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Biomarker Baseline Survey on the Vøring Plateau

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Preface

The environmental baseline survey in the Vøring area in 2005 was managed and conducted by DNV in accordance to SFT guidelines. An optional study on biomarker baseline levels in deep-sea fish and amphipods was subcontracted to IRIS Biomiljø and results of this study is presented here.

IRIS field work was prepared and conducted by: Lars Petter Myhre, Rolf C. Sundt and Jan Fredrik Børseth.

Taxonomy of amphipod species was performed by Anne Helene Tandberg.

Chemical analyses in fish liver and amphipod tissue were coordinated by Kjell Birger Øysæd. Atle Nævdal made the PAH analyses by GC/MS and Stig Westerlund the metal analyses by ICP/MS. Chemical analyses of PCB and PFOS in fish liver and amphipod tissue was subcontracted to NILU.

Biomarker analyses were conducted by: Lars Petter Myhre, Atle Nævdal and Nadia Aarab. Micronuclei and DNA adduct analyses were subcontracted to University of Vilnius and University of Stockholm respectively. A major delay of this report is due to methodological problems with the DNA adduct analysis. *Sigma* stopped the production of one of the analytical enzymes, thus the lab in Stockholm had to find an alternative solution and to test this against the old procedure before continuing their analyses.

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Stavanger, 30. March 2007

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Summary

The environmental baseline survey in the Vøring area in 2005 was managed and conducted by DNV in accordance to SFT guidelines. An optional study on biomarker baseline levels in deep-sea fish and amphipods was subcontracted to IRIS Biomiljø and results of this study is presented here.

Special deep-sea traps were developed for the purpose of collecting fresh and biological samples from depths around 1,100 meter. With the use of these new traps it was possible to obtain intact tissue samples of deep-sea fish and amphipods for later measurements of enzyme activities in i.e. liver and muscle tissue. Baseline levels of different biomarkers of contamination were determined at two different areas in the Norwegian Sea.

Additionally, baseline levels of body burden of PAH, PCB, PFOS and metals were measured in the collected deep-sea organisms.

The baseline levels of selected chemicals and biomarkers presented in this report represent important background information for possible follow-up environmental monitoring campaigns in the future.

1 Introduction

The main objective of the present study was to measure the background levels of different biomarkers in indigenous fish and amphipod species inhabiting two deep-water zones in the Norwegian Sea. Fish and amphipods samples were collected in the period 23rd June to 1st July 2005 at two sites in the Vøring area. The study organisms were collected by means of baited traps remotely controlled by acoustic release equipment.

When designing the analysis program, a relevant set of chemicals as well as biological parameters (biomarkers of sub-lethal environmental damage) was chosen. Special attention was given to biomarkers that respond to environmental presence of drill cutting associated chemicals (e.g. metals) and on polyaromatic hydrocarbons. Biomarkers used during the ICES-BECPELAG workshop and suggested in guidelines from the Joint Monitoring and Environmental Assessment Programme (under the framework of the OSPAR Commission) were prioritised in the analyses program (e.g. PAH metabolites in fish bile and DNA adduct in fish liver). Biological samples for analyses of contaminant background levels were collected from a subset of the catch.

2 Field methods and biological material

2.1 The study area

2.1.1 Description of sampling areas

It was decided to collect study organisms from two locations (**Error! Reference source not found.**). The two sampling areas were characterized by clay bottom and depths of approximately 1.100 m. Within each sampling area fish and amphipods were collected.

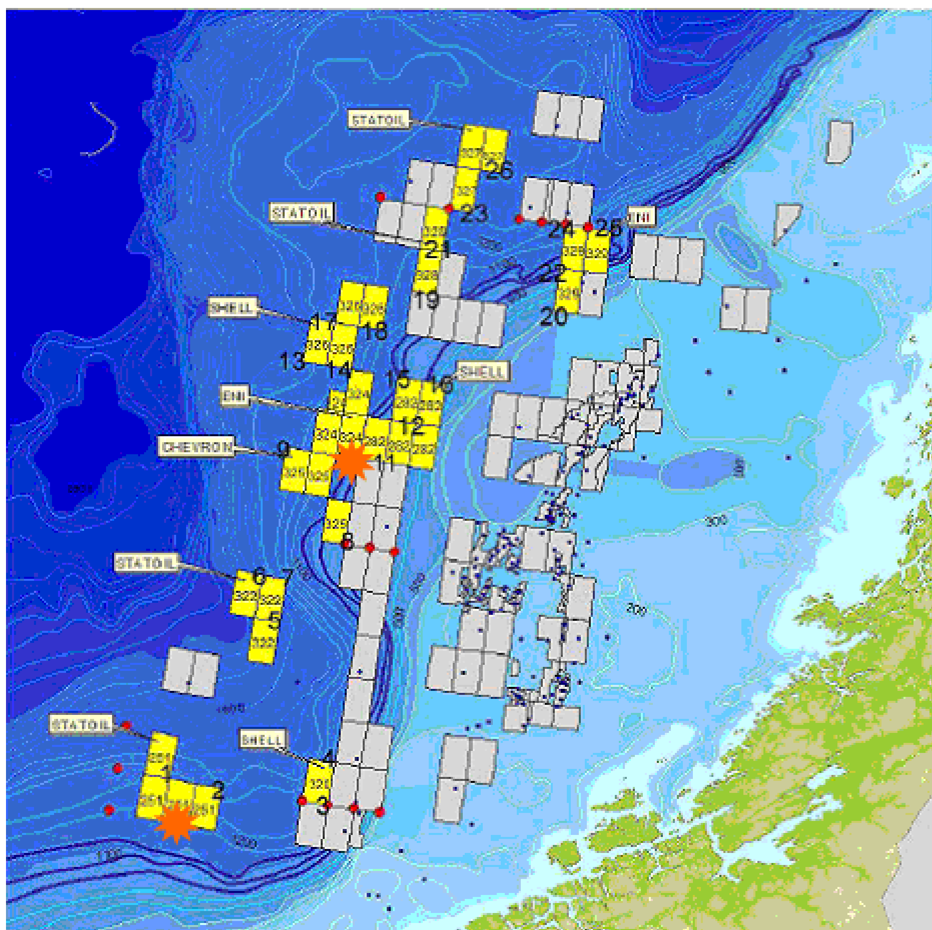


Figure 1. Map of the sampling area. The collection of deep-sea organisms were made at the two sites indicated by stars in orange colour. Site 1 is the Southern one and site 2 the Northern.

2.2 Survey vessel

The support vessel m/v Polarbjørn from Rieber Shipping A/S was hired for the field work. The vessel carried cranes and provided a suitable in-door lab facility for the biological sampling (wet-lab). Technical specifications and information of the vessel are given below (**Error! Reference source not found.**).

Table 1. Vessel specification and description of m/v Polarbjørn

Length Overall	80.7 m
Breadth Moulded	16.0 m
Draft max	7.25 m
Endurance	30 days between ports
Scientific Berths	100
Crew	20
Constructed	2001
Call signal	LARY5
IMO No	IMO 9233997
Propulsion	kW
Bow Thrusters	kW
Class	+1A1, icebreaker

Table 2. Scientific crew

Name	**Company**	**Task**
Tor Jensen	DNV	Project leader
Tormod Glette Hansen	DNV	Sediment
Øyvind Fjukmoen	DNV	Sediment
Håkon Eilif Larsen	DNV	Sediment
Amund Ulfsnes	DNV	Sediment
Andreas Parscow	DNV	Sediment
Rolf C. Sundt	RF-Akvamiljø	Biomarkers
Jan Fredrik Børseth	RF-Akvamiljø	Biomarkers

2.3 Equipment and procedures for animal collection

2.3.1 Trap rig and retrieval mechanism

The retrieval mechanism design generally used for shallow sampling are fitted with floating buoys for hook in. This traditional design is disproportionately complicated to

operate under deep water conditions. The substantial projected area of surface line would lead to demand of extremely dimensioned bottom weight and floating elements in order to avoid drifting of gear.

2.3.2 Acoustic releasers

The design used in the present study is based on rigs without surface line and with acoustic releasers that are activated to retrieve the gear. An acoustic releaser (Sonardyne OM – 7710C, provided by Seatronics) was fitted inside the centre of the rig and connected to the weight. 100 kg round steel plates (two plates à 50 kg for easy handling) connected to the releaser hook by a chain.

Communication with the releasers (arming, releasing and depth recordings) were commenced by a portable deck unit (7663/7 LCU Psion).

Figure 2

Figure 2. Acoustic releaser unit to be fitted inside trap rig center axis.

Figure 3. Portable deck unit for communication with releasers.

2.3.3 Acoustic transponders

To enhance accurate positioning of gear all rigs were fitted with acoustic mini SSBL transponders (Simrad NST 342/N). The transponders were attached on the rope below the doughnut with hose clamps. To avoid wear on the rope, an armed plastic hose was fitted before attachment.

The vessels transponder system (Kongsberg, Simrad –HIP AP, Apos software) was used under deployment and a portable deck unit (Sonardyne 7769 transceiver, provided by Seatronics) was used as backup system.

Figure 4. Acoustic transponder unit to be fitted on rope between rig and lowest buoy.

2.3.4 Buoyancy units

Buoyancy units (total 100 kg buoyancy) consisted of 2 times 25 kg round units and a 50 kg “doughnut” (both unit types certified to 2,000 metres water pressure). A 20 meter rope was fitted between the to 25 kg units to ease the retrieving operation. The separate buoy was retrieved by a heaving line fitted with a 2 kg treble hook and the line connected to the crane hook for hosing of the rig.

2.3.5 Trap selection and bait

In advance of the survey different equipment was evaluated to increase the probability of getting enough specimens of fish and invertebrates. It was also of importance that the animals were caught as locally as possible and that the specimens were brought to the surface in a good condition, preferably alive. These considerations led to the decision of using baited traps as catching technique. It was decided to use three different traps; large ocean-going fish-traps and two types of smaller traps for invertebrates.

Both fish traps and invertebrate traps were baited with a combination frozen herring (*Clupea harengus*) and mackerel (*Scomber scomber*). The fish used for bait was purchased in frozen condition and thawed before loaded into the traps. To avoid depletion of bait some of it was put in containers inaccessible for the amphipod scavengers. This procedure extends the duration of the bait and thus prolonged the traps active fishing time.

2.3.6 Fish traps

The large fish trap had been developed at the Norwegian Institute of Marine Research and was originally designed to catch gadoid species such as haddock and cod. The robust design and large volume of this trap allowed it to be left out in periods of bad weather without reducing the fish quality. It was made up of a steal and aluminium frame enclosed in a 2 cm mesh net and could be collapsed during transport. The fish pot is a two compartment trap with two caching units in the lower sections and with a storing compartment at the top of the trap (Figure 5). Height/width/length was 113*100*152 cm, respectively and entrance opening 100*56 cm and 15*25 cm (outer and inner diameter, respectively).

Figure 5. Trap used for catching fish mounted on rig.

2.3.7 Bottle traps

The bottle traps were made of perforated baited soda bottles (volume 1.5 L) where the bottleneck was cut free and assembled backwards (Figure 6). This bottle-traps has previously been successfully used for collection of hagfish in deep waters. Traps were attached to frame inside the fish trap.

Figure 6. Bottle trap used for catching invertebrates.

2.3.8 Drain pipe traps

The traps are made up from a 15 cm perforated drain pipe fitting "access pipe with screw opening" with a 26 mm one way funnel entrance. Entrance is fitted with splintered 1 mm plankton mesh to avoid animals escaping once inside the trap (Figure 7). Traps were attached to frame inside the fish trap.

Figure 7. Drain pipe trap used for catching invertebrates. Entrance at the right, baiting and removal of catch is done through screw lid on the bottom.

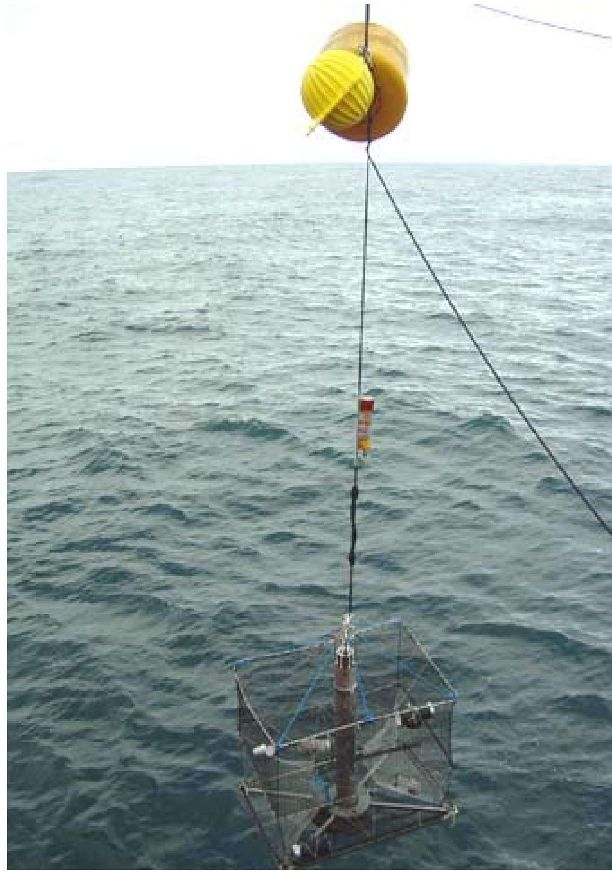


Figure 8. Complete rig arrangement ready for deployment.

2.3.9 Study areas

On each of the areas 5 – 7 rigs were deployed (see Table 3). The first rig was deployed at the position given in Table 3. and the subsequent were deployed approximately 100 metres from the position on a transect 90º on the expected current direction. The traps were placed out in the evening and picked up the morning after.

Table 3. Area information.

Area no.	Date	No of rigs deployed	Bottom time	Depth	Position
1	25th June	2	~9 h	1110 m	63º 25. 000 N 03º 15. 000 E
1	26th June	5	~23 h	1110 m	63º 25. 000 N 03º 15. 000 E
2	30th June	5	~30 h	1057 m	65º 33. 000 N 04º 27. 000 E

Figure 9. Five rigs ready for deployment.

2.4 Rig performance and catch

2.4.1 Sink speed and drift of rigs

By means of transducer readings connected to GPS, positioning drift was tracked after deployment (Figure 10). Rigs typically drifted approximately 150 m from the drop point to the sea bed. Typical sink time to 1,100 metres was approximately 20 minutes (~ 0,91 metres / minute). Typical float time from 1,100 metres was approximately 25 minutes (~ 0,75 metres / minute).

