of the sample replicates) 10 µl of 0,32 µM resorufin standard was added. Then 200 µl of 7-ethoxyresorufin solution (reaction solution) and, to initiate the reaction, 25 µl NADPH solution was added to all the wells except for those containing standards.

Microtiterplate for EROD analysis:

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0	0,01	0,02	0,04	0,08	0,16	0,32	0,64	Blank	Blank	Blank	Blank	
B	0	0,01	0,02	0,04	0,08	0,16	0,32	0,64	Blank	Blank	Blank	Blank	
C	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	
D	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	
E	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	
F	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	*
G	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	*
H	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	*

s1-12: sample 1-sample 12 *10 ul 0,32 uM resorufin added

The plate was read immediately in a plate reader at exitation wavelenght 530 nm and emission wavelenght 590 nm, in 8 steps for appr. 4 minutes (238 sec.) of duration. The EROD values could be calculated based on the formula (Nilsen et al., 1998):

$$\text{pmol resorufin/min/mg protein} = F_S/\text{min} \times R/F_R \times 1/V_S \times 1/C_S$$

F_S/min = Increase in fluorescence per minute.
R = Amount of resorufin added as internal standard (pmol).
F_R = Increase in fluorescence due to resorufin standard.
V_S = Sample volume (ml).
C_S = Protein concentration of sample (mg/ml).

EROD analysed by HPLC

The sculpin results were dominantly negative which shows that the method used was not sensitive enough for the sculpin analysis. The sculpin samples were, therefore, reanalysed at the National

Veterinary Institute in Norway by HPLC detection as described in Ruus et al., 2002, with some modifications. Description of the method is given in attachment 4.

2.2.5 CYP1A protein

CYP1A protein content was analysed by an enzyme-linked immunosorbent assay (ELISA) using Anti-fish (CP226) as primary antibody and Sigma goat anti rabbit IgG HRP conjugated (GAR-HRP) as secondary antibody. Description of the method is given in attachment 5. The results (absorbance at 450 nm) were normalized to the total protein content by dividing by the total protein content measured (see section 2.2.3 and attachment 6). The method is semi-quantitative and the results are presented as abs/mg protein.

2.2.6 Preparation of bile samples

The gall bladder with content had been frozen in cryo tubes, and the first step was to remove the bile out of the bladder. This was done with a syringe by which the content of the bladder was transferred into new Eppendorf tubes. Several of the gall bladders however, contained insufficient amounts of bile to be analysed.

For the preparation of the bile samples to be analysed using HPLC with fluorescence detection, 0,05 ml of deionized water, 20 µl of sample, 20 µl of β-glucuronidase/aryl sulfatase, and 10 µl internal standard (triphenylamin) was added to the sample tubes. After mixing on a whirl mixer the samples were incubated at 37ºC for one hour. Then the samples were mixed again and centrifugated at 4000 *x* g for ten minutes. The supernatant was transferred to HPLC vials and put in a freezer (-20ºC) until analysis the next day.

The remaining bile (if any) was analysed for relative biliverdin content (see below).

2.2.7 PAH-metabolites in bile

The concentration of PAH metabolites in bile was analysed by HPLC detection. The HPLC detector used in analysing the prepared bile samples, was a Waters High Performance Liquid Chromatograph consisting of: Waters 600 pump controller, Waters 470 Scanning Fluorescence Detector, Waters 717 Autosampler, Waters 490 Programmable multiwavelength detector and a Waters column oven. 25 µl of sample was injected into the column. The mobile phase consisted of acetonitril (ACN, Rathburn HPLC-grade), starting with 40% ACN in H_2O changing to 100% ACN during the running of one sample. The flow rate was 1 ml/min. The analysing time per sample was 33 minutes. The temperature of the column was 35ºC and there was a continuing degassing with helium during the analysis. The fluorescence was measured with an excitation wavelength at 282 nm and an emission wavelength at 375 nm.
A standard curve of 11 standard solutions, each containing different concentrations of the metabolites that were analysed: 2-OH-naphtalene, 1-OH-fenantrene, 1-OH-pyrene, 3-OH-B(a)P and the internal standard: triphenylamin, was run prior to the samples, and one of the standards was run between every ten samples. The results reflect by retention times and peak heights the presence and the concentrations of the different metabolites. An example of the chromatogram for one of the standard solutions is shown in attachment 3C. The sample concentrations are determined compared to the standard curve and the level of the internal standard (triphenylamin). An example of a typical chromatogram from running of a sample is shown in attachment 3C. To six of the samples however, which were prepared one day later than the other, internal standard was not added, and thus the results were found by running the standard curve again and comparing the values of the samples

with the standard curve without adjusting for the internal standard. Results showing a peak less than three times the background "noise" were excluded.

Normalization for biliverdin content in the bile

The remaining bile (if any) was analysed for relative biliverdin content by measuring the absorbance at 380nm of the bile diluted in ethanol using a spectrophotometer, since biliverdin has one of its two major peaks at this wavelenght. The concentrations of PAH metabolites found by the HPLC analyses were normalized for the biliverdin content by dividing the results by the absorbance at 380nm.

2.2.8 Vitellogenin in blood samples

Analysis of vitellogenin in cod

Blood samples from cod were analysed using **competitive ELISA** (enzyme-linked immunosorbent assay) as described in Scott & Hylland (2002).

The 96 well microtiter plates were coated by adding 100 µl of standard cod antigen (Vtg), in the concentration 50 ng/ml, to the wells in the microtitre plate, except for the blanks and NSB (Non-Specific Binding) wells, to which only coating buffer was added. The plates were sealed and incubated at 4ºC over night.

Microtiterplate for the analyses of vitellogeninin cod:

	1	2	3	4	5	6	7	8	9	10	11	12	Sample diluted
A	blank	std. 2	std. 5	std. 7	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	1:10
B	blank	std. 2	std. 5	std. 8	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	1:10
C	NSB	std. 3	std. 5	std. 8	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	1:10
D	NSB	std. 3	std. 6	std. 8	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	1:10
E	std. 1	std. 3	std. 6	std. 9	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	1:100
F	std. 1	std. 4	std. 6	std. 9	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	1:100
G	std. 1	std. 4	std. 7	std. 10	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	1:100
H	std. 2	std. 4	std. 7	std. 10	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	1:100

RF1-RF8: cod sample 1- cod sample 8.

Then the plates were washed three times with TTBS (Tris-buffered saline solution (TBS) with 0,05% Tween-20). A 250 µl blocking solution (1% Bovine Serum Albumin (BSA) in TBS without Tween-20) was added to block any remaining protein-binding sites in the wells, and the plates were incubated for 60 min. at room temperature. The plates were again washed three times and were then ready for primary antibody incubation. The samples were diluted 1:10 and 1:100 in TBS with 0,1% BSA. 100 µl TBS was added the blanks and 50 µl of TBS to the NSB wells. To all the other wells 50 µl standard or diluted sample were added. For each sample, an aliquot of sample, diluted 1:10, was added to 4 wells and the sample diluted 1:100 was added to 4 other wells. Primary antibody 50 µl of polyclonal cod antibody (CS-1)[8] was added to each well, except for the blanks. The plates were sealed and incubated at 4ºC over night.

The plates were washed three times with TTBS. Then 100 µl of secondary antibody (goat anti rabbit/HRP conjugate) was added at a dilution 1:15000 in TBS with 0,1% BSA. Only TBS with

[8] Polyclonal (rabbit) antibody against antigen of Atlantic cod (*Gadus morhua*).

0,1% BSA was added to the blanks. The plates were sealed and incubated for 6 hours at 4ºC. After washing 5 x 1,5 min. in TTBS the plates were ready for color development. 100 µl of TMB-plus solution (0,04 % O-phenylene-diamine in 150 mM phosphate, 50 mM citrate buffer, pH 5,7, with 0,012 % hydrogen peroxide) was added to all the wells and incubated in the dark for 8-12 min. before adding the stop solution (1 M H_2SO_4). The absorbance was read in a plate-reader at 450 nm and the concentration of vitellogenin was calculated using the sigmoidal standard curve, the line was fitted using the equation: $y = (A-D)/(1+(x/C)^B)-D$.

Analysis of vitellogenin in sculpin and dab

Since there was no sculpin and dab antibody serum available, sculpin and dab samples had to be tested against the available antibody serums (Arctic char, sea-bream, wolffish and cod) to see how the sculpin and dab antigens reacted against them. For this purpose a **capture ELISA** test was used (Scott & Hylland, 2002; Specker & Anderson, 1994).

Determination of optimal antibody and dilutions

To find the optimal concentrations of antibody, 4 microtiter plates were coated by adding 100 µl of samples diluted 1:1000 in coating buffer. The samples were taken from three sculpins and three dabs: One reproductionally mature female (U4 and S9), one less reproductionally mature female (U5 and S7) and one male (U21 and S13) of each species. For each plate two columns of each of the selected samples were added. The plates were covered with plate-sealer and incubated at 4ºC over the night.

Microtiter plate for the optimalization study:

	1	2	3	4	5	6	7	8	9	10	11	12	Dilution of primary antibody
A	U4	U4	U5	U5	U21	U21	S9	S9	S7	S7	S13	S13	1:1000
B	U4	U4	U5	U5	U21	U21	S9	S9	S7	S7	S13	S13	1:2000
C	U4	U4	U5	U5	U21	U21	S9	S9	S7	S7	S13	S13	1:4000
D	U4	U4	U5	U5	U21	U21	S9	S9	S7	S7	S13	S13	1:8000
E	U4	U4	U5	U5	U21	U21	S9	S9	S7	S7	S13	S13	1:16000
F	U4	U4	U5	U5	U21	U21	S9	S9	S7	S7	S13	S13	1:32000
G	U4	U4	U5	U5	U21	U21	S9	S9	S7	S7	S13	S13	1:64000
H	U4	U4	U5	U5	U21	U21	S9	S9	S7	S7	S13	S13	1:128000

U: sculpin, S: dab.

Then the plates were washed three times with PBS (phosphate buffered saline solution) with 0,05% Tween and blocking solution (2% Bovine Serum Albumin (BSA) in PBS) was added. After an incubation for 60 min. at room temperature and an additional washing procedure, 100 µl of the four different antibody serums diluted in 1% BSA in PBS were added to the four different plates. To each row on the plate were added different dilutions of the antibody, the dilutions doubling for each row from 1:1000 (row A) to 1:128 000 (row H). The plates were sealed and incubated at 37ºC for 2 hours and after an additional washing procedure the secondary antibody could be added. 100 µl of goat anti rabbit/HRP conjugate diluted 1:3000 in 1% BSA in PBS was added to each well and the plates were sealed and incubated at 37ºC for 1 hour. Then the plates were washed five times with PBS solution with Tween and the developing procedure was done in the same way as for the competitive ELISA except that the developing reaction lasted 14 min. for all the plates before

adding the stop solution. The plates were read in the plate-reader at 450 nm and from the results the best suitable primary antibody and the best suitable dilution could be chosen.

Parallelism between the response of standard and sample

The chosen antibody serums were CS-3 (wolffish) and CS-1 (cod). To test, if the reaction of sculpin and dab were parallel to the cod, one reproductionally mature female (RF10, U4 and S9)and one reproductionally immature male (RF13, U21 and S13) of the three species were chosen. Two plates were coated with samples from those individuals with two columns for each individual. The samples were diluted, the dilution doubled for each row from 1:1000 (row A) to 1:128000 (row H).

Microtiterplate for the study of parallellism:

	1	2	3	4	5	6	7	8	9	10	11	12	Plate coated w. sample diluted:
A	RF10	RF10	RF13	RF13	U4	U4	U21	U21	S9	S9	S13	S13	1:1000
B	RF10	RF10	RF13	RF13	U4	U4	U21	U21	S9	S9	S13	S13	1:2000
C	RF10	RF10	RF13	RF13	U4	U4	U21	U21	S9	S9	S13	S13	1:4000
D	RF10	RF10	RF13	RF13	U4	U4	U21	U21	S9	S9	S13	S13	1:8000
E	RF10	RF10	RF13	RF13	U4	U4	U21	U21	S9	S9	S13	S13	1:16000
F	RF10	RF10	RF13	RF13	U4	U4	U21	U21	S9	S9	S13	S13	1:32000
G	RF10	RF10	RF13	RF13	U4	U4	U21	U21	S9	S9	S13	S13	1:64000
H	RF10	RF10	RF13	RF13	U4	U4	U21	U21	S9	S9	S13	S13	1:128000

RF: cod, U: sculpin, S: dab

A capture ELISA test was performed, as described above, using the CS-3 antibody as primary antibody for one plate at a dilution of 1:32000 and the CS-1 antibody as the primary antibody for the other plate at a 1:16000 dilution. The secondary antibody was Goat anti Rabbit/HRP conjugate diluted 1:12000 for both plates. The results were plotted to see if the shape of the curves of sculpin and dab were parallel to the cod curve. If the curves were parallel, the cod standard curve could be used as a standard to get the relative concentrations of vitellogenin in dab and sculpin.

2.2.9 Statistics

The results were tested for significant differences between means by using one-way ANOVA and correlation between parameters was analysed using linear regression. All analyses were made by the computer program SYSTAT, and significance level was set at 95% ($P<0,05$). When significant difference was found, normal distribution of data was tested with a probability plot.

3 Results of the fish study

Table 3.1 shows the number and size of the fish caught, along with the Condition Factor Index (CFI), Liversomatic Index (LSI) and Gonadosomatic Index (GSI). Sculpins from three periods were analysed: January, March-May and July, while cod and dab were analysed from two periods: April(-May) and July and flounder was only from February. Each fish species from one location was analysed (either Kaldbak or Kirkjubø) except dab, which was analysed from two locations (Kaldbak and Kirkjubø) in July. The individual data for the fish are given in attachment 6.

The length of the sculpins varied between 16,5–32,5 cm, and the weight between 66–600 g. The length of the cod was from 35-58 cm and weighed 212-4050 g. The length and weight of the dabs varied between 24,5-38 cm and 200-750 g, respectively and the flounders were between 29-42 cm and 250-1334 g.

Table 3.1 Mean lenght and weight of fish caught in Kaldbak and Kirkjubø from January to July 2002.

Species	Location	Date	n		Length, cm	Weight, g	CFI[a]	LSI[b]	GSI[c]
Sculpin	Kaldbak	Jan. '02	4	Female	29,5±1,9	496±43,2	1,9±0,3	4,1±1,7	12,3±7,0
			2	Male	28,5±0,7	353±29,7	1,5±0,0	1,6±0,9	1,9±0,6
		Mar.-May '02	9	Female	25,0±5,0	283±145	1,6±0,3	1,3±0,3	1,6±1,5
			3	Male	24,5±2,3	230±94,9	1,5±0,3	1,2±0,1	1,0±0,6
		July '02	8	Female	25,6±3,8	289±111	1,6±0,2	2,1±1,4	1,0±0,5
			2	Male	27,0±5,7	384±306	1,7±0,4	1,8±0,6	1,1±0,4
Cod	Kaldbak	Apr.-May '02	6	Female	44,6±9,8	965±624	1,0±0,1	2,4±2,7	0,5±0,3
			6	Male	42,0±4,8	699±206	0,9±0,1	1,3±0,8	1,5±1,8
		July '02	6	Female	37,5±9,1	577±422	0,9±0,0	1,4±0,5	0,4±0,4
			6	Male	42,8±16,7	1226±1476	1,1±0,1	1,9±0,9	0,3±0,6
Dab	Kaldbak	July '02	6	Female	30,1±3,4	311±87,8	1,1±0,2	1,5±0,5	2,5±2,6
			3	Male	28,0±0,5	226±5,3	1,0±0,0	1,7±0,4	0,4±0,3
	Kirkjubø	Apr. '02	4	Female	35,3±3,0	595±179	1,3±0,2	1,7±0,2	14,2±3,3
			4	Male	29,5±1,7	262±62,4	1,0±0,1	0,9±0,2	0,6±0,4
		July '02	11	Female	32,0±2,3	371±93,7	1,1±0,1	1,7±0,7	1,2±0,5
			2	Male	30,5±1,4	340±48,1	1,2±0,0	1,5±0,1	1,0±1,4
Flounder	Kirkjubø	Feb. '02	0	Female	-	-	-	-	-
			6	Male	32,8±4,8	530±403	1,3±0,3	2,7±1,8	10,2±9,2

[a] Condition Factor index: total weight/total length^3
[b] Liversomatic Index: (liver weight/total weight)x100
[c] Gonadosomatic Index: (gonad weight/total weight)x100

As can be seen from the standard deviations there is great variation in the weights of the different groups, and for cod also in the length. This is due to some large, outlying individuals, which skew the sample pool. For the sculpins the variation in the weight can be due to the large variations in stomach content which in some specimens could be a significant part of the weight. The length is therefore a better variable to look at when comparing the size. Graphs of length versus weight for the different species can be seen in attachment 7.

The Gonadosomatic Index (GSI) is the weight of the gonads divided by the whole weight of the fish multiplied by 100, and the seasonal course of the GSI can be used as a measure of the reproductional cycle of the fish (Sabarowski et al., 1997).

Figure 3.1 and Table 3.1 shows the GSI of the fish sampled and the periods with increased gonad development can be distinguished. The female sculpins from January, the male flounders from February and the female dabs from April had increased gonadal development, whereas in cod, only males from April-May showed a slightly increased gonadal development. The GSI was significantly higher in female than in male dab from April-May (P=0,0002). In sculpins from January the difference between males and females was not significant.

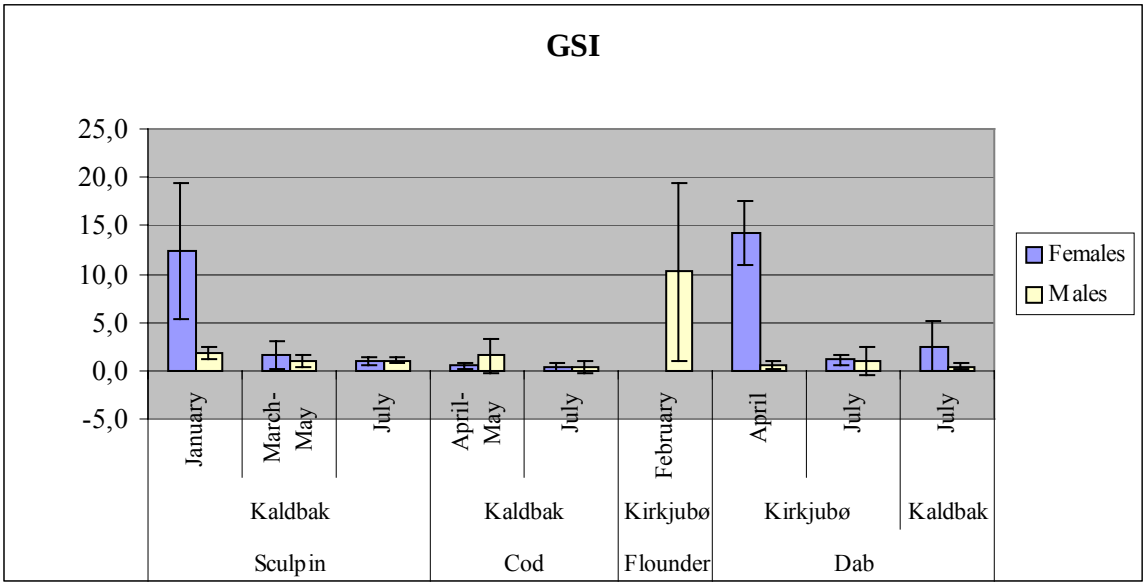


Figure 3.1 GSI in fish species from Kaldbak and Kirkjubø

3.1 P4501A induction

Induction of the P4501A (CYP1A) system was measured as catalytic activity by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay, and in sculpin and cod also as CYP1A protein content by ELISA test.

3.1.1 P4501A activity

Table 3.2 and Figure 3.2 show the mean values of EROD activity in the fish samples. As seen from the standard deviations variations are very large.

Table 3.2 Mean CYP1A activity in fish from Kaldbak and Kirkjubø measured by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay.

Species	Location	Sampling period	Gender	n	EROD (pmol/min/mg protein)	
					mean±std.dev	range*
Sculpin	Kaldbak	January	Female	4	0,4±0,2	(0,2-0,7)
			Male	2	7,1	(0,1-14,1)
		March-May	Female	9	2,1±4,6	(0,0-14,3)
			Male	3	0,2±0,1	(0,1-0,2)
		July	Female	8	7,2±18,2	(0,1-52,2)
			Male	2	0,3	(0,3-0,4)
Cod	Kaldbak	April-May	Female	6	65,2±110,0	(1,3-286,8)
			Male	6	64,3±78,2	(0,3-218,5)
		July	Female	6	42,0±54,9	(2,8-151,9)
			Male	6	36,5±28,9	(2,0-83,6)
Dab	Kaldbak	July	Female	6	139,2±159,8	(3,8-420,8)
			Male	3	27,5±12,2	(19,7-41,5)
	Kirkjubø	April	Female	4	3,2±1,5	(1,5-5,1)
			Male	4	83,0±27,7	(43,1-106,0)
		July	Female	11	199,4±263,5	(30,6-868,3)
			Male	2	94,9	(67,2-122,7)
Flounder	Kirkjubø	February	Male	6	23,8±15,2	(1,2-37,3)

*range: min-max values

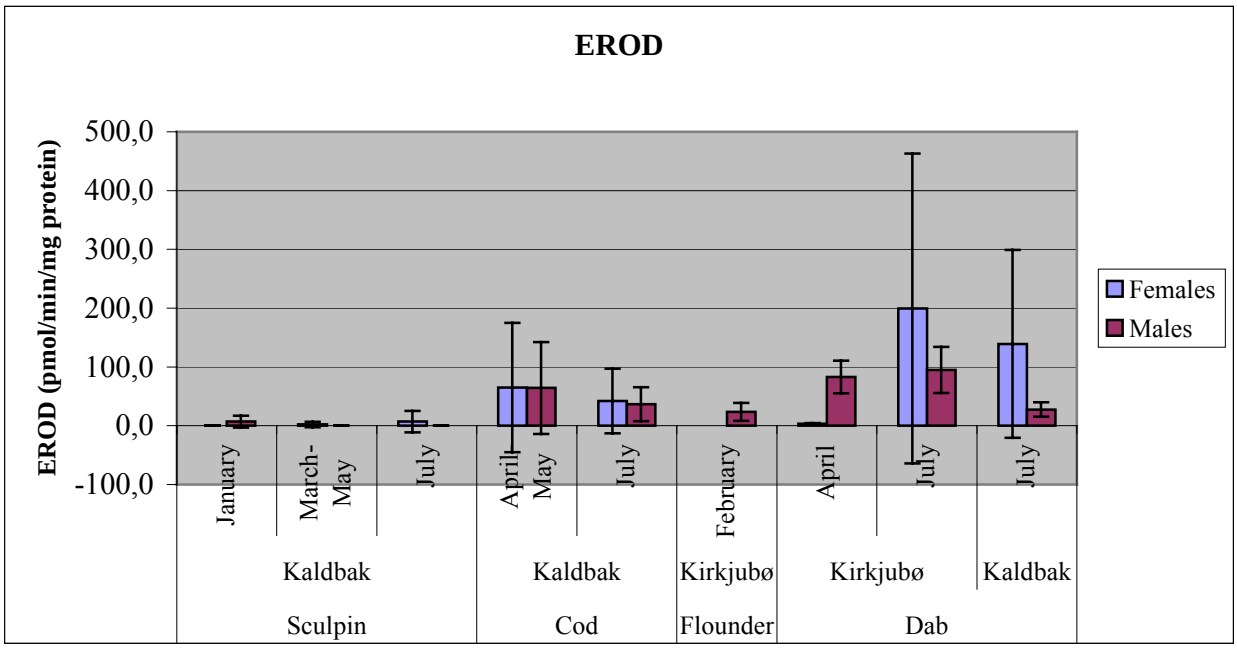


Figure 3.2 Mean CYP1A activity in fish from Kaldbak and Kirkjubø measured by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay.

EROD activity was analysed in sculpin from Kaldbak in January, March-May and July. Of the fish species analysed sculpin showed the lowest EROD activities. The single values ranged from 0,0 to 52,2 pmol/min/mg protein in females and from 0,1 to 14,3 pmol/min/mg protein in males. The females had lowest mean activity in January and highest in July. Males had highest activity in

January, the values being at the female July level, while male March-May and July levels were at the female January level. The number of samples is, however, low, and variability high, and the difference in EROD activity between females and males is not statistically significant in any season (P>0,05).

EROD activity was analysed in cod from Kaldbak in April-May and July. In cod there appeared to be no difference in EROD activity between females and males. The levels seemed to be higher in April-May than in July, but not significantly higher. The levels were higher than in sculpins, ranging from 0,3-218,5 pmol/min/mg protein in males and 1,3-286,8 pmol/min/mg protein in females.

Generally, the highest EROD activities were found in dab, with the highest mean activity in females from Kirkjubø in July. The highest single value of 868,3 pmol/min/mg protein was found in a female dab caught in Kirkjubø in July. However, the values show large variation and the lowest values for dabs in this period on this location was 30,59 pmol/min/mg protein, in a female. The female dabs seemed to have lower activity in April than in July, (although the difference was not significant) the activity in males was at the same level both periods. The EROD activity in dab ranged from 1,5-868,3 pmol/min/mg protein in females and 19,7-122,7 pmol/min/mg protein in males.

In July dabs were analysed from two stations: Kaldbak and Kirkjubøur, and in both females and males the mean activities were higher in Kirkjubø than in Kaldbak. The differences between locations were not significant in either of the sex groups, but in males the difference was almost significant (P=0,06).

Flounder was only analysed from Kirkjubø in February and all specimens were males. The EROD activity ranged from 1,2-37,3 pmol/min/mg protein with a mean activity of 23,8 pmol/min/mg protein.

3.1.2 CYP1A protein

CYP1A protein was analysed in sculpin and cod. The results are shown in Table 3.3 and the number of individuals analysed (n) is in some of the groups lower than the number sampled, because of lack of liver tissue.

Table 3.3 CYP1A protein content in sculpin and cod from Kaldbak measured by ELISA test.

Species	Location	Sampling period	Gender	n	CYP1A (abs/mg protein)	
					mean±std.dev	range
Sculpin	Kaldbak	January	Female	4	0,084±0,04	(0,045-0,146)
			Male	2	0,162	(0,141-0,183)
		March-May	Female	9	0,159±0,03	(0,132-0,212)
			Male	3	0,182±0,04	(0,148-0,226)
		July	Female	3	0,128±0,02	(0,109-0,152)
			Male	2	0,150	(0,145-0,154)
Cod	Kaldbak	April-May	Female	6	0,058±0,01	(0,036-0,073)
			Male	5	0,068±0,06	(0,054-0,068)
		July	Female	5	0,067±0,01	(0,058-0,081)
			Male	6	0,077±0,02	(0,053-0,098)

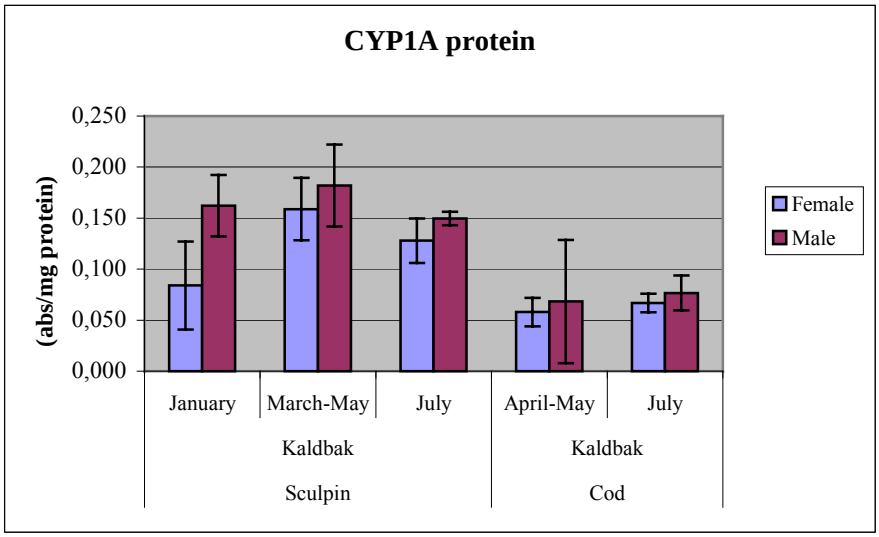


Figure 3.3 Content of CYP1A protein in sculpin and cod measured by ELISA test.

As shown above the mean values of CYP1A protein are generally higher in sculpins than in cod. For both cod and sculpins the mean values were lower in females than in males, but the difference between sexes was not significant (P>0,05). The level in females in January was significantly lower than in females in March-May (P=0,01).

3.1.3 EROD vs. CYP1A protein

Since the EROD and CYP1A protein analyses are measurements of the catalytic activity and concentration of the same proteins the two parameters would be expected to be correlated, if the catalytic activity has not been destroyed or suppressed. Figure 3.4 and Figure 3.5 show EROD activity versus CYP1A protein activity in sculpins and cod respectively.

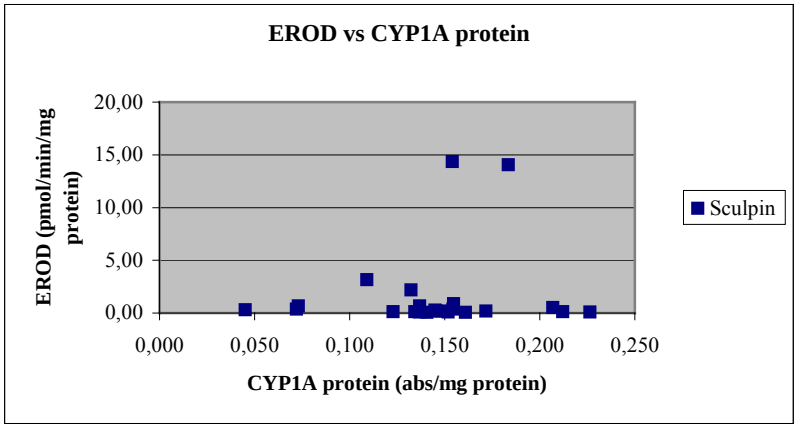


Figure 3.4 The correlation between CYP1A activity and CYP1A protein content in sculpin. (CYP1A activity was measured by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay and CYP1A protein content by ELISA test.)

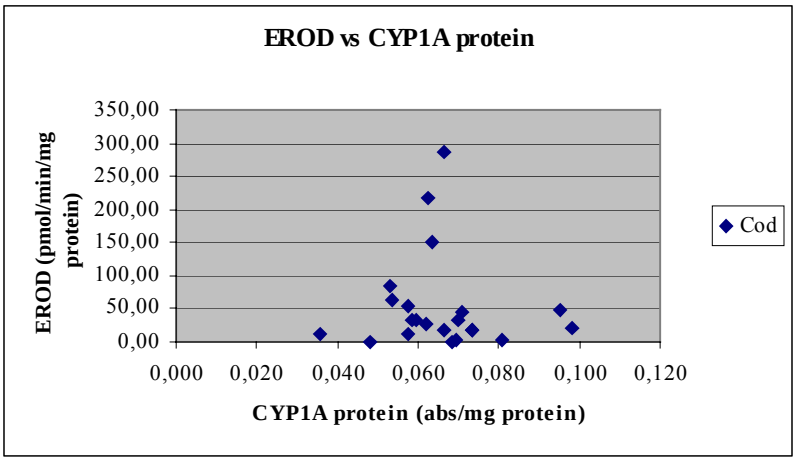


Figure 3.5 The correlation between CYP1A activity and CYP1A protein in cod. (CYP1A activity was measured by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay and CYP1A protein content by ELISA test.)

As seen in the figures the two parameters do not seem to be correlated in either of the species (r^2=0,003 in cod and r^2=0,018 in sculpin).The low EROD results in sculpin compared to the CYP1A protein results indicate that the catalytic activity has been destroyed in the sculpin samples and hence that the analysis of CYP1A protein is a better indicator of CYP1A induction in these samples than EROD.

3.1.4 CYP1A induction versus Gonadosomatic Index

The CYP1A induction can be affected by the gonadal maturation (Krüner & Westernhagen, 1999; Lange et al., 1998; 1999; Edwards et al, 1988; Lindström-Seppä & Stegeman, 1995). This is most likely due to the presence of sex steroids, which have been found to suppress the MFO expression (Förlin et al.,1984; Stegeman & Woodin, 1984). Hence the MFO activity will be low in pre-spawning and spawning females, when the GSI is high.

To investigate the correlation between these parameters, the EROD activity has been plotted versus the GSI for the different species in their spawning seasons. Figure 3.6 shows the EROD activity versus GSI in flounder from February.

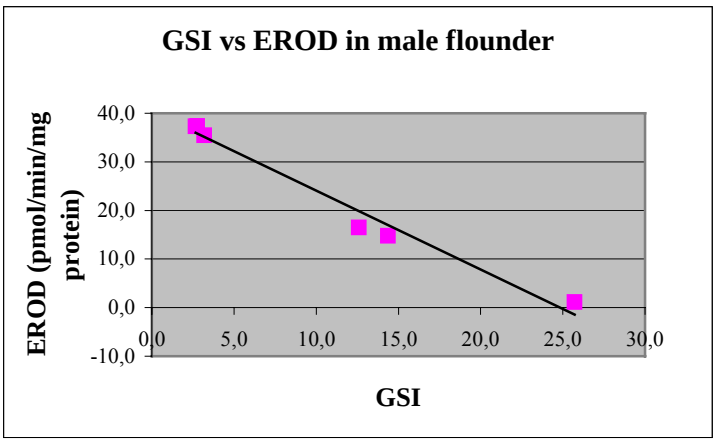


Figure 3.6 CYP1A activity (measured by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay), versus GSI in male flounder in February 2002.

As the figure shows there is significant negative correlation between EROD and GSI in flounder (P=0,0002; r^2=0,98) (Figure 3.6).

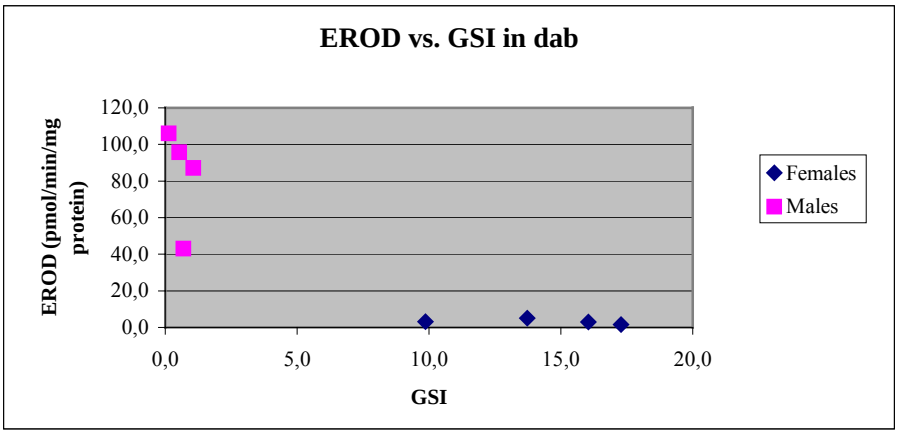


Figure 3.7 CYP1A activity (measured by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay versus GSI in dab from April 2002

In dab from April the results of EROD activity versus GSI are grouped into males and females, where the females have high GSI and low EROD activity and males have low GSI and high EROD activity (Figure 3.7). The values of EROD and GSI in female dab are not significantly correlated, although the EROD activity is low when the GSI is high and vice versa. The difference in EROD activity in female dab in April and July is not significant (P>0,05), while the GSI is significantly higher in April than in July (P=0,0000).

In sculpins the CYP1A protein content has been plotted against GSI instead of EROD, as these results seem to be more reliable than the EROD results as a measure of the MFO activity in sculpins. The CYP1A protein content and GSI are significantly correlated when lumping females and males (r^2=0,93 and P=0,002) and for females only (r^2=0,96 and P=0,02).

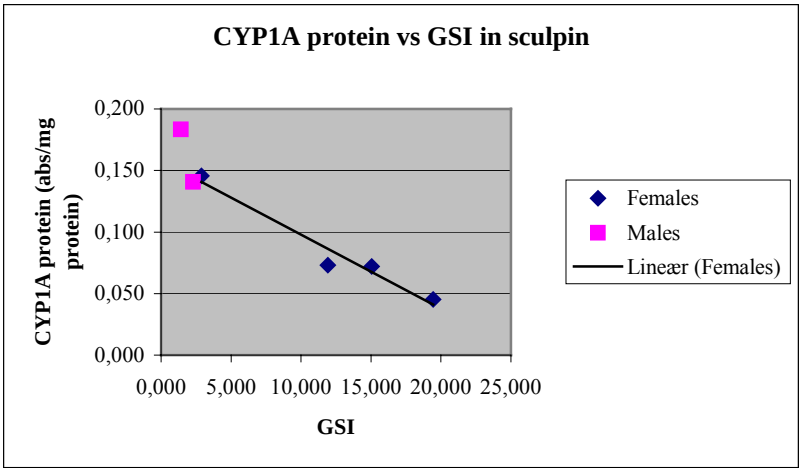


Figure 3.8 CYP1A protein content (measured by ELISA test) versus GSI in sculpins from January 2002.

3.2 PAH metabolites

Of the PAH metabolites analysed (2-OH-naphtalene, 1-OH-fenantrene, 1-OH-pyrene, 3-OH-B(a)P) only 1-OH-pyrene was found in non-negligible amounts. OH-pyrene is the major biotransformation product of pyrene in teleosts (Beyer, 1996). Table 3.4 and Figure 3.9 show the content of 1-OH-pyrene found in the fish.

Table 3.4 1-OH-pyrene (measured by HPLC detection) in the bile of fish from Kaldbak and Kirkjubø

Species	**Location**	**Sampling period**	**Gender**	**n**	**1-OH-pyrene** (µg/kg)			**n**	**1-OH-pyrene** norm. (µg/kg/abs 380nm)
					mean±std.dev	range			mean±std.dev
Sculpin	Kaldbak	January	Female	4	43,7±24,1	(15,2-72,8)		4	2,81±0,7
			Male	1	18,3	-		1	1,9
		March-May	Female	4	18,7±12,2	(4,5-33,7)		3	0,9±0,6
			Male	2	5,3	(4,7-5,9)		1	0,5
		July	Female	7	21,4±29,3	(1,8-81,0)		4	0,3±0,4
			Male	1	23,0	-		1	1,5
Cod	Kaldbak	April-May	Female	6	14,0±12,7	(4,6-39,2)		6	0,7±0,4
			Male	6	10,5±3,6	(5,0-15,0)		4	0,6±0,4
		July	Female	3	10,4±10,8	(4,0-22,8)		3	0,6±0,5
			Male	5	11,1±12,6	(3,5-33,4)		4	0,9±1,1
Dab	Kaldbak	July	Female	6	14,1±17,9	(3,8-50,1)		5	0,2±0,2
			Male	3	27,8±24,3	(13,2-55,8)		3	0,4±0,2
	Kirkjubø	April	Female	2	2,4	(2,2-2,6)		1	0,7
			Male	3	8,3±9,2	(2,9-18,9)		2	3,7
		July	Female	7	4,8±4,3	(0,9-11,2)		5	0,5±0,6
			Male	2	2,0	(1,2-2,7)		1	0,4
Flounder	Kirkjubø	February	Male	1	322,0	-		1	6,1

Several of the gall bladders contained too small amounts of bile to be analysed, and, therefore, the numbers of individuals analysed (n) is somewhat lower in most of the groups than the number sampled.

