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Water Column Monitoring 2004

Summary Report

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REPORT

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Abstract

This report presents results from the Water Column Monitoring 2004, carried out in collaboration between NIVA and RF-Akvamiljø, with sub-contractors. The objective of the survey was to assess the extent to which discharges from Statfjord B affect organisms living in the water column. The study was designed to monitor bioaccumulation and biomarker responses in organisms held in cages in the vicinity of the platform. Furthermore, feral saithe were caught at locations with increasing distance from the platform. The results from the survey indicate that caged organisms have been exposed to low levels of produced water components, compared to results from 2001 (BECPELAG). The reason for this is not clear, but several possible explanations are proposed. However, mussels accumulated PAHs, with levels following the expected gradient with distance from the platform. There was no clear signal from the biological effects, possibly with the exception of micronucleus formation in mussel haemocytes. Concentrations of PAH-metabolites in bile of caged cod and feral saithe were both low, suggesting low exposure levels. There were no differences between locations in the levels of hepatic DNA adducts in the saithe, but there were individuals at each of the locations that had elevated levels of adducts. There were no obviously contaminant-related effects on liver histology in the saithe.

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Preface

This is the final report of the Water Column Monitoring, 2004. The monitoring has been carried out in collaboration between the Norwegian Institute for Water Research (NIVA) and RF-Akvamiljø, with several sub-contractors: The Centre for Environment, Fisheries and Aquaculture Sciences (CEFAS), the laboratory for Cell Biology at the University of the Basque Country (UPV/EHU), The Institute of Applied Environmental Research at Stocholm University and the University of Vilnius. Other contributors to the monitoring (coordinated directly from Statoil ASA and OLF) have been Ocean Climate A/S and the Norwegian Insititute of Marine Research.

This was the second regular Water Column Monitoring survey in the North Sea, based on guidelines established as a result of findings from the BECPELAG workshop (2001). The efforts of a number of people are aknowledged:

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Summary

The Water Column Monitoring (WCM) 2004 has been carried out in collaboration between the Norwegian Institute for Water Research (NIVA) and RF-Akvamiljø, with several sub-contractors, the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), the laboratory for Cell Biology at the University of the Basque Country (UPV/EHU), the Institute of Applied Environmental Research at Stocholm University and the University of Vilnius.

The objective of the WCM survey 2004 was to assess the extent to which discharges from the Statfjord B platform affect organisms living in the water column. The study was designed to monitor bioaccumulation and biomarker responses in organisms (cod and blue mussels) held in cages in the vicinity of the Statfjord B platform. In addition, feral saithe were caught at locations with increasing distance from the platform.

The results from the 2004 Water Column Monitoring survey indicate that caged organisms has been exposed to low levels of produced water components during August and September 2004. Comparing these results with results from the BECPELAG workshop in 2001, it is evident that accumulated levels of PAHs were a factor of 5 or more lower in 2004 compared to 2001. The reason for this difference is not clear, but there are a number of possible explanations. None can, however, fully explain the observed situation. One issue is whether ocean current patterns have deviated or deviate through the year, and the second whether stratification differed between the two periods. There is also limited knowledge of the amounts of particles in the water during the two periods, a fact which would also affect bioavailability. However, caged blue mussels did accumulate PAHs, especially 2- and 3-ring components, during the deployment. Bioaccumulation levels followed the expected gradient with highest levels in mussels caged 500 m from the platform decreasing in mussels held at 1000 m and 10 000 m from the platform. The lowest levels were found in 0-time mussels and in mussels held at the reference location.

There was no clear signal from the biological effects, possibly with the exception of micronucleus formation. The results indicate a weak sex-dependence of Benzo(a)pyrene hydroxylase in mussels, but no relationship with PAH exposure. Furthermore, results indicate a gradient of hemocyte micronuclei formation in mussel caged at different distances from the Statfjord B oil platform, although only mussels caged at 500 m had significantly increased levels compared to mussels caged at the reference location. This parameter shows promise in indicating possibly genotoxic impacts at low exposure levels. There were histological changes in some mussels, most of which was presumably associated with gametogenesis, but some unexplained effects were observed in mussels caged 500 m from the platform.

Concentrations of PAH-metabolites in bile of Atlantic cod suggest that exposure levels have been very low, at least during the last week prior to sampling (due to rapid excretion). The results did indicate that the cod had been exposed to PAHs prior to deployment, highlighting the need to keep all experimental organisms under clean conditions prior to such deployments. Biological responses, such as cytochrome P4501A activity (EROD) and glutathione S-transferase activity (GST) reflected the low exposure. There were only minor differences between cod held at different locations and no gradient in responses.

As for cod, levels of PAH metabolites in bile from feral saithe were low and there were no differences between locations. Furthermore, there were no differences between locations in the levels of hepatic DNA adducts in the saithe, but there were individuals at each of the locations that had clearly elevated concentrations of adducts. There were no obviously contaminant-related effects on liver histology in the saithe.

1. Introduction

The Water Column Monitoring (WCM) 2004 has been carried out in collaboration between the Norwegian Institute for Water Research (NIVA) and RF-Akvamiljø, with several sub-contractors. The sub-contractors have been The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), the laboratory for Cell Biology at the University of the Basque Country (UPV/EHU), the Institute of Applied Environmental Research at Stocholm University and the University of Vilnius.

Organisms living in the water column around offshore oil and gas production facilities are predominantly exposed to chemicals through discharge of production water (OLF 2000). The amount and composition of produced water (PW) varies from field to field (Røe 1998), but is generally a mixture of:

- Formation water contained naturally in the reservoir.
- Injected water used for secondary oil recovery.
- Treatment chemicals added during production.

Typically, produced water contains dissolved inorganic salts, minerals and heavy metals together with dissolved and dispersed oil components and other organic compounds. The specific chemical composition varies between reservoirs and within a reservoir as production proceeds. A target chemical characterisation of four offshore oil production platforms in the North Sea showed that the major organic components were BTEX (benzene, toluene, ethylbenzene and xylene), NPD (naphtalenes, phenanthrenes and dibenzothiophenes), PAHs (polyaromatic hydrocarbons), organic acids, alkylphenols (APs) and phenols (Røe and Johnsen 1996; Utvik 1999). As a natural consequence of well exploitation, oil content in the reservoirs will decrease and the need to inject water will increase, thus eventually leading to increase in the discharges of PW. A recent report estimate that the total discharges of PW in the Norwegian sector of the North Sea will increase from approximately 130 million m^3/year in 2002 to 180 million m^3/year in 2011 followed by stabilisation and decrease in discharges (SFT 2004).

Some of the organic chemicals found in PW are relatively resistant to biodegradation, have a bioaccumulation potential and may be toxic to organisms in receiving waters (Brendehaug et al. 1992; Tollefsen et al. 1998; Taban and Børseth 2000; Aas et al. 2000a). This applies in particular to groups of chemicals such as alkylphenols (APs) and polycyclic aromatic hydrocarbons (PAHs) that are known to produce various toxic effects including reproductive disturbances, mutagenicity and carcinogenicity (Landahl et al. 1990; Bechmann 1999; Lye 2000; Meier et al. 2002). Recent studies from the ICES workshop "Biological effects of contaminants in the pelagic ecosystem (BECPELAG)" indicate that toxic compounds are detectable several kilometres away from a North Sea oil production platform using *in vitro* bioassays (Thomas et al. in press; Tollefsen et al. in press) and biomarkers (Balk et al. in press; Regoli et al. in press; Aas et al. in press). Although there is reason to assume that many of the chemicals that are present in PW effluents may produce biological responses, the ability to assess the potential for adverse effects are limited by the lack of sufficient *in situ* monitoring data using biological effects methods with endpoints reflecting long term (ecological) effects.

Biological indicators or markers (biomarkers) have been developed to measure the biological response related to an exposure to, or the toxic effect of, an environmental chemical (Peakall 1992). Some biomarkers are specific in terms of their ability to detect and assess the potential for effects through a specific toxic mechanism, whereas others give information about larger groups of chemicals with more diverse mechanisms of action. Common for all of the methods is the capability of performing time-integrating response assessment to complex mixtures over extended periods of time, which is often required in environmental monitoring. Since most of these methods are highly sensitive and responses occur at lower concentrations and/or prior in time to more adverse effects at a higher

organisation level, the methods have become convenient early-warning tools for assessing the potential for long term (ecological) effects. The use of biomarkers in sentinel species or specific caging systems with keystone species has consequently facilitated the implementation of such methods in various environmental monitoring programs in freshwater, marine and estuarine areas. A combination of laboratory and field validation of the different biomarker and effects-based methods has greatly improved the knowledge of the potential and limitations of these methods and made it possible to link responses of biomarker signals to the potential for more adverse effects at the ecological level (Collier et al. 1992a; Elliot et al. 2003; Bechmann et al. in prep).

1.1 Objective

The objective of the WCM survey 2004 was to assess the extent to which discharges from an oil production platform affect organisms living in the water column. To fulfil this objective, the survey was designed as described below (chapter 1.2).

Produced water discharges, which are the most pronounced contributor to pollution of the water column, contain polycyclic aromatic hydrocarbons (PAHs), alkylphenols, decalins, organic acids and a range of inorganic chemicals (Utvik, 1999). Some of the relevant chemicals are reported to produce biological responses in controlled laboratory experiments that may ultimately cause long term (ecological) effects. Controlled caging experiments using well documented species and methods of effect have been used as the best suited monitoring system to assess the extent of influence from oil or gas production platforms (SFT, 2003).

1.2 Description of methods

This study was designed to monitor bioaccumulation and biomarker responses in organisms held in cages in the vicinity of the Statfjord B platform. All cages were deployed for 6 weeks. Six rigs were initially deployed. Four of the rigs were placed in an expected plume, 500 m, 1000 m, 2500 m and 10000 m, respectively away from the platform. Two rigs were regarded as reference, with the intention of sampling one (and have one as backup). One of the reference cages was lost. The 2500 m cage was lost, due to collision with the supply wessel Normann Draupner, August 10th, but later retrieved. Samples of fish bile and mussels for PAH- and NPD- body burden was taken from this cage, although the samples will not represent a full exposure history and may be contaminated during retrieval (see below). Zero-time samples were taken for both blue mussels and Atlantic cod for determination of pre-exposure levels of contaminants and biomarker responses. Feral saithe were collected (by lures on hand held equipment) in the vicinity of the 500 m, 1000 m and 10 000 m locations.

Details regarding geographical positions for the deployment stations and CTD profile of the water column can be found in the cruise report (**Appendix A**). Maps showing Statfjord B and the stations for deployment is presented in **Appendix H**.

Devices for monitoring polar chemicals, POCIS passive samplers were also deployed with the cages. Methods and results from these samplers are described elsewhere (Tollefsen et al. 2005).

The monitoring approach was based on the reported chemical profile of selected production platforms and the experience from the BECPELAG workshop (Hylland et al. *in press*). The methods applied are presented in **Table 1**. In addition, gill EROD activity was measured in a selection of individuals of caged cod.

Table 1. Overview of biological and chemical analyses.

Caged cod:		
method	**matrix**	**No. of samples**
general health criteria	whole fish	
EROD	liver	(4 x 25) + 25
GST	liver	(4 x 25) + 25
vitellogenin	blood plasma	(4 x 25) + 25
PAH-metabolites, FF	bile	(4 x 25) + 25
PAH-metabolites, GC/MS	bile	30

Caged mussels		
method	**matrix**	**No. of samples**
general health criteria	soft tissue	
BaPH	hepatopancreas	(4 x 20) + 20
lysosomal stability	hematocytes	(4 x 20) + 20
histopathology	hepatopancreas	4 x 15
PAH concentration	whole mussel	(5 x 3) + 3 pools
lipid content	whole mussel	(5 x 3) + 3 pools
micronucleus	hematocytes	(4 x 10) + 20

Feral saithe		
method	**matrix**	**No. of samples**
DNA adducts	liver	75
histopathology	liver	75
PAH-metabolites, FF	bile	54
PAH-metabolites, GC/MS	bile	15

Additional methods		
method	**matrix**	**No. of samples**
micronucleus	cod	(4 x 10) + 20

1.2.1 PAH concentration (mussel body burden)

The chemical composition of produced water is dominated by low molecular PAHs (naphthalenes, phenanthrenes, dibenzothiophenes, commonly denoted NPDs), decalins and their alkylated homologues (Utvik, 1999). High molecular PAHs such as benzopyrene, pyrene, chrysene, fluorene are also present in effluents of produced water from production platforms in the North Sea, although at lower concentrations than the more low-molecular weight PAHs. Many of these chemicals have also been detected in caged organisms deployed downstream discharge points (Røe, 1998). This applies in particular to alkylated NPDs, which have been found in higher concentrations than their non-alkylated sister compounds in organisms and passive sampling devices (Røe, 1998; Ruus et al., in press). Although the different compounds represent variable degree of health risk to the aquatic fauna, measurement of their body burden in caged animals are commonly used to assess the exposure situation in a specified area.

1.2.2 General biological observations

General biological data as body length, weight and sex is usually recorded in environmental monitoring studies and is used in the interpretation of biomarker data. For the interpretation of

biomarkers of reproductive disturbance such as vitellogenin (vtg), the information about sex is crucial for interpretation. A relationship between length and weight can be used as an estimate of the condition of the individual (at least for more or less “cylindrical” fish).

1.2.3 PAH-metabolites in fish bile

The potential adverse effects of PAHs have resulted in many years of concentration monitoring in water, sediment and biota. However, the extensive biotransformation of PAHs by fish greatly prevents the accumulation of these compounds in extra-hepatic tissues (Stein et al. 1987). Consequently, tissue levels of parent PAH do usually not provide an adequate assessment of the PAH exposure level (Varanasi 1989). The metabolites concentrate in the gall bladder of fish following biotransformation. Analysis of PAH metabolites in the fish bile constitutes a very sensitive method for assessment of PAH exposure in laboratory and field studies (Beyer et al. 1998; Aas et al. 2001). PAH metabolites are commonly determined by semi-quantitative screening analysis (so called fixed fluorescence; Aas et al. 2000b) or by quantitative determination of specific metabolites by GC/MS (Jonsson et al. 2003; Jonsson et al. 2004).

1.2.4 EROD

CYP1A induction, and in particular EROD, has been widely used as a biomarker for planar organic compounds, including some PAHs, PCBs and dioxins. (Goksøyr and Förlin 1992; Bucheli and Fent 1995). PAHs from petrogenic sources have been shown to be inducers of CYP1A in a range of fish species. Both laboratory based investigations (Upshall et al. 1993; Celander et al. 1994) and field work (Payne et al. 1984; Collier et al. 1992b; George et al. 1995; McDonald et al. 1995; Stagg et al. 1995) have shown indications of induction, though the field studies have generally focused on severely polluted areas.

1.2.5 GST

Glutathione S-transferase (GST) is also a part of the detoxification system and is evolutionary developed by organisms in order to convert lipophlic compounds into more hydrophilic and thereby more easily excretable metabolites. Two major types of reactions exist: phase I, which involves hydrolysis, oxidation and reduction, and phase II, which involves conjugation. GST catalyses conjugation of glutathione to compounds with electrophilic centres (SH group of glutathione neutralises the electrophilic site). The compounds may otherwise be harmful as they may react with macromolecules controlling cell growth, such as DNA, RNA and proteins. It is therefore of great importance that the animal is capable of neutralises and excrete these compounds. Changes in the activity of GST may reflect exposure to xenobiotics.

1.2.6 Benzo(a)pyrene hydroxylase activity

Benzo(a)pyrene hydroxylase (BaPH), commonly referred to as aryl hydrocarbon hydroxylases (AHH), represents an enzymatic activity commonly grouped as mixed function oxidases (MFOs). These enzymes metabolise selected PAHs and consequently alter potentially harmful chemical to non-toxic and readily excretable end products. The BaPH have also an ability to convert moderately toxic chemicals to highly reactive metabolites, as seen with the conversion of benzo(a)pyrene (BaP) to quinone derivatives that may interact with DNA to form DNA-adducts, which may potentially lead to permanent cellular damage and cancer. BaPH has been shown to be induced by a variety of PAHs in mussels and consequently been proposed as a biomarker for the exposure to and the potential for adverse biological effects of certain types of PAHs (Michel et al., 1994; Sole et al., 1998). Measurement of BaPH in sentinel species such as the blue mussel has consequently been used to determine the effects of PAHs in several environmental monitoring studies including the BECPELAG workshop (Burgeot et al., in press).

1.2.7 Vitellogenin

In unexposed fish, the synthesis of vitellogenin (vtg) takes place in the liver of oviparous females under the stimulation of endogenous estradiol (Tata and Smith, 1979). Male and juvenile fish of most species, which only have low levels of circulating estrogens, do not produce appreciable levels of vtg. However, these fish exhibit considerable levels of hepatic estrogen receptors and the genetic machinery required for protein synthesis, and are thus capable of producing high levels of vtg when exposed to exogenous estrogens. Induction of this female typical protein in male and juvenile fish has therefore been widely used as a sensitive biomarker for exposure to xenoestrogens (Sumpter and Jobling, 1995). The use of vtg as a biomarker for xenoestrogens in ecologically relevant fish species has since then been employed for coastal and freshwater environmental monitoring (Hylland et al., 1998; Hylland et al., 1999) and for monitoring of areas that are effected by discharged from oil production activities (Scott et al., In press). Recent studies with freshwater species such as zebrafish and rainbow trout suggest that induction of vtg occur at concentrations of xenoestrogens that also produce alteration in sexual development when exposed during sensitive windows of embryonal and larval development (Jobling et al., 1996; Orn et al., 2003)

1.2.8 Lysosomal stability

Membrane integrity has been found to be affected by a range of stressors, including metals and organic chemicals. One of the most well-established methods to determine changes in membrane integrity is through measurements on the lysosomal membrane stability. The method uses one of a range of available dyes, e.g. neutral red, which will accumulate in the lysosomal compartment of cells. A reduction in membrane integrity will cause the dye to leak back into the cytosol, an effect which can then be quantified. The method is most commonly used with circulating cells, e.g. haemocytes in blue mussels, but methods exist to use a similar method on tissues.

1.2.9 DNA adducts

The detoxification of genotoxicants by the inducible cytochrome P450 mixed function oxygenase systems often results in the production of reactive chemical intermediates that are highly electrophilic and can covalently bind to the bases of DNA forming adducts. Thus, the presence of DNA adducts has been taken as evidence of exposure to specific genotoxicants.

1.2.10 Micronucleus formation

Chromosomal rearrangements, such as micronuclei (MN), are recognised as a consequence of genome instability (Fenech et al., 1999). 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; Kramer, 1998; Zoll-Moreux).

1.2.11 Histology

Histological biomarkers provide powerful 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). Following studies of this type, it is generally accepted that certain
liver lesions in marine fish can be induced by environmental contaminants and that these represent an ecologically relevant biological endpoint of exposure to pollution. Guidelines for the diagnosis of pathological changes in the liver have been produced (Feist et al., 2004). In addition, a number of other organ systems may exhibit toxicopathic changes related to exposure to contaminants (Hylland et al., 2003).

In mussel, histopathological biomarkers are generally analysed in the digestive gland. The digestive gland of molluscs is the main center for metabolic regulation, participating in the mechanisms of immune defense and homeostatic regulation of the internal medium, as well as in the processes of detoxification and elimination of xenobiotics (Moore and Allen, 2002). The battery of biomarkers selected can be grouped as follows: biomarkers of metal exposure (autometallographic localization of metals in lysosomes) and biomarkers of general stress (histopathological alterations).

Histopathological changes in the cell and tissue organization of the digestive, reproductive and immune systems, as well as the incidence of parasites in these tissues are sensitive biomarkers which may respond to a diverse variety of xenobiotics and natural stressors. Such biomarkers have been shown to be responsive in molluscs and fishes. In the digestive gland of marine molluscs, different stressors provoke changes in the phasic activity and atrophy of the digestive alveoli (Lowe et al., 1981; Couch, 1984; Vega et al., 1989; Cajaraville et al., 1992). The changes can be measured with the aid of quantitative microscopy (planimetry), as changes in the mean epithelial thickness (MET), mean diverticular radius (MDR), mean luminal radius (MLR) and the ratios MLR/MET and MET/MDR (Vega et al., 1989). In addition, there is clear evidence of severe changes in the distribution and relative occurrence of cell types in the digestive gland of molluscs. Digestive cells are usually much more abundant than basophilic cells but, under conditions of exposure to pollutants, an apparent increase in the relative numbers of basophilic cells (measured as volume density of basophilic cells) occurs. This so-called “cell-type replacement” (Marigómez et al., 1998) may provoke disturbances in digestion and metabolism.

2. Material and Methods

Atlantic cod and blue mussel that originated from local fish and shellfish farmers were transported to the Statfjord C field and deployed in cages as described in the survey report (**Appendix A**). After 6 weeks of field exposure, cages with animals were retrieved, biological data on length, weight, and sex was measured and biological samples obtained. The rig at 2500 metres was lost due to collision with a vessel. Organisms from this cage were frozen without dissection at -20ºC onboard the vessel. Samples of fish bile and mussels for PAH- and NPD- body burden was retrieved from this station, nevertheless. Feral saithe were collected (by lures on hand held equipment) in the vicinity of the 500 m, 1000 m and 10 000 m locations. There are no saithe from the reference location.

2.1 PAH concentrations in blue mussel

Twenty to thirty whole blue mussels were excised from their shell and transferred to solvent cleaned and high temperature treated glass containers. The wet weight of tissue was recorded and tissues were frozen and transported to NIVA on dry-ice. The samples were stored at -20^{o}C until analyses.

The biological matter was homogenised, added internal standards (napthalene d8, acenaphthene d8, phenanthrene d10, chrysene d12, perylene d12 and athracene d10) and saponified. The compounds were extracted with n-pentane and dried over sodium sulphate. The extraction volume was reduced and the extracts were cleaned by GPC and solvent exchanged to cyclohexane. The extracts were then analysed by GC/MS with the MS detector operating in selected ion monitoring mode (SIM) and analyte concentrations in the standard solutions were in the range 5-1000 ng/µl. The GC was equipped with a 30 m column with a stationary phase of 5% phenyl polysiloxane (0.25 mm i.d. and 0.25 µm film thickness), and an injections operated in splitless mode. The initial column temperature was 60°C, which after two minutes was raised to 250°C at a rate of 7°C/min and thereafter raised to 310°C at a rate of 15°C/min. The injector temperature was 300°C, the transfer line temperature was 280°C, the MS source temperature was 230°C and the column flow rate was 1.2 ml/min. Quantification of individual components was performed by using the internal standard method. The alkylated homologues were quantified by baseline integration of the established chromatographic pattern and the response factors were assumed equal within each group of homologues.

The chemical analyses were performed at NIVAs laboratory, accredited by the Norwegian Accreditation as a testing laboratory according to the requirements of NS-EN ISO/IEC 17025 (2000). Analytical standards are also certified by the participation in international calibration tests, including QUASIMEME twice per year.

2.2 Length, weight, sex and condition

The length and weight of cod and mussel were measured with fixed mounted balances in the lab facility onboard and in the onshore laboratory, respectively. Fish were sexed by visual examination of gonads and liver weight was recorded. Condition was determined as the ratio between total weight and the cube of the fork length of the fish.

2.3 PAH-metabolites in fish bile

2.3.1 Fixed fluorescence

Fixed Fluorescence (FF) is a semi-quantitative and semi-qualitative screening method for direct fluorescence detection of groups of PAH metabolites (Aas *et al*. 2000b). 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, optimised 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 at 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.

2.3.2 GC/MS

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.

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.

2.4 EROD

2.4.1 Liver

7-ethoxyresorufin-*O*-deethylase (EROD) is a specific cytochrome P450 (CYP) reaction where ethoxyresorufin is used as a substrate (Burke and Mayer, 1974). Cytochrome P450 1A catalyse the deethylation of 7-ethoxyresorufin to resorufin. Cytohrome P450 1A activity in microsome fractions was quantified from the amount of resorufin produced.

Preparation of microsomes was performed on ice with pre-cooled equipment and solutions. The cryo-preserved liver samples were homogenized in a potassium phosphate buffer (0.1 M, pH 7.8) containing KCl (0.15 M), DTT (1mM) and glycerol (5% v/v), using a Potter-Elvehjem Teflon-glass homogeniser. The homogenate was centrifuged (10 000 g; 30 min) before the supernatant was re-centrifuged (50 000 g; 120 min). The microsome fraction was obtained by resuspending the resulting pellet in potassium phosphate buffer (0.1 M, pH 7.8) containing KCl (0.15 M), DTT (1 mM), EDTA (1 mM) and glycerol (20% v/v).

EROD activity was assayed fluorimetrically by a method adapted for plate-reader as follows: samples of microsomes were diluted to 2 mg/ml in buffer and pipetted (50 µl) onto a plate. Pre-prepared resorufin standards (duplicates) were then added to subsequent wells. Reaction mixture (200 µl,

containing 0.1 M potassium phosphate buffer, pH 8.0, and 3 µM 7-ethoxyresorufin) was added to the sample wells, before NADPH-solution (2.4 mM in final well volume 275 µl) was added to initiate the reaction. Transformation of 7-ethoxyresorufin to resorufin was read in 8 steps on the plate reader. Excitation was at 530 nm and fluorescence emission was measured at 590 nm.

The EROD activity was normalised to protein content in the microsome fraction (denoted as pmol/min/mg microsomal protein), determined according to the Lowry protein assay (Lowry et al. 1951) adapted to measurement by a plate reader. Protein standard was bovine gamma globulin.

2.4.2 Gills

As mentioned, 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.

Gill arcs are removed from the fish and stored in Hepes-Cortland (HC) buffer on ice (cod gills can be stored for 3-4 days). From each individual 10 duplicates with 2mm long primary filaments in a 12 well microplate in HC buffer. The HC buffer is exchanged with reaction buffer (HC containing ethoxyresorufin and dicumarol as protease inhibitor) and the samples are transferred to a 96 well plate. After 10 or 30 min for exposed fish and 30 or 50 for control fish the activity levels are measured. During this time CYP1A in the filaments convert ethoxyresorufin to resorufin. The amount of resorfin in each well is measured by fluorecens in a plate reader (ex 540 nm, em 590 nm). The more CYP1A present in the gills the higher activity measured expressed as pmol resorfin/filament/minute. For more info see http://publications.uu.se/theses/abstract.xsql?dbid=3913.

2.5 Glutathion-S-transferase (GST) activity

The method used is based on Winston et al. (1998) and Regoli and Winston (1999), except that buffers were optimised for cod tissues. Liver tissue was homogenised with a Potter-Elvehjem glass/teflon homogeniser in four volumes of 100 mM KH_2PO_4 buffer, pH 7.5, 2.5% NaCl. The homogenate was centrifuged at 100 000 × g for 1 h, and cytosolic fractions were aliquoted and stored at –80°C.

Reagents (20 mM CDNB, 20 mM GSH) and cytosol samples (50 µL) were transferred to U-shaped 96 microwell plates, each containing 3 replicates of the samples, a negative and a positive control (cod sample). Each microplate were stored on ice until transferred to the automatic pipetting robot (Tomtec Quadra 96 model 320) operating board for further dilutions and mixing. (Tomtecs special tips are designed to aspirate multiple reagents in one pass using air gap separation; witch makes it possible to mix all the reagents directly into the microplate wells without further pipetting). Different dilutions are selected for each sample to obtain a linear signal, but normally a 5 fold cytosol dilution in phosphate buffer (100mM KH_2PO_4/K_2HPO_4, pH 7.4, 24°C) will be carried out before new tips are changed to perform a final 1:20 dilution of samples and reagents in the reading microplate. After the samples and reagents have been mixed (15 sec.) the plate is transferred to the microplatereader were the absorbance is measured at 340 nm during 1 minute run at 24°C. The enzyme activity can be estimated and normalised against the sample protein concentration.

The activity calculation: $(\Delta \text{ Absorbance-blank})/ (9.6x [\text{Protein}]_{well})$, where 1.7 is the molar extinction coefficient ($\in$) for the CDNB-GSH conjugate (in $mM^{-1}cm^{-1}$). GST activities were expressed as moles of substrate converted per minute per mg of protein in the cytosol.

The total protein concentrations of the samples where determined by a procedure based on the Bradford method (Bradford, 1976).

2.6 Protein determination

Analyses at RF-Akvamiljø used the Bradford assay for protein normalisation, whereas analyses at NIVA used the Lowry assay to determine the concentration of protein in samples. The Bradford assay relies on the fact that protein binds to Coomassie Brilliant Blue G-250 and changes colour. Coomassie Blue exists in two colour forms, red and blue. Upon binding protein, the red form is converted to the blue form. The protein-dye complex absorbs light at 595 nm of test solution (protein solution + Coomassie) as compared to a set of standard protein solutions (bovine serum albumin, BSA).

Biomarker analyses at NIVA were normalised to protein concentration using Lowry's method adapted for plate-readers (Lowry et al., 1951) with bovine gammaglobulin as standard.

2.7 Benzo(a)pyrene hydroxylase activity

Benzo(a)pyrene hydroxylase activity was determined in the microsomal fraction of hepatopancreas by a method modified from Michel et al. (1994). Essentially, frozen hepatopancreas was homogenised in 5 volumes of ice-cold 0.1M potassium phosphate buffer (pH 7.8) containing 0.15 M KCl, one tablet Complete™ protease inhibitor (Boehringer-Mannheim) per 100 ml, 1mM dithiothreitol and 5% glycerol. The homogenate was centrifuged at 10 000 *g* (4 ^{o}C, 30 min.), whereupon the supernatant was removed and subjected to centrifugation at 50 000 *g* (4^{o}C, 120 min.). After centrifugation, the supernatant was removed and the pellet resuspended in ice-cold homogenisation buffer added 15% glycerol (in total 20% glycerol) and 1mM EDTA to obtain a microsomal fraction.

For analysis, 100 µl of the microsmal fraction was added to 700 µl 0.05M sodium phosphate buffer (pH 7.3) containing 2 mg/ml BSA and 40 µl of a BaP solution of 2 mM BaP and 82 µM (100 MBq/L) ^{14}C-BaP in acetone. The solution was divided into two glass tubes and one tube added 80 µl sodium phosphate buffer containing 10 mM NADPH, whereas the other was added 80 µl sodium phosphate buffer. Both tubes were mixed and incubated on an orbital shaker (20^{o}C, 20 min.) before the reaction was terminated by adding 1 ml of stop solution containing 15% 1M KOH and 85 % DMSO. Non-metabolised BaP was removed by 2 sequential extraction steps with 5 ml n-hexane/cyclohexane for 30 min. Following extraction, 700 µL of the water phase was removed for liquid scintillation counting using a standard ^{14}C protocol.

2.8 Vitellogenin

Blood samples were taken from the caudal artery by means of pre-cooled syringes containing heparin (10000 IU/ml, Sigma) and the protease inhibitor Aprotinin (5 TIU/ml, Sigma) and centrifuged at approximately 2000 g (10 min at 4°C). The supernatant was carefully decanted, aliquots prepared and samples snap-frozen in liquid nitrogen. Plasma samples were transported to NIVA on dry-ice and stored at -80°C until analysis. Vitellogenin was determined in plasma from caged cod using a competitive ELISA with cod vitellogenin as standard and competing antigen. The analyses were done using a kit from Biosense Laboratories AS (Bergen, Norway) with anti-cod antiserum and cod vitellogenin as standard, according to the instructions of the manufacturer.

2.9 Lysosomal membrane stability

The mussels from the pre-exposure group and field groups were brought to the lab in Stavanger on ice. The mussels were acclimatised in the lab in aquaria with fresh supply of sea-water for two days prior to sampling (to alleviate stress during transport). Haemolymph samples were obtained from 19 individuals at each field station and 26 individuals from the pre-exposure group.

0.4 ml haemolymph was sampled from each mussel and mixed with filtered sea water at the ratio 2:1. 40 µl haemolymph/seawater-mixture was pipetted out on microscope-slides, and incubated in a light-proof box for 20 min before 35 µl neutral red (concentration 0.1 µg/µl) was added. All analyses were performed blind. For a detailed description of the method see Lowe (1994).

NR is selectively taken up by haemolymph cells and this adds an extra stress to the membranes. After some time, from 15 to 200 minutes, depending of the health status of the mussels, the membrane will start to burst and NR will leak out in the cytosol. This causes the form of the cells to change from irregular to round shaped. The time from NR is added the cells and until they become round and perish is observed visually with a microscope (**Figure 1**). The cells are observed repeatedly at 15, 30, 60, 90, 120, 150 and 180 minutes of incubation with NR. The endpoint of the analysis is when 50% of all cells become round and die. This method is perceived as a general health-parameter, and has been shown to respond to PAH/oil-exposed mussels.

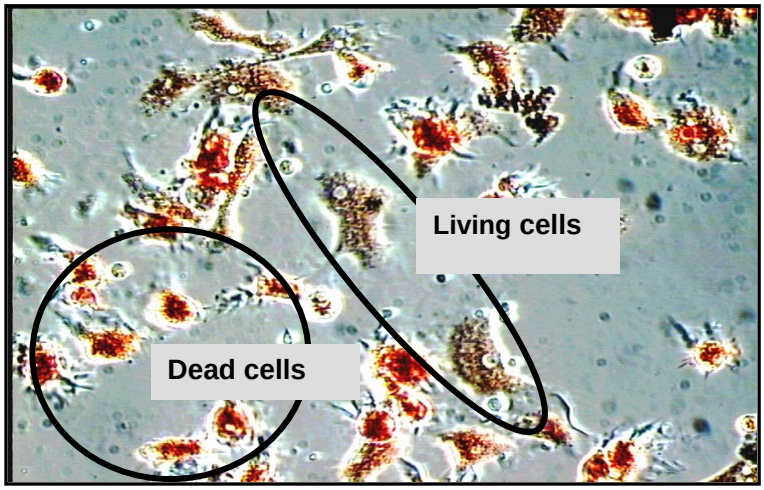


Figure 1. Microscope view (400× magnification) of living and dead mussel haemolymph cells

2.10 DNA adducts

Deep-frozen liver tissue pieces from cod were semi-thawed. DNA was extracted and purified according to Dunn *et al*., 1987; Reichert and French 1994, with minor modifications as described by Ericson *et al*. 1998 and Ericson and 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 (Aas *et al*. 2000a). 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). In addition, several quality control experiments were performed parallel to the analysis of the samples.

2.11 Micronucleus formation

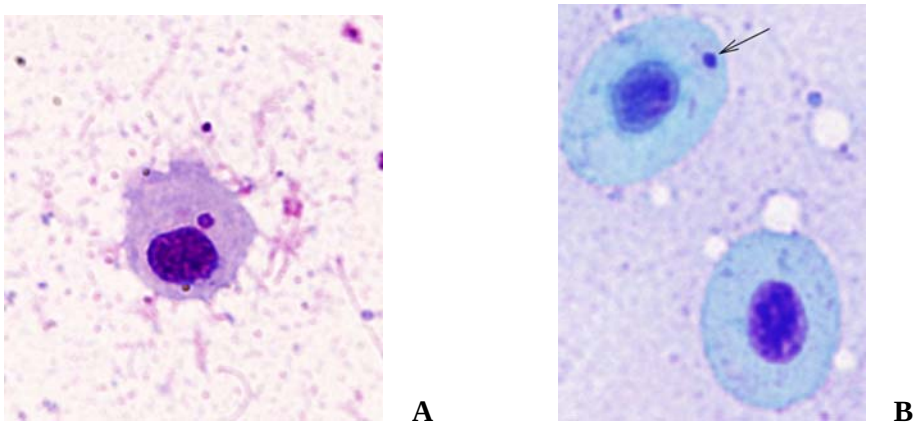
The analysis of micronuclei was performed in both cod and mussels.

The small piece of cod liver was directly smeared on slides, air-dried and fixed in methanol for 15 min. Spread on the slides and air-dried mussel hemolymph was also fixed for 15 min. in methanol.

The slides were then shipped and cytogenetic analysis was done in Institute of Ecology at Vilnius University (Lithuania). Slides were stained with 5% Giemsa solution for 10-20 min. Blind scoring of micronuclei was performed on coded slides without knowledge of the exposure status of the samples to eliminate technical variability.

The frequency of micronuclei in liver cells or haemocytes was determined by scoring at a 1000× magnification using Olympus BX 51 or Nikon Eclipse 50i bright-field microscopes. A total of 25000-36000 cells (2000-5000 cells from each specimen) were examined in each caged experimental group of cod and 72000 cells in cod before caging. In some mussel slides, the deficiency of appropriate cells for the micronuclei analysis was noted. Nevertheless, 500 haemocytes was a minimum amount of cells suitable for the analysis. Therefore, in mussels micronuclei were counted in 500-2000 haemocytes from each 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 of 1/3-1/50 (for fish) or 1/3-1/20 (for mussels) of the main nucleus and clearly detached from it were interpreted as micronuclei (**Figure 2**). 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.



A **B**

Figure 2. Micronucleated haemocytes in mussels (A) and cod liver cell with micronuclei (B); 1000× magnification.

2.12 Histology

2.12.1 Saithe

Livers from saithe were received dispatched to the CEFAS Weymouth Laboratory, UK, to be processed for histology.

Upon receipt, samples were transferred to 70 % industrial methylated spirit (IMS) for processing to wax in a vacuum infiltration processor using standard protocols. Sections were cut at 3-5 μm on a rotary microtome and resulting tissue sections were mounted onto glass slides before staining with haematoxylin and eosin (H & E). Stained sections were analysed by light microscopy (Nikon Eclipse E800) and digital images were taken using the LuciaG™ Screen Measurement System (Nikon, UK).

Liver pathology criteria were derived from those specified by the International Council for the Exploration of the Sea (ICES) (Anon, 1999) and by Feist et al. (2004). The presence of parasites was also recorded. A three-point classification system for hepatocyte vacuolation, based upon that described by Stentiford et al. (2003) was used. This scale (Vac I-III) represented a progressive increase in the vacuolation of liver cells.

2.12.2 Mussels

Digestive glands were dissected, fixed in Davidson solution on board and transferred to the laboratory at the University of the Basque Country. Then, fixed tissues were dehydrated in alcohols and embedded in paraffin. Histological sections (7 µm) were cut with the aid of a rotary microtome, stained with haematoxylin-eosin (H/E) and mounted.

Histopathological examination was carried out under the light microscope. Prevalence of parasites, hemocyte infiltration and general condition of the digestive epithelium, the interstitial connective tissue and the gonad tissue were systematically recorded.

As an indication of whether cell-type replacement occurred or not, the volume density of basophilic cells (VvBAS) in the digestive gland of mussels was determined by means of stereology. A Weibel graticule (M-168; Weibel, 1979) was superimposed onto paraffin sections stained with H/E with the aid of a drawing tube attachment. Randomly selected 5 fields were counted in 10 individuals per experimental group (20x objective). The volume density of basophilic cells was calculated as $V_{VBAS} = V_{BAS}/V_{DT}$ where V = volume; BAS = basophilic cell and DT = digestive gland epithelium.

In order to quantify the structure of the digestive tubules, a planimetric procedure was applied on paraffin sections of digestive gland tissue (Vega et al. 1989). A total of 25 tubule sections per individual were recorded in an image analysis system (Visilog 5.4 Noesis) attached to an Olympus BX50 light-microscope. Five parameters were obtained: MET (mean epithelial thickness), MDR (mean diverticular radius), MLR (mean luminal radius), MET/MDR ratio and MLR/MET ratio.

Gonad Index (GI) was determined in 10 individuals per experimental group according to the classification proposed by Seed (1969). Gonad development stages (1: resting gonad; 2: early gametogenesis; 3: advanced gametogenesis; 4: mature gonad; 5: spawning gonad; and 6: post-spawning gonad) were determined after histological examination of histological sections (7 µm) stained with hematoxylin-eosin.

Intralysosomal metal levels were determined on paraffin sections by autometallography (AMG; Soto et al. 1998). Briefly, paraffin sections (7 µm) were dewaxed in xylene, hydrated in ethanol-water mixtures and left in an oven at 37ºC until completely dried. Tissue sections were covered with a photographic emulsion (Ilford Nuclear Emulsion L4) under safety light conditions. After drying for 45 min in total darkness, sections were rinsed in a developer bath (1:5, b/w Ultrathin Tetenal) for 15 min, rinsed in a stop bath (1% acetic acid) for 1 min, and finally rinsed in a fixative bath (1:10, b/w Agefix Agfa) for 10 min (Soto et al. 1998). Sections were mounted in Kaiser's glycerol gelatine (Merck). Metals were developed as black silver deposits (BSD) and quantified by means of image analysis (BMS, Sevisan, Bilbo). The volume density of BSD (VDBSD) was calculated by stereology as $VD_{BSD}=V_{BSD}/V_{Ti}$, where V_{BSD} is volume of BSD and V_{Ti} is volume of tissue (Soto and Marigómez 1997).

2.13 Statistical methods

Biological responses in individual mussel or fish were subjected to analysis of variance (ANOVA) to clarify whether there were differences between groups (Sokal & Rohlf, 1981). Prior to analyses, homogenity of variances was checked using the Levene's test. Variables were transformed as appropriate to attain homodascicity. Where this was not possible, the non-parametric Kruskal-Wallis analysis was used (Sokal & Rohlf, 1981). Where the parametric ANOVA indicated significant differences, groups were compared using Tukey's post-hoc test. The level of significance for rejection of H_0: "no difference between groups" was set to 0.05.

3. Results

3.1 Exposure – tissue levels of NPDs, PAHs in blue mussels

Data for PAHs are presented on a wet-weight basis. Due to the small sample sizes (n=3 pools in each group), statistical evaluation was not performed. Groups with no overlapping values can be regarded as different. The lipid content of the blue mussels does appear to have decreased somewhat during the exposure period, possibly most at the reference location (**Figure 3**). Results for NPD and PAH concentrations in blue mussels give a clear picture; the concentrations of PAHs are very low in the population used for deployment, which makes it possible to detect even small increases. Sum PAHs increase about threefold in mussels exposed at 500 m and 1000 m, and twofold in mussels caged at 10 000 m (**Table 2**, **Figure 4**). For the lighter PAHs, typically found in produced water effluents, there were clear signals and gradients (**Figure 5**, **Figure 6**); there was even a minor increase in mussels kept at the reference locations, presumably reflecting the general background level in the northern North Sea.

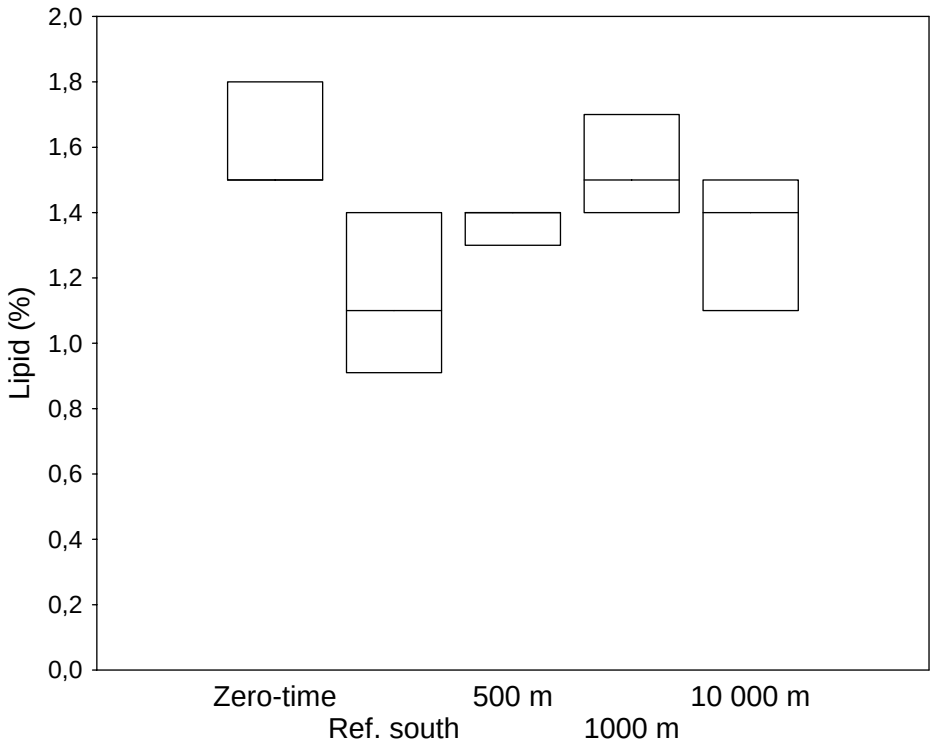


Figure 3. Lipid content (%) of mussels from the groups indicated. The figure shows median and min./max.

Table 2. Body burden (ng/g wet wt.) of naphthalene, dibenzothiophene, their alkylated homologues (of which some components are specified) and 16 EPA PAHs in blue mussels from the different experimental groups (**a.**; Results are given as median and min./max), and analytical detection limits (**b.**).

a.

	Zero-time			Ref. south			500 m			1000 m			2500 m			10 000 m		
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
Total dry matter (g/kg)	**175**	119	178	**159**	152	188	**175**	174	180	**182**	167	193	**121**	94.2	121	**168**	161	181
Lipid (%)	**1.5**	1.5	1.8	**1.1**	0.91	1.4	**1.4**	1.3	1.4	**1.5**	1.4	1.7	**0.89**	0.58	0.93	**1.4**	1.1	1.5
Biphenyl	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	0.32	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	0.33
Naphthalene	**<1.5**	<1.5	<1.5	**<1.5**	<1.5	<1.5	**<1.5**	<1.5	<1.5	**<1.5**	<1.5	<1.5	**1.9**	<1.5	2.1	**<1.5**	<1.5	<1.5
C1-Naphthalenes	**<2**	<2	<2	**<2**	<2	<2	**<2**	<0.5	<2	**<0.5**	<0.5	0.68	**7**	5.3	7	**<2**	<2	<2
C2-Naphthalenes	**<2**	<2	<2	**<2**	<2	<2	**<2**	<2	2.4	**<2**	<2	<2	**8.9**	6.4	9	**<2**	<2	<2
C3-Naphthalenes	**<5**	<5	<5	**<5**	<5	<5	**14**	14	17	**9.5**	9.2	14	**23**	17	25	**8**	5.3	8.2
Acenaphthylene	**<0.2**	<0.2	0.23	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2
Acenaphthene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2
Fluorene	**0.33**	<0.2	0.57	**0.27**	<0.2	0.28	**0.2**	<0.2	0.31	**0.21**	<0.2	0.33	**0.51**	0.24	0.51	**0.21**	<0.2	0.3
Anthracene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2
Phenanthrene	**<0.5**	<0.5	<0.5	**<0.5**	<0.5	<0.5	**0.82**	0.68	0.84	**0.62**	0.6	0.93	**1.4**	0.99	1.4	**<0.5**	<0.5	0.66
C1-Phenanthrenes	**<2**	<2	<2	**<2**	<2	<2	**5**	4.7	6.3	**4.1**	3.7	4.2	**4.9**	3.6	5.1	**2.1**	<2	3.1
C2-Phenanthrenes	**<2**	<2	<2	**3.4**	2.8	3.8	**26**	25	29	**22**	21	23	**17**	14	20	**13**	11	20
C3-Phenanthrenes	**<2**	<2	<2	**2.2**	2.2	3	**19**	17	19	**15**	15	16	**11**	8.4	12	**11**	8.8	14
Dibenzotiophene	**<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	**<0.5**	<0.5	<0.5
C1-Dibenzotiophenes	**<2**	<2	<2	**<2**	<2	<2	**2.5**	2.4	2.6	**<2**	<2	<2	**2.2**	<2	2.7	**<2**	<2	<2
C2-Dibenzotiophenes	**<2**	<2	<2	**<2**	<2	2	**16**	16	17	**13**	12	13	**10**	7.2	11	**7.9**	7.6	9.5
C3-Dibenzotiophenes	**<2**	<2	<2	**<2**	<2	2.1	**20**	18	20	**16**	15	16	**11**	7.7	12	**9.8**	9.6	15
Fluoranthene	**0.33**	0.33	0.34	**0.35**	0.32	0.37	**0.69**	0.51	0.77	**0.61**	0.53	0.84	**<0.2**	<0.2	0.24	**0.56**	0.48	0.7
Pyrene	**0.32**	0.3	0.36	**0.34**	0.33	0.38	**0.64**	0.59	0.75	**0.72**	0.66	0.9	**<0.2**	<0.2	<0.2	**0.57**	0.48	0.9
Benzo(a)anthracene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2
Chrysene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	0.3	**0.34**	0.33	0.41	**0.45**	0.43	0.45	**0.3**	0.28	0.33	**0.31**	0.28	0.46
Benzo(b)fluoranthene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	0.24	**0.21**	<0.2	0.23	**0.21**	<0.2	0.22	**<0.2**	<0.2	0.28	**0.29**	<0.2	0.3
Benzo(j,k)fluoranthene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2
Benzo(e)pyrene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**0.5**	0.45	0.54	**0.34**	<0.2	0.49	**0.2**	<0.2	0.22	**0.39**	0.31	0.41
Benzo(a)pyrene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2
Indeno(1,2,3-cd)pyrene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2
Dibenz(a,c/a,h)anthracene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2
Benzo(ghi)perylene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2

Table 2 continued.

	Zero-time			Ref. south			500 m			1000 m			2500 m			10 000 m		
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
Specified NPDs																		
2-methyl-naphthalene	**<0.9**	<0.9	<0.9	**<0.9**	<0.9	<0.9	**<0.9**	<0.9	<0.9	**<0.9**	<0.9	<0.9	**6.3**	5.1	7.2	**<0.9**	<0.9	<0.9
1-methyl-naphthalene	**<0.5**	<0.5	<0.5	**<0.5**	<0.5	<0.5	**<0.5**	<0.5	<0.5	**<0.5**	<0.5	<0.5	**6**	4.2	6.1	**<0.5**	<0.5	<0.5
2,6-dimethyl-naphthalene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**0.22**	<0.2	0.22	**<0.2**	<0.2	0.25	**1**	0.67	1.2	**<0.2**	<0.2	<0.2
2,3,5-trimethyl-naphthalene	**<0.2**	<0.2	<0.2	**<0.2**	<0.2	<0.2	**0.49**	0.48	0.58	**0.32**	0.31	0.45	**0.73**	0.5	0.84	**0.26**	0.22	0.3
1-methyl-phenanthrene	**<0.2**	<0.2	<0.2	**0.28**	0.24	0.35	**1.5**	1.4	1.8	**1.1**	1.1	1.4	**1.4**	1.1	1.6	**0.57**	0.53	0.88

Table 2 continued.
b. Analytical detection limits

Compound	ng/g wet wt.
Biphenyl	0.2
Naphthalene	1.5
C1-Naphthalenes	2
C2-Naphthalenes	2
C3-Naphthalenes	5
Acenaphthylene	0.2
Acenaphthene	0.2
Fluorene	0.2
Anthracene	0.2
Phenanthrene	0.5
C1-Phenanthrenes	2
C2-Phenanthrenes	2
C3-Phenanthrenes	2
Dibenzotiophene	0.5
C1-Dibenzotiophenes	2
C2-Dibenzotiophenes	2
C3-Dibenzotiophenes	2
Fluoranthene	0.2
Pyrene	0.2
Benzo(a)anthracene	0.2
Chrysene	0.2
Benzo(b)fluoranthene	0.2
Benzo(j,k)fluoranthene	0.2
Benzo(e)pyrene	0.2
Benzo(a)pyrene	0.2
Indeno(1,2,3-cd)pyrene	0.2
Dibenz(a,c/a,h)anthracene	0.2
Benzo(ghi)perylene	0.2

Compound	ng/g wet wt.
2-methyl-naphthalene	0.9
1-methyl-naphthalene	0.5
2,6-dimethyl-naphthalene	0.2
2,3,5-trimethyl-naphthalene	0.2
1-methyl-phenanthrene	0.2

Note that the accuracy of values <0.5 is lower than for values >0.5.

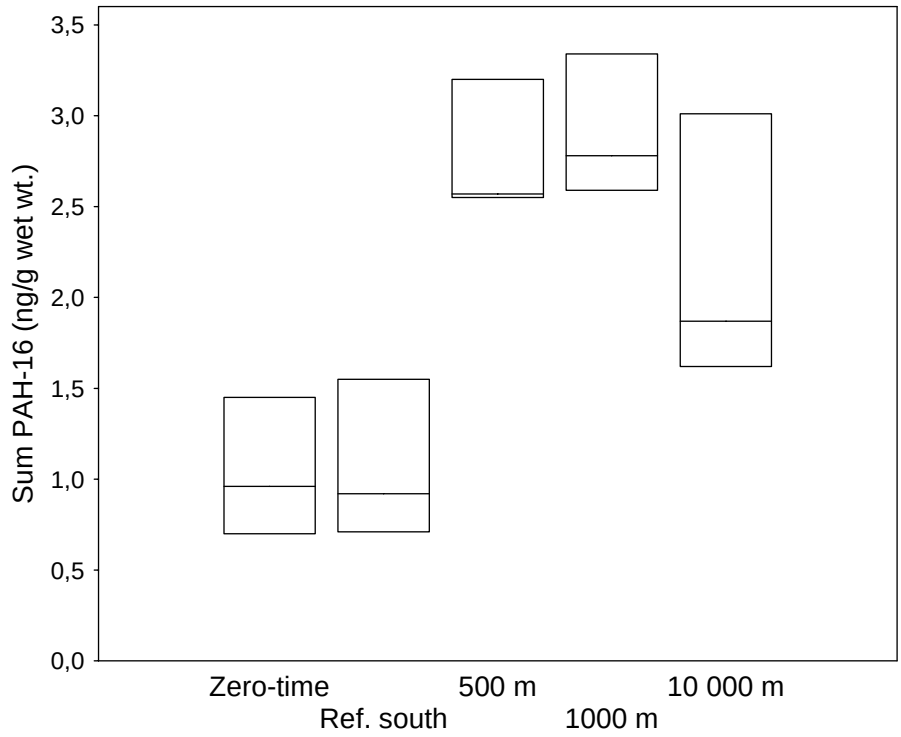


Figure 4. Body burden of Sum-PAH (EPA 16; ng/g wet wt.) in mussels from the groups indicated. The figure shows median and min./max.

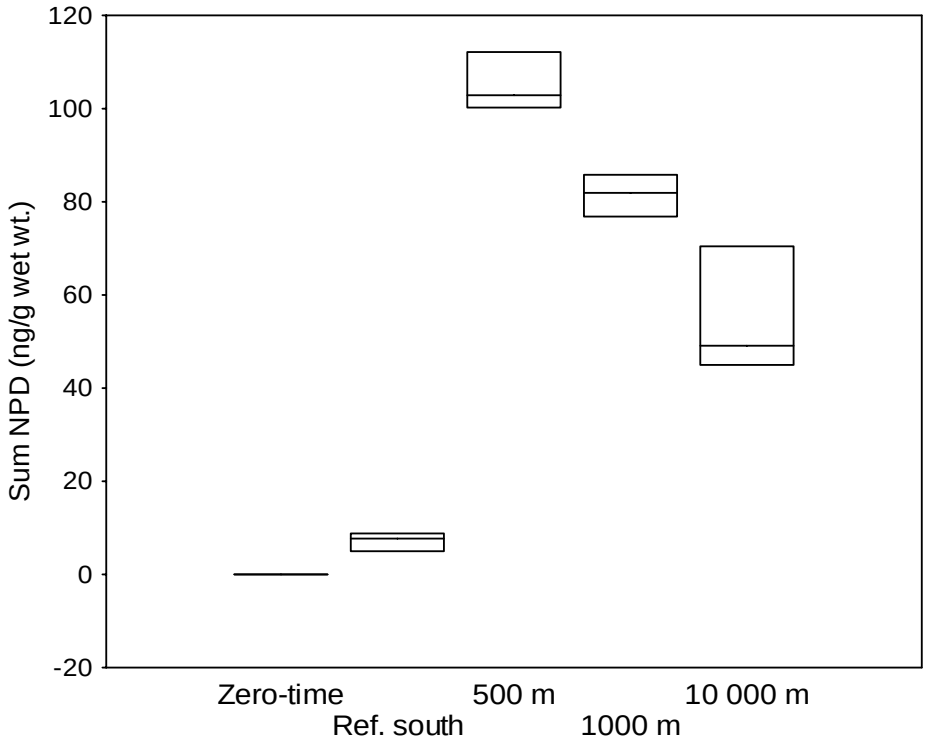


Figure 5. Body burden of Sum-NPDs (ng/g wet wt.) in mussels from the groups indicated. The figure shows median and min./max.

