Species differences in sensitivity to environmental pollutants in Gadidae in the Oslofjord

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Abstract

Related species have been assumed to have a similar sensitivity to exposure to environmental pollution. Atlantic cod (*Gadus morhua*) is an important commercial fish and a key-species in monitoring. With the recent decline in Atlantic cod populations in the Oslofjord and a change in dominating species, knowledge about sensitivity in species related to Atlantic cod that are also present in the Oslofjord may be important for future monitoring and management of populations. The Oslofjord is divided into the urban inner Oslofjord and a less polluted outer Oslofjord.

This study aimed to identify location differences between the urban and more polluted inner Oslofjord and the less polluted outer Oslofjord, to identify differences between species in the Gadidae family (Atlantic cod, whiting (*Merlangius merlangus*), Norway pout (*Trisopterus esmarkii*) and haddock (*Melanogrammus aeglefinus*)), to identify seasonal differences in exposure and effects of contaminants in the Oslofjord, and to identify differences in effect of exposure to contaminants between tissues. This was done by sampling 8 fish from each species in the inner and outer Oslofjord in the spring and fall. Concentrations of polycyclic aromatic hydrocarbon (PAH) metabolites were quantified in the bile and mercury concentration was determined in liver samples to get estimates of exposure to environmental pollution. Hepatic, gill, and heart cytochrome P4501A (CYP1A) activity was determined from the ethoxyresorufin *O*-deethylase (EROD) assay to get estimates of effect of exposure to environmental pollution as the activity can be induced by exposure to contaminants such as PAHs.

The study identified significantly higher concentrations of PAH-metabolites in bile in the inner Oslofjord. Species differences and seasonal differences varied among the three groups of PAH metabolites, indicating differences in exposure and sources through the year. There was identified a location difference in mercury exposure in Norway pout in the inner Oslofjord, which could be due to differences in age between locations. Atlantic cod had higher hepatic mercury concentrations than one or more species in both locations in both seasons, which could be due to differences in age or diet between species.

There was significantly lower hepatic CYP1A activity in spring in the inner Oslofjord, which may be due to inhibition of CYP1A activity by reproductive hormones or other CYP1A

inhibitors. There were no species differences in hepatic CYP1A activity. However, there are known differences in baseline activity, which could indicate a difference in sensitivity, as the activity was not induced to the same degree in all species. The hepatic CYP1A activity was higher in fall than in spring, with median activity almost one order of magnitude higher in fall. This is not in accordance with activity determined in previous years and could indicate that there is a new source of contamination that induces the activity. Gill CYP1A activity was significantly different between Norway pout and Atlantic cod, whiting, and haddock in the inner Oslofjord and between Norway pout and Atlantic cod and whiting in fall. The gill CYP1A activity was higher in the spring and showed a positive correlation with exposure to pyrene, indicating that gill CYP1A activity could be a good biomarker for exposure to water-borne contaminants. There were species differences in heart CYP1A activity, with significantly higher activity in Atlantic cod than in whiting in the inner Oslofjord in spring, higher activity in Atlantic cod than in whiting and Norway pout in fall, higher activity in Atlantic cod than in haddock in the outer Oslofjord in fall, higher activity in Atlantic cod in haddock and whiting in the inner Oslofjord and higher activity in haddock than in Norway pout in the outer Oslofjord in fall. Hepatic CYP1A activity was generally the highest of the three tissues, followed by gill CYP1A activity. To correctly interpret results and identify species differences, estimates of baseline activity in more species and tissues needs to be determined.

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Abbreviations

ANOVA – Analysis of variance

BSA – Bovine serum albumin

CH_3Hg – Methyl mercury

CYP1A – Cytochrome P4501A

DTT – DL-dithiothreitol

EROD – Ethoxyresorufin *O*-deethylase

GSI – Gonad somatic index

Hg – Mercury

K2HPO4 – Dibasic potassium phosphate

KCl – Potassium chloride

KH2PO4 – Monobasic potassium phosphate

MeOH - Methanol

Na2HPO4 – Disodium phosphate, dibasic

NADPH – Nicotinamide adenine dinucleotide phosphate

NaH2PO4 – Monosodium phosphate, monobasic

PAH – Polycyclic aromatic hydrocarbons

Reagent A – Copper tartrate solution

Reagent B – Folin solution

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1 Introduction

1.1 The Oslofjord

The Oslofjord can be separated into an inner and outer fjord due to the topography of the seafloor a little north of Drøbak. Here, a sill that is approximately 20 m deep and 1 km wide creates a natural separation of the fjord (Thaulow et al. 2014). The inner Oslofjord is associated with urban pollution that stems from industry, sewage, rivers, runoff from land as well as pollution from boat traffic such as cruise ships, cargo transport and the large number of recreational boats. The Oslofjord is also associated with runoff from agriculture that is found along the entire coast that also can affect the fjord negatively (Arvnes et al. 2019). Due to the shallow sill that separates the inner and outer Oslofjord, the water exchange in the inner Oslofjord is limited. Vestfjorden has on average a yearly deep water renewal and Bunnefjorden has on average a deep water renewal every three years (Stigebrandt et al. 2002). The limited water exchange may periodically lead to anoxic conditions in the bottom layers of basins in the inner Oslofjord. Low oxygen in bottom layers may cause increased filtration of water through the gills of e.g. fish to keep oxygen uptake stable, which could lead to increased exposure to water-soluble contaminants (Schmieder and Weber 1992). The limited water exchange also causes the pollution that enters the inner Oslofjord to be less likely to be transported out of the fjord and persistent compounds can be accumulated and essentially trapped in the inner basin (Wania 1999). Due to the connection to Skagerrak and the lesser influx of urban contamination that is associated with the outer Oslofjord, this is area has been found to be a less contaminated area than the inner Oslofjord (K. Hylland, pers. comm.).

From 2006 to 2011 a remediation project was completed in the inner Oslofjord to attempt to reduce the amount of bioavailable contaminants in the sediments. The project was successful, and available contaminant levels in the sediments after the project was finished was lower than before the remediation project started (Størdal 2020). However, monitoring of the inner Oslofjord from 2013-2019 reported that there has been an increase in contaminants in the sediments after the project was completed, possibly due to runoff from land and overload of wastewater systems (Størdal 2020).

1.2 Species of interest

In the present study, four species from the family Gadidae (codfish) were studied: Atlantic cod (*Gadus morhua*), whiting (*Merlangius merlangus*), Norway pout (*Trisopterus esmarkii*) and haddock (*Melanogrammus aeglefinus*). Atlantic cod has been of great importance for both commercial fisheries and recreational fishing in the Oslofjord (Kleiven et al. 2016). Recruitment of Atlantic cod has declined in both the inner and outer Oslofjord since the year 2000 (Espeland and Knutsen 2019). Some results suggest that cod larvae in both the inner and outer Oslofjord may currently not be dominated by contributions from the local, stationary spawning population but rather fish originating from the migrating North Sea population (Knutsen et al. 2018). The assumed stationary nature of the Atlantic cod population in the inner Oslofjord has made it an important species in monitoring programs, as it reflects local exposure to a larger degree than other more migratory species. While it is not known whether whiting spawns in the inner Oslofjord, this species has been the dominating species in the inner Oslofjord for the past few years (Staalstrøm et al. 2021). Spawning grounds has been identified in the North Sea and the Skagerrak for Norway pout (Nash et al. 2012), but there are areas in the Oslofjord where mature individuals have been found close to the spawning period. Spawning grounds for haddock are found in the North Sea and towards Skagerrak (González-Irusta and Wright 2016), and local fishermen have identified spawning areas outside Larvik (Anon 2022). If these species are migrating out of the fjord to spawn, the populations may be less appropriate monitoring species for the Oslofjord than a stationary Atlantic cod population, as they would not only be exposed to contaminants from the Oslofjord. Whiting, haddock and Norway pout are also commercially important fish species (Anon 2013, Anon 2017), and knowledge about sensitivity to environmental pollutants are important for management of populations.

Other factors such as diet and habitat can also affect the exposure of a species to contaminants. Some contaminants are water-soluble, while some bind to sediment and/or accumulate in organisms and can biomagnify in food chains. Diet or habitat differences may therefore cause differences in exposure between species. When comparing diet preferences for Atlantic cod, whiting and haddock, the diet is most similar during earlier life stages and gets more differentiated with age (Rowlands et al. 2008). Fish is an important part of the diet of adult Atlantic cod, whiting, and haddock but not for Norway pout, whose diet consists mainly of krill (Bromley et al. 1997). Adult whiting and Atlantic cod predate on other fish, such as 0-and 1-group of Atlantic cod, herring and whiting, while the diet of adult haddock

consists mostly of bottom-dwelling organisms and occasionally smaller fish (Hislop et al. 1991, Bromley et al. 1997).

Studying related species that are all present in the same fjord could give insight into differences and similarities between the species. There has been an assumption that related fish species may have similar sensitivity to environmental contaminants due to evolutionary similarities (van den Hurk et al. 2017). Identification of more sensitive species or species with the same profile as important monitoring species such as Atlantic cod is important for future monitoring and if it one day will be necessary to replace Atlantic cod in monitoring programs.

1.3 Environmental pollution

Potentially toxic substances produced by human activities often end up in the marine environment in the end due to precipitation and runoff from land or through direct exposure (Stegeman and Hahn 1994, Macdonald and Bewers 1996). The inner Oslofjord is surrounded by urban areas and the Oslo harbour. Exposure of contaminants from wastewater (e.g. pesticides, pharmaceuticals and synthetic hormones), oil spills (e.g. polycyclic aromatic hydrocarbons (PAHs) and metals), combustion of fossil fuel (e.g. PAHs, benzene, dioxins), industrial activity (e.g. metals and dioxins), boat traffic (e.g. PAHs and antifouling) and agriculture (e.g. pesticides and fertilizer) (Durand et al. 2004, Neff et al. 2006, Nriagu 2011, Arvnes et al. 2019, Rizzo et al. 2020) are all sources of contamination to the inner Oslofjord. The effects of contaminants on marine fish has been extensively researched, and there are ongoing monitoring studies to investigate temporal trends in areas such as the Oslofjord (Engesmo et al. 2020, Staalstrøm et al. 2021). In this study, PAH metabolites in bile and hepatic mercury concentration were chosen as indicators of exposure to environmental pollution in fish. PAHs are a key group of contaminants in the exposure in the inner Oslofjord and the concentration of PAH metabolites in bile can give valuable information about recent exposure. Mercury accumulates in tissues and in the food web (Harley et al. 2015) and can provide information about the total exposure to bioaccumulating contaminants that a population has been exposed to up until point of sampling.

1.3.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of contaminants, and all contain one or more aromatic groups that are connected. There is a large variety of sources of

the compounds but the most important sources for the marine environment are pyrogenic (combustion processes) and petrogenic (fossil fuel) sources. 2-and 3-ringed polycyclic aromatic hydrocarbons are mainly found in crude oil, for instance from oil spills or offshore activities (Neff et al. 2006), while larger compounds such as pyrene and benzo[a]pyrene are found in combustion products from either natural or anthropogenic sources such as sewage, runoff from roads (Durand et al. 2004), and smelters (Næs and Oug 1998).

The bioavailability of PAHs is modulated by complex interactions with organic material, inorganic and organic particles, and large PAHs can have half-lives ranging from months to years in sediments in marine environments (Wilcock et al. 1996), while smaller 2- and 3-ring PAHs are more water soluble (Pearlman et al. 1984) and do not accumulate in sediments to the same extent. The toxicity of PAHs can be acute and caused by the parent compound or caused by activation of compounds during biotransformation (Cerniglia 1984).

Fish can take up PAHs through their diet by ingesting PAHs that are present in their prey or by ingesting particulate matter that contains PAHs, and through the gills as some PAHs are soluble in water (Balk et al. 1984). Water-soluble PAHs can also be taken up in the intestine and stomach of marine fish as they drink water. PAHs are metabolised into more readily excreted compounds by phase I and phase II biotransformation enzymes. The membrane bound cytochrome P-450 monooxygenase system is a key component of first step of biotransformation, the oxygenation, of PAHs (Cerniglia 1984) which may result in inactivation of PAHs, or as mentioned above, an activation of the compound into a more toxic intermediate substance. The oxidized compound can then be further metabolised before the compound is excreted through the bile.

Quantifying PAH-metabolites in bile is an efficient method to investigate recent exposure in the organism. However, one issue is that the gall bladder is emptied into the intestine after a fish feeds and sampling of fish after a recent feeding can make it challenging to collect a bile sample. A simple and rapid method of measuring specific PAH metabolites is the Fixed wavelength Fluorescence (FF) (Ariese et al. 2005, Beyer et al. 2010). This method is based on the metabolites having intrinsic fluorescence at specific excitation/emission wavelength pairs, which the FF method utilizes.

1.3.2 Mercury

Elemental mercury is naturally found in the earth's crust. Through human activity the amount of available mercury has increased drastically. Anthropogenic sources of mercury are mining, combustion of fossil fuels, and waste from different industries such as hydroelectric and paper industries (Anon 2019). Deposition of elemental mercury in the oceans occurs mainly through atmospheric deposition (Mason et al. 1994), and this is relevant for the inner and outer Oslofjord as well. Runoff from land, fossil fuel combustion and waste disposal (Pirrone et al. 2010) are other sources of mercury in sediment and biota close to urban areas such as the inner Oslofjord, but will have less of an impact on areas with lower human activity. Inorganic mercury is capable of long-range transport, and the concentration of mercury in sediments and biota will not necessarily reflect inputs from a local source.

Inorganic mercury is poorly absorbed in the gut when ingested, and will readily be excreted through faeces and urine (Clarkson 1997). In the oceans inorganic mercury can be metabolised into organic mercury, methyl mercury (CH_3Hg), by sulphate-reducing bacteria (Jensen and Jernelöv 1969). Methylmercury is readily absorbed from the gut because of the hydrophobic methyl group that is bound to the single mercury atom. This increased lipophilicity facilitates transport through cell walls or tight junctions between cells, which increases the uptake of mercury in the gastrointestinal tract. The presence of the hydrophobic methyl group enables the compound to be stored in lipid-rich tissues (Harley et al. 2015). However, it does also have an affinity for proteins, due to interactions with thiol groups. Elevated levels of methylmercury have been shown to have a similarly neurotoxic effect on Atlantic cod as documented in mammals (Berg et al. 2010) and can cause oxidative stress. In this study, mercury was used as an indication of general pollution in the inner Oslofjord, but it will be taken into account that both areas will receive inorganic atmospheric mercury.

Mercury contamination in the Oslo harbour peaked around the 1940-1950s (Lepland et al. 2010). Remediation projects such as the Oslo harbour remediation project (2006-2008), where parts of the heavily polluted Oslo harbour were dredged or capped to avoid resuspension of contaminants that were accumulated in the sediments, has resulted in a decrease in the pollution levels in sediments in the inner Oslofjord after the project was completed (Størdal 2020).

1.5 Evaluating environmental pollution - cytochrome P4501A activity

The cytochrome P-450 monooxygenase system has a broad substrate specificity. One of the well-studied proteins in this family is the cytochrome P4501A (CYP1A) enzyme, which is an important enzyme in the biotransformation of pharmaceuticals and many aromatic xenobiotic compounds in fish (one isoform in most species), as well as an important enzyme in metabolism of endogenous compounds. The CYP1A enzyme can also be induced by a group of planar, aromatic compounds, such as dioxin-like polychlorinated bisphenyls (PCBs) and PAHs (Goksoyr and Forlin 1992, Nebert et al. 2004). These compounds bind to the aryl hydrocarbon receptor, leading to increased expression of mRNA and higher levels, and hence activity, of the CYP1A enzyme in the membranes (Goksøyr 1995, Rowlands and Gustafsson 1997, Berg et al. 1998).

Gender differences in EROD activity of sexually mature individuals has been observed in other teleost species (Mathieu et al. 1991, Lindström-Seppä and Stegeman 1995). This could be caused by the reproductive hormone oestradiol acting as an inhibitor of the CYP1A enzyme in female fish (Larsen et al. 1992). Maturity of the fish sampled may therefore be a confounding factor when comparing CYP1A activity between spring and fall. When analysing the results, it is important to keep in mind the gender and maturity stage of the fishes that are being compared. Metals, such as cadmium, copper, and mercury, have also been found to reduce the CYP1A activity, either through catalytic inhibition of the enzyme or through reduction of CYP1A protein (Whyte et al. 2000).

CYP1A activity is assumed to be proportional to ethoxyresorufin *O*-deethylase (EROD) activity that can be quantified measuring the rate of the deethylation of the synthetic enzyme substrate 7-ethoxyresorufin to resorufin. Increased EROD activity is often used as an early warning sign of PAH exposure (Goksoyr and Forlin 1992).

1.6 Study design and comparing related species

In this study, I chose to quantify CYP1A activity in several tissues and to determine the concentration of three PAH-metabolites in bile and hepatic mercury concentration in a few replicates from four related species. The study design was chosen to be able to both quantify the general exposure levels to contaminants in the fjord, determined by the concentration of three groups of PAH-metabolites in bile and mercury concentration in liver, and to quantify

the effect of the exposure by using the established biomarker of effect, the EROD assay. The decision to sample fish from both the inner and outer Oslofjord was made because of the natural design of the fjord with two close areas with different pollution profiles. The decision to sample species during both spring and fall was made to be able to examine seasonal differences in both exposure and natural seasonal variation in responses.

The number of replicates for each species was set to eight individuals from each species at each location during each season to have enough samples to get an estimate of the natural variation in the different populations of the four species, as well as a feasible workload while sampling.

1.7 Aims and hypotheses

The aim of this thesis is to investigate whether pollution in the inner Oslofjord affects fish species present, if there are any species differences, tissue differences or seasonal differences in pollution-related responses. Responses will be measured by cytochrome P4501A (CYP1A) activity in liver, gills, and heart. Exposure will be evaluated through concentrations of bile PAH-metabolites and hepatic Hg. Results from the inner (urban, polluted) and outer (largely unpolluted) Oslofjord will be compared.

The main aim is divided into the following testable hypotheses:

1. **H_0** – No difference in exposure to PAHs between locations, species, and seasons.
    - H_0 – No difference between species and locations in spring
    - H_0 – No difference between species and locations in fall
    - H_0 – No difference between species and seasons in the inner Oslofjord
    - H_0 – No difference between species and seasons in the outer Oslofjord

2. **H_0** – No difference in exposure to mercury between locations, species, and seasons.
    - H_0 – No difference between species and locations in spring
    - H_0 – No difference between species and locations in fall
    - H_0 – No difference between species and seasons in the inner Oslofjord
    - H_0 – No difference between species and seasons in the outer Oslofjord

3. **H_0** – No difference in CYP1A activity between location, species, and seasons in each of the three tissues
    - H_0 – No difference between species and locations in spring in liver
    - H_0 – No difference between species and locations in fall in liver
    - H_0 – No difference between species and seasons in the inner Oslofjord in liver
    - H_0 – No difference between species and seasons in the outer Oslofjord in liver
    - H_0 – No difference between species and locations in spring in gills
    - H_0 – No difference between species and locations in fall in gills
    - H_0 – No difference between species and seasons in the inner Oslofjord in gills
    - H_0 – No difference between species and seasons in the outer Oslofjord in gills
    - H_0 – No difference between species and locations in spring in heart
    - H_0 – No difference between species and locations in fall in heart
    - H_0 – No difference between species and seasons in the inner Oslofjord in heart
    - H_0 – No difference between species and seasons in the outer Oslofjord in heart

4. **H_0** – No difference in CYP1A activity between the tissues for each of the four species in each location
    - H_0 – No difference between tissues for Atlantic cod in the inner Oslofjord
    - H_0 – No difference between tissues for Atlantic cod in the outer Oslofjord
    - H_0 – No difference between tissues for Whiting in the inner Oslofjord
    - H_0 – No difference between tissues for Whiting in the outer Oslofjord
    - H_0 – No difference between tissues for Norway pout in the inner Oslofjord
    - H_0 – No difference between tissues for Haddock in the outer Oslofjord

5. **H_0** – No correlation between exposure to environmental pollution and CYP1A activity
    - H_0 – There is no correlation between exposure to PAH and CYP1A activity
    - H_0 – There is no correlation between exposure to Hg and CYP1A activity

2 Materials and methods

2.1 Collection of fish

Sampling was performed following trawling in the inner Oslofjord at Midtmeie, see Figure 1, and outer Oslofjord in Holmestrandsfjorden, see Figure 2, during March 1st to March 3rd and August 30th to September 1st 2021 using the research vessel Trygve Braarud.

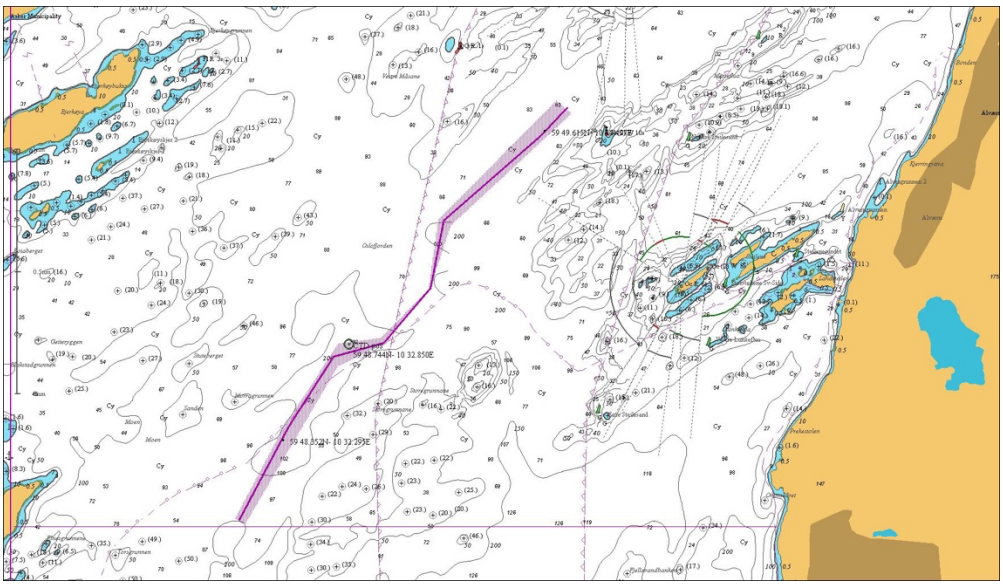

Figure 1. The trawling location in the inner Oslofjord, at Midtmeie (Steilene).

Figure 2. The trawling location in the outer Oslofjord, in Holmestrandsfjorden.

Bottom trawling was performed at both locations during both seasons at 120-70 m depth in the outer Oslofjord and 110-90 meters depth in the inner Oslofjord. The trawling duration was 30-40 minutes at 1.5 knots. One or more trawls was performed at each location. Table 1 shows hydrography data from the two seasons.

Table 1. Temperature, salinity, and oxygen saturation at the top and bottom of the water column in the inner Oslofjord during spring and the inner and outer Oslofjord during fall. There was no data available from the outer Oslofjord during spring.

Season	*Location*	*Temperature (°C)*		*Salinity (PSU)*		*Oxygen saturation (%)*	
		Top	Bottom	Top	Bottom	Top	Bottom
Spring	Midtemeie	6.2	7.7	31.2	33.6	71.1	78.6
	Holmestrandsfjorden	-	-	-	-	-	-
Fall	Midtmeie	17.6	7.5	23.9	33.2	91.7	41.6
	Holmestrandsfjorden	17.8	7.4	20.2	34.7	79.6	64.1

2.2 Sampling

A total of 49 fish were sampled during the period of March 1st to March 3rd and 56 fish were sampled during the period of August 31st to September 2nd. Atlantic cod (*Gadus morhua*), whiting (*Merlangius merlangus*) and Norway pout (*Trisopterus esmarkii*) were sampled from the inner and outer Oslofjord, and haddock (*Melanogrammus aeglefinus*) was only sampled in the outer Oslofjord. Norway pout was only sampled in the inner Oslofjord during the spring sampling, as not enough fish were collected during trawling in the outer Oslofjord, which lead to the decision that haddock would be included as an additional species from the outer Oslofjord. However, this issue was not encountered during sampling in the fall, and Norway pout was sampled in both fjords and haddock only in the outer Oslofjord. The number of fish from each species collected at the two locations during the two sampling periods is shown in Table 2.