As the measurement of the concentration of metabolites in bile can be influenced by differences in bile density (caused by concentration of bile in the gall bladder after prolonged periods of starvation), the results were normalized by dividing the results by the absorbance at 380nm (see section 2.2.7). The normalized results are shown in Table 3.4 (grey shaded), but as this resulted in even less number of samples (n) due to small amounts of bile fluid, those results are not treated further.

Only one of the flounders had enough bile to be analysed and the 1-OH-pyrene concentration was much higher in this individual than other groups. The concentration in flounder was 322,0 µg/kg which is nearly 3,5 times higher than the second highest single value of 72,8 µg/kg found in a female sculpin in January. However, when looking at the normalized data (Table 3.4, grey shaded), the difference between the level in flounder and in sculpins in January is not as big, indicating that the bile fluid in this particular flounder is highly concentrated.

Disregarding the flounder, the highest values of 1-OH-pyrene were found in sculpins, ranging from 4,5-72,8 µg/kg. In male groups the numbers analysed were only 1 or 2 and although the mean

values seem to be higher than in the female groups the male values were within the range of the females.

The range of 1-OH-pyrene in cod was between 3,5-39,2 µg/kg, and there did not seem to be any difference between males and females or season.

In dab the values of 1-OH-pyrene ranged between 1,2-18,9 in Kirkjubø and between 3,8-55,8 in Kaldbak. There seemed not to be any difference between the values in males and females in any of the groups.

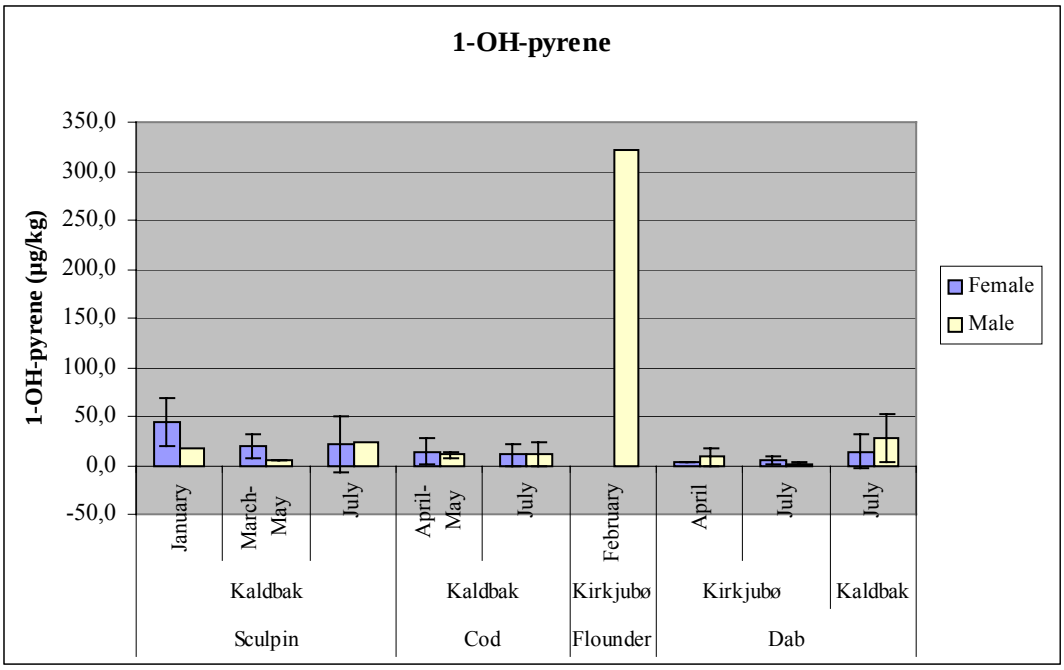


Figure 3.9 Mean concentrations and standard deviations of 1-OH-pyrene in the fish samples (measured by HPLC detection.

In July dab from both Kaldbak and Kirkjubø was analysed and the 1-OH-pyrene content was significantly higher in dabs from Kaldbak than in dab from Kirkjubø ($P<0,05$).

3.2.1 CYP1A induction versus pyrene

PAH compounds metabolized by the cytochrome P450 enzyme system may be excreted by the organism via the bile. Hence, if the PAH exposure has occurred recently a correlation between CYP1A induction and concentration of PAH metabolites in bile would be expected.

The correlation between EROD or CYP1A protein and 1-OH-pyrene in the fish groups not affected by spawning are shown in Table 3.5 and Table 3.6.

Table 3.5 Correlation between CYP1A activity and 1-OH-pyrene for different fish groups. (CYP1A activity measured by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay and 1-OH-pyrene by HPLC detection).

EROD vs pyrene			f(x)	r^2	P(2 tail)
Cod	Kaldbak	April-May	-0,0108x + 12,994	0,01	0,74
		July	-0,104x + 16,294	0,18	0,29
Dab	Kaldbak	July	-0,0515x + 23,91	0,13	0,34
	Kirkjubø	July	0,0164x + 1,9834	0,41	0,06

Table 3.6 Correlation between CYP1A protein and 1-OH-pyrene for different fish groups. (CYP1A protein measured by ELISA test and 1-OH-pyrene by HPLC detection).

CYP1A protein vs pyrene			f(x)	r^2	P(2 tail)
Sculpin	Kaldbak	March-May	0,0015x + 0,1455	0,56	0,09
		July	0,0003x + 0,1198	0,23	0,52
Cod	Kaldbak	April-May	-0,0006x + 0,0671	0,34	0,07
		July	0,0007x + 0,0654	0,23	0,27

The PAH metabolite content in the bile and the CYP1A induction in the liver does not seem to be correlated for the species caught in either of the locations. Only the correlation between EROD and OH-pyrene in dab from Kirkjubø in July was nearly significant (r^2=0,41; P=0,06) (Figure 3.10), as well as the correlation between CYP1A protein and OH-pyrene in sculpin from Kaldbak in March-May (r^2=0,56; P=0,09) (Figure 3.11).

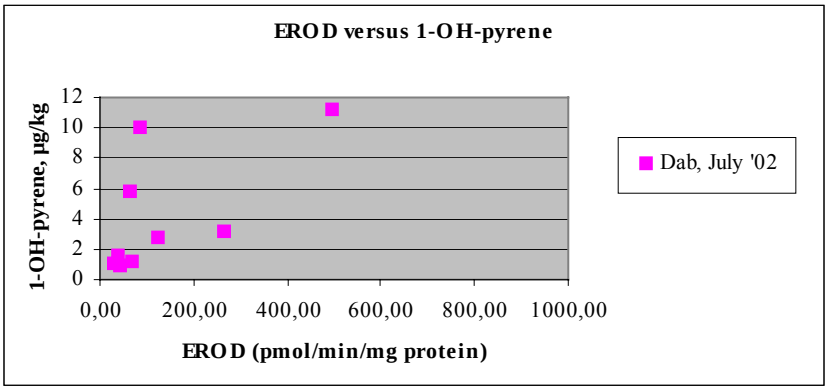


Figure 3.10 The correlation between 1-OH-pyrene and EROD activity in dab from Kirkjubø. (CYP1A activity measured by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay and 1-OH-pyrene by HPLC detection).

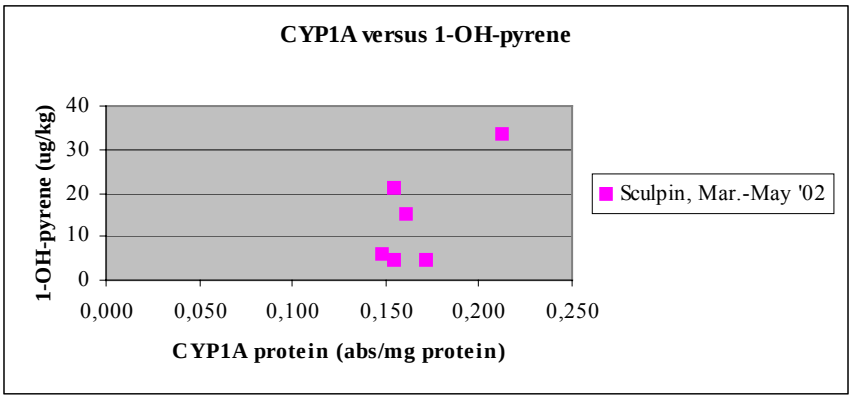


Figure 3.11 The correlation between 1-OH-pyrene and CYP1A protein content in sculpin from Kaldbak in March-May. (CYP1A protein measured by ELISA test and 1-OH-pyrene by HPLC detection)

3.3 Vitellogenin

The results from the vitellogenin analyses in cod are given in Table 3.7 and Figure 3.12. Two samples from the group of females in April-May were discarded because their optical density values were below the standard curve, both when diluted 100x and 10x. These should have been reanalysed undiluted, but this was not done.

The levels seem to be higher in July than in April-May and the variations seem to be larger in July. There was no significant difference between females and males.

Table 3.7 Vitellogenin in cod from Kaldbak measured by ELISA test.

Sampling period	**Gender**	**n**	**Vtg** (ng/ml)	
			mean±std.dev.	(range)
April-May	Female	4	1978±347	(1563-2394)
	Male	6	1758,3±452	(1163-2445)
July	Female	6	3240±3101	(784-8790)
	Male	6	2595±2017	(798-6070)

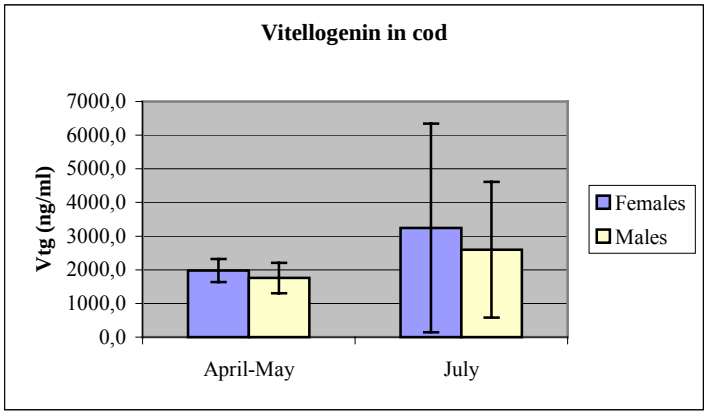


Figure 3.12 Vitellogenin in cod from Kaldbak

Since there was no sculpin and dab antibody serum available, only cod were analysed for vitellogenin. Sculpin and dab antigens were however tested against the available antiserums to see which of them gave the best reaction. Against the two antigens, which gave the best response (cod and wolffish), the sculpin and dab antigens were tested along with cod antigens to see if the response matched the cod response and a relative value could be measured. The response in dab did match the cod response, whereas the sculpin response did not. The results are given in attachment 8. Relative vitellogenin, values therefore, can be measured in dab using cod antibody, but this was not done because of lack of time and resources.

Invertebrate study

4 Methods

4.1 Sampling of invertebrates

Invertebrates were sampled on 6 places on the Faroe Islands: Hvannasund, Svínáir, Kaldbak, Velbastaður, Kirkjubøur and Trongisvágur (see Map 1 on page 32). Sampling was performed three times, in Desember 2001/February 2002, March/May 2002 and in June/August 2002. Approximately 50 limpets (*Patella vulgata*), blue mussels (*Mytilus edulis*), dogwhelks (*Nucella lapillus*) and approximately 100 periwinkles (*Littorina obtusata*) were sampled by hand in the littoral zone, wearing talcum-free gloves, and put in PE plastic bags (Minigrip®). The limpets were loosened from the rock with a knife. The samples were stored in a freezer (-20ºC) until further preparation. The blue mussels from the two last sample periods, however, were placed in a small container with sea water for appr. 24 hours in order to empty their stomachs, before they were put in plastic bags and frozen. This depuration procedure was not followed for the samples from the first sampling period.

Individuals of all species were not found at all stations. In Kirkjubøur horse mussels (*Modiolus modiolus*) were sampled instead of blue mussels, and since they are living in the sub-littoral the sampling was done by scuba diving. All the horse mussels were placed in a container with sea water for approximately 24 hours before the sample preparation. The species sampled at each station are shown in Table 4.1.

Table 4.1 Invetrebrate species sampled at the different stations. Sampling was performed in Dec´01/Feb´02, Mar-May´02, June-Aug´02.

	Hvannasund	**Svínáir**	**Kaldbak**	**Kirkjubøur**	**Velbastaður**	**Trongisvágur**
Blue mussels (*Mytilus edulis*)	(x)	x	x			x
Limpets (*Patella vulgata*)	x	x	x	x	x	x
Dogwhelks (*Nucella lapillus*)	x	x	x	x	x	(x)
Periwinkles (*Littorina sp*)	x	x	(x)			x
Horse mussels (*Modiolus modiolus*)				x		

The brackets mark that the species were not found at all three sampling periods.
Grey shadow: Not analysed.

The individuals of the samples were divided into two size groups, each group constituting one pooled sample. The length of the shell for each individual was measured and the soft part was removed from the shell. For the periwinkles only the largest and the smallest individuals for each group were measured. For the blue mussels and horse mussels the adductor muscles were cut and the mussels were set on a piece of water-absorbing paper for 5-10 minutes to drain sea-water, which has been trapped in the shells, before removing the soft part from the shell. For dogwhelks and periwinkles the shell had to be crushed before the soft part was removed. The weight of the pooled samples was recorded.

4.2 Analysis of invertebrate samples

The analyses of **invertebrates** were performed by NIVA (Norwegian Institute for Water Reseach). The invertebrate samples were analysed for 23 different PAHs (for the individual compounds, see attachment 10B) and the metals Ag, As, Ba, Cd, Cr, Cu, Ni, Pb, Sr, Zn and Hg, along with analysis of dry matter and fat content. For each group (each species each station each season) two pooled samples were analysed except for the periwinkles, where only one pooled sample was analysed for each station each season.

4.2.1 PAH

The analysis for PAH in invertebrate tissue was performed by gas chromatograph/mass selective detector (GC/MSD). Prior to the analysis the samples were saponified with KOH/methanol and PAHs were extracted with pentane. The method is described in attachment 9B.

4.2.2 Metals

All the metals, except mercury and chromium were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The samples were digested with nitric acid prior to analysis by Perkin-Elmer Sciex ELAN 6000 ICP-MS. Mercury was analysed by Perkin-Elmer FIMS-400 and P-E amalgam system. Chromium was analysed by atom absorbtion using Perkin Elmer-AAnalyst 700. Description of the methods is found in attachment 9C-9E.

4.3 Analysis of cod livers

Additionally 20 samples of **cod liver** (stored at -80ºC), from the fish study, were analysed for DNA adducts by the ^{32}P-postlabeling assay (section 4.3.1) and livers (stored at 20ºC) from four individuals of cod, representing different levels EROD activity, were analysed for dioxin (PCDD/PCDF) and PCB (non-and mono–ortho chlorinated PCBs and marker PCBs: CB 28, 52, 101, 118, 138, 153 and 180) (section 4.3.2).

4.3.1 DNA adducts

Liver samples from 20 cods from Kaldbak were measured for DNA adducts by the ^{32}P-postlabelling assay by Institute of Applied Environmental research (ITM) at Stockholm University, Sweden. Description of the method is given in attachment 11.

4.3.2 Polychlorinated biphenyls and dioxins

Four individual cod liver samples, representing the range of EROD activities found, were analysed for dioxin (PCDD/PCDF) and PCB (non-and mono–ortho chlorinated PCBs and marker PCBs: CB 28, 52, 101, 118, 138, 153 and 180) by ERGO Forschungsgesellshaft in Hamburg, Germany. The analyses were performed using high resolution gaschromatography and high resolution mass spectrometry (HRGC/MS), and description of the methods can be seen in attachment 12.

The results of the analyses for dioxins and non-and mono–ortho chlorinated PCBs were calculated to toxic equivalents (TEQs) by dividing the concentrations by toxic equivalency factors (TEFs)[9] for these compounds in fish (Van den Berg et al., 1998).

[9] TEFs have been established by WHO, as a measure of the toxicity of dioxins and dioxin-like compounds for humans/mammals, birds and fish, based on excisting litterature data (Van den Berg et al., 1998).

5 Results of invertebrate study

5.1 PAH in invertebrates

All the invertebrate samples were analysed for 23 different PAHs (see attachment 10B). The figures below show the PAH content in the different species as the sum of the concentration of PAH found and the sum of KPAHs[10] on a wet weight basis. Results for the individual pooled samples can be seen in attachment 10B. The PAH concentrations were also calculated on a lipid weight basis, but this did not change the pattern of accumulation to any extent and is thus not shown here.

The PAH content in periwinkles (*Littorina obtusata*) from two stations were analysed. Periwinkles from Hvannasund were analysed from all three seasons, whereas in Svínáir only periwinkles from July were measured.

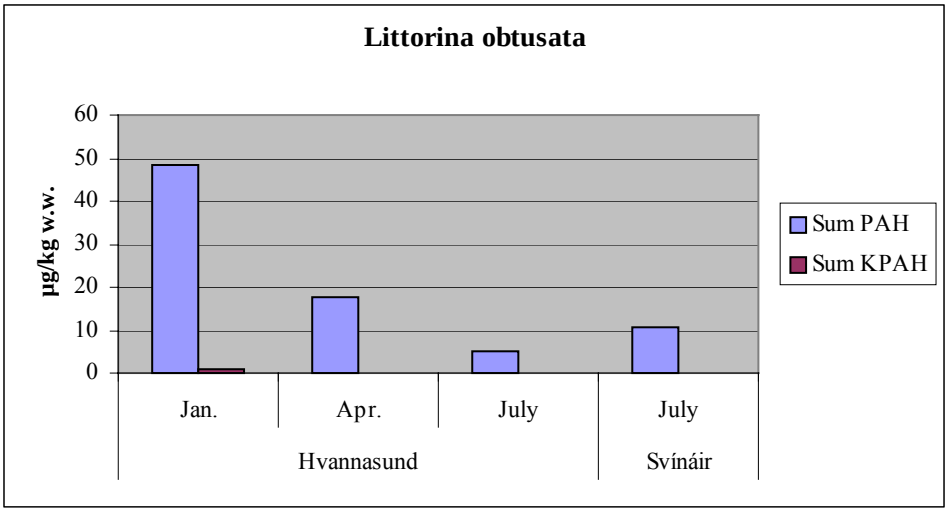


Figure 5.1 Sum of PAHs and KPAHs in periwinkles (*Littorina obtusata*), analysed by GC/MSD.

As Figure 5.1 shows, the content of PAH in periwinkles varied seasonally with highest levels in January and lowest in July. Some of the PAHs measured in January in Hvannasund were KPAHs whereas KPAHs were not found at this station in other periods or, in Svínáir in July. The sampling of periwinkles in Hvannasund was, however, performed closer to the innermost part of the fjord in January than in the other periods, and are, therefore, probably more exposed to local pollution. Comparing the two stations shows that the levels in Svínáir seem to be higher than in Hvannasund in July, although the difference is small.

PAHs in dogwhelks (*Nucella lapillus*) were analysed from the same stations and periods as the periwinkles (Figure 5.2). The levels of PAH in dogwhelks were also found to be highest in January and lowest in July, although the differences between April and July were small. In dogwhelks the levels were lower in Svínáir than in Hvannasund in July. The concentrations seemed to be a little lower than in periwinkles. There were not measured any compounds from the KPAH group in dogwhelks.

[10] KPAH: PAH compounds with potential carcinogen properties for humans according to IARC (1987). Listed in IARC's categories 2A or 2B (likely or potential carcinogens).

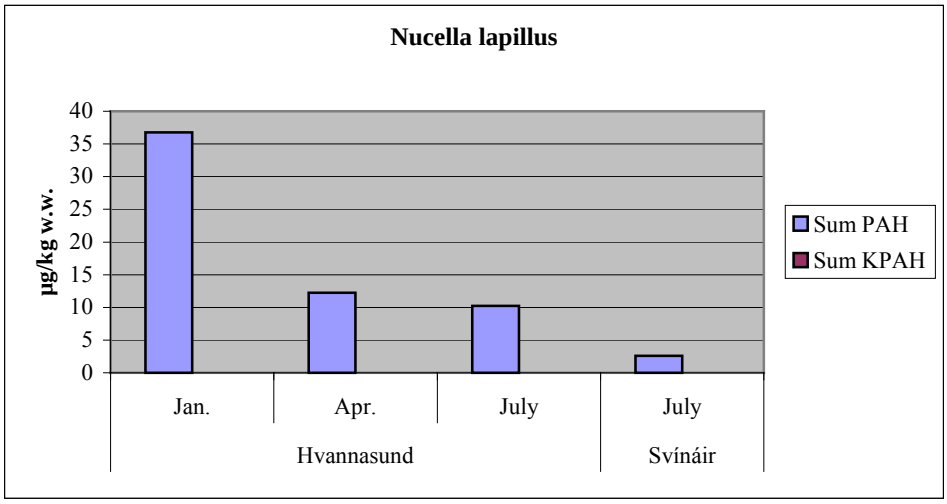


Figure 5.2 Sum of PAHs and KPAHs in dogwhelks (*Nucella lapillus*), analysed by GC/MSD.

Limpets (*Patella vulgata*) were analysed at four stations in all three seasons (see Figure 5.3). The highest values were found in Trongisvágur, with the highest level of sum PAHs in May. The analyses from Hvannasund showed the same seasonal pattern as in dogwhelks and periwinkles, although the values were somewhat lower in limpets. KPAHs were found in Hvannasund in January and in Trongisvágur in all three seasons, however at low concentrations. The highest concentration of KPAHs was found in Trongisvágur in July, although this was not the period with highest concentration of sum PAH.

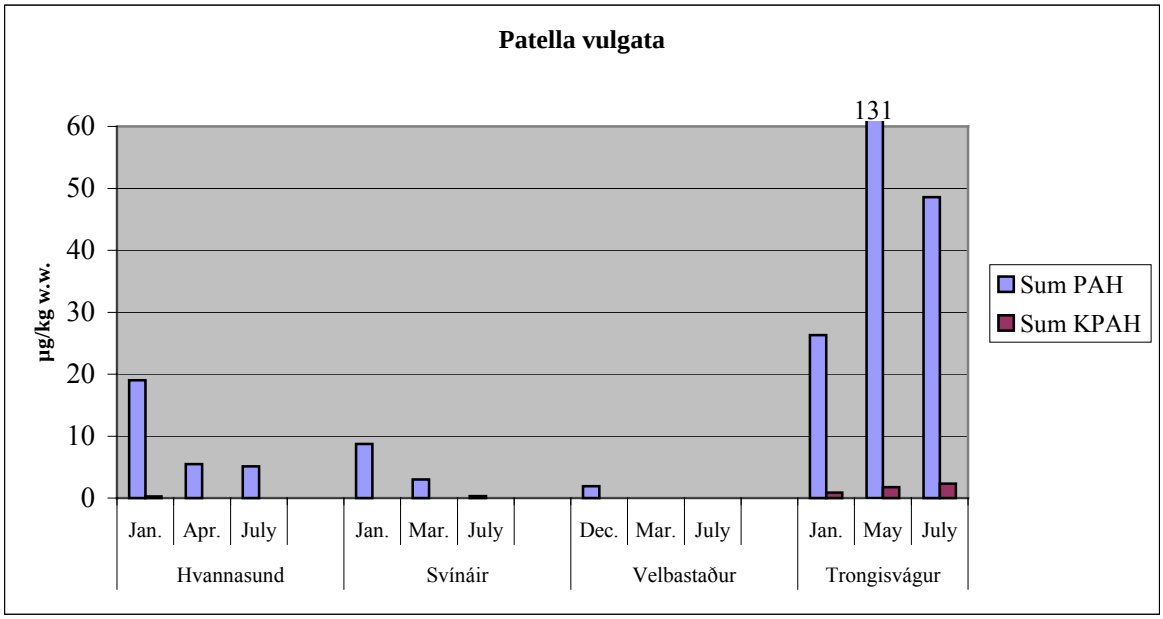


Figure 5.3 Sum of PAHs and KPAHs in limpets (*Patella vulgata*), analysed by GC/MSD.

PAH was analysed in blue mussels (*Mytilus edulis*) from the same stations and seasons as in limpets, except that blue mussels from Kaldbak were analysed, instead of from Velbastaður (no mussels were found in Velbastaður), and that no blue mussels from Hvannasund in July were analysed (not found). As shown in Figure 5.4 the concentrations of PAHs in blue mussels are roughly 10 fold higher than in the other species analysed. The relative PAH accumulation between stations show the same pattern as in limpets, with highest levels in Trongisvágur and May being the month with highest values at this location. KPAHs were found at all stations all seasons except in Svínáir in July. The highest values of KPAHs were found in Trongisvágur in January, although this was not the month with highest level of sum PAH.

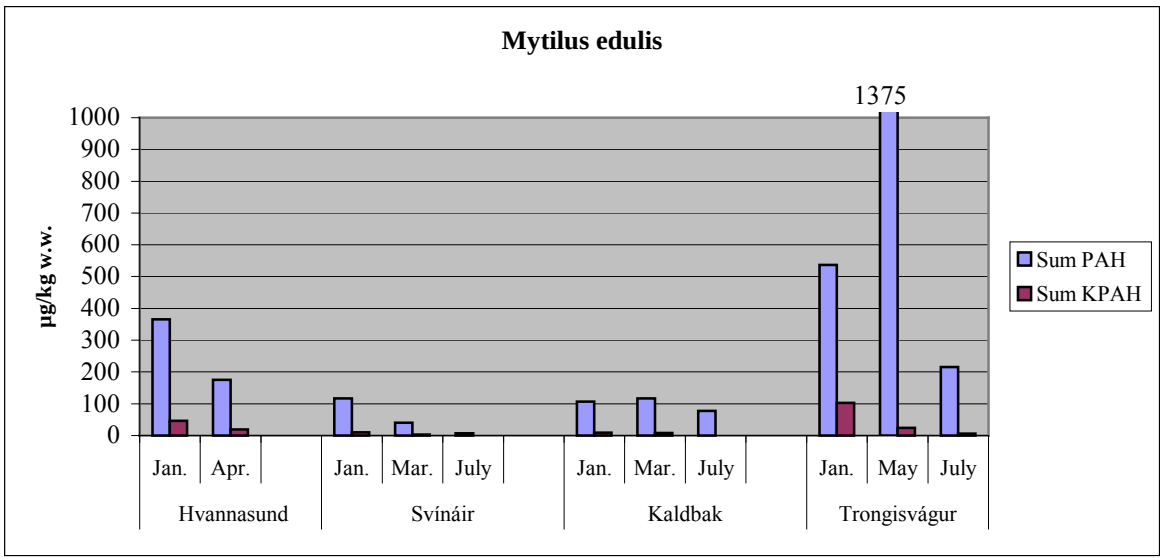


Figure 5.4 Sum of PAHs and KPAHs in blue mussels (*Mytilus edulis*), analysed by GC/MSD.

Horse mussels (*Modiolus modiolus*) were only sampled in Kirkjubøur. The PAH concentrations are highest in February and lowest in May, and are at the same level as in periwinkles, dogwhelks and limpets and lower than in blue mussels. Kirkjubøur and Velbastaður are two stations not far from each other, and can probably be regarded as one station. Comparing the concentrations in horse mussel with the concentrations in limpets from Velbastaður, show that horse mussels seem to accumulate PAHs to a higher level than limpets.

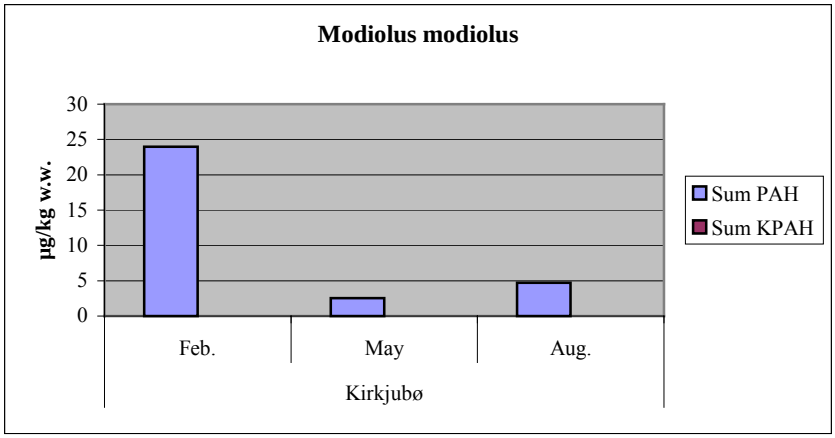


Figure 5.5 Sum of PAHs and KPAHs in horse mussels (*Modiolus modiolus*), analysed by GC/MSD.

5.1.1 Fat content

The fat content was lowest in spring for all the species except in periwinkles and horse mussels where the fat content was lowest in winter and summer respectively.

5.2 Metals in invertebrates

All the invertebrate samples were analysed for 10 metals. Seven of the metals (Ba, Cd, Cr, Cu, Hg, Pb and Zn) are used in monitoring of pollution from oil/gas-fields in the Norwegian off-shore sector (Nilssen, 1999) and only these will be treated here. The results from all the metal analysis for the individual pooled samples can be seen in attachment 10C.

The metal results did not show a general pattern of seasonal variation, but differences between seasons varied with species and metals. The figures below show the concentrations of metals as means for all three seasons for the different species at the different stations expressed on a wet weight basis. The concentrations were also calculated on a dry weight basis, but that did not change the distribution pattern to any extent. The dry matter content and fat content can be seen in attachment 10A.

The concentrations of the various metals were found at very different levels. The concentrations of barium are shown in Figure 5.6. Barium was generally found at very low concentrations. As shown by the standard deviations the values varied between seasons, especially in periwinkles from Hvannasund, limpets from Svínáir and in horse mussels from Kirkjubø (periwinkles and dogwhelks from Svínáir were only analysed in July). None of the species analysed seem to accumulate barium to a greater extent than the others, and none of the stations seem to yield higher barium concentrations than the others.

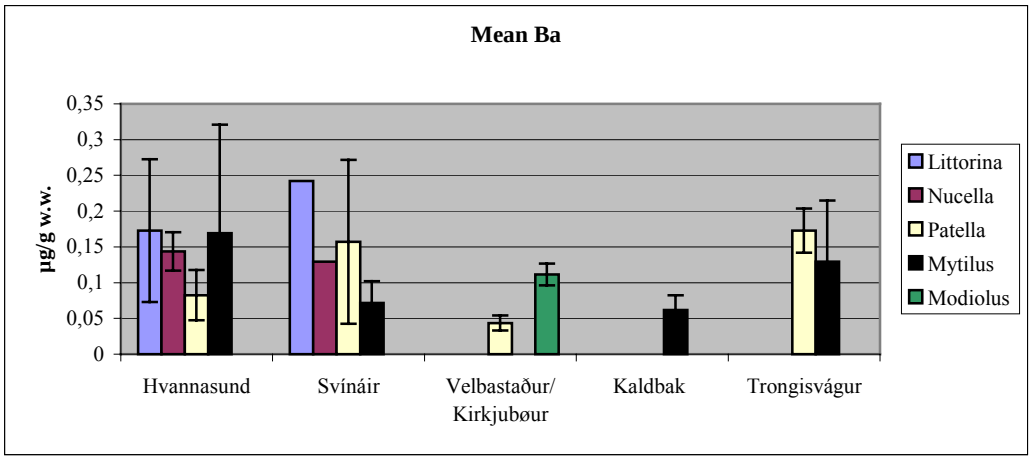


Figure 5.6 Concentrations of barium (means of seasons) in different species at each location, analysed by ICP-MS.

Figure 5.7 shows the concentrations of cadmium for the species at each stations. The highest levels were found in dogwhelks from Hvannasund and in limpets and horse mussels from Velbastað and Kirkjubø respectively. In blue mussels on the other hand, the cadmium concentration was very low at all stations. In Hvannasund the dogwhelks had a much higher concentration of cadmium than the other species, whereas in Svínáir the concentrations in dogwhelks were at the same level as in

periwinkles and limpets. Velbastaður and Kirkjubøur seem to be the stations with highest cadmium concentrations.

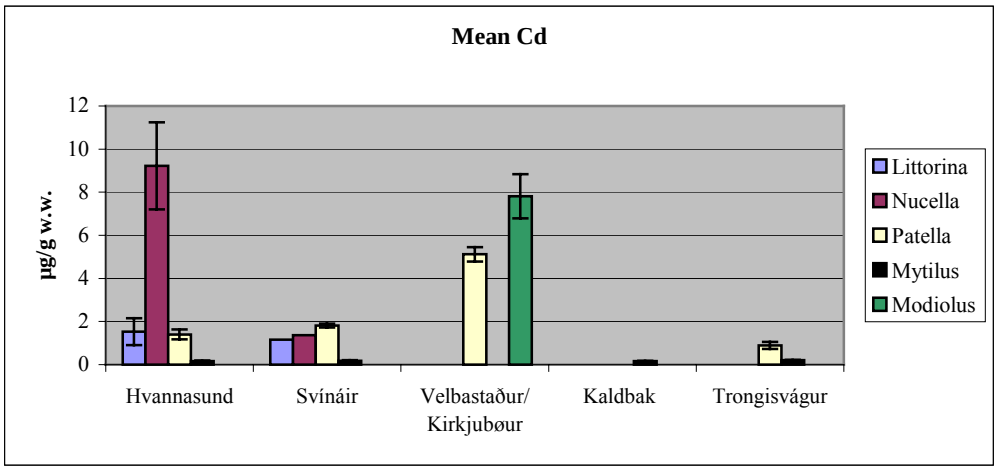


Figure 5.7 Concentrations of cadmium (means of seasons) in different species at each location, analysed by ICP-MS.

As Figure 5.8 shows the levels of chromium concentrations generally were low in the species analysed. The highest values were found in periwinkles, but the difference between seasons was large in Hvannasund (large standard deviations). The concentrations seemed to be highest in Hvannasund and Trongisvágur and lowest in Velbastaður, although the differences were not large. The concentration pattern between species seemed to be the same in Hvannasund and Trongisvágur (limpets and blue mussels) but a different pattern was seen in Svínáir.

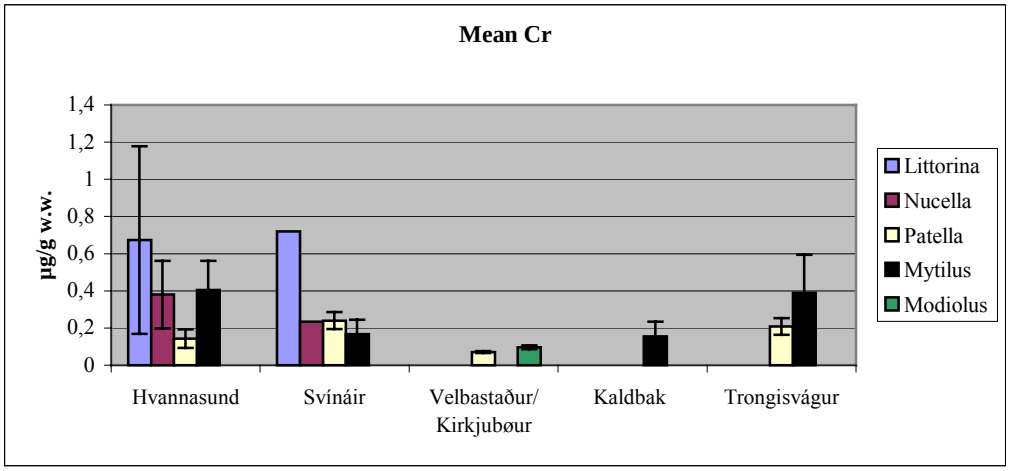


Figure 5.8 Concentrations of chromium (means of seasons) in different species at each location, analysed by atom absorbtion, AAnalyst 700.

The accumulation of copper obviously differed between the species analysed (Figure 5.9). Periwinkles and dogwhelks clearly accumulate copper to a much greater extent than the other species analysed. The concentrations in limpets, blue- and horse mussels were generally low and there seemed not to be any difference in copper exposure between the stations.

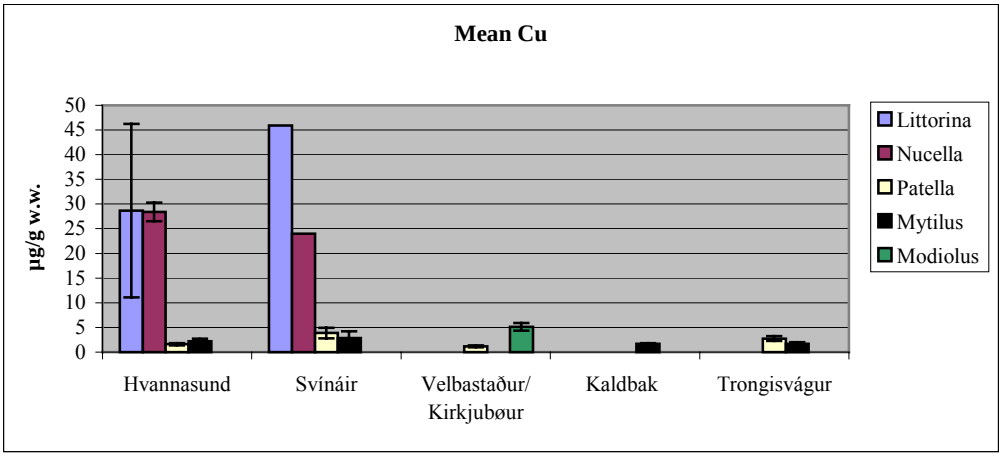


Figure 5.9 Concentrations of copper (means of seasons) in different species at each location, analysed by ICP-MS.

Mercury was found in low concentrations at all stations (Figure 5.10). Dogwhelks seem to accumulate mercury to the greatest extent. Hvannasund and Svínáir showed the same pattern of accumulation between species, but in Trongisvágur the content in blue mussels was higher, compared with the content in limpets, than in Hvannasund and Svínáir. When looking at the concentrations in blue mussels the Hg exposure was highest in Trongisvágur, but when looking at the accumulation in limpets the exposure was highest in Hvannasund. Mercury was however accumulated in very low concentrations, as mentioned above.

