NTNU
Department of Materials Science and Engineering

SINTEF

Correlation between life stage, lipid composition and accumulation of polycyclic aromatic hydrocarbons (PAH) in lumpsucker *(Cyclopterus Lumpus)* embryos exposed to produced water

Korrelasjon mellom livsstadie, lipidinnhold og akkumulering av polysykliske aromatiske hydrokarboner (PAH) i rognkjeks (*Cyclopterus lumpus*) embryo eksponert for produsert vann

Bachelor thesis

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Prologue

This thesis is a cumulutaion of our bachelor's degree in chemical engineering at NTNU. All of the experimental work presented in this thesis was performed at SINTEF Ocean by the students and SINTEF Ocean staff.

As we have had the most interesting project we could ever imagine for our thesis, we would like to thank SINTEF Ocean for giving us the opportunity to take part of it. Hereof, huge gratitude goes to Dr. Bjørn Henrik Hansen, whom linked us to this project. We would also declare our appreciation to Dr. Lisbet Sørensen, who found our assistance useful for this project and gave us the best guidances and advice. As our supervisors they have given us the best information and help during the project. Furthermore we would like to thank the labstuff and students for the kindly receivement and general advice.

Finally, we owe a huge thank to our supervisors at NTNU, Eirik Sundby and Lene Østby, for great advisement and proofreading.

We are very grateful for the time we had at SINTEF Ocean. This project gave us the opportunity to get an insight in the exciting world of science - what a fun time it was!

Hannah Marie Knutsen
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Abstract

The goal of this bachelor project was to study the correlation between life stage, lipid composition and bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in early life stages (ELS) of the fish species lumpsucker (*Cyclopterus Lumpus*) exposed to produced water (PW). The current work is a part of the PW-Exposed project, lead by SINTEF Ocean, which will investigate and provide a detailed understanding of the chemical composition and toxicological impacts of PW discharges from oil producing platforms along the Norwegian continental shelf (NCS). As a part of the bigger project, this bachelor thesis is based on experiments of PW and ELS of lumpsuckers, with the purpose of identifying the most sensitive life stage in the development of the lumpsucker embryo.

Offshore oil and gas industry annually contribute to massive discharges of PW on NCS. PW contains polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs, which are known to be toxic to early life stages (ELS) of fish. The study was performed on lumpsucker embryos exposed at different live stages during the embryo stage and monitored until hatching. Three exposure experiments, where accumulation and depuration (dep) of PAHs and alkyl PAHs were observed, was performed at three potentially sensitive stages; during fertilization ("dep1"), 36 hours post fertilization (hpf) ("dep2") and 10 days post fertilization (dpf) ("dep3"). Embryos were sampled at seven timepoints after exposure (0, 6, 12, 24, 48, 96 and 192 hours after exposure (hpe)).

In order to evaluate the bioaccumulation of PAHs, egg samples from the different sampling points were homogenized, extracted with solvent and the extracts purified by solid-phase extraction (SPE). The PAHs were analysed by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). It is not enough research of ELS of fish in this area, this due to development of techniques and methods for studies of PAH body burden concentration in ELS of fish that recently became sensitive enough for small biotic samples (100 mg) exposed to low concentration of contaminants. The total lipid content (by mass) of egg samples was determined by preforming a modified Folch extraction.

The project revealed that lumpsucker embryos exposed to PW displayed increased internal body burden concentration of PAHs and alkyl PAHs. This thesis focuses on three compounds accumulated in the embryos; naphthalene (2-ring PAH), C1-phenanthrene (3-ring alkyl PAH) and dibenzothiophene (3-ring PAH). The highest accumulation of PAHs was observed in the embryos exposed during the earliest life stage (0 hpf), with decreased body burden observed in embryos exposed at later life stages (36 hpf and 10 dpf). After transfer to clean water, the body burden decreased as expected in all exposure scenarios. Naphthalene had the highest body burden, followed by dibenzothiophene and lastly C1-phenanthrene, this applies to all of the exposures. Total extractable lipid content remained more or less constant throughout the development of ELS lumpsucker, for this reason normalizing the body burden concentrations to lipid content revealed the same results as the body burden concentration for the depurations. The bioconcentration factor (BCF) calculated for the 'compounds at focus' revealed that dibenzothiphene was the most bioaccumulated compound in embryos exposed at 0 hpf, for embryos exposed at 36 hpf and 10 dpf, naphthalene accumulated the most. The biotransformation and the development of embryos affected the results. Video analysis performed on embryos after 17 dpf by Drs' Bjørn Henrik Hansen and Julia Farkas revealed that embryos exposed during the earliest life stages (36 hpf and 10 dpf) had a significantly reduced heart rate.

The current project was beneficial the PW-Exposed project and the field of science due to improvement of techniques and methods for studiying PAH body burden concentration in a new species of fish ELS exposed to low concentration of PW. Furthermore, the study conducted has revealed new information regarding bioaccumulation and sensitivity at different life stages of lumpsucker embryos. The PW-Exposed project is not finished and will be further investigated by SINTEF Ocean along with partners.

Sammendrag

Hensikten med denne bacheloroppgaven var å studere korrelasjonen mellom livsstadier, lipidsammensetning og bioakkumulering av polysykliske aromatiske hydrokarboner (PAH) i fiskearten rognkjeks (*Cyclopterus lumpus*). Arbeidet er en del av et prosjekt ledet av SINTEF Ocean kalt "PW-Exposed". "PW-Exposed"-prosjektet forsker på å få en detaljert forståelse av den kjemiske sammensetningen og den toksikologiske innvirkningen utslippet av produsert vann har på norsk kontinentalsokkel. Som en del av et større arbeid er denne bacheloroppgaven basert på eksperimenter med produsert vann og tidlige livsstadier av rognkjeks, med det formål å identifisere det mest sensitive livsstadiet i utviklingen av rognkjeks-emrbyo.

Offshore olje- og gassindustri bidrar årlig til et stort utslipp av produsert vann på norsk kontinentalsokkel. Produsert vann inneholder polysykliske aromatiske hydrokarboner (PAH-er) og alkyl PAH-er, som er kjente for å være toksiske mot fisk i tidlig livsstadium. Det ble studert rognkjeks embryoer eksponert for produsert vann på tre forskjellige livsstadier i embryonalutviklingen og overvåket til klekking. Eksponeringsforsøk, hvor opptak og utskilling av PAH-er og alkyl PAH-er ble observert, ble utført ved tre potensielt sensitive stadier; under fertilisering ("dep1"), 36 timer etter fertilisering ("dep2") og 10 dager etter fertilisering ("dep3"). Prøvetakning av embryoer ble gjort ved sju tidspunkt etter endt eksponering (0, 6, 12, 24, 48, 96 og 192 timer etter endt eksponering).

For å kunne evaluere bioakkumulasjonen av PAH, ble prøver med egg fra forskjellige prøvetaknings-tidspunkt homogenisert, ekstrahert med løsemiddel og ekstraktet ble renset ved hjelp av en fastfase ekstraksjon (SPE). PAH-er ble analysert ved hjelp av en gass kromatograf koblet med et tandem massespektrometri (GC-MS/MS). Fiskeembryo er lite forsket på innenfor dette feltet på grunn av at teknikker og metoder for studering av PAH body burden konsentrasjon i fisk i tidlig livsstadium nylig har blitt sensitive nok for små biotiske prøver (100 mg) eksponert for kontaminanter i lave konsentrasjoner. Det totale lipidinnhold i prøvene ble funnet ved å utføre en modifisert Folch ekstraksjon.

I dette prosjektet så man at rognkjeks-embryoene eksponert for produsert vann får en økt innvendig "body burden" konsentrasjon av PAH-er og alkyl PAH-er. I tillegg til å se på total PAH (tPAH) i embryo, hadde oppgaven fokus på tre komponenter; naftalen (2-ring PAH), C1-fenantren (3-ring alkyl PAH) og dibenzotiofen (3-ring PAH). Den høyeste akkumuleringen av PAH-er ble observert i embryoer eksponert under det tidligste livsstadiet (0 timer etter fertilisering), med en synkende body burden observert i embryoer i senere livsstadier (36 timer etter fertilisering og 10 dager etter fertilisering). Som forventet sank body burden i alle eksponerings scenarioene etter overføring til rent vann. Av komponentene i fokus hadde naftalen høyeste body burden, etterfulgt av dibenzotiofen og til slutt C1-fenantren i alle eksponeringsforsøkene. Totalt ekstraherbart lipidinnhold holdte seg jevt gjennom hele utviklingen av rognkjeks-embryoene, dette førte til at normalisering av body burden konsentrasjoner til lipidinnhold ga samme resultater som body burden konsentrasjonene for tPAH i hvert eksponeringsforsøk. Biokonsentrasjons-faktoren viste at dibenzotiofen hadde den høyeste bioakkumuleringen hos emrbyoer eksponert 0 timer etter fertilisering blandt komponentene i fokus, hos embryoer eksponert ved 36 timer etter fertilisering og 10 dager etter fetilisering er det naftalen som er den mest akkumulerte komponenten. Biotransformasjon og utvikling av embryoet påvirket resultatet. Videoanalyser på

rognkjeks-embryo gjennomført 17 dager etter fertilisering av Dr. Bjørn Henrik Hansen og Julia Farkas viste at embryoer eksponert under de tidligste livsstadiene (36 timer etter fertilisering og 10 dager etter fertilisereing) hadde en betydelig redusert hjertefrekvens.

Denne oppgaven har vært gunstig for "PW-Exposed"-prosjektet og dets forskningsområde på grunn av forbedringene av benyttede teknikker og metoder brukt for å studere PAH body burden konsentrasjoner i en ny type fiskeart eksponert for lave konsentrasjoner av produsert vann. Videre har studiet i oppgaven avduket ny informasjon angående bioakkumulering og sensitivitet ved forskjellige livsstadier hos rognkjeksembryoer. "PW-Exposed"-prosjektet er enda ikke fullført, og videre forskning vil bli gjort på dette feltet av SINTEF Ocean i sammarbeid med partnere.

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1 Abbreviations

AhR	Aryl hydrocarbon receptor
BB	Body burden
BCF	Bioconcentration factor
BSD	Blue sac disease
Cyp	Cytochrome P450
Cyp1a	Cytochrome P4501A
DCM	Dichlormethane
Dep	Depuration
dpe	Days post exposure
dpf	Days post fertilization
ELS	Early life stages
GC-MS/MS	Gas chromatography/tandem mass spectrometry
hpe	Hours post exposure
hpf	Hours post fertilization
HR	Heart rate
K_{ow}	Octanol-water coefficient
log K_{ow}	n-octanol-water partition coefficient
MRM	Multiple reaction monitoring
MS	Mass spectrometry
NCS	Norwegian continental shelf
PAH	Polycyclic aromatic hydrocarbon
PW	Produced water
scm	Standard cubic metres
SPE	Solid phase extraction
STD	Standard Deviation
tPAH	Total polycyclic aromatic hydrocarbon

2 Introduction

2.1 Background

Since the discovery of Ekofisk in 1969, the Norwegian economy have been highly dependent on the oil and gas industry. It is considered to be the main reason for the development of the current welfare system we have. World-wide, Norway is one of the largest provider of oil and gas. Norway is also a major provider of fish, due to the large coast which are well suited for fisheries activities [3].

The offshore oil and gas industry affect the marine environment by discharges of oil and produced water (PW). Annually PW contributes to a discharge of 130-150 million standard cubic metres (scm) in the Norwegian continental shelf (NCS) [4]. Discharges of oil and PW causes a release of compounds, for instance polycyclic aromatic hydrocarbons (PAHs) and alkyl PAHs. These compounds are known to be toxic to early life stages (ELS) of fish, such as lumpsucker (*Cyclopterus Lumpus*) embryos. The toxicological impact of PW on lumpsuckers are yet to be fully investigated. Recent studies shows that lumpsuckers uses the offshore oil and gas platforms as a habitat and spawning site [5]. This makes the lumpsucker appropriate for toxicity testing and environmental monitoring related to PW.

2.2 The current work

This current work is a part of the PW-Exposed project, lead by SINTEF Ocean. The PW-Exposed project investigates the chemical and toxicological impact of PW discharges from platforms along NCS. The purpose of this bachelor thesis is to identify the most sensitive life stage in the development of the lumpsucker embryo.

The toxicological impact PAHs have on lumpsuckers have not been fully investigated. The current work will look at the difference the impact PAHs have on lumpsuckers at three potentially sensitive stages. Looking at the difference of the affection PAHs and alkyl PAHs has at different time points through the development of the fish embryo is something that have never been done before. In addition to disclose the experiment, description of theory of produced water, polycyclic aromatic hydrocarbons, bioaccumulation, biotransformation and analytical techniques is embodied in the thesis. The report is compiled by the main purpose of this thesis, listed under:

- Investigation of the correlation between different life stages of PW-exposed lumpsucker embryos, (0 hours post fertilization (hpf), 36 hpf and 10 days post fertilization (dpf)).
- Investigation of PAH bioaccumulation and elimination in different life stages of PW-exposed lumpsucker embryos (0 hpf, 36 hpf, 10 dpf).
- Investigation of lipid composition in the different life stages of PW-exposed lumpsucker embryos (0 hpf, 36 hpf and 10 dpf).

3 Theory

3.1 Lumpsucker

The fish specie studied in this thesis, lumpsucker, is a semipelagic fish specie which is wildly distributed throughout the boreal region of the North Atlantic Ocean and the Barents Sea [6] [7]. The specie has been an important resource in the Norwegian salmon farming industry, being utilized as a "cleaning fish" [6]. Further, the lumpsucker is considered an ideal candidate for toxicity testing and environmental monitoring due to its characteristic [7]. Their robust egg shells is at interest as it can withstand mechanical stress. Another characteristic of the egg is its lipid-rich content, meaning the embryo has a high potential for accumulation of lipofilic pollutants, such as PAHs [7].

Several studies have shown that the lumpsucker spend much of its life far from land, but most workers have assumed that its distribution is inshore. The breeding occurs in the distributions area in the Norwegian sea, the Barent sea, the White sea and in the Greenland sea [8]. The spawning areas for lumpsucker are considered especially vulnerable to oil discharges as larvae and juvenile lumpsuckers are poor swimmers, having limited possibilities to escape potential oil spill and PW discharges [6] [5]. Lumpsuckers are harvested in winter/spring, and their availability is 3-4 times per year [9] [7].

Spawning occurs at 0-40 m depth [8]. After spawning, larvae and juvenile lumpsuckers remains in the costal area in shallow water, where they have access to seaweed. After a year they descend to deeper water - offshore. Furthermore, recent studies show that fish and invertebrate, such as the lumpsucker, also uses the offshore oil and gas platforms for a habitat and spawning site. The fact that the spawning finds place around oil spills, the lumpsucker is appropriate for toxicity testing and environmental monitoring [5].

Figure 2: **Lumpsucker fish caught in Blyth in the North Sea [1].**

3.2 Produced water

Worldwide, the NCS is probably the most studied area considering the environmental impacts of offshore oil and gas production. Discharges from oily water, primarily from the PW, are known to affect the marine environment negatively. As the biggest contribution, PW amounted 134 million scm in 2018 [4]. In 2007, annual discharge PW had a peak at 162 million scm. In the following years, the amount has varied between 130-150 million scm [4] [10].

PW refers to the water that accompanies the crude oil up to the platform. It contains an aqueous mix of formation water, injected water, oil and/or gas from the well. The formation water is the natural sea water that has been trapped in geologic formations holding oil and gas. Injected water is production chemicals, freshwater and brine water that sometimes are injected into the formations to increase the safety of operations and the recovery rates [11]. Various treatment technologies decrease the oil content in the PW before being discharged to the sea, but still, PW is one of the major sources of contaminants entering the sea [12]. The regulatory threshold allows up to 30 mg/L oil content in PW discharged to the sea from Norwegian installations. In 2017, the oil content averaged 12,1 mg/L across the NCS [4] [13].

PW discharges have been reduced over the years, thereof the quantity of oil spilled into the sea. Due to lack of knowledge of possible long term impact of PW, PW discharge have been a target for concern and ascendance considering the large overall discharge volumes [11] [13]. To monitor the ecological effects of PW discharges, several studies and experiment have been compiled [13].

The chemical composition of PW is complex, and contains a mixture of dissolved and particulate, organic and inorganic compounds [11]. The physical and chemical composition of PW varies widely from field to field, depending on the composition of the reservoir oil and gas phases, geochemistry of the hydrocarbon bearing formation, dept of the well, in addition to geologic age. To address the environmental risk from discharge from a specific well, a region study is needed [12]. Inorganic compounds reported in PW include salt and inorganic ions like sodium and chloride, magnesium, sulfate, iodide, potassium, bromide and bicarbonate [11]. The focus in this thesis will be on the organic compounds, more specifically, the PAHs. Other organic compounds reported in PW are saturated hydrocarbons, monocyclic aromatic hydrocarbons, in addition to oxygenated compounds, like ketones, acids and phenols.

PAH is given high priority due to its potential carcinogen effects, among several toxic and mutagenic effects [14]. It is therefore of high priority to environmental discharge regulation [13].

3.2.1 PAH in produced water

PAHs are aromatic hydrocarbons containing two or more benzene rings fused together in various configurations, and are considered hydrophobic molecules [15]. In PW, PAHs are of one of the compound groups of greatest environmental concern because of their known toxic effects to marine organisms. Alkyl PAH remains less studied than its unsubstituted congeners, although evidence suggest that they are even more toxic [11]. Studies shows a good correlation between toxicity to ELS of fish and the measured aqueous concentration of total PAH (tPAH) [16] [17].

Some studies have proven that PAHs cause mutagenic, carcinogenic and teratogenic effects on a wide range of organisms [18] [19].

PAHs containing up to four benzene rings are called light PAHs, while heavy PAHs contains more than four rings. Heavy PAHs are more stable then light, and are considered to be more toxic. The only two components in PAHs, hydrogen and carbon, are arranged in both complex and simple ring systems. The characteristics of different PAHs varies chemical, toxicological and physical depending on the arrangement of the rings. They can occur as a ring linkage pattern, however, most PAHs will occur as a hybrid comprising various structural components, naphthalene, pyrene, phenanthrene and dibenz(a,h)anthracene for instance [20]. Some PAHs are heteroaromatic compounds. This occurs when one of the ring carbons is replaced by a nitrogen, sulfur or oxygen atom (for instance pyridine, dibenzothiophene or dibenzofuran) [3].

The concentration of tPAH in PW will vary from field to field, but typically it ranges from 0,040 to 3 mg/L [11]. Even though heavy PAHs are considered the most toxic, lighter PAHs, such as the 2-rings and 3-rings PAH like naphthalene, phenanthrene and their substituted homologues, are more water soluble, hence tPAH concentration in PW primarily consist of them. Heavier PAHs, 4-6-rings, are rarely found in treated PW, and they are often associated with dispersed oil droplets because of their low aqueous solubilities [11]. For this reason, the thesis will focus on light PAHs, in which naphthalene (2-ring), dibenzothiophene (3-ring) and C1-phenanthrene (alkylated 3-ring) will be at focus. See Figure 3 for the structure of naphthalene and dibenzothiophene, and Figure 4 for the structures of C1-phenanthrene.

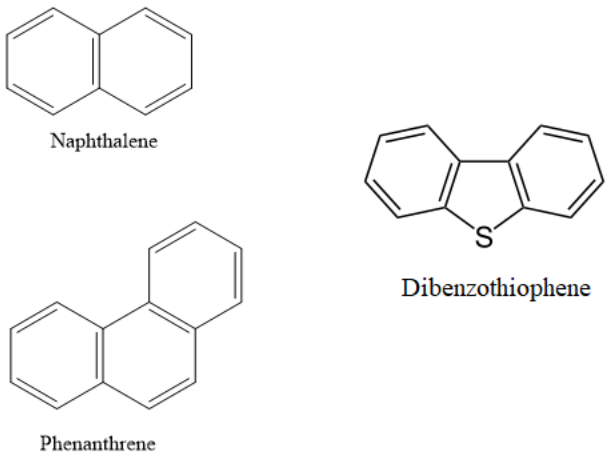


Figure 3: **Structure of naphthalene, phenanthrene and dibenzothiophene. Picture is made in paint.**

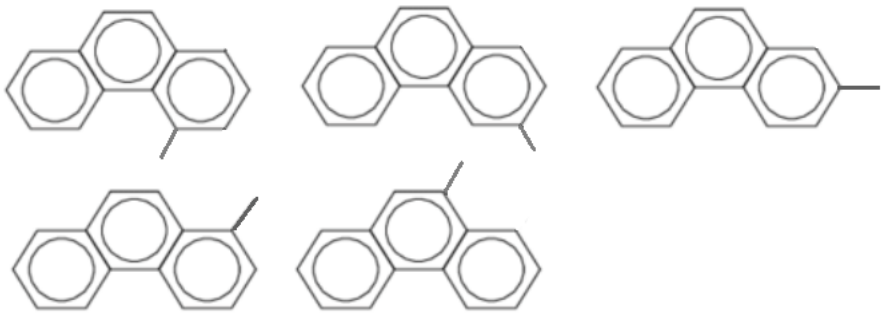


Figure 4: **Illustration of the different C1-phenanthrenes (1-metylphenanthrene, 2-metylphenanthrene, 3-metyl phenanthrene, 4-metylphenanthrene and 9-metylphenanthrene). Picture is made in paint.**

3.2.2 Toxicity effects of PAHs on fish embryos

The effects of PAH in PW on the environment has been reported in a high number of reviews and scientific papers, and many investigations conclude with PAH being of concern due to it's ecotoxicological effects to ELS of marine species [16] [17] [18] [19]. Fish embryos exposed to PAHs may show a characteristic suite of malformations, such as edema, spinal curvature, cardiac dysfunction and reduction in the size of craniofacial structures, the jaw for instance. Figure 5 shows a healthy lumpsucker compared to a PW exposed lumpsucker with this characteristic suite of deformities. In addition to the malformations, PAH exposed fish ELS may also display DNA damage, oxidative stress, embryotoxicity or cardiac function defects, reduction of the heart rate (bradycardia) and an irregular heartbeat (cardiac arrhytmia) [21]. Some PAHs are in a high priority class of contaminants because they are known to be potent carcinogens [13].

Figure 5: **Upper picture shows a healthy lumpsucker, bottom picture shows a PW exposed lumpsucker with jaw deformations, spine deformations, deformed suction cup and circulatory blockage of tail vein. The pictures are from another experiment preformed by SINTEF Ocean. (Photo: Bjørn Henrik Hansen, SINTEF Ocean.)**

Blue sac disease (BDS) is a disease related to PAH exposed fish ELS. BSD is associated with a lot of the malformitations mentioned above (e.g. craniofacial and spinal malformation, neuronal cell death, reduced growth, hemorrhaging, yolk sac edema, impaired swimming and early mortality) [22]. it is also often referred to as "dioxin-like" toxicity, as it often is seen in fish exposed to dioxins, polychlorinated biphenyls and PAHs [3] [23]. BSD is also associated with Cyp1a (cytochrome P4501A) induction.

The Cyp1a plays an important role in the metabolic activation of carcinogenic PAH [3]. PAHs are known to be an Aryl hydrocarbon receptor (AhR) agonists, meaning that they have high affinity to the receptor and activates it by binding themselves to it [13]. This binding initiates transcription of the Cyp1a gene, respectively leading to toxicity and cancer [15] [3]. For a more detailed description of the metabolism, see chapter 3.4.

Many teleosts have two AhR genes, AhR1 and AhR2, due to genome duplication. Mammals only have one AhR. The AhR1 in fish is the ortholog of the mammalian AhR, which has been shown by gene mapping and phylogenetic analysis. The AhR2 is the divergent, and appears to be the predominant form in fish. Mammalian AhR and fish AhR1 have some similarities, for instance their structure, high-affinity dioxin-binding, transcriptional activation of target genes and properties, such as binding to AhR response elements [24] [25].

Former studies have demonstrated that size and structural differences in PAH have different toxicity effect on ELS of zebrafish [21] [26]. Accordingly, the effects of the individual compounds of PAHs have been difficult to separate [3]. Incardona et al. [21] revealed that the three-ring PAHs, phenanthrene and dibenzothiophene, were adequate to cause the characteristic suite of malformations and genetic ablation of cardiac function. These defects have secondary consequences for late stages of kidney development, cardiac morphogenesis, formation of the craniofacial skeleton and neural tube structure. This toxicity were directly proportional to the amount of phenanthrene or dibenzothiophene in the mixture the fish embryos were exposed to. The study also showed that smaller petrogenic PAHs toxicity is often caused by an AhR independent pathway (see chapter 3.4 for a detailed description). [21]. Cardiac ion channels are critical for cardiac functions, such as rhythmicity and contractility [27]. If targeted by three-ring PAHs, embryonic cardiac defects might be caused in Cyp1a- and Ahr-independent ways, demonstrated by another study of Incardona et al. [24].

Incardona et al. [21] also described that the 4-ring PAH pyrene induced a different syndrome of anemia, neuronal cell death and peripheral vascular defects. A mild bradycardia was induced by naphthalene (2-ring), a 5-6 % reduction at 39 or 78 µM. Thus, different compounds of PAH have specific and distinct effects on ELS of fish [21].

3.3 Bioaccumulation of organic compounds in marine species

The term bioaccumulation is describing the uptake of a certain compound, or groups of compounds, in an organism or specified tissues, where the uptake is in relation to the exposure concentration and relative to inherent clearance rates [3] [28]. Direct uptake through water

(through the gills or the skin) is found to be the predominant bioaccumulation process for fish, uptake through ingestion of particles or food is also common [29] [28]. In embryos the uptake through ingestion is not present, though.

Bioaccumulation of organic compounds in an organism depends on environmental factors such as temperature, oxygen content, pH of the water and salinity [3] [28]. To the specific organism, factors like organism size, membrane permeability, ingestion rate, extraction efficiency and osmoregulation must be taken into account [3]. Passive diffusion across membranes is the main accumulation of dissolved fraction in the lipid phase of marine organisms. Hence, uptake and elimination in the organism might also be affected by its behavior, external stressors, nutrition etc. [3].

The hydrophobicity of organic compounds is expressed by the octanol-water coefficient K_{ow} [10]. The definition of *log* K_{ow} is the ratio of the concentration of a compound in n-octanol and water at equilibrium, whereof compounds with a high *log* K_{ow} have low affinity for water, and is directly proportional to molecular weight [30]. It is established that all compounds with a log K_{ow} above 3 have the potential to bioaccumulate. It is difficult to detect compounds with log $K_{ow} >$ 6-7, due to their restricted water solubility [10] [3]. A high K_{ow} indicates a high tendency to partition into the organic phase [31]. See Appendix 1 for the values of PAH compounds.

Is is suggested that the lipid in biological membranes in marine organisms is linked to the uptake of hydrophobic contaminants [3]. A study performed by Øverjordet et al. [31] where one lipid-rich and one lipid-poor stage of the arctic copepod (*Calanus hyperboreus*) were exposed to crude oil water-soluble fraction (WSF), found that the estimated steady-state bioconcentration factor (BCF) for the lipid-rich stage was much higher than for the lipid-poor, meaning that the bioconcentration potential was clearly higher for the lipid-rich stage [31].

3.3.1 Bioaccumulation of PAHs in fish embryos and larvae

Studies of PAHs and alkyl PAHs in fish embryos and other marine organisms have observed that bioaccumulation are directly linked to lipid content in biological membranes. For this reason it is by interest to find the concentration of tPAH in the lipid content. It is also observed that alkyl PAHs have an higher BCF than the parent PAHs in adult fish [3]. The toxicity of organic compounds is yet to be fully understood. Using the internal body residues have been suggested as a more effective admission than external surrounding concentration, when determine the environmental hazard of PAH [28]. By internal body residues means the accumulation fraction found through body burden [10].

In order to find the concentration of body burden in lipid content, the content of lipid in the body burden (BB) samples must be found through calculation (Equation 1).

$$Lipid\ content\ in\ BB\ sample = \frac{lipid\ weight}{egg_{lipid\ sample}} * egg_{BB\ sample}$$

(Equation 1)

Further, the lipid content in the body burden sample can be used to find the concentration of tPAH in lipid content (Equation 2).

$$C_{BB} = \frac{C_{tPAH}}{Lipid\ content\ in\ BB\ sample}$$

(Equation 2)

C_{BB} = Body burden concentration of tPAH in lipid content.
C_{tPAH} = Body burden concentration of PAH in given sample (ng/sample).

In PW, the majority of PAHs present are light PAHs, as they are the most water-soluble compounds [32]. Being highly water-soluble means the compounds accumulate less than the more hydrophobic PAHs (the heavier PAHs) since the uptake of PAH is related to the hydrophobicity of the PAH [33] [10]. Furthermore, the light PAHs are expected to metabolize to a great extent compared to more hydrophobic compounds [33]. Importantly, the more readily a compound is metabolized, the less it bioaccumulates [28]. A study preformed by Baussant et al. [10] found that naphthalene and its alkylated congeners represented the major fraction of tPAH in PW exposed fish tissue, followed by dibenzothiophene. Together they represented approximately 90 % of tPAH [10]. The content of PAH compounds depends on the type of PW, however, the the major concentration of PAH in marine organism consists of light PAHs as PW mostly consists of light PAHs.