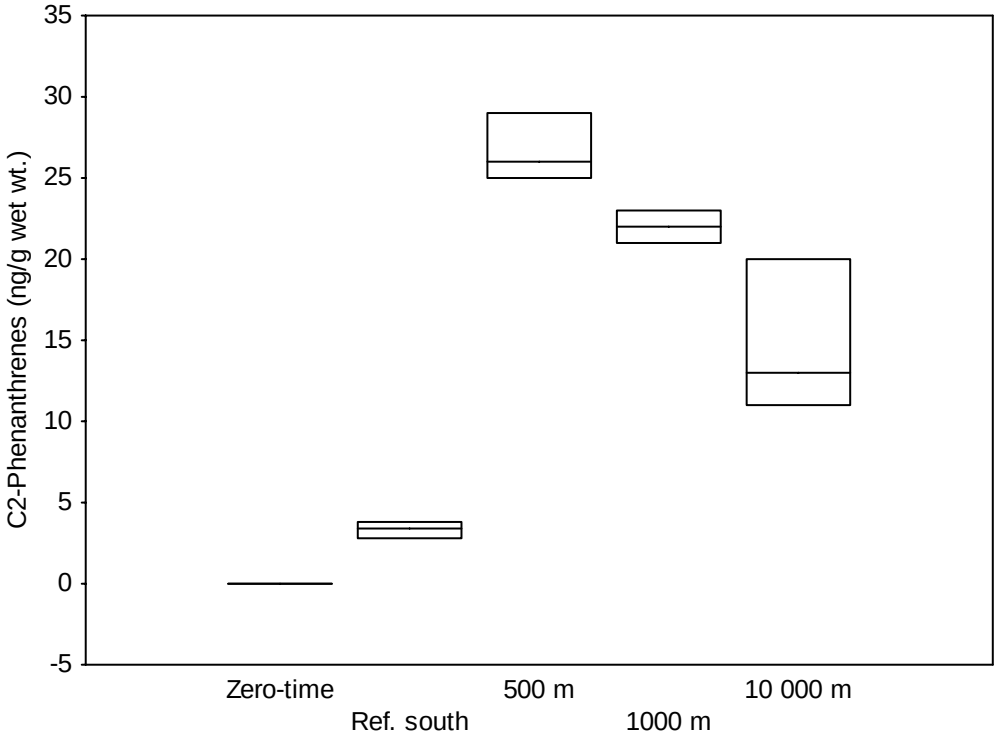


Figure 6. Body burden of C2-phenanthrenes (ng/g wet wt.) in mussels from the groups indicated. The figure shows median and min./max.

3.2 Cod - length, weight, condition and sex

The body length and weight distribution in the different groups are shown in **Figure 7** and **Figure 8**. The difference in mean values between groups is relatively small. Fish from the 2500 metres group is somewhat smaller than the other field groups; as was noted earlier, these fish were sampled at a different time point and possibly received different treatment. The 2500 m group will consequently be omitted in all statistical analyses in the following.

Not suprisingly, the experimental cod has lost some weight during the exposure period, but there were no differences between groups. Differences in (log-transformed) condition index ([Weight (g)/Length $(cm)^3$]×100) were found between the experimental groups (one-way ANOVA; $p<0.000001$). Tukey HSD test showed that the condition of the fish at zero-time was higher than in all other groups ($p<0.003$; Figure 5).The ratio between males and females may be characterised as normal although there is a surprising overrepresentation of males (**Figure 10**). This would obviously have no bearing on whether those individuals were exposure to produced water effluent or caging.

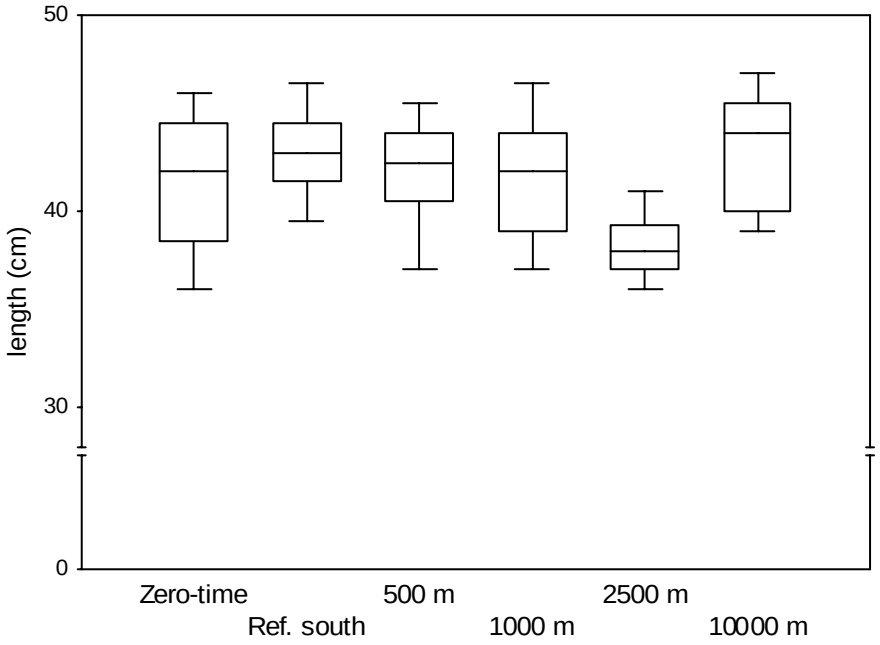


Figure 7. Length (cm) of cod in the different groups. Note axis break. The figure shows median, quartiles and 10/90-percentiles.

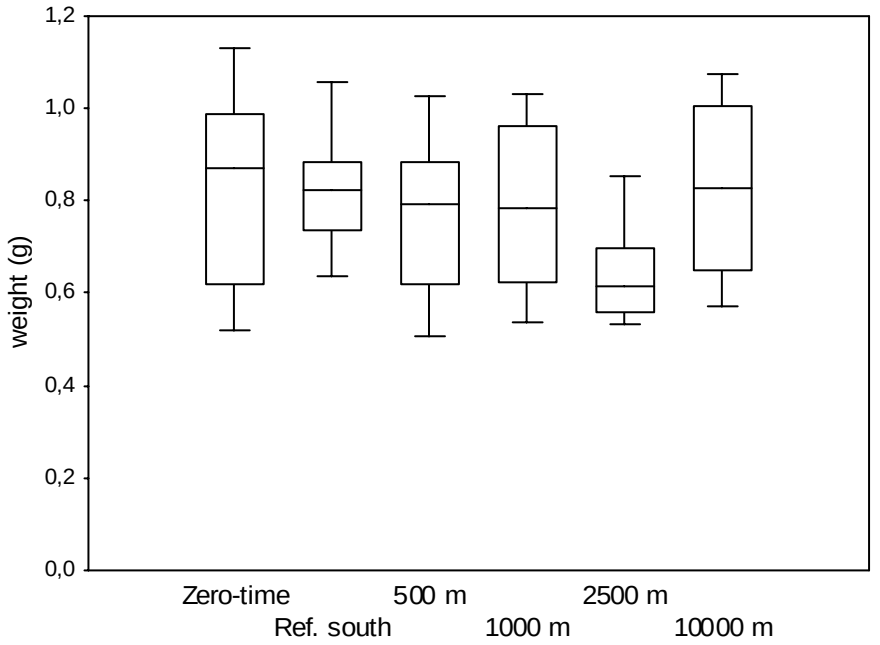


Figure 8. Weight (kg) of cod in the different groups. The figure shows median, quartiles and 10/90-percentiles.

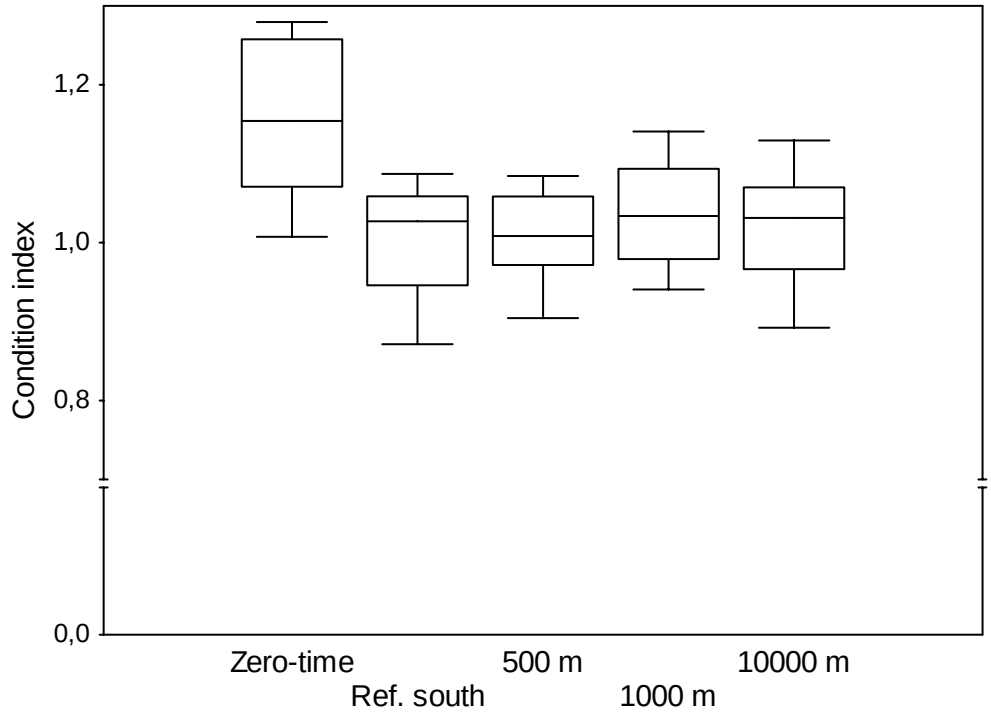


Figure 9. Condition of cod in the indicated groups. Note axis break. The figure shows median, quartiles and 10/90-percentiles.

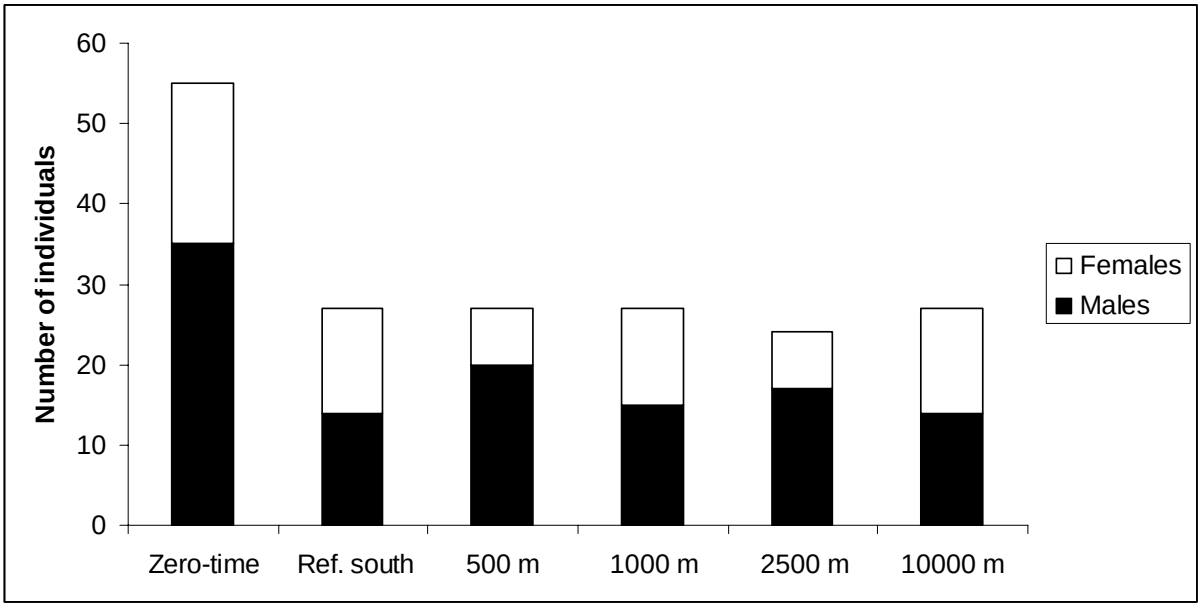


Figure 10. Sex ratios of cod in the groups indicated.

3.3 Cod - PAH-metabolites in bile

3.3.1 Fixed fluorescence

Fluorecence levels in bile from field exposed fish represent background levels. The most striking result is the relatively high fluorescence levels found in the pre-exposure group. Presence of 5 ring structures suggests PAHs from combustion processes (not typical petrogenic) and possible sources could be local ship traffic or, more probable, emissions from generators or outboard engines at the fish farm.

Significant differences in fixed fluorescence between the groups were found for all three wavelength pairs 290/334 nm (**Figure 11.**; one-way ANOVA on log-transformed data), 341/383 nm (**Figure 12.**; one-way ANOVA on log-transformed data) and 380/430 nm (**Figure 13.**; Kruskal-Wallis test).

The 290/334 nm fluorescence was higher in the zero-time group than all other groups (which were not significantly different; Tukey HSD; $p<0.0004$). The 341/383 nm fluorescence was higher in the zero-time group, than all other groups (Tukey HSD; $p<0.00002$), and lower at the reference station (Ref. south), than in all other groups (Tukey HSD; $p<0.002$). No difference in the 341/383 nm fluorescences were found between the 500 m, 1000 m and 10 000 m stations. The 380/430 fluorescence at zero-time was significantly higher than in all other groups (Multiple comparisons; $p<0.03$), and significantly lower at 1000 m than both at zero-time (Multiple comparisons; $p<0.000001$) and at the reference station (Ref. south; Multiple comparisons; $p<0.03$).

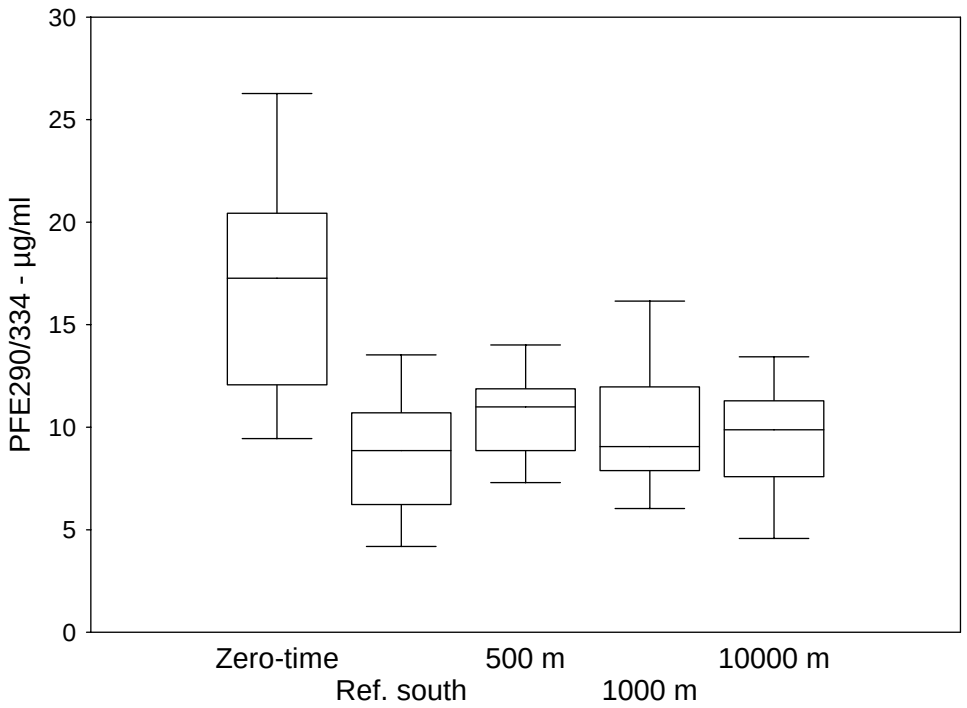


Figure 11. Fixed wavelength (290/334 nm) fluorescence levels in bile from cod in the groups indicated, expressed as pyrene fluorescence equivalents, PFE µg/g . The wavelength pair 290/334 nm identifies 2-3 ring structures. The figure shows median, quartiles and 10/90-percentiles.

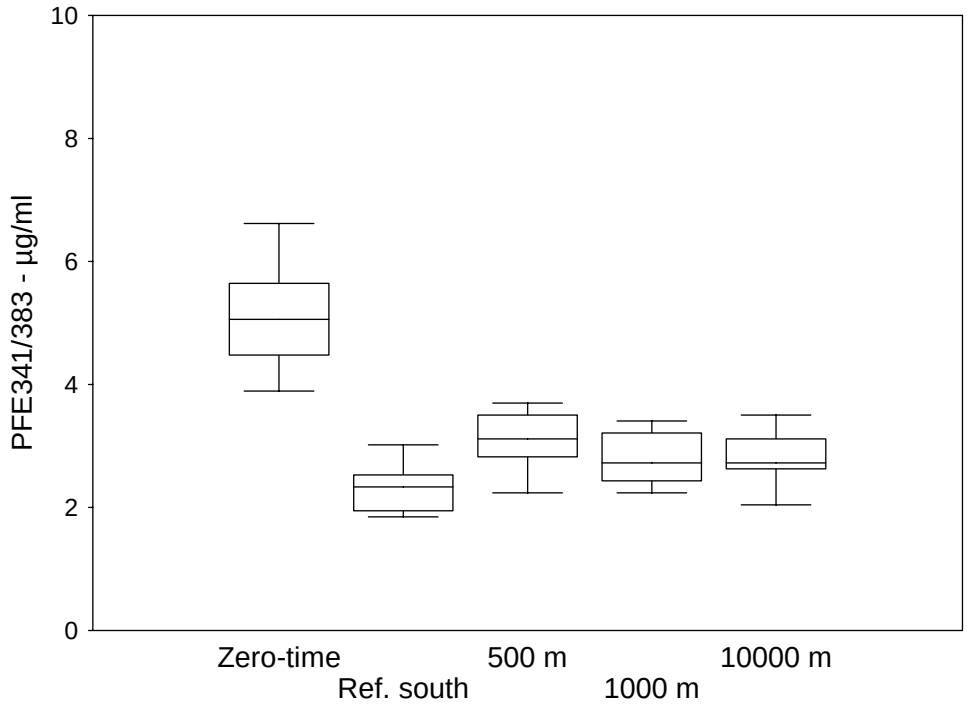


Figure 12. Fixed wavelength (341/383 nm) fluorescence levels in bile from cod in the groups indicated, expressed as pyrene fluorescence equivalents, PFE µg/g . The wavelength pair 341/383 nm identifies 4 ring structures. The figure shows median, quartiles and 10/90-percentiles.

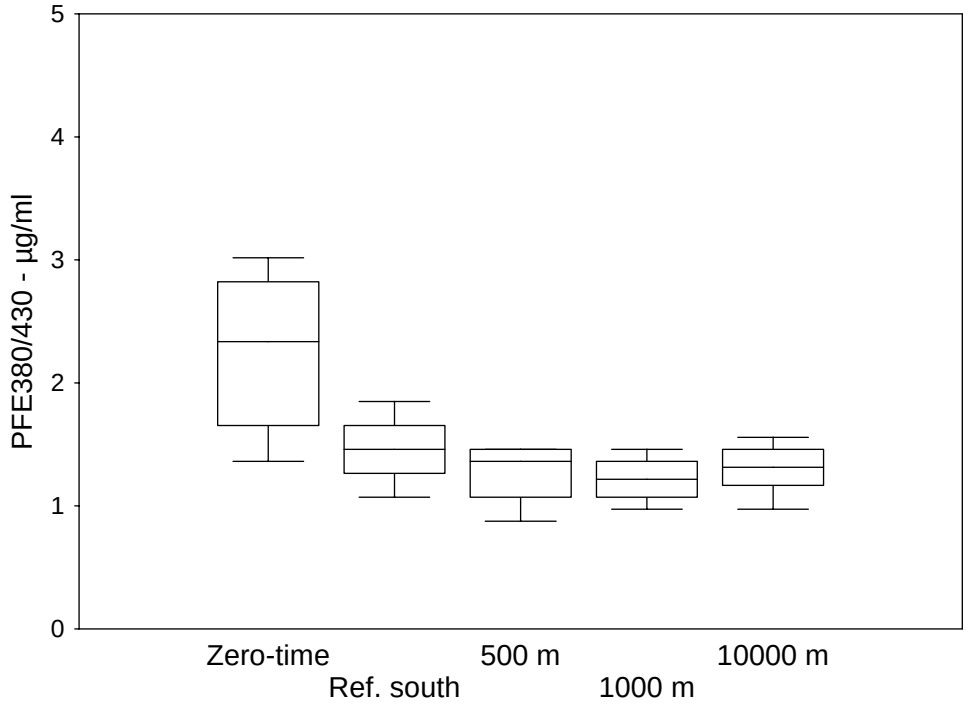


Figure 13. Fixed wavelength (380/430 nm) fluorescence levels in bile from cod in the groups indicated, expressed as pyrene fluorescence equivalents, PFE µg/g . The wavelength pair 380/430 nm identifies 5 ring structures. The figure shows median, quartiles and 10/90-percentiles.

3.3.2 GC/MS

There were only minor differences between cod caged at different locations (**Figure 14**). There were, however, obvious differences between 0-time cod and caged cod. 0-time cod had higher levels of many components, but lower levels of obvious produced water components.

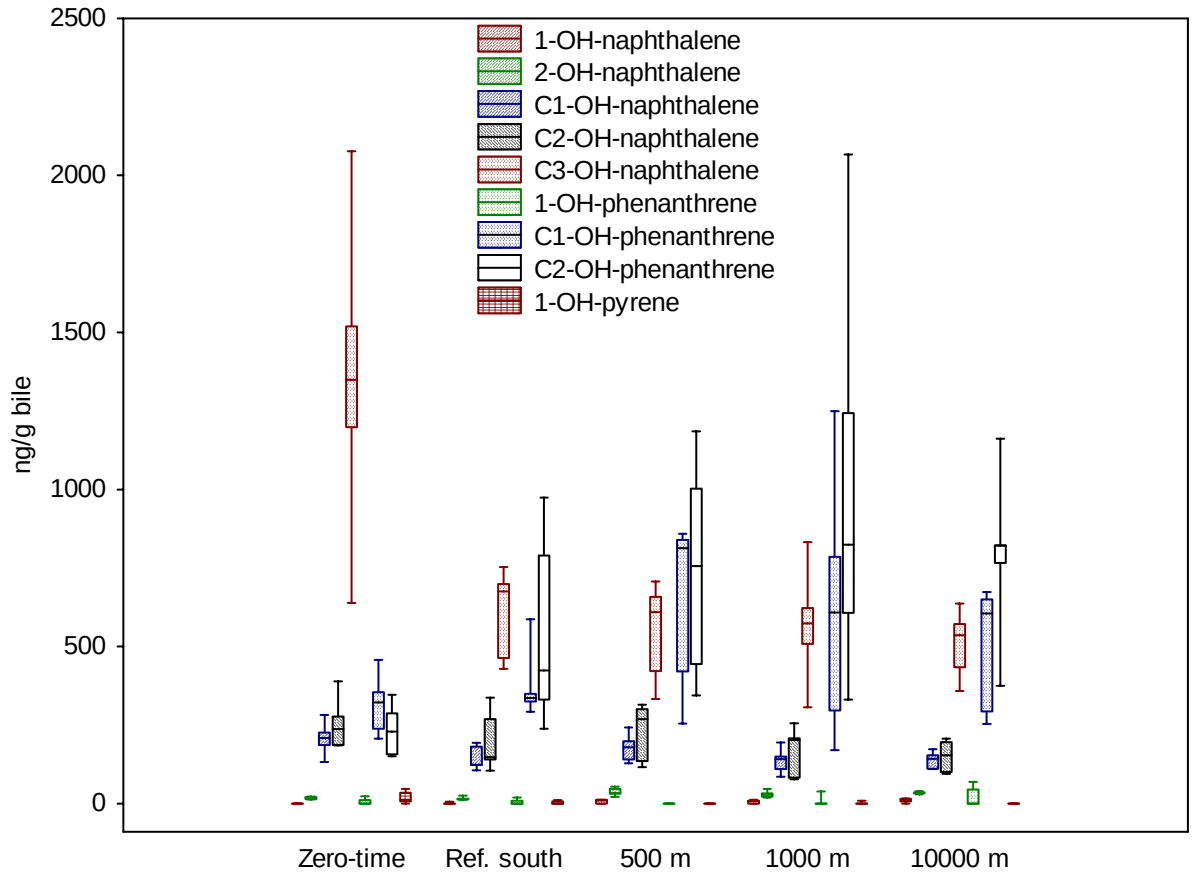


Figure 14. Concentrations (ng/g bile) of PAH-metabolites in caged cod from the groups indicated. The figure shows median, quartiles and 10/90 percentiles of five individuals from each group.

Concentrations of 2-OH-naphthalene were significantly lower at Ref. south than at the 500 m and 10 000 m locations ($p<0.05$; Kruskal-Wallis followed by multiple comparisons). Concentrations of C3-OH-naphthalene were significantly higher in the zero-time group, than in all other groups ($p<0.003$; one-way ANOVA, followed by Tukey HSD). Concentrations of C2-OH-phenenthrene were significantly lower in the zero-time group, than at the 1000 m location ($p<0.04$; Kruskal-Wallis followed by multiple comparisons).

3.4 Cod - EROD

3.4.1 Liver

No differences were found between groups in a two-way ANOVA on hepatic EROD activity (log-transformed) with sex and location as predictors (**Figure 15**). The variability in female cod in some of the cages was large, possibly reflecting a different endocrine status for those individuals.

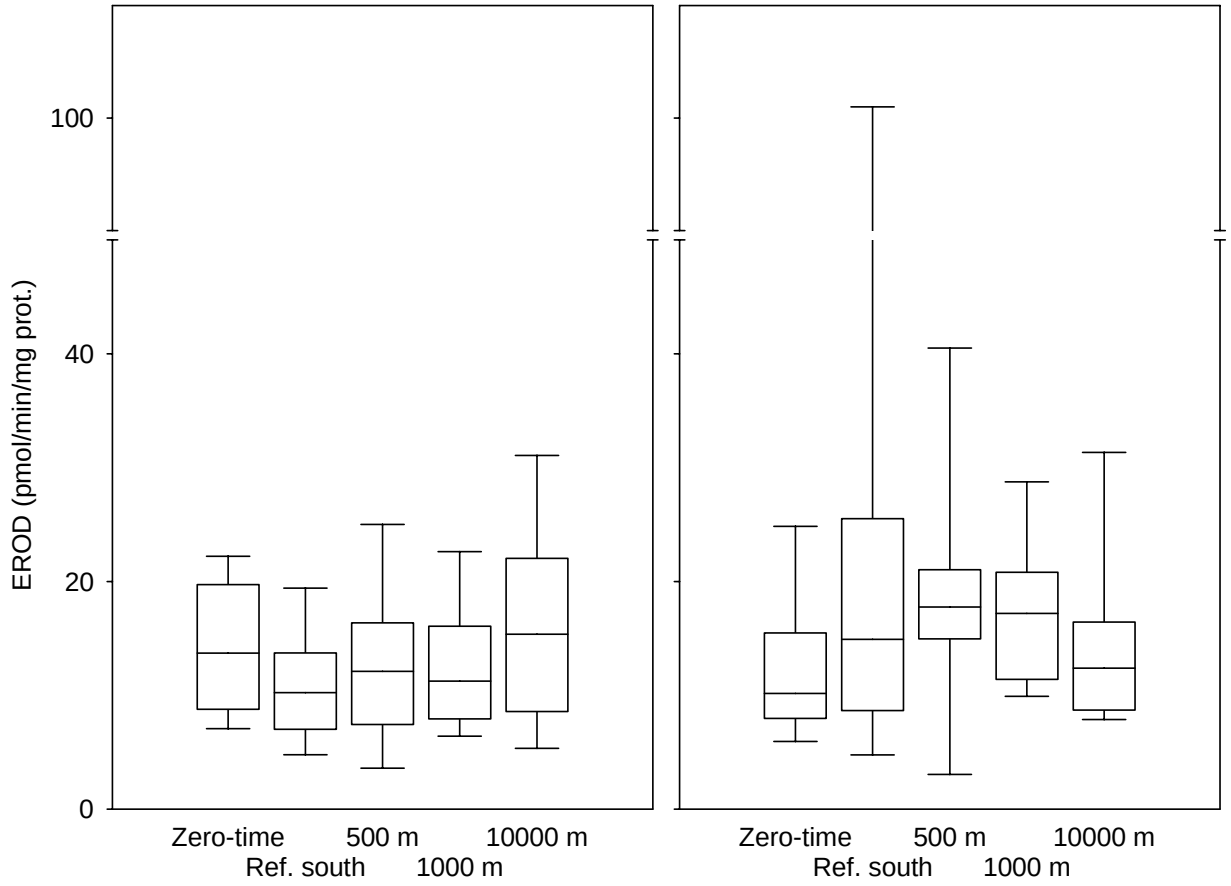


Figure 15. Hepatic EROD activity (pmol/min./mg prot.) in cod from the indicated groups. Note axis break. The figure shows median, quartiles and 10/90-percentiles. Males – left panel; females – right panel.

3.4.2 Gills

Significant differences were found in gill EROD activity in caged cod between the experimental groups (one-way ANOVA; $p<0.03$). Tukey HSD test showed that the gill EROD activity at the 1000 m station was higher than at the 10000 m station ($p<0.04$) (Figure 8).

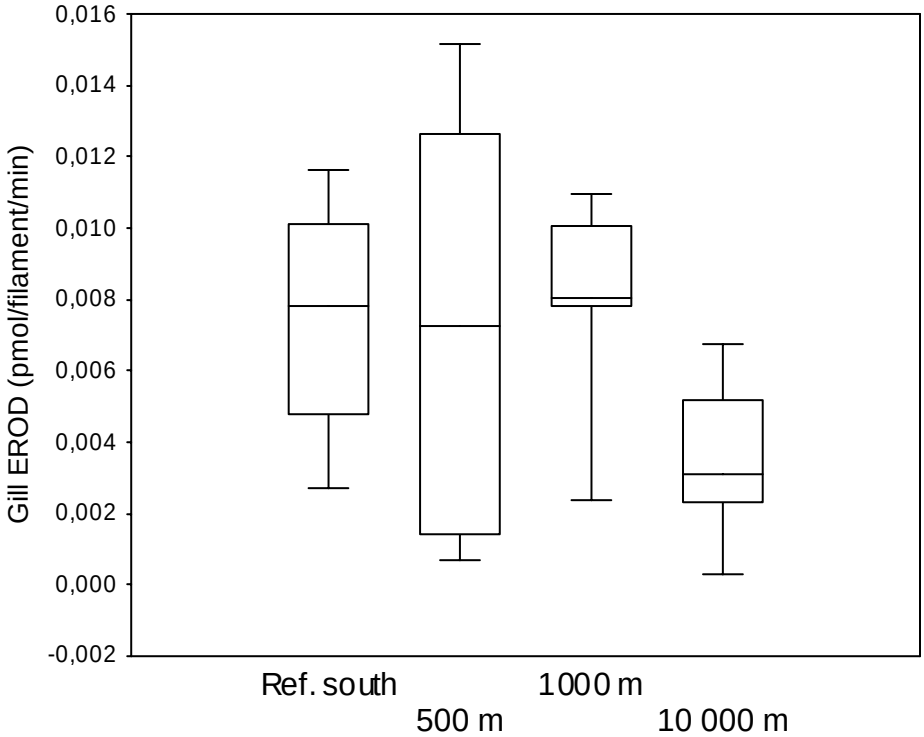


Figure 16. EROD activity (pmol/filament/min) in gills from cod from the groups indicated. The figure shows median, quartiles and 10/90-percentiles. Gill-EROD was an additional method to the original programme and zero-time samples were not retreived.

3.5 Cod – hepatic GST

Significant differences were found in hepatic GST activity in caged cod between the experimental groups (two-way ANOVA with experimental group and sex , as well as their interaction, as predictors; $p<0.001$). Tukey HSD test showed that the GST activity in males at the 1000 m station was lower than in the zero-time group (both sexes; $p<0.03$ and $p<0.008$ in males and females, respectively) (**Figure 17**).

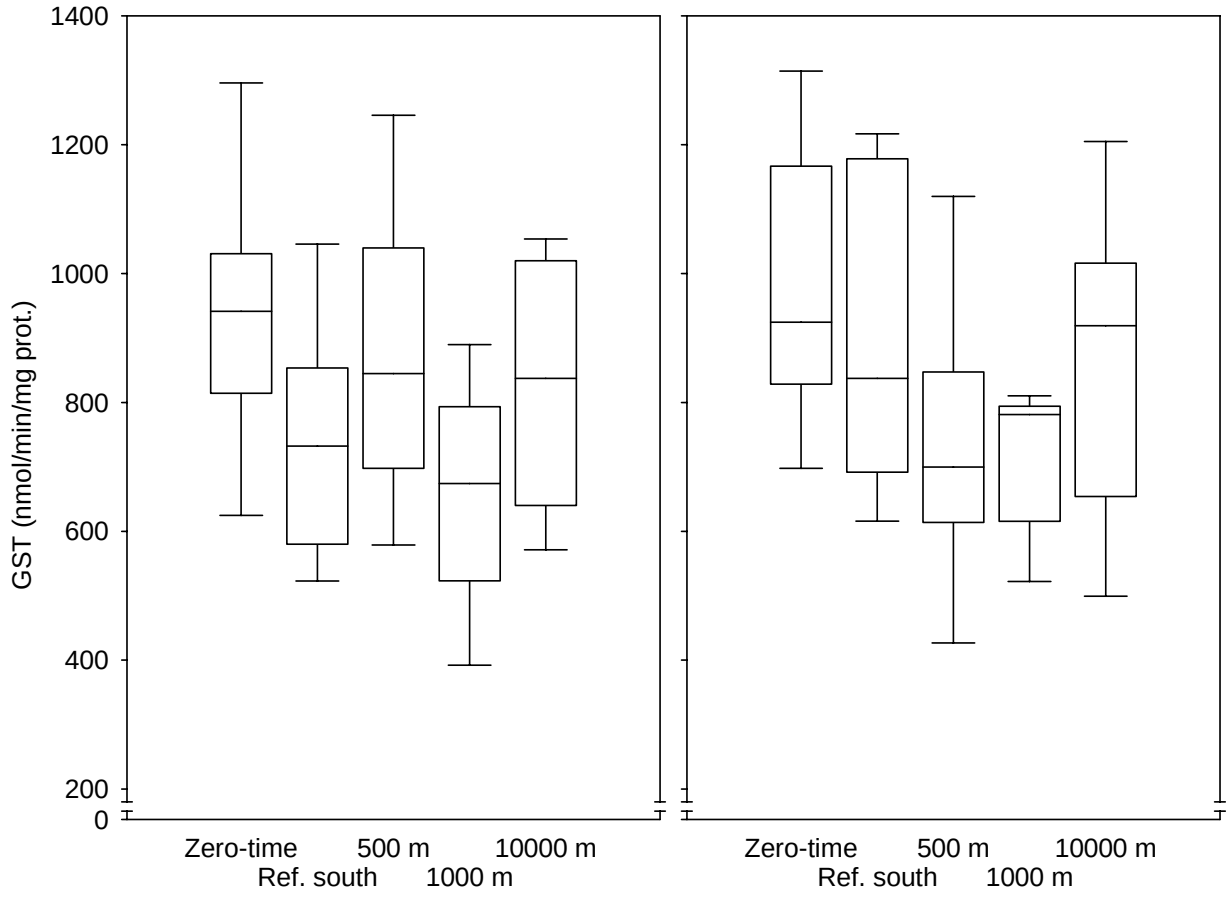


Figure 17. Hepatic Glutathione S-transferase, GST, (nmol/min/mg prot.) activity in cod from the indicated groups. Note axis break. The figure shows median, quartiles and 10/90-percentiles. Males – left panel; females – right panel.

3.6 Cod – vitellogenin

The ranges of plasma concentrations of vitellogenin were large for both male and female cod caged at the different locations (**Figure 18**). There were no significant differences between groups for any sex. Interestingly, the variability was by far largest for cod sampled prior to deployment (directly from the farm), possibly reflecting the complex mixture of substances found in commercial cod feeds, or exposure to endogenous estrogens from the females. Another interesting observation is the apparently depressed vitellogenin concentrations found in cod from the two locations closest to the platform.

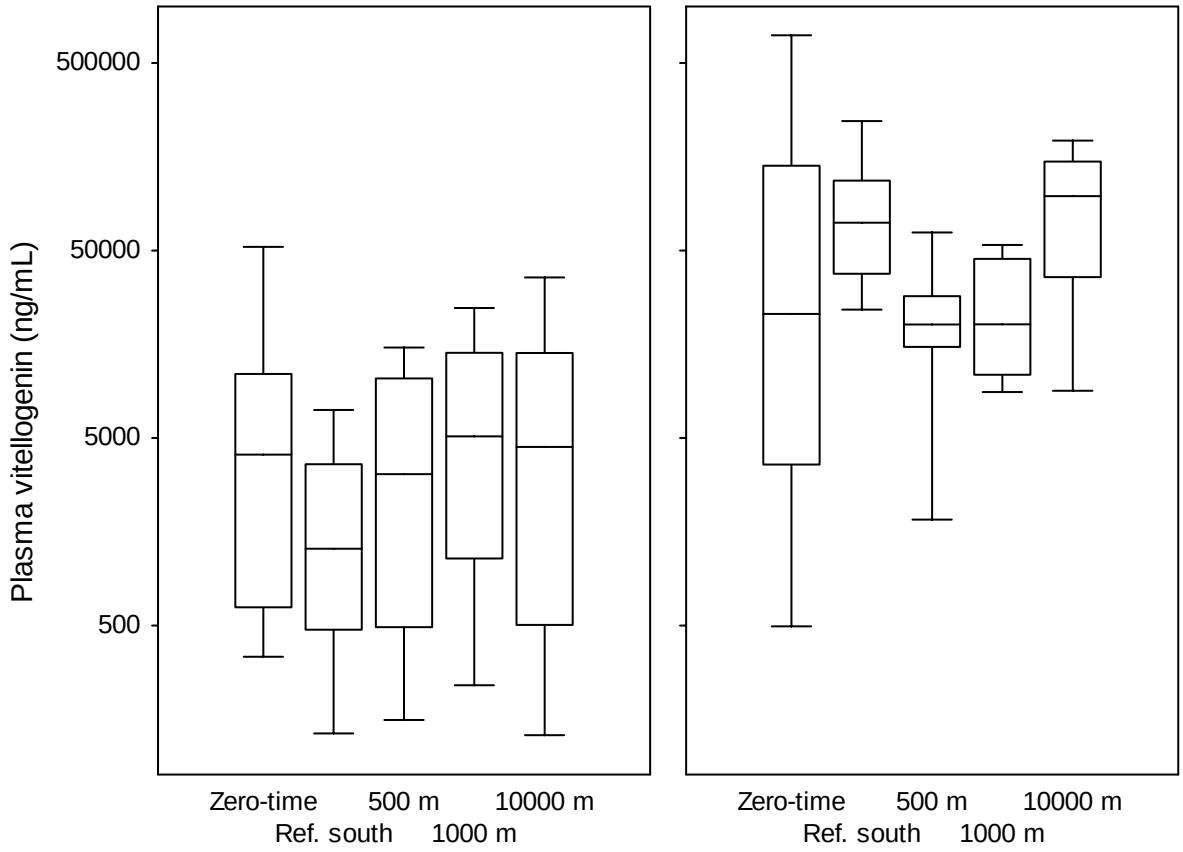


Figure 18. Plasma vitellogenin (ng/mL) in caged cod from the groups indicated. Note logarithmic scale. The figure shows median, quartiles and 10/90-percentiles. Males – left panel; females – right panel.

3.7 Cod - micronucleus formation

The lowest frequency of micronuclei (0.14 MN/1000 cells) was observed in cod caged in the largest distance (10000 meters) from the Statfjord B platform. In cod sampled prior to the caging experiment, the MN mean equals to 0.21 MN/1000 cells. A bit higher level of MN induction (0.27 MN/1000 cells) was found in fish caged at the reference site. The highest incidences of cells with MN were registered in cod from cages, which were immersed near the platform (**Table 3**).

Table 3. The frequency of micronuclei (MN/1000 liver cells) in Atlantic cod caged in Statfjord B platform zone

Exposure/MN	**Zero-time**	**Ref. south**	**500 m**	**1000 m**	**10000 m**
Number of cod	20	10	10	10	10
Number of cells	74000	30500	26500	36000	25500
Mean	**0.2100**	**0.2700**	**0.4900**	**0.3800**	**0.1444**
SD	0.2469	0.4165	0.5705	0.6494	0.2242
SE	0.0552	0.1317	0.1804	0.2054	0.0747
Max.	1.0	1.0	1.5	2.0	0.5

There were no significant differences in micronucleus formation in liver cells for cod caged at different distances from the Statfjord B platform (Kruskal-Wallis; **Figure 19**). Levels of micronuclei were close to baseline levels in cod from all groups, although cod kept at the 500-m location had higher median response than cod kept at other locations or sampled prior to deployment.

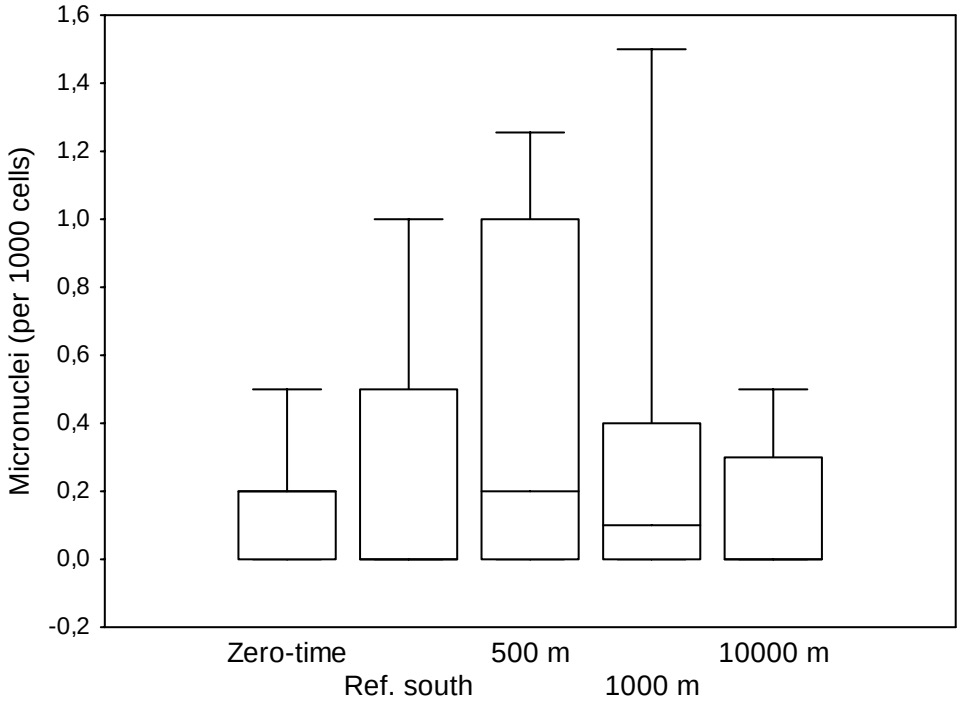


Figure 19. Frequency of micronuclei (MN/1000 cells) in cod liver cells from the indicated groups. The figure shows median, quartiles and 10/90-percentiles.

3.8 Blue mussels - benzo(a)pyrene hydroxylase activity

There was a significant difference between male and female mussels in the activity of benzo(a)pyrene hydroxylase in the hepatopancreas (two-way ANOVA, $p<0.02$), but no difference in activity between locations (**Figure 20**).

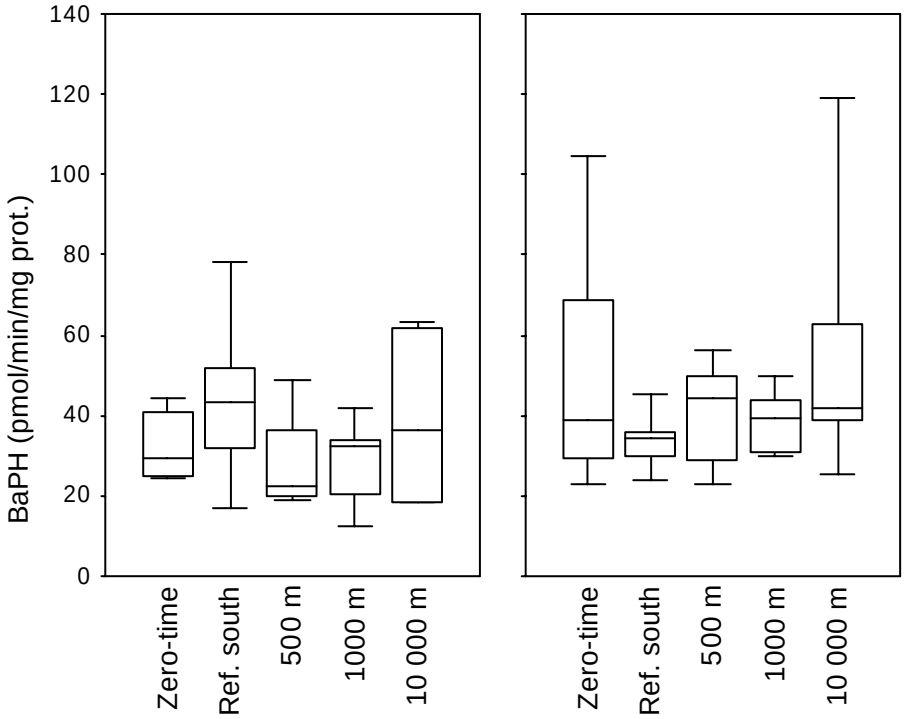


Figure 20. Activity of benzo(a)pyrene hydroxylase, BaPH, (pmol/min/mg prot) in mussels from the groups indicated. The figure shows median, quartiles and 10/90-percentiles. Males – left panel; females – right panel.

3.9 Blue mussels - lysosomal membrane stability

There were no differences in the lysosomal membrane stability between mussels sampled prior to exposure or mussels exposed at different locations (one-way ANOVA; **Figure 21**). The observed lysosomal responses are within the normal range of retention times usually observed for blue mussels in unexposed areas. It is not known whether transport to the onshore laboratory prior to analysis has influenced the results. The ideal would have been to analyse the NRRT directly on the vessel and for pre exposure group at the mussel-farm before the study. Another aspect is the salinity differences between the mussel-farm to the north-sea and to the lab. Mussels are known to be stressed by transport and the change in salinity that inevitably occurs when transferring them to an onshore lab.

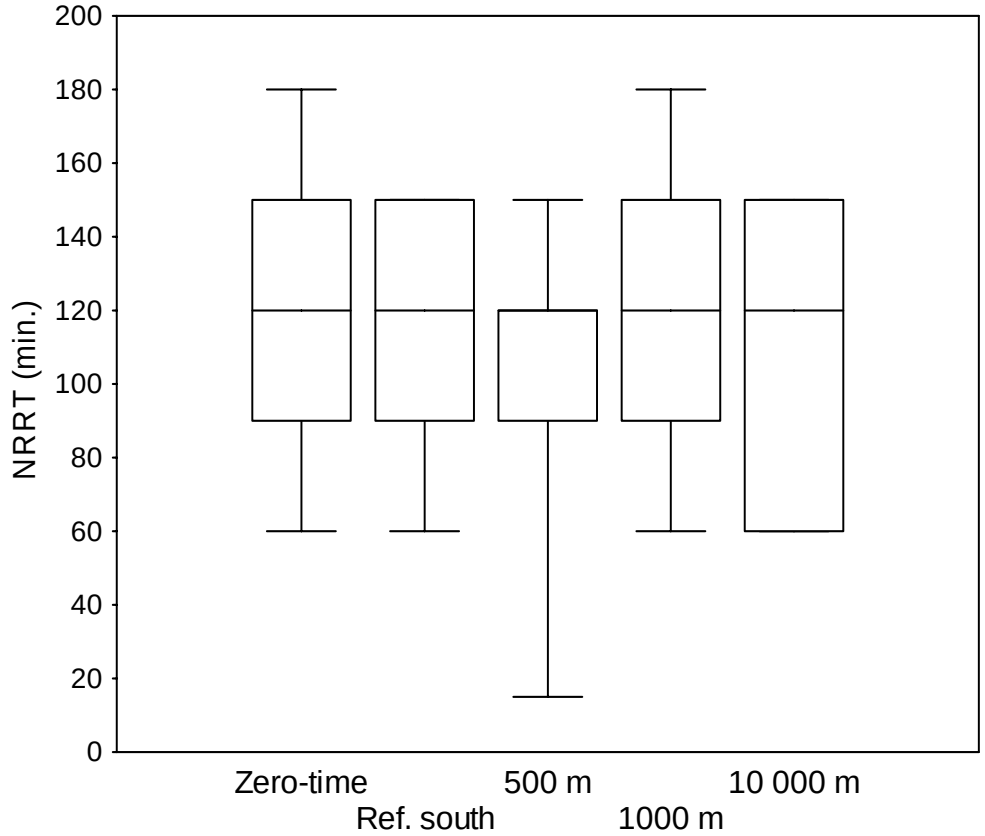


Figure 21. Labilisation period (given as Neutral Red Retention Time , NRRT; defined as the time from the addition of Neutral read to 50% of the cells are dead; min.) of lysosomal membrane in haemolymph cells from mussels from the different experimental groups. The figure shows median, quartiles and 10/90-percentiles.

3.10 Blue mussels - micronucleus formation

There were significant differences in micronucleus formation in haemocytes from mussels caged at different locations or sampled prior to deployment ($p<0.0002$ in a one-way ANOVA; **Table 4**, **Figure 22**). A follow-up Tukey HSD analysis indicated that micronucleus formation was significantly higher in mussels caged at the 500 m location compared to 0-time sampled mussels ($p<0.0002$) and mussels caged at the reference location ($p<0.05$) and at the 10 000 m location ($p<0.003$).

Table 4. Frequency of micronuclei (MN/1000 haemocytes) in mussels from the groups indicated.

Exposure/MN	**Zero-time**	**Ref. south**	**500 m**	**1000 m**	**10000 m**
Number of mussels	20	10	10	10	10
Mean	**2.13**	**4.00**	**7.66**	**4.80**	**2.58**
SD	2.125	3.238	3.782	3.216	2.330
SE	0.4752	1.024	1.196	1.017	0.7368
Max.	6.0	10.0	14.0	10.0	6.7

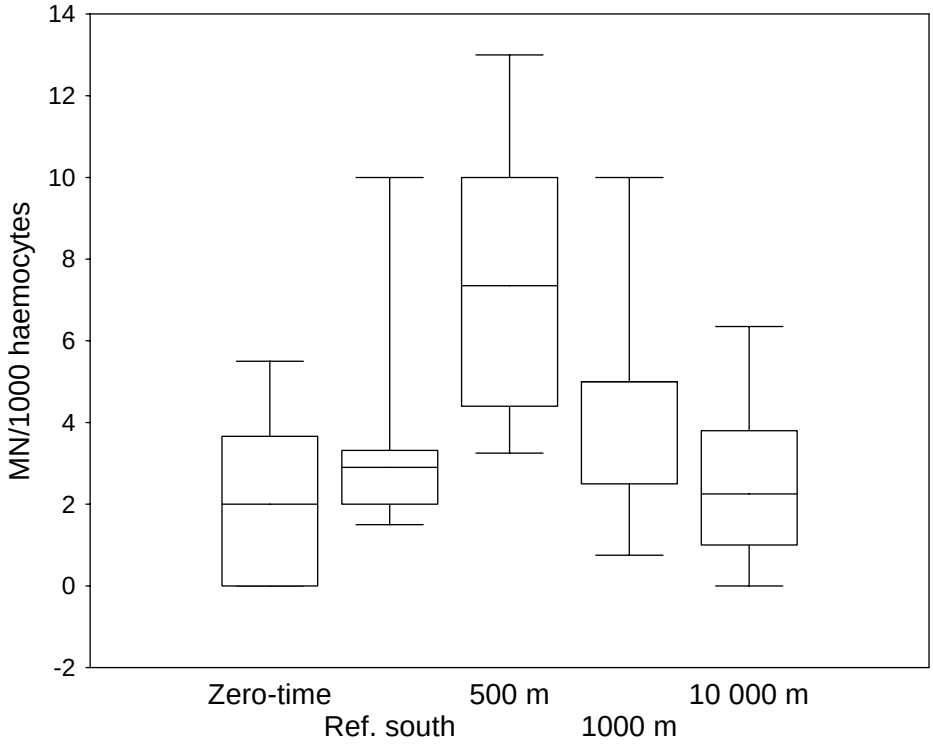


Figure 22. Frequency of micronuclei (MN/1000 haemocytes) in mussels from the groups indicated. The figure shows median, quartiles and 10/90-percentiles.

3.11 Blue mussels - histology

The Gonad Index was different in mussels caged at different locations (**Figure 23**). Mature gametes were observed in most mussels, both male and female. Female gonads at zero-time were at phases 6 and 1, whereas in caged mussels the dominant stages were 5 and 6 with the exception of mussels from 1000 m station where also phase 3 and 1 were identified. Accordingly, gonads in zero-time male mussels were in phases 5 and 6 and those from caged mussels were at 4 and 5 stages, although gonads at stage 3 were identified in mussels from the 500 m sampling station and gonads at the stage 6 in mussels from 1000 and 10000 m sampling stations. Most mussels presented a highly developed reserve connective tissue, with large numbers of adipogranular and vesicular cells.

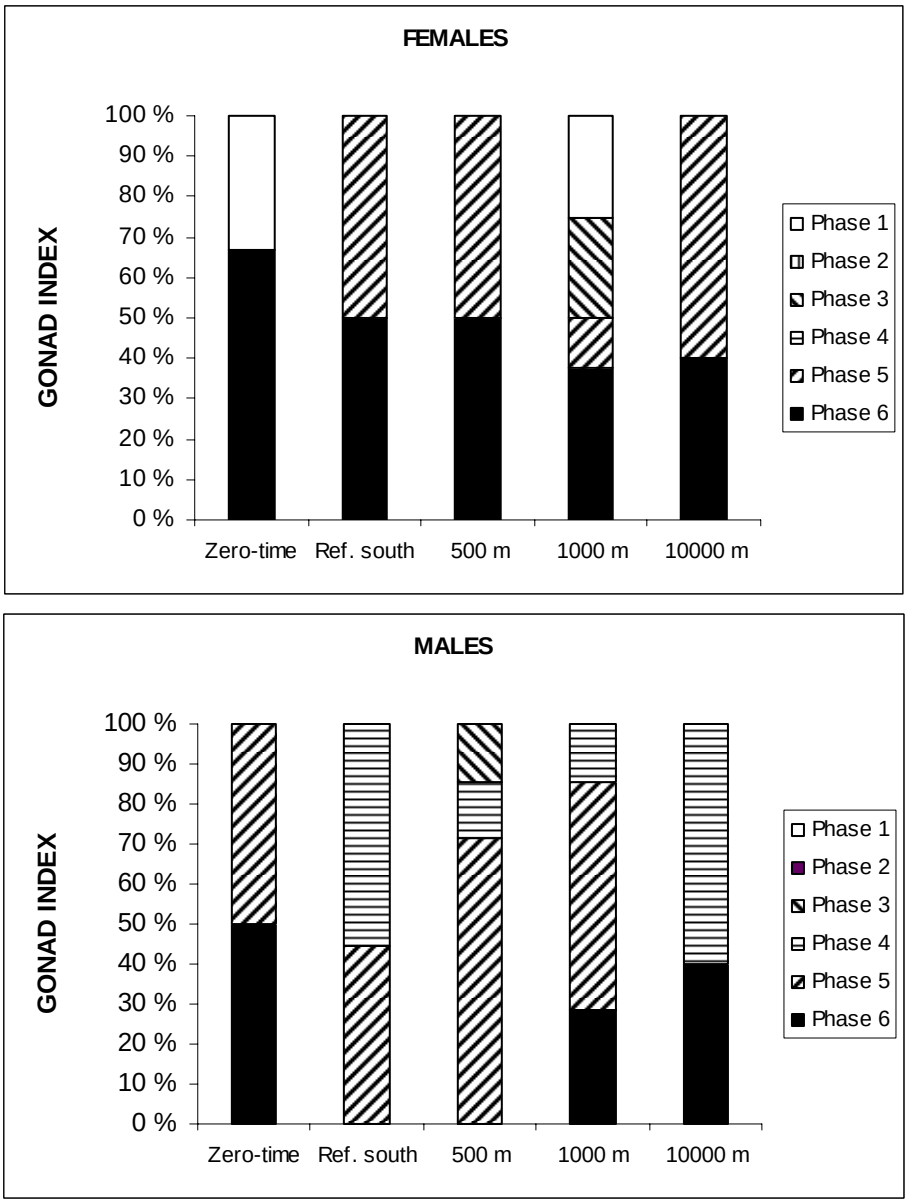


Figure 23. Gonad index recorded in female (top) and male (bottom) mussels from the groups indicated.