Table 2. Number of fish of each species collected during the sampling of the inner and outer Oslofjord during spring and fall in 2021

		Atlantic cod	*Whiting*	*Norway pout*	*Haddock*
Spring	Inner	8	8	8	-
	Outer	8	9	-	8
Fall	Inner	8	8	8	-
	Outer	8	8	8	8

2.2.1 Sampling procedure

The fish were killed by a blow to the head. The length, in cm, and weight, in g, of the fish was measured (Appendix A). Then a sample of gill lamellae was collected by dissecting out two or more of the branchial arches and cutting off the filaments with a scissor. A bile sample was collected using a syringe and transferred to an Eppendorf tube and stored at -20°C. The liver was weighed (g) (Appendix A) and three liver samples were collected. The gender of the fish was determined (male, female, or juvenile), and the gonads were weighed (g) (Appendix A). Then a sample of the heart was collected. The stomach was removed after all samples were collected and the weight (g) of the gutted fish was measured (Appendix A). All samples were collected using tweezers, scissors, and a scalpel, cleaned between each fish. The samples were placed in cryotubes and stored in liquid nitrogen. Afterwards the samples were transported back to the University of Oslo and stored at -80°C.

2.2.3 Gonadally somatic index (GSI) and maturity

The gonadosomatic index (GSI) is a measure of maturity of fish that changes with the reproductive cycle. GSI is defined as:

$$GSI = \frac{gonad\ weight\ (g)}{gutted\ weight\ (g)} * 100$$

Equation 1

The gutted weight was calculated by adding the weight of the gonads and the liver to the weight of the gutted fish. The GSI was calculated for all fish (Appendix A) and used as a measure of maturity and to assess if the fish was juvenile or maturing/mature. A GSI > 1% for males and >2.5% for females were set as the threshold value for mature or maturing fish, fish with a GSI value below either of these values were assigned as juvenile individuals (Appendix A). The threshold values were based on preliminary manuals for determination of

gonad maturity for two of the relevant species (Bucholtz et al. 2008, Bucholtz et al. 2008) as well manual determination of gonadal maturity during sampling.

2.3 Preparation of tissue samples

The samples were kept on ice during the entire processing to avoid degradation of the biomolecules.

2.3.1 Preparation of buffers

A 0.2 M solution of sodium dihydrogen phosphate monohydrate (NaH_2PO_4 x H_2O) was prepared by dissolving 27.6 g of NaH_2PO_4 x H_2O (137.99 g/mol) in 1000 mL of distilled water. Then a 0.2 M solution of sodium phosphate, dibasic (Na_2HPO_4) was prepared by dissolving 28.4 g of Na_2HPO_4 (141.96 g/mol) in 1000 mL of distilled water. It was made sure that all the substance in both solutions was dissolved before progressing. To make a 0.1 M sodium phosphate buffer, 23 mL of the 0.2 M NaH_2PO_4 x H_2O solution, 230 mL of the 0.2 M Na_2HPO_4 solution and 5.59 g of KCl (74.56 g/mol) was dissolved in distilled water to a total volume of 500 mL. It was made sure that the buffer was properly mixed, and all substance was dissolved before storing the buffer at 4°C.

A homogenisation buffer was prepared by mixing 190 mL of the sodium phosphate buffer, 30.85 mg of 1 mM DL-dithiothreitol (DTT) (154.25 g/mol) and 10 mL of glycerol (92.09 g/mol). It was made sure that all substances were fully dissolved before storing the buffer at 4°C. The buffer was remade as needed. A microsomal buffer was prepared by mixing 160 mL of the 0.1 M sodium phosphate buffer, 0.4 mL of 0.5 M EDTA solution and 39.6 mL glycerol (92.09 g/mol). The buffer was mixed well and stored at 4°C.

2.3.2 Tissue homogenisation

All samples were homogenised using a Precellys 24 system with a Cryolys cooling unit (Bertin technologies). For the homogenisation of liver and heart samples, 15 ceramic beads were added to each Precellys tube. For gill samples, 5 metal beads were added to each tube. After the tissue samples had thawed on ice, 0.3-0.5 g of tissue sample were added to each Precellys tube, and the tubes were labelled accordingly. Homogenisation buffer was added to a total volume of 1 mL in each tube. If the sample was smaller than 0.15 g, homogenisation buffer was added to a total volume of 0.75 mL to ensure an equal tissue to buffer ratio. The

Cryolys cooling unit (5 bar system 70 L/min) and the Precellys 24 was turned on and liquid nitrogen was added to the Cryolys. When the Precellys 24 had reached a temperature below 10°C, the Precellys tubes were added to the machine in a balanced setup and the second run setting (6000 rpm, 3 x 10 sec, 5 sec pauses) was started.

2.3.3 Sub-cellular separation

When the homogenisation in the Precellys 24 was finished, the tubes were removed and the Precellys 24 and the Cryolys were turned off. Because the Precellys tubes fit in the Heraeus Multifuge 3 S-R, they were moved directly to the centrifuge in the same balanced setup. The run parameters were set to 12000 rpm for 30 minutes at 4°C with 10000 x g. When the machine had cooled down to 4°C, the centrifuge was started. This process removed all cell debris and the mitochondria.

After 30 minutes the tubes were transferred to ice and the Heraeus Multifuge 3 S-R was turned off. The supernatant of each sample was transferred to a clean, labelled Eppendorf tube. For gills and hearts, the S9-fraction was not processed further, and all samples were distributed in clean and labelled cryotubes and stored at -80°C. The liver samples were processed further. Approximately the same amount of the S9-fraction, the supernatant, was transferred from the Precellys tube to each Eppendorf tube to ensure a balanced setup in the thermo scientific Sorwall MTX 150 micro-ultracentrifuge. The Eppendorf tubes were transferred to the MTX 150 centrifuge and the following run parameters were set: 100000 x g for 60 minutes at 4°C and 38800 rpm. The vacuum was sealed and when the machine reached 4°C, the centrifuge was started. This process separated the S9 fraction into the microsomal fraction (the pellet) and the cytosolic fraction (the supernatant).

To obtain the microsomal fraction, the supernatant was removed, and the resulting pellet was suspended in microsomal buffer to a total volume of 500 µL and homogenised using a Teflon pestle. The microsomal fraction was then distributed to labelled cryotubes and stored at -80°C.

2.3.4 Reference samples

To prepare reference samples, 48 random liver samples were homogenised in the same way as described above. All samples were centrifuged in the Heraeus Multifuge 3 S-R to obtain the

S9 fraction, and the supernatants (the S9 fractions) were mixed in a large tube. 12 Eppendorf tubes were then filled with 1 mL each of the S9 fraction and separated further into a cytosolic and microsomal fraction as described previously. The supernatants, the cytosolic fraction, were collected in a large tube and mixed. As described above, microsomal buffer was added to each remaining pellet (the microsomal fraction) to a total volume of 500 µL and were homogenised with a Teflon pestle. The microsomal fractions were transferred to a larger tube and mixed well. The resulting reference samples at three different stages of sub-cellular separation were then transferred as 300 µL aliquots to labelled cryotubes and stored at -80°C.

2.4 Quantification of total mercury concentration in liver samples

Total mercury concentration in tissues was quantified using a DMA-80 total mercury analyser. Because of mercury's high affinity for gold, mercury was released from the sample and moved to the catalyst section of the furnace with a carrier gas. Other compounds were eliminated, and mercury was trapped in a second furnace where it was heated up and released into a spectrophotometer where the concentration was quantified by absorption at 253.65 nm.

2.4.1 Preparation of samples

Preparation of samples was done by thawing liver samples on ice and weighing in 15-20 mg of sample in triplicate in sterile nickel vessels using plastic tweezers. The exact weight of each of the replicates was noted down for further use. The tweezer was washed in 0.1 M HCl between samples to avoid contamination. Each run included 11 samples in triplicate, 3 empty vessels, two certified reference samples, DORM-4, and DOLT-4, each weighing approximately 10 mg, and one internal reference sample, FISK, weighing approximately 3 mg.

2.4.2 Preparation of the DMA-80 mercury analyser

The valves for the oxygen supply to the DMA-80 mercury analyser was opened and adjusted to a supply of 4 bar of oxygen to the machine (the carrier gas) and left for 15 minutes. Then the DMA-80 (direct mercury analyser) was turned on and a new experiment was created. For the tab “links”, the standard calibration method was chosen (”Standard.m80”), which was the settings prepared by the supplier. In the tab “Sample”, the ID and weight of all the samples were added in the corresponding order that they were going to be placed in the machine. The

experiment was then saved with date and initials, and all the vessels were placed in the correct order in the machine. To start the analysis, the “play” button on the screen was pressed.

When the analysis was finished, the results were saved and exported to an excel file. The oxygen supply was turned off before the machine was turned off. The nickel vessels were removed from the machine and soaked in distilled water. After soaking they were scrubbed clean, soaked in 70% ethanol, and rinsed again in distilled water before they were placed in a rack and covered with aluminium foil. The nickel vessels were burnt in a muffle at 500-550 degrees for two hours and left to cool down over night before being taken out and marked as clean.

2.4.4 Calculation of mercury concentration

Calculations were performed by the DMA-80 mercury analyser by dividing the determined amount of mercury by the amount weighed into each nickel vessel at the beginning. This resulted in concentrations of mercury in ng/mg wet weight sample. To get one concentration of mercury per sample, the average of the three replicates per tissue sample was calculated from the concentrations given by the DMA-80 mercury analyser (Appendix B).

2.4.5 Reference samples

The mercury concentration in the certified reference samples, DOLT-5 and DORM-4, and internal reference sample, FISK, was quantified in all rounds. Plots of the change in concentration of mercury in each of the reference samples were made and are presented in Figure 3, Figure 4 and Figure 5. The certified values for the references are as following: DOLT-5 0.44 +/- 0.18 mg/kg, DORM-4 0.412 +/- 0.036 mg/kg and TORSK 2.255-2.312 mg/kg. The measured concentrations are close to or within the reference area for the samples. Prior to use the bottles should be rotated to mix the content well. This was not done and could have caused a shift in the results.

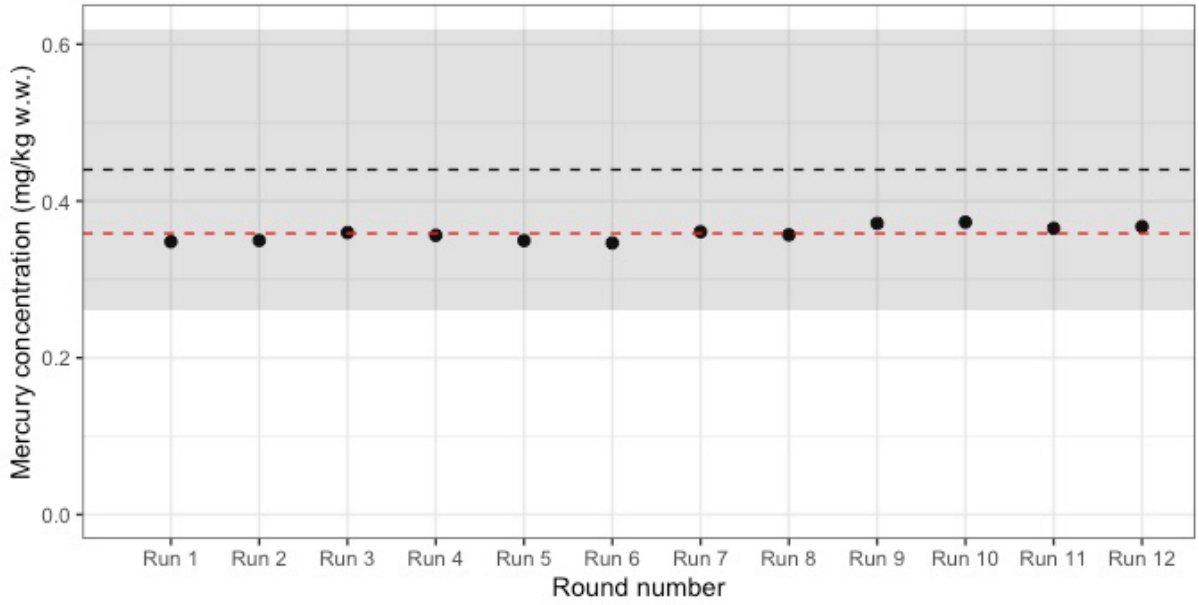

Figure 3. Mercury concentration in DOLT-5 certified reference samples. The concentrations are reported as mg/kg wet weight. The dashed red line represents the mean concentration calculated from all samples. The dashed black line represents the mean certified value, and the shaded area is the given range.

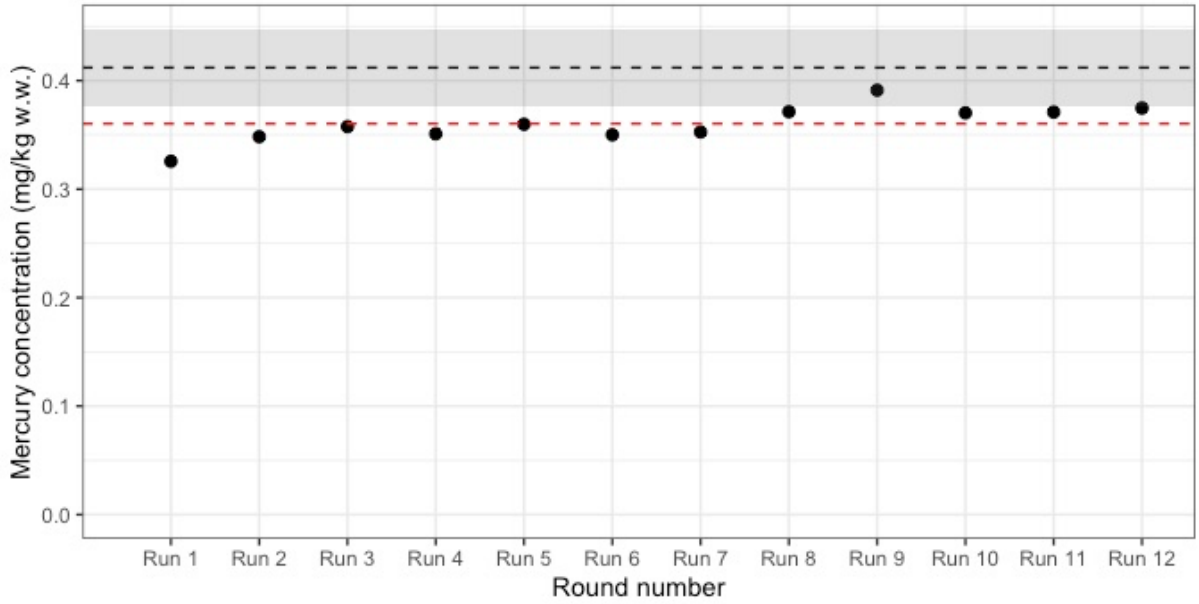

Figure 4. Mercury concentration in DORM-4 certified reference samples. The concentrations are reported as mg/kg wet weight. The dashed red line represents the mean concentration calculated from all samples. The dashed black line represents the mean certified value, and the shaded area is the range.

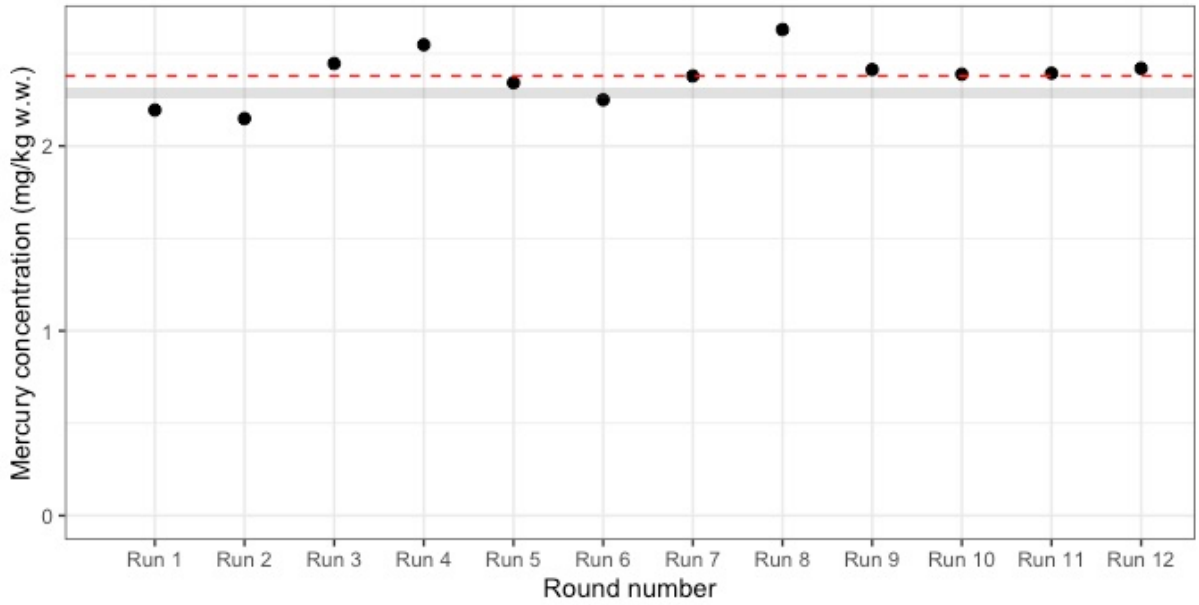

Figure 5. Mercury concentration in FISK internal reference samples. The concentrations are reported as mg/kg wet weight. The dashed red line represents the mean concentration calculated from all samples. The grey area shows the expected range of concentrations for the internal standard.

2.5 Protein analysis, Lowry´s assay

Protein concentration was determined in the microsomal fraction of liver samples and the S9-fraction of gill and heart samples using a modified Lowry's assay (Lowry et al. 1951). The resulting protein concentrations were used to standardise the activity of the cytochrome P450A1 enzyme.

2.5.1 Preparation of buffers and dilution of standards and samples

Tris buffer was prepared by dissolving 4.44 g of Trizma base (121.14 g/mol) and 2.65 g Trizma HCl (157.60 g/mol) in distilled water to a total volume of 500 mL. The buffer was mixed well to ensure that all substance was dissolved. Then the pH was adjusted to 8 with a PHM 92 Lab pH meter (produced by Radiometer Copenhagen). The buffer was stored at 4°C.

4 dilutions of bovine gamma globulin protein standard (BSA standard) (200 mg/mL) were prepared in Tris buffer by first preparing a 1.6 mg/mL solution. The 1.6 mg/mL solution was prepared by mixing 80 µL of 200 mg/mL BSA with 9.92 mL Tris buffer. Then the 1.6 mg/mL solution was diluted 1:1 with Tris buffer in three steps to get four dilutions with the concentrations 1.6 mg/mL, 0.8 mg/mL, 0.4 mg/mL, and 0.2 mg/mL.

The homogenized tissue samples were thawed on ice and then diluted 10, 30, 50 or 80 times in Tris buffer, depending on the sample. Tissue type and fraction were important factors in determining the necessary dilution. This was done to ensure that all resulting absorbances would be no more than 20% higher than the absorbance from the 1.6 mg/mL BSA standard, so that it would be possible to calculate the protein concentration from the known linear relationship between the concentration and measured absorbance within the range of the concentrations of the BSA standard.

2.5.2 Preparation of the 96-well microplate

A new and sterile clear Thermo Scientific Nunc 167008 Nunclon 96-Well MicroWell Plate was used for each round of analysis. 20 µL of blank (Tris buffer), 4 standards, 18 samples and a reference sample were added in quadruplicate to the clean, dry 96-well microplate. 25 µL of the alkaline copper tartrate solution (Reagent A) was then added to each well, and lastly 200 µL of the folin solution (Reagent B) was added to each well. The plate was agitated gently by hand for 5 seconds to mix the content in the wells of the plate before the plate was incubated for 15 minutes at room temperature. After the incubation, the absorbance at 750 nm was measured using a BioTek Synergy Mx Microplate Reader SMA. The plate reader was prepared with the following settings: optics position – top, sensitivity - 100.

If a sample had an absorbance more than 20% higher than the absorbance measured for the 1.6 mg/mL concentration standard, the sample had to be further diluted and analysed again. All results were blank subtracted.

2.5.3 Calculation of protein concentrations from measured absorbance

A standard curve was prepared based on the four concentrations of the BSA standard. A linear relationship between the protein concentration and the measured absorbance was assumed within those known protein concentrations. A linear regression was performed, and the regression line was forced through zero. The resulting function for the regression line was then used for protein calculation in the samples that were analysed on the same plate. The mean of the four measurements was calculated for each sample and the mean absorbance for each sample was divided by the slope number of the corresponding standard curve. The concentration was then corrected for the dilution in the well (12.5) and the dilution prior to

analysis (10, 30, 50 or 80, depending on the sample). The resulting protein concentration for each of the samples were given in mg protein/mL (Appendix C).

2.5.4 Reference samples

The protein concentration in all reference samples was calculated as described above. The protein concentrations were plotted for all the rounds of analysis. One figure was made for the reference samples of the microsomal fraction, Figure 6, and one for the reference samples of the S9 fraction samples, Figure 7.

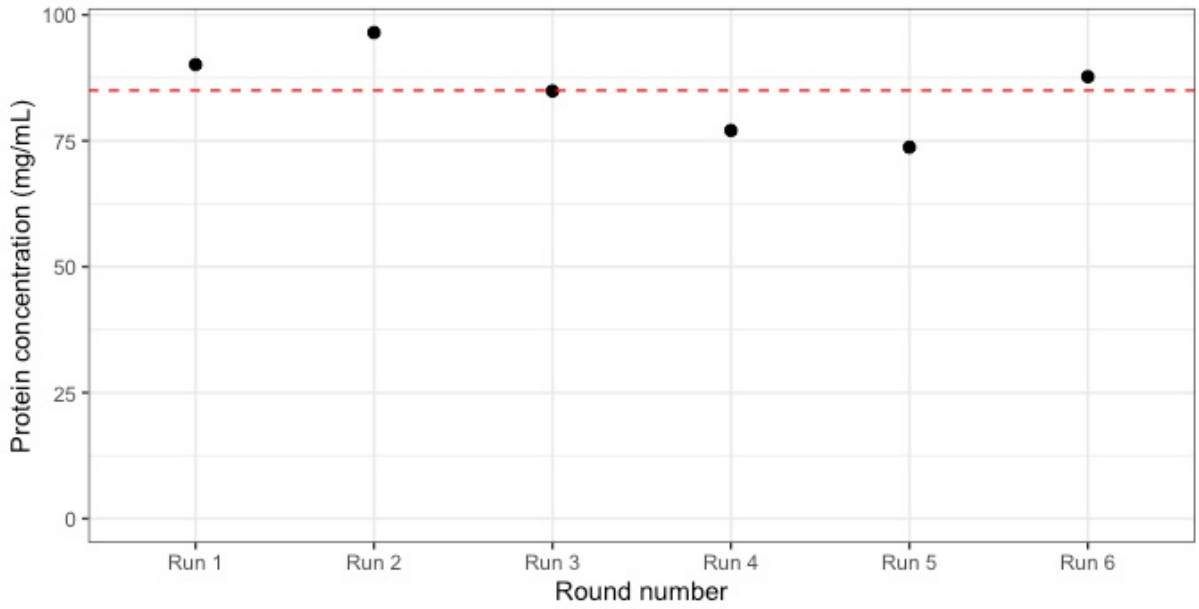

Figure 6. Protein concentration in the microsomal fraction reference samples. The concentration is reported as mg/mL. The dashed red line represents the mean protein concentration of all the samples.

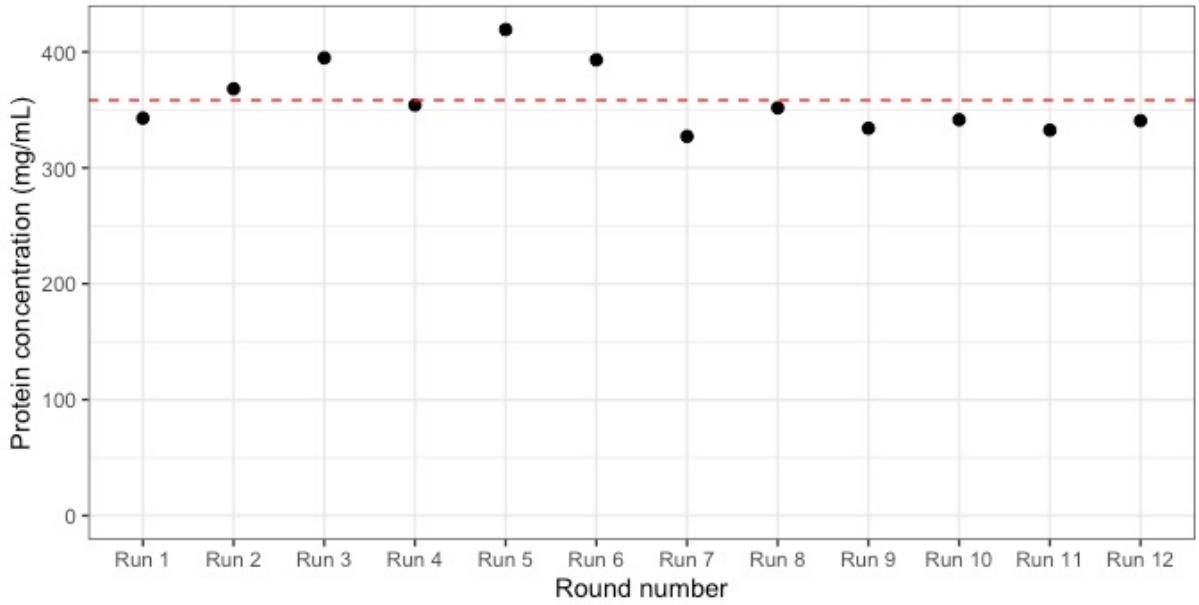

Figure 7. Protein concentration in the S9 fraction reference samples. The concentration is reported as mg/mL. The dashed red line represents the mean protein concentration of all the samples.