Studies of PAH body burden concentration in ELS of fish have been unreported until recently due to the absence of techniques sensitive enough to handle such low concentrations and small samples like fish eggs. Consequently, ELS metabolism is yet to be fully investigated in which it affects the accumulation to the individual fish species differently. The correlation between increased development and increased metabolism is already noticed [3]. However, when previously not considering the ELS metabolism as a bioaccumulation factor, studies have shown Cyp1a induction has occurred as early as three days post fertilization (dpf) in haddock embryos [34]. The Cyp1a induction is reviewed in chapter 3.4.

3.3.2 Bioconcentration factor

The BCF is generally referred to as the relationship between the concentration of a compound in the organism (C_{BB}) and the surrounding medium, for instance water (C_W), when these are at equilibrium or so-called "steady-state". The BCF allows comparison of the accumulation of PAH in different organisms [3]. There are many ways to calculate the BCF, but in this thesis, the BCF will be calculated by Equation 3.

$$BCF = \frac{C_{BB}}{C_W}$$

(Equation 3)

C_W = Concentration of PAH in sea water solution.

When comparing literature data based on the BCF, considering the diversity of exposure designs, analytical methods and reporting methods applied is necessary information. While handling fish eggs, some studies choose to normalize the body burden concentration normalized to lipid weight, while others normalize it to dry weight or wet weight of the whole sample or per individual (per egg) [3]. The current study has normalized the body burden concentrations to lipid weight before calculating BCF.

3.3.3 Estimating bioaccumulation

Log K_{ow} is found to correlate well with BCF, meaning the coefficient express to which extent a compound bioaccumulates [3]. Devillers et al. [35] used seven linear and non linear models in order to calculate BCF based on log K_{ow}. The study found that smaller compounds (log $K_{ow} <$ 6) obtained with different models was equivalent, and that for log $K_{ow} > 6$, the bilinear models was superior to the other studied models [35]. A difficulty with the study by Devillers et al. [35] is that the study disregarded any influenced by the biotransformation in the species [35]. Franke et al. [36] proposes that basing the bioaccumulation on log K_{ow} alone is inadequate. This is supported by others, because some processes are not included in these models, such as uptake- and elimination kinetics, uptake process, cell membrane diffusion and metabolism rate. Additionally, a linear relationship between lipid uptake and log K_{ow} is not a describing model for PAH bioaccumulation [3]. The models do not simulate the bioconcentration of the contaminants in the marine biota, as they ignore the biotransformation of PAHs and other chemicals.

However, the plot of BCF based on K_{ow} is currently the most competent. Devillers et al. [35] found that the correlation between log K_{ow} and BCF for compounds with a log K_{ow} below 6 have been established as successful. The study have compared seven models calculating BCF based on log K_{ow} with the same results, where the BCF values calculated by the linear models are equivalent to those experimentally observed [35]. Though, the exact relationship is not known [3]. As the elimination process is the most challenging factor to model, a suggestion is assumed; by measuring the net elimination rate and the levels of metabolites in an organism one might be able infer this factor. Although there are currently no tools available to differ between the different elimination processes [3].

3.4 Biotransformation of PAH

Biotransformation is a metabolism where xenobiotics get detoxified and transformed into more easily excreted compounds in organisms [3]. Xenobiotics is another word for chemicals foreign to the normal metabolism of an organism. Without the biotransformation, several xenobiotics would reach toxic levels in an organism [37]. Further, the biotransformation depends on the organism

and the compound type. While some organic compounds are resistant to metabolism, some compounds are more easily metabolized - PAHs and alkyl PAHs belong to the latter [3].

The PAH biotransformation of parent compounds influence the elimination of PAHs, as well as driven diffusion or rate of excretion [10] [38]. Parent PAHs are more able to diffuse through the gill membrane than polar PAH metabolites transformed during the metabolism, hence parent PAHs is favored for this elimination and elimination by excretion is favored for the polar PAH metabolites [38]. The metabolism of PAHs involves several reactions, like oxy-reduction, hydrolysis and conjugation - primarily to increase the polarity, the water solubility and facilitating the excretion of the oxygenated products [39]. More detailed description of the enzymatic reactions further down in this chapter.

Studies of metabolism of PAH in fish embryos have been underreported so far. Both small samples and use of radiolabeled chemicals in the uptake studies have been an issue, in which radiolabeled chemicals lead to co-determination of PAHs and their metabolites [3]. Studies of crude oil exposed pink salmon and pacific herring ELS, studied by Mathew et al. [40], observed the decline in PAH body burden over time. As the larvae develop (the metabolism included) some adult fish is reported to have eliminated up to 99 % of parent PAH during a day being exposed [3]. When determining exposure doses the biotransformation of xenobiotic compounds is the most perplex and confusing factor [3].

Barron et al. [41] found that alkyl PAHs turned out to be better AhR agonists than non-alkylated, and that heavier PAHs (5-6 ring) were more active agonists than lighter [41]. The AhR initiates transcription of many genes, such as Cyp and Cyp1a, once it is activated. The Cyp1a genes activity often causes oxidative stress and cellular damage, this leads to acute toxicity [3]. The process of the activating of Cyp1a gene produces hydrophilic PAH metabolites by degradation, and is controlled by enzymatic systems [10]. These hydrophilic metabolites is eliminated by excretion as they are less able to diffuse through membranes [3]. The products from PAH metabolism might be more reactive and develop to products with toxicity effects [39].

Some studies show that specific toxicity mechanisms can be divided into two groups: 1. Toxicity induced by activation of the AhR, and 2. Toxicity induced by pathway circumventing the AhR [3]. Frohlish et al. [39] shows that mechanisms for metabolism by enzymatic activation of PAHs, consist of three pathways: 1. CYP1A1/1B1 and epoxide hydrolase pathway (pathway 1) 2. CYP peroxidase pathway (pathway 2) 3. aldo-keto reductase pathway (pathway 3) (see Figure 6) [39]. The figure has taken benzo(a)pyrene as an indicator of PAHs, a commonly used indicator when studying the toxicity of PAH [39].

Adduction of DNA, that might lead to DNA mutations, is caused by pathway 1. This process of PAH metabolism occurs in both invertebrates and vertebrates, in which it occurs in three enzymatic reactions [39].

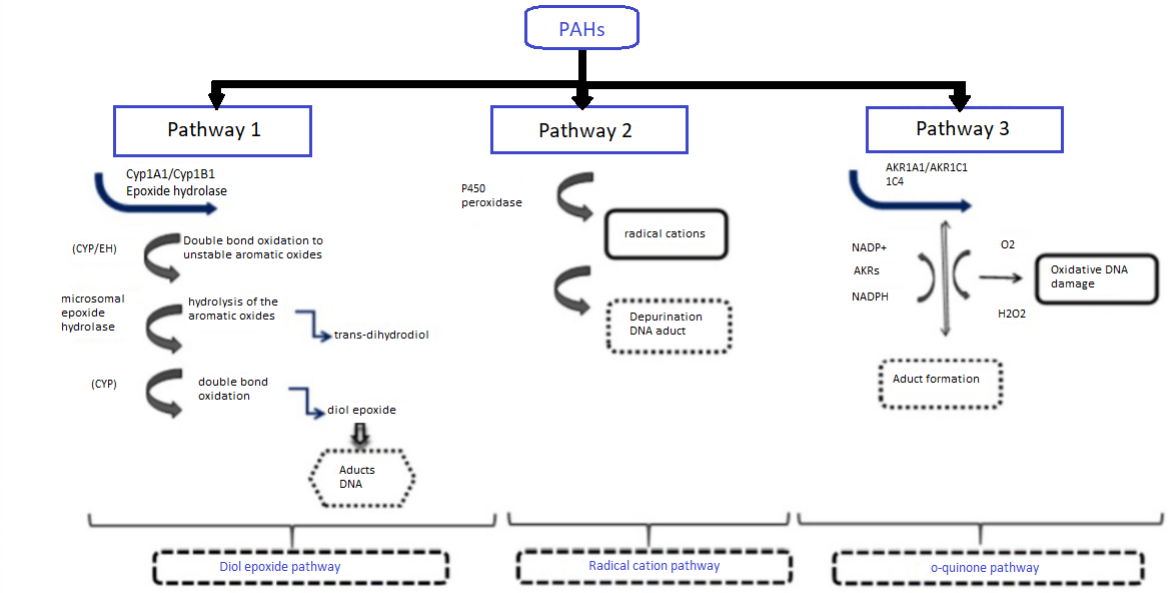


Figure 6: **Illustration of the major pathways for PAH metabolism and potentially harmful mechanisms [39]**

The enzymatic reactions

In the first reaction of the metabolism of PAHs, functionalization with hydroxy or epoxy groups are used to form hydroxylated PAHs, also known as reactive oxygen species (ROS). Cyp monooxygenase may catalyze this transformation, depending on the species. In Figure 6, Cyp catalyze double bond oxidation resulting in unstable aromatic oxides [39]. Reacting functional groups, -OH, -NH2, -COOH for instance, is added to the PAH molecule, resulting in unstable aromatic oxides. ROS are known to damage DNA, proteins and membranes [3].

Secondly, the hydroxylated PAHs are then transformed further by conjugation reactions. In Figure 6, the conjugation presented is hydrolysis by the microsomal epoxide hydrolase, formating the hydroxylated PAHs to trans-dihydrodiol. The PAH conjugates formed are highly water-soluble, sulphates for instance. From here most PAHs, like trans-dihydrodiol, will be excreted after conjugation. The rest of them will be further transformed by a double bond oxidation, catalyzed by Cyp. This process generates the diol-epoxide, leading to adduction of DNA as the diol-epoxide covalently binds to DNA [3] [39].

In biotransformation of xenobiotics the liver is accordingly the main organ involved in almost all organisms [3]. Hence, studies of PAH metabolites in fish bile have proven that some metabolites can be directly excreted in bile (via the gallbladder) as unconjugated hydrophobic metabolites [42].

3.5 Analytical techniques

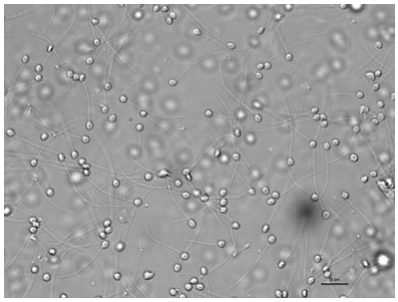
3.5.1 Pre-treatment and sample storage of lumpsucker

To achieve successful results, the treatment of biological samples is important. The most important factors are sampling strategy, preservation and storage conditions. Small biota samples are sensitive to issues like contamination, decomposition and loss of analytes. Following, biological samples should be kept in temperature controlled and cleaned containers and prior to treatment, minimal handling of samples should occur [43].

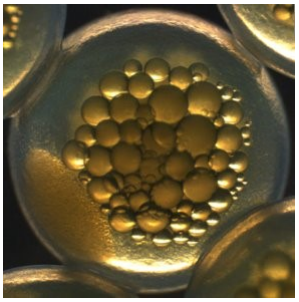
3.5.2 Exposure experiment

Based on earlier lumpsucker experiments performed at SINTEF Ocean, the importance of a continuous flow of sea water has turned out to be a determining factor of hatching success. Henceforth, a flow-through system is necessary for such experiments [44]. The exposure solution utilized in the experiment is another factor affecting the results and hatching success. By choosing an exposure concentration of PW low enough low enough to gain hatching success, the experiment is able to investigate the sensitivity of the fish embryos. Earlier lumpsucker experiments performed by SINTEF Ocean have already investigated the hatching success of lumpsucker embryos were exposed to PW of different concentrations, where a concentration of 20 µg/L achieved hatching success [44].

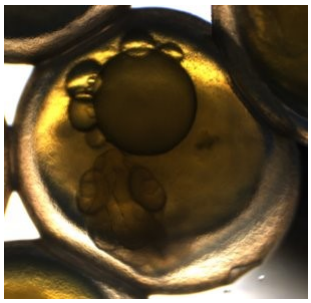
The times of exposure are selected in order to evaluate potentially sensitivity in different ELS of the lumpsucker. The first exposure at 0 hpf (dep1) gives the opportunity to evaluate the sensitivity during fertilization, while the exposure at 36 hpf (dep2) gives the opportunity to evaluate the sensitivity once the eggs become hard. The last exposure is at 10 dpf (dep3), with regard to the heart beat, whereof predicted start will be around day 9 or 10 [44]. The figure below (Figure 23) display potentially sensitive stages.



(a) During fertilization: Milt from lumpsucker.



(b) Embryo 36 hpf.



(c) Embryo 10 dpf.

Figure 7: **Potentially sensitive stages. hpf = hours post fertilization, dpf = days post fertilization. (Photo: Dag Altin, BioTrix (a), Bjørn Henrik Hansen, SINTEF Ocean (b and c)).**

3.5.3 Body burden

The definition of body burden is an organism's internal time-varying exposure concentration of a pollutant, and it is determined by the uptake and elimination balance. The dynamic balance between uptake and elimination is influenced by factors like type of species, type of compound, metabolism, temperature and ventilation rates [45].

Considering the environmental risk of chronic discharge, a realistic approach would be to look at estimated effects based on body burden in marine organisms ex situ, rather than looking at the external sediment or water concentration. The internal body concentration is used to study toxic effects of PW and crude oil [45]. Sensitive and precise analysis of low concentration of PAHs and alkyl PAHs accumulated in the lumpsucker eggs is necessary to fully understand the toxicity. [46] In many cases, the only way for the chemicals to exert their toxic effect, are if they enter the body. The uptake rates and bioaccumulation levels of substances in the tissues can relate to toxicity, and therefore the body burden and toxicity relationship can be used to estimate the impact [45].

Currently there are several existing methods established for extraction and analysis of accumulated organic pollutants in larger biota samples (sample size >1g). Downscaling of the method to be suitable for smaller samples (< 0,1g) seems to be a challenge. In ecotoxicity experiments the sample size is normally limited because the target organism is small (for instance

fish embryos). Economy and environmental perspectives, in addition to limited sample size, makes techniques on micro-extraction for biotic samples desirable, the consumption of solvents and chemicals needed for sample preparation will be reduced [43].

The method used in this project consist of extraction and clean-up of PAH in lumpsucker egg samples, a method for quantitatively results. A challenge associated with analysis of lipid rich biota samples are co-extraction of naturally occurring, non-target biological compounds. These compounds can cause negatively impact on the GC separation and resolution of target analytes and must be removed. Hence, a clean-up step is added. Though it is a challenge to develop an efficient clean-up method, due to the matrix components, especially for organic compounds in lipid rich matrices. They are in many cases chemically similar to the desired analyte, regarding molecular size, polarity and functional groups [43]. In this project, a SPE column comprising silica (5 g) were used for the extraction clean-up. SPE columns are time and cost efficient, in addition to a being a reproducible method and provides a clean extract and trueness [47]. Prior to analysis there is a need for solvent concentration, and this is obtained by evaporation approaches. This includes a heat block and/or a stream of inert gas (typically N_2). This evaporation can cause a loss of volatile compounds, such as naphthalenes [43].

Traditionally, crude oil and PW analysis is performed by a gas chromatograph coupled to a universal detector (for instance a flame ionization detector) or a mass spectrometer. For known target molecules analysis, and low concentration samples with challenging matrices, it have become more common with GC coupled with tandem mass spectrometry [3].

3.5.4 GC-MS/MS

A variety of techniques have been used for determination of alkyl PAH and their unsubstituted homologous, and GC-MS (gas chromatography coupled with a mass spectrometry) has been one of the most common techniques [48]. Recently, GC-MS/MS has become more common due to its sensitivity. Tandem mass spectrometry is an analytical technique that is well suited to detect contaminants at trace levels in challenging matrices [3]. Compared to the GC-MS method, which needs several grams of biotic sample, the GC-MS/MS method allows analysis of very small biotic sample sizes (100mg) to obtain the same sensitivity using low levels of analyte. GC-MS/MS has been clearly proven as the most applicable method as it is able to analyze tPAH body burden below 0,5 µg/g [48]. Sørensen et al. [46] reveals that GC-MS/MS is suitable detecting PAHs in crude oil exposed fish eggs [46]. As well as crude oil, PW contains petrogenetic compounds, and therefore this method is suitable for detecting PAH and alkyl PAH in PW exposed eggs [33].

GC-MS/MS is a gas chromatograph coupled with a tandem mass spectrometer (quadrupole mass spectrometer). The tandem mass spectrometry consist of two mass analyzers coupled in series (see Figure 8). The ion source ionize the analyte molecule. In the first mass analyzer, the molecular fragmentation ions from the ionizing are separated, and these molecules are in the collision cell bombared with an inert gas (commonly used are N_2 and Ar; in this project N_2 was used) to subject to further fragmentation. In the second mass analyzer, the fragments are separated prior to detection. Every compounds show a specific fragmentation pattern throughout the analysis, which leads to a selective detection. In recent years, the GC-MS/MS method have been developed.

The detection limits GC-MS/MS offers are considerably improved over GC-MS instruments. Other advantages with GC-MS/MS are increased signal-to-noise ratio and the possibility to filter out background noise. Several improvements have been accomplished the last two decades, such as increased sensitivity of analytical instrumentation; mass detectors are more efficient and by higher resolutions, volume injectors are larger (for instance the programmable temperature vaporizer) and back-flushing by post-column to remove unwanted matrix components. The limit of detection (LOD) for trace analytes have been significantly lowered, due to these improvements. The quantification in GC-MS/MS is based on fragmentations from the molecular ions [3] [49].

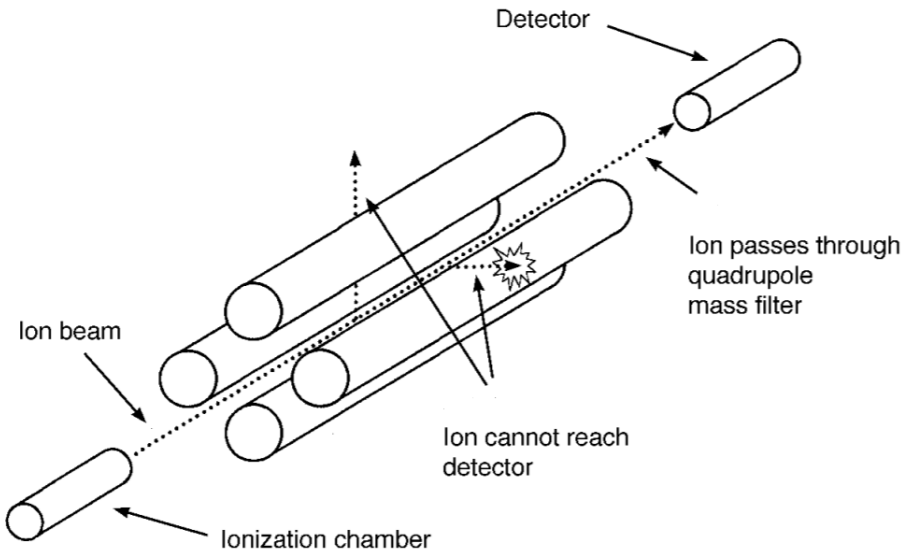


Figure 8: **Quadrupole mass spectrometry [2].**

As there is not enough available analytical standards to quantify each alkyl PAH in body burden of PW exposed organisms, one have to consider a wider range of analytes. These are commonly referred to as alkyl PAH clusters. As the GC-MS/MS method has been developed, it can determine several alkyl PAH clusters by multiple reaction monitoring (MRM). This method take advantage of the individual homologues' specific MRM transitions to identify them. Currently, this method gives the most precisely quantification of an alkyl cluster as the sum of all the integrals is based on the MRM transitions for the fragmentation of the molecular ion ($[M]^+$) in order to form the dominating product for different types of Cx-PAHs; $[M–X]^+$ [48].

In the current study 22 alkyl PAH clusters, whom are commonly analyzed in PW and crude oil derived samples, were looked at. The alkyl clusters are as following: C1-C4 naphthalenes, C1-C3 fluorenes, C1-C4 phenanthrenes, C1-C4 dibenzothiophenes, C1-C3 pyrenes and C1-C4 chrysenes.

The definition of LOD is the average concentration in blanks + three times standard deviation (STD) [3]. LOQ (limit of quantification) is defined as the lowest level in calibration curve where signal to noice ratio was >10. LOQ = 3 x LOD [46]. The values of LOD and LOQ are given in table S2, Appendix 2, given in concentration (ng/sample). In order to compare the LOD/LOQ values to the concentrations of PAHs and alkyl PAHs in dep1 (Table S3, Appendix 3), dep2 (Table S4, Appendix 4), dep3 (Table S5, Appendix 5) and ctrl (Table S6, Appendix 6), one have to divide the concentration (ng/sample) with the number of eggs. See Equation 4:

$$C_{PAH/egg}\left(\frac{ng}{egg}\right) = \frac{(^{ng}/_{sample})}{\#egg}$$

(Equation 4)

3.5.5 Lipid extraction

The method for lipid extraction used in this project is called the Folch extraction, and was established in 1957 [50]. Since then it has been one of the most popular method for isolating lipid from biological samples, by taking advantage of the biphasic solvent system of chloroform/methanol/water [51]. It is a simple method for isolation and purification of total lipides from lumpsucker tissues. This technique involve homogenizing the lumpsucker tissue with a mixture of 2:1 chloroform-methanol, and the extract being washed by addition of 0,2 times its volume and 0,9 % NaCl in Milli-Q water. In the washing procedure, the proportions between chloroform, methanol and water are 8:4:3 by volume, considering that the extract contains all the water from the tissue. It is important that this proportion is kept constant. After the washing the resulting mixture is allowed to separate into two phases, and the lower phase is the total pure lipid extract [50].

4 Experimental section

The work described in chapter 4.1 and 4.4 is performed by SINTEF staff, while the work in chapter 4.2 and 4.3 is performed by the students.

4.1 Exposure experiment

The principle of the current experiment is based on earlier experiments of lumpsucker. The exposure lasted for 48 hours, and after the exposure the eggs was placed in a recovery rig for depuration. The recovery rig had a flow-though system. Lumpsucker eggs and sperm ("milk") was obtained from Marine Harvest. The eggs was transported on ice to the Sealab, where the fertilization of the eggs occured on the same day as the arrival. The exposure concentration contained nominally 20 µg/L for all of the exposure experiments, excluding C0-C3-naphthalene [44].

The selected time points of sampling was 0, 6, 12, 24, 48, 96 and 192 hpe. The last sampling was 720 hpf, being the tentatively hatching day, and the possibility that the eggs hatched before was present. Still, sampling right ahead hatching would come to benefit when analyzing the accumulation and elimination of PAH.

4.1.1 Preparation of exposure solutions

The preparation of PW extract exposure solutions was performed the day before each exposure experiment. Before each exposure the total extract was reconstructed and diluted.

DCM extract (7 mL of 250 mL total) was spiked in the bottom of a glass bottle (2 L). The glass bottle was pre-rinsed with MilliQ-water (three times), dried (50 °C) and autoclaved. The bottle was placed in a wather bath (40 °C) and an inert gas of N_2 was blown into the bottle for 30 minutes.

Further, the bottle was filled with the appropriate volume (1,4 L) of sterile filtered seawater and placed on an ultrasonic bath (most powerful, filled maximally with water) for 30 minutes. During the re-construction, the bottle was shaken gently 3 times.

Following, dilutions (2 µg/L) was made in 7 x 1 L bottles. This was done by mixing 200 mL stock and 800 mL sterile filtered seawater. The seawater used for the dilutions was pre-aerated (with sterile air by bubbling trough a glass pipette for 5 minutes). To transfer the amount of stock to each bottle, pre-cleaned cylinders was used.

Chemical characterization of the stock solution was done by removing a sample (200 mL) from one bottle - this sample was acidified and extracted in according to SINTEF standard operation procedures.

The solutions (20 µg/L) were placed at 10 °C over night to acclimatize.

4.1.2 Preservation and recovery system

The experiments were conducted in a temperature-controlled room (10°C) using a hatching tank, also referred to as a recovery rig, that contained 112 separate chambers. The tank had a continuous flow of sea water (5L/min, 10°C). The flow of sea water went through a filter (1 µm filtered) before flowing into the rig.

The recovery system consisted of a tank with 16 rows and 7 columns of holes. See Figure 9 below. Each hole held a recovery chamber, which meant a tube where both ends had a plankton mesh. For this reason, the recovery chambers could be submerged into the water, and vice versa. The recovery chamber suited 60 eggs (1 unit).

Figure 9: **Photo of the recovery rig. After exposure, the samples were placed in the recovery rig for depuration. (Photo: Bjørn Henrik Hansen).**

4.1.3 Fertilization and exposure

All eggs were fertilized the day they arrived. The total amount of eggs was 60 eggs x 128 units, comprising four replicates to each sampling timepoint. The selected time points for the three exposure experiments were at 0 hpf, 36 hpf and 10 dpf. Each exposure lasted 48 hours with replenishing of exposure solution after 24 hours.

The procedure below applies to the exposure experiment 36 hpf (dep2), 10 days post fertilization (dpf) (dep3) and the controll samples. The first exposure experiment, 0 hpf (dep1), was performed during fertilization and the amount of eggs required were 60 eggs x 28 units. The exposure experiment 0 hpf (dep1) has its own procedure where the exposure of PW is performed

during fertilization (see next paragraph).

The fertilization procedure involved placing the eggs in a separate jar and fill it with filtered seawater (100 mL) and spermia (100 µL), then gently mix the solution for two minutes. Next, the eggs were spread across a plate holding circle templates whereof desired amount of eggs fitted. In short time, the eggs would stick to each other and form firm circles. After 30 minutes the egg circles were placed in individual recovery chambers in the recovery rig until the exposure.

Exposure during fertilization
The eggs were placed into a separate glass jar, in which the exposure solution (100 ml) and spermia (100 µL) were added. The solution was gently mixed for two minutes, then the eggs were spread across a premade plate holding circle templates whereof desired amount of eggs fitted. In short time, the eggs would stick to each other and form firm circles. As the exposure would last for 48 hours, 32 premade caviar glasses consisting fresh exposure solutions were already placed in a box filled with filtered sea water. After 30 minutes, the eggs had sticked together and a circle of eggs were placed in each glass to continue the exposure. 24 hours after the fertilization, the solution was replenished by gently pouring out the solution and refill the glass with the same exposure concentration of PW (semi-static regime).

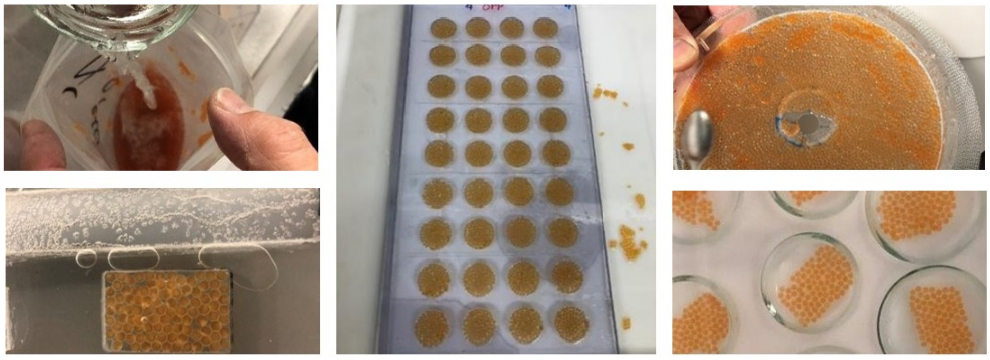


Figure 10: **Photos of the fertilization of lumpsucker eggs. (Photo: Bjørn Henrik Hansen, SINTEF Ocean)**

Exposure 36 hours post fertilization
36 hours post fertilization (hpe), the second exposure started, referred to as dep2. Eggs from the second row of the recovery rig (see Figure 9) were placed in caviar glasses holding fresh exposure solution. Like dep1, they were kept in the solution for 48 hours with replenishing of the solution after 24 hours in the same manner as for dep1 (semi-static regime).

Exposure 10 days post fertilization
10 days post fertilization (dpf), the third exposure started, referred to as dep3. Eggs from the third row of the recovery rig were placed in caviar glasses holding fresh exposure solution. Like dep1 and dep2, they were kept in the solution for 48 hours with replenishing of the solution after

24 hours in the same manner.

Control samples
The control samples, being unexposed to the exposure solution of PW, were fertilized the same day as the the rest of the samples along with dep2 and dep3. The samples were placed in the recovery rig right away, staying in the recovery rig the entire period. The sampling timepoints were in relation to the sampling timepoints of the samples exposed to PW.

4.1.4 Sampling procedure

After 48 hours of exposure, the first sampling found place. This sampling was called '0 hpe'. The rest of the samples were transferred to the recovery rig for depuration. The other samples were taken after 6, 12, 24, 48, 96, 192 hpe, and 720 hpf. The control samples were taken 0, 48, 96, 240, 288 and 720 hpf.

When sampling, triplicate samples were taken from the specific row, including triplicate controls. The fourth replicate was left behind in the recovery rig for the investigation of the effects on survival (16 dpf) and the hatching success. The sample was placed in a petri dish, where the sample was divided in three; 1. Body burden analysis (tentatively 25 eggs), 2. lipid analysis (tentatively 10 eggs), 3. The rest of the sample (to another analysis going to be performed by another student). Dead and unfertilized eggs was removed.