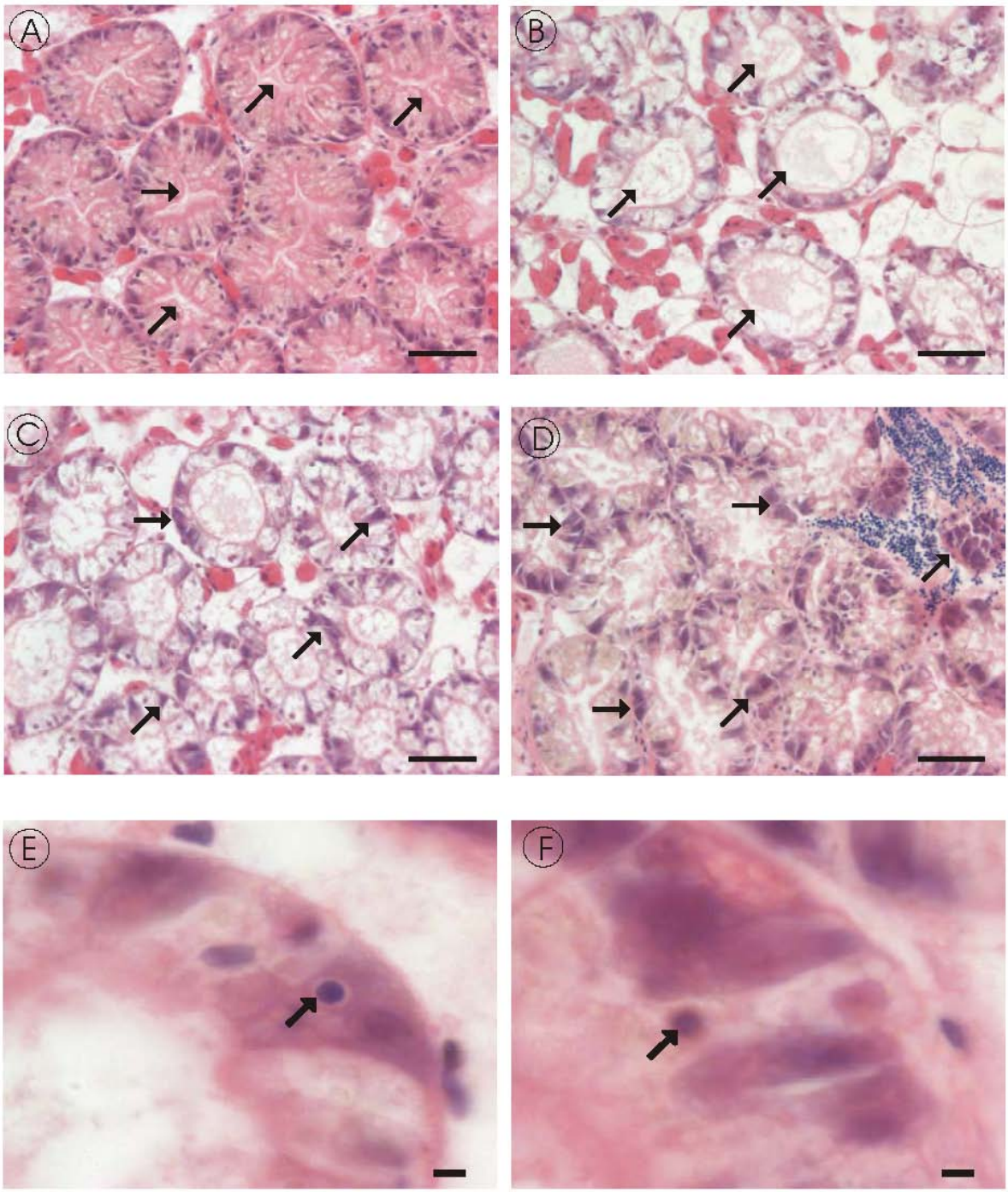


Figure 24. Haematoxylin-eosin stained paraffin-sections of mussel digestive gland from 10000 m (A), Zero-time (B), 1000 m (C), 500 m (D), 10000 m (E-F). Note (arrows) narrow lumen in (A) and wide lumen in (B) and (C). Also note (arrows) basophilic cells in (C) and (D). Note intracellular parasites in (E) and (F). Scale bars: (A-D): 100 µm; (E, F): 10 µm.

In previous laboratory experiments it has been demonstrated that in mussel digestive cell lysosomes BSD-extent increases after one day exposure to metals (Marigómez et al., 2002). Presently, BSD-extent found in digestive gland tissue was very low and therefore there was no need to quantify it by

image analysis. This low BSD-extent indicated a correspondingly low exposure to bioavailable metals. Bioavailability of metals was low at all sampling sites.

The relative proportion of basophilic cells increases under environmental stress conditions in molluscan digestive gland (Marigómez et al., 1998). Basophilic cells were conspicuous and apparently very abundant in all the studied mussels, although they were especially abundant in digestive gland of mussels collected at the 500 m sampling station (**Figure 24**). Stereological analysis showed that "0 Time" mussels and mussels from the reference location appeared to have lower Vv_{BAS} values than mussels collected 500 m away from the rig, but the differences were not statistically significant (one-way ANOVA; **Figure 25**). However, all the Vv_{BAS} values presently recorded were higher (nearly two-fold) in comparison with data from last year (WCM survey 2003), which indicates that all mussels were somewhat stressed.

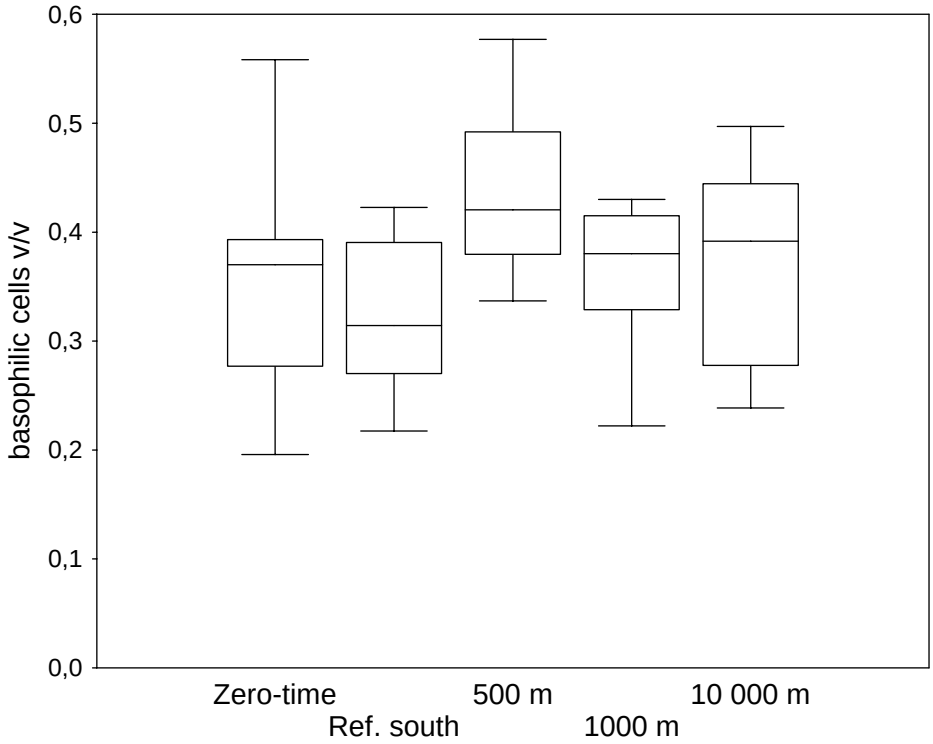


Figure 25. Volume density (v/v) of basophilic cells in digestive gland epithelium from mussels from the different experimental groups. The figure shows median, quartiles and 10/90-percentiles.

Mussels from 0-time exhibited a reduced mean epithelial thickness (MET) and increased mean luminal radius (MLR, **Figure 26**) when compared with results from the Water Column Monitoring 2003 and when compared with the rest of the stations. This is also illustrated in **Figure 24**, where the digestive tubules of mussels fro the zero-time showed a thinner epithelium and a wider lumen than mussels from other stations. Accordingly, the parameters MLR/MET (which relates tubule lumen width (MLR) to epithelial thickness) and MET/MDR (which relates epithelial thickness to mean diverticular radius) showed high and low values, respectively (**Figure 26**). These results suggest that zero-time mussels were subjected to some stress source (Vega et al., 1989), which most likely could be due to the reproductive cycle since these mussels were at a post-spawning stage (**Figure 23**).

Mussels sampled close to the platform exhibited lower MET and lower MDR values than mussels from other sampling stations along the expected pollution gradient. Accordingly, only MET values

corresponding to 10000 and 1000 sampling points were significantly higher than those recorded at 0-time (**Figure 26**).

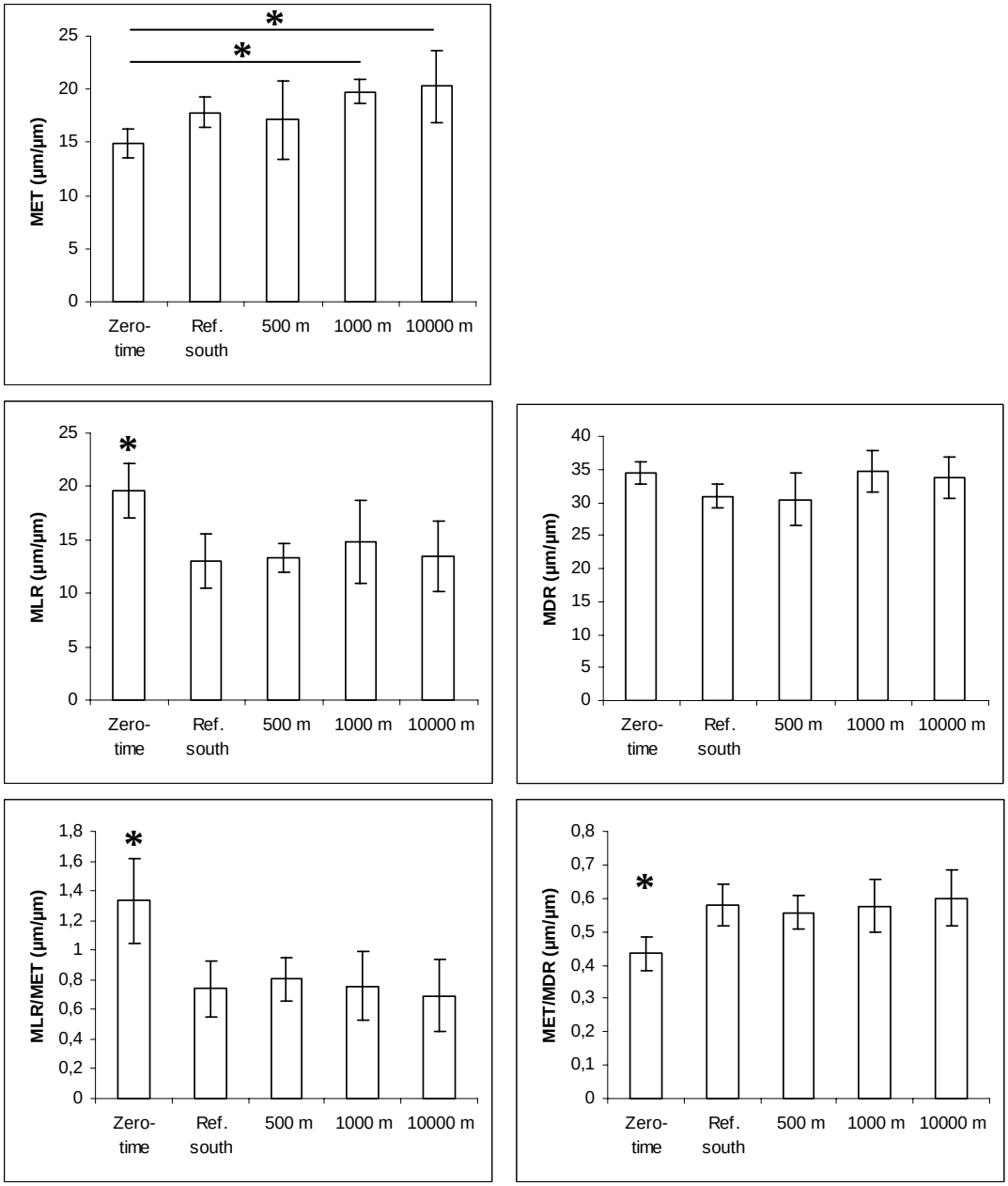


Figure 26. Planimetric parameters (mean epithelial thickness (MET), mean luminal radius (MLR), mean diverticular radius (MDR), MLR/MET, MET/MDR) indicative of the structure of digestive tubules in mussel digestive gland. Error bars indicate standard deviation. Asterisks indicate significant differences ($P<0.05$) between pairs of means according to de Duncan's test after one-way ANOVA. One single asterisk indicates that this group is different from all other groups. Asterisks above lines indicate differences between groups connected by the lines.

Prevalence of parasites, hemocyte infiltration and general condition of the digestive epithelium were examined. Overall, no significant parasitic infestation or pathological lesion was found in any case. A

weak incidence of intracellular parasites (**Figure 24 E-F**), were found in mussels collected at 10 000 (30%) and 1000 m (20%) downstream Statfjord B.

3.12 Saithe – PAH-metabolites

3.12.1 Fixed fluorescence

There were no significant differences between the three locations in the levels of bile metabolites in saithe deriving from exposure to 2- and 3-ring PAHs (the 290/334 nm wave length pair; one-way ANOVA; **Figure 27**).

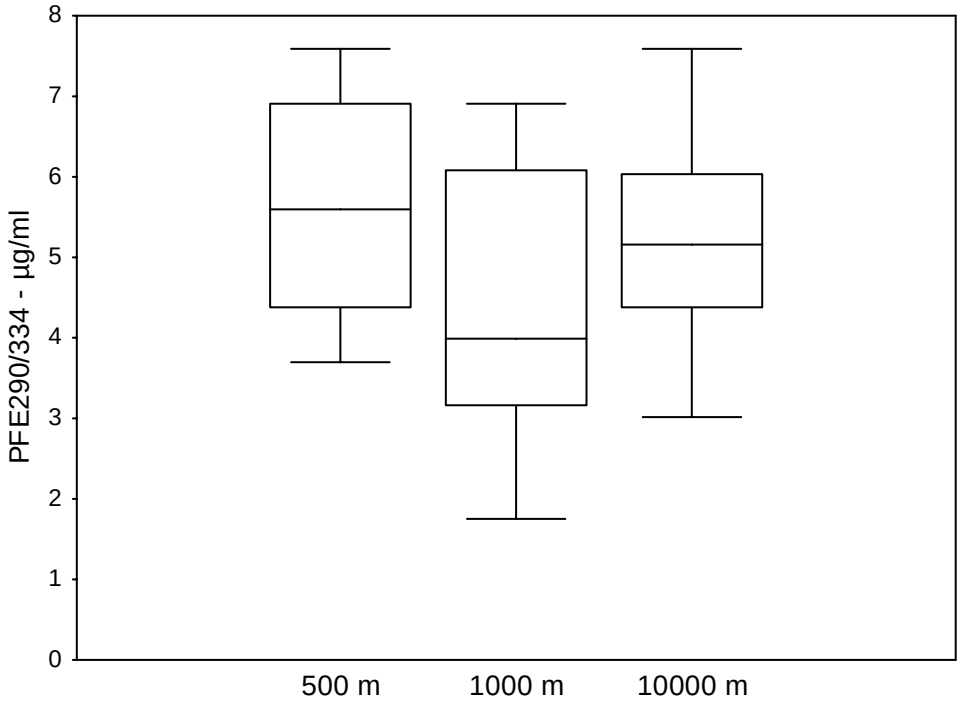


Figure 27. Fixed wavelength (290/334 nm) fluorescence levels in feral saithe caught 500 m, 1000 m and 10000 m away from the Statfjord B platform. The results are expressed as pyrene fluorescence equivalents, PFE µg/g. The wavelength pair 290/334 nm identifies 2-3 ring structures. The figure shows median, quartiles and 10/90-percentiles.

There were significant differences in the levels of 4-ring PAH metabolites (the 341/383 nm wave length pair) in saithe collected at the three locations (one-way ANOVA; $p<0.002$; **Figure 28**). The concentration of metabolites was lower in saithe collected in the vicinity of the 1000-m location, than at the 500-m location (Tukey HSD; $p<0.003$) and the 10 000-m location (Tukey HSD; $p<0.05$).

As for 2- and 3-ring metabolites there were no differences for 5-ring PAH metabolites (the 380/430 nm wave length pair) sampled from saithe collected at the three locations (one-way ANOVA; **Figure 29**).

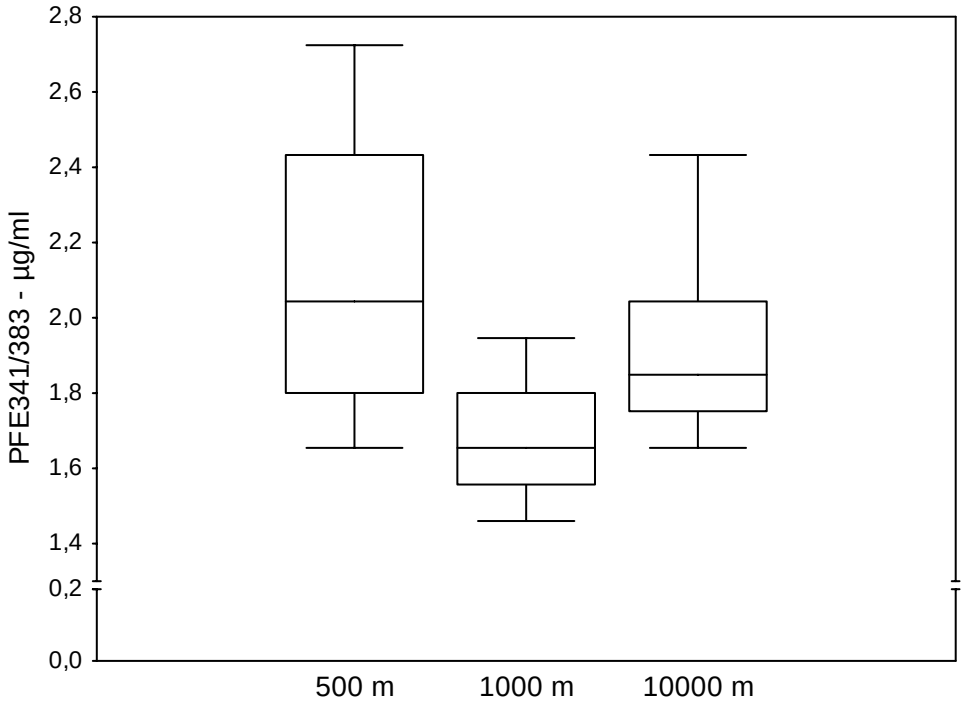


Figure 28. Fixed wavelength (341/383 nm) fluorescence levels in feral saithe caught 500 m, 1000 m and 10000 m away from the Statfjord B platform. The results are expressed as pyrene fluorescence equivalents, PFE µg/g. The wavelength pair 341/383 nm identifies 4 ring structures. The figure shows median, quartiles and 10/90-percentiles.

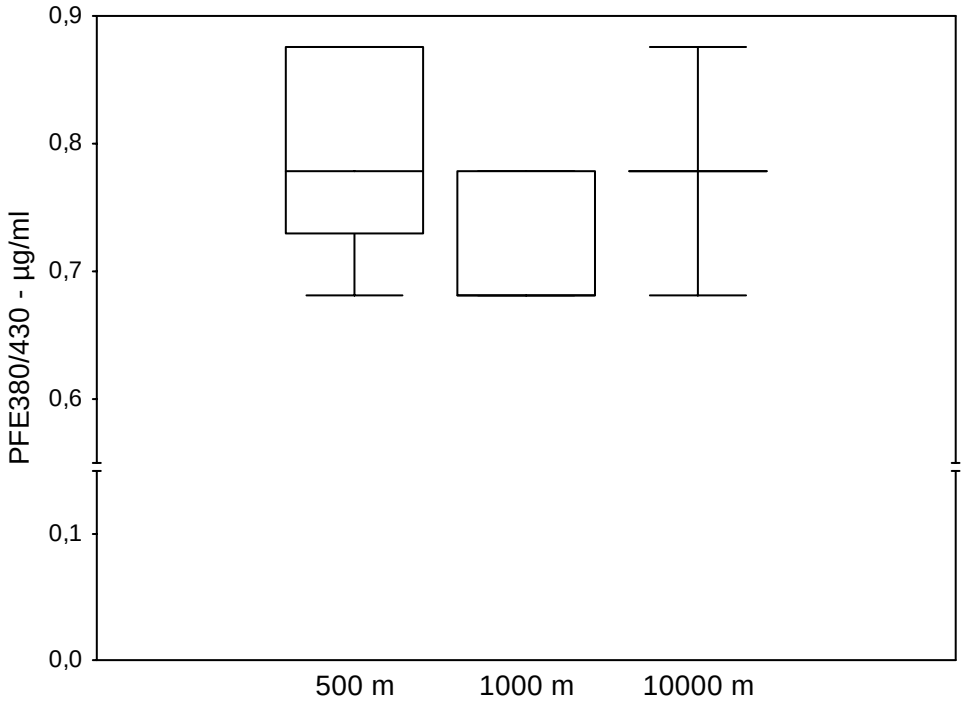


Figure 29. Fixed wavelength (380/430 nm) fluorescence levels in feral saithe caught 500 m, 1000 m and 10000 m away from the Statfjord B platform. The results are expressed as pyrene fluorescence equivalents, PFE µg/g. The wavelength pair 380/430 nm identifies 5 ring structures. The figure shows median, quartiles and 10/90-percentiles.

3.12.2 GC/MS

The C3-OH-naphthalene metabolites dominates the samples from feral saithe collected at three locations in the Statfjord region (**Figure 30**), but the concentrations were not significantly different between the three locations (one-way ANOVA). Only 2-OH-napthalene concentrations differed between locations (Kruskal-Wallis; $p<0.007$). Multiple comparisons showed that 2-OH-napthalene concentrations were higher at the 10 000-m location, than at the 500-m location ($p<0.05$).

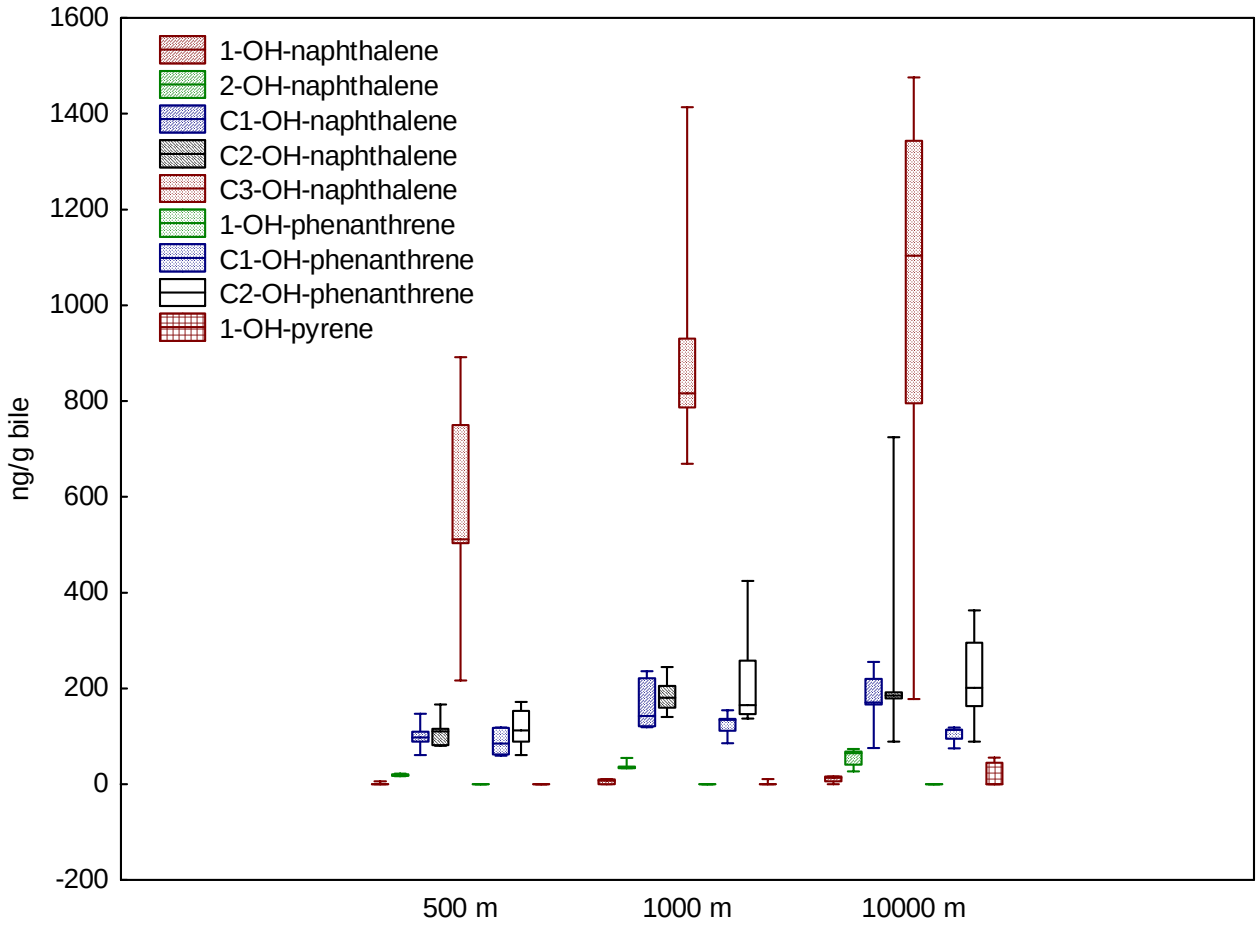


Figure 30. Concentrations (ng/g bile) of PAH-metabolites in feral saithe caught 500 m, 1000 m and 10 000 m downstream the Statfjord B platform. Results are shown as median, quartiles and 10/90 percentiles.

3.13 Saithe - histology

There were no clear differences between saithe collected at the three locations in the frequency of hepatic pathologies, although fish sampled closest to the platform had somewhat elevated incidence of inflammatory pathologies (**Table 5**).

Table 5. Summary of pathologies (% prevalence) detected in the livers of saithe captured at 500 m, 1,000 m and 10 000 m from the Statfjord B oil platform. Key: NP (no pathology detected), MMA (melanomacrophage aggregates) and VAC (mean vacuolation status of hepatocytes, based on a three point scale where 3 is highest).

Site	NP	Necrosis	MMA	Inflammation	Granuloma	Fibrosis	VAC
500 m	50	0	8.3	41.7	4.2	4.2	2.92
1000 m	68.9	0	3.4	31.0	6.9	0	2.93
10000 m	65.2	4.3	4.3	34.8	0	0	2.91

The saithe appeared generally healthy at all three locations with lipid-rich livers, although some lesions could be seen in some individuals (**Figure 31**).

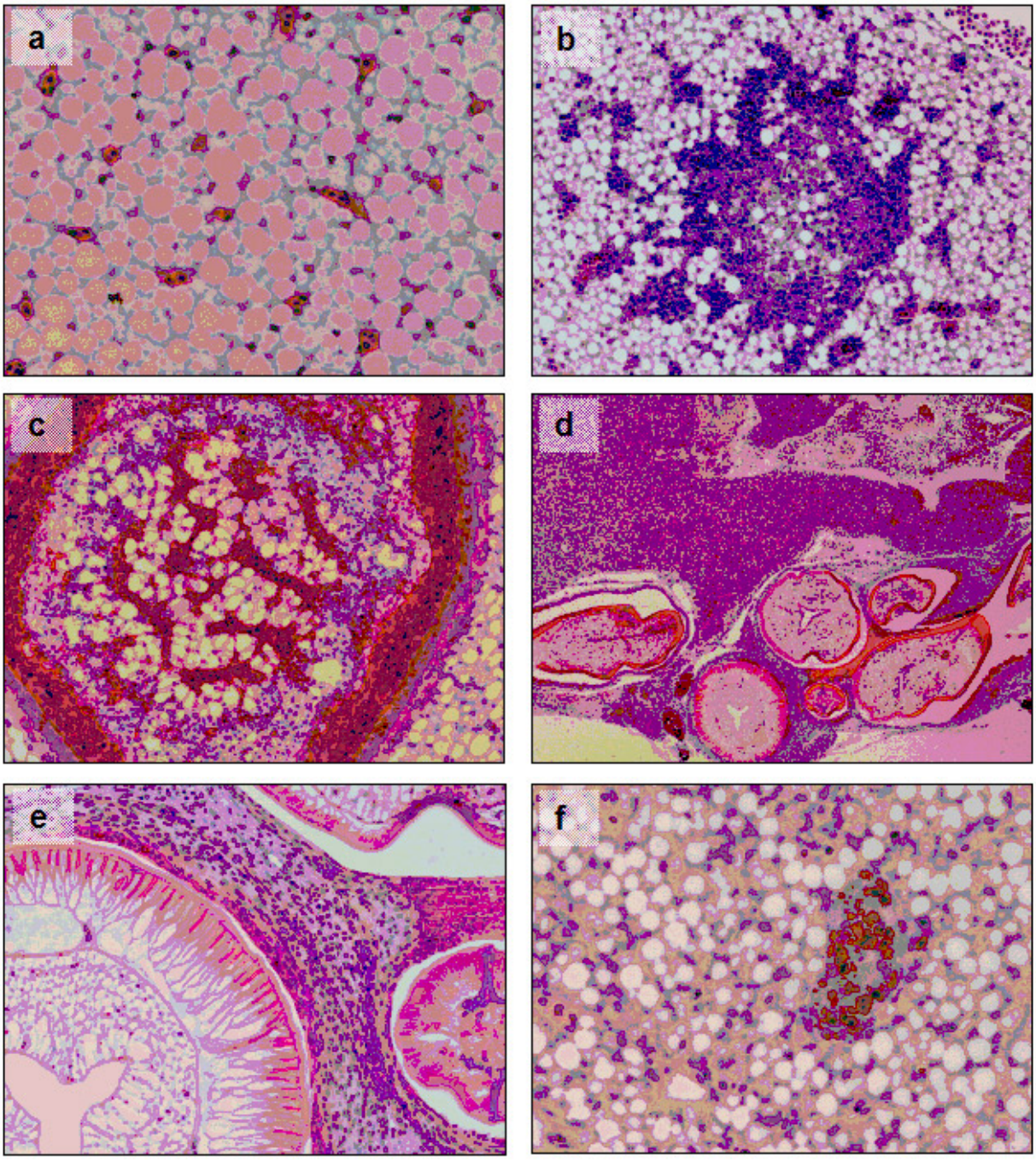


Figure 31. Liver histopathology of saithe captured approximately 500 m, 1,000 m and 10 000 m from the Statfjord B platform. (a) Normal liver showing pronounced fatty vacuolation. (b) Inflammatory focus with necrotic hepatocytes. (c) Granulomatous lesion, containing necrotic hepatocytes. (d). Anisakid nematodes on the surface of the liver. (e) Inflammatory response to nematodes on the surface of the liver. (f) Melanomacrophage aggregate (MMA) amongst normal fatty hepatocytes.

3.14 Saithe - DNA-adducts

There were no significant differences in hepatic DNA adducts in saithe collected at three locations in the Tampen area (**Figure 32**). Some individuals collected at all three locations had clearly elevated concentrations of hepatic DNA adducts indicating past exposure to genotoxic substances. Note that the results are preliminary and only reflect a limited sample.

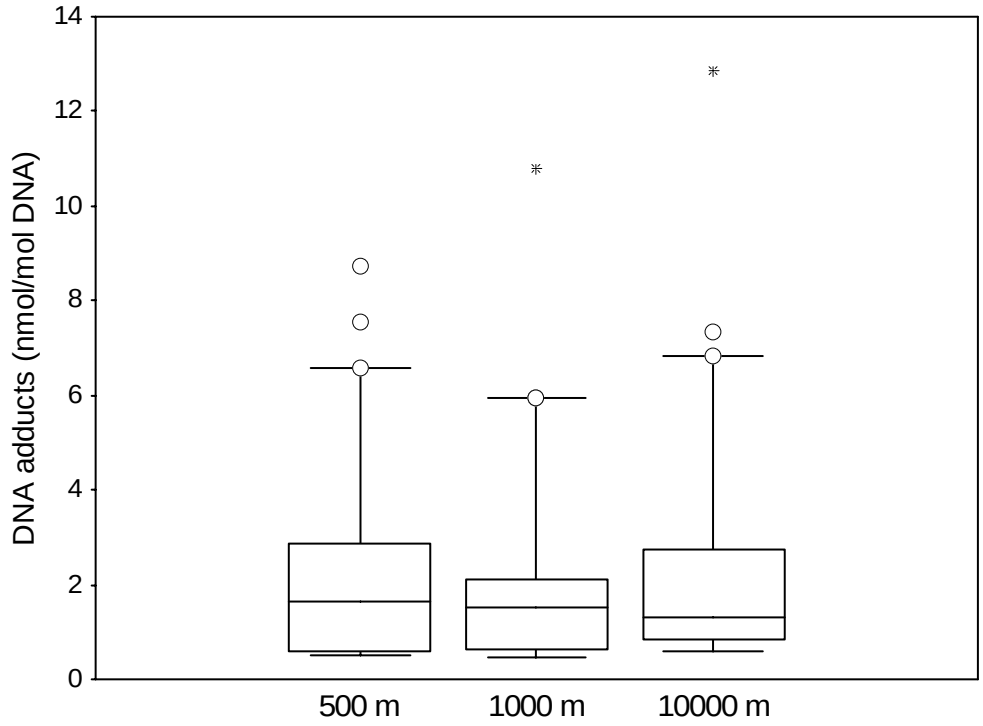


Figure 32. Hepatic DNA adducts (nmol adducts/mol DNA) in saithe collected in the vicinity of the three indicated locations. Results are shown as median, quartiles and 10/90 percentiles. Outliers (○) and extremes (∗) are superimposed.

4. Discussion

4.1 Tissue levels of PAHs in blue mussels – exposure

The results from the 2004 Water Column Monitoring survey indicate that caged organisms has been exposed to low levels of produced water components during August and September 2004. Comparing this years results with results from the BECPELAG workshop in 2001, it is evident that accumulated levels of PAHs were a factor of 5 or more lower in 2004 compared to 2001 (**Figure 33**).

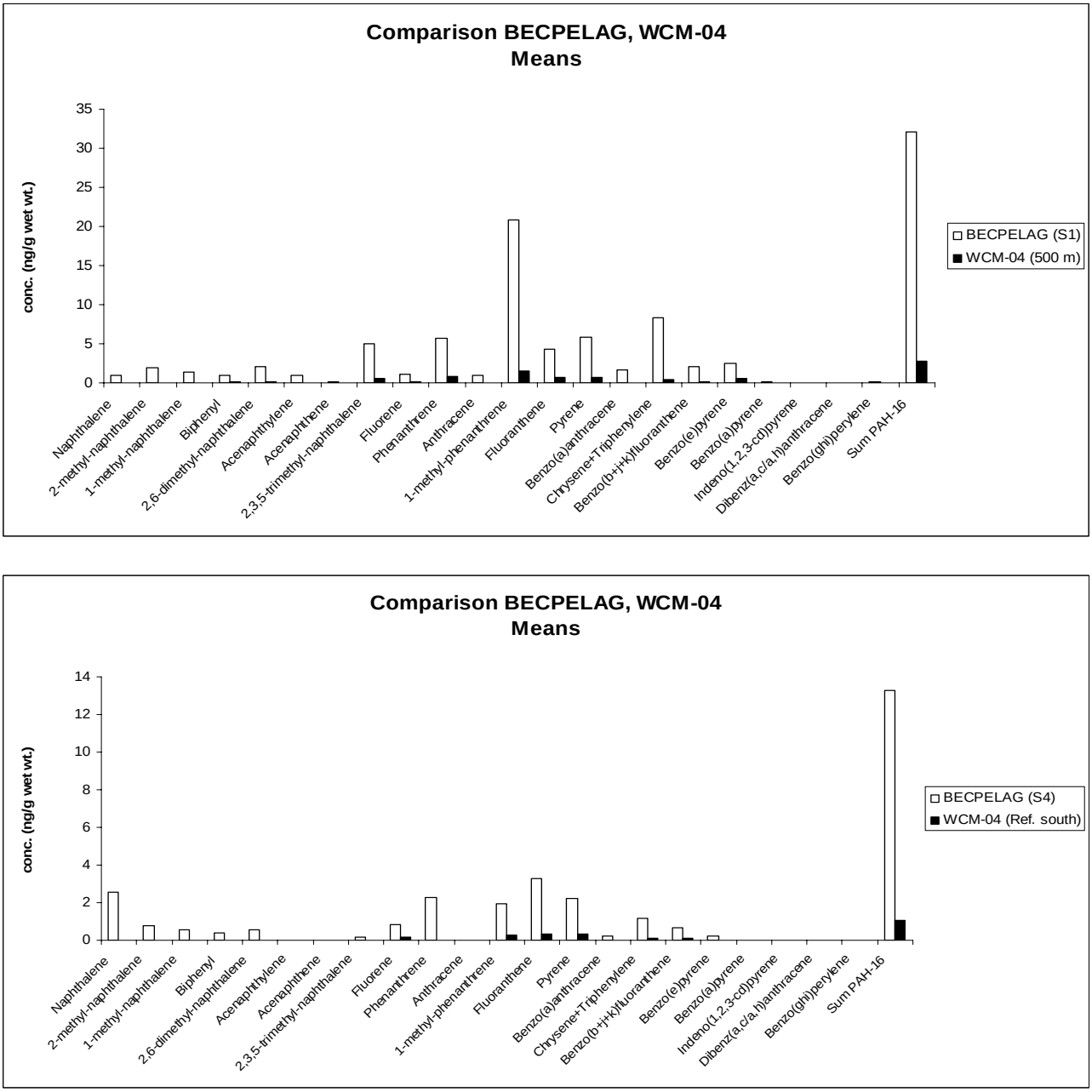


Figure 33. Concentrations (ng/g wet wt.) of PAHs and specific NPDs in blue mussels caged 500 m (**top**) from the Statfjord B platform and at the reference station (**bottom**) during the BECPELAG workshop (2001) and the Water Column Monitoring survey 2004.

The reason for this difference is not clear as inputs from Statfjord B was almost identical in the two periods. Furthermore there was actually no input in the last week prior to sampling in 2001, which would decrease levels further. On the other hand, although the total discharge of PAHs was higher in 2004, that 2001, the concentrations in the effluent was higher in 2001. The DREAM model was not run for the deployment period (2004), but wind-data suggest no anomalities in this time-frame.

There are a number of possible explanations, but none can fully explain the observed situation. The same laboratory has analysed all samples from both series, analytical results have been checked and double-checked. There is unfortunately no data on 0-time mussels from 2001, but other data does not suggest that there is a major difference in baseline levels of mussels used in the two years. Finally, there is a question of whether the plume has actually reached the cages to the same extent in the two periods. There is modelling data available for 2001, but not for 2004. There are really two issues; one is whether current patterns have deviated or deviate through the year, the second whether stratification differed between the two periods. Both factors would obviously affect exposure. A deeper pycnocline would cause a larger dilution of the effluent due to a larger volume of water available for immediate dilution. Caging was done in May-June in 2001 and in August-September in 2004. The pycnocline will generally be deeper in autumn, which would cause a larger dilution volume and lower exposure levels for pelagic (and caged) organisms. In addition, there is limited knowledge of the amounts of particles in the water during the two periods, a fact which would also affect bioavailability (although blue mussels would accumulate both from water and particles). In future surveys, every effort should be made to include a comprehensive hydrography programme and assessment of relevant factors before, during and after caging. One option is using *in situ* detectors and loggers.

4.2 Effect responses in caged mussels

Chemistry data indicated that there was an exposure of blue mussels to PAHs, although lower exposure than during the BECPELAG survey. There was no clear responses from the biological effects, possibly with the exception of micronucleus formation. Benzo(a)pyrene hydroxylase has been the method used in the programme to indicate effects from PAHs in the water column. The results from WCM 2004 indicate a weak sex-dependence of the response, but no relationship with PAH exposure. There was also no response in lysosomal stability, a method which is generally assumed to indicate toxic stress. In contrast to the biochemical assays such as BaPH, lysosomal stability is very sensitive to the recent treatment of the mussels. During this year's programme, mussels were transported to the lab and acclimated there for two days prior to analysis. The subsequent analysis indicated healthy mussels with high membrane integrity. The most relevant components in produced water effluent have a short biological half-life, even in molluscs, and it is conceivable that a two-day "depuration" is too much considering the low exposure level, especially for a tissue such as circulating cells. Based on experiences from the two recent Water Column Monitoring surveys (2003 and 2004), furure use of lysosomal stability should be conducted as early as possible to avoid depuration of chemicals and loss of the ability to detect effects.

The current results indicated a gradient of hemocyte micronuclei formation in mussel caged at different distances from the Statfjord B oil platform, although only mussels caged at 500 m had significantly increased levels compared to mussels caged at the reference location. This parameter has not been included in the Water Column Monitoring programme earlier, but shows promise in indicating possibly genotoxic impacts at low exposure levels.

The histological studies with blue mussels indicated that the mussels were not exposed to metals, an expected result with current knowledge of the low content of metals in produced water from Statfjord B. The mussels were generally seen as healthy with gonad and storage tissue in good condition. Some of the histological endpoints indicated that the mussels were somewhat stressed, which may have been associated with the strat of gametogenesis, but there was an unresolved effect that could not be accounted for (either an effect of pollution or another factor) for and which was most clearly expressed

at the 500 m location. A more extensive histological study could presumably contribute towards understanding both physiological changes and changes associated with produced water exposure.

4.3 Exposure and effect responses in caged cod

The results for PAH-metabolites in the bile of Atlantic cod suggest that exposure levels have been very low, at least during the last week prior to sampling (due to rapid excretion; data from the industry indicate that discharges were lowest towards the end of the caging period). The results did indicate that the cod had been exposed to PAHs prior to deployment, highlighting the need to keep all experimental organisms under clean conditions prior to such deployments. Biological responses, e.g. cytochrome P4501A activity (EROD) and glutathione S-transferase activity (GST) reflected the low exposure. There were only minor differences between cod held at different locations and no gradient in responses. The observed EROD activity can be compared with other monitoring activities, e.g. the Norwegian JAMP (Joint Assessment and Monitoring Programme), in which cod is sampled at a number of coastal locations and analysed for EROD on a yearly basis (in September-October). Although temperature will affect the EROD response, it is clear that the activities observed in most of the cod caged during this survey would be within a background range (cf Ruus et al., 2003). The levels are lower that those observed during the BECPELAG workshop at the same locations (Förlin & Hylland, in press).

4.4 Exposure and effect responses in feral saithe

Saithe were collected at three locations in the Tampen region, although not at a reference site. A similar design was used during the BECPELAG workshop, but it is likely that saithe will move within the entire region. Results from both BECPELAG and this survey bear this out in that there were no major differences between locations. As for cod, levels of PAH metabolites in bile were low and there were no differences between locations.

During BECPELAG, some results did indicate tissue changes in saithe close to the platform and there was an indication of increased frequency of inflammatatory responses at the 500-m location in this survey. During the current survey, the saithe was however clearly in good condition at all locations with lipid-rich livers. The sampling method used during the survey reported here (lure) may not be optimal as that could select for the most active individuals in the populations, whereas e.g. trawling would be more likely to collect a more representative sample.

There were also no differences between locations in the levels of hepatic DNA adducts, but there were individuals at each of the locations that had clearly elevated concentrations of adducts. A recent study has indicated elevated concentrations of DNA adducts in haddock collected from the Tampen region compared to haddock from other areas of the North Sea. It is not possible to compare species directly, and without a reference group it is not possible to assess the presence of adducts in saithe from Tampen.

5. Conclusions

Exposure of caged organisms to produced water components, i.e. PAHs, was lower than in previous years (2001) and lower than expected from the known inputs. This would likely also apply to other compounds than PAH. Possible explanations include differences in hydrographic regime (currents, stratification), differences in particle density and differences in baseline PAH levels in the blue mussels used for caging.

Caged blue mussels did accumulate PAHs, especially 2- and 3-ring components, during the deployment. Bioaccumulation levels followed the expected gradient with highest levels in mussels caged 500 m from the platform decreasing in mussels held at 1000 m and 10 000 m from the platform. The lowest levels were found in 0-time mussels and in mussels held at the reference location. There was a significant increase in micronucleus formation in haemocytes of mussels caged at 500 m from the platform compared to reference and 0-time groups. It is known that micronucleus formation is commonly observed as a response to PAH exposure. There were histological changes in some mussels, most of which was presumably associated with gametogenesis (a natural reproduction process), but some unresolved effect that could not be accounted for (either an effect of pollution or another factor) were observed in mussels caged 500 m from the platform.

Exposure of caged Atlantic cod to produced water components was low, as indicated by low accumulation of bile metabolites. There were no obvious differences in PAH-metabolite levels between cod held 500 m from the Statfjord B platform and reference cod. 0-time cod had higher levels of some components than all groups following deployment, again highlighting the importance of controlling exposure levels for fish to be used in caging experiments. In accordance with the low exposure levels there were no clear effect responses in cod. However, since bile is emptied on a regular basis (weekly), this exposure marker may not properly describe any exposure in the early phases of the deployment.

There were no obvious differences in any effect parameter in the saithe collected at three locations in the Tampen region. PAH-metabolite levels indicate that exposure is moderate at all three locations, although there is limited previous data on baseline levels in this species. Results for hepatic DNA adducts indicate no differences between sampling locations, although elevated concentrations of adducts were found in some individuals at all three locations. There were no obviously contaminant-related effects on liver histology in the saithe.

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Appendix A. Cruise report

Rolf C. Sundt

Water Column Monitoring 2004
Cruise report

Report RF – 2005 / 036

Rev.no:	01	Date:	29.02.2005

Project number:	715 1713
Project title:	Water Column Monitoring 2004
Project Leader:	Rolf C. Sundt
Client(s):	Statoil on behalf of OLF WCM coordination group
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1 First survey – Deployment of organisms

1.1 Research vessel and scientific personnel

The live fish carrier Seigrunn departured Austevoll 5th Aug 13:00 and arrived back in Austevoll 8th Aug 06.00. The scientific personnel onboard were: Rolf C. Sundt (RF-Akvamiljø), Bjørn Serigstad (Ocean Climate a/s), and Dag Altin (Biotrix). The client was represented by Karl Henrik Bryne (Statoil).

1.2 Transfer of cod and mussels to the vessel

During fish transfer the vessel was moored to the fish farm. The fish was lifted by hand from the farms net pens in landing nets and distributed to cages summerged in the vessels fish well. To secure good water quality in the well, care was taken when choosing locality for the initial filling of the tank.

The general impression was that the fish was of good quality as delivered from the farme and that it coped well with the transport to Statfjord, no lethality was observed.

Figure 1. Fish and mussels were transported to field in cages submerged in the vessels well.

Mussels were transported on ice from the shellfish farm in the Trondheim Fjord to Flesland by air and from there to Austevoll and Mekjarvik by car. Mussels arrived onboard the boat at 14:30 and RF-Akvamiljø lab Mekjarvik 21:00 5th Aug.

On board the vessel the mussels were wrapped in protective nets before mounting on the cages.

Figure 2. Prior to mounting on the cages, mussels were wrapped in protective nets.

Figure 3. Preparation of cage with fish, mussels and SPMDs prior to deployment.

1.3 Stations and rig equipment

Stations were marked according to Table 1. Locations are given in same table. Five rigs containing fish and mussels were deployed. Four of the rigs were located at 500, 1000, 2500 and 10 000 metres from the platform along a transect in the dominating current direction (East South East). In addition a reference station was placed at an expectedly clean location approximately 38 km South East of Oseberg South.

Figure 4. Deployment of cage containing fish and mussels, note the orange primary buoyancy elements.

Table 1. Distance from platformand and geographical coordinates.

Code	**Distance from Statfjord B (m)**	**Positions**	
301->	500	N 61°12,3	E 01°50,3
201->	1000	N 61°12,1	E 01°50,7
500->	2500	N 61°11,8	E 01°52,1
101->	10 000	N 61°10,0	E 01°59,5
401->	Ref. south	N 60º 07,0	E 01° 52,5

The cage deployed 2500 meter from Statfjord B was lost due to collision with the supply wessel Normann Draupner 10 Aug. Fish and mussels were frozen round onboard the vessel and transported to the Ågotnes base. Required equipment for sample preservation was not available onboard the supply wessel. Bile was sampled for PAH analysis and biological data were recorded.

1.4 Monitoring of environmental data

CTD profiles were made during the rig deployment operation with a SBE "Seabird 901" probe. Temperature, conductivity, salinity, Σth, sound h. and sound m. were recorded from surface to 20 metres. Detailes given in Appendix 1.

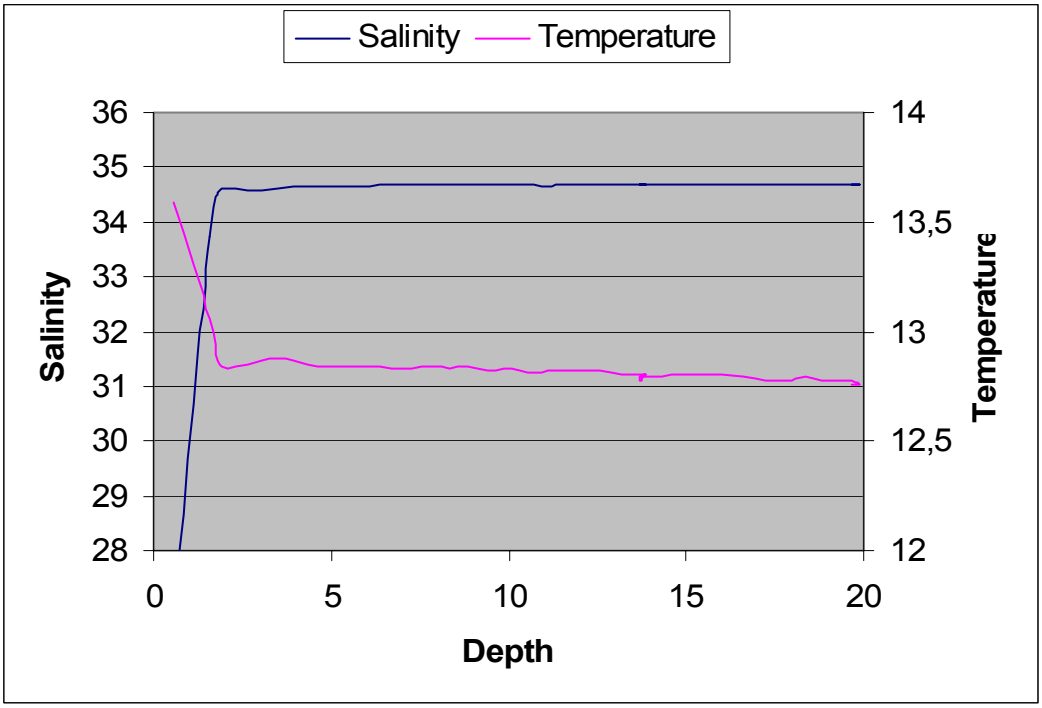

Figure 5. CTD profile recorded 17th Sept. (temperature given in ºC and salinity in ‰).

1.5 PAH monitoring

In order to examine potential PAH contamination in the storage well system onboard the wessel, samples were collected. Five samples were taken from the surface of the main tank and stored in 5 litres tinted glass bottles with HCl added. The bottles were wrapped in aluminium foil and kept refrigerated until analyzes.

1.6 Pre-exposure sampling of cod and mussels

Pre-exposure sampling of cod was conducted 5 August on the fish farm that delivered fish to the experiment. Sampling was carried out in the fish farm by personnel from RF-Akvamiljø (Rolf C Sundt, Lars Petter Myhre and Anne Helene Tandberg) and NIVA (Sigurd Øxnevad and Eivind Farmen Finne). Samples were taken according to the list in Table 3.

Mussels for the pre-exposure sampling arrived 5 Aug at the RF-Akvamiljø facility in Mekjarvik and were kept in clean sea water for 4 days prior to sampling. Sampling was conducted 9 Aug by personnel from RF-Akvamiljø (Jan Fredrik Børseth and Anne Helene Tandberg). Samples were taken according to the list in Table 4.

Figure 6. Pre-exposure sampling of cod was conducted at the fish farm.

Table 2. Overview of samples collected from caged cod and from pre-exposure.

Matrix	**Number of samples**	**Recipient**	**Preservation**	**Methode**
Bloodplasma	27	NIVA	Cryotubes, (l) N_2	Vitellogenin
Bloodplasma	27	NIVA	Cryotubes, (l) N_2	Proteomics
Gills	20	RF	Buffer, on ice.	Gill EROD
Gills	20	RF	Buffer, on ice.	Na+/K+ ATPase
Bile	27	RF	Cryotubes, (l) N_2	PAH met. GCMS
Liver	27	NIVA/RF	Cryotubes, (l) N_2	EROD, GST
Liver	27	RF	Cryotubes, (l) N_2	DNA adducts
Liver	27	NIVA (CEFAS)	Buffered formalin	Histopat./chem.
Liver	20	RF	Smear on slides	Micronucleus

Table 3. Overview of samples collected from feral saithe.

Matrix	**Number of samples**	**Recipient**	**Preservation**	**Methode**
Bile	25	RF	Cryotubes, (l) N_2	PAH metabolites
Liver	25	RF	Cryotubes, (l) N_2	DNA adducts
Liver	25	NIVA (CEFAS)	Buffered formalin	Histopat./chem.

Table 4. Overview of samples collected from mussels.

Matrix	**Number of samples**	**Recipient**	**Preservation**	**Methode**
Haemolymphe	20	RF	Direct analyzis	Lysosomal stability
Hepatopancreas	20	NIVA	Cryotubes, (l) N_2	BaPH
Hepatopancreas	20	NIVA (Bilbao)	Cryotubes, (l) N_2	Histopat./chem.
Hepatopancreas	20	NIVA (Bilbao)	Cryotubes, (l) N_2	Histopat.
Hepatopancreas	20	NIVA (CEFAS)	Davidsons fix	Histopat.
Whole animals	5x3 pools	NIVA	Heated glass/-20°C	PAH, lipid

2 Second survey – Sampling

2.1 Objectives

The objectives were to collect cages with mussels, fish and SPMDs and obtain biological samples for effect related monitoring of discharges from the Statfjord C platform. (Water Colunm Monitoring 2004). In addition samples for analysis of EROD and Na/K ATPase in fish gills were collected for graduate student Alexandra Abrahamson at NFH.

The survey was conducted by personnel from Ocean Climate (Bjørn Serigstad), RF-Akvamiljø (Rolf Sundt, Lars Petter Myhre and Anne Helene Tandberg) and NIVA (Eivind Farmen Finne). The client was represented by Karl Henrik Bryne (Statoil).