2.6 Ethoxyresorufin *O*-deethylase (EROD) assay

The activity of the cytochrome P4501A (CYP1A) enzyme was quantified by measuring the deethylation of ethoxyresorufin to resorufin (Burke and Mayer 1974, Eggens and Galgani 1992). Because the production of resorufin over time is proportional to CYP1A activity, this procedure measured the change in fluorescence of resorufin, measured at the wavelength pair 530/590 nm, over time.

2.6.1 Preparation of buffers and stock solutions

A 1 M di-potassium hydrogen phosphate (K_2HPO_4) stock solution was prepared by dissolving 43.545 g K_2HPO_4 (174.18 g/mol) in distilled water to a final volume of 250 mL. A 1 M potassium dihydrogen phosphate (KH_2PO_4) stock solution was prepared by dissolving 34.0225 g of KH_2PO_4 (136.09 g/mol) in distilled water to a final volume of 500 mL. A 0.1 M potassium phosphate buffer with a pH of 8 was prepared by mixing 94 mL of 1 M K_2HPO_4 stock solution and 6 mL of the 1 M KH_2PO_4 stock solution and adding distilled water to a total volume of 1 L. The pH was measured and adjusted to 8 using a PHM 92 Lab pH meter (Radiometer Copenhagen). The solution was stored at 4°C.

The rest of the solutions were prepared in the dark, as the substances were light sensitive.

A 0.5 mM resorufin ethyl ether (7-ethoxyresorufin) stock solution was prepared by dissolving 5 mg of resorufin ethyl ether (241.24 g/mol) in 41.45 mL DMSO (78.13 g/mol). This was done by adding 0.5 mL DMSO to the delivered resorufin ethyl ether vial and mixing well with a pipette. The 0.5 mL mix was transferred to a large tube. The vial was rinsed 10 times with 0.5 mL DMSO, until several of the washes came back clear. Each wash was transferred to the same tube. DMSO was added to the total volume of 41.45 mL and the solution was mixed well to ensure that all the substance had dissolved. The 0.5 mM stock solution was then distributed in 0.2 mL aliquots in Eppendorf tubes (same amount of Eppendorf tubes as rounds needed to analyse all samples) and the rest was distributed in tubes at larger volumes. All tubes were labelled with date of preparation, content, method of dilution and end concentration. The tubes were stored at -20°C.

A 1 mM resorufin sodium salt stock solution, the standard, was prepared by dissolving 11.8 mg of resorufin sodium salt (213.19 g/mol) in 50 mL of DMSO (78.13 g/mol). After the substance was completely dissolved, the solution was distributed as 50 µL aliquots in Eppendorf tubes (same amount of Eppendorf tubes as rounds needed to analyse all samples) and the rest was distributed in tubes at larger volumes. All tubes were labelled with content, concentration, date prepared and method of dilution. The 1 mM resorufin sodium salt stock solution was stored at -20°C.

A 50 mM solution of β-NADPH solution was prepared by dissolving 50 mg of β-NADPH (833.4 g/mol) in 1.2 mL of potassium phosphate buffer. The solution was distributed as 100 µL aliquots in Eppendorf tubes and all tubes were labelled with content, concentration, date prepared and method of dilution. The 50 mM stock solution was stored at -20°C.

2.6.2 Preparation of working solutions for the analysis

All steps were performed in the dark and on ice. For each planned plate, one tube for each of the stock solutions, resorufin standard, 7-ethoxyresorufin and β-NADPH, were thawed on ice in the dark.

A reaction solution was prepared from the 0.5 mM 7-ethoxyresorufin stock solution by mixing 180 µL of the 7-ethoxyresorufin stock with 30 mL of potassium phosphate buffer. A dilution series of the resorufin standard was prepared in several steps. First the 1 mM

resorufin sodium salt stock solution was diluted to 10 µM by mixing 14 µL of the stock solution with 1386 µL of potassium phosphate buffer. The 10 µM solution was further diluted to 0.64 µM by mixing 128 µL of the 10 µM solution with 1872 µL of the reaction solution. A dilution series of 1:1 dilution was prepared to achieve seven standards with the concentrations: 0.64 µM, 0.32 µM, 0.16 µM, 0.08 µM, 0.04 µM, 0.02 µM and 0.01 µM. All concentrations had a volume of 1 mL, except the lowest concentration, which had a volume of 2 mL. Lastly, the 50 mM β-NADPH stock solution was diluted to a 2.4 mM solution by mixing 96 µL of the 50 mM stock with 1904 µL of potassium phosphate buffer to achieve a total volume of 2 mL 2.4 mM β-NADPH.

2.6.3 Plate reader settings

New and sterile black Greiner bio-one, 655078, 96-well polystyrene microplates were used for each round of the analysis. A BioTek Synergy Mx Microplate Reader SMA was set up with the correct protocol prior to plate preparation to ensure that the protocol could be started immediately after the reaction in the wells on the plate was started.

The plate reader settings were the same for the two types of plates that were prepared. A plate containing all concentrations of the standard was prepared once a day, and from this plate a standard curve could be constructed. The rest of the plates contained only one standard, blanks, a reference sample, and samples. Both types of plates measured fluorescence at the excitation wavelength of 530 nm and emission wavelength 590 nm with the slit set to 20 nm, optics position: top, and auto sensitivity. The difference was that the plate with only standards was only measured in one step. For the plate with tissue samples, the plate was shaken for 5 seconds to mix the content in the wells before a kinetic read was performed. On this plate, change in fluorescence over time was measured for 15 minutes with a total of 16 measurements. The plate reader then plotted these measurements over time and found the value for Vmax, where the change in fluorescence was the largest. From this, an estimate of the maximum value for CYP1A activity in each sample could be calculated.

2.6.4 Preparing the 96-well microplates

Each day one plate was prepared that contained the seven concentrations of the standard from the dilution series. On this plate, 50 µL of potassium phosphate buffer was added in triplicate to the plate, followed by 75 µL of potassium phosphate buffer added in triplicate. Then 200

μL of reaction solution was added to all six wells. Then, with increasing concentration, 275 μL of each of the seven standard concentrations were added in triplicate. Lastly, 25 μL of β-NADPH was added in triplicate to the first three wells to a total volume of 275 μL. The plate was added to the plate reader and measurement was started immediately after the β-NADPH was added.

For the plates also containing samples, 50 μL of the 0.1 M potassium phosphate buffer, the 0.02 μg/mL standard, 21 samples and one reference sample was added in triplicate to the plate. Then, 200 μL of the reaction mix was added to all wells before lastly, 25 μL of β-NADPH was added to all wells. The plate was added to the plate reader and the protocol was started immediately after the addition of the β-NADPH.

2.6.5 Calculation of the CYP1A-activity from the measured fluorescence

The measure of fluorescence in the samples from the plate reader was given as Vmax (mRFU/min). These values were first divided by 1000 to get the results in RFU/min. Then a standard curve was constructed from the fluorescence measurements from the plate with the seven standards. Each standard curve was only used for the samples that were analysed the same day. A linear regression was performed, and the regression line was forced through zero. The equation for the regression line is given on the form $y = OD\ min^{-1} \times a$. The fluorescence for each sample is divided by the slope number for the respective standard curve. Then, to account for the dilution of the sample in each well, the value is multiplied by 5.5. Lastly all measures of CYP1A activity were standardized by dividing by the corresponding protein concentration for each sample. The resulting measures of CYP1A activity had the unit: nmol/min/mg protein (Appendix D).

2.6.6 Reference samples

The mean CYP1A activity in the reference samples was calculated as explained above. The CYP1A activity in the reference samples were plotted for all the rounds of analysis. One figure for the reference samples of the microsomal fraction, Figure 8, and one for the reference samples of the S9 fraction samples, Figure 9.

For the reference samples for the microsomal fraction, an estimate for CYP1A activity is missing from run 6 as the wrong reference sample was used. However, the reference had an

activity that does not deviate from the other S9 references, and the fluorescence measures on the plate were therefore assumed to be accurate. For the reference samples for the S9 fraction, an estimate is missing from run 12 due to it deviating from the other reference samples. The CYP1A activity of the samples analysed on this plate has been corrected by multiplying the activity of each sample by the average CYP1A activity in the reference samples from run 1 to run 11, divided by the CYP1A activity in the reference from run 12.

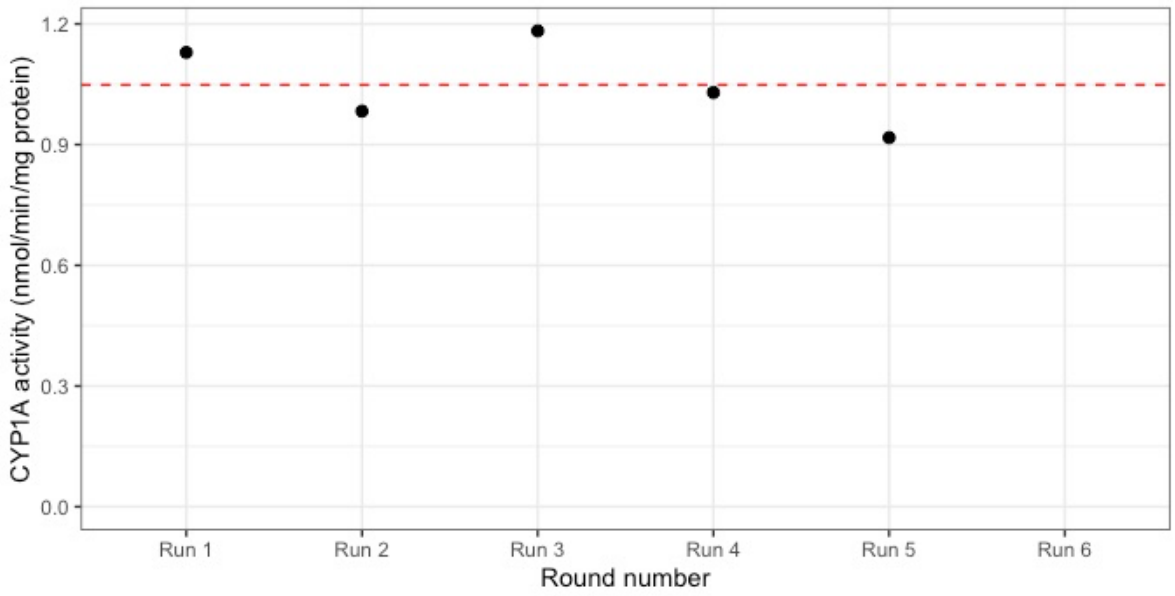

Figure 8. CYP1A activity in the microsomal fraction reference samples. The activity is reported as nmol/min/ mg protein. The dashed red line represents the mean CYP1A from all the samples.

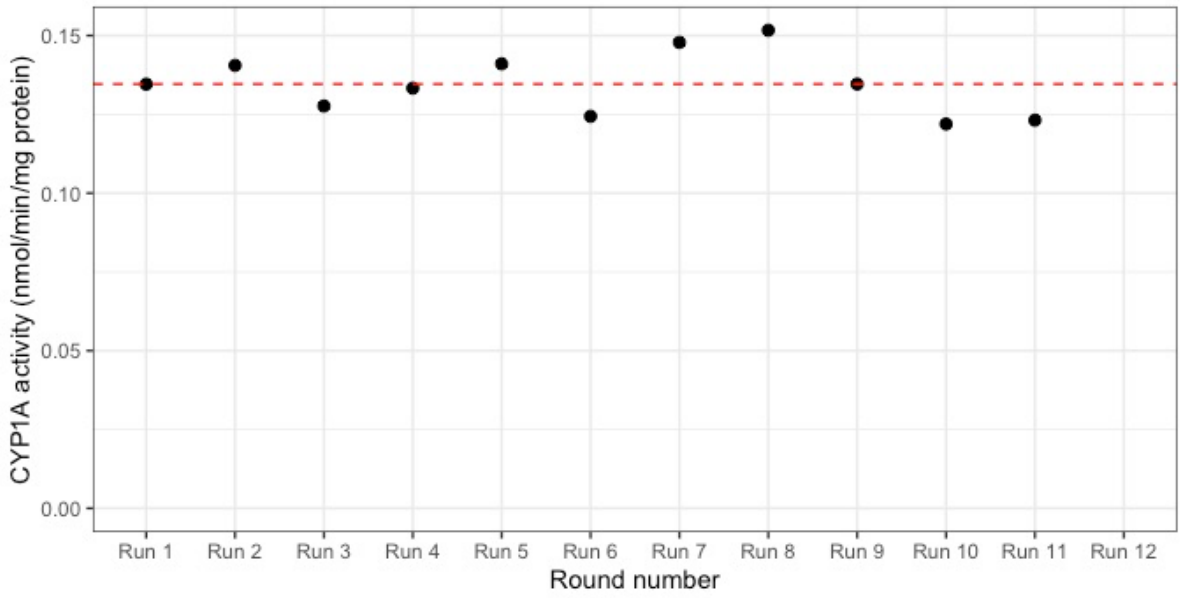

Figure 9. CYP1A activity in the S9 fraction reference samples. The activity is reported as nmol/min/mg protein. The dashed red line represents the mean CYP1A activity from all the samples.

2.7 Measurement of PAH-metabolites in bile samples

Procedure for quantification of PAH metabolites in bile by using a spectrofluorometer to measure fixed wavelength Fluorescence (FF) at excitation/emission wavelength pairs that are specific to different PAHs (Aas et al. 2000, Beyer et al. 2010).

2.7.1 Preparation of solutions and sample dilution

Blanks and a dilution series of a standard was included on all plates. The undiluted standard had known concentrations of 1-OH-Pyrene (2.5 µg/mL), 3-OH-Benzo[a]pyrene (0.625 µg/mL) and the 2- and 3-rings: 1-OH-naphthalene (6.25 µg/mL), 2-OH-naphthalene (6.25 µg/mL) and 1-OH-phenanthrene (2.5 µg/mL). The standard was diluted with a 50% MeOH (32.04 g/mol) solution (diluted with distilled water) in steps to achieve a dilution series of 1:500, 1:1000 and 1:5000 for the standard.

Prior to the analysis a test was performed to investigate how much the samples should be diluted to minimize the matrix effects. Four dilutions were tested on five different samples: 1:1000, 1:2000, 1:4000 and 1:8000. The 1:4000 dilution gave the lowest values without any negative replicates and was the dilution used in the analysis of all the samples.

2.7.2 Preparation of the 96-well quartz plate and measurement of fluorescence

Prior to measuring the fluorescence all the samples included in the run were diluted 1:4000 in a dilution series. The plate used for all rounds of the analysis was a clear quartz 96 well microplate. On all plates 200 µL of blanks (50% MeOH diluted in distilled water), the standard diluted 1:500, 1:1000 and 1:5000 and 20 samples diluted 1:4000 was added in quadruplicate. After all the solutions were added to the plate, fluorescence at three specific excitation/emission wavelength pairs was measured in one step with a BioTek Synergy Mx Microplate Reader SMA. The wavelength pairs were as following: 2- and 3- rings: 290/335 nm, pyrene metabolites: 341/383 nm and for 3-OH-benzo[a]pyrene: 379/425 nm. The set up on the plate reader was as follows: slit – 13.5 nm, optics position: top, sensitivity: auto.

2.7.3 Cleaning procedure of the quarts plate

The plate was cleaned by emptying out the wells and rinsing all wells with the 50% MeOH solution. The plate was rinsed 6 times with MeOH and was left upside down on paper towels to let the residual solution drain out of the wells before the analysis was repeated.

2.7.4 Calculation of concentration of PAH metabolites from measured fluorescence

The results were exported to Excel, and a standard curve based on the measured fluorescence for the three concentrations of the standard was constructed for each of the wavelength pairs and linear regression was performed. The measured fluorescence for each sample was then divided by the corresponding slope number from the regression line and corrected for the 1:4000 dilution prior to analysis by multiplying the concentration with 4000. The resulting concentration of 2- and 3- ring PAHs, pyrene metabolites, and 3-OH-benzo[a]pyrene was given in µg/mL bile (Appendix E).

2.8 Statistical analysis

Statistical analysis of the results was performed in RStudio (version 4.1.2 – 2009-2022). The significance level was set to 0.05. When appropriate, data were $\log_{10}$-transformed. The Shapiro-Wilks test (Shapiro and Wilk 1965) was performed to test for normal distribution. All data that did not fulfil the normality requirement was $\log_{10}$-transformed. Levene´s test (Levene 1960) was performed to test homogeneity of variance. Data that had homogenous variances was analysed using parametric tests. The tests included one- and two-way analysis of variance (ANOVA), followed by a Tukey HSD post hoc test to test what groups were

statistically significantly different. The non-parametric Kruskal-Wallis test (Kruskal and Wallis 1952) was performed on the data with non-homogenous variances, followed by a pairwise Wilcoxon rank sum test (Wilcoxon 1945) to calculate pairwise comparisons between groups. The reported p-values from the Wilcoxon rank sum test were corrected using Bonferroni correction. Correlations were expressed by the non-parametric Spearman's rank correlation coefficient and corrected using Bonferroni correction.

3 Results

3.1 Length and Gonad somatic index (GSI)

3.1.1 GSI

Mature individuals were sampled only during spring in all four species, and the mature individuals were mainly females, as depicted in Figure 10 and Figure 11. For Atlantic cod and Haddock, juvenile individuals were also sampled during spring. During fall, only juvenile individuals were sampled.

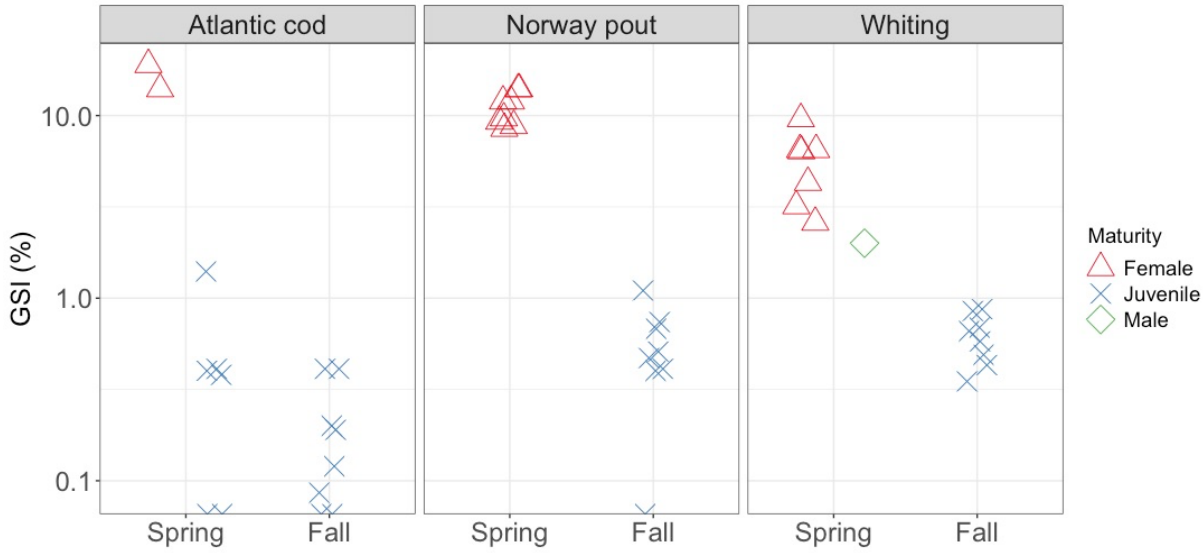

Figure 10. The gonad somatic index of the fish sampled in the inner Oslofjord. The fish are separated by species and season. The colours represent the maturity and gender of the fish; red – female, blue – juvenile, and green – male.

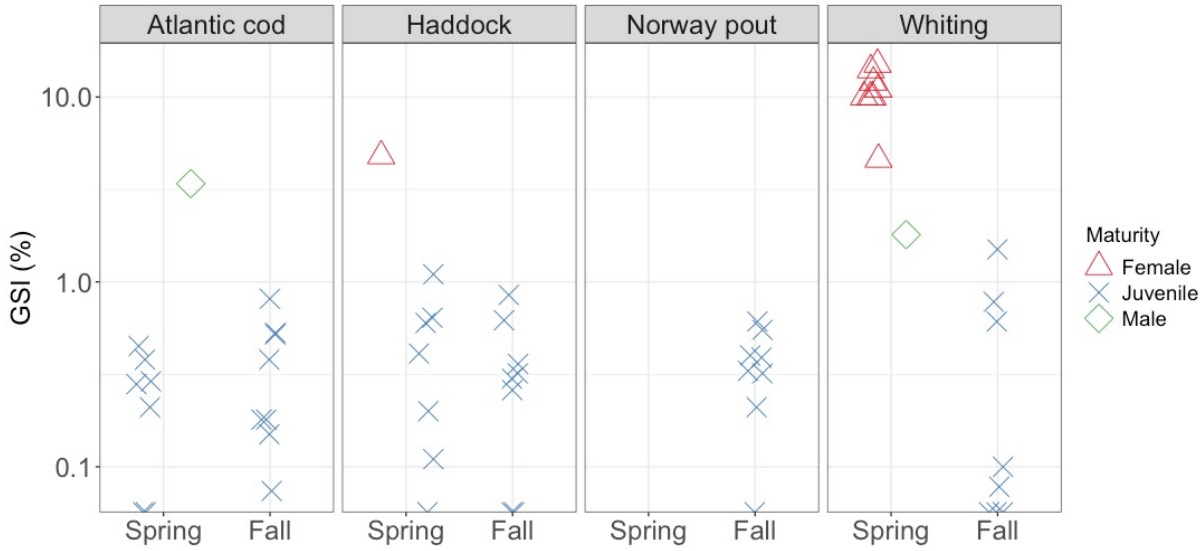

Figure 11. The gonad somatic index of the fish sampled in the outer Oslofjord. The fish are separated by species and season. The colours represent the maturity and gender of the fish; red – female, blue – juvenile, and green – male.

3.1.2 Fish length

The mean length of each species at each location and season was estimated, and the results are presented in Table 3.

Table 3. Mean length (cm) of the fish sampled at each location in each season.

Species	Spring		Fall	
	Inner	Outer	Inner	Outer
Atlantic cod	36.1	33.3	36.3	37.2
Whiting	30.3	27.4	29.8	25.6
Norway pout	21.5	-	22.6	18.1
Haddock	-	26.9	-	29.1

3.2 Polycyclic aromatic hydrocarbon (PAH) metabolites in bile

The concentrations of 2- and 3-ring metabolites, pyrene metabolites, and 3-OH-benzo[a]pyrene were investigated separately.

3.2.1 Concentration of 2- and 3-ringed PAH metabolites in bile

The concentration of 2- and 3-ring PAH metabolites (μg/mL) in bile was determined in Atlantic cod, whiting and haddock (Figure 12).