Prior to transferring into sterile vials, a photo was taken of the selected eggs and the tube next to each other, with the sample name visible. The photo was taken with regard to be able to count the eggs in each sample. The tube was flash-freezed in liquid nitrogen once the sample had been put into it. After the sampling was completed, the tubes were transported to a freezer and stored at - 80°C prior to extraction.

4.2 Body Burden extraction and clean up-SPE

4.2.1 Reagents, solvents and equipment

All reagents used during the experiments in this project was purchased from Teknolab, Sigma-Aldrich and VWR. The glassware and Na_2SO_4 were baked at 450 °C for 3 hours to remove any organic residue. Volumetric equipment, glass tops, teflon inserts, spatulas and other equipment which cannot be baked, were washed thoroughly three times with DCM before usage.
Chemicals

- 10 %DCM in *n*-hexane (Suprasolv)
- 50 % DCM in *n*-hexane (Suprasolv)
- DCM
- Na_2SO_4 (baked)
- SIS PAH A703 (see Appendix 7 for content)
- RIS-standard A705 (see Appendix 7 for content)

Equipments

- IKA T10 basic homogenizer workcenter
- Vortexer (e.g. Labinco or Lab dancer)
- Centrifuge with fittings
- Chromabond SPE columns (SiOH, 3 mL, 500 mg, glass column)
- Vacuum manifold for SPE
- Equipment for evaporation with heat block/bath and N_2 blow down

Body Burden extraction
The entire sample, tentatively consisting of 25 lumpsucker eggs, was weighted in a glass vial (4 mL), then the weight was zeroed. The sample was transferred to a kimax tube (12 mL), the glass vial was weighted again and the weight loss was noted. The weight loss equals the weight of the sample. 50% DCM in *n*-hexane solvent (4 mL) was then added to the sample using a pipette. A surrogate internal standard (SIS) (SIS-ID: A703, 100 µL) was added using an injector. Na_2SO_4 (0,1-0,2g) was added to the mixture, and then the eggs were crushed using a glass rod. The glass rod was cleaned with DCM within every new tube. After this, the sample was mixed with a vortexer for 30 sec, and then centrifuged (2 min, 2000 rpm). The supernatant was transferred into a new glass tube (12 mL). 50 % DCM in *n*-hexane solvent (2 mL) was added to the sample tube using a pipette. The sample was mixed on a vortexer and then centrifuged again (2 min, 2000 rpm). This step was repeated two more times to make sure the salt pellet had been washed of. The supernatant was transferred to a new kimax tube (12 mL) between every centrifugation. The combined extract was evaporated to $\sim$ 0,5 mL under a gentle flow of an inert gas (N_2) before the next step.

Clean-up-SPE
Chromabond solid phase extraction (SPE) columns (SiOH, 3 mL, 500 mg) was conditioned with *n*-hexane (6 mL) under vacuum. Importantly, the column could not run dry. The sample was transferred to the column and cleansed out of the sample tube with *n*-hexane (2-3 mL). Then the sample was eluted with additionally 10 % DCM in *n*-hexane (3 x 2 mL), and then evaporated to approximately 0,2 mL. The sample, including wash volume, was transferred to a GC-vial. Next step, immediately prior to analysis, the volume was adjusted to 400 µL through evaporation and a recovery internal standard (RIS) was added (RIS-ID: A705, 100 µL).

4.2.2 PAH and alkyl PAH analysis

An Agilent 7890B gas chromatograph coupled with an Agilent 7010B triple quadrupole mass spectrometer fitted with an electron ionization source and a collision cell (GC-MS/MS) was used for the analysis of PAH and alkyl PAH (Agilent Technologies, Santa Clara, CA, USA). The technique consist of two mass analyzers, whereof two Agilent J&W HP-5MS UI GC-columns (30 m x 0,25 mm x 0,25 µm) coupled in series through a purged ultimate union.

Figure 11: **The pictures show the gas chromatography–tandem mass spectrometry analysis instrument, placed in the laboratory at SINTEF Ocean (Photo: Kaja Skarpnord 30.04.19).**

Helium was used as a carrier gas. The gas was of high purity (6,0) and at constant flow (1,2 mL/min). At 310 °C, samples (1 µL) were injected splitless. A temperature grading program was set, the oven was kept at 40 °C for 1 min, then the temperature increased by 40 °C/min until it reached 110 °C, and then it increased by 6 °C/min to 220 °C, and finally by 4 °C/min to 325 °C. For 5 min the temperature was held at 330 °C, and the first column was back-flushed. The ion transfer line temperature was kept at 300 °C, the ion source temperature was 230 °C and the quadrupole temperature was 150 °C. The electron ionization ran at 70 eV.

As a collision gas, N_2 was used at a flow of 1,5 mL/min. As a quench gas, helium was used at a flow of 2,25 mL/min. To identify the target analyte (PAH/alkyl PAH) two unique MRM transitions were used, and quantified by the most intense peak. MRM was used to determine alkyl PAH clusters, using transitions from the molecular ion (as described in chapter 3.5.3). To monitor the system performance, standards were run for each 12 sample injections, a variation of 25 % was accepted, and no more. [48]

To quantify parent PAH, a quadratic regression of a 12-level calibration curve (0,01-250 ng/mL) was used. Alkyl substituted PAH homologue groups were quantified by the response factor calculated for a methyl-substituted PAH reference compound [48]. When quantifying the C1-phenanthrene cluster, the integrals of the five different C1-phenanthrenes are summarized [7].

Further, Agilent MassHunter Quantitative analysis b.08.00 were used to calculate the integrals of the chromatograms. The program provided the concentrations of PAHs and alkyl PAHs in the samples. The compounds analyzed are listed in Table 1.

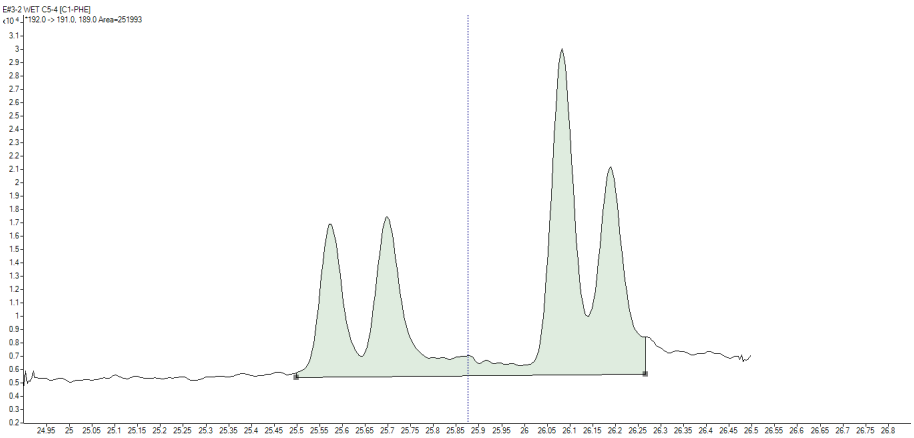


Figure 12: **Illustration of a C1-phenanthrene chromatogram from MassHunter Quantitative analysis. The picture is a screenshot from the program.**

Table 1: **The table shows all the compounds analyzed with the GC-MS/MS in this bachelor project. NAP = naphthalene, FLU = Fluorene, PHE = phenanthrene, FLA/PYR = fluoranthene/Pyrene, CHR = chrysene.**

	Parent PAH	**Alkylated PAH**
2-ring	- Benzothiophene - Naphthalene - Biphenyl	- C1-NAP - C2-NAP - C3-NAP - C4 NAP
3-ring	- Acenaphthylene - Acenaphthene - Dibenzofuran - Fluorene - Phenanthrene - Anthracene - Dibenzothiophene	- C1-FLU - C2-FLU - C3-FLU - C1-PHE - C2-PHE - C3-PHE - C4-PHE - C1-DBT - C2-DBT - C3-DBT - C4-DBT
4-ring	- Fluoranthene - Pyrene - Benzo[a]anthracene - Chrysene	- C1-FLA/PYR - C2-FLA/PYR - C3-FLA/PYR - C1-CHR - C2-CHR - C3-CHR - C4-CHR
5-ring	- Benzo[b]fluoranthene - Benzo[k]fluoranthene - Benzo[e]pyrene - Benzo[a]pyrene - Perylene - Dibenz[ah]anthracene	
6-ring	- Indeno[1,2,3-cd]pyrene - Benzo[ghi]perylene	

4.3 Lipid extraction by Folch method

4.3.1 Reagents, solvents and equipment

All reagents used during the experiments in this project was purchased from Teknolab, Sigma-Aldrich and VWR. The glassware were baked at 450 °C for 3 hours to remove any organic residue. Volumetric equipment, glass tops, teflon inserts, spatulas and other equipment which cannot be baked, were washed thoroughly three times with DCM before usage.
Chemicals

- 2:1 chloroform-methanol solution
- 0,9 % NaCl in MilliQ water
- Isopropanol

Equipment

- IKA T10 basic Ultra Turrax with IKA S10N-5G stainless steel knife (0,5 cm diameter)
- Vortex
- Eppendorf pipettes
- Heat block with N_2 evaporator
- Centrifuge with inserts for 12 mL kimax tubes

4.3.2 Lipid extraction

The entire sample, tentatively consisting of approximately 10 lumpsucker eggs, was weighted in a glass vial (4 mL). The weight was zeroed, and the sample was transferred to a kimax tube (12 mL). The glass vial was weighted again, and the weight loss was noted. The weight loss equals the weight of the sample. 2:1 chloroform-methanol solution (4 mL) was added using a graded glass pipette. The sample was homogenized using a homogenizator knife (1 minute, 20 000 rpm). The homogenizator knife was cleansed with 2:1 chloroform-methanol solution between every homoganizations.

After the homogenization, the sample was centrifuged (10 minutes, 2 000 rpm), followed by transmission of the supernatant to a new kimax tube (12 mL). It was important that the precipitate did not follow. According to the Folch extraction, this step was done once. Premeditatedly this step was done twice after some testing, for a better result.

Using an Eppendorf pipette, 0,9 % NaCl in MilliQ solution (1 mL) was added to the organic extract. Then the sample was centrifuged (5 minutes, 2 000 rpm). The upper aqueous phase, including potentially remaining precipitate, was then removed. The organic phase was transferred into a new kimax tube, and then evaporated to dryness in a heat block under a gentle flow of N_2. The tube with lipid was weighted after reaching room temperature. The lipid were washed out from the kimax tube with isopropanol, and transferred to GC-vial glasses for storing. A freezer holding -20 degrees was a suitable place.

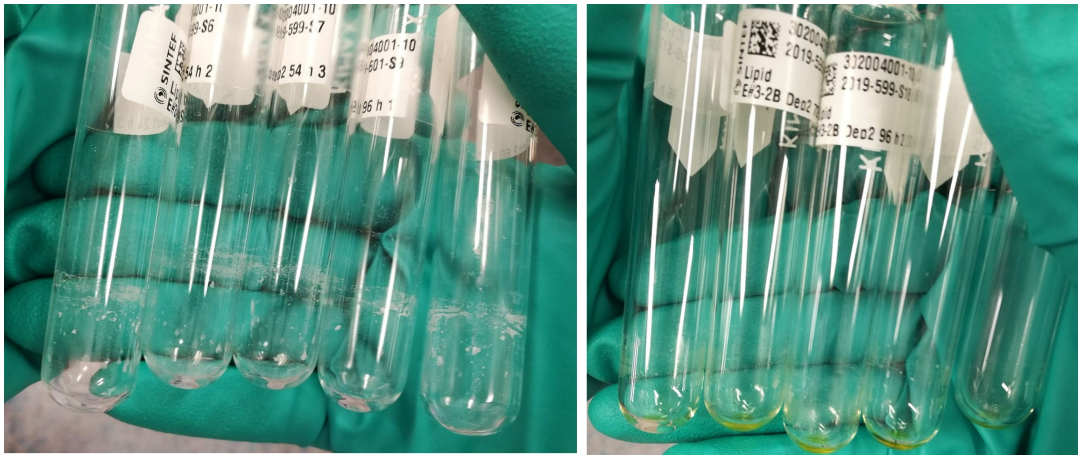


Figure 13: **Originally Folch extract method resulted in a dirty kimax tube (to the left) V.S. the result after adding an extra step (to the right) (Photo: Hannah Marie Knutsen 08/03-2019).**

4.4 Larvae endpoint analysis/ Image analysis

In addition to the experiment described in the report, the samples incubating until hatching was a part of another experiment performed as a part of the PW-Exposed project. After hatching, macroscope images and video filming was performed for the deformation, biometric and cardiotoxicity analyses.

5 Results

The results presented in this chapter will contain the concentration, uptake and elimination of tPAHs and of the compounds at focus; naphthalene, sum of C1-phenanthrene and dibenzothiophene. The results of the lipid content from the Folch extraction, the body burden concentrations normalized to lipid content and the bioconcentration factors are also represented. These dataes were collected during two months with laboratory experiments performed by the students. In addition to the lipid content and PAH body burden in lumpsucker, several toxicity endpoints was measured during the experiments. These measurements are not a part of the bachelor project. However, to put the chemical results in light of the overall project and discuss the impacts, a selection of endpoint measurements by Drs' Julia Farkas and Bjørn Henrik Hansen will be presented.

5.1 Body burden

Each exposure lasted for 48 hours, from 0-48 hpf (dep1), 36-84 hpf (dep2) and 10-12 dpf (dep3). A wide range of alkyl PAHs and parent PAHs was detected, identified and quantified in the GC-MS/MS analysis of lumpsucker eggs exposed to PW. The exposure concentration was set to 20 µg/L for all of the exposure experiments. The actual water concentration for all of the exposure experiments was measured, see Appendix 13. The results from the analysis is presented by various graphic representations. Other graphic representations of body burden concentrations of compounds at focus, including all the parallels, are displayed in Appendix 15. The concentrations of the compounds in embryos exposed at 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3) are listed in Appendix 3-5 Table S3-S5.

During the laboratory work, triplicate sets of samples were sampled, extracted and analyzed for each timepoint in each exposure experiment, as explained in chapter 4.1.4. The average concentrations of the parallels were calculated and presented in this chapter with standard derivation (see Appendix 8).

5.1.1 Body burden concentration of total PAHs

The body burden of tPAH in the three exposure experiments (0 hpf (dep1), 36 hpf (dep2), and 10 dpf (dep3)) at 0 hpe is compared to each other and to the control group at 0 hpf in Figure 14. Parent, and alkyl, PAH concentrations in the studied depurations are summarized in Table S8 in Appendix 8.

As seen in Figure 14, the embryos exposed at the earliest life stage (0 hpf, dep1) have the highest body burden of tPAH at 0 hpe. The body burden of embryos exposed at 0 hpe is 1,9 ± 0,3 ng/egg, the body burden of embryos exposed at 36 hpf is 1,3 ± 0,2 ng/egg, and 0,36 ± 0,07 ng/egg for the embryos exposed at 10 dpf. The body burden in the control samples is almost not present, with the concentration of 0,01 ± 0,01 ng/egg .

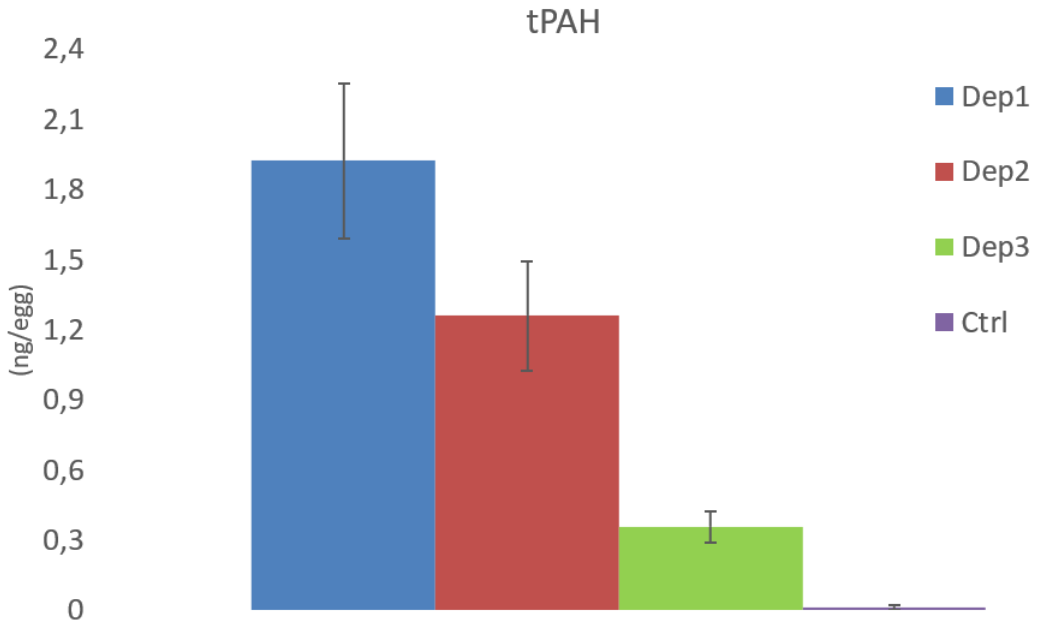


Figure 14: **Graphic illustration of concentration of accumulated tPAH at 0 hpe in embryos exposed at 0 hpf (dep1), embryos exposed at 36 hpf (dep2) and embryos exposed at 36 dpf (dep3) and after ended exposure and corresponding control samples (ctrl). hpe = hours post exposure, dpf = days post exposure.**

In addition, the body burden concentration and the elimination of tPAH in the three exposure experiments, 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3) was compared to each other at each of the 7 sampling timeponts (0, 6, 12, 24, 48, 96 and 192 hpe) in Figure 15.

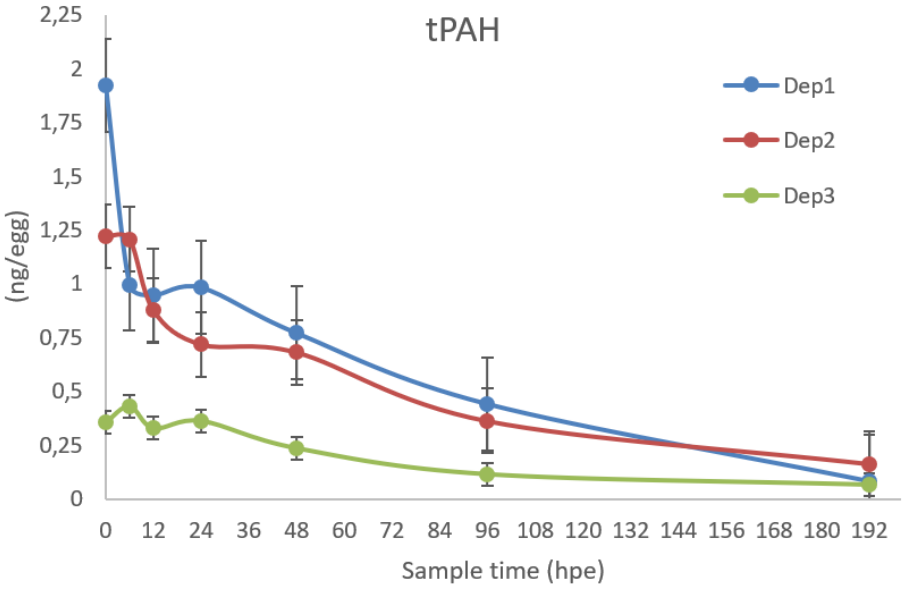


Figure 15: **The figure illustrate the average uptake of tPAH in lumpsucker eggs exposed at 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3), and the elimination after the samples were placed in the recovery tank after exposure ended. The values of the graph is from Table S8 in Appendix 8. hpe = hours post exposure, hpf =hours post fertilization, dpf = days post fertilization.**

Figure 15 accentuates that the greatest elimination in embryos exposed at 0 hpf (dep1) is from time point 0 to 6 hpe as the curve descends. From 6 to 24 hpe there is as good as no elimination, however the curve shows a low increase. From 24 to 192 hpe the curve is also nearly linear, meaning from the concentration of 0,9 ± 0,2 ng/egg to 0,08 ± 0,02 ng/egg.

There is a low decrease in tPAH in embryos exposed at 36 hpf (dep2) from timepoint 0 to 6 hpe (see Figure 15). As the curve decline the most from 6 to 12 hpe, accordingly the elimination rate is highest from 6 to 12 hpe. From 12 hpe the concentration decrease slowly to 0,16 ± 0,02 ng/egg at 192 hpe.

The elimination of tPAH for embryos exposed at 10 dpf (dep3) differs from the embryos exposed at 0 hpf and 36 hpf, due to the increasing and decreasing of the concentrations (see Figure 15). The curve increases to 6 hpe, then declines to 12 hpe, followed by increase until 24 hpe. From this timepoint on the concentration only decreases, with a final concentration of 0,066 ± 0,005 ng/egg at 192 hpe.

5.1.2 Compounds at focus

This thesis focuses at the compounds naphthalene, C1-phenanthrene and dibenzothiophene. Naphthalene is the compound of highest body burden concentration in the PW exposed lumpsucker. C1-phenanthrene and dibenzothiophene have a high concentration compared to many other compounds, as seen in Appendix 3-5, in Table S3-S5. These compounds will be referred to as 'compounds at focus'.

Naphthalene

The body burden concentration of naphthalene in the three exposure experiments, 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3) is compared to each other for each of the 7 timeponts of sampeling (0, 6, 12, 24, 48, 96 and 192 hpe) in Figure 16.

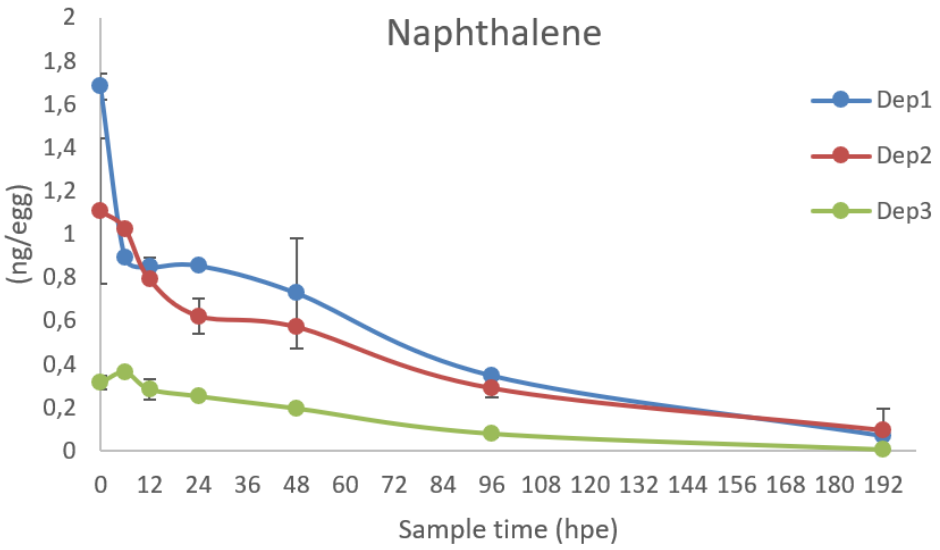


Figure 16: **The figure illustrates the average uptake of naphthalene in lumpsucker embryos after exposure, and the elimination after the sample were placed in the recovery tank for each exposure experiment. The concentration of naphthalene in embryos exposed at 0 hpf (dep1), embryos exposed at 36 hpf (dep2) and embryos exposed at 10 dpf (dep3) is represented in Table S9 in Appendix 8. hpe = hours post exposure, hpf = hours post fertilization, dpf = days post fertilization.**

Figure 16 shows that the body burden of naphthalene decreases for each exposure experiment, where embryos exposed at 0 hpf (dep1) holds the greatest concentration of 1,68 ± 0,06 ng/egg, and constitutes 87 % of tPAH. Embryos exposed at 36 hpf (dep2) holds a concentration of 1,1 ± 0,3 ng/egg, and contributes to 83 % of tPAH, while embryos exposed at 10 dpf (dep3) comprises of 0,31 ± 0,03 ng/egg and constitutes 88 % of tPAH at 0 hpe (see Appendix 8, Table S9 for naphthalene body burden concentrations, and Table S8 for tPAH body burden concentrations). Embryos exposed at 0 hpf has the steepest curve of elimination from timepoint 0 to 6 hpe, and a

gradually decreasing curve from 24 to 192 hpe. The curve of embryos exposed at 36 hpf is more even and linear than for 0 hpf, and the steepest time point is from 6 to 12 hpe. There is a difference in the concentration of 0 hpf (dep1) and 36 hpf (dep2) against 10 dpf (dep3). The curve of the eggs exposed at 10 dpf is almost completely linear, readily apparent holding an even elimination, with a little increase at 6 hpe. All of the exposure experiments is virtually holding the same concentration at 192 hpe, where the body burden of embryos exposed at 0 hpf is 0,07 ± 0,02 ng/egg, the embryos exposed at 36 hpf holds the body burden of 0,10 ± 0,01 ng/egg, and the concentration of 0,008 ± 0,002 ng/egg for the eggs exposed at 10 dpf.

C1-phenanthrene

The body burden concentration of sum of C1-phenanthrene in the three exposure experiments, 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3), was compared to each other at each of the 7 timeponts of sampeling (0, 6, 12, 24, 48, 96 and 192 hpe) in Figure 17.

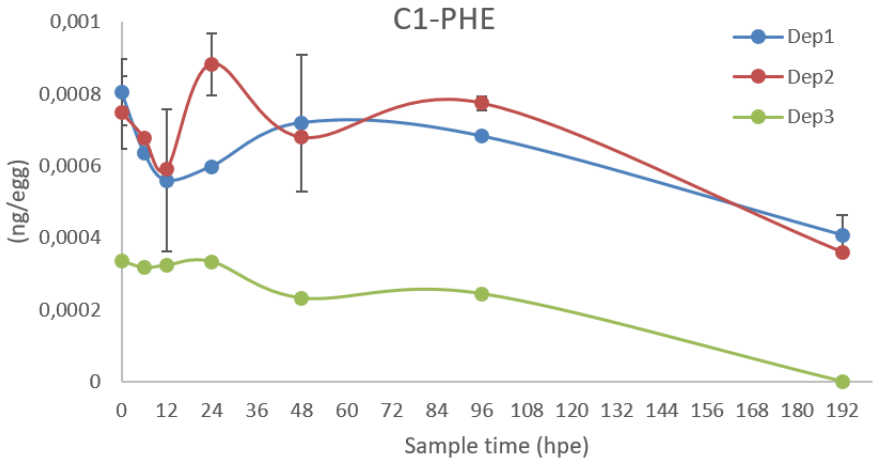


Figure 17: **The figure illustrates the average uptake of the sum of C1-phenanthrene in lumpsucker embryos after exposure, and the elimination after the sample were placed in the recovery tank for each exposure experiment. The concentration of C1-phenanthrene in embryos exposed at 0 hpf (dep1), embryos exposed at 36 hpf (dep2) and embryos exposed at 10 dpf (dep3) is represented in Table S10 in Appendix 8. hpe = hours post exposure, hpf = hours post fertilization, dpf = days post fertilization.**

Embryos exposed at 0 hpf has the greatest body burden concentration of sum of C1-phenanthrene at 0 hpe (Figure 17), 0,00081 ± 0,00009 ng/egg and comprise 0,00052 % of tPAH. Embryos exposed at 36 hpf holds a body burden of 0,0007 ± 0,0001 ng/egg and constitutes 0,0011 % of tPAH, while embryos exposed at 10 dpf consists of 0,000336 ± 0,000008 ng/egg and constitutes 0,001 % of tPAH at 0 hpe (see Appendix 8, Table S10 for body burden concentrations of sum of C1-phenanthrene, and Table S8 for tPAH body burden concentrations).

The curve of 0 hpf (dep1) shows a great elimination from 0 to 12 hpe by the decline of the curve,

then the curve peaks until 48 hpe, followed by another decline in the curve. 36 hpf (dep2) also shows a great elimination from 0 to 12 hpe, but thenceforth has a variable uptake process that constitute ups and downs until it steadily decreases from 96 to 192 hpe. 10 dpf (dep3), on the other hand, shows a more gradually decreasing curve the whole time.

Dibenzothiophene

The body burden concentration of dibenzothiophene in the three exposure experiments, 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3) was compared to each other for each of the 7 timepoints of sampling (0, 6, 12, 24, 48, 96 and 192 hpe) in Figure 18.