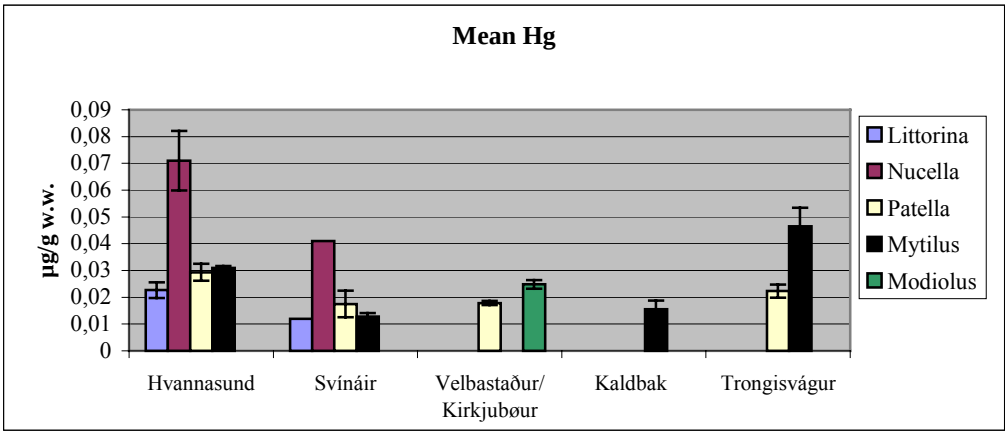


Figure 5.10 Concentrations of mercury (means of seasons) in different species at each location, analysed by FIMS 400 and amalgam system.

Figure 5.11 shows the accumulation of lead in the various species. Horse mussels seemed to have the largest accumulation of lead, although the accumulation generally was low in all species analysed. The second highest accumulation was seen in blue mussels in Hvannasund and Trongisvágur, where the blue mussels had higher concentration than the limpets. In Svínáir however, the accumulation was low and at the same level in all four species analysed. The highest exposure of lead seemed to occur in Kirkjubø, Trongisvágur and Hvannasund although the levels generally were low.

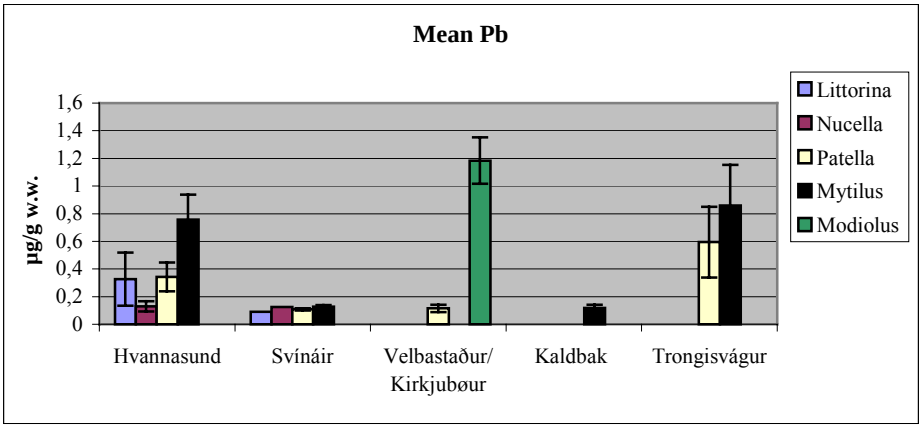


Figure 5.11 Concentrations of lead (means of seasons) in different species at each location, analysed by ICP-MS.

Zinc was generally found in very high concentrations in the species analysed, at all stations (Figure 5.12). The highest accumulation seemed to occur in dogwhelks followed by blue mussels and horse mussels whereas the accumulation in periwinkles and limpets was found to be lower. The highest exposure of zinc seemed to occur in Trongisvágur although differences between stations were not large.

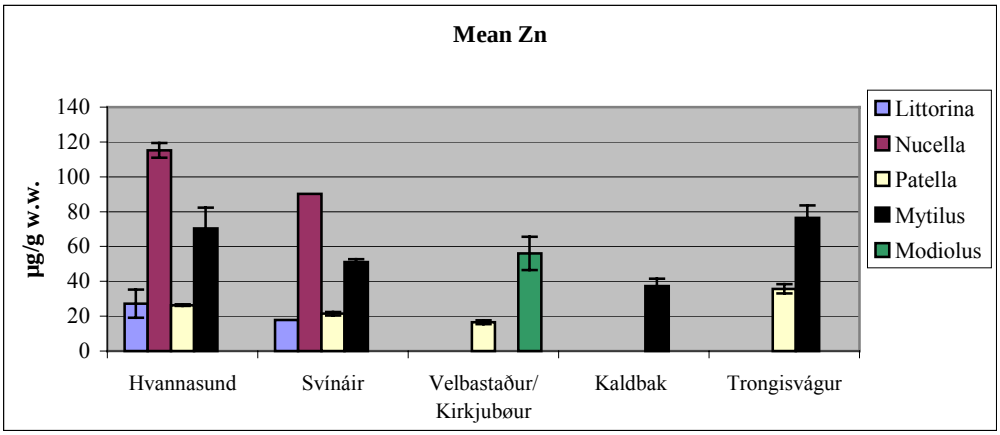


Figure 5.12 Concentrations of zinc (means of seasons) in different species at each location, analysed by ICP-MS.

5.3 Results of additional analyses in fish liver

5.3.1 DNA adducts

The formation of DNA adducts in liver cells has shown to be correlated to long-term exposure of PAH. Liver samples of 20 of the cods were analysed for DNA adducts by the ^{32}P-postlabeling assay. DNA adducts were not detected in any of the samples analysed (see Table 5.1).

Table 5.1 DNA adducts in cod samples

Fish nr.	Gender	Sampling period	DNA adducts (nmol/mol nucleotides)
Rf 1	F	Apr.-May	<1.4
Rf 2	M	Apr.-May	<1.1
Rf 3	F	Apr.-May	<1.3
Rf 4	M	Apr.-May	<1.3
Rf 5	M	Apr.-May	<1.3
Rf 6	M	Apr.-May	<1.4
Rf 7	M	Apr.-May	<1.2
Rf 8	F	Apr.-May	<0.9
Rf 9	M	Apr.-May	<1.2
Rf 10	F	Apr.-May	<1.0
Rf 11	F	Apr.-May	<1.3
Rf 12	F	Apr.-May	<1.1
Rf 13	M	July	<0.9
Rf 14	F	July	<1.0
Rf 15	M	July	<1.0
Rf 17	M	July	<1.0
Rf 19	F	July	<0.7
Rf 20	M	July	<1.0
Rf 23	F	July	<0.9
Rf 24	M	July	<0.7

The limit of detection for quantification of DNA adducts was considered as a spot-specific (area/zone), corresponding to 1.5 times the representative background (spot/area/zone) on the same autoradiogram.

5.3.2 PCB and dioxin in cod

Along with PAHs, dioxins and certain PCB compounds (the non-and mono–ortho substituted congeners) are known to be inducers of CYP1A (Parke, 1990; Stegeman et al., 1992), and the levels of PCB (non-and mono–ortho PCBs and marker PCBs) and dioxins (PCDD/PCDF) were analysed in the liver of four individual cods, which had been found to have different levels of EROD activity. The concentrations of PCDD/PCDFs and non- and mono-ortho PCBs have been calculated in toxicity equivalents (TEQ) for fish according to the WHO-system (Van den Berg et al., 1998) and the levels are shown in Figure 5.13.

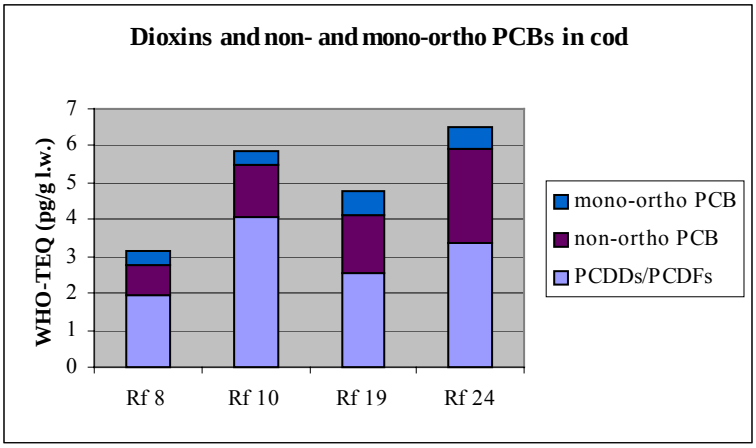


Figure 5.13 Dioxins and non- and mono-ortho PCBs (analysed by HRGC/MS) in four individuals of cod, shown as TEQ for fish. Rf 8-24: ID of the cods analysed (see attachment 6).

Figure 5.13 shows the proportions of the TEQ of the different compound groups within the four individuals. The level of total TEQ was highest in Rf 24, which also had the highest levels of dioxin like PCBs, whereas the highest level of dioxin (PCDD/PCDF) was found in Rf 10. The lipid content in the fish was: Rf 8: 47,0%, Rf 10:35,3%, Rf 19: 35,7%, Rf 24: 40,8%.

In order to see if the CYP1A induction found is a response to the levels of dioxins and PCBs have been plotted against the EROD activity and CYP1A protein content.

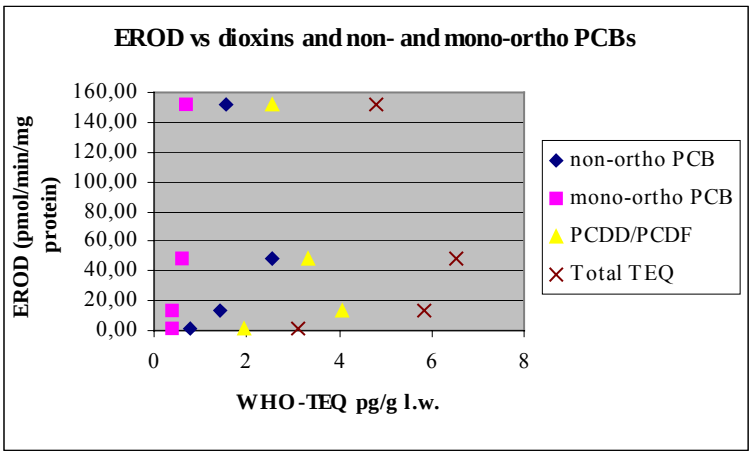


Figure 5.14 Dioxins and non- and mono-ortho PCBs (analysed by HRGC/MS) versus CYP1A activity (measured by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay) in four individual cods.

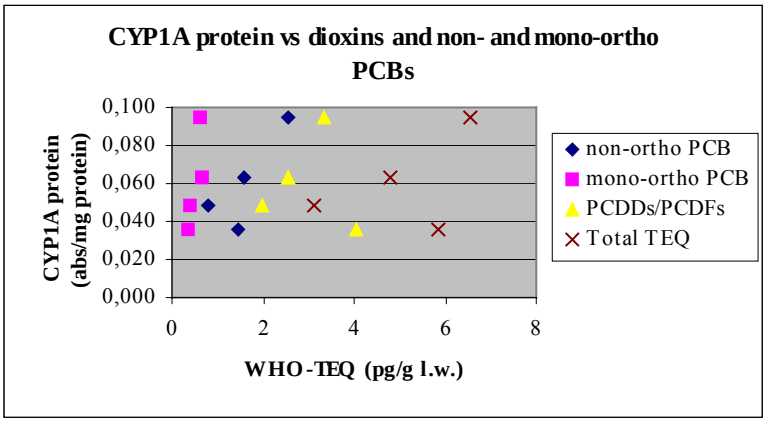


Figure 5.15 Dioxins and non- and mono-ortho PCBs (analysed by HRGC/MS) versus CYP1A protein (measured by ELISA test) in four individual cods.

As shown in Figure 5.14 and Figure 5.15 neither EROD activity nor CYP1A protein content seem to be correlated to either of the compound groups measured or to the total TEQ.

CYP1A protein is however found to be correlated to the concentration of marker PCBs[11] (r^2=0,92; P(2 tail)=0,04), although only one of the congeners included in marker PCBs (CB 118) is among the PCBs believed to induce CYP1A (Figure 5.16). EROD is not found to be correlated to marker PCBs.

[11]The sum of the concentrations of seven congeners: CB 28, 52, 101, 118, 138, 153, 180.

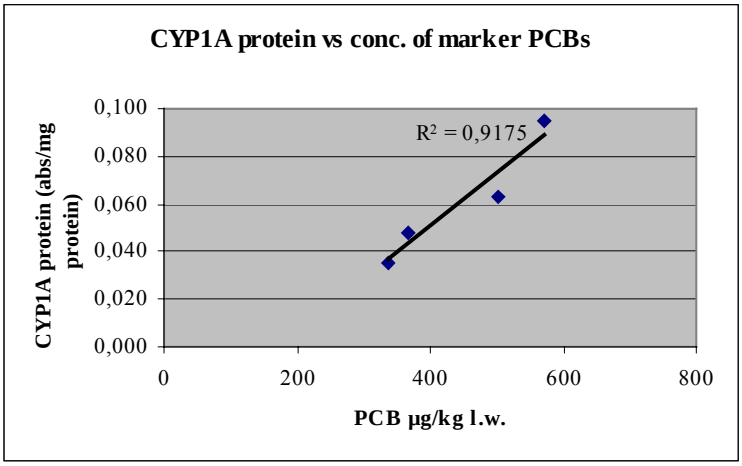


Figure 5.16 Correlation between CYP1A protein (measured by ELISA test) content and concentration of marker PCBs (analysed by HRGC/MS) in four individuals of cod.

6 Discussion

The results show, by analyses of biomarkers, the levels of PAH contamination in fish in the coastal zone of the Faroe Islands, along with the concentrations of PAH and metals in selected invertebrate species.

Discussion of fish results

The plan was to sample fish over three seasons in one year to be able to determine seasonal differences. Because of difficulties with the sampling, only sculpins were sampled in all three seasons, whereas dab and cod only were sampled in spring and summer and the only flounders analysed were those from Kirkjubø in late winter (February). This means that data on seasonal variation are limited for cod and dab and seasonal differences cannot be determined in flounder in this study.

Variation
The results generally showed large variations within the groups. Especially the EROD results, measuring the catalytic activity of the CYP1A system, varied within the groups, but also the data on concentration of pyrene-metabolites and the vitellogenin from July. The large variations are probably, in part, due to the small sample sizes. The sample sizes were between 6 and 12 individuals in each group, split on species, location and season. Moreover, these groups have furthermore been divided into females and males. Larger sample sizes would facilitate statistical analyses. Vandermeulen & Mossman (1995) also found large variation in the groups when analysing for MFO activities in winter flounder (*Pleuronectes americanus*) although they had 30 individuals (males and females) in their groups. They explained the variation as individual physiological differences or differences in inductive response.

Variability in metabolite content in bile can be affected by inter-individual exposure or by differences in the feeding status of the fish (Varanasi & Collier, 1991; Beyer, 1996; Ariese et al., 1997) resulting in differences in bile density. Normalising for bile density by dividing the PAH metabolite concentration found in the bile with the absorbance of the bile at 380 nm, reduced variation for some groups, but in other groups the variation increased. However, many of the fish did not have a sufficient amount of bile to allow the measurement of the absorbance at 380nm and the sample numbers for normalized data thus became very small, making it difficult to compare them to the un-normalized data. It has been shown that errors are introduced by normalizing the data (Aas et al., 2000b), as the coefficient of variation (Std.dev./mean) increases by normalisation with respect to biliverdin concentrations. This is more pronounced for groups with low PAH exposure.

Variation between seasons was seen in CYP1A induction in connection with spawning. The levels of biomarkers, not influenced by spawning, did not vary significantly between seasons.

Influence of spawning
The CYP1A induction was influenced by gonadal development in female sculpin and dab and in male flounder with low levels of EROD activity or CYP1A protein content in groups with increased GSI. Cod did not show elevated GSI in either of the two sampling periods and was thus not influenced by spawning.

An inverse relationship has been shown to occur between GSI and EROD in the periods of developing ovaries in flounder (Khan & Payne, 2002) and in dab (Lange et al., 1998). This was confirmed in this study for dab and flounder and in sculpin with respect to GSI and CYP1A protein content. In April the EROD activity in female dab was only 3,8% of that in males, and 1,6% of the activity in females in July. In male dab the April activity was 87% of that in July. In dab the GSI in males was significantly lower than in females in April. This is, however, normal, as shown by Saborowski at al. (1997). They found that maximum GSI in male dab did not exceed 3,0 at any of the monthly sampling dates during the reproduction cycle.

Lindström-Seppä & Stegeman (1995) found that GSI in pre-spawning flounder was three times higher in females than in males and EROD activity in females was only 3,5% of that in males. This is similar to our results in dab and can be explained by the fact that hormonal factors such as oestradiol suppress CYP1A induction in female liver (Lindström-Seppä & Stegeman, 1995). Our results for male flounder show GSI levels ranging from 2,7 to 25,7 (Figure 3.1 and attachment 6) and an inverse relationship between CYP1A activity and GSI. This indicates that androgens also can suppress CYP1A induction, although previous studies on this have shown unclear results. Some have shown suppression of CYP1A induction by androgens others have not (Andersson & Förlin, 1992; Stegeman & Woodin, 1984). The role of androgens on the CYP1A expression probably differs between species (Andersson & Förlin, 1992).

It can be concluded, that CYP1A induction is suppressed in specimens with increased gonadal development and, therefore, sampling of specimens for measurements of CYP1A induction are should not be performed during the pre-spawning and spawning periods.

The reproductive cycle is divided into four periods: Pre-spawning, spawning, post-spawning and resting[12] (Sabarowski et al., 1997). The sampling in the present study was performed only 1-3 times a year for each species. As we do not have monthly samples it is difficult or impossible to distinguish between different reproductive periods, and the maximum level of GSI cannot be determined with any certainty. An increase in GSI for one species can reflect both the pre-spawning and spawning period.

According to Joensen & Tåning (1969) the spawning period in flounder and sculpin is from winter to early spring, whilst spawning in dab starts in April (peaks in May). This is consistent with the findings of an increased GSI in female sculpin in January, in female dab in April and in male flounder in February. Monthly measurements of GSI in dab from the same area as in this study, were performed in 1996-97 by Dam (2000). The results showed that the spawning season seemed to peak in March (or February) in 1996 and even earlier in 1997 (January - February). Hence the time of spawning can vary between years. Our results show a GSI of approximately 14 in female dab in April. This is at the same level as the peak in GSI shown by Sabarowski et al. (1997) indicating that dab from April in our study were at or near at their peak in GSI and thus in the beginning of their spawning period.

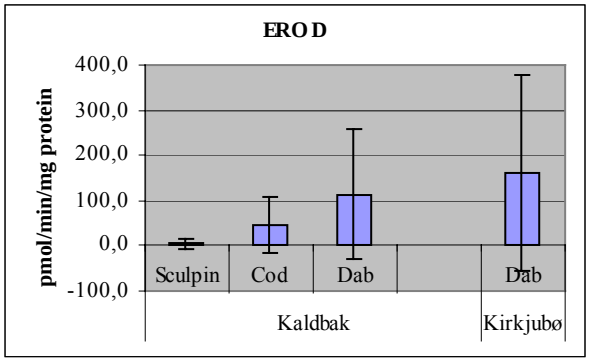
[12] The reproductive cycle in dab can be classified into four periods: Pre-spawning, spawning, post-spawning and resting. The pre-spawning period is characterised by an increase in ovary weight (and thereby in GSI), whereas the spawning period is characteized by a decrease in GSI due to the release of eggs. The beginning of the spawning period is defined as the maximum GSI of females. In the post-spawning period the gonad tissue is resorbed an remains in a state of resting until the pre-spawning period (Sabarowski et al., 1997).

Biomarker levels

The levels of biomarkers, not influenced by gonadal developement, did not vary significantly between seasons and the mean levels of the biomarkers measured, after excluding the individuals with elevated GSI, are summarized in Table 6.1 and Figure 6.1. The flounders measured were all influenced by gonadal development and are not included in the figure and table below.

Table 6.1 The mean values for biomarkers measured, when individuals with elevated GSI (>2) have been excluded. CYP1A activity measured by EROD analysis, CYP1A protein content measured by ELISA test, 1-OH-pyrene concentration measured by HPLC analysis.

		EROD (pmol/min/mg protein)	**CYP1A protein** (abs/ mg protein)	**1-OH-pyrene** (µg/kg)	**Vitellogenin** (ng/ml)
Kaldbak	Sculpin	4,2±11,5	0,14±0,04	18,6±21,2	
	Cod	46,8±63,0	0,07±0,02	11,8±10,2	2541±1983
	Dab	113,5±143,1		20,5±20,4	
Kirkjubø	Dab	159,7±216,1		5,2±5,5	



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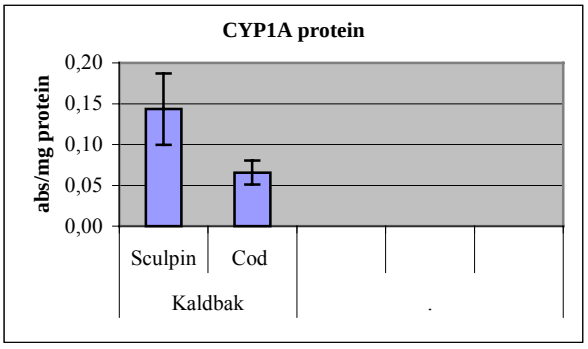

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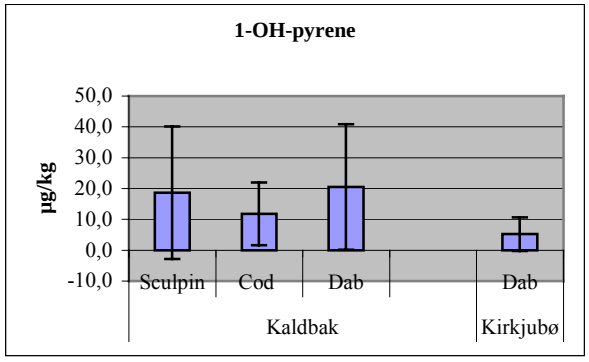

C

Figure 6.1 The mean values for the PAH biomarkers measured, when individuals with elevated GSI (>2) have been excluded. **A**: CYP1A activity (measured by EROD analysis), **B**: CYP1A protein content (measured by ELISA test), **C**: 1-OH-pyrene concentration (measured by HPLC analysis).

CYP1A induction

Dab seems to be the species most sensitive to measurements of CYP1A activity by EROD analysis, followed by cod, whereas the activity in sculpins, generally, was very low. When using the fluorescence plate-reader method, most of the EROD results in sculpins were undetectable. This was also observed by Stephensen et al. (2000), who analysed EROD activity in sculpins on Iceland by the fluorescence plate-reader method. Others have found elevated levels of EROD in sculpin (e.g Ruus et al., 2002, using HPLC detection method). The results of the measurements of CYP1A protein content, on the other hand, were high (relative to the content in cod), indicating that the catalytic activity had been destroyed or suppressed. The loss of catalytic activity could be due to non-optimal freezing, transport and storage conditions (Stephensen et al., 2000) since EROD activity has been shown to be sensitive to such conditions (Förlin & Andersson, 1985). If the lack of catalytic activity is due to the freezing, transport and storage conditions, the sculpins seem to be more sensitive than the other species analysed in this project, as all fish samples were treated the same way and the other species showed higher EROD activity.

Other contaminants such as heavy metals (e.g. cadmium) (Goksøyr et al., 1989; Beyer et al., 1997; Sandvik et al., 1997) or some organochlorines can also inhibit the CYP1A activity. Heavy metals can affect the heme synthesis, resulting in distorted forms of cytochromes P450 with no enzymatic activity. The distorted forms of cytochrome P450 will however still be detected by immunochemical analysis of CYP1A protein (Goksøyr et al., 1989).

Some PCBs have been shown to be able to inhibit the catalytic activity of induced P-450 by acting as an alternate substrate (Gooch et al., 1989). Gooch et al. (1989) found that the EROD activity decreased dramatically when concentrations of the coplanar PCB congener 3,3',4,4' TCB (CB 77) exceeded 2 mg/kg.

Sculpin caught in the same area as in the present study, have been analysed for PCB concentration in liver from 1999 to 2001 in connection with the AMAP[13] project and relatively large concentrations have been found with the sum of seven PCB congeners ranging between 1-3 mg/kg of lipids for the size group corresponding to the present study (Hoydal et al., 2003). Of the congeners analysed in the AMAP study only three are in the group of coplanar PCBs (CB 105,118, and 156). The sum of the concentrations of these three congeners was 0,02 and 0,06 mg/kg w.w. in two pooled samples in 2001 in the size group corresponding to the present study. These levels are much lower than the effect levels reported by Gooch et al., indicating that the low levels of CYP1A activity in sculpin are not due to inhibition by the concentration of PCB in the liver. However, sculpins have not been analysed for PCB in 2002 and CB 77 was not among the congeners analysed in 2001. Further investigation are needed to reveal the relationship between EROD inhibition and PCB in sculpins.

The EROD activity in flounder at reference stations have been reported to be 91$\pm$41 pmol/min/mg protein (Addison & Edwards, 1988) and 39$\pm$19 pmol/min/mg protein (Stegeman et al., 1988) in Langesundfjorden and 4$\pm$1 pmol/min/mg protein (Beyer et al., 1996) in Sørfjorden in Norway. Our results in flounder are comparable to Stegeman et al. (1988) although one should bear in mind that our results are influenced by spawning and hence the exposure is possibly underestimated.

[13] The Arctic Monitoring and Assessment Program

The EROD results in female dab from July, both from Kaldbak and Kirkjubø, exceed these levels. In males the results seem to be comparable. However, EROD has been found to be 3-4 times higher in dab than in flounder when sampled under same conditions (Krüner & Westernhagen, 1999). Taking this into consideration the dab results seem to be comparable to the results of Stegeman et al. (1988). Ruus et al. (2003) reported levels of EROD in dab ranging from 123-529 pmol/min/mg protein. The mean level from Kaldbak in our study is much lower and the mean levels in Kirkjubø are at the lower end of this range. However, variations are large and some of the individual results are at the higher end of or above this range. The earlier reported higher EROD activity in male compared with female dab (Krüner & Westernhagen, 1999; Lange et al., 1999; Goksøyr et al., 1992) was not found in this study.

Ruus et al. (2003) reported EROD concentrations in cod from reference stations in the range 9-95 pmol/min/mg protein. Our mean levels in cod are at the same level or lower, although a few individual values are above that range (see attachment 6). A study of the pollution status of the harbour of Tórshavn included analyses of EROD activity in cod (the coastal stage "reyðfiskur") in June, and found a mean concentration of 68 pmol/min/mg protein (Dam & Danielsen, 2002). Comparing this with the present results from July shows, that the EROD activity in cod from Kaldbak is lower. This was expected, because the harbour of Tórshavn has considerable industrial activity and cod from the harbour of Tórshavn should, therefore, be more exposed to EROD inducers than cod from Kaldbak

No correlation was found between the EROD activity and CYP1A protein content in cod and sculpin. Lack of correlation between these two parameters indicate that the catalytic activity has been destroyed or suppressed, since they measure the content and catalytic activity of the same proteins. As mentioned above, this indicates that the low EROD activity in sculpin is due to catalytically inactive enzymes, which also explains the lack of correlation between the EROD measurements and the CYP1A protein. It is more puzzling that there was no correlation between these parameters in cod, as several individuals had high EROD activities but relative low CYP1A protein content. Goksøyr et al. (1989) found good correlation between EROD activity and ELISA CYP1A protein in dab, but not in flounder and plaice (*Pleuronectes platessa*).

The EROD activity was generally higher in females than in males in the species analysed, except in the spawning season, when the CYP1A induction was inhibited in females, probably due to increased levels of steroid hormones. This is not in accordance with the general finding that males have higher EROD activity than females. For the CYP1A protein contents, on the other hand, the mean values were higher in males than in females in all the groups analysed.

PAH metabolites

1-OH-pyrene was the only metabolite detected in bile of the fish analysed. OH-pyrene is the major biotransformation product of pyrene in teleosts (Beyer, 1996). The pyrene metabolite concentration in sculpin, cod and dab are low compared to data from Norway in 1997-2001 (Ruus et al., 2003) (normalized data). In the study of the pollution status of the harbour of Tórshavn PAH metabolites were also analysed in cod. The results showed a mean concentration of 1-OH- pyrene of 66 µg/kg (ranging between 45-120 µg/kg) (Dam & Danielsen, 2002). As expected, this is at a much higher level than the results of the present study, which had a mean around 11µg/kg in July (ranging between 3,5-33,4 µg/kg).

Only one flounder had a sufficient amount of bile to allow analysis and the concentration of OH-pyrene found was markedly higher than in the other species. Since there was only one measurement it is impossible to say if the OH-pyrene content is higher in flounders in general or only in this individual. The EROD activity was low in this individual, and combined with the findings of a high density of biliverdin in the bile sample, this indicates that the pyrene metabolites do not stem from a recent exposure but are rather a result of concentration of the bile fluid and of PAH metabolites in it. When the fish has been fasting for a long period the bile fluid gets more concentrated and this affects the results of PAH metabolite concentration in the bile (Varanasi & Collier, 1991; Beyer, 1996; Ariese et al., 1997).

DNA adducts in cod

There were not detected any DNA adducts in the liver of the cod analysed. This is in accordance with the low levels of metabolites found in the bile, as the formation of DNA adducts is correlated with concentrations of PAH metabolites in bile (Aas et al., 2000a). In the study of the pollution status of the harbour of Tórshavn, DNA adducts were found in the range 2,7-31 nmol/mol, with a mean level of 15 nmol/mol (Dam & Danielsen, 2002). DNA adducts have been shown to reflect the cumulative uptake of PAH in fish (Meador et al., 1995; French et al., 1996) and as no DNA adducts were detected in this study, it indicates that the cod analysed has not been exposed to heavy PAHs to a great extent.

EROD versus pyrene

No correlation between EROD activity and OH-pyrene content in bile was found in any of the species analysed. Correlation between the two parameters would indicate recent contamination of PAH (Ruus et al., 2003). Aas et al. (2000a) found significant correlation between EROD activity and PAH metabolites in bile in cod exposed to crude oil. Cod exposed to 1 ppm crude oil in water for 30 days, showed a rapid increase in PAH metabolites in bile, measured by fixed wavelength fluorescence analysis, during the first 3 days, followed by a slower increase until day 30. Significant difference between control and exposed groups was seen after 12 hours of exposure. EROD activity for the same groups showed the same pattern of a rapid increase during the first 3 days followed by a slower increase until day 30. After one week depuration in clean water the PAH metabolite content was much reduced, although still significantly higher than in the control group, whereas EROD had only decreased by 50% (Aas et al., 2000a).

The lack of correlation between CYP1A activity and PAH metabolites in bile, can be due to other inducing agents, since EROD activity also can be induced by other planar molecules such as dioxins and planar PCBs (non- and mono-ortho PCB) in addition to PAH. Also the time elapsed since the exposure can be an important factor explaining the lack of correlation, since the concentration of PAH metabolites in bile can decrease rapidly due to evacuation of the bile from the gall bladder in connection with digestion of food (Aas et al., 2000a; Varanasi et al., 1989) and PAH metabolites in gall probably have a shorter half life than EROD (when dealing with single exposure) (Aas et al., 2000a). On the other hand, chronic exposure to CYP1A inducers, probably can lead to normal EROD levels, due to adaption (Bello et al., 2001)

PCB/dioxin in cod

Analysis of the livers of four individual cods for dioxins and planar PCBs - expected to induce CYP1A (non- and mono-ortho PCBs) - did not show any correlation with EROD activity, or with

CYP1A protein content. Only the concentration of the marker PCBs[14], which are not expected to induce CYP1A showed correlation with CYP1A protein content. This correlation may be explained, by a common source of both planar and non-planar PCBs, where the metabolic active compounds have been metabolized and excreted, whilst the more persistent congeners (such as CB 153) are retained in the lipids of the organism and give a cumulated picture of the exposure history. However since there are only four samples, the statistical basis is weak and it cannot be ruled out that a correlation would have been found if the exposure of dioxins and planar PCBs was higher.
A fact that makes the matter more complicated is that certain PCBs also can inhibit the catalytic activity of CYP1A (Gooch et al., 1989), but this would however not influence the measurement of CYP1A protein concentration.

Vitellogenin
Vitellogenin (Vtg) levels were only measured in cod, because this was the only species, where antibodies were available. The vitellogenin concentrations did not differ significantly between sexes or seasons. This should be expected, as the fish are believed to be immature and no gonadal maturation should be going on in the females. The vitellogenin levels are however very high. Hylland and Haux (1997) analysed vitellogenin in cod exposed to sewage effluents and seawater. The vitellogenin in their unexposed control group did not exceed 100 ng/ml, whereas the mean result of the present study is 2430 ng/ml, with individual concentrations ranging from 800 ng/ml to almost 9000 ng/ml. In Apr.-May the mean level was 1846 ng/ml and in July 2917 ng/ml. Mean levels of vitellogenin in cod from the harbour of Tórshavn was 4948 ng/ml (Dam & Danielsen, 2002). This is much higher than the results from Kaldbak, and indicates, as was expected, that cod from Kaldbak has been exposed to xenoestrogens to a much lesser extent as the cod from the harbor of Tórshavn. Further investigations are needed to explain why the level of vitellogenin in juvenile cod from the Faroese area have been found to be at a much higher level than in other areas.

Comparison of locations
Dab was analysed from both Kaldbak and Kirkjubø in July making it possible to compare locations. The results from the two locations show that the OH-pyrene concentrations in dab from July were higher in Kaldbak than in Kirkjubø. For EROD activity the mean values were higher in Kirkjubø than in Kaldbak. This is in accordance with the observed lack of correlation between EROD activity and level of OH-pyrene and indicates that the PAH contamination that created the pyrene metabolites is not due to recent exposure, and EROD activity may have been induced by other contaminants, for instance PCBs.

Discussion of invertebrate results

PAH
Generally the seasonal differences in PAH concentration in the different species showed the same pattern within the stations, but varied between the stations.

For all the species the seasonal pattern seemed to be that the highest PAH content was found in winter (Dec.-Feb.) with lower levels in spring (Mar.-May) and lowest levels in summer (June-Aug.). This pattern was seen for all the stations except in Trongisvágur, where the highest content

[14] The sum of the concentrations of seven congeners: CB 28, 52, 101, 118, 138, 153, 180

was found in May, and in Kaldbak where the content in blue mussel was at the same level all the seasons analysed.

The seasonal patterns of the PAH concentrations did not seem to be related to the lipid content or water content and the seasonal differences in accumulation mainly seem to be related to exposure at the location, and not to physiological differences in the organisms. The results of the PAH analysis in invertebrates is summerized in the table below:

Mean of sum PAH in invertebrates in µg/kg v.v. at the different locations

	Hvannasund	**Svínáir**	**Kaldbak**	**Kirkjubøur/ Velbastaður**	**Trongisvágur**
Littorina	**23,7**±22,3	**10,73**	na	na	na
Nucella	**19,7**±13,3	**2,58**	na	na	na
Patella	**9,9**±8,2	**4,03**±3,9	na	**0,63**±1,10	**68,7**±50,0
Mytilus	**270,2**±114,5	**54,61**±50,7	**100,4**±49,07	nf	**709,0**±541,0
Modiolus	nf	nf	nf	**10,42**±10,69	nf

nf: Species not found at the location
na: not analysed

When looking at PAH, Trongisvágur seem to be the most polluted area followed by Hvannasund. The differences in PAH pollution, probably, reflect the human activities going on in and around the fjord and how sheltered the location is. Locations more exposed to surfe should have a faster removal of compounds, due to enhanced dilution and evaporation.

The highest PAH accumulation was found in blue mussels. Horse mussels and the snails had lower levels. Mussels have been shown to accumulate KPAHs to a greater extent than snails (Næs et al., 1995; 1998; Knutzen & Sortland, 1982) and should be better indicators of PAH pollution. Horse mussels have been shown to accumulate a larger fraction of the KPAHs found in the ambient environment than the blue mussels (Næs et al., 1995; Næs et al., 1998). In this study KPAHs were not found in horse mussels, but in blue mussels at all stations and in periwinkles in Hvannasund and in limpets in Hvannasund and Trongisvágur. The results indicate that Kirkjubøur is a relatively "clean" station, not contaminated by heavy PAHs. Horse mussels and blue mussels were not sampled at the same stations, and their relative uptake cannot be directly compared in this study. The accumulated fraction of PAHs being KPAHs in limpets and periwinkles was lower than the corresponding fraction in blue mussels, which is in accordance with the results of Næs et al. (1995) who showed that limpets and periwinkles accumulate a relatively larger fraction of the low molecular PAHs than the high molecular PAHs. The difference in habitat preference has been shown to be a stronger predictor than the feeding mode in the uptake of PAHs (Næs et al., 1998). In the present study blue mussels had a much higher PAH accumulation than snails, although all live in the littoral zone. Blue mussels from Hvannasund were, however, found partly buried in soft sediment and this could probably lead to a higher uptake from the sediment.

PAH and metals have previously been analysed in blue mussels from Svínáir and limpets from Kirkjubø in 1996-97 (Dam, 2000). The total PAH content in blue mussel was found to be 3,8 µg/kg w.w. in June and 22,3 µg/kg w.w. in Desember (Dam, 2000). These results show as in the present study, that the levels are higher in winter than in summer, but the levels in 1996 were much lower than in the present study, in which the mean levels were 6,8 µg/kg w.w. in July and 117,5 µg/kg w.w. in January. One explanation of this could be the lower fat content in the specimens from 1996-97. In 1996-97 the fat content was 2 % of wet weight of the soft parts in September and less than

1% in June and Desember ’96 and March ’97 (Dam, 2000). The lowest fat content was, however, found in Desember ’96 (aproximately 0,1-0,2 %) when the highest PAH content was found. In the present study the fat content in blue mussels from Svínáir was 2,7% both in January and July and 2% in March. However, since the PAH results from the present study not seemed to be related to the fat content, the difference can probably rather be explained by an enhanced industrial activity in or around the fjord (e.g. in connection with sea-farming) during the last years.

In limpets from 1996 the total PAH level was found in the range 0,6-5,7 µg/kg w.w. with the highest level in June and the lowest in March (Dam, 2000). In the present study limpets from Kirkjubøur were not analysed but from Velbastaður which is located 4 km to the northwest along the coast from Kirkjubøur (see Map 1 on page 32). The mean total PAH content in limpets from Velbastaður was 1,9 µg/kg w.w. in Desember 2001, whereas no PAHs were detected in March and July. From these two analyses the mussel results indicate an increase in PAH pollution whereas the limpet results do not. The disagreement in the results could be due to local variations or differences in sensitivity between the species.

Metals

Although a large number of samples have been analysed representing different species, locations and seasons, it is difficult to pick out the place with highest exposure of metals and the most suitable species to be used as test organism in monitoring activities, partly, because all species have not been analysed at all stations all seasons, but also because the relative accumulation compared between species within the stations is not always the same.

The results show, that for the metals copper, zinc and partly for mercury (in Hvannasund and Svínáir) the accumulation ratio between species seems to be similar for the different stations. For barium, cadmium (in Hvannasund and Svínáir), chromium and lead, on the other hand, the accumulation ratio compared between species seems to differ from station to station.

The levels of cadmium were highest in limpets and horse mussels fromVelbastaður and Kirkjubøur and in dogwhelks from Hvannasund. In 1996 cadmium levels were found to be much higher in limpets from Velbastað and Kirkjubø (7-10 mg/kg w.w.) than in limpets from Kaldbak (around 2 mg/kg w.w.) whereas the copper content was approximately two times as high in limpets from Kaldbak (3,5 mg/kg w.w.) as in Velbastað and Kirkjubø (1-1,5 mg/kg w.w.) (Dam, 2000). Limpets from Kaldbak were not analysed in the present study, but in limpets from Velbastaður the mean level of cadmium was 5,12 mg/kg w.w (31,5 mg/kg d.w.) and the mean level of copper was 1,1 mg/kg w.w (7,1 mg/kg d.w.) which are at the same level as in 1996, although somewhat lower.

