Source Origin of Polycyclic Aromatic Hydrocarbons (PAHs) in Sediment, and Fate of Organic Contaminants in Dragonfly Larvae (*Aeshnidae)* from Highway Sedimentation Ponds and Natural Ponds

Viviane Girardin

Master Thesis in Toxicology

Department of Biosciences
Faculty of Mathematics and Natural Sciences

UNIVERSITY OF OSLO

November 2018

2018

Source Origin of Polycyclic Aromatic Hydrocarbons (PAHs) in Sediment, and Fate of Organic Contaminants in Dragonfly Larvae (*Aeshnidae*) from Highway Sedimentation Ponds and Natural Ponds

Viviane Girardin

http://www.duo.uio.no/

Print: Reprosentralen, Universitetet i Oslo

Acknowledgements

The presented thesis was carried out at the Department of Biosciences at the University in Oslo, and is associated with the project Reducing Highway Runoff Pollution (REHIRUP). The project invests in research and development with the goal of presenting optimal solutions for design, operation and maintenance of runoff treatment. REHIRUP is a joint collaboration between the Norwegian Public Roads Administration (NPRA), Swedish Transport Administration, and the Danish Road Directorate. The Norwegian Institute for Water Research (NIVA) is a partner in part of the project.

I would like to thank my supervisors, Professors Merete Grung, Sondre Meland, and Katrine Borgå for their help and support. I would like to express my gratitude to Sondre for going above and beyond in his role as a supervisor, being always available to answer my questions, and for the extra help with statistics. Merete for all her help in the lab and for several constructive feedbacks. A huge thank you to my mentors Kjersti Kronvall and Lene Sørlie Heier from the Norwegian Public Roads Administration for their tremendous support, and for sharing their knowledge with me.

Thank you Alfhild Kringstad and Katharina Løken, and many others from NIVA for the great help and patience in and out of the lab. Professors Ketil Hylland and Tom Andersen for always being very welcoming whenever I asked for advice. Amalie Liane for the tips, for showing me the way to the ponds, and for the material you left for me when you were done. Sofie Lindman, my dear field buddy, for the company in so many field trips, and whom inspired me to be a more organized person. Øyvind Grotmol for his patience and time checking my statistics. Clare McEnally, Patricia Decourt, and Mariana Paz for helping me during many field trips. Thank you so much for proofreading (a thousand times) this thesis, and the amazing support Clare! Thank you Pati, “bestifrendi”, for turning what it would have been the worse field trip into the funniest, most memorable of them all. And an extra round of applause for Mariana, who not only proved to be an amazing larva whisperer, but also took such good care of my kids, becoming my daughter’s idol.

And my family: My daughter and son, Helena and Johan, thank you for your patience and love. I could not have asked for better kids. I love you! (You guys could have slept through the night, and made less of a mess at home, but ok). My awesome mom! Thank you for absolutely everything! And last, but not least; Thank you Joachim for being there for me. For your patience, solicited and unsolicited advices, support, and love. I love you!

I am now ready for a 48hr nap…

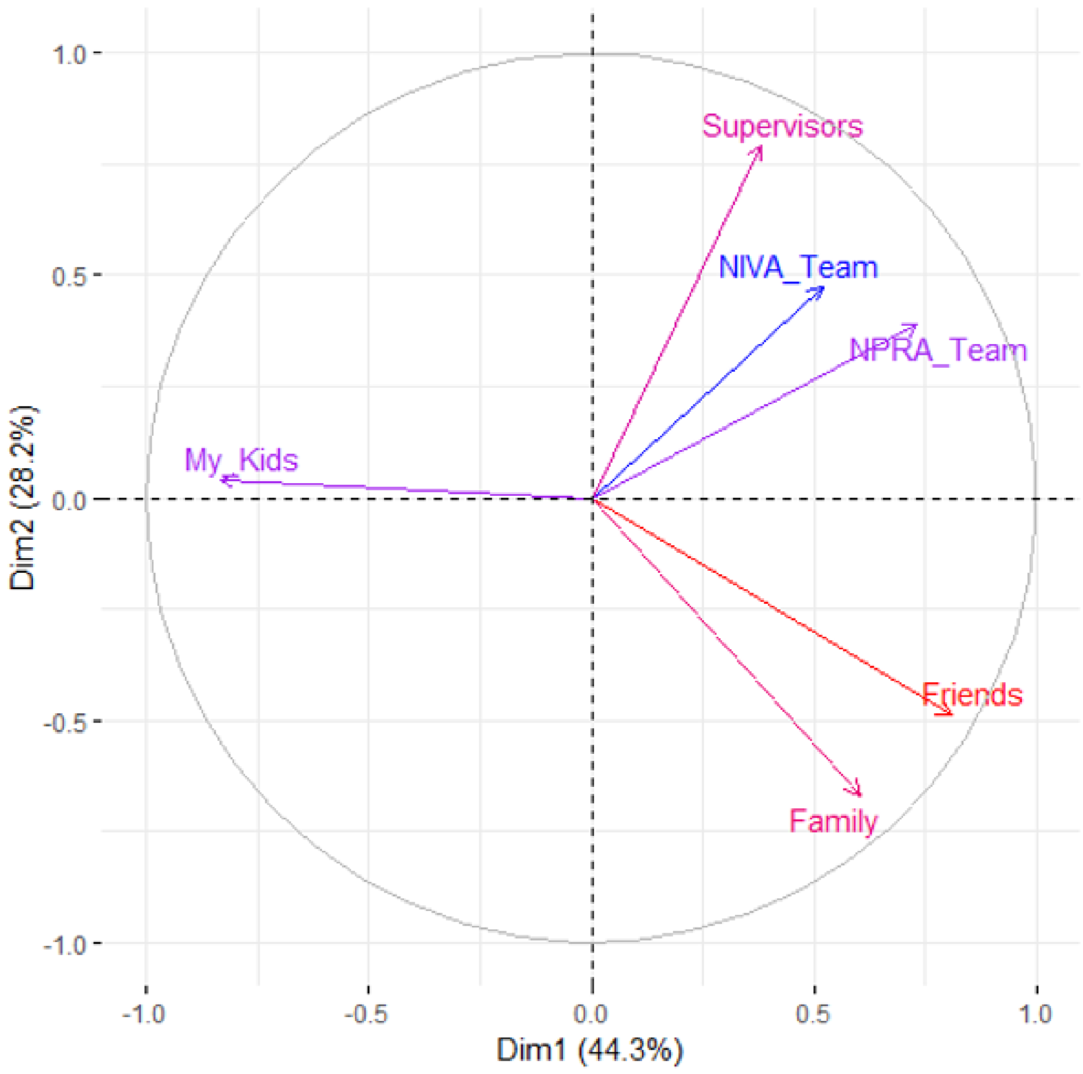


Figure 1 - Thank you all!

Abstract

Road and tunnel wash runoff contain a mixture of organic and inorganic contaminants that threatens the quality of natural water bodies, and the health of the organisms dependent on these waters. A variety of treatment solutions can be established to reduce potential runoff impact. The most common mitigation adopted in Norway is the installation of nature-based sedimentation ponds. A variety of organisms migrate to these ponds over time, and are thus at risk of exposure to high levels of traffic-related contaminants. Dragonflies, with their aquatic life stage, can potentially transfer these substances back to the terrestrial environment.

This aim of this study was to assess the source origin and fate of polycyclic aromatic hydrocarbons (PAHs), and two types of organobromine compounds used as flame retardants, polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) in three natural and seven highway sedimentation ponds in Norway. Sediment samples were used to determine source origin of PAHs. The concentrations of organic contaminants were analyzed in dragonfly larvae to investigate their potential role as pollutant vectors across ecosystems.

Parent and alkylated PAHs in sediment were measured, and the results were used to characterize the source of PAHs. Distribution patterns of selected PAHs showed similar patterns in all sedimentation ponds, and distinct patterns in natural ponds. Specific PAH ratios indicated that sedimentation ponds are dominated by petrogenic PAHs, whereas natural ponds showed pyrogenic dominance. Moreover, the addition of alkylated PAHs resulted in significant changes in the environmental quality standard values related to sediment pollution.

PAHs, PBDEs and HBCD were quantified in sediment, and larval exuvia and tissue. Haemolymph was also analyzed for PAH metabolites. The results indicated that dragonfly larvae accumulate PAHs in the exuvia, but not sufficiently enough to avoid bioaccumulation. 1-hydroxypyrene was detected only in some of the samples and at very low concentrations, and thus it is not clear whether larvae are able to metabolize PAHs at low levels or if the metabolite has come from others sources. Nevertheless, the results suggest that metabolites are not suitable biomarkers for PAH exposure in dragonfly larvae. Levels of BFRs were detected at very low concentrations, and the results were qualified. Overall, there was no indication of bioaccumulation of BFRs.