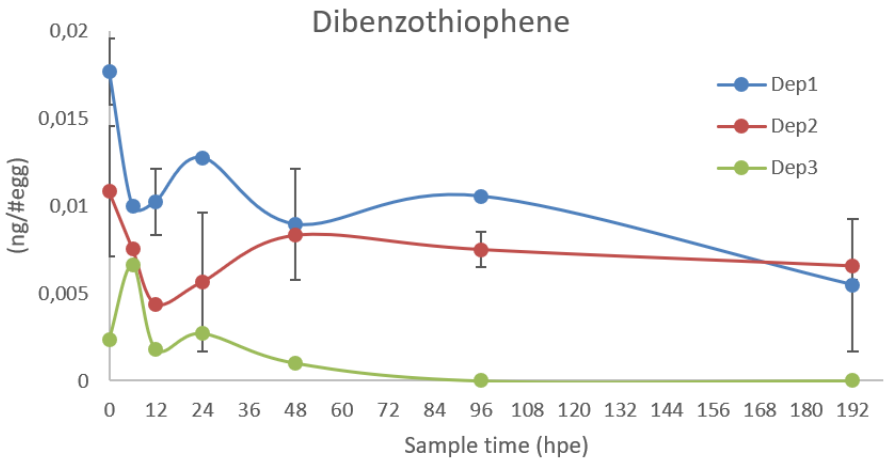


Figure 18: **The figure illustrates the average uptake of dibenzothiophene in lumpsucker embryos after exposure, and the elimination after the sample were placed in the recovery tank for each exposure experiments. The concentration of dibenzothiophene in embryos exposed at 0 hpf (dep1), embryos exposed at 36 hpf (dep2) and embryos exposed at 10 dpf (dep3) is represented in Table S11 in Appendix 8. hpe = hours post exposure, hpf = hours post fertilization, dpf = days post fertilization.**

By studying the change in body burden of dibenzothiophene as presented in Figure 18 it is readily apparent that all of the curves are characterized by increase and declines, still having an overall decrease for each exposure experiment. In addition, the elimination of dibenzothiophene is lower than for the already mentioned compounds. The concentration at timepoint 0 hpe is the highest for embryos exposed at 0 hpf (dep1) in this compound as well. With a concentration of 0,018 ± 0,002 ng/egg, the embryos constitutes 0,09 % of tPAH. 0 hpf is followed by embryos exposed at 36 hpf (dep2), containing 0,011 ± 0,004 ng/egg, and comprises 0,012 % of tPAH, and embryos exposed at 36 hpf (dep3) has the concentration 0,002 ± 0,00 ng/egg, constituting 0,005 % of tPAH (see Appendix 8, Table S11 for dibenzothiophene body burden concentrations, and Table S8 for tPAH body burden concentrations).

Control samples

The control samples were below LOQ for all of the compounds above. For this reason, no graphic

illustration was made. See Appendix 2, Table S2 for LOQ values, see Appendix 6, Table S6 for control body burden concentrations for each compound analyzed.

5.2 Lipid content

The lipid content in the three exposure experiments (0 hpf (dep1), 36 hpf (dep2 and 10 dpf (dep3)) was measured and compared to each other and to the control (ctrl) from timepoint 0 hpf and to the end of each exposure, given as a function of time (hpf) in Figure 19.

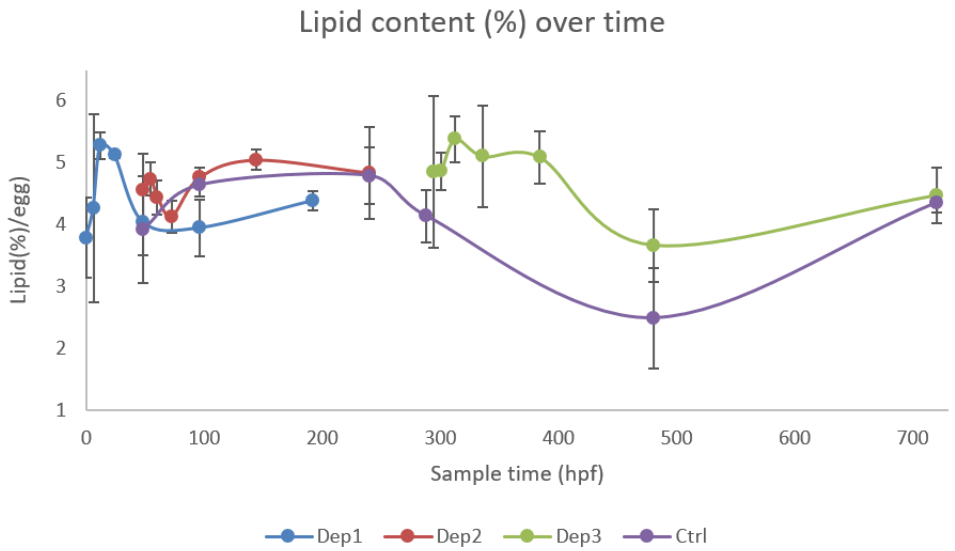


Figure 19: **Graphic illustration of lipid content (mg/egg) in embryos exposed at 0 hpf (dep1), 36 hpf (dep2), 10 dpf (dep3) and control samples (ctrl) as function of hpf. hpf = hours post fertilization, dpf = days post fertilization.**

The lipid content in the three exposure experiments (0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3)) are compared to each other, given as a function of time (hpe) in Figure 20.

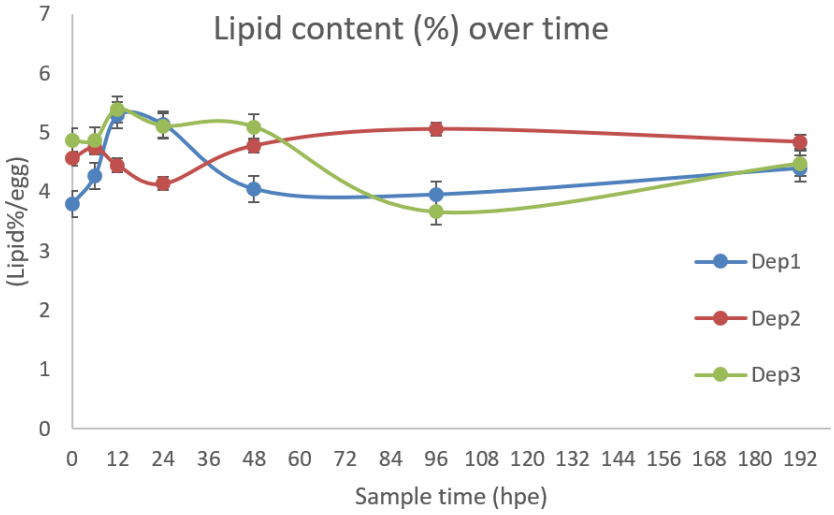


Figure 20: **Graphic illustration of the lipid content (mg/egg) in embryos exposed at 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3) in each exposure experiment as function of hpe. hpe = hours post exposure, hpf = hours post fertilization, dpf = days post fertilization.**

By conducting and comparing lipid profiles for the three different life stages for embryos exposed at 0 hpf, 36 hpf, 10 dpf and control (see Figure 19 and 20), the values indicate that total extractable lipid content maintains quite the same throughout the development of ELS lumpsucker. The average extractable lipid content supports this as the content is 4,8 ± 0,6 % mg/egg for embryos exposed at 0 hpf, 4,9 ± 0,3 % mg/egg for embryos exposed at 3 hpf, and 5,0 ± 0,5 % mg/egg for embryos exposed at 10 dpf. Lipid extraction (Folch) for embryos exposed at 0 hpf, 36 hpf, 10 dpf and control samples is to be found in Appendix 9-12, Table S13-S16.

5.3 Body burden concentration normalized to lipid content

The concentration of PAHs and alkyl PAHs are measured in the embryos, whereof the normalized value of body burden concentration to lipid content is calculated from Equation 1 and 2 in chapter 3.3.1. The three exposure experiments show a significant different pattern of PAH and alkyl PAH uptake. Notably, the last exposure (10 dpf (dep3)) showed a distinct reduction. Figure 21 shows a graphic illustration of the body burden concentration normalized to lipid content over time is compared for the three potentially sensitive life stages (0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3)).

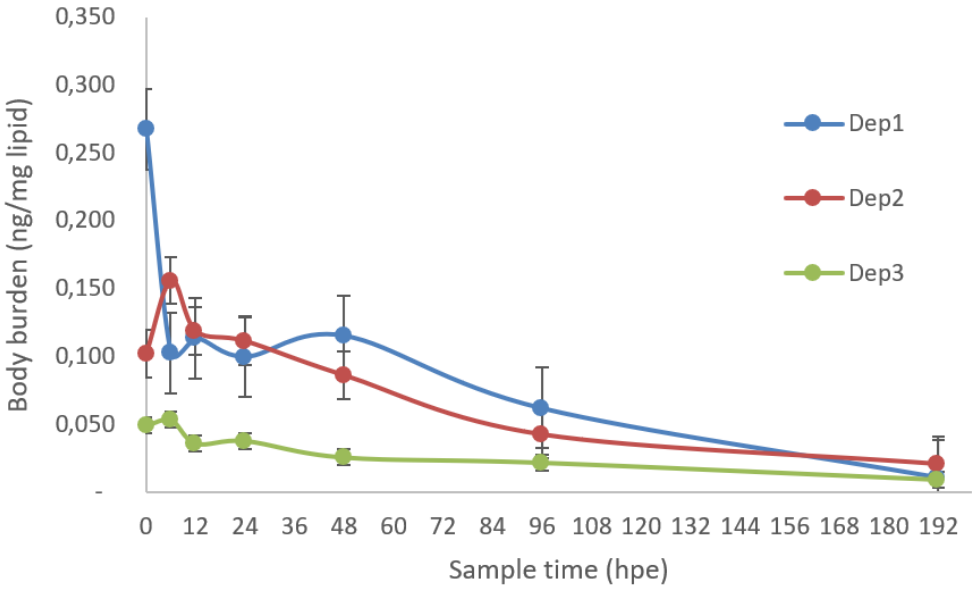


Figure 21: **Graphic illustration of body burden concentration of tPAH normalized to the lipid content for embryos exposed at 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3). hpe = hours post exposure, hpf = hours post fertilization, dpf =days post fertilization.**

From the calculations and graphic illustrations (Figure 21), the tPAH body burden in enbryos exposed at 0 hpe is clearly the highest at 0 hpe among the exposure experiments, with the concentration of 0,27 ± 0,03 tPAH in lipid content (ng/mg). Embryos exposed at 36 hpf holds the concentration of 0.10 ± 0,005 ng/mg, while embryos exposed at 10 dpf represents the lowest concentration; 0,05 ± 0,01 ng/mg (see Table S12 in Appendix 8).

The concentrations in embryos exposed at 0 hpf indicate great elimination of tPAHs after being placed in the recovery rig. Already at 6 hpe the concentration decrease to 0,103 ± 0,008 tPAH in lipid content (ng/mg), then steadily decreasing to 0.011 ± 0,008 ng/mg at 192 hpe.

In embryos exposed at 36 hpf, the concentration increase at 6 hpe (0,156 ± 0,01 ng/mg), followed by decrease. Prior hatching, the last concentration measured is 0,021 ± 0,002 ng/mg.

In the beginning of embryos exposed at 10 dpf the body burden concentration of tPAH is low compared to embryos exposed at 0 and 36 hpf. At 6 hpe the concentration measured is 0,053 ± 0,005 ng/mg, an increase of the concentration at 0 hpe. From 6 hpe to 192 hpe the concentration decreases to 0,009 ± 0,002 ng/mg tPAH in lipid content. This concentration is the lowest measured for all of the exposure experiments.

5.4 Bioaccumulation of compounds at focus

The BCFs are calculated using Equation 3, the values used for this equation are found in Appendix 3-5and 9-11, Table S3-S5 and S13-S15. BCFs for the 'compounds at focus' are given in Table 2.

Table 2: **Bioconcentration factor (BCF) of compounds at focus for embryos exposed at 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3), given with standard deviation, at 0 hpe. hpe = hours post exposure. hpf = hours post fertilization. dpf = days post fertilization.**

		BCF (L/kg)		
Compounds	Log Kow	Dep1 (0 hpf)	Dep2 (36 hpf)	Dep3 (10 dpf)
Naphthalene	3.17	864 ± 136	498 ± 126	296 ± 57
C1-Phenanthrene	4.89	5,0 ± 0,9	4,0 ± 0,4	1,8 ± 0,3
Dibenzothiophene	4.17	879 ± 157	467 ± 147	82 ± 64

The compound with the highest bioconcentration factor (BCF) among embryos exposed at 0 hpf (dep1) is dibenzothiophene, with a concentration of 879 ± 157 L/kg, meaning that dibenzothiophene bioaccumulates the most at 0 hpe. Naphthalene follows with a concentration of 864 ± 136 L/kg, and lastly C1-phenanthrene bioaccumulates the least with a concentration of 5,0 ± 0,9 L/kg.

Naphthalene bioaccumulates the most for 36 hpf (dep2), with a BCF of 498 ± 126 L/kg. Dibenzothiophene follows with a BCF of 467 ± 147 L/kg. C1-phenanthrene has the lowest BCF, 4,0 ± 0,4 L/kg, meaning it bioaccumulates the least. Compared to the embryos exposed at 0 hpf (dep1), the bioaccumulation in embryos exposed at 36 hpf (dep2) is more or less halved for naphthalene and dibenzothiophene.

For embryos exposed at 10 dpf (dep3), naphthalene bioaccumulates the most with a BCF of 296 ± 57 L/kg. The BCF of dibenzothiophene here has decreased by five times of the BCF in embryos exposed at 36 hpf, with a concentration of 82 ± 64 L/kg in embryos exposed at 10 dpf. Also here, C1-phenanthrene has the lowest BCF and accumulates the least 10 hpf, with a concentration of 1,8 ± 0,3 L/kg.

5.5 Effects of PW toxicity

This section of the results is based on work accomplished by Drs' Bjørn Henrik Hansen and Julia Farkas and the results are preliminary.

5.5.1 Effects on survival

Preliminary (16 dpf) results show no impact on fertilization rates or on embryonic survival (see Figure 22). This is the given timepoint for the investigation of effects on embryonic survival.

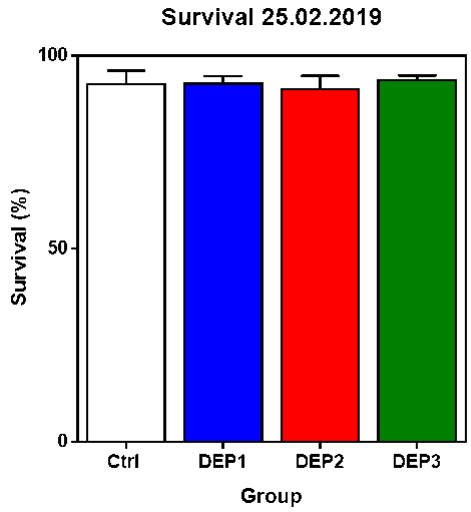
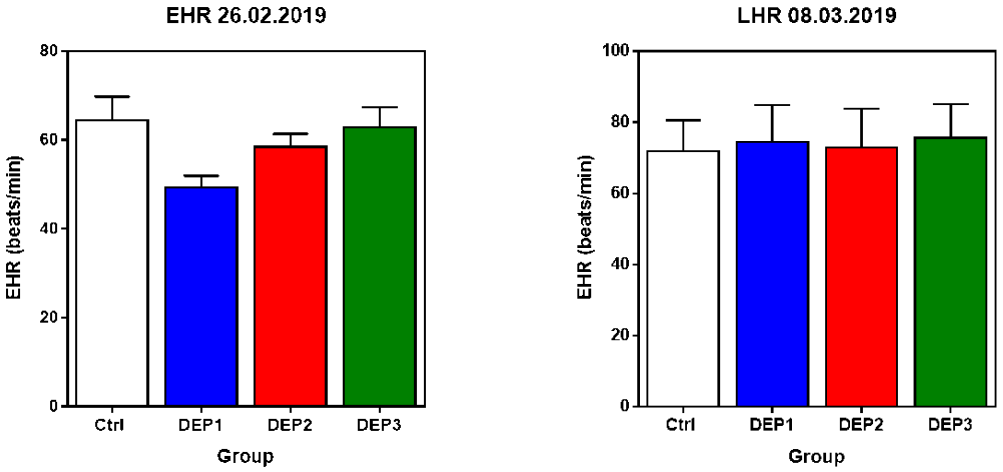


Figure 22: **Illustrates survival of embryos exposed at 0 hpf (dep1), 36 hpf (dep2) and 10 dpf (dep3) at 16 dpf. hpf = hours post exposure, dpf = days post fertilization.**

5.5.2 Heart activity - bradycardia

Heart rate (HR) analyses after 17 dpf were accomplished (see Figure 23). For embryos exposed during fertilization (dep1) and 36 hpf (dep2) the HR are significantly reduced. For embryos exposed 10 dpf (dep3) no significant differences are observed compared to the control group. After hatching new HR analyses were performed, but no significant differences between the groups are spotted in HR for larvae.



(a) 17 dpf

(b) After hatching

Figure 23: **Illustrations of HR analyses for embryos exposed at 0 hpf (dep1), 36 hpf (dep2), 10 dpf (dep3) and control samples (ctrl) at 17 dpf (a) and after hatching (b). hpf = hours post fetilization, dpf = days post fertilization.**

6 Discussion

6.1 Methods used in the current work

6.1.1 Body burden extraction and clean-up SPE

To fully understand the toxicity, it is necessary with a sensitive and precise analysis of low concentration of alkyl PAH and parent PAH accumulated in lumpsucker embryos [46]. The toxicity can be related to uptake rates and bioaccumulation levels of substances in the tissues. To estimate the impact of PAHs, the relationship between body burden and toxicity is utilized [45].

The method used for the body burden consists of a homogenization, an extraction and a clean-up step [43]. The extraction step brought some difficulties regarding the crushing of the lumpsucker eggs. Although the method is well established for other fish eggs, the homogenizing knife could not manage to crush the lumpsucker eggs due to their robust egg shells. Experiencing that the homogenizing step was very time consuming and inefficiently, the knife was replaced with a glass rod. The glass rod did not contaminate the samples as it was cleansed with DCM and dried within every new sample. This adjustment resulted in a more efficient egg crushing. Despite the usage of a glass rod, a few eggs would still not break. The number of uncrushed eggs were noted. Although, some uncrushed eggs may have been unnoticed and unlisted. This may have lead to a lower concentration of PAH in the current samples, although the uncrushed eggs still are expected to contribute in some amounts to the concentration. Samples containing uncrushed eggs are marked with a (c) in Appendix 3-6, Table S3-S6.

The usage of the evaporator was experienced as time consuming. This step allowed the students to handle other tasks in the meanwhile while the samples evaporated. This resulted in some samples being to much evaporated, or worse, completely evaporated. The samples regarding are marked with (e) in Appendix 3-6, Table S3-S6. This may have caused a loss of light compounds, such as the naphthalenes. Still, naphthalene is the most accumulated compound due to its presence in PW.

6.1.2 GC-MS/MS

The GC-MS/MS is more ideal than the previous analysis method, GC-MS, due to its sensitivity [3]. This current work demonstrates that the GC-MS/MS is sensitive enough to detect the PAH concentrations (20 µg) in this experiments, although some concentrations fell outside the LOQ.

To describe the accumulation of alkyl PAH in PW exposed lumpsuckers precisely, one have to look at the body burden, and count on a wider range of analytes (alkyl PAH clusters) than accessible as analytical standards. The quantification of the group of alkyl PAH clusters is made based on chromatographic patterns of the molecular ion of these compound groups. It is not possible to determine accurate response factor for each alkyl PAH cluster, with this lack of analytical standards for most of them. A solution to this problem is to use the parent PAH as a "representative" for the alkyl homologous for each group, or to use several fragment ions for each group [3].

6.1.3 Lipid extraction

A technique that allows small sample sizes is desirable, because of the small sample size of lumpsucker embryos. In addition, smaller sample sizes reduces the consumption of solvents and chemical needed for the preparation of the micro-extraction. This is both economical and more sustainable [43] The miniaturized Folch extraction protocols fulfills these requirement by allowing a sample size $<$ 200 mg [50]. The method takes advantage of the biphasic system of chloroform:methanol:water with the proportion of 8:4:3 by volume, considering the extract contains all the water from the tissue [51]. As a result of this, the extract embodied in the lower is pure lipid [50].

In order to find the lipid content, the lipid extraction must be what is left in the preweighted tube, thus the water phase and the precipitate must to be thoroughly removed. The distinction between the water- and lipid phase became clearer after adding 0,9 % NaCl-MilliQ-solution and centrifuging, which made it easier to remove the water phase.

To produce a good result, the sample has to be as clean as possible before the evaporation, due to the extra weight the dirt and precipitate will bring to the lipid weight. During the laboratory work, it was experienced a difficulty regarding producing samples clean enough, and for that reason, an extra step was added to the procedure. The extra step consisted of transferring the lipid supernatant to a new kimax tube and centrifuge twice, while originally this was done once only. The extra step resulted in a significantly cleaner sample with less dirt and precipitate, which led to a more accurate and reliable result (see Figure 13). Samples regarding precipitate are marked with (d) in Appendix 9-12, Table S13-S16.

As for the homogenizing step, the egg crushed easily in the chloroform/methanol solvent. The step by Folch method was appropriate for the lumpsucker eggs, unlike in the homogenizing step for the body burden analysis.

6.2 Body burden and lipid content

The data presented in chapter 5 confirms that the three potentially sensitivity stages (embryos exposed at 0 hpf, 36 hpf and 10 dpf) were affected by the PW exposure differently. The embryos exposed during fertilization accumulated significantly more PAHs from the PW than those exposed later in the development.

6.2.1 Body Burden concentration of PAHs

Body burden of total PAHs

In the current study, Body burden concentrations of tPAH and of the compounds at focus were studied. The elimination process increases with the development of the fish embryo, due to the biotransformation of xenobiotic compounds. Because the biotransformation is not fully developed in the lumpsucker embryos exposed at at 0 hpf or 36 hpf, 10 dpf (dep3) helds the greatest elimination rate. This is readily apparent in the graphic illustrations of the average uptake of tPAH (see Figure 15), since embryos exposed at 0 hpf (dep1) and 36 hpf (dep2) have a greater concentration from the beginning - one of the reasons embryos exposed at 10 dpf (dep3) starts

with a lower concentration is that the elimination process already had started before the eggs were exposed to PW. Consequently, the lower uptake of PAHs is due to the elimination.

Dep1 was exposed during fertilization. At this point the eggs shells were soft compared to the the shell at 36 hpf (dep2) and 10 dpf (dep3). As the hypothesis claims, the eggs accumulate more PAHs and alkyl PAHs in their early stages as the concentration is higher for embryos exposed at 0 hpf compared to 36 hpf and 10 dpf. Hence, the results matches the hypothesis. Analyzing the results and figure 14, embryos exposed at 0 hpf (dep1) accumulates significantly more tPAH than the other depurations. Studying figure 15, embryos exposed at 36 hpf (dep2) have a lower concentration of tPAH than 0 hpf, and 10 dpf a lower concentration than embryos exposed at 36 hpf. During the development of the embryos, the egg shells hardened, presumable 36-38 hpf [7]. Due to this, embryos exposed at 36 hpf presumably decreased its accumulation. Embryos exposed at 10 dpf is explained in the same manner, with egg shells robust enough to withstand mechanical stress.

Body burden of compounds at focus
The lab results revealed that the body burden concentration of naphthalene was the highest. Though it is established that heavier PAHs bioaccumulates more than lighter PAHs, light PAHs is significantly more present in produced water due to the water solubility. Thereof, naphthalene with the lowest molecular weight of the studied compounds had the highest body burden of all the 'dep's'. C1-phenanthrene has the highest molecular weight of the compounds at focus, and was the least present in the PW. Thereby, the body burden of C1-phenanthrene was the lowest. Dibenzothiophene is a lighter compound than C1-phenanthrene. However, it has three rings, while naphthalene consist of two rings only, which makes it a lot heavier than naphthalene. Hence, the body burden of dibenzothiophene was higher than C1-phenanthrene, but far less than the concentration of naphthalene.

Elimination of PAHs
Studies show some adult fish have metabolized up to 99 % of parent PAH during a day being exposed [3]. Elimination of PAHs can be both passive and active (biotransformation). It is expected that after ended exposure, the concentration of tPAHs will decrease, also in organisms with reduced metabolism [31]. Experiments done on fish embryos shows that the metabolism is a way of eliminating PAHs [3]. When determining exposure doses, the biotransformation of xenobiotic compounds is the most perplex and confusing factor. The proposed cause of elimination is biotransformation, which is a metabolism where xenobiotics gets detoxified and transformed into more easily excreted compounds in the lumpsucker [3]. The elimination started once the exposure ended; 2 dpf.

6.2.2 Lipid content

Some studies reveal that the lipid content in the biological membrane in the fish is directly linked to the uptake of hydrophobic compounds, such as PAHs and alkyl PAHs [38]. The lumpsucker embryos and larvae did not vary much in the content of lipid, neither over time nor in comparison of the exposure experiments. Some variations occurred in the samples that were prepared in the beginning by the students. A reason for the variation could be that the method was not optimized

at first, as described in chapter 4.3. In the first samples prepared, the homogenizing and transmission to a new kimax tube was done once. This lead to a dirty sample containing precipitate and particles. The dirt impacted the weight, leading to an inaccurate result. The samples regarding are listed in Table S9-S12 in Appendix 13-16.

The three life stages analyzed (0 hpf, 36 hpf and 10 dpf) in chapter 5.2, shows that there is no significant difference in the lipid content. Due to this, nothing certain can be said about the correlation between the three life stages, the correlation between exposure and lipid content, or the correlation between life stages and the lipid content. Although the lipid content did not vary much, the composition of the lipid may vary both over time or in the different exposure experiments performed, although, this was not tested in the bachelor project.

6.2.3 Bioconcentration of PAHs

It is of interest to look at the BCF because it allows comparison of the bioconcentration of compounds in different samples or organisms [3]. In order to find the BCF, the the body burden concentration was normalized to the lipid weight, using Equation 2. It is established that all compounds with a log K_{ow} above 3 have the potential to bioaccumulate [10], as all the compounds analyzed do have. Because the embryos did not have any uptake of compounds via dietary exposure, the bioaccumulation is a result of diffusion only [33].

The BCF should be calculated at steady-state [3], but in this case it was not possible, due to short exposure time, the presumably metabolism in the embryos and the exposure experiment was performed with a semi-static renewal regime. Still, the BCF was estimated in order to give an impression and to compare the accumulation of the different PAHs and alkyl PAHs in the all of the exposure experiments.

Due to the water-solubility of light PAHs, the composition of tPAH mainly consists of PAHs and alkyl PAHs with a log K_{ow} below 4. While all the exposure experiments have a significant high body burden concentration of naphthalene, it is not the most bioaccumulated compound for embryos exposed at 0 hpf. The BCF is a little higher for dibenzothiophene than for naphthalene. Dibenzothiophene is a heavier PAH than naphthalene, heavier compounds are less water-soluble and more soluble in lipid. Heavy PAHs will accumulate more in lipid rich organisms, such as the lumpsucker. C1-phenanthrene is a heavier compound than naphthalene and dibenzothiophene, still it the least bioaccumulated compound in all of the exposure experiments. Sørensen et al. [52] suggests that different compounds have different metabolisms in ELS of fish [52]. The low BCF of C1-phenanthrene can be explained by this hypothesis, as the reason the bioaccumulation is so low compared to naphthalene and dibenzothiophene may be due to the metabolism of this compound starting earlier. Researchers are still trying to figure this out, and nothing certain can be said.

Log K_{ow} is found to correlate well with BCF [3]. When estimating the bioaccumulation, a plot of BCF based on log K_{ow} is currently the most competent [3]. This model have some weaknesses, due to the exclusion of some processes (e.g. uptake- and elimination kinetics, uptake process, cell membrane diffusion and metabolism rate). Devillers et al. [35] found that the correlation between log K_{ow} and BCF for compounds with a log K_{ow} below 6 have been established as successful. As seen in the Table S1 in Appendix 1, naphthalene has a log K_{ow} of 3,17, dibenzothiophene has a

log K_{ow} value of 4,17, and C1-phenanthrene has the log K_{ow} value of 4,85. As all these compounds has a log K_{ow} below 6, a plot of BCF based on log K_{ow} could be a way of estimating the bioaccumulation. Although in this thesis, the BCF is calculated in order to compare the bioaccumulation of 'compounds at focus' to each other and to other studies. No plot of BCF based on log K_{ow} is made, due to the lack of time.

Sørensen et al. [52] compared the uptake of parent and alkylated PAHs in Atlantic cod (*Gadus morhua*) and haddock embryos exposed to dispersed crude oil at a range of environmentally relevant concentrations (10-600 µg oil/L seawater) until hatching [52]. In Appendix 5, Table S11, the BCF of cod and haddock is represented. The haddock and cod are less lipid rich than the lumpsucker, and for this reason are expected to bioaccumulate less [7]. As the results provided, the BCF results of lumpsucker embryos were significant lower than the BCF results of haddock embryos and cod embryos. A suggested reason for the difference is that the lumpsucker embryos was exposed to PW with a semi-static renewal regime, while the cod and haddock embryos was exposed with a continuous flow-through exposure regime. The semi-static renewal regime involved batchwise renewal of the test solution after 24 hours, resulting in the exposure values sinking through the day. The continuous flow-trough exposure regime maintained the exposure values throughout the whole exposure period, leading to a higher exposure than a semi-static renewal regime [53].

6.3 Effects of produced water toxicity

The results achieved in this section are preliminary and not yet fully analyzed. Still, the observed results can demonstrate the relation between body burden and toxicity. The results from the body burden analysis displayed that the embryos exposed at 0 hpf holds the greatest body burden of tPAHs, and it is expected that they are affected the most by the toxicity effects from the PAHs. Embryos exposed at 36 hpf has the second highest body burden of tPAHs. They are expected to be affected a little less than those exposed at 0 hpf, but more affected than the embryos exposed at 10 dpf, as these has the lowest t PAH body burden concentration.