There are large differences between the different molluscan species with respect to the accumulation of metals. Dogwhelks and periwinkles had a much higher copper content than the other species analysed. Also zinc was found in highest concentrations in dogwhelks. In 1997 dogwhelks and limpets from “Gamlarætt”, a location between Kirkjubøur and Velbastaður, were analysed for cadmium and copper content (Dam, 2000). The results showed that the dogwhelks accumulated around 6 times as much cadmium as limpets (157,8 and 24,5 mg/kg d.w. respectively), and 19 times as much copper (265,0 and 14,2 mg/kg d.w. respectively) when the results were presentent on a dry weight basis. The levels in limpets from Velbastaður in the present study show higher cadmium levels and lower copper levels than in “Gamlarætt” in 1997 (see above). Dogwhelks from Velbastaður or Kirkjubø were not analysed in the present study, but the different

accumulation in these two species found in the study from 1996 (Dam, 2000) is confirmed, as the dry weight results of dogwhelks and limpets from Hvannasund showed that the copper content in dogwhelks was 13 times the content in limpets, and the cadmium content in dogwhelks was around 5 times the content in limpets.

The cadmium content in blue mussels was low in the present study (0,18 mg/kg w.w. in blue mussels from Svínáir) and the copper content varied between 1,42 and 4,39 in blue mussels for all the stations (mean copper level in Svínár 1,68 mg/kg w.w.). The cadmium and copper levels in blue mussel from the study from 1996 were at the same level as in the present study, although the cadmium results were a little higher (cadmium: 0,22-0,32 mg/kg w.w. and copper: 1,6-3,2 mg/kg w.w.) (Dam, 2000). The copper content in blue mussel from several locations around the Faroe Island were compared in Feb-March 1996. In that study the highest copper content was found in blue mussels from Svínáir (3 mg/kg w.w.) and the lowest level was found in Trongisvágur (approximately 1,5 mg/kg w.w) (Dam, 2000). This is in accordance with the results of the present study as the mean copper content in blue mussel from Svínáir was 2,8 mg/kg w.w and from Trongisvágur 1,7 mg/kg w.w.

The concentration of lead was highest in horse mussels from Kirkjubø. Since the concentration of lead in limpets from the near by site Velbastaður was much lower it appears that horse mussels may accumulate lead to a greater extent than limpets. The analyses of lead in the study from 1996 showed similar levels as in the present study.

The mercury levels in limpets were generally low in the present study and at the same level as in blue mussels, except in Trongisvágur where the blue mussels had higher concentration than limpets. The mercury content was at the same level as found in the study from 1996 (Dam, 2000).

Although the feeding mode is not the important factor when regarding PAH uptake (Næs et al., 1998) it could possibly be relevant for the metal uptake, since these are mainly found as ionic compounds and thus are not free to diffuse over biological surfaces. Given that the mussels are filter-feeders, limpets and periwinkles are grazers, and dogwhelks are carnivores feeding on other invertebrates, the uptake of cadmium, mercury, and zinc, and possibly also the uptake of lead, could indicate connection to different feeding modes since the uptake of these metals are similar for limpets and periwinkles but different for dogwhelks and mussels. The accumulation of zinc in the two mussel species are quite similar relative to the accumulation in limpets, although the habitats are different and they are found at different depths. The accumulation of zinc in the species which feed by grazing (herbivores) is also similar, indicating that the feeding mode is significant for the uptake. The relative high concentration of zinc in dogwhelks can be due to bioaccumulation effects as it feeds on other invertebrates.

The pattern for mercury uptake seems to be the same as for zinc, except that the uptake in mussels is at the same level as in periwinkles and limpets. However in Trongisvágur the uptake in blue mussels relative to limpets shows the same ratio as for zinc.

Cadmium concentration in dogwhelks is high in Hvannasund (41 mg/kg d.w.) in comparison to Svínáir (5,6 mg/kg d.w.), however these concentrations are still low compared to dogwhelks in "Gamlarætt" with 157,8 mg/kg d.w.) (Dam, 2000). The diet of dogwhelks can possibly be very variable, dependent on available prey. The metal exposure from ingestion of food will thus vary to the same extent. The cadmium accumulation in blue mussels is, however, very low, and in horse

mussels high. Feeding mode seems, therefore, not to be a determining factor for the uptake of cadmium. The same seems to apply for barium, chromium and copper.

The organism accumulating cadmium to the greatest extent seems to be dogwhelks and probably horse mussels, whereas blue mussels accumulate very little. Periwinkles seem to be accumulate chromium to high concentrations and copper is also accumulated to high levels by periwinkles as well as dogwhelks. For mercury the accumulation is highest in dogwhelks and for lead horse mussels and blue mussels have the highest accumulation. The accumulation of zinc is highest in dogwhelks followed by blue mussels and horse mussels. These results can be taken into account when selecting species for analysis for metal pollution. However, to what extent the accumulation in the different species reflect the metal levels in the ambient environment is not known from this study.

According to Molvær et al. (1997) the environmental condition of fjords and coastal waters can be classified by the concentration of pollutants in organisms living in the location in question. The locations are classified into five classes, from insignificantly-slightly polluted (class I) to very strongly polluted (classV). Table 6.2 shows the result of the classification of the locations[15] from the present study by the levels of metals and PAHs in blue mussels.

Table 6.2 Results of classification of the environmental condition of locations, by levels of metals and PAHs in blue mussels according to Molvær et al. (1997).

Blue mussel:		Cd	Cr	Cu	Hg	Pb	Zn	sum PAH	sum KPAH	B(a)P
Hvannasund	Jan.	I	II	II	I	II	II	III	III	III
	Apr.	I	I	II	II	II	III	II	II	II
Svínáir	Jan.	I	I	II	I	I	II	II	I	I
	Mar.	I	I	II	I	I	II	I	I	I
	July	I	I	I	I	I	II	I	I	I
Kaldbak	Jan.	I	I	II	I	I	II	II	I	I
	Mar.	I	I	II	I	I	I	II	I	I
	July	I	I	I	I	I	II	II	I	I
Trongisvágur	Jan.	I	II	II	II	II	III	III	IV	III
	May	I	I	I	II	II	III	III	II	II
	July	I	I	I	II	II	III	III	I	I

Class I: Insignificantly-slightly polluted
Class II: Moderatly polluted
Class III: Markedly polluted
Class IV: Strongly polluted
Class V: Very strongly polluted

From the table it is shown that the Hvannasund and Trongisvágur are moderately to markedly polluted with regard to PAH, whereas Svínáir and Kaldbak are slightly to moderately polluted. The same is found for the metals although some of them are at a class I level in Hvannasund and Trongisvágur also. Svínáir and Kaldbak are only insignificantly to slightly polluted by metals except for copper and zinc, by which they are moderately polluted. Both zinc and copper are regularly analysed in connection with monitoring of pollution from fish-farming, since zinc is added to the feed of farmed salmon and trout, and the net-cages surrounding the fish are treated with copper to prevent fouling. Studies have shown that the level of copper and zinc is elevated in the

[15] Only locations where blue mussels were found.

close vicinity of fish-farms (Olsen, 2002). As a conclusion Hvannasund and Trongisvágur are moderately to markedly polluted, whereas Kaldbak and Svínáir are slightly to moderatly polluted with regard to metals and PAHs.

7 Conclusion

The present investigation shows the level of several biomarkers in different fish species and conclusions with respect to the suitability of the different species as test organisms for the different biomarker analyses can also be drawn from the results.

The levels of CYP1A induction and the content of 1-OH-pyrene in bile, seem to be at levels comparable to or lower than the levels reported from clean reference sites in Norway, although there are large variations in the results with some high individual levels. The measurements of DNA adducts in cod liver were all below detection limit. The vitellogenin levels in cod were relative high.

According to this study, the species best suited for biomarker analyses is dab (*Limanda limanda*) or cod (*Gadus morhua*). Dab seems to be the species most sensitive to measurements of CYP1A activity of the species analysed. The MFO measurements in dab are however, highly influenced by spawning and differ between males and females. If dab is to be used as indicator organism in biomarker analyses, sampling should be performed in the non-spawning (resting) period (July or Aug.-Sept.).
Cod seems to be somewhat less sensitive to CYP1A induction showing lower levels than dab, but are immature when living near the coast and thus not influenced by seasonal differences in the reproduction cycle or difference between the sexes. It also speaks in favour of choosing cod that in this study all the biomarker analyses have been performed on cod, whereas CYP1A protein content and vitellogenin were not analysed in dab. No vitellogenin and CYP1A protein data are thus available for dab near the Faroese coast at present.

Sculpins did not respond well to the analysis of CYP1A activity, probably due to an enhanced sensibility to protein denaturation during sampling, freezing and storage and are thus not among the best suited indicator organisms for PAH exposure, although they showed good response to the other parameters analysed.

The results showed large variation and a larger number of samples and individual analyses are required in order to determine the baseline levels of the biomarkers, which is a necessary prerequisit for detection of small scale changes in biomarker levels in future assessments.

It is, therefore, proposed that one of the species responding well to the biomarkers analysed in the present study (cod or dab), is subjected to further investigations with a larger number of individuals analysed and with efforts directed at minimizing the influence of parameters that affect variability. One way to minimize the effects from influencing parameters, is to collect samples once in a year in a season not influenced by gonadal development.

The different analyses of the fish did not seem to be correlated, showing the importance of analysing several biomarkers to get an adequate picture of the PAH exposure. It is particularly important to support the analyses of CYP1A activity with analyses of CYP1A protein content, since the results of CYP1A activity can be underestimated due to protein denaturation or inhibition of the catalytic activity. Also the analyses of DNA adducts and the results of PAH metabolites in bile complement each other by giving information of long- and short term exposure to PAH.

The analyses of ambient levels of PAH and selected metals in invertebrates provide a suite of background data to which results from future chemical analyses of these parameters can be compared.

The levels of PAH vary between the stations, probably due to local contamination. With regard to PAH blue mussel seem to be the species best suited as indicator organism, since it accumulates PAH to the greatest extent. For the metals it is not possible to pick one species which is best suited. Different species accumulate different metals to various extents depending on local factors, where availability of prey may be among the important ones. Thus it is not feasible to base the chemical analyses in future investigations on one species only, but rather a selection of species. The actual species chosen for a coastal zone chemical pollution assessment will depend on the characteristics of the location in question.

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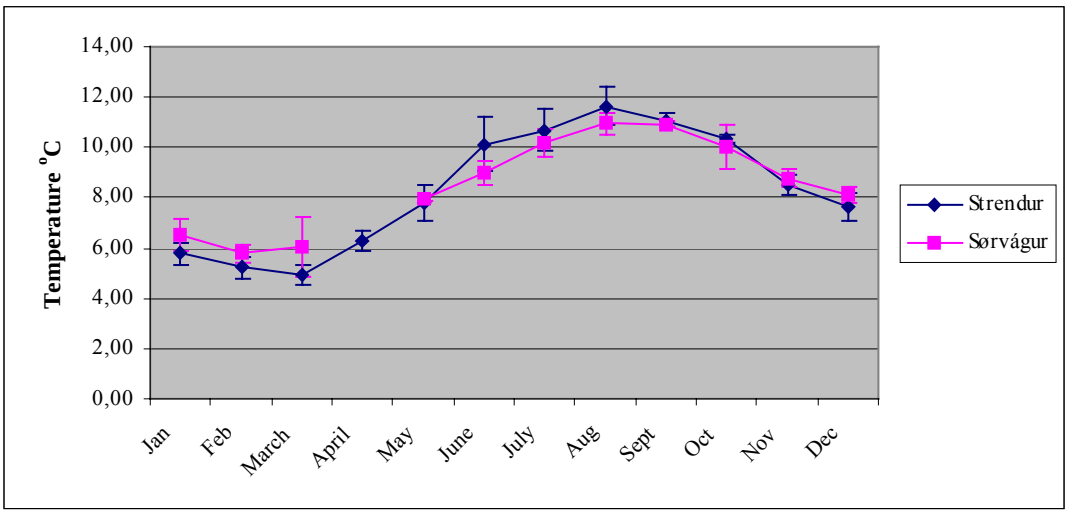
List of attachments

Attachment 1:

Sea-temperatures:
The figure is showing the sea temperature at to locations on the Faroe Islands (Strendur and Sørvágur) during 2002, based on measurements performed by The Faroese Office of Public works.



Mean sea-temparature at "Strendur" and "Sørvágur" in 2002 (data obtained from The Faroese Office of Public Works).

Strendur	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
mean	**5,8**	**5,2**	**4,9**	**6,3**	**7,8**	**10,1**	**10,7**	**11,6**	**11,1**	**10,3**	**8,5**	**7,7**
std.dev.	*0,42*	*0,42*	*0,39*	*0,39*	*0,71*	*1,06*	*0,84*	*0,73*	*0,26*	*0,19*	*0,41*	*0,56*

Sørvágur	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Mean	**6,5**	**5,8**	**6,1**		**8,0**	**9,0**	**10,2**	**11,0**	**10,9**	**10,0**	**8,8**	**8,1**
Std.dev.	*0,64*	*0,37*	*1,18*		*0,11*	*0,51*	*0,56*	*0,43*	*0,15*	*0,88*	*0,35*	*0,30*

Difference*	-0,73	-0,56	-1,14		-0,22	1,14	0,46	0,69	0,14	0,34	-0,29	-0,44

*Strendur ÷ Sørvágur

Attachment 2:

Overview of reagents and solutions used in the biomarker analyses:

Protein:

0,1 M Tris buffer: 12,70 g Trizma base (Sigma T-1503) $C_4H_{11}NO_3$, FW 121,1 mol/g
2,36 g Trizma Hydrochloride (Sigma T-3253) $C_4H_{11}NO_3 \cdot HCl$, FW 157,6 mol/g
in dH_2O to 1 liter, pH 7,96 at 5ºC.

Reagent A: alkaline copper tartrate solution (BioRad protein assay reagent A, catalog 500-0113)
Reagent B: diluted Folin reagent (BioRad protein assay reagent B, catalog 500-0114)

Bovine gamma globulin protein standard (BioRad)

EROD:

0,1 M potassium-phosphate buffer: 8,71g K_2HPO_4
30 ml 0,1M KH_2PO4
in dH_2O to 500 ml, pH 7,91-8,03.

50 nM NADPH (Sigma N-1630) in buffer

0,2 mM 7-ethoxyresorufin (Sigma E-3763) in DMSO

10 µM resorufin (Sigma R-3257) in DMSO (for standards solutions)

Vitellogenin:

Competitive ELISA:

0,05 M Carbonate/bicarbonate coatingbuffer: 1 coating buffer capsule (Fluka, cat.no.21851) in dH_2O

TTBS with Tween-20, pH 8,7: 17,4g Trizma base (Sigma T-1503)
8,8g Trizma Hydrochloride (Sigma T-3253)
292,4g NaCl (Sigma S-7653)
in 10 liter dH_2O + 5ml Tween-20 (Polyoxyethylene sorbitan, Sigma P-1379)

Blocking solution: 1% BSA (Bovine serum albumin, Sigma A-7906) in TTBS (without Tween-20)

Stop solution: 1M H_2SO_4

Primary antibody: Rabbit anti-cod Vtg, Polyclonal antibody, CS-1 (Biosense) diluted 1:100 000 in 0,1% TTBS/BSA.

Secondary antibody: Goat anti-rabbit IgG Horseraddish Peroxidase Conjugate (GAR-HRP, AmDEX AX01-0301X) diluted 1:15 000 in 0,1% TTBS/BSA.

Developing solution: TMB plus substrate (Kem-en-Tech 4390A)

Capture ELISA:

0,05 M Carbonate/bicarbonate coating buffer: 1 coating buffer capsule (Fluka, cat.no.21851) in 100 ml dH_2O

PBS (Phosphate buffered saline), pH 7,2: 8,90g NaHPO4
2,72g NaHPO4 (3,13g · H_2O)
87,66g NaCl
in in 10 liter dH_2O

Blocking solution: 2% BSA in PBS

Primary antibodies: Rabbit anti-cod Vtg, Polyclonal antibody, CS-1 (Biosense)
Rabbit anti-wolffish Vtg, Polyclonal antibody, CS-3 (Biosense)
Rabbit anti-Arctic char Vtg, Polyclonal antibody, PO-1 (Biosense)
Rabbit anti-sea bream Vtg, Polyclonal antibody, PO-2 (Biosense)
diluted 1:1000 – 1:128 000 in 1% BSA in PBS.

CYP1A protein:

0,05 M Carbonate/bicarbonate coatingbuffer: 1 coating buffer capsule (Fluka, cat.no.21851) in dH_2O

TTBS: 20 mM Tris-buffer, pH 8.5,
with 0.5 M NaCl (292 g), to 10 L in dH_2O;
with 5 mL Tween- 20

Blocking solution: 1% BSA in TTBS (without Tween-20)

Antibody-buffer: TTBS med 0.1% BSA

Stop solution: 3 N HCl eller 3 N H_2SO_4

Primary antibody: anti-fisk CYP1A antibody (CP226) (Biosense C02401201).

Secondary antibody: Goat anti-rabbit IgG Horseraddish Peroxidase Conjugate (GAR-HRP, AmDEX AX01-0301X) diluted 1:15 000 in 0,1% TTBS/BSA.

Developing solution: TMB plus substrate (Kem-en-Tech 4390A).

Attachment 3A:

Protein results:

Sample ID	Mean, µg/mL	Std Dev	CV	Dil.Factor	Protein, mg/ml
U1	599,2	7.342	1.225	5	2996
U2	878,2	5.507	0.627	5	4391
U3	823,3	30.97	3.762	5	4116,5
U4	834,5	13.07	1.566	5	4172,5
U5 re.an.	805,5	9.534	1.184	10	8055
U6	712,5	33.07	4.642	5	3562,5
U7	899,4	11.71	1.301	5	4497
U8 re.an.	644,4	22.22	3.448	10	6444
U9	641,6	9.116	1.421	5	3208
U10	916,7	42.84	4.673	5	4583,5
U11	614,8	*****	*****	5	3074
U12	503,1	2.596	0.516	5	2515,5
U13	549	16.49	3.003	5	2745
U14	827,6	29.37	3.549	5	4138
U15	606,1	9.828	1.621	5	3030,5
U16	617,4	25.70	4.162	5	3087
U17	1171	7.930	0.677	5	5855
U18	976,5	30.31	3.104	5	4882,5
U19	969,5	31.40	3.239	5	4847,5
U20 re.an.	605,5	27.73	4.580	10	6055
U21 re.an.	744,8	14.36	1.929	10	7448
U22	873,5	9.828	1.125	5	4367,5
U23 re.an.	610,5	17.28	2.830	10	6105
U24	806,9	51.07	6.329	5	4034,5
ref.G.m.	577,6	13.32	2.306	5	2888
ref.L.l.	786,1	11.90	1.513	5	3930,5

Sample ID	Mean, µg/mL	Std Dev	CV	Dil.Factor	Protein, mg/ml
SK1	352,8	13.82	3.916	7,000	2469,6
SK2	611,7	19.60	3.204	7,000	4281,9
SK3	506,2	24.71	4.881	7,000	3543,4
SK4	513,3	19.24	3.748	7,000	3593,1
SK5	626,7	16.17	2.581	7,000	4386,9
SK6	481,8	9.401	1.951	7,000	3372,6
RF1	750,8	26.77	3.565	7,000	5255,6
RF2 re.an.	318,6	2.715	0.852	7	2230,2
RF3	609,9	20.13	3.301	7,000	4269,3
RF4	483,1	23.63	4.892	7,000	3381,7
RF5	486,7	13.65	2.804	7,000	3406,9
RF6	598,4	4.062	0.679	7,000	4188,8
RF7	361,7	13.12	3.626	7,000	2531,9
RF8	458,3	8.124	1.772	7,000	3208,1
RF9	380,3	13.12	3.449	7,000	2662,1
RF10	219	1.535	0.701	7,000	1533
RF11	665,7	17.30	2.599	7,000	4659,9
RF12	531,9	14.65	2.753	7,000	3723,3
RF13	421,1	8.124	1.929	7,000	2947,7
RF14	482,3	12.57	2.606	7,000	3376,1
RF15	540,8	13.65	2.523	7,000	3785,6
RF16	483,1	13.82	2.860	7,000	3381,7
RF17	554,9	14.81	2.668	7,000	3884,3
RF18	648	18.61	2.872	7,000	4536
ref.G.m.	576,2	18.61	3.230	7,000	4033,4
ref.L.l.	767,7	35.97	4.686	7,000	5373,9

Sample ID	Mean, µg/mL	Std Dev	CV	Dil.Factor	Protein, mg/ml
RF19	436,3	4.147	0.951	7	3054,1
RF20	342,2	11.30	3.303	7	2395,4
RF21	849,8	16.59	1.952	7	5948,6
RF22	634,4	18.08	2.849	7	4440,8
RF23	378,3	24.43	6.458	7	2648,1
RF24	377	13.44	3.564	7	2639
S7	517,7	19.07	3.683	7	3623,9
S8	508,7	11.52	2.264	7	3560,9
S9	847,1	24.63	2.908	7	5929,7
S10	546,7	14.11	2.580	7	3826,9
S11	541,2	19.20	3.547	7	3788,4
S12	611,8	7.678	1.255	7	4282,6
S13	390,6	1.920	0.491	7	2734,2
S16	614,5	11.52	1.874	7	4301,5
S31	643,5	22.11	3.436	7	4504,5
S32	760,2	31.46	4.139	7	5321,4
S33	659,8	29.03	4.400	7	4618,6
ref.G.m.	553,4	9.598	1.734	7	3873,8
ref.L.l.	762	7.837	1.028	7	5334

Sample ID	Mean, µg/mL	Std Dev	CV	Dil.Factor	Protein, mg/ml
U25	646,1	15.44	2.390	7	4522,7
U26	484,4	1.619	0.334	7	3390,8
U27	854,5	19.89	2.328	7	5981,5
U28	875	18.39	2.101	7	6125
S34	584,8	9.914	1.695	7	4093,6
S35	673,2	14.84	2.204	7	4712,4
S36	857,3	9.847	1.149	7	6001,1
S37	765,7	23.96	3.129	7	5359,9
S38	803,1	22.84	2.844	7	5621,7
S39	638,6	28.78	4.507	7	4470,2
S41	618	5.837	0.944	7	4326
S42	479,7	22.95	4.785	7	3357,9
S43	325,5	12.85	3.948	7	2278,5
S44	710,5	18.67	2.628	7	4973,5
S45	412,4	7.419	1.799	7	2886,8
S46	382,5	1.619	0.423	7	2677,5
S47	666,6	1.619	0.243	7	4666,2
S48	519,9	11.33	2.180	7	3639,3
S49	587,2	5.837	0.994	7	4110,4
S50	530,2	12.22	2.305	7	3711,4
S51	666,6	16.43	2.465	7	4666,2
S52	275,9	9.847	3.569	7	1931,3
S53	506,8	5.837	1.152	7	3547,6
ref.G.m.	579,7	21.42	3.694	7	4057,9
ref.L.l.	787,2	11.67	1.483	7	5510,4

Attachment 3B:

EROD results:

kode	EROD (pmol/min/mg protein)	CV(%)
U1	-1,56	-16,29
U2	-1,06	-27,87
U3	-0,74	-74,50
U4	-1,11	-23,21
U5	-0,74	-15,78
U6	6,50	43,16
U7	-1,30	-9,22
U8	-0,81	-13,41
U9	-1,58	-19,38
U10	-1,04	-24,58
U11	-2,06	-23,66
U12	-2,26	-26,71
U13	-0,35	-24,69
U14	-0,17	-36,08
U15	0,51	30,27
U16	-0,18	-81,98
U17	4,30	23,00
U18	-0,16	-24,49
U19	-0,12	-84,69
U20	-0,37	-29,64
U21	-0,05	-78,42
U22	1,15	37,02
U23	-0,10	-77,46
U24	-0,17	-68,16
U25	7,96	64,36
U26	-0,04	-587,42
U27	0,17	43,24
U28	-0,07	-61,97
SK1	1,16	46,00
SK2	14,78	24,36
SK3	37,30	37,73
SK4	16,51	10,73
SK5	37,34	38,72
SK6	35,43	13,26

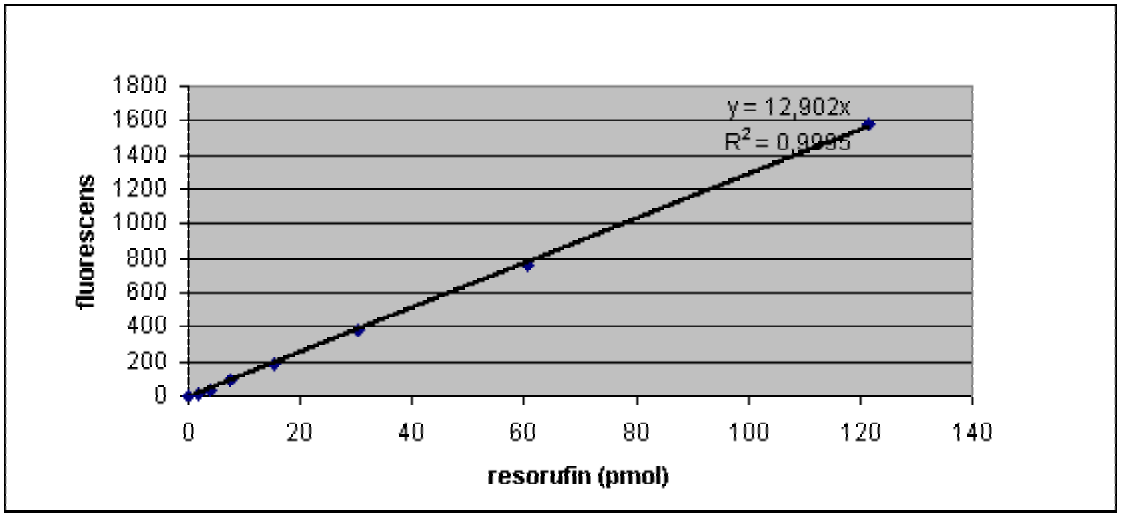
kode	EROD (pmol/min/mg protein)	CV(%)
S7	3,22	21,66
S8	3,05	11,85
S9	1,49	19,07
S10	43,10	26,37
reS11	87,11	24,64
reS12	95,63	29,93
S13	105,95	11,04
S16	5,09	36,32
S31	3,81	36,43
S32	19,72	32,68
reS33	222,75	3,81
S34	64,85	27,62
reS35	420,77	12,64
S36	21,14	46,63
S37	41,54	30,88
S38	9,29	37,11
reS39	113,53	46,63
reS41	205,87	30,88
reS42	46,66	37,11
S43	43,45	42,24
reS44	264,61	27,07
S45	38,11	24,64
S46	30,59	29,93
S47	52,48	4,06
S48	122,67	29,29
reS49	868,32	9,16
reS50	494,47	25,38
reS51	84,86	42,24
S52	63,60	16,81
S53	67,17	16,08

Grey shaded re-analysed by HPLC fluorescense detection

kode	EROD (pmol/min/mg protein)	CV(%)
RF1	18,47	29,53
RF2	39,20	21,02
RF3	18,45	12,76
RF4	0,26	46,48
RF5	32,19	10,88
RF6	32,02	6,16
RF7	218,47	16,86
RF8	1,35	20,88
RF9	63,61	5,81
RF10	12,83	4,63
reRF11	286,83	27,28
RF12	53,29	3,66
RF13	45,15	10,25
RF14	25,96	12,53
RF15	20,86	6,59
RF16	13,02	20,55
RF17	18,78	46,26
RF18	32,92	7,92
RF19	151,92	10,77
RF20	83,55	6,29
RF21	1,99	29,70
RF22	2,83	10,26
RF23	25,15	6,78
RF24	48,44	27,84

Example of standard curve for EROD measurements:

antatt mengde (pmol)	µM	replikate målinger (over tid)								gjennomsnitt	beregnet mengde (pmol)	korrigert fluorescens
0	0	23	23	23	23	23	22	22	22	22,5	0	0
0	0	23	22	23	23	22	22	22	22			
2,75	0,01	36	36	36	36	35	35	35	35	35,5625	1,897199454	13,0625
2,75	0,01	37	36	36	36	35	35	35	35			
5,5	0,02	62	61	61	61	61	60	60	60	61,9375	3,794398907	39,4375
5,5	0,02	64	64	64	64	63	62	62	62			
11	0,04	112	111	110	110	108	107	108	108	110,6875	7,588797814	88,1875
11	0,04	114	114	112	113	112	110	111	111			
22	0,08	212	210	208	209	206	203	205	205	210,625	15,17759563	188,125
22	0,08	218	216	215	216	213	210	212	212			
44	0,16	411	408	404	405	401	395	400	397	407	30,35519126	384,5
44	0,16	419	417	413	415	409	403	408	407			
88	0,32	796	790	783	785	776	763	772	770	785,625	60,71038251	763,125
88	0,32	805	801	796	799	790	776	785	783			
176	0,64	1568	1559	1542	1551	1529	1503	1520	1520	1603	121,420765	1580,5
176	0,64	1701	1692	1682	1682	1659	1636	1654	1650			



slope (fra graf)

12,902 13,0278

forventet FU av spike (3.2 nmol)

41,69

Example of the EROD measurments of sample U1:

kode	0	34	68	102	136	170	204	238	alle tider	**slope**	**intercept**	**r-sq**	unntatt to første	**slope**	**intercept**	**r-sq**
U1	-2	-3	-4	-4	-5	-6	-6	-5		-0,015	-2,5	0,793		-0,010	-3,4	0,514
U1	-1	-1	-2	-3	-3	-5	-4	-4		-0,016	-0,9	0,810		-0,013	-1,5	0,584
U1	-3	-4	-5	-4	-5	-7	-6	-6		-0,013	-3,3	0,716		-0,011	-3,8	0,439
U1	30	30	29	29	28	27	28	27		-0,013	30,2	0,860		-0,012	29,9	0,700
U1	26	26	25	25	24	22	23	23		-0,016	26,2	0,813		-0,015	26,1	0,631
U1	26	26	25	26	24	23	24	24		-0,011	26,2	0,642		-0,010	26,0	0,386

F_S/min = Increase in fluorescence per minute = mean of the slopes for the different measurements for one sample/60

reagens	**antatt kons (mM)**	**absorbans**	**beregnet kons**	**kommentar**
7-ER	0,01375	0,06		absorbans ved 450 nm
Resorufin	0,01	0,51	0,006899	absorbans ved 572 nm, ekstinksjonskoeffisient 73.2
NADPH	2,5			

fortynning i brønn (alle volum i µL)		
buffer	200	
prøve	50	
NADPH	25	
spike	10	gjelder bare halvparten av prøvene

Example of EROD calculations:

kode	0-verdi (protein)	forventet FU : målt FU (intercept)	forventet FU : målt FU (0-verdi)	ikke-korrigert slope (alle verdier)	korrigert intercept	korrigert slope (minus 2 første)	quench-korrigert EROD (pmol/min/mL)	protein (µg/ml)	fortynning	EROD (pmol/min/mg protein)
U1	-2	1,4000	1,4212	-0,0141	1,3817	-0,0118	-2	2996	0,33	-1,56
U2	-3	1,2949	1,2894	-0,0142	1,2948	-0,0129	-2	4391	0,33	-1,06
U3	-2	1,3376	1,3448	-0,0102	1,3149	-0,0081	-1	4117	0,33	-0,74
U4	-4	1,3570	1,3744	-0,0132	1,3503	-0,0120	-2	4173	0,33	-1,11
U5	-8	1,4933	1,4889	-0,0153	1,4870	-0,0143	-2	8055	0,33	-0,74
U6	5	1,4786	1,5068	0,0540	1,4611	0,0550	8	3563	0,33	6,50
U7	-4	1,5314	1,5252	-0,0149	1,5295	-0,0137	-2	4497	0,33	-1,30
U8	-1	1,4933	1,5252	-0,0130	1,4767	-0,0123	-2	6444	0,33	-0,81
U9	0	1,2486	1,2383	-0,0170	1,2628	-0,0147	-2	3208	0,33	-1,58
U10	-2	1,3909	1,4052	-0,0130	1,3949	-0,0122	-2	4584	0,33	-1,04
U11	0	1,5008	1,5252	-0,0161	1,4865	-0,0148	-2	3074	0,33	-2,06
U12	1	1,3619	1,3744	-0,0166	1,3366	-0,0148	-2	2516	0,33	-2,26

EROD (pmol/min/mg protein)= "quench-korrigert EROD (pmol/min/mL)"/("protein (mg/ml)"/1000)/"fortynning"

quench-korrigert EROD (pmol/min/mL)= ("korrigert slope (minus 2 første)"/ "slope (fra graf)")*60*20*("forventet FU:målt FU (0-verdi)")

korrigert slope (minus 2 første)= mean of: "slope(minus 2 første)"

forventet FU:målt FU (0-verdi)= "slope (fra graf)"/(mean: U1d-U1f – mean: U1a-U1c)

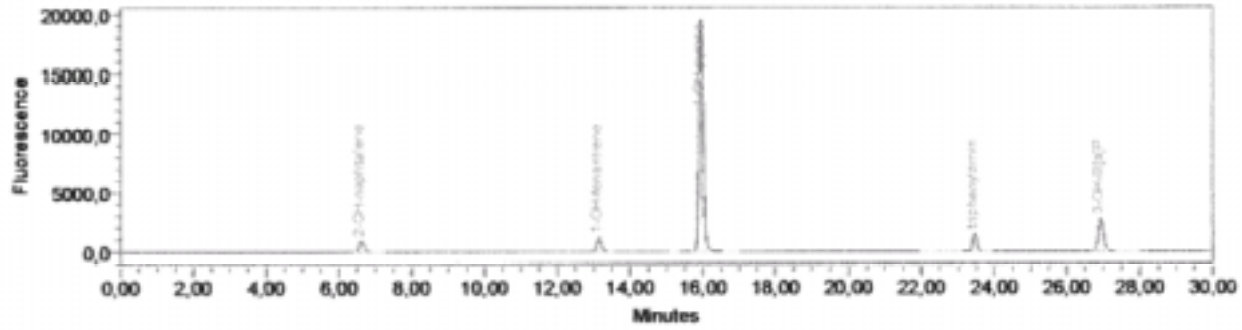
Attachment 3C:

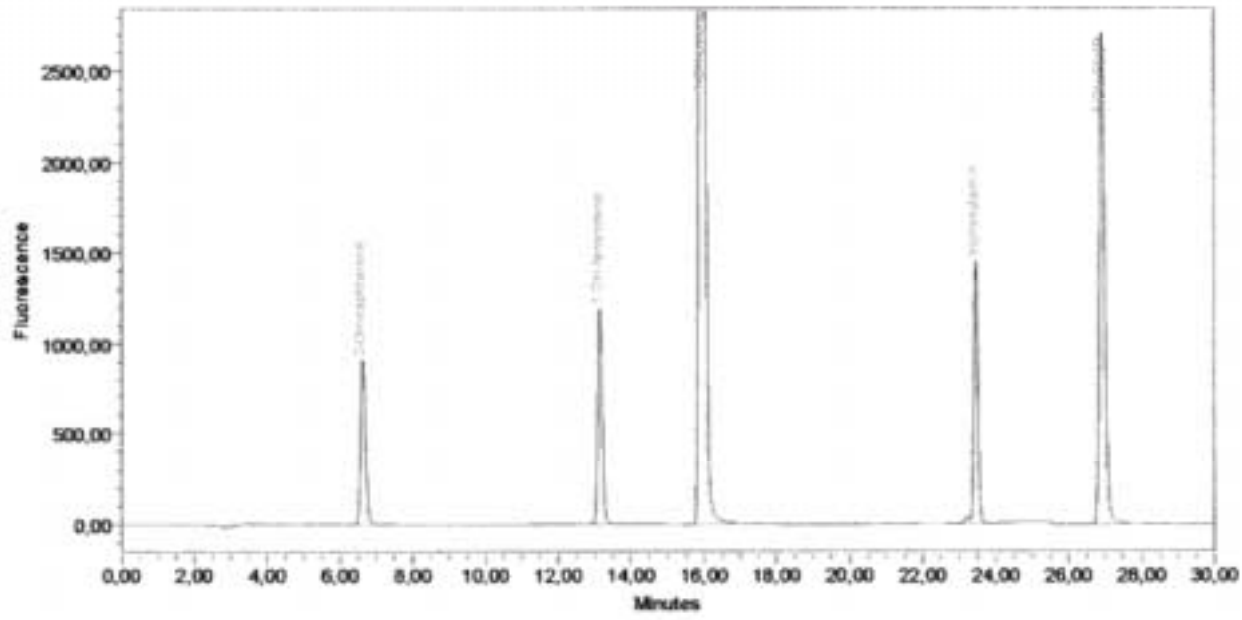
Measurement of PAH metabolites by HPLC

std. 2

SampleWeight 1,00000

Sample Type:	Standard	Acquired By:	System
Vial:	2	Date Acquired:	05.09.2002 15:12:43
Injection #:	1	Acq. Method Set:	PAH metabolitter
Injection Volume:	25,00 ul	Date Processed:	06.09.2002 08:37:48
Run Time:	30,0 Minutes	Processing Method:	020905 origo PAH m
Sample Set Name:	020905 PAHm stdrekke	Channel Name:	474 Ch1





Peak Results

	Name	RT	Area	Height	Concentration	Units	Control Value	% Deviation
1	2-OH-naphtalene	6,622	8560564	906901	618,820			
2	1-OH-fenantrene	13,143	10079789	1192945	69,100			
3	1-OH-pyrene	15,950	159100654	19527535	256,060			
4	triphenylamin	23,480	11211276	1451550	272,210			
5	3-OH-BjaP	26,935	27558983	2707004	171,220			

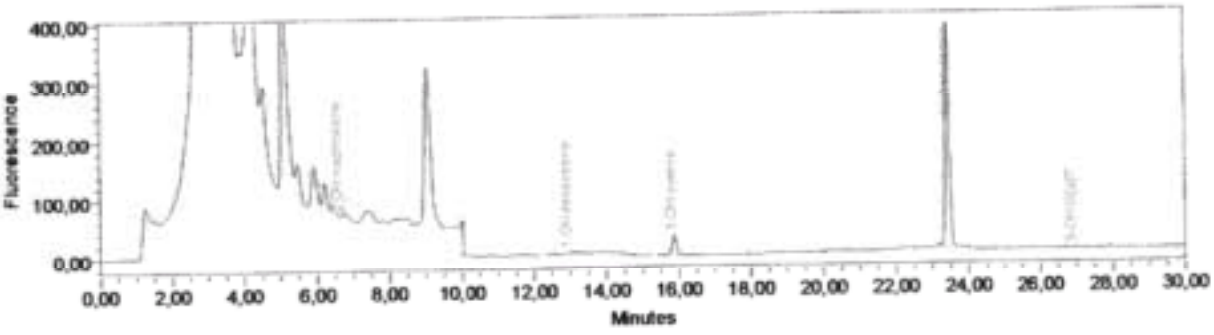
Example of chromatogram from running of a standard (standard 2)