Abbreviations

AADT	Annual Average Daily Traffic
ACE	Acenaphthene
ACY	Acenaphthylene
ANTH	Anthracene
BaA	Benzo[a]anthracene
BaP	Benzo[a]pyrene
BbjF	Benzo[b,j]fluoranthene
BeP	Benzo[e]pyrene
BFR	Brominated Flame Retardants
BghiPER	Benzo[ghi]perylene
BkF	Benzo[k]fluoranthene
CAS	Chemical Abstracts Service
CHR	Chrysene
DachA	Dibenz[ac/ah]antracene
DIB	Dibenzothiophene
FLO	Fluorene
FLUORA	Fluoranthene
GC/MS	Gas chromatography/Mass spectrometry
GPC	Gel Permeation Chromatography

HBCD	Hexabromocyclododecane
HMW	High Molecular Weight
HPLC	High Performance Liquid Chromatography
I123P	Indeno [1,2,3-cd]pyrene
IS	Internal Standard
LMW	Low Molecular Weight
NAPH	Napthalene
NIVA	Norwegian Institute for Water Research
NPRA	Norwegian Public Roads Admnistration
PAH	Polycyclic Aromatic Hydrocarbon
PBDE	Polybrominated Diphenyl Ethers
PER	Perylene
PHEN	Phenanthrene
PYR	Pyrene
RBSP	River Basin-Specific Pollutants
SRM	Standard Reference Material
WFD	Water Framework Directive

Table of Contents

1 Introduction

Over five million vehicles are driven on Norway's almost 100.000 km of roads and tunnels (Statistics Norway, 2017a, 2017b), releasing into the environment a complex mixture of inorganic and organic contaminants. Examples of common traffic-related contaminants are the metals cadmium, copper, lead, nickel and zinc, as well as organic compounds such as benzene and polycyclic aromatic hydrocarbons (PAHs). Sources include fuel emission, tire and asphalt wear, and oil spills (Kose et al., 2007; Meland et al., 2010b). In addition, detergents are used in tunnel wash activities, and de-icing salts are used during the winter (Åstebøl & Hvitved-Jacobsen, 2014; Meland, 2010).

Some of these compounds may photolyse, volatilize or be transported by wind when released in open highways. Others might remain in the air or settle on the ground, being eventually washed off by rain (Mangani et al., 2005; Ngabe et al., 2000). Tunnels are periodically washed for maintenance and safety purposes (Meland et al., 2010a). Road and tunnel wash runoff can eventually reach natural water bodies, threatening the water quality and health of all organisms dependent directly or indirectly on these systems.

In October 2000 the European Commission implemented the EU Water Framework Directive (WFD) after a restructuring process of the European Water Policy. The former fragmented policies were consolidated into one piece of framework, and the main goal is for all EU countries to achieve "good status" for all waters by a set deadline, which is currently 2027 (European Commission, 2016). Norway is one of the non-EU countries that has integrated the WFD into its legislation (Norwegian Ministry of Climate and Environment, 2011).

Several priority substances under the Annex II of Directive 2008/105/EC (European Commission, 2008) are common in road runoff, including lead, nickel, copper, zinc, and all eight priority PAHs (Table 1). An additional eight PAHs have been declared River Basin-Specific Pollutants (RBSP) in Norway (Committee of Directorates for the Water Framework Directive, 2018). Such pollutants are identified to be of regional or local relevancy. Countries that declare RBSP also provide environmental quality standards, monitoring, and regulatory programs for the selected compounds (Piha et al., 2010).

Table 1 – List of compounds connected to road pollution and included in the EU list of priority substances. Table adapted from the list of priority substances in the field of water policy -Annex II of Directive 2008/105/EC.

CAS number	EU number[1]	Name of priority substance	Sources from road and tunnel
		PAHs	
120-12-7	204-371-1	Anthracene	Incomplete combustion, tire and asphalt wear, leaching/spill of oil/petrol/grease (Meland et al., 2010b)
91-20-3	202-049-5	Naphthalene	
206-44-0	205-912-4	Fluoranthene	
50-32-8	200-028-5	Benzo(a)pyrene	
205-99-2	205-911-9	Benzo(b)fluoranthene	(sources include all PAHs)
191-24-2	205-883-8	Benzo(g,h,i)perylene	
207-08-9	205-916-6	Benzo(k)fluoranthene	
193-39-5	205-893-2	Indeno(1,2,3-cd)pyrene	
		River Basin-Specific PAHs	
208-96-8		Acenaphthylene	
83-32-9		Acenaphthene	
86-73-7		Fluorene	
85-01-8		Phenanthrene	
129-00-0		Pyrene	
50-32-8		Benzo[a]anthracene	
218-01-9		Chrysene	
53-70-3		Dibenz(ac/ah)anthracene	
		Other organic compounds	
71-43-2	200-753-7	Benzene	Emission from fuel (Skov et al., 2001)
		Inorganic compounds	
7439-92-1	231-100-4	Lead and its compounds	Car bodies, tires, brake pads, fuel, fuel additives, lubricants, bitumen (Folkeson, 2009; Meland, 2010)
7440-02-0	231-111-4	Nickel and its compounds	
7440-43-9	231-152-8	Cadmium and its compounds	

[1] European Inventory of Existing Commercial Substances (EINECS) or European List of Notified Chemical Substances (ELINCS).

A variety of solutions have been designed to treat runoff before its discharge into nearby aquatic environments. Nature-based solutions aim to approach environmental issues by using methods and technologies inspired by nature (European Commission, 2015). The most common solution applied in Norway is the construction of nature-based sedimentation ponds. These low-cost, sustainable drainage systems are built to retain and improve the quality of the runoff before its discharge into natural water bodies (Meland, 2015; Meland, 2016b). The treatment method follows, in principle, the Stoke's law, meaning that particles are expected to sediment at a certain rate according to gravity forces, and particle size. As a result, contaminants bound to particles gradually settle, while runoff moves along the pond. In practice, the rate of sedimentation will depend on other factors such as turbidity and particle shape (Åstebøl & Hvitved-Jacobsen, 2014). Moreover, water-soluble contaminants in the runoff are diluted (Bækken et al., 2005), and decomposition by chemical and biological processes is expected to occur as in natural ponds.

In addition to their function as a filter for contaminants, sedimentation ponds also play a role as wetland areas. A large number of organisms migrate to these ponds over time, increasing biodiversity in zones of high traffic density. This is of special significance given the significant reduction in global wetlands in the last century. Remaining wetlands may occupy only about 9% of the world's area (Zedler & Kercher, 2005).

The importance of natural ponds as biodiversity hotspots has been ignored in the past, but there has been an increasing understanding of their ecological contribution (Céréghino et al., 2008). For instance, in a study comparing data from five different European countries, Davies et al.(2008) concluded that natural ponds were the most species-rich habitats for plants and macroinvertebrates at a regional level, when compared to ditches, lakes, rivers and streams.

The contribution of artificial ponds to aquatic biodiversity has also gained attention in recent years (Le Viol et al., 2009; Sun et al., 2018). In a study investigating the key factors driving biodiversity, Sun et al. (2018) identified a total of 96 taxa in 12 sedimentation ponds in Norway, including six species listed on the Norwegian Red List. In another study, Brittain et al. (2017) identified taxa richness ranging from 67 to 128 in twelve Norwegian sedimentation ponds. Many factors may determine species richness, such as vegetation density and pond size (Brittain et al., 2017; Sun et al., 2018).

Sedimentation ponds are, however, often designed only to treat polluted water and control floods, and ecological potential is not considered. (Clevenot et al., 2018). As a result, biota might inaccurately perceive these ponds as suitable habitats. Furthermore, bioaccumulative contaminants that are expected to settle can potentially be transported out of the ponds by organisms with both aquatic and terrestrial life stages, and further transported to other ecosystems by trophic transfer. Buckland-Nicks et al. (2014) demonstrated that dragonflies retain mercury throughout their life cycle. Dragonflies are, therefore, potential vectors of bioaccumulative contaminants from the aquatic to terrestrial environments.

1.1 Odonata – Dragonflies

The Order *Odonata* comprises a group of hemimetabolous, predatory insects. It is divided into two suborders; dragonflies (Anisoptera) and damselflies (Zygoptera). The main characteristics distinguishing Odonata from other insects are their very large eyes (in proportion to their head), their long and thin abdomen, and a characteristic lower lip (labium) that extends in order to catch prey (Corbet, 1999).

Dragonfly larvae are in general more robust than damselfly larvae. Dragonflies' abdomen terminates in five short, stiff appendages at the tip of the abdomen, whereas in damselflies there are three long, soft appendages. These differences make it quite easy to distinguish between the two suborders.

There are about 6000 dragonfly species around the world. Most are associated with warm climates, but some species are also found in the colder, northern countries (Askew, 2004). About 50 species have been registered in Norway, according to the Norwegian Biodiversity Information Center (2013).

1.1.1 Dragonflies as model organisms

Odonata play a key ecological role as they are intermediate predators in both terrestrial and aquatic ecosystems (Combes et al., 2012). Dragonfly larvae have a diverse diet, including other odonates (Crumrine, 2010). They are also prey for other animals such as fish (Stoks & Córdoba-Aguilar, 2012).