In the present study, it is difficult to separate the effects the individual compounds of PAH have. The embryos were exposed to the same mixture of PAHs and alkylated PAHs, where naphthalene had the significantly highest concentration in all of the exposed embryos. Accordingly, it may be suggested that naphthalene is the main reason for the toxicity effects observed in the embryos, however this not certain.

6.3.1 Effects on survival

Figure 22 reveals that 16 dpf preliminary there are no impacts on fertilization rates or on embryonic survival in all of the exposure experiments and the control samples. The results displayed that the exposure concentration of PW is low enough to gain successful survival at the timepoint for the investigation of survival effects. At this timepoint, the biotransformation had developed a lot, and the elimination process were efficient. The flow system developed to this project also contributed to the survival [44].

6.3.2 Heart rate

In the current study, the heartbeat occurred normally in the all of the lumpsucker larvae after hatching, but 17 dpf the embryos exposed during fertilization (dep1) and at 36 hpf (dep2) displayed bradycardia.
Studying zebrafish embryos exposed to different mixtures of PAHs, Incardona et al. [21] found that phenanthrene or dibenzothiophene produced dose-dependent reduction in heart rate, while fluorene exposed zebrafish embryos showed significant bradycardia. Naphthalene induced a mild, non-dose-dependent bradycardia. The study of zebrafish embryos cannot state the different compound's function in the lumpsucker, though, as fish species gets affected differently.

7 Conclusion

This experiment investigated bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) and alkyl PAHs in early life stages (ELS) of the fish specie lumpsucker (*Cyclopterus Lumpus*). As the results affirmed, the bioaccumulation did decrease with the developmental stages of the fish specie lumpsucker. The more the embryo developed, the greater the metabolism and the elimination of PAHs and alkyl PAHs. Accordingly, the overall bioaccumulation of PAHs and alkyl PAHs in the lumpsucker embryo was at its highest in the first exposure experiment (embryos exposed during fertilization, dep1). The fact that the lumpsucker eggs become hard after approximately 36-38 hpf is also a current hypothesis explaining why the PAH bioaccumulation decrease as the embryos develop. Their robust egg will prevent uptake of compounds such as PAHs.

At 0 hours post exposure (hpe) the uptake in embryos exposed during fertilization held the greatest concentration of total PAH (tPAH), 1,9 ± 0,3 ng/egg, due to the eggs had not began to hardened yet and the metabolism in the fish had not been fully develop. This hypothesis was supported by the rest of the study, where embryos exposed 36 hours post fertilization (hpf) held the second highest concentration of 1,3 ± 0,2 ng/egg at 0 hpe, as the metabolism still was underdeveloped, but the eggs had become completely hard egg. Embryos exposed 10 days post fertilization (dpf) had an increased metabolism which resulted in the lowest concentration at 0 hpe, 1,3 ± 0,2 ng/egg. The body burden of naphthalene was the highest of all compounds in all the exposure experiments, comprising 87 % of tPAH in embryos exposed at 0 hpf, 83 % in embryos exposed at 36 hpf and 88 % in embryos exposed at 10 dpf. The most water-soluble compounds will be the most present in the produced water, such as naphthalene. Dibenzothiophene gained the second highest tPAH body burden among the 'compounds at focus', comprising 0,09 % of tPAH in embryos exposed at 0 hpf, 0,012 % in embryos exposed at 36 hpf and 0,005 % in embryos exposed at 10 dpf. The tPAH body burden of sum of C1-phenanthrene was the lowest among the three compounds, constituting of 0,0005 % of tPAH in embryos exposed at 0 hpf, 0,001 % in embryos exposed at 36 hpf and 0,001 % in embryos exposed at 10 dpf.

The greatest discovery in this thesis was the biotransformation in ELS of lumpsucker affecting the bioaccumulation to a great extent as the elimination process increased with the growing metabolism in the fish embryo. The suggested cause of elimination is biotransformation, likewise the robust egg shell of lumpsucker embryos - completely hard after approximately 36-38 hours post fertilization. Thus, the field is yet to be fully understood.

The lipid content has been proven directly linked to the uptake of hydrophobic compounds. The lipid content most probably affected the bioaccumulation of tPAH, due to the less water-soluble compounds, such as heavy PAHs, accumulate more in lipid-rich organisms. Because the lipid content maintained quite the same throughout the development of the lumpsucker ELS there is no proof of the hypothesis of correlation between lipid content and PAH accumulation.

The bioconcentration factor (BCF) calculated for the 'compounds at focus' shows that in embryos exposed at 0 hpf, dibenzothiophene accumulated the most, followed by naphthalene right below, and C1-phenanthrene lastly with a significantly lower BCF than the other two compounds. In embryos exposed at 36 hpf and 10 dpf naphthalene was the most accumulated compound, followed

by dibenzothiophene and lastly C1-phenanthrene. This is comprehended by the presence of light PAHs in produced water, because even though heavier PAHs accumulate to a greater extend in lipid-rich organisms (e.g. lumpsuckers), light PAHs accumulated more.

To summarize the results in this thesis it is stated that the eggs accumulate PAHs and alkyl PAHs, due to the log K_{ow} value. The investigation of the correlation between life stage, lipid content and accumulation was the main purpose of the thesis, and the correlation between life stage and accumulation was evident from the analyzes. The current work could not prove any correlation with regard to lipid content, as the lipid content did not differ particularly throughout the period. The results and founds in this thesis gives better insight of the influence produced water (PW) has on lumpsucker ELS.

Recommendations for further research

The chemical analysis was at interest in this thesis, in which the body burden concentration of tPAH and the bioaccumulation were at focus. Studying the results, the biotransformation was suggested as a distinct factor affecting the concentration of tPAH in the embryos. When looking at at BCF for the 'compounds at focus', C1-phenanthrene was the least accumulated compound, which contradicts the hypothesis that heavier PAHs accumulates more than lighter PAHs. There is no particular reason for this, the suggested cause is that the metabolism for C1-phenanthrene starts earlier than for naphthalene and dibenzothiophene.

As a recommendation for further research, in order to achieve a better understanding of PAHs and alkyl PAHs influence of marine species, the investigation of metabolism in ELS of fish for several species and distinct effects of PAHs and alkyl PAHs should be examined and compared. Associated with the recommendations, studying various PAHs, the malformations as a result of PW exposure, or studying the exposure for a longer time is suggested. Another suggestion is to investigate several toxicity endpoints through measurements. By measuring the Cyp-induction during the same period with the same exposure regime, the impact different exposure times has on the Cyp-induction can be determined, for instance. PW discharges is of concern for the marine environment, and this needs to be investigated more in the future.

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Appendices

S1 Table. Properties of PAH analytes and their GC-MS/MS analytical conditions.

Analyte	Short	Mw (g/mol)	log Kow	MRM Quant	CE (V)	MRM Qual	CE (V)
Biphenyl	BIP	154.2	4.01	154-153	20	154-152	30
Benzothiophene	BT	134.2	3.12	134-89	30	134-90	30
Dimethylbenzothiophene, 2,5-	BT-2,5	162.3	4.08	162-161	15	161-128	20
Trimethylbenzothiophene, 2,5,7-	BT-2,5,7	176.3	4.63	176-161	15	176-175	20
Naphthalene	NAP	128.2	3.17	128-102	25	128-78	25
Methylnaphthalene, 2-	NAP-2	142.2	3.72	142-141	20	142-115	40
Methylnaphthalene, 1-	NAP-1	142.2	3.72	142-141	20	142-115	40
Dimethylnaphthalene, 2,6&2,7-	NAP-2,6&2,7	156.2	4.26	156-141	20	141-115	25
Dimethylnaphthalene, 1,4-	NAP-1,4	156.2	4.26	156-141	20	141-115	25
Dimethylnaphthalene, 1,3&2,3-	NAP-1,3&2,3	156.2	4.26	156-141	20	141-115	25
Trimethylnaphthalene, 1,3,7-	NAP-1,3,7	170.3	4.81	170-155	15	155-153	15
Trimethylnaphthalene, 2,3,5-	NAP-2,3,5	170.3	4.81	170-155	15	155-153	15
Trimethylnaphthalene, 1,2,3-	NAP-1,2,3	170.3	4.81	170-155	20	155-153	20
Tetramethylnaphthalene, 1,2,5,6-	NAP-1,2,5,6	184.3	4.96	184-169	20	169-154	15
Tetramethylnaphthalene, 1,4,6,7-	NAP-1,4,6,7	184.3	4.96	184-169	20	169-154	15
Acenaphthylene	ACY	152.2	3,94	152-151	25	152-150	45
Acenaphthene	ACE	154.2	4.15	154-153	25	153-152	25
Dibenzofuran	DBF	168.2	4,12	168-139	30	139-89	45
Fluorene	FLU	166.2	4.02	166-165	25	165-164	25
Ethylfluorene, 9-	FLU-9et	194.3	5.09	180-165	25	165-164	20
Methylfluorene, 1-	FLU-1	180.3	4.97	194-165	20	165-164	20
Propylfluorene, 9-n-	FLU-9pro	208.3	5.13	208-165	30	165-164	30
Dibenzothiophene	DBT	184.3	4.17	184-139	45	184-152	25
Methyldibenzothiophene, 4-	DBT-4	198.3	4.71	198-197	20	197-165	25
Ethyldibenzothiophene, 4-	DBT-4et	212.3	5.20	212-197	20	197-165	25
Propyldibenzothiophene, 4-n-,	DBT-4pro	226.4	5.69	226-197	20	197-165	25
Butyldibenzothiophene, 4-n-	DBT-4but	240.4	6.19	240-197	30	197-165	30
Phenanthrene	PHE	178.2	4.35	178-176	45	178-177	30
Anthracene	ANT	178.2	4.35	178-176	45	178-177	30
Methylphenanthrene, 3-	PHE-3	192.3	4.89	192-191	25	191-189	25
Methylphenanthrene, 2-	PHE-2	192.3	4.89	192-191	25	191-189	25
Methylphenanthrene, 9-	PHE-9	192.3	4.89	192-191	25	191-189	25
Methylphenanthrene, 1-	PHE-1	192.3	4.89	192-191	25	191-189	25
Dimethylphenanthrene, 3,6-	PHE-3,6	206.3	5.44	206-189	45	206-191	20
Dimethylphenanthrene, 1,7-	PHE-1,7	206.3	5.44	206-189	45	206-191	20
Dimethylphenanthrene, 1,2-	PHE-1,2	206.3	5.44	206-191	20	206-189	45
Trimethylphenanthrene, 2,6,9-	PHE-2,6,9	220.3	5.99	220-205	20	205-189	35

S1 Table continued

Analyte	Short	Mw (g/mol)	log Kow	MRM Quant	CE (V)	MRM Qual	CE (V)
Trimethylphenanthrene, 1,2,6-	PHE-1,2,6	220.3	5.99	220-205	20	205-189	35
Trimethylphenanthrene, 1,2,7-	PHE-1,2,7	220.3	5.99	220-205	20	205-189	35
Tetramethylphenanthrene, 1,2,6,9-	PHE-1,2,6,9	234.3	6.53	234-219	20	234-203	35
Fluoranthene	FLA	202.3	4.93	202-200	40	202-201	25
Pyrene	PYR	202.1	4.93	202-200	45	202-201	25
Methylfluoranthene, 2-	FLA-2	216.3	5.48	216-215	30	215-213	40
Methylpyrene, 1-	PYR-1	216.3	5.48	216-215	30	215-189	30
Dimethylpyrene, 4,5-	PYR-4,5	230.3	6.03	230-215	30	215-189	30
Propylpyrene, 1-n-	PYR-1pro	244.3	6.46	215-189	40	230-215	30
Ethylpyrene, 1-	PYR-1et	230.3	5.97	215-189	35	215-213	45
Butylpyrene, 1-n-	PYR-1but	258.3	6.95	215-189	40	215-213	40
Benz[a]anthracene	BAA	228.3	5.52	228-226	45	226-224	45
Chrysene	CHR	228.3	5.52	228-226	45	228-227	25
Methylchrysene, 1-	CHR-1	242.3	6.07	242-241	20	242-239	40
Ethylchrysene, 6-	CHR-6et	256.3	6.56	256-241	15	256-239	45
Propylchrysene, 6-n-	CHR-6pro	270.4	7.05	241-239	35	270-241	20
Butylchrysene, 6-n-	CHR-6but	284.4	7.54	241-239	40	284-241	25
Benzo[b]fluoranthene	BBF	252.3	6,11	252-250	45	250-248	45
Benzo[k]fluoranthene	BKF	252.3	6,11	252-250	45	250-248	45
Benzo[e]pyrene	BEP	252.3	6,11	252-250	45	250-248	45
Benzo[a]pyrene	BAP	252.3	6,11	252-250	45	250-248	45
Perylene	PER	252.3	6,11	252-250	45	250-248	45
Indeno[1,2,3-cd]pyrene	IND	276.3	6.70	276-274	45	274-272	45
Dibenz[a,h]anthracene	DBA	278.3	6.70	278-276	45	276-274	45
Benzo[g,h,i]perylene	BGP	276.3	6.70	276-274	45	274-272	45
Naphthalene-*d*8		136.2		136-108	25	136-131	25
Biphenyl-*d*10		164.3		164-122	40	164-131	30
Acenapthylene-*d*8		160.2		160-158	30	160-132	30
Anthracene-*d*10		188.3		188-161	35	188-181	40
Pyrene-*d*10		212.3		212-208	40	212-210	35
Perylene-*d*12		264.4		264-262	40		
Indeno[1,2,3,*cd*]pyrene-*d*12		288.4		288-282	50	288-286	40

S1 Table continued

Analyte	Short	Mw (g/mol)	log Kow	MRM Quant	CE (V)	MRM Qual	CE (V)
C1-benzothiophenes	C1-BT	148.3	3.54	148-133 148-147	15 15		
C2- benzothiophenes	C2-BT	162.3	4.08	162-147 162-161	15 15		
C3- benzothiophenes	C3-BT	176.3	4,63	176-161 176-175	15 20		
C4- benzothiophenes	C4-BT	190.4	5.18	190-161 190-175	15 15		
C2-naphthalenes	C2-NAP	156.2	4.26	156-141	20		
C3- naphthalenes	C3-NAP	170.3	4.81	170-141 170-155	20 15		
C4- naphthalenes	C4-NAP	184.3	4.96	184-141 184-155 184-169	20 20 20		
C1-fluorenes	C1-FLU	180.3	4.97	180-179	30 30		
C2- fluorenes	C2-FLU	194.3	5.11	194-165 194-179	30 30		
C3- fluorenes	C3-FLU	208.3	5.66	208-179 208-193	30 30		
C1- fluorenes	C1-DBT	198.3	4.71	206-191	20		
C2- fluorenes	C2-DBT	212.3	5.26	220-191 220-205	25 20		
C3- fluorenes	C3-DBT	226.4	5.81	234-191 234-205 234-219	25 25 20		
C4- fluorenes	C4-DBT	240.4	6.35	198-197	20		
C2-phenanthrenes	C2-PHE	206.3	5.44	212-197 212-211	20 20		
C3- phenanthrenes	C3-PHE	220.3	5.99	226-197 226-211	20 20		
C4- phenanthrenes	C4-PHE	234.3	6.53	240-197 240-211 240-225	30 25 20		
C1-pyrenes	C1-PYR	216.3	5.48	216-215	30		
C2- pyrenes	C2-PYR	230.3	6.03	230-215	30		
C3- pyrenes	C3-PYR	244.3	6.57	244-215 244-229	30 30		
C1-chrysenes	C1-CHR	242.3	6.07	242-241	20		

C2- chrysenes	C2-CHR	256.3	6.62	256-241	15		
C3- chrysenes	C3-CHR	270.4	7.16	270-241 270-255	20 20		
C4- chrysenes	C4-CHR	284.4	7.71	284-241 284-255 284-269	20 20 20		

Table S2: LOD and LOQ in compounds. LOQ = 3*LOD

Analyte	LOD (ng/sample)	LOQ (ng/sample)
Benzothiophene	1,746	5,239
Naphthalene	0,003	0,010
C1-NAP	0,027	0,080
C2-NAP	0,011	0,034
C3-NAP	0,015	0,044
C4-NAP	0,025	0,075
Biphenyl	0,003	0,010
Acenaphthylene	0,148	0,444
Acenapthene	0,186	0,558
Dibenzofuran	0,003	0,010
Fluorene	0,809	2,426
C1-FLU	0,004	0,011
C2-FLU	0,008	0,023
C3-FLU	0,012	0,037
Phenanthrene	2,648	7,943
Anthracene	3,697	11,090
C1-PHE	0,004	0,013
C2-PHE	0,017	0,050
C3-PHE	0,004	0,011
C4-PHE	0,028	0,083
Dibenzothiophene	0,003	0,010
C1-DBT	0,001	0,004
C2-DBT	0,002	0,005
C3-DBT	0,000	0,001
C4-DBT	0,002	0,005
Fluoranthene	0,512	1,536
Pyrene	0,257	0,770
C1-FLA/PYR	0,002	0,005
C2-FLA/PYR	0,001	0,003
C3-FLA/PYR	0,001	0,004
Benz[a]anthracene	0,300	0,900
Chrysene	0,188	0,564
C1-CHR	0,001	0,003
C2-CHR	0,001	0,004
C3-CHR	0,003	0,008
C4-CHR	0,003	0,009
Benzo[b]fluoranthene	0,311	0,933
Benzo[k]fluoranthene	0,548	1,645
Benzo[e]pyrene	0,388	1,164
Benzo[a]pyrene	0,505	1,514
Perylene	0,569	1,707
Indeno[1,2,3-cd]pyrene	0,009	0,026
Dibenz[ah]anthracene	0,504	1,511
Benzo[ghi]perylene	0,517	1,552

Table S3: Raw data for dep1. (e)= sample evaporated too much, (c)= uncrushed eggs.

Sample ID, E#3-2B	DEP1 0h 1	DEP1 0h 2 **/Ctrl0h2	DEP1 0h 3 **/Ctrl0h3	DEP1 0h 4 *	DEP1 6h 2	DEP1 6h 3	DEP1 12h 1	DEP1 12h 3	DEP1 24h 1	DEP1 24h 3	DEP1 48h 1	DEP1 48h 2	DEP1 48h 3 (c)	DEP1 96h 1	DEP1 96h 2	DEP1 96h 3 (e) (c)	DEP1 192 1	DEP1 192 2 (c)	DEP1 192 3 (c)
Sample time (hours)	0	0	0	0	6	6	12	12	24	24	48	48	48	96	96	96	192	192	192
Hours after fertilization	48	48	48	48	54	54	60	60	72	72	96	96	96	144	144	144	240	240	240
Sample # eggs	22	24	24	23	25	24	20	19	24	26	23	21	23	28	26	20	23	25	20
mLipidBB	8,241936967	6,221005304	7,302595351	6,96787471	9,830209971	9,607965781	8,677001181	8,000512101	8,997401744	10,666689	5,621287542	6,49347277	7,929532227	7,590783389	6,850742919	6,948676717	7,776157473	7,886949705	6,897238865
Average:				7,183		9,719		8,339		9,832			6,681			7,130			7,520
Analyte	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**
Benzothiophene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Naphthalene	1,666	1,785	1,657	1,627	0,850	0,936	1,105	0,594	0,731	0,980	0,737	0,723	0,569	0,321	0,315	0,411	<LOQ	0,052	0,092
C1-NAP	0,019	0,021	0,033	0,017	0,009	0,011	0,013	0,007	0,009	0,013	0,012	0,012	0,009	0,010	0,010	0,013	0,006	0,006	0,009
C2-NAP	0,005	0,005	0,004	0,004	0,002	0,003	0,004	0,002	0,003	0,004	0,004	0,004	0,003	0,003	0,003	0,004	0,002	0,002	0,003
C3-NAP	0,003	0,003	0,002	0,002	0,002	0,002	0,002	0,001	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,003	0,001	0,001	0,002
C4-NAP	0,004	0,003	0,003	0,003	0,002	0,004	0,003	0,002	0,003	0,003	0,003	0,004	0,003	0,003	0,003	0,004	0,002	0,002	0,004
Biphenyl	0,098	0,097	0,089	0,089	0,043	0,053	0,068	0,038	0,044	0,070	0,064	0,058	0,035	0,044	0,036	0,059	0,001	0,014	0,032
Acenaphthylene	0,009	<LOD	<LOD	0,009	<LOD	0,007	<LOD	<LOD	<LOD	0,008	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Acenapthene	0,014	0,010	0,010	0,009	<LOD	<LOD	0,009	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Dibenzofuran	0,021	0,019	0,020	0,017	0,006	0,009	0,011	0,006	0,005	0,015	0,006	0,006	<LOD	0,004	0,002	0,009	<LOD	<LOD	<LOD
Fluorene	0,050	0,052	0,047	0,041	<LOD	<LOD	<LOD	<LOD	<LOD	0,039	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-FLU	0,001	0,001	0,001	0,001	0,000	0,001	0,001	0,000	0,000	0,000	0,000	0,001	0,000	0,000	0,000	0,001	0,000	0,000	0,000
C2-FLU	0,001	0,001	0,001	0,001	0,000	0,002	0,001	0,000	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,000	0,000	0,001
C3-FLU	<LOD	<LOD	0,001	<LOD	<LOD	0,001	0,001	<LOD	<LOD	<LOD	<LOD	0,001	<LOD	0,000	<LOD	<LOD	<LOD	<LOD	<LOD
Phenanthrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-PHE	0,001	0,001	0,001	0,001	0,000	0,001	0,001	0,000	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,000	0,000	0,001
C2-PHE	0,001	0,001	0,002	0,001	0,001	0,002	0,001	<LOD	0,001	0,001	0,001	0,002	0,001	0,001	0,001	0,002	<LOD	<LOD	0,001
C3-PHE	0,001	0,001	0,001	0,001	0,000	0,003	0,001	0,000	0,001	0,001	0,001	0,001	0,001	0,000	0,000	0,001	<LOD	0,000	0,000
C4-PHE	<LOD	<LOD	<LOD	<LOD	<LOD	0,005	0,001	<LOD	<LOD	<LOD	<LOD	0,002	<LOD	<LOD	0,001	<LOD	<LOD	<LOD	0,002
Dibenzothiophene	0,019	0,019	0,019	0,014	0,008	0,012	0,013	0,007	0,009	0,017	0,011	0,011	0,005	0,010	0,006	0,016	<LOD	0,004	0,007
C1-DBT	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
C2-DBT	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	0,000
C3-DBT	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
C4-DBT	0,000	<LOD	0,000	<LOD	<LOD	0,000	0,000	<LOD	<LOD	0,000	<LOD	0,000	0,000	0,000	0,000	0,000	<LOD	<LOD	0,000
Fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-FLA/PYR	0,000	0,000	0,000	0,000	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	0,000
C2-FLA/PYR	0,000	0,000	0,000	0,000	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	0,000
C3-FLA/PYR	0,000	0,000	0,000	0,000	0,000	0,001	0,000	<LOD	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	<LOD	0,000
Benz[a]anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Chrysene	0,010	0,008	0,014	<LOQ	<LOQ	0,012	<LOD	<LOD	<LOD	<LOD	<LOD	0,010	<LOD	0,007	<LOD	0,010	<LOD	<LOD	<LOD
C1-CHR	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	<LOD	0,000
C2-CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C3-CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C4-CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[b]fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[k]fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[e]pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[a]pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Perylene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Indeno[1,2,3-cd]pyrene	0,000	<LOD	0,001	<LOD	<LOD	0,002	<LOD	<LOD	0,001	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Dibenz[ah]anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[ghi]perylene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table S4: Raw data for dep2. (e)= sample evaporated too much, (c)= uncrushed eggs.

Sample ID, E#3-2B	DEP2 48h 1	DEP2 48h 2	DEP2 48h 3	DEP2 48h 4	DEP2 54h 1	DEP2 54h 2	DEP2 54h 3	DEP2 60h 1	DEP2 60h 2	DEP2 72h 1 (e)	DEP2 72h 2	DEP2 72h 3	DEP2 96h 1	DEP2 96h 2	DEP2 96h 3 (c)	DEP2 144h 1 (c)	DEP2 144h 2 (e)	DEP2 144h 3	DEP2 240h 1	DEP2 240h 2	DEP2 240h 3
Sample time (hours)	0	0	0	0	6	6	6	12	12	24	24	24	48	48	48	96	96	96	192	192	192
Hours after fertilization	36	36	36	36	42	42	42	48	48	60	60	60	84	84	84	132	132	132	240	240	240
Sample # eggs	23	25	25	26	21	19	19	23	21	25	20	20	24	23	20	20	25	23	19	23	22
Analyte	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**
Benzothiophene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Naphthalene	1,218	0,607	<LOD	1,068	0,965	1,141	0,972	0,749	0,835	0,706	0,600	0,563	0,567	0,671	0,480	0,313	0,278	0,279	0,098	0,106	0,083
C1-NAP	0,017	0,012	<LOD	0,015	0,013	0,019	0,016	0,011	0,013	0,014	0,014	0,013	0,013	0,014	0,010	0,010	0,012	0,009	0,008	0,008	0,007
C2-NAP	0,004	0,003	<LOD	0,004	0,004	0,004	0,003	0,003	0,003	0,003	0,003	0,003	0,003	0,004	0,003	0,004	0,003	0,003	0,003	0,003	0,003
C3-NAP	0,002	0,002	<LOD	0,002	0,002	0,003	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002
C4-NAP	0,004	0,003	<LOD	0,004	0,004	0,004	0,003	0,003	0,003	0,007	0,003	0,003	0,003	0,004	0,003	0,004	0,004	0,003	0,003	0,003	0,003
Biphenyl	0,080	0,052	0,106	0,073	0,053	0,076	0,050	0,051	0,048	0,042	0,033	0,038	0,049	0,075	0,045	0,051	0,018	0,040	0,045	0,044	0,035
Acenaphthylene	<LOD	<LOD	0,010	0,006	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,007	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Acenapthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Dibenzofuran	0,008	0,002	0,015	0,005	<LOD	0,007	<LOD	0,000	<LOD	0,004	<LOD	<LOD	0,004	0,008	<LOD	0,001	<LOD	<LOD	<LOD	0,001	<LOD
Fluorene	<LOD	<LOD	0,041	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-FLU	0,000	0,000	0,001	0,000	0,000	0,001	0,000	0,000	0,000	0,001	0,000	0,000	0,000	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000
C2-FLU	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,003	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,000	0,001	0,000
C3-FLU	<LOD	<LOD	0,001	0,001	<LOD	<LOD	<LOD	<LOD	0,001	0,001	<LOD	<LOD	<LOD	0,001	0,001	<LOD	0,001	0,001	<LOD	<LOD	<LOD
Phenanthrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-PHE	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,000	0,000	0,000
C2-PHE	0,002	0,001	0,001	0,002	0,001	0,002	0,001	0,001	0,001	0,002	0,001	0,002	0,001	0,002	0,001	0,001	0,002	0,001	<LOD	0,001	0,001
C3-PHE	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,004	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,000	0,000	0,000	0,000
C4-PHE	0,001	<LOD	0,001	<LOD	<LOD	<LOD	<LOD	0,001	<LOD	0,004	<LOD	<LOD	<LOD	<LOD	0,001	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Dibenzothiophene	0,011	0,006	0,017	0,009	0,006	0,013	0,004	0,005	0,003	0,012	0,002	0,003	0,008	0,013	0,003	0,009	0,007	0,006	0,007	0,008	0,005
C1-DBT	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
C2-DBT	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
C3-DBT	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
C4-DBT	0,000	0,000	0,000	0,000	<LOD	<LOD	<LOD	<LOD	0,000	0,000	<LOD	<LOD	<LOD	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	<LOD
Fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,015	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-FLA/PYR	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,002	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
C2-FLA/PYR	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,003	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD
C3-FLA/PYR	0,000	<LOD	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,002	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Benz[a]anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Chrysene	0,011	0,008	0,011	0,010	0,011	0,011	0,010	0,009	<LOD	0,017	<LOD	<LOD	0,008	0,011	0,010	0,011	<LOD	<LOD	<LOD	<LOD	<LOD
C1-CHR	6,16E-05	<LOD	5,83E-05	9,17E-05	7,91E-05	1,01E-04	6,10E-05	6,03E-05	6,64E-05	3,43E-04	6,79E-05	5,98E-05	5,58E-05	7,86E-05	8,73E-05	6,48E-05	5,09E-05	4,97E-05	<LOD	<LOD	<LOD
C2-CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	7,27E-05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C3-CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,51E-04	2,28E-04	<LOD	<LOD	<LOD	<LOD	1,44E-04	1,33E-04	2,15E-04
C4-CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,59E-04	<LOD	<LOD	<LOD	<LOD	1,61E-04	<LOD
Benzo[b]fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[k]fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[e]pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[a]pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Perylene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Indeno[1,2,3-cd]pyrene	<LOD	<LOD	<LOD	<LOD	0,000	0,005	<LOD	<LOD	<LOD	0,003	<LOD	<LOD	<LOD	<LOD	<LOD	0,001	<LOD	<LOD	<LOD	<LOD	<LOD
Dibenz[ah]anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[ghi]perylene	<LOD	<LOD	<LOD	<LOD	<LOD	0,030	0,028	<LOD	<LOD	<LOD	0,029	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table S5: Raw data for dep3. (e)= sample evaporated too much, (c)= uncrushed eggs.