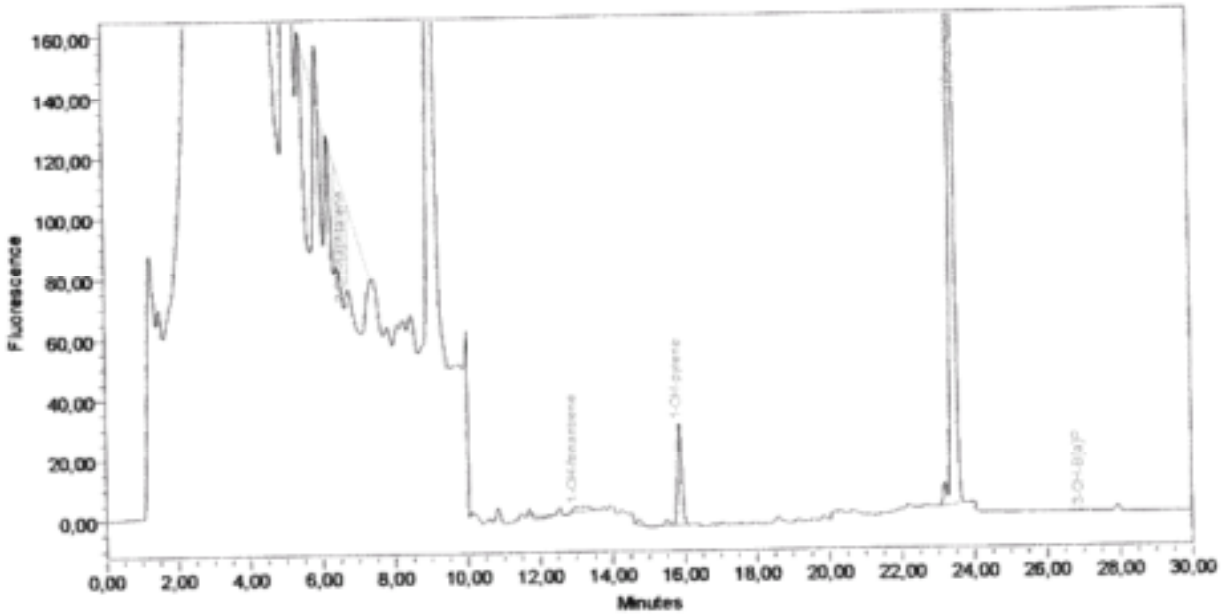
s35

SampleWeight 0,02000

Sample Type:	Unknown
Vial:	31
Injection #:	1
Injection Volume:	25,00 ul
Run Time:	30,0 Minutes
Sample Set Name:	2002 Coastal Baseline

Acquired By:	System
Date Acquired:	06.09.2002 04:38:09
Acq. Method Set:	PAH metabolitter
Date Processed:	11.09.2002 10:50:15
Processing Method:	020905 origo PAH m
Channel Name:	474 Ch1





Peak Results

	Name	RT	Area	Height	Concentration	Units	Control Value	% Deviation
1	2-OH-naphtalene	6,625	689528	40594				
2	1-OH-fenantrene	12,965	40677	1542	1,666			
3	1-OH-pyrene	15,891	271665	33473	7,791			
4	triphenylamin	23,443	2988647	381815	24,980			
5	3-OH-BjaP	26,932	2140	313	0,375			

A typical chromatogram from running af a sample (sample s35 – dab 35)

Attachment 3C:

Measurment of PAH metabolites in bile by HPLC:

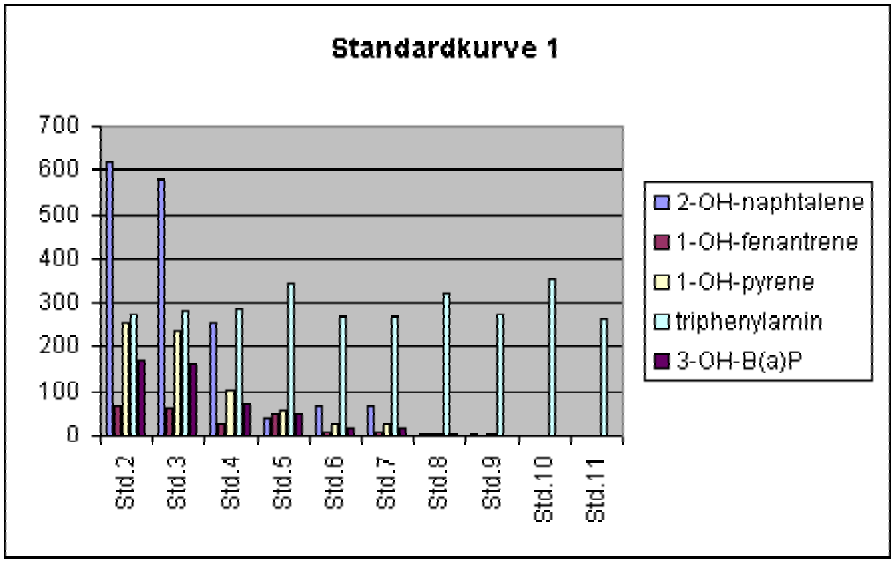
	2-OH-napht.	1-OH-fen.	1-OH-pyr.	triphen.	3-OH-B(a)P	Abs 380nm	normalized
Sk 1			322	24,98	2,552	52,961	6,0791
S 7			2,58	24,98	0,296	3,927	0,657
S 10			2,898	24,98	-2,331	25,957	0,1116
S 11			3,072	24,98	0,113		
S 13			18,85	24,98		2,6035	7,2406
S 16			2,226	24,98			
S 31			50,11	24,98		97,137	0,5158
S 32			14,26	24,98	0,337	60,742	0,2348
S 33			12,65	24,98	0,409		
S 34			5,263	24,98	0,406	40,097	0,1313
S 35			7,791	24,98	0,375	41,309	0,1886
S 36			55,8		2,609	94,627	0,5896
S 37			13,22	24,98	0,724	42,218	0,3132
S 38			3,795	24,98	0,304	18,079	0,2099
S 39			5,084	24,98		29,694	0,1712
S 43			0,939	24,98	0,289	21,917	0,0428
S 44			3,211	24,98		13,837	0,2321
S 45			1,614	24,98	0,282	4,08	0,3956
S 46			1,086	24,98	0,296	4,08	0,2662
S 48			2,746	24,98	0,385	7,752	0,3542
S 50			11,19	24,98	0,285	7,191	1,5554
S 51			9,992	24,98	0,582		
S 52			5,803	24,98	0,268		
S 53			1,164	24,98	0,268		
U 1			36,83			10,659	3,4548
U 3		4,449	50,11		0,787	27,876	1,7977
U 4			15,16		1,269	5,292	2,8647
U 5			72,75		0,936	24,846	2,928
U 6			18,35		0,943	9,828	1,867
U 7			33,74	24,98	0,42	21,412	1,5758
U 8			15,37	24,98	0,329	23,836	0,6448
U 10			4,709	24,98	0,284	8,772	0,5368
U 16			21,21	24,98	1,68	41,749	0,508
U 17			4,515	24,98	0,218		

	2-OH-napht.	1-OH-fen.	1-OH-pyr.	triphen.	3-OH-B(a)P	Abs 380nm	normalized
U 18			5,879	24,98	0,21		
U 19			12,94	24,98	0,325	13,837	0,935318
U 20			81,04	24,98	1,249		
U 21			22,99	24,98	0,326	14,948	1,537798
U 22			39,25	24,98	0,179		
U 23			5,344	24,98	0,27	22,22	0,240504
U 25			1,784	24,98	0,123		
U 26			6,346	24,98	0,332	75,3	0,084276
U 27			2,91	24,98	0,338	21,513	0,135267
RF 1			11,01	24,98	0,641	16,665	0,660426
RF 2			11,57	24,98	0,392		
RF 3			13,22	24,98	0,391	11,716	1,128542
RF 4			7,486	24,98	0,458	25,957	0,2884
RF 5			12,06	24,98	0,361	10,1	1,193663
RF 6			12,18	24,98	0,522	19,594	0,621466
RF 7			14,96	24,98	0,536	31,007	0,482568
RF 8			7,429	24,98	0,494	17,271	0,430143
RF 9			4,981	24,98	0,264		
RF 10			39,21	24,98	0,478	30,906	1,268589
RF 11			8,871	24,98	0,525	21,715	0,408519
RF 12			4,555	24,98	0,365	21,715	0,209763
RF 13			6,832	24,98	0,326	17,372	0,393277
RF 14			22,83	24,98	0,385	20,099	1,135927
RF 15			33,42	24,98	0,382	12,827	2,605442
RF 17			4,736	24,98	0,167		
RF 19			4,019	24,98	0,342	13,13	0,306093
RF 20			3,455	24,98	0,45	10,807	0,3197
RF 23			4,209	24,98	0,347	17,978	0,234119
RF 24			7,195	24,98	0,264	22,624	0,318025

Grey shaded: no internal standard added, calculated from standard curve 2

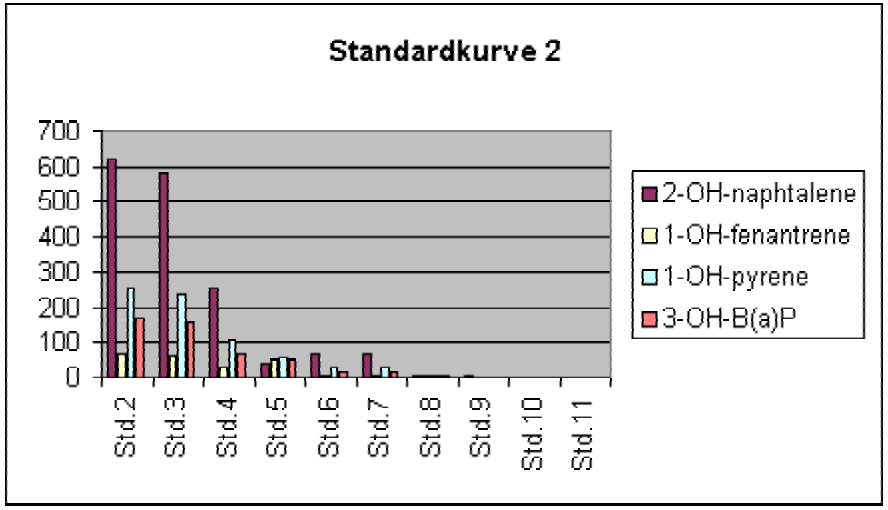
3-OH-B(a)P: Results not included, since the peaks were not 3 times the background levels

Std. kurve 1	C, ug/kg	2-OH-naphtalene	1-OH-fenantrene	1-OH-pyrene	triphenylamin	3-OH-B(a)P
Std.2	180	618,82	69,1	256,1	272,2	171,22
Std.3	90	579,07	64,66	239,6	282,3	160,22
Std.4	45	253,76	28,33	105	288,3	70,21
Std.5	18	40,48	48,61	57,13	345,4	51,5
Std.6	9	65,54	7,32	27,12	267,8	18,13
Std.7	4,5	65,54	7,32	27,12	267,8	18,13
Std.8	1,8	3,95	4,74	5,57	324	5,02
Std.9	0,9	5,98	0,67	2,47	274,7	1,65
Std.10	0,45	0,97	1,16	1,37	353,2	1,23
Std.11	0,18	1,31	0,15	0,54	263,2	0,36



Std. kurve 2

Std.2	180	618,82	69,1	256,1		171,22
Std.3	90	579,07	64,66	239,6		160,22
Std.4	45	253,76	28,33	105		70,21
Std.5	18	40,48	48,61	57,13		51,5
Std.6	9	65,54	7,32	27,12		18,13
Std.7	4,5	65,54	7,32	27,12		18,13
Std.8	1,8	3,95	4,74	5,57		5,02
Std.9	0,9	5,98	0,67	2,47		1,65
Std.10	0,45	0,97	1,16	1,37		1,23
Std.11	0,18	1,31	0,15	0,54		0,36



Attachment 3D:

Vitellogenin in cod:

Sample ID	Mean value	Std Dev	CV	mean OD	Dil.Factor
RF1	1563	416,4	26,64	2,713	100
RF2	1954	340,2	17,41	2,596	100
RF3	5,98E+06	8,53E+06	142,7	0,2645	100
RF4	1163	339,8	29,23	2,85	100
RF5	1956	790,9	40,43	2,613	100
RF6	2445	1072	43,84	2,501	100
RF7	1497	707,2	47,23	2,75925	100
RF8	2394	694,3	29	2,49625	100
RF9	1535	555,9	36,22	2,601	100
RF10	1,69E+05	60786	35,87	0,43125	100
RF11	2066	668,9	32,37	2,48925	100
RF12	1889	572,3	30,3	2,52425	100
RF13	6070	7463	122,9	2,1365	100
RF14	2169	1035	47,72	2,47875	100
RF15	2560	928,3	36,27	2,39875	100
RF16	4967	1678	33,79	2,04475	100
RF17	797,9	249,3	31,25	2,15775	100
RF18	783,5	262,5	33,5	2,164	100
RF19	1589	460,7	28,99	1,9265	100
RF20	1257	610,1	48,54	2,02475	100
RF21	1177	156,2	13,26	2,028	100
RF22	1142	133,5	11,68	2,038	100
RF23	8790	1299	14,78	1,1745	100
RF24	3707	783	21,12	1,5695	100

Grey shaded: not included (to low measurements)

Attachment 4:

Method for EROD analysis by HPLC performed by the National Veterinary Institute in Norway

The 1,5 ml incubation mixture contained 0,1 M sodium phosphate buffer (pH 7,8), 5 µl 7-ethoxyresorufin(0,5 mM dissolved in DMSO) and 10 µl NADPH (10 mM). Reactions were initiated by adding 100 µl of microsomal protein solutions (corresponding to appr. 0,4 mg protein) and stopped with 1,5 ml of ice-cold methanol. The incubation time was 20 min. The vials were centrifuged at 3100 rpm for 30 min and the supernatant was transferred to HPLC vials. Resorufin was then quantified against known standards by the use of HPLC (5 µl injections, mobile phase: 40:60 v/v acetonitrile:water, flow:1.0 ml/min). The column used was a Symmetry® C18 (3.9 x 150 mm, 5 µm; Waters, Milford, MA, USA) and resorufin was detected on a Shimadzu RF-10A XL (Shimadzu, Kyoto, Japan) fluorescence detector. Excitation was at 535 nm and fluorescence emission was measured at 585 nm.
(Ruus et al., 2002)

EROD in sculpin analysed by HPLC:

Ulke	Picomol "målt"	picomol/ml	protein (µg/ml):	EROD-aktivitet picomol/min mg protein:
U1	0,003	0,6	2996	0,300400534
U2	0,001	0,2	4391	0,068321567
U3	0,009	1,8	4117	0,655897
U4	0,005	1	4173	0,359496705
U5	0,005	1	8055	0,186219739
U6	0,167	33,4	3563	14,06315789
U7	0,002	0,4	4497	0,133422282
U8	0,001	0,2	6444	0,046554935
U9	0,001	0,2	3208	0,093516209
U10	0,003	0,6	4584	0,196356496
U11	0,001	0,2	3074	0,097592713
U12	0,001	0,2	2516	0,119260584
U13	0,006	1,2	2745	0,655737705
U14	0,007	1,4	4138	0,507491542
U15	0,022	4,4	3031	2,177858439
U16	0,009	1,8	3087	0,874635569
U17	0,28	56	5855	14,34671221
U18	0,003	0,6	4883	0,184331797
U19	0,002	0,4	4848	0,123775142
U20	0,002	0,4	6055	0,09909166
U21	0,007	1,4	7448	0,281954887
U22	0,046	9,2	4368	3,159702347
U23	0,004	0,8	6105	0,196560197
U24	0,005	1	4035	0,371793283
U25	0,787	157,4	4523	52,20332987
U26	0,012	2,4	3391	1,061696355
U27	0,007	1,4	5982	0,351082504
U28	0,004	0,8	6125	0,195918367
Blank	**0,001***			

*** Blank er trukket fra prøvene**

Attachment 5:

Measurement of CYP1A protein with ELISA (enzyme-linked immunosorbent assay):

100 µl of coating buffer was added to the A1-D1 wells of the 96 well microtiter plates whereas 100 µl of reference sample was added to the E12-H12 wells. To the rest of the wells 100 µl of diluted sample was added (sample 1 in A2-D2, sample 2 in E2-H2, sample 3 in A3-D3 etc.) The plates were sealed and incubated at 4ºC over the night.
Then the plates were washed three times with TTBS (Tris-buffered saline solution (TBS) with 0,05% Tween-20). A 300 µl blocking solution (TBS with 1% Bovine Serum Albumin (BSA)) was added to all wells, and the plates were incubated for 30-60 min. at room temperature. The plates were again washed three times with TTBS. Then 100 µl of primary antibody (Anti-fish (CP226)) diluted 1:1000 in TTBS with 0,1% BSA, was added to all the wells and the plates were sealed and incubated at 4ºC over the night. The plates were washed three times with TTBS and 100 µl of secondary antibody (Sigma goat anti rabbit IgG HRP conjugated (GAR-HRP)) diluted 1:3000 in TTBS with 0,1% BSA, was added to all wells. The plates were sealed and incubated at 4ºC for 6-8 hours. After washing 5 times in TTBS, 100 µl of TMB-plus solution (0,04 % O-phenylene-diamine in 150 mM phosphate, 50 mM citrate buffer, pH 5,7, with 0,012 % hydrogen peroxide) was added to all the wells for color development, and the plates were incubated in the dark for 8-12 min. before adding the stop solution (1 M H_2SO_4). The absorbance was read in a platereader at 450 nm.

Primary antibody: Anti-fish (CP226)
Secondary antibody: Sigma goat anti rabbit IgG, HRP conjugated (GAR-HRP)

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank	s. 1	s. 3	s. 5	s. 7	s. 9	s. 11	s. 13	s. 15	s. 17	s. 19	s. 21
B	blank	s. 1	s. 3	s. 5	s. 7	s. 9	s. 11	s. 13	s. 15	s. 17	s. 19	s. 21
C	blank	s. 1	s. 3	s. 5	s. 7	s. 9	s. 11	s. 13	s. 15	s. 17	s. 19	s. 21
D	blank	s. 1	s. 3	s. 5	s. 7	s. 9	s. 11	s. 13	s. 15	s. 17	s. 19	s. 21
E	Ref.	s. 2	s. 4	s. 6	s. 8	s. 10	s. 12	s. 14	s. 16	s. 18	s. 20	s. 22
F	Ref.	s. 2	s. 4	s. 6	s. 8	s. 10	s. 12	s. 14	s. 16	s. 18	s. 20	s. 22
G	Ref.	s. 2	s. 4	s. 6	s. 8	s. 10	s. 12	s. 14	s. 16	s. 18	s. 20	s. 22
H	Ref.	s. 2	s. 4	s. 6	s. 8	s. 10	s. 12	s. 14	s. 16	s. 18	s. 20	s. 22

s.1-s.22: sample 1-sample 22

Results of CYP1A protein measurments:

kode	Abs				mean	corrected for blank	cv (%)
Bl	0,239	0,243	0,252	0,224	0,24	0,00	4,9
Ref. RF11	0,905	0,909	0,897	0,897	0,90	0,66	0,7
ref. RF11	0,902	0,848	0,869	0,878	0,87	0,63	2,6
Ref. RF11	0,905	0,909	0,897	0,897	0,90	0,66	0,7
Ref. U10	1,923	1,913	1,752	1,928	1,88	1,64	4,5
Ref. U10	1,658	1,709	1,654	1,607	1,66	1,42	2,5
Ref. U10	1,923	1,913	1,752	1,928	1,88	1,64	4,5
RF1	0,897	0,912	0,933	0,878	0,91	0,67	2,6
RF2							
RF3	0,999	0,986	0,951	0,95	0,97	0,73	2,6
RF4	0,9	0,9	0,924	0,965	0,92	0,68	3,3
RF5	0,834	0,812	0,816	0,873	0,83	0,59	3,3
RF6	0,758	0,833	0,874	0,824	0,82	0,58	5,8
RF7	0,843	0,851	0,876	0,891	0,87	0,63	2,6
RF8	0,714	0,69	0,763		0,72	0,48	5,2
RF9	0,778	0,769	0,772	0,785	0,78	0,54	0,9
RF10	0,514	0,639	0,669	0,56	0,60	0,36	12,0
RF11	0,94	0,92	0,88	0,881	0,91	0,67	3,3
RF12	0,774	0,894	0,799	0,789	0,81	0,57	6,7
RF13	0,972	0,952	0,99	0,874	0,95	0,71	5,4
RF14	0,897	0,863	0,83	0,845	0,86	0,62	3,4
RF15	0,911	0,927	1,479	1,562	1,22	0,98	28,6
RF16	0,809		0,765	0,876	0,82	0,58	6,8
RF17	0,908	1,001	1,01		0,97	0,73	5,8
RF18	0,975	0,946	0,935	0,903	0,94	0,70	3,2
RF19	0,863	0,861	0,887	0,881	0,87	0,63	1,5
RF20	0,764		0,784	0,757	0,77	0,53	1,8
RF21	0,904	0,926	0,942	0,97	0,94	0,70	3,0
RF22	0,859		1,186	1,104	1,05	0,81	16,2
RF23							
RF24	1,276	1,243	1,189	1,06	1,19	0,95	8,0

kode	Abs				mean	corrected for blank	cv (%)
U1	0,707	0,664	0,677	0,715	0,69	0,45	3,5
U2	1,563	1,694	1,735	1,596	1,65	1,41	4,9
U3	0,947	0,946	1,008	0,975	0,97	0,73	3,0
U4	0,883	0,977	1,034	0,946	0,96	0,72	6,6
U5	1,771	1,677	1,724	1,606	1,69	1,46	4,2
U6	1,925	2,11	2,12	2,142	2,07	1,83	4,8
U7	2,414	2,37	2,249	2,41	2,36	2,12	3,3
U8	1,788	1,839	1,923	1,849	1,85	1,61	3,0
U9	2,56	2,486	2,606	2,36	2,50	2,26	4,3
U10	1,965	1,811	2,029	2,02	1,96	1,72	5,2
U11	1,653	1,618	1,61	1,546	1,61	1,37	2,8
U12	1,613	1,54	1,551	1,627	1,58	1,34	2,8
U13	1,7	1,609	1,622	1,504	1,61	1,37	5,0
U14	2,358	2,211	2,433	2,232	2,31	2,07	4,6
U15	1,574	1,499	1,59	1,584	1,56	1,32	2,7
U16	1,854	1,671	1,86	1,759	1,79	1,55	5,0
U17	1,837	1,706	1,777	1,8	1,78	1,54	3,1
U18	1,735	1,647	1,804	1,69	1,72	1,48	3,9
U19	1,531	1,48	1,451	1,409	1,47	1,23	3,5
U20	1,734	1,694	1,78	1,823	1,76	1,52	3,2
U21	1,743	1,639	1,716	1,657	1,69	1,45	2,9
U22	1,325	1,27	1,33	1,397	1,33	1,09	3,9
U23							
U24	1,687	1,838	1,81	1,795	1,78	1,54	3,7
U25							
U26							
U27							

Calculations of CYP1A protein results:

kode	protein (µg/ml)	coating bf	prøve (µL)	totalvolum (mL)	absorbance	CYP1A (abs/mg protein)
U1	2996	4985	17	5	0,45	0,045
U2	4391	4990	11	5	1,41	0,141
U3	4117	4990	12	5	0,73	0,073
U4	4173	4990	12	5	0,72	0,072
U5	8055	4995	6	5	1,46	0,146
U6	3563	4985	14	5	1,83	0,183
U7	4497	4990	11	5	2,12	0,212
U8	6444	4990	8	5	1,61	0,161
U9	3208	4985	16	5	2,26	0,226
U10	4584	4990	11	5	1,72	0,172
U11	3074	4985	16	5	1,37	0,137
U12	2516	4980	20	5	1,34	0,134
U13	2745	4980	18	5	1,37	0,137
U14	4138	4990	12	5	2,07	0,207
U15	3031	4985	16	5	1,32	0,132
U16	3087	4985	16	5	1,55	0,155
U17	5855	4990	9	5	1,54	0,154
U18	4883	4990	10	5	1,48	0,148
U19	4848	4990	10	5	1,23	0,123
U20	6055	4990	8	5	1,52	0,152
U21	7448	4995	7	5	1,45	0,145
U22	4368	4990	11	5	1,09	0,109
U23	6105	4990	8	5		
U24	4035	4990	12	5	1,54	0,154
U25	4523	4990	11	5		
U26	3391	4985	15	5		
U27	5982	4990	8	5		
U28	6125	4990	8	5		

kode	protein (µg/ml)	coating bf	prøve (µL)	totalvolum (mL)	absorbance	CYP1A (abs/mg protein)
RF1	5256	4990	10	5	0,67	0,067
RF2	2230	4980	22	5		
RF3	4269	4990	12	5	0,73	0,073
RF4	3382	4985	15	5	0,68	0,068
RF5	3407	4985	15	5	0,59	0,059
RF6	4189	4990	12	5	0,58	0,058
RF7	2532	4980	20	5	0,63	0,063
RF8	3208	4985	16	5	0,48	0,048
RF9	2662	4980	19	5	0,54	0,054
RF10	1533	4965	33	5	0,36	0,036
RF11	4660	4990	11	5	0,67	0,067
RF12	3723	4985	13	5	0,57	0,057
RF13	2948	4985	17	5	0,71	0,071
RF14	3376	4985	15	5	0,62	0,062
RF15	3786	4985	13	5	0,98	0,098
RF16	3382	4985	15	5	0,58	0,058
RF17	3884	4985	13	5	0,73	0,073
RF18	4536	4990	11	5	0,70	0,070
RF19	3054	4985	16	5	0,63	0,063
RF20	2395	4980	21	5	0,53	0,053
RF21	5949	4990	8	5	0,70	0,070
RF22	4441	4990	11	5	0,81	0,081
RF23	2648	4980	19	5		
RF24	2639	4980	19	5	0,95	0,095

Attachment 6:

Fish data

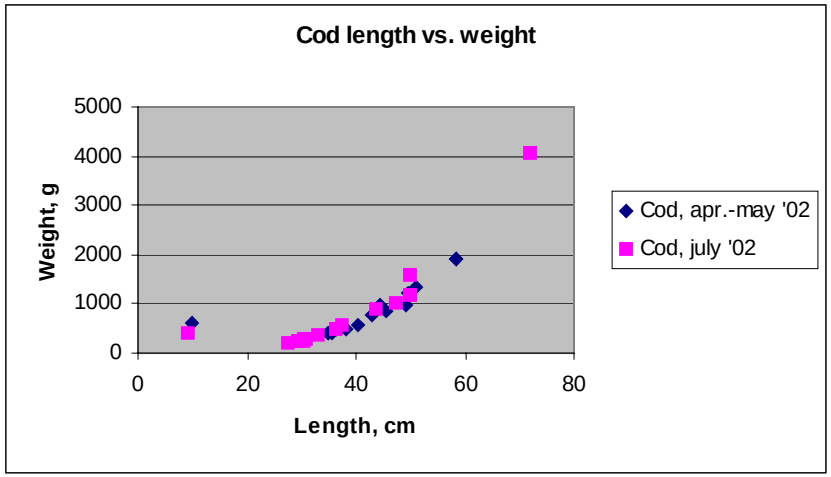
Species	ID	Date	Location	Length (cm)	Weight (g)	Gender	Gall (g)	Liver (g)	Gonads (g)	Protein (µg/ml)	EROD (pmol/min/mg protein)	1-OH-pyrene (µg/kg)	Abs 380nm	Vtg (ng/ml)	CYP1A protein (abs/mg protein)
Gadus morhua	Reyðfiskur 1	22-04-2002	Kaldbak	35	424	F	0,74	5,12	0,91	5256	18,47	11,01	16,67	1563	0,067
Gadus morhua	Reyðfiskur 2	22-04-2002	Kaldbak	36,2	496	M	0,36	13,82	9,23	2230	39,20	11,57		1954	
Gadus morhua	Reyðfiskur 3	22-04-2002	Kaldbak	51	1322	F	0,75	12,50	8,66	4269	18,45	13,22	11,72		0,073
Gadus morhua	Reyðfiskur 4	01-05-2002	Kaldbak	49	994	M	1,66	5,73	31,06	3382	0,26	7,49	25,96	1163	0,068
Gadus morhua	Reyðfiskur 5	01-05-2002	Kaldbak	43	758	M	0,86	8,56	-	3407	32,19	12,06	10,10	1956	0,059
Gadus morhua	Reyðfiskur 6	01-05-2002	Kaldbak	37,6	528	M	0,48	7,26	-	4189	32,02	12,18	19,59	2445	0,058
Gadus morhua	Reyðfiskur 7	06-05-2002	Kaldbak	40,5	550	M	0,81	4,37	22,58	2532	218,47	14,96	31,01	1497	0,063
Gadus morhua	Reyðfiskur 8	06-05-2002	Kaldbak	49,5	1236	F	1,54	29,15	4,64	3208	1,35	7,43	17,27	2394	0,048
Gadus morhua	Reyðfiskur 9	06-05-2002	Kaldbak	45,5	870	M	0,35	10,99	0,39	2662	63,61	4,98		1535	0,054
Gadus morhua	Reyðfiskur 10	06-05-2002	Kaldbak	58,5	1918	F	2,13	149,53	21,27	1533	12,83	39,21	30,91		0,036
Gadus morhua	Reyðfiskur 11	07-05-2002	Kaldbak	38	502	F	0,54	5,41	1,86	4660	286,83	8,87	21,72	2066	0,067
Gadus morhua	Reyðfiskur 12	07-05-2002	Kaldbak	35,5	388	F	0,27	3,68	0,85	3723	53,29	4,56	21,72	1889	0,057
Gadus morhua	Reyðfiskur 13	10-07-2002	Kaldbak	43,5	884	M	0,57	16,00	-	2948	45,15	6,83	17,37	6070	0,071
Gadus morhua	Reyðfiskur 14	10-07-2002	Kaldbak	30,5	242	F	0,22	3,27	0,77	3376	25,96	22,83	20,10	2169	0,062
Gadus morhua	Reyðfiskur 15	10-07-2002	Kaldbak	33	365	M	0,47	4,86	-	3786	20,86	33,42	12,83	2560	0,098
Gadus morhua	Reyðfiskur 16	11-07-2002	Kaldbak	36,5	470	F	0,22	9,40	0,13	3382	13,02			4967	0,058
Gadus morhua	Reyðfiskur 17	11-07-2002	Kaldbak	27,5	212	M	0,11	2,44	-	3884	18,78	4,74		797,9	0,073
Gadus morhua	Reyðfiskur 18	11-07-2002	Kaldbak	29,5	250	F	0,07	2,37	0,31	4536	32,92			783,5	0,070
Gadus morhua	Reyðfiskur 19	12-07-2002	Kaldbak	47,5	1014	F	1,19	19,94	3,04	3054	151,92	4,02	13,13	1589	0,063
Gadus morhua	Reyðfiskur 20	16-07-2002	Kaldbak	72	4050	M	1,10	113,49	61,64	2395	83,55	3,46	10,81	1257	0,053
Gadus morhua	Reyðfiskur 21	16-07-2002	Kaldbak	30,5	270	M	0,15	3,09	-	5949	1,99			1177	0,070
Gadus morhua	Reyðfiskur 22	16-07-2002	Kaldbak	30,8	288	F	0,13	3,25	0,59	4441	2,83			1142	0,081
Gadus morhua	Reyðfiskur 23	16-07-2002	Kaldbak	50	1198	F	1,25	9,96	13,92	2648	25,15	4,21	17,98	8790	
Gadus morhua	Reyðfiskur 24	17-07-2002	Kaldbak	50	1576	M	1,46	48,53	4,69	2639	48,44	7,20	22,62	3707	0,095
Platichtys flesus	Skrubba 1	05-02-2002	Kirkjubø	42	1334	M	1,69	83,96	343,00	2470	1,16	321,95	52,96		
Platichtys flesus	Skrubba 2	05-02-2002	Kirkjubø	34	514	M	0,45	11,97	73,87	4282	14,78				
Platichtys flesus	Skrubba 3	05-02-2002	Kirkjubø	30	344	M	0,25	8,19	9,20	3543	37,30				
Platichtys flesus	Skrubba 4	05-02-2002	Kirkjubø	30,5	394	M	0,08	8,70	49,60	3593	16,51				
Platichtys flesus	Skrubba 5	05-02-2002	Kirkjubø	29	250	M	0,20	3,76	6,90	4387	37,34				
Platichtys flesus	Skrubba 6	05-02-2002	Kirkjubø	31,5	346	M	0,20	6,14	11,08	3373	35,43				

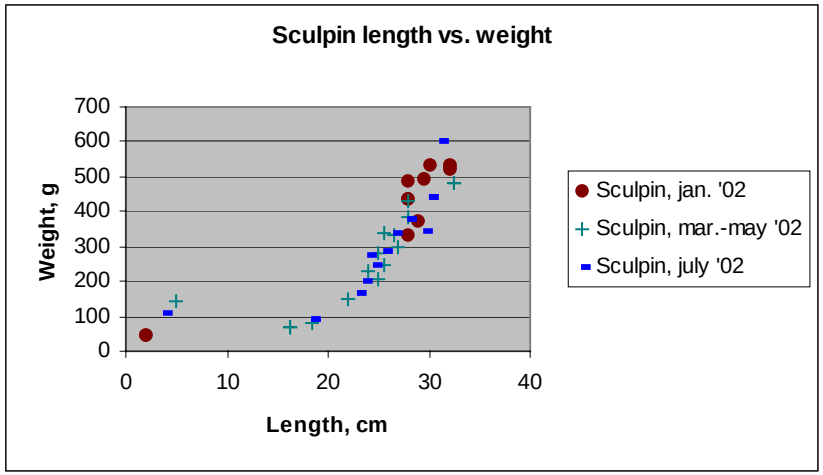
Species	ID	Date	Location	Length (cm)	Weight (g)	Gender	Gall (g)	Liver (g)	Gonads (g)	Protein (µg/ml)	EROD (pmol/min/mg protein)	1-OH-pyrene (µg/kg)	Abs 380nm	Vtg (ng/ml)	CYP1A protein (abs/mg protein)
Myoxocephalus scorpius	ulka 1	15-01-2002	Kaldbak	28	488	F	0,70	23,69	94,91	2996	0,30	36,83	10,66		0,045
Myoxocephalus scorpius	ulka 2	15-01-2002	Kaldbak	29	374	M	0,27	8,24	8,58	4391	0,07				0,141
Myoxocephalus scorpius	ulka 3	15-01-2002	Kaldbak	30	536	F	0,84	27,98	63,86	4117	0,66	50,11	27,88		0,073
Myoxocephalus scorpius	ulka 4	16-01-2002	Kaldbak	28	438	F	0,32	20,88	65,90	4173	0,36	15,16	5,29		0,072
Myoxocephalus scorpius	ulka 5	16-01-2002	Kaldbak	32	520	F	0,79	7,88	14,96	8055	0,19	72,75	24,85		0,146
Myoxocephalus scorpius	ulka 6	23-01-2002	Kaldbak	28	332	M	0,21	3,04	4,67	3563	14,06	18,35	9,83		0,183
Myoxocephalus scorpius	Ulka 7	23-03-2002	Kaldbak	25,5	244	F	0,21	3,47	3,09	4497	0,13	33,74	21,41		0,212
Myoxocephalus scorpius	Ulka 8	19-04-2002	Kaldbak	32,5	482	F	0,54	3,45	26,28	6444	0,05	15,37	23,84		0,161
Myoxocephalus scorpius	Ulka 9	22-04-2002	Kaldbak	22	148	M	0,03	1,73	0,48	3208	0,09				0,226
Myoxocephalus scorpius	Ulka 10	22-04-2002	Kaldbak	25	208	M	0,40	2,30	3,08	4584	0,20	4,71	8,77		0,172
Myoxocephalus scorpius	Ulka 11	01-05-2002	Kaldbak	28	430	F	0,15	4,49	4,96	3074	0,10				0,137
Myoxocephalus scorpius	Ulka 12	01-05-2002	Kaldbak	25,5	336	F	0,09	4,77	4,42	2516	0,12				0,134
Myoxocephalus scorpius	Ulka 13	01-05-2002	Kaldbak	24	232	F	0,15	3,43	1,65	2745	0,66				0,137
Myoxocephalus scorpius	Ulka 14	01-05-2002	Kaldbak	16,2	66	F	0,09	0,73	0,62	4138	0,51				0,207
Myoxocephalus scorpius	Ulka 15	06-05-2002	Kaldbak	28	384	F	0,15	5,52	4,55	3031	2,18				0,132
Myoxocephalus scorpius	Ulka 16	06-05-2002	Kaldbak	27	298	F	0,28	4,37	3,23	3087	0,87	21,21	41,75		0,155
Myoxocephalus scorpius	Ulka 17	06-05-2002	Kaldbak	18,5	78	F	0,08	1,16	1,15	5855	14,35	4,52			0,154
Myoxocephalus scorpius	Ulka 18	07-05-2002	Kaldbak	26,5	334	M	0,33	4,14	3,89	4883	0,18	5,88			0,148
Myoxocephalus scorpius	Ulka 19	10-07-2002	Kaldbak	26,6	340	F	0,28	17,90	3,85	4848	0,12	12,94	13,84		0,123
Myoxocephalus scorpius	Ulka 20	10-07-2002	Kaldbak	18,5	90	F	0,07	1,17	0,43	6055	0,10	81,04			0,152
Myoxocephalus scorpius	Ulka 21	10-07-2002	Kaldbak	23	168	M	0,33	2,33	1,40	7448	0,28	22,99	14,95		0,145
Myoxocephalus scorpius	Ulka 22	10-07-2002	Kaldbak	24,5	246	F	0,26	4,39	1,50	4368	3,16	39,25			0,109
Myoxocephalus scorpius	Ulka 23	11-07-2002	Kaldbak	29,5	342	F	0,57	3,11	5,82	6105	0,20	5,34	22,22		
Myoxocephalus scorpius	Ulka 24	11-07-2002	Kaldbak	31	600	M	0,10	13,34	8,08	4035	0,37				0,154
Myoxocephalus scorpius	Ulka 25	12-07-2002	Kaldbak	23,5	200	F	0,24	6,48	1,61	4523	52,20	1,78			
Myoxocephalus scorpius	Ulka 26	12-07-2002	Kaldbak	30	440	F	0,48	6,82	7,52	3391	1,06	6,35	75,30		
Myoxocephalus scorpius	Ulka 27	12-07-2002	Kaldbak	28	378	F	0,50	6,18	4,20	5982	0,35	2,91	21,51		
Myoxocephalus scorpius	Ulka 28	18-07-2002	Kaldbak	24	276	F	-	3,45	2,16	6125	0,20				