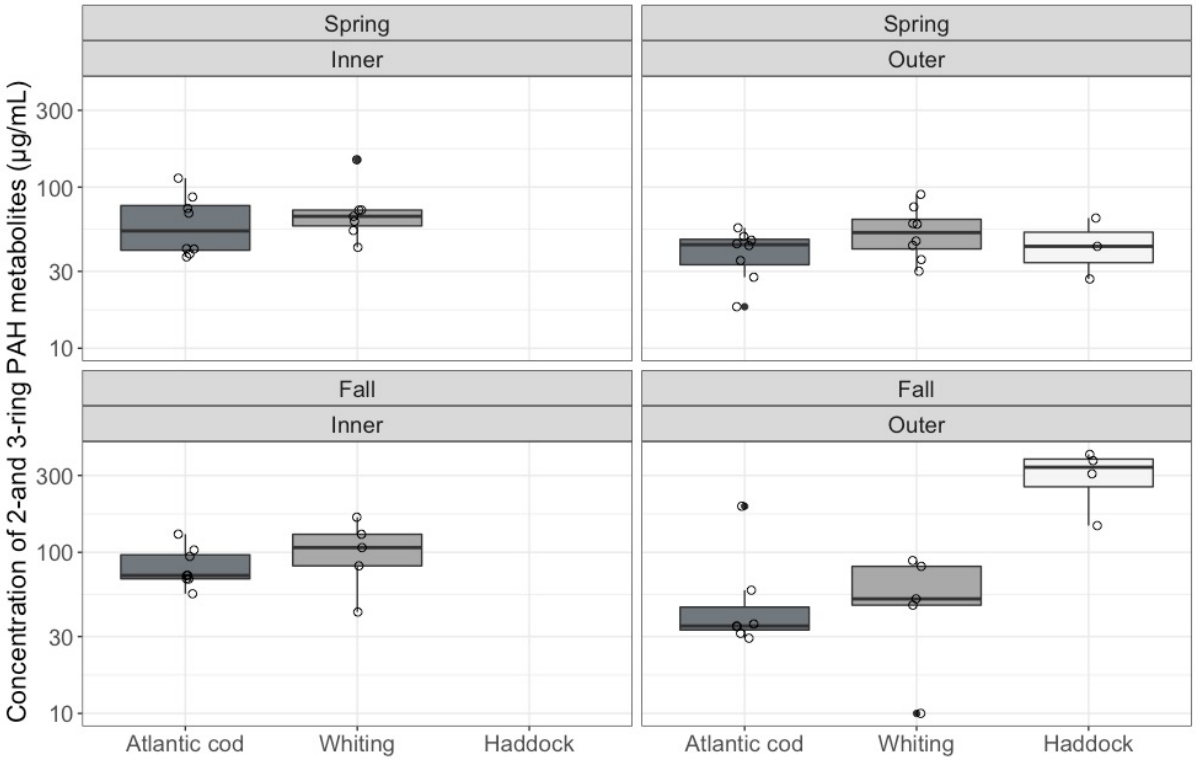

*Figure 12. Concentrations of 2-and-3-ring PAH metabolites in bile of Atlantic cod, whiting, and haddock. The colours of the boxes indicate the different species. The thick black line in each box indicates the median value. The boxes represent the first and third quartile, the whiskers extend from the quartile to the respectively smallest and largest value no further than 1.5 * inter-quartile range (IQR) from the quartile. Outliers and are plotted individually as filled black dots. Black circles represent the datapoints.*

A two-way ANOVA was performed to test for differences in concentrations of 2- and 3-ring PAH metabolites in bile by location and species in spring. There was a significant effect of location ($F(1,27) = 5.94$, $p = 0.02$) on group means of Atlantic cod and whiting in the inner and outer Oslofjord in spring. There was no significant interaction effect ($F(1,27) = 0.23$, $p = 0.6$) or effect of species ($F(1,27) = 3.00$, $p = 0.1$) on group means. There was a significant difference in concentration of 2- and 3-ring PAH metabolites between the inner and outer Oslofjord in spring (Tukey, $p = 0.02$). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in concentrations of 2- and 3-ring PAH metabolites in bile by location and species in fall. There was a significant effect of location ($F(1,21) = 7.11$, $p = 0.01$) on group means of Atlantic cod and whiting in the inner and outer Oslofjord in fall. There was no significant interaction effect ($F(1,21) = 0.20$, $p = 0.7$) or effect of species ($F(1,21) = 0.093$, $p = 0.8$) on group means. There was a significant

difference in concentration of 2- and 3-ring PAH metabolites between the inner and outer Oslofjord in spring (Tukey, $p = 0.01$). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in concentrations of 2- and 3-ring PAH metabolites in bile by season and species in the inner Oslofjord. There was a significant effect of season ($F(1,24) = 4.38$, $p = 0.05$) on group means of Atlantic cod and whiting in spring and fall in the inner Oslofjord. There was no significant interaction effect ($F(1,24) < 0.01$, $p = 1$) or effect of species ($F(1,24) = 1.31$, $p = 0.3$) on group means. There was a significant difference in concentration of 2- and 3-ring PAH metabolites between spring and fall in the inner Oslofjord (Tukey, $p = 0.05$). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in concentrations of 2- and 3-ring PAH metabolites in bile by season and species in the outer Oslofjord. There was a significant interaction effect ($F(2,29) = 8.37$, $p = 0.01$) on group means of Atlantic cod, whiting, and haddock in spring and fall in the outer Oslofjord. There was a significant difference in concentration of 2- and 3-ring PAH metabolites between haddock and Atlantic cod in fall (Tukey, $p < 0.01$), between whiting and haddock in fall (Tukey, $p < 0.01$) and between haddock in spring and fall (Tukey, $p < 0.01$). Levene's test was performed, and the assumption was met.

3.2.2 Concentration of pyrene metabolites in bile

The concentration of pyrene metabolites (μg/mL) in bile was determined in Atlantic cod, whiting and haddock (Figure 13).

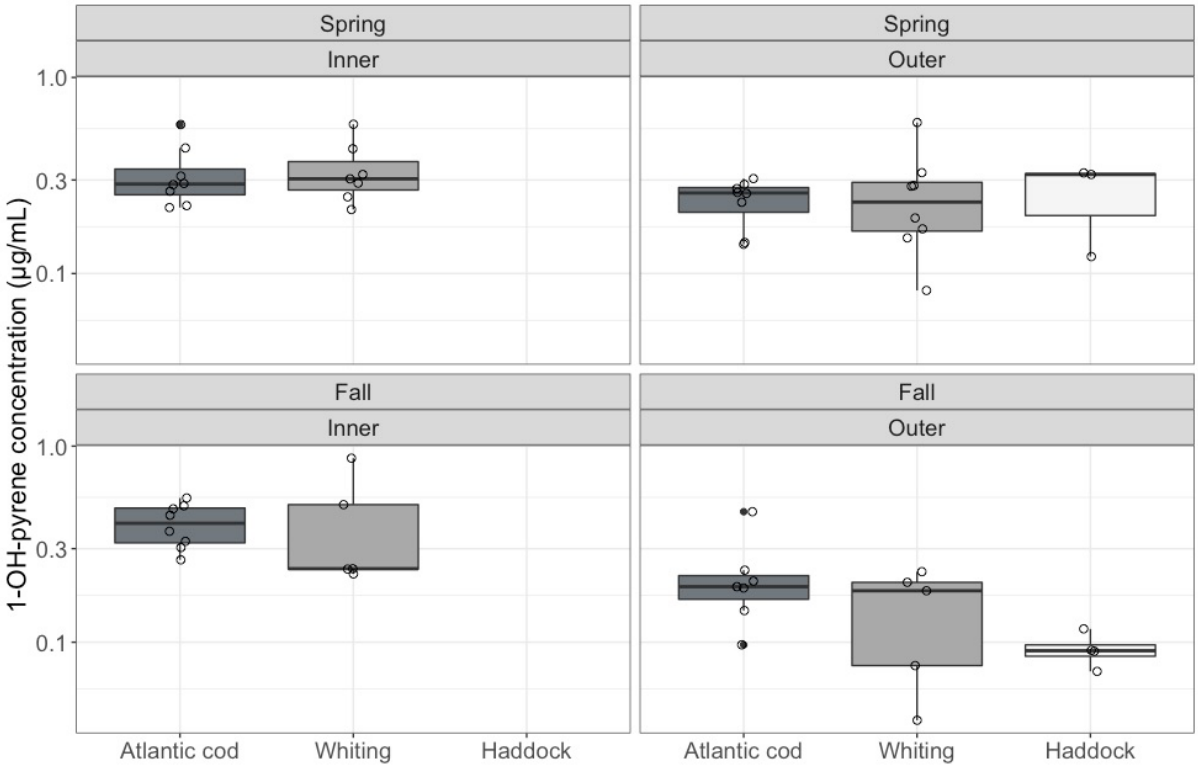

*Figure 13. Concentrations of pyrene metabolites in bile of Atlantic cod, whiting, and haddock. The colours of the boxes indicate the different species. The thick black line in each box indicates the median value. The boxes represent the first and third quartile, the whiskers extend from the quartile to the respectively smallest and largest value no further than 1.5 * IQR from the quartile. Outliers and are plotted individually as filled black dots. Black circles represent the datapoints.*

A two-way ANOVA was performed to test for differences in concentrations of pyrene metabolites in bile by location and species in spring. There was a significant effect of location ($F(1,21) = 17.02$, $p < 0.01$) on group means of Atlantic cod and whiting in the inner and outer Oslofjord in spring. There was no significant interaction effect ($F(1,21) = 0.82$, $p = 0.4$) or effect of species ($F(1,21) = 1.90$, $p = 0.2$) on group means. There was a significant difference in concentration of pyrene metabolites between the inner and outer Oslofjord in spring (Tukey, $p < 0.01$). While there were no species differences, whiting in the outer Oslofjord has a wider distribution than the other species in the outer Oslofjord (Figure 13). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in concentrations of pyrene metabolites in bile by location and species in fall. A two-way ANOVA indicated a significant effect of location ($F(1,27) = 5.15$, $p = 0.03$) on group means of Atlantic cod and whiting in the inner and outer Oslofjord in spring. There was no significant interaction effect ($F(1,27) = 0.05$, $p = 0.8$) or effect of species ($F(1,27) = 0.01$, $p = 0.9$) on group means. There was a

significant difference in concentration of pyrene metabolites between the inner and outer Oslofjord in fall (Tukey, $p < 0.01$). While there were no species differences, whiting in the inner and outer Oslofjord has a wider distribution than the other species in the respective locations (Figure 13). Levene's test was performed, and the assumption was met.

A two-way ANOVA indicated no significant interaction effect ($F(1,24) = 0.27$, $p = 0.6$), effect of species ($F(1,24) = 0.02$, $p = 0.9$) or effect of season ($F(1,24) = 1.53$, $p = 0.2$) on group means of Atlantic cod and whiting in spring and fall in the inner Oslofjord. While there were no species differences, whiting in fall in the inner Oslofjord has a wider distribution than Atlantic cod in both seasons and whiting in spring in the inner Oslofjord (Figure 13). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in concentrations of pyrene metabolites in bile by season and species in the outer Oslofjord. There was a significant effect of season ($F(1,29) = 8.04$, $p = 0.01$) on group means of Atlantic cod, whiting, and haddock in spring and fall in the outer Oslofjord. There was no significant interaction effect ($F(2,29) = 1.61$, $p = 0.2$) or effect of species ($F(2,29) = 1.62$, $p = 0.2$) on group means. There was a significant difference in concentration of pyrene metabolites in bile between the spring and fall in the outer Oslofjord (Tukey, $p = 0.01$). While there were no species differences, whiting in the inner and outer Oslofjord has a wider distribution than the other species in the respective locations (Figure 13). Levene's test was performed, and the assumption was met.

3.2.3 Concentration of 3-OH-benzo[a]pyrene in bile

The concentration of 3-OH-benzo[a]pyrene (μg/mL) in bile was determined in Atlantic cod, whiting and haddock (Figure 14).

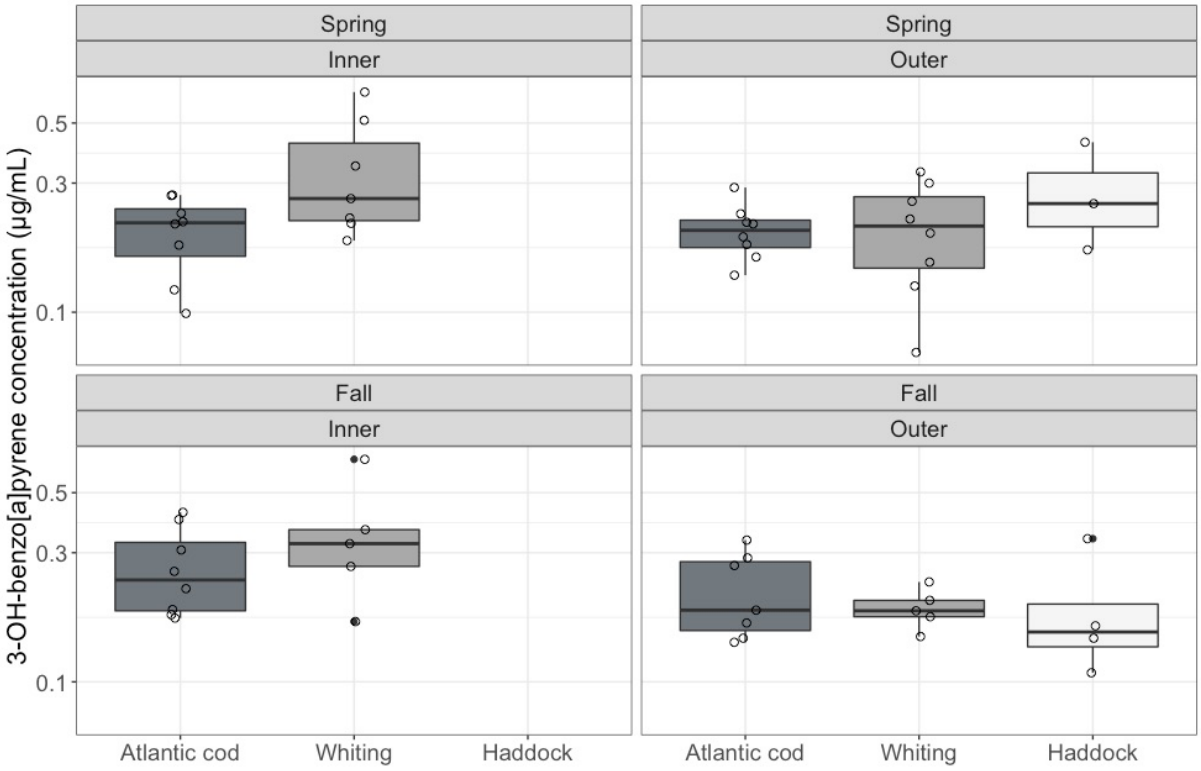

*Figure 14. Concentrations of 3-OH-benzo[a]pyrene in bile of Atlantic cod, whiting, and haddock. The colours of the boxes indicate the different species. The thick black line in each box indicates the median value. The boxes represent the first and third quartile, the whiskers extend from the quartile to the respectively smallest and largest value no further than 1.5 * IQR from the quartile. Outliers and are plotted individually as filled black dots. Black circles represent the datapoints.*

A two-way ANOVA indicated no significant interaction effect ($F(1,27) = 3.37$, $p = 0.08$), effect of species ($F(1,27) = 2.03$, $p = 0.2$), or effect of location ($F(1,27) = 2.13$, $p = 0.2$) on group means of Atlantic cod and whiting in the inner and outer Oslofjord in spring. While there were no species differences, whiting in the outer Oslofjord has a wider distribution than the other species in the outer Oslofjord (Figure 14). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in 3-OH-benzo[a]pyrene concentrations in bile by location and species in fall. A two-way ANOVA indicated a significant effect of location ($F(1,21) = 5.49$, $p = 0.03$) on group means of Atlantic cod and whiting in the inner and outer Oslofjord in spring. There was no significant interaction effect ($F(1,21) = 1.41$, $p = 0.3$) or effect of species ($F(1,21) = 0.25$, $p = 0.6$) on group means. There was a significant difference in concentration of pyrene metabolites in bile between the inner and outer Oslofjord in fall (Tukey, $p = 0.03$). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in 3-OH-benzo[a]pyrene concentrations in bile by season and species in the inner Oslofjord. There was a significant effect of species ($F(1,24) = 5.56$, $p = 0.03$) on group means of Atlantic cod and whiting in spring and fall in the inner Oslofjord. There was no significant interaction effect ($F(1,24) = 0.56$, $p = 0.5$) or effect of season ($F(1,24) = 0.94$, $p = 0.3$) on group means. There was a significant difference in concentration of 3-OH-benzo[a]pyrene between Atlantic cod and whiting in the inner Oslofjord (Tukey, $p = 0.03$). Levene's test was performed, and the assumption was met.

A two-way ANOVA indicated no significant interaction effect ($F(2,29) = 0.99$, $p = 0.4$), effect of species ($F(2,29) = 0.25$, $p = 0.8$), or effect of season ($F(1,29) = 0.20$, $p = 0.7$) on group means of Atlantic cod, whiting, and haddock in the spring and fall in the outer Oslofjord in spring. While there were no species differences, whiting in spring in the outer Oslofjord has a wider distribution than the other species in spring in the outer Oslofjord (Figure 14). Levene's test was performed, and the assumption was met.

3.2.4 Norway pout

Due to difficulties in collecting bile samples from Norway pout, only a total of three bile samples were collected from all four locations. The results were not included due to large variability.

3.3 Total mercury concentration in liver

Total mercury (Hg) concentration (µg/kg wet weight) was determined in samples of liver of Atlantic cod, whiting, Norway pout and haddock in the inner and outer Oslofjord in spring and fall (Figure 15).

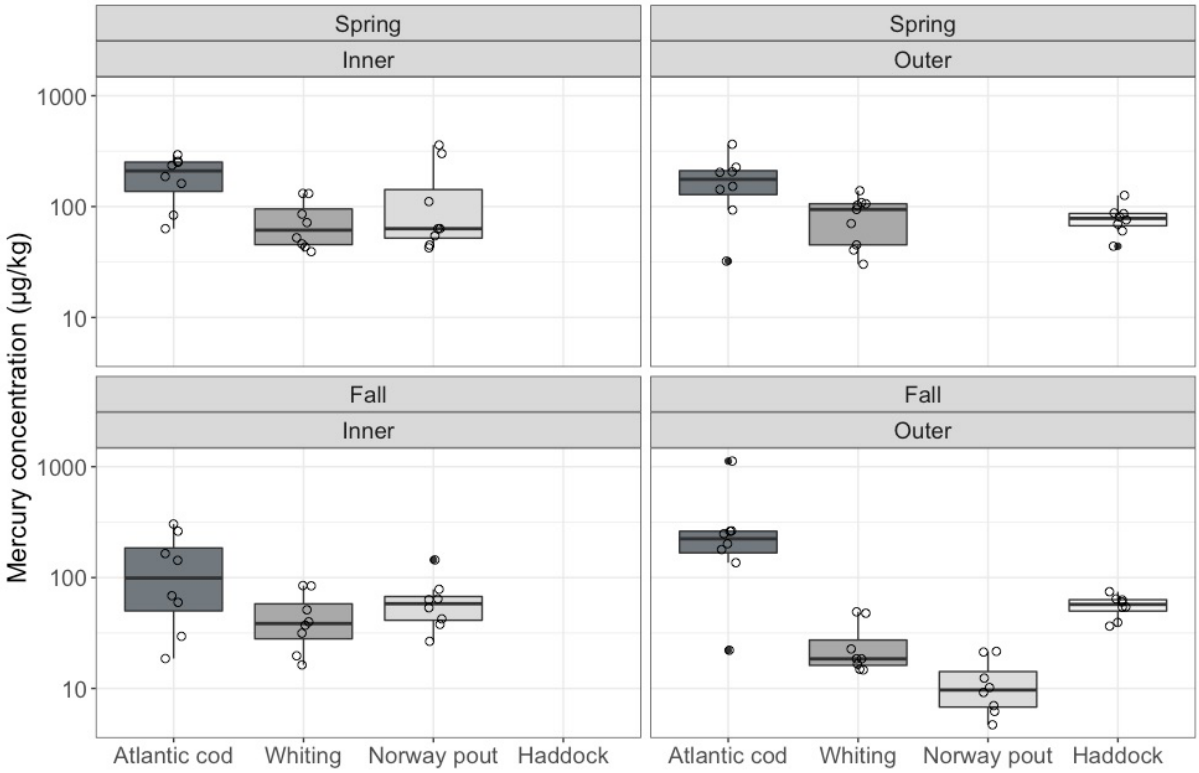

*Figure 15. Concentrations of mercury in liver samples of Atlantic cod, whiting, Norway pout, and haddock. The colours of the boxes indicate the different species. The thick black line in each box depicts the median value. The boxes represent the first and third quartile, the whiskers extend from the quartile to the respectively smallest and largest value no further than 1.5 * IQR from the quartile. Outliers are plotted individually as filled black dots. Black circles represent the datapoints.*

A two-way ANOVA was performed to test for differences in concentration of mercury in liver by location and species in spring. There was a significant effect of species ($F(1,29) = 15.88$, $p < 0.01$) on group means of Atlantic cod and whiting in the inner and outer Oslofjord in spring. There was not a significant interaction effect ($F(1,29) = 0.30$, $p = 0.6$) or effect of location ($F(1,29) = 0.07$, $p = 0.8$) on group means. There was a significant difference in mercury concentration between whiting and Atlantic cod (Tukey, $p < 0.01$). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in concentration of mercury in liver by location and species in fall. There was a significant interaction effect ($F(2,42) = 11.13$, $p < 0.01$) on group means of Atlantic cod, whiting and Norway pout in the inner and outer Oslofjord in fall. There was a significant difference in mercury concentration between Norway pout and Atlantic cod in the outer Oslofjord (Tukey, $p < 0.01$), between whiting and Atlantic cod in the outer Oslofjord (Tukey, $p < 0.01$), and between Norway pout in the inner

and outer Oslofjord (Tukey, $p < 0.01$). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in concentration of mercury in liver by seasons and species in the inner Oslofjord. There was a significant effect of species ($F(2,42) = 6.47$, $p < 0.01$) and seasons ($F(1,42) = 7.67$, $p < 0.01$) on group means of Atlantic cod, whiting, and Norway pout in spring and fall in the inner Oslofjord. There was not a significant interaction effect ($F(2,42) = 0.051$, $p = 0.9$) on group means. There was a statistically significant difference in mercury concentration between whiting and Atlantic cod (Tukey, $p < 0.01$) and between spring and fall (Tukey, $p < 0.01$). Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in concentration of mercury in liver by seasons and species in the outer Oslofjord. There was a significant interaction effect ($F(2,43) = 5.60$, $p < 0.01$) on group means of Atlantic cod, whiting and haddock in the spring and fall in the outer Oslofjord. There was a significant difference in mercury concentration between haddock and Atlantic cod in fall (Tukey, $p < 0.01$), between whiting and Atlantic cod in fall (Tukey, $p < 0.01$) and between whiting in spring and fall (Tukey, $p < 0.01$). Levene's test was performed, and the assumption was met.

3.4 CYP1A activity in liver, heart, and gills

CYP1A activity (nmol/min/mg protein) was determined in samples of liver, gills and heart of Atlantic cod, whiting, Norway pout, and haddock.

3.4.1 Hepatic CYP1A activity

Hepatic CYP1A activity (nmol/min/mg protein) was determined in samples of Atlantic cod, whiting, Norway pout, and haddock (Figure 16).

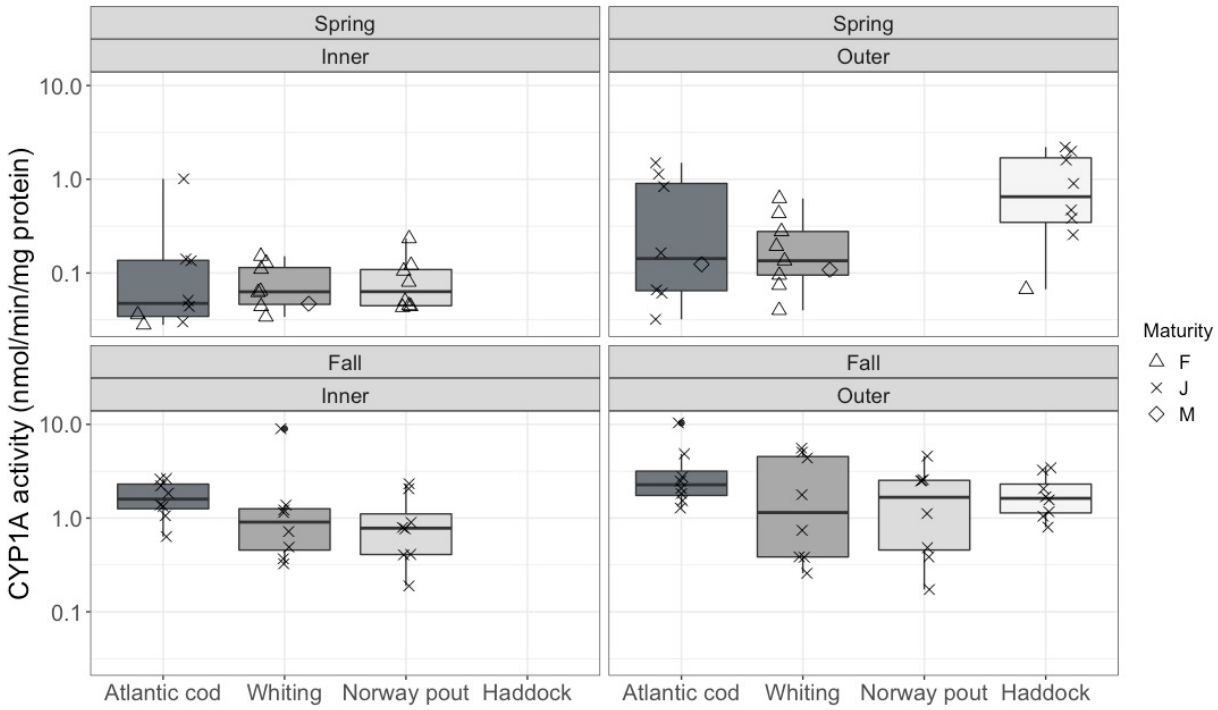

*Figure 16. Hepatic CYP1A activity in Atlantic cod, whiting, Norway pout, and haddock. The colours of the boxes indicate the different species. The thick black line in each box depicts the median value. The boxes represent the first and third quartile, the whiskers extend from the box to the smallest and largest value < 1.5 * inter-quartile range (IQR) from the boxes. Outliers and are plotted individually as filled black dots. The maturity of the datapoints is indicated as: triangle – female, cross – juvenile and diamond - male.*

A two-way ANOVA was performed to test for differences in hepatic CYP1A activity between locations and species in spring. There was a significant effect of location ($F(1,29) = 5.62$, $p = 0.02$) on group means of Atlantic cod and whiting in spring. There was no statistically significant interaction effect ($F(1,29) = 0.062$, $p = 0.8$) or effect of species ($F(1,29) = 0.27$, $p = 0.6$) on group means. There was a significant difference in hepatic CYP1A activity between the inner and outer Oslofjord (Tukey, $p = 0.02$). A Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in hepatic CYP1A activity between locations and species in fall. The test showed no indication of a significant interaction effect ($F(2,42) = 0.09$, $p = 0.9$), effect of species ($F(2,42) = 3.36$, $p = 0.06$), or effect of location ($F(1,42) = 2.00$, $p = 0.2$) on group means. A Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in hepatic CYP1A activity between species and seasons in the inner Oslofjord. There was a significant effect of season ($F(1,42) =$

118.18, $p < 0.01$) on group means of Atlantic cod, whiting and Norway pout in the spring and fall in the inner Oslofjord. There was no statistically significant interaction effect ($F(2,42) = 0.74$, $p = 0.5$) or effect of species ($F(2,42) = 0.95$, $p = 0.4$) on group means. There was a significant difference in hepatic CYP1A activity between spring and fall (Tukey, $p < 0.01$). Levene's test was performed, and the assumption was met.