Many consider odonates strong bio-indicators of environmental quality (Villalobos-Jiménez et al., 2016). Some studies, however, suggest that odonates are only good bio-indicators for certain habitats, and it depends on the type of vegetation. In addition, many species are rather tolerant to pollution. Thus landscape, vegetation, and species tolerance are important features when choosing Odonata as bio-indicators (Ferreras-Romero et al., 2009; Samways & Steytler, 1996). Since individual species have individual habitat needs and tolerance levels, finding many different species in the same habitat is a reliable indicator of a healthy ecosystem (Corbet, 1999).

1.1.2 Life cycle

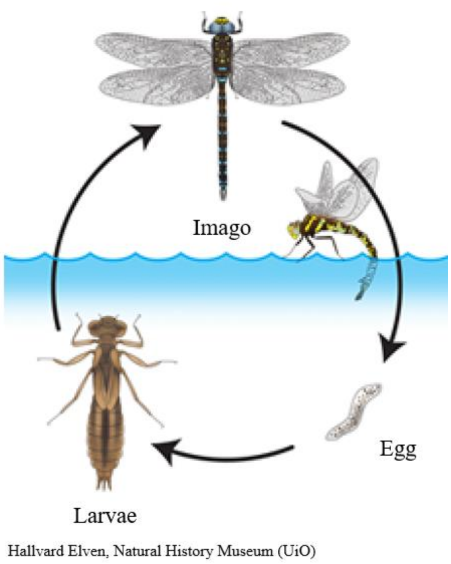


Figure 2 - Life cycle of a dragonfly

Dragonflies have a complex life cycle. Eggs are either deposited into plant tissue or in water. The egg stage lasts between two to four weeks after being laid. Embryological development may stop due to changes in physiology or environment, in which case hatching is delayed for several months (Askew, 2004). In some situations, the larva can be fully developed inside the egg, but hatching is delayed until a hatching stimulus is received, i.e. an appropriate level of oxygen in the environment or a favorable temperature (Corbet, 1999). The aquatic larval stage can last from months to years, and may undergo as many as 10 molts through their development. The length will depend on weather and food availability, and varies between species. (Stoks & Córdoba-Aguilar, 2012). In the last molt, the larva leaves the water to complete metamorphosis into the terrestrial adult stage (*Figure 3*). This process often occurs early in the morning in temperate regions (Askew, 2004).

Figure 3 - Final molting of a dragonfly at Vassum sedimentation pond. August 2017. Photo: Sondre Meland

1.2 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) form a significant family of hundreds of ubiquitous organic compounds structured by two or more aromatic rings fused together.

PAHs can be formed during natural processes, but it is widely understood that their release are mostly due to anthropogenic action. Tire and asphalt wear, and incomplete combustion of fuel are the main sources of PAHs from traffic (Meland et al., 2010b). The use of extender oils in tire production were regulated in Europe from 1st of January 2010 due to the high likelihood of PAH formation during tire production (European Commission, 2009).

PAHs can be generally grouped according to their origin; petrogenic (from petroleum derivate), pyrogenic (from incomplete combustion of organic substances), biogenic and diagenetic (from biological and geological processes respectively). Petrogenic and pyrogenic types are the most relevant in terms of road runoff, and therefore the main focus in this thesis.

These organic compounds are found in complex mixtures, with those formed from combustion processes being mainly parent PAHs (without alkyl groups, heteroatoms or hydroxides), whereas many of the alkylated forms are associated with oils (Zhendi Wang et al., 2008).

Physicochemical properties vary depending on molecule size (the number of carbon centers) and how the rings are linked (Bjørseth, 1983). Generally, PAHs with two to three aromatic rings are considered low molecular weight (LMW), and those with four or more rings are considered high molecular weight (HMW) (Canadian Council of Ministers of the Environment, 1999). Commonly, volatility decreases with increase in molecule size; most LMW species are volatile, 4-ring PAHs are often semi-volatile, whereas HMW species are mostly bound to particles in the environment (Srogi, 2007). Due to their hydrophobicity, all PAHs tend to adsorb to particles and sediment in the aquatic environment (V. Carrasco Navarro, 2013).

Toxic effects linked to PAHs include reduction of growth, altered behavior (Vignet et al., 2014b; Vignet et al., 2014a), and mortality (Clément et al., 2005; Mayer & Holmstrup, 2008). Several PAHs are known to be potentially carcinogenic and mutagenic (Aas et al., 2000; Penning, 1993; Shaw & Connell, 2001). Moreover, some PAHs exhibit photo-induced toxicity (Bowling et al., 1983; Newsted & Giesy, 1987), and some alkylated forms are reported to be more toxic than their parental forms (Marvanova et al., 2008; Turcotte et al., 2011). Consequently, several PAHs have been added to the list of substances of concern in environmental risk assessment and monitoring.

In benthic invertebrates (fresh- and saltwater), the main mechanism of PAH toxicity is narcosis (Burgess, 2009). Apparent paralysis, and curved spines, were observed in dragonfly larvae (*Ophiogomphus* species) during toxicity tests with fluoranthene under UV-light performed by Spehar et al. (1999).

Vertebrates can metabolize PAHs (D'Adamo et al., 1997; Takeuchi et al., 2009), and these contaminants have been shown to not biomagnify (Wan et al., 2007). Nevertheless, PAHs remain a concern due to the toxic effects linked to their metabolites. Moreover, not all invertebrates metabolize PAHs efficiently (V. Carrasco Navarro, 2013), and bioaccumulation can vary substantially among species (Ruus et al., 2005).

Table 2- Table of information on detected PAHs[2]

PAHs	Molecular weight (g/mol)	Solubility (mg/L)	Kow	CAS number	Maximum allowed concentration in freshwater (µg/L)[3]	Number of rings	Common sources	Priority list[4]	IARC Classification
Naphthalene	128	31	3.37	91-20-3	130	2	Petro (parent and alkylated)	E, U	2B
Dibenzothiophene	184	1.47	4.49	132-65-0	NA	3	Petro	-	-
Acenaphthylene	152	3.9	4.1	208-96-8	33	3	Petro	U, N	-
Acenaphthene	152	3.9	3.9	83-32-9	3.8	3	Petro	U, N	3
Fluorene	166	1.69	4.18	86-73-7	33.9	3	Petro	U, N	3
Phenanthrene	178	1.10	4.57	85-01-8	6.7	3	Pyro Petro	U, N	3
Anthracene	178	1.0	4.54	120-12-7	0.1	3	Pyro	E,U	3
Fluoranthene	202	0.26	5.22	206-44-0	0.12	4	Pyro	E,U	3
Pyrene	202	0.135	5.18	129-00-0	0.023[5]	4	Pyro	U, N	3
Benzo[*a*]anthracene	228	0.009 – 0.014	5.6	50-32-8	0.018	4	Pyro	C, U, N	2B
Benzo[*e*]pyrene	252	0.005	6.44	192-97-2	NA	5	Pyro	-	
Benzo[*a*]pyrene	252	0.003	6		0.18	5	Pyro	C, E, U	1
Perylene	252	0.0004	6.4	198-55-0	NA	5	Natural	-	
Indeno [*1,2,3-cd*]pyrene	276	0.00019	6.6	193-39-5	0.063	6	Pyro	C, E, U	2B
Dibenz[*ac/ah*]antracene	278	0.0005	6.5	53-70-3	0.014	5	Pyro	C, U, N	2A
Benzo[*ghi*]perylene	276	0.00026	7.1	191-24-2	0.084	6	Pyro	C, E, U	3
Chrysene	228	0.002	5.86	218-01-9	0.07	4	Pyro	C, U, N	2B
Benzo[k]fluoranthene	252	0.0007– 0.008	6	207-08-9	0.14	5	Pyro	C, E, U	2B
Benzo[b]fluoranthene	252	0.0014	5.8	205-99-2	0.14	5	Pyro	C, E, U	2B

[2] Sources:(Agency for Toxic Substances and Disease Registry, 1995; Committee of Directorates for the Water Framework Directive, 2018; European Commission, 2008; Lerda, 2011; National Center for Biotechnology Information; NCBI; Stogiannidis & Laane, 2015; Stout et al., 2004)

[3] According to the Norwegian environmental quality standards, and set according to average annual values.

[4] Enlisted in specific priority lists: C- IARC classification; E - European priority pollutant as defined by the European Commission; U - U.S. EPA 16 ; N – Norwegian river basin specific pollutants

[5] This value was not stated, and the average annual value was used instead as suggested by the original document

1.2.1 PAH metabolites

All animals produce enzymes capable of converting lipophilic xenobiotics into more water-soluble products in order to facilitate excretion. They are more abundant in the liver of vertebrates, whereas in invertebrates they are found mostly in the tissues associated with digestion (Livingstone, 1998). Biotransformation of PAHs generally starts with oxidation. This is often catalyzed by the family of monooxygenase enzymes cytochrome P450 (Stegeman & Lech, 1991). These enzymes are found in a wide array of organisms, from bacteria to mammals (Nelson & Strobel, 1987).