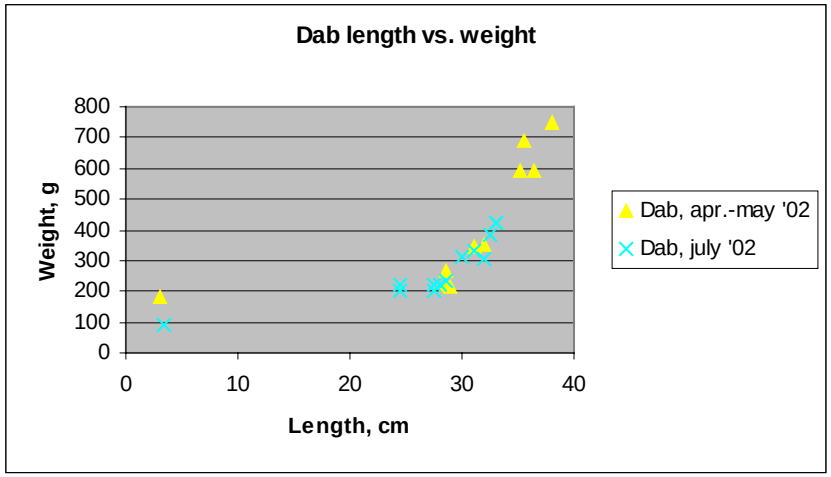
Species	ID	Date	Location	Length (cm)	Weight (g)	Gender	Gall (g)	Liver (g)	Gonads (g)	Protein (µg/ml)	EROD (pmol/min/mg protein)	1-OH-pyrene (µg/kg)	Abs 380nm	Vtg (ng/ml)	CYP1A protein (abs/mg protein)
Limanda limanda	Sandsprøka 31	11-07-2002	Kaldbak	27,5	200	F	0,49	4,70	3,82	4505	3,81	50,11	97,14		
Limanda limanda	Sandsprøka 32	11-07-2002	Kaldbak	27,5	224	M	0,40	4,10	0,65	5321	19,72	14,26	60,74		
Limanda limanda	Sandsprøka 33	11-07-2002	Kaldbak	24,5	220	F	0,19	2,56	1,79	4619	222,75	12,65			
Limanda limanda	Sandsprøka 34	11-07-2002	Kaldbak	31	332	F	0,73	3,68	4,81	4094	64,85	5,26	40,10		
Limanda limanda	Sandsprøka 35	11-07-2002	Kaldbak	32	308	F	0,39	3,12	5,29	4712	420,77	7,79	41,31		
Limanda limanda	Sandsprøka 36	12-07-2002	Kaldbak	28,5	232	M	0,49	4,77	0,35	6001	21,14	55,80	94,63		
Limanda limanda	Sandsprøka 37	16-07-2002	Kaldbak	28	222	M	0,53	2,83	1,78	5360	41,54	13,22	42,22		
Limanda limanda	Sandsprøka 38	16-07-2002	Kaldbak	32,5	386	F	0,56	7,03	29,78	5622	9,29	3,80	18,08		
Limanda limanda	Sandsprøka 39	16-07-2002	Kaldbak	33	420	F	1,10	5,88	6,93	4470	113,53	5,08	29,69		
Limanda limanda	Sandsprøka 7	06-04-2002	Kirkjubø	35,5	692	F	1,11	13,24	68,26	3624	3,22	2,58	3,93		
Limanda limanda	Sandsprøka 8	06-04-2002	Kirkjubø	36,5	592	F	0,57	9,57	94,99	3561	3,05		23,26		
Limanda limanda	Sandsprøka 9	06-04-2002	Kirkjubø	38	750	F		14,01	129,62	5930	1,49				
Limanda limanda	Sandsprøka 10	06-04-2002	Kirkjubø	32	348	M	1,22	2,41	2,40	3827	43,10	2,90	25,96		
Limanda limanda	Sandsprøka 11	06-04-2002	Kirkjubø	28,5	220	M	0,20	2,41	2,34	3788	87,11	3,07			
Limanda limanda	Sandsprøka 12	06-04-2002	Kirkjubø	29	212	M		1,71	1,13	4283	95,63				
Limanda limanda	Sandsprøka 13	06-04-2002	Kirkjubø	28,5	268	M	0,57	2,81	0,36	2734	105,95	18,85	2,60		
Limanda limanda	Sandsprøka 16	11-04-2002	Kirkjubø	31	344	F	0,52	5,09	47,21	4302	5,09	2,23			
Limanda limanda	Sandsprøka 41	23-07-2002	Kirkjubø	35	458	F	0,19	4,86	4,45	4326	205,87				
Limanda limanda	Sandsprøka 42	23-07-2002	Kirkjubø	32,5	432	F	0,87	7,48	6,64	3358	46,66		21,01		
Limanda limanda	Sandsprøka 43	23-07-2002	Kirkjubø	35	466	F	0,41	9,58	7,37	2279	43,45	0,94	21,92		
Limanda limanda	Sandsprøka 44	23-07-2002	Kirkjubø	33,5	358	F	0,88	4,45	5,53	4974	264,61	3,21	13,84		
Limanda limanda	Sandsprøka 45	23-07-2002	Kirkjubø	33	458	F	1,13	12,95	2,99	2887	38,11	1,61	4,08		
Limanda limanda	Sandsprøka 46	23-07-2002	Kirkjubø	33,5	440	F	1,73	11,91	4,01	2678	30,59	1,09	4,08		
Limanda limanda	Sandsprøka 47	23-07-2002	Kirkjubø	32	420	F	1,17	6,72	5,82	4666	52,48				
Limanda limanda	Sandsprøka 48	23-07-2002	Kirkjubø	29,5	306	M	0,46	4,55	-	3639	122,67	2,75	7,75		
Limanda limanda	Sandsprøka 49	23-07-2002	Kirkjubø	30	258	F		2,85	4,25	4110	868,32		7,19		
Limanda limanda	Sandsprøka 50	23-07-2002	Kirkjubø	30	288	F	0,49	3,15	4,58	3711	494,47	11,19			
Limanda limanda	Sandsprøka 51	23-07-2002	Kirkjubø	28,5	204	F	0,20	2,19	0,26	4666	84,86	9,99			
Limanda limanda	Sandsprøka 52	23-07-2002	Kirkjubø	29	300	F	0,02	6,15	2,21	1931	63,60	5,80			
Limanda limanda	Sandsprøka 53	23-07-2002	Kirkjubø	31,5	374	M	0,16	5,92	7,55	3548	67,17	1,16			

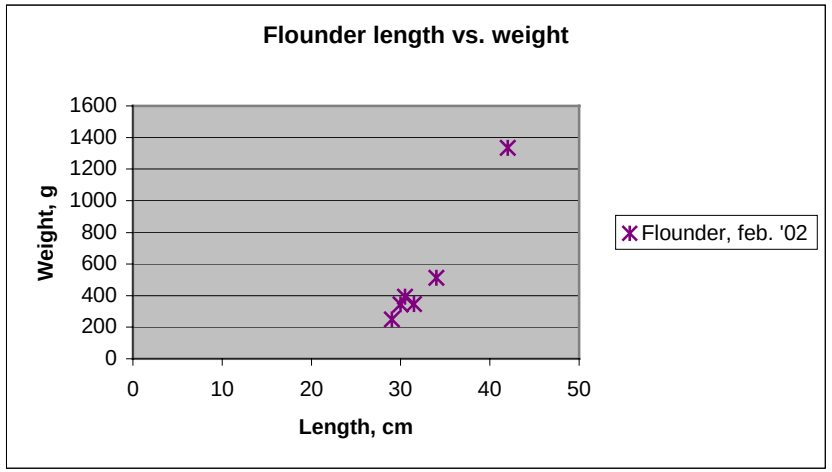
Attachment 7:

Curves of length versus weight for the different fish species:





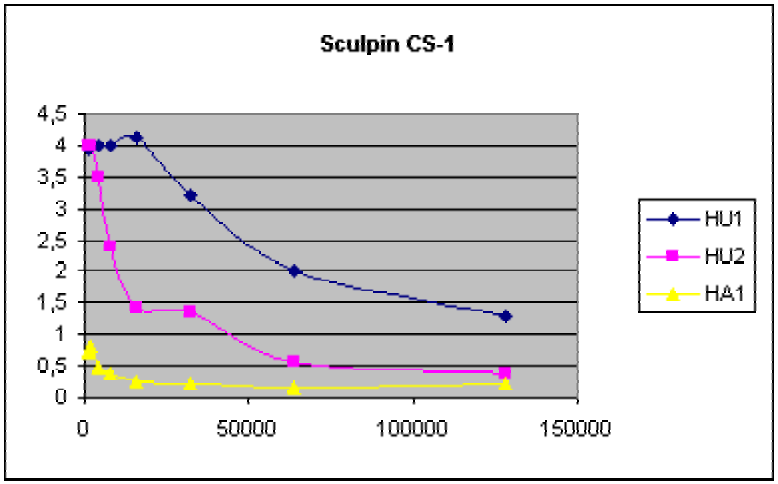


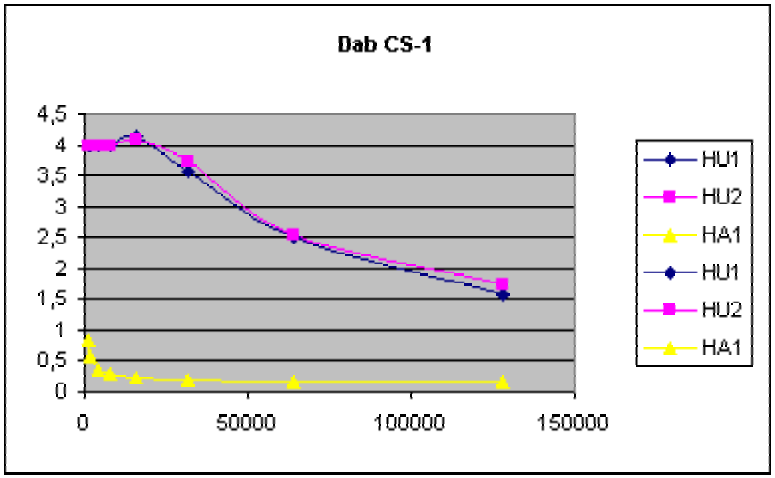


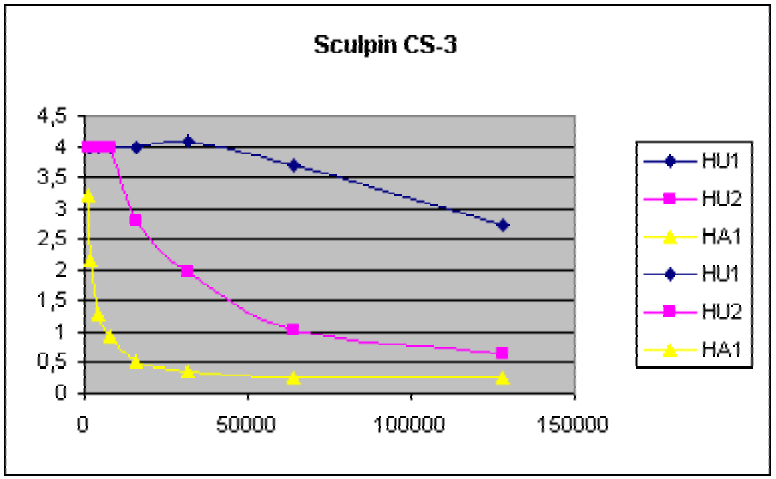
Attachment 8:

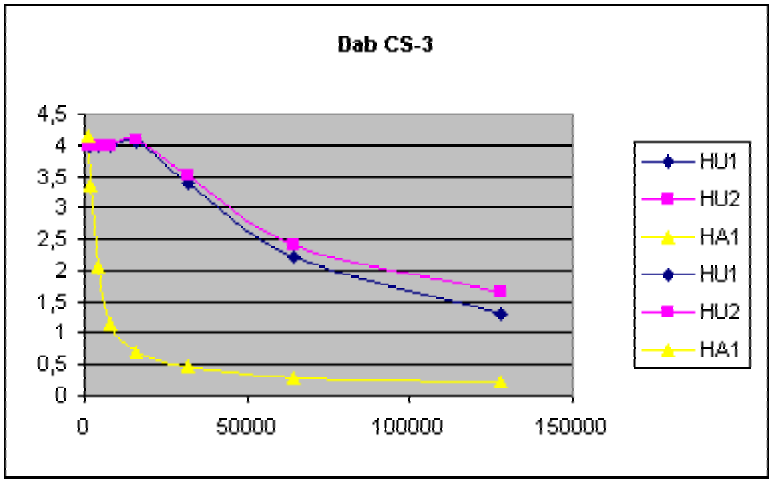
Vitellogenin in sculpin and dab

Optimalization:

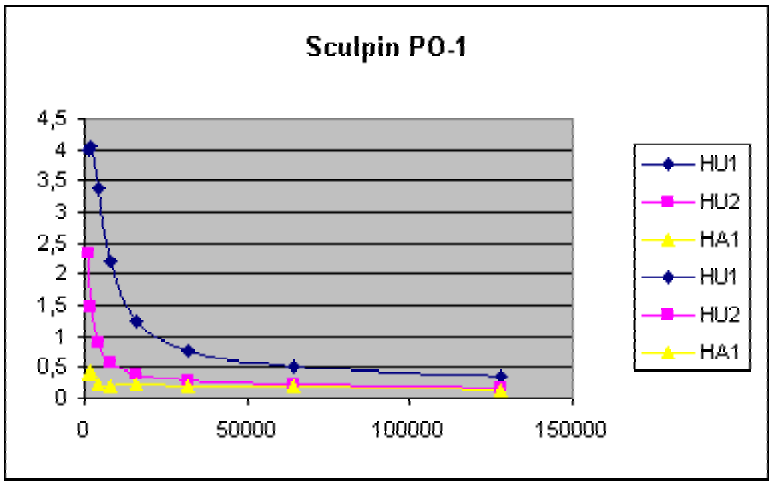


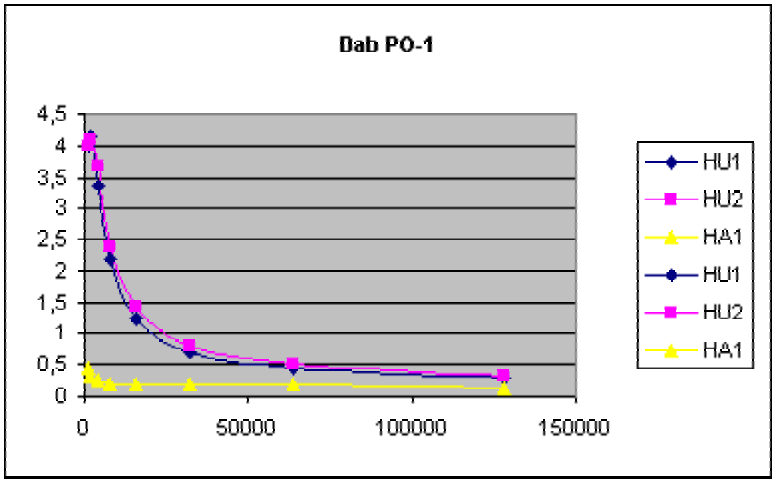


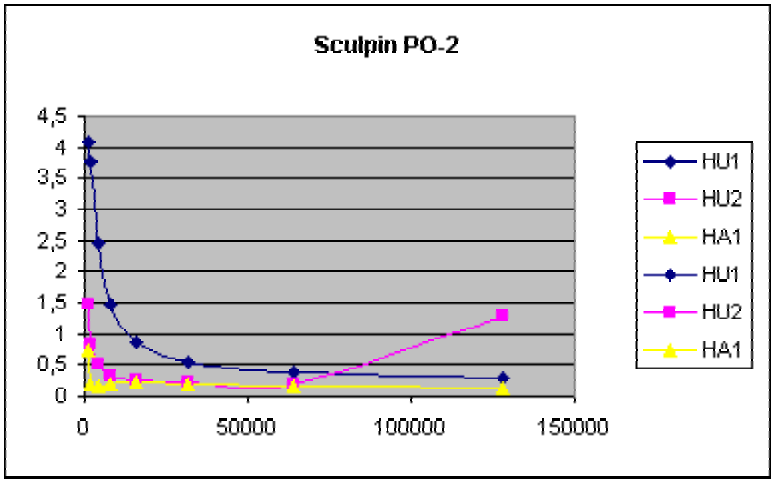


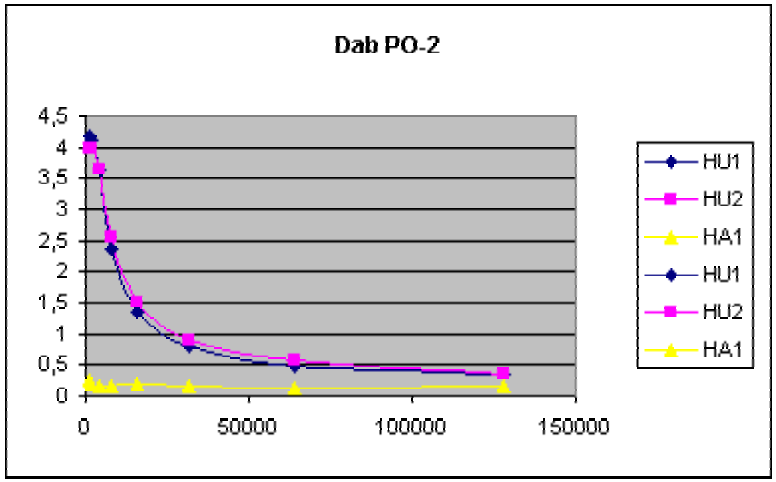


HU1: Reproductionally mature female HU2: Not as reproductionally mature female HA1: Reproductionally immature male



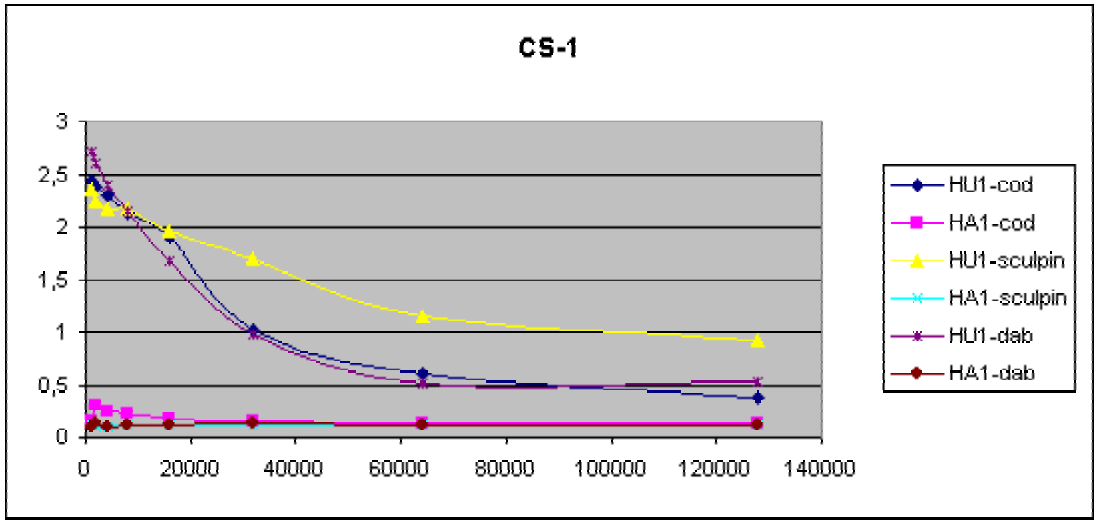


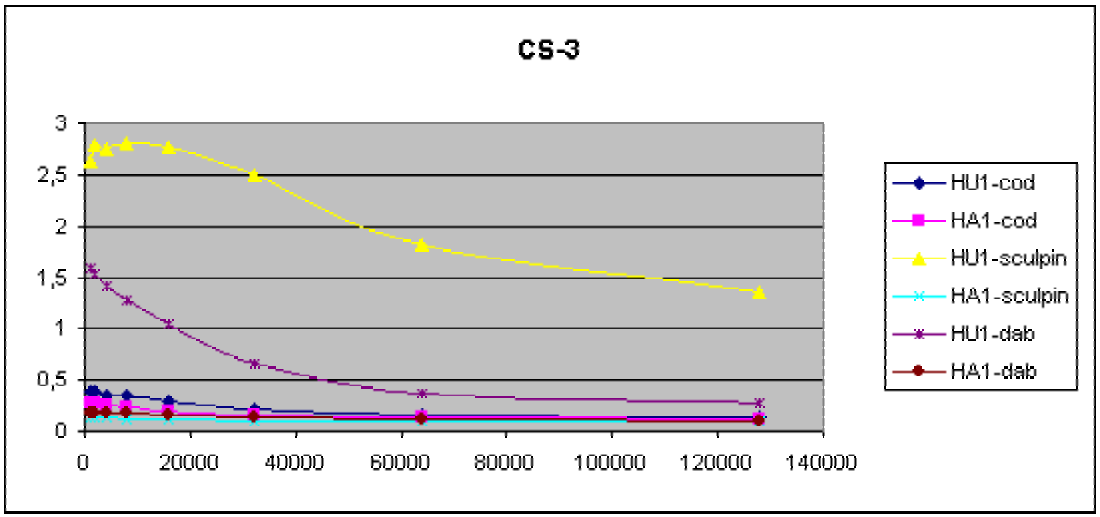




HU1: Reproductionally mature female HU2: Not as reproductionally mature female HA1: Reproductionally immature male

Parallellism:





HU1: Reproductionally mature female HA1: Reproductionally immature male

Attachment 9:

Methods for analyses of PAH and metals in invertebrate samples at NIVA (Norwegian Institute for Water Research).

A: Analysis of dry matter content:

NIVA-metode nr.	Analysevariabel:	Måleenhet:	Labdatakode:
B 3-1	**Totalt tørrstoffinnhold og gløderest**	mg/l, mg/kg	**TTS, TGR, TTS-V, TGR-V**

Tittel:

Bestemmelse av totalt tørrstoffinnhold og dets gløderest i vann, slam, sedimenter og biologisk materiale.

Anvendelsesområde:

Denne metoden benyttes ved bestemmelse av totalt innhold av tørrstoff og dets gløderest i alle typer vann, slam og sedimenter, samt biologisk materiale. I vann er nedre bestemmelsesgrense 0,02 g/l, i faste prøver er grensen avhengig av innveid prøvemengde.

Prinsipp:

Tørrstoffinnholdet bestemmes ved at en kjent mengde prøve tørkes til tørrhet ved 105 °C, og den gjenværende rest veies. Deretter glødes dette ved 550 °C, og den gjenværende rest veies. 550 °C er en hensiktsmessig temperatur for destruering av organisk materiale uten at vesentlige mengder uorganisk stoff går tapt. Gløderesten av tørrstoff for slam, sedimenter og biologisk materiale oppgis på tørrvektbasis.

Instrument(er):

Thermaks 4115 varmeskap, Naber Multitherm N11/R glødeovn, Sartorius R 200 D vekt.
Aluminiumskåler minimum 20 ml.

Måleusikkerhet:

Tre sedimentprøver benyttet til kontroll av repeterbarheten ga følgende standardavvik for TTS og TGR: 2,3 og 5,0, 5,2 og 3,6, 5,7 og 3,5. For TTS-V, 9 bestemmelser ved dobbeltanalyse av naturlig prøve ga middelavvik mellom parallellene på 3,2 %, md standardavvik 5,2 %.

Referanser:

NS 4764. Tørrstoff og gløderest i vann, slam og sedimenter. 1980, 1. utgave.

B: Analysis of PAH:

NIVA-metode nr.	Analysevariabel:	Måleenhet:	Labdatakode:
H 2-4	**Polyaromatiske hydrokarboner**	µg/kg t.v.	**PAH-B, PAH16-B**

Tittel:

Ekstraksjon og opparbeiding av PAH i biologisk materiale.

Anvendelsesområde:

Metoden benyttes for bestemmelse av PAH i biologisk materiale fra det vandige miljø som fisk, muslinger og krabbe. Deteksjonsgrensen avhenger av prøvemengden. Denne metoden benyttes sammen med metode H 2-1.

Prinsipp:

Prøvene tilsettes indre standarder. Biologisk materiale forsåpes først med KOH/metanol. Deretter ekstraheres PAH med pentan. Ekstraktene gjennomgår så ulike renseprosesser for å fjerne forstyrrende stoffer. Tilslutt analyseres ekstraktet med GC/MSD. PAH identifiseres med MSD ut fra retensjonstider og forbindelsenes molekylioner. Kvantifisering utføres ved hjelp av de tilsatte indre standarder.

Instrument(er):

Hewlett Packard modell 5890 Series II, med column injector og HP autosampler 7673. Systemet er utstyrt med HD modell 5970 B masseselektiv detektor, og kolonne HP-5 MS 30 m x 0,25 mm i.d. x 0,25 µm.

Måleusikkerhet:

Se NIVA-dokument nr. Y – 3.

Referanser:

Grimmer, G. og Bøhnke, H., 1975. Jour. of the AOAC, Vol. 58, No. 4.

C: Analysis of heavy metals:

NIVA-metode nr.	Analysevariabel:	Måleenhet:	Labdatakode:
E 8-3	**Metaller, -MS**	µg/l	**Me/MS**

Tittel:

Grunnstoffbestemmelse med ICP-MS.

Anvendelsesområde:

Metoden angir bestemmelse av en rekke elementer i ferskvann, salpetersyreoppsluttet biota og sedimenter: Li, (Be, B, Na, Mg), Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, (Ga, Ge), As, Se, (Rb), Sr, (Y, Zr), Nb, Mo, Ag, Cd, (In), Sn, Sb, (Cs), Ba, (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Ta, W), Re, Tl, Pb, Bi, Th og U, de i parentes er ikke akkreditert. Konsentrasjonsområdet for metoden for de ulike elementene uten fortynning av prøven kan fås oppgitt ved laboratoriet.

Prinsipp:

Prøver konservert med salpetersyre introduseres med en peristaltisk pumpe og overføres til en aerosol i forstøveren. Denne blir ført til argonplasmaet som atomiserer og ioniserer prøven. Etter plasmaet passerer prøven to seriekoblete koner i et område med redusert trykk hvor plasmagassen fjernes. Ionestrømmen fokuseres med en elektrisk ionelinse før den introduseres til det kvadrupole massespektrometeret for separasjon basert på masse/ladningsforholdet. Ionene måles med en pulstellingsdetektor basert på en diskret dynode multiplikator.

Instrument(er):

Perkin-Elmer Sciex ELAN 6000 ICP-MS, utstyrt med P-E autosampler AS-90, AS-90b prøvebrett og P-E Rinsing Port Kit.

Måleusikkerhet:

Se NIVA-dokument Y-3.

Referanser:

Perkin-Elmer: ELAN 6000 Hardware Guide, Part No. 0993-8969, og ELAN 6000 Software Guide, Part No. 0993-8968.

D: Analysis of mercury:

NIVA-metode nr.	Analysevariabel:	Måleenhet:	Labdatakode:
E 4-3	**Kvikksølv**	ng/l, µg/g	**Hg/L, Hg-Sm, Hg-B, Hg/H**

Tittel:

Bestemmelse av kvikksølv i vann, slam og sedimenter og biologisk materiale med Perkin-Elmer FIMS-400.

Anvendelsesområde:

Metoden omfatter bestemmelse av kvikksølv i renvann, samt avløpsvann, biologisk materiale slam og sedimenter oppsluttet i salpetersyre. Biologiske prøver, slam og sediment frysetørres fortrinnsvis. Ved tørking av prøver i varmeskap må ikke temperaturen overstige 80°C. Nedre grense er for renvann 1,0 ng/l, oppsluttet renvann 10 ng/l, avløpsvann 0,1 µg/l, faste prøver 0,005 µg/g.

Prinsipp:

Kvikksølv må foreligge på ionisk form i prøveløsningen for at kalddampteknikk skal kunne benyttes. Når reduksjonsmiddelet ($SnCl_2$) blandes med prøven blir det ioniske kvikksølvet omformet til metallisk kvikksølv (Hg). En inert bæregass (argon) transporterer kvikksølvet til spektrofotometeret. En fordel med denne teknikken er den gode separasjonen av analytten fra matrisen, slik at ikke-spesifikk bakgrunnsabsorpsjon og matriseinterferenser er minimale. Kvikksølvet oppkonsentreres i et amalgameringssystem.

Instrument(er):

Perkin-Elmer FIMS-400 med P-E AS-90 autosampler og P-E amalgamsystem.

Måleusikkerhet:

6 målinger av Drøbaksjøvann tilsatt 20 ng/l Hg ga middelverdi 21,1 og standardavvik 0,52 ng/l. Tilsvarende for faste materialer: 10 målinger av DORM-1 (fiskemuskel) 0,798 ± 0,074 µg/g, ga 0,835 og 0,054 µg/g, 7 målinger av MESS-2 (sediment) 0,092 ± 0,009 µg/g, ga 0,086 og 0,003 µg/g.

Referanser:

B. Welz, M. Melcher, H.W. Sinemus, D. Maier: Pico-trace determination of mercury using the amalgamation technique. Norsk Standard, NS 4768. Vannundersøkelse. Bestemmelse av kvikksølv ved kalddamp atomabsorpsjonsspektrometri Oksidasjon med salpetersyre. 1. Utg. 1989.

E: Analysis of cromium:

NIVA-metode nr.	Analysevariabel:	Måleenhet:	Labdatakode:
E 2-5	**Metaller, grafittovn atomabsorpsjon**	µg/l	**Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sr, V, Zn**

Tittel:

Bestemmelse av metaller ved atomabsorpsjon – atomisering i Perkin-Elmer AAnalyst 700 grafittovn.

Anvendelsesområde:

Denne metoden skal benyttes når metallkonsentrasjonene i løsningene er så lave at de ikke kan bestemmes ved atomisering i flamme uten oppkonsentrering. Prøvene kan være naturlig vann, ekstrakter, eller oppslutninger av slam, sedimenter og biologisk materiale. Tabell 1 angir nedre og øvre grense (µg/l) for bestemmelse av de enkelte metaller med grafittovn, når det injiseres et prøvevolum på 20 µl direkte i grafittrøret.

Prinsipp:

En passende mengde prøve (20-50 µl), konservert med salpetersyre, overføres til et grafittrør som oppvarmes elektrotermisk. Ved trinnvis øking av temperaturen etter et program tilpasset for hvert enkelt metall, gjennomføres tørking, foraskning og atomisering. Som lyskilde benyttes en hulkatodelampe, der katoden inneholder det metallet som skal bestemmes, eller en elektrodeløs lampe (EDL). Lampene avgir et linjespektrum som er spesifikt for lampen og det metallet som skal bestemmes. Lyset absorberes selektivt av dette elementets atomer når det passerer gjennom den atomiserte prøven. Metallkonsentrasjonen bestemmes ved å jevnføre prøvens absorbans med kjente kalibreringsløsningers absorbans.

Instrument(er):

Perkin-Elmer atomabsorpsjonsspektrometer AAnalyst 700, tilkoblet grafittovn av typen HGA og prøveveksler AS 800. Instrumentet styres med Dell Pentium 2 PC, og resultater skrives ut på en Laserjet 1100.

Måleusikkerhet:

Se NIVA-dokument Y-3.

Referanser:

Norsk Standard, NS 4780. Metalller i vann, slam og sedimenter. Elektrotermisk atomisering i grafittovn. Generelle prinsipper og retningslinjer. 1. Utg. 1988. NS 4781.

Attachment 10A:

Invertebrate biological data

Species	ID of pooled sample	Date	Location	n	Sample weight, g	Mean length, cm	Dry matter	Lipid pr.w.w.
							%	%
Littorina obtusata	L. Hv. 1A	29.01.02	Hvannasund	100	25,9	1,2-1,8*	27,9	0,41
Littorina obtusata	L. Hv. 2A	09.04.02	Hvannasund	100	30,4	1,2-1,7*	21,2	1,28
Littorina obtusata	L. Hv. 3A	22.07.02	Hvannasund	100	30,8	1,3-1,7*	22,9	1,59
Littorina obtusata	L. Sv. 3A	05.07.02	Svínáir	100	37,8	1,2-1,8*	24,6	1,51
Nucella lapillus	Nl. Hv. 1A	29.01.02	Hvannasund	35	29,4	2,8	25,3	2,33
Nucella lapillus	Nl. Hv. 1B	29.01.02	Hvannasund	37	22,7	2,5	24,8	2,48
Nucella lapillus	Nl. Hv. 2A	09.04.02	Hvannasund	52	45,3	2,9	21	1,99
Nucella lapillus	Nl. Hv. 2B	09.04.02	Hvannasund	50	34,6	2,7	21,5	1,89
Nucella lapillus	Nl. Hv. 3A	22.07.02	Hvannasund	46	44,4	2,9	21,4	2,05
Nucella lapillus	Nl. Hv. 3B	22.07.02	Hvannasund	46	35,1	2,6	22,1	2,45
Nucella lapillus	Nl. Sv. 3A	05.07.02	Svínáir	32	72,9	3,7	23,5	2,47
Nucella lapillus	Nl. Sv. 3B	05.07.02	Svínáir	33	34,7	2,9	25,8	2,4
Patella vulgata	Pv. Hv. 1A	29.01.02	Hvannasund	30	179,0	4,7	16,1	0,83
Patella vulgata	Pv. Hv. 1B	29.01.02	Hvannasund	30	190,9	4,7	15,7	0,87
Patella vulgata	Pv. Hv. 2A	09.04.02	Hvannasund	30	185,7	4,7	15,5	0,71
Patella vulgata	Pv. Hv. 2B	09.04.02	Hvannasund	30	153,5	4,4	16,5	1
Patella vulgata	Pv. Hv. 3A	22.07.02	Hvannasund	28	214,6	4,9	17,1	1,65
Patella vulgata	Pv. Hv. 3B	22.07.02	Hvannasund	30	150,4	4,4	16,6	1,25
Patella vulgata	Pv. Ve. 1A	19.12.01	Velbastaður	30	161,7	4,4	15,8	1,03
Patella vulgata	Pv. Ve. 1B	19.12.01	Velbastaður	35	132,3	4,0	15	0,83
Patella vulgata	Pv. Ve. 2A	19.03.02	Velbastaður	22	178,9	5,0	15,7	0,89
Patella vulgata	Pv. Ve. 2B	19.03.02	Velbastaður	22	128,1	4,6	15,4	0,78
Patella vulgata	Pv. Ve. 3A	05.07.02	Velbastaður	25	152,2	4,4	17,9	1,34
Patella vulgata	Pv. Ve. 3B	05.07.02	Velbastaður	25	122,8	4,1	17,8	1,35
Patella vulgata	Pv. Sv. 1A	25.01.02	Svínáir	25	175,2	4,7	16,6	1,34
Patella vulgata	Pv. Sv. 1B	25.01.02	Svínáir	25	123,6	4,3	16,9	1,34
Patella vulgata	Pv. Sv. 2A	19.03.02	Svínáir	25	174,6	5,0	17,2	1,09
Patella vulgata	Pv. Sv. 2B	19.03.02	Svínáir	25	132,7	4,5	16,6	0,99
Patella vulgata	Pv. Sv. 3A	05.07.02	Svínáir	19	132,4	4,6	19	1,64
Patella vulgata	Pv. Sv. 3B	05.07.02	Svínáir	20	98,5	4,2	18,4	1,42
Patella vulgata	Pv. Tr. 1A	27.01.02	Trongisvágur	25	187,8	4,9	16,4	1
Patella vulgata	Pv. Tr. 1B	27.01.02	Trongisvágur	26	128,7	4,3	17,3	1,09
Patella vulgata	Pv. Tr. 2A	18.05.02	Trongisvágur	20	120,2	4,6	18,9	0,94
Patella vulgata	Pv. Tr. 2B	18.05.02	Trongisvágur	20	93,1	4,3	20,5	1,03
Patella vulgata	Pv. Tr. 3A	20.07.02	Trongisvágur	25	121,2	4,3	18,8	1,25
Patella vulgata	Pv. Tr. 3B	20.07.02	Trongisvágur	25	91,9	4,0	18,7	1,14
Mytilus edulis	Me. Hv. 1A	29.01.02	Hvannasund	7	30,9	5,8	16,8	2,25
Mytilus edulis	Me. Hv. 1B	29.01.02	Hvannasund	10	15,6	4,3	16,3	2,53
Mytilus edulis	Me. Hv. 2A	09.04.02	Hvannasund	19	156,5	6,4	16,2	2,08
Mytilus edulis	Me. Hv. 2B	09.04.02	Hvannasund	23	63,0	4,6	14,9	1,36
Mytilus edulis	Me. Sv. 1A	25.01.02	Svínáir	25	89,7	5,2	18	2,87
Mytilus edulis	Me. Sv. 1B	25.01.02	Svínáir	25	45,5	4,4	17,9	2,6
Mytilus edulis	Me. Sv. 2A	19.03.02	Svínáir	25	95,8	5,4	15,6	2,06
Mytilus edulis	Me. Sv. 2B	19.03.02	Svínáir	25	57,4	4,7	15,1	2,03
Mytilus edulis	Me. Sv. 3A	05.07.02	Svínáir	25	68,7	4,7	18,4	2,64
Mytilus edulis	Me. Sv. 3B	05.07.02	Svínáir	25	51,9	4,3	18,1	2,75

Species	ID of pooled sample	Date	Location	n	Sample weight, g	Mean length, cm	Dry matter	Lipid pr.w.w.
Mytilus edulis	Me. Ka. 1A	02.01.02	Kaldbak	25	50,0	4,4	16,7	2,22
Mytilus edulis	Me. Ka. 1B	02.01.02	Kaldbak	25	37,4	4,2	17,3	1,85
Mytilus edulis	Me. Ka. 2A	22.03.02	Kaldbak	25	95,4	5,2	18,9	2,08
Mytilus edulis	Me. Ka. 2B	22.03.02	Kaldbak	25	41,7	4,0	16,6	1,6
Mytilus edulis	Me. Ka. 3A	16.07.02	Kaldbak	15	82,1	6,3	18,8	2,29
Mytilus edulis	Me. Ka. 3B	16.07.02	Kaldbak	15	51,1	5,5	18,8	2,21
Mytilus edulis	Me. Tr. 1A	27.01.02	Trongisvágur	25	49,4	4,6	14,5	1,64
Mytilus edulis	Me. Tr. 1B	27.01.02	Trongisvágur	26	35,4	4,2	14,5	1,52
Mytilus edulis	Me. Tr. 2A	18.05.02	Trongisvágur	25	84,2	5,2	15,6	1,72
Mytilus edulis	Me. Tr. 2B	18.05.02	Trongisvágur	25	52,0	4,3	16,5	1,94
Mytilus edulis	Me. Tr. 3A	20.07.02	Trongisvágur	25	66,4	4,9	15,2	1,79
Mytilus edulis	Me. Tr. 3B	20.07.02	Trongisvágur	25	43,4	4,2	15,6	1,46
Modiolus modiolus	Mm. Ki. 1A	05.02.02	Kirkjubø	11	111,8	8,3	17,4	1,6
Modiolus modiolus	Mm. Ki. 1B	05.02.02	Kirkjubø	10	94,0	7,1	20	1,87
Modiolus modiolus	Mm. Ki. 2A	18.05.02	Kirkjubø	12	240,5	9,6	16,8	1,93
Modiolus modiolus	Mm. Ki. 2B	18.05.02	Kirkjubø	16	209,5	8,0	17,8	1,52
Modiolus modiolus	Mm. Ki. 3A	11.08.02	Kirkjubø	8	149,9	9,3	14,2	1,23
Modiolus modiolus	Mm. Ki. 3B	11.08.02	Kirkjubø	11	141,6	7,7	16,4	1,76

*range

n=Number of individuals in pooled sample.