A Levene's tests was performed, and the assumption of homogenic variance was not met when comparing hepatic CYP1A activity between species and locations in the outer Oslofjord. Tests for species and seasonal differences were performed separately using the non-parametric Kruskal-Wallis test.

A Kruskal-Wallis test was performed to compare mean CYP1A activity between the species Atlantic cod, whiting and haddock in the outer Oslofjord in spring. The test did not indicate a significant effect of species on hepatic CYP1A activity in liver ($H(2) = 4.97$, $p = 0.08$).

A Kruskal-Wallis test was performed to compare hepatic CYP1A activity between the species Atlantic cod, whiting, Norway pout, and haddock in the outer Oslofjord in fall. The test indicated no significant effect of species on hepatic CYP1A activity in liver ($H(3) = 2.28$, $p = 0.5$).

Kruskal-Wallis tests were performed for each of the species Atlantic cod, whiting and haddock to compare hepatic CYP1A activity between seasons in the outer Oslofjord. The tests revealed that there was a significant effect of season on hepatic CYP1A activity for Atlantic cod ($H(1) = 10.6$, $p < 0.01$), for whiting ($H(1) = 7.79$, $p < 0.01$), but not for haddock ($H(1) = 2.82$, $p = 0.09$). For Atlantic cod and whiting two Wilcoxon signed rank pairwise tests were carried out for the groups, with p-values adjusted using the Bonferroni correction. There was a significant difference in hepatic CYP1A activity between spring and fall in the outer Oslofjord for Atlantic cod ($p < 0.01$) and for whiting ($p < 0.01$).

In the inner Oslofjord in spring, whiting and Norway pout are mainly mature female fish that have been sampled, while for Atlantic cod, only two of the fish are mature female fish. All fish sampled in fall are juvenile fish. All species show the same pattern of higher hepatic CYP1A activity in fall than in spring in the inner Oslofjord (Figure 16).

In the outer Oslofjord in spring, whiting are mainly mature female fish that have been sampled, while Atlantic cod and haddock are mainly juvenile fish. All fishes sampled in fall are juvenile fish. There seems to be a pattern of higher hepatic CYP1A activity in fall than in spring for Atlantic cod and whiting in the outer Oslofjord, however distributions are wide (Figure 16).

A Spearman's rank correlation was computed to test for correlation between hepatic CYP1A activity and maturity, measured as GSI (Table 4). There was no correlation between CYP1A activity in the liver and GSI in any of the species.

Table 4. Spearman's rank correlation between CYP1A activity in liver and GSI. $P < 0.05$. P-values corrected using Bonferroni correction for multiple correlations.

Group	Maturity	Spearman's rank correlation coefficient	p-value
Atlantic cod spring inner Oslofjord	Only juvenile, excluding two females	-0.20	0.8
Atlantic cod spring outer Oslofjord	Only juvenile, excluding one male	0.27	0.6
Atlantic cod fall inner Oslofjord	Only juvenile	-0.25	0.5
Atlantic cod fall outer Oslofjord	Only juvenile	0.00	1
Whiting spring inner Oslofjord	Ony female, excluding one male	0.47	0.3
Whiting spring outer Oslofjord	Only female, excluding one male	0	1
Whiting fall inner Oslofjord	Only juvenile	-0.095	0.8
Whiting fall outer Oslofjord	Only juvenile	-0.39	0.3
Norway pout spring inner Oslofjord	Only female	-0.13	0.7
Norway pout fall inner Oslofjord	Only juvenile	-0.45	0.3
Norway pout fall outer Oslofjord	Only juvenile	0.095	0.8
Haddock spring outer Oslofjord	Only juvenile, excluding one female	0.71	0.07
Haddock fall outer Oslofjord	Only juvenile	-0.51	0.2

3.4.2 Gill CYP1A activity

Gill CYP1A activity (nmol/min/mg protein) was determined in samples of Atlantic cod, whiting, Norway pout, and haddock (Figure 17).

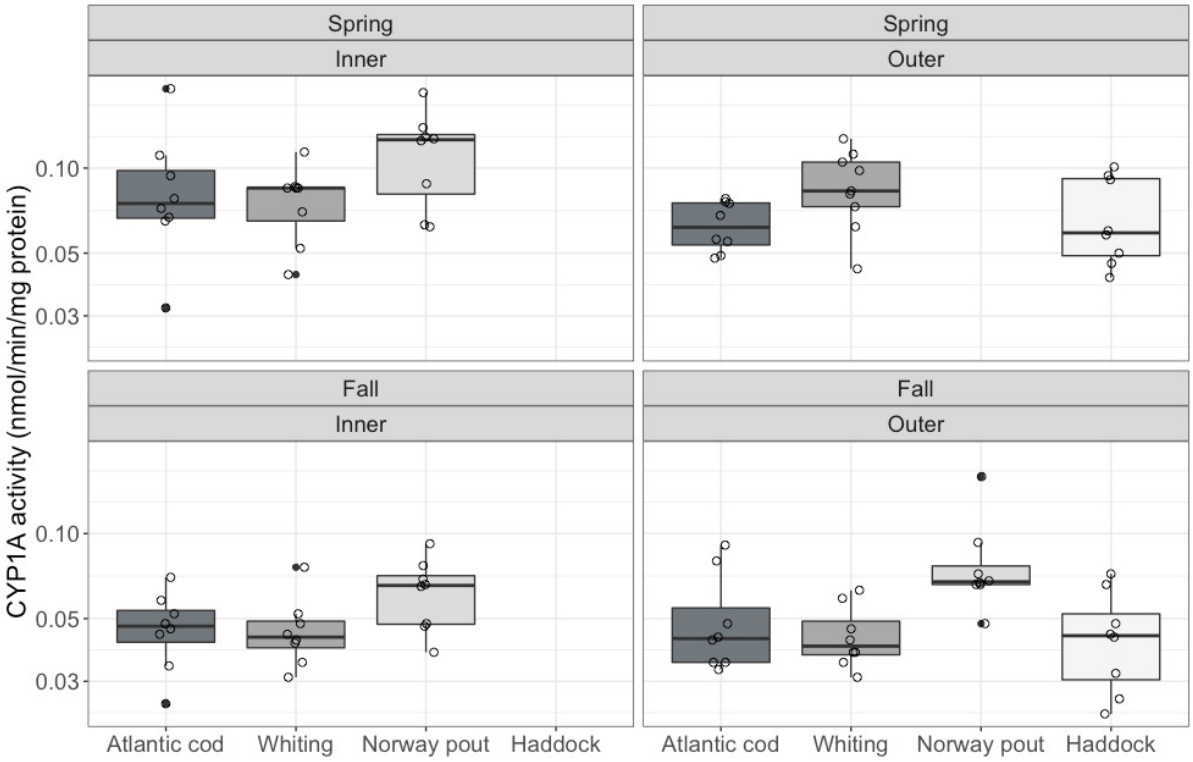

*Figure 17. Gill CYP1A activity in Atlantic cod, whiting, Norway pout, and haddock. The colours of the boxes indicate the different species. The thick black line in each box depicts the median value. The boxes represent the first and third quartile, the whiskers extend from the box to the smallest and largest value < 1.5 * IQR from the boxes. Outliers and are plotted individually as filled black dots. Black circles represent the datapoints.*

A two-way ANOVA was performed to test for differences in gill CYP1A activity between species and locations in spring. The two-way ANOVA did not indicate a significant interaction effect ($F(1,29) = 2.13$, $p = 0.16$), effect of location ($F(1,29) = 0.20$, $p = 0.66$) or effect of species ($F(1,29) = 0.94$, $p = 0.34$) on group means of Atlantic cod and whiting in the inner and outer Oslofjord in spring. A Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in gill CYP1A activity between locations and species in fall. There was a significant effect of species ($F(2,42) = 8.98$, $p < 0.01$) on group means of Atlantic cod, whiting and Norway pout in the inner and outer Oslofjord in. There was no significant interaction effect ($F(2,42) = 0.69$, $p = 0.5$) or effect of location ($F(1,42) = 0.67$, $p = 0.4$) on group means. There was a significant difference in gill CYP1A activity between Norway pout and Atlantic cod (Tukey, $p = 0.01$) and between whiting and Norway pout (Tukey, $p < 0.01$). A Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in gill CYP1A activity between species and seasons in the inner Oslofjord. There was a significant effect of species ($F(2,42) = 4.25$, $p = 0.02$) and season ($F(1,42) = 28.03$, $p < 0.01$) on group means of Atlantic cod, whiting and Norway pout in the spring and fall in the inner Oslofjord. There was no significant interaction effect ($F(2,42) = 0.038$, $p = 1$) on group means. There was a statistically significant difference in gill CYP1A activity between Norway pout and whiting (Tukey, $p = 0.03$) and between spring and fall (Tukey, $p < 0.01$). A Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in gill CYP1A activity between species and seasons in the outer Oslofjord. There was a significant effect of season ($F(1,43) = 24.29$, $p < 0.01$) on group means of Atlantic cod, whiting and haddock in the spring and fall in the outer Oslofjord. There was no significant interaction effect ($F(2,43) = 1.53$, $p = 0.23$) or effect of species ($F(2,43) = 1.17$, $p = 0.32$) on group means. There was a significant difference in gill CYP1A activity between spring and fall (Tukey, $p < 0.01$). A Levene's test was performed, and the assumption was met.

3.4.3 Heart CYP1A activity

Heart CYP1A activity (nmol/min/mg protein) was determined in samples of Atlantic cod, whiting, Norway pout, and haddock (Figure 18).

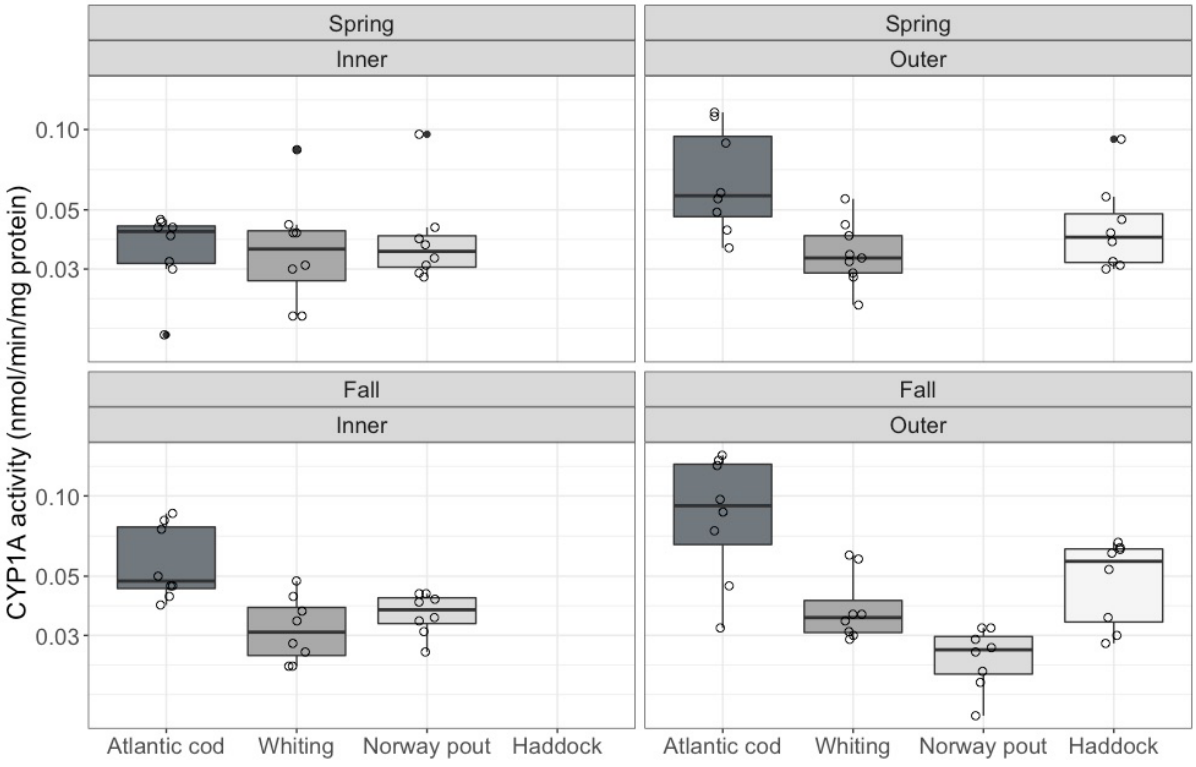

*Figure 18. Heart CYP1A activity in Atlantic cod, whiting, Norway pout, and haddock. The colours of the boxes indicate the different species. The thick black line in each box depicts the median value. The boxes represent the first and third quartile, the whiskers extend from the box to the smallest and largest value < 1.5 * IQR from the boxes. Outliers and are plotted individually as filled black dots. Black circles represent the datapoints.*

A two-way ANOVA was performed to test for differences in heart CYP1A activity between species and locations in spring. There was a significant interaction effect ($F(1,29) = 5.28$, $p = 0.03$) on group means of Atlantic cod and whiting in the inner and outer Oslofjord in spring. There was a significant difference in heart CYP1A activity between Atlantic cod in the inner and outer Oslofjord (Tukey, $p = 0.02$), and between whiting and Atlantic cod in the outer Oslofjord (Tukey, $p = 0.01$). A Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in heart CYP1A activity between locations and species in fall. There was a significant interaction effect ($F(2,42) = 4.05$, $p < 0.01$) on group means of Atlantic cod, whiting and Norway pout in the inner and outer Oslofjord in. There was a significant difference in heart CYP1A activity between whiting and Atlantic cod in the inner Oslofjord (Tukey, $p = 0.02$), between Atlantic cod and Norway pout in the outer Oslofjord (Tukey, $p < 0.01$), and between whiting and Atlantic cod in the outer Oslofjord (Tukey, $p < 0.01$). A Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in heart CYP1A activity between species and seasons in the inner Oslofjord. There was a significant interaction effect ($F(2,42) = 3.30$, $p = 0.05$) on group means of Atlantic cod, whiting and Norway pout in the spring and fall in the inner Oslofjord. There was a statistically significant difference in heart CYP1A activity between Atlantic cod and whiting in fall (Tukey, $p = 0.02$). A Levene's test was performed, and the assumption was met.

A two-way ANOVA was performed to test for differences in heart CYP1A activity between species and seasons in the outer Oslofjord. There was a significant effect of species ($F(2,43) = 14.01$, $p < 0.01$) on group means of Atlantic cod, whiting and haddock in the spring and fall in the outer Oslofjord. There was no significant interaction effect ($F(2,43) = 0.24$, $p = 0.79$) or effect of season ($F(1,43) = 2.33$, $p = 0.13$) on group means. There was a significant difference in heart CYP1A activity between haddock and Atlantic cod (Tukey, $p < 0.01$) and between whiting and Atlantic cod (Tukey, $p = 0.01$). A Levene's test was performed, and the assumption was met.

3.4.4 Tissue differences in Atlantic cod

A one-way ANOVA was performed to test for tissue differences in CYP1A activity in Atlantic cod in the inner Oslofjord in spring. The test did not indicate a significant effect of tissues on group means ($F(2,21) = 2.73$, $p = 0.09$). However, liver has a wider distribution than the other tissues. Levene's test was performed, and the assumption was met.

A one-way ANOVA was performed to test for tissue differences in CYP1A activity in Atlantic cod in the inner Oslofjord in fall. There was a statistically significant effect of tissues on group means ($F(2,21) = 213.1$, $p < 0.01$). There was a statistically significant difference in CYP1A activity between liver and gills (Tukey, $p < 0.01$) and between liver and heart (Tukey, $p < 0.01$). Levene's test was performed, and the assumption was met.

A Levene's test was performed, and the assumption was not met. Therefore, the non-parametric Kruskal-Wallis test was performed to compare CYP1A activity between tissues in Atlantic cod in the outer Oslofjord in spring. The test did not indicate a statistically significant effect of tissues on CYP1A activity ($H(2) = 4.3438$, $p = 0.1$). Liver has a wider distribution than the other tissues.

A one-way ANOVA was performed to test for tissue differences in CYP1A activity in Atlantic cod in the outer Oslofjord in fall. There was a statistically significant effect of the tissue on group means ($F(2,21) = 126.4$ $p < 0.01$). There was a statistically significant difference in CYP1A activity between liver and gills (Tukey, $p < 0.01$) and between liver and heart (Tukey, $p < 0.01$). Levene's test was performed, and the assumption was met.

3.4.5 Tissue differences in whiting

A one-way ANOVA was performed to test for tissue differences in CYP1A activity in whiting in the inner Oslofjord in spring. There was a statistically significant effect of the tissue on group means ($F(2,21) = 6.76$, $p < 0.01$). There was a statistically significant difference in CYP1A activity between heart and gills (Tukey, $p = 0.01$) and between liver and heart (Tukey, $p = 0.02$). A Levene's test was performed, and the assumption was met.

Normality checks and Levene's tests were performed, and none of the assumptions were met. Therefore, the non-parametric Kruskal-Wallis test was performed to compare CYP1A activity between tissues in whiting in the inner Oslofjord in fall. The test revealed that there was a statistically significant effect of tissues on CYP1A activity ($H(2) = 17.38$, $p < 0.01$). A Wilcoxon signed rank pairwise test was carried out, with p-values adjusted using the Bonferroni correction. There was a statistically significant difference in CYP1A activity between liver and heart ($p < 0.01$) and between liver and gills ($p < 0.01$). Liver has a wider distribution than the other tissues.

A Levene's test was performed, and the assumption was not met. Therefore, the non-parametric Kruskal-Wallis test was performed to compare CYP1A activity between tissues in whiting in the outer Oslofjord in spring. The test revealed that there was a statistically significant effect of tissues on CYP1A activity ($H(2) = 16.97$, $p < 0.01$). A Wilcoxon signed rank pairwise test was carried out, with p-values adjusted using the Bonferroni correction. There was a statistically significant difference in CYP1A activity between gills and heart ($p < 0.01$), and between liver and heart ($p < 0.01$). Liver has a wider distribution than the other tissues.

A Levene's test was performed, and the assumption was not met. Therefore, the non-parametric Kruskal-Wallis test was performed to compare CYP1A activity between tissues in whiting in the outer Oslofjord in fall. The test revealed that there was a statistically significant

effect of tissues on CYP1A activity ($H(2) = 16.29$, p-value < 0.01). A Wilcoxon signed rank pairwise test was carried out, with p-values adjusted using the Bonferroni correction. There was a statistically significant difference in CYP1A activity between liver and gills ($p < 0.01$), and between liver and heart ($p < 0.01$). Liver has a wider distribution than the other tissues.

3.4.5 Tissue differences in Norway pout

A one-way ANOVA was performed to test for tissue differences in CYP1A activity in Norway pout in the inner Oslofjord in spring. There was a statistically significant effect of the tissue on group means ($F(2,21) = 9.43$, $p < 0.01$). There was a statistically significant difference in CYP1A activity between heart and gills (Tukey, $p < 0.01$) and between liver and heart (Tukey, $p = 0.03$). A Levene's test was performed, and the assumption was met.

A Levene's tests were performed, and the assumption was not met. Therefore, the non-parametric Kruskal-Wallis test was performed to compare CYP1A activity in tissues in Norway pout in the inner Oslofjord in fall. The test revealed that there was a statistically significant effect of the tissue on CYP1A activity ($H(2) = 19.29$, p-value < 0.01). A Wilcoxon signed rank pairwise test was carried out, with p-values adjusted using the Bonferroni correction. There was a statistically significant difference in CYP1A activity between liver and gills ($p < 0.01$), between liver and heart ($p < 0.01$), and between heart and gills ($p = 0.01$). Liver has a wider distribution than the other tissues.

A Levene's tests were performed, and the assumption was not met. Therefore, the non-parametric Kruskal-Wallis test was performed to compare CYP1A activity between tissues in Norway pout in the outer Oslofjord in fall. The test revealed that there was a statistically significant effect of tissues on CYP1A activity $H(2) = 20.50$, $p < 0.01$). A Wilcoxon signed rank pairwise test was carried out, with p-values adjusted using the Bonferroni correction. There was a statistically significant difference in CYP1A activity between liver and gills ($p < 0.01$), between liver and heart ($p < 0.01$), and between heart and gills ($p < 0.01$). Liver has a wider distribution than the other tissues.

3.4.5 Tissue differences in haddock

A Levene's test was performed, and the assumption was not met. Therefore, the non-parametric Kruskal-Wallis test was performed to compare CYP1A activity between tissues in

haddock in the outer Oslofjord in spring. The test revealed that there was a statistically significant effect of tissues on CYP1A activity ($H(2) = 15.93$, $p < 0.01$). A Wilcoxon signed rank pairwise test was carried out for the group, with p-values adjusted using the Bonferroni correction. There was a statistically significant difference in CYP1A activity between liver and gills ($p < 0.01$), and between liver and heart ($p < 0.01$). Liver has a wider distribution than the other tissues.

A one-way ANOVA was performed to test for tissue differences in CYP1A activity in haddock in the outer Oslofjord in spring. There was a statistically significant effect of the tissue on group means ($F(2,21) = 183.3$, $p < 0.01$). There was a statistically significant difference in CYP1A activity between liver and gills (Tukey, $p < 0.01$), and between liver and heart (Tukey, $p < 0.01$). Levene's test was performed, and the assumption was met.

3.5 Correlations between exposure and CYP1A activity

3.5.1 Exposure to PAH and CYP1A activity

For Atlantic cod, whiting and haddock, Spearman's rank correlations were computed to assess the relationship between each of the PAH metabolites and CYP1A activity in each of the three tissues. Juvenile and female fish were tested separately, see Table 5 to Table 8. There was no correlation between 2-and 3-ring PAH metabolites and CYP1A activity in any of the tissues for all species. There was negative correlation between concentration of pyrene metabolites in bile and heart CYP1A activity in juvenile Atlantic cod ($r(27) = -0.48$, $p = 0.01$), and juvenile whiting ($r(14) = -0.46$, $p = 0.01$). There was a positive correlation between concentration of pyrene metabolites in bile and gill CYP1A activity in juvenile haddock ($r(13) = 0.75$, $p = 0.05$).

3.5.2 Exposure to Hg and CYP1A activity

Spearman's rank correlation was computed to assess the relationship between mercury concentration in liver and CYP1A activity in each of the three tissues. Due to expected differences between juvenile and mature female fish, correlation was tested separately for the two groups. There was a positive correlation between mercury concentration and CYP1A activity in heart for juvenile ($r(14) = 0.54$, $p = 0.02$) and female Norway pout ($r(6) = 0.79$, $p = 0.02$). But no correlation in other species or in other tissues, see Table 5 to Table 8.