The primary PAH products catalyzed in phase I are arene epoxides that can be further transformed through several possible pathways (*Figure 4*). Some metabolic products will be excreted, whereas others will become carcinogenic, and mutagenic products (Dreij, 2005). Reactive electrophilic compounds may form DNA adducts by binding to nucleophilic sites in the DNA, thus increasing carcinogenicity and toxic potential. Moreover, the geometry of the molecule affects whether they will be metabolized to reactive forms, with a higher potential to produce DNA adducts (Ewa & Danuta, 2017).

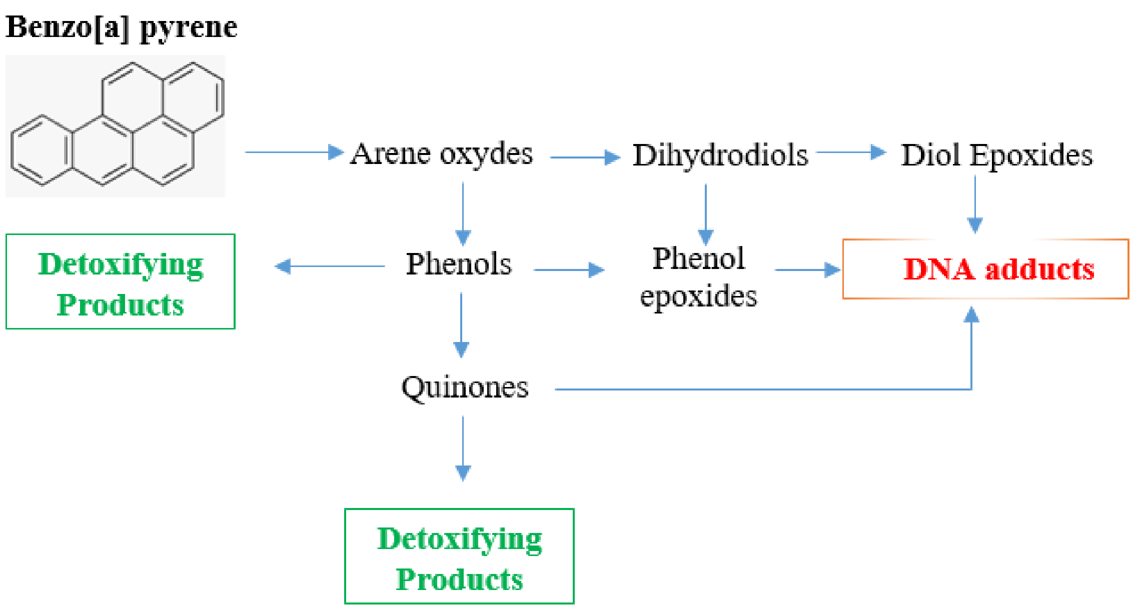


Figure 4 - Simplified scheme of possible metabolic pathways of benzo[a]pyrene in mammals. Adapted from (Antonova et al., 2015)

Metabolites can be used as biomarkers for PAH exposure in vertebrates (Giessing et al., 2003; Grung et al., 2009; Sundt et al., 2011; Whyte et al., 2000). Metabolism efficiency, however, varies among invertebrates (Rust et al., 2004; Stroomberg et al., 2004; Van Brummelen et al., 1996). To the author's knowledge, this is the first study investigating the biotransformation of PAHs in dragonflies.

1.3 Brominated flame retardants (BFRs)

Free radicals have, in general, very strong affinity to halogens. If present during the combustion process, halogens effectively decrease the ability for the flame to propagate by reacting with the free radicals produced during the fire. Of all the organohalogens, organobromine has the best ratio of stability to affinity with oxidizing agents, and a low decomposing temperature. Consequently, organobromine is the most popular choice of organohalogen for use as flame retardant (Guerra et al., 2011).

Brominated flame retardants (BFRs) form a group of synthetic brominated hydrocarbons. They are added to the surface of a range of products such as textiles, furniture, appliances, and computers in order to reduce flammability. These compounds are divided into three subgroups according to how they are incorporated into the polymers; additive, reactive, and polymeric. Additive BFRs are mixed with the other polymer components. Reactive BFRs chemically bond to the polymers. Polymeric BFRs are the most stable of the three groups, having their bromine atoms incorporated into the backbone of the polymers (Guerra et al., 2011). Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) fall into the group of additives, and can easily leach into the environment (de Wit, 2002; Guerra et al., 2011). They enter the environment through various sources, including emissions from manufacturers, and volatilization from products (Streets et al., 2006).

PBDEs are compounds containing 2-10 bromine atoms bound to a diphenyl ether molecule. There are 209 PBDE congeners (Agency for Toxic Substances and Disease Registry, 2017). Tetra-, penta-, hexa-, hepta- and decabromodiphenyl ether are listed in the elimination list of the Stockholm convention under Annex A (Stockholm Convention).

HBCD has similar physicochemical properties to PBDEs. Studies have indicated HBCD as a potential endocrine disruptor, reprotoxic, and neurotoxic (Gorga et al., 2013). HBCD has been included in the 2018 Norwegian priority list (Committee of Directorates for the Water Framework Directive, 2018; Norwegian Environmental Agency, 2016).

PBDEs and HBCD are known for being persistent (de Wit, 2002; Jansson et al., 1987), and for their bioaccumulative/ biomagnification properties (Kelly et al., 2008). Previous studies have, however, detected lower levels of PBDEs in Odonata larvae than is predicted from their trophic position, and efficiency as predators (Grung et al., 2018; Van Praet et al., 2012).

BFRs are common in urban zones (Dodder et al., 2002; Van Praet et al., 2012), and have been previously detected in runoff sediment samples (Dybwad, 2015).

1.4 Sedimentation ponds

Sedimentation ponds are drainage technologies with the purpose of capturing and treating road runoff in a sustainable manner (Fletcher et al., 2014). Some other common technologies are infiltration into road embankments (Boivin et al., 2008), and compact or underground retention basins (J. Andersson et al., 2018).

In Norway, the cleaning strategy is planned according to the AADT (Annual Average Daily Traffic), and the vulnerability of the aquatic systems receiving the runoff (Norwegian Public Roads Administration, 2018). Infiltration is considered sufficient for areas where AADT is below 3000, and also for areas with an AADT up to 30000 if environmental impact is considered low. The runoff must be treated in cases when a medium to high impact is expected (Meland et al., 2016a). According to the latest NPRA guidelines for road construction from July 2018 (Norwegian Public Roads Administration, 2018), future cleaning measures may be divided into two steps. The first step's main function is to remove particle-bound contaminants by sedimentation. The second step's function is removal of dissolved contaminants, often by infiltration.

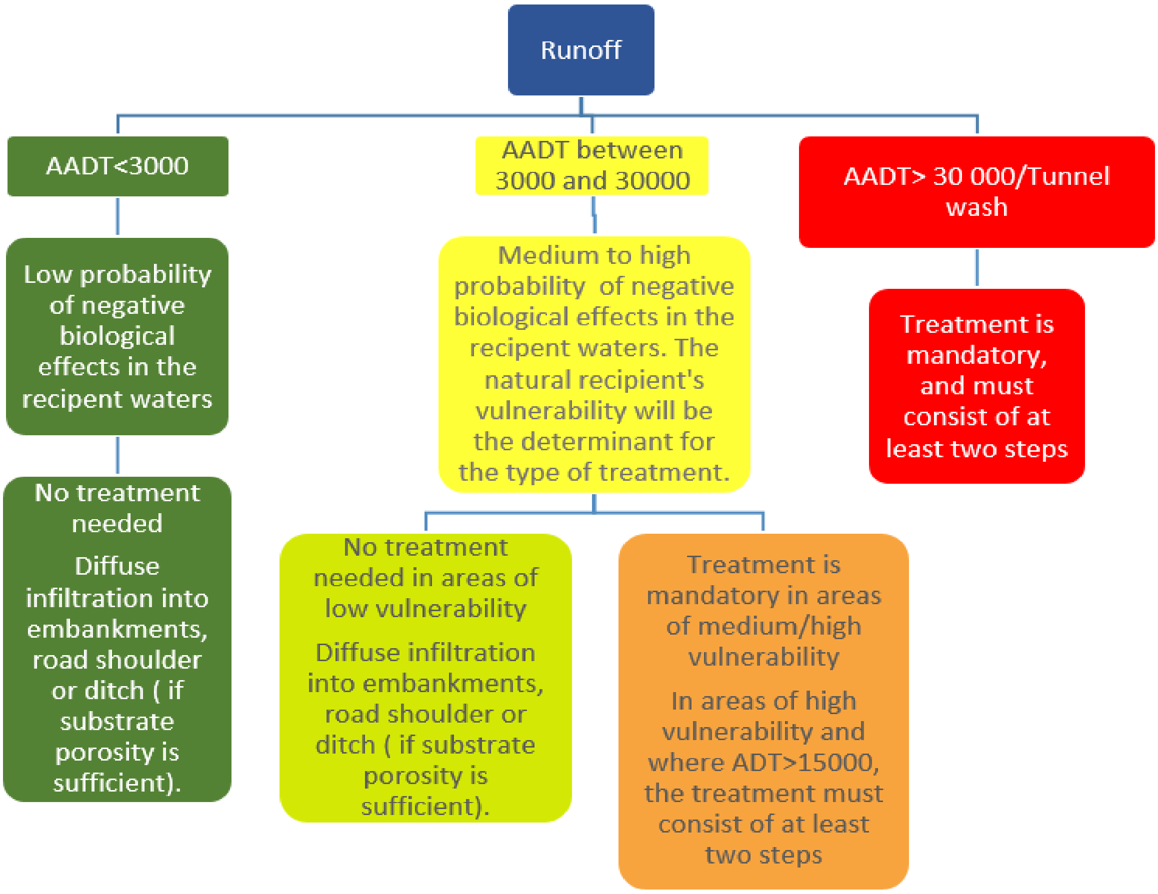


Figure 5 - Cleaning strategy in Norway for road construction. Adapted from (Meland et al., 2016a)

Wet sedimentation ponds are designed to temporarily stop water runoff from moving to natural water bodies, allowing contaminants adhered to the particles to sediment, thus avoiding heavy discharges of contaminants in natural water bodies. They are one of the most common runoff treatment systems in Norway and Sweden (Meland, 2016b). By 2013, the NPRA had built 161 ponds (Paus et al., 2013).