Attachment 10B:

PAH in invertebrate tissue (µg/kg v.v):

	ID	Naftalen	2-Metylnaftalen	1-Metylnaftalen	Bifenyl	2,6-Dimetylnaftalen	Acenaftylen	Acenaften	2,3,5-Trimetylnaftalen	Fluoren	Fenantren	Antracen	1-Metylfenantren	Fluoranten	Pyren	Benz(a)antracen	Chrysen+trifenylen	Benzo(b+j,k)fluoranten	Benzo(e)pyren	Benzo(a)pyren	Perylene	Indeno(1,2,3cd)pyren	Dibenz(a,c7a,h)antracen	Benzo(ghi)perylen	Sum PAH	Sum KPAH
	Rings	2	2	2	2	2	2	2	2	2 (3)	3	3	3	3(4)	4	4	4+4	4	5	5	5	5(6)	6	6		
Littorina obtusata	L. Hv. 1A	3,2	4,1	1,5	<0,5	0,84	<0,5	0,62	1	0,5	8,9	0,85	7,5	2,4	15	0,71	1,3	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	48,42	0,71
	L. Hv. 2A	2,2	2,3	0,98	<0,5	<0,5	<0,5	2,1	<0,5	<0,5	4	<0,5	2,4	0,59	3,1	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	17,67	0
	L. Hv. 3A	<1,1	0,98	<0,5	<0,5	<0,5	<0,5	1,5	<0,5	<0,5	1,7	<0,5	0,87	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	5,05	0
	L. Sv. 3A	6	1,6	0,7	<0,5	<0,5	<0,5	1,7	<0,5	<0,5	0,73	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	10,73	0
Nucella lapillus	Nl. Hv. 1A	7,6	4,6	3,3	1,2	0,93	<0,5	<0,5	2,3	1,4	7,8	<0,5	3,8	1,7	3,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	38,13	0
	Nl. Hv. 1B	<3,0	4,9	3,2	1,1	1,6	<0,5	<0,5	3	1,6	9,1	0,52	5,1	2,1	3,2	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	35,42	0
	Nl. Hv. 2A	2,1	2,1	2	<0,5	<0,5	<0,5	<0,5	0,68	<0,5	4,6	<0,5	1,7	0,95	0,53	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	14,66	0
	Nl. Hv. 2B	1,1	1,4	1,4	<0,5	<0,5	<0,5	<0,5	0,67	<0,5	3,2	<0,5	1,2	0,83	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	9,8	0
	Nl. Hv. 3A	<1,1	1,5	1,7	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	3,4	<0,5	1,2	1,1	0,69	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	9,59	0
	Nl. Hv. 3B	0,67	1,4	1,2	<0,5	<0,5	<0,5	<0,5	0,54	<0,5	3,4	<0,5	1,3	1,4	0,97	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	10,88	0
	Nl. Sv. 3A	0,8	0,74	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,73	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	2,27	0
	Nl. Sv. 3B	<1,4	0,95	0,54	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	1,4	<0,5	<0,5	<0,5	<0,6	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	2,89	0
Patella vulgata	Pv. Hv. 1A	0,65	0,83	<0,5	<0,5	1,1	<0,5	<0,5	<0,5	<0,5	3,1	<0,5	5,3	<0,5	2,8	<0,5	0,71	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	14,49	0
	Pv. Hv. 1B	0,98	0,58	<0,5	<0,5	0,61	<0,5	<0,5	1,1	0,53	3,7	<0,5	8,4	<0,5	4,8	0,51	1,6	<1,0	0,73	<0,5	<0,5	<0,5	<0,5	<0,5	23,54	0,51
	Pv. Hv. 2A	0,65	0,67	<0,5	<0,5	0,62	<0,5	0,62	<0,5	<0,5	1,9	<0,5	3,9	<0,5	1,9	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	10,26	0
	Pv. Hv. 2B	0,71	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,71	0
	Pv. Hv. 3A	<0,6	<0,5	<0,5	<0,5	<0,5	<0,5	0,66	<0,5	<0,5	1,1	<0,5	2	<0,5	1,2	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	4,96	0
	Pv. Hv. 3B	<0,6	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	1,2	<0,5	2,8	<0,5	1,3	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	5,3	0
	Pv. Ve. 1A	1,1	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	1,1	0
	Pv. Ve. 1B	2,7	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	2,7	0
	Pv. Ve. 2A	<0,7	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0	0
	Pv. Ve. 2B	<0,7	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0	0
	Pv. Ve. 3A	<0,7	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0	0
	Pv. Ve. 3B	<0,7	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0	0
	Pv. Sv. 1A	4,5	1,1	0,67	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,64	<0,5	0,99	<0,5	0,82	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	8,72	0
	Pv. Sv. 1B	6,4	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,59	<0,5	1,1	<0,5	0,69	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	8,78	0
	Pv. Sv. 2A	0,7	0,6	0,6	<0,5	<0,5	<0,5	0,6	<0,5	<0,5	0,5	<0,5	0,8	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	3,8	0
	Pv. Sv. 2B	0,83	0,7	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,71	<0,5	<0,7	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	2,24	0
	Pv. Sv. 3A	<0,7	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,63	<0,5	<0,7	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,63	0
	Pv. Sv. 3B	<0,7	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,7	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0	0

	ID	Naftalen	2-MetylNaftalen	1-MetylNaftalen	Bifenyl	2,6-Dimetyl naftalen	Acenaftylen	Acenaften	2,3,5-Trimetyl naftalen	Fluoren	Fenantren	Antracen	1-Metylfenantren	Fluoranten	Pyren	Benz(a)antracen	Chrysen+ trifenylen	Benzo(b+j,k) fluoranten	Benzo(e)pyren	Benzo(a)pyren	Perylene	Indeno(1,2,3cd) pyren	Dibenz(a,c7a,h) antracen	Benzo(ghi) perylen	Sum PAH	Sum KPAH
	Rings	2	2	2	2	2	2	2	2	2 (3)	3	3	3	3(4)	4	4	4+4	4	5	5	5	5(6)	6	6		
Patella vulgata	Pv. Tr. 1A	0,96	0,84	0,67	<0,5	0,74	<0,5	<0,5	0,71	0,57	4,5	<0,5	5,9	1,1	4,3	0,79	<0,5	<1,0	1,4	<0,5	<0,5	<0,5	<0,5	<0,5	22,48	0,79
	Pv. Tr. 1B	3,3	1,1	0,8	<0,5	0,77	<0,5	<0,5	0,79	0,6	5,4	0,5	4,78	0,86	5,4	0,92	2,1	<1,0	2,2	<0,5	<0,5	<0,5	<0,5	0,65	30,17	0,92
	Pv. Tr. 2A	0,92	2,3	1	<0,5	4,6	<0,5	<0,5	17	3,5	19	1,8	37	3,5	21	1	4,6	<1,0	1,9	<0,5	<0,5	<0,5	<0,5	0,53	119,7	1
	Pv. Tr. 2B	1,7	3,4	1,4	<0,5	9,7	<0,5	<0,5	14	3,6	25	2	44	4,3	23	1	5,5	1,5	2	<0,5	<0,5	<0,5	<0,5	0,64	142,7	2,5
	Pv. Tr. 3A	0,82	1,8	1,5	0,84	2,1	<0,5	1,3	1,1	1,6	8,9	0,65	10	3	5,6	0,95	2,5	1,9	2,4	<0,5	<0,5	0,84	<0,5	0,86	48,66	3,69
	Pv. Tr. 3B	2	2	1,5	0,82	1,2	<0,5	1,3	0,97	1,6	11	0,96	11	2	5,7	1	2,4	<1,0	2,4	<0,5	<0,5	<0,5	<0,5	0,68	48,53	1
Mytilus edulis	Me. Hv. 1A	1,9	5	3,3	4,3	15	4,5	0,64	23	7,7	29	5,3	59	28	84	13	15	18	14	3,5	4,1	3,8	0,64	4	346,7	38,9
	Me. Hv. 1B	2,6	4,2	2,4	2,5	7,2	2,8	1,3	18	7,3	28	4,9	62	36	98	20	23	21	18	6	5,4	6,5	0,96	5,4	383,5	54,5
	Me. Hv. 2A	2,4	4,1	2,5	2,1	5,9	2,4	0,56	11	4,5	17	3,3	45	17	50	7,6	9,5	9,7	8,4	2	2,8	1,8	<0,5	2,1	211,7	21,1
	Me. Hv. 2B	1,7	2,7	1,6	1,4	2,7	1,4	<0,5	6,6	3,1	11	2,2	26	12	31	5,7	7,9	8,1	6,5	1,7	1,9	1,9	<0,5	1,8	138,9	17,4
	Me. Sv. 1A	0,62	1,4	0,9	1,2	4,6	0,8	<0,5	10	3,5	12	2,6	24	7,9	23	2,1	4,1	5,4	4,7	0,73	2,1	1,1	<0,5	1,7	114,5	9,33
	Me. Sv. 1B	0,84	1,4	0,88	1,4	3,7	0,8	<0,5	12	3,4	13	2,2	25	7,8	24	2,3	4	6	5,1	0,68	2,3	1,3	<0,5	1,7	119,8	10,3
	Me. Sv. 2A	0,65	1,6	1,1	1,2	1,4	0,53	<0,5	2,2	1,2	3,9	0,7	6,3	3,2	5	0,78	1,6	1,8	2,2	<0,5	0,9	<0,5	<0,5	0,65	36,91	2,58
	Me. Sv. 2B	0,57	1,5	0,98	1	1,7	0,58	<0,5	2,3	1,3	4	1,1	7,4	3,7	5,8	1	1,8	2,8	2,8	<0,5	1,2	0,56	<0,5	0,75	42,84	4,36
	Me. Sv. 3A	2,3	0,95	0,54	<0,5	<0,5	<0,5	<0,5	<0,5	0,64	1,2	<0,5	0,87	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	6,5	0
	Me. Sv. 3B	2,4	0,89	0,52	<0,5	<0,5	<0,5	<0,5	<0,5	0,61	1,3	<0,5	0,91	0,51	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	7,14	0
	Me. Ka. 1A	0,73	1,4	0,84	1,3	4,4	<0,5	<0,5	7	2,3	4,8	1,1	8,9	5,2	10	1,3	<0,5	2,4	2,4	<0,5	0,76	<0,5	<0,5	0,69	55,52	3,7
	Me. Ka. 1B	0,72	2	1,1	1,4	4,2	1,2	2,3	4,7	9	18	5,4	12	38	32	7,1	4,9	6,2	4	0,8	1,3	0,88	<0,5	1,3	158,5	15
	Me. Ka. 2A	1,4	2,7	1,7	1,5	7,6	s0,76	s0,52	9,7	4,4	12	2,5	16	24	23	4	4,3	4	3,2	<0,5	0,73	<0,5	<0,5	<0,5	s124,01	8
	Me. Ka. 2B	m	m	m	m	m	m	0,61	14	6,6	11	2,5	14	22	22	4,8	5	3,9	2,8	<0,5	0,66	<0,5	<0,5	<0,5	109,9	8,7
	Me. Ka. 3A	4	12	8	15	42	23	<0,5	3,7	2,2	3,3	<0,5	6,3	2,6	3,5	0,52	0,86	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	127	0,52
	Me. Ka. 3B	1,3	1,3	0,72	0,6	1,4	0,5	<0,5	2,2	1,1	3,5	0,89	7,6	2,6	3,2	<0,5	0,71	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	27,62	0
	Me. Tr. 1A	1,4	5,6	3,3	3	8,2	5,6	2,2	9,6	19	72	15	33	140	110	43	29	49	27	7,7	5,5	4,4	1,5	4,8	599,8	106
	Me. Tr. 1B	<1,0	4,2	2,2	2,2	6,6	1,3	1,7	8,2	12	56	12	27	98	83	34	26	51	21	8,2	5,7	6,3	1,2	5,6	473,4	101
	Me. Tr. 2A	<1,0	7,5	4,3	6,1	70	i	i	150	66	200	23	400	60	220	12	31	9	9	1,1	2,5	1,3	<0,5	1,4	1274	23,4
	Me. Tr. 2B	2,1	9,5	5,5	7,5	87	12	i	210	77	220	28	440	65	240	12	34	10	9,8	1,6	2,4	1,2	<0,5	1,5	1476	24,8
	Me. Tr. 3A	1,1	8,9	6,3	5,5	12	1,2	5,2	12	13	40	3	37	23	23	3,6	3,1	2	2,7	<0,5	0,79	<0,5	<0,5	0,81	204,2	5,6
	Me. Tr. 3B	4,9	6,9	5,7	4,3	8,4	s0,82	6,1	17	13	50	3,7	42	24	24	2,6	4,3	2,3	3	0,55	1	0,53	<0,5	1	s226,1	5,98
Modiolus modiolus	Mm. Ki. 1A	10	3,5	2,8	0,89	0,59	<0,5	1,1	<0,5	1,5	2,1	<0,5	<0,5	0,75	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	23,23	0
	Mm. Ki. 1B	5,9	4,7	3,8	1,1	1,1	<0,5	1,5	<0,5	2,3	3	<0,5	<0,5	0,74	0,61	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	24,75	0
	Mm. Ki. 2A	0,86	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,69	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	1,55	0
	Mm. Ki. 2B	0,89	0,9	0,65	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,54	<0,5	<0,5	0,53	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	3,51	0
	Mm. Ki. 3A	1,1	2,4	1,6	1,1	0,82	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	7,02	0
	Mm. Ki. 3B	1,3	0,64	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<1,0	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	2,44	0

Grey shaded: PAH compounds with potential carcinogen properties for humans according to IARC (1987). Listed in IARC's categories 2A or 2B (likely or potential carcinogens) - KPAHs

Attachment 10C:

Metals in invertebrate tissue

	ID	Dry matter %	Lipid-% % pr.w.w.	Ag µg/g w.w.	As µg/g w.w.	Ba µg/g w.w.	Cd µg/g w.w.	Cr µg/g w.w.	Cu µg/g w.w.	Hg µg/g w.w.	Ni µg/g w.w.	Pb µg/g w.w.	Sr µg/g w.w.	Zn µg/g w.w.
Littorina obtusata	L. Hv. 1A	27,9	0,41	0,095	7,93	0,284	0,82	1,17	48,8	0,021	s0,052	0,48	77,1	36,1
	L. Hv. 2A	21,2	1,28	0,14	5,98	0,144	1,8	0,69	20,9	0,026	0,56	0,39	47	20,4
	L. Hv. 3A	22,9	1,59	0,164	6,4	0,0905	1,98	0,16	16,3	0,021	0,436	0,11	36,8	24,9
	L. Sv. 3A	24,6	1,51	0,094	11	0,242	1,16	0,72	45,9	0,012	s0,179	0,09	59,2	17,9
Nucella lapillus	Nl. Hv. 1A	25,3	2,33	0,136	9,75	0,118	9,95	0,84	25,3	0,073	s0,253	0,16	35,5	114
	Nl. Hv. 1B	24,8	2,48	0,139	10,8	0,207	8,43	0,34	28,8	0,076	<0,0026	0,17	51,8	111
	Nl. Hv. 2A	21	1,99	0,183	13,4	0,127	12,5	0,2	30,3	0,079	<0,0026	0,14	54	113
	Nl. Hv. 2B	21,5	1,89	0,16	11	0,0986	10	0,34	30,8	0,081	<0,0025	0,13	44,5	113
	Nl. Hv. 3A	21,4	2,05	0,12	9,31	0,114	8,18	0,24	30,9	0,066	s0,023	0,09	33,8	123
	Nl. Hv. 3B	22,1	2,45	0,081	8,45	0,197	6,25	0,32	24,3	0,051	s0,029	0,09	33,5	117
	Nl. Sv. 3A	23,5	2,47	0,048	29,4	0,115	1,79	0,3	25,9	0,042	0,118	0,12	14,4	87,2
	Nl. Sv. 3B	25,8	2,4	0,03	21,3	0,144	0,932	0,17	22,1	0,04	s0,133	0,13	16,9	93,2
Patella vulgata	Pv. Hv. 1A	16,1	0,83	0,05	4,42	0,183	1,59	0,15	1,32	0,028	0,194	0,46	15,8	24,6
	Pv. Hv. 1B	15,7	0,87	0,039	4,71	0,0633	0,971	0,24	1,44	0,038	0,336	0,19	11,5	28
	Pv. Hv. 2A	15,5	0,71	0,055	4,07	0,0617	1,56	0,09	1,5	0,027	0,281	0,44	12,2	24,2
	Pv. Hv. 2B	16,5	1	0,062	4,47	0,0649	1,78	0,1	1,75	0,027	0,298	0,47	11,4	27,5
	Pv. Hv. 3A	17,1	1,65	0,049	3,66	0,0541	1,21	0,17	1,77	0,027	0,293	0,25	10,6	25,8
	Pv. Hv. 3B	16,6	1,25	0,05	3,58	0,0684	1,29	0,11	1,79	0,029	0,273	0,25	11,4	27,9
	Pv. Ve. 1A	15,8	1,03	0,11	3,72	0,0335	6,27	0,08	1,04	0,019	0,118	0,08	14,8	15,9
	Pv. Ve. 1B	15	0,83	0,087	3,21	0,0303	4,18	0,05	0,921	0,017	0,121	0,11	14,1	19,7
	Pv. Ve. 2A	15,7	0,89	0,079	3,37	0,0462	4,29	0,04	1,1	0,015	0,215	0,11	12,7	15,5
	Pv. Ve. 2B	15,4	0,78	0,1	3,31	0,0471	5,2	0,11	1,14	0,019	0,22	0,1	15,9	16
	Pv. Ve. 3A	17,9	1,34	0,094	4,26	0,0583	5,22	0,05	1,43	0,019	0,324	0,16	12,1	17,8
	Pv. Ve. 3B	17,8	1,35	0,094	3,9	0,0461	5,56	0,09	1,39	0,018	0,335	0,13	14,6	14,6
	Pv. Sv. 1A	16,6	1,34	0,04	7,73	0,0486	1,56	0,13	2,51	0,024	0,166	0,1	10,6	22
	Pv. Sv. 1B	16,9	1,34	0,034	8,87	0,0501	1,85	0,25	3,11	0,022	0,267	0,1	11,2	22,4
	Pv. Sv. 2A	17,2	1,09	0,029	7,6	0,115	1,79	0,32	3,91	0,012	0,686	0,1	11,4	19,9
	Pv. Sv. 2B	16,6	0,99	0,027	7,73	0,173	1,95	0,24	3,73	0,015	0,42	0,13	11,3	20,7
	Pv. Sv. 3A	19	1,64	0,041	7,76	0,497	1,9	0,39	5,89	0,015	0,915	0,12	11,9	21,4
	Pv. Sv. 3B	18,4	1,42	0,035	6,99	0,0582	1,82	0,11	4,03	0,017	0,319	0,1	10,5	22,4
	Pv. Tr. 1A	16,4	1	0,038	4,93	0,138	0,726	0,13	2,26	0,028	0,196	0,46	14,5	35,5
	Pv. Tr. 1B	17,3	1,09	0,034	4,51	0,259	0,76	0,3	2,51	0,02	0,253	0,46	19,2	38,4
	Pv. Tr. 2A	18,9	0,94	0,031	4,98	0,159	0,862	0,16	2,99	0,021	0,271	0,56	21,3	35
	Pv. Tr. 2B	20,5	1,03	0,035	5,28	0,204	0,865	0,34	3,61	0,026	0,404	1,22	21,6	40
	Pv. Tr. 3A	18,8	1,25	0,051	4,94	0,0927	1,13	0,12	2,32	0,019	0,246	0,37	15	31,7
	Pv. Tr. 3B	18,7	1,14	0,046	4,84	0,184	1	0,2	2,77	0,02	0,331	0,5	18,4	33,8

	ID	Dry matter %	Lipid-% % pr.w.w.	Ag µg/g w.w.	As µg/g w.w.	Ba µg/g w.w.	Cd µg/g w.w.	Cr µg/g w.w.	Cu µg/g w.w.	Hg µg/g w.w.	Ni µg/g w.w.	Pb µg/g w.w.	Sr µg/g w.w.	Zn µg/g w.w.
Mytilus edulis	Me. Hv. 1A	16,8	2,25	0,005	3,93	0,0988	0,125	0,32	2,13	0,031	0,157	0,89	16,4	66,5
	Me. Hv. 1B	16,3	2,53	0,004	3,89	0,454	0,146	0,71	2,99	0,03	0,635	0,88	12	57,4
	Me. Hv. 2A	16,2	2,08	<0,0025	6,22	0,0612	0,171	0,3	1,72	0,033	0,184	0,64	6,2	80,7
	Me. Hv. 2B	14,9	1,36	0,003	6,88	0,062	0,2	0,29	1,87	0,03	0,226	0,62	5,85	77
	Me. Sv. 1A	18	2,87	<0,0025	5,59	0,0438	0,165	0,15	2,23	0,012	0,101	0,12	8,98	54,3
	Me. Sv. 1B	17,9	2,6	0,002	5,1	0,141	0,155	0,23	2,77	0,014	0,269	0,13	9	51
	Me. Sv. 2A	15,6	2,06	0,003	4,99	0,109	0,156	0,3	4,25	0,013	0,28	0,14	7,02	46,9
	Me. Sv. 2B	15,1	2,03	<0,0024	5,17	0,0622	0,163	0,16	4,39	0,015	0,135	0,14	7,49	55,2
	Me. Sv. 3A	18,4	2,64	<0,0024	5,33	0,0356	0,22	0,05	1,65	0,011	0,071	0,1	7,51	46,2
	Me. Sv. 3B	18,1	2,75	<0,0025	5,33	0,0374	0,201	0,11	1,7	0,012	0,115	0,14	8,39	52,6
	Me. Ka. 1A	16,7	2,22	0,004	2,49	0,0618	0,18	0,14	1,65	0,017	0,139	0,12	7,76	33,8
	Me. Ka. 1B	17,3	1,85	0,004	2,61	0,0988	0,165	0,24	1,85	0,02	0,244	0,12	10,4	36,9
	Me. Ka. 2A	18,9	2,08	0,003	3,16	0,0513	0,145	0,16	1,46	0,015	0,129	0,14	11,4	38
	Me. Ka. 2B	16,6	1,6	0,005	2,85	0,0798	0,147	0,26	2,05	0,017	0,321	0,14	10,3	30,8
	Me. Ka. 3A	18,8	2,29	0,004	2,57	0,0449	0,18	0,07	1,48	0,011	0,0942	0,11	9,98	45,3
	Me. Ka. 3B	18,8	2,21	0,004	2,58	0,0342	0,15	0,06	1,57	0,013	0,104	0,08	9,43	38,9
	Me. Tr. 1A	14,5	1,64	0,003	2,41	0,213	0,208	0,47	1,91	0,053	0,257	1,33	40	93,8
	Me. Tr. 1B	14,5	1,52	0,024	2,33	0,239	0,242	0,78	2,26	0,056	0,501	0,99	20,7	75,5
	Me. Tr. 2A	15,6	1,72	0,003	2,59	0,0648	0,207	0,33	1,56	0,046	0,41	0,9	12,8	71,7
	Me. Tr. 2B	16,5	1,94	<0,0024	2,53	0,0629	0,21	0,18	1,58	0,04	0,12	0,79	10,3	72
	Me. Tr. 3A	15,2	1,79	0,003	2,25	0,126	0,198	0,25	1,42	0,041	0,17	0,56	11,4	73,6
	Me. Tr. 3B	15,6	1,46	0,004	2,11	0,0714	0,198	0,33	1,49	0,043	0,22	0,58	13	71,7
Modiolus modiolus	Mm. Ki. 1A	17,4	1,6	0,074	3,07	0,125	8,84	0,09	4,02	0,026	0,885	1,25	8,13	64,5
	Mm. Ki. 1B	20	1,87	0,128	2,96	0,0991	8,25	0,12	4,47	0,02	0,817	1,14	7,68	62,9
	Mm. Ki. 2A	16,8	1,93	0,376	3,08	0,113	7,36	0,08	5,63	0,027	0,754	1,11	6,23	49,4
	Mm. Ki. 2B	17,8	1,52	0,244	3,12	0,0784	5,91	0,09	5,51	0,025	0,632	0,91	6,91	41,2
	Mm. Ki. 3A	14,2	1,23	0,671	3,27	0,159	10,3	0,12	5,12	0,031	1,03	1,86	6,66	65,7
	Mm. Ki. 3B	16,4	1,76	0,387	3,04	0,0939	6,17	0,08	6,01	0,02	0,628	0,83	5,63	52,4

Attachment 11:

Measurement of DNA adducts with ^{32}P-postlabelling assay:

Liver tissue samples were semi-thawed and the DNA extracted and purified according to Dunn et al., 1987; Reichert & French 1994, slightly modified as described in Ericson et al. 1998 and Ericson & Balk 2000. DNA adducts were enriched using the Nuclease P1 method, 0.8 µg Nuclease P1/µg DNA, and a 45 min incubation period (Reddy and Randerath 1986; Beach and Gupta 1992). Finally the DNA adducts were radiolabelled using 5´-[γ-^{32}P]triphosphate([γ-^{32}P]ATP) and T_4 polynucleotide kinase. Separation and clean up of adducts was performed by multidirectional thin-layer chromatography (TLC) on laboratory produced polyethyleneimine cellulose sheets, described as suitable for adducts formed from large hydrophobic xenobiotics, such as 4- to 6- ring, PAHs (Reichert and French 1994; Ericson *et al*. 1999). Adducts were located and quantified by storage phosphor imaging technology (PhosphorImagerTMSI and ImageQuant 5.0). In addition, several quality control experiments were performed parallel to the analysis of the cod samples. All these quality assurance experiments strongly suggested a faultless assay for the DNA adduct measurements.

The detection limit of DNA adducts was calculated for each individual sample, from the actual background signal which was selected from a representative area of their respective autoradiogram. A spot-specific (area/zone), corresponding to 1.5 times the representative background (spot/area/zone) on the same autoradiogram was considered as the limit of detection, and limit of quantification of DNA adducts.

References

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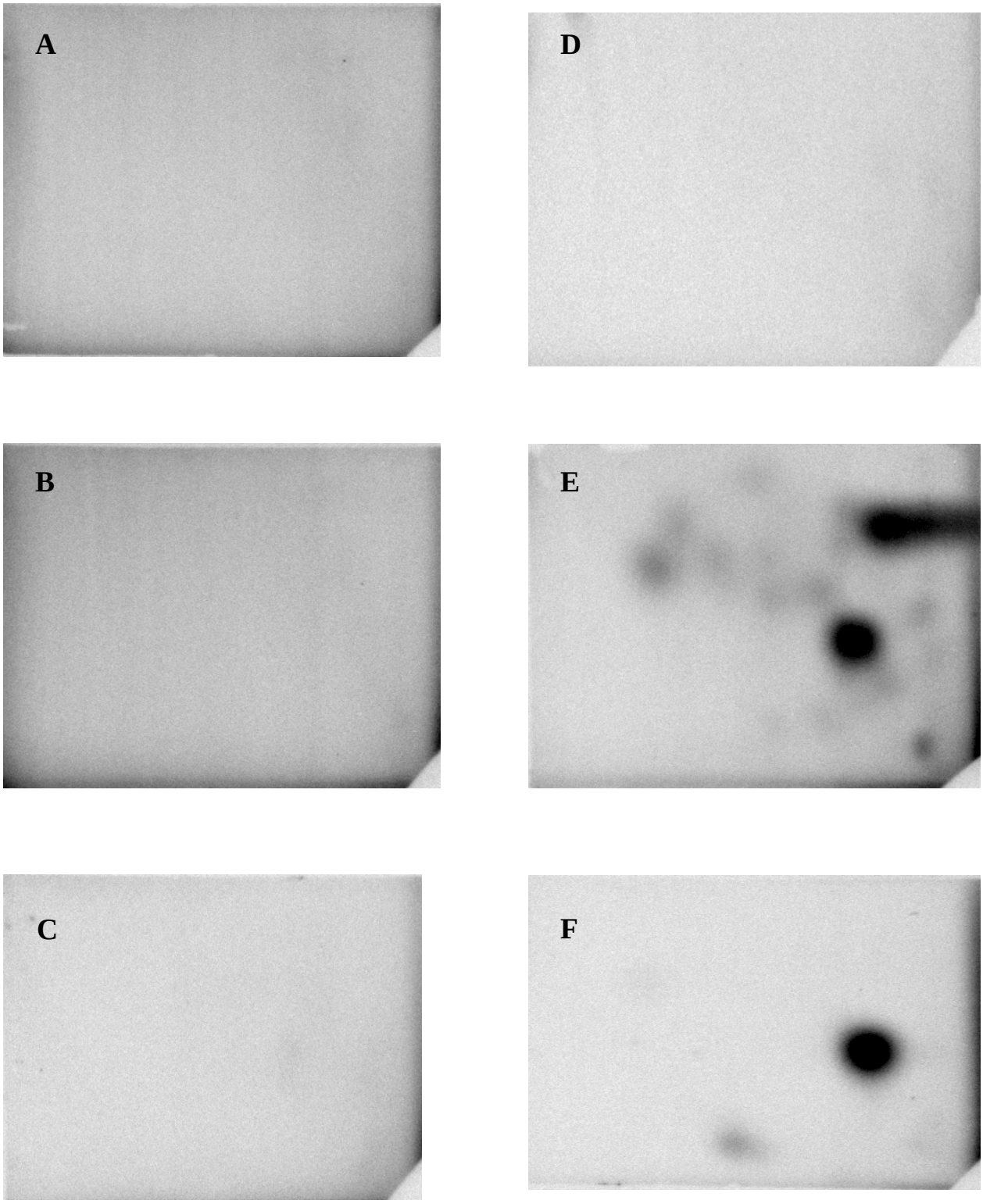

Autoradiograms from the DNA adduct analysis. Figures a, b, and c show chromatograms from fish nr. 6, 12 and 23 respectively. Figures d, e and f show the controls; d: Standard DNA, negative control, e: Bequalm liver standard (from perch injected with BaP), f: Standard BaPDE adduct.

DNA adduct levels in liver of Cod (*Gadus morhua*) from Kaldbak, The Faroe Islands

(Reyðfiskur)
031203

Results: DNA adduct levels in all samples were below the detections limits.

Fish nr.	DNA adducts (nmol/mol nucleotides)	For mean calculations
1	<1.4	0,7
2	<1.1	0,55
3	<1.3	0,65
4	<1.3	0,65
5	<1.3	0,65
6	<1.4	0,7
7	<1.2	0,6
8	<0.9	0,45
9	<1.2	0,6
10	<1.0	0,5
11	<1.3	0,65
12	<1.1	0,55
13	<0.9	0,45
14	<1.0	0,5
15	<1.0	0,5
17	<1.0	0,5
19	<0.7	0,35
20	<1.0	0,5
23	<0.9	0,45
24	<0.7	0,35
	Average	**0,54**
	Stdev	0,11

Attachment 12:

Methods for analyses of non- and mono-ortho PCBs and dioxins (PCDD/PCDF) in cod liver samples at ERGO Forschungsgesellshaft (Hamburg, germany):

5 Analytical Procedures

In the following the analytical procedures for the analysis of PCDDs/PCDFs, WHO-PCBs and Marker-PCBs are shown.

5.1 PCDDs/PCDFs and WHO-PCBs

We would like to mention, that the measurements are done *by high resolution mass spectrometry (HRMS)*, which guarantees high specificity and high sensitivity.

Prior the extraction following ^{13}C-UL-labeled internal standards are added to the sample:

Internal standards (^{13}C-UL), PCDDs/PCDFs			
PCDDs		PCDFs	
2,3,7,8	-Tetra-CDD	2,3,7,8	-Tetra-CDF
1,2,3,7,8	-Penta-CDD	1,2,3,7,8	-Penta-CDF
		2,3,4,7,8	-Penta-CDF
1,2,3,4,7,8	-Hexa-CDD	1,2,3,4,7,8	-Hexa-CDF
1,2,3,6,7,8	-Hexa-CDD	1,2,3,6,7,8	-Hexa-CDF
1,2,3,7,8,9	-Hexa-CDD	1,2,3,7,8,9	-Hexa-CDF
		2,3,4,6,7,8	-Hexa-CDF
1,2,3,4,6,7,8	-Hepta-CDD	1,2,3,4,6,7,8	-Hepta-CDF
		1,2,3,4,7,8,9	-Hepta-CDF
1,2,3,4,6,7,8,9	-Octa-CDD	1,2,3,4,6,7,8,9	-Octa-CDF

	Internal standards (^{13}C-UL), WHO-PCBs		
	Compound		IUPAC Code
Non-ortho PCBs	3,3´,4,4´	-Tetra-CB	PCB 77
	3,4,4´,5	-Tetra-CB	PCB 81
	3,3´,4,4´,5	-Penta-CB	PCB 126
	3,3´,4,4´,5,5´	-Hexa-CB	PCB 169
Mono-ortho PCBs	2,3,3´,4,4´	-Penta-CB	PCB 105
	2,3,4,4´,5	-Penta-CB	PCB 114
	2,3´,4,4´,5	-Penta-CB	PCB 118
	2´,3,4,4´,5	-Penta-CB	PCB 123
	2,3,3´,4,4´,5	-Hexa-CB	PCB 156
	2,3,3´,4,4´,5´	-Hexa-CB	PCB 157
	2,3´,4,4´,5,5´	-Hexa-CB	PCB 167
	2,3,3´,4,4´,5,5´	-Hepta-CB	PCB 189

After spiking, the samples are extracted/solved with appropriate solvents for ultratrace-analyses (e.g. nanograde) by using a solid / lipid extraction.

The clean up is done on a multicolumn system (involving carbon-on-glasfibre). The measurement is done by means of high resolution gaschromatography and high resolution mass spectrometry (HRGC/HRMS) with VG-AutoSpec and/or Finnigan MAT 95 XL using DB-5 capillary columns.

For each component 2 isotope masses are measured. The quantification is carried out by the use of internal/external standard mixtures (isotope dilution method). Following PCDDs/PCDFs and PCBs are determined and reported.

PCDDs/PCDFs			
PCDDs		PCDFs	
2,3,7,8	-Tetra-CDD	2,3,7,8	-Tetra-CDF
1,2,3,7,8	-Penta-CDD	1,2,3,7,8	-Penta-CDF
		2,3,4,7,8	-Penta-CDF
1,2,3,4,7,8	-Hexa-CDD	1,2,3,4,7,8	-Hexa-CDF
1,2,3,6,7,8	-Hexa-CDD	1,2,3,6,7,8	-Hexa-CDF
1,2,3,7,8,9	-Hexa-CDD	1,2,3,7,8,9	-Hexa-CDF
		2,3,4,6,7,8	-Hexa-CDF
1,2,3,4,6,7,8	-Hepta-CDD	1,2,3,4,6,7,8	-Hepta-CDF
		1,2,3,4,7,8,9	-Hepta-CDF
1,2,3,4,6,7,8,9	-Octa-CDD	1,2,3,4,6,7,8,9	-Octa-CDF

	WHO-PCBs		
	Compound		IUPAC Code
Non-ortho PCBs	3,3´,4,4´	-Tetra-CB	PCB 77
	3,4,4´,5	-Tetra-CB	PCB 81
	3,3´,4,4´,5	-Penta-CB	PCB 126
	3,3´,4,4´,5,5´	-Hexa-CB	PCB 169
Mono-ortho PCBs	2,3,3´,4,4´	-Penta-CB	PCB 105
	2,3,4,4´,5	-Penta-CB	PCB 114
	2,3´,4,4´,5	-Penta-CB	PCB 118
	2´,3,4,4´,5	-Penta-CB	PCB 123
	2,3,3´,4,4´,5	-Hexa-CB	PCB 156
	2,3,3´,4,4´,5´	-Hexa-CB	PCB 157
	2,3´,4,4´,5,5´	-Hexa-CB	PCB 167
	2,3,3´,4,4´,5,5´	-Hepta-CB	PCB 189

In addition to the single results, calculations of the toxicity equivalents (TEQ) according to the WHO-system are carried out.

5.2 Marker-PCBs

Within the scope of the investigation, the PCBs 28, 52, 101, 118, 138, 153 and 180 are determined. Before the extraction the following ^{13}C-UL-labeled internal standards are added to the sample:

2,4,4'-Tri-PCB (PCB-28)	^{13}C-UL
2,2',5,5'-Tetra-PCB (PCB-52)	^{13}C-UL
2,2',4,5,5'-Penta-PCB (PCB-101)	^{13}C-UL
2,2',3,4,4',5'-Hexa-PCB (PCB-138)	^{13}C-UL
2,2',4,4',5,5'-Hexa-PCB (PCB-153)	^{13}C-UL
2,2',3,4,4',5,5'-Hepta-PCB (PCB-180)	^{13}C-UL

After the spiking, the samples are extracted with appropriate solvents for ultratrace-analyses (e.g. nanograde). In the following, a column clean up is performed. The measurement is done by means of high resolution gaschromatography and mass spectrometry (HRGC/MS) using DB-5 capillary columns.

For each substance 2 isotope masses are measured. The quantification is carried out with the use of internal/external standard mixtures.