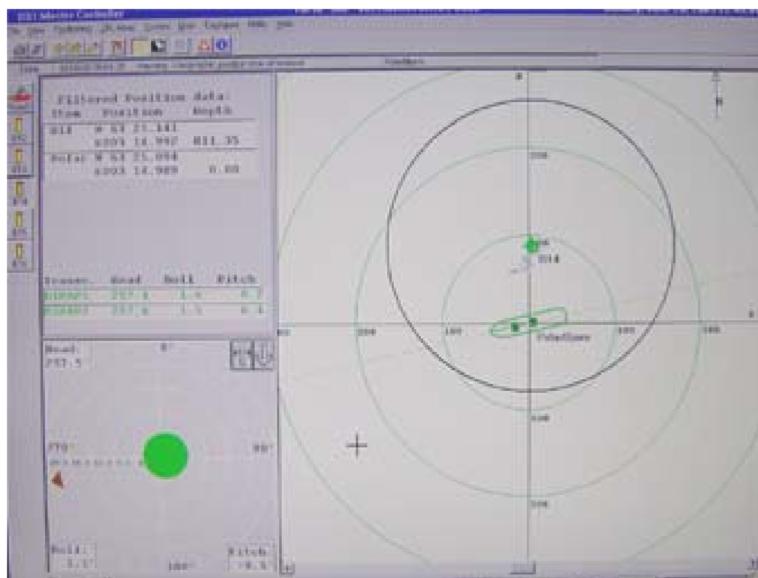


Figure 10. Screen picture of device used for tracking of gear showing vessel and gear drift from sea bed.

2.4.2 Catch success

Fish

Based on previous experience, it is known that the crustacean traps catch hagfish when present. Lack of hagfish in the catches lead us to the conclusion that these species were not abundant in the area. Explanations might be that the bottom substrate is unfavourable, or the hagfish may face too high competition on prey from scavenging amphipods.

However, the fish catch consisted exclusively of Arctic rockling, *Onogadus argentatus* (Figure 11). The bottle traps did not collect any hagfish but were instead full of amphipods.

Table 4. Distribution of fish catch among traps during survey shows patchy distribution.

Fish species	Area 1	Area 2	Sum
Onogadus argentatus	1+2+2+0+0+8+4	1+1+3+1+1	24

Figure 11. Arctic rockling, *Onogadus argentatus*.

Invertebrates

The catch of invertebrates consisted only of amphipods. The most abundant species were *Eurythenes gryllus* (giant amphipods) and *Anonyx magox*. Both species are big enough for individual sampling and both species were present in sufficient numbers for complete sampling in all rigs. Therefore these two species were selected as target species. In addition to the two big amphipod species several other smaller species were caught in abundance in all traps (see Figure 12). Each rig setting (two bottle traps and two drain pipe traps) caught from 250 to 1250 gram amphipods.

Figure 12. Selection of amphipods from catch. The two lower species *Eurythenes gryllus*, giant amphipods (right) and *Anonyx magox* (left) were individually sampled for biomarker and chemical analysis.

2.5 Procedure for biological sampling

The main goal of the biological sampling was to obtain a maximum of 25 specimens of each target species from each of the two study sites. Captured animals (fish and invertebrates) were brought to a indoor wet lab facility for dissecting. All samples for biomarker analyses were taken at an individual specimen basis.

2.5.1 Fish sampling

Before sampling the fish was killed with a sharp blow to the head. Length and weight were measured and external signs of disease were examined. The first gill at the left side was excised and fixed in formalin (only site 2). The abdominal cavity was opened and the gallbladder was removed. The bile was drained into a cryovial and frozen in liquid N_2. The liver was cut free, weighed and then split. Two cryovials with liver were frozen in N_2 (DNA adducts and EROD analysis) and excess liver was put in plastic bags (for metal analysis) and preburned scintillation gasses (for PAH analysis) and transferred to a -20˚C freezer. The sex of fish was determined and the gonadal maturity was recorded. A slice of gonad and liver were put in histology cassettes and transferred to buffered formalin (for histology). A piece of liver was smeared on microscopic slide and dried prior to methanol fixation (for micronucleus analyses). Lastly, a muscle sample for body burden assessment was cut out, wrapped in aluminium foil and transferred to a -20˚C freezer. After the end of cruise the N_2 preserved samples were transferred to a -80˚C freezer for storage until analyses.

Figure 13. Fish sampling in the wet-laboratory.

2.5.2 Invertebrates sampling

As noted above the two amphipod species *Eurythenes gryllus* (giant amphipods) and *Anonyx magox* were selected as target species based on their abundance and large size.

Size data on all individually sampled *Eurythenes gryllus* specimen were recorded. Haemolymph was obtained by means of a heparinised syringe and dispersed on microscopic slides (for micronucleus analysis). Head was cut of and body cavity organs (mainly hepatopancreas) were squeezed out into a cryovial and frozen in N_2. Separate specimens were opened ventrally with a scalpel and intestine transferred to formalin in histology cassette. Pools of specimens were trimmed for legs and leg cover and transferred to heat treated glass (for PAH analysis).

After the end of cruise the N_2 preserved samples were transferred to a -80˚C freezer for storage until analyses.

2.6 Biological samples collected

2.6.1 Fish samples

Table 5. Samples of collected from the deep water fish Arctic rockling (*Onogadus argentatus*)

Matrix	Analysis method	Preservation	St. 1	St. 2	Sum
Bile	PAH met. (FF and GC/MS)	(l) N_2	17	7	25
Liver	EROD	(l) N_2	17	7	25
Liver	DNA adducts	(l) N_2	17	7	25
Liver	PAH by GC/MS	÷ 20	17	7	25
Liver	PCB & PFOS	÷ 20	17	7	25
Liver	Histology	Formalin	17	7	25
Liver	Micronucleus	Slide	10	7	17
Liver	Metals	÷ 20	17	7	25
Liver	Histology	Formalin	17	7	25
Gonad	Histology	Formalin	17	7	25
Gills	Histology	Formalin	-	7	7
Muscle	PAH	÷ 20	-	7	7

2.6.2 Invertebrate samples

Individual samples were collected from the two most abundant amphipod species; *Eurythenes gryllus* and *Anonyx magox*.

Table 6. Samples of collected from the giant amphipod *Eurythenes gryllus*.

Matrix	Analysis method	Preserved	St. 1	St. 2	Sum
Haemolymph	Micronuclei	Slides	9	10	19
Hepatopancreas	GST	(l) N_2	25	25	50
Gut	Histology	Formalin	10	10	20
Whole organism	Metals	÷ 20	3 pools	3 pools	6 pools
Whole organism	PAH	÷ 20	3 pools	3 pools	6 pools

Table 7. Samples of collected from the amphipod *Anonyx magox*.

Matrix	Analysis method	Preserved	St. 1	St. 2	Sum
Hepatopancreas	GST	(l) N_2	25	25	50
Gut	Histology	Formalin	10	10	20
Whole organism	Metals	÷ 20	3 pools	3 pools	6 pools
Whole organism	PAH	÷ 20	3 pools	3 pools	6 pools

3 Analytical methods

3.1 Body burden

3.1.1 Polycyclic aromatic hydrocarbons

The methodology for extractions of PAH in water and tissue for GC/MS analyses followed the descriptions provided by Skadsheim et al. (2000a) and Baussant et al. (2001a). Raw data of PAHs analysed are listed in the Appendix. The 16 PAHs which

are included in the EPA list of priority pollutants were analysed including benzo(b+k)fluoanthene and the alkylated congeners of naphthalene (C1-C3), phenanthrene/anthracene (C1-C2) and chrysene (C1-C2). In addition, both the parental and C1-C2 congeners of dibenzothiophene were determined.

Detection limits. The detection limit in the GC/MS analyses was at ca 1 μg/kg sample. Some symbols means as follows: mi = matrix interference, mi spike = matrix interference from spike, *< = a quantity detected under the quantification level in the analyses, values may be included in the data set but then in parentheses (). In some appendix tables values derived from analyses with matrix interference and tentative readings under the detection limit are shaded in gray as an alternative to the symbols described above. nd = not detected.

3.1.2 Polychlorinated biphenyls and PFOS

Here follows a short description of the method NILU-O-2 used for PCB analysis:

Sample preparation: Samples are spiked with ^{13}C-labelled analogs of the analytes and extracted with a method suitable for the actual matrix.

Sample clean-up: For the majority of the samples, the most of the sample matrix are removed with size exclusion chromatography (GPC) followed by cleanup using silica and alumina oxide. Prior to analysis, the samples are spiked with a recovery control standard.

Quantification: High resolution GC is used for separations. The main part of the components of interest are analysed using high resolution MS, while some of the chlorinated pesticides are analysed using low resolution MS. The added ^{13}C-labelled isomers are used as internal standard for selected groups. Additionally, the recoveries of the added internal standard compounds are determined.

Quality assurance: The following conditions must be fulfilled for an unequivocal identification and quantification of the O-2 components:

The retention time must be in a window of +3 to 0 s compared to the corresponding ^{13}C-labelled isomer.

The isotope ratio of the two monitored isotopes must be within ±20% of the theoretical value.

The signal/noise must be >3/1 for quantification.

The recovery of the added ^{13}C labelled internal standards must be within a certain range.

NILU-O-2 is according to the directives given by the European Commission for determination of dioxin-like PCBs for the official control of foodstuffs (2002/69/EC) and feedstuffs (2002/70/EC). The method is also according to the guidelines given by the European Commission's for Residues Monitoring in the European Union (document no. SANCO/3103/2000).

3.1.3 Metals in Tissue

The tissue sample had been stored in a freezer in polypropylene vials or plastic bags after the dissection of the fish. The liver sample was then digested with a mixture of HNO_3 and H_2O_2 in a microwave oven system. The other tissue samples were digested only HNO_3 About 1 gram tissue sample was placed in a Teflon vessel, 5 ml 15 M HNO_3 and 1ml 9 M H_2O_2 vas added. The Teflon vessel was sealed and placed in the microwave oven. The samples were then diluted to 50 ml in 50 ml polypropylene test tubes. The samples were the diluted further and In was added to act as internal standard. The final metal determination was performed using an ICP-MS (See below).

ICP-MS metal determination of metals. For the determination of the metals other than Hg a VG-PQ2+ ICP MS was used. I peak jump procedure was used with as many masses of each element to be able to evaluate possible isobaric interferences. In the procedure Indium was used as internal standard to compensate for instrumental drift. In evaluating the results the mass with the highest abundance and lowest interferences was used for the quantification. The operating conditions can be found in the table below.

Determination of Hg. Mercury was determined using a Perkin-Elmer flow-injection system. The Hg was detected with the cold vapour technique by atomic absorption in a gas cell.

3.2 Biotransformation markers

3.2.1 Aromatic metabolites

Fixed fluorescence anaysis. FF is a semi-quantitative and semi-qualitative screening method for direct fluorescence detection of groups of PAH metabolites (Aas et al. 2000). Bile samples were diluted 1:1600 in methanol:water (1:1). Slit widths were set at 2.5 nm for both excitation and emission wavelengths, and samples were analysed in a quartz cuvette. All bile samples were analysed by FF at the wavelength pairs 290/335, 341/383 and 380/430 nm, optimized for the detection of 2-3 ring, 4-ring and 5-ring PAH metabolites, respectively. The fluorescence signal was transformed into pyrene fluorescence equivalents through a standard curve made by pyrene (Sigma St Louis, USA). Pyrene was measured in the same fluorometer, with the same cuvette, same solvent, and with the same slit settings as the bile samples. It was, however, measured at the optimal wavelength pair of pyrene, 332/374 nm (ex/em). The concentration of PAH metabolites in bile samples was expressed as µg pyrene fluorescence equivalents (PFE) /ml bile.

GC/MS sample preparation. Fish bile was prepared for analysis as described by Jonsson et al. (2003; 2004). Briefly, 25–30 µl of bile was weighed accurately into a micro centrifuge vial. Internal standards (2,6-dibromophenol, 3-fluorophenanthrene and 1-fluoropyrene) and β-glucuronidase (3000 units) in sodium acetate buffer (0.4 M, pH = 5) were added and the solution left at 40°C for 2 hours. The OH-PAHs were extracted with ethylacetate (4 times 0.5 ml), the combined extract dried with anhydrous sodium sulphate and concentrated to 0.5 ml. Trimethylsilyl (TMS) ethers of OH-PAHs were prepared by addition of 0.2 ml BSTFA and heating for two hours at 60°C. TPA

was added as a GC-MS performance standard before transferring the prepared samples to capped vials.

GC-MS analysis. Trimethylsilyl ethers of OH-PAHs (TMS-OH-PAHs) in fish bile samples were analysed by a GC-MS system consisting of a HP5890 series II Gas chromatograph, Finnigan A200S autosampler and a Finnigan MAT SSQ7000 mass spectrometer (Thermo Finnigan, Huddinge, Sweden). The system was controlled by a DEC station 5000. Helium was used as carrier gas and the applied column was CP-Sil 8 CB-MS, 50 m x 0.25 mm and 0.25 µm film-thickness (Instrument Teknikk A.S., Oslo, Norway). Samples and calibration standards (1 µl) were injected on a split/splitless injector with splitless mode on for one minute. The temperatures for the injector, transfer-line and ion source were held at 250°C, 300°C and 240°C, respectively, and the GC oven temperature programme was as follows: 80°C to 120°C at 15°C min^{-1}, 120°C to 300°C at 6°C min^{-1} and held at 300°C for 30 min. Mass spectra were obtained at 70 eV in selected ion mode (SIM). Based on the fragmentation pattern of non-alkylated TMS-O-PAHs (Jonsson et al. 2003) and studies performed by Krahn et al. (1992) and Yu et al. (1995) the molecular ions were selected for determination of both alkylated and non-alkylated TMS-O-PAHs.

3.2.2 EROD activity

EROD activity is an indirect measure of the catalytic activity of the CYP1A1 enzyme. The measure is based on the CYP1A catalytic formation of the product resorufin from the substrate ethoxyresorufin. The presence of resorufin is detected by fluorescence measurement (excitation 535 nm, emission 585 nm). NADPH is combined with buffer, biological sample, and ethoxyresorufin to perform a co-enzyme role, delivering oxidative power to the reaction. Resorufin is added as internal standard. Samples should be not be thawed more than once (for sample preparation) prior to analysis. Ethoxyresorufin and NADPH are added in excess to the reaction.

Liver samples were homogenised in phosphate buffer (pH 7.4, 0.5 g liver to 2 ml buffer) and centrifuged at 12000g for 20 min. The supernatant was further centrifuged at 100.000g for 1 hour to give microsome fraction. Samples were stored in -80°C until analysis. EROD activity was measured on a Perkin Elmer LS50B luminescence spectrometer in cuvettes according to protocol described in Nilsen (1998). Hepatic S-12 fraction of cod exposed intraperitoneally to 5 mg kg^{-1} benzo[a]pyrene for 3-5 days served as a positive control in all analytical series. EROD activities are normalised to protein concentration in the sample.

Protein concentrations in the liver samples were measured according to the method described in Bradford (1976). Samples were diluted and pipetted out on a microplate in 4 replicates. A dilution series of 8 standards was made from a 5 % Bovine Serum Albumin (BSA) solution, and all standards were run in 3 replicates, on each microplate analysed. A 1:5 solution of Bradford reagents (Bio-Rad Laboratories) in distilled water was prepared shortly before use and added to each well. After 5 minutes reaction time, the absorbance was measured at 595 nm (Labsystems, Multiscan RC).