Unlike dry basins, the wet type is built considering a minimum and a maximum volume to avoid drought during dry seasons or overflow during wet seasons. The dimensions are calculated to have the capacity to collect at least 80% of the particles predicted to reach the system (Norwegian Public Roads Administration, 2018). Submerged inlets and outlets are often chosen to avoid having ice blocking water flow (Meland, 2016b). In addition, submerged outlets facilitate the retention of oils and volatile contaminants that will most likely be at the surface (J. Andersson et al., 2018).

Wet sedimentation ponds in Norway are often divided into two basins that might be either entirely or partially separated. Runoff will reach the first, smaller basin (forebay), where most of the coarse particles are expected to settle near the pond inlet. Finer particles are expect to settle in the larger, main basin (Åstebøl & Hvitved-Jacobsen, 2014; Auckland Regional Council, 2003).

Since larger particles will take a greater volume, the addition of a forebay facilitates maintenance and improves the performance of the pond structure (J. Andersson et al., 2018).

Nevertheless, systems with a fixed volume have some drawbacks; poor flow patterns can lead to stagnation zones, an ineffectiveness when it comes to retention of fine suspended particles and hydrophilic pollutants, and potential remobilization of contaminants (Wong et al., 1999) .

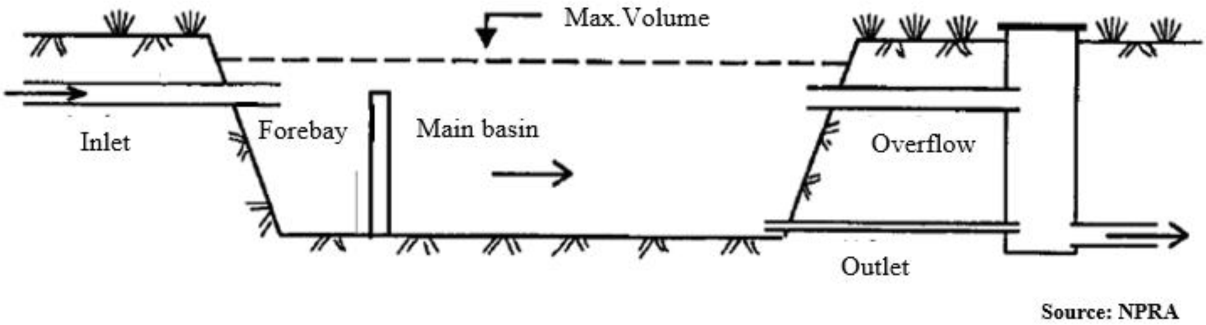


Figure 6 - Sketch of a wet sedimentation pond, and a recently built pond.

1.5 Objectives

This study aimed to determine the fate of selected BFRs and PAHs in dragonfly larvae, and the source origin of PAHs from road runoff. Sediment and dragonfly samples were collected from seven sedimentation ponds, as well as three natural ponds not directly affected by road runoff. Detected contaminants were quantified in larvae of different sizes, and in different body regions to determine whether dragonfly larvae are able to excrete organic compounds by metabolic processes and/ or by using molting as a mechanism of depuration. The fate of PAHs in dragonfly larvae determines whether these organisms are a concern regarding the transfer of PAHs through trophic transfer and across ecosystems.

The origin of PAHs was investigated in sediment samples to determine whether their sources in sedimentation ponds differ from natural ponds that are not directly affected by road runoff.

The following hypotheses were tested:

1. *Contaminant levels are higher in sediment and dragonfly larvae from sedimentation ponds than from natural ponds.*

2. *PAHs detected in all sedimentation ponds come from similar sources and thus present similar patterns. PAHs detected in natural ponds come from similar sources and thus present similar patterns*.

3. *Dragonflies metabolize PAH, and therefore metabolites can be used as a potential biomarker to study PAH exposure in dragonflies.*

4. *Organic contaminants accumulate in dragonfly larvae exuvia, significantly reducing the bioaccumulation in tissues.*

5. *Concentrations of contaminants in earlier instars do not differ from concentrations in later instars, indicating that organic contaminants are eliminated by the larvae.*

2 Materials and methods

2.1 Study sites

A total of seven sedimentation ponds and three natural ponds were analysed for this study (Table 3). The map below gives an overview of their location. Appendix F gives more details about each pond.

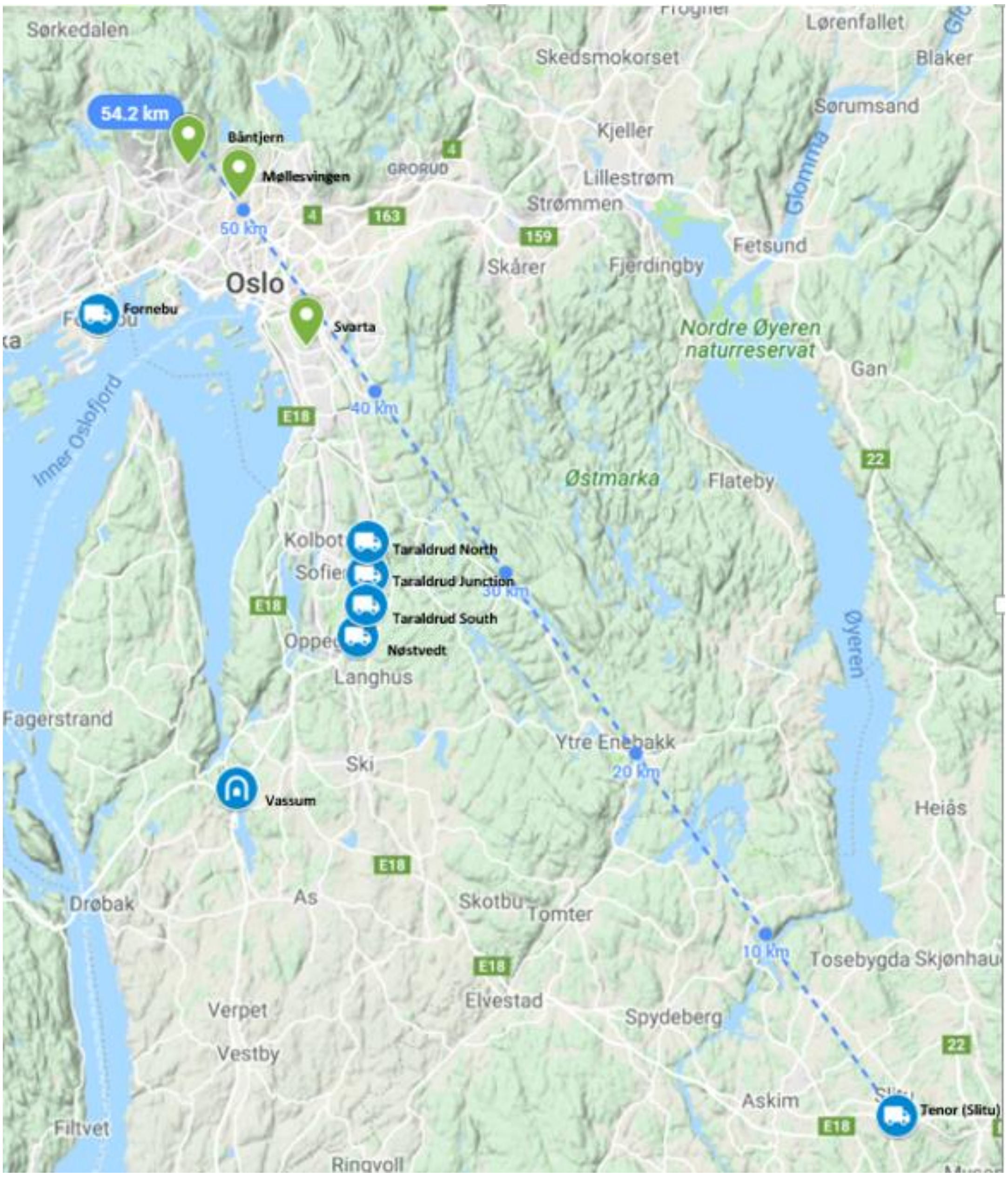


Figure 7 - Map of sedimentation ponds – Green icons represent natural ponds, blue icons represent sedimentation ponds. Tunnel icon represents sedimentation pond receiving tunnel wash runoff.