The vessel Segrunn departed Flesland 16th Sept. 18:00 and arrived Flesland 18th Sept. 12:00.

2.2 Equipment and logistics

RF-Akvamiljø was responsible for the accomplishment of the logistic part of the survey and brought necessary equipment. Equipment and chemicals were brought onboard the vessel together by the sampling personell. Sample facilities were established in a 10 ft steel container mounted the after-deck.

2.3 Accomplishment

All cages, with the exception of one (2500 metres from the platform) that was lost, were picked up without any major problems. Based on visual inspection, both mussels and fish apperantly were in good shape. A few individual fish had worn fins exspectedly due to contact with the net walls.

Most fish stommacs investigated were emty, some individuals had been eating fouling from the cages (mainly Bryozoa spp.) Since no typically pelagic prey organisms were recorded, the effect of the attractant light in uncertain.

Aftre retrieveing the cages, the organisms were stored in the vessels fish well until sampling. Mussels were packed in ice for transportation to the lab in Stavanger. Cod was carried to the sampling lag in groups of five individuals. Detailes regarding distribution of sampling tasks are given in Appendix 2.

Feral saithe was cauth by lures on hand held equipment.

2.4 Conclusion

The accomplishment of the second survey was on the whole successful with respect to pick-up of cages and sampling. However, the loss of the 2500 metres cage means that there is only material for bile analysis from this station

The rest of tre sampling of the fish and mussels went on as planned. Extra transport and storing for the mussels must be considered when results are interpreted. Future surveys should preferably be conducted earlier in the autumn, when the weather condition in the North Sea is more stable.

As suggested for the 2003 Water Column Monitoring the program should be commenced at leased a month earlier. There are two major reasons for this.

The water temperature in August is causing at least two potentially serious problems. The probability of microbial infections and physiological stress during fish handling increases with temperature. This could potentially both cause lethality and disturb biomarker signals. Lower temperature would also allow non destructive blood sampling, which would make possible a better basis for interpretation of biomarker data (especially Vtg). An alternative would be to undertake 0-sampling at the same time as the main sampling. The letter would require a storage facility for the 0-sampling group.

The probability of bad weather conditions causing mechanical stress on the caged organisms and possible delays due to operational limitations during bad weater conditions.

3 Overall evaluation of field work

- As pointed out in the 2003 The deployment period should be earlier in the season. This gives more stable weather conditions and less risk for loss of rigs by collition with vessels due to reduced visibility, rough sea etc.
- No damage of rig equipment due to rough weather conditions (several storms).
- Very few emty mussel shells in the stockings, proper mounting of stockings
- Deployment of rigs mainly evaluated as successful. One exception: loss of one rig due to coillision with vessel.
- Scarce time with 1-2 days for the sample survey. Scarce survey time easily reduce the quality of the sampling work.
- Scarce time also makes it difficult to perform onboard screening biomarker as with the NRRT method.
- Sufficient time for fish sampling, mussel sampling is more time consuming.
- Concern regarding possible contamination of PAH when sampling lab is mounted close to the exhaust outlet.

Appendix 1.

CTD data

Salinity	Temperature	Density	Depth	Date	Time
26,81	13,59	19,95	0,55	17.09.2004	17:21:19
33,75	13,06	25,421	1,55	17.09.2004	17:21:20
34,53	12,87	26,063	1,83	17.09.2004	17:21:21
34,6	12,83	26,127	2,1	17.09.2004	17:21:22
34,58	12,85	26,109	2,64	17.09.2004	17:21:23
34,62	12,88	26,139	3,52	17.09.2004	17:21:24
34,65	12,85	26,171	4,32	17.09.2004	17:21:25
34,66	12,84	26,184	4,88	17.09.2004	17:21:26
34,66	12,84	26,188	5,54	17.09.2004	17:21:27
34,66	12,84	26,189	6,09	17.09.2004	17:21:28
34,67	12,83	26,201	6,68	17.09.2004	17:21:29
34,68	12,83	26,212	7,26	17.09.2004	17:21:30
34,67	12,84	26,206	7,83	17.09.2004	17:21:31
34,68	12,83	26,216	8,35	17.09.2004	17:21:32
34,67	12,84	26,21	8,87	17.09.2004	17:21:33
34,67	12,82	26,217	9,39	17.09.2004	17:21:34
34,68	12,83	26,224	9,85	17.09.2004	17:21:35
34,67	12,82	26,219	10,31	17.09.2004	17:21:36
34,67	12,81	26,223	10,71	17.09.2004	17:21:37
34,66	12,82	26,215	11,08	17.09.2004	17:21:38
34,68	12,82	26,233	11,32	17.09.2004	17:21:39
34,67	12,82	26,225	11,63	17.09.2004	17:21:40
34,67	12,82	26,228	12,25	17.09.2004	17:21:41
34,68	12,81	26,241	12,92	17.09.2004	17:21:42
34,68	12,8	26,245	13,48	17.09.2004	17:21:43
34,68	12,8	26,248	13,88	17.09.2004	17:21:44
34,69	12,79	26,256	13,69	17.09.2004	17:21:45
34,68	12,78	26,25	13,68	17.09.2004	17:21:46
34,68	12,78	26,25	13,77	17.09.2004	17:21:47
34,7	12,79	26,264	13,8	17.09.2004	17:21:48
34,7	12,79	26,265	13,92	17.09.2004	17:21:49
34,7	12,79	26,267	14,32	17.09.2004	17:21:50
34,7	12,8	26,267	14,87	17.09.2004	17:21:51
34,69	12,8	26,262	15,39	17.09.2004	17:21:52
34,68	12,8	26,258	16,01	17.09.2004	17:21:53
34,68	12,79	26,261	16,64	17.09.2004	17:21:54
34,67	12,78	26,259	17,26	17.09.2004	17:21:55
34,68	12,78	26,269	17,67	17.09.2004	17:21:56
34,67	12,78	26,261	17,88	17.09.2004	17:21:57
34,67	12,79	26,262	18,34	17.09.2004	17:21:58
34,69	12,78	26,281	18,84	17.09.2004	17:21:59
34,67	12,78	26,269	19,44	17.09.2004	17:22:00
34,69	12,77	26,287	19,77	17.09.2004	17:22:01
34,68	12,77	26,281	19,77	17.09.2004	17:22:02
34,69	12,76	26,29	19,89	17.09.2004	17:22:03
34,7	12,76	26,296	19,64	17.09.2004	17:22:04

Appendix 2.

Distribution of sampling tasks among crew.

Personell	Task / matrix	Treatment	Sample distr.	Preservation	Analysis
A*	Fish supply				
A*	Control tagging				
A	Length				
A	Weigth				
A	Record fish quality				
A	Sex				
B	Blood	centrifugation	Plasma 1	N_2	VTG
			Plasma 2	N_2	*Proteom*
B	Gills, two arches	-	50 ml tube	buffer on ice	*EROD*
	Gills, two filarments	buffer		N_2	Na, K ATPase
C	Open abdomen				
C	Bile	-	One tube	N_2	
C	Liver weight				EROD/GST
C	Liver	-	Lever 1	N_2	*EROD, GST,*Cyp 1a
C	Liver	-	Lever 2	N_2	DNA adducts
C	Gonad weight				
D	Read sex to A				
D	Liver	Smear on slide		MeOH, dry	Micronucleus
D	Liver	Cassette		Formalin	Histopat.
A	Water sampling				
A	CTD				
B	SPMD samplers				

Appendix 3.

Sampling information fish.

Sampling WCM 2004 - Caged cod

Date: 17.09.2004 Group: 10000m

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
101	40,0	0,7020	0,0725	F	
102	41,0	0,6150	0,0582	M	
103	41,0	0,6725	0,0750	M	Green liver
104	39,0	0,6435	0,0628	M	
105	41,5	0,8180	0,0998	M	
106	47,0	0,9440	0,8300	F	
107	37,5	0,5438	0,0760	M	
108	43,0	0,8295	0,0972	M	
109	37.5	0,5028	0,0543	M	
110	44,0	0,9040	0,1025	M	
111	45,5	1,0640	0,1104	F	
112	39,5	0,5998	0,0570	M	
113	45,5	0,9105	0,0930	F	
114	42,0	0,7325	0,0858	M	
115	44,0	0,9030	0,0105	F	
116	47,0	1,0070	0,0970	F	
117	47,0	1,1025	0,1290	M	
118	49,5	1,0385	0,1045	F	
119	44,0	0,9115	0,1025	F	
120	44,0	0,9335	0,1136	M	
121	48,5	1,1605	0,1280	F	
122	40,0	0,5720	0,0480	M	
123	40,0	0,6555	0,0721	F	
124	46,5	1,0735	0,1159	F	
125	44,0	1,0240	0,1065	F	
126	39,5	0,6490	0,0620	M	
127	44,0	0,7460	0,0640	F	

Sampling WCM 2004 - Caged cod

Date: 17.09.2004 Group: 1000m

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
201	45,0	0,9510	0,1048	F	
202	47,5	1,1250	0,1130	F	
203	41,0	0,7575	0,0773	M	
204	42,0	0,7045	0,0725	M	
205	36,0	0,4788	0,0450	M	
206	42,0	0,7980	0,0924	M	
207	37,0	0,4752	0,0355	M	
208	49,5	1,1440	0,1222	F	
209	42,0	0,7095	0,0945	M	
210	44,0	0,9600	0,0985	M	
211	36,5	0,5592	0,0488	M	
212	46,0	0,9665	0,0910	F	
213	39,5	0,6035	0,0642	M	
214	46,5	0,9985	0,1104	F	
215	46,5	1,0300	0,1080	F	
216	39,0	0,5830	0,0718	M	
217	42,5	0,8395	0,1040	M	
218	38,0	0,6260	0,0660	M	
219	42,0	0,7850	0,0860	F	Green liver
220	43,0	0,8220	0,0688	F	
221	44,0	0,9645	0,1100	M	
222	43,5	0,8830	0,0860	F	
223	39,0	0,5370	0,0430	M	
224	42,0	0,6970	0,0710	F	
225	38,5	0,6910	0,0700	M	
226	43,0	0,7930	0,0750	F	
227	40,0	0,6640	0,0613	F	

Sampling WCM 2004 - Caged cod

Date: 17.09.2004 Group: 500m

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
301	44,0	1,0420	0,1055	M	
302	45,5	0,9385	0,0935	F	
303	38,0	0,5570	0,0500	M	
304	37,0	0,5086	0,0510	M	
305	46,0	1,0250	0,1102	M	
306	45,0	0,9225	0,1040	F	
307	44,0	0,7920	0,0800	F	
308	42,0	0,7200	0,0730	M	
309	44,0	0,8265	0,0920	M	
310	40,5	0,5950	0,0560	M	
311	39,0	0,6590	0,0830	M	
312	44,0	0,8675	0,0918	M	
313	42,5	0,7525	0,0847	M	
314	43,0	0,7570	0,0933	M	
315	42,0	0,7970	0,0940	M	
316	41,0	0,7475	0,0702	M	
317	45,5	0,8520	0,0850	F	
318	42,0	0,7350	0,0712	M	
319	42,5	0,8110	0,0813	M	
320	39,5	0,6215	0,0460	F	
321	41,0	0,5932	0,0920	F	
322	44,0	0,8835	0,0890	F	
323	43,0	0,7925	0,0500	M	
324	35,5	0,4752	0,1112	M	
325	45,5	1,0175	÷	M	
326	47,0	1,0990	0,0150	M	histologi marked 602
327	36,5	0,4840	0,0480	M	

Sampling WCM 2004 - Caged cod

Date: 18.09.2004 kl. 01:10 Group: Ref. South

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
401	43,5	0,7680	0,0630	M	
402	42,5	0,7705	0,0842	M	
403	41,5	0,7515	0,0692	F	
404	39,0	0,6385	0,0745	M	
405	49,5	1,1645	0,1360	F	
406	41,0	0,7380	0,0745	M	
407	43,5	0,8715	0,0945	F	
408	39,5	0,7000	0,0857	M	
409	43,0	0,8255	0,1029	F	
410	42,5	0,6690	0,0631	M	
411	44,0	0,8750	0,1001	M	
412	50,0	1,1825	0,1234	F	
413	46,5	0,9585	0,1068	F	
414	42,5	0,7815	0,0976	F	
415	45,0	0,9510	0,0945	M	
416	39,5	0,5084	0,0417	F	skull deformed
417	42,0	0,6080	0,0572	M	
418	46,0	0,8915	0,0912	F	
419	42,0	0,7765	0,0928	F	
420	46,0	1,0580	0,1351	F	
421	42,0	0,7740	0,0870	M	
422	44,0	0,8855	0,0980	M	
423	44,5	0,8225	0,0760	F	
424	40,0	0,8220	0,0940	M	
425	44,0	0,8730	0,0840	M	
426	44,0	0,8540	0,0918	F	
427	41,0	0,7360	0,0728	M	

Sampling WCM 2004 - Caged cod

Cage lost, fish frozen round on vessel. Liver partly dissolved - weight uncertain.

Date:	31.09.2004		Group:	2500m	
Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
501	44,0	0,981	0,0880	F	Only bile
502	39,5	0,674	0,0683	M	Only bile
503	39,0	0,655	0,0447	M	Only bile
504	36,0	0,560	0,0421	M	Only bile
505	38,0	0,593	0,0535	M	Only bile
506	38,0	0,634	0,0675	M	Only bile
507	37,0	0,625	0,0360	F	Only bile
508	42,5	1,008	0,0990	F	Only bile
509	40,0	0,707	0,0800	M	Only bile
510	37,0	0,606	0,0565	F	Only bile
511	38,0	0,600	0,0480	M	Only bile
512	37,0	0,602	0,0460	M	Only bile
513	36,0	0,582	0,0295	F	Only bile
514	39,0	0,750	0,0774	M	Only bile
515	39,5	0,633	0,0406	F	Only bile
516	41,0	0,855	0,0717	F	Only bile
517	37,0	0,533	0,0361	M	Only bile
518	39,0	0,686	0,0170	M	Only bile
519	36,5	0,494	0,0110	M	Only bile
520	38,5	0,743	0,0680	M	Only bile
521	37,0	0,561	0,0600	M	Only bile
522	37,0	0,540	0,0470	M	Only bile
523	37,0	0,557	0,0450	M	Only bile
524	33,0	0,476	0,0300	M	Only bile

Sampling WCM 2004 - Cod, Pre-exposure sampling

Date:	05.08.2004		Group:	0-sampling	
Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
1	41,00	0,90		M	
2	45,50	0,97		M	
3	41,00	0,93		M	
4	36,00	0,56		M	
5	42,00	0,52		M	
6	38,00	0,38		F	
7	46,00	1,09		F	
8	42,50	0,55		M	
9	42,50	0,98		M	
10	44,00	0,99		F	
11	37,50	0,61		M	
12	40,50	0,85		M	
13	44,50	1,07		F	histopat. L, K, G
14	41,00	0,87		M	histopat. L, K, G
15	35,50	0,45		M	histopat. L, K, G
16	46,50	1,13		F	histopat. L, K, G
17	42,00	0,94		M	histopat. L, K, G
18	41,50	0,72		M	histopat. L, K, G
19	39,50	0,66		F	histopat. L, K, G
20	39,50	0,78		M	histopat. L, K, G
21	46,00	1,18		F	histopat. L, K, G
22	47,50	1,14		F	histopat. L, K, G
23	35,50	0,50		F	histopat. L, K, G
24	47,00	1,08		F	histopat. L, K, G
25	42,50	0,74		M	histopat. L, K, G
26	46,50	1,22		M	histopat. L, K, G
27	45,50	1,02		F	histopat. L, K, G
28	44,00	0,98		F	histopat. L, K, G
29	36,50	0,52		M	histopat. L, K, G
30	34,00	0,46		M	histopat. L, K, G
31	37,50	0,67		M	histopat. L, K, G
32	46,00	1,18		F	histopat. L, K, G
33	37,50	0,56		M	histopat. L, K, G
34	43,50	0,95		F	histopat. L, K, G
35	42,00	0,95		F	histopat. L, K, G
36	45,50	1,06		M	histopat. L, K, G
37	39,00	0,66		M	histopat. L, K, G
38	41,50	0,89		F	histopat. L, K, G
39	44,00	0,97		F	histopat. L, K, G
40	42,00	1,02		F	histopat. L, K, G
41	44,50	0,91		M	histopat. L, K, G
42	46,00	1,09		F	
43	42,50	0,80		M	
44	36,50	0,58		M	
45	39,50	0,71		M	
46	39,50	0,75		M	
47	38,50	0,62		M	
48	43,50	0,95		M	
49	42,50	0,96		M	

50	36,00	0,51		M	
51	46,50	1,27		F	
52	39,50	0,82		M	
53	38,00	0,69		M	
54	36,00	0,53		M	
55	39,50	0,78		M	

QA: AHST

Sampling WCM 2004 - Feral saithe (*Pollachius virens*)

Date: 06.08.2004 Group: 500m / 1000m / 10000m

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
500m-1	44,5	0,97		F	No bile
2	66,0	2,20		M	Pollock (*Pollachius pollachius*)
3	55,0	1,66		F	No bile
4	49,0	0,98		F	No bile
5	46,0	1,01		M	No bile
6	45,0	0,83		F	No bile
7	55,0	1,35		M	
8	58,0	1,88		M	No bile
9	52,0	1,43		F	No bile
10	45,0	0,79		F	No bile
11	47,0	0,98		M	
12	51,0	1,12		F	No bile
13	47,0	0,64		M	No bile
14	45,5	0,85		F	
15	41,0	0,70		M	
16	49,0	1,05		F	
17	50,0	1,16		M	
18	53,0	1,48		M	
19	48,0	1,37		F	
20	41,0	0,71		F	No bile
21	44,0	0,95		F	
22	41,0	0,73		M	
23	44,0	0,88		F	No bile
24	46,0	1,07		M	
1000m-25	44,0	0,77		M	No bile
26	47,0	0,92		M	
27	48,0	1,18		F	
28	56,0	1,65		M	No bile
29	45,0	0,92		F	
30	54,0	1,33		F	
31	54,0	1,24		M	
32	50,0	1,12		M	
33	44,0	0,71		M	No bile
34	52,0	1,30		M	
35	51,0	1,18		F	
36	57,0	1,58		M	
37	42,0	0,70		M	

38	43,0	0,78		M	
39	54,0	1,47		M	
40	46,0	0,96		M	
41	44,0	0,82		F	
42	47,0	0,94		F	
43	52,0	1,20		M	No bile
44	44,0	0,78		F	
45	45,0	0,92		F	
46	46,0	0,78		M	
47	47,0	0,90		M	
48	42,0	0,67		F	
49	43,0	0,65		F	
50	47,0	0,83		M	
51	50,0	1,02		M	No bile
52	48,0	0,95		F	
53	52,0	1,26		M	
54	46,0	0,92		F	No bile
10000m-55	50,0	1,05		M	No bile
56	51,0	1,38		F	
57	45,0	1,23		M	
58	59,0	1,80		F	
59	49,0	0,90		F	
60	44,0	0,83		M	No bile
61	60,0	1,85		F	
62	56,0	1,22		F	
63	55,0	1,54		M	
64	56,0	1,47		F	No bile
65	65,0	2,26		F	
66	55,0	1,20		F	
67	53,0	1,18		F	
68	48,0	0,93		M	
69	50,0	1,16		M	
70	70,0	2,85		M	No bile
71	47,0	1,05		M	No bile
72	53,0	1,38		F	No bile
73	43,0	0,74		M	
74	59,0	1,76		M	No bile
75	52,0	1,25		F	
76	49,0	1,14		F	No bile
77	51,0	1,15		F	
78	57,0	1,50		F	
79	54,0	1,52		M	

QA: AHST

Appendix 5.

Sampling data mussels

Sampling WCM 2004 - Mussel

Stylus removed from digestive (all groups)

Date: 21.09.2004 10000m

Mussel No.	Length (mm)	Weight-g	Comments
101	59,0	16,75	
102	59,0	16,62	
103	54,0	17,40	
104	58,0	19,92	
105	57,0	16,31	
106	57,0	18,37	
107	67,0	23,17	
108	59,0	18,94	
109	73,0	34,73	
110	65,0	26,58	
111	62,0	27,43	
112	62,0	23,25	
113	70,0	32,53	
114	72,0	35,44	
115	66,0	28,79	
116	61,0	24,04	
117	66,0	30,20	
118	54,0	18,55	
119	80,0	42,90	
120	73,0	40,92	

Sampling WCM 2004 - Mussel

Date:	21.09.2004		1000m
Mussel No.	Length (mm)	Weight-g	Comments
201	57,0	19,17	
202	62,0	27,96	
203	60,0	21,05	
204	65,0	26,33	
205	64,0	31,66	
206	60,0	21,62	
207	58,0	24,98	
208	65,0	27,50	
209	65,0	27,89	
210	69,0	33,34	
211	77,0	41,94	
212	56,0	17,07	
213	60,0	19,70	
214	66,0	27,57	
215	75,0	43,28	
216	52,0	16,04	
217	53,0	13,06	
218	55,0	17,35	
219	57,0	16,78	
220	60,0	24,42	

Sampling WCM 2004 - Mussel

Date:	21.09.2004		500m
301	64,0	29,76	
302	57,0	20,19	
303	62,0	25,24	
304	65,0	24,67	
305	58,0	17,23	
306	60,0	22,39	
307	69,0	19,14	
308	51,0	16,17	
309	69,0	24,53	
310	70,0	34,14	
311	72,0	35,83	
312	60,0	26,38	
313	70,0	38,09	
314	66,0	31,54	
315	71,0	33,44	
316	56,0	16,00	
317	61,0	26,25	
318	63,0	26,63	
319	68,0	37,57	
320	59,0	21,86	

Sampling WCM 2004 - Mussel

Date:	21.09.2004		Ref south
Mussel no.	Length (mm)	Weight-g	Comments
401	65,0	25,21	
402	70,0	38,46	
403	60,0	20,83	
404	63,0	24,80	
405	68,0	32,67	
406	55,0	15,75	
407	64,0	20,48	
408	61,0	21,83	
409	65,0	24,22	
410	71,0	34,56	
411	53,0	15,30	
412	55,0	15,74	
413	70,0	32,80	
414	76,0	42,11	
415	57,0	20,61	
416	66,0	28,41	
417	58,0	17,11	
418	66,0	26,05	
419	58,0	19,39	
420	63,0	22,08	

Sampling WCM 2004 - Mussel

Date:	11.08.2004		0-sampling
Mussel no.	Length (mm)	Weight-g	Comments
1	73,0	32,54	
2	65,0	25,32	
3	73,0	33,01	
4	65,0	26,02	
5	73,0	33,25	
6	68,0	31,52	
7	70,0	25,68	
8	68,0	28,63	
9	73,0	29,88	
10	63,0	24,35	
11	70,0	32,12	
12	71,0	33,72	
13	62,0	23,08	
14	66,0	23,86	
15	68,0	29,04	
16	67,0	26,27	
17	71,0	27,13	
18	65,0	24,79	
19	59,0	18,12	
20	60,0	20,02	
			QA: AHST

Appendix B. Data report – University of the Basque Country (Bilbao)

Water Column Survey – 2004 (WCS2004)

Work Contracter NIVA

UNIVERSITY OF THE BASQUE COUNTRY

Tissue-Level Biomarkers and Histopathology in Caged Blue Mussel

Final Report

UPV/EHU, Bilbo (Basque Country)

15 January 2005

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Water Column Survey – 2004 (WCS2004)

Work Contractor NIVA
UNIVERSITY OF THE BASQUE COUNTRY (Bilbao)

Tissue-Level Biomarkers and Histopathology in Caged Blue Mussel

FINAL REPORT, 15 JANUARY 2005

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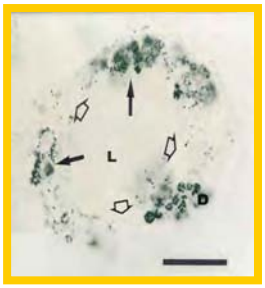
INTRODUCTION

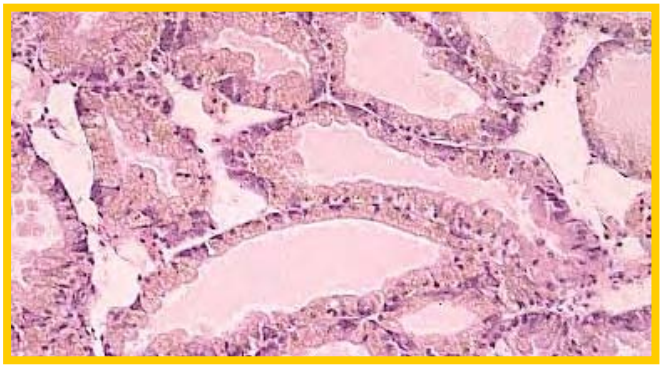
The mussel *Mytilus* sp. is the most widely used sentinel species in pollution monitoring programs aimed to study the health of coastal and estuarine environments (Goldberg, 1975; UNEP/RAMOGE, 1999; Cajaraville et al., 2000). Actually, the Mussel Watch (Goldberg, 1975) is the oldest pollution biomonitoring program still in progress worldwide. Mussels possess an extraordinary ability to uptake chemical compounds from the environment and accumulate them in their tissues. Moreover, mussels are ubiquitous and abundant and due to their sessile life style they cannot escape from pollutants. Thus, mussels endure exposure to xenobiotics responding in a broad range of ways that can be measured as biomarkers. Finally, mussels are easy to handle, and are very suitable organisms for transplant experiments since they are easily kept caged at sites of interest.

SELECTED BIOMARKERS IN BLUE MUSSEL

Biomarkers were analyzed in mussel digestive gland. The digestive gland of molluscs is the main center for metabolic regulation, participating in the mechanisms of immune defense and homeostatic regulation of the internal medium, as well as in the processes of detoxification and elimination of xenobiotics (Moore and Allen, 2002). The battery of biomarkers selected can be grouped as follows: biomarkers of metal exposure (autometallographic localization of metals in lysosomes), and biomarkers of general stress (histopathological alterations).



BSD-extent. Autometallography (AMG) reveals metal deposits in cells; further, it allows a cost/effective determination of metal levels in biological tissues (Marigómez et al., 2002). This technique has been applied in various investigations, to demonstrate the association of metals in cells of fish and molluscs with environmental levels of metals. AMG deposits, which appear specifically confined to the lysosomal compartment in molluscan digestive cells, are measured presently by image analysis of paraffin sections. In molluscs, a close parallel has been found between AMG deposits (black silver deposit-extent, or BSD-extent) in target cell compartments and metal concentrations determined by atomic absorption spectrophotometry in the soft tissues (Soto & Marigómez, 1997).



Tissue-level biomarkers. Histopathological changes in the cell and tissue organization of the digestive, reproductive and immune systems, as well as the incidence of

parasites in these tissues, are sensitive biomarkers, which respond to a diverse variety of xenobiotics and natural stressors. Such biomarkers have been shown to be responsive in molluscs and fishes. In the digestive gland of marine molluscs, different stressors provoke changes in the phasic activity and atrophy of the digestive alveoli (Lowe et al., 1981; Couch, 1983; Vega et al., 1989; Cajaraville et al., 1992). The changes can be measured with the aid of quantitative microscopy (planimetry), as changes in the mean epithelial thickness (MET), mean diverticular radius (MDR), mean luminal radius (MLR) and the ratios MLR/MET and MET/MDR (Vega et al., 1989). In addition, there is clear evidence of severe changes in the distribution and relative occurrence of cell types in the digestive gland of molluscs. Digestive cells are usually much more abundant than basophilic cells but, under conditions of exposure to pollutants, an apparent increase in the relative numbers of basophilic cells (measured as volume density of basophilic cells) occurs. This so-called “cell-type replacement” (Marigómez et al., 1998) may provoke disturbances in digestion and metabolism.

MATERIAL & METHODS

EXPERIMENTAL DESIGN AND SPECIMEN COLLECTION

Experimental sets of blue mussels (*Mytilus edulis*) were deployed in cages at different stations in North Sea around an oil rig. Blue mussel was caged in 4 stations. In addition, a "0 Time·" sample (code 001-020) was obtained just before deploying.

Code	Distance from Oil rig (m)
101-120	10000
201-220	1000
301-320	500
401-420	*South Reference*

20 mussels were collected per sampling site and fixed in Davidson solution. Processing is described in detail below.

BIOMARKERS AND COMPLEMENTARY MEASURES IN BLUE MUSSEL

Histo(patho)logical Examination and Tissue-level biomarkers

Digestive glands were dissected, fixed in Davidson solution on board and transferred to the laboratory. Then, fixed tissues were dehydrated in alcohols and embedded in paraffin. Histological sections (7 μm) were cut with the aid of a rotary microtome, stained with haematoxylin-eosin (H/E) and mounted.

Histopathological examination was carried out under the light microscope. Prevalence of parasites, hemocyte infiltration and general condition of the digestive epithelium, the interstitial connective tissue and the gonad tissue were systematically recorded.

As an indication of whether cell-type replacement occurred or not, the volume density of basophilic cells (V_{vBAS}) in the digestive gland of mussels was determined by means of stereology. A Weibel graticule (M-168; Weibel, 1979) was superimposed onto paraffin sections stained with H/E with the aid of a drawing tube attachment. Randomly selected 5 fields were counted in 10 individuals per experimental group (20x objective). The volume density of basophilic cells was calculated as $V_{VBAS} = V_{BAS}/V_{DT}$ where V = volume; BAS = basophilic cell and DT = digestive gland epithelium.

In order to quantify the structure of the digestive tubules, a planimetric procedure was applied on paraffin sections of digestive gland tissue (Vega et al. 1989). A total of 25 tubule sections per individual were recorded in an image analysis system (Visilog 5.4 Noesis) attached to an Olympus BX50 light-microscope. Five parameters were obtained: MET (mean epithelial thickness), MDR (mean diverticular radius), MLR (mean luminal radius), MET/MDR ratio and MLR/MET ratio.

Gonad Index (GI) was determined in 10 individuals per experimental group according to the classification proposed by Seed (1969). Gonad development stages (1: resting gonad; 2: early gametogenesis; 3: advanced gametogenesis; 4: mature gonad; 5: spawning gonad; and 6: post-spawning gonad) were determined after histological examination of histological sections (7 μm) stained with hematoxylin-eosin.

Biomarkers of metal exposure: BSD extent

Intralysosomal metal levels were determined on paraffin sections by autometallography (AMG; Soto et al. 1998). Briefly, paraffin sections (7 μm) were dewaxed in xylene, hydrated in ethanol-water mixtures and left in an oven at 37ºC until completely dried. Tissue sections were covered with a photographic emulsion (Ilford Nuclear Emulsion L4) under safety light conditions. After drying for 45 min in total darkness, sections were rinsed in a developer bath (1:5, b/w Ultrathin Tetenal) for 15 min, rinsed in a stop bath (1% acetic acid) for 1 min, and finally rinsed in a fixative bath (1:10, b/w Agefix Agfa) for 10 min (Soto et al. 1998). Sections were mounted in Kaiser's glycerol gelatine (Merck). Metals were developed as black silver deposits (BSD) and quantified by means of image analysis (BMS, Sevisan, Bilbo). The volume density of BSD (VD_{BSD}) was calculated by stereology as $VD_{BSD}=V_{BSD}/V_{Ti}$, where V_{BSD} is volume of BSD and V_{Ti} is volume of tissue (Soto and Marigómez 1997).

Statistical analysis

The statistical analyses were made using SPSS v 11.5 (SPSS Inc., Chicago, Illinois). Significant ifferences in the different biomarkers between experimental groups were tested by one-way analysis of variance (ANOVA). Significant differences among means were determined after the Duncan's test. In all the cases 95% significance level was established.

RESULTS AND DISCUSSION

Gonad development

Gonad Index exhibited differences between sampling sites (Fig 1). Mature gametes were observed in most mussels, both male and female. However, gametogenesis seemed to be a little bit delayed in mussels kept in cages offshore. Female gonads at "0" Time were at phases 6 and 1, whereas in caged mussels the dominant stages were 5 and 6 with the exception of mussels from 1000 m station where also phase 3 and 1 were identified. Accordingly, gonads in "0" Time male mussels were in phases 5 and 6 and those from caged mussels were at 4 and 5 stages, although gonads at stage 3 were identified in mussels from the 500 m sampling station and gonads at the stage 6 in mussels from 1000 and 10000 m sampling stations. Most mussels presented a highly developed reserve connective tissue, with large numbers of adipogranular and vesicular cells.

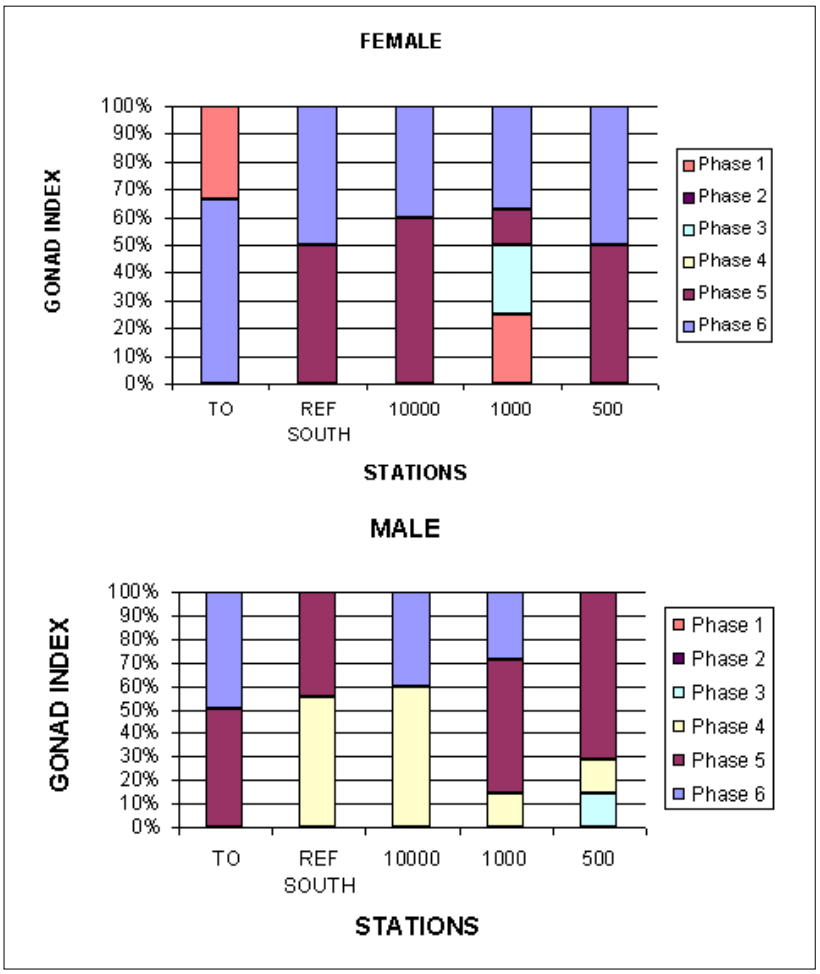


Fig 1. Gonad index recorded in female and male mussels at "0" Time (T0), Reference South (REF SOUTH), and 10000, 1000 and 500 m away from the rig

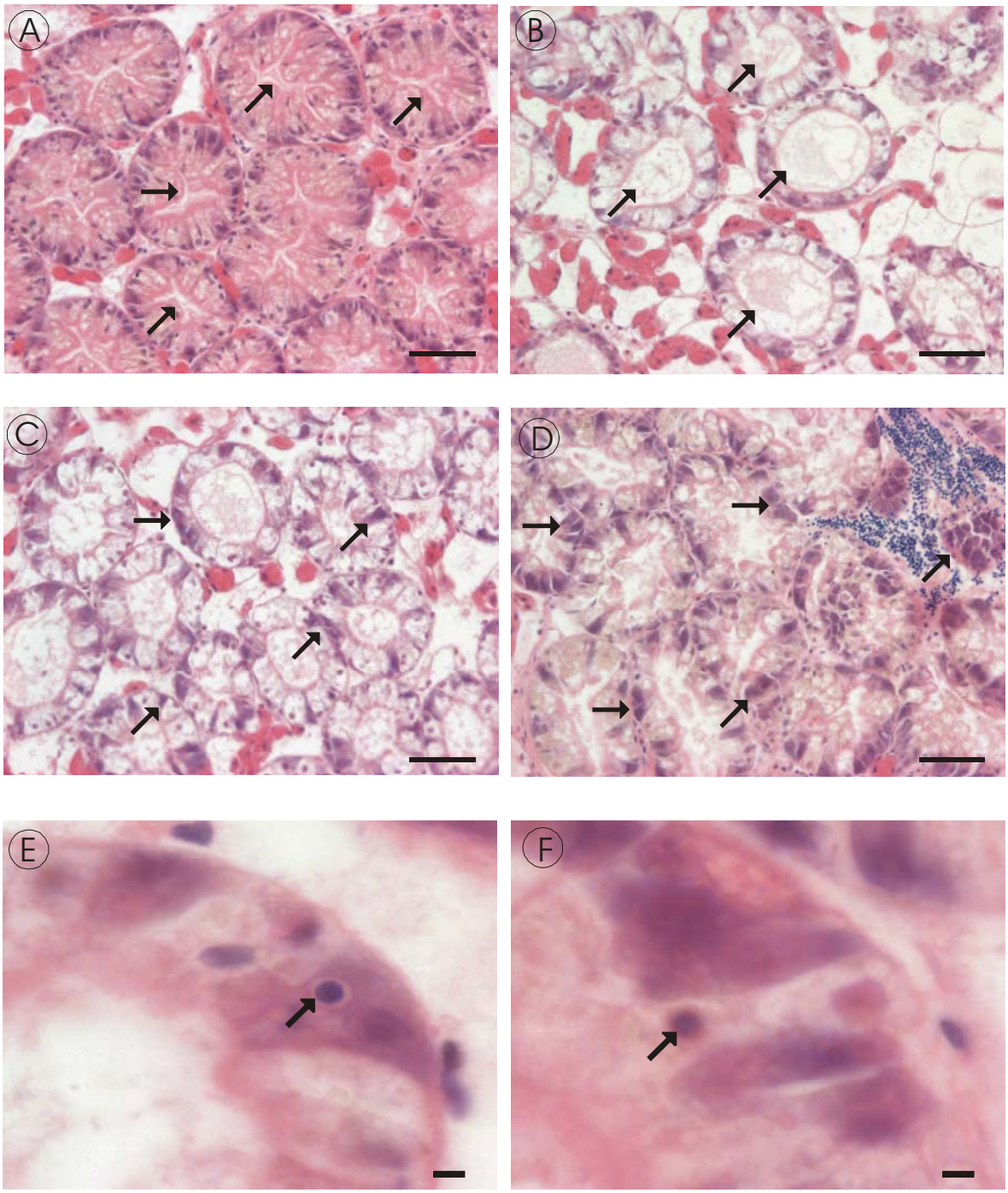


Fig 2. Haematoxylin-eosin stained paraffin-sections of mussel digestive gland from (A) 10000 m. B) "0" Time, (C) 1000 m. (D) 500 m.(E-F) 10000 m. Note (see arrows) narrow lumen in (A) and wide lumen in (B & C). Note (see arrows) basophilic cells in (C) and (D). Note intracellular parasites in (E) and (F). Scale bars: (A-D): 100 μm; (E, F): 10 μm.

Autometallography

In previous laboratory experiments it has been demonstrated that in mussel digestive cell lysosomes BSD-extent increases after one day exposure to metals (Marigómez et al., 2002). Presently, BSD-extent found in digestive gland tissue was very low and therefore there was no need to quantify by image analysis. This low BSD-extent indicated a correspondingly low bioavailability of metals. In conclusion, it seems that metal availability was low at all sampling sites.

Cell-Type Replacement

The relative proportion of basophilic cells increases under environmental stress conditions in molluscan digestive gland (Marigómez et al., 1998). Basophilic cells were conspicuous and apparently very abundant in all the studied mussels, although they were especially abundant in digestive gland of mussels collected at the 500 m sampling station (Fig. 2). Thus, stereological analysis revealed that "0 Time" mussels and mussels from the South Reference site exhibited lower Vv_{BAS} values than mussels collected 500 m away from the rig (Fig 3). However, all the Vv_{BAS} values presently recorded were higher (nearly two-fold) in comparison with data from previous years (WCS 2003) and therefore some stress source might be envisaged in all mussels. Vv_{BAS} values were statistically different between mussels from the South Reference and those collected 500 m away from the rig.

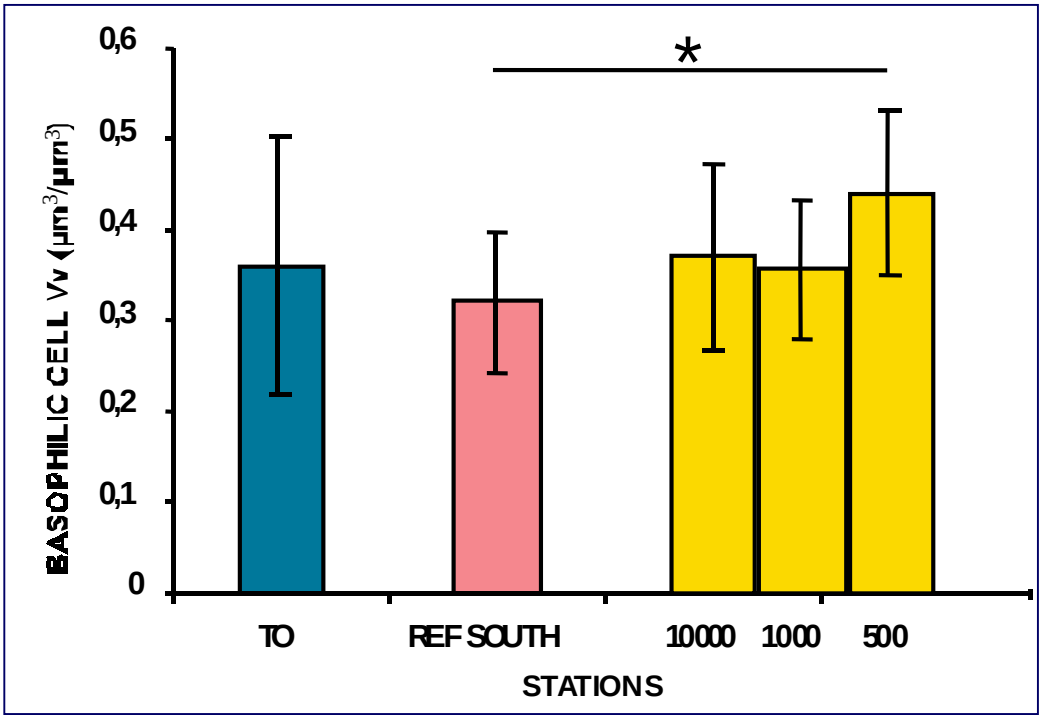


Fig 3. Volume density of basophilic cells in mussel digestive gland epithelium. Vertical segments show standard deviations. Asterisks indicate significant differences ($P<0.05$) between pairs of means (between groups connected by the lines) according to Duncan's test after one-way ANOVA.

Quantitative Structure of Digestive Tubules

Mussels from the "0" Time exhibited a reduced mean epithelial thickness (MET) and increased mean luminal radius (MLR, Fig. 4) when compared with previous results (WCS2003) and when compared withn the rest of the stations. This is also illustrated in Fig. 2, where the digestive tubules of mussels fro the “0” Time showed a thinner epithelium and a wider lumen than mussels from other stations. Accordingly, the parameters MLR/MET (which relates tubule lumen width (MLR) to epithelial thickness) and MET/MDR (which relates epithelial thickness to mean diverticular radius) showed high and low values, respectively (Fig. 4). These results suggest that "0" Time mussels were subjected to some stress source (Vega et al., 1989), which most likely could be due to the reproductive cycle since these mussels were at a post-spawning stage (Fig. 1).

Mussels sampled close to the platform exhibited lower MET and lower MDR values than mussels from other sampling stations along the expected pollution gradient. Accordingly, only MET values corresponding to 10000 and 1000 sampling points were significantly higher than those recorded at "0" Time (Fig. 4).

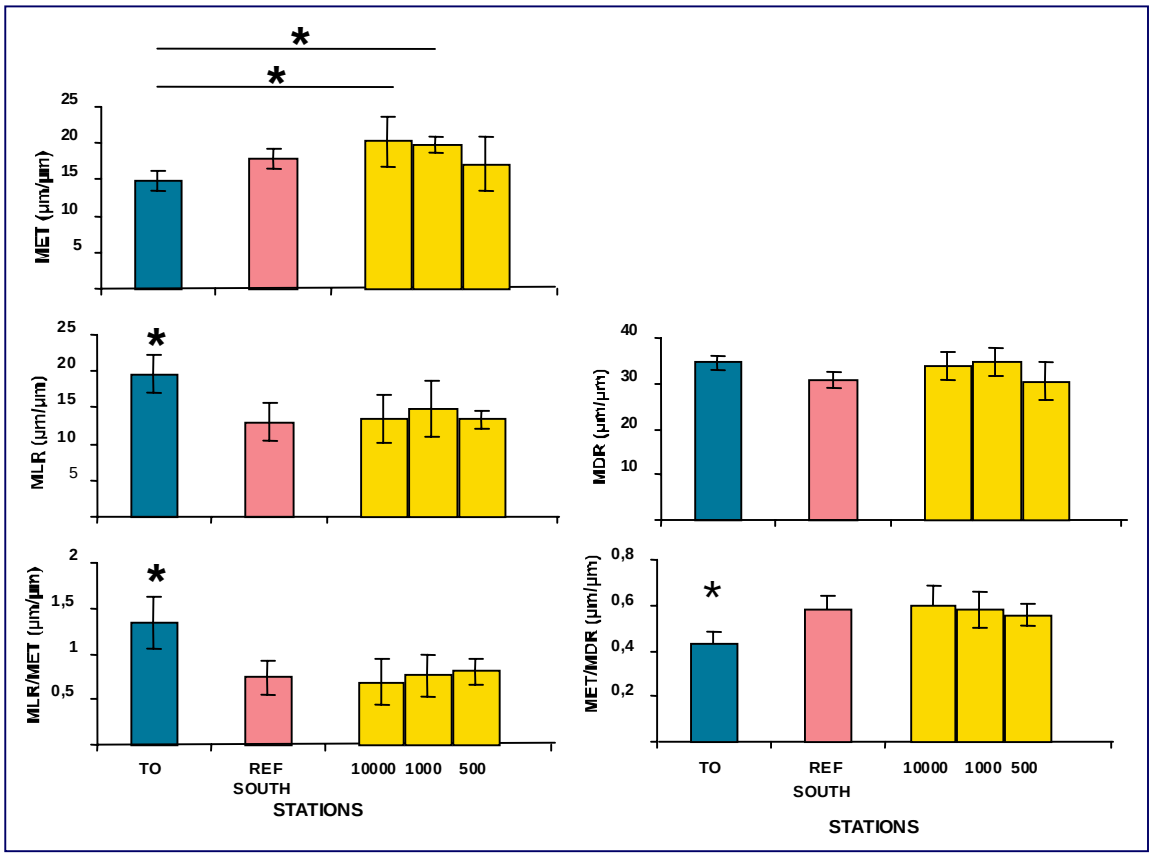


Fig 4. Planimetric parameters (MET, MLR, MDR, MLR/MET, MET/MDR) indicative of the structure of digestive tubules in mussel digestive gland. Vertical segments show standard deviations. Asterisks indicate significant differences (P<0.05) between pairs of means according to de Duncan's test after one-way ANOVA. One single asterisk indicates that this group is different from every other one. Asterisks above lines indicate differences between groups connected by the lines.

Histopathology of the digestive gland

Prevalence of parasites, hemocyte infiltration and general condition of the digestive epithelium were examined. Overall, no significant parasitic infestation or pathological lesion was found in any case. Exceptionally, a weak incidence of intracellular parasites (Fig. 2 E-F), were found in mussels collected at 10000 (30%) and 1000 m (20%) away from the rig.

CONCLUSIONS

- Biomarkers of metal exposure (BSD-extent in digestive cell lysosomes) indicated that mussels were not exposed to significant levels of metal pollution.

- "0" Time mussels do not seem to be an adequate reference for comparison purposes. In the present study mussels collected at "0" Time did not show a good condition of the digestive gland according to tissue level biomarkers and histopathology (low MET, high MLR/MET, high VvBAS).

- "0" Time sample did not provide any comparable information for WCS2004 purposes. For further surveys, we do not see the need to spend any effort in processing and analyzing this sample because it is not useful at all for comparative purposes. Similar conclusions were attained in WSC2003. The only reason to keep this sample, just in case, is that it might be valuable as inner control or to check sentinel quality if anomalous results were obtained.

- Histological observations of gonad tissue revealed that both gonad and reserve tissue were in good condition in all sampling sites, including "0" Time. It seems, however, that caging might produce a slight delay in gametogenesis in both males and females.

- Vv_{BAS} and planimetric parameters indicated that mussels were not fully healthy but, even so, more marked distress was recorded in mussels from the 500 m sampling station in comparison with 1000 and 10000 m sampling stations.

- Overall, our present results have a limited value for diagnosis since the measured parameters were very limited without complementary information provided by other biomarkers and measures. Biomarkers must be applied in a fully informative battery and presently we lacked frozen material for cryotomy and biochemistry, so that other complementary biomarkers such as peroxisome proliferation, lysosomal responses and neutral lipid accumulation could not be measured. If proper samples were available it would be very interesting to complete the battery of biomarkers.

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ANNEXE

CELL-TYPE REPLACEMENT MUSSEL DIGESTIVE GLAND EPITHELIUM

Basophilic cells volume density

ANOVA

BASOFILA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7,832E-02	4	1,958E-02	1,900	,127
Within Groups	,464	45	1,031E-02		
Total	,542	49			

Homogeneous Subsets

BASOFILA

Duncan[a]

		Subset for alpha = .05	
GRUPO	N	1	2
5,00	10	,31974760	
3,00	10	,35536474	,35536474
1,00	10	,36033136	,36033136
2,00	10	,37002431	,37002431
4,00	10		,44072134
Sig.		,321	,092

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10,000.

QUANTITATIVE STRUCTURE OF DIGESTIVE TUBULES IN MUSSEL

MET

ANOVA

MET

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	93,133	4	23,283	3,835	,018
Within Groups	121,418	20	6,071		
Total	214,550	24			

Post Hoc Tests
Homogeneous Subsets

MET

Duncan[a]

GRUPO	N	Subset for alpha = .05	
		1	2
1,00	5	14,92859	
4,00	5	17,09606	17,09606
5,00	5	17,81992	17,81992
3,00	5		19,79353
2,00	5		20,26463
Sig.		,093	,075

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5,000.

MDR

ANOVA

MDR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	83,286	4	20,821	2,486	,076
Within Groups	167,525	20	8,376		
Total	250,810	24			

MDR

Duncan[a]

GRUPO	N	Subset for alpha = .05
		1
4,00	5	30,45525
5,00	5	30,84792
2,00	5	33,75467
1,00	5	34,53129
3,00	5	34,65801
Sig.		,050

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5,000.

MET/MDR

ANOVA

METMDR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8,860E-02	4	2,215E-02	4,970	,006
Within Groups	8,914E-02	20	4,457E-03		
Total	,178	24			

Post Hoc Tests
Homogeneous Subsets

METMDR

Duncan[a]

		Subset for alpha = .05	
GRUPO	N	1	2
1,00	5	,43373042	
4,00	5		,55747435
3,00	5		,57639511
5,00	5		,57981724
2,00	5		,60060080
Sig.		1,000	,361

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5,000.

MLR

ANOVA

MLR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	149,890	4	37,472	4,533	,009
Within Groups	165,329	20	8,266		
Total	315,218	24			

Post Hoc Tests
Homogeneous Subsets

MLR

Duncan[a]

		Subset for alpha = .05	
GRUPO	N	1	2
5,00	5	13,02795	
4,00	5	13,35922	
2,00	5	13,48999	
3,00	5	14,86446	
1,00	5		19,60264
Sig.		,366	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5,000.

MLR/MET

ANOVA

MLRMET

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,394	4	,348	6,994	,001
Within Groups	,997	20	4,983E-02		
Total	2,390	24			

Homogeneous Subsets

MLRMET

Duncan[a]

		Subset for alpha = .05	
GRUPO	N	1	2
2,00	5	,69218569	
5,00	5	,74083682	
3,00	5	,75963086	
4,00	5	,80423415	
1,00	5		1,332647
Sig.		,476	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5,000.

Appendix C. Data report – CEFAS (Weymouth)

Saithe histopathology NIVA Water Column Survey 2004

NIVA reference: LID/ARU, J.No. 1650/04; S.No. 24215

Objective: To undertake histopathological analysis of liver from wild-caught saithe as submitted, using standard histological techniques.

Histopathology report

Saithe (*Pollachius virens*) (n=79) were collected as part of the NIVA Water Column Monitoring Survey in 2004. Fish were collected from within 500m, 1000m and 10,000m of an industrial oil installation. Livers from saithe were received dispatched to the CEFAS Weymouth Laboratory, UK where they were processed for histology.

Upon receipt, samples were transferred to 70 % industrial methylated spirit (IMS) for processing to wax in a vacuum infiltration processor using standard protocols. Sections were cut at 3-5 μm on a rotary microtome and resulting tissue sections were mounted onto glass slides before staining with haematoxylin and eosin (H & E). Stained sections were analysed by light microscopy (Nikon Eclipse E800) and digital images were taken using the LuciaG™ Screen Measurement System (Nikon, UK).

Liver pathology criteria were derived from those specified by the International Council for the Exploration of the Sea (ICES) (Anon, 1999) and by Feist et al. (2004). The presence of parasites was also recorded. A three-point classification system for hepatocyte vacuolation, based upon that described by Stentiford et al. (2003) was used. This scale (Vac I-III) represented a progressive increase in the vacuolation of liver cells.

Results

The liver from the majority of saithe examined was highly vacuolated (Vac III) with hepatocytes containing large single or multiple vacuoles (Fig. 1). The overall appearance was of a lipid storage liver. In some specimens, the vacuoles were reduced in size (Vac II), with larger amounts of basophilic cytoplasm. No significant differences were observed in the vacuolation status of saithe captured from the different locations (see Table 1).

A relatively high proportion of fish captured from each location contained regions of inflammation depicted by infiltration by leukocytes (Fig. 2) or in some cases, pronounced lesions reminiscent of visceral granulomatosis (Fig. 3). The cause of such lesions could not be determined though several fish from each site were shown to be infected by anasakid nematodes (Fig. 4). Nematodes were commonly seen at the surface of the liver, occasionally bisecting the liver parenchyma and often associated with inflammatory host responses (Fig. 5). In certain cases, the liver of fish from each site was seen to contain melanomacrophage aggregates

(Fig. 6). No other significant pathologies (including those associated with the carcinogenic pathway) were recorded in the liver of saithe captured from the three sites and no apparent significant difference was observed between sites. A summary of liver pathology prevalence at the three sites is given in Table 1.

Site	NP	Necrosis	MMA	Inflammation	Granuloma	Fibrosis	VAC
500m	50	0	8.3	41.7	4.2	4.2	2.92
1000m	68.9	0	3.4	31.0	6.9	0	2.93
10000m	65.2	4.3	4.3	34.8	0	0	2.91

Table 1. Summary of pathologies detected in the livers of saithe captured at 500m, 1,000m and 10,000m from oil platform. Key: NP (no pathology detected), MMA (melanomacrophage aggregates) and VAC (mean vacuolation status of hepatocytes, based on a three point scale where 3 is highest). Inflammatory pathologies (including granuloma, MMA and fibrosis) were slightly elevated in fish captured closest to the platform.