3.2.3 GST activity

The determinations of the total protein concentrations has two purposes, firstly, the protein concentration is used to make an appropriate dilution of GST samples prior to analyses. Secondly, the protein concentration is needed for normalisation for GST results. The total protein concentrations of the samples where determined by a procedure based on the Bradford method (Bradford, 1976).

Total glutathione-S-transferase (GST) specific activities were measured using 1-chloro-2,4-dinitrobenzene (CDNB, $C_6H_3ClN_2O_4$) as a substrate and glutathione-S-hydroxylase (GSH, $C_{10}H_{17}N_3O_6S$) as a co-factor (Habig et al. 1974; Habig and Jakoby 1981).

Before analysis, the cytosol was diluted in phosphate buffer (pH 7.4), in order to obtain linear signal with time during the spectrometric measurements. The standard assay conditions are described in Table 4. After the addition of all the components, the cuvette (plastic) was capped, the content rapidly mixed and the increase in absorbance due to the formation of the conjugate product (GST-CDNB) was recorded by continuous registration for 1 minute at 340 nm, on a Perkin-Elmer UV/VIS spectrometer (Lambda 2S).

When GST activities were quantified, the blank value was deducted and a molar extinction coefficient (∈) for glutathione-CDNB of 9.6 mM^{-1} cm^{-1} was used. GST activities were expressed as moles of substrate converted per minute per mg of protein in the cytosol.

3.3 Genotoxicity markers

3.3.1 DNA adducts

DNA adducts were analysed in the liver of Arctic rockling (*Onogadus argentatus*) sampled at two different locations. They were analysed with the ^{32}P postlabelling assay, which is the most sensitive and frequently applied technique for detecting PAH-DNA adducts in marine organisms (Reichert *et al.*, 1998). DNA adducts are widely used as, and considered to be highly relevant biomarker for PAH exposure to fish (Ericson *et al.*, 1998).

Chemicals. Standard DNA (salmon sperm, D-1626), spermidin (S-2626), RNase A (R-4642), micrococcal endonuclease (N-3755) and spleen phosphodiesterase (P-9041) were obtained from Sigma Chemical Company, St. Louis, MO, USA. RNase T1 (109 193), proteinase K (1000144), α-amylase (102814), T_4-polynucleotidekinase (3´-phosphatase free, 838 292) and phenol (1814303) were purchased from Roche Diagnostics, Scandinavia AB, Bromma, Sweden. Nuclease P_1 (7160) was bought from Yamasa Corporation, Diagnostics Department, Chuo-Ku, Tokyo, Japan, and later Sigma-Aldrich Sweden AB, Stockholm, Sweden. Radiolabelled ATP ([γ-^{32}P]ATP) with specific activity 3000 Ci/mmol (110 TBq/mmol) were obtained from Amersham Biosciences, Uppsala, Sweden. The benzo[a]pyrene standard adduct, 7R, 8S, 9S-trihydroxy, 10R-(N^2-deoxyguanosyl-3´-phosphate)-7,8,9,10-tetrahydro-benzo(a)-pyrene (BaPDE-dG-3´p), was obtained from Midwest Research Institute, Kansas City, MO, USA. Cellulose

(MN-301) was purchased from Machery-Nagel, Düren, Germany. Vinyl strips (PVC foil, 0.2 mm thickness), used for the groundwork of the polyethyleneimine cellulose sheets were obtained from Andren & Söner, Stockholm, Sweden. Scintillation fluid (Ultima gold) was purchased from CIAB, Lidingö, Sweden. All other solvents and chemicals for DNA purification and adduct analysis were purchased from common commercial sources and were of analytical purity.

DNA adduct analysis. Tissue samples were semi-thawed and the DNA extracted and purified according to Dunn *et al*., 1987; Reichert and French 1994, slightly modified as described in Ericson and Balk, 2000. DNA adducts were enriched using the Nuclease P1 method, 0.41 µg Nuclease P1/µg DNA, and a 45 min incubation period (Reddy and Randerath 1986; Beach and Gupta 1992). The DNA adducts were radiolabelled using 5´-[γ-^{32}P]triphosphate([γ-^{32}P]ATP) and T_4 polynucleotide kinase. Separation and cleanup of adducts was performed by a modified multidirectional thin-layer chromatography (TLC) on laboratory produced polyethyleneimine cellulose sheets that serve as anionic exchanger support. After elution, adducts were then located on the sheets and quantified by storage phosphor imaging technology (PhosphorImager^{TMSI} and ImageQuant 5.0). In addition, several quality control experiments were performed in parallel to the analysis of the various fish tissue samples.

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

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

3.3.2 Micronuclei

The MN test is among the most widely used tools in eco-genotoxicology. Micronuclei are chromatin-containing structures that are surrounded by a membrane and have no detectable link to the cell nucleus. Cytogenetic damage can result in the formation of MN-containing lagging whole chromosomes or chromosome fragments. Thus, MN assay provide the evidence of DNA breakage and spindle dysfunction caused by clastogens and aneuploidogenic poisons (Heddle et al., 1983, 1991; MacGregor, 1991; Seelbach et al., 1993; Zoll-Moreux, Ferrier, 1999).

Micronuclei analysis was done in liver immature erythrocytes of 17 specimens of *Onogadus argentatus* and in haemolymph of 19 *Eurythenes gryllus*. The narrow possibility to analyze micronuclei formation in 8 specimens of *Anonyx magox* was restricted to insufficient amount (50-300 cells) of cells suitable for the analysis.

The small piece of liver was directly smeared on slides, air-dried and fixed in methanol for 15 min. Slides were stained with 5% Giemsa solution for 10-20 min. Haemolymph from amphipods was withdrawn and cells placed on the clean microscopic slides, dried and fixed in methanol for 15 min. To minimize technical variation the blind scoring of micronuclei was performed on coded slides without knowledge on sample location and species-dependence.

The frequency of micronuclei in liver cells was determined by scoring at a 1000× magnification using Olympus BX 51 or Nikon Eclipse 50i bright-field microscopes. 3000-5000 cells were counted for the detection of micronuclei in each fish and 1000 haemocytes – in each amphipod specimen.

Only cells with intact cellular and nuclear membrane were scored. Round or ovoid-shaped non-refractory particles with colour and structure similar to chromatin, with a diameter 1/3-1/50 of the main nucleus and clearly detached from it were interpreted as micronuclei (Fig. 1). In general, colour intensity of MN should be the same or less than of the main nuclei. Particles with colour intensity higher than of the main nuclei were not counted as MN.

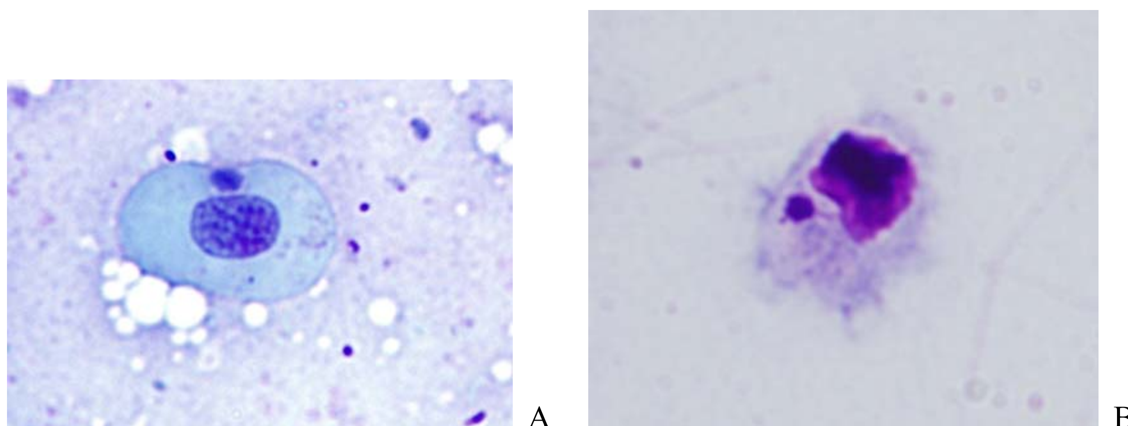


Figure 14. Micronuclei in immature erythrocyte of *Onogadus argentatus* (A)and in haemocyte of *Eurythenes gryllus* (B); 1,000× magnification.

The means of micronuclei per 1000 studied cells, standard errors and P values were calculated using PRISM statistical package. Mann-Whitney U-test was employed.

3.4 Histological markers

Histological biomarkers provide tools to detect and characterise the biological endpoints of toxicant and carcinogen exposure (Hinton et al., 1992; Moore & Simpson, 1992). As such, the utility of histological lesions as sensitive and reliable indicators of the health of wild fish populations has been demonstrated in several European and North American studies (Kranz & Dethlefsen, 1990; Myers et al., 1998; Köhler, 1991,1992; Lang et al., 1999). Several laboratory and mesocosm studies have also demonstrated causal links between exposure to xenobiotics and the development of toxicopathic hepatic lesions (Malins et al., 1985a; Malins et al., 1985b; Moore & Myers, 1994, Aarab et al, 2004).

Histopathological characteristics of specific organs express condition and represent time-integrated endogenous and exogenous impacts on the organism stemming from alterations at lower levels of biological organisation (Chavin, 1973; Stebbing, 1985; Swee et al 1996). Therefore, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters. As an integrative parameter, histological response provides a better evaluation of an organism health than a single biochemical parameter (Segner & Braunbeck, 1988; Swee et al., 1996).

Wax section

After fixation in formalin buffer, the liver, gills and gonads from *Onogadus argentatus* were dehydrated through a series of graded ethanol solutions (50-99%) followed by toluene. Histological sections (3µm thick) were cut on a microtome, mounted on slides, dried at 37 C for 24h, and stored at room temperature until staining. The three organs were stained with Heamtoxylin and Eosin and were then examined for histopathological alterations.

Histological specimens of the three tissues from two different stations were evaluated by light microscopy Zeiss for abnormalities. Each slide was examined at both low (40X) and high magnification (400X) without knowledge stations.

3.5 Statistics

The statistical software JMP 3.2.6 from SAS Institute Inc. was used to treat data. Most frequently, the statistical tests applied were the Kruskal-Wallis non-parametric test followed by all pair Tukey-Kramer test.

4 Results and discussion

4.1 Collection of deep-sea organisms

The catch was typically between 0-8 fishes in each trap with an average of 2 fishes per trap. The traps caught fish in the size range of 190-620 gram with average weight of 373 gram. An issue that could be considered is to modify the mesh size of the trap. Smaller mesh size could result in better catch by preventing escape possibilities for smaller species.

No hagfish were collected in the bottle traps. Instead these traps were loaded with large amphipods. The lack of hagfish catch could be due to the depth in the study area (1,100 m). Earlier studies in deeper waters (700 m) have shown that these traps are highly effective for hagfish collection.

To simplify the onboard operation during the hauling of traps, some minor changes should be performed with the trap equipment, but overall the newly developed rig system performed satisfactory.

4.2 Body burden

4.2.1 Polycyclic aromatic hydrocarbons

Table 8-11 show the level of polychlorinated biphenyls in fish liver and whole body samples of amphipods collected at the two sites in the Norwegian Sea.

There are relatively large natural variations between species so the data presented here, both for amphipods and fish, are most likely reflecting the background levels in these species at these locations.

Table 8. Concentrations of polychlorinated biphenyls in fish liver

O. argentatus PAH (ug/kg)

Label liver/ biota	Naphthalene	C1-Naphthalene	C2-Naphthalene	C3-Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	C1-Phen/Anthr	C2-Phen/Anthr	Dibenzothiophene	C1-Dibenzothiophene	C2-Dibenzothiophene
Fish 5	25,8	10,5	12,4	45,5	0,0	0,0	0,0	5,2	0,0	0,0	0,0	0,0	0,0	0,0
Fish 7	78,0	16,5	32,1	74,6	0,0	0,0	13,5	12,3	0,0	0,0	0,0	0,0	0,0	0,0
Fish 9	38,1	13,1	15,5	60,7	0,0	0,0	6,1	7,5	0,0	0,0	0,0	0,0	0,0	0,0
Fish 10	29	17	18	69	0,0	0,0	4,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 11	37,8	18,5	17,7	107	0,0	0,0	4,3	5,4	0,0	0,0	0,0	0,0	0,0	0,0
Fish 12	21,2	12,6	9,8	69,3	0,0	0,0	0,0	5,1	0,0	0,0	0,0	0,0	0,0	0,0
Fish 14	28	19	19	81	0,0	0,0	5,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 15	24	45	31	139	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 16	17	14	14	75	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 17	11	17	8,7	85	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 1,2,3	56	13	25	41	0,0	0,0	7	9	0,0	0,0	0,0	0,0	28	0,0
Fish 4,6,8,13	52	15	25	51	0,0	0,0	9	9	0,0	0,0	0,0	0,0	17	0,0
Fish 5,9,12	28,4	12,1	12,6	58,5				5,5						
Site 1 mean	*34,28*	*17,21*	*18,49*	*73,60*	*0,00*	*0,00*	*4,15*	*4,57*	*0,00*	*0,00*	*0,00*	*0,00*	*3,71*	*0,00*
Fish 1	6,3	19,0	17,8	54,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 2	9,8	24,7	21,7	49,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 3	6,4	15,9	16,7	71,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 4	7,0	15,2	15,4	47,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 5	56	17	29	59	0,0	0,0	8	13	0,0	0,0	0,0	0,0	13	0,0
Fish 6	4,5	12,1	12,2	40,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 7	51	13	25	51	0,0	0,0	10	10	0,0	0,0	0,0	0,0	31	0,0
Site 2 mean	*20,09*	*16,70*	*19,66*	*52,93*	*0,00*	*0,00*	*2,62*	*3,24*	*0,00*	*0,00*	*0,00*	*0,00*	*6,20*	*0,00*

Table 9. Concentrations of polychlorinated biphenyls in fish liver

O. argentatus PAH (ug/kg)

Label liver/ biota	Fluoranthene	Pyrene	Benzo(a)anthracene	Chrysene/Triphenylene	C1-Chrysene	C2-Chrysene	Benzo(b,j)fluoranthene	Benzo(k)fluoranthene	Benzo(b,j,k)fluoranthene	Benzo(a)pyrene	Indeno(1,2,3-cd)pyrene	Benzo(g,h,i)perylene	Dibenzo(a,h)anthracene
Fish 5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 10	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 11	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 12	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 14	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 15	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 16	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 17	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 1,2,3	4,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 4,6,8,13	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 5,9,12													
Site 1 mean	*0,33*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*
Fish 1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 2	7,4	4,1	3,9	5,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Fish 7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Site 2 mean	*1,05*	*0,58*	*0,56*	*0,84*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*	*0,00*

Table 10. Concentrations of polychlorinated biphenyls in amphipods.