Table 3 – General information of study sites

Pond	Coordinates	AADT[6]	Size (m2)[7]	Vegetation
Nøstvedt	59.77153, 10.83268	35500	Forebay 40; Main 340	Dense around the main pond, light around forebay Moderate aquatic
Vassum	59.70988, 10.73669	41000	Forebay 68; Main 363	Very dense around the main pond, moderate around forebay Moderate aquatic
Fornebu	59.90115, 10.62591	23193	Forebay 145; Main 480	Very dense around the whole pond Moderate aquatic
Taraldrud North	59.80933, 10.84031	42900	780	Dense around the whole pond Moderate aquatic
Taraldrud South	59.78405, 10.84002	42200	474	Dense around the whole pond Moderate aquatic
Taraldrud Junction	59.79662, 10.84075	42200	1400	Dense around the whole pond Moderate aquatic
Tenor (Slitu)	59.57755, 11.26207	12000	Forebay 175; Main 480	Very dense around the whole pond Dense aquatic
Båntjern	59.96119, 10.69742	NA	500	Dense around the whole pond Moderate aquatic
Møllesvingen	59.94762, 10.73823	NA	320	Very dense around the whole pond Dense aquatic
Svarta	59.88792, 10.79212	NA	1200	Dense around the whole pond Dense aquatic

[6] Brittain et al. (2017)
[7] Sedimentation ponds (Norwegian Public Roads Administration, 2010); reference ponds (Strand, 2006, 2008)

2.1.1 Sedimentation ponds

Nøstvedt

Nøstvedt is located in the Follo district in Akershus county. It receives runoff from the south stretch of the E6 highway (Ringnes – Oslo), and treated water is discharged at Gjersjø river and Tussetjernet lake.

Figure 8 - Nøstvedt sedimentation pond. Source: NPRA

Vassum

The pond receives runoff from the road area around it (E6 road), as well as from tunnel wash from the tunnels Nordby, Vassum and Smihagan (COWI 2012). Water is discharged into the Årung river which is an important spawning growing area for sea trout (Meland 2010).

Figure 9 - Vassum's main basin. Source: NPRA

Fornebu

Fornebu sedimentation pond is located in an urban zone in Bærum, just outside of Oslo. It receives water from RV166, and discharges treated water into the Storøykilen nature reserve, in the inner Oslofjord.

Figure 10 - Fornebu sedimentation pond. Source: NPRA

Taraldrud North

The pond is located in Ski, Akershus. It receives runoff from E6 South (Ringnes – Oslo) and a 26000m^2 stretch of road connecting Taraldrud and Oslo. Water is discharged into lake Snipetjernet.

Figure 4 - Taraldrud North. 2014
Source: NPRA

Taraldrud South

Located in Ski, Akershus, Taraldrud South receives runoff from E6 south (Ringnes - Oslo), and a stretch of 54500m2 of road between Taraldrud and Oslo. Treated water is discharged at Gjersjø river and Tussetjernet lake.

Figure 11 - Taraldrud South, 2014
Source: NPRA

Taraldrud Junction

The pond is located in the vicinity of Taraldrud North. It also receives runoff from the E6, and discharges at Snipetjernbekken.

Figure 12- Taraldrud Junction, 2018
Photo: Sofie Lindeman

Tenor (Slitu)

The pond is located at Slitu, a village in Eidsberg, Østfold. It receives runoff from the E18 (stretch Mormarken - Sekkelsten), and discharges at Lekumelva, in the Glomma watercourse.

Figure 13 - Tenor sedimentation pond in 2018 (left)) and 2014 (top). Sources: Sofie Lindman and NPRA

2.1.2 Reference ponds

Båntjern

The pond is privately owned, and located in a dead-end, residential street in the district of Vestre Aker, on the west side of Oslo. The pond is on average 4 m deep, and covers an area of $500m^2$.

Figure 14- Båntjern pond. Source naturarv.no

Møllesvingen

The pond is located at Møllesvingen 20, a residential street in the district of Nordre Aker, in the north side of Oslo. Its original name is Bergdammen, but in this thesis it is referred to as Møllesvingen. Its size is 320 m^2, with an average depth of 2 m. The land is owned by the municipality of Oslo (Naturarv.no).

Figure 15 - Møllesvingen pond. Source: Naturarv.no

Svarta

Located in the Nordstrand district, in the southern part of Oslo, Svarta covers an area of 1200 m^2, with an average depth of approximately 1 m (Strand 2008). The pond and its surroundings are often used for leisure.

Figure 16 -Svartadamen. Photo: Gunnar Pedersen (nodblad.no)

2.2 Sampling

Samples were collected in June 2018, with the exception of the small larvae and sediment from Svarta, which were collected in June 2017. All samples were transported to the lab in incinerated glass jars. Sediment samples were collected from the upper 5cm of each location with a van Veen grab. Each sediment sample consisted of material collected from five different spots which were combined and mixed. Water was collected directly into jars. Water variables such as pH, temperature and conductivity were measured using a multiparameter display system (YSI-650MDS). Dragonfly larvae were sampled using a dip net. All sampled material was directly transported to NIVA in Styrofoam boxes with ice. Once in the lab, larvae were rinsed with distilled water, pat dried, and killed by introducing a scalpel to the head. They were then weighed, measured, and the haemolymph was extracted.

Samples were stored in incinerated jars, with the exception of haemolymph, which was kept in glass capillaries inside centrifuge tubes. Material was kept at -20 ºC until further analysis.

2.3 Laboratory

Water samples were sent to Eurofins Environment Testing Norway for analysis of total nitrogen and phosphor. Sediment and larvae were analysed at the Norwegian Institute for Water Research (NIVA). All the laboratory work was performed by the author, except for the instrumental analysis, and part of the sediment preparation.

2.3.1 Dragonfly Larvae

Through observation of their body and head shape, larvae were identified to be from the Genus *Aeshna,* as described by Brooks & Cham (2009) - *Figure 17*. Individuals were divided into two groups: *Big* and *Small (Figure 18). Big* dragonflies were those larger than 3.4 cm, and with minimum wing pad length of 4.3 mm. It was inferred that larvae in group *Big* were in instars F1-F0, and group *Small* were within instars F11 and F02. Stages were determined by using the minimum and maximum size values for larval and wing pad length for *Aeshna cyanea* as determined by Goretti et al. (2001). *Aeshna cyanea* individuals were recorded in 10 of 12 sedimentation ponds mapped by Brittain et al. (2017) in Oslo, Akershus and Østfold. Moreover, out 59 individuals sampled from five ponds by Meland (2018), including the ponds Svarta and Båntjern, 43 were of this species. The other identified species were *Aeshna juncea* (14) and *Aeshna grandis* (2).

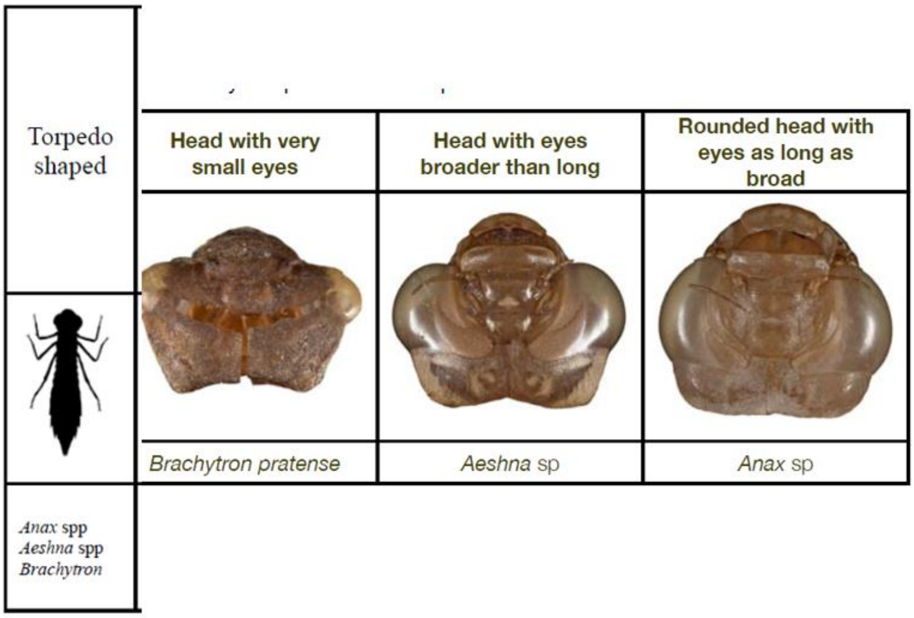


Figure 17 - Body and head shape were used to determine larvae family and genus. Image: Brooks & Cham (2009)