SampleID, E#3-2B :	DEP3 288h 1	DEP3 288h 2	EP3 288h 3/4	EP3 288h 3/4	DEP3 294h 1	DEP3 294h 2	DEP3 300h 1	DEP3 300h 2 (e)	DEP3 300h 3 (e)	DEP3 312h 1	DEP3 312h 2	DEP3 312h 3 (e)	DEP3 336h 1	DEP3 336h 2	DEP3 336h 3	DEP3 384h 1	DEP3 384h 2	DEP3 480h 1	DEP3 480h 2	DEP3 480h 3
Hours after exposure	0	0	0	0	6	6	12	12	12	24	24	24	48	48	48	96	96	192	192	192
Hours after fertilization	240	240	240	240	246	246	252	252	252	264	264	264	288	288	288	336	336	432	432	432
Sample # eggs	23	21	20	19	24	20	23	24	21	18	18	24	18	25	28	20	21	22	21	23
Analyte	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**
Benzothiophene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Naphthalene	0,355	0,265	0,317	0,324	0,410	0,317	0,268	0,288	0,302	0,220	0,254	0,286	0,221	0,199	0,173	0,074	0,088	0,010	0,006	0,008
C1-NAP	0,007	0,005	0,006	0,007	0,007	0,006	0,006	0,006	0,006	0,006	0,007	0,007	0,006	0,005	0,005	0,005	0,005	0,003	0,003	0,003
C2-NAP	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,001	0,001	0,001
C3-NAP	0,001	0,001	0,001	0,002	0,001	0,001	0,001	0,001	0,001	0,002	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001
C4-NAP	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,001	0,002	<LOD
Biphenyl	0,028	0,016	0,025	0,023	0,041	0,026	0,024	0,023	0,030	0,020	0,017	0,030	0,028	0,025	0,021	0,017	0,023	0,022	0,018	0,014
Acenaphthylene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Acenapthene	<LOD	<LOD	<LOD	<LOD	0,0133	<LOD	<LOD	<LOD	<LOD	0,0112	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,012	0,013	0,010
Dibenzofuran	0,003	<LOD	<LOD	<LOD	0,0181	<LOD	<LOD	<LOD	<LOD	0,0285	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,002	0,011	0,011	0,010
Fluorene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-FLU	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	<LOD	<LOD
C2-FLU	0,001	0,001	0,000	0,001	0,000	0,001	0,001	0,001	0,000	<LOD	0,001	0,000	<LOD	0,000	0,000	<LOD	0,000	<LOD	0,000	<LOD
C3-FLU	0,001	0,001	<LOD	<LOD	0,001	0,001	<LOD	0,001	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000	0,001	<LOD	<LOD	<LOD	<LOD
Phenanthrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-PHE	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	<LOD	<LOD
C2-PHE	0,001	0,001	0,001	<LOD	<LOD	0,001	<LOD	0,001	0,001	0,001	<LOD	0,001	<LOD	<LOD	0,001	<LOD	0,001	<LOD	<LOD	<LOD
C3-PHE	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	0,000	0,000	0,000	<LOD	0,001	<LOD
C4-PHE	0,001	<LOD	<LOD	<LOD	0,002	<LOD	<LOD	0,002	0,001	0,002	<LOD	<LOD	<LOD	<LOD	0,001	<LOD	0,002	0,002	0,002	<LOD
Dibenzothiophene	0,004	<LOD	0,002	0,001	0,007	<LOD	0,002	0,002	0,001	<LOD	<LOD	0,003	0,000	0,002	0,000	<LOD	<LOD	<LOD	<LOD	<LOD
C1-DBT	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
C2-DBT	0,000	0,000	0,000	<LOD	0,000	0,000	<LOD	0,000	0,000	<LOD	0,000	0,000	0,000	<LOD	0,000	0,000	<LOD	<LOD	<LOD	<LOD
C3-DBT	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD
C4-DBT	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	<LOD	0,000	0,000	<LOD	<LOD	<LOD	0,000	<LOD
Fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-FLA/PYR	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	0,000	<LOD	<LOD	<LOD	0,000	0,000	0,000	<LOD	<LOD	0,000	<LOD
C2-FLA/PYR	0,000	<LOD	0,000	0,000	0,000	0,000	<LOD	<LOD	<LOD	0,000	0,000	0,000	<LOD	0,000	<LOD	0,000	<LOD	<LOD	0,000	<LOD
C3-FLA/PYR	0,000	0,000	0,000	0,000	0,000	0,000	0,000	<LOD	0,000	0,000	0,000	0,000	<LOD	<LOD	0,000	<LOD	<LOD	<LOD	0,000	<LOD
Benz[a]anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Chrysene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-CHR	4,84E-05	5,25E-05	6,53E-05	5,96E-05	4,67E-05	6,43E-05	4,49E-05	<LOD	5,99E-05	6,88E-05	<LOD	<LOD	<LOD	<LOD	<LOD	5,27E-05	<LOD	<LOD	6,44E-05	<LOD
C2-CHR	<LOD	<LOD	<LOD	<LOD	7,88E-05	7,25E-05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4,96E-05	<LOD	<LOD	8,07E-05	1,01E-04	6,05E-05
C3-CHR	<LOD	<LOD	<LOD	<LOD	1,46E-04	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,42E-04	1,50E-04	1,33E-04
C4-CHR	0,000	0,000	<LOD	<LOD	0,000	0,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[b]fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[k]fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[e]pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[a]pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Perylene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Indeno[1,2,3-cd]pyrene	0,000	<LOD	<LOD	<LOD	0,008	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,008	0,008	0,009
Dibenz[ah]anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[ghi]perylene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table S6: Raw data for ctrl. (e)= sample evaporated too much, (c)= uncrushed eggs.

Sample ID, E#3-2B:	Ctrl 0h 1	DEP1 0h 2 **/Ctrl0h2	DEP1 0h 3 **/Ctrl0h3	Ctrl 0h 4	Ctrl 48h 1	Ctrl 48h 3	Ctrl 96h 1	Ctrl 96h 2 (c)	Ctrl 96h 3 (c)	Ctrl 240h 1	Ctrl 240h 2	Ctrl 240h 3	Ctrl 288h 1	Ctrl 288h 2	Ctrl 288h 3	Ctrl 480h 1	Ctrl 480h 2	Ctrl 480h 3
Sample time (hours)	0	0	0	0	48	48	96	96	96	240	240	240	288	288	288	480	480	480
Hours after fertilization	0	0	0	0	48	48	96	96	96	240	240	240	288	288	288	480	480	480
Sample # eggs	23	15	31	26	22	28	22	23	21	21	20	18	25	21	23	22	20	26
Analyte	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**	**ng/egg**
Benzothiophene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Naphthalene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-NAP	<LOD	0,001857554	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C2-NAP	<LOD	0,000760896	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C3-NAP	<LOD	0,001413048	<LOD	<LOD	<LOD	<LOD	0,000688187	<LOD	0,000727764	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C4-NAP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Biphenyl	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Acenaphthylene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Acenapthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,010338601	<LOD	0,009247135	0,008566551	0,009637664	<LOD
Dibenzofuran	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,004680696	0,004624697	0,006536144	0,004664563	0,005693497	0,0039448
Fluorene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-FLU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C2-FLU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C3-FLU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000701183	0,00081325	<LOD	0,000704799	<LOD
Phenanthrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-PHE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C2-PHE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,09451126	<LOD	<LOD	<LOD	<LOD
C3-PHE	<LOD	0,000283176	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000188298	<LOD	<LOD	<LOD	<LOD	0,025512376	0,00024363	<LOD	0,000193979	<LOD
C4-PHE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,001442118	<LOD	<LOD	0,002378758	0,422516871	0,004037178	0,002635238	0,003295165	0,001546678
Dibenzothiophene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1-DBT	<LOD	9,67962E-05	<LOD	<LOD	<LOD	<LOD	<LOD	6,71135E-05	<LOD	<LOD	7,48045E-05	<LOD	<LOD	9,91084E-05	6,1087E-05	8,31848E-05	<LOD
C2-DBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,004227305	<LOD	<LOD	<LOD	<LOD	<LOD
C3-DBT	<LOD	3,31779E-05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2,4953E-05	<LOD	<LOD	2,24034E-05	1,6499E-05	0,002803695	2,4733E-05	2,15436E-05	2,86052E-05	<LOD
C4-DBT	<LOD	0,000143334	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	8,8602E-05	<LOD	<LOD	<LOD	8,87309E-05	0,012616502	0,000136676	9,98944E-05	0,00012021	<LOD
Fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,195330705	<LOD	<LOD	<LOD	<LOD
Pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,277617976	<LOD	<LOD	<LOD	<LOD
C1-FLA/PYR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,067976853	7,16011E-05	<LOD	<LOD	<LOD
C2-FLA/PYR	<LOD	7,72268E-05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,031267196	<LOD	<LOD	<LOD	<LOD
C3-FLA/PYR	<LOD	9,54525E-05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	5,07565E-05	0,074239846	6,9358E-05	<LOD	<LOD	5,80143E-05
Benz[a]anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,4159241	<LOD	<LOD	<LOD	<LOD
Chrysene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,581852877	<LOD	<LOD	<LOD	<LOD
C1-CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4,27883E-05	0,054097358	4,94686E-05	<LOD	<LOD	<LOD
C2-CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000109351	0,220045077	0,000168096	0,000130312	0,000142024	7,5233E-05
C3-CHR	<LOD	0,000241256	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000226628	0,401500031	0,00030181	0,000241325	0,000257598	0,00014082
C4-CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000183147	0,357841956	0,000260304	0,000185848	0,000230914	<LOD
Benzo[b]fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	14,3500802	<LOD	<LOD	<LOD	<LOD
Benzo[k]fluoranthene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	14,00110628	<LOD	<LOD	<LOD	<LOD
Benzo[e]pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzo[a]pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,24208994	<LOD	<LOD	<LOD	<LOD
Perylene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	10,80634702	<LOD	<LOD	<LOD	<LOD
Indeno[1,2,3-cd]pyrene	<LOD	0,002877773	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,000650373	<LOD	<LOD	0,007945044	14,20083213	0,009594334	0,009170542	0,008077271	<LOD
Dibenz[ah]anthracene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	25,20408268	<LOD	<LOD	<LOD	<LOD
Benzo[ghi]perylene	<LOD	0,041352211	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	29,33888976	<LOD	<LOD	<LOD	<LOD

Table S7: Internal standards used for quantification of PAHs and alkyl PAHs

Internal standards (IS):	IS concentration (ng/mL)
Recovery NAP-d8	250,8
Recovery ACE-d10	106,4
Recovery PHE-d10	50,00
Recovery CHR-d12	48,6
Recovery PER-d12	50,8

Table S8: Average concentration of tPAH from each depuration, given with standard deviation *. hpe = hours post exposure.

tPAH			
Sample time (hpe)	**Dep1 (ng/egg)**	**Dep2 (ng/egg)**	**Dep3 (ng/egg)**
0	1,9 ± 0,3	1,3±0,2	0,36 ± 0,07
6	0,9 ± 0,2	1,2 ± 0,2	0,44 ± 0,07
12	0,9 ± 0,2	0,9 ± 0,2	0,33 ± 0,06
24	0,9 ± 0,2	0,7 ± 0,1	0,36 ± 0,06
48	0,7 ± 0,1	0,7 ± 0,1	0,24 ± 0,05
96	0,44 ± 0,07	0,36 ± 0,06	0,12 ± 0,02
192	0,08 ± 0,02	0,16 ± 0,02	0,066 ± 0,005

Table S9: Average concentration of naphthalene from each depuration, given with standard deviation *. hpe = hours post exposure.

Naphthalene			
Sample time (hpe)	**Dep1 (ng/egg)**	**Dep2 (ng/egg)**	**Dep3 (ng/egg)**
0	1,68 ± 0,06	1,1 ± 0,3	0,31 ± 0,03
6	0,89 ± 0,04	1,03 ± 0,08	0,36 ± 0,05
12	0,8 ± 0,3	0,79 ± 0,04	0,29 ± 0,01
24	0,9 ± 0,1	0,62 ± 0,06	0,25 ± 0,03
48	0,73 ± 0,08	0,57 ± 0,08	0,20 ± 0,02
96	0,35 ± 0,04	0,29 ± 0,02	0,081 ± 0,007
192	0,07 ± 0,02	0,10 ± 0,01	0,008 ± 0,002

Table S10: Average concentration of C1-phenanthrene from each depuration, given with standard deviation *. hpe = hours post exposure.

C1-phenanthrene			
Sample time (hpe)	**Dep1 (ng/egg)**	**Dep2 (ng/egg)**	**Dep3 (ng/egg)**
0	0,00081 ± 0,00009	0,0007 ± 0,0001	0,000336 ± 0,000008
6	0,0006 ± 0,0002	0,00068 ± 0,00009	0,0003167 ± 0,0000006
12	0,0006 ± 0,0002	0,00059 ± 0,00002	0,000323 ± 0,000007
24	0,00060 ± 0,00006	0,0009 ± 0,0004	0,000333 ± 0,000006
48	0,00072 ± 0,00007	0,0007 ± 0,0002	0,000232 ± 0,000006
96	0,0007 ± 0,0002	0,0008 ± 0,0001	0,000244 ± 0,000001
192	0,0004 ± 0,0001	0,00036 ± 0,00003	0,00 ± 0,00

Table S11: Average concentration of dibenzothiophene from each depuration, given with standard deviation *. hpe = hours post exposure.

Dibenzothiophene			
Sample time (hpe)	**Dep1 (ng/egg)**	**Dep2 (ng/egg)**	**Dep3 (ng/egg)**
0	0,018 ± 0,002	0,011 ± 0,004	0,002 ± 0,00
6	0,010 ± 0,002	0,008 ± 0,004	0,0066 ± 0,0000
12	0,010 ± 0,003	0,004 ± 0,001	0,00183 ± 0,00009
24	0,013 ± 0,004	0,006 ± 0,004	0,003 ± 0,000
48	0,009 ± 0,003	0,008 ± 0,004	0,0010 ± 0,0001
96	0,011 ± 0,004	0,0075 ± 0,0009	0,00 ± 0,00
192	0,005 ± 0,002	0,007 ± 0,001	0,00 ± 0,00

Table S12: Average BB concentration of tPAH* normalized to lipid content** from each depuration, given with standard deviation. hpe = hours post exposure.

Body burden (ng tPAH/mg lipid)			
Sample time (hpe)	**Dep1 (ng tPAH/mg lipid)**	**Dep2 (ng tPAH/mg lipid)**	**Dep3 (ng tPAH/mg lipid)**
0	0,27 ± 0,03	0,10 ± 0,05	0,05 ± 0,01
6	0,103 ± 0,008	0,156 ± 0,01	0,053 ± 0,005
12	0,11 ± 0,03	0,12 ± 0,01	0,036 ± 0,003
24	0,099 ± 0,009	0,111 ± 0,006	0,038 ± 0,009
48	0,12 ± 0,03	0,086 ± 0,008	0,026 ± 0,005
96	0,06 ± 0,01	0,04 ± 0,01	0,022 ± 0,004
192	0,011 ± 0,008	0,021 ± 0,002	0,009 ± 0,002

* Values calculated from appendix 3-6.

** Values calculated from appendix

Table S13: Folch extraction analysis of dep1. (c) = uncrushed eggs, (d) = dirty sample

			Lipid									BB				
SINTEF-ID	Sample ID	hpe	eggs	weight1	weight2	weight (egg)	weight (lipid)	Lipid content (%)/egg	Average (%)	STD		Lipid content in BB sample	Average, Lipid cont. in BB sample	STD	weight (egg)	Lipid content in BB(%)/egg
2019-598-S1	E#3-2B Dep1 0 h 1	0	14	11179,82	11184,81	104,39	4,99	4,7802				8,24194			172,42	4,7802
2019-598-S2	E#3-2B Dep1 0 h 2	0	14	11147,43	11150,97	118,77	3,54	2,9806				6,22101			208,72	2,9806
2019-598-S3	E#3-2B Dep1 0 h 3	0	12	10971,16	10974,56	88,62	3,40	3,8366				7,30260			190,34	3,8366
2019-598-S4	E#3-2B Dep1 0 h 4	0	15	10930,15	10934,00	107,75	3,85	3,5731	3,7926	0,64900015		6,96787	7,18335308	0,72583792	195,01	3,5731
2019-598-S5	E#3-2B Dep1 6 h 1	6	15	11000,24	11004,26	188,73	4,02	2,1300				4,02000			188,73	2,1300
2019-598-S6	E#3-2B Dep1 6 h 2	6	12	10953,03	10957,41	85,25	4,38	5,1378				9,83021			191,33	5,1378
2019-598-S7	E#3-2B Dep1 6 h 3	6	18	11092,04	11099,37	132,09	7,33	5,5492	4,2724	1,52414659		9,60797	7,81939192	2,68810743	173,14	5,5492
2019-598-S9	E#3-2B Dep1 12 h 1	12	12	10953,50	10959,02	101,64	5,52	5,4309				8,67700			159,77	5,4309
2019-598-S10	E#3-2B Dep1 12 h 2	12	12	10980,09	10984,93	88,81	4,84	5,4498				9,41296			172,72	5,4498
2019-598-S11	E#3-2B Dep1 12 h 3	12	10	11062,62	11066,88	85,53	4,26	4,9807	5,2872	0,21683082		8,00051	8,69682376	0,57679896	160,63	4,9807
2019-598-S13	E#3-2B Dep1 24 h 1	24	12	10846,99	10852,05	97,45	5,06	5,1924				8,99740			173,28	5,1924
2019-598-S14	E#3-2B Dep1 24 h 2	24	10	10920,77	10924,51	73,75	3,74	5,0712				7,91916			156,16	5,0712
2019-598-S15	E#3-2B Dep1 24 h 3	24	15	11157,98	11163,49	107,14	5,51	5,1428	5,1355	0,04975912		10,66669	9,19441862	1,13029042	207,41	5,1428
2019-598-S17	E#3-2B Dep1 48 h 1	48	10	11037,31	11039,78	74,25	2,47	3,3266				5,62129			168,98	3,3266
2019-598-S18	E#3-2B Dep1 48 h 2	48	14	11018,03	11022,31	101,36	4,28	4,2226				6,49347			153,78	4,2226
2019-598-S19	E#3-2B Dep1 48 h 3	48	12	11154,54	11158,80	92,78	4,26	4,5915	4,0469	0,53112793		7,92953	6,68143085	0,9516633	172,70	4,5915
2019-598-S21	E#3-2B Dep1 96 h 1	96	14	11099,13	11103,20	108,12	4,07	3,7643				7,59078			201,65	3,7643
2019-598-S22	E#3-2B Dep1 96 h 2	96	12	11165,11	11168,33	91,80	3,22	3,5076				6,85074			195,31	3,5076
2019-598-S23	E#3-2B Dep1 96 h 3	96	12	11064,37	11068,48	89,55	4,11	4,5896	3,9539	0,46160188		6,94868	7,13006768	0,32821942	151,40	4,5896
2019-598-S25	E#3-2B Dep1 192 h 1	192	17	11000,39	11006,17	127,26	5,78	4,5419				7,77616			171,21	4,5419
2019-598-S26	E#3-2B Dep1 192 h 2	192	13	11154,79	11158,76	95,04	3,97	4,1772				7,88695			188,81	4,1772
2019-598-S27	E#3-2B Dep1 192 h 3	192	13	10985,89	10990,43	101,48	4,54	4,4738	4,3976	0,15832806		6,89724	7,52012	0,44275657	154,17	4,4738
	E#3-2B Dep1 A		5	10988,62	10990,48	21,49	1,86	8,6552								
	E#3-2B Dep1 B		5	11078,31	11080,11	20,59	1,80	8,7421								
	E#3-2B Dep1 C		5	11061,44	11062,88	21,75	1,44	6,6207								
	E#3-2B Dep1 D		5	11132,03	11133,43	20,76	1,40	6,7437								
	E#3-2B Dep1 E		5	11027,71	11029,55	21,72	1,84	8,4715								
	E#3-2B Dep1 F		5	10952,16	10954,07	21,40	1,91	8,9252	7,900644791	0,96045405						
Sum average									4,8483	0,56890608						

Table S14: Folch extraction analysis of dep2. (c) = uncrushed eggs, (d) = dirty sample

			Lipid									BB				
SINTEF-ID	Sample ID	hpe	eggs	weight1	weight2	weight (egg)	weight (lipid)	Lipid content (%)/egg	Average (%)	STD		Lipid content in BB sample	Average, Lipid cont. in BB sample	STD	weight (egg)	Lipid content in BB(%)/egg
2019-599-S1	E#3-2B Dep2 48 h 1	0	10	11179,82	11184,81	76,17	3,60	4,7263				8,720914			184,52	4,7263
2019-599-S2	E#3-2B Dep2 48 h 2	0	10	11147,43	11150,97	81,66	2,89	3,5391				7,014072			198,19	3,5391
2019-599-S3	E#3-2B Dep2 48 h 3	0	11	10971,16	10974,56	80,94	3,95	4,8802				8,711082			178,50	4,8802
2019-599-S4	E#3-2B Dep2 48 h 4	0	11	10930,15	10934,00	80,53	4,10	5,0913	4,5592	0,6030551		9,484528	8,48264882	0,90407847	186,29	5,0913
2019-599-S5	E#3-2B Dep2 54 h 1	6	13	11000,24	11004,26	106,55	4,82	4,5237				7,677168			169,71	4,5237
2019-599-S6	E#3-2B Dep2 54 h 2	6	11	10953,03	10957,41	86,64	4,42	5,1016				7,689086			150,72	5,1016
2019-599-S7	E#3-2B Dep2 54 h 3	6	12	11092,04	11099,37	102,73	4,73	4,6043	4,7432	0,2555403		6,912439	7,42623093	0,36333806	150,13	4,6043
2019-599-S9	E#3-2B Dep2 60 h 1	12	14	10953,50	10959,02	105,37	5,01	4,7547				7,935551			166,90	4,7547
2019-599-S10	E#3-2B Dep2 60 h 2	12	17	10980,09	10984,93	124,96	5,63	4,5054				6,848722			152,01	4,5054
2019-599-S11	E#3-2B Dep2 60 h 3	12	10	11062,62	11066,88	73,53	3,00	4,0800	4,4467	0,2785626		8,152999	7,64575723	0,57053762	199,83	4,0800
2019-599-S13	E#3-2B Dep2 72 h 1	24	11	10846,99	10852,05	89,17	3,50	3,9251				7,078894			180,35	3,9251
2019-599-S14	E#3-2B Dep2 72 h 2	24	12	10920,77	10924,51	96,67	4,35	4,4998				6,527925			145,07	4,4998
2019-599-S15	E#3-2B Dep2 72 h 3	24	16	11157,98	11163,49	114,06	4,55	3,9891	4,1380	0,2571811		5,907899	6,50490617	0,47833368	148,10	3,9891
2019-599-S17	E#3-2B Dep2 96 h 1	48	12	11037,31	11039,78	91,78	4,55	4,9575				8,328116			167,99	4,9575
2019-599-S18	E#3-2B Dep2 96 h 2	48	13	11018,03	11022,31	94,23	4,51	4,7862				8,405935			175,63	4,7862
2019-599-S19	E#3-2B Dep2 96 h 3	48	14	11154,54	11158,80	105,70	4,85	4,5885	4,7774	0,1507917		7,009787	7,91461292	0,64059672	152,77	4,5885
2019-599-S21	E#3-2B Dep2 144 h 1	96	12	11099,13	11103,20	93,79	4,54	4,8406				7,014031			144,90	4,8406
2019-599-S22	E#3-2B Dep2 144 h 2	96	12	11165,11	11168,33	90,91	4,63	5,0929				9,799343			192,41	5,0929
2019-599-S23	E#3-2B Dep2 144 h 3	96	13	11064,37	11068,48	98,32	5,14	5,2278	5,0538	0,1604908		8,757657	8,52367709	1,14907223	167,52	5,2278
2019-599-S25	E#3-2B Dep2 240 h 1	192	11	11000,39	11006,17	86,70	4,01	4,6251				7,035769			152,12	4,6251
2019-599-S26	E#3-2B Dep2 240 h 2	192	12	11154,79	11158,76	87,08	4,63	5,3169				8,808591			165,67	5,3169
2019-599-S27	E#3-2B Dep2 240 h 3	192	13	10985,89	10990,43	91,27	4,18	4,5798	4,8406	0,3373118		7,624481	7,822947	0,73723162	166,48	4,5798
	E#3-2B Dep2 A		5	11138,58	11140,20	19,67	1,62	8,2359								
	E#3-2B Dep2 B		5	11063,98	11065,05	21,26	1,07	5,0329								
	E#3-2B Dep2 C		5	10972,04	10973,37	21,22	1,33	6,2677								
	E#3-2B Dep2 D		5	10630,17	10631,82	21,28	1,65	7,7538								
	E#3-2B Dep2 E		5	10920,38	10921,98	20,23	1,60	7,9090								
	E#3-2B Dep2 F		5	11171,83	11173,34	22,91	1,51	6,5910	6,9651	1,1171078						
Sum average									4,9405	0,3030444						

Table S15: Folch extraction analysis of dep3. (c) = uncrushed eggs, (d) = dirty sample

			Lipid								BB				
SINTEF-ID	Sample ID	hpe	eggs	weight1	weight2	weight (egg)	weight (lipid)	Lipid content (%)/egg	Average (%)	STD	Lipid content in BB sample	Average, lipid cont. in BB sample	STD	weight (egg)	Lipid content in BB(%)/egg
2019-600-S1	E#3-2B Dep3 288 h 1	0	13	11141,49	11146,31	94,98	4,82	5,0748			8,25967			184,52	4,4763
2019-600-S2	E#3-2B Dep3 288 h 2	0	14	11143,21	11148,93	104,35	5,72	5,4816			8,31935			198,19	4,1977
2019-600-S3	E#3-2B Dep3 288 h 3	0	11	11150,14	11154,17	80,68	4,03	4,9950			7,19736			178,50	4,0321
2019-600-S4	E#3-2B Dep3 288 h 4	0	16	11024,35	11030,59	160,58	6,24	3,8859	4,8593	0,591507614	5,28251	7,2647225	1,22839975	186,29	2,8356
2019-600-S5	E#3-2B Dep3 294 h 1	6	9	10946,70	10950,03	68,77	3,33	4,8422			8,96442			169,71	5,2822
2019-600-S6	E#3-2B Dep3 294 h 2	6	10	11076,50	11080,30	72,30	3,80	5,2559			7,49383			150,72	4,9720
2019-600-S7	E#3-2B Dep3 294 h 3	6	15	11092,43	11097,36	109,10	4,93	4,5188	4,8723	0,301665318	10,23325	8,89716667	1,11937415	150,13	6,8163
2019-600-S9	E#3-2B Dep3 300 h 1	12	11	11029,74	11033,81	83,34	4,07	4,8836			8,72945			166,90	5,2303
2019-600-S10	E#3-2B Dep3 300 h 2	12	11	11065,14	11069,56	77,02	4,42	5,7388			9,99177			152,01	6,5731
2019-600-S11	E#3-2B Dep3 300 h 3	12	17	11146,75	11153,30	118,21	6,55	5,5410	5,3878	0,365537806	8,7542	9,15847333	0,58931635	199,83	4,3808
2019-600-S13	E#3-2B Dep3 312 h 1	24	10	11037,73	11041,46	75,25	3,73	4,9568			6,79579			180,35	3,7681
2019-600-S14	E#3-2B Dep3 312 h 2	24	13	10985,26	10989,28	96,12	4,02	4,1823			5,53691			145,07	3,8167
2019-600-S15	E#3-2B Dep3 312 h 3	24	10	11041,02	11045,57	73,53	4,55	6,1880	5,1090	0,825857184	11,94027	8,09099	2,7699473	148,10	8,0623
2019-600-S17	E#3-2B Dep3 336 h 1	48	10	11115,26	11119,26	72,52	4,00	5,5157			7,81688			167,99	4,6532
2019-600-S18	E#3-2B Dep3 336 h 2	48	13	11020,04	11025,20	98,22	5,16	5,2535			9,62601			175,63	5,4808
2019-600-S19	E#3-2B Dep3 336 h 3	48	15	10971,41	10976,85	120,61	5,44	4,5104	5,0932	0,425782647	10,24899	9,23062667	1,03151531	152,77	6,7088
2019-600-S21	E#3-2B Dep3 384 h 1	96	11	11062,72	11065,72	75,36	3,00	3,9809			5,72373			144,90	3,9501
2019-600-S22	E#3-2B Dep3 384 h 2	96	Missing	11010,87	11013,47	81,44	2,60	3,1925			4,89352			192,41	2,5433
2019-600-S23	E#3-2B Dep3 384 h 3	96	11	10972,63	10975,82	83,40	3,19	3,8249	3,6661	0,34087553	6,50622	5,70782333	0,65847809	167,52	3,8838
2019-600-S25	E#3-2B Dep3 480 h 1	192	10	11103,14	11106,23	79,98	3,09	3,8635			6,25572			152,12	4,1124
2019-600-S26	E#3-2B Dep3 480 h 2	192	16	11024,85	11030,47	121,39	5,62	4,6297			7,02049			165,67	4,2376
2019-600-S27	E#3-2B Dep3 480 h 3	192	15	11128,01	11133,59	112,86	5,58	4,9442	4,4791	0,453866961	8,42537	7,233860	0,89851372	166,48	5,0609
	E#3-2B Dep3 A		5	11105,75	11106,31	22,88	0,56	2,4476							
	E#3-2B Dep3 B		5	11141,13	11142,77	21,28	1,64	7,7068							
	E#3-2B Dep3 C		5	11072,09	11073,22	15,75	1,13	7,1746							
	E#3-2B Dep3 D		5	11023,18	11024,35	21,41	1,17	5,4647							
	E#3-2B Dep3 E		5	10975,35	10977,08	21,99	1,73	7,8672							
	E#3-2B Dep3 F		5	11113,91	11115,57	22,24	1,66	7,4640	6,3541	1,918179088					
Sum average									4,9776	0,503625968					