Table 5. Spearman's rank correlation between determined concentrations of PAH metabolites, mercury, and CYP1A activity in juvenile Atlantic cod. Significance level p< 0.05; n.s., not significant. P-values corrected using Bonferroni correction for multiple correlation.

Correlation parameters	Atlantic cod	
	Juvenile (n = 29)	
	Spearman's rank correlation coefficient	p-value
2-and 3-ring PAH metabolites vs. hepatic CYP1A activity	0.19	n.s.
2-and 3-ring PAH metabolites vs. gill CYP1A activity	-0.035	n.s.
2-and 3-ring PAH metabolites vs. heart CYP1A activity	-0.25	n.s.
Pyrene metabolites vs. hepatic CYP1A activity	-0.0016	n.s.
Pyrene metabolites vs. gill CYP1A activity	-0.14	n.s.
Pyrene metabolites vs. heart CYP1A activity	-0.48	**0.01**
3-OH-benzo[a]pyrene vs. hepatic CYP1A activity	0.11	n.s.
3-OH-benzo[a]pyrene vs. gill CYP1A activity	-0.33	n.s.
3-OH-benzo[a]pyrene vs. heart CYP1A activity	-0.18	n.s.
Hg concentration vs. hepatic CYP1A activity	0.19	n.s.
Hg concentration vs. gill CYP1A activity	0.16	n.s.
Hg concentration vs. heart CYP1A activity	-0.029	n.s.

Table 6. Spearman's rank correlation between determined concentrations of PAH metabolites, mercury, and CYP1A activity in juvenile and female whiting. Significance level p< 0.05; n.s., not significant. P-values corrected using Bonferroni correction for multiple correlation.

Correlation parameters	Whiting			
	Juvenile (n = 16)		Female (n = 15)	
	Spearman's rank correlation coefficient	p-value	Spearman's rank correlation coefficient	p-value
2-and 3-ring PAH metabolites vs. hepatic CYP1A activity	-0.067	n.s.	0.055	n.s.
2-and 3-ring PAH metabolites vs. gill CYP1A activity	-0.0061	n.s.	0.53	n.s.
2-and 3-ring PAH metabolites vs. heart CYP1A activity	-0.21	n.s.	-0.050	n.s.
Pyrene metabolites vs. hepatic CYP1A activity	-0.091	n.s.	-0.17	n.s.
Pyrene metabolites vs. gill CYP1A activity	0.23	n.s.	0.48	n.s.
Pyrene metabolites vs. heart CYP1A activity	-0.46	**0.01**	-0.15	n.s.
3-OH-benzo[a]pyrene vs. hepatic CYP1A activity	-0.27	n.s.	-0.51	n.s.
3-OH-benzo[a]pyrene vs. gill CYP1A activity	-0.11	n.s.	0.14	n.s.
3-OH-benzo[a]pyrene vs. heart CYP1A activity	-0.58	n.s.	0.29	n.s.
Hg concentration vs. hepatic CYP1A activity	0.25	n.s.	0.22	n.s.
Hg concentration vs. gill CYP1A activity	0.14	n.s.	-0.42	n.s.
Hg concentration vs. heart CYP1A activity	-0.36	n.s.	-0.079	n.s.

Table 7. Spearman's rank correlation between determined concentrations of PAH metabolites, mercury, and CYP1A activity in juvenile haddock. Significance level p< 0.05; n.s., not significant. P-values corrected using Bonferroni correction for multiple correlation.

Correlation parameters	Haddock	
	Juvenile (n = 15)	
	Spearman's rank correlation coefficient	p-value
2-and 3-ring PAH metabolites vs. hepatic CYP1A activity	0.39	n.s.
2-and 3-ring PAH metabolites vs. gill CYP1A activity	-0.54	n.s.
2-and 3-ring PAH metabolites vs. heart CYP1A activity	-0.29	n.s.
Pyrene metabolites vs. hepatic CYP1A activity	-0.39	n.s.
Pyrene metabolites vs. gill CYP1A activity	0.75	**0.05**
Pyrene metabolites vs. heart CYP1A activity	0.090	n.s.
3-OH-benzo[a]pyrene vs. hepatic CYP1A activity	-0.21	n.s.
3-OH-benzo[a]pyrene vs. gill CYP1A activity	0.21	n.s.
3-OH-benzo[a]pyrene vs. heart CYP1A activity	-0.59	n.s.
Hg concentration vs. hepatic CYP1A activity	-0.20	n.s.
Hg concentration vs. gill CYP1A activity	-0.24	n.s.
Hg concentration vs. heart CYP1A activity	-0.23	n.s.

Table 8. Spearman's rank correlation between determined concentrations of PAH metabolites, mercury, and CYP1A activity in juvenile and female Norway pout. Significance level p< 0.05; n.s., not significant. P-values corrected using Bonferroni correction for multiple correlation.

Correlation parameters	Norway pout			
	Juvenile (n = 16)		Female (n = 8)	
	Spearman's rank correlation coefficient	p-value	Spearman's rank correlation coefficient	p-value
Hg concentration vs. hepatic CYP1A activity	-0.091	n.s.	-0.55	n.s.
Hg concentration vs. gill CYP1A activity	-0.33	n.s.	-0.43	n.s.
Hg concentration vs. heart CYP1A activity	0.54	**0.02**	0.79	**0.02**

4 Discussion

4.1 GSI

Few mature Atlantic cod were collected in the spring. Whiting had the same distribution of mature male and female fish in the inner and outer Oslofjord in the spring. Only mature female Norway pout were collected in the inner Oslofjord in spring. For haddock, only one mature female fish was collected in the spring. Only juvenile fish were collected in the fall.

An explanation for the collection of few mature cod in the spring is that the population in the inner Oslofjord, previously believed to be stationary, may now be dominated by North Sea cod, which is less stationary. Mature individuals could therefore have migrated to spawning grounds in the Skagerrak in the spring. As haddock has a wider spawning season lasting until May/June, the lack of mature fish in the spring could be due to the early sampling time and the fish could still be maturing or due to larger spawning related migrations out of the fjord.

Norway pout was not sampled in the outer Oslofjord in the spring due to few individuals being collected. This could be due to Norway pout not spawning in the outer Oslofjord, and that mature individuals are migrating to a different location in the sampling period. Another explanation is that Norway pout form dense schools and that no schools of Norway pout were encountered during sampling.

4.2 PAH exposure

4.2.1 2- and 3-ring PAH metabolites

The concentration of 2- and 3-ring PAH metabolites in bile was significantly higher in the inner than in the outer Oslofjord in both seasons. This indicates that the fish has been exposed to more 2-and 3-ring contaminants in the inner Oslofjord. As 2- and 3-ring PAHs are of petrogenic origin, the higher concentrations in the inner Oslofjord may be explained by more boat traffic in the inner than in the outer Oslofjord, and therefore more potential spills of oil, as well as accidental oil spills from the urban area. These results are also in accordance with previous monitoring of PAH metabolites in Atlantic cod (Lundsør et al. 2018), which reported higher concentrations of 2- and 3-ring PAH metabolites in the inner Oslofjord than in the outer Oslofjord in fall.

The concentrations of 2- and 3-ring PAH metabolites in bile had few species differences, except for a significant difference between haddock and Atlantic cod and haddock and whiting in the outer Oslofjord in fall, where haddock had significantly higher concentrations in bile than the two other species. This indicates that haddock in the outer Oslofjord in fall was exposed to higher levels of 2-and 3-ring PAHs than Atlantic cod and whiting.

There were significantly higher concentrations in fall than in the spring in both locations. As 2- and 3-ring PAHs are predominantly petrogenic, the main source is oil. The higher concentrations of metabolites found in bile in fall could possibly be explained by the increased boat traffic in the summer and early fall and therefore an increased possibility of accidental spills of oil from the boats. The higher concentrations of 2- and 3-ring PAH metabolites in the inner Oslofjord can also be due to more boat traffic in the inner Oslofjord than the outer, leading to more exhaust, as well as oil spills from the harbour. Monitoring studies have also found that the highest concentrations of PAH metabolites in bile in both the inner and outer Oslofjord were concentrations of 2- and 3-ring PAH metabolites (Lundsør et al. 2016, Lundsør et al. 2018), which indicates that the Oslofjord is to a larger degree exposed to petrogenic PAHs. This was also found in this study.

4.2.2 Pyrene metabolites

The concentrations of pyrene metabolites in bile were higher in the inner than in the outer Oslofjord in both spring and fall. Pyrene is a pyrogenic PAH, and releases to the marine environment is associated with combustion products, runoff from land and sewage. Therefore, the higher concentrations of pyrene metabolites in bile in the inner Oslofjord could be explained by the increased urban pollution and runoff from land and the increased boat traffic in the inner Oslofjord. This is in accordance with previous monitoring studies that also found higher concentrations of 4-ringed PAH metabolites in the inner Oslofjord (Magnusson et al. 2004, Lundsør et al. 2018). However, the exposure to pyrene, and 4-ring PAHs in general, seems to vary, as some years showed no difference between the inner and outer Oslofjord in concentration of these metabolites in bile (Lundsør et al. 2016)

There were no species differences in any locations or seasons, which indicates that the species were recently exposed to similar concentrations of pyrene.

The concentration of pyrene metabolites in bile was only higher in spring than in fall in the outer Oslofjord, and there was no seasonal variance in the inner Oslofjord. This could indicate that the input of pyrene to the inner Oslofjord is stable through the year, while the outer Oslofjord is more seasonally dependent. This could be due to a larger variation in level of exposure. However, all determined concentrations of pyrene metabolites in this study were in accordance with results from flounder (*Platichthys flesus*) sampled in the southern North Sea by Eggens et al. (1996) and flounder (*Platichthys flesus*) sampled in coastal areas of the Baltic sea in Kammann (2007), indicating that the exposure to pyrene in the Oslofjord is similar to exposure in other coastal areas.

4.2.3 3-OH-benzo[a]pyrene

There was a difference in concentration of 3-OH-benzo[a]pyrene in bile between the inner and outer Oslofjord in the fall, with a higher concentration in the inner Oslofjord. There was no indication of a difference between locations in spring. This differs from previous monitoring of bile samples in Atlantic cod, which found no difference in concentration of 5- and 6- ringed PAH metabolites between the inner and outer Oslofjord in fall (Lundsør et al. 2016, Lundsør et al. 2018). As with pyrene, benzo[a]pyrene is a pyrogenic PAH, and releases to the marine environment are associated with combustion products, runoff from land and sewage. Therefore, the difference in concentrations of 3-OH-benzo[a]pyrene between the inner and outer Oslofjord in fall could indicate increased exposure to benzo[a]pyrene in the inner Oslofjord due to urban pollution and runoff from land as well as the increased boat traffic in summer and early fall in the inner Oslofjord. As benzo[a]pyrene is a larger PAH, it is less water soluble than smaller PAH and can be accumulated in the sediments or bound to particulate matter and may persist in the environment and be available to the biota longer than water-soluble PAHs (Abdel-Shafy and Mansour 2016).

Whiting had a higher concentration of 3-OH-benzo[a]pyrene in bile than Atlantic cod in the inner Oslofjord when seasons were combined. This could be due to sampling of mature individuals of whiting, who may have a different diet than juvenile Atlantic cod.

There was no indication of seasonal differences in either location. This indicates that the exposure to benzo[a]pyrene is similar in the two seasons. There were also lower concentrations of 3-OH-benzo[a]pyrene than 2-and 3-ring PAHs, which is in accordance with

results from monitoring of the inner Oslofjord (Lundsør et al. 2018) and studies comparing polluted and unpolluted areas in western Norway (Aas et al. 2001).

4.3 Mercury exposure

There was a location difference in mercury concentration in Norway pout in the inner and outer Oslofjord in fall. Previous research has found that there is a positive relationship between mercury concentration and length of fish (Jackson 1991, Evans et al. 1993, Piraino and Taylor 2009). As mercury is accumulated over time, a difference in hepatic mercury concentration could be due to sampling of older and longer fish. The difference could be explained by 4.5 cm difference in mean length of Norway pout between the locations. There were no other location differences in mercury. Mercury concentrations in the sediments in the inner Oslofjord has been found to be increasing in the later years due to new inputs from land or sewage (Størdal 2020) as well as a long-term increasing trend of mercury accumulation in Atlantic cod (Schøyen et al. 2021). The lack of difference between the two locations may be explained by less stationary populations and the exposure therefore not being specific to each location or increasing contamination in both locations.

There were species differences in both locations and during both seasons. Atlantic cod had higher concentrations than whiting, Norway pout, and haddock in both locations and both seasons. These species differences could be due to differences in feeding preference or age (Chouvelon et al. 2014), as e.g. Norway pout has a shorter lifespan than the other species, as well as a difference in diet, and may not accumulate mercury to the same concentrations as the other species. Haddock feeds on bottom-dwelling animals and may to a larger extent be exposed to mercury bound to sediments. However, as the diet is usually at a lower trophic level than the diet of Atlantic cod and whiting, this could explain the lower hepatic mercury concentrations in haddock (Hammerschmidt and Fitzgerald 2006).

There was a seasonal difference in hepatic mercury concentration in the inner Oslofjord, and a seasonal difference in hepatic mercury concentration in whiting in the outer Oslofjord. The difference in hepatic mercury concentration could be due to length differences (Sackett et al. 2013). The Atlantic cod sampled in spring and fall in the inner Oslofjord had similar mean lengths, this was also the case for whiting and Norway pout sampled in the inner Oslofjord. Length differences can therefore not explain the differences in mercury concentrations in

these species. However, whiting sampled in the outer Oslofjord had a 1.9 cm difference in mean length between spring and fall which could explain the apparent seasonal difference in mercury concentration for whiting in the outer Oslofjord. Mercury concentration is also dependent on other factors, such as fat loss after spawning (Riisgård and Famme 1988), which could explain the higher observed mercury concentrations in mature female whiting and Norway pout in spring prior to spawning.

4.4 CYP1A activity: species, season, and location

4.4.1 Liver

There was a significant location difference in hepatic CYP1A activity in spring when Atlantic cod and whiting were combined, with higher activity in the outer Oslofjord. Also, inducibility of the CYP1A monooxygenase system has been showed to be impaired after prolonged exposure to contaminants (Celander and Förlin 1995). As the concentration of 2- and 3-ring PAH metabolites and pyrene metabolites are higher in the inner Oslofjord in spring, impairment of inducibility in the inner Oslofjord may be a cause for the higher CYP1A activity in the outer Oslofjord, the reference area, in spring. There was no difference between the locations in fall when Atlantic cod, whiting and Norway pout were combined. All determined concentrations of PAH metabolites were higher in the inner than in the outer Oslofjord in fall. Earlier studies have found higher hepatic CYP1A in the inner than in the outer Oslofjord in fall (Magnusson et al. 2004), although no difference between locations have been found in monitoring projects from more recent years (Lundsør et al. 2018).

There were no indications of species differences between both locations and seasons, though some species had wide distributions of hepatic CYP1A activity. Previous studies have found EROD activity in Atlantic cod from reference areas to be at approximately 60 pmol/min/mg protein (Goksøyr et al. 1994), while other sources define the baseline hepatic CYP1A activity in Atlantic cod at 145 pmol/min/mg protein and baseline hepatic activity in haddock between 215 and 421 pmol/min/mg protein in microsome subfractions (Hylland et al. 2012). Using these values as measures of baseline activity, the lack of species differences may imply that Atlantic cod and haddock are affected differently by exposure to contaminants, as exposure to PAHs was similar.

There was a significant seasonal difference in hepatic CYP1A activity in the inner Oslofjord, with higher hepatic CYP1A activity in fall when activity in Atlantic cod, whiting and Norway pout were combined. There was also a significant seasonal difference in hepatic CYP1A activity for Atlantic cod and whiting in the outer Oslofjord, with higher activity in fall. When comparing hepatic CYP1A activity determined in this study to baseline hepatic CYP1A activity reported by Hylland et al. (2012), the CYP1A activity determined in Atlantic cod in this study was elevated in all locations except the inner Oslofjord in spring. Hepatic CYP1A activity in Atlantic cod collected in the outer Oslofjord in spring had a wide distribution and not all samples had higher activity than reported baseline, however the mean value was still higher than baseline values. Haddock has a larger variation in baseline hepatic CYP1A activity than Atlantic cod but mean hepatic CYP1A activity in the outer Oslofjord in spring and fall both exceed the reported baseline activity. However, the distribution of the activity in the samples collected in spring are wide and some samples have lower activity than the upper limit of the baseline.

Atlantic cod is known to have a low seasonal variation in baseline hepatic CYP1A activity (Nahrgang et al. 2013), and seasonal variation can therefore be assumed to be based on differences in induction or inhibition of the enzyme. However, the activity in fall was almost one order of magnitude higher than in spring in all species except haddock. Such high levels of hepatic CYP1A activity in both the inner and outer Oslofjord has not been observed (Lundsør et al. 2016, Lundsør et al. 2018, Schøyen et al. 2021). The levels in both the inner and outer Oslofjord were unexpectedly high in the fall and this could perhaps indicate a novel source of exposure that has not been identified that can induce hepatic CYP1A activity.

There was no indication of a seasonal difference in hepatic CYP1A activity in haddock. The higher hepatic CYP1A activity in haddock could therefore be explained by the higher baseline activity as well as induction by PAHs which were quantified in the bile. Due to the higher baseline activity, hepatic CYP1A activity in haddock does not appear to be elevated to the same extent as the other species in the outer Oslofjord in fall. As there were no species differences in hepatic CYP1A activity, this may indicate that haddock responds differently to exposure than Atlantic cod.

Fish in both locations and seasons were exposed to PAHs in different concentrations, but only 2- and 3-ring PAH metabolites were found in higher concentrations in fall than in spring in

the inner Oslofjord and pyrene metabolites had higher concentrations in spring than fall in the outer Oslofjord. While elevated hepatic CYP1A activity is used as an indicator of exposure to PAHs, the pattern of hepatic CYP1A induction does not match the pattern observed in the analysis of PAH metabolites in bile. There is a possibility that the higher concentrations of mercury and subsequent inhibition of hepatic CYP1A activity could explain the lower hepatic CYP1A activity in spring and the discrepancy between determined concentrations of PAH metabolites in bile and hepatic CYP1A activity (Eggens et al. 1992).

Another important factor when considering differences in hepatic CYP1A activity is the maturity. Mature female fish have been shown to have lower hepatic CYP1A activity than mature males, spent females and juvenile individuals (Elskus et al. 1992, Eggens et al. 1995). There is a difference in maturity between seasons. In whiting and Norway pout, only mature female fish were sampled in the spring and juvenile fish in fall. For Atlantic cod and haddock, few mature female individuals were sampled in spring. When comparing hepatic CYP1A activity between species, this difference must be considered. However, regardless of maturity status, the hepatic CYP1A activity was higher in fall than in spring for Atlantic cod, whiting and Norway pout. A combination of both mercury exposure and maturity could explain the lower hepatic CYP1A activity in the spring, as maturity (GSI) alone showed no correlation with hepatic CYP1A activity in any of the species. For haddock, the lowest determined value of hepatic CYP1A activity in the outer Oslofjord in spring is from the only mature female fish sampled. This is not considered an outlier, but it is notable that all the juvenile haddock samples had a higher hepatic CYP1A activity than the mature female. Additionally, there are other contaminants present in both the inner and outer Oslofjord that have not been examined in this study. The cumulative effect of the total exposure to inducers and inhibitors may explain the pattern observed in hepatic CYP1A activity.

4.4.2 Gills

There was not a difference in gill CYP1A activity between the locations in spring or fall. Other studies have found increased gill CYP1A activity between polluted areas and reference areas in both marine and freshwater fish (Jönsson et al. 2003, Abrahamson et al. 2007), though the activity was expressed as pmol resorufin/filament/min. As this study has determined gill CYP1A activity in nmol/min/mg protein, it is not possible to compare results from these studies with the present study. The use of the same amount of tissue, not the same number of filaments per sample, could have affected the amount of CYP1A available in each

sample in a way that does not reflect a possible induction, but rather that different amounts of gill filaments have been analysed. The presence of PAH metabolites in bile in all locations indicate exposure to PAHs. Gills have been found to be sensitive to water-borne inducers (Abrahamson et al. 2007). Activity in gills could therefore provide a good understanding of water-borne inducers, whereas internal organs are exposed to bioaccumulated and lipid soluble contaminants.

There were some species differences in CYP1A activity. Norway pout had higher gill CYP1A activity than whiting in the inner and outer Oslofjord in fall, and higher than Atlantic cod in fall when both locations were combined. This could reflect a difference in susceptibility to inducers between the species (Jönsson et al. 2009).

There were seasonal differences in gill CYP1A activity in both the inner and outer Oslofjord, with higher activity in spring than in fall. As gills are in direct contact with the water, this seasonal difference could be due to a larger exposure to water-borne PAHs (Jönsson et al. 2009) in the spring than in fall and a subsequent induction. As some of the PAH-metabolites showed a similar pattern of higher concentrations in spring, this may explain the seasonal difference in gill CYP1A activity.

4.4.3 Heart

There was a location difference in heart CYP1A activity between the inner and outer Oslofjord in Atlantic cod pout in spring. The heart CYP1A activity was higher in the outer Oslofjord. Otherwise, there were no location differences in either season. Other studies on Atlantic cod (Husoy et al. 1994, Husøy et al. 1996) have found that the heart is sensitive to induction of CYP1A by environmental contaminants and that activity in polluted areas were elevated compared to reference areas. The higher activity in the outer Oslofjord may therefore be due to the presence of inhibitors in the inner Oslofjord. Other studies (Stegeman et al. 1991, Yawetz et al. 1998) have found that some species show induced concentrations of CYP1A enzymes while still maintaining a low catalytic activity. The activity of CYP1A in the heart may therefore not always reflect the effect of exposure.

There were some species differences in heart CYP1A activity. Atlantic cod had higher heart CYP1A activity than whiting in the outer Oslofjord in spring, and in the inner and outer Oslofjord in fall. Atlantic cod also had higher heart CYP1A activity than Norway pout in the

outer Oslofjord in fall. These species differences in heart CYP1A induction may be due to differences in diet or feeding behaviour (Husøy et al. 1996)

There were no seasonal differences in the inner or outer Oslofjord. This could imply that there is a low seasonal variability in heart CYP1A activity. Previous studies have found that while the concentrations of cytochrome 450 monooxygenase proteins in the heart can be high, the catalytic activity remains relatively low compared to other tissues (Stegeman et al. 1982, Stegeman et al. 1989). This may imply that protein synthesis is induced while the catalytic activity is inhibited (Husøy et al. 1996).

4.5 CYP1A activity: tissue differences

The liver is considered the tissue that contributes most to CYP1A-catalysed metabolism, due to the relatively high CYP1A activity compared to other tissues (Lech and Bend 1980, Binder et al. 1984), however studies have found that other tissues contribute to the biotransformation of CYP1A specific substrates in a considerable degree due to differences in exposure routes and sensitivity to specific contaminants (Van Veld et al. 1997, Levine and Oris 1999). In this study CYP1A activity in the three tissues liver, gills and heart was compared. In fall, liver had significantly different CYP1A activity than both heart and gills in both locations for all species. In the outer Oslofjord in spring, hepatic CYP1A activity was higher than both gill and heart CYP1A activity in haddock, and higher than heart CYP1A activity in whiting. In the inner Oslofjord in spring, hepatic CYP1A activity was higher than heart CYP1A activity in whiting and Norway pout. Gill CYP1A activity was higher than heart CYP1A activity in the outer Oslofjord in fall in Atlantic cod and Norway pout, in the inner and outer Oslofjord in spring in whiting and in the inner Oslofjord in spring in Norway pout.
The lack of difference between hepatic and gill CYP1A activity in the inner and outer Oslofjord in spring for most species could be due to inhibition of hepatic CYP1A.
Compared to liver and gills, heart CYP1A activity indicated little location or seasonal differences that were observed in the other tissues. This could indicate species specific levels of activity, and low induction of catalytic activity. However, the CYP1A activity in heart has been shown to be inducible when exposed to agonists (Schlezinger and Stegeman 2000).

Gills are in direct contact with water-borne contaminants and gill CYP1A activity could therefore be a better measure of exposure to water-soluble PAHs than hepatic CYP1A activity

as the liver is also exposed to bioaccumulated and lipid-soluble contaminants. However, more location and seasonal differences were observed in hepatic CYP1A activity. This could be because the liver is also exposed to bioaccumulating compounds. The two measures of effect of exposure may therefore provide insight to the effect of compounds with different routes of exposure as well as different loads of exposure.