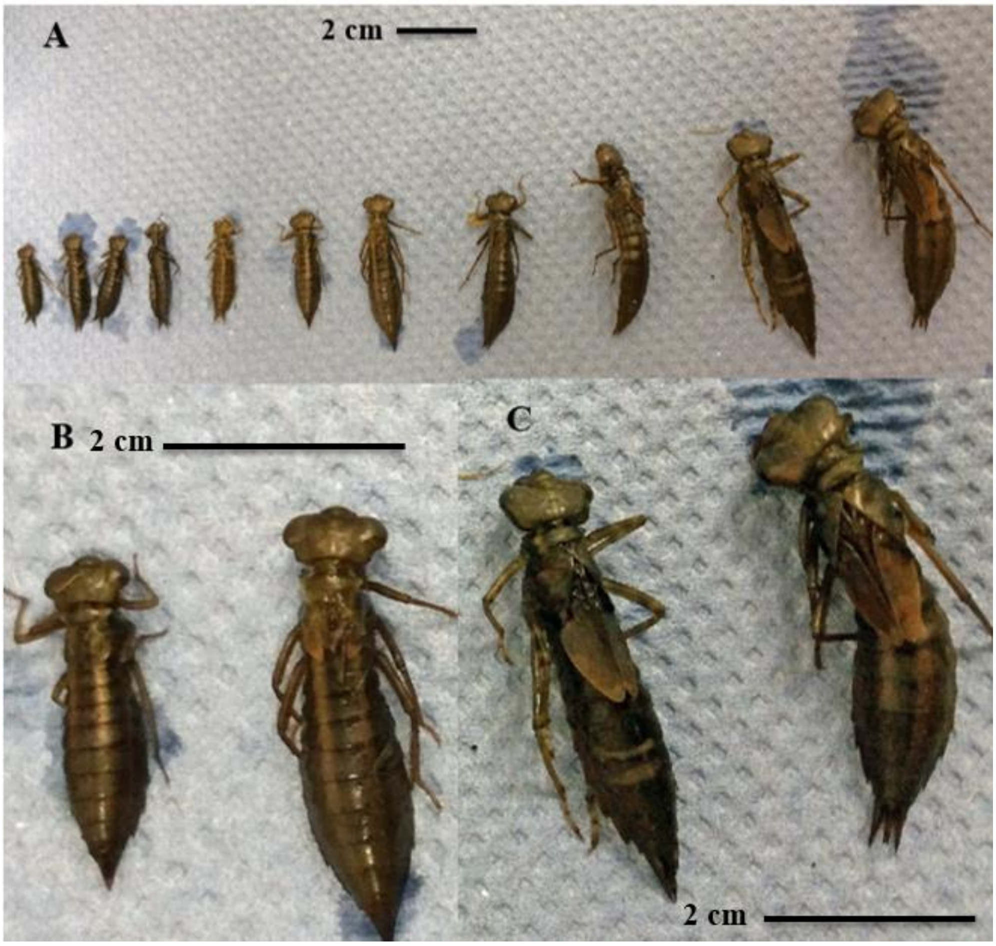


Figure 18 - Larvae were divided into two groups according to their length and wing pad length. A –Larvae ranging from approximately 2 cm to 4.5 cm. B – Examples of larvae from group *Small*. C – Examples of larvae from group *Big*.

Larvae in the *Big* group had exuvia and tissue analysed separately, and haemolymph extracted for determination of PAH metabolites. *Small* larvae were analysed as a whole. Individuals were pooled in order to obtain enough material to detect contaminants (Table 4).

Table 4 -Number of larvae pooled from each pond

Ponds	*Big*	*Small*
Nøstvedt	17	10
Taraldrud North	12	6
Taraldrud South	15	2
Taraldrud Junction	6	11
Tenor	10	9
Fornebu	15	3
Vassum	12	7
Båntjern	10	13
Svarta	9	4
Møllesvingen	11	0
Total # of larvae (Sedimentation)	87	48
Total # of larvae (natural ponds)	30	17
Total # of larvae	**117**	**65**

Extraction of haemolymph

The middle leg of the larvae was removed with a tweezer, and a glass capillary (Hilgenbert, 80 mm length, 0.4 mm outer circumference, 0.04 mm wall thickness) was inserted. Applying gentle pressure, the haemolymph was released and collected in the capillary. The volume of haemolymph extracted was measured by its weight.

Dissection

Exuvia and the internal tissues were separated in frozen larvae by lifting the wing pads with a tweezer. Exuvia was cracked open in the area between the thorax and abdomen. From there, the abdomen was opened using a carbon steel surgical blade. Internal tissues were scraped out with a metal spoon. The labium was removed in order to get access to the head, and to reach the rest of the internal tissues. Wing pads were pulled off to get access to the wings, which were already developed in some individuals. Tissue and exuvia were transferred to separately marked extraction glasses to be analysed separately.

Figure 19 - Developed wings of larva in its last stage

2.3.2 Extraction method

Samples (sediment/ larvae) were weighed, and then freezer-dried for 48 hrs (larvae - 2-8 g wet weight/ sediment 5 g wet weight). Dried contents were homogenized with a glass stirring rod, and approximately 15 mL of cyclohexane: dichloromethane (90:10), and 50 µL of PAH and BFR internal standards were added.

Samples were placed in an ultrasonic bath for 1 hour to improve extraction efficiency, and then centrifuged for 5 minutes at 3000 RPM. The extraction process was repeated two times.

Extractions were then concentrated to 1 mL in an automated solvent evaporation system (TurboVap LV) at 37^{o}C. Half of the final extract (0.5 mL) was used for PAH, and the other half for BFR analysis.

BFRs:

Sulphuric acid (approx. 2 mL) was added to the samples to remove the impurities in the extract. Samples were vortexed and centrifuged for 5 minutes at 2000 RPM. Sulphuric acid was then removed with a glass pipette. This process was repeated several times, until the sulphuric acid showed very little or no colour. The clean extract was rinsed with Milli-Q® water to remove any residual H_2SO_4. After centrifugation (5 minutes at 2000 RPM), the extract was removed with a glass pipette, and transferred to 0.9 mL vials to be concentrated to approximately 100 µL for further analysis.

PAHs:

Extracts were transferred to 2 mL vials and concentrated to approx. 100 µL. 400 µL of ethyl acetate (LS-MS graded) was added. Extracts were transferred to Eppendorf tubes with centrifuge tubes filters (0.2 µM nylon filters), centrifuged for 1 min at 13000 RPM, and transferred to vials to be cleaned by Gel Permeation Chromatography to exclude high-molecular compounds such as proteins and fat.

A small amount of cyclohexane was added, and the extracts were once again concentrated to approximately 1 mL in an automated solvent evaporation system (TurboVap LV) at 37^{o}C. The samples were further concentrated to approx. 100 µL, and transferred to 0.9 mL vials for analysis.

2.3.3 PAH metabolites

Haemolymph samples were transferred to Eppendorf tubes (approximately 10 µL). This procedure was done whilst keeping the haemolymph on ice whenever possible, and keeping the samples away from direct light. 10 µL of internal standard was added to each sample. 50 µL of MillQ® water, followed by 20 µL of β-glucoronidase/ aryl sulfatase, was added to the samples and well mixed with the help of pipette tips.

Samples were set on a heating block at 37^{o}C for 1 hr, and then 200 µL of methanol was added and mixed well. Samples were then centrifuged for 10 minutes at 13000 RPM.

Supernatants were transferred to 300 µL vials and kept in a freezer at -20^{o}C until analysis.

2.3.4 Analysis

PAHs and BFRs

Sediment and dragonfly larvae extracts were analyzed by gas chromatography/mass spectrometry in selected ion monitoring mode (Agilent GC 6890/MSD 5973; Agilent Technologies, Wilmington, DE, USA). The internal standard method was used for quantification of individual components. See appendices B2 and B4 for information on instrument setup.

PAH metabolites

Haemolymph extract was analyzed by high performance liquid chromatography (HPLC) using Waters 2695 Separations Module and 2475 fluorescence detector. The internal standard method was used for quantification of individual components. See appendix B3 for instrument setup.

2.3.5 Quality Assurance

Measures were taken in order to increase the validity and precision of the analyses.

Internal standard (IS) of PAHs and BDEs were added to all samples before the sample preparation stage. ISs are known concentrations of the target compounds (or compounds that behave similarly) added to the analytes with the purpose of accounting for any loss of analyte during sample preparation.

Blank samples containing only IS and solvent were also treated with the samples, and analysed with each sample series. Blank samples were used to trace contamination and material loss.

Standard Reference Material® (SRM) was used to quality-control samples. SRMs are matrices containing well-characterized concentrations of contaminants, and are used to verify the accuracy of the analysis method and the traceability of the contaminants of interest. The SRM used was obtained from the National Institute of Standards and Technology (NIST). In cases where no commercial SRM was available, a reference material prepared by NIVA was used.