Amphipods	PAH (ug/kg) Naphthalene	C1-Naphthalene	C2-Naphthalene	C3-Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	C1-Phen/Anthr	C2-Phen/Anthr	Dibenzothiophene	C1-Dibenzothiophene	C2-Dibenzothiophene
Eurythenes gryllus														
Pool 1	0,0	8,1	10,0	33,0	0,0	0,0	0,0	6,3	0,0	0,0	0,0	0,0	0,0	0,0
Pool 2	0,0	6,7	9,3	20,0	0,0	0,0	0,0	6,8	0,0	0,0	0,0	0,0	0,0	8,4
Pool 3	23,0	11,0	15,0	32,0	0,0	0,0	0,0	5,3	0,0	0,0	0,0	0,0	0,0	0,0
Site 1 mean	*7,7*	*8,6*	*11,4*	*28,3*	*0,0*	*0,0*	*0,0*	*6,1*	*0,0*	*0,0*	*0,0*	*0,0*	*0,0*	*2,8*
Pool 1	22,0	11,0	21,0	51,0	0,0	0,0	0,0	6,2	0,0	0,0	6,3	0,0	5,2	0,0
Pool 2	0,0	6,8	9,8	23,0	0,0	0,0	0,0	4,9	0,0	5,3	6,7	0,0	0,0	5,9
Pool 3	0,0	6,8	11,0	22,0	0,0	0,0	0,0	5,4	0,0	0,0	0,0	0,0	0,0	5,0
Site 2 mean	*7,3*	*8,2*	*13,9*	*32,0*	*0,0*	*0,0*	*0,0*	*5,5*	*0,0*	*1,8*	*4,3*	*0,0*	*1,7*	*3,6*
Anonyx magox														
Pool 1	0,0	8,9	9,9	28,0	0,0	0,0	0,0	27,8	0,0	0,0	0,0	0,0	0,0	0,0
Pool 2	0,0	8,0	8,5	20,0	0,0	0,0	0,0	5,1	0,0	0,0	0,0	0,0	0,0	0,0
Pool 3	0,0	7,0	6,7	23,0	0,0	0,0	0,0	4,4	0,0	0,0	0,0	0,0	0,0	0,0
Site 1 mean	*0,0*	*8,0*	*8,4*	*23,7*	*0,0*	*0,0*	*0,0*	*12,4*	*0,0*	*0,0*	*0,0*	*0,0*	*0,0*	*0,0*
Pool 1	0,0	7,8	9,3	25,0	0,0	0,0	0,0	6,2	0,0	0,0	0,0	0,0	0,0	0,0
Pool 2	0,0	9,2	11,0	30,0	0,0	0,0	0,0	7,5	0,0	0,0	0,0	0,0	0,0	0,0
Pool 3	0,0	7,2	8,2	25,0	0,0	0,0	0,0	6,3	0,0	0,0	0,0	0,0	0,0	0,0
Site 2 mean	*0,0*	*8,1*	*9,5*	*26,7*	*0,0*	*0,0*	*0,0*	*6,7*	*0,0*	*0,0*	*0,0*	*0,0*	*0,0*	*0,0*

Table 11. Concentrations of polychlorinated biphenyls in amphipods.

Amphipods PAH (ug/kg)

	Fluoranthene	Pyrene	Benzo(a)anthracene	Chrysene/Triphenylene	C1-Chrysene	C2-Chrysene	Benzo(b,j)fluoranthene	Benzo(k)fluoranthene	Benzo(b,j,k)fluoranthene	Benzo(a)pyrene	Indeno(1,2,3-cd)pyrene	Benzo(g,h,i)perylene	Dibenzo(a,h)anthracene
Eurythenes gryllus													
Pool 1	11,8	6,1	0,0	23,2	7,3	6,0	20,3	4,3	22,2	0,0	0,0	0,0	0,0
Pool 2	10,9	5,1	0,0	36,1	12,0	13,0	42,6	9,7	51,3	0,0	7,9	6,6	0,0
Pool 3	13,8	7,0	0,0	28,0	10,0	11,0	29,9	6,4	35,8	0,0	4,9	6,1	0,0
Site 1 mean	*12,2*	*6,1*	*0,0*	*29,1*	*9,8*	*10,0*	*30,9*	*6,8*	*36,4*	*0,0*	*4,3*	*4,2*	*0,0*
Pool 1	11,5	6,4	0,0	27,2	10,0	11,0	23,8	4,8	28,3	0,0	0,0	5,2	0,0
Pool 2	11,8	7,1	5,6	81,3	24,0	18,0	102,0	30,6	132,0	6,4	13,8	10,6	0,0
Pool 3	9,3	6,5	0,0	46,6	24,0	21,0	87,1	25,0	113,0	4,8	14,6	12,9	0,0
Site 2 mean	*10,9*	*6,7*	*1,9*	*51,7*	*19,3*	*16,7*	*71,0*	*20,1*	*91,1*	*3,7*	*9,5*	*9,6*	*0,0*
Anonyx magox													
Pool 1	0,0	0,0	0,0	0,0	0,0	0,0	6,7	0,0	9,2	0,0	0,0	0,0	0,0
Pool 2	0,0	0,0	0,0	11,8	6,4	0,0	18,1	0,0	22,0	0,0	0,0	0,0	0,0
Pool 3	0,0	0,0	0,0	6,3	0,0	0,0	7,1	0,0	9,1	0,0	0,0	0,0	0,0
Site 1 mean	*0,0*	*0,0*	*0,0*	*6,0*	*2,1*	*0,0*	*10,6*	*0,0*	*13,4*	*0,0*	*0,0*	*0,0*	*0,0*
Pool 1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,1	0,0	0,0	0,0	0,0
Pool 2	0,0	0,0	0,0	0,0	0,0	0,0	4,1	0,0	4,8	0,0	0,0	0,0	0,0
Pool 3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,4	0,0	0,0	0,0	0,0
Site 2 mean	*0,0*	*0,0*	*0,0*	*0,0*	*0,0*	*0,0*	*1,4*	*0,0*	*4,4*	*0,0*	*0,0*	*0,0*	*0,0*

4.2.2 Polychlorinated biphenyls and PFOS

Table 12-19 show the level of polychlorinated biphenyls in fish liver and whole body samples of amphipods collected at the two sites in the Norwegian Sea. In addition, Table 20 shows the level of PFOS in these organisms.

There are relatively large natural variations between species so the data presented here, both for amphipods and fish, are most likely reflecting the background levels in these species at these locations.

Table 12. Concentrations of polychlorinated biphenyls in fish liver.

O. argentatus

	PCB (ng/g)	IUPAC:	18	28	31	33	37		47	52	66	74	
Label liver/ biota	PeCB	HCB	2,2',5-TriCB	2,4,4'-TriCB	2,4',5-TriCB	2',3,4-TriCB	3,4,4'-TriCB	Sum-TriCB	2,2',4,4'-TetCB	2,2',5,5'-TetCB	2,3',4,4'-TetCB	2,4,4',5-TetCB	Sum-TetCB
Fish 7	2,35	26,8	0,49	1,92	2,28	0,12	<0,01	6,37	3,34	9,15	5,72	6,21	41,5
Fish 10	2,63	28,1	0,55	2,07	2,21	0,17	<0,01	6,25	2,81	8,32	5,5	6,06	39
Fish 11	2,24	26,4	0,51	1,93	2,15	0,11	<0,01	6,41	5	13,3	7,41	10,4	58,9
Fish 14	1,95	22,7	0,61	1,39	1,68	0,11	<0,01	5	2,48	9,16	3,21	2,54	31,1
Fish 15	2,25	28,4	0,34	1,62	1,75	0,08	0,02	5,06	4,06	10,1	6,13	8,03	45,9
Fish 16	2,05	21,3	0,93	1,57	1,61	0,11	<0,01	5,33	1,54	6,02	2,77	2,5	23,7
Fish 17	2,26	24,4	0,85	1,81	1,81	0,1	<0,01	6,05	2,52	8,31	3,76	3,47	32,6
Fish 1,2,3	2,38	27,7	0,74	1,97	2,09	0,12	<0,01	6,31	4,2	12	6,44	6,68	47,8
Fish 4,6,8,13	2,92	33,6	0,64	2,2	2,42	0,14	<0,01	7,75	5,18	14,4	7,95	9	59,9
Fish 5,9,12	2,3	26,6	0,6	1,5	1,7	0,1	0,0	5,7	4,2	12,1	5,4	7,1	46,6
Site 1 mean	*2,33*	*26,60*	*0,62*	*1,80*	*1,97*	*0,12*	*0,02*	*6,03*	*3,53*	*10,29*	*5,43*	*6,20*	*42,70*
Fish 1	1,93	19	0,42	1,73	1,27	0,08	0,01	4,41	2,16	5,28	4,22	3,12	23,9
Fish 2	1,91	20,7	0,55	1,85	1,73	0,07	<0,01	5,94	3	9,58	5,41	5,67	38,7
Fish 3	2,31	28,6	0,48	2,23	2,39	0,08	0,02	7,37	4,43	12,4	8,59	8,66	55,4
Fish 4	2,14	27,7	0,3	2,2	2,19	0,1	0,01	6,89	4,96	13,1	8,22	9,34	55,4
Fish 5	2,36	26,7	0,56	2,25	2,29	0,12	0,02	7,28	3,88	9,97	7,57	7,68	47,7
Fish 6	2,53	31,2	1,2	2,47	2,11	0,09	<0,01	7,14	2,43	9,88	4,6	4,04	36,2
Fish 7	2,48	22,7	0,42	2,05	2,34	0,1	0,02	6,65	4,75	14,8	8,86	11	64,3
Site 2 mean	*2,24*	*25,23*	*0,56*	*2,11*	*2,05*	*0,09*	*0,02*	*6,53*	*3,66*	*10,72*	*6,78*	*7,07*	*45,94*

Table 13. Concentrations of polychlorinated biphenyls in fish liver.

O. argentatus

Sample	99 2,2',4,4',5-PenCB	101 2,2',4,5,5'-PenCB	105 2,3,3',4,4'-PenCB	114 2,3,4,4',5-PenCB	118 2,3',4,4',5-PenCB	122 2',3,3',4,5-PenCB	123 2',3,4,4',5-PenCB	Sum-PenCB
Fish 7	22,7	22,5	12,2	1,21	36,9	<0,03	0,32	130
Fish 10	20	19,7	11,1	1,01	33,6	0,04	0,31	118
Fish 11	40,2	43,2	20,4	2,02	63,2	<0,02	0,41	223
Fish 14	16	17,2	7,81	0,64	20,6	<0,01	0,17	86,5
Fish 15	34,5	32,3	17,7	1,6	51,8	0,02	0,34	187
Fish 16	7,36	10,8	3,74	0,38	11,6	<0,01	0,16	48,1
Fish 17	15,6	17,5	7,8	0,73	23,2	<0,01	0,2	87,4
Fish 1,2,3	30	31,8	14,1	1,41	44,7	0,02	0,34	164
Fish 4,6,8,13	38,9	39,8	20,1	1,9	56,9	0,02	0,45	216
Fish 5,9,12	36,6	32,9	18,4	1,6	48,5	0,0	0,3	189,0
Site 1 mean	*26,19*	*26,77*	*13,34*	*1,25*	*39,10*	*0,02*	*0,30*	*144,90*
Fish 1	16,3	16,9	7,46	0,65	23,3	<0,02	0,24	81,4
Fish 2	23,5	25,5	11,8	1,18	35,9	0,04	0,39	133
Fish 3	32,1	34,5	17,1	1,69	51	0,03	0,5	182
Fish 4	37,8	32,5	16,3	1,81	53,3	0,03	0,44	189
Fish 5	27	27,6	15	1,5	45,7	0,03	0,47	159
Fish 6	13,7	16,9	6,65	0,58	19,6	<0,02	0,24	81,2
Fish 7	34,5	39	17	1,9	59,4	0,03	0,49	206
Site 2 mean	*26,41*	*27,56*	*13,04*	*1,33*	*41,17*	*0,03*	*0,40*	*147,37*

Table 14. Concentrations of polychlorinated biphenyls in fish liver.

O. argentatus

Sample	128	138	141	149	153	156	157	167	
	2,2',3,3',4,4'-HexCB	2,2',3,4,4',5'-HexCB	2,2',3,4,5,5'-HexCB	2,2',3,4',5',6-HexCB	2,2',4,4',5,5'-HexCB	2,3,3',4,4',5-HexCB	2,3,3',4,4',5'-HexCB	2,3',4,4',5,5'-HexCB	Sum-HexCB
Fish 7	9,85	63,9	5,26	17,9	88,2	4,81	1,04	1,83	2,53
Fish 10	8,42	56,5	4,51	15,6	74,2	4,26	0,95	1,68	218
Fish 11	16,5	116	9,35	28,5	161	8,59	1,85	2,55	454
Fish 14	6,04	41,4	3,42	12,6	58,4	2,88	0,69	0,79	169
Fish 15	15,1	104	7,92	24,7	144	7,41	1,59	2,12	398
Fish 16	2,86	17,8	1,69	7,52	25,9	1,17	0,28	0,59	78,4
Fish 17	6,09	41,3	3,17	11,3	60,2	3,04	0,71	0,88	166
Fish 1,2,3	12,5	87,1	5,88	23,7	119	5,89	1,35	2,11	342
Fish 4,6,8,13	16,6	112	8,05	29	156	8,04	1,79	2,48	443
Fish 5,9,12	15,7	104,0	8,4	19,7	152,0	7,4	1,6	1,8	403,0
Site 1 mean	*10,97*	*74,40*	*5,76*	*19,05*	*103,89*	*5,35*	*1,18*	*1,68*	*267,39*
Fish 1	5,45	34,2	2,36	9,86	60,4	2,55	0,65	1,19	151
Fish 2	9,28	61,4	5,21	18,9	86,7	4,6	0,97	1,79	252
Fish 3	13,6	88,8	6,96	22,9	121	6,54	1,46	2,58	351
Fish 4	14,5	107	5,73	19,5	154	6,66	1,49	2,53	405
Fish 5	11,8	77,4	5,69	23,1	108	5,89	1,33	2,55	311
Fish 6	4,87	31,8	2,71	13,1	44,1	2,24	0,51	0,86	136
Fish 7	13,4	103	8,88	32,3	149	8,06	1,79	3,57	421
Site 2 mean	*10,41*	*71,94*	*5,36*	*19,95*	*103,31*	*5,22*	*1,17*	*2,15*	*289,57*

Table 15. Concentrations of polychlorinated biphenyls in fish liver.