Attachment 13A:

PCDDs/PCDFs in cod liver						
Values in: pg/g (ppt) **lipid based**						
Analysis-No.: H-03-08-0473 Reyðfiskur 8, Livur, Kaldbak,						
	Concentration	WHO-TEF	WHO-TEQ	LOD	**WHO-TEF(fish)**	**WHO-TEQ(fish)***
2.3.7.8-Tetra-CDD	0,32	1,000	0,318		1,000	0,318
1.2.3.7.8-Penta-CDD	0,15	1,000	0,152		1,000	0,152
1.2.3.4.7.8-Hexa-CDD	n.d.	0,100	0,009	(0,09)	0,500	< 0,045
1.2.3.6.7.8-Hexa-CDD	2,54	0,100	0,254		0,010	0,025
1.2.3.7.8.9-Hexa-CDD	0,34	0,100	0,034		0,010	0,003
1.2.3.4.6.7.8-Hepta-CDD	0,74	0,010	0,007		0,001	0,001
OCDD	n.d.	0,0001	< 0,001	(1,23)	<0,0001	< 0,000
2.3.7.8-Tetra-CDF	10,62	0,100	1,062		0,050	0,531
1.2.3.7.8-Penta-CDF	3,51	0,050	0,176		0,050	0,176
2.3.4.7.8-Penta-CDF	0,71	0,500	0,354		0,500	0,354
1.2.3.4.7.8-Hexa-CDF	1,50	0,100	0,150		0,100	0,150
1.2.3.6.7.8-Hexa-CDF	1,08	0,100	0,108		0,100	0,108
1.2.3.7.8.9-Hexa-CDF	n.d.	0,100	0,006	(0,06)	0,100	< 0,006
2.3.4.6.7.8-Hexa-CDF	0,86	0,100	0,086		0,100	0,086
1.2.3.4.6.7.8-Hepta-CDF	0,26	0,010	0,003		0,010	0,003
1.2.3.4.7.8.9-Hepta-CDF	0,08	0,010	0,001		0,010	0,001
OCDF	n.d.	0,0001	< 0,001	(0,12)	<0,0001	< 0,000
Total 2.3.7.8-PCDD	4,09		0,775			0,545
Total 2.3.7.8-PCDF	18,63		1,946			1,415
Total 2.3.7.8-PCDD/PCDF	22,72		2,721			1,960
Total non-ortho PCB	538		15,554			0,787
Total mono-ortho PCB	76113		11,199			< 0,381
TEQ (WHO) based on PCDD/PCDF in consideration of 100 % detection limit			2,721			1,960
I - TEQ (NATO-CCMS) in consideration of 100 % detection limit			2,646			
TEQ (WHO) based on PCDD/PCDF, non-ortho- and mono-ortho-PCB in consideration of 100 % LOD, lipid based			**29,474**			**3,127**

TEQ, TEF (WHO) = Toxic equivalent resp. -factor by WHO for humans & mammals
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed
(M) = maximum value, contains possible outside contamination
small differences on totals caused by computercalculations

PCDDs/PCDFs in cod liver						
Values in: ng/kg (ppt) **fresh weight based**						
Analysis-No.: H-03-08-0473 Renofisk 8, Livur, Kaldbak, 06.02.2002,						
	Concentration	WHO-TEF	WHO-TEQ	LOD	**WHO-TEF(fish)**	**WHO-TEQ(fish)***
2.3.7.8-Tetra-CDD	0,15	1,000	0,150		1	0,150
1.2.3.7.8-Penta-CDD	0,07	1,000	0,071		1	0,071
1.2.3.4.7.8-Hexa-CDD	n.d.	0,100	0,004	(0,04)	0,5	< 0,021
1.2.3.6.7.8-Hexa-CDD	1,19	0,100	0,119		0,01	0,012
1.2.3.7.8.9-Hexa-CDD	0,16	0,100	0,016		0,01	0,002
1.2.3.4.6.7.8-Hepta-CDD	0,35	0,010	0,003		0,001	0,000
OCDD	n.d.	0,0001	< 0,001	(0,58)	<0,0001	< 0,000
2.3.7.8-Tetra-CDF	4,99	0,100	0,499		0,05	0,250
1.2.3.7.8-Penta-CDF	1,65	0,050	0,083		0,05	0,083
2.3.4.7.8-Penta-CDF	0,33	0,500	0,167		0,5	0,167
1.2.3.4.7.8-Hexa-CDF	0,70	0,100	0,070		0,1	0,070
1.2.3.6.7.8-Hexa-CDF	0,51	0,100	0,051		0,1	0,051
1.2.3.7.8.9-Hexa-CDF	n.d.	0,100	0,003	(0,03)	0,1	< 0,003
2.3.4.6.7.8-Hexa-CDF	0,41	0,100	0,041		0,1	0,041
1.2.3.4.6.7.8-Hepta-CDF	0,12	0,010	0,001		0,01	0,001
1.2.3.4.7.8.9-Hepta-CDF	0,04	0,010	< 0,001		0,01	0,000
OCDF	n.d.	0,0001	< 0,001	(0,05)	<0,0001	< 0,000
Total 2.3.7.8-PCDD	1,92		0,364			0,256
Total 2.3.7.8-PCDF	8,76		0,915			0,665
Total 2.3.7.8-PCDD/PCDF	10,68		1,279			0,921
Total non-ortho PCB	253		7,310			0,370
Total mono-ortho PCB	35773		5,264			< 0,179
TEQ (WHO) based on PCDD/PCDF in consideration of 100 % detection limit			1,279			0,921
I - TEQ (NATO-CCMS) in consideration of 100 % detection limit			1,244			
TEQ (WHO) based on PCDD/PCDF, non-ortho- and mono-ortho-PCB in consideration of 100 % LOD, fresh weight based			**13,853**			**1,470**

TEQ, TEF (WHO) = Toxic equivalent resp. -factor by WHO for humans & mammals
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed
(M) = maximum value, contains possible outside contamination
small differences on totals caused by computercalculations

***WHO-TEF(fish) inserted and WHO-TEQ(fish) calculated after recieving the results from the laboratory. Other results calculated by the laboratory.**

PCDDs/PCDFs in cod liver

Values in: pg/g (ppt) **lipid based**

Analysis-No.: H-03-08-0474 Reyðfiskur 10, Livur, Kaldbak,

	Concentration	WHO-TEF	WHO-TEQ	LOD	WHO-TEF(fish)	WHO-TEQ(fish)*
2.3.7.8-Tetra-CDD	1,11	1,000	1,113		1,000	1,113
1.2.3.7.8-Penta-CDD	0,24	1,000	0,243		1,000	0,243
1.2.3.4.7.8-Hexa-CDD	n.d.	0,100	0,009	(0,09)	0,500	< 0,046
1.2.3.6.7.8-Hexa-CDD	2,25	0,100	0,225		0,010	0,023
1.2.3.7.8.9-Hexa-CDD	0,37	0,100	0,037		0,010	0,004
1.2.3.4.6.7.8-Hepta-CDD	1,41	0,010	0,014		0,001	0,001
OCDD	2,73	0,0001	< 0,001		<0,0001	< 0,000
2.3.7.8-Tetra-CDF	30,59	0,100	3,059		0,050	1,529
1.2.3.7.8-Penta-CDF	4,07	0,050	0,203		0,050	0,203
2.3.4.7.8-Penta-CDF	1,12	0,500	0,558		0,500	0,558
1.2.3.4.7.8-Hexa-CDF	0,97	0,100	0,097		0,100	0,097
1.2.3.6.7.8-Hexa-CDF	1,05	0,100	0,105		0,100	0,105
1.2.3.7.8.9-Hexa-CDF	n.d.	0,100	0,008	(0,08)	0,100	< 0,008
2.3.4.6.7.8-Hexa-CDF	1,22	0,100	0,122		0,100	0,122
1.2.3.4.6.7.8-Hepta-CDF	0,19	0,010	0,002		0,010	0,002
1.2.3.4.7.8.9-Hepta-CDF	n.d.	0,010	0,001	(0,07)	0,010	< 0,001
OCDF	n.d.	0,0001	< 0,001	(0,30)	<0,0001	< 0,000
Total 2.3.7.8-PCDD	8,12		1,641			1,430
Total 2.3.7.8-PCDF	39,20		4,155			2,625
Total 2.3.7.8-PCDD/PCDF	47,32		5,796			4,055
Total non-ortho PCB	663		28,709			0,000
Total mono-ortho PCB	74619		10,305			< 0,000

TEQ (WHO) based on PCDD/PCDF in consideration of 100 % detection limit	5,796	4,055
I - TEQ (NATO-CCMS) in consideration of 100 % detection limit	5,677	
TEQ (WHO) based on PCDD/PCDF, non-ortho- and mono-ortho-PCB in consideration of 100 % LOD, lipid based	**44,810**	**4,055**

TEQ, TEF (WHO) = Toxic equivalent resp. -factor by WHO for humans & mammals
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed
(M) = maximum value, contains possible outside contamination
small differences on totals caused by computercalculations

PCDDs/PCDFs in cod liver

Values in: pg/g (ppt) **fresh weight based**

Analysis-No.: H-03-08-0474 Reyðfiskur 10, Livur, Kaldbak,

	Concentration	WHO-TEF	WHO-TEQ	LOD	WHO-TEF(fish)	WHO-TEQ(fish)*
2.3.7.8-Tetra-CDD	0,39	1,000	0,393		1,000	0,393
1.2.3.7.8-Penta-CDD	0,09	1,000	0,086		1,000	0,086
1.2.3.4.7.8-Hexa-CDD	n.d.	0,100	0,003	(0,03)	0,500	< 0,016
1.2.3.6.7.8-Hexa-CDD	0,80	0,100	0,080		0,010	0,008
1.2.3.7.8.9-Hexa-CDD	0,13	0,100	0,013		0,010	0,001
1.2.3.4.6.7.8-Hepta-CDD	0,50	0,010	0,005		0,001	0,000
OCDD	0,96	0,0001	< 0,001		<0,0001	< 0,000
2.3.7.8-Tetra-CDF	10,80	0,100	1,080		0,050	0,540
1.2.3.7.8-Penta-CDF	1,44	0,050	0,072		0,050	0,072
2.3.4.7.8-Penta-CDF	0,39	0,500	0,197		0,500	0,197
1.2.3.4.7.8-Hexa-CDF	0,34	0,100	0,034		0,100	0,034
1.2.3.6.7.8-Hexa-CDF	0,37	0,100	0,037		0,100	0,037
1.2.3.7.8.9-Hexa-CDF	n.d.	0,100	0,003	(0,03)	0,100	< 0,003
2.3.4.6.7.8-Hexa-CDF	0,43	0,100	0,043		0,100	0,043
1.2.3.4.6.7.8-Hepta-CDF	0,07	0,010	0,001		0,010	0,001
1.2.3.4.7.8.9-Hepta-CDF	n.d.	0,010	< 0,001	(0,02)	0,010	< 0,000
OCDF	n.d.	0,0001	< 0,001	(0,11)	<0,0001	< 0,000
Total 2.3.7.8-PCDD	2,87		0,579			0,505
Total 2.3.7.8-PCDF	13,84		1,467			0,927
Total 2.3.7.8-PCDD/PCDF	16,70		2,046			1,431
Total non-ortho PCB	234		10,134			0,000
Total mono-ortho PCB	26340		3,638			< 0,000

TEQ (WHO) based on PCDD/PCDF in consideration of 100 % detection limit	2,046	1,431
I - TEQ (NATO-CCMS) in consideration of 100 % detection limit	2,004	
TEQ (WHO) based on PCDD/PCDF, non-ortho- and mono-ortho-PCB in consideration of 100 % LOD, fresh weight based	**15,818**	**1,431**

TEQ, TEF (WHO) = Toxic equivalent resp. -factor by WHO for humans & mammals
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed
(M) = maximum value, contains possible outside contamination
small differences on totals caused by computercalculations

***WHO-TEF(fish) inserted and WHO-TEQ(fish) calculated after recieving the results from the laboratory. Other results calculated by the laboratory.**

PCDDs/PCDFs in cod liver

Values in: np/g (ppt) lipid based
Analysis-No.: H-03-08-0475 Reyðfiskur 19, Livur, Kaldbak,

	Concentration	WHO-TEF	WHO-TEQ	LOD	WHO-TEF(fish)	WHO-TEQ(fish)*
2.3.7.8-Tetra-CDD	0,76	1,000	0,761		1,000	0,761
1.2.3.7.8-Penta-CDD	0,14	1,000	0,144		1,000	0,144
1.2.3.4.7.8-Hexa-CDD	n.d.	0,100	0,003	(0,03)	0,500	< 0,015
1.2.3.6.7.8-Hexa-CDD	1,46	0,100	0,146		0,010	0,015
1.2.3.7.8.9-Hexa-CDD	0,45	0,100	0,045		0,010	0,004
1.2.3.4.6.7.8-Hepta-CDD	0,43	0,010	0,004		0,001	0,000
OCDD	n.d.	0,0001	< 0,001	(0,91)	<0,0001	< 0,000
2.3.7.8-Tetra-CDF	17,28	0,100	1,728		0,050	0,864
1.2.3.7.8-Penta-CDF	2,64	0,050	0,132		0,050	0,132
2.3.4.7.8-Penta-CDF	0,50	0,500	0,250		0,500	0,250
1.2.3.4.7.8-Hexa-CDF	1,49	0,100	0,149		0,100	0,149
1.2.3.6.7.8-Hexa-CDF	0,90	0,100	0,090		0,100	0,090
1.2.3.7.8.9-Hexa-CDF	n.d.	0,100	0,003	(0,03)	0,100	< 0,003
2.3.4.6.7.8-Hexa-CDF	1,06	0,100	0,106		0,100	0,106
1.2.3.4.6.7.8-Hepta-CDF	0,25	0,010	0,003		0,010	0,003
1.2.3.4.7.8.9-Hepta-CDF	n.d.	0,010	< 0,001	(0,04)	0,010	< 0,000
OCDF	n.d.	0,0001	< 0,001	(0,11)	<0,0001	< 0,000
Total 2.3.7.8-PCDD	3,24		1,103			0,940
Total 2.3.7.8-PCDF	24,12		2,461			1,597
Total 2.3.7.8-PCDD/PCDF	27,36		3,564			2,537
Total non-ortho PCB	1240		37,136			0,000
Total mono-ortho PCB	121019		16,626			< 0,000

TEQ (WHO) based on PCDD/PCDF in consideration of 100 % detection limit	3,564	2,537
I - TEQ (NATO-CCMS) in consideration of 100 % detection limit	3,493	
TEQ (WHO) based on PCDD/PCDF, non-ortho- and mono-ortho-PCB in consideration of 100 % LOD, lipid based	**57,326**	**2,537**

TEQ, TEF (WHO) = Toxic equivalent resp. -factor by WHO for humans & mammals
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed
(M) = maximum value, contains possible outside contamination
small differences on totals caused by computercalculations

PCDDs/PCDFs in cod liver

Values in: pg/g (ppt) fresh weight based
Analysis-No.: H-03-08-0475 Reyðfiskur 19, Livur, Kaldbak,

	Concentration	WHO-TEF	WHO-TEQ	LOD	WHO-TEF(fish)	WHO-TEQ(fish)*
2.3.7.8-Tetra-CDD	0,27	1,000	0,272		1,000	0,272
1.2.3.7.8-Penta-CDD	0,05	1,000	0,051		1,000	0,051
1.2.3.4.7.8-Hexa-CDD	n.d.	0,100	0,001	(0,01)	0,500	< 0,005
1.2.3.6.7.8-Hexa-CDD	0,52	0,100	0,052		0,010	0,005
1.2.3.7.8.9-Hexa-CDD	0,16	0,100	0,016		0,010	0,002
1.2.3.4.6.7.8-Hepta-CDD	0,15	0,010	0,002		0,001	0,000
OCDD	n.d.	0,0001	< 0,001	(0,32)	<0,0001	< 0,000
2.3.7.8-Tetra-CDF	6,17	0,100	0,617		0,050	0,308
1.2.3.7.8-Penta-CDF	0,94	0,050	0,047		0,050	0,047
2.3.4.7.8-Penta-CDF	0,18	0,500	0,089		0,500	0,089
1.2.3.4.7.8-Hexa-CDF	0,53	0,100	0,053		0,100	0,053
1.2.3.6.7.8-Hexa-CDF	0,32	0,100	0,032		0,100	0,032
1.2.3.7.8.9-Hexa-CDF	n.d.	0,100	0,001	(0,01)	0,100	< 0,001
2.3.4.6.7.8-Hexa-CDF	0,38	0,100	0,038		0,100	0,038
1.2.3.4.6.7.8-Hepta-CDF	0,09	0,010	0,001		0,010	0,001
1.2.3.4.7.8.9-Hepta-CDF	n.d.	0,010	< 0,001	(0,01)	0,010	< 0,000
OCDF	n.d.	0,0001	< 0,001	(0,04)	<0,0001	< 0,000
Total 2.3.7.8-PCDD	1,16		0,394			0,335
Total 2.3.7.8-PCDF	8,61		0,878			0,570
Total 2.3.7.8-PCDD/PCDF	9,77		1,272			0,905
Total non-ortho PCB	443		13,258			0,000
Total mono-ortho PCB	43204		5,935			< 0,000

TEQ (WHO) based on PCDD/PCDF in consideration of 100 % detection limit	1,272	0,905
I - TEQ (NATO-CCMS) in consideration of 100 % detection limit	1,247	
TEQ (WHO) based on PCDD/PCDF, non-ortho- and mono-ortho-PCB in consideration of 100 % LOD, fresh weight based	**20,465**	**0,905**

TEQ, TEF (WHO) = Toxic equivalent resp. -factor by WHO for humans & mammals
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed
(M) = maximum value, contains possible outside contamination
small differences on totals caused by computercalculations

***WHO-TEF(fish) inserted and WHO-TEQ(fish) calculated after recieving the results from the laboratory. Other results calculated by the laboratory.**

PCDDs/PCDFs in cod liver

Values in: pg/g (ppt) **lipid based**

Analysis-No.: H-03-08-0476 Reyðfiskur 24, Livur, Kaldbak,

	Concentration	WHO-TEF	WHO-TEQ	LOD	WHO-TEF(fish)	WHO-TEQ(fish)*
2.3.7.8-Tetra-CDD	0,85	1,000	0,849		1,000	0,849
1.2.3.7.8-Penta-CDD	0,26	1,000	0,255		1,000	0,255
1.2.3.4.7.8-Hexa-CDD	n.d.	0,100	0,009	(0,09)	0,500	< 0,043
1.2.3.6.7.8-Hexa-CDD	0,83	0,100	0,083		0,010	0,008
1.2.3.7.8.9-Hexa-CDD	0,16	0,100	0,016		0,010	0,002
1.2.3.4.6.7.8-Hepta-CDD	n.d.	0,010	0,002	(0,19)	0,001	0,000
OCDD	n.d.	0,0001	< 0,001	(0,87)	<0,0001	< 0,000
2.3.7.8-Tetra-CDF	27,96	0,100	2,796		0,050	1,398
1.2.3.7.8-Penta-CDF	2,76	0,050	0,138		0,050	0,138
2.3.4.7.8-Penta-CDF	0,99	0,500	0,494		0,500	0,494
1.2.3.4.7.8-Hexa-CDF	0,36	0,100	0,036		0,100	0,036
1.2.3.6.7.8-Hexa-CDF	0,47	0,100	0,047		0,100	0,047
1.2.3.7.8.9-Hexa-CDF	n.d.	0,100	0,007	(0,07)	0,100	< 0,007
2.3.4.6.7.8-Hexa-CDF	0,61	0,100	0,061		0,100	0,061
1.2.3.4.6.7.8-Hepta-CDF	n.d.	0,010	0,002	(0,17)	0,010	< 0,002
1.2.3.4.7.8.9-Hepta-CDF	n.d.	0,010	0,001	(0,07)	0,010	< 0,001
OCDF	n.d.	0,0001	< 0,001	(0,15)	<0,0001	< 0,000
Total 2.3.7.8-PCDD	2,09		1,214			1,158
Total 2.3.7.8-PCDF	33,15		3,583			2,185
Total 2.3.7.8-PCDD/PCDF	35,24		4,796			3,343
Total non-ortho PCB	1022		51,263			0,000
Total mono-ortho PCB	125275		17,199			< 0,000

TEQ (WHO) based on PCDD/PCDF in consideration of 100 % detection limit	**4,796**	3,343
I - TEQ (NATO-CCMS) in consideration of 100 % detection limit	4,670	
TEQ (WHO) based on PCDD/PCDF, non-ortho- and mono-ortho-PCB in consideration of 100 % LOD, lipid based	**73,258**	**3,343**

TEQ, TEF (WHO) = Toxic equivalent resp. -factor by WHO for humans & mammals
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed
(M) = maximum value, contains possible outside contamination
small differences on totals caused by computercalculations

PCDDs/PCDFs in cod liver

Values in: pg/g (ppt) **fresh weight based**

Analysis-No.: H-03-08-0476 Reyðfiskur 24, Livur, Kaldbak,

	Concentration	WHO-TEF	WHO-TEQ	LOD	WHO-TEF(fish)	WHO-TEQ(fish)*
2.3.7.8-Tetra-CDD	0,35	1,000	0,346		1,000	0,346
1.2.3.7.8-Penta-CDD	0,10	1,000	0,104		1,000	0,104
1.2.3.4.7.8-Hexa-CDD	n.d.	0,100	0,004	(0,04)	0,500	< 0,018
1.2.3.6.7.8-Hexa-CDD	0,34	0,100	0,034		0,010	0,003
1.2.3.7.8.9-Hexa-CDD	0,07	0,100	0,007		0,010	0,001
1.2.3.4.6.7.8-Hepta-CDD	n.d.	0,010	0,001	(0,08)	0,001	0,000
OCDD	n.d.	0,0001	< 0,001	(0,35)	<0,0001	< 0,000
2.3.7.8-Tetra-CDF	11,41	0,100	1,141		0,050	0,570
1.2.3.7.8-Penta-CDF	1,13	0,050	0,056		0,050	0,056
2.3.4.7.8-Penta-CDF	0,40	0,500	0,202		0,500	0,202
1.2.3.4.7.8-Hexa-CDF	0,15	0,100	0,015		0,100	0,015
1.2.3.6.7.8-Hexa-CDF	0,19	0,100	0,019		0,100	0,019
1.2.3.7.8.9-Hexa-CDF	n.d.	0,100	0,003	(0,03)	0,100	< 0,003
2.3.4.6.7.8-Hexa-CDF	0,25	0,100	0,025		0,100	0,025
1.2.3.4.6.7.8-Hepta-CDF	n.d.	0,010	0,001	(0,07)	0,010	< 0,001
1.2.3.4.7.8.9-Hepta-CDF	n.d.	0,010	< 0,001	(0,03)	0,010	< 0,000
OCDF	n.d.	0,0001	< 0,001	(0,06)	<0,0001	< 0,000
Total 2.3.7.8-PCDD	0,85		0,495			0,472
Total 2.3.7.8-PCDF	13,53		1,462			0,891
Total 2.3.7.8-PCDD/PCDF	14,38		1,957			1,364
Total non-ortho PCB	417		20,915			0,000
Total mono-ortho PCB	51112		7,017			< 0,000

TEQ (WHO) based on PCDD/PCDF in consideration of 100 % detection limit	**1,957**	1,364
I - TEQ (NATO-CCMS) in consideration of 100 % detection limit	1,905	
TEQ (WHO) based on PCDD/PCDF, non-ortho- and mono-ortho-PCB in consideration of 100 % LOD, fresh weight based	**29,889**	**1,364**

TEQ, TEF (WHO) = Toxic equivalent resp. -factor by WHO for humans & mammals
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed
(M) = maximum value, contains possible outside contamination
small differences on totals caused by computercalculations

Attachment 13B:

non-ortho and mono-ortho PCB in cod liver		
Values in:	pg/g (ppt)	**lipid based**
Analysis-No.:	H-03-08-0473	Reyðfiskur 8, Livur, Kaldbak, 06.02.2002

non-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ (fish)
3,4,4',5-Tetra-CB	81	7	0,0001	0,001	0,0005		0,004
3,3',4,4'-Tetra-CB	77	326	0,0001	0,033	0,0001		0,033
3,3',4,4',5-Penta-CB	126	150	0,1000	14,960	0,005		0,748
3,3',4,4',5,5'-Hexa-CB	169	56	0,0100	0,561	0,00005		0,003
Total non-ortho PCB		538		**15,554**			**0,787**

mono-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ (fish)
2,3,3',4,4'-Penta-CB	105	16414	0,0001	1,641	<0,000005		< 0,082
2,3,4,4',5-Penta-CB	114	1177	0,0005	0,588	<0,000005		< 0,006
2,3',4,4',5-Penta-CB	118	46907	0,0001	4,691	<0,000005		< 0,235
2',3,4,4',5-Penta-CB	123	418	0,0001	0,042	<0,000005	(M)	< 0,002
2,3,3',4,4',5,-Hexa-CB	156	6500	0,0005	3,250	<0,000005		< 0,033
2,3,3',4,4',5'-Hexa-CB	157	1810	0,0005	0,905	<0,000005		< 0,009
2,3',4,4',5,5'-Hexa-CB	167	2300	0,00001	0,023	<0,000005		< 0,012
2,3,3',4,4',5,5'-Hepta-CB	189	588	0,0001	0,059	<0,000005		< 0,003
Total mono-ortho PCB		76113		**11,199**			< **0,381**

WHO-TEF = toxic factor, WHO-TEQ = toxic equvalent by WHO for humans & mammals
TEQ (WHO) in consideration of 100% detection limit
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed,
(M) = maximum value, contains outside contamination
Small differences on totals result from computerroundings

non-ortho and mono-ortho PCB in cod liver		
Values in:	pg/g (ppt)	fresh weight based
Analysis-No.:	H-03-08-0473	Reyðfiskur 8, Livur, Kaldbak, 06.02.2002

non-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ (fish)
3,4,4',5-Tetra-CB	81	3	0,0001	< 0,001	0,0005		0,002
3,3',4,4'-Tetra-CB	77	153	0,0001	0,015	0,0001		0,015
3,3',4,4',5-Penta-CB	126	70	0,1000	7,031	0,005		0,352
3,3',4,4',5,5'-Hexa-CB	169	26	0,0100	0,264	0,00005		0,001
Total non-ortho PCB		253		**7,310**			**0,370**

mono-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ (fish)
2,3,3',4,4'-Penta-CB	105	7714	0,0001	0,771	<0,000005		< 0,0386
2,3,4,4',5-Penta-CB	114	553	0,0005	0,277	<0,000005		< 0,0028
2,3',4,4',5-Penta-CB	118	22046	0,0001	2,205	<0,000005		< 0,1102
2',3,4,4',5-Penta-CB	123	196	0,0001	0,020	<0,000005	(M)	< 0,0010
2,3,3',4,4',5,-Hexa-CB	156	3055	0,0005	1,528	<0,000005		< 0,0153
2,3,3',4,4',5'-Hexa-CB	157	851	0,0005	0,425	<0,000005		< 0,0043
2,3',4,4',5,5'-Hexa-CB	167	1081	0,00001	0,011	<0,000005		< 0,0054
2,3,3',4,4',5,5'-Hepta-CB	189	276	0,0001	0,028	<0,000005		< 0,0014
Total mono-ortho PCB		35773		**5,264**			< **0,1789**

WHO-TEF = toxic factor, WHO-TEQ = toxic equvalent by WHO for humans & mammals
TEQ (WHO) in consideration of 100% detection limit
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed,
(M) = maximum value, contains outside contamination
Small differences on totals result from computerroundings

***WHO-TEF(fish) inserted and WHO-TEQ(fish) calculated after recieving the results from the laboratory. Other results calculated by the laboratory.**

non-ortho and mono-ortho PCB in cod liver		
Values in:	pg/g (ppt)	lipid based
Analysis-No.:	H-03-08-0474	Reyðfiskur 10, Livur, Kaldbak, 06.05.2002

non-ortho PCB

	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
3,4,4',5-Tetra-CB	81	13	0,0001	0,001	0,0005		0,006
3,3',4,4'-Tetra-CB	77	288	0,0001	0,029	0,0001		0,029
3,3',4,4',5-Penta-CB	126	278	0,1000	27,837	0,005		1,392
3,3',4,4',5,5'-Hexa-CB	169	84	0,0100	0,842	0,00005		0,004
Total non-ortho PCB		663		**28,709**			**1,431**

mono-ortho PCB

	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
2,3,3',4,4'-Penta-CB	105	13402	0,0001	1,340	<0,000005		< 0,067
2,3,4,4',5-Penta-CB	114	1028	0,0005	0,514	<0,000005		< 0,005
2,3',4,4',5-Penta-CB	118	48479	0,0001	4,848	<0,000005		< 0,242
2',3,4,4',5-Penta-CB	123	768	0,0001	0,077	<0,000005	(M)	< 0,004
2,3,3',4,4',5,-Hexa-CB	156	5236	0,0005	2,618	<0,000005		< 0,026
2,3,3',4,4',5'-Hexa-CB	157	1616	0,0005	0,808	<0,000005		< 0,008
2,3',4,4',5,5'-Hexa-CB	167	3425	0,00001	0,034	<0,000005		< 0,017
2,3,3',4,4',5,5'-Hepta-CB	189	667	0,0001	0,067	<0,000005		< 0,003
Total mono-ortho PCB		74619		**10,305**			< **0,373**

WHO-TEF = toxic factor, WHO-TEQ = toxic equvalent by WHO for humans & mammals
TEQ (WHO) in consideration of 100% detection limit
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed,
(M) = maximum value, contains outside contamination
Small differences on totals result from computerroundings

non-ortho and mono-ortho PCB in cod liver		
Values in:	pg/g (ppt)	fresh weight based
Analysis-No.:	H-03-08-0474	Reyðfiskur 10, Livur, Kaldbak, 06.05.2002

non-ortho PCB

	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
3,4,4',5-Tetra-CB	81	4	0,0001	< 0,001	0,0005		0,002
3,3',4,4'-Tetra-CB	77	102	0,0001	0,010	0,0001		0,010
3,3',4,4',5-Penta-CB	126	98	0,1000	9,826	0,005		0,491
3,3',4,4',5,5'-Hexa-CB	169	30	0,0100	0,297	0,00005		0,001
Total non-ortho PCB		234		**10,134**			**0,505**

mono-ortho PCB

	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
2,3,3',4,4'-Penta-CB	105	4731	0,0001	0,473	<0,000005		< 0,0237
2,3,4,4',5-Penta-CB	114	363	0,0005	0,181	<0,000005		< 0,0018
2,3',4,4',5-Penta-CB	118	17113	0,0001	1,711	<0,000005		< 0,0856
2',3,4,4',5-Penta-CB	123	271	0,0001	0,027	<0,000005	(M)	< 0,0014
2,3,3',4,4',5,-Hexa-CB	156	1848	0,0005	0,924	<0,000005		< 0,0092
2,3,3',4,4',5'-Hexa-CB	157	570	0,0005	0,285	<0,000005		< 0,0029
2,3',4,4',5,5'-Hexa-CB	167	1209	0,00001	0,012	<0,000005		< 0,0060
2,3,3',4,4',5,5'-Hepta-CB	189	235	0,0001	0,024	<0,000005		< 0,0012
Total mono-ortho PCB		26340		**3,638**			< **0,1317**

WHO-TEF = toxic factor, WHO-TEQ = toxic equvalent by WHO for humans & mammals
TEQ (WHO) in consideration of 100% detection limit
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed,
(M) = maximum value, contains outside contamination
Small differences on totals result from computerroundings

***WHO-TEF(fish) inserted and WHO-TEQ(fish) calculated after recieving the results from the laboratory. Other results calculated by the laboratory.**

non-ortho and mono-ortho PCB in cod liver		
Values in:	pg/g (ppt)	lipid based
Analysis-No.:	H-03-08-0475	Reyðfiskur 19, Livur, Kaldbak, 12.07.2002

non-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
3,4,4',5-Tetra-CB	81	34	0,0001	0,003	0,0005		0,017
3,3',4,4'-Tetra-CB	77	745	0,0001	0,075	0,0001		0,075
3,3',4,4',5-Penta-CB	126	361	0,1000	36,068	0,005		1,803
3,3',4,4',5,5'-Hexa-CB	169	99	0,0100	0,990	0,00005		0,005
Total non-ortho PCB		1240		**37,136**			**1,900**

mono-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
2,3,3',4,4'-Penta-CB	105	24226	0,0001	2,423	<0,000005		< 0,121
2,3,4,4',5-Penta-CB	114	1526	0,0005	0,763	<0,000005		< 0,008
2,3',4,4',5-Penta-CB	118	76585	0,0001	7,658	<0,000005		< 0,383
2',3,4,4',5-Penta-CB	123	1165	0,0001	0,117	<0,000005	(M)	< 0,006
2,3,3',4,4',5,-Hexa-CB	156	8341	0,0005	4,171	<0,000005		< 0,042
2,3,3',4,4',5'-Hexa-CB	157	2713	0,0005	1,357	<0,000005		< 0,014
2,3',4,4',5,5'-Hexa-CB	167	5650	0,00001	0,056	<0,000005		< 0,028
3,3',4,4',5,5'-Hepta-CB	189	812	0,0001	0,081	<0,000005		< 0,004
Total mono-ortho PCB		121019		**16,626**			< **0,605**

WHO-TEF = toxic factor, WHO-TEQ = toxic equvalent by WHO for humans & mammals
TEQ (WHO) in consideration of 100% detection limit
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed,
(M) = maximum value, contains outside contamination
Small differences on totals result from computerroundings

non-ortho and mono-ortho PCB in cod liver		
Values in:	pg/g (ppt)	fresh weight based
Analysis-No.:	H-03-08-0475	Reyðfiskur 19, Livur, Kaldbak, 12.07.2002

non-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
3,4,4',5-Tetra-CB	81	12	0,0001	0,001	0,0005		0,006
3,3',4,4'-Tetra-CB	77	266	0,0001	0,027	0,0001		0,027
3,3',4,4',5-Penta-CB	126	129	0,1000	12,876	0,005		0,644
3,3',4,4',5,5'-Hexa-CB	169	35	0,0100	0,353	0,00005		0,002
Total non-ortho PCB		443		**13,258**			**0,678**

mono-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
2,3,3',4,4'-Penta-CB	105	8649	0,0001	0,865	<0,000005		< 0,043
2,3,4,4',5-Penta-CB	114	545	0,0005	0,272	<0,000005		< 0,003
2,3',4,4',5-Penta-CB	118	27341	0,0001	2,734	<0,000005		< 0,137
2',3,4,4',5-Penta-CB	123	416	0,0001	0,042	<0,000005	(M)	< 0,002
2,3,3',4,4',5,-Hexa-CB	156	2978	0,0005	1,489	<0,000005		< 0,015
2,3,3',4,4',5'-Hexa-CB	157	969	0,0005	0,484	<0,000005		< 0,005
2,3',4,4',5,5'-Hexa-CB	167	2017	0,00001	0,020	<0,000005		< 0,010
2,3,3',4,4',5,5'-Hepta-CB	189	290	0,0001	0,029	<0,000005		< 0,001
Total mono-ortho PCB		43204		**5,935**			< **0,216**

WHO-TEF = toxic factor, WHO-TEQ = toxic equvalent by WHO for humans & mammals
TEQ (WHO) in consideration of 100% detection limit
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed,
(M) = maximum value, contains outside contamination
Small differences on totals result from computerroundings

***WHO-TEF(fish) inserted and WHO-TEQ(fish) calculated after recieving the results from the laboratory. Other results calculated by the laboratory.**

non-ortho and mono-ortho PCB in cod liver		
Values in:	pg/g (ppt)	**lipid based**
Analysis-No.:	H-03-08-0476	Reyðfiskur24, Livur, Kaldbak, 17.07.2002

non-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
3,4,4',5-Tetra-CB	81	13	0,0001	0,001	0,0005		0,006
3,3',4,4'-Tetra-CB	77	410	0,0001	0,041	0,0001		0,041
3,3',4,4',5-Penta-CB	126	502	0,1000	50,246	0,005		2,512
3,3',4,4',5,5'-Hexa-CB	169	98	0,0100	0,975	0,00005		0,005
Total non-ortho PCB		1022		**51,263**			2,564

mono-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
2,3,3',4,4'-Penta-CB	105	22288	0,0001	2,229	<0,000005		< 0,111
2,3,4,4',5-Penta-CB	114	1492	0,0005	0,746	<0,000005		< 0,007
2,3',4,4',5-Penta-CB	118	82287	0,0001	8,229	<0,000005		< 0,411
2',3,4,4',5-Penta-CB	123	1046	0,0001	0,105	<0,000005	M	< 0,005
2,3,3',4,4',5,-Hexa-CB	156	8812	0,0005	4,406	<0,000005		< 0,044
2,3,3',4,4',5-Hexa-CB	157	2644	0,0005	1,322	<0,000005		< 0,013
2,3',4,4',5,5'-Hexa-CB	167	5639	0,00001	0,056	<0,000005		< 0,028
2,3,3',4,4',5,5'-Hepta-CB	189	1067	0,0001	0,107	<0,000005		< 0,005
Total mono-ortho PCB		125275		**17,199**			< **0,626**

WHO-TEF = toxic factor, WHO-TEQ = toxic equivalent by WHO for humans & mammals
TEQ (WHO) in consideration of 100% detection limit
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed,
(M) = maximum value, contains outside contamination
Small differences on totals result from computerroundings

non-ortho and mono-ortho PCB in cod liver		
Values in:	pg/g (ppt)	**fresh weight based**
Analysis-No.:	H-03-08-0476	Reyðfiskur 24, Livur, Kaldbak, 17.07.2002

non-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
3,4,4',5-Tetra-CB	81	5	0,0001	0,001	0,0005		0,003
3,3',4,4'-Tetra-CB	77	167	0,0001	0,017	0,0001		0,017
3,3',4,4',5-Penta-CB	126	205	0,1000	20,500	0,005		1,025
3,3',4,4',5,5'-Hexa-CB	169	40	0,0100	0,398	0,00005		0,002
Total non-ortho PCB		417		**20,915**			**1,046**

mono-ortho PCB	IUPAC-No.	Concentration	WHO-TEF	WHO-TEQ	WHO-TEF(fish)	LOD	WHO-TEQ(fish)
2,3,3',4,4'-Penta-CB	105	9094	0,0001	0,909	<0,000005		< 0,045
2,3,4,4',5-Penta-CB	114	609	0,0005	0,304	<0,000005		< 0,003
2,3',4,4',5-Penta-CB	118	33573	0,0001	3,357	<0,000005		< 0,168
2',3,4,4',5-Penta-CB	123	427	0,0001	0,043	<0,000005	M	< 0,002
2,3,3',4,4',5,-Hexa-CB	156	3595	0,0005	1,798	<0,000005		< 0,018
2,3,3',4,4',5-Hexa-CB	157	1079	0,0005	0,539	<0,000005		< 0,005
2,3',4,4',5,5'-Hexa-CB	167	2301	0,00001	0,023	<0,000005		< 0,012
2,3,3',4,4',5,5'-Hepta-CB	189	435	0,0001	0,044	<0,000005		< 0,002
Total mono-ortho PCB		51112		**7,017**			< **0,256**

WHO-TEF = toxic factor, WHO-TEQ = toxic equivalent by WHO for humans & mammals
TEQ (WHO) in consideration of 100% detection limit
n.d. = not detected, limit of detection (LOD) in ( ), n.a. = not analysed,
(M) = maximum value, contains outside contamination
Small differences on totals result from computerroundings

***WHO-TEF(fish) inserted and WHO-TEQ(fish) calculated after recieving the results from the laboratory. Other results calculated by the laboratory.**

Attachment 13C:

8.1.1 Polychlorinated Biphenyls (PCB) in cod liver

Values in: µg/kg (ppb) **lipid based**

	Reyðfiskur 8	Reyðfiskur 10	Reyðfiskur 19	Reyðfiskur 24
PCB #28	2	5	3	6
PCB #52	7	14	5	19
PCB #101	26	32	23	46
PCB #118	47	48	77	82
PCB #138	100	90	129	143
PCB #153	137	99	202	202
PCB #180	46	50	63	73
total	366	338	501	572

Polychlorinated Biphenyls (PCB) in cod liver

Values in: µg/kg (ppb) **fresh weight based**

	Reyðfiskur 8	Reyðfiskur 10	Reyðfiskur 19	Reyðfiskur 24
PCB #28	1	2	1	2
PCB #52	3	5	2	8
PCB #101	12	11	8	19
PCB #118	22	17	27	47
PCB #138	47	32	46	58
PCB #153	65	35	72	83
PCB #180	22	18	22	30
total	172	119	179	247

n.d. = not detected
n.a. = not analysed
(M) = maximum value, contains possible outside contamination
small differences on totals result from computerroundings