Atlantic cod is known to have low variability in baseline hepatic CYP1A activity and has been an important species in monitoring, while haddock has a higher and more variable hepatic CYP1A baseline activity. Whiting had similar responses to Atlantic cod in CYP1A activity in both liver and gills in both locations during both seasons. CYP1A activity in the tissues of Norway pout and haddock were to a larger degree different to Atlantic cod than whiting. This could be due to Norway pout having a shorter lifespan and therefore the sampled fish were younger than fish of the other species. Differences could also be due to differences in baseline CYP1A activity, as indicated by haddock. Knowledge about baseline levels and differences between baseline CYP1A activity and induced CYP1A activity is important and can vary among species and within species. This is important to be able to compare and draw conclusions from field data.

4.6 Correlations

4.6.1 Correlation between CYP1A activity and PAHs

There was a negative correlation between pyrene metabolite concentrations in bile and heart CYP1A activity in juvenile Atlantic cod and whiting. This discrepancy between estimates of exposure and effect can be explained by inhibition by heavy metals or reproductive hormones that can cause a lower hepatic activity than expected. Studies have found correlation between exposure to low-molecular weight PAHs (such as phenanthrene and naphthalene) and hepatic CYP1A activity in salmon (Stagg et al. 2000), 2-and 3- ring PAHs and hepatic CYP1A activity in Atlantic cod (Aas et al. 2000), an increase in CYP1A activity with increasing concentrations of PAHs in laboratory experiments (Oris and Roberts 2007) and a positive correlation between concentration of benzo[a]pyrene and gill CYP1A activity in zebrafish and rainbow trout (Jönsson et al. 2009).

There was a positive correlation between pyrene metabolite concentrations in bile and gill CYP1A activity in juvenile haddock. The gills are directly exposed to contaminants dissolved

in the water. The positive correlation may also indicate exposure to other water-borne contaminants that are known to induce gill CYP1A activity, such as benzo[a]pyrene, as has been shown in Jönsson et al. (2009), though a correlation was not indicated in this study.

The bile in the gall bladder is emptied into the intestine as the fish feed, and recent feeding could have affected the content and volume of bile in the gall bladder (Collier and Varanasi 1991, Brumley et al. 1998, Aas 2000). The time the fish were sampled during the day could have affected the bile contents and given less accurate estimates of recent exposure to PAHs. Still, PAH metabolites in bile is a valuable indicator of recent exposure, especially when paired with measures of effect such as CYP1A activity. Together they can give an indication of the levels of exposure that the fish are currently experiencing, as well as the effect of the cumulative exposure.

4.6.2 Correlation between CYP1A activity and mercury concentration

Hepatic mercury concentration showed a positive correlation to heart CYP1A activity in both female and juvenile Norway pout. The hepatic mercury concentration showed no correlation to hepatic or gill CYP1A activity in any of the species. This could indicate that inner organs such as the heart may be affected by bioaccumulated compounds. However, as the mercury concentrations are measured in the liver, the correlation does not indicate a direct relationship between mercury and heart CYP1A activity.

Other in vivo studies have also found no correlation between hepatic mercury concentration and hepatic CYP1A, while still observing a trend of inhibited hepatic CYP1A activity in areas with high mercury contamination (Guilherme et al. 2008). Due to the wide variety of inducers and inhibitors that fish are exposed to, the lack of correlation between exposure and measures of effect may not imply that there is no other relationship between mercury exposure and CYP1A activity, but rather that induction and inhibition is a complex process. This is important to be aware of when analysing results.

4.7 Differences in sensitivity between related species

Related species are often assumed to have a similar sensitivity to environmental pollution (van den Hurk et al. 2017). However, some studies have investigated this and found that fish family is not always the best indicator of predicted response or toxicity of contaminants in a

species (Tuulaikhuu et al. 2017) and some have found that fish in the same family may exhibit differences in sensitivity to specific environmental pollutants (Maldonado, Nowicki et al. 2019, Spurgeon, Lahive et al. 2020, Bai, Lian et al. 2021). As seen in this study, differences in baseline CYP1A activity may hide differences in sensitivity to pollution and differences in diet and age can be important for interpretation of the results. Species-specific knowledge is therefore important to be able to correctly interpret results, determine the effect of exposure, and to identify sensitive species.

4.8 Conclusions

The results of this study demonstrated that:

There were differences in exposure to PAHs between locations, species, and seasons.

- There were location differences in exposure for 2- and 3-ring PAHs and pyrene in spring, and 2- and 3-ring PAHs, pyrene, and benzo[a]pyrene in fall.
- There were species differences in exposure to 2- and 2-ring PAHs in the outer Oslofjord and benzo[a]pyrene in the inner Oslofjord.
- There were seasonal differences in exposure to 2- and 3-ring PAHs in the inner and outer Oslofjord, to pyrene in the outer Oslofjord.

There were differences in exposure to mercury between locations, species. and seasons.

- There were location differences in the fall.
- There were species differences in exposure in all seasons and locations.
- There were seasonal differences in the inner and outer Oslofjord.

There were some location, species, and seasonal differences in CYP1A activity in each of the three tissues.

- There were location differences in hepatic CYP1A activity in spring but not in fall. There were no species differences in hepatic CYP1A activity. There were seasonal differences in hepatic CYP1A activity in the inner and outer Oslofjord.
- There were no location differences in gill CYP1A activity. There were species differences in fall, and in the inner Oslofjord. There were seasonal differences in the inner and outer Oslofjord.

- There were location differences in heart CYP1A activity in the spring. There were species differences in both locations and seasons. There were no seasonal differences in the inner or outer Oslofjord.

There were differences in CYP1A activity in the three tissues for each of the four species.

- There were differences between the tissues in Atlantic cod in the inner and outer Oslofjord in fall but not in spring. There were differences in CYP1A activity between the tissues in whiting in the inner and outer Oslofjord in fall and in spring.
- There were differences in CYP1A activity between the tissues in Norway pout in the inner Oslofjord in spring and fall and in the outer Oslofjord in fall.
- There were differences in CYP1A activity between the tissues in haddock in the outer Oslofjord in spring and fall.

There were correlations between exposure to environmental pollution and CYP1A activity.

- There were negative correlations between the concentration of pyrene metabolites in bile and heart CYP1A activity in whiting and Norway pout.
- There was a positive correlation between the concentration of pyrene metabolites in bile and gill CYP1A activity in haddock.
- There was a negative correlation between hepatic mercury concentration and heart CYP1A activity in Norway pout.

4.9 Future perspectives

This study has shown the importance of combining estimates of exposure with estimates of effect to be able to understand the mechanisms present in the species. As the exposure is not always reflected in the effect, and vice-versa, the analyses provide important information about the toxicants present in the environment and available to the species, and some effects of the cumulative toxic burden on different species. This can give relevant information about important fish stocks in the Oslofjord and help us to determine how to best manage current and future issues.

Knowledge of the baseline CYP1A activity in different fish species is essential to explain similarities or differences in patterns of CYP1A activity. To further investigate species differences in sensitivity to environmental pollutants that induce or inhibit the CYP1A

monooxygenase system, good estimates of baseline CYP1A activity for all species are needed. As whiting is the dominating species in the inner Oslofjord, this species could be an important fish to monitor in the Oslofjord in the future, and its similarities in response to Atlantic cod calls for further research on the baseline levels found in this species. As the hepatic CYP1A concentrations could seem to be inhibited in mature female fish in the spring, sampling of fish is recommended to be performed in periods without reproduction. Gill CYP1A activity may be a good measure of effects of water-soluble CYP1A inducers during the whole year. There is a need for estimates of baseline activity in several species and tissues to be able to compare and determine if there are any species differences and to determine sensitivity to environmental pollution.

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List of chemicals

Chemical	Producer no.	Producer
Bovine serum albumin	A7030	Sigma-Aldrich
DC Protein Assay Reagent B	5000114	BioRad
DF Protein Assay Reagent A	5000113	BioRad
Di-potassium hydrogen phosphate anhydrous	1.05104	MERCK
Dimethyl sulfoxide	1.02952	MERCK
DL-Dithiothreitol	43819	Fluka Analytical
DOLT-5	45047	National Research Council Canada
DORM-4	42844	National Research Council Canada
Glycerol bidistilled	24388.364	VWR International LTD
Hydrochloric acid 35%	2024.323	VWR International LTD
Methanol	1.06009.2511	MERCK
Potassium chloride	1.04936	MERCK
Potassium dihydrogen phosphate	1.04873	MERCK
Resorufin ethyl ether	E3763	Sigma-Aldrich
Resorufin sodiou salt	R0012	Tokyo Chemical Industry CO., LTD
Sodium dihydrogen phosphate monohydrate	1.06346	MERCK
Sodium phosphate dibasic	S5136	Sigma-Aldrich
Trizma® base	T1503	Sigma-Aldrich
Trizma® hydrochloride	T3253	Sigma-Aldrich
β-NADPH	481973	EMD Millipore corp., USA

Appendix A

Table 9. Field data showing ID, species, season, location, sex (manually determined during dissection), total weight, length, weight of liver and gonads, weight without gut, GSI and maturity (determined by GSI).

ID	Species	Season	Location	Sex	Total weight (g)	Length (cm)	Weight liver (g)	Weight gonad (g)	Gutted weight (g)	GSI (%)	Maturity
1H	Whiting	Spring	Inner	F	247	33	6.6	10.3	219	4.3	F
2H	Whiting	Spring	Inner	F	231	33.5	8.6	5.8	206	2.6	F
3H	Whiting	Spring	Inner	F	210	31	5.2	6.5	191	3.2	F
4H	Whiting	Spring	Inner	F	200	30	8.2	12.1	170	6.4	F
5H	Whiting	Spring	Inner	F	189	30	6.5	11.3	157	6.5	F
6H	Whiting	Spring	Inner	F	163	27.5	5.8	15	135	9.6	F
7H	Whiting	Spring	Inner	F	185	30	3.3	11.4	162	6.5	F
8H	Whiting	Spring	Inner	M	161	27.5	3.9	3	144	2	M
1NP	Norway pout	Spring	Inner	F	60	22	3.7	6.8	48	12	F
2NP	Norway pout	Spring	Inner	F	71	22	1.4	9.2	57	14	F
3NP	Norway pout	Spring	Inner	F	96	22	4	8.6	80	9.3	F
4NP	Norway pout	Spring	Inner	F	53	19.5	2.4	4.2	43	8.5	F
5NP	Norway pout	Spring	Inner	F	151	26.5	5	17.2	121	12	F
6NP	Norway pout	Spring	Inner	F	79	21.5	3.8	7.3	64	9.7	F
7NP	Norway pout	Spring	Inner	F	44	18.5	1.4	6.1	35	14	F
8NP	Norway pout	Spring	Inner	F	69	20	3.5	5.8	57	8.8	F
1T	Atlantic cod	Spring	Inner	F	655	40.5	10.5	82.5	505	14	F
2T	Atlantic cod	Spring	Inner	F	367	36	5.6	1.3	335	0.38	J
3T	Atlantic cod	Spring	Inner	F	775	44.5	11.8	132.5	571	19	F
4T	Atlantic cod	Spring	Inner	M	344	34.5	3.2	0	313	0	J
5T	Atlantic cod	Spring	Inner	F	294	33	2.7	3.8	261	1.4	J

Table 9. (continued)

ID	Species	Season	Location	Sex	Total weight (g)	Length (cm)	Weight liver (g)	Weight gonad (g)	Gutted weight (g)	GSI (%)	Maturity
6T	Atlantic cod	Spring	Inner	F	189	29.5	2.1	0.7	171	0.4	J
7T	Atlantic cod	Spring	Inner	M	380	36	4.5	0	341	0	J
8T	Atlantic cod	Spring	Inner	F	361	35	2.9	1.3	310	0.41	J
16H	Whiting	Spring	Outer	F	222	30	8.9	15.3	108	12	F
17H	Whiting	Spring	Outer	F	131.5	27.5	4.7	6.5	131	4.6	F
18H	Whiting	Spring	Outer	F	131	26	5.2	19.1	100	15	F
19H	Whiting	Spring	Outer	F	157	27	3.8	20.5	124	14	F
20H	Whiting	Spring	Outer	F	200	30	7.5	19	162	10	F
21H	Whiting	Spring	Outer	F	169	27	7.4	17.6	135	11	F
22H	Whiting	Spring	Outer	M	120	25.5	2.8	2	109	1.8	M
23H	Whiting	Spring	Outer	F	122	26	3	11.7	100	10	F
24H	Whiting	Spring	Outer	F	153	28	3.4	14.8	124	10	F
10HY	Haddock	Spring	Outer	F	108.6	24.5	1.9	0.2	96	0.2	J
11HY	Haddock	Spring	Outer	F	102	22.5	2.2	0.1	85.7	0.11	J
12HY	Haddock	Spring	Outer	F	188	28	5.9	1.1	164	0.64	J
13HY	Haddock	Spring	Outer	F	112	23	2.2	0.6	97	0.6	J
14HY	Haddock	Spring	Outer	M	166	27.5	2.1	0.6	144	0.41	J
15HY	Haddock	Spring	Outer	F	201	29.5	5.9	2	173	1.1	J
16HY	Haddock	Spring	Outer	F	168	27	4.8	7.4	142	4.8	F
17HY	Haddock	Spring	Outer	F	148	33	NA	0.6	129	0	J
16T	Atlantic cod	Spring	Outer	M	378	36	3.1	0	341	0	J
17T	Atlantic cod	Spring	Outer	M	332	33	4.3	10.2	290	3.4	M
18T	Atlantic cod	Spring	Outer	F	262	31.5	2.3	0.5	240	0.21	J
19T	Atlantic cod	Spring	Outer	M	165	27.5	1.1	0	147	0	J
20T	Atlantic cod	Spring	Outer	F	354	35	3.5	0.9	316	0.28	J
21T	Atlantic cod	Spring	Outer	F	365	35	5.4	1	339	0.29	J

Table 9. (continued)

ID	Species	Season	Location	Sex	Total weight (g)	Length (cm)	Weight liver (g)	Weight gonad (g)	Gutted weight (g)	GSI (%)	Maturity
22T	Atlantic cod	Spring	Outer	F	377	35.5	2.9	1.5	330	0.45	J
23T	Atlantic cod	Spring	Outer	F	288	33	2.8	1	259	0.38	J
25H	Whiting	Fall	Inner	F	186.9	29	8.6	0.6	161	0.35	J
26H	Whiting	Fall	Inner	F	259.3	33	16.9	2.1	221.4	0.87	J
27H	Whiting	Fall	Inner	F	151.3	27	4.6	0.7	137.6	0.49	J
28H	Whiting	Fall	Inner	F	125.3	27	3.2	0.5	113.7	0.43	J
29H	Whiting	Fall	Inner	F	346	36.5	11.6	2.7	305	0.85	J
30H	Whiting	Fall	Inner	F	192.5	29.5	14.5	1.2	165	0.66	J
31H	Whiting	Fall	Inner	F	168.1	27.5	10.5	0.9	144	0.58	J
32H	Whiting	Fall	Inner	F	178.8	29	12	1.1	147	0.69	J
25NP	Norway pout	Fall	Inner	F	81.9	22	5.2	0.8	69.5	1.1	J
26NP	Norway pout	Fall	Inner	F	124.6	23.5	9.2	0.6	108.9	0.51	J
27NP	Norway pout	Fall	Inner	F	168.5	26.5	12	1.2	148.5	0.74	J
28NP	Norway pout	Fall	Inner	F	83	23	21	0.4	77	0.41	J
29NP	Norway pout	Fall	Inner	F	90.6	22	6.8	0.4	78.9	0.47	J
30NP	Norway pout	Fall	Inner	M	71	21	3.6	0	64.1	0	J
31NP	Norway pout	Fall	Inner	F	77.9	21.5	5.7	0.5	67.1	0.68	J
32NP	Norway pout	Fall	Inner	F	80	21	8.4	0.3	65.7	0.4	J
25T	Atlantic cod	Fall	Inner	M	626	43	8.3	0.5	570	0.086	J
26T	Atlantic cod	Fall	Inner	M	463	38	7.9	0.5	403	0.12	J
27T	Atlantic cod	Fall	Inner	M	341	33.5	13.5	0.6	301	0.19	J
28T	Atlantic cod	Fall	Inner	F	637	45	7.8	2.4	575	0.41	J
29T	Atlantic cod	Fall	Inner	F	402.6	35	5.5	1.5	358.4	0.41	J
30T	Atlantic cod	Fall	Inner	M	241.2	30	7.5	0	211.2	0	J

Table 9. (continued).

ID	Species	Season	Location	Sex	Total weight (g)	Length (cm)	Weight liver (g)	Weight gonad (g)	Gutted weight (g)	GSI (%)	Maturity
31T	Atlantic cod	Fall	Inner	M	288.5	33.5	2.6	0	259.6	0	J
32T	Atlantic cod	Fall	Inner	F	287.5	32	4.1	0.5	242.9	0.2	J
33H	Whiting	Fall	Outer	F	202.3	30	4.6	2.6	164.9	1.5	J
34H	Whiting	Fall	Outer	M	188.3	27	4.4	1.3	160.7	0.78	J
35H	Whiting	Fall	Outer	F	76.1	22	2.1	0	69.6	0	J
36H	Whiting	Fall	Outer	M	106.4	25	4.9	0.1	92.4	0.1	J
37H	Whiting	Fall	Outer	M	152	27.5	5.7	0.1	122.5	0.078	J
38H	Whiting	Fall	Outer	M	119	25	7.4	0	101.3	0	J
39H	Whiting	Fall	Outer	F	106.4	24.5	4.2	0.6	93.5	0.61	J
40H	Whiting	Fall	Outer	M	83.3	23.5	3.2	0	73.8	0	J
33NP	Norway pout	Fall	Outer	F	35.2	17	3.5	0.2	29	0.61	J
34NP	Norway pout	Fall	Outer	F	36.5	17	3.8	0.2	32.3	0.55	J
35NP	Norway pout	Fall	Outer	F	53	19	7.2	0.2	43.3	0.39	J
36NP	Norway pout	Fall	Outer	M	35.2	16.5	3.1	0	29.2	0	J
37NP	Norway pout	Fall	Outer	F	31.8	17	2.1	0.1	28.1	0.33	J
38NP	Norway pout	Fall	Outer	F	35.8	17	3.5	0.1	27.7	0.32	J
39NP	Norway pout	Fall	Outer	F	80.1	21	7.8	0.3	67.5	0.4	J
40NP	Norway pout	Fall	Outer	F	53	20	3.5	0.1	45	0.21	J
1HY	Haddock	Fall	Outer	F	212.8	28	13	0.6	184	0.3	J
2HY	Haddock	Fall	Outer	F	168	25.5	9.2	0.4	145.5	0.26	J
3HY	Haddock	Fall	Outer	F	401.8	35	31.6	3.1	328	0.85	J
4HY	Haddock	Fall	Outer	F	446.4	36.5	31.1	2.5	370.6	0.62	J
5HY	Haddock	Fall	Outer	F	216	28.5	16.4	0.7	177.5	0.36	J
6HY	Haddock	Fall	Outer	M	180.4	26.5	8.8	0	149.5	0	J
7HY	Haddock	Fall	Outer	F	208.8	27.8	14.8	0.6	173	0.32	J
8HY	Haddock	Fall	Outer	M	140	25	9.5	0	119.4	0	J
33T	Atlantic cod	Fall	Outer	M	807	46	16.3	1.1	726	0.15	J

Table 9. (continued)

ID	Species	Season	Location	Sex	Total weight (g)	Length (cm)	Weight liver (g)	Weight gonad (g)	Gutted weight (g)	GSI (%)	Maturity
34T	Atlantic cod	Fall	Outer	F	385	36	8	2.8	337	0.81	J
35T	Atlantic cod	Fall	Outer	M	474.3	38	7.9	0.7	389	0.18	J
36T	Atlantic cod	Fall	Outer	F	197.5	29	3.1	0.3	162.5	0.18	J
37T	Atlantic cod	Fall	Outer	F	373.6	35	4.9	1.8	332	0.53	J
38T	Atlantic cod	Fall	Outer	M	483.6	38	24.9	0.3	381.8	0.074	J
39T	Atlantic cod	Fall	Outer	F	438.6	37.5	3.6	2	379.4	0.52	J
40T	Atlantic cod	Fall	Outer	F	474.6	38	6.9	1.6	407.2	0.38	J

Appendix B

Table 10. Hepatic mercury concentration (µg/kg).

ID	Species	Season	Location	Mercury concentration (µg/kg)
1H	Whiting	Spring	Inner	52.4
2H	Whiting	Spring	Inner	71.7
3H	Whiting	Spring	Inner	85.5
4H	Whiting	Spring	Inner	43.3
5H	Whiting	Spring	Inner	39.2
6H	Whiting	Spring	Inner	46.1
7H	Whiting	Spring	Inner	131.2
8H	Whiting	Spring	Inner	131.4
1NP	Norway pout	Spring	Inner	111.0
2NP	Norway pout	Spring	Inner	359.3
3NP	Norway pout	Spring	Inner	63.1
4NP	Norway pout	Spring	Inner	54.5
5NP	Norway pout	Spring	Inner	300.6
6NP	Norway pout	Spring	Inner	63.3
7NP	Norway pout	Spring	Inner	42.5
8NP	Norway pout	Spring	Inner	45.0
1T	Atlantic cod	Spring	Inner	83.6
2T	Atlantic cod	Spring	Inner	161.5
3T	Atlantic cod	Spring	Inner	250.5
4T	Atlantic cod	Spring	Inner	256.1
5T	Atlantic cod	Spring	Inner	186.6
6T	Atlantic cod	Spring	Inner	63.2
7T	Atlantic cod	Spring	Inner	235.4
8T	Atlantic cod	Spring	Inner	293.6
16H	Whiting	Spring	Outer	70.3
17H	Whiting	Spring	Outer	108.5
18H	Whiting	Spring	Outer	94.2
19H	Whiting	Spring	Outer	139.1
20H	Whiting	Spring	Outer	106.0
21H	Whiting	Spring	Outer	45.1
22H	Whiting	Spring	Outer	30.2

Table 10. (continued)

ID	Species	Season	Location	Mercury concentration (µg/kg)
23H	Whiting	Spring	Outer	102.8
24H	Whiting	Spring	Outer	40.6
10HY	Haddock	Spring	Outer	86.3
11HY	Haddock	Spring	Outer	60.3
12HY	Haddock	Spring	Outer	80.6
13HY	Haddock	Spring	Outer	75.9
14HY	Haddock	Spring	Outer	126.2
15HY	Haddock	Spring	Outer	43.9
16HY	Haddock	Spring	Outer	87.6
17HY	Haddock	Spring	Outer	69.6
16T	Atlantic cod	Spring	Outer	226.5
17T	Atlantic cod	Spring	Outer	93.1
18T	Atlantic cod	Spring	Outer	152.2
19T	Atlantic cod	Spring	Outer	203.7
20T	Atlantic cod	Spring	Outer	142.7
21T	Atlantic cod	Spring	Outer	32.2
22T	Atlantic cod	Spring	Outer	364.8
23T	Atlantic cod	Spring	Outer	205.8
25H	Whiting	Fall	Inner	19.7
26H	Whiting	Fall	Inner	51.2
27H	Whiting	Fall	Inner	16.3
28H	Whiting	Fall	Inner	37.1
29H	Whiting	Fall	Inner	84.2
30H	Whiting	Fall	Inner	31.5
31H	Whiting	Fall	Inner	39.9
32H	Whiting	Fall	Inner	84.7
25NP	Norway pout	Fall	Inner	63.2
26NP	Norway pout	Fall	Inner	78.4
27NP	Norway pout	Fall	Inner	64.2
28NP	Norway pout	Fall	Inner	144.3
29NP	Norway pout	Fall	Inner	42.4
30NP	Norway pout	Fall	Inner	37.8

Table 10. (continued)

ID	Species	Season	Location	Mercury concentration (µg/kg)
31NP	Norway pout	Fall	Inner	53.4
32NP	Norway pout	Fall	Inner	26.6
25T	Atlantic cod	Fall	Inner	304.1
26T	Atlantic cod	Fall	Inner	29.5
27T	Atlantic cod	Fall	Inner	261.9
28T	Atlantic cod	Fall	Inner	59.7
29T	Atlantic cod	Fall	Inner	164.7
30T	Atlantic cod	Fall	Inner	18.6
31T	Atlantic cod	Fall	Inner	68.6
32T	Atlantic cod	Fall	Inner	143.0
33H	Whiting	Fall	Outer	22.7
34H	Whiting	Fall	Outer	18.5
35H	Whiting	Fall	Outer	49.1
36H	Whiting	Fall	Outer	14.9
37H	Whiting	Fall	Outer	47.7
38H	Whiting	Fall	Outer	18.5
39H	Whiting	Fall	Outer	14.7
40H	Whiting	Fall	Outer	16.6
33NP	Norway pout	Fall	Outer	9.2
34NP	Norway pout	Fall	Outer	7.0
35NP	Norway pout	Fall	Outer	4.7
36NP	Norway pout	Fall	Outer	10.2
37NP	Norway pout	Fall	Outer	21.6
38NP	Norway pout	Fall	Outer	6.2
39NP	Norway pout	Fall	Outer	21.3
40NP	Norway pout	Fall	Outer	12.4
1HY	Haddock	Fall	Outer	39.4
2HY	Haddock	Fall	Outer	54.3
3HY	Haddock	Fall	Outer	62.8
4HY	Haddock	Fall	Outer	54.0
5HY	Haddock	Fall	Outer	74.6
6HY	Haddock	Fall	Outer	36.5

Table 10. (continued)

ID	Species	Season	Location	Mercury concentration (µg/kg)
7HY	Haddock	Fall	Outer	64.2
8HY	Haddock	Fall	Outer	60.4
33T	Atlantic cod	Fall	Outer	1123.8
34T	Atlantic cod	Fall	Outer	248.3
35T	Atlantic cod	Fall	Outer	263.4
36T	Atlantic cod	Fall	Outer	136.2
37T	Atlantic cod	Fall	Outer	178.9
38T	Atlantic cod	Fall	Outer	22.1
39T	Atlantic cod	Fall	Outer	201.9
40T	Atlantic cod	Fall	Outer	261.4

Appendix C

Table 11. Protein concentrations (mg/mL) in liver, gill, and heart samples.