Table 5 - Information on internal standards and quality control samples

Matrix	IS- PAH 2 µg/mL (toluene)	IS- BDE 90 ng/mL (isooctane)	IS- OH-PAH [8] 16 µg/mL (triphenylamine)	Quality control samples
Sediment	Naphthalene-d_8 Biphentyl-d_{10} Acenaphtylene-d_8 Pyrene-d_{10} Dibenzothiophene-d_{10} benzo(a)anthracene-d_{12} Perylene-d_{12}	BDE-30 BDE-119 BDE-181	-	NIST®SRM® 1944
Larvae	Same as above	Same as above	-	NIST®SRM® 2974a
Haemolymph	-	-	1-OH-PHEN 1-OH-PYR 3-OH-BaP	Reference material prepared at NIVA

2.4 Experimental study

An exposure experiment had been planned to expose dragonfly larvae to water spiked with pyrene and BDE-47. The goal was to investigate whether pyrene and BDE-47 would bioaccumulate or depurate via exuvia, and if pyrene would be metabolized. PBDEs are well-known for their bioaccumulative nature, but have been previously detected in dragonfly larvae at lower levels than predicted according to their trophic position (Grung et al., 2018).

A minimum of 80 larvae would be placed in individual glasses and exposed to a nominal concentration of 5µg/L of pyrene (solubility 0.135mg/L), and 0.5µg/L of BDE-47 (solubility 10 µg/L). There would be a minimum of five time points and four replicates.

[8] PHEN – Phenanthrene , PYR – Pyrene, BaP – Benzo[*a*]pyrene

A total of 100 larvae were collected from different sedimentation ponds, and taken to a climate room at NIVA (14 ^{o}C) where the experiment would occur. They were supposed to be kept in this room for a week for depuration before the beginning of the experiment.

Many of the larvae were, however, found dead within 24 hrs of being captured, and the experiment had to be cancelled. Some were found decapitated. Larvae collected in the autumn of 2017 were kept in the same container for days with no incidents, but larvae collected in the spring of 2018 behaved aggressively. Surviving larvae were released into an urban pond in the proximity of NIVA.

2.5 PAH source apportionment

The relative abundance of PAHs in the environment can be used as fingerprints to estimate their source of origin, and some alkylated PAHs can be used as specific source markers (Law & Biscaya, 1994; Neff et al., 2004; Stogiannidis & Laane, 2015; Zhendi Wang & Fingas, 1997; Webster, 2010). Fingerprinting can be also achieved by calculating concentration ratios of specific PAHs (Brown & Peake, 2005; De Luca et al., 2005).

The relative abundance of certain PAHs in sediment was qualified and quantified, and ratios were calculated to estimate the sources of the PAHs detected in the sediment. Svarta was not included in all tests due to insufficient data. The abbreviations used for PAHs are described on the table below:

Table 6 - list of PAH abbreviations

List of PAH Abbreviations

NAPH	Naphthalene	**BaA**	Benzo[a]anthracene
ACY	Acenaphthylene	**I123P**	Indeno [1,2,3-cd]pyrene
ACE	Acenaphthene	**DachA**	Dibenz[ac/ah]antracene
FLO	Fluorene	**BghiPER**	Benzo[ghi]perylene
ANTH	Anthracene	**CHR**	Chrysene
FLUORA	Fluoranthene	**BkF**	Benzo[k]fluoranthene
PYR	Pyrene	**BbjF**	Benzo[b,j]fluoranthene
PHEN	Phenanthrene	**DIB**	Dibenzothiophene
BaP	Benzo[a]pyrene	**BeP**	Benzo[e]pyrene
		PER	Perylene

Ratios of low molecular weight (2-3 rings) to high molecular weight (4-6 rings) were calculated. Petrogenic PAH mixtures contain higher levels of low molecular weight (LMW) in relation to high molecular weight (HMW). Therefore, values of LMW/ HMW>1 suggest a prevalence of petrogenic PAHs, whilst PAH pyrogenesis is reflected at values below 1 (Brown & Peake, 2005; De Luca et al., 2005). The LMW/HMW ratio was calculated as described by De Luca et al. (2003):

$$\frac{NAPH + ACE + ACY + FLO + PHEN + ANTH}{PYR + FLUORA + BaA + CHRY + BbjF + BkF + BaP + I123P + DachA + BghiPER}$$

Ratios of phenanthrene to anthracene (PHEN/ANTH) and fluoranthene to pyrene (FLUORA/PYR) were also used. Anthracene and fluoranthene are produced during high-temperature processes, but are less thermodynamically stable during the slow process of fossil fuel formation (Neff et al., 2004). Values of FLUORA/PYR below 1 indicate mainly petrogenic origin. Values approaching, or above 1, indicate pyrogenic dominance. High PHEN/ANTH ratios often indicate dominance of petrogenic sources, and low ratios of pyrogenic sources (De Luca et al., 2003; Neff et al., 2004; Stogiannidis & Laane, 2015). For this study PHEN/ANTH >10 were interpreted as mainly petrogenic, and <10 as mainly pyrogenic as described by Wang et al. (1999).

Petrogenic PAH mixtures are often characterized by the dominance of the alkylated homologs of naphthalene, fluorene, phenanthrene, dibenzothiophene, and chrysene (Zhendi Wang & Fingas, 1997). Thus, it is useful to use alkylated data when available. Pyrogenic Index (PI) and its precursor Fossil Fuel Pollution Index (FFPI) were calculated as described in Z. Wang et al. (1999), and Boehm and Farrington (1984), respectively:

$$PI = \frac{ACY+ACE+FLUORA+PYR+ANTH+BaP+BkF+BeP+PER+BghiPER+I123P+DachA+BbF+BaA}{\Sigma NAPH_{(C0 - C4)} + \Sigma PHEN_{(C0 - C4)} + \Sigma DIB_{(C0 - C3)} + \Sigma FLO_{(C0 - C3)} + \Sigma CHRY_{(C0 - C3)}}$$

$$FFPI = \frac{\Sigma NAPH_{(C0 - C4)} + ½*(\Sigma PHEN_{(C0, C1)}) + \Sigma DIB_{(C0 - C4)} + \Sigma PHEN_{(C2 - C4)}}{TPAH^{9}}$$

PI values below 0.8 indicate PAH petrogenic dominance, and values above 0.8 are most likely dominated by petrogenic PAHs. FFPI suggests that values closer to 1 indicate petrogenic predominance, and closer to 0 indicates pyrogenic dominance.

[9] Total PAH-16 + PER + BeP

2.6 Statistical analysis and data handling

Data were processed using Microsoft Excel version 2013 for Windows. Statistical analyses were performed using RStudio (version 1.1.456 - 2009-2018). R packages used are specified in appendix D3.

For dragonfly data, only PAHs which had at least 80% of the concentration quantified were used. Observations reported as “less than” (<) were substituted with half of its value. For sediment data, different substitution methods (as described in Wood, Beresford & Copplestone (2011)) were tested, with no significant difference in the overall results. Consequently, [(<) /2] was also applied for variables containing less than 80% of observations detected, when appropriate.

LOQ and LOD values were low for BFRs in sediment, and for that reason they were treated as 0. There was also no significant difference in the overall results.

BFRs were detected at very low concentrations in dragonfly larvae, and the results were mostly below the level of detection. For that reason BFR results in larvae were qualified only. All measures above the LOD were characterized as “above” and below the LOD as “below”. Levels below the LOQ, but above the LOD were included as “above”. LOQ values can be stated as analyte being present in the sample, but the exact concentration is uncertain. A value below the LOD, on the other hand, means that the analyte was not detected. LOD values imply, therefore, that either the analyte is not present, or that they are present at such low concentrations that they cannot be distinguished from the noise.

Shapiro-Wilk test for normal distribution was performed. Data were log-transformed when normality assumptions were not met. Wilcoxon rank sum test was performed when the assumption of normality was still not met after data transformation. Welch's t-test was performed whenever the normality assumption was met. Welch’s t-test has been shown to be more robust against type-I error than the Student’s t-test when samples sizes are unequal, and/or small (Moser & Stevens, 1992; Ruxton, 2006). Significance level was set to $\alpha = 0.05$.

Multivariate analysis was performed using Principal Component Analysis (PCA) to explore structure between variables. Variables were log transformed in order to improve the normal distribution, and reduce the impact of outliers. Data were standardized (centered and rescaled).

3 Results

3.1 Water

A Principal Component Analysis (PCA) biplot was performed (*Figure 20*). Raw data are presented in appendix E1. The first two dimensions captured 66% of the total variation.

Points clustered together are similar in characteristics. Overall, the analysis indicates that sedimentation ponds have similar water characteristics, with the exception of Fornebu. PCA indicates that Møllesvingen and Svarta are similar (and similar also to Fornebu). Taraldrud North and Junction share high pH and O_2 values. Båntjern stands alone on the top left of the PCA due to particularly high levels of nitrogen and phosphorous, combined with low temperature. Svarta, Nøstvedt and Fornebu share similarities in relation to temperature (relatively high) and O_2 (relatively low). Vassum lies very close to the center. That is, there were no particularly high or low variances detected in the variables from Vassum.

The PCA captured a positive correlation between O_2, pH, redox potential and conductivity, and these are all in general higher in sedimentation ponds. A strong positive correlation between nitrogen and phosphorus (higher in natural ponds), and between O_2 and pH was detected. Temperature was negatively correlated to redox potential, pH, O_2, and conductivity.