O. argentatus

	170	180	183	187	189		194	206	209		
Sample	2,2',3,3',4,4',5-HepCB	2,2',3,4,4',5,5'-HepCB	2,2',3,4,4',5',6-HepCB	2,2',3,4',5,5',6-HepCB	2,3,3',4,4',5,5'-HepCB	Sum-HepCB	2,2',3,3',4,4',5,5'-OctCB	2,2',3,3',4,4',5,5',6-NonCB	DecaCB	Sum 7 PCB	Sum PCB
Fish 7	11,4	32,8	7,17	22,4	0,44	94,3	3,23	1,07	0,68	255	530
Fish 10	9,15	26,9	5,55	16,9	0,35	75,4	2,53	0,89	0,57	221	461
Fish 11	19,7	60,3	12,5	38,3	0,77	165	5,91	1,8	1,07	460	916
Fish 14	7,06	20,7	4,2	13,2	0,24	57,5	2,26	0,74	0,48	169	352
Fish 15	18	52,2	11	32,5	0,65	143	5,02	1,73	1,06	396	787
Fish 16	2,55	7,74	1,64	6,24	0,09	23	0,65	0,28	0,22	81,4	180
Fish 17	7,11	21,6	4,31	11,9	0,25	56	1,97	0,68	0,41	174	351
Fish 1,2,3	13,3	44	9,16	29,5	0,5	121	4,27	1,13	0,79	341	687
Fish 4,6,8,13	18,9	56,7	11,3	35,7	0,79	156	5,21	2,19	1,17	438	891
Fish 5,9,12	17,5	51,7	10,5	29,9	0,7	137,0	4,6	1,8	1,1	402,0	788,0
Site 1 mean	*12,47*	*37,46*	*7,73*	*23,65*	*0,47*	*102,82*	*3,56*	*1,23*	*0,76*	*293,74*	*594,30*
Fish 1	5,46	19,5	4,19	8,04	0,21	48,3	2,72	0,45	0,31	161	313
Fish 2	10,1	30,4	5,94	17,6	0,37	82,6	2,83	0,86	0,38	251	516
Fish 3	15,2	44,7	8,88	27,8	0,59	122	3,6	1,21	0,59	355	724
Fish 4	18,8	56,9	11	32,9	0,67	148	5	1,66	0,73	419	811
Fish 5	14,1	39,8	8	25,8	0,56	113	3,46	1,26	0,69	311	643
Fish 6	4,2	13,8	3,03	10,5	0,17	41,7	1,5	0,35	0,29	139	305
Fish 7	19,7	62	12,1	37,8	0,79	169	5,62	2,2	1,06	429	877
Site 2 mean	*12,51*	*38,16*	*7,59*	*22,92*	*0,48*	*103,51*	*3,53*	*1,14*	*0,58*	*295,00*	*598,43*

Table 16. Concentrations of polychlorinated biphenyls in two deep-sea amphipod species.

Amphipods	PCB ng/g	IUPAC:	18	28	31	33	37		47	52	66	74	
	PeCB	HCB	2,2',5-TriCB	2,4,4'-TriCB	2,4',5-TriCB	2',3,4-TriCB	3,4,4'-TriCB	Sum-TriCB	2,2',4,4'-TetCB	2,2',5,5'-TetCB	2,3',4,4'-TetCB	2,4,4',5-TetCB	Sum-TetCB
Eurythenes gryllus													
Pool 1	0,7	47,5	1,1	1,3	3,9	0,3	0,1	9,2	12,3	56,9	9,9	20,4	141,0
Pool 2	0,3	5,0	0,3	1,3	1,2	0,3	0,1	4,3	2,6	10,5	6,0	4,8	42,6
Pool 3	0,2	2,9	0,2	0,7	0,6	0,1	0,1	2,3	3,3	15,9	5,5	5,1	45,3
Site 1 mean	*0,4*	*18,5*	*0,6*	*1,1*	*1,9*	*0,2*	*0,1*	*5,3*	*6,0*	*27,8*	*7,1*	*10,1*	*76,3*
Pool 1	0,3	4,2	0,3	1,0	0,8	0,2	0,1	2,9	2,9	12,4	5,4	5,0	39,5
Pool 2	0,2	4,1	0,2	0,9	0,7	0,2	0,1	2,7	1,7	8,2	4,0	3,6	28,9
Pool 3	0,2	3,3	0,2	0,8	1,1	0,1	0,1	3,4	5,8	24,2	8,6	10,3	71,6
Site 2 mean	*0,2*	*3,9*	*0,3*	*0,9*	*0,9*	*0,2*	*0,1*	*3,0*	*3,5*	*14,9*	*6,0*	*6,3*	*46,7*
Anonyx magox													
Pool 1	0,4	2,8	0,0	0,2	0,2	0,0	0,0	0,6	0,2	0,3	0,5	0,4	2,8
Pool 2	0,5	4,9	0,0	0,3	0,3	0,1	0,1	1,1	0,4	0,9	0,9	0,7	5,3
Pool 3	0,5	4,3	0,1	0,4	0,4	0,1	0,0	1,2	1,6	5,5	3,7	3,2	21,3
Site 1 mean	*0,5*	*4,0*	*0,0*	*0,3*	*0,3*	*0,1*	*0,0*	*1,0*	*0,7*	*2,2*	*1,7*	*1,4*	*9,8*
Pool 1	0,4	3,7	0,0	0,3	0,3	0,1	0,0	1,0	0,3	0,8	0,8	0,6	4,4
Pool 2	0,4	4,3	0,1	0,4	0,2	0,1	0,0	0,9	0,8	2,5	1,6	1,3	8,7
Pool 3	0,4	3,4	0,1	0,3	0,3	0,1	0,0	1,1	0,3	0,9	0,7	0,5	4,5
Site 2 mean	*0,4*	*3,8*	*0,1*	*0,3*	*0,3*	*0,1*	*0,0*	*1,0*	*0,5*	*1,4*	*1,0*	*0,8*	*5,9*

Table 17. Concentrations of polychlorinated biphenyls in two deep-sea amphipod species.

Amphipods	99	101	105	114	118	122	123	
	2,2',4,4',5-PenCB	2,2',4,5,5'-PenCB	2,3,3',4,4'-PenCB	2,3,4,4',5-PenCB	2,3',4,4',5-PenCB	2',3,3',4,5-PenCB	2',3,4,4',5-PenCB	Sum-PenCB
Eurythenes gryllus								
Pool 1	89,2	130,0	27,0	4,0	127,0	<0,06	1,5	509,0
Pool 2	18,7	30,5	10,2	1,0	32,5	0,1	0,5	132,0
Pool 3	31,9	47,3	14,6	1,4	46,0	0,1	0,4	192,0
Site 1 mean	*46,6*	*69,3*	*17,3*	*2,1*	*68,5*	*0,1*	*0,8*	*277,7*
Pool 1	20,7	35,3	11,3	1,1	39,1	<0,04	0,4	140,0
Pool 2	15,5	19,3	6,5	0,7	23,9	0,1	0,3	91,0
Pool 3	50,3	72,0	21,3	2,5	78,4	0,1	1,0	304,0
Site 2 mean	*28,8*	*42,2*	*13,0*	*1,4*	*47,1*	*0,1*	*0,6*	*178,3*
Anonyx magox								
Pool 1	1,1	1,5	0,7	0,1	2,0	0,0	0,0	7,9
Pool 2	2,1	2,5	1,2	0,1	3,6	0,0	0,1	14,3
Pool 3	15,7	26,5	9,7	0,8	32,9	<0,02	0,4	112,0
Site 1 mean	*6,3*	*10,2*	*3,9*	*0,3*	*12,8*	*0,0*	*0,2*	*44,7*
Pool 1	1,6	2,0	1,0	0,1	2,9	0,0	0,1	11,0
Pool 2	7,0	9,6	3,9	0,4	10,9	<0,02	0,1	42,3
Pool 3	1,6	2,0	0,9	0,1	2,7	0,0	0,0	10,8
Site 2 mean	*3,4*	*4,5*	*1,9*	*0,2*	*5,5*	*0,0*	*0,1*	*21,4*

Table 18. Concentrations of polychlorinated biphenyls in two deep-sea amphipod species.

Amphipods	128 2,2',3,3',4,4'-HexCB	138 2,2',3,4,4',5'-HexCB	141 2,2',3,4,5,5'-HexCB	149 2,2',3,4',5',6-HexCB	153 2,2',4,4',5,5'-HexCB	156 2,3,3',4,4',5-HexCB	157 2,3,3',4,4',5'-HexCB	167 2,3',4,4',5,5'-HexCB	Sum-HexCB
Eurythenes gryllus									
Pool 1	24,0	247,0	21,4	132,0	330,0	18,1	3,4	8,5	1058,0
Pool 2	8,6	57,2	5,6	28,5	77,8	4,0	0,9	2,2	250,0
Pool 3	18,4	124,0	9,4	56,6	176,0	8,2	1,8	3,7	535,0
Site 1 mean	*17,0*	*142,7*	*12,1*	*72,4*	*194,6*	*10,1*	*2,0*	*4,8*	*614,3*
Pool 1	9,1	63,0	4,3	35,4	88,2	3,9	0,9	2,4	277,0
Pool 2	5,6	45,6	3,1	25,6	60,8	2,8	0,6	1,7	195,0
Pool 3	20,5	151,0	13,1	76,9	213,0	10,6	2,1	5,4	666,0
Site 2 mean	*11,7*	*86,5*	*6,8*	*46,0*	*120,7*	*5,8*	*1,2*	*3,2*	*379,3*
Anonyx magox									
Pool 1	0,5	3,0	0,3	1,4	4,1	0,2	0,1	0,1	13,2
Pool 2	0,9	5,5	0,6	2,6	7,4	0,4	0,1	0,2	23,8
Pool 3	8,6	58,7	5,4	28,0	84,4	4,7	0,9	2,6	256,0
Site 1 mean	*3,3*	*22,4*	*2,1*	*10,7*	*32,0*	*1,8*	*0,4*	*1,0*	*97,7*
Pool 1	0,7	4,2	0,4	1,9	5,8	0,3	0,1	0,2	18,0
Pool 2	3,8	26,4	1,9	9,1	37,2	1,8	0,4	0,7	106,0
Pool 3	0,7	4,1	0,4	1,9	5,7	0,3	0,1	0,2	18,0
Site 2 mean	*1,7*	*11,6*	*0,9*	*4,3*	*16,2*	*0,8*	*0,2*	*0,4*	*47,3*

Table 19. Concentrations of polychlorinated biphenyls in two deep-sea amphipod species.

Amphipods	170	180	183	187	189		194	206	209		
	2,2',3,3',4,4',5-HepCB	2,2',3,4,4',5,5'-HepCB	2,2',3,4,4',5',6-HepCB	2,2',3,4',5,5',6-HepCB	2,3,3',4,4',5,5'-HepCB	Sum-HepCB	2,2',3,3',4,4',5,5'-OctCB	2,2',3,3',4,4',5,5',6-NonCB	DecaCB	Sum 7 PCB	Sum PCB
Eurythenes gryllus											
Pool 1	43,2	138,0	29,4	101,0	1,5	405,0	12,8	4,4	1,6	1030,0	2140,0
Pool 2	9,7	30,5	6,2	22,6	0,3	88,5	2,7	0,9	0,5	240,0	521,0
Pool 3	28,5	92,2	18,9	59,0	1,2	254,0	11,0	3,1	1,7	503,0	1045,0
Site 1 mean	*27,1*	*86,9*	*18,2*	*60,9*	*1,0*	*249,2*	*8,8*	*2,8*	*1,3*	*591,0*	*1235,3*
Pool 1	8,8	29,1	5,7	20,9	0,4	83,9	2,4	0,7	0,4	268,0	547,0
Pool 2	7,2	23,5	4,8	16,5	0,3	67,5	1,9	0,6	0,4	182,0	388,0
Pool 3	27,3	87,2	18,5	62,9	1,0	254,0	8,7	3,6	1,2	627,0	1312,0
Site 2 mean	*14,5*	*46,6*	*9,6*	*33,4*	*0,5*	*135,1*	*4,3*	*1,6*	*0,7*	*359,0*	*749,0*
Anonyx magox											
Pool 1	0,4	1,3	0,3	1,1	0,0	4,1	0,1	0,0	0,0	12,4	28,6
Pool 2	0,9	2,5	0,6	2,0	0,0	7,9	0,2	0,1	0,1	22,7	52,7
Pool 3	11,4	36,7	7,3	25,4	0,4	102,0	3,2	0,9	0,5	245,0	497,0
Site 1 mean	*4,2*	*13,5*	*2,7*	*9,5*	*0,2*	*38,0*	*1,2*	*0,3*	*0,2*	*93,4*	*192,8*
Pool 1	0,6	1,9	0,4	1,5	0,0	5,8	0,2	0,1	0,1	18,0	40,5
Pool 2	5,3	18,1	3,5	12,2	0,2	49,0	2,2	0,5	0,3	105,0	210,0
Pool 3	0,7	2,0	0,5	1,5	0,0	6,0	0,2	0,1	0,1	17,8	40,7
Site 2 mean	*2,2*	*7,3*	*1,4*	*5,0*	*0,1*	*20,2*	*0,9*	*0,2*	*0,1*	*46,9*	*97,1*

Table 20. PFOS in Arctic rockling, *Onogadus argentatus*..

Sample	PFOS (ng/g)	Lipid content (%)
Fish 7	<0,17	67,1
Fish 10	<0,17	78,4
Fish 11	<0,19	67,4
Fish 14	<0,19	57,7
Fish 15	<0,17	58,2
Fish 16	<0,23	73,8
Fish 17	0,29	70,8
Fish 1,2,3	0,13	66,1
Fish 4,6,8,13	<0,17	72,1
Fish 5,9,12	0,2	59,8
Site 1 mean	*0,22*	*67,14*
Fish 1	<0,19	63,7
Fish 2	0,2	70,5
Fish 3	<0,18	70,5
Fish 4	0,16	64,2
Fish 5	0,33	71,6
Fish 6	0,27	76,9
Fish 7	<0,29	62,8
Site 2 mean	*0,24*	*68,60*

Table 21. PFOS in amphipods.

Sample	PFOS (ng/g)	Lipid content (%)
Eurythenes gryllus		
Pool 1	0,5	11,5
Pool 2	1,7	12,8
Pool 3	0,2	8,9
Site 1 mean	*0,8*	*11,1*
Pool 1	1,2	13,8
Pool 2	0,4	13,8
Pool 3	0,6	8,5
Site 2 mean	*0,7*	*12,0*
Anonyx magox		
Pool 1	0,3	4,0
Pool 2	0,5	4,9
Pool 3	0,5	7,6
Site 1 mean	*0,4*	*5,5*
Pool 1	0,5	8,7
Pool 2	0,5	7,8
Pool 3	<0,22	8,9
Site 2 mean	*0,5*	*8,5*

4.2.3 Metals in tissue

For the Arctic rockling, Cu and Cd levels in liver are almost one magnitude higher compared to what we find in Atlantic cod frequently studied in our experiments here at IRIS Biomiljø (Table 7). The levels of Cu and Cd in control fish are typically 1 mg/kg and 0.1 mg/kg respectively.

For the amphipods it is observed two high values (sample G22 and B13) for Zn (Table 8). This might be caused by leakages from galvanised parts in the trap system. However, the most interesting part of the data set is the high Cd levels. It looks like Cd is naturally accumulated in the amphipods. This fact makes them potentially useful as indicators of Cd discharges.

There are relatively large natural variations between species so the data presented here, both for amphipods and fish, are most likely reflecting the background levels in these species at these locations.