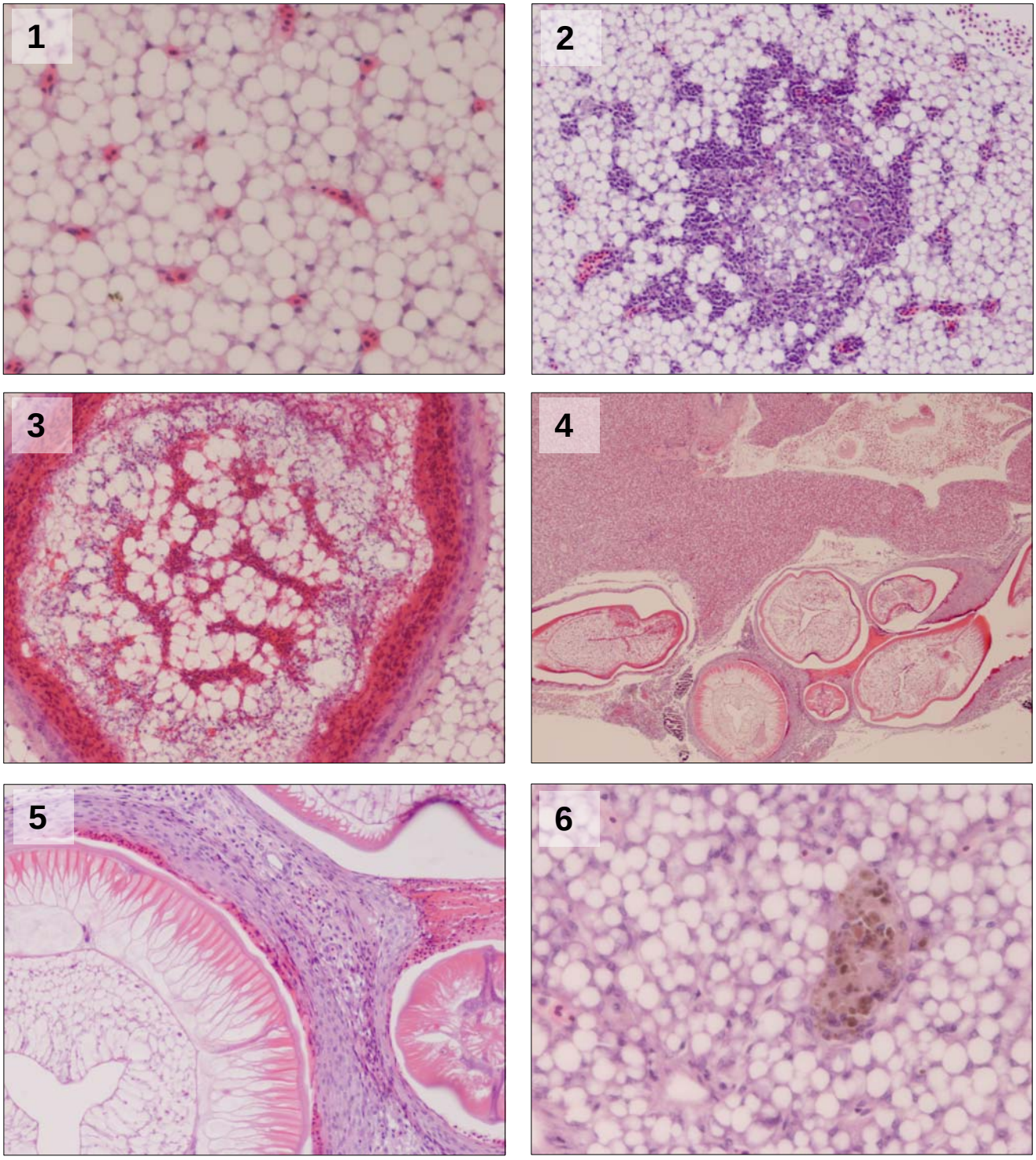
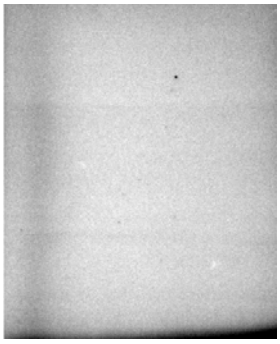


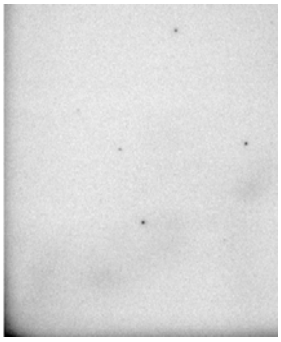
Fig 1-6. Liver Histopathology of saithe captured at 500m, 1,000m and 10,000m from oil platform. (1) Normal liver showing pronounced fatty vacuolation. (2). Inflammatory focus with necrotic hepatocytes. (3). Granulomatous lesion, containing necrotic hepatocytes. (4). Anasakid nematodes at the surface of the liver. (5). Inflammtory response to nematodes at the surface of the liver. (6). Melanomacrophage aggregate (MMA) amongst normal fatty hepatocytes.

Appendix D. Data report – ITM, Stockholms Universitet (Stockholm)

location	fish number	DNA adducts (nmol/mol DNA)	location	fish number	DNA adducts (nmol/mol DNA)	location	fish number	DNA adducts (nmol/mol DNA)
500 m	**1**	**≤1,53**	1000 m	25	**≤2,14**	10000 m	55	**0,823**
500 m	3	**1,64**	1000 m	26	**5,93**	10000 m	**56**	**5,17**
500 m	4	**6,59**	1000 m	**27**	**0,219**	10000 m	57	**≤1,02**
500 m	5	**7,56**	1000 m	28	**≤0,899**	10000 m	58	**0,865**
500 m	6	**1,16**	1000 m	29	**1,35**	10000 m	59	**7,35**
500 m	7	**1,01**	1000 m	30	**1,54**	10000 m	60	**1,92**
500 m	8	**2,45**	1000 m	31	**5,94**	10000 m	61	**≤1,14**
500 m	9	**2,65**	1000 m	32	**≤1,84**	10000 m	62	**12,9**
500 m	10	**0,278**	1000 m	33	**1,55**	10000 m	63	**≤2,60**
500 m	11	**2,88**	1000 m	34	**2,97**	10000 m	64	**2,06**
500 m	12	**1,79**	1000 m	35	**2,09**	10000 m	65	**≤1,42**
500 m	13	**≤0,447**	1000 m	36	**1,52**	10000 m	66	**1,75**
500 m	14	**1,26**	1000 m	37	**10,8**	10000 m	67	**5,07**
500 m	15	**1,32**	1000 m	38	**≤3,67**	10000 m	68	**1,07**
500 m	16	**2,62**	1000 m	39	**1,67**	10000 m	69	**2,23**
500 m	17	**≤1,21**	1000 m	40	**3,97**	10000 m	70	**1,26**
500 m	18	**≤1,15**	1000 m	41	**≤0,737**	10000 m	71	**6,84**
500 m	19	**8,74**	1000 m	42	**≤1,31**	10000 m	72	**≤1,49**
500 m	20	**≤0,995**	1000 m	43	**3,02**	10000 m	73	**0,913**
500 m	21	**≤1,09**	1000 m	44	**1,15**	10000 m	74	**3,14**
500 m	22	**2,83**	1000 m	45	**≤1,21**	10000 m	75	**2,75**
500 m	23	**3,32**	1000 m	46	**≤2,09**	10000 m	76	**1,09**
500 m	24	**3,53**	1000 m	47	**≤2,00**	10000 m	77	**1,33**
			1000 m	48	**≤1,14**	10000 m	78	**0,375**
			1000 m	49	**≤1,03**	10000 m	79	**1,13**
			1000 m	52	**1,70**			
			1000 m	54	**2,11**			

WCM 2004
500m


1 ≤1,53nmol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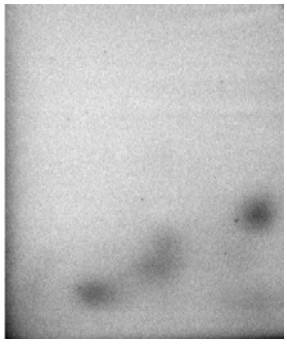
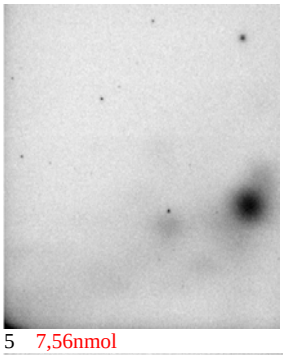
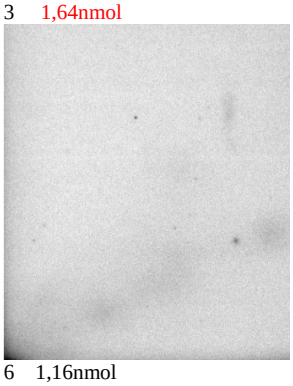
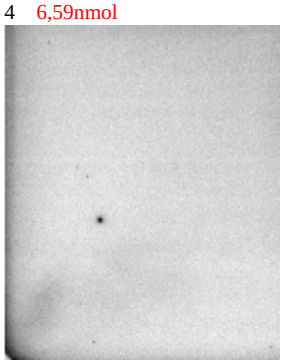
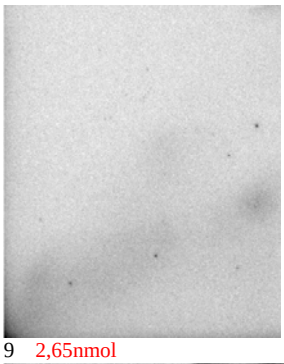

4 6,59nmol


5 7,56nmol

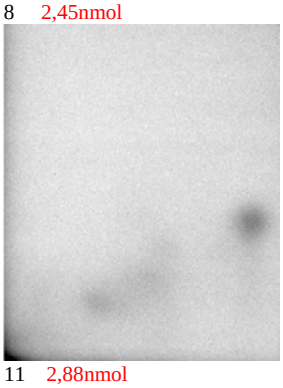

6 1,16nmol

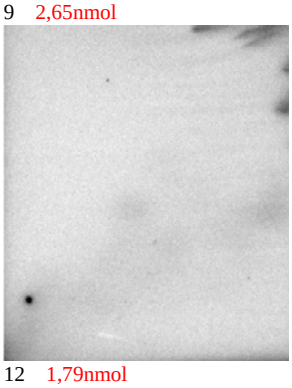

7 1,01nmol

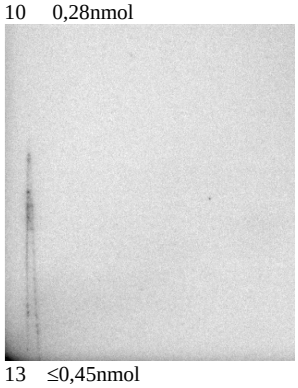
8 2,45nmol

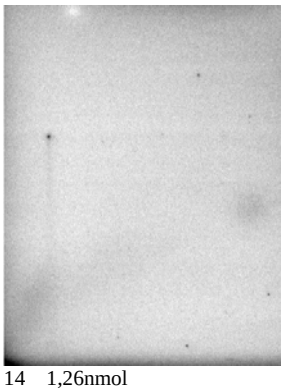

9 2,65nmol

10 0,28nmol

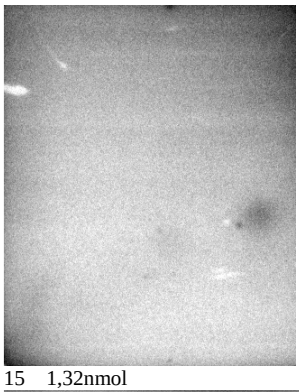

11 2,88nmol


12 1,79nmol

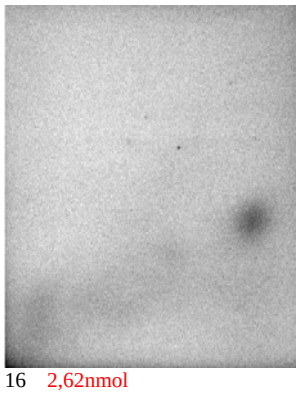

13 ≤0,45nmol



14 1,26nmol

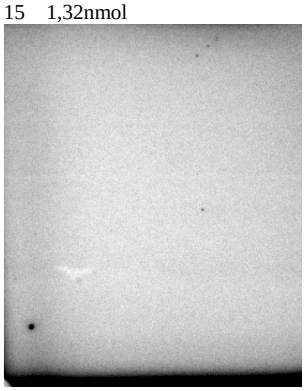


15 1,32nmol

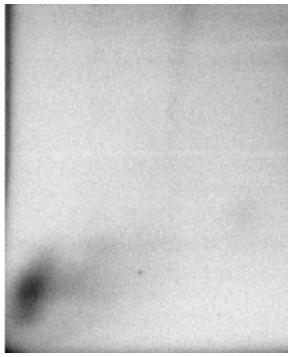


16 2,62nmol

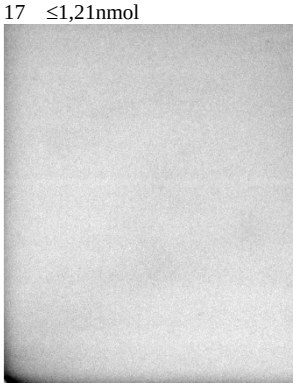
17 ≤1,21nmol



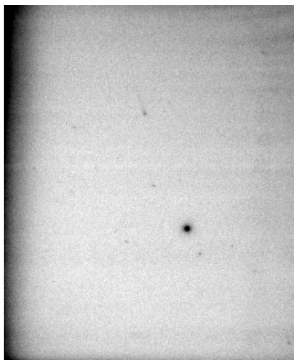
18 ≤1,15nmol



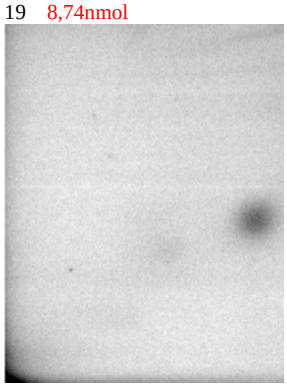
19 8,74nmol



20 ≤0,99nmol

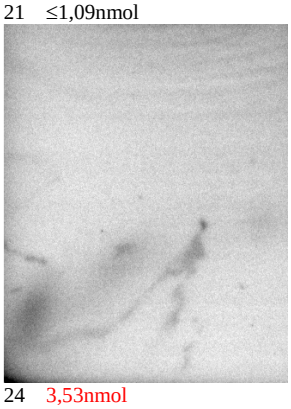


21 ≤1,09nmol



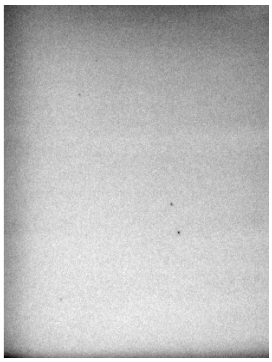
22 2,83nmol

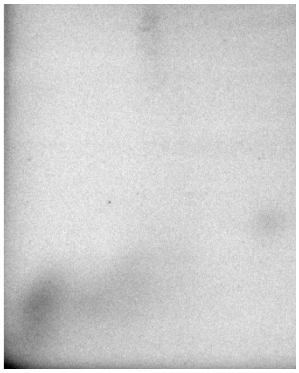
23 3,32nmol

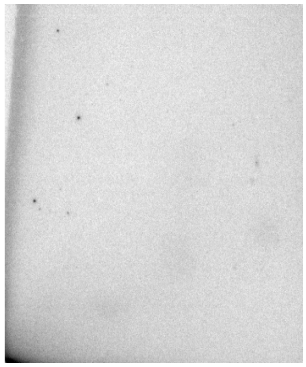


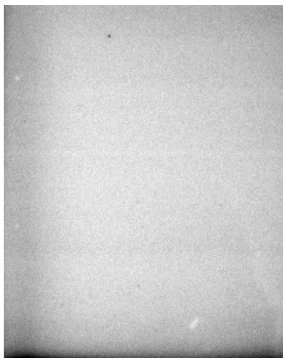
24 3,53nmol

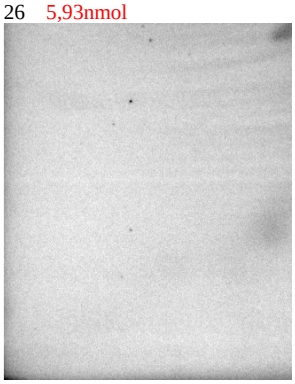
WCM 2004
1000m

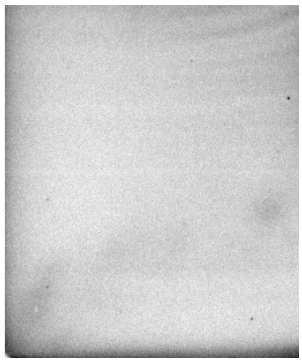

25 ≤1,48nmol

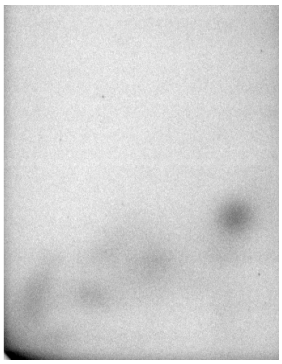

26 5,93nmol

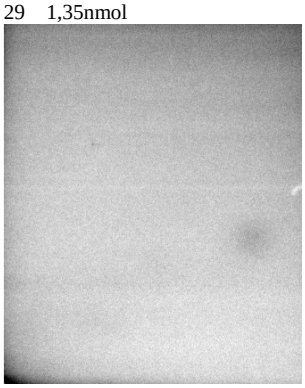

27 0,22nmol

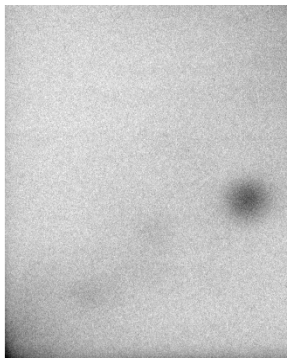

28 ≤0,90nmol

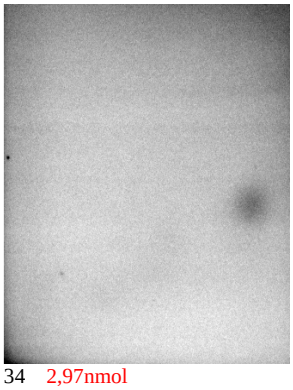

29 1,35nmol

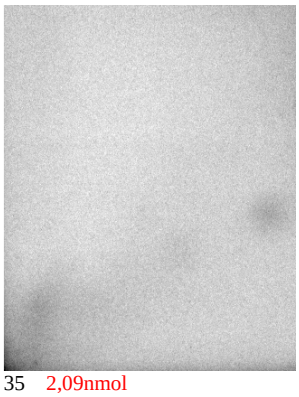

30 1,54nmol

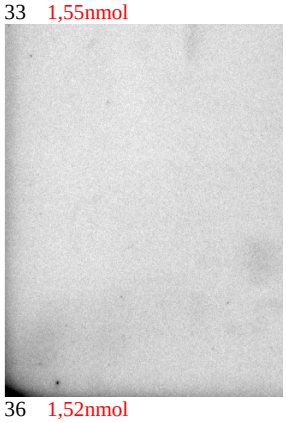

31 5,94nmol

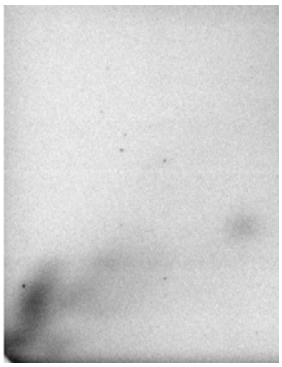

32 ≤1,84nmol


33 1,55nmol


34 2,97nmol


35 2,09nmol


36 1,52nmol



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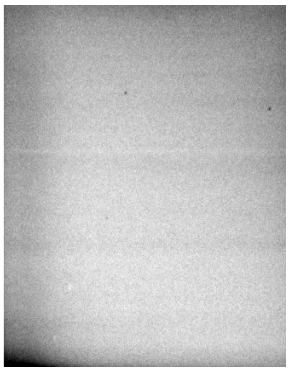
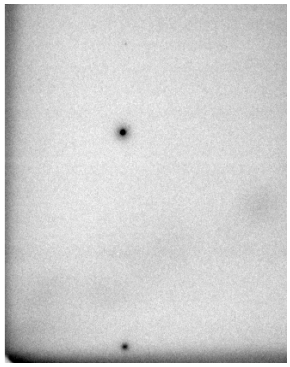
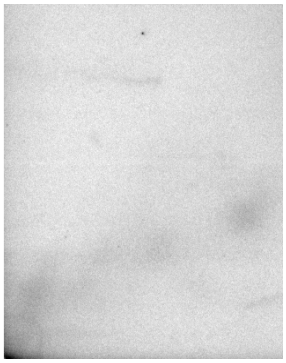


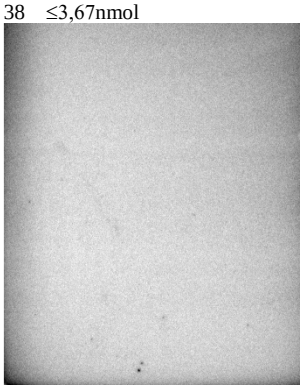
38 ≤3,67nmol



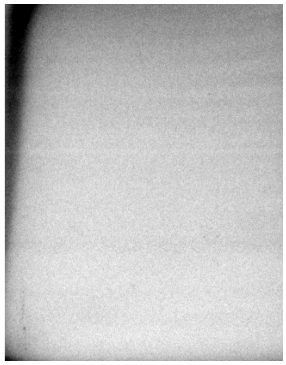
39 1,62nmol



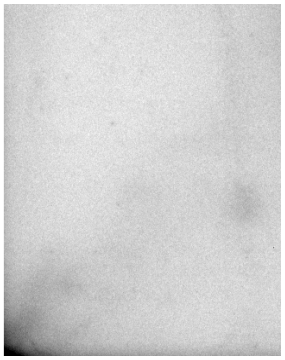
40 3,97nmol



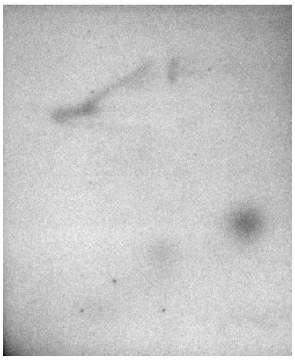
41 ≤0,74nmol



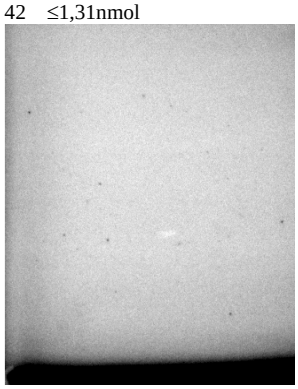
42 ≤1,31nmol



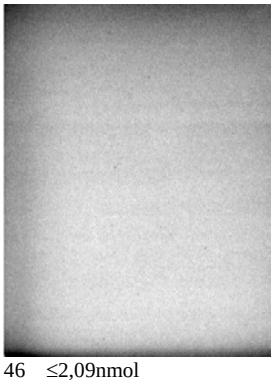
43 3,02nmol



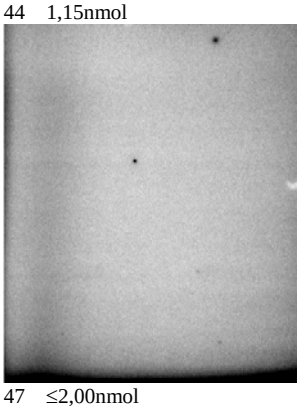
44 1,15nmol



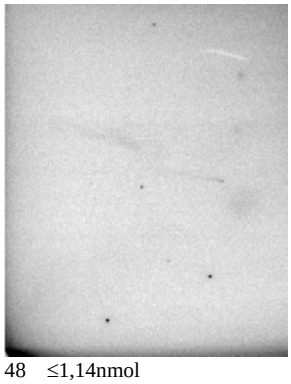
45 ≤1,21nmol



46 ≤2,09nmol



47 ≤2,00nmol



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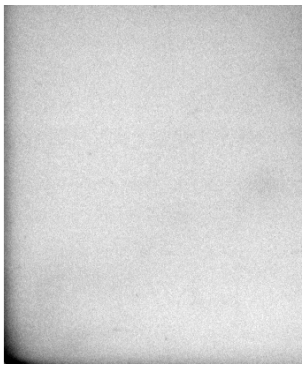
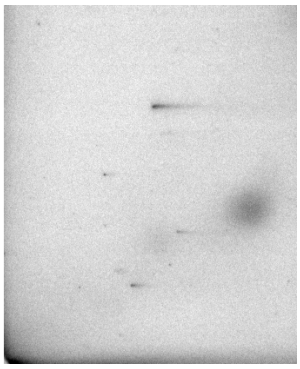
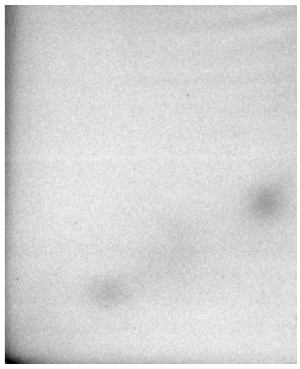


49 ≤1,03nmol

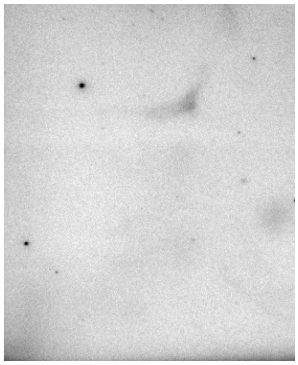


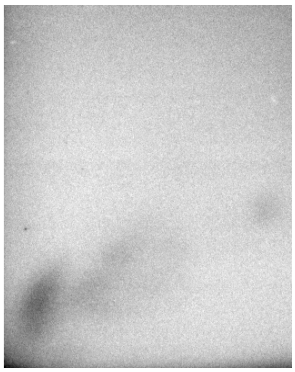
52 1,7nmol

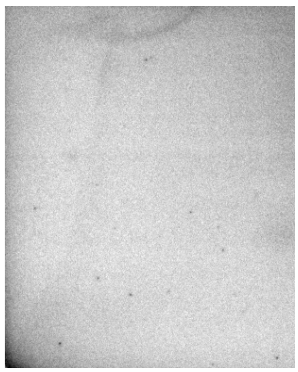


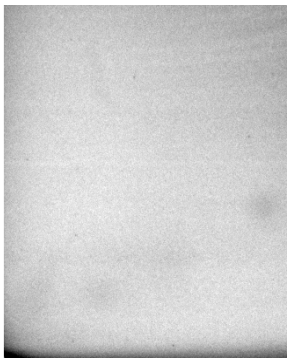
54 2,11nmol

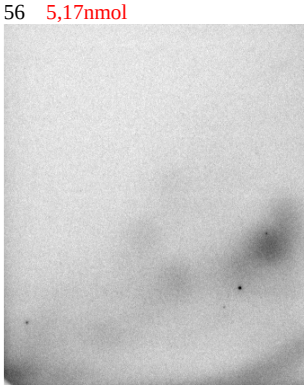
WCM 2004
10000m

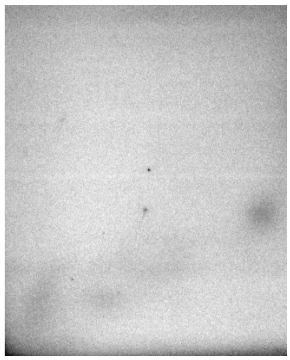

55 0,82nmol


56 5,17nmol

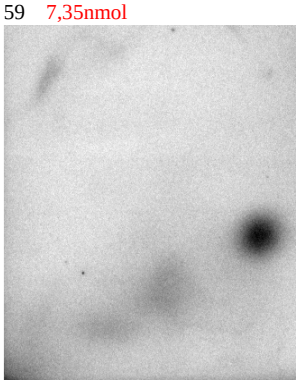

57 ≤1,02nmol


58 0,86nmol

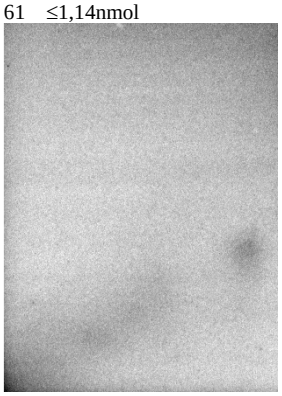

59 7,35nmol

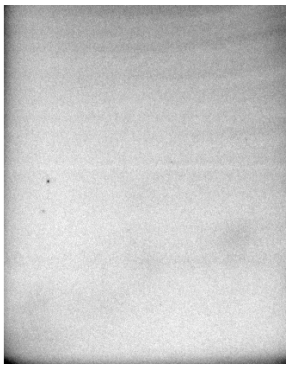

60 1,92nmol

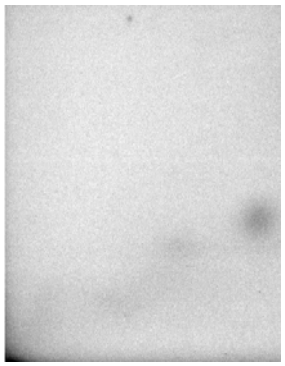
61 ≤1,14nmol

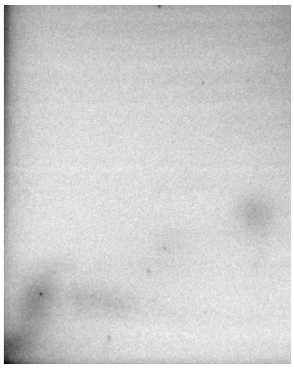

62 12,86nmol

63 ≤2,60nmol

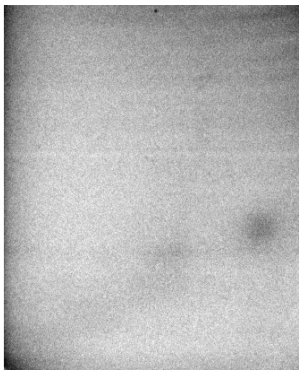

64 2,06nmol


65 ≤1,42nmol

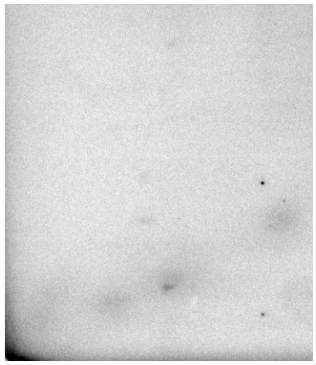

66 1,75nmol



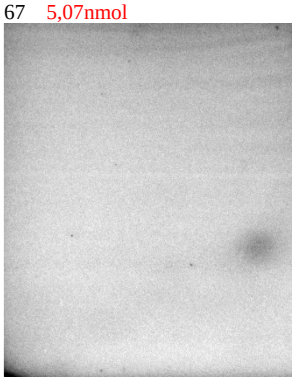
67 5,07nmol



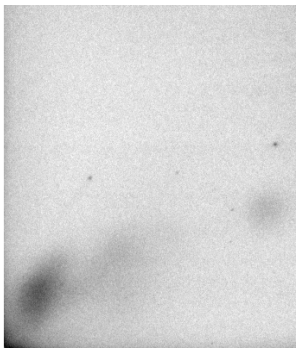
68 1,07nmol



69 2,23nmol

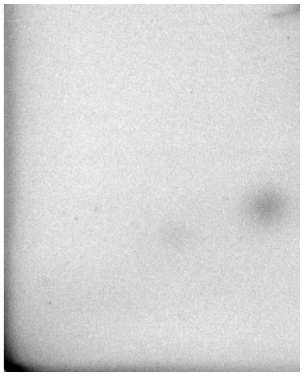


70 1,26nmol

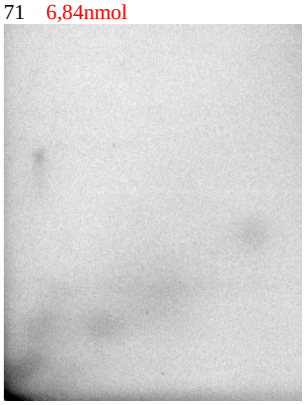


71 6,84nmol

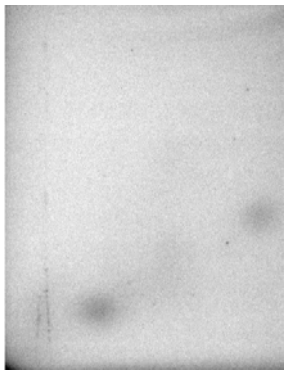
72 ≤1,49nmol



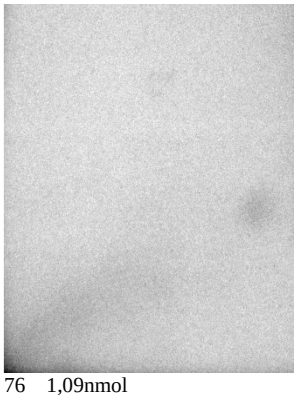
73 0,91nmol



74 3,14nmol

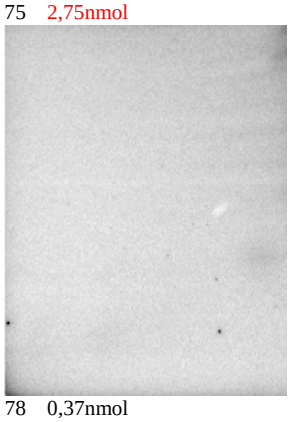


75 2,75nmol

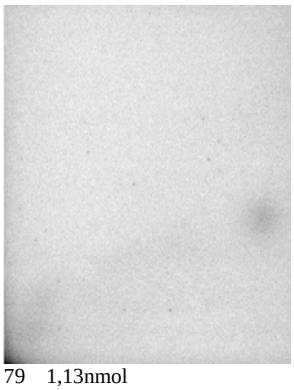


76 1,09nmol

77 1,33nmol

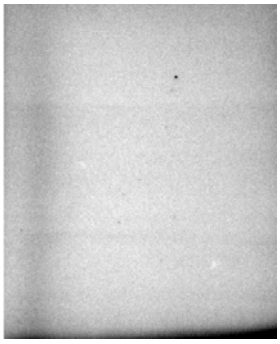


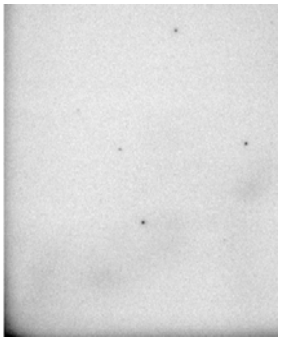
78 0,37nmol

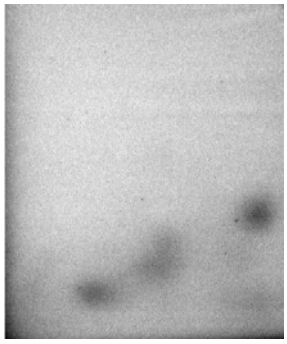


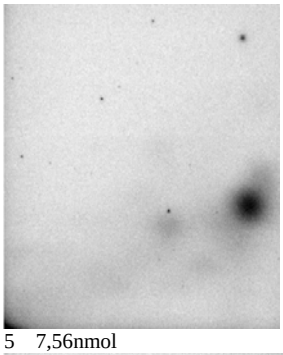
79 1,13nmol

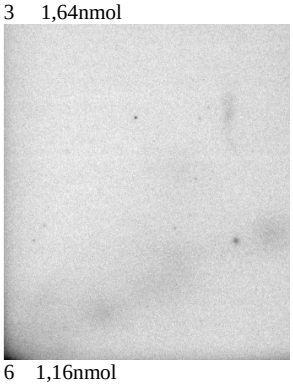
WCM 2004
500m

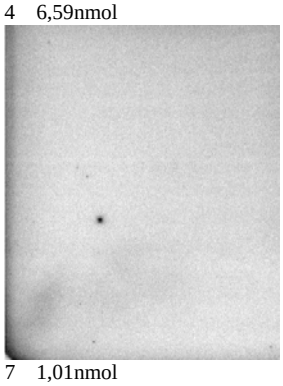

1 ≤1,53nmol


3 1,64nmol

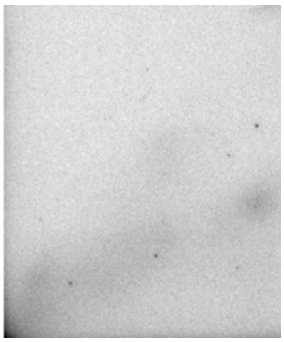

4 6,59nmol

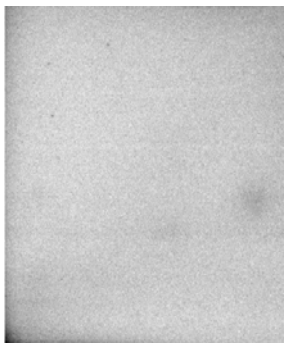

5 7,56nmol

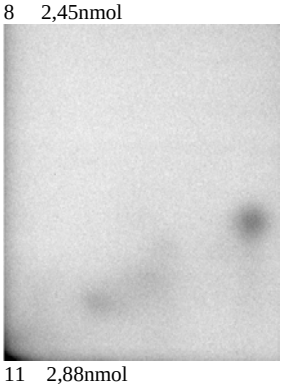

6 1,16nmol

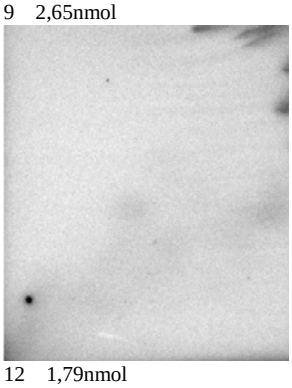

7 1,01nmol

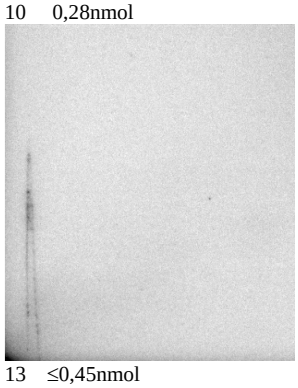
8 2,45nmol

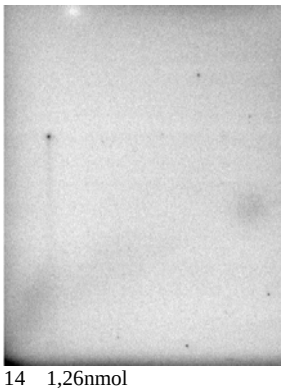

9 2,65nmol


10 0,28nmol

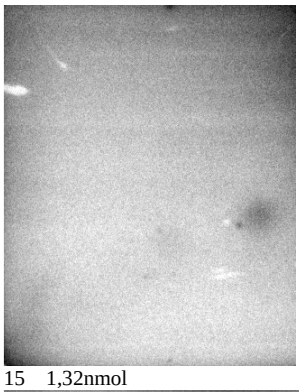

11 2,88nmol


12 1,79nmol

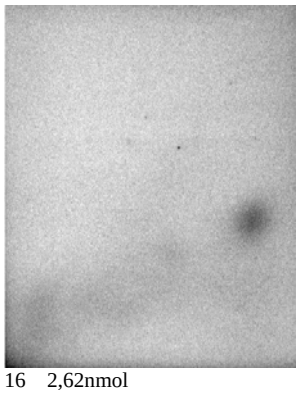

13 ≤0,45nmol



14 1,26nmol

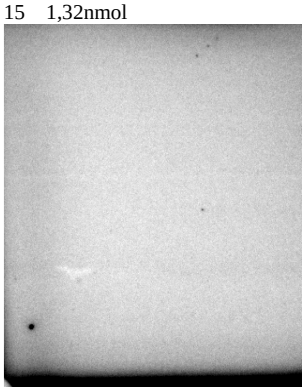


15 1,32nmol

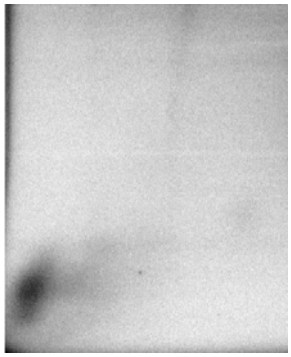


16 2,62nmol

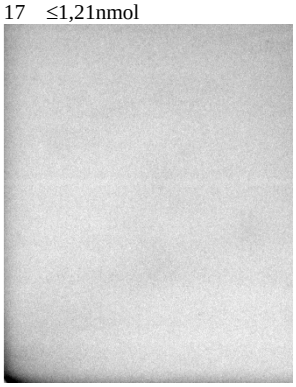
17 ≤1,21nmol



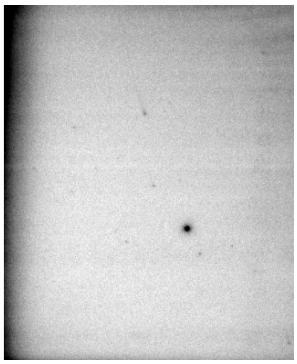
18 ≤1,15nmol



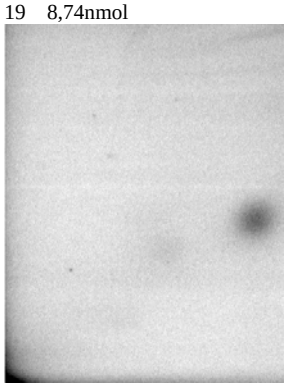
19 8,74nmol



20 ≤0,99nmol

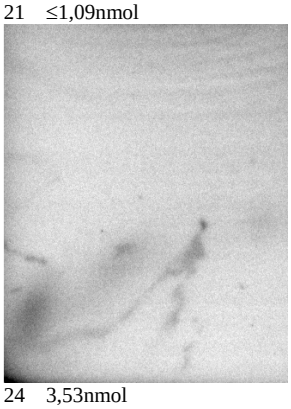


21 ≤1,09nmol



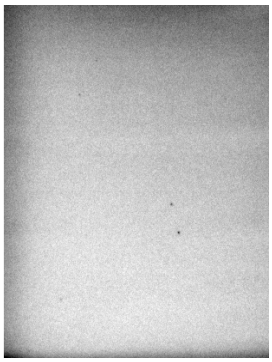
22 2,83nmol

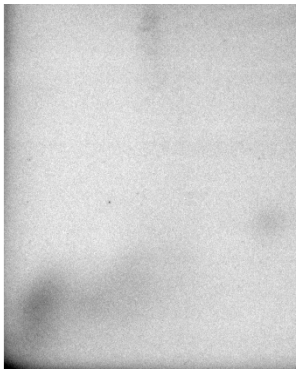
23 3,32nmol

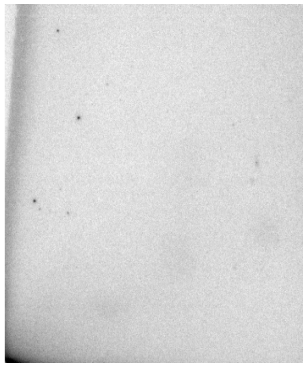


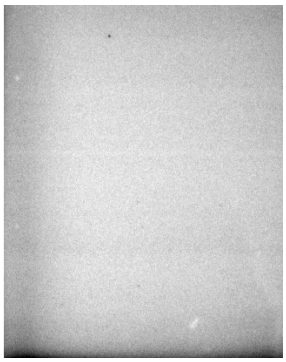
24 3,53nmol

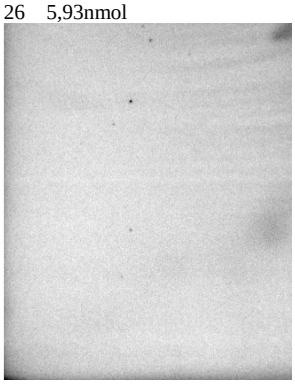
WCM 2004
1000m

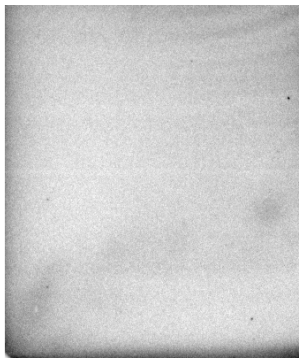

25 ≤1,48nmol

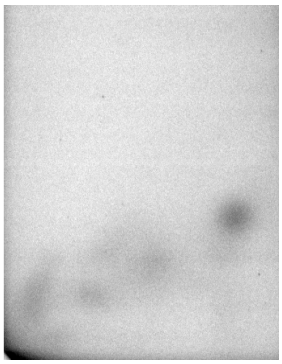

26 5,93nmol

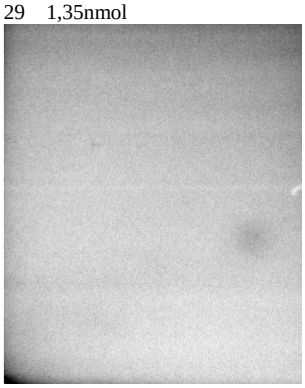

27 0,22nmol

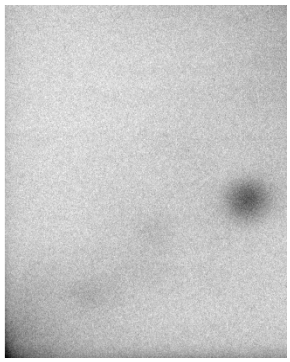

28 ≤0,90nmol

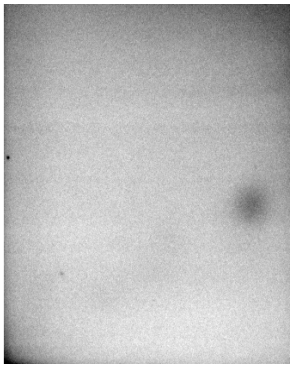

29 1,35nmol

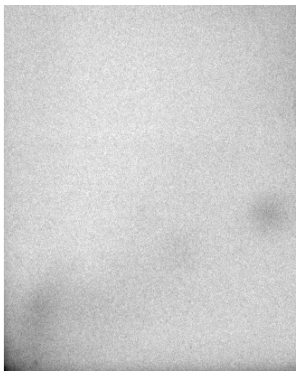

30 1,54nmol

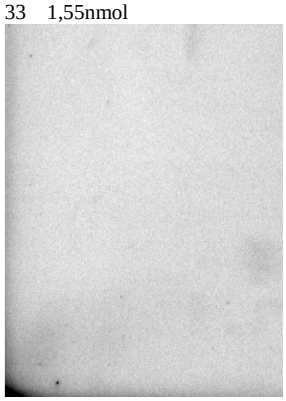

31 5,94nmol

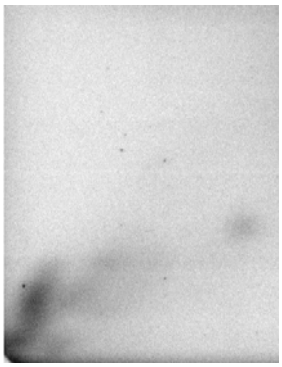

32 ≤1,84nmol


33 1,55nmol

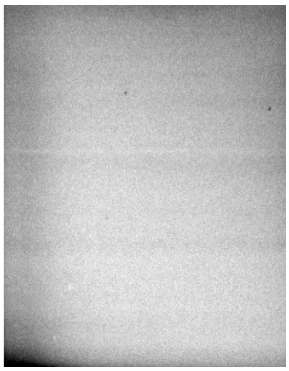

34 2,97nmol


35 2,09nmol

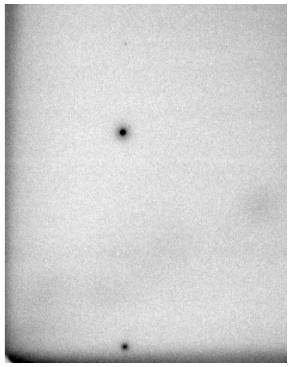

36 1,52nmol



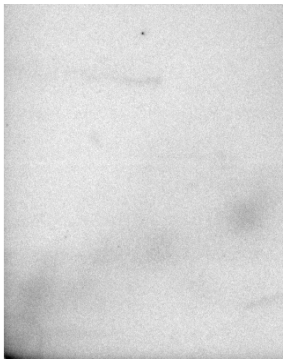
37 10,80nmol



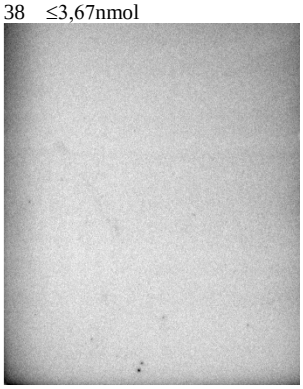
38 ≤3,67nmol



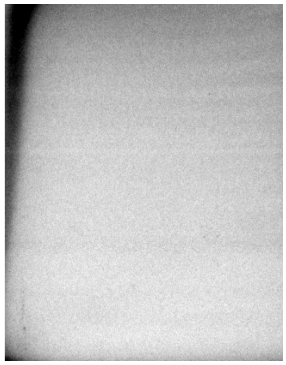
39 1,62nmol



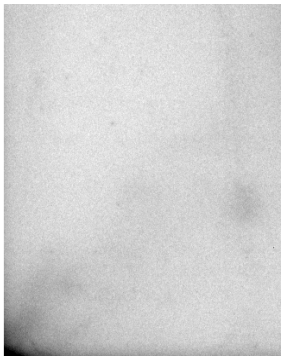
40 3,97nmol



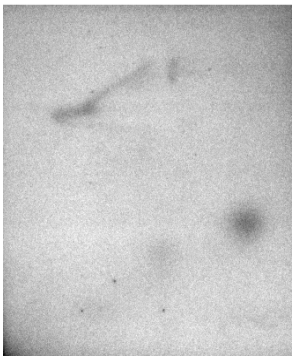
41 ≤0,74nmol



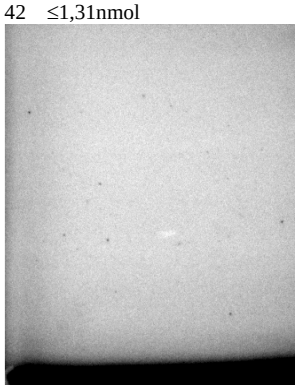
42 ≤1,31nmol



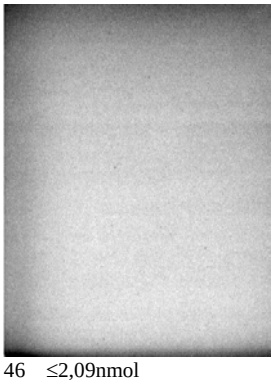
43 3,02nmol



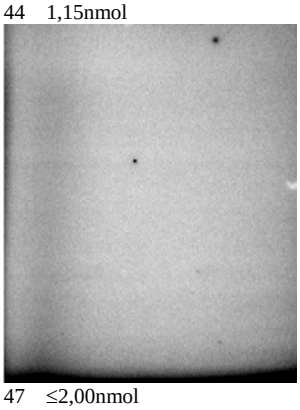
44 1,15nmol



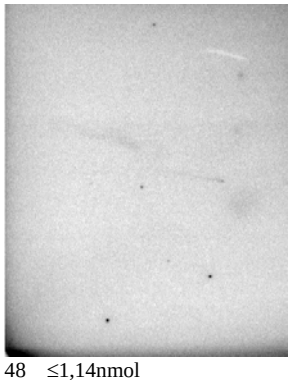
45 ≤1,21nmol



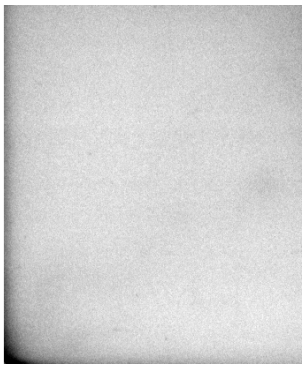
46 ≤2,09nmol



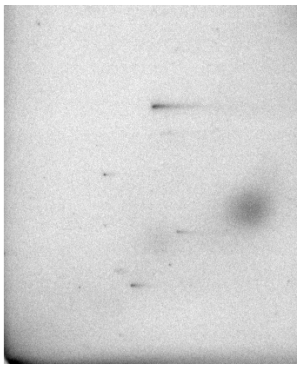
47 ≤2,00nmol



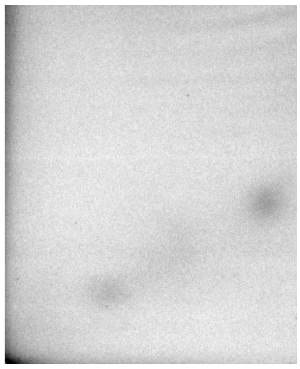
48 ≤1,14nmol



49 ≤1,03nmol

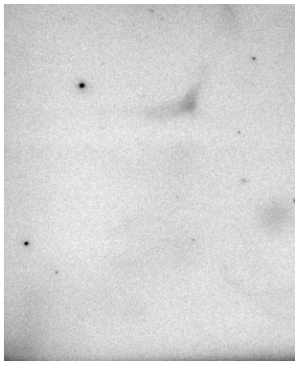


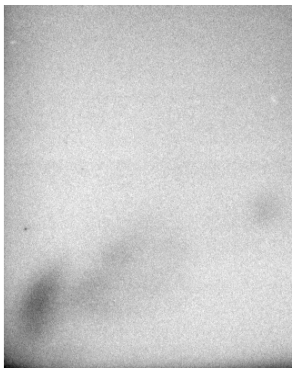
52 1,7nmol



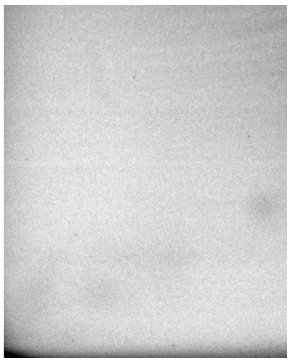
54 2,11nmol

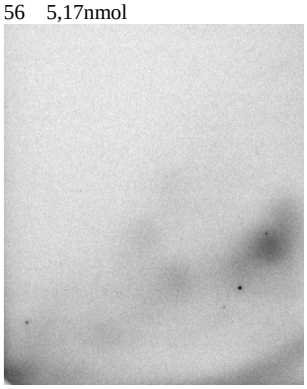
WCM 2004
10000m

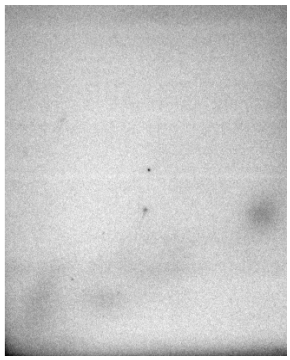

55 0,82nmol


56 5,17nmol

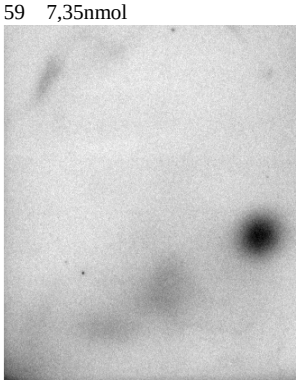
57 ≤1,02nmol


58 0,86nmol

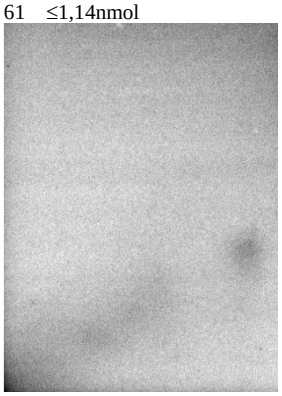

59 7,35nmol

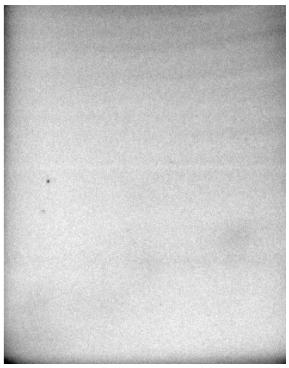

60 1,92nmol

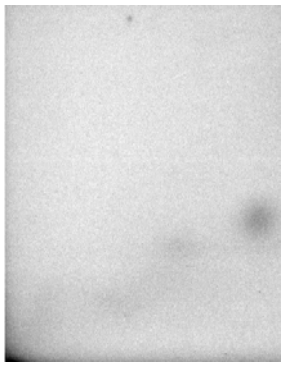
61 ≤1,14nmol

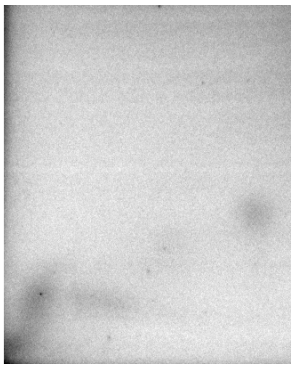

62 12,86nmol

63 ≤2,60nmol

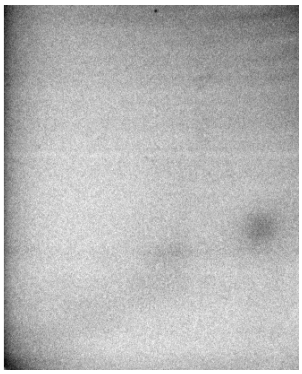

64 2,06nmol


65 ≤1,42nmol

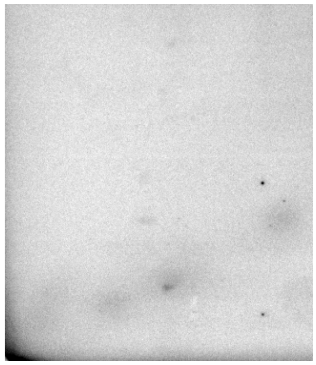

66 1,75nmol



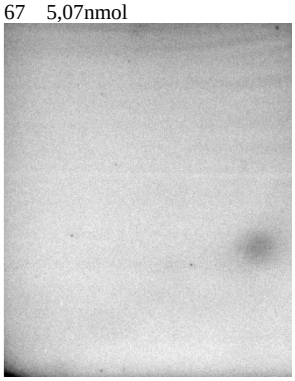
67 5,07nmol



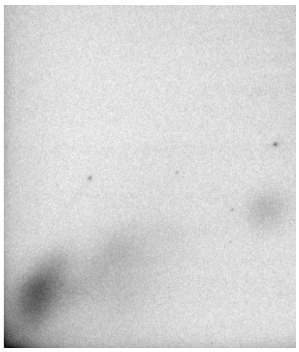
68 1,07nmol



69 2,23nmol

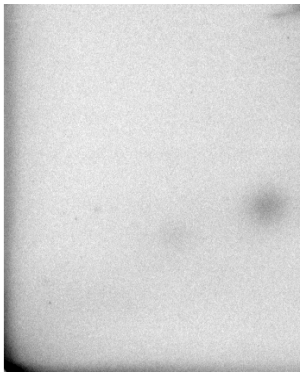


70 1,26nmol

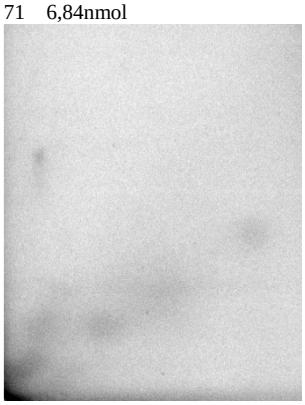


71 6,84nmol

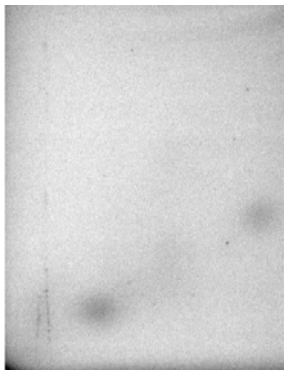
72 ≤1,49nmol



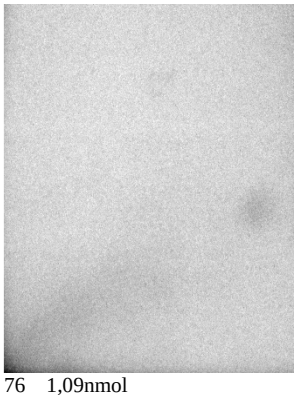
73 0,91nmol



74 3,14nmol

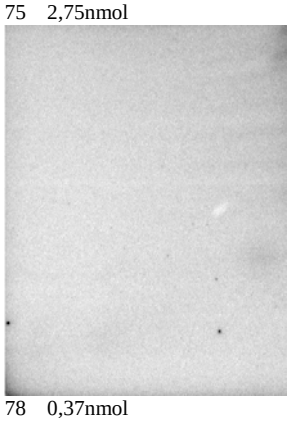


75 2,75nmol

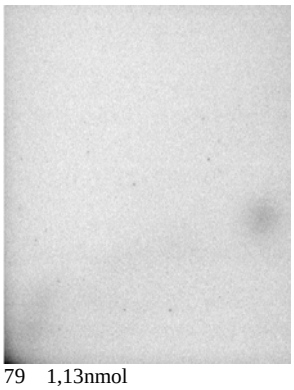


76 1,09nmol

77 1,33nmol



78 0,37nmol



79 1,13nmol

Appendix E. Data report – NIVA (Oslo)

Appendix E.