ID	Species	Season	Location	Liver	Gills	Heart
1H	Whiting	Spring	Inner	331.72	140.35	380.15
2H	Whiting	Spring	Inner	309.93	153.62	236.85
3H	Whiting	Spring	Inner	314.44	141.99	337.88
4H	Whiting	Spring	Inner	517.23	198.27	393.80
5H	Whiting	Spring	Inner	411.44	153.75	338.47
6H	Whiting	Spring	Inner	458.40	175.51	186.67
7H	Whiting	Spring	Inner	427.44	229.98	186.73
8H	Whiting	Spring	Inner	391.29	119.02	409.51
1NP	Norway pout	Spring	Inner	172.35	168.26	252.59
2NP	Norway pout	Spring	Inner	401.12	103.64	236.42
3NP	Norway pout	Spring	Inner	445.65	112.39	212.27
4NP	Norway pout	Spring	Inner	340.41	86.51	164.20
5NP	Norway pout	Spring	Inner	432.48	175.99	340.26
6NP	Norway pout	Spring	Inner	249.45	87.10	259.16
7NP	Norway pout	Spring	Inner	255.83	64.43	110.63
8NP	Norway pout	Spring	Inner	346.02	110.97	148.51
1T	Atlantic cod	Spring	Inner	479.66	187.32	355.27
2T	Atlantic cod	Spring	Inner	307.75	219.02	514.47
3T	Atlantic cod	Spring	Inner	420.76	203.17	347.26
4T	Atlantic cod	Spring	Inner	378.84	188.76	332.38
5T	Atlantic cod	Spring	Inner	352.83	131.55	402.25
6T	Atlantic cod	Spring	Inner	343.22	208.85	204.57
7T	Atlantic cod	Spring	Inner	376.15	167.31	407.69
8T	Atlantic cod	Spring	Inner	189.15	238.85	424.01
16H	Whiting	Spring	Outer	379.01	105.77	352.84
17H	Whiting	Spring	Outer	367.22	175.67	198.38
18H	Whiting	Spring	Outer	386.29	149.45	341.34
19H	Whiting	Spring	Outer	406.73	186.43	287.09
20H	Whiting	Spring	Outer	443.70	157.24	300.69

Table 11. (continued)

ID	Species	Season	Location	Liver	Gills	Heart
21H	Whiting	Spring	Outer	383.92	129.23	296.98
22H	Whiting	Spring	Outer	214.77	139.97	277.39
23H	Whiting	Spring	Outer	340.65	149.20	188.92
24H	Whiting	Spring	Outer	579.52	143.78	209.84
10HY	Haddock	Spring	Outer	507.37	190.31	239.39
11HY	Haddock	Spring	Outer	420.77	106.49	140.51
12HY	Haddock	Spring	Outer	277.70	167.73	344.22
13HY	Haddock	Spring	Outer	393.42	177.81	216.58
14HY	Haddock	Spring	Outer	424.63	134.42	270.31
15HY	Haddock	Spring	Outer	327.42	121.33	359.67
16HY	Haddock	Spring	Outer	481.90	164.96	393.08
17HY	Haddock	Spring	Outer	302.66	114.84	254.39
16T	Atlantic cod	Spring	Outer	432.60	186.75	371.65
17T	Atlantic cod	Spring	Outer	386.59	154.18	389.12
18T	Atlantic cod	Spring	Outer	374.47	239.20	283.92
19T	Atlantic cod	Spring	Outer	343.92	112.39	227.14
20T	Atlantic cod	Spring	Outer	491.68	197.47	239.88
21T	Atlantic cod	Spring	Outer	350.73	163.47	401.50
22T	Atlantic cod	Spring	Outer	427.01	202.02	282.83
23T	Atlantic cod	Spring	Outer	417.91	163.47	470.39
25H	Whiting	Fall	Inner	64.22	154.56	234.95
26H	Whiting	Fall	Inner	35.22	245.04	319.21
27H	Whiting	Fall	Inner	98.82	228.61	260.85
28H	Whiting	Fall	Inner	87.50	196.03	319.55
29H	Whiting	Fall	Inner	75.27	221.51	292.64
30H	Whiting	Fall	Inner	62.54	218.64	341.31
31H	Whiting	Fall	Inner	83.92	247.05	253.62
32H	Whiting	Fall	Inner	91.76	244.75	458.66
25NP	Norway pout	Fall	Inner	112.97	129.73	331.64
26NP	Norway pout	Fall	Inner	95.44	221.80	153.61
27NP	Norway pout	Fall	Inner	56.68	189.69	220.53
28NP	Norway pout	Fall	Inner	105.29	290.37	191.53

Table 11. (continued)

ID	Species	Season	Location	Liver	Gills	Heart
29NP	Norway pout	Fall	Inner	77.65	191.21	245.31
30NP	Norway pout	Fall	Inner	78.14	140.02	155.23
31NP	Norway pout	Fall	Inner	65.04	209.96	342.22
32NP	Norway pout	Fall	Inner	71.59	158.10	275.30
25T	Atlantic cod	Fall	Inner	107.39	274.43	369.23
26T	Atlantic cod	Fall	Inner	86.96	229.18	391.73
27T	Atlantic cod	Fall	Inner	98.96	251.38	338.89
28T	Atlantic cod	Fall	Inner	146.46	189.09	437.45
29T	Atlantic cod	Fall	Inner	118.71	229.99	360.54
30T	Atlantic cod	Fall	Inner	73.90	274.02	235.93
31T	Atlantic cod	Fall	Inner	122.98	274.31	322.45
32T	Atlantic cod	Fall	Inner	129.36	296.35	492.79
33H	Whiting	Fall	Outer	106.99	222.08	323.71
34H	Whiting	Fall	Outer	102.52	236.46	306.49
35H	Whiting	Fall	Outer	61.67	208.43	179.18
36H	Whiting	Fall	Outer	54.09	307.34	190.42
37H	Whiting	Fall	Outer	67.28	214.91	271.02
38H	Whiting	Fall	Outer	35.99	253.36	344.58
39H	Whiting	Fall	Outer	79.73	191.71	333.11
40H	Whiting	Fall	Outer	68.62	247.34	292.12
33NP	Norway pout	Fall	Outer	42.54	167.78	127.53
34NP	Norway pout	Fall	Outer	31.89	144.33	203.52
35NP	Norway pout	Fall	Outer	84.77	127.76	215.23
36NP	Norway pout	Fall	Outer	22.85	64.29	108.75
37NP	Norway pout	Fall	Outer	59.15	120.21	85.62
38NP	Norway pout	Fall	Outer	43.01	95.71	106.22
39NP	Norway pout	Fall	Outer	25.36	179.91	198.77
40NP	Norway pout	Fall	Outer	45.84	112.28	141.10
1HY	Haddock	Fall	Outer	78.12	255.37	244.83
2HY	Haddock	Fall	Outer	77.27	214.42	193.80
3HY	Haddock	Fall	Outer	65.62	262.05	340.40
4HY	Haddock	Fall	Outer	83.71	204.58	410.78

Table 11. (continued)

ID	Species	Season	Location	Liver	Gills	Heart
5HY	Haddock	Fall	Outer	73.53	250.76	312.08
6HY	Haddock	Fall	Outer	66.92	167.78	329.85
7HY	Haddock	Fall	Outer	58.39	246.19	258.94
8HY	Haddock	Fall	Outer	77.16	172.08	285.42
33T	Atlantic cod	Fall	Outer	44.67	246.59	450.14
34T	Atlantic cod	Fall	Outer	102.30	212.33	457.22
35T	Atlantic cod	Fall	Outer	55.78	239.81	505.19
36T	Atlantic cod	Fall	Outer	94.15	291.52	329.13
37T	Atlantic cod	Fall	Outer	43.85	233.85	407.97
38T	Atlantic cod	Fall	Outer	112.75	218.80	381.94
39T	Atlantic cod	Fall	Outer	144.68	208.47	314.47
40T	Atlantic cod	Fall	Outer	125.53	185.83	249.65

Appendix D

Table 12. Hepatic, gill, and heart CYP1A activity (nmol/min/mg protein).

ID	Species	Season	Location	Hepatic CYP1A activity	Gill CYP1A activity	Heart CYP1A activity
1H	Whiting	Spring	Inner	0.062	0.114	0.041
2H	Whiting	Spring	Inner	0.044	0.070	0.044
3H	Whiting	Spring	Inner	0.064	0.085	0.020
4H	Whiting	Spring	Inner	0.128	0.052	0.020
5H	Whiting	Spring	Inner	0.110	0.085	0.031
6H	Whiting	Spring	Inner	0.151	0.085	0.084
7H	Whiting	Spring	Inner	0.034	0.042	0.041
8H	Whiting	Spring	Inner	0.047	0.086	0.030
1NP	Norway pout	Spring	Inner	0.050	0.062	0.039
2NP	Norway pout	Spring	Inner	0.044	0.127	0.096
3NP	Norway pout	Spring	Inner	0.121	0.129	0.028
4NP	Norway pout	Spring	Inner	0.043	0.088	0.037
5NP	Norway pout	Spring	Inner	0.045	0.063	0.043
6NP	Norway pout	Spring	Inner	0.080	0.139	0.033
7NP	Norway pout	Spring	Inner	0.105	0.185	0.031
8NP	Norway pout	Spring	Inner	0.233	0.125	0.029
1T	Atlantic cod	Spring	Inner	0.028	0.067	0.043
2T	Atlantic cod	Spring	Inner	0.044	0.072	0.017
3T	Atlantic cod	Spring	Inner	0.036	0.078	0.040
4T	Atlantic cod	Spring	Inner	0.051	0.094	0.045
5T	Atlantic cod	Spring	Inner	0.135	0.191	0.032
6T	Atlantic cod	Spring	Inner	0.030	0.032	0.030
7T	Atlantic cod	Spring	Inner	0.141	0.111	0.046
8T	Atlantic cod	Spring	Inner	1.010	0.065	0.043
16H	Whiting	Spring	Outer	0.193	0.098	0.029
17H	Whiting	Spring	Outer	0.624	0.044	0.044
18H	Whiting	Spring	Outer	0.432	0.105	0.022
19H	Whiting	Spring	Outer	0.135	0.062	0.032
20H	Whiting	Spring	Outer	0.095	0.083	0.033
21H	Whiting	Spring	Outer	0.074	0.112	0.028
22H	Whiting	Spring	Outer	0.108	0.127	0.034

Table 12. (continued)

ID	Species	Season	Location	Hepatic CYP1A activity	Gill CYP1A activity	Heart CYP1A activity
23H	Whiting	Spring	Outer	0.277	0.081	0.040
24H	Whiting	Spring	Outer	0.040	0.073	0.055
10HY	Haddock	Spring	Outer	0.255	0.050	0.030
11HY	Haddock	Spring	Outer	0.901	0.091	0.041
12HY	Haddock	Spring	Outer	2.206	0.046	0.038
13HY	Haddock	Spring	Outer	1.992	0.041	0.046
14HY	Haddock	Spring	Outer	0.470	0.060	0.092
15HY	Haddock	Spring	Outer	1.603	0.094	0.032
16HY	Haddock	Spring	Outer	0.067	0.058	0.056
17HY	Haddock	Spring	Outer	0.384	0.101	0.031
16T	Atlantic cod	Spring	Outer	0.061	0.056	0.058
17T	Atlantic cod	Spring	Outer	0.124	0.075	0.042
18T	Atlantic cod	Spring	Outer	0.164	0.078	0.055
19T	Atlantic cod	Spring	Outer	0.836	0.076	0.089
20T	Atlantic cod	Spring	Outer	0.032	0.049	0.036
21T	Atlantic cod	Spring	Outer	1.135	0.048	0.049
22T	Atlantic cod	Spring	Outer	0.066	0.068	0.116
23T	Atlantic cod	Spring	Outer	1.498	0.055	0.112
25H	Whiting	Fall	Inner	8.979	0.076	0.048
26H	Whiting	Fall	Inner	1.220	0.035	0.023
27H	Whiting	Fall	Inner	0.368	0.044	0.037
28H	Whiting	Fall	Inner	0.489	0.048	0.028
29H	Whiting	Fall	Inner	0.325	0.042	0.026
30H	Whiting	Fall	Inner	1.143	0.052	0.034
31H	Whiting	Fall	Inner	0.719	0.031	0.042
32H	Whiting	Fall	Inner	1.375	0.041	0.023
25NP	Norway pout	Fall	Inner	0.767	0.092	0.040
26NP	Norway pout	Fall	Inner	0.410	0.038	0.043
27NP	Norway pout	Fall	Inner	0.901	0.066	0.031
28NP	Norway pout	Fall	Inner	0.794	0.048	0.026
29NP	Norway pout	Fall	Inner	0.189	0.047	0.041
30NP	Norway pout	Fall	Inner	2.058	0.069	0.035

Table 12. (continued)

ID	Species	Season	Location	Hepatic CYP1A activity	Gill CYP1A activity	Heart CYP1A activity
31NP	Norway pout	Fall	Inner	0.405	0.077	0.043
32NP	Norway pout	Fall	Inner	2.326	0.065	0.034
25T	Atlantic cod	Fall	Inner	2.643	0.034	0.046
26T	Atlantic cod	Fall	Inner	0.634	0.058	0.081
27T	Atlantic cod	Fall	Inner	1.379	0.052	0.046
28T	Atlantic cod	Fall	Inner	1.059	0.046	0.075
29T	Atlantic cod	Fall	Inner	1.844	0.070	0.042
30T	Atlantic cod	Fall	Inner	2.612	0.025	0.086
31T	Atlantic cod	Fall	Inner	1.333	0.044	0.039
32T	Atlantic cod	Fall	Inner	2.211	0.048	0.050
33H	Whiting	Fall	Outer	0.386	0.063	0.036
34H	Whiting	Fall	Outer	0.741	0.031	0.034
35H	Whiting	Fall	Outer	5.557	0.059	0.058
36H	Whiting	Fall	Outer	4.380	0.038	0.060
37H	Whiting	Fall	Outer	5.046	0.046	0.036
38H	Whiting	Fall	Outer	1.772	0.038	0.030
39H	Whiting	Fall	Outer	0.258	0.035	0.029
40H	Whiting	Fall	Outer	0.382	0.042	0.031
33NP	Norway pout	Fall	Outer	2.574	0.048	0.029
34NP	Norway pout	Fall	Outer	0.385	0.068	0.015
35NP	Norway pout	Fall	Outer	0.173	0.066	0.020
36NP	Norway pout	Fall	Outer	2.486	0.159	0.032
37NP	Norway pout	Fall	Outer	4.572	0.067	0.027
38NP	Norway pout	Fall	Outer	1.116	0.093	0.032
39NP	Norway pout	Fall	Outer	2.515	0.072	0.022
40NP	Norway pout	Fall	Outer	0.483	0.066	0.026
1HY	Haddock	Fall	Outer	1.565	0.043	0.064
2HY	Haddock	Fall	Outer	1.166	0.066	0.061
3HY	Haddock	Fall	Outer	1.046	0.026	0.067
4HY	Haddock	Fall	Outer	0.800	0.048	0.053
5HY	Haddock	Fall	Outer	3.439	0.032	0.030
6HY	Haddock	Fall	Outer	3.254	0.072	0.063

Table 12. (continued)

ID	Species	Season	Location	Hepatic CYP1A activity	Gill CYP1A activity	Heart CYP1A activity
7HY	Haddock	Fall	Outer	1.685	0.023	0.035
8HY	Haddock	Fall	Outer	2.055	0.044	0.028
33T	Atlantic cod	Fall	Outer	10.362	0.048	0.074
34T	Atlantic cod	Fall	Outer	2.753	0.035	0.032
35T	Atlantic cod	Fall	Outer	4.840	0.033	0.046
36T	Atlantic cod	Fall	Outer	1.280	0.035	0.097
37T	Atlantic cod	Fall	Outer	2.476	0.043	0.136
38T	Atlantic cod	Fall	Outer	1.821	0.080	0.130
39T	Atlantic cod	Fall	Outer	1.534	0.042	0.087
40T	Atlantic cod	Fall	Outer	2.076	0.091	0.142

Appendix E

Table 13. Concentrations of PAH metabolites in bile (μg/mL).

ID	Species	Season	Location	2- and 3-rings	Pyrene metab.	3-OH-benzo[a]pyrene
1H	Whiting	Spring	Inner	72.0	0.433	0.263
2H	Whiting	Spring	Inner	148.0	0.212	0.651
3H	Whiting	Spring	Inner	61.3	0.304	0.184
4H	Whiting	Spring	Inner	42.3	0.246	0.223
5H	Whiting	Spring	Inner	-	-	-
6H	Whiting	Spring	Inner	72.1	0.577	0.347
7H	Whiting	Spring	Inner	53.8	0.289	0.512
8H	Whiting	Spring	Inner	65.9	0.320	0.213
1NP	Norway pout	Spring	Inner	-	-	-
2NP	Norway pout	Spring	Inner	-	-	-
3NP	Norway pout	Spring	Inner	6.3	0.105	0.131
4NP	Norway pout	Spring	Inner	-	-	-
5NP	Norway pout	Spring	Inner	-	-	-
6NP	Norway pout	Spring	Inner	-	-	-
7NP	Norway pout	Spring	Inner	-	-	-
8NP	Norway pout	Spring	Inner	-	-	-
1T	Atlantic cod	Spring	Inner	73.8	0.437	0.270
2T	Atlantic cod	Spring	Inner	86.9	0.575	0.271
3T	Atlantic cod	Spring	Inner	38.5	0.222	0.177
4T	Atlantic cod	Spring	Inner	36.9	0.263	0.121
5T	Atlantic cod	Spring	Inner	41.5	0.314	0.212
6T	Atlantic cod	Spring	Inner	41.3	0.284	0.232
7T	Atlantic cod	Spring	Inner	68.9	0.288	0.216
8T	Atlantic cod	Spring	Inner	113.7	0.217	0.099
16H	Whiting	Spring	Outer	-	-	-
17H	Whiting	Spring	Outer	46.3	0.192	0.153
18H	Whiting	Spring	Outer	75.4	0.281	0.257
19H	Whiting	Spring	Outer	58.9	0.279	0.125
20H	Whiting	Spring	Outer	35.5	0.152	0.196
21H	Whiting	Spring	Outer	90.2	0.589	0.330
22H	Whiting	Spring	Outer	43.5	0.327	0.300

Table 13. (continued)

ID	Species	Season	Location	2- and 3-rings	Pyrene metab.	3-OH-benzo[a]pyrene
23H	Whiting	Spring	Outer	59.5	0.082	0.071
24H	Whiting	Spring	Outer	30.07	0.169	0.221
10HY	Haddock	Spring	Outer	64.3	0.320	0.425
11HY	Haddock	Spring	Outer	-	-	-
12HY	Haddock	Spring	Outer	-	-	-
13HY	Haddock	Spring	Outer	26.9	0.122	0.170
14HY	Haddock	Spring	Outer	42.9	0.326	0.252
15HY	Haddock	Spring	Outer	-	-	-
16HY	Haddock	Spring	Outer	-	-	-
17HY	Haddock	Spring	Outer	-	-	-
16T	Atlantic cod	Spring	Outer	27.6	0.144	0.137
17T	Atlantic cod	Spring	Outer	43.4	0.285	0.289
18T	Atlantic cod	Spring	Outer	55.9	0.256	0.212
19T	Atlantic cod	Spring	Outer	35.0	0.231	0.190
20T	Atlantic cod	Spring	Outer	46.8	0.271	0.215
21T	Atlantic cod	Spring	Outer	44.5	0.305	0.178
22T	Atlantic cod	Spring	Outer	18.1	0.141	0.160
23T	Atlantic cod	Spring	Outer	49.4	0.259	0.231
25H	Whiting	Fall	Inner	82.4	0.237	0.267
26H	Whiting	Fall	Inner	165.2	0.868	0.664
27H	Whiting	Fall	Inner	-	-	-
28H	Whiting	Fall	Inner	42.6	0.236	0.324
29H	Whiting	Fall	Inner	107.0	0.503	0.365
30H	Whiting	Fall	Inner	129.4	0.223	0.167
31H	Whiting	Fall	Inner	-	-	-
32H	Whiting	Fall	Inner	-	-	-
25NP	Norway pout	Fall	Inner	-	-	-
26NP	Norway pout	Fall	Inner	43.8	0.242	0.214
27NP	Norway pout	Fall	Inner	191.9	0.066	0.079
28NP	Norway pout	Fall	Inner	-	-	-
29NP	Norway pout	Fall	Inner	-	-	-
30NP	Norway pout	Fall	Inner	-	-	-
31NP	Norway pout	Fall	Inner	-	-	-

Table 13. (continued)

ID	Species	Season	Location	2- and 3-rings	Pyrene metab.	3-OH-benzo[a]pyrene
32NP	Norway pout	Fall	Inner	-	-	-
25T	Atlantic cod	Fall	Inner	72.0	0.327	0.177
26T	Atlantic cod	Fall	Inner	55.3	0.263	0.185
27T	Atlantic cod	Fall	Inner	94.3	0.544	0.398
28T	Atlantic cod	Fall	Inner	71.7	0.444	0.256
29T	Atlantic cod	Fall	Inner	68.5	0.304	0.172
30T	Atlantic cod	Fall	Inner	103.4	0.479	0.307
31T	Atlantic cod	Fall	Inner	68.3	0.496	0.423
32T	Atlantic cod	Fall	Inner	129.7	0.368	0.221
33H	Whiting	Fall	Outer	-	-	-
34H	Whiting	Fall	Outer	51.4	0.076	0.200
35H	Whiting	Fall	Outer	-	-	-
36H	Whiting	Fall	Outer	-	-	-
37H	Whiting	Fall	Outer	81.8	0.202	0.174
38H	Whiting	Fall	Outer	10.0	0.040	0.147
39H	Whiting	Fall	Outer	88.9	0.229	0.234
40H	Whiting	Fall	Outer	47.0	0.183	0.183
33NP	Norway pout	Fall	Outer	-	-	-
34NP	Norway pout	Fall	Outer	-	-	-
35NP	Norway pout	Fall	Outer	-	-	-
36NP	Norway pout	Fall	Outer	-	-	-
37NP	Norway pout	Fall	Outer	-	-	-
38NP	Norway pout	Fall	Outer	-	-	-
39NP	Norway pout	Fall	Outer	-	-	-
40NP	Norway pout	Fall	Outer	-	-	-
1HY	Haddock	Fall	Outer	-	-	-
2HY	Haddock	Fall	Outer	-	-	-
3HY	Haddock	Fall	Outer	370.7	0.071	0.108
4HY	Haddock	Fall	Outer	-	-	-
5HY	Haddock	Fall	Outer	405.3	0.091	0.338
6HY	Haddock	Fall	Outer	146.5	0.117	0.145
7HY	Haddock	Fall	Outer	307.1	0.090	0.161
8HY	Haddock	Fall	Outer	-	-	-

Table 13. (continued)

ID	Species	Season	Location	2- and 3-rings	Pyrene metab.	3-OH-benzo[a]pyrene
33T	Atlantic cod	Fall	Outer	193.3	0.463	0.287
34T	Atlantic cod	Fall	Outer	35.9	0.233	0.269
35T	Atlantic cod	Fall	Outer	29.3	0.145	0.140
36T	Atlantic cod	Fall	Outer	-	-	-
37T	Atlantic cod	Fall	Outer	31.4	0.097	0.145
38T	Atlantic cod	Fall	Outer	58.2	0.192	0.165
39T	Atlantic cod	Fall	Outer	34.6	0.205	0.334
40T	Atlantic cod	Fall	Outer	34.9	0.189	0.184