Table 22. Body burden of metals in Arctic rockling, *Onogadus argentatus.*

Station	*Onogadus argentatus* liver number	Vanadium	Chromium	Cobalt	Nickel	Copper	Zinc	Arsenic	Cadmium	Barium	Lead	Hg
						(mg/kg vet weight)						
St1	7	0,03	1,12	0,038	<0.2	10,47	20,8	8,1	1,19	0,20	0,01	0,036
St1	11	0,07	1,09	0,044	<0.2	7,01	18,6	15,3	0,83	0,07	0,01	0,062
St1	10	0,11	1,03	0,019	<0.2	8,81	22,4	8,2	0,79	0,10	0,01	0,030
St1	14	0,11	0,94	0,018	<0.2	8,90	30,3	8,2	0,75	0,18	0,01	0,056
St1	17	0,13	0,98	0,016	<0.2	10,41	26,1	6,2	1,52	0,21	<0.01	0,065
St1	15	0,11	0,93	0,032	<0.2	4,27	12,4	11,5	1,46	0,09	<0.01	0,052
St1	16	0,06	0,93	0,015	<0.2	6,37	18,9	4,3	0,16	0,08	<0.01	0,024
St1	1,2,3	0,15	1,03	0,037	<0.2	13,14	30,5	8,9	1,87	0,16	0,10	0,048
St1	4,6,8,13	0,14	1,00	0,041	<0.2	12,74	26,8	18,3	1,42	0,13	0,01	0,047
St1	5,9,12	0,13	0,82	0,045	<0.2	7,01	18,3	26,6	1,06	0,12	0,05	0,053
	Average	0,10	0,99	0,031	<0.2	8,91	22,52	11,56	1,10	0,13	0,03	0,05
St2	2	0,07	1,06	0,025	<0.2	12,18	28,4	29,7	2,02	0,06	0,01	0,035
St2	1	0,12	1,00	0,045	<0.2	5,41	17,8	39,9	0,26	0,57	0,02	0,076
St2	3	0,14	1,16	0,017	<0.2	8,79	22,2	9,0	1,49	0,06	0,01	0,048
St2	4	0,22	0,83	0,062	<0.2	10,55	24,0	14,8	0,96	0,12	0,02	0,043
St2	5	0,18	0,96	0,016	<0.2	11,07	23,6	5,6	0,99	0,08	<0.01	0,053
St2	7	0,14	0,93	0,028	<0.2	4,84	12,9	9,2	0,72	0,23	<0.01	0,024
St2	6	0,12	0,87	0,033	<0.2	6,36	16,1	17,6	0,18	0,12	<0.01	0,029
	Average	0,14	0,97	0,032	<0.2	8,46	20,72	17,98	0,95	0,18	0,01	0,04

Table 23. Body burden of metals in the amphipods, *Eurythenes gryllus* and *Anonyx magox*.

Station	Amphipod sample no.	Vanadium	Chromium	Cobalt	Nickel	Copper	Zinc	Arsenic	Cadmium	Barium	Lead	Hg
						(mg/kg vet weight)						
	Eurythenes gryllus											
St1	1	0,22	0,43	0,056	0,6	9,57	46,6	4,6	19,98	0,68	0,05	0,083
St1	2	0,23	0,56	0,089	1,1	47,36	31,2	6,0	18,29	0,98	0,02	0,044
St1	3	0,17	0,13	0,043	0,5	12,07	34,3	5,9	13,04	0,64	0,01	0,197
	Average	0,21	0,37	0,063	0,70	23,00	37,35	5,50	17,10	0,77	0,03	0,108
St2	G23	0,33	0,28	0,119	1,6	64,55	52,9	6,2	26,88	1,56	0,04	0,408
St2	G22	0,34	0,57	0,184	2,8	22,09	195,3	4,3	11,08	2,76	0,29	0,082
St2	G21	0,49	0,49	0,135	3,1	17,16	33,4	3,0	3,49	1,67	0,03	0,133
	Average	0,39	0,45	0,146	2,49	34,60	93,85	4,49	13,82	1,99	0,12	0,208
	Anonyx magox											
St1	B13	0,33	0,27	0,157	1,8	23,80	163,5	7,1	4,79	1,68	1,81	0,071
St1	B12	0,92	0,23	0,179	2,2	32,25	42,1	7,7	11,36	1,42	1,26	0,090
St1	B11	0,30	0,20	0,169	2,5	19,97	48,3	6,4	5,23	1,71	1,71	0,064
	Average	0,52	0,23	0,169	2,15	25,34	84,64	7,07	7,13	1,60	1,59	0,075
St2	B23	0,90	0,37	0,217	2,0	20,47	34,5	8,5	9,98	1,08	0,03	0,049
St2	B22	1,07	0,25	0,221	2,3	17,95	36,1	9,0	5,62	1,08	0,02	0,039
St2	B21	0,61	0,18	0,208	2,5	14,93	32,9	7,4	4,25	1,29	0,04	0,051
	Average	0,86	0,27	0,215	2,26	17,79	34,50	8,32	6,62	1,15	0,03	0,047
	Detection limit	0,05	0,05	0,005	0,2	0,02	0,05	0,1	0,01	0,1	0,01	0,001

4.3 Biotransformation markers

4.3.1 Aromatic metabolites analysed by fixed wavelength fluorescence

Sample no. 11 (site 1) is showing peak values within the 2-3 and 4 ring aromatics and is from the statistical analyses indicated as a possible outlier sample in the total dataset. Figure 15 shows the results for all samples. There are no significant differences between the two sites and all levels are low an represent expected background levels for fish from non-contaminated area.

As shown in Figure 16, sample 16 (site 1) is showing a peak in the 2 ring area. The same peak appears in samples 3 and 5 (site 2). These individual observations are sometimes seen and might be related to metabolites from internal breakdown of hormones in the fish or from occasionally small contents of aromatics in ingested food.

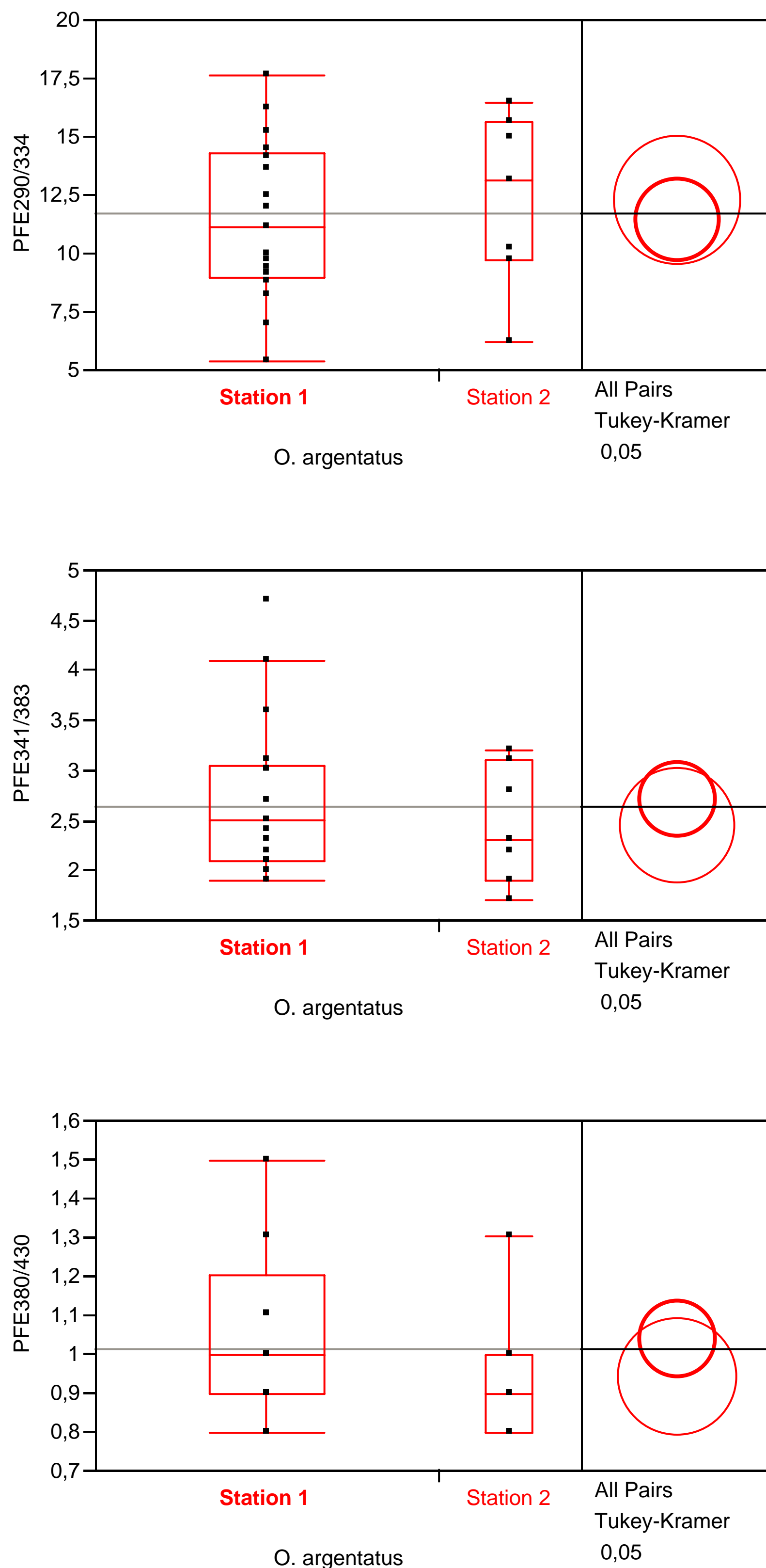


Figure 15. Aromatic metabolites in fish bile as measured by fixed fluorescence method at three different wavelength ranges (naphthalene, phenanthrene and chrysene ranges).

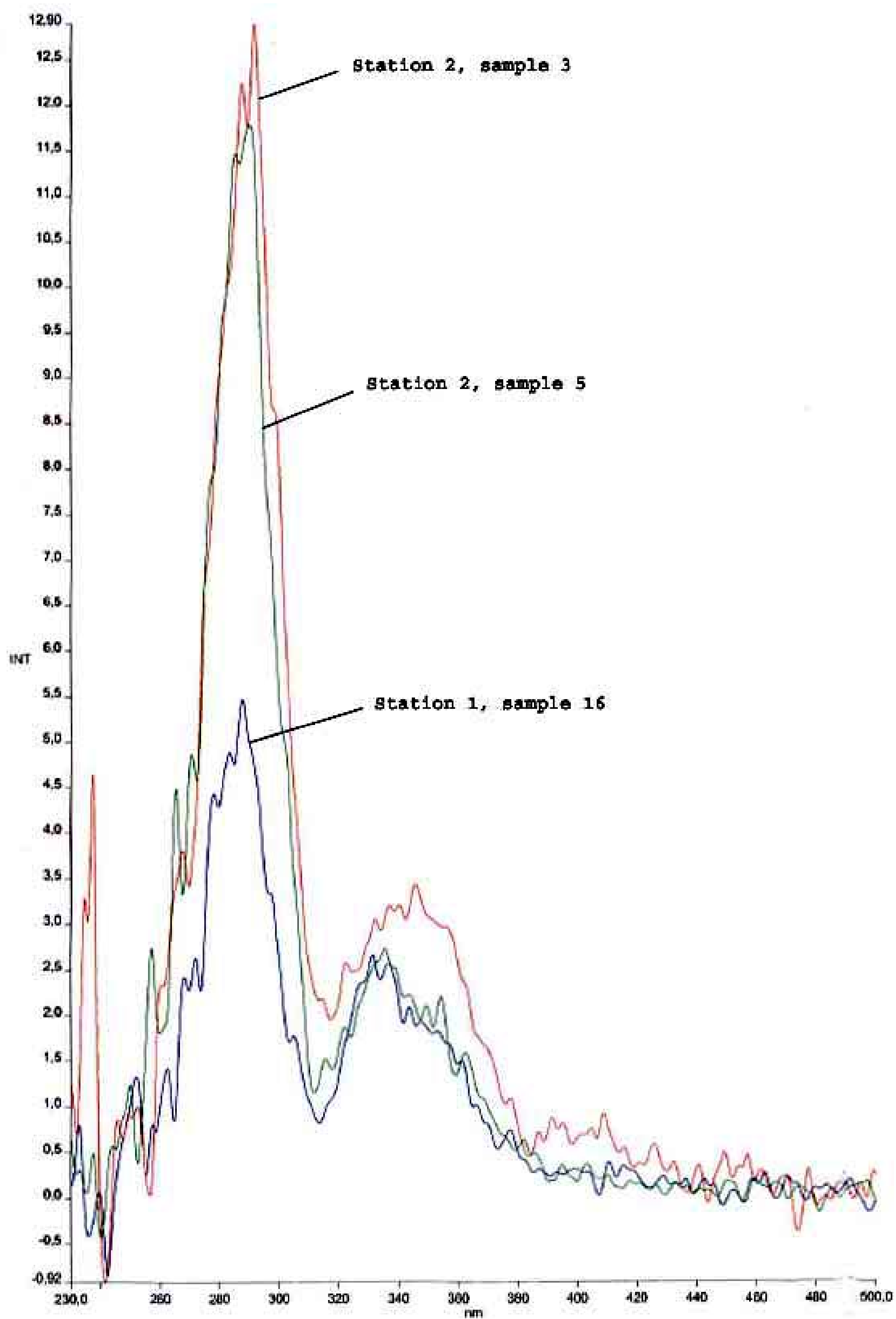


Figure 16. Fluorescence scan of selected bile samples showing slightly elevated levels of metabolites as measured in the wavelength that represents 2-ring aromatic metabolites as napthalenes.

4.3.2 Aromatic metabolites analysed by GC/MS

The results from GC/MS analyses of PAH metabolites in fish bile is shown in Figure 17. Only the PAH metabolite 2-OH-naphtalene shows significant different levels between the two study sites. Two of the phenanthrene metabolites and the pyrene metabolite show a trend of higher values at site 2.