Data report

Water Column Monitoring 2004

NIVA (Oslo)

Photo: Hylland, Lang

Table 1. Body burden (µg/kg wet wt.) of naphthalene, dibenzothiophene, their alkylated homologues (of which some components are specified) and 16 EPA PAHs in individual blue mussels from the different experimental groups. Note that more than normal uncertainty (for the laboratory) must be taken into consideration for values <0.5.

Group	10 000 m	10 000 m	10 000 m
Total dry matter (g/kg)	181	161	168
Lipid (%)	1.5	1.1	1.4
All conc. are µg/kg wet wt.			
Biphenyl	<0.2	0.33	<0.2
Naphthalene	<1.5	<1.5	<1.5
C1-Naphthalenes	<2	<2	<2
C2-Naphthalenes	<2	<2	<2
C3-Naphthalenes	8.2	8	5.3
Acenaphthylene	<0.2	<0.2	<0.2
Acenaphthene	<0.2	<0.2	<0.2
Fluorene	<0.2	0.3	0.21
Anthracene	<0.2	<0.2	<0.2
Phenanthrene	0.66	<0.5	<0.5
C1-Phenanthrenes	3.1	<2	2.1
C2-Phenanthrenes	20	11	13
C3-Phenanthrenes	14	8.8	11
Dibenzotiophene	<0.5	<0.5	<0.5
C1-Dibenzotiophenes	<2	<2	<2
C2-Dibenzotiophenes	9.5	7.6	7.9
C3-Dibenzotiophenes	15	9.6	9.8
Fluoranthene	0.7	0.48	0.56
Pyrene	0.9	0.48	0.57
Benzo(a)anthracene	<0.2	<0.2	<0.2
Chrysene	0.46	0.31	0.28
Benzo(b)fluoranthene	0.29	0.3	<0.2
Benzo(j,k)fluoranthene	<0.2	<0.2	<0.2
Benzo(e)pyrene	0.41	0.39	0.31
Benzo(a)pyrene	<0.2	<0.2	<0.2
Indeno(1,2,3-cd)pyrene	<0.2	<0.2	<0.2
Dibenz(a,c/a,h)anthracene	<0.2	<0.2	<0.2
Benzo(ghi)perylene	<0.2	<0.2	<0.2
Sum PAH-16	3.01	1.87	1.62
Sum NPD	70.46	45	49.1
2-methyl-naphthalene	<0.9	<0.9	<0.9
1-methyl-naphthalene	<0.5	<0.5	<0.5
2,6-dimethyl-naphthalene	<0.2	<0.2	<0.2
2,3,5-trimethyl-naphthalene	0.3	<0.2	0.22
1-methyl-phenanthrene	0.88	0.53	0.57

Table 1. continued

Group	1000 m	1000 m	1000 m
Total dry matter (g/kg)	182	193	167
Lipid (%)	1.4	1.7	1.5
All conc. are µg/kg wet wt.			
Biphenyl	<0.2	<0.2	<0.2
Naphthalene	<1.5	<1.5	<1.5
C1-Naphthalenes	0.68	<0.5	<0.5
C2-Naphthalenes	<2	<2	<2
C3-Naphthalenes	14	9.2	9.5
Acenaphthylene	<0.2	<0.2	<0.2
Acenaphthene	<0.2	<0.2	<0.2
Fluorene	0.21	0.33	<0.2
Anthracene	<0.2	<0.2	<0.2
Phenanthrene	0.93	0.62	0.6
C1-Phenanthrenes	4.2	4.1	3.7
C2-Phenanthrenes	22	23	21
C3-Phenanthrenes	15	16	15
Dibenzotiophene	<0.5	<0.5	<0.5
C1-Dibenzotiophenes	<2	<2	<2
C2-Dibenzotiophenes	13	13	12
C3-Dibenzotiophenes	16	16	15
Fluoranthene	0.53	0.84	0.61
Pyrene	0.66	0.9	0.72
Benzo(a)anthracene	<0.2	<0.2	<0.2
Chrysene	0.45	0.43	0.45
Benzo(b)fluoranthene	<0.2	0.22	0.21
Benzo(j,k)fluoranthene	<0.2	<0.2	<0.2
Benzo(e)pyrene	<0.2	0.49	0.34
Benzo(a)pyrene	<0.2	<0.2	<0.2
Indeno(1,2,3-cd)pyrene	<0.2	<0.2	<0.2
Dibenz(a,c/a,h)anthracene	<0.2	<0.2	<0.2
Benzo(ghi)perylene	<0.2	<0.2	<0.2
Sum PAH-16	2.78	3.34	2.59
Sum NPD	85.81	81.92	76.8
2-methyl-naphthalene	<0.9	<0.9	<0.9
1-methyl-naphthalene	<0.5	<0.5	<0.5
2,6-dimethyl-naphthalene	0.25	<0.2	<0.2
2,3,5-trimethyl-naphthalene	0.45	0.32	0.31
1-methyl-phenanthrene	1.4	1.1	1.1

Table 1. continued

Group	500 m	500 m	500 m
Total dry matter (g/kg)	175	180	174
Lipid (%)	1.4	1.3	1.4
All conc. are µg/kg wet wt.			
Biphenyl	<0.2	0.32	<0.2
Naphthalene	<1.5	<1.5	<1.5
C1-Naphthalenes	<0.5	<2	<2
C2-Naphthalenes	<2	<2	2.4
C3-Naphthalenes	14	14	17
Acenaphthylene	<0.2	<0.2	<0.2
Acenaphthene	<0.2	<0.2	<0.2
Fluorene	0.31	<0.2	0.2
Anthracene	<0.2	<0.2	<0.2
Phenanthrene	0.82	0.68	0.84
C1-Phenanthrenes	5	4.7	6.3
C2-Phenanthrenes	25	26	29
C3-Phenanthrenes	17	19	19
Dibenzotiophene	<0.5	<0.5	<0.5
C1-Dibenzotiophenes	2.4	2.5	2.6
C2-Dibenzotiophenes	16	16	17
C3-Dibenzotiophenes	20	20	18
Fluoranthene	0.51	0.69	0.77
Pyrene	0.59	0.64	0.75
Benzo(a)anthracene	<0.2	<0.2	<0.2
Chrysene	0.34	0.33	0.41
Benzo(b)fluoranthene	<0.2	0.21	0.23
Benzo(j,k)fluoranthene	<0.2	<0.2	<0.2
Benzo(e)pyrene	0.45	0.54	0.5
Benzo(a)pyrene	<0.2	<0.2	<0.2
Indeno(1,2,3-cd)pyrene	<0.2	<0.2	<0.2
Dibenz(a,c/a,h)anthracene	<0.2	<0.2	<0.2
Benzo(ghi)perylene	<0.2	<0.2	<0.2
Sum PAH-16	2.57	2.55	3.2
Sum NPD	100.22	102.88	112.14
2-methyl-naphthalene	<0.9	<0.9	<0.9
1-methyl-naphthalene	<0.5	<0.5	<0.5
2,6-dimethyl-naphthalene	0.22	<0.2	0.22
2,3,5-trimethyl-naphthalene	0.49	0.48	0.58
1-methyl-phenanthrene	1.5	1.4	1.8

Table 1. continued

Group	Ref. south	Ref. south	Ref. south
Total dry matter (g/kg)	188	152	159
Lipid (%)	1.4	1.1	0.91
All conc. are µg/kg wet wt.			
Biphenyl	<0.2	<0.2	<0.2
Naphthalene	<1.5	<1.5	<1.5
C1-Naphthalenes	<2	<2	<2
C2-Naphthalenes	<2	<2	<2
C3-Naphthalenes	<5	<5	<5
Acenaphthylene	<0.2	<0.2	<0.2
Acenaphthene	<0.2	<0.2	<0.2
Fluorene	<0.2	0.27	0.28
Anthracene	<0.2	<0.2	<0.2
Phenanthrene	<0.5	<0.5	<0.5
C1-Phenanthrenes	<2	<2	<2
C2-Phenanthrenes	3.8	3.4	2.8
C3-Phenanthrenes	3	2.2	2.2
Dibenzotiophene	<0.5	<0.5	<0.5
C1-Dibenzotiophenes	<2	<2	<2
C2-Dibenzotiophenes	2	<2	<2
C3-Dibenzotiophenes	<2	2.1	<2
Fluoranthene	0.37	0.32	0.35
Pyrene	0.34	0.33	0.38
Benzo(a)anthracene	<0.2	<0.2	<0.2
Chrysene	<0.2	<0.2	0.3
Benzo(b)fluoranthene	<0.2	<0.2	0.24
Benzo(j,k)fluoranthene	<0.2	<0.2	<0.2
Benzo(e)pyrene	<0.2	<0.2	<0.2
Benzo(a)pyrene	<0.2	<0.2	<0.2
Indeno(1,2,3-cd)pyrene	<0.2	<0.2	<0.2
Dibenz(a,c/a,h)anthracene	<0.2	<0.2	<0.2
Benzo(ghi)perylene	<0.2	<0.2	<0.2
Sum PAH-16	0.71	0.92	1.55
Sum NPD	8.8	7.7	5
2-methyl-naphthalene	<0.9	<0.9	<0.9
1-methyl-naphthalene	<0.5	<0.5	<0.5
2,6-dimethyl-naphthalene	<0.2	<0.2	<0.2
2,3,5-trimethyl-naphthalene	<0.2	<0.2	<0.2
1-methyl-phenanthrene	0.35	0.28	0.24

Table 1. continued

Group	2500 m	2500 m	2500 m
Total dry matter (g/kg)	94.2	121	121
Lipid (%)	0.58	0.89	0.93
All conc. are µg/kg wet wt.			
Biphenyl	<0.2	<0.2	<0.2
Naphthalene	<1.5	1.9	2.1
C1-Naphthalenes	5.3	7	7
C2-Naphthalenes	6.4	8.9	9
C3-Naphthalenes	17	25	23
Acenaphthylene	<0.2	<0.2	<0.2
Acenaphthene	<0.2	<0.2	<0.2
Fluorene	0.51	0.51	0.24
Anthracene	<0.2	<0.2	<0.2
Phenanthrene	0.99	1.4	1.4
C1-Phenanthrenes	3.6	4.9	5.1
C2-Phenanthrenes	14	17	20
C3-Phenanthrenes	8.4	11	12
Dibenzotiophene	<0.5	<0.5	<0.5
C1-Dibenzotiophenes	<2	2.2	2.7
C2-Dibenzotiophenes	7.2	11	10
C3-Dibenzotiophenes	7.7	11	12
Fluoranthene	0.24	<0.2	<0.2
Pyrene	<0.2	<0.2	<0.2
Benzo(a)anthracene	<0.2	<0.2	<0.2
Chrysene	0.28	0.3	0.33
Benzo(b)fluoranthene	0.28	<0.2	<0.2
Benzo(j,k)fluoranthene	<0.2	<0.2	<0.2
Benzo(e)pyrene	0.22	0.2	<0.2
Benzo(a)pyrene	<0.2	<0.2	<0.2
Indeno(1,2,3-cd)pyrene	<0.2	<0.2	<0.2
Dibenz(a,c/a,h)anthracene	<0.2	<0.2	<0.2
Benzo(ghi)perylene	<0.2	<0.2	<0.2
Sum PAH-16	2.3	4.11	4.07
Sum NPD	70.59	101.3	104.3
2-methyl-naphthalene	5.1	6.3	7.2
1-methyl-naphthalene	4.2	6.1	6
2,6-dimethyl-naphthalene	0.67	1	1.2
2,3,5-trimethyl-naphthalene	0.5	0.84	0.73
1-methyl-phenanthrene	1.1	1.4	1.6

Table 1. continued

Group	Zero-time	Zero-time	Zero-time
Total dry matter (g/kg)	178	175	119
Lipid (%)	1.5	1.5	1.8
All conc. are µg/kg wet wt.			
Biphenyl	<0.2	<0.2	<0.2
Naphthalene	<1.5	<1.5	<1.5
C1-Naphthalenes	<2	<2	<2
C2-Naphthalenes	<2	<2	<2
C3-Naphthalenes	<5	<5	<5
Acenaphthylene	<0.2	<0.2	0.23
Acenaphthene	<0.2	<0.2	<0.2
Fluorene	0.33	<0.2	0.57
Anthracene	<0.2	<0.2	<0.2
Phenanthrene	<0.5	<0.5	<0.5
C1-Phenanthrenes	<2	<2	<2
C2-Phenanthrenes	<2	<2	<2
C3-Phenanthrenes	<2	<2	<2
Dibenzotiophene	<0.5	<0.5	<0.5
C1-Dibenzotiophenes	<2	<2	<2
C2-Dibenzotiophenes	<2	<2	<2
C3-Dibenzotiophenes	<2	<2	<2
Fluoranthene	0.33	0.34	0.33
Pyrene	0.3	0.36	0.32
Benzo(a)anthracene	<0.2	<0.2	<0.2
Chrysene	<0.2	<0.2	<0.2
Benzo(b)fluoranthene	<0.2	<0.2	<0.2
Benzo(j,k)fluoranthene	<0.2	<0.2	<0.2
Benzo(e)pyrene	<0.2	<0.2	<0.2
Benzo(a)pyrene	<0.2	<0.2	<0.2
Indeno(1,2,3-cd)pyrene	<0.2	<0.2	<0.2
Dibenz(a,c/a,h)anthracene	<0.2	<0.2	<0.2
Benzo(ghi)perylene	<0.2	<0.2	<0.2
Sum PAH-16	0.96	0.7	1.45
Sum NPD	0	0	0
2-methyl-naphthalene	<0.9	<0.9	<0.9
1-methyl-naphthalene	<0.5	<0.5	<0.5
2,6-dimethyl-naphthalene	<0.2	<0.2	<0.2
2,3,5-trimethyl-naphthalene	<0.2	<0.2	<0.2
1-methyl-phenanthrene	<0.2	<0.2	<0.2

Table 2. Benzo(a)pyrene hydroxylase activity (and sex) in individual blue mussels from the different experimental groups.

Group	Sample no.	BaPH (pmol/min/mg prot.)	SEX (results from UPV/EHU)
0-time	1	24.77	H
0-time	2	44.39	H
0-time	3	29.39	M
0-time	4	28.23	H
0-time	5	23.93	M
0-time	6	23.05	M
0-time	7	40.07	H
0-time	8	29.52	H
0-time	9	24.69	H
0-time	10		M
0-time	11	32.11	
0-time	12	37.69	M
0-time	13	82.86	M
0-time	14	38.77	M
0-time	15	64.55	
0-time	16	59.98	M
0-time	17	41.07	H
0-time	18	68.95	M
0-time	19	104.56	M
0-time	20	59.06	
0-time	21		
0-time	22		
0-time	23		
0-time	24		
0-time	25		
0-time	26		
10 000 m	101	66.16	M
10 000 m	102	59.55	
10 000 m	103	42.59	H
10 000 m	104	63.51	H
10 000 m	105	41.24	M
10 000 m	106	72.35	
10 000 m	107	62.70	M
10 000 m	108	118.90	M
10 000 m	109	18.53	H
10 000 m	110	51.96	M
10 000 m	111	38.59	
10 000 m	112	77.18	
10 000 m	113	30.13	H
10 000 m	114	25.54	M
10 000 m	115	36.31	H
10 000 m	116	61.73	H
10 000 m	117	41.64	M
10 000 m	118	37.77	M
10 000 m	119	18.30	H
10 000 m	120	39.11	M

Table 2. continued

Group	Sample no.	BaPH (pmol/min/mg prot.)	SEX (results from UPV/EHU)
1000 m	201	54.82	
1000 m	202	38.55	
1000 m	203	30.81	M
1000 m	204	48.15	
1000 m	205	34.13	H
1000 m	206	39.26	M
1000 m	207	22.25	H
1000 m	208	58.20	
1000 m	209	41.66	H
1000 m	210	43.80	M
1000 m	211		H
1000 m	212	32.60	H
1000 m	213	20.43	H
1000 m	214	29.84	M
1000 m	215	12.42	H
1000 m	216	49.71	M
1000 m	217	32.58	H
1000 m	218	33.82	H
1000 m	219	31.42	
1000 m	220	18.34	H
500 m	301	40.61	M
500 m	302	22.88	M
	303		
500 m	304	30.92	M
500 m	305	49.05	H
500 m	306	42.23	
500 m	307	48.40	M
500 m	308	51.70	M
500 m	309	56.17	M
500 m	310	18.73	H
500 m	311	8.29	
500 m	312	23.80	H
500 m	313	11.92	
500 m	314	27.32	M
500 m	315	48.19	M
500 m	316	62.79	
500 m	317	39.84	
500 m	318	31.56	
500 m	319	21.10	H
500 m	320	29.93	

Table 2. continued

Group	Sample no.	BaPH (pmol/min/mg prot.)	SEX (results from UPV/EHU)
Ref. south	401	16.85	H
Ref. south	402	50.05	M
Ref. south	403	43.41	H
Ref. south	404	22.55	M
Ref. south	405	36.13	M
Ref. south	406	71.78	
Ref. south	407	78.47	H
Ref. south	408	33.41	M
Ref. south	409	43.76	H
Ref. south	410	30.13	M
Ref. south	411	42.45	
Ref. south	412	40.56	M
Ref. south	413	34.03	M
Ref. south	414	25.53	M
Ref. south	415	51.85	H
Ref. south	416	34.25	M
Ref. south	417	34.43	
Ref. south	418	18.75	
Ref. south	419	32.13	H
Ref. south	420	35.46	M

Table 3. Length (cm), weight (kg), condition index, liver weight (kg), sex, EROD (pmol/min/mg prot.) and plasma vitellogenin (ng/ml) for individual cod from the different experimental groups.

Group	Sample no.	Length (cm)	Weight (kg)	Condition index	Weight liver (kg)	Sex	EROD (pmol/min/mg prot.)	VTG (ng/mL)
0-time	1	41.0	0.900	1.31		M	22.22	
0-time	2	45.5	0.970	1.03		M	12.04	
0-time	3	41.0	0.930	1.35		M	21.48	
0-time	4	36.0	0.560	1.20		M	14.74	
0-time	5	42.0	0.520	0.70		M	18.10	
0-time	6	38.0	0.380	0.69		F	16.13	
0-time	7	46.0	1.090	1.12		F	24.85	
0-time	8	42.5	0.550	0.72		M	28.10	
0-time	9	42.5	0.980	1.28		M	21.31	
0-time	10	44.0	0.990	1.16		F	10.75	
0-time	11	37.5	0.610	1.16		M	15.25	
0-time	12	40.5	0.850	1.28		M	12.70	
0-time	13	44.5	1.070	1.21		F	32.58	
0-time	14	41.0	0.870	1.26		M	16.41	
0-time	15	35.5	0.450	1.01		M	8.78	
0-time	16	46.5	1.130	1.12		F	1.06	
0-time	17	42.0	0.940	1.27		M	10.00	
0-time	18	41.5	0.720	1.01		M	2.99	
0-time	19	39.5	0.660	1.07		F	9.03	
0-time	20	39.5	0.780	1.27		M	7.08	
0-time	21	46.0	1.180	1.21		F	12.14	
0-time	22	47.5	1.140	1.06		F	8.47	
0-time	23	35.5	0.500	1.12		F	14.85	
0-time	24	47.0	1.080	1.04		F	5.96	
0-time	25	42.5	0.740	0.96		M	10.31	
0-time	26	46.5	1.220	1.21		M	7.31	
0-time	27	45.5	1.020	1.08		F	7.50	
0-time	28	44.0	0.980	1.15		F	9.58	
0-time	29	36.5	0.520	1.07		M	19.74	
0-time	30	34.0	0.460	1.17		M	8.31	
0-time	31	37.5	0.670	1.27		M		

Table 3. continued

Group	Sample no.	Length (cm)	Weight (kg)	Condition index	Weight liver (kg)	Sex	EROD (pmol/min/mg prot.)	VTG (ng/mL)
0-time	32	46.0	1.180	1.21		F		237000.00
0-time	33	37.5	0.560	1.06		M		3017.00
0-time	34	43.5	0.950	1.15		F		35191.00
0-time	35	42.0	0.950	1.28		F		6259.00
0-time	36	45.5	1.060	1.13		M		523.80
0-time	37	39.0	0.660	1.11		M		322.00
0-time	38	41.5	0.890	1.25		F		10698.00
0-time	39	44.0	0.970	1.14		F		495.00
0-time	40	42.0	1.020	1.38		F		964.70
0-time	41	44.5	0.910	1.03		M		340.60
0-time	42	46.0	1.090	1.12		F		702000.00
0-time	43	42.5	0.800	1.04		M		8479.00
0-time	44	36.5	0.580	1.19		M		727.50
0-time	45	39.5	0.710	1.15		M		1835.00
0-time	46	39.5	0.750	1.22		M		52258.00
0-time	47	38.5	0.620	1.09		M		8211.00
0-time	48	43.5	0.950	1.15		M		4782.00
0-time	49	42.5	0.960	1.25		M		6256.00
0-time	50	36.0	0.510	1.09		M		343.00
0-time	51	46.5	1.270	1.26		F		46224.00
0-time	52	39.5	0.820	1.33		M		3372.00
0-time	53	38.0	0.690	1.26		M		14823.00
0-time	54	36.0	0.530	1.14		M		56615.00
0-time	55	39.5	0.780	1.27		M		13504.00

Table 3. continued

Group	Sample no.	Length (cm)	Weight (kg)	Condition index	Weight liver (kg)	Sex	EROD (pmol/min/mg prot.)	VTG (ng/mL)
10 000 m	101	40.0	0.702	1.10	0.0725	F	12.88	154000.00
10 000 m	102	41.0	0.615	0.89	0.0582	M	22.04	278000.00
10 000 m	103	41.0	0.673	0.98	0.0750	M	5.23	2732.00
10 000 m	104	39.0	0.644	1.08	0.0628	M	19.59	904.80
10 000 m	105	41.5	0.818	1.14	0.0998	M	13.98	130.10
10 000 m	106	47.0	0.944	0.91	0.8300	F	9.72	8942.00
10 000 m	107	37.5	0.544	1.03	0.0760	M	5.79	503.30
10 000 m	108	43.0	0.830	1.04	0.0972	M	16.65	106.90
10 000 m	109	37.5	0.503	0.95	0.0543	M	14.85	2721.00
10 000 m	110	44.0	0.904	1.06	0.1025	M	8.58	9361.00
10 000 m	111	45.5	1.064	1.13	0.1104	F	12.39	53389.00
10 000 m	112	39.5	0.600	0.97	0.0570	M	61.83	14219.00
10 000 m	113	45.5	0.911	0.97	0.0930	F	9.60	46288.00
10 000 m	114	42.0	0.733	0.99	0.0858	M	5.35	6462.00
10 000 m	115	44.0	0.903	1.06	0.0105	F	13.64	193000.00
10 000 m	116	47.0	1.007	0.97	0.0970	F	8.31	36044.00
10 000 m	117	47.0	1.103	1.06	0.1290	M	8.89	6228.00
10 000 m	118	49.5	1.039	0.86	0.1045	F	3.85	149000.00
10 000 m	119	44.0	0.912	1.07	0.1025	F	7.87	97527.00
10 000 m	120	44.0	0.934	1.10	0.1136	M	15.90	265.80
10 000 m	121	48.5	1.161	1.02	0.1280	F	21.72	21708.00
10 000 m	122	40.0	0.572	0.89	0.0480	M	31.04	24177.00
10 000 m	123	40.0	0.656	1.02	0.0721	F	35.53	103000.00
10 000 m	124	46.5	1.074	1.07	0.1159	F	31.34	320000.00
10 000 m	125	44.0	1.024	1.20	0.1065	F	8.71	5379.00
10 000 m	126	39.5	0.649	1.05	0.0620	M	31.08	35943.00
10 000 m	127	44.0	0.746	0.88	0.0640	F	16.44	127000.00

Table 3. continued

Group	Sample no.	Length (cm)	Weight (kg)	Condition index	Weight liver (kg)	Sex	EROD (pmol/min/mg prot.)	VTG (ng/mL)
1000 m	201	45.0	0.951	1.04	0.1048	F	17.35	53505.00
1000 m	202	47.5	1.125	1.05	0.1130	F	21.37	62550.00
1000 m	203	41.0	0.758	1.10	0.0773	M	16.07	5105.00
1000 m	204	42.0	0.705	0.95	0.0725	M	12.04	240.30
1000 m	205	36.0	0.479	1.03	0.0450	M	26.70	24686.00
1000 m	206	42.0	0.798	1.08	0.0924	M	9.76	1923.00
1000 m	207	37.0	0.475	0.94	0.0355	M	4.31	28.01
1000 m	208	49.5	1.144	0.94	0.1222	F	9.91	50248.00
1000 m	209	42.0	0.710	0.96	0.0945	M	6.87	7011.00
1000 m	210	44.0	0.960	1.13	0.0985	M	6.42	7571.00
1000 m	211	36.5	0.559	1.15	0.0488	M	20.66	30804.00
1000 m	212	46.0	0.967	0.99	0.0910	F	13.85	40076.00
1000 m	213	39.5	0.604	0.98	0.0642	M	9.68	1139.00
1000 m	214	46.5	0.999	0.99	0.1104	F	20.24	8790.00
1000 m	215	46.5	1.030	1.02	0.1080	F	17.04	31722.00
1000 m	216	39.0	0.583	0.98	0.0718	M	7.93	4586.00
1000 m	217	42.5	0.840	1.09	0.1040	M	8.56	277.50
1000 m	218	38.0	0.626	1.14	0.0660	M	14.47	14238.00
1000 m	219	42.0	0.785	1.06	0.0860	F	3.69	11876.00
1000 m	220	43.0	0.822	1.03	0.0688	F	28.76	7949.00
1000 m	221	44.0	0.965	1.13	0.1100	M	15.08	5340.00
1000 m	222	43.5	0.883	1.07	0.0860	F	18.83	23698.00
1000 m	223	39.0	0.537	0.91	0.0430	M	22.62	21138.00
1000 m	224	42.0	0.697	0.94	0.0710	F	30.92	9887.00
1000 m	225	38.5	0.691	1.21	0.0700	M	11.26	4843.00
1000 m	226	43.0	0.793	1.00	0.0750	F	9.91	15998.00
1000 m	227	40.0	0.664	1.04	0.0613	F	12.89	16702.00

Table 3. continued

Group	Sample no.	Length (cm)	Weight (kg)	Condition index	Weight liver (kg)	Sex	EROD (pmol/min/mg prot.)	VTG (ng/mL)
500 m	301	44.0	1.042	1.22	0.1055	M	10.64	19849.00
500 m	302	45.5	0.939	1.00	0.0935	F	40.50	27778.00
500 m	303	38.0	0.557	1.02	0.0500	M	26.85	1058.00
500 m	304	37.0	0.509	1.00	0.0510	M	14.73	7402.00
500 m	305	46.0	1.025	1.05	0.1102	M	12.45	11460.00
500 m	306	45.0	0.923	1.01	0.1040	F	21.04	18033.00
500 m	307	44.0	0.792	0.93	0.0800	F	17.76	28508.00
500 m	308	42.0	0.720	0.97	0.0730	M	9.13	13062.00
500 m	309	44.0	0.827	0.97	0.0920	M	13.13	193.80
500 m	310	40.5	0.595	0.90	0.0560	M	2.21	25.40
500 m	311	39.0	0.659	1.11	0.0830	M	7.63	3223.00
500 m	312	44.0	0.868	1.02	0.0918	M	4.46	2262.00
500 m	313	42.5	0.753	0.98	0.0847	M	2.76	6835.00
500 m	314	43.0	0.757	0.95	0.0933	M	14.85	634.40
500 m	315	42.0	0.797	1.08	0.0940	M	7.25	3070.00
500 m	316	41.0	0.748	1.08	0.0702	M	7.25	10893.00
500 m	317	45.5	0.852	0.90	0.0850	F	3.06	20142.00
500 m	318	42.0	0.735	0.99	0.0712	M	10.86	201.10
500 m	319	42.5	0.811	1.06	0.0813	M	12.49	3196.00
500 m	320	39.5	0.622	1.01	0.0460	F	14.96	1833.00
500 m	321	41.0	0.593	0.86	0.0920	F	17.91	15299.00
500 m	322	44.0	0.884	1.04	0.0890	F	15.57	62343.00
500 m	323	43.0	0.793	1.00	0.0500	M	23.17	9896.00
500 m	324	35.5	0.475	1.06	0.1112	M	11.77	119.50
500 m	325	45.5	1.018	1.08	÷	M	34.61	6661.00
500 m	326	47.0	1.099	1.06	0.0150	M	17.90	345.30
500 m	327	36.5	0.484	1.00	0.0480	M	18.34	17312.00

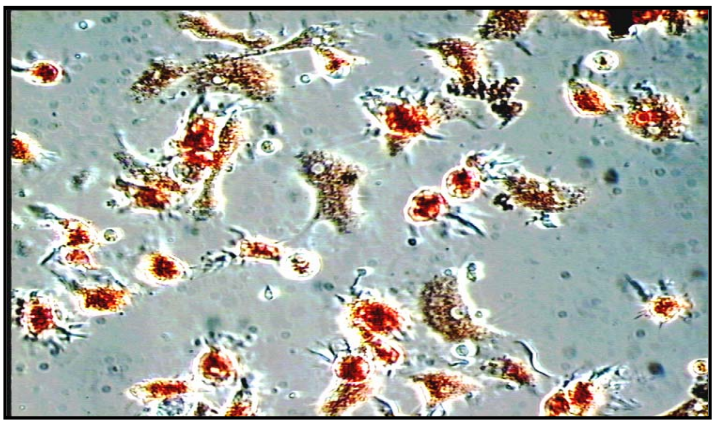
Table 3. continued

Group	Sample no.	Length (cm)	Weight (kg)	Condition index	Weight liver (kg)	Sex	EROD (pmol/min/mg prot.)	VTG (ng/mL)
Ref. south	401	43.5	0.768	0.93	0.0630	M	12.58	132.90
Ref. south	402	42.5	0.771	1.00	0.0842	M	8.99	3621.00
Ref. south	403	41.5	0.752	1.05	0.0692	F	14.92	24180.00
Ref. south	404	39.0	0.639	1.08	0.0745	M	7.22	734.50
Ref. south	405	49.5	1.165	0.96	0.1360	F	4.77	70418.00
Ref. south	406	41.0	0.738	1.07	0.0745	M	7.03	1976.00
Ref. south	407	43.5	0.872	1.06	0.0945	F	16.14	65090.00
Ref. south	408	39.5	0.700	1.14	0.0857	M	17.02	46.95
Ref. south	409	43.0	0.826	1.04	0.1029	F	4.61	71649.00
Ref. south	410	42.5	0.669	0.87	0.0631	M	19.41	7053.00
Ref. south	411	44.0	0.875	1.03	0.1001	M	11.66	2697.00
Ref. south	412	50.0	1.183	0.95	0.1234	F	<1	318000.00
Ref. south	413	46.5	0.959	0.95	0.1068	F	<1	37584.00
Ref. south	414	42.5	0.782	1.02	0.0976	F	10.88	55022.00
Ref. south	415	45.0	0.951	1.04	0.0945	M	11.50	5265.00
Ref. south	416	39.5	0.508	0.82	0.0417	F	30.16	78855.00
Ref. south	417	42.0	0.608	0.82	0.0572	M	13.73	1695.00
Ref. south	418	46.0	0.892	0.92	0.0912	F	5.90	245000.00
Ref. south	419	42.0	0.777	1.05	0.0928	F	15.72	118000.00
Ref. south	420	46.0	1.058	1.09	0.1351	F	25.52	9374.00
Ref. south	421	42.0	0.774	1.04	0.0870	M	8.11	729.90
Ref. south	422	44.0	0.886	1.04	0.0980	M	2.61	872.30
Ref. south	423	44.5	0.823	0.93	0.0760	F	8.67	34718.00
Ref. south	424	40.0	0.822	1.28	0.0940	M	5.50	475.00
Ref. south	425	44.0	0.873	1.02	0.0840	M	4.80	248.00
Ref. south	426	44.0	0.854	1.00	0.0918	F	9.04	178000.00
Ref. south	427	41.0	0.736	1.07	0.0728	M	24.00	14872.00

Table 3. continued

Group	Sample no.	Length (cm)	Weight (kg)	Condition index	Weight liver (kg)	Sex	EROD (pmol/min/mg prot.)	VTG (ng/mL)
2500 m	501	44.0	0.981		0.0880	F		
2500 m	502	39.5	0.674		0.0683	M		
2500 m	503	39.0	0.655		0.0447	M		
2500 m	504	36.0	0.560		0.0421	M		
2500 m	505	38.0	0.593		0.0535	M		
2500 m	506	38.0	0.634		0.0675	M		
2500 m	507	37.0	0.625		0.0360	F		
2500 m	508	42.5	1.008		0.0990	F		
2500 m	509	40.0	0.707		0.0800	M		
2500 m	510	37.0	0.606		0.0565	F		
2500 m	511	38.0	0.600		0.0480	M		
2500 m	512	37.0	0.602		0.0460	M		
2500 m	513	36.0	0.582		0.0295	F		
2500 m	514	39.0	0.750		0.0774	M		
2500 m	515	39.5	0.633		0.0406	F		
2500 m	516	41.0	0.855		0.0717	F		
2500 m	517	37.0	0.533		0.0361	M		
2500 m	518	39.0	0.686		0.0170	M		
2500 m	519	36.5	0.494		0.0110	M		
2500 m	520	38.5	0.743		0.0680	M		
2500 m	521	37.0	0.561		0.0600	M		
2500 m	522	37.0	0.540		0.0470	M		
2500 m	523	37.0	0.557		0.0450	M		
2500 m	524	33.0	0.476		0.0300	M		

Appendix F. Data report – RF (Stavanger)



Rolf C. Sundt

Water Column Monitoring 2004 - Analyses report

Report RF – 2005 / 041

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Introduction

The present report raw data from analysis conducted at RF Akvamiljø are given; discussion and conclusions are presented in the summary report.

Lysosomal response was measured with the NRRT method according to (Lowe and Pipe 1994), measured as the retention of the neutral red probe in haemocytes of the mussels. This biomarker method is characterised as a general health parameter and is earlier shown to respond to PAH and crude oil exposed mussels (Camus et al. 2000; Fernley et al. 2000).

CYP1A induction, and in particular EROD, has been widely used as a biomarker for planar organic compounds, including some PAHs, PCBs and dioxins. (Goksøyr and Förlin 1992; Bucheli and Fent 1995). PAHs from petrogenic sources have been shown to be inducers of CYP1A in a range of fish species. Both laboratory based investigations (Upshall et al. 1993; Celander et al. 1994) and field work (Payne et al. 1984; Collier et al. 1992; George et al. 1995; McDonald et al. 1995; Stagg et al. 1995) have shown indications of induction, though the field studies have generally focused on severely polluted areas.

Glutathione S-transferase (GST) is also a part of the detoxification system and is evolutionary developed by organisms in order to convert lipophlic compounds into more hydrophilic and thereby more easily excretable metabolites. Two major types of reactions exist: Phase I, which involves hydrolysis, oxidation and reduction, and Phase II, which involves conjugation or synthetic processes. GST catalyses one of these Phase II processes, that is conjugation of glutathione to compounds with electrophilic centres (-SH group of glutathione neutralises the electrophilic site). These electrophilic centres may otherwise be harmful as they may react with macromolecules controlling cell growth, such as DNA, RNA and proteins. It is therefore of great importance that the animal is capable of neutralises and excrete these compounds. Changes in the activity of GST may reflect exposure to xenobiotics.

The potential adverse effects of PAHs have resulted in many years of monitoring their concentrations in water, sediment and biota. However, the extensive biotransformation of PAHs by fish greatly prevents the accumulation of these compounds in extra-hepatic tissues (Stein et al. 1987). Consequently, tissue levels of parent PAH do usually not provide an adequate assessment of the PAH exposure level (Varanasi 1989). The metabolites concentrates in the gall bladder of fish following biotransformation. Analysis of PAH metabolites in the fish bile constitutes a very sensitive method for assessment of PAH exposure in laboratory and field studies (Beyer et al. 1998; Aas et al. 2001). PAH metabolites are commonly determined by semi-quantitative screening analysis (Aas et al. 2000) or by quantitative determination of specific metabolites (Jonsson et al. 2003; Jonsson et al. 2004). The method to use will depend on the questions to be answered, the compound of interest and the required level of detail.

1 Material and methods

Atlantic cod, *Gadus morhua*, and blue mussel, *Mytilus edulis*, that originated from local fish and shellfish farmers were transported to the Statfjord C field and deployed in cages as described in the survey report (Sundt 2004). After 6 weeks of field exposure, cages with animals were picked up, biological data on length, weight, and sex was measured and biological samples obtained. The rig at 2500 metres was lost due to collision with a vessel. Organisms from this cage were frozen round at 20 ºC onboard the vessel. Due to insufficient sample handling, only besides

1.1 Length, weight and sex

The length and weight of cod and mussel were measured with fixed mounted balances in the lab facility onboard and in onshore lab respectively. Fish were sexed by visual examination of gonads and liver weight was recorded. Raw data given in Appendix 5.

1.2 PAH metabolites in cod - 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)(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.

2 Lysosomal membrane stability in mussels

The mussels from the pre exposure group and field groups were brought from to the lab in Stavanger on ice. The mussels were further acclimatised in the lab in aquaria with fresh supplies of sea-water for two days prior to sampling (to prevent influence of travel-stress). Haemolymph samples were obtained of 19 individuals at each field station and 26 individuals from the pre exposure group.

0.4 ml haemolymph was sampled from each mussel and mixed with filtered sea water at the ration 2:1. 40 µl haemolymph/seawater-mixture was pipetted out on microscope-slides, and incubated in a light-proof box for 20 min before 35 µl neutral red (concentration 0.1 µg/µl) was added. All analyses were performed blind, for detailed description of method see Lowe (1994).

NR is selectively taken up by haemolymph cells and this adds an extra stress to the membranes. After some time, from 15 to 200 minutes, depending of the health status of the mussels, the membrane will start to burst and NR will leak out in the cytosol. This causes the form of the cells to change from irregular to round shaped. The time from NR is added the cells and until they round up and perish is observed visually with a microscope (Figure x). The cells are observed repeatedly at 15, 30, 60, 90, 120, 150 and 180 minutes of incubation with NR. The endpoint of the analysis is when 50% of all cells are rounded up and dead. This method is perceived as a general health-parameter, and has been shown to respond to PAH/oil-exposure of mussels.

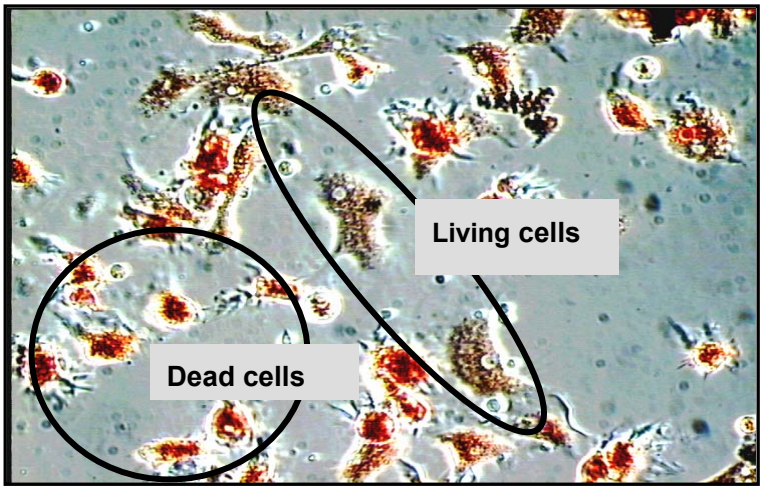

Figure 1. Microscope view (400 X magnification) of living and dead mussel haemolymph cells.

2.1 EROD activity in gills

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.

Gill arcs are removed from the fish and stored in Hepes-Cortland (HC) buffer on ice (cod gills can be stored for 3-4 days). From each individual 10 duplicates with 2mm long primary filaments in a 12 well microplate in HC buffer. The HC buffer is exchanged with reaction buffer (HC containing ethoxyresorufin and dicumarol as protease inhibitor) and the samples are transferred to a 96 well plate. After 10 / 30 min for exposed fish and 30 / 50 for control fish the activity levels are measured. During this time CYP1A in the filaments convert resorufin from ethoxyresorufin. The amount of resorfin in each well is measured by fluorecens in a plate reader (ex 540 nm, em 590 nm). The more CYP1A present in the gills the higher activity measured expressed as pmol resorfin/filament/minute. For more info see http://publications.uu.se/theses/abstract.xsql?dbid=3913

2.2 Glutathion-S-transferase (GST) activity

The method is based on Winston *et al.* (1998) and Regoli and Winston (1999), except that buffers were adjusted for cod. Liver tissue is homogenised with a Potter-Elvehjem glass/Teflon homogeniser in four volumes of 100 mM KH_2PO_4 buffer, pH 7.5, 2.5% NaCl. The homogenate is centrifuged at 100 000 × g for 1 h, and cytosolic fractions were aliquoted and stored at –80^{o}C.

Reagents (20 mM CDNB, 20 mM GSH) and cytosol samples (50 µL) are transferred to U-shaped 96 microwell plates, each containing 3 replicates of 22 samples, a negative and a positive control (cod sample). Each microplate are stored on ice until transferred to the automatic pipetting robot (Tomtec Quadra 96 model 320) operating board for further dilutions and mixing. Tomtecs special tips are designed to aspirate multiple reagents in one pass using air gap separation; witch makes it possible to mix all the reagents directly into the microplate wells without further pippeting. Different dilutions are selected for each specie to obtain a linear signal, but normally a 5 fold cytosol dilution in phosphate buffer (100mM KH_2PO_4/K_2HPO_4, pH 7.4, 24°C) are carried out before new tips are changed to perform a final 1:20 dilution of samples and reagents in the reading microplate. After the samples and reagents have been mixed together (15 sec.) the plate is transferred to the microplatereader were the absorbance is measured at 340nm during 1 minute run at 24°C. The enzyme activity are estimated and normalized against the sample protein concentration.

The activity calculation: (Δ Absorbance-blank)/ (9.6x $[\text{Protein}]_{well}$), were 1.7 is the molar extinction coefficient ($\in$) for the CDNB-GSH conjugate (in $mM^{-1}cm^{-1}$). GST activities were expressed as moles of substrate converted per minute per mg of protein in the cytosol.

The total protein concentrations of the samples where determined by a procedure based on the Bradford method (Bradford, 1976).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.

2.3 Protein determination (Bradford method)

The Bradford assay relies on the fact that protein binds to Coomassie Brilliant Blue G-250 and changes colour. Coomassie Blue exists in two colour forms, red and blue. Upon binding protein, the red form is converted to the blue form. The protein-dye complex absorbs light at 595 nm of your test solution (protein solution + Coomassie) as compared to a set of standard protein solutions (bovine serum albumin, BSA).

Protein standards (0,625-10 mg/mL, Bovine Serum Albumin, BSA) and cytosol samples (50 µL) are transferred to U-shaped 96 well microplates, each containing 3 replicates of 23 samples and a blank sample. Each microplate are stored on ice until transferred to the automatic pipetting robot (Tomtec Quadra 96 model 320) operating board for further dilutions and mixing. Tomtecs special tips are designed to aspirate multiple reagents in one pass using air gap separation; witch makes it possible to mix all the reagents directly into the microplate wells without further pippeting. The sample cytosol and the standards are first diluted 1:20 in distilled water before new tips are changed and a final 1:25 diluted samples and standards are mix together with a final 1:5 fold diluted BioRad Protein Assay Dye. The dye is mixed together with the sample cytosol and standard in the microplate wells 5 times before extension for 5 minutes. After another 5 times mixing the microplate is transferred to the microplate reader were the absorbance is measured at 595nm. The sample protein concentration is estimated from the standard curve.

3 Results

3.1 Length, weight and sex

The body length and weight distribution in the different experimental groups are shown in Figure 2 and 3. The difference in mean values between groups is relative small. Fish from the 2500 metres group is somewhat lighter than the other field groups and might be due to the different treatment for this group. It seems like the cod have lost weight during the exposure period. The distribution of male and females should be characterised as normal (Fig. 4). Raw data given in Appendix 5.

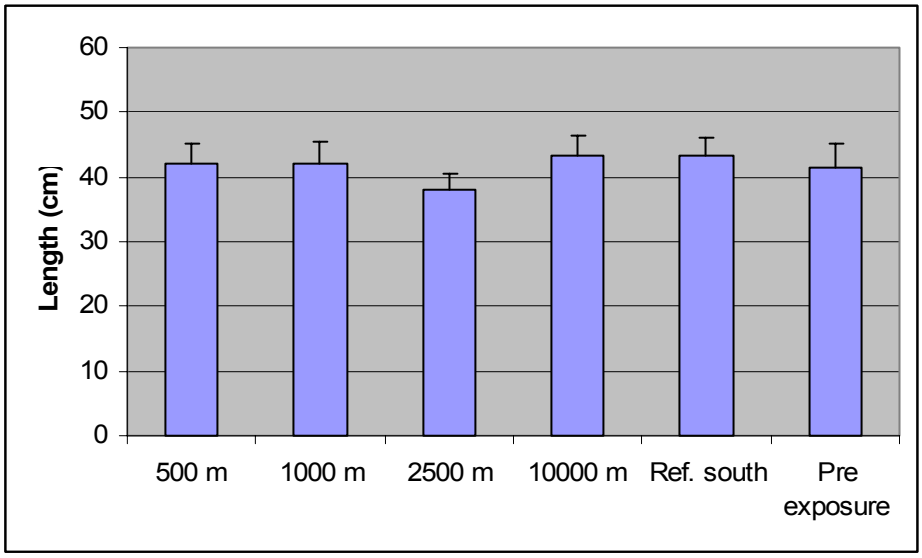

Figure 2. Length and SD of cod in the different experimental groups.

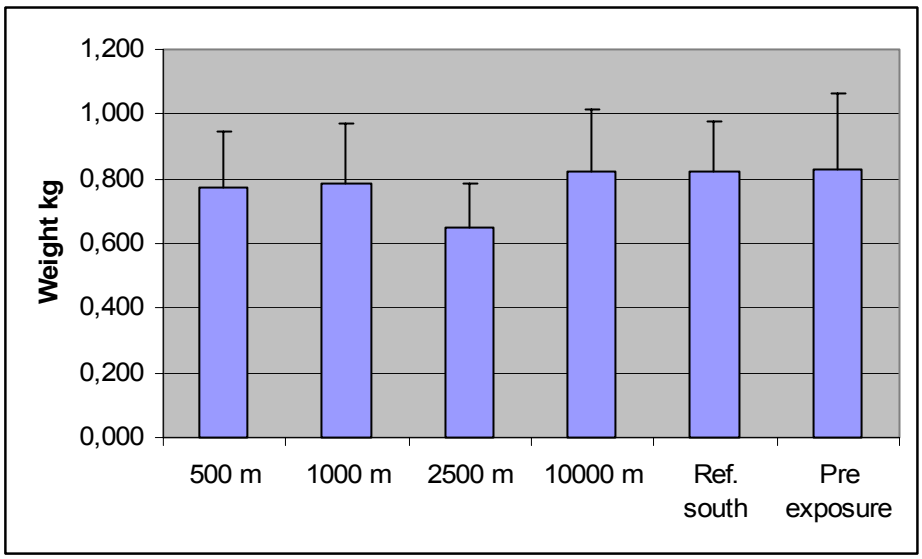

Figure 3. Weight and SD of cods in the different experimental groups.

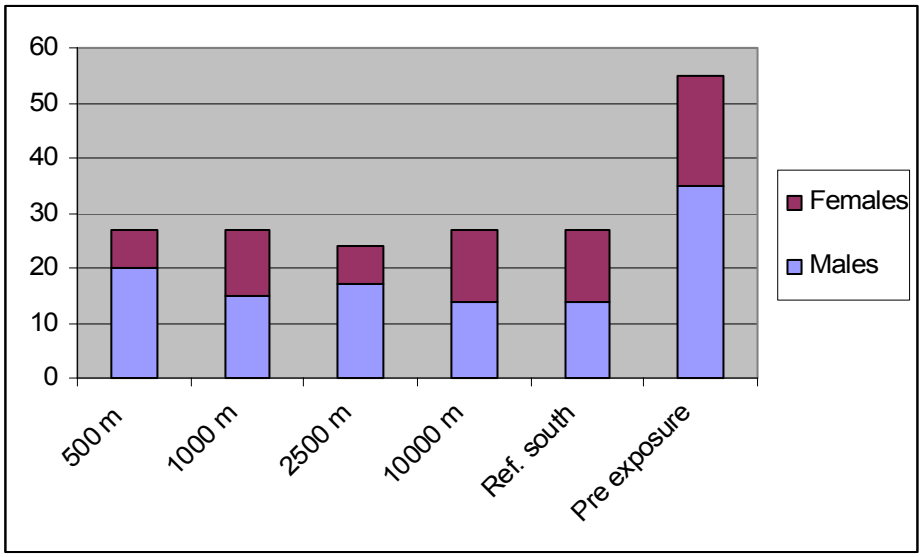

Figure 4. Distribution of male and female cod in different experimental groups.

3.2 Bile metabolites in cod by Fixed Fluorescence

Fluorecence levels in field samples represent background levels.

The most striking result is the relatively high fluorescence levels found pre exposure group. Presence of 5 ring structures suggests PAHs from combustion processes (not typical petrogenic) and possible sources could be local ship traffic and emissions from the electricity generator situated on the fish farm. Decreasing gradient in fluorescence levels of 2-4 ring structures (Figure 5-7) with distance from the platform found.

Samples from the 2500 m station were frozen at -20ºC. Due to the different sample handling care must be taken when comparing data from this group with the other.

Raw data given in Appendix 4.

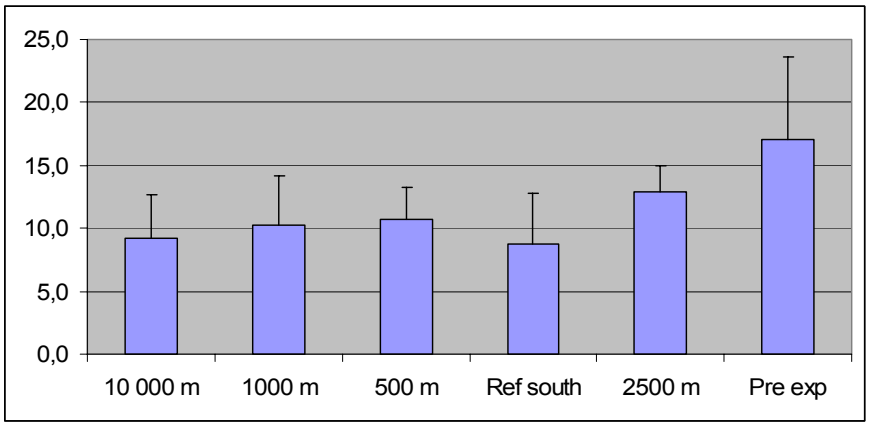

Figure 5 Fixed wavelength (290/334nm) fluorescence levels in cod expressed as pyrene fluorescence equivalents, µg/g (mean and SD). The wavelength pair 290/334nm identifies 2-3 ring structures.

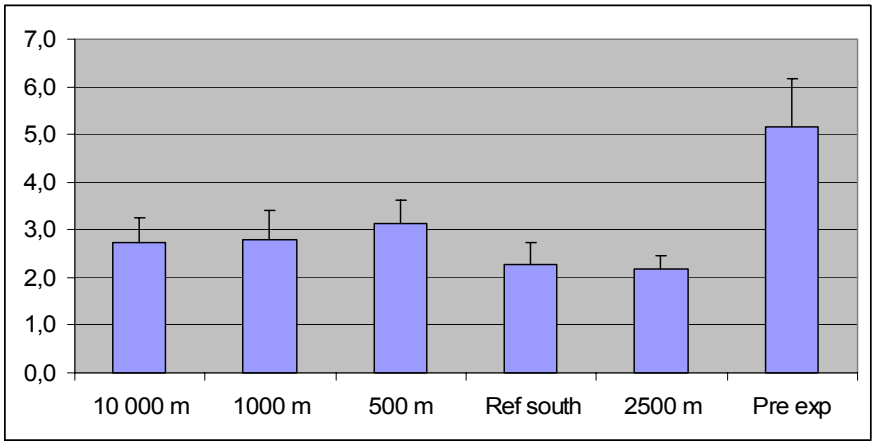

Figure 6. Fixed wavelength (341/383nm) fluorescence levels in cod expressed as pyrene fluorescence equivalents, µg/g (mean and SD). The wavelength pair 341/383nm identifies 4 ring structures.