Table S16: Folch extraction analysis of ctrl. (c) = uncrushed eggs, (d) = dirty sample

			Lipid									BB				
SINTEF-ID	Sample ID	hpe	eggs	weight1	weight2	weight (egg)	weight (lipid)	Lipid content (%)/egg	Average (%)	STD		Lipid content in BB sample	Average, lipid cont. in BB sample	STD	weight (egg)	Lipid content (%)/egg
2019-601-S1	E#3-2B Ctrl 0 h 1	0	10	11023,86	11026,96	62,29	3,10	4,9767				10,0728849			202,40	4,9767
2019-601-S2	E#3-2B Ctrl 0 h 2	0	9	11156,44	11159,34	108,81	2,90	2,6652				3,8911865			146,00	2,6652
2019-601-S3	E#3-2B Ctrl 0 h 3	0	7	11180,63	11183,02	64,73	2,39	3,6923				9,1021597			246,52	3,6923
2019-601-S4	E#3-2B Ctrl 0 h 4	0	14	11104,87	11109,39	102,72	4,52	4,4003	3,9336	0,86212177		9,5711176	8,159337176	2,48801194	217,51	4,4003
2019-601-S5	E#3-2B Ctrl 48 h 1	48	11	11146,58	11150,05	77,07	3,47	4,5024				7,4307616			165,04	4,5024
2019-601-S6	E#3-2B Ctrl 48 h 2	48	11	11039,76	11043,47	82,00	3,71	4,5244				8,5732671			189,49	4,5244
2019-601-S7	E#3-2B Ctrl 48 h 3	48	12	11159,25	11163,68	90,00	4,43	4,9222	4,6497	0,19293182		10,5507833	8,851604016	1,28885929	214,35	4,9222
2019-601-S9	E#3-2B Ctrl 96 h 1	96	12	10793,11	10797,95	88,95	4,84	5,4413				11,0707858			203,46	5,4413
2019-601-S10	E#3-2B Ctrl 96 h 2	96	11	11069,19	11073,38	93,62	4,19	4,4755				7,7910190			174,08	4,4755
2019-601-S11	E#3-2B Ctrl 96 h 3	96	10	10980,46	10984,04	79,96	3,58	4,4772	4,7980	0,45484466		7,7630840	8,874962962	1,55272312	173,39	4,4772
2019-601-S13	E#3-2B Ctrl 240 h 1	240	15	11021,80	11027,06	111,80	5,26	4,7048				7,5658372			160,81	4,7048
2019-601-S14	E#3-2B Ctrl 240 h 2	240	11	11018,43	11021,76	90,12	3,33	3,6951				5,8045905			157,09	3,6951
2019-601-S15	E#3-2B Ctrl 240 h 3	240	16	10956,19	10961,02	119,87	4,83	4,0294	4,1431	0,42000169		5,9453283	6,438585342	0,79915554	147,55	4,0294
2019-601-S17	E#3-2B Ctrl 288 h 1	288	12	10974,24	10977,49	92,48	3,25	3,5143				6,4377974			183,19	3,5143
2019-601-S18	E#3-2B Ctrl 288 h 2	288	10	11130,89	11132,05	76,15	1,16	1,5233				2,4117032			158,32	1,5233
2019-601-S19	E#3-2B Ctrl 288 h 3	288	12	10851,98	10854,15	88,58	2,17	2,4498	2,4958	0,81345879		4,3720919	4,407197491	1,64383349	178,47	2,4498
2019-601-S21	E#3-2B Ctrl 480 h 1	480	14	10929,67	10934,45	111,08	4,78	4,3032				7,3554681			170,93	4,3032
2019-601-S22	E#3-2B Ctrl 480 h 2	480	15	10945,16	10949,88	112,37	4,72	4,2004				6,5438177			155,79	4,2004
2019-601-S23	E#3-2B Ctrl 480 h 3	480	14	10945,53	10950,55	109,66	5,02	4,5778	4,3605	0,15929521		9,6641638	7,854483218	1,32184262	211,11	4,5778
	E#3-2B Ctrl-A		5	11142,43	11144,27	22,32	1,84	8,2437								
	E#3-2B Ctrl A II		5	11107,37	11109,01	21,05	1,64	7,7910								
	E#3-2B Ctrl-B		5	11026,76	11028,48	22,14	1,72	7,7687								
	E#3-2B Ctrl B II		5	10969,37	10970,90	21,78	1,53	7,0248	7,7071	0,43713452						
Sum average									4,5840	0,47711264						

Table S17: Average concentration (ng/L) of compound in seawater solution

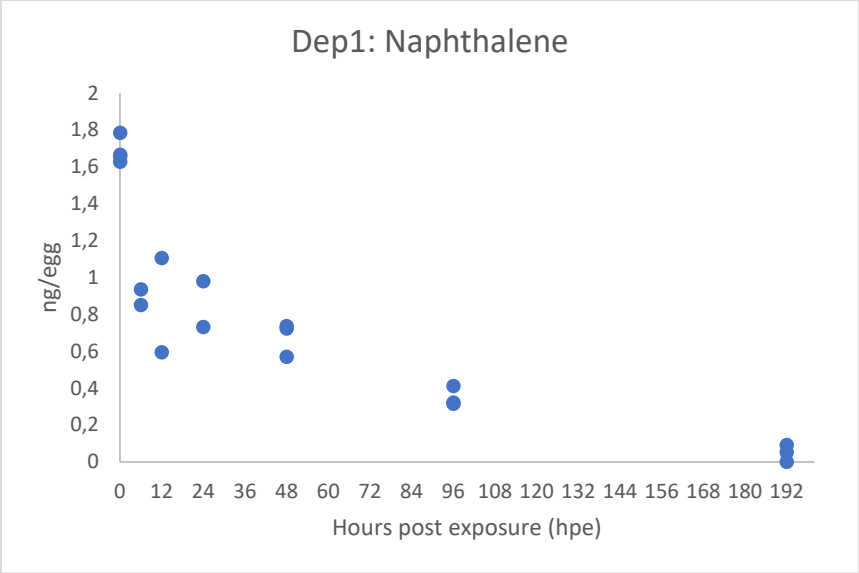
Compound	Dep1	Dep2	Dep3
NAP	6411	6381	3128
C1-PHE	529	545	528
DBT	66	67	63

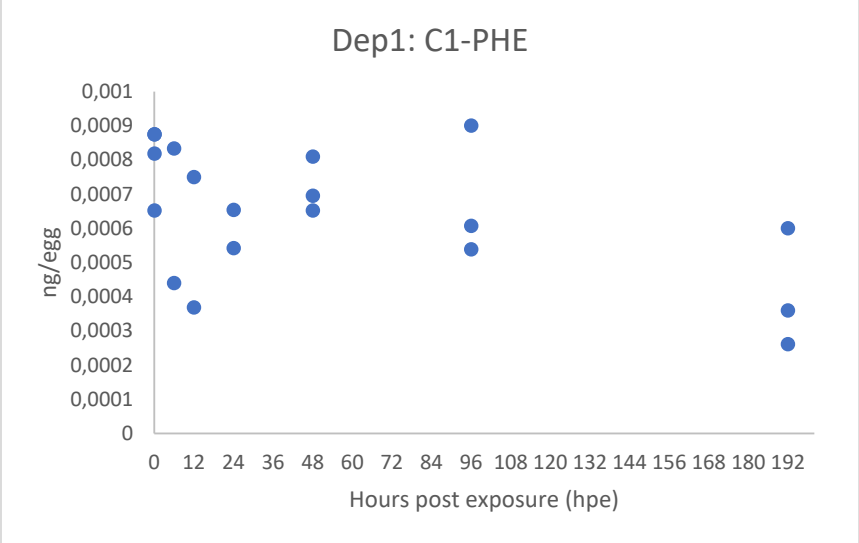
NAP = naphthalene
C1-PHE = C1-phenanthrene
DBT = dibenzothiophene

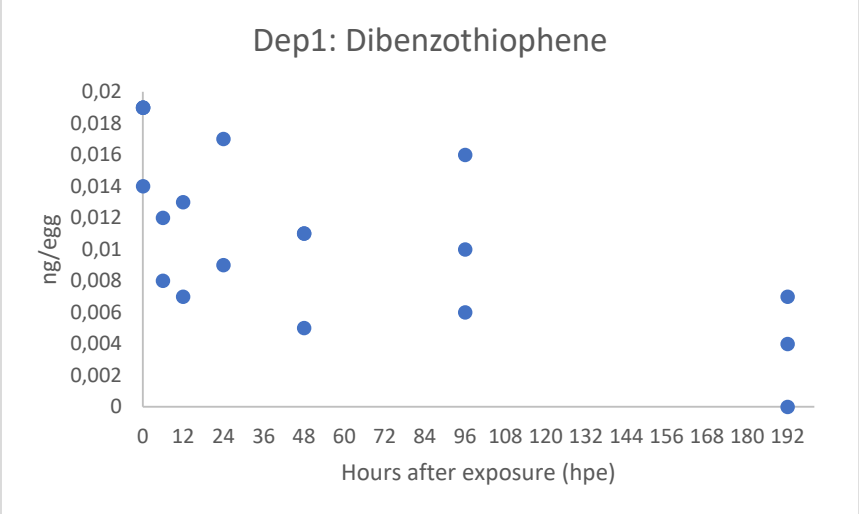
Table S18: Bioconcentration factor (BCF) values, given with standard deviation, calculated by Lisbet Sørensen, from research article "Oil droplet fouling and differential toxicokinetics of polycyclic aromatic hydrocarbons in embryos of Atlantic haddock and cod". SD = standard deviation (STD)

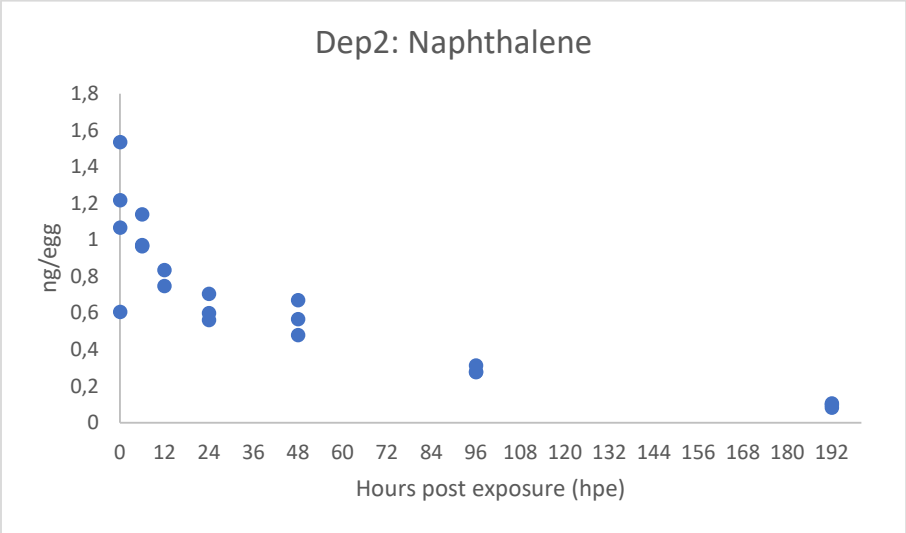
BCF (LKG lipid)	COD	HADDOCK
Naphthalene	3 406	2 194
Dibenzothiophene	92 867	114 881
Methylphenanthrene, 3-	70 134	72 715
Methylphenanthrene, 2-	76 959	98 346
Methylphenanthrene, 9-	95 592	130 771
Methylphenanthrene, 1-	78 882	81 888
C1-phenanthrene (average)	80 392	95 930
SD (ng/L)		
Naphthalene	295	308
Dibenzothiophene	10 796	19 837
Methylphenanthrene, 3-	10 753	10 829
Methylphenanthrene, 2-	12 215	23 031
Methylphenanthrene, 9-	14 040	22 167
Methylphenanthrene, 1-	12 702	18 140
C1-phenanthrene (average)	24 935	37 746

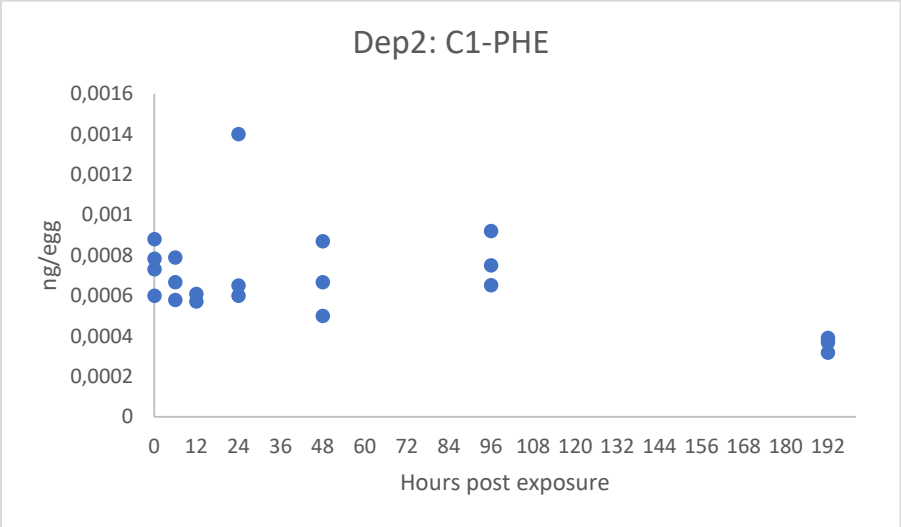
Body burden concentration [ng/egg] in dep 1, dep2 and dep3

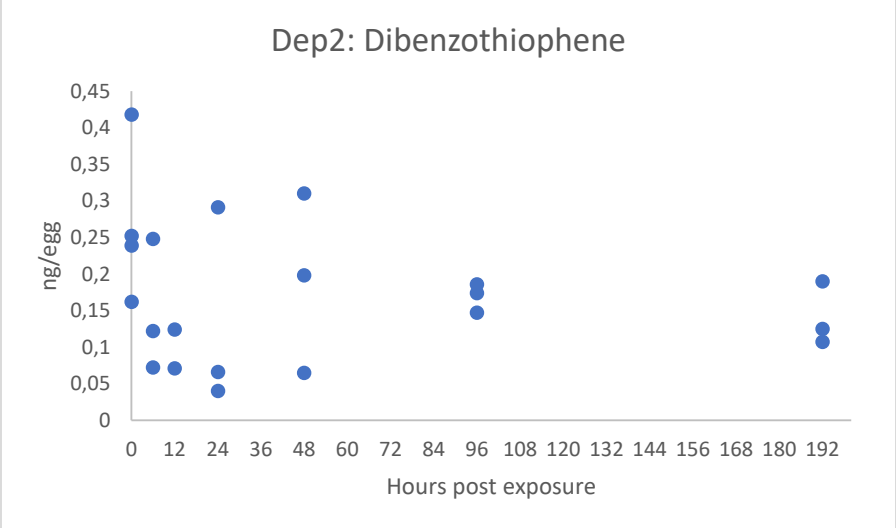


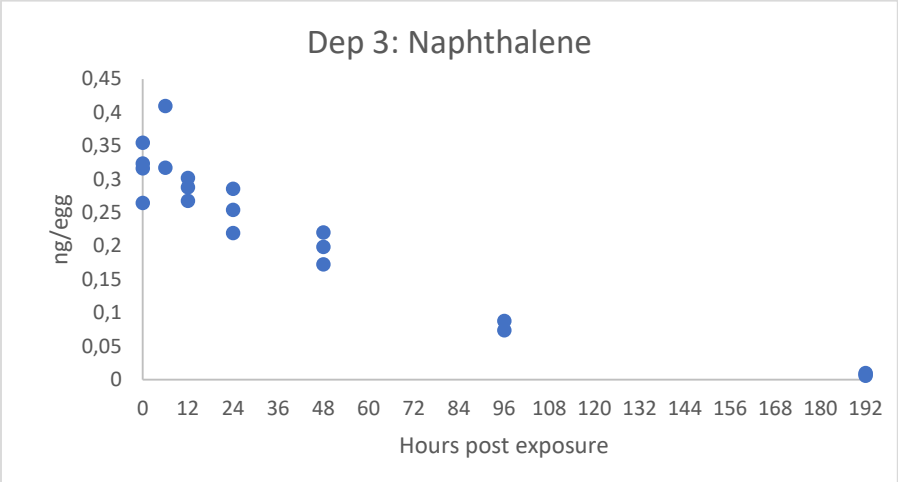


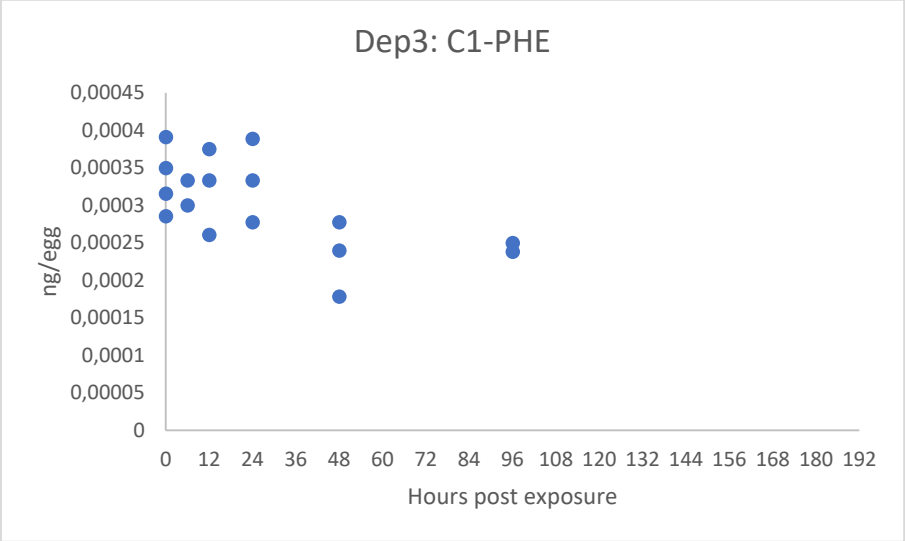


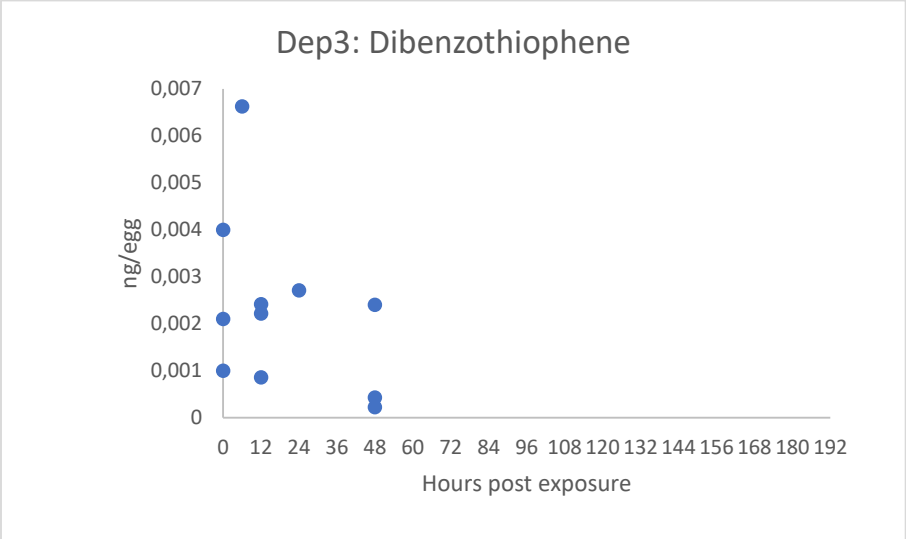

Dep2: Naphthalene
ng/egg
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)


Dep2: C1-PHE
ng/egg
0,0016
0,0014
0,0012
0,001
0,0008
0,0006
0,0004
0,0002
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure

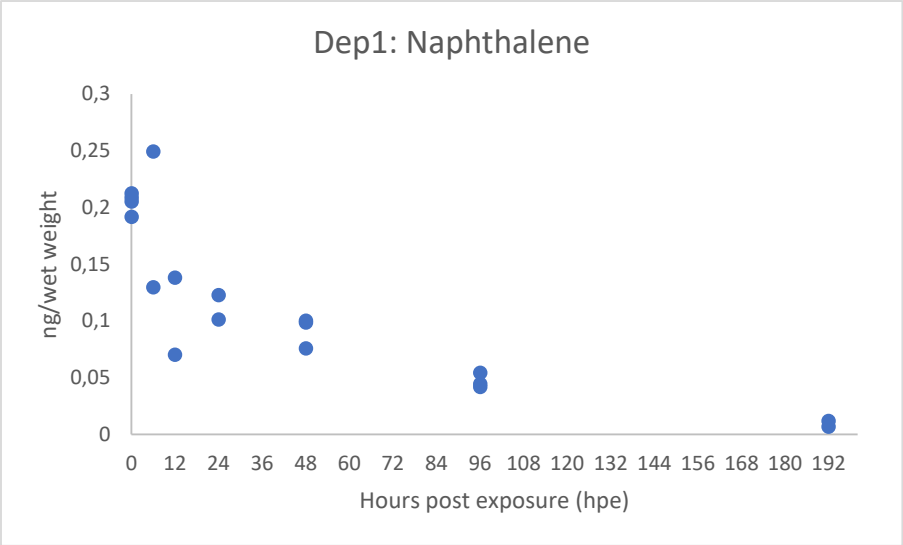

Dep2: Dibenzothiophene
ng/egg
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure

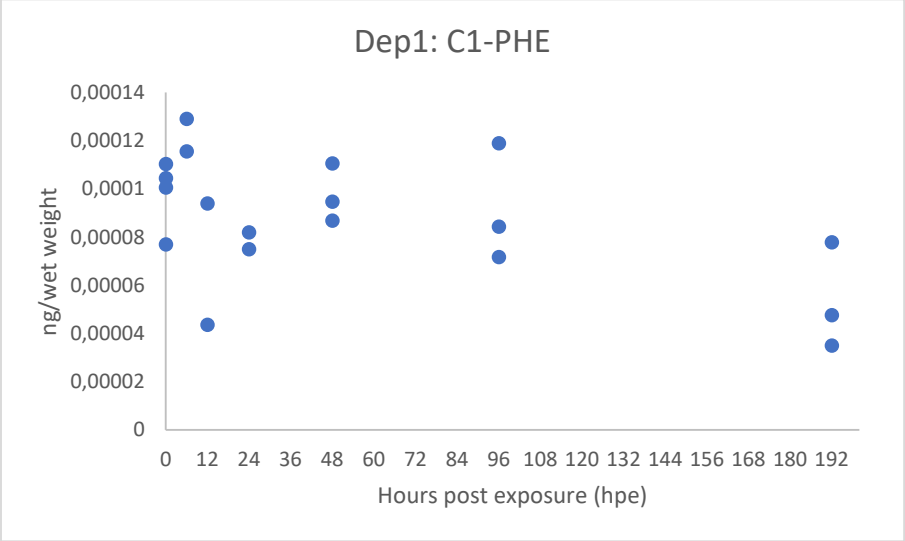

Dep 3: Naphthalene
ng/egg
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure

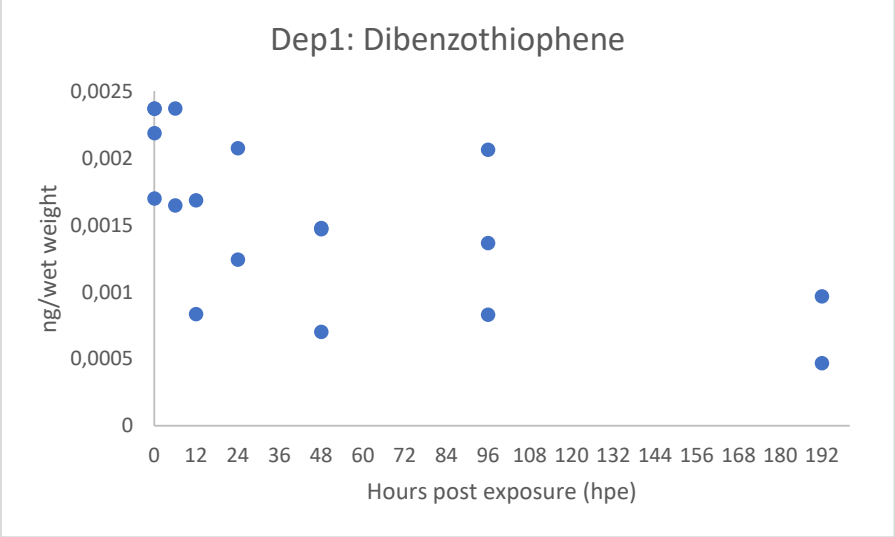

Dep3: C1-PHE
ng/egg
0,00045
0,0004
0,00035
0,0003
0,00025
0,0002
0,00015
0,0001
0,00005
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure

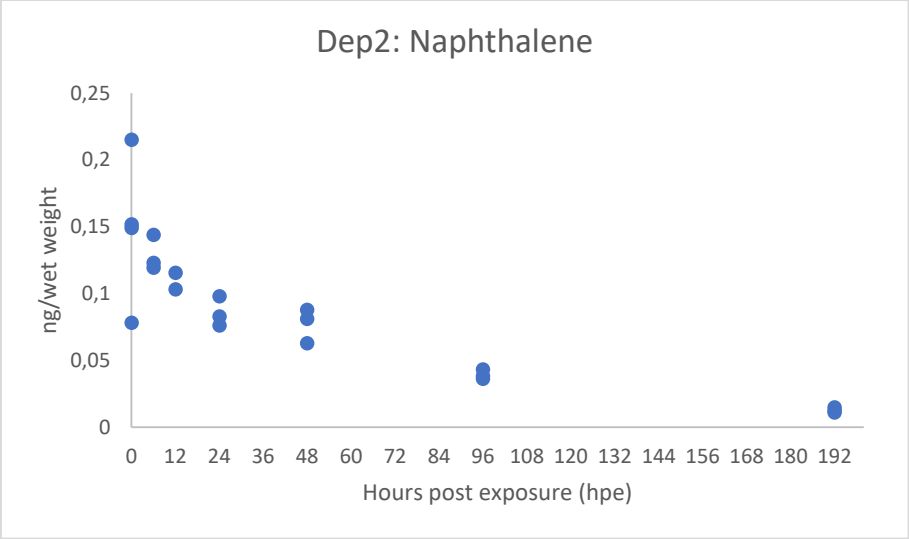

Dep3: Dibenzothiophene
ng/egg
0,007
0,006
0,005
0,004
0,003
0,002
0,001
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure

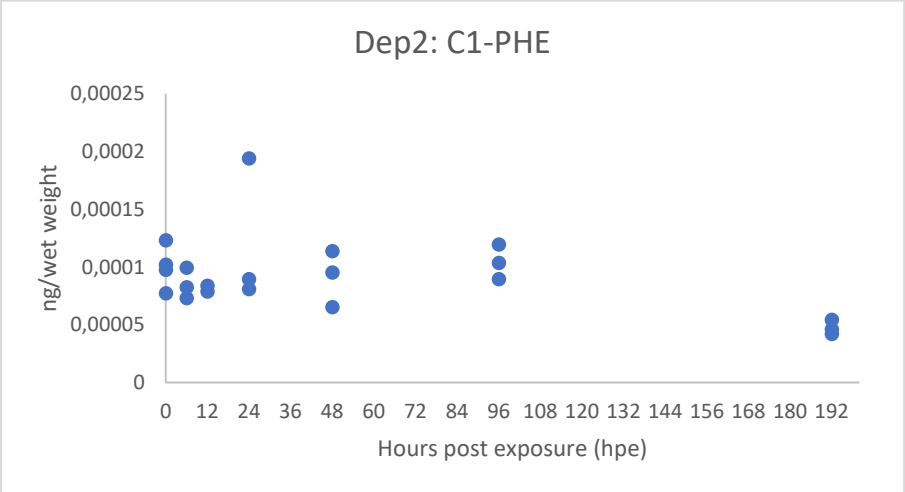
Body burden concentration [ng/wet weight] in dep 1, dep2 and dep3