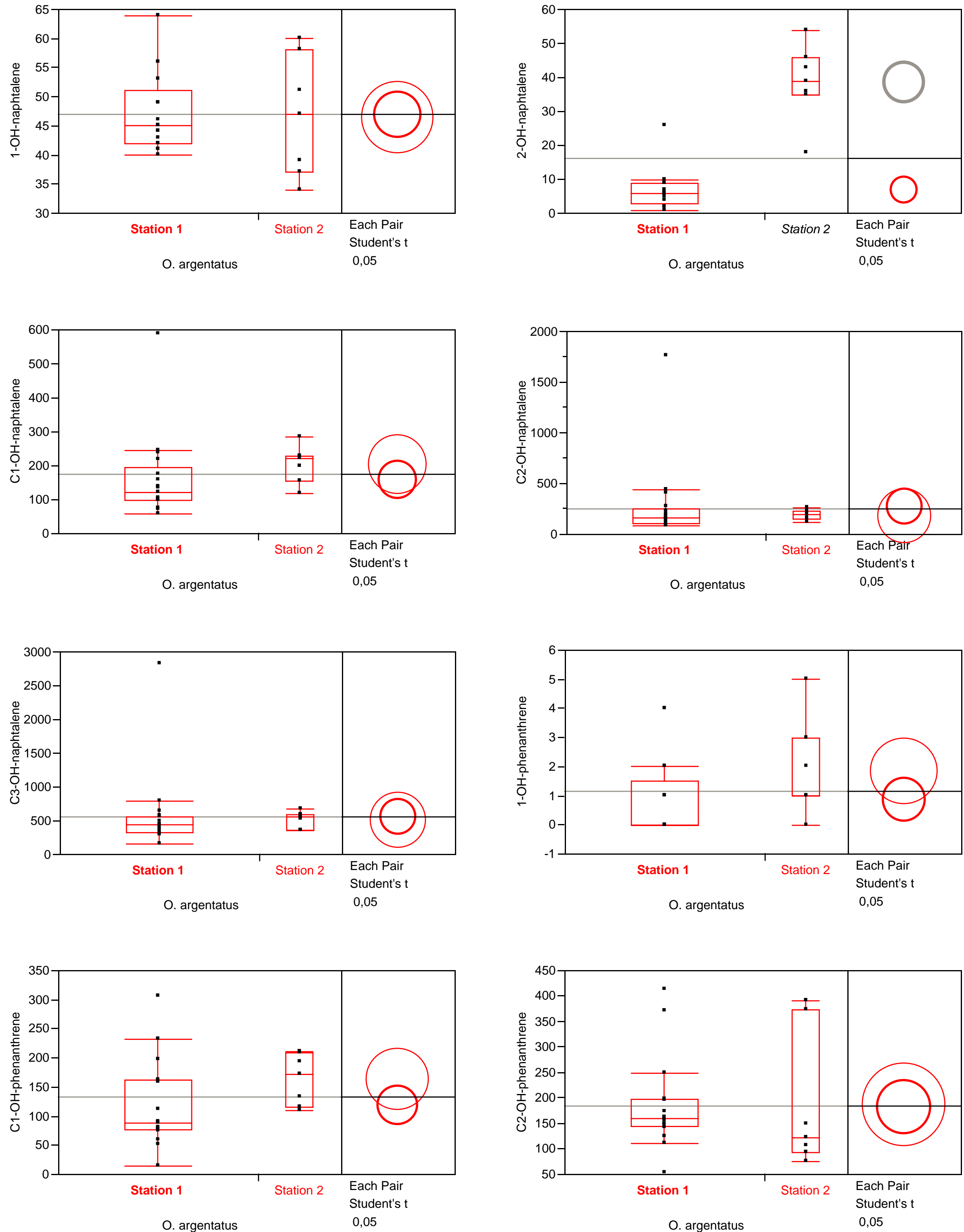


Figure 17. PAH metabolites in fish bile measured by GC/MS.

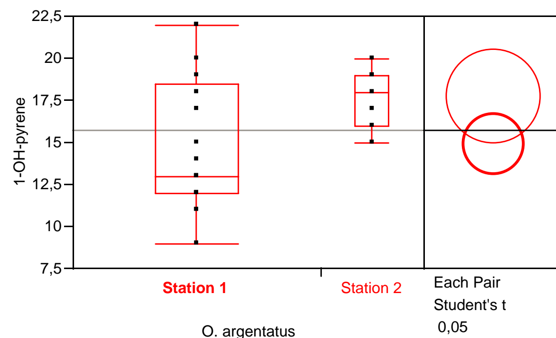


Figure 17 (continued). PAH metabolites in fish bile measured by GC/MS.

4.3.3 EROD and GST activities

Results for the hepatic activity of the detoxification enzyme EROD is shown in Figure 18. There was no significant difference between the two study sites and the average level is considered typically low compared to those reported in other gadoid species exposed to contaminants.

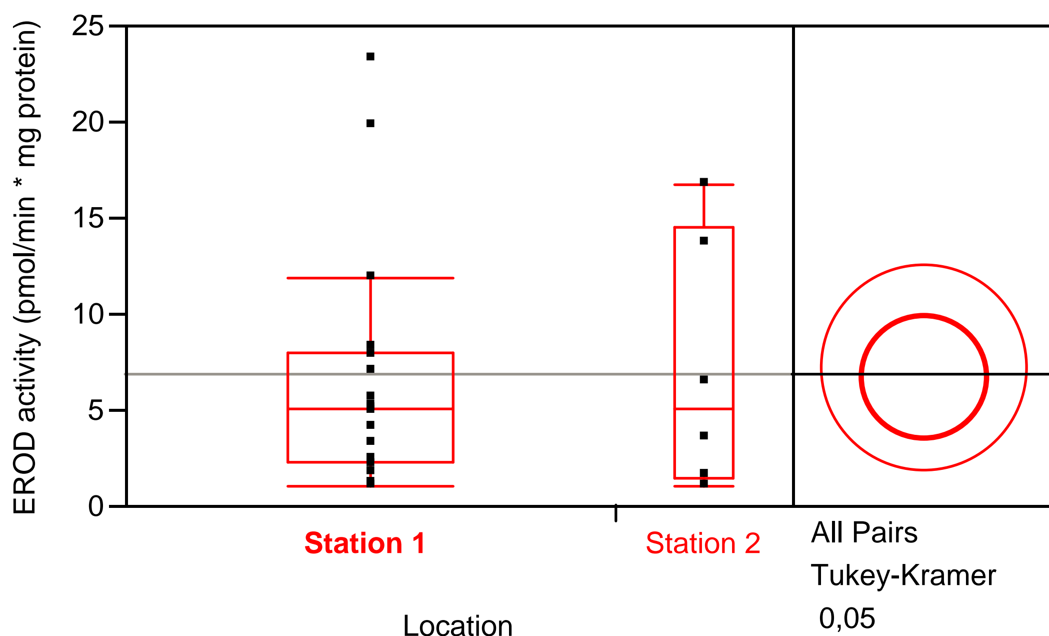


Figure 18. Hepatic activity of EROD.

The GST activity measured in whole body samples of amphipods were below the detection limit of the present method. Measurement of this biomarker have never been conducted in these specific species before. Nevertheless, GST is a very universal detoxification enzyme and by a more specific dissection of, i.e. the digestive organs of

the largest amphipod specimens, this might values above detection limit in possible future studies.

4.4 Genotoxicity markers

4.4.1 DNA adducts

None of the samples analysed in this study showed DNA adduct levels above the detection limits, see Table 1 for all results. Figure 1 shows some autoradiograms from the results, all are spotless, indicating zero adduct levels. It also shows controls, which are processed in parallel with the samples in all analysis.

The analytical work showed that labelling and TLC migration of the standard adduct B[a]PDE-dG-3´p, on the polyethyleneimine cellulose sheets showed normal labelling frequency as well as expected migration and spot shape on the sheets (se Figure 1). Pure salmon sperm was used as a negative control, and did not show any spots, indicative of false adducts. In addition, results from positive control sample (adducted liver tissue from perch) processed in parallel served as quality assurance for all analytical steps in the ^{32}P-postlabeling method (see Figure 1). Based on this, it is unlikely that the low adduct levels observed in this field study were due to any kind of artefact.

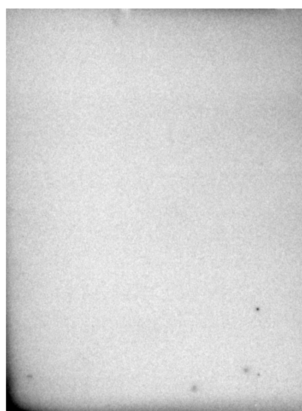
DNA adducts have been analysed in this species before. Aas *et al*. (2003) studied DNA adducts in 6 Arctic rocklings (*Gaidropsarus argentatus; now Onogadus argentatus)* caught west of Svalbard in 1999-2001. Five had DNA adduct levels below the detection limits, and one individual had just detectable levels of 0.75 nmol add/mol normal nucleotides.

Table 24. DNA adduct levels (nmol adducts/mol normal nucleotides) in liver of Arctic rockling (*Onogadus argentatus*) sampled at two different stations.

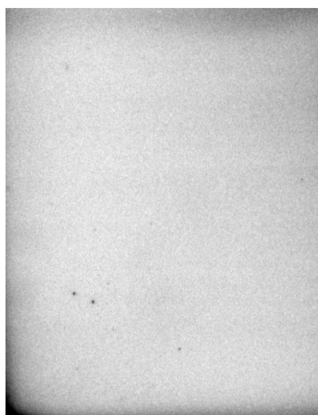
	sample nr.	DNA nr	DNA adducts (nmol add/mol norm. nucleotides)	For calculating average value*
Station 1	1	06175	≤0.906	0.45
	2	06176	≤0.427	0.21
	3	06177	≤0.651	0.33
	4	06178	≤2.44	1.22
	5	06621	≤1.24	0.61
	6	06222	≤0.515	0.26
	7	06223	≤0.608	0.30
Average				**0.48**
Stdev				**0.35**
Confidence level(95,0%)				**0.33**
Station 2	1	06179	≤0.828	0.41
	2	06180	≤0.431	0.22
	3	06181	≤0.782	0.40
	4	06182	≤0.658	0.33
	5	06224	≤0.566	0.28
	6	06225	≤0.509	0.26
	7	06226	≤0.469	0.24
Average				**0.30**
Stdev				**0.08**
Confidence level(95,0%)				**0.07**

* ≤ means the adduct levels were below or equal to the detection limits, and the value given is the background value / detection limit of that particular autoradiogram. If any adducts should be present in that sample, their value could range from zero up to the background value. Therefore an average of zero and the background is found (the value*0.5), and that number used in calculations.

Station 1



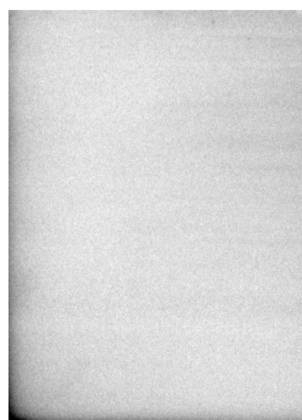
Sample 1: ≤0.91 nmol



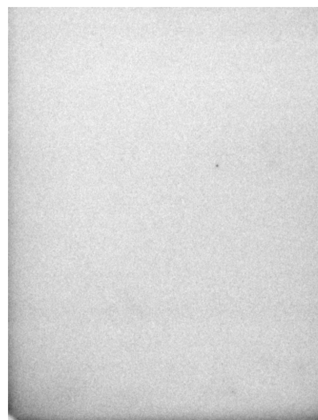
Sample 3: ≤0.65 nmol

Sample 7: ≤0.61 nmol

Station 2

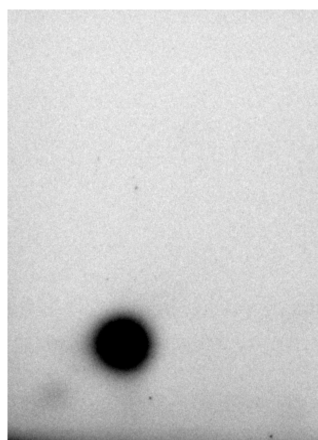


Sample 1: ≤0.83 nmol

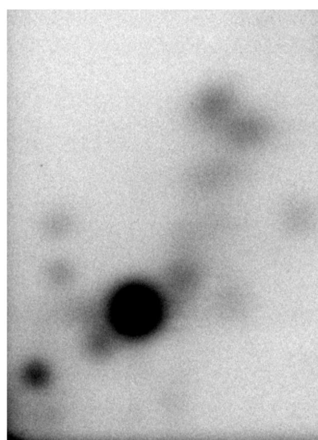


Sample 2: ≤0.43 nmol

Sample 6: ≤0.51 nmol



The standard B[a]P adduct



The positive control of adducted liver of perch

Figure 19. Autoradiograms and DNA adduct levels in liver samples of Arctic rockling (*Onogadus argentatus).* Numbers under the autoradiograms represent sample number (fish), and DNA adducts levels (nmol add/mol normal nucleotides). ≤ indicates DNA adduct levels were below the detection limits which is given for each autoradiogram.

4.4.2 Micronuclei

The level of micronuclei was a bit higher in amphipods compared to fish, especially in specimens inhabiting the second location. Comparison of environmental genotoxicity levels between fish and amphipod species inhabiting the same location did not revealed significant inter-specific differences.

The frequency of micronuclei in *Onogadus argentatus* from station 1 varied in the range from 0.0 to 0.67 MN/1000 erythrocytes, in fish from station 2 – from 0.0 to 1.0 MN/1000 erythrocytes. The mean of MN in fish from first station was equal to 0.47 MN/1000 erythrocytes, in fish from second station – 0.23 MN/1000 erythrocytes (**Error! Reference source not found.**).

In *Eurythenes gryllus* collected from first and second stations, the variation of MN frequency ranged from 0.0 to 2.0 MN/1000 cells. Whilst the mean values of MN in the amphipod species were different - 0.52 MN/1000 cells in those from first station and 0.43 MN/1000 cells in *E. gryllus* from the second location (Fig.2).

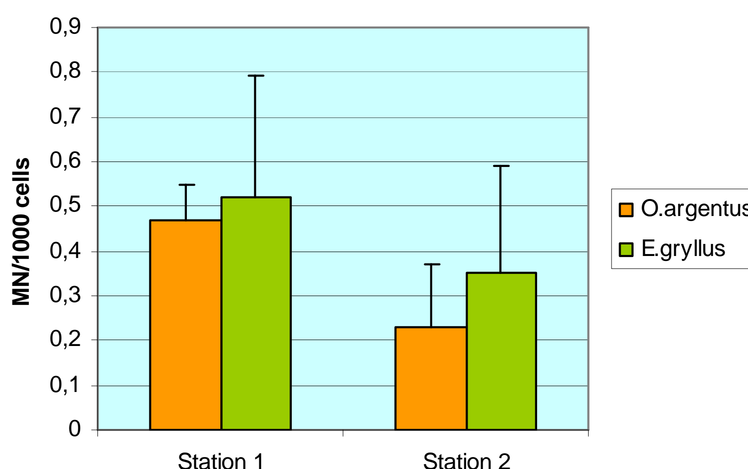


Figure 20. Micronuclei frequency in blood cells of Arctic rockling and *Eurythenes gryllus*.

Non-parametric Mann-Whitney U-test did not show significant inter-location differences between *O. argentatus* (P=0.0878) as well as between *E. gryllus* collected from first and second locations (P=0.6607).

Individual analysis showed comparatively homogenic distribution of micronuclei frequency in fish from the first location. Half of studied specimens of *O. argentatus* possessed 0.67 MN/1000 cells. In one specimen, micronuclei were not found. Whereas in fish collected from second station, there were no MN in four fish individuals. Only one fish with MN frequency of 1.0 MN/1000 cells was analysed in group from second location (see Appendix).