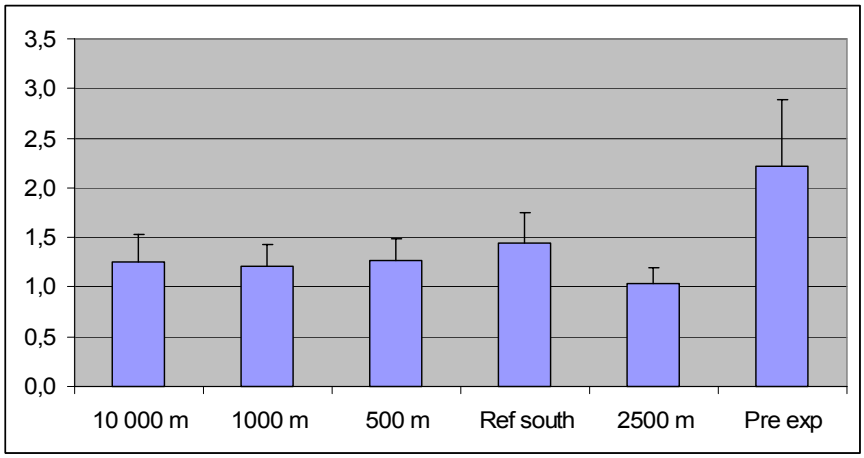

Figure 7. Fixed wavelength (380/430nm) fluorescence levels in cod expressed as pyrene fluorescence equivalents, µg/g (mean and SD). The wavelength pair 380/430nm identifies 5 ring structures.

3.3 Bile metabolites in cod by GCMS

Metabolite levels from field samples are very low compared to levels experienced at other similar field studies like in the BECPELAG workshop at Statfjord B.

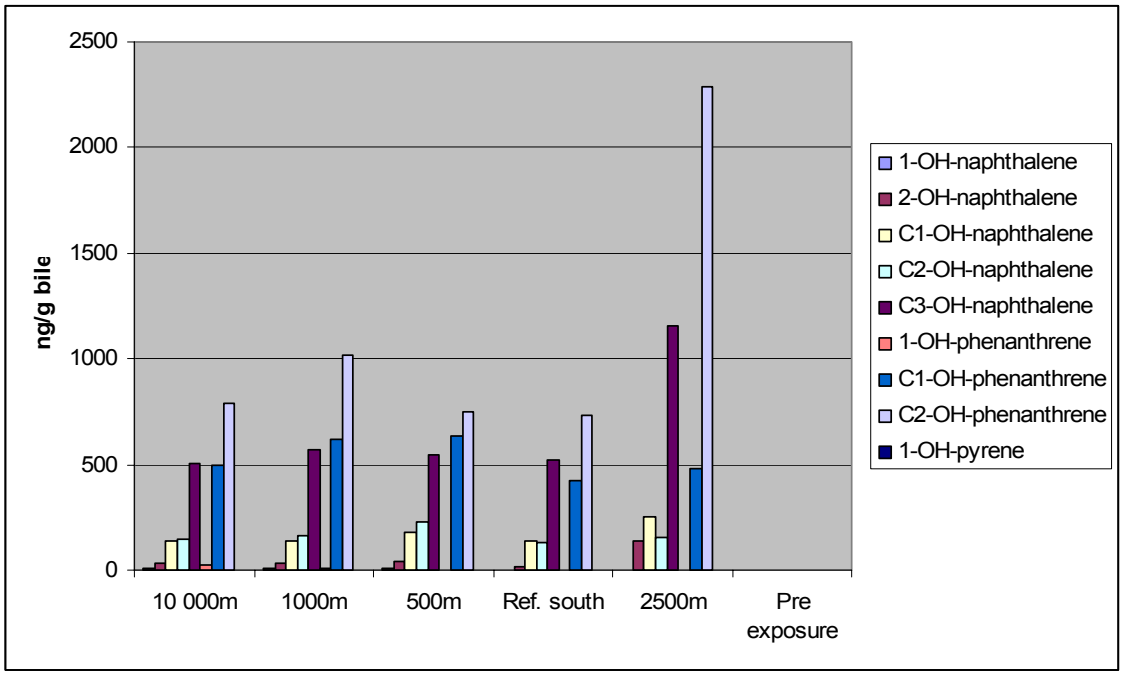

Figure 8. PAH metabolites in caged cod given in ng/g bile as mean of five selected individuals from each of the groups. See Appendix 4 for sample selection.

3.4 Bile metabolites in feral saithe by Fixed Fluorescence

Low levels of PAH metabolites were detected from all fish analysed (fluorescence levels detected represents background). Raw data given in Appendix 5.

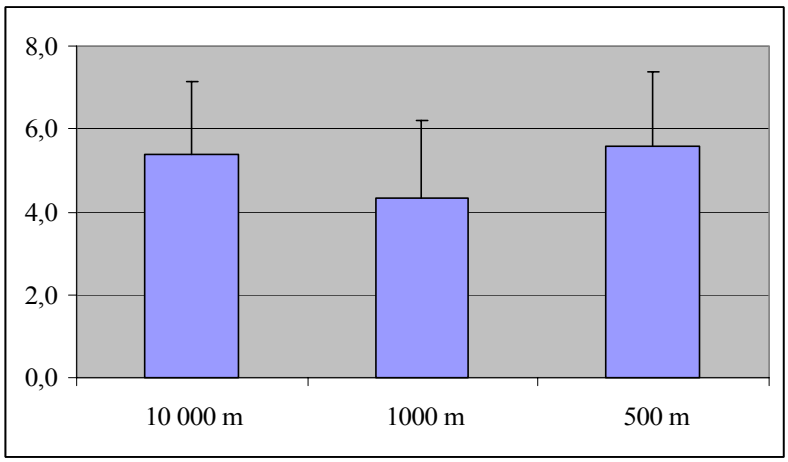

Figure 9 Fixed wavelength (290/334nm) fluorescence levels given in feral saithe expressed as pyrene fluorescence equivalents, µg/g (mean and SD). The wavelength pair 290/334nm identifies 2-3 ring structures.

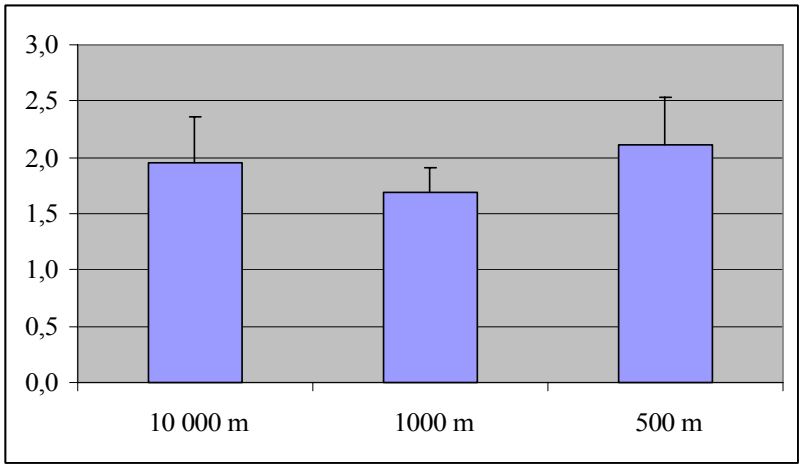

Figure 10. Fixed wavelength (341/383nm) fluorescence levels in cod expressed as pyrene fluorescence equivalents, µg/g (mean and SD). The wavelength pair 341/383nm identifies 4 ring structures.

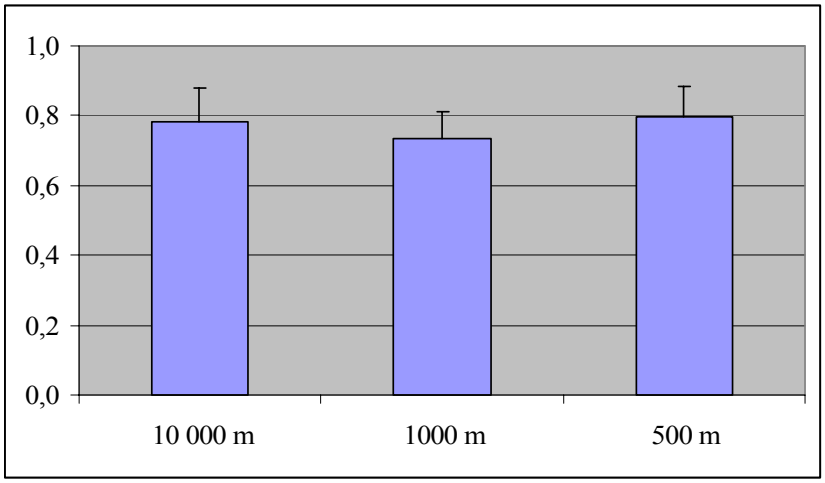

Figure 11. Fixed wavelength fluorescence (380/430nm) levels in cod expressed as pyrene fluorescence equivalents, µg/g (mean and SD). The wavelength pair 380/430nm identifies 5 ring structures.

3.5 Bile metabolites in feral saithe by GCMS

The low levels of PAH metabolites detected by FF were confirmed by GCMS analysis. (fluorescence levels detected represents background).

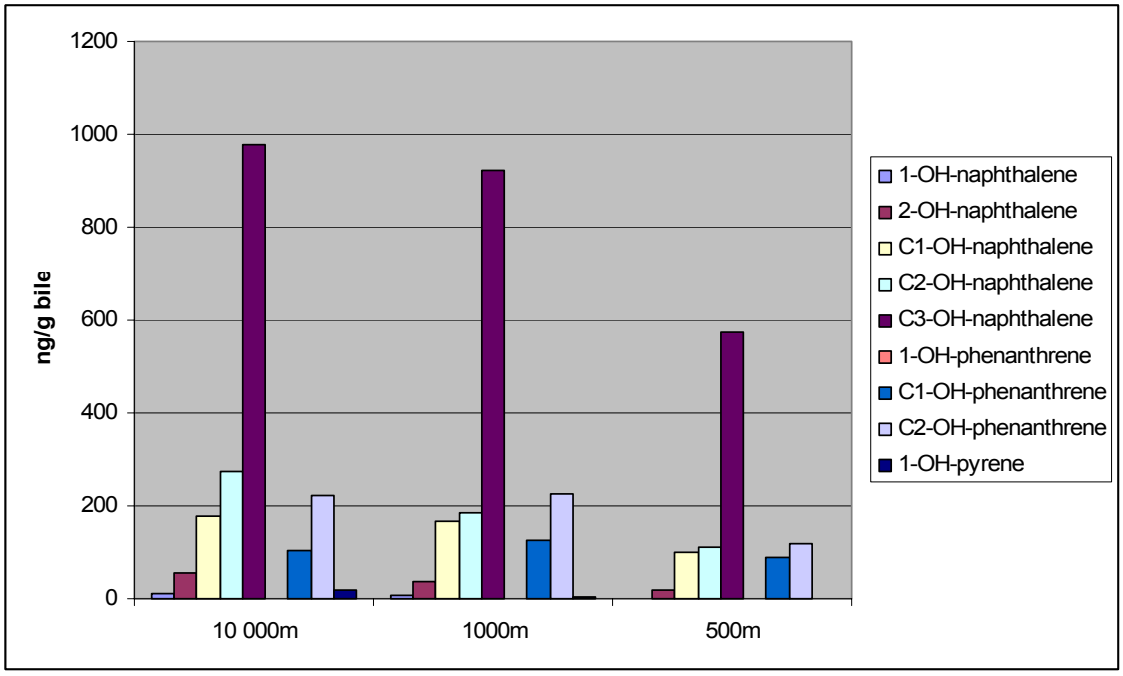

Figure 12. PAH metabolites in feral saithe given in ng/g bile as mean of five selected individuals from each of the groups. See Appendix 5 for sample selection.

3.6 Lysosomal response in mussels

Lysosomal response is within the normal range of retention time usually observed for blue mussels in unexposed areas. There is no statistical evidence that any of the groups are differing from the others. From the means some trends can be seen, mainly that the 500 metres group are the mussels that seem to be the worst in health. It is unknown weather transport to the onshore lab influenced the results. The ideal would have been to analyse the NRRT directly on the vessel and for pre exposure group at the mussel-farm before the study. Another aspect is the salinity differences between the mussel-farm to the north-sea and to the lab. Mussels are known to be stressed by transport and change in salinity that inevitably occurs when transferring them to an onshore lab.

Raw data given in Appendix 6.

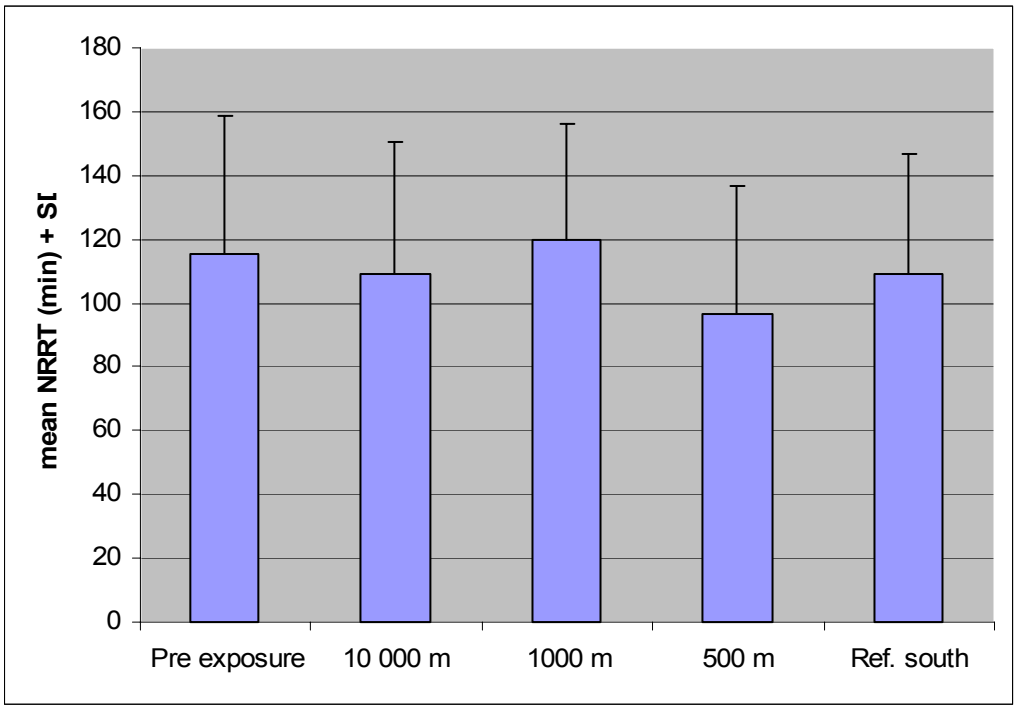

Figure 13. Mean labialization period (defined as 50% dead cells) and SD of lysosomal membrane in mussel haemolymph cells.

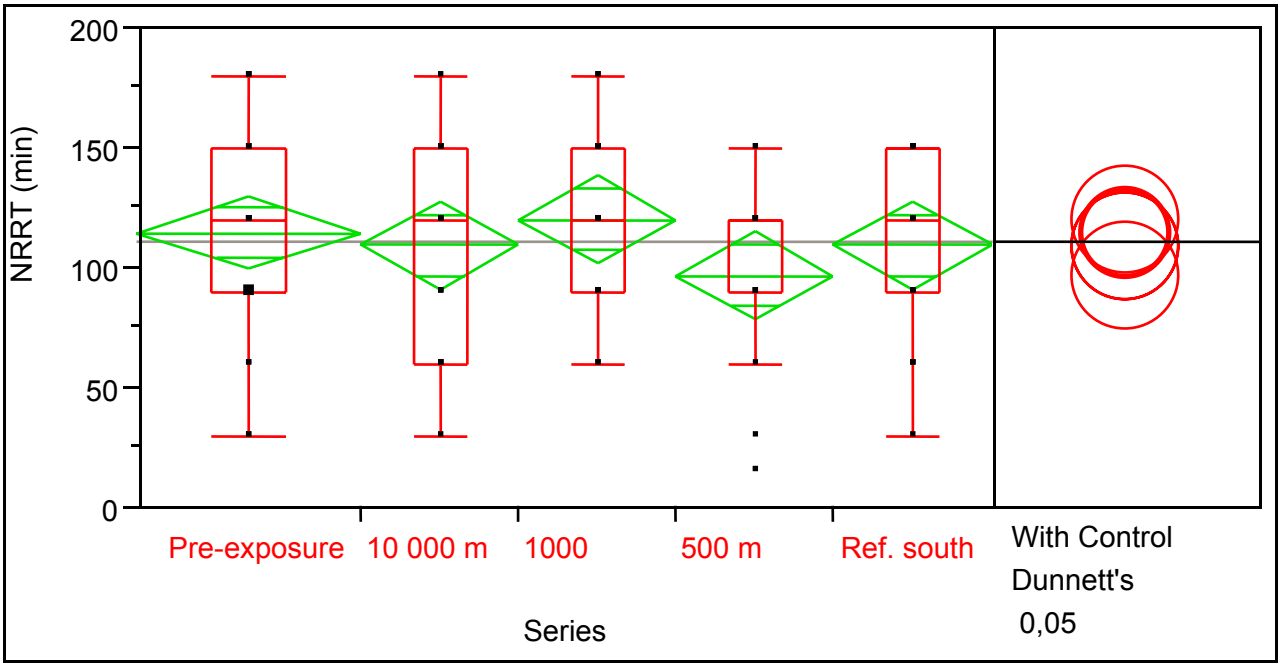

Figure 14. Statistics on labialization period of lysosomal membrane in mussel haemolymph cells show no significant differences among groups.

3.7 EROD activity in cod gills

EROD activity in cod gills show a great deal of intra group variation typically seen in field studies. Some of the variation is expected to be caused by limited measuring accuracy due to the low activity present. Raw data given in Appendix 7.

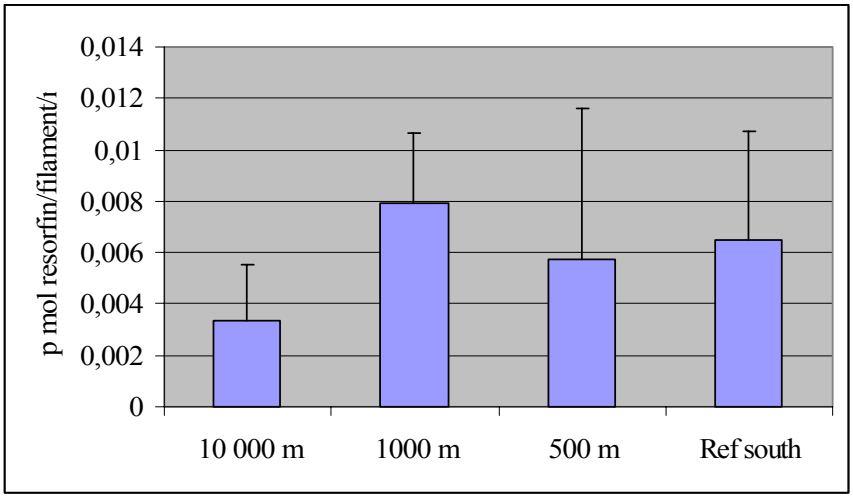

Figure 15. EROD activity in cod gills.

3.8 GST activity in cod liver

Hepatic glutathion-S-transferase (GST) activity in cod is shown in Figure 16. After Post Hoc Test (homogeneity of variance, Levenne in SPSS) Tukey test was preformed. Except for a statistically significant difference (p=0,0002) between the pre exposure and the 1000m groups, no significant difference among groups were found.

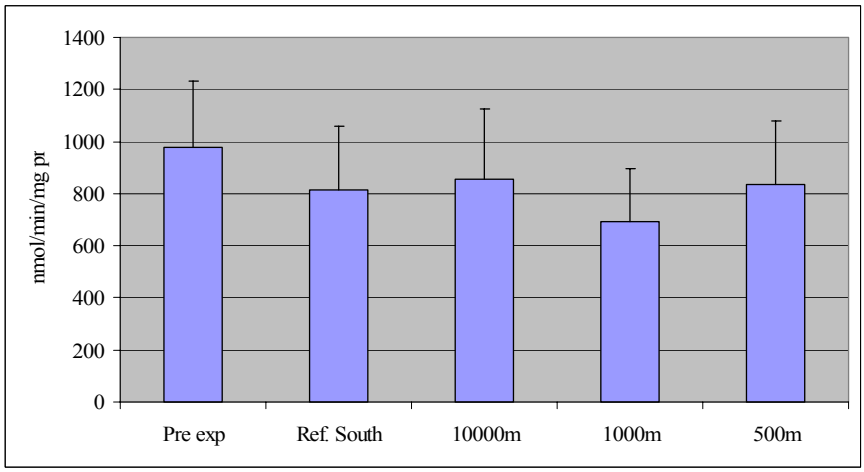

Figure 16. Hepatic GST activity measured in cod. Data is given as mean and SD of 27 individuals in each group.

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Appendix 1

Sampling information caged cod.

Sampling WCM 2004 - Caged cod

Date: 17.09.2004 Group: 10000m

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
101	40,0	0,7020	0,0725	F	
102	41,0	0,6150	0,0582	M	
103	41,0	0,6725	0,0750	M	Green liver
104	39,0	0,6435	0,0628	M	
105	41,5	0,8180	0,0998	M	
106	47,0	0,9440	0,8300	F	
107	37,5	0,5438	0,0760	M	
108	43,0	0,8295	0,0972	M	
109	37.5	0,5028	0,0543	M	
110	44,0	0,9040	0,1025	M	
111	45,5	1,0640	0,1104	F	
112	39,5	0,5998	0,0570	M	
113	45,5	0,9105	0,0930	F	
114	42,0	0,7325	0,0858	M	
115	44,0	0,9030	0,0105	F	
116	47,0	1,0070	0,0970	F	
117	47,0	1,1025	0,1290	M	
118	49,5	1,0385	0,1045	F	
119	44,0	0,9115	0,1025	F	
120	44,0	0,9335	0,1136	M	
121	48,5	1,1605	0,1280	F	
122	40,0	0,5720	0,0480	M	
123	40,0	0,6555	0,0721	F	
124	46,5	1,0735	0,1159	F	
125	44,0	1,0240	0,1065	F	
126	39,5	0,6490	0,0620	M	
127	44,0	0,7460	0,0640	F	

Sampling WCM 2004 - Caged cod

Date: 17.09.2004 Group: 1000m

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
201	45,0	0,9510	0,1048	F	
202	47,5	1,1250	0,1130	F	
203	41,0	0,7575	0,0773	M	
204	42,0	0,7045	0,0725	M	
205	36,0	0,4788	0,0450	M	
206	42,0	0,7980	0,0924	M	
207	37,0	0,4752	0,0355	M	
208	49,5	1,1440	0,1222	F	
209	42,0	0,7095	0,0945	M	
210	44,0	0,9600	0,0985	M	
211	36,5	0,5592	0,0488	M	
212	46,0	0,9665	0,0910	F	
213	39,5	0,6035	0,0642	M	
214	46,5	0,9985	0,1104	F	
215	46,5	1,0300	0,1080	F	
216	39,0	0,5830	0,0718	M	
217	42,5	0,8395	0,1040	M	
218	38,0	0,6260	0,0660	M	
219	42,0	0,7850	0,0860	F	Green liver
220	43,0	0,8220	0,0688	F	
221	44,0	0,9645	0,1100	M	
222	43,5	0,8830	0,0860	F	
223	39,0	0,5370	0,0430	M	
224	42,0	0,6970	0,0710	F	
225	38,5	0,6910	0,0700	M	
226	43,0	0,7930	0,0750	F	
227	40,0	0,6640	0,0613	F	

Sampling WCM 2004 - Caged cod

Date: 17.09.2004 Group: 500m

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
301	44,0	1,0420	0,1055	M	
302	45,5	0,9385	0,0935	F	
303	38,0	0,5570	0,0500	M	
304	37,0	0,5086	0,0510	M	
305	46,0	1,0250	0,1102	M	
306	45,0	0,9225	0,1040	F	
307	44,0	0,7920	0,0800	F	
308	42,0	0,7200	0,0730	M	
309	44,0	0,8265	0,0920	M	
310	40,5	0,5950	0,0560	M	
311	39,0	0,6590	0,0830	M	
312	44,0	0,8675	0,0918	M	
313	42,5	0,7525	0,0847	M	
314	43,0	0,7570	0,0933	M	
315	42,0	0,7970	0,0940	M	
316	41,0	0,7475	0,0702	M	
317	45,5	0,8520	0,0850	F	
318	42,0	0,7350	0,0712	M	
319	42,5	0,8110	0,0813	M	
320	39,5	0,6215	0,0460	F	
321	41,0	0,5932	0,0920	F	
322	44,0	0,8835	0,0890	F	
323	43,0	0,7925	0,0500	M	
324	35,5	0,4752	0,1112	M	
325	45,5	1,0175	÷	M	
326	47,0	1,0990	0,0150	M	histologi marked 602
327	36,5	0,4840	0,0480	M	

Sampling WCM 2004 - Caged cod

Date: 18.09.2004 kl. 01:10 Group: Ref. South

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
401	43,5	0,7680	0,0630	M	
402	42,5	0,7705	0,0842	M	
403	41,5	0,7515	0,0692	F	
404	39,0	0,6385	0,0745	M	
405	49,5	1,1645	0,1360	F	
406	41,0	0,7380	0,0745	M	
407	43,5	0,8715	0,0945	F	
408	39,5	0,7000	0,0857	M	
409	43,0	0,8255	0,1029	F	
410	42,5	0,6690	0,0631	M	
411	44,0	0,8750	0,1001	M	
412	50,0	1,1825	0,1234	F	
413	46,5	0,9585	0,1068	F	
414	42,5	0,7815	0,0976	F	
415	45,0	0,9510	0,0945	M	
416	39,5	0,5084	0,0417	F	skull deformed
417	42,0	0,6080	0,0572	M	
418	46,0	0,8915	0,0912	F	
419	42,0	0,7765	0,0928	F	
420	46,0	1,0580	0,1351	F	
421	42,0	0,7740	0,0870	M	
422	44,0	0,8855	0,0980	M	
423	44,5	0,8225	0,0760	F	
424	40,0	0,8220	0,0940	M	
425	44,0	0,8730	0,0840	M	
426	44,0	0,8540	0,0918	F	
427	41,0	0,7360	0,0728	M	

Sampling WCM 2004 - Caged cod

Cage lost, fish frozen round on vessel. Liver partly dissolved - weight uncertain.

Date:	31.09.2004		Group:	2500m	
Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
501	44,0	0,981	0,0880	F	Only bile
502	39,5	0,674	0,0683	M	Only bile
503	39,0	0,655	0,0447	M	Only bile
504	36,0	0,560	0,0421	M	Only bile
505	38,0	0,593	0,0535	M	Only bile
506	38,0	0,634	0,0675	M	Only bile
507	37,0	0,625	0,0360	F	Only bile
508	42,5	1,008	0,0990	F	Only bile
509	40,0	0,707	0,0800	M	Only bile
510	37,0	0,606	0,0565	F	Only bile
511	38,0	0,600	0,0480	M	Only bile
512	37,0	0,602	0,0460	M	Only bile
513	36,0	0,582	0,0295	F	Only bile
514	39,0	0,750	0,0774	M	Only bile
515	39,5	0,633	0,0406	F	Only bile
516	41,0	0,855	0,0717	F	Only bile
517	37,0	0,533	0,0361	M	Only bile
518	39,0	0,686	0,0170	M	Only bile
519	36,5	0,494	0,0110	M	Only bile
520	38,5	0,743	0,0680	M	Only bile
521	37,0	0,561	0,0600	M	Only bile
522	37,0	0,540	0,0470	M	Only bile
523	37,0	0,557	0,0450	M	Only bile
524	33,0	0,476	0,0300	M	Only bile

Sampling WCM 2004 - Cod, Pre-exposure sampling

Date:	05.08.2004		Group:	0-sampling	
Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
1	41,00	0,90		M	
2	45,50	0,97		M	
3	41,00	0,93		M	
4	36,00	0,56		M	
5	42,00	0,52		M	
6	38,00	0,38		F	
7	46,00	1,09		F	
8	42,50	0,55		M	
9	42,50	0,98		M	
10	44,00	0,99		F	
11	37,50	0,61		M	
12	40,50	0,85		M	
13	44,50	1,07		F	histopat. L, K, G
14	41,00	0,87		M	histopat. L, K, G
15	35,50	0,45		M	histopat. L, K, G
16	46,50	1,13		F	histopat. L, K, G
17	42,00	0,94		M	histopat. L, K, G
18	41,50	0,72		M	histopat. L, K, G
19	39,50	0,66		F	histopat. L, K, G
20	39,50	0,78		M	histopat. L, K, G
21	46,00	1,18		F	histopat. L, K, G
22	47,50	1,14		F	histopat. L, K, G
23	35,50	0,50		F	histopat. L, K, G
24	47,00	1,08		F	histopat. L, K, G
25	42,50	0,74		M	histopat. L, K, G
26	46,50	1,22		M	histopat. L, K, G
27	45,50	1,02		F	histopat. L, K, G
28	44,00	0,98		F	histopat. L, K, G
29	36,50	0,52		M	histopat. L, K, G
30	34,00	0,46		M	histopat. L, K, G
31	37,50	0,67		M	histopat. L, K, G
32	46,00	1,18		F	histopat. L, K, G
33	37,50	0,56		M	histopat. L, K, G
34	43,50	0,95		F	histopat. L, K, G
35	42,00	0,95		F	histopat. L, K, G
36	45,50	1,06		M	histopat. L, K, G
37	39,00	0,66		M	histopat. L, K, G
38	41,50	0,89		F	histopat. L, K, G
39	44,00	0,97		F	histopat. L, K, G
40	42,00	1,02		F	histopat. L, K, G
41	44,50	0,91		M	histopat. L, K, G
42	46,00	1,09		F	
43	42,50	0,80		M	
44	36,50	0,58		M	
45	39,50	0,71		M	
46	39,50	0,75		M	
47	38,50	0,62		M	
48	43,50	0,95		M	

49	42,50	0,96		M	
50	36,00	0,51		M	
51	46,50	1,27		F	
52	39,50	0,82		M	
53	38,00	0,69		M	
54	36,00	0,53		M	
55	39,50	0,78		M	

QA: AHST

Appendix 2

Sampling information feral saithe

Sampling WCM 2004 - Feral saithe (*Pollachius virens*)

Date: 06.08.2004 Group: 500m / 1000m / 10000m

Fish no.	Length (cm)	Weight-kg	Liver (kg)	Sex - M/F	Comments
500m-1	44,5	0,97		F	No bile
2	66,0	2,20		M	Pollock (*Pollachius pollachius*)
3	55,0	1,66		F	No bile
4	49,0	0,98		F	No bile
5	46,0	1,01		M	No bile
6	45,0	0,83		F	No bile
7	55,0	1,35		M	
8	58,0	1,88		M	No bile
9	52,0	1,43		F	No bile
10	45,0	0,79		F	No bile
11	47,0	0,98		M	
12	51,0	1,12		F	No bile
13	47,0	0,64		M	No bile
14	45,5	0,85		F	
15	41,0	0,70		M	
16	49,0	1,05		F	
17	50,0	1,16		M	
18	53,0	1,48		M	
19	48,0	1,37		F	
20	41,0	0,71		F	No bile
21	44,0	0,95		F	
22	41,0	0,73		M	
23	44,0	0,88		F	No bile
24	46,0	1,07		M	
1000m-25	44,0	0,77		M	No bile
26	47,0	0,92		M	
27	48,0	1,18		F	
28	56,0	1,65		M	No bile
29	45,0	0,92		F	
30	54,0	1,33		F	

31	54,0	1,24		M	
32	50,0	1,12		M	
33	44,0	0,71		M	No bile
34	52,0	1,30		M	
35	51,0	1,18		F	
36	57,0	1,58		M	
37	42,0	0,70		M	
38	43,0	0,78		M	
39	54,0	1,47		M	
40	46,0	0,96		M	
41	44,0	0,82		F	
42	47,0	0,94		F	
43	52,0	1,20		M	No bile
44	44,0	0,78		F	
45	45,0	0,92		F	
46	46,0	0,78		M	
47	47,0	0,90		M	
48	42,0	0,67		F	
49	43,0	0,65		F	
50	47,0	0,83		M	
51	50,0	1,02		M	No bile
52	48,0	0,95		F	
53	52,0	1,26		M	
54	46,0	0,92		F	No bile
10000m-55	50,0	1,05		M	No bile
56	51,0	1,38		F	
57	45,0	1,23		M	
58	59,0	1,80		F	
59	49,0	0,90		F	
60	44,0	0,83		M	No bile
61	60,0	1,85		F	
62	56,0	1,22		F	
63	55,0	1,54		M	
64	56,0	1,47		F	No bile
65	65,0	2,26		F	
66	55,0	1,20		F	
67	53,0	1,18		F	
68	48,0	0,93		M	
69	50,0	1,16		M	
70	70,0	2,85		M	No bile
71	47,0	1,05		M	No bile
72	53,0	1,38		F	No bile
73	43,0	0,74		M	
74	59,0	1,76		M	No bile
75	52,0	1,25		F	
76	49,0	1,14		F	No bile
77	51,0	1,15		F	
78	57,0	1,50		F	
79	54,0	1,52		M	

QA: AHST

Appendix 3.

Sampling information mussels.

Sampling WCM 2004 - Mussel

Stylus removed from digestive (all groups)

Date: 21.09.2004 10000m

Mussel No.	Length (mm)	Weight-g	Comments
101	59,0	16,75	
102	59,0	16,62	
103	54,0	17,40	
104	58,0	19,92	
105	57,0	16,31	
106	57,0	18,37	
107	67,0	23,17	
108	59,0	18,94	
109	73,0	34,73	
110	65,0	26,58	
111	62,0	27,43	
112	62,0	23,25	
113	70,0	32,53	
114	72,0	35,44	
115	66,0	28,79	
116	61,0	24,04	
117	66,0	30,20	
118	54,0	18,55	
119	80,0	42,90	
120	73,0	40,92	

Sampling WCM 2004 - Mussel

Date:	21.09.2004		1000m
Mussel No.	Length (mm)	Weight-g	Comments
201	57,0	19,17	
202	62,0	27,96	
203	60,0	21,05	
204	65,0	26,33	
205	64,0	31,66	
206	60,0	21,62	
207	58,0	24,98	
208	65,0	27,50	
209	65,0	27,89	
210	69,0	33,34	
211	77,0	41,94	
212	56,0	17,07	
213	60,0	19,70	
214	66,0	27,57	
215	75,0	43,28	
216	52,0	16,04	
217	53,0	13,06	
218	55,0	17,35	
219	57,0	16,78	
220	60,0	24,42	

Sampling WCM 2004 - Mussel

Date:	21.09.2004		500m
301	64,0	29,76	
302	57,0	20,19	
303	62,0	25,24	
304	65,0	24,67	
305	58,0	17,23	
306	60,0	22,39	
307	69,0	19,14	
308	51,0	16,17	
309	69,0	24,53	
310	70,0	34,14	
311	72,0	35,83	
312	60,0	26,38	
313	70,0	38,09	
314	66,0	31,54	
315	71,0	33,44	
316	56,0	16,00	
317	61,0	26,25	
318	63,0	26,63	
319	68,0	37,57	
320	59,0	21,86	

Sampling WCM 2004 - Mussel

Date:	21.09.2004		Ref south
Mussel no.	Length (mm)	Weight-g	Comments
401	65,0	25,21	
402	70,0	38,46	
403	60,0	20,83	
404	63,0	24,80	
405	68,0	32,67	
406	55,0	15,75	
407	64,0	20,48	
408	61,0	21,83	
409	65,0	24,22	
410	71,0	34,56	
411	53,0	15,30	
412	55,0	15,74	
413	70,0	32,80	
414	76,0	42,11	
415	57,0	20,61	
416	66,0	28,41	
417	58,0	17,11	
418	66,0	26,05	
419	58,0	19,39	
420	63,0	22,08	

Sampling WCM 2004 - Mussel

Date:	11.08.2004		0-sampling
Mussel no.	Length (mm)	Weight-g	Comments
1	73,0	32,54	
2	65,0	25,32	
3	73,0	33,01	
4	65,0	26,02	
5	73,0	33,25	
6	68,0	31,52	
7	70,0	25,68	
8	68,0	28,63	
9	73,0	29,88	
10	63,0	24,35	
11	70,0	32,12	
12	71,0	33,72	
13	62,0	23,08	
14	66,0	23,86	
15	68,0	29,04	
16	67,0	26,27	
17	71,0	27,13	
18	65,0	24,79	
19	59,0	18,12	
20	60,0	20,02	
			QA: AHST

Appendix 4

Raw data from Fixed Fluorecence and bileverdin in caged cod. Dilution: Bileleverdin, 400X and FF, 1600X.* Analyzed twice

Group	**Fish no**	**Biliverdin-abs**	**Biliverdin mg/ml**	**290/335**	$PFE_{290/334}$ - µg/ml	**341/383**	$PFE_{341/383}$ - µg/ml	**380/430**	$PFE_{380/430}$ - µg/ml	**Scan**	**Comments**
	Solvent	0	0	0,1	0,1	1,3	1,3	0,6	0,6		
10 000 m	101	0,0195	0,4446	7,7	7,5	2,8	2,7	1,2	1,2		
10 000 m	102	0,022	0,5016	10,1	9,8	3	2,9	1,4	1,4	X	
10 000 m	103	0,0075	0,1710	3,1	3,0	2	1,9	1,1	1,1	X	GCMS
10 000 m	104	0,03	0,6840	9,6	9,3	2,8	2,7	1,2	1,2		
10 000 m	105	0,0205	0,4674	10,5	10,2	2,8	2,7	1,2	1,2		
10 000 m	106	0,0595	1,3566	10,6	10,3	3	2,9	1,5	1,5		
10 000 m	107	0,017	0,3876	13,8	13,4	2,9	2,8	1,3	1,3		GCMS
10 000 m	108	no bile									
10 000 m	109	0,005	0,1140	8,7	8,5	3,6	3,5	1,8	1,8	X	GCMS
10 000 m	110	0,06	1,3680	13,3	12,9	2,8	2,7	1,2	1,2		
10 000 m	111	0,027	0,6156	6,2	6,0	2,3	2,2	1	1,0		
10 000 m	112	0,0115	0,2622	9,7	9,4	3,1	3,0	1,4	1,4		
10 000 m	113	0,02	0,4560	10,2	9,9	3,6	3,5	1,6	1,6		
10 000 m	114	0,004	0,0912	4,7	4,6	2	1,9	0,8	0,8		
10 000 m	115	0,018	0,4104	11,8	11,5	2,8	2,7	1,2	1,2		
10 000 m	116	0,027	0,6156	11,6	11,3	3,4	3,3	1,5	1,5		
10 000 m	117	0,052	1,1856	16,3	15,9	3,2	3,1	1,5	1,5	X	GCMS
10 000 m	118	0,028	0,6384	9,2	9,0	2,6	2,5	1,2	1,2		
10 000 m	119	0,018	0,4104	10,5	10,2	3	2,9	1,4	1,4		
10 000 m	120	0,011	0,2508	8,8	8,6	2,7	2,6	1,5	1,5		
10 000 m	121	0,035	0,798	14,1	13,7	3,2	3,1	1,6	1,6		
10 000 m	122	0,005	0,114	11,1	10,8	3,6	3,5	1,8	1,8	X	GCMS
10 000 m	123	0,018	0,4104	12,8	12,5	2,7	2,6	1	1,0		

10 000 m	124	0,02	0,456	7,5	7,3	2,5	2,4	1,1	1,1		
10 000 m	125	0,017	0,3876	3,8	3,7	2,1	2,0	0,9	0,9		
10 000 m	126	0,01	0,228	7,8	7,6	3,3	3,2	1,4	1,4		
10 000 m	127	0,035	0,798	10,9	10,6	2,8	2,7	1,4	1,4		
1000 m	201	0,02	0,456	6,9	6,7	2,6	2,5	1,2	1,2		
1000 m	202	0,019	0,4332	6,2	6,0	2,4	2,3	1,1	1,1	X	
1000 m	203	0,03	0,684	9,2	9,0	2,8	2,7	1,2	1,2		
1000 m	204	0,01	0,228	9,3	9,1	3,1	3,0	1,5	1,5		
1000 m	205	0,032	0,7296	12,3	12,0	3,1	3,0	1,3	1,3	X	GCMS
1000 m	206	0,0365	0,8322	9,4	9,1	2,8	2,7	1,3	1,3		
1000 m	207	0,031	0,7068	10,4	10,1	3,3	3,2	1,2	1,2		
1000 m	208	0,021	0,4788	5,9	5,7	2,3	2,2	1	1,0		
1000 m	209	0,0115	0,2622	13	12,7	2,3	2,2	1	1,0		
1000 m	210	0,026	0,5928	8,1	7,9	3,3	3,2	1,4	1,4		
1000 m	211	0,014	0,3192	8,2	8,0	2,6	2,5	1,1	1,1	X	
1000 m	212	0,0285	0,6498	11,4	11,1	2,7	2,6	1,3	1,3		
1000 m	213	0,008	0,1824	5,9	5,7	2,7	2,6	1,2	1,2		
1000 m	214	0,018	0,4104	7,6	7,4	3	2,9	1,4	1,4		
1000 m	215	0,021	0,4788	9,1	8,9	2,5	2,4	1,1	1,1		
1000 m	216	0,039	0,8892	12,9	12,6	3,5	3,4	1,4	1,4		
1000 m	217	0,049	1,1172	21,4	20,8	2,4	2,3	1,1	1,1	X	GCMS
1000 m	218	0,02	0,4560	9,2	9,0	3,5	3,4	1,5	1,5		
1000 m	219	0,021	0,4788	9,3	9,1	2,9	2,8	1,5	1,5		
1000 m	220	0,025	0,5700	10,5	10,2	2,9	2,8	1,3	1,3		
1000 m	221	0,018	0,4104	6,9	6,7	2,3	2,2	0,9	0,9		
1000 m	222	0,019	0,4332	9,3	9,1	2,1	2,0	0,9	0,9		
1000 m	223	0,038	0,8664	16,6	16,2	4,1	4,0	1,6	1,6		GCMS
1000 m	224	0,029	0,6612	20,8	20,2	4,8	4,7	1,8	1,8	X	GCMS
1000 m	225*	0,016	0,3648	71	69,1	1,9	1,8	0,9	0,9	X	GCMS
1000 m	226	0,0265	0,6042	11,2	10,9	2,5	2,4	1,1	1,1		
1000 m	227	0,032	0,7296	13,9	13,5	3,3	3,2	1,3	1,3		

500 m	301	0,0285	0,6498	9,8	9,5	3,3	3,2	1,5	1,5		
500 m	302	0,021	0,4788	10,1	9,8	2,9	2,8	1,1	1,1		
500 m	303	0,016	0,3648	12,2	11,9	3,5	3,4	1,4	1,4		
500 m	304	0,0185	0,4218	11,2	10,9	3,5	3,4	1,5	1,5	X	
500 m	305	0,055	1,254	14,4	14,0	3,6	3,5	1,2	1,2		
500 m	306	0,0095	0,2166	5,9	5,7	2,2	2,1	0,9	0,9	X	
500 m	307	0,017	0,3876	11,5	11,2	3,4	3,3	1,3	1,3		
500 m	308	0,012	0,2736	7,5	7,3	2,9	2,8	1,4	1,4		
500 m	309	0,01	0,228	8,1	7,9	2,6	2,5	1,1	1,1		
500 m	310	0,016	0,3648	11,8	11,5	2,3	2,2	0,9	0,9		GCMS
500 m	311	0,018	0,4104	11,3	11,0	3,6	3,5	1,5	1,5		
500 m	312	0,0435	0,9918	11,2	10,9	3,2	3,1	1,4	1,4		
500 m	313	0,0235	0,5358	10,8	10,5	3,2	3,1	1,4	1,4	X	
500 m	314	0,022	0,5016	12,2	11,9	3,6	3,5	1,4	1,4		
500 m	315	0,044	1,0032	7,5	7,3	2,3	2,2	0,9	0,9		GCMS
500 m	316	0,015	0,3420	12	11,7	3,7	3,6	1,4	1,4		
500 m	317	0,0375	0,8550	9,1	8,9	3	2,9	1,1	1,1		
500 m	318	0,024	0,5472	12,8	12,5	3,2	3,1	1,4	1,4		
500 m	319	0,049	1,1172	16,5	16,1	3,8	3,7	1,5	1,5		GCMS
500 m	320	0,0315	0,7182	11,8	11,5	3,1	3,0	1,1	1,1		
500 m	321	0,026	0,5928	13,7	13,3	3,8	3,7	1,6	1,6		GCMS
500 m	322	0,011	0,2508	5,8	5,6	2,4	2,3	1	1,0		
500 m	323	0,026	0,5928	9,9	9,6	3,2	3,1	1,2	1,2		
500 m	324	0,0135	0,3078	9	8,8	2,8	2,7	1,2	1,2		
500 m	325	0,053	1,2084	16,2	15,8	3,7	3,6	1,5	1,5		GCMS
500 m	326	0,016	0,3648	11,4	11,1	3,7	3,6	1,7	1,7		
500 m	327	0,0155	0,3534	12,8	12,5	4	3,9	1,5	1,5		
Ref south	401	0,0295	0,6726	10,1	9,8	2,4	2,3	1,7	1,7	X	
Ref south	402	0,021	0,4788	5,2	5,1	1,9	1,8	1,2	1,2	X	
Ref south	403	0,01	0,228	4,3	4,2	2	1,9	1,3	1,3		
Ref south	404	0,0165	0,3762	5,8	5,6	2,1	2,0	1,3	1,3		

Ref south	405	0,011	0,2508	6,4	6,2	2,4	2,3	1,5	1,5		
Ref south	406	0,018	0,4104	3,4	3,3	1,6	1,6	0,9	0,9	X	
Ref south	407	0,038	0,8664	9,1	8,9	2,3	2,2	1,4	1,4		
Ref south	408	0,044	1,0032	10,5	10,2	2,4	2,3	1,5	1,5		GCMS
Ref south	409	0,0325	0,741	8,8	8,6	2,4	2,3	1,6	1,6	X	
Ref south	410	0,01	0,228	6,1	5,9	2,5	2,4	1,5	1,5		
Ref south	411	0,036	0,8208	10,1	9,8	2	1,9	1,4	1,4		
Ref south	412	0,032	0,7296	9	8,8	2,2	2,1	1,5	1,5		
Ref south	413	0,02	0,456	13,9	13,5	3,2	3,1	1,9	1,8	X	GCMS
Ref south	414	0,021	0,4788	8,4	8,2	2,6	2,5	1,6	1,6		
Ref south	415	0,021	0,4788	7,2	7,0	2,2	2,1	1,4	1,4		
Ref south	416	0,053	1,2084	17,5	17,0	3,1	3,0	1,8	1,8		GCMS
Ref south	417	0,021	0,4788	8,8	8,6	2	1,9	1,1	1,1		
Ref south	418	0,023	0,5244	18,2	17,7	3,5	3,4	2,1	2,0		GCMS
Ref south	419	0,0275	0,627	12,3	12,0	2,7	2,6	1,7	1,7	X	
Ref south	420	0,068	1,5504	11	10,7	2,4	2,3	1,4	1,4		
Ref south	421	0,021	0,4788	10,5	10,2	2,4	2,3	1,6	1,6		
Ref south	422	0,0195	0,4446	10,6	10,3	2,6	2,5	1,6	1,6		
Ref south	423	0,0305	0,6954	12,3	12,0	2,7	2,6	1,9	1,8		
Ref south	424	0,022	0,5016	13,4	13,0	2,5	2,4	1,7	1,7		
Ref south	425	0,027	0,6156	3,3	3,2	1,8	1,8	1,1	1,1	X	
Ref south	426	0,046	1,0488	7,4	7,2	2	1,9	1,3	1,3	X	GCMS
Ref south	427	0,038	0,8664	9,5	9,2	2,3	2,2	1,8	1,8		
	solvent	0	0	0,2	0,2	1,3	1,3	0,6	0,6		
2500 m	501	0,046	1,0488	12,6	12,3	2,3	2,2	1,1	1,1	X	
2500 m	502	0,018	0,4104	11,5	11,2	2,5	2,4	1,2	1,2		
2500 m	503	0,0095	0,2166	15,4	15,0	2,2	2,1	1,2	1,2	X	
2500 m	504	0,006	0,1368	11,9	11,6	1,7	1,7	0,8	0,8		
2500 m	505	0,0135	0,3078	11,2	10,9	2,7	2,6	1,3	1,3		
2500 m	506	0,010	0,228	13	12,7	2,8	2,7	1,5	1,5		
2500 m	507	0,008	0,1824	14,3	13,9	2,9	2,8	1,2	1,2		

2500 m	508	0,023	0,5244	14,9	14,5	2,2	2,1	1,0	1,0		
2500 m	509	0,021	0,4788	9,8	9,5	2,0	1,9	1,0	1,0	X	
2500 m	510	0,011	0,2508	11,8	11,5	2,1	2,0	0,9	0,9		
2500 m	511	0,0235	0,5358	16,0	15,6	2,0	1,9	1,0	1,0		
2500 m	512	0,007	0,1596	12,3	12,0	2,2	2,1	1,1	1,1		
2500 m	513	0,000	0,00	17,8	17,3	2,3	2,2	1,2	1,2		
2500 m	514	0,000	0,00	15,1	14,7	2,5	2,4	1,0	1,0		
2500 m	515	0,000	0,00	10	9,7	2,0	1,9	1,0	1,0	X	
2500 m	516	0,000	0,00	13,7	13,3	2,2	2,1	1,1	1,1		
2500 m	517	0,000	0,00	12,2	11,9	2,3	2,2	1,0	1,0		
2500 m	518	0,000	0,00	10,6	10,3	2,1	2,0	1,0	1,0		
2500 m	519	0,000	0,00	15,6	15,2	2,0	1,9	0,9	0,9		
2500 m	520	0,000	0,00	13,8	13,4	2	1,9	0,9	0,9	X	
2500 m	521	0,000	0,00	10,6	10,3	2,5	2,4	1,3	1,3		
2500 m	522	0,000	0,00	14,8	14,4	1,8	1,8	0,8	0,8		
2500 m	523	0,000	0,00	11,8	11,5	2,2	2,1	1,0	1,0		
2500 m	524	0,000	0,00	16,4	16,0	1,9	1,8	0,9	0,9	X	
Pre exp.	Cod 1	0,01	0,228	11,8	11,5	4	3,9	1,2	1,2	X	
Pre exp.	Cod 2	0,025	0,57	17,6	17,1	5,2	5,1	2,6	2,5		
Pre exp.	Cod 3	0,038	0,8664	26	25,3	6	5,8	3,1	3,0	X	
Pre exp.	Cod 4	0,049	1,1172	21	20,4	5,7	5,5	3,5	3,4		GCMS
Pre exp.	Cod 5	0,04	0,912	21	20,4	5	4,9	2,7	2,6		
Pre exp.	Cod 6	0,004	0,0912	6,1	5,9	3,7	3,6	1	1,0		GCMS
Pre exp.	Cod 7	0,041	0,9348	29	28,2	6,3	6,1	3,2	3,1	X	
Pre exp.	Cod 8	0,048	1,0944	21	20,4	5,3	5,2	2,9	2,8		
Pre exp.	Cod 9	0,0135	0,3078	20,6	20,0	8	7,8	2,1	2,0		GCMS
Pre exp.	Cod 10	0,007	0,1596	9,5	9,2	5,1	5,0	1,5	1,5		
Pre exp.	Cod 11	0,0135	0,3078	10,3	10,0	3,7	3,6	1,5	1,5		
Pre exp.	Cod 12	0,023	0,5244	13,5	13,1	5,7	5,5	1,8	1,8		
Pre exp.	Cod 13	0,033	0,7524	13,8	13,4	4	3,9	2,1	2,0		
Pre exp.	Cod 14	0,033	0,7524	18,9	18,4	6,1	5,9	2,4	2,3		

Pre exp.	Cod 15	0,0045	0,1026	9,7	9,4	4,8	4,7	1,4	1,4		
Pre exp.	Cod 16	0,026	0,5928	27	26,3	4,6	4,5	2,4	2,3		
Pre exp.	Cod 17	0,0175	0,3990	26	25,3	6,8	6,6	3	2,9		
Pre exp.	Cod 18	0,0715	1,6302	17,9	17,4	4,1	4,0	2,7	2,6		
Pre exp.	Cod 19	0,015	0,3420	10,4	10,1	5	4,9	1,7	1,7	X	
Pre exp.	Cod 20	0,011	0,2508	12,4	12,1	5,4	5,3	1,7	1,7		
Pre exp.	Cod 21	0,016	0,3648	14,2	13,8	5,1	5,0	1,9	1,8		
Pre exp.	Cod 22	0,028	0,6384	20,6	20,0	5,8	5,6	2,9	2,8		
Pre exp.	Cod 23	0,035	0,7980	18,7	18,2	5,2	5,1	2,7	2,6		
Pre exp.	Cod 24	0,008	0,1824	12,9	12,6	7	6,8	1,8	1,8		GCMS
Pre exp.	Cod 25	0,054	1,2312	13,7	13,3	4,2	4,1	2,4	2,3		
Pre exp.	Cod 26	0,027	0,6156	32	31,1	5,6	5,4	3,1	3,0	X	GCMS

Appendix 5

Raw data from Fixed Fluorecence and bileverdin in feral saithe. Dilution: Bileleverdin, 400X and FF, 1600X.*

Group	**Sanple no**	**Biliverdin-abs**	**Biliverdin mg/ml**	**290/335**	$PFE_{290/334}$ - µg/ml	**341/383**	$PFE_{341/383}$ - µg/ml	**380/430**	$PFE_{380/430}$ - µg/ml	**Scan**	**Comments**
	Solvent	0	0	0,1	0,1	1,4	1,4	0,6	0,6		
500 m	Sei 4	0,013	0,2964	5,7	5,5	2,2	2,1	1	1,0		GCMS
500 m	Sei 7	0,018	0,4104	4,9	4,8	1,9	1,8	0,9	0,9	X	
500 m	Sei 10	0,007	0,1596	6,3	6,1	2,2	2,1	0,8	0,8		GCMS
500 m	Sei 11	0,008	0,1824	3,8	3,7	2	1,9	0,8	0,8		
500 m	Sei 14	0,004	0,0912	4,2	4,1	1,7	1,7	0,7	0,7		
500 m	Sei 15	0,0065	0,1482	8,9	8,7	2,9	2,8	0,9	0,9		GCMS
500 m	Sei 16	0,006	0,1368	7,2	7,0	2,8	2,7	0,9	0,9	X	GCMS
500 m	Sei 17	0,016	0,3648	7	6,8	2,7	2,6	0,8	0,8		
500 m	Sei 18	0,004	0,0912	4,8	4,7	1,8	1,8	0,8	0,8		
500 m	Sei 19	0,006	0,1368	2,5	2,4	1,7	1,7	0,7	0,7		GCMS
500 m	Sei 22	0,006	0,1368	7,8	7,6	1,9	1,8	0,7	0,7		
500 m	Sei 24	0,013	0,2964	5,8	5,6	2,3	2,2	0,8	0,8		
1000 m	Sei 26	0,013	0,2964	3,9	3,8	1,9	1,8	0,7	0,7	X	
1000 m	Sei 27	0,002	0,0456	6,3	6,1	1,9	1,8	0,7	0,7		
1000 m	Sei 29	0,0095	0,2166	4	3,9	2	1,9	0,8	0,8		
1000 m	Sei 30	0,012	0,2736	4,4	4,3	1,9	1,8	0,8	0,8		
1000 m	Sei 31	0,0035	0,0798	3,2	3,1	1,4	1,4	0,7	0,7		
1000 m	Sei 32	0,004	0,0912	7,4	7,2	1,6	1,6	0,7	0,7		GCMS
1000 m	Sei 34	0,0135	0,3078	4,2	4,1	2	1,9	0,9	0,9	X	
1000 m	Sei 35	0,004	0,0912	3,4	3,3	1,6	1,6	0,7	0,7		
1000 m	Sei 36	0,013	0,2964	3,7	3,6	1,8	1,8	0,8	0,8		
1000 m	Sei 37	0,002	0,0456	1,8	1,8	1,6	1,6	0,7	0,7		
1000 m	Sei 38	0,0025	0,057	1,3	1,3	1,5	1,5	0,7	0,7		
1000 m	Sei 39	0,002	0,0456	0,9	0,9	1,5	1,5	0,7	0,7		GCMS

1000 m	Sei 40	0,006	0,1368	2,5	2,4	1,7	1,7	0,8	0,8	X	GCMS
1000 m	Sei 41	0,003	0,0684	4,7	4,6	1,6	1,6	0,8	0,8		
1000 m	Sei 42	0,004	0,0912	6,6	6,4	1,6	1,6	0,7	0,7		
1000 m	Sei 44	0,005	0,114	6,9	6,7	1,7	1,7	0,7	0,7		
1000 m	Sei 45	0,0115	0,2622	7,1	6,9	2,5	2,4	1	1,0		GCMS
1000 m	Sei 46	0,003	0,0684	3,1	3,0	1,6	1,6	0,7	0,7		
1000 m	Sei 47	0,003	0,0684	5,3	5,2	1,7	1,7	0,8	0,8		
1000 m	Sei 48	0,009	0,2052	6,2	6,0	1,8	1,8	0,8	0,8		
1000 m	Sei 49	0,005	0,1140	3,9	3,8	1,6	1,6	0,7	0,7		
1000 m	Sei 50	0,004	0,0912	3,3	3,2	1,5	1,5	0,7	0,7		
1000 m	Sei 52	0,012	0,2736	4,4	4,3	1,8	1,8	0,7	0,7	X	
1000 m	Sei 53	0,009	0,2052	8,1	7,9	1,8	1,8	0,8	0,8		GCMS
10000 m	Sei 56	0,018	0,4104	5,7	5,5	1,8	1,8	0,8	0,8	X	
10000 m	Sei 57	0,02	0,4560	4,5	4,4	1,7	1,7	0,8	0,8		
10000 m	Sei 58	0,0185	0,4218	7,8	7,6	2	1,9	0,9	0,9		
10000 m	Sei 59	0,012	0,2736	9,7	9,4	1,8	1,8	0,8	0,8	X	GCMS
10000 m	Sei 61	0,016	0,3648	7,6	7,4	2,1	2,0	0,8	0,8		
10000 m	Sei 62	0,013	0,2964	5,9	5,7	2,2	2,1	0,8	0,8		
10000 m	Sei 63	0,013	0,2964	6,2	6,0	2,2	2,1	0,8	0,8		
10000 m	Sei 65	0,008	0,1824	5,3	5,2	1,7	1,7	0,7	0,7		
10000 m	Sei 66	0,011	0,2508	3,8	3,7	1,8	1,8	0,7	0,7		GCMS
10000 m	Sei 67	0,027	0,6156	7,4	7,2	3,4	3,3	1,1	1,1		GCMS
10000 m	Sei 68	0,016	0,3648	5,2	5,1	2,5	2,4	0,9	0,9		GCMS
10000 m	Sei 69	0,005	0,1140	3,1	3,0	1,8	1,8	0,7	0,7		
10000 m	Sei 73	0,013	0,2964	3,7	3,6	1,8	1,8	0,8	0,8		
10000 m	Sei 75	0,012	0,2736	5,1	5,0	1,9	1,8	0,8	0,8		
10000 m	Sei 77	0,0245	0,5586	5	4,9	1,9	1,8	0,8	0,8		
10000 m	Sei 78	0,015	0,342	5,5	5,4	1,9	1,8	0,8	0,8		
10000 m	Sei 79	0,0095	0,2166	2,8	2,7	1,6	1,6	0,7	0,7		GCMS

Appendix 6.