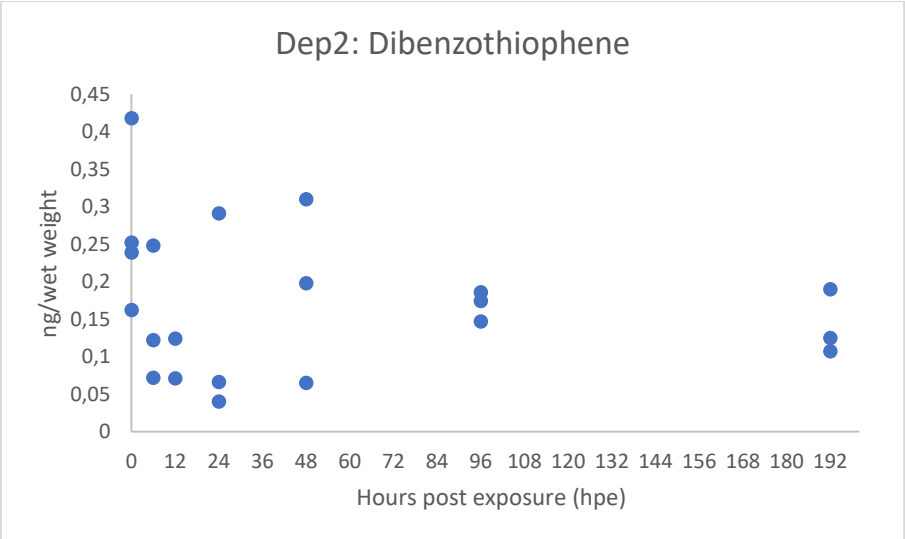


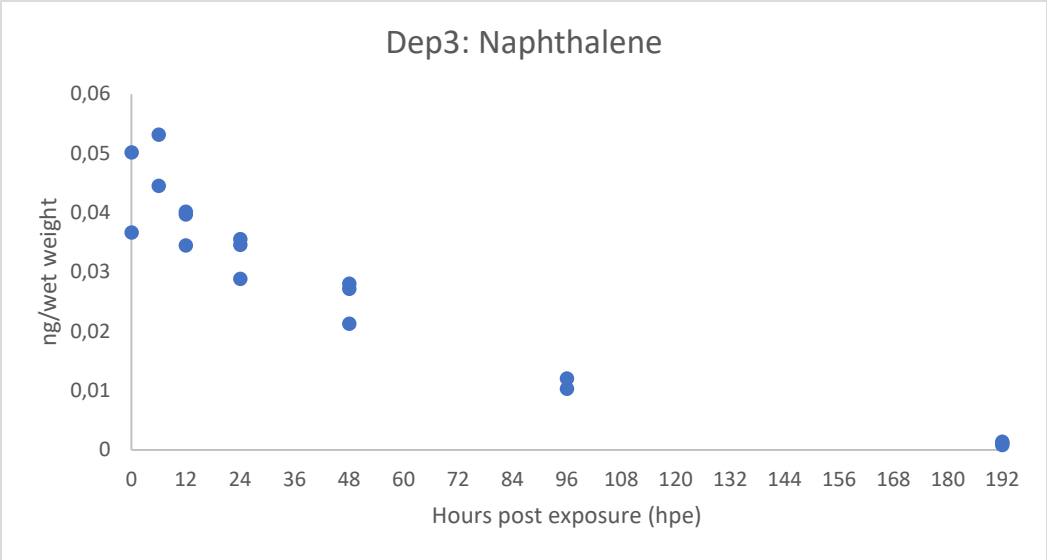


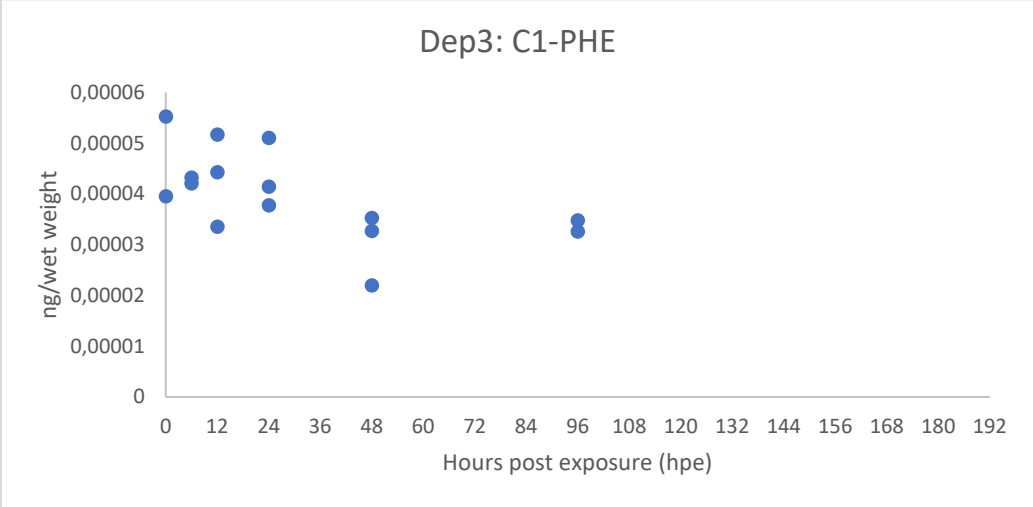


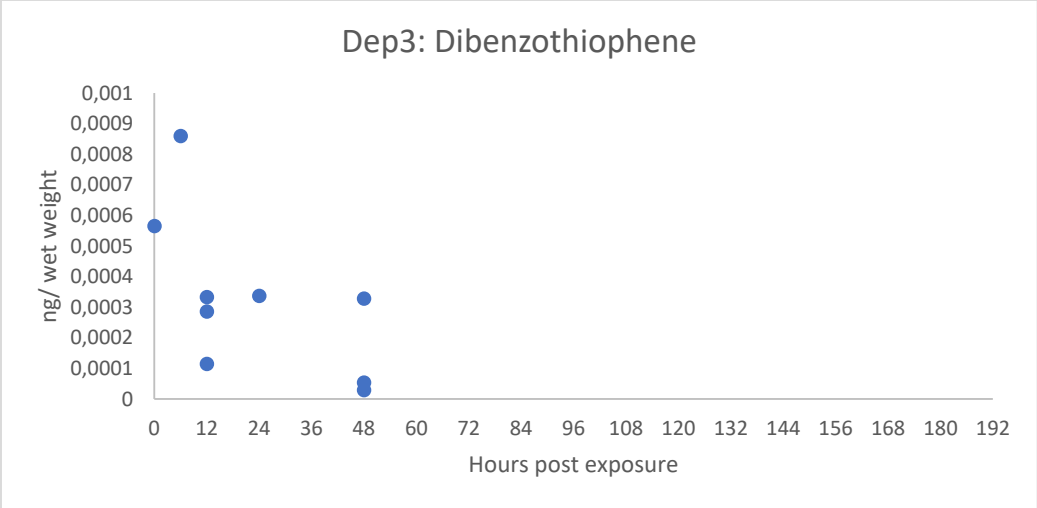

Dep2: Naphthalene
ng/wet weight
0,25
0,2
0,15
0,1
0,05
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)


Dep2: C1-PHE
ng/wet weight
0,00025
0,0002
0,00015
0,0001
0,00005
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)

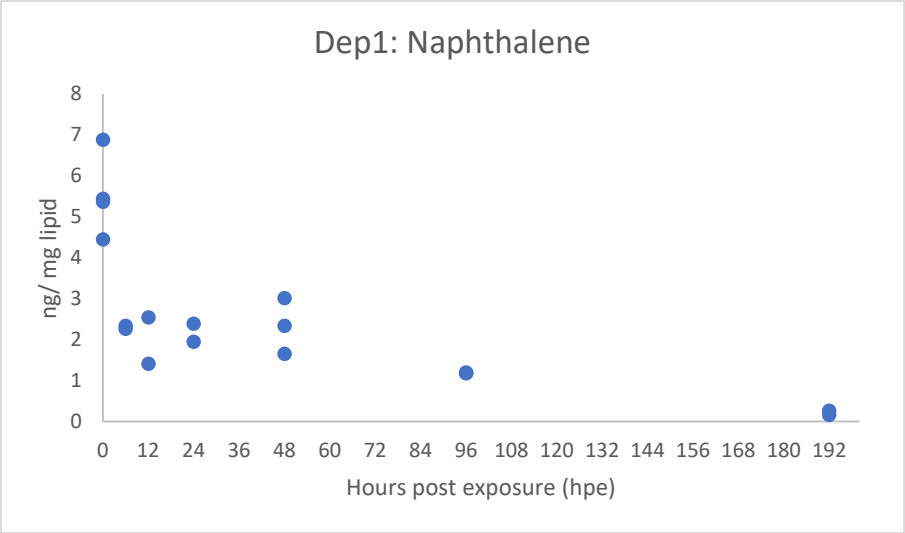

Dep2: Dibenzothiophene
ng/wet weight
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)

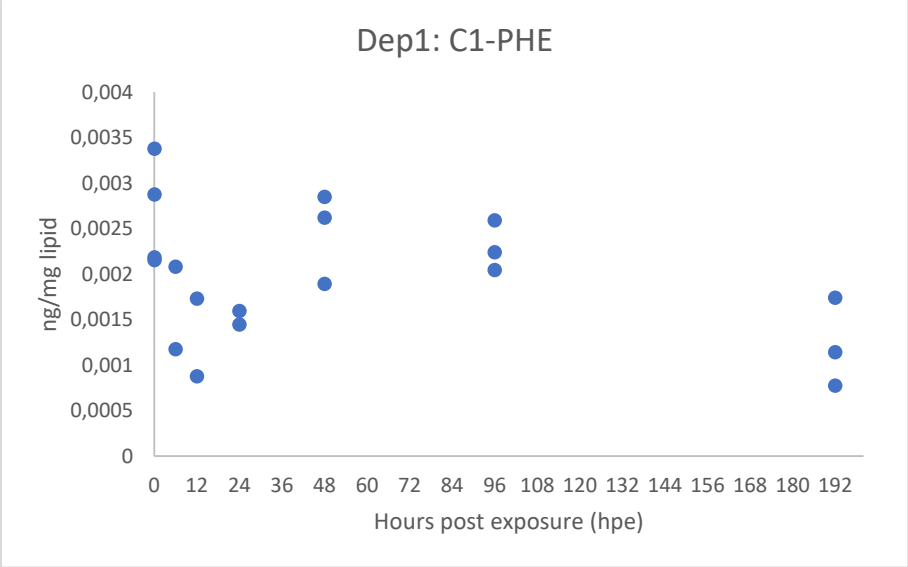

Dep3: Naphthalene
ng/wet weight
0,06
0,05
0,04
0,03
0,02
0,01
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)

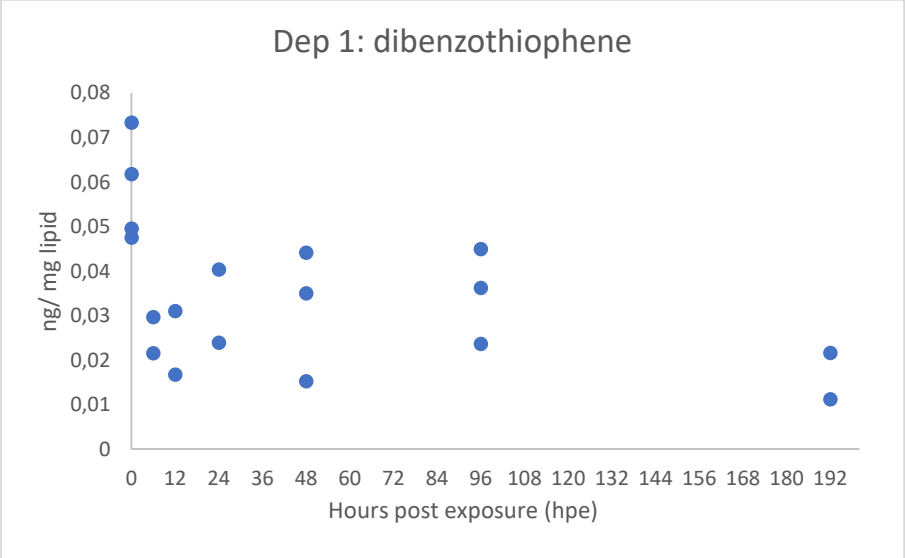

Dep3: C1-PHE
ng/wet weight
0,00006
0,00005
0,00004
0,00003
0,00002
0,00001
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)

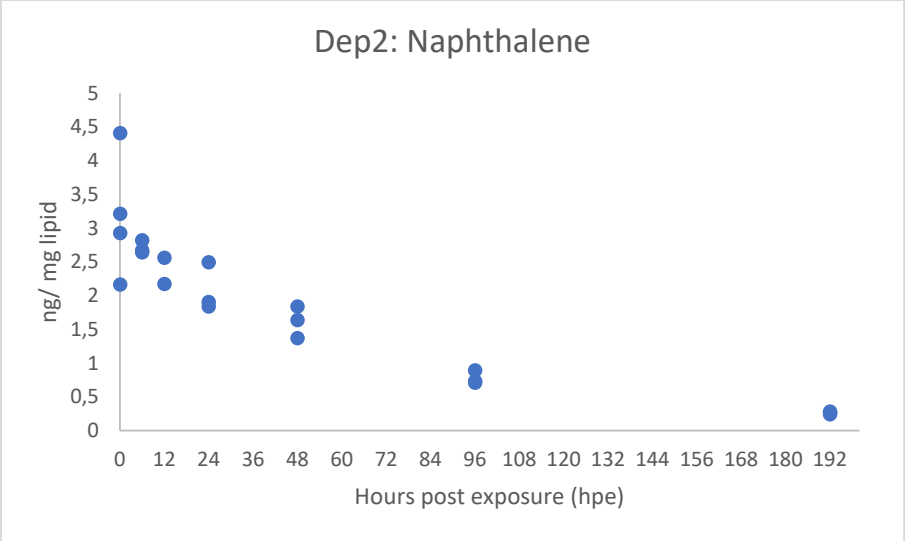

Dep3: Dibenzothiophene
ng/ wet weight
0,001
0,0009
0,0008
0,0007
0,0006
0,0005
0,0004
0,0003
0,0002
0,0001
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure

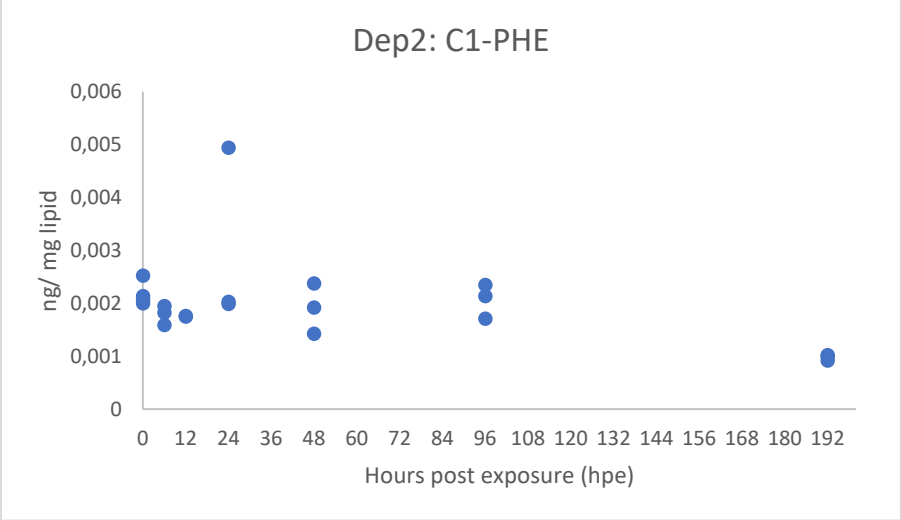
Body burden concentration [ng/mg lipid] in dep 1, dep2 and dep3

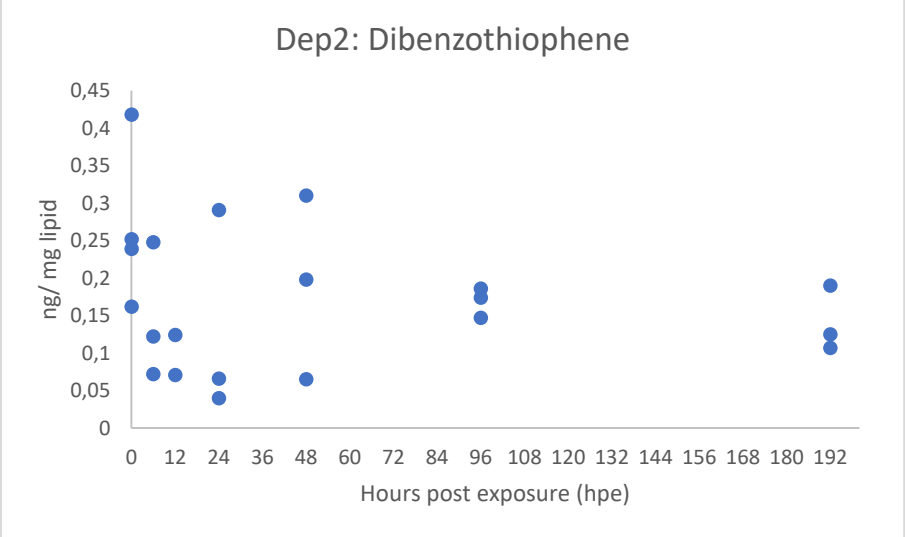


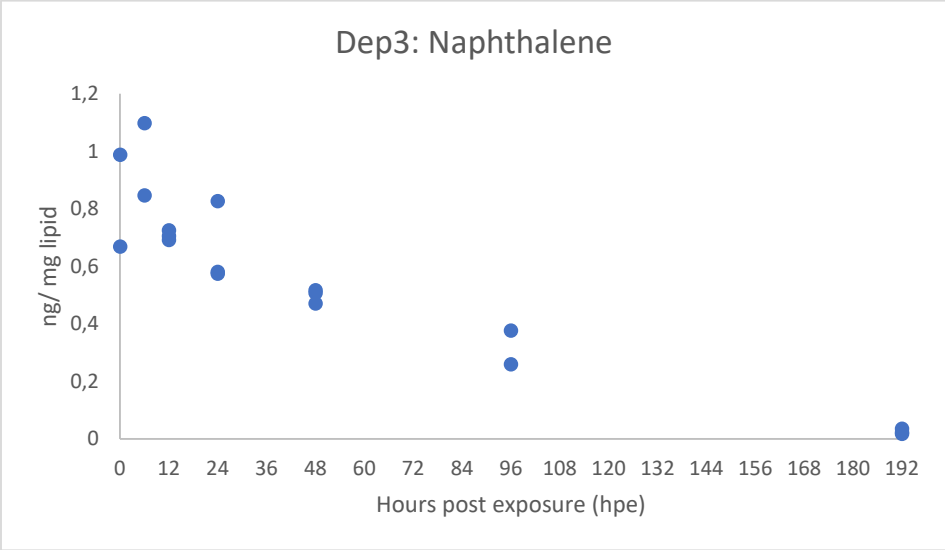


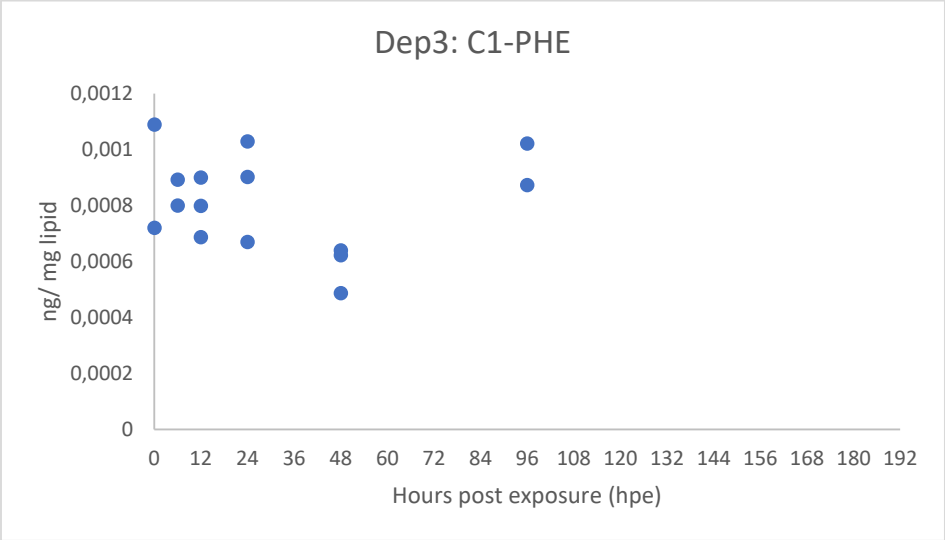


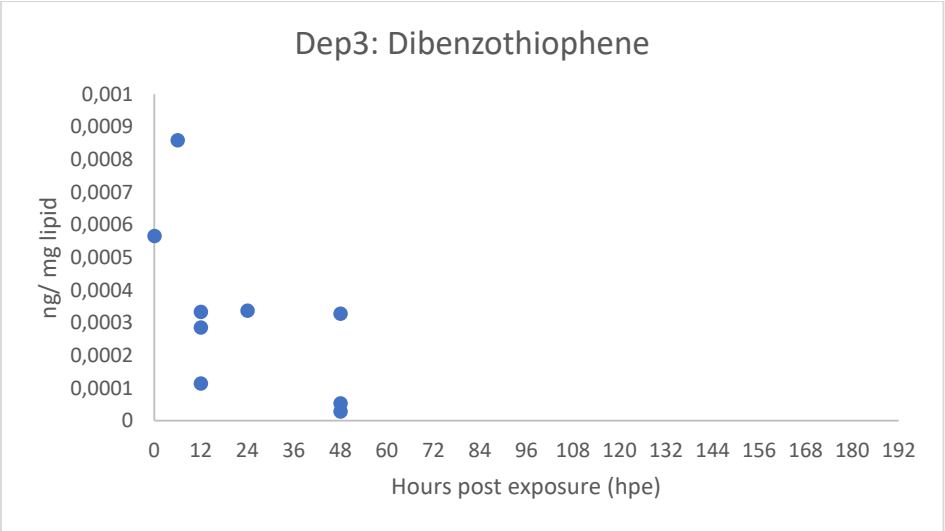

Dep2: Naphthalene
ng/ mg lipid
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)


Dep2: C1-PHE
ng/ mg lipid
0,006
0,005
0,004
0,003
0,002
0,001
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)


Dep2: Dibenzothiophene
ng/ mg lipid
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)


Dep3: Naphthalene
ng/ mg lipid
1,2
1
0,8
0,6
0,4
0,2
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)


Dep3: C1-PHE
ng/ mg lipid
0,0012
0,001
0,0008
0,0006
0,0004
0,0002
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)


Dep3: Dibenzothiophene
ng/ mg lipid
0,001
0,0009
0,0008
0,0007
0,0006
0,0005
0,0004
0,0003
0,0002
0,0001
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192
Hours post exposure (hpe)

Harmful discharges of produced water

Discharges from the oil and gas platforms cause toxicity effects on marine organisms!

The Norwegian continental shelf (NCS) is considered especially vulnerable to oil discharges due to the activity several fish species have in the area. Several species, the lumpsucker (*Cyclopterus Lumpus*) for instance, use the the NCS for spawning areas [1]. Lumpsuckers are vulnerable for oil discharges, as larvae and juvinile lumpsuckers are poor swimmers, having limited possibilities to escape potential oil spill and produced water discharges [2] [3]. Produced water annually contributes to a discharge of 130-150 million standard cubic metres on the NCS [4]. The discharges consist of compounds such as polycyclic aromatic hydrocarbons (PAHs) and alkyl PAHs, which are known to be toxic to early life stages of fish - the impacts of produced water is up for research [3]. Even though the regulatory threshold allows up to 30 mg/L oil content in produced water, studies have found that the amount causes serious mutagenic and toxic effects on marine organisms [4] [5].

The fish species lumpsucker and the toxicity effects of PAHs on lumpsucker is not fully investigated. It is at interests to study the uptake and elimination of PAHs and alkyl PAHs in the lumpsucker, especially in early life stages. For this reason a study of lumpsucker embryos exposed to produced water was conducted, lead by SINTEF Ocean. The body burden of total PAHs (tPAHs) in the lumpsucker embryos was analyzed, as body burden is a way to measure the amount of pollutants in an organism over time [6].

Figure 1: Picture of a lumpsucker fish [7].

The investigation

Three potentially sensitive life stages of lumpsucker embryos was exposed to produced water for 48 hours, then placed in a hatching tank with a flow of sea water until hatching. Samples for body burden extraction was analyzed at 7 timepoints. Uptake and elimination of tPAHs was found by a body burden extraction and a clean-up extraction, followed by a tPAH analysis performed by a gas chromatograph coupled with a mass spectrometer.

The results reveal that embryos exposed at the earliest life stage has the greatest body burden of tPAHs while the tPAH body burden in embryos exposed at the latest stage of the development was the lowest. The body burden concentration of tPAH in the all the embryos decreased a lot before hatching.

Produced water in lumpsucker embryos

As the study states, the lumpsucker embryos are more vulnerable to PAHs at the earliest life stages. At this timepoint, the eggshells are soft and the metabolism in the fish embryo is not fully developed yet, resulting in a higher body burden of tPAH. Even though the fish embryo metabolism eliminates PAH compounds with greater scope as the embryos develop, as the concentration of tPAH decreases, the impacts of PAH are still harmful to the lumpsucker.

The awareness of the harmful discharges from produced water have resulted in many investigations of the impacts. Some have found that PAHs cause teratogenic, carcinogenic and mutagenic effects in a variety of marine organisms [3]. This study has displayed that lumpsucker embryos achieve a body burden concentration of the known toxic compound in produced water, PAH.

NTNU
Department of Materials Science and Engineering

SINTEF

References

[1] Victoria L.G.Todd, Edward W. Lavallin, and Peter I. Macreadie. Quantitative analysis of fish and invertebrate assemblage dynamics in association with a north sea oil and gas installation complex. *Marine Environmental Research*, 142:69 – 79, 2018.

[2] Marianne Frantzen, Bjørn H. Hansen, Perrine Geraudie, Jocelyn Palerud, Inger-Britt Falk-Petersen, Gro H. Olsen, and Lionel Camus. Acute and long-term biological effects of mechanically and chemically dispersed oil on lumpsucker (cyclopterus lumpus). *Marine Environmental Research*, 105:9, 2015.

[3] Lisbet Sørensen. Identification and measurement of crude oil derived organic compounds bioaccumulating in early life stages of atlantic cod (gadus morhua) and haddock (melanogrammus aeglefinus). *University of Bergen*, (1):3–386, 2017.

[4] norskoljeoggass.no. *Environmental report*, 2018. `https://www.norskoljeoggass.no/globalassets/dokumenter/miljo/miljorapporter/environmental-report-2018.pdf` (Accessed: 2019-03-29).

[5] Torgeir Bakke, Jarle Klungsøyr, and Steinar Sanni. Environmental impacts of produced water and drilling waste discharges from the norwegian offshore petroleum industry. *Marine Environmental Research*, 92:154 – 169, 2013.

[6] study.com. *Body Burden: Definition, Chemicals Testing.* `https://study.com/academy/lesson/body-burden-definition-chemicals-testing.html` (Accessed: 2019-05-15).

[7] Picture of a lumpsucker fish. *Reddit*, 2019.

Risikoanalyse

Jobbe med DCM

Uønsket hendelse +	Analyseres		Risiko
Liten eksponering for DCM	Ja [FERDIG]		Vurdér risiko...
Søl av DCM	Ja [FERDIG]		Vurdér risiko...
Søl av DCM utenfor avtrekksskap	Ja [FERDIG]		Vurdér risiko...
Stor eksponering av DCM	Ja [FERDIG]		Vurdér risiko...
Inhalere DCM	Ja [FERDIG]		Vurdér risiko...

Jobbe med n-hexan

Uønsket hendelse +	Analyseres		Risiko
Liten eksponering for n-hexan	Ja [FERDIG]		Vurdér risiko...
Søl av n-hexan	Ja [FERDIG]		Vurdér risiko...
Stor eksponering av n-hexan	Ja [FERDIG]		Vurdér risiko...
Inhalering av n-hexan	Ja [FERDIG]		Vurdér risiko...

Jobbe med sentrifuge

Uønsket hendelse +	Analyseres		Risiko
Nedsatt hørsel	Ja [FERDIG]		Vurdér risiko...

Jobbe med ultralydbad

Uønsket hendelse +	Analyseres		Risiko
Nedsatt hørsel	Ja [FERDIG]		Vurdér risiko...

Jobbe i kjølig rom

Uønsket hendelse +	Analyseres		Risiko
Kan bli syk	Ja [FERDIG]		Vurdér risiko...
Kan bli forkjølet	Ja [FERDIG]		Vurdér risiko...

Jobbe med kloroform

Uønsket hendelse +	Analyseres		Risiko
Søle kloroform	Ja [FERDIG]		Vurdér risiko...
Inhalere kloroform	Ja [FERDIG]		Vurdér risiko...

Jobbe med metanol

Uønsket hendelse +	Analyseres		Risiko
Liten eksponering for metanol	Ja [FERDIG]		Vurdér risiko...
Stor eksponering for metanol	Ja [FERDIG]		Vurdér risiko...

Body burden ekstraksjon

Uønsket hendelse +	Analyseres		Risiko
Skjære seg på homogeniserer med kniv	Ja [FERDIG]		Vurdér risiko...

Rognekjeks eksponeringseksperiment

Uønsket hendelse +	Analyseres		Risiko
Jobbe med flytende nitrogen	Ja [FERDIG]		Vurdér risiko...