In amphipods, the individual variation of MN frequency was much higher. Comparatively high frequency of micronuclei were detected in three specimens from first group and in two individuals from the second location. In all other amphipods, micronuclei were not detected (see Appendix).

Exceptionally high coefficient of variation (213.49%) was found in amphipods and in fish (163.14%) collected from the second location. The value of 54.66% was defined in fish from the first location.

The results of our study showed very similar levels of micronuclei in both, in cod *O. argentatus* and in amphipods *E. gryllus* from the deep-sea zones of the Norwegian Sea. Moreover, higher values of MN were observed in both species inhabiting first location.

The outputs of the current study revealed that micronuclei frequency in immature erythrocytes of Arctic rockling equal to 0.47 and 0.23 MN/1000 cells. The last value could be suspected as baseline level in the fish species from the deep-sea zones in the Norwegian Sea. The level of MN in Atlantic cod from the coastal sites in the North Sea ranged from 0.14 to 0.50 MN/1000 erythrocytes. The MN frequency of 0.50 MN/1000 erythrocytes was observed in cod peripheral blood from heavily polluted fjord of Karmsund zone and in deep-layers of northern part of the North Sea (Baršienė et al., unpublished data). In Atlantic cod from hatcheries, which was used for control in experimental studies (Akvamiljø), the frequencies of MN in immature erythrocytes varied in a range between 0.13 MN/1000 erythrocytes and 0.33 MN/1000 erythrocytes (Baršienė et al., 2005, 2006, unpublished data).

Thus, in comparison to Atlantic cod, the Arctic rockling *O. argentatus* from the first location showed approximately 2-4-fold higher response. The explanation of the finding could be due to inter-specific differences in responses, mitotic activity as well as to differences in habitats pollution or other stressful conditions in deep-sea zones. Differential inter-species response has been shown after exposure to various contaminants in European minnow *Phoxinus phoxinus* and mollie *Poecilia latipinna* (Ayllon, Garcia-Vazquez, 2000), brown trout *Salmo trutta*, and European eel *Anguilla anguilla* (Rodriguez-Cea et al., 2003). Inter-specific differences in MN induction by pollutants have been earlier described in marine fish (Baršienė et al., 2005; 2006).

It should be stressed that there are no literature data on micronuclei levels in any species from deep-sea areas. Some data on DNA damage were presented for deep-sea hydrothermal-vent organisms in studies of David Dixon and his co-authors (Dixon et al., 2001, 2002, 2003, 2004). They have analysed chromosome sets in vent and non-vent mollusc, annelid and arthropod species. The similarities in chromosome number and genome size in deep-sea and non deep-sea organisms belonging to the same phylogenetic group and weak divergence between them has been shown. But in deep-sea organisms, higher rate of cell division was detected. Furthermore was stated that adaptation to stressful life in deep-sea zones was accompanied by changes in chromosome structure or DNA composition (Dixon et al., 2001). Consider results presented by Dixon et al. (2001) and our study, we can assume, that erythropoiesis is more intensive in cod *O. argentatus*, compared to Atlantic cod. Consequently, the spontaneous level of MN can reach higher level in deep-sea dwelling cod than in cod from non deep-sea zones. It is not yet known what extent of DNA repair is in deep-sea

fish species or what is the physiological barrier to their survival in the extreme marine environment.

Nonetheless, in nowadays marine environment become a sink for various mutagenic/genotoxic compounds and complex environmental mixtures are distributed along coastal, offshore areas, and evidently in deep-sea zones also, that can have adverse health effects on indigenous biota. Industrial wastes, preferably from chemical, metal, pulp and paper industries and petroleum refining include genotoxic compounds. The genotoxicity of numerous organic substances in aquatic environment is associated with particulate matter; others are distributed in water soluble fraction (Claxton, Houk, 1998). Some studies have shown that mutagenic PAHs can compose 10-20%, or sometimes up to 70-80% of sediment mutagenic activity (La Rocca et al., 1996; White et al., 1998).

Relatively few studies have addressed the question of how the indigenous organisms are influenced by exposure to reactive oxygen species, DNA-damaging substances. Comparatively high background levels of DNA damage have been reported in Atlantic deep-sea mussel *Bathymodiolus azoricus*, which was considered as not fully resistant to their habitat conditions (Dixon et al., 2000). The progressive declines in the efficiency of DNA repair capacity after oxidative stress have been elucidated during the lifespan of the mussels. Only young specimens exhibited higher resistance to the stress than older mussels and were able to repair DNA damage (Dixon et al.,2003).

In overall it is seen evident relevance of micronucleus test in fish, even dwelling in unusual deep-sea habitat. Although in amphipods we meet some difficulties, anywhere, the further elaboration and adoption of the approach could be successfully performed. Accumulation of data on correlation between cytogenetic impairments and environmental pollution should demonstrate the usefulness of the approach for the detection of mutagenic substances within complex mixture of pollutants, which usually exists in stressful field conditions. Consider data on DNA damage in deep-sea mollusks, the use of MN test in these organisms should be promising and relevant approach.

The outputs of the current study revealed the same level of genotoxic effects in Arctic rockling *O. argentatus* and in amphipods *E. gryllus.*

Slightly higher levels of micronuclei were detected in both organisms inhabiting the first location. The frequency of micronuclei in liver immature erythrocytes of *O. argentatus* equaled to 0.47 MN/1000 cells, in *E. gryllus* – to 0.52 MN/1000 cells. The value of MN in fish from the second location was 0.23, in amphipods – 0.35 MN/1000 cells.

The micronuclei frequency of 0.23 MN/1000 cells could be reputed as baseline level in fish from the deep-sea zones in the Norwegian Sea

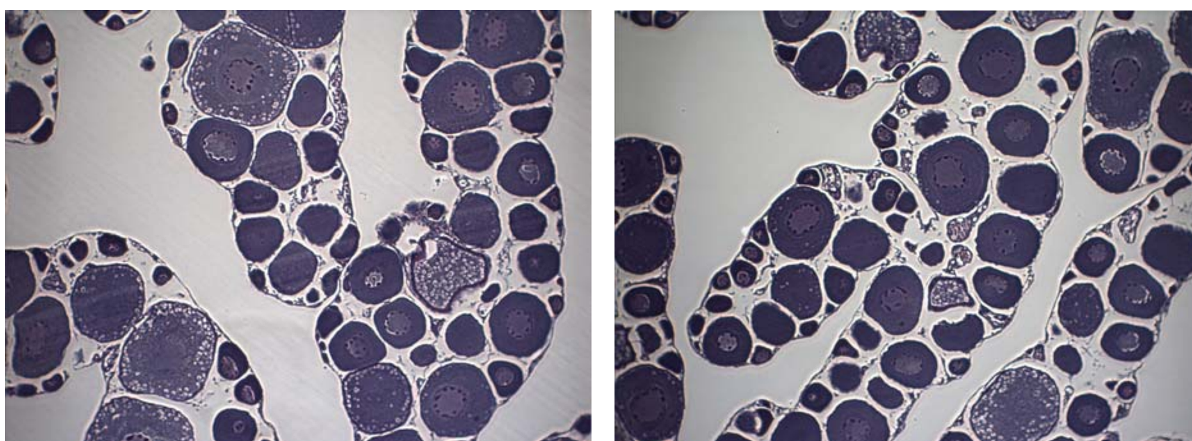
Further studies of organisms from the deep-sea region in the Norwegian Sea can continue using Arctic rockling, amphipods *E. gryllus* and available species of filter-feeding mollusks. Testing of mutagenicity/genotoxicity in complex mixtures of deep-sea waters will allow accumulating data indicating the existence of identified and unidentified substances and potentially ecological risk.

4.5 Histological markers

4.5.1 Gonad histology

The general conclusion is that there is no basis for making any diagnoses in this material. The photos in Figure 21- illustrate healthy looking ovary fish collected from both stations 1 and 2. Histological sections of fish gonads displayed numerous ovarian follicles filled with mature ovocytes, associated with reduced connective tissue, indicating pre-spawning stage. In fish collected from station 2, the ovarian follicles appeared reduced in size and empty of ovocytes. These results have been observed in the mantle of mussels (Aarab, 2004) and turbot exposed to PAH and mixture of PAH and alkylphenols.

ST2 F1-2 x10



ST2 F1-2 x40

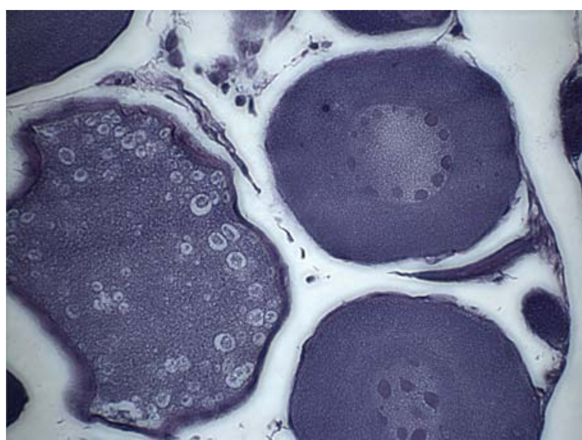
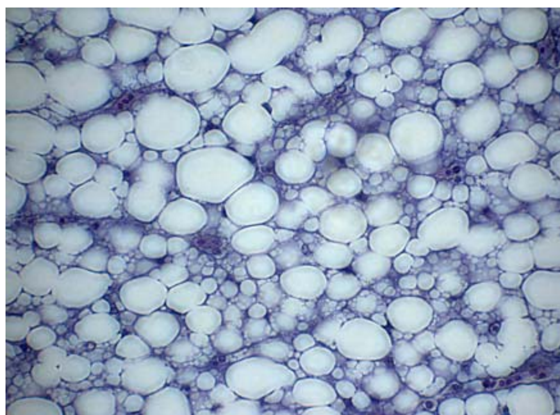


Figure 21. Gonad histology of Arctic rockling, *Onogadus argentatus*. ST: station, N: Number of individual.

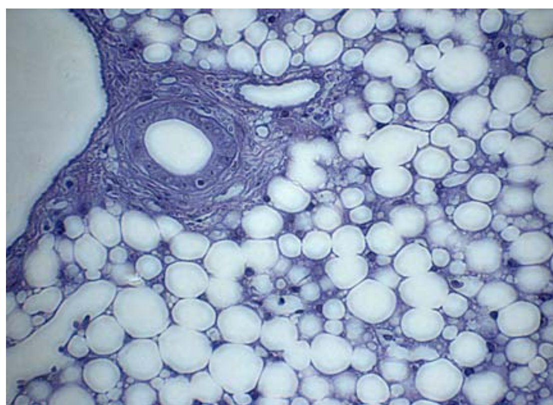
4.5.2 Liver histology

The pictures shown as examples are from individual no. 8 and 12 (Figure 22). The vacuolar aspect of the hepatocytes is explained by their rich dissolved glycogen content. In the center of the image one can see a transverse section of bile duct, lined by a simple epithelium and some connective tissue (arrow). Sinusoids containing red blood cells are obvious (arrowheads). PV: portal veinule.

ST1 N8 x40



ST1 N12 x4



ST1 N8 x10

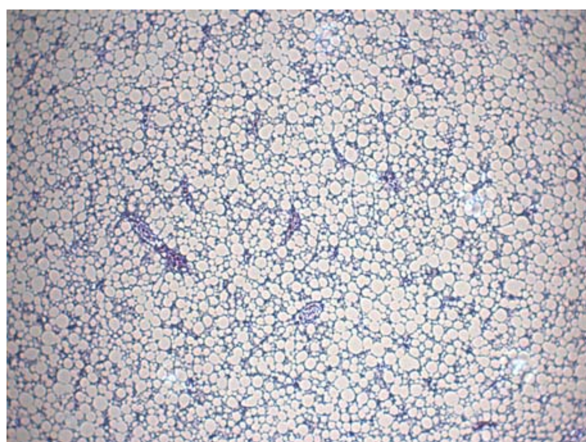


Figure 22. Histology of liver of Arctic rockling, *Onogadus argentatus* collected at station 1. ST: station , N: Number of individual.

4.5.3 Gill histology

Fish gills have a very efficient counter current mechanism in which the water drawn through fish gills and circulating blood runs in opposite direction. There is exchange of gases at every point of contact and there is no gaseous equilibrium between water and blood.

Primary lamellae. Primary lamellae have a cartilaginous core. Each primary lamella is covered by an epithelium secreting mucus coat that protects against injury or infection. Primary lamellae also have chloride cells responsible for ionic regulation. Primary lamellae give off secondary lamellae on either side to increase the surface area for exchange of gases. The primary lamellae functioning can be changed by the exposure to some contaminant like PAH (David Lowe, 2006).

Secondary lamellae. Secondary lamellae are arranged perpendicular to the primary lamella. Secondary lamellae are covered by simple squamous epithelium with few interspersed chloride cells. Every lamella has blood capillaries that connect afferent and efferent arterioles. A marginal capillary is located at the apex of each secondary lamella. Endothelial cells of blood capillaries are modified to form pillar cells that control the capillary diameter and thus regulate blood flow. The results from the fish sampling from station 2 showed presence of aneurysms as well as clubbing of the lamellar and epithelial lifting (Figure 23). Epithelial lifting and fusion of the secondary lamellae were found in more than 60% of fish sampled from station 2.

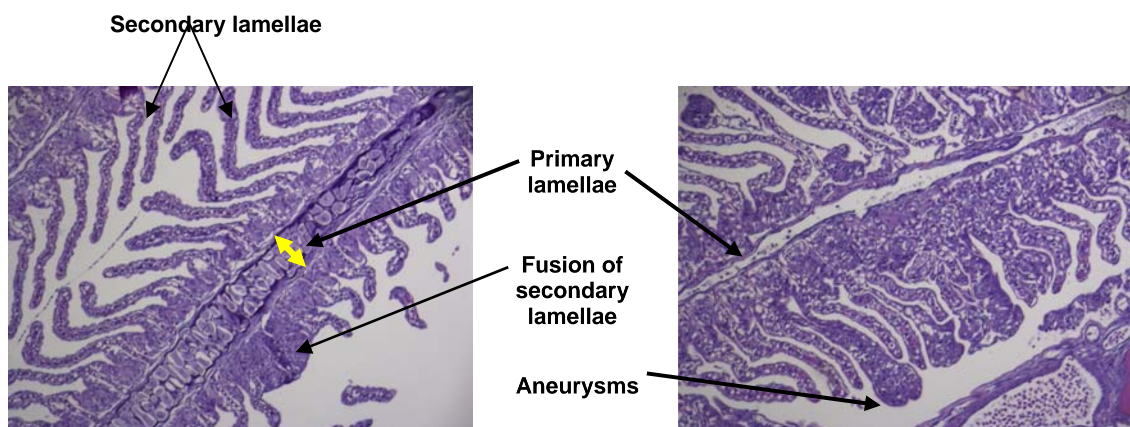


Figure 23. Histological section of gills from *Onogadus argentatus* sampled from station 2 showed severe changes, with epithelial hyperplasia and fusion of secondary lamellae, dilation of capillary and aneurysms.

5 References

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Appendix