Lysosomal response data for mussels

	Pre exposure	10 000 m	1000 m	500 m	Ref. south
1	90	150	60	120	90
2	90	150	120	150	120
3	120	60	120	90	30
4	180	120	90	30	60
5	90	60	120	120	60
6	120	150	90	90	150
7	150	150	60	90	150
8	150	120	150	15	60
9	90	90	90	150	120
10	60	30	180	120	90
11	120	60	120	15	150
12	90	150	90	120	120
13	90	60	150	90	150
14	30	120	150	120	150
15	180	90	180	60	90
16	120	90	90	120	120
17	180	120	150	120	150
18	150	180	150	90	120
19	120	120	120	120	90
20	120				
21	150				
22	30				
23	120				
24	120				
25	180				
26	60				
Mean (min)	115,38	108,95	120,00	96,32	108,95
St. Dev	43,01	41,49	36,06	40,41	37,70

Appendix 7.

Gill EROD data cod

Group		Duplikatmean	mean	stdev
10 000 m		0,005505097	0,003358	0,002158
		0,005204648		
		0,000856139		
		0,000271252		
		0,002526034		
		0,003119398		
		0,002322595		
		0,003661902		
		0,00675587		
1000 m		0,008027364	0,007942	0,002683
		0,008985223		
		0,002390408		
		0,007840878		
		0,010070231		
		0,007917168		
		0,010104137		
		0,010934846		
		0,005204648		
500 m		0,015165925	0,005731	0,005908
		0,0088808		
		0,000693813		
		-0,000595863		
		-0,000922363		
		0,00140395		
		0,007231975		
		0,007068725		
		0,012651875		
ref south		0,003795563	0,006505	0,004199
		0,002705415		
		0,008839988		
		0,011345875		
		0,011639725		
		0,007656425		
		0,005770888		
		-0,001232538		
		0,008023738		

Appendix 8.

GST raw data for cod.

Fish no.	DO1/min	DO2/min	blank	Snitt Do	(DO - DO blanc)/mn	Prot(mg/ml)	dilution	Final prot kons.	Qty in well (10µl S9)	Aktivity nmole/mn/mg proteiner
Cod 201	1,33E-01	1,89E-01	6,50E-03	1,61E-01	1,54E-01	3,85E+00	5	0,770	0,039	705,80
Cod 202	1,11E-01	1,16E-01	6,50E-03	1,14E-01	1,07E-01	3,59E+00	5	0,718	0,036	525,47
Cod 203	1,95E-03	1,62E-01	6,50E-03	8,18E-02	7,53E-02	3,38E+00	5	0,676	0,034	392,21
Cod 204	1,64E-01	1,45E-01	6,50E-03	1,55E-01	1,48E-01	3,05E+00	5	0,610	0,031	854,56
Cod 205	1,66E-01	1,41E-01	6,50E-03	1,54E-01	1,47E-01	3,88E+00	5	0,776	0,039	667,81
Cod 206	1,23E-01	1,43E-01	6,50E-03	1,33E-01	1,27E-01	3,13E+00	5	0,626	0,031	711,69
Cod 207	1,39E-01	1,23E-01	6,50E-03	1,31E-01	1,24E-01	3,25E+00	5	0,650	0,033	674,09
Cod 208	1,55E-01	1,53E-01	6,50E-03	1,54E-01	1,48E-01	3,21E+00	5	0,642	0,032	810,49
Cod 209	1,08E-01	1,18E-01	6,50E-03	1,13E-01	1,06E-01	3,58E+00	5	0,716	0,036	523,22
Cod 210	9,22E-02	-6,00E-04	6,50E-03	4,58E-02	3,93E-02	2,95E+00	5	0,590	0,030	234,61
Cod 211	1,30E-01	1,40E-01	6,50E-03	1,35E-01	1,29E-01	2,11E+00	5	0,422	0,021	1072,29
Cod 212	1,47E-01	1,74E-01	6,50E-03	1,61E-01	1,54E-01	3,76E+00	5	0,752	0,038	721,20
Cod 213	8,31E-02	8,33E-02	6,50E-03	8,32E-02	7,67E-02	2,13E+00	5	0,426	0,021	634,05
Cod 214	1,48E-01	1,84E-01	6,50E-03	1,66E-01	1,60E-01	3,54E+00	5	0,708	0,035	794,91
Cod 215	9,18E-02	1,10E-01	6,50E-03	1,01E-01	9,45E-02	4,50E+00	5	0,900	0,045	369,70
Cod 216	7,30E-02	9,50E-02	6,50E-03	8,40E-02	7,75E-02	2,65E+00	5	0,530	0,027	514,85

Cod 217	1,36E-01	1,38E-01	6,50E-03	1,37E-01	1,31E-01	3,38E+00	5	0,676	0,034	680,47
Cod 218	9,45E-02	8,83E-02	6,50E-03	9,14E-02	8,49E-02	1,68E+00	5	0,336	0,017	889,83
Cod 219	1,37E-01	1,57E-01	6,50E-03	1,47E-01	1,41E-01	3,19E+00	5	0,637	0,032	777,55
Cod 220	1,97E-01	1,79E-01	6,50E-03	1,88E-01	1,82E-01	4,07E+00	5	0,814	0,041	785,97
Cod 221	1,91E-01	1,82E-01	6,50E-03	1,86E-01	1,80E-01	3,99E+00	5	0,798	0,040	793,56
Cod 222	1,59E-01	1,66E-01	6,50E-03	1,62E-01	1,56E-01	3,49E+00	5	0,698	0,035	785,13
Cod 223	1,58E-01	1,54E-01	6,50E-03	1,56E-01	1,49E-01	3,70E+00	5	0,740	0,037	710,31
Cod 224	1,44E-01	1,52E-01	6,50E-03	1,48E-01	1,42E-01	2,11E+00	5	0,422	0,021	1181,98
Cod 225	1,69E-01	1,59E-01	6,50E-03	1,64E-01	1,57E-01	4,60E+00	5	0,920	0,046	601,57
Cod 226	1,49E-01	1,78E-01	6,50E-03	1,63E-01	1,57E-01	3,48E+00	5	0,696	0,035	793,53
Cod 227	7,15E-02	1,18E-01	6,50E-03	9,46E-02	8,81E-02	2,97E+00	5	0,594	0,030	522,01
Date:	17.09.2004		Group:	500m						
Fish no.	DO1/min	DO2/min	DO Blanc	MoyenDO	DO-DO B	Prot(mg/ml)	dilution	Final prot kons.	Qty in well (10µl S9)	Aktivity nmole/mn/mg proteiner
Cod 301	1,67E-01	1,66E-01	6,50E-03	1,66E-01	1,60E-01	3,39E+00	5	6,78E-01	0,0339	828,953
Cod 302	1,51E-01	1,41E-01	6,50E-03	1,46E-01	1,40E-01	4,00E+00	5	8,00E-01	0,04	615,675
Cod 303	1,48E-01	1,84E-01	6,50E-03	1,66E-01	1,60E-01	4,78E+00	5	9,56E-01	0,0478	587,614
Cod 304	1,45E-01	1,46E-01	6,50E-03	1,46E-01	1,39E-01	3,54E+00	5	7,08E-01	0,0354	691,239
Cod 305	1,93E-01	2,22E-01	6,50E-03	2,08E-01	2,01E-01	3,20E+00	5	6,41E-01	0,032032001	1105,353
Cod 306	1,15E-01	1,21E-01	6,50E-03	1,18E-01	1,12E-01	3,20E+00	5	6,41E-01	0,032032001	613,801
Cod 307	1,29E-01	1,30E-01	6,50E-03	1,29E-01	1,23E-01	2,55E+00	5	5,11E-01	0,025536794	847,386
Cod 308	1,60E-01	1,43E-01	6,50E-03	1,52E-01	1,45E-01	1,96E+00	5	3,92E-01	0,019597195	1305,611

Cod 309	1,71E-01	1,62E-01	6,50E-03	1,67E-01	1,60E-01	2,90E+00	5	5,79E-01	0,028951856	974,564
Cod 310	1,95E-01	2,01E-01	6,50E-03	1,98E-01	1,91E-01	3,25E+00	5	6,50E-01	0,032496706	1035,195
Cod 311	1,15E-01	1,19E-01	6,50E-03	1,17E-01	1,11E-01	1,54E+00	5	3,07E-01	0,015356332	1268,294
Cod 312	1,09E-01	1,28E-01	6,50E-03	1,19E-01	1,12E-01	1,89E+00	5	3,78E-01	0,018894712	1044,375
Cod 313	8,17E-02	8,65E-02	6,50E-03	8,41E-02	7,76E-02	1,71E+00	5	3,42E-01	0,017116067	798,137
Cod 314	8,53E-02	7,56E-02	6,50E-03	8,05E-02	7,40E-02	3,02E+00	5	6,04E-01	0,03022157	430,794
Cod 315	1,32E-01	1,32E-01	6,50E-03	1,32E-01	1,25E-01	3,01E+00	5	6,01E-01	0,030069174	733,124
Cod 316	1,28E-01	1,27E-01	6,50E-03	1,28E-01	1,21E-01	3,74E+00	5	7,49E-01	0,037427952	570,168
Cod 317	6,90E-02	7,25E-02	6,50E-03	7,07E-02	6,42E-02	2,65E+00	5	5,30E-01	0,0265	426,759
Cod 318	1,74E-01	1,64E-01	6,50E-03	1,69E-01	1,62E-01	3,32E+00	5	6,65E-01	0,033237116	860,544
Cod 319	1,55E-01	1,18E-01	6,50E-03	1,36E-01	1,30E-01	1,87E+00	5	3,74E-01	0,018703481	1223,435
Cod 320	1,58E-01	1,30E-01	6,50E-03	1,44E-01	1,38E-01	3,46E+00	5	6,92E-01	0,034602343	699,743
Cod 321	1,54E-01	1,48E-01	6,50E-03	1,51E-01	1,45E-01	3,06E+00	5	6,12E-01	0,030621315	831,365
Cod 322	1,77E-01	1,99E-01	6,50E-03	1,88E-01	1,81E-01	2,85E+00	5	5,70E-01	0,028512547	1119,881
Cod 323	1,78E-01	1,69E-01	6,50E-03	1,74E-01	1,67E-01	3,95E+00	5	7,89E-01	0,039470937	746,208
Cod 324	1,73E-01	1,68E-01	6,50E-03	1,71E-01	1,64E-01	4,11E+00	5	8,21E-01	0,041061881	704,235
Cod 325	2,30E-01	2,01E-01	6,50E-03	2,16E-01	2,09E-01	3,74E+00	5	7,49E-01	0,037427952	984,622
Cod 326	1,24E-01	1,48E-01	6,50E-03	1,36E-01	1,30E-01	3,53E+00	5	7,07E-01	0,035338919	645,233
Cod 327	1,25E-01	1,52E-01	6,50E-03	1,38E-01	1,32E-01	2,53E+00	5	5,06E-01	0,025319617	917,724
Date:	18.09.2004	kl. 01:10	Group:	Ref. South						
Fish no.	DO1/min	DO2/min	DO Blanc	MoyenDO	DO-DO B	Prot(mg/ml)	dilution	Final prot kons.	Qty in well (10µl S9)	Aktivity nmole/mn/mg proteiner

Cod 401	1,41E-01	1,44E-01	6,50E-03	0,142595	1,36E-01	4,58E+00	5	0,916	0,046	522,98
Cod 402	1,09E-01	1,07E-01	6,50E-03	0,108038	1,02E-01	2,39E+00	5	0,478	0,024	748,27
Cod 403	1,57E-01	1,57E-01	6,50E-03	0,156978	1,50E-01	1,96E+00	5	0,392	0,020	1352,75
Cod 405	1,72E-01	1,45E-01	6,50E-03	0,158947	1,52E-01	2,28E+00	5	0,456	0,023	1178,25
Cod 406	1,60E-01	1,28E-01	6,50E-03	0,144101	1,38E-01	3,22E+00	5	0,645	0,032	751,14
Cod 407	1,44E-01	1,61E-01	6,50E-03	0,152223	1,46E-01	3,71E+00	5	0,742	0,037	691,77
Cod 408	1,41E-01	1,33E-01	6,50E-03	0,137066	1,31E-01	2,84E+00	5	0,568	0,028	810,02
Cod 409	1,50E-01	1,55E-01	6,50E-03	0,152402	1,46E-01	4,17E+00	5	0,834	0,042	615,88
Cod 410	1,16E-01	1,08E-01	6,50E-03	0,111845	1,05E-01	2,73E+00	5	0,546	0,027	679,32
Cod 411	1,04E-01	1,01E-01	6,50E-03	0,102371	9,59E-02	2,91E+00	5	0,582	0,029	579,92
Cod 412	1,68E-01	1,80E-01	6,50E-03	0,174049	1,68E-01	2,50E+00	5	0,500	0,025	1179,80
Cod 413	1,63E-01	1,75E-01	6,50E-03	0,16917	1,63E-01	3,12E+00	5	0,624	0,031	917,51
Cod 414	1,14E-01	1,07E-01	6,50E-03	0,110179	1,04E-01	2,91E+00	5	0,582	0,029	627,48
Cod 415	1,39E-01	1,31E-01	6,50E-03	0,134952	1,28E-01	2,16E+00	5	0,432	0,022	1045,87
Cod 416	1,57E-01	1,39E-01	6,50E-03	0,148266	1,42E-01	3,39E+00	5	0,679	0,034	735,54
Cod 417	1,78E-01	1,27E-01	6,50E-03	0,152683	1,46E-01	3,02E+00	5	0,603	0,030	853,47
Cod 418	2,01E-01	1,98E-01	6,50E-03	0,199444	1,93E-01	2,79E+00	5	0,558	0,028	1216,78
Cod 419	1,58E-01	1,72E-01	6,50E-03	0,165032	1,59E-01	3,35E+00	5	0,671	0,034	832,00
Cod 420	1,67E-01	1,51E-01	6,50E-03	0,158816	1,52E-01	3,20E+00	5	0,640	0,032	837,61
Cod 421	1,23E-01	1,37E-01	6,50E-03	0,130001	1,24E-01	3,04E+00	5	0,607	0,030	716,05
Cod 422	1,03E-01	1,11E-01	6,50E-03	0,106762	1,00E-01	1,96E+00	5	0,391	0,020	902,48

Cod 423	1,24E-01	1,02E-01	6,50E-03	0,113115	1,07E-01	2,23E+00	5	0,445	0,022	842,69
Cod 424	1,37E-01	1,47E-01	6,50E-03	0,142131	1,36E-01	1,98E+00	5	0,395	0,020	1208,14
Cod 425	1,34E-01	1,44E-01	6,50E-03	0,13883	1,32E-01	5,36E+00	5	1,073	0,054	434,25
Cod 426	1,90E-01	1,85E-01	6,50E-03	0,187583	1,81E-01	5,82E+00	5	1,164	0,058	547,53
Cod 427	1,35E-01	1,12E-01	6,50E-03	0,123584	1,17E-01	3,89E+00	5	0,778	0,039	529,64
Fish no.	DO1/min	DO2/min	DO Blanc	MoyenDO	DO-DO B	Prot mg/ml	dilution	Final prot kons.	Qty in well (10µl S9)	Aktivity nmole/mn/mg proteiner
Cod 1	1,97E-01	2,04E-01	6,50E-03	0,200404	0,193903926	4,19E+00	5	0,838499412	0,041924971	814,197
Cod 2	1,86E-01	2,29E-01	6,50E-03	0,207692	0,20119224	4,84E+00	5	0,968420014	0,048421001	731,464
Cod 3	1,52E-01	1,69E-01	6,50E-03	0,16064	0,154140204	4,34E+00	5	0,868527358	0,043426368	624,853
Cod 4	2,04E-01	2,08E-01	6,50E-03	0,206072	0,199571665	3,85E+00	5	0,769141286	0,038457064	913,562
Cod 5	1,94E-01	2,12E-01	6,50E-03	0,202968	0,196467759	3,71E+00	5	0,74204795	0,037102397	932,191
Cod 6	2,24E-01	2,52E-01	6,50E-03	0,237984	0,231483552	4,74E+00	5	0,947705991	0,0473853	859,987
Cod 7	1,52E-01	1,80E-01	6,50E-03	0,165958	0,159457508	3,43E+00	5	0,68522061	0,03426103	819,332
Cod 8	2,08E-01	1,76E-01	6,50E-03	0,191914	0,185413808	6,78E+00	5	1,355627679	0,067781384	481,556
Cod 9	2,23E-01	2,03E-01	6,50E-03	0,212934	0,206434481	4,01E+00	5	0,802118066	0,040105903	906,128
Cod 10	2,04E-01	2,14E-01	6,50E-03	0,209182	0,202682266	5,19E+00	5	1,037886295	0,051894315	687,561
Cod 11	2,41E-01	2,34E-01	6,50E-03	0,237595	0,231094782	4,28E+00	5	0,855597468	0,042779873	950,968
Cod 12	2,52E-01	2,20E-01	6,50E-03	0,235604	0,229103808	3,27E+00	5	0,654359229	0,032717961	1232,712
Cod 13	2,39E-01	1,97E-01	6,50E-03	0,218132	0,211631737	3,20E+00	5	0,639208508	0,031960425	1165,692
Cod 14	2,02E-01	1,65E-01	6,50E-03	0,183278	0,176777954	3,14E+00	5	0,628708655	0,031435433	989,975
Cod 15	2,31E-01	2,59E-01	6,50E-03	0,244949	0,238449254	3,24E+00	5	0,647795336	0,032389767	1295,996

Cod 16	1,24E-01	1,24E-01	6,50E-03	0,124118	0,117618014	2,97E+00	5	0,593486054	0,029674303	697,764
Cod 17	1,91E-01	2,09E-01	6,50E-03	0,200115	0,193615401	3,28E+00	5	0,655982011	0,032799101	1039,186
Cod 18	1,57E-01	1,73E-01	6,50E-03	0,164907	0,15840735	3,06E+00	5	0,612719021	0,030635951	910,247
Cod 19	1,14E-01	1,31E-01	6,50E-03	0,122071	0,115571101	2,07E+00	5	0,414084127	0,020704206	982,666
Cod 20	1,23E-01	1,36E-01	6,50E-03	0,129891	0,123391304	2,11E+00	5	0,421440804	0,02107204	1030,845
Cod 21	1,40E-01	1,48E-01	6,50E-03	0,144047	0,13754708	2,89E+00	5	0,577930825	0,028896541	837,956
Cod 22	1,77E-01	1,59E-01	6,50E-03	0,168198	0,161698016	2,44E+00	5	0,487396351	0,024369818	1168,067
Cod 23	1,54E-01	1,42E-01	6,50E-03	0,14768	0,141180459	1,48E+00	5	0,296339649	0,014816982	1677,375
Cod 24	1,37E-01	1,25E-01	6,50E-03	0,130896	0,124395545	2,53E+00	5	0,5054638	0,02527319	866,483
Cod 25	1,39E-01	1,27E-01	6,50E-03	0,13288	0,126380202	2,32E+00	5	0,463563212	0,023178161	959,877
Cod 26	1,65E-01	1,27E-01	6,50E-03	0,14597	0,139470374	2,42E+00	5	0,483194703	0,024159735	1016,261
Cod 27	1,95E-01	1,65E-01	6,50E-03	0,18	0,173500039	2,80E+00	5	0,559495015	0,027974751	1091,814
Cod 28	1,76E-01	1,80E-01	6,50E-03	0,177696	0,171195561	2,29E+00	5	0,458519499	0,022925975	1314,559
Cod 29	1,06E-01	1,12E-01	6,50E-03	0,108676	0,102175938	2,31E+00	5	0,462817119	0,023140856	777,293
Cod 30	2,33E-01	1,72E-01	6,50E-03	0,202315	0,195815338	2,30E+00	5	0,460458605	0,02302293	1497,275
Date:	17.09.2004		Group:	10000m						
Fish no.	DO1/min	DO2/min	DO Blanc	MoyenDO	DO-DO B	Prot mg/ml	dilution	Final prot kons.	Qty in well (10µl S9)	Aktivity nmole/mn/mg proteiner
Cod 101	1,60E-01	1,99E-01	6,50E-03	1,80E-01	1,73E-01	2,95E+00	5	0,590	0,029	1033,61
Cod 102	1,08E-01	1,31E-01	6,50E-03	1,20E-01	1,13E-01	1,90E+00	5	0,380	0,019	1049,78
Cod 103	1,82E-01	1,74E-01	6,50E-03	1,78E-01	1,72E-01	2,87E+00	5	0,573	0,029	1053,86
Cod 104	1,61E-01	1,57E-01	6,50E-03	1,59E-01	1,52E-01	3,35E+00	5	0,670	0,033	801,59

Cod 105	1,67E-01	1,62E-01	6,50E-03	1,64E-01	1,58E-01	2,72E+00	5	0,545	0,027	1019,99
Cod 106	1,45E-01	1,59E-01	6,50E-03	1,52E-01	1,46E-01	2,77E+00	5	0,554	0,028	925,17
Cod 107	1,18E-01	8,98E-02	6,50E-03	1,04E-01	9,73E-02	3,00E+00	5	0,599	0,030	571,47
Cod 108	1,39E-01	1,51E-01	6,50E-03	1,45E-01	1,38E-01	3,81E+00	5	0,762	0,038	640,20
Cod 109	1,35E-01	1,39E-01	6,50E-03	1,37E-01	1,30E-01	1,64E+00	5	0,327	0,016	1403,21
Cod 110	1,26E-01	1,26E-01	6,50E-03	1,26E-01	1,20E-01	2,11E+00	5	0,422	0,021	997,04
Cod 111	1,36E-01	1,55E-01	6,50E-03	1,45E-01	1,39E-01	2,03E+00	5	0,406	0,020	1205,18
Cod 112	1,21E-01	1,10E-01	6,50E-03	1,16E-01	1,09E-01	2,13E+00	5	0,427	0,021	901,71
Cod 113	2,33E-01	2,29E-01	6,50E-03	2,31E-01	2,25E-01	4,16E+00	5	0,833	0,042	949,51
Cod 114	1,25E-01	1,40E-01	6,50E-03	1,33E-01	1,26E-01	3,55E+00	5	0,710	0,035	625,47
Cod 115	1,28E-01	1,41E-01	6,50E-03	1,35E-01	1,28E-01	2,45E+00	5	0,491	0,025	919,21
Cod 116	1,31E-01	1,36E-01	6,50E-03	1,34E-01	1,27E-01	2,64E+00	5	0,528	0,026	848,20
Cod 117	7,48E-02	5,60E-02	6,50E-03	6,54E-02	5,89E-02	1,84E+00	5	0,368	0,018	563,09
Cod 118	9,51E-02	7,92E-02	6,50E-03	8,71E-02	8,06E-02	4,90E+00	5	0,980	0,049	289,48
Cod 119	1,44E-01	1,50E-01	6,50E-03	1,47E-01	1,40E-01	2,43E+00	5	0,486	0,024	1016,40
Cod 120	1,50E-01	1,66E-01	6,50E-03	1,58E-01	1,52E-01	3,54E+00	5	0,709	0,035	753,09
Cod 121	1,14E-01	9,38E-02	6,50E-03	1,04E-01	9,74E-02	3,34E+00	5	0,668	0,033	513,59
Cod 122	1,53E-01	1,47E-01	6,50E-03	1,50E-01	1,44E-01	3,64E+00	5	0,727	0,036	696,16
Cod 123	1,38E-01	1,38E-01	6,50E-03	1,38E-01	1,32E-01	4,64E+00	5	0,929	0,046	499,27
Cod 124	1,39E-01	1,41E-01	6,50E-03	1,40E-01	1,33E-01	1,73E+00	5	0,347	0,017	1352,23
Cod 125	1,44E-01	1,63E-01	6,50E-03	1,54E-01	1,47E-01	2,93E+00	5	0,586	0,029	884,88

Cod 126	1,35E-01	1,46E-01	6,50E-03	1,40E-01	1,34E-01	2,70E+00	5	0,540	0,027	872,90
Cod 127	1,73E-01	1,69E-01	6,50E-03	1,71E-01	1,64E-01	4,43E+00	5	0,885	0,044	654,07

Appendix G. Data report – University of Vilnius (Vilnius)

Report on micronuclei evaluation
in Water Column Monitoring

Reporting Period: - November 2004- January 2005

Responsible Person – Dr. habil. Janina Baršienė

January 2005

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Introduction

Marine organism could be exposed to mutagenic compounds via several routes. In oil drilling areas can be distributed potentially genotoxic polycyclic aromatic hydrocarbons, alkylphenols. Nevertheless a scarcity of studies on toxicological response levels in marine biota remains and the lack of data related to biotransformation and genotoxicity of oil are recognized as great challenges (Pacheco, Santos, 2001; Maria et al., 2002a; Brown, Steinert, 2003). Some of PAHs are classified as a promutagenes (Johnson, 1992). Hazardous effects of of these compounds arise as a result of oxidative biotransformation producing highly DNA-reactive metabolites. These metabolites are recognized as carcinogenic, mutagenic and cytotoxic compounds (Torres-Bugarin et al., 1998; Woodhead et al., 1999). Mechanisms of PAH metabolic transformation have been studied and genotoxic potency of metabolites was confirmed in various fish species (Metcalfe, 1988; Pacheco, Santos, 1997, 2001; Venier et al., 1997; Harvey et al., 1999; White, 2002; Maria et al., 2002a, 2002b; Gravato, Santos, 2002, 2003; Brown, Steinert, 2003; Teles et al., 2003). Most of above mentioned studies have been performed in laboratory conditions. Whilst, a little attention has been paid to oil genotoxic effects *in situ*, especially in aquatic organisms inhabiting contaminated environment (Reichert et al., 1998; Harvey et al., 1999; Vasseur, Cossu-Leguille, 2003; Moore et al., 2004). The assessment of cytogenetic damage has been presented as very important step in identification of oil pollution hazards. There are some studies that described increase of environmental genotoxicity in zones affected by oil spill (Parry et al., 1997; Harvey et al., 1999; Pietrapiana et al., 2002; Baršienė et al., 2004). Moreover, the genotoxicity related to transfer of oil pollutants from marine edible organisms via the food chain to mammals has been observed (Lemiere et al., 2004).

Extensive chromosomal rearrangements, such as micronuclei (MN), are well recognized as a consequence of genome instability (Fenech et al., 1999*).* 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; Kramer, 1998; Zoll-Moreux, Ferrier, 1999).

Fish and mussels have often been considered as the “sentinel” organisms in marine ecosystem health assessment. These organisms are widely distributed in aquatic habitats and have a great

commercial and recreational value. Analysis of interspecies variation in fish, mussel and crab DNA damage caused by genotoxic compounds *in situ* showed species specific susceptibility to environmental genotoxins (Bihari, Fafandel, 2004). Thus, the multi-species approach in impact and risk assessment should be linked to harmful effects of pollution in sentinel organisms to their ecological consequences. In the context of ecological genotoxicity or ecosystem health, genotoxicity biomarkers are also being used to link cytogenetic damage through to higher levels, i.e. pathology with reduced reproductive success of marine organisms inhabiting zones affected by incidental or operational oil spills from all activities related to transport or processing of petroleum products.

The main objective of the present study was to evaluate the micronuclei formation in liver cells of cod and in hemolymph of blue mussels caged in different distance from the Statfjord B oil platform. Peculiarities of cytogenetic damage were described regarding distance from pollution source and the relevance and reliability of MN test in monitoring of genotoxic effects occurred in oil drilling zone were shortly presented.

Materials and methods

The analysis of micronuclei was performed in 60 cod and 60 mussel specimens. The background levels of micronuclei incidences were evaluated in 20 fish and in 20 mussel individuals (before the caging experiment). The level of micronuclei induction was detected in fish and mussels caged in 500, 1000 and 10,000 meters from the Statfjord B oil platform (10 cod and 10 mussel specimens from the each caged group) in comparison to those caged in comparatively clean southern reference zone (10 cod and 10 mussel individuals).

Micronuclei induction in cod liver and in mussel haemolymph was the endpoints of environmental genotoxicity. In total, 60 liver samples from fish and 60 hemolymph preparations from mussels were analyzed for the micronuclei formation.

The small piece of liver was directly smeared on slides, air-dried and fixed in methanol for 15 min. Spread on the slides and air-dried mussel hemolymph was also fixed 15 min. in methanol. After that the slides were shipped and cytogenetic analysis was done in Institute of Ecology of Vilnius University. Slides were stained with 5% Giemsa solution for 10-20 min. To minimize technical variation the blind scoring of micronuclei was performed on coded slides without knowledge of the exposure status of the samples.

The frequency of micronuclei in liver cells or haemocytes was determined by scoring at a 1000× magnification using Olympus BX 51 or Nikon Eclipse 50i bright-field microscopes. A total of 25000-36000 cells (2000-5000 cells from each specimen) were examined in each caged experimental group of cod and 72000 cells in cod before caging. In some mussel slides, the deficiency of appropriate cells for the micronuclei analysis was marked. Nevertheless, 500 haemocytes was a minimum amount of cells suitable for the analysis. Therefore, in mussels micronuclei were counted in 500-2000 haemocytes from each 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 either 1/3-1/50 (for fish) or 1/3-1/20 (for mussels) 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.

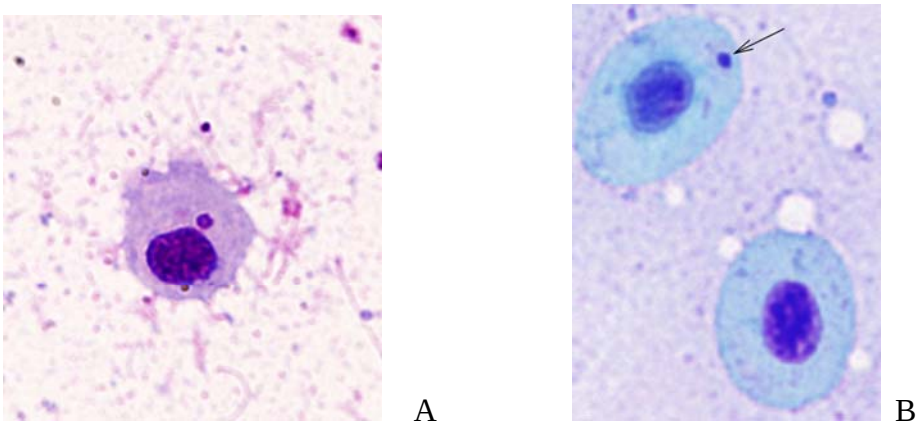


Fig. 1. Micronucleated haemocytes in mussels (A) and cod liver cell with micronuclei (B); 1000× magnification.

The average means of micronuclei per 1000 studied liver cells, standard errors and P values were calculated using PRISM statistical package. Mann-Whitney U-test and ANOVA Single Factor analysis were employed. The frequency of MN was expressed as index of MN number per 1000 cells.

Results

Atlantic cod

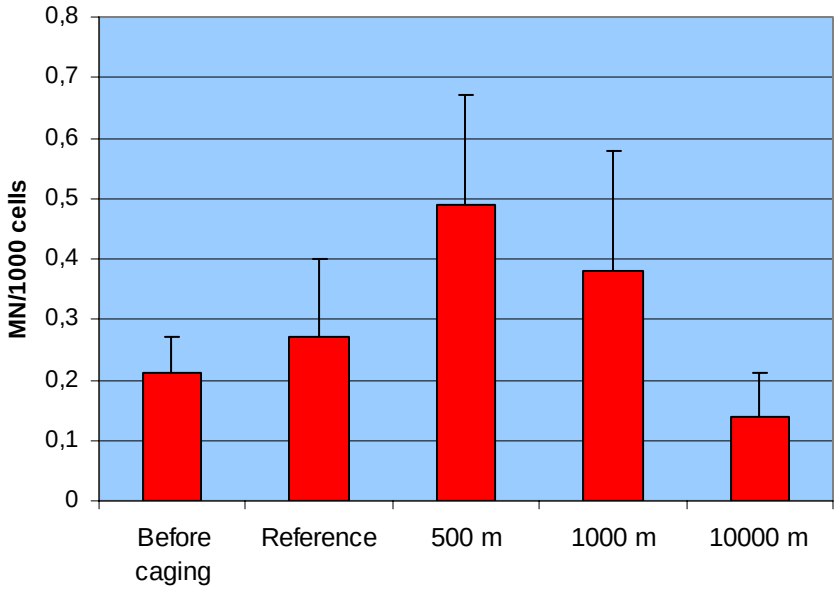
The lowest frequency of micronuclei (0.14 MN/1000 cells) was observed in cod caged in the largest distance (10000 meters) from the Statfjord B platform. In cod before the caging experiment, the MN mean equals to 0.21 MN/1000 cells. A bit higher level of MN induction (0.27 MN/1000 cells) was found in fish caged in reference site. The highest incidences of cells with MN were registered in cod from cages, which were immersed near the platform (Table 1).

Table 1. The frequency of micronuclei (MN/1000 liver cells) in Atlantic cod caged in Statfjord B platform zone

Exposure/MN	Before caging	Reference site	500m-caging	1000m -caging	10000m-caging
Number of cod	20	10	10	10	10
Number of cells	74000	30500	26500	36000	25500
Mean	**0.2100**	**0.2700**	**0.4900**	**0.3800**	**0.1444**
SD	0.2469	0.4165	0.5705	0.6494	0.2242
SE	0.0552	0.1317	0.1804	0.2054	0.0747
Maximum	1.0	1.0	1.5	2.0	0.5

The non-parametric Mann-Whitney U-test did not show MN induction in cod liver cells in regard to caging distance from Statfjord B platform. The levels of cytogenetic damage in cod from reference group, also before caging and caged in 10000 meters groups were very similar to a baseline level in the species inhabiting unpolluted areas of the North Sea (Fig. 2). The highest differentiation in response was marked between cod from 500-meters and 10000-meters caging groups, whilst differences were far away (P=0.2428) of borderline to statistical significance.

Fig. 2 Frequency of micronuclei in cod liver cells after caging in Statfjord B platform zone



Blue mussels

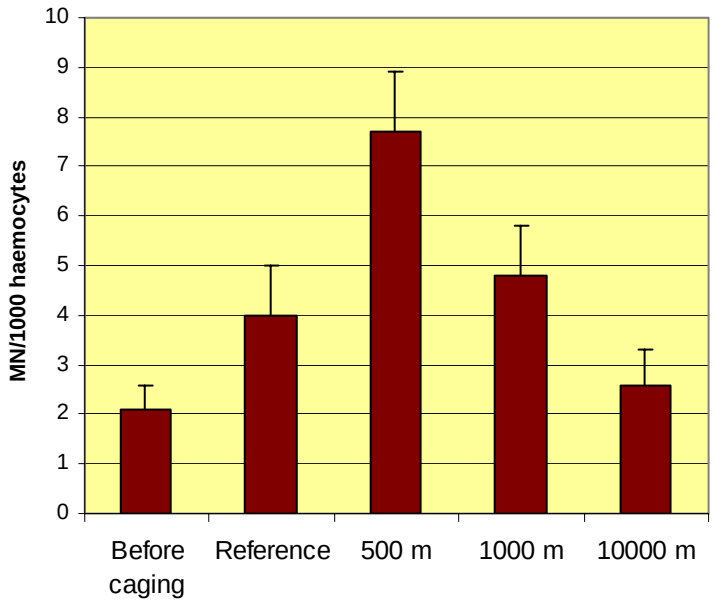
The lowest average values of MN (2.13 MN/1000 cells) were detected in haemocytes of mussels before caging experiment and from those, caged in 10000 meters from the oil platform (Table 2). Significant increase of MN induction (P=0.0004) was found in mussels from the cage at distance of 500 meters and from the cage immersed at 1000 meters (P=0.0247). Mussels from the southern reference site also showed increased level of genotoxicity, but MN incidences were significantly lower (P=0.0185) than in group caged at 500 meters from the oil platform. Thus, the significant inter-location differences in response were observed.

Table 2. The frequency of micronuclei (MN/1000 haemocytes) in mussels caged in Statfjord B platform zone

Exposure/MN	Before caging	Reference site	500m-caging	1000m -caging	10000m-caging
Number of mussels	20	10	10	10	10
Mean	**2.13**	**4.00**	**7.66**	**4.80**	**2.58**
SD	2.125	3.238	3.782	3.216	2.330
SE	0.4752	1.024	1.196	1.017	0.7368
Maximum	6.0	10.0	14.0	10.0	6.7

The average values of MN in mussels exposed in closest site from the oil platform reached 7.66 MN/1000 cells and 4.8 MN/1000 cells in the mussels caged at 1000 meters. The gradient of MN incidences was observed in mussels from three exposure cages (at 500m>1000m>10000m) (Fig.3).

Fig. 3 Frequency of micronuclei in haemocytes of mussels caged in oil platform zone



Results of analysis of variance indicated that MN frequency was dependent on exposure location in the nearby of the Statfjord B oil platform (F=6.996, P=0.0001 (ANOVA Single Factor).

Discussion

The key objective of the current study was to estimate environmental genotoxicity rate in liver cells of cod and haemocytes of mussels caged in the North Sea oil platform Statfjord B. Target organ liver was used as a tissue directly affected by oil metabolic components, which can act as mutagenic agents in fish (Stegeman, 1993). Hepatic micronuclei approach has been applied in fish and was considered a sensitive tool for the evaluation of environmental genotoxicity in contaminated sites (Williams, Metcalfe, 1992; Rao et al., 1997; Arcand-Hoy, Metcalfe, 2000; Pietrapiana et al., 2002). Previous results have been confirmed this parameter being sensitive biomarker for the assessment genotoxic effects in moderately contaminated locations *in situ* (Arcand-Hoy, Metcalfe, 2000). Moreover, it was concluded that hepatic micronucleus assay is a more sensitive system for the assessment of genotoxicity in fish than micronucleus assay on peripheral blood erythrocytes (Telli-Karakoc et al., 2001).

Mussels were analyzed as sensitive organisms to the action of environmental pollutants. Oil spills can result in wide distribution of petroleum hydrocarbons in marine environment seriously impacting DNA of filter-feeding bivalve populations (Hamoutene et al., 2002). High level of bioaccumulation of water-soluble alkylated PAHs has been described in inter-tidal mussels inhabiting Halifax Harbour (Hellou et al., 2005), Venice Lagoon (Wetzel, Van Vleet, 2004). Increase of genotoxicity in mussels from oil spill areas primarily depends on action of water soluble components (Carls et al., 2001). Significant elevation of micronuclei level in mussels 30 days post-oil spill and persistence of the cytogenetic damage up to 100 days later has been described (Parry et al., 1997). Cells with micronuclei were found to increase in the gills or hemolymph of marine molluscs treated with benzo(a)pyrene (Burgeot et al., 1995; Venier et al., 1997; Siu et al., 2004), dimethylbenz(a)antracene (Bolognesi et al., 1996). The results of the Comet and MN assays have been presented evidences on clear dose- and time-dependent responses to benzo(a)pyrene exposure in mytiliid bivalve *Perna viridis*. Besides was concluded, that the assays provide a convenient, highly sensitive, non-invasive monitoring tools of genotoxin assessments in marine environment (Siu et al., 2004).

Anaphase aberrations in fish embryos were correlated with concentrations of PAHs within the oil trajectory following the Exon Voldez oil spill in Prince William Sound in March 1989 (Hose, Brown, 1998). More frequently chromosomal aberrations and malformations have been observed in cod (*Gadus morhua*) and pollock (*Pollachius virens*) embryos from the oil spill area (Longwell, 1977). Cytogenetic damage has been described in molluscs inhabiting marine port and oil terminal areas in the

Baltic Sea (Baršienė, Baršytė Lovejoy, 2000; Baršienė, 2002). Elevated levels of micronuclei incidences were detected in flounder (*Platychthys flesus*) and blue mussels in 8 months after oil spill from Butinge oil terminal in the Baltic Sea (Baršienė et al., 2004). Higher frequency of DNA adducts have been described in marine organisms 12-17 months after the Sea Empress oil spill (Harvey et al., 1999). Philippe et al. (1993) reported that increased values of MN could also be related to an overall genetic instability, as cells with unstable karyotype tend to compensate by the way of chromosome elimination and MN formation.

This study showed a gradient of micronuclei induction in hemolymph of mussels caged in different distance from the Statfjord B oil platform. 2-3-fold increase of MN was detected in mussels immersed at 500 and 1000 meters from the platform, when compared with mussel group before the caging experiment. Similar pattern of MN induction was found in cod liver cells, whilst the response was at the lower level than in mussels. This finding related to assessed level of environmental genotoxicity in fish and mussels caged in oil drilling zone is important as present information on potential ecological risk with regard to marine pollution by oil or produced water effluents. The higher response in filter-feeding mussels reflects action of water-soluble phase of genotoxic compounds, which are possibly distributed in this study zone. On the other hand, lower response in cod could be addressed to high metabolizing potency of genotoxic PAHs in fish (Pacheco, Santos, 2001, 2002). Nevertheless, inter-specific pattern of tolerance to caging stress also can influence different levels of environmental genotoxicity in cod and mussels.

It is known that oil contains potentially genotoxic components (Klekowski et al., 1994), therefore the elevation in MN frequency in mussels and cod after exposure is an obvious response to action of genotoxic substances of the crude oil. The highest level of an original PAH concentrations in liver have been observed in Atlantic cod 3 days after the start of exposure (Aas et al., 2000). An increased level of micronuclei was also observed in cod after 3-day treatment with different concentrations of crude oil. It should be stressed also on earlier finding of our experimental work that showed the highest level of oil genotoxicity in cod liver and mussel gill cells after 14-day treatment and efficient recovery after 2 weeks. The previous caging experiment at Troll B platform have demonstrated the significant increase of cytogenetic damage in cod depending on the distance of their immersion.

Dose-dependent induction of micronuclei in Atlantic cod liver and induction by low environmentally relevant concentrations of North Sea crude oil (0.06 and 0.25 ppm) was also confirmed earlier in our experimental work. Furthermore, the significant induction of micronuclei in Atlantic cod

(Barents Sea population) was observed after treatment with low concentrations of crude oil at a low temperature. These results and data of the current study elicit that micronucleus test is very sensitive response to oil contaminants and may potentially offer an early warning of pollution-induced damage on health of indigenous wildlife species. Nevertheless the ecologically relevant information should be obtained by assessment of contaminant effects in sensitive indigenous species *in situ*.

Laboratory bioassays provide a first step in evaluation of contaminant biological effects, whilst their results cannot be directly extrapolated to the field conditions. Field studies on contaminant exposure usually provide information of complex masked interrelationships and interpretation of results is often complicated. The caging of indigenous organisms in gradient from certain pollution source could be used as the bridge between laboratory and field studies. Comparison of environmental genotoxicity in caged and wild indigenous mussels from different sites of the Ligurian coast showed the specificity of pollutant accumulation and response to various PAHs and heavy metals (Bolognesi et al., 2004).

The micronucleus test relevance in fish and mussels was confirmed by several large-scale genotoxicity studies in marine coastal areas, which we have conducted in the North and Baltic seas. A correlation between cytogenetic impairments and environmental pollution demonstrated the usefulness of the approach in detection of mutagens within complex mixture of pollutants, which usually exists in uncontrolled field conditions. Future research should focus on identifying both the short- and long-term consequences of genotoxicity in exposed fish populations. An assessment of cytogenetic damage in early life stages and in mature organisms (including somatic and gonadal cells) is recommended.

It should be stressed also that some studies on MN formation have no points on seasonal variations in poikilotherm organisms as well as on identification of baseline level. The seasonal fluctuations and low water temperature could result in the decreased metabolic rate and decreased activation of potentially genotoxic compounds of crude oil (Harvey et al., 1999). Moreover MN baseline level can vary depending upon age, sex, season, temperature, oxygen factors and criteria of MN identification. All these factors must be taken into account when using MN assay under the field conditions at different times of the year.

Conclusions

The current study results showed a gradient of micronuclei induction in hemolymph of mussels caged in different distance from the Statfjord B oil platform. 2-3-fold increase of MN was detected in mussels immersed at 500 and 1000 meters from the platform, when compared with mussel group before the caging experiment. Similar pattern, but lower level of MN induction was found in cod liver cells.

The assessed level of environmental genotoxicity in fish and mussels caged in oil platform zone have indicated the existence of potential ecological risk potentially caused by oil pollution or by produced water effluents.

The higher response in filter-feeding mussels reflects action of water-soluble phase of genotoxic compounds, which are possibly distributed in this study zone. Lower response in cod can arise as a result of high metabolizing rate of genotoxic PAHs in fish, or due to inter-species tolerance to caging stress.

The used micronucleus test in cod and mussels is very sensitive, simple, cost-effective and rapid method to detect environmental genotoxicity level in oil platform zones. The end-point is well characterized and can easily be evaluated in fish or mussels after 3 or more days exposure in certain oil spill areas.

The standard protocol of MN assay in cod and mussels should include at least 12-15 specimens per caging group/site that should provide sufficient data base in order to reach statistical power and ensure the reliability of response in exposure gradient.

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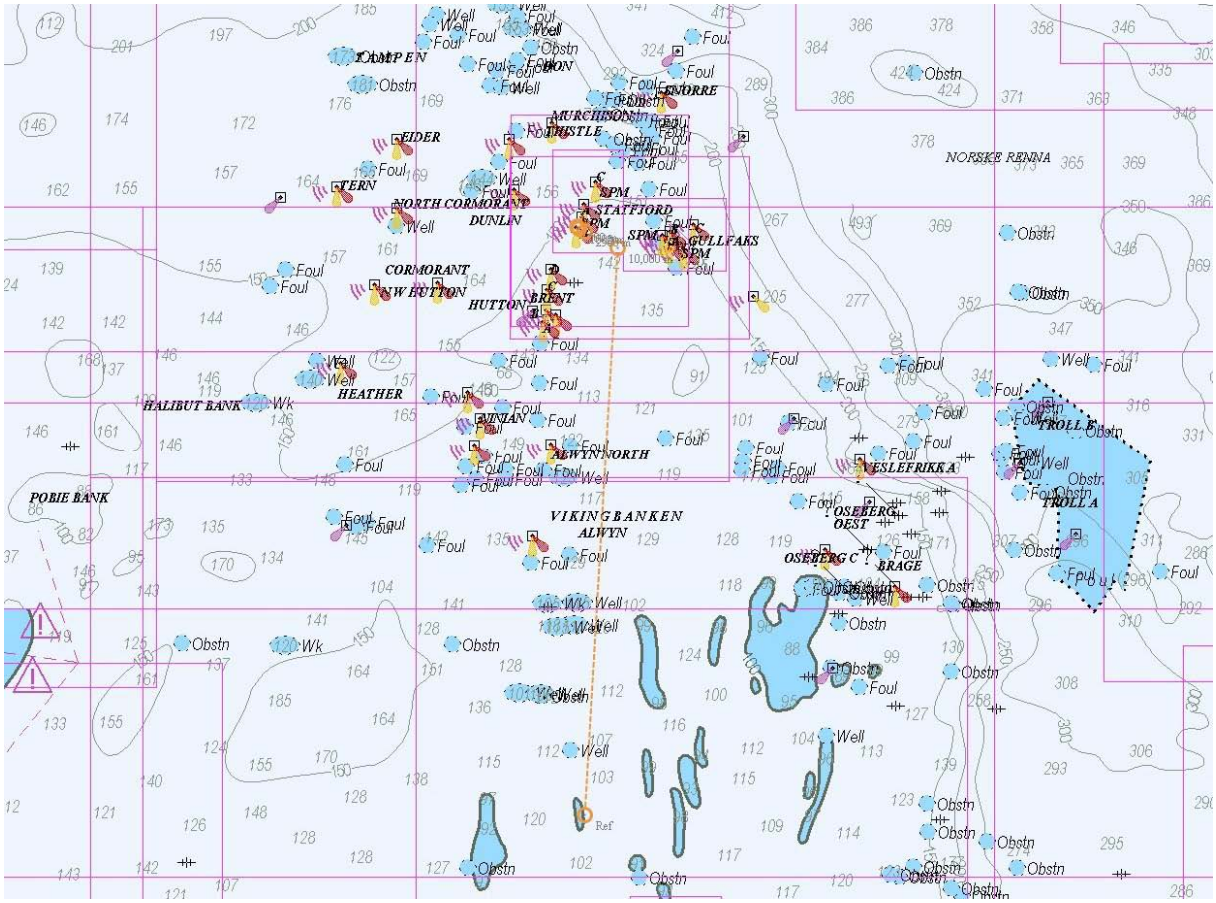
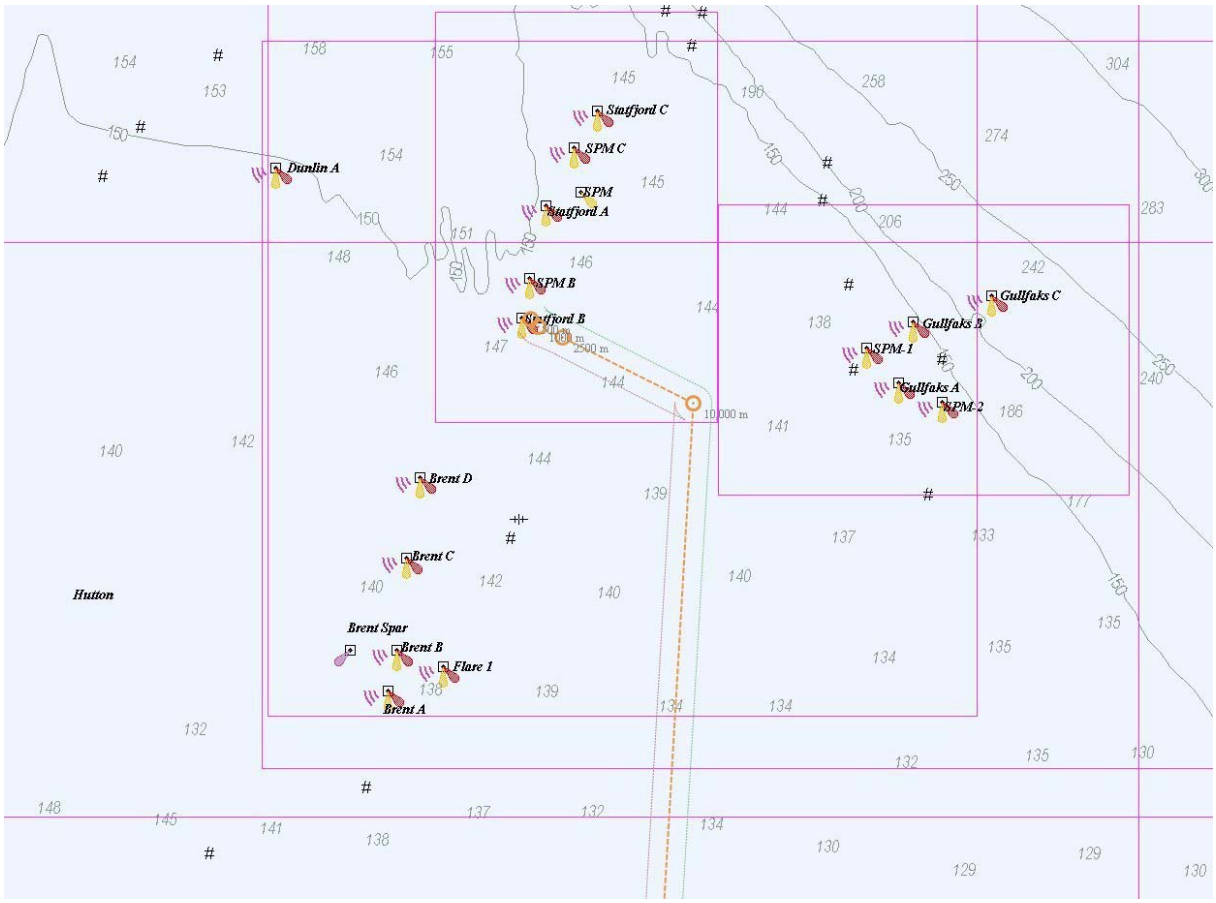
Appendix H. Maps




Statfjord C
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Dunlin A
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Statfjord B
Gullfaks C
Gullfaks B
SPM-1
Gullfaks A
SPM-2
Brent D
Brent C
Hutton
Brent Spar
Brent B
Flare 1
Brent A
10000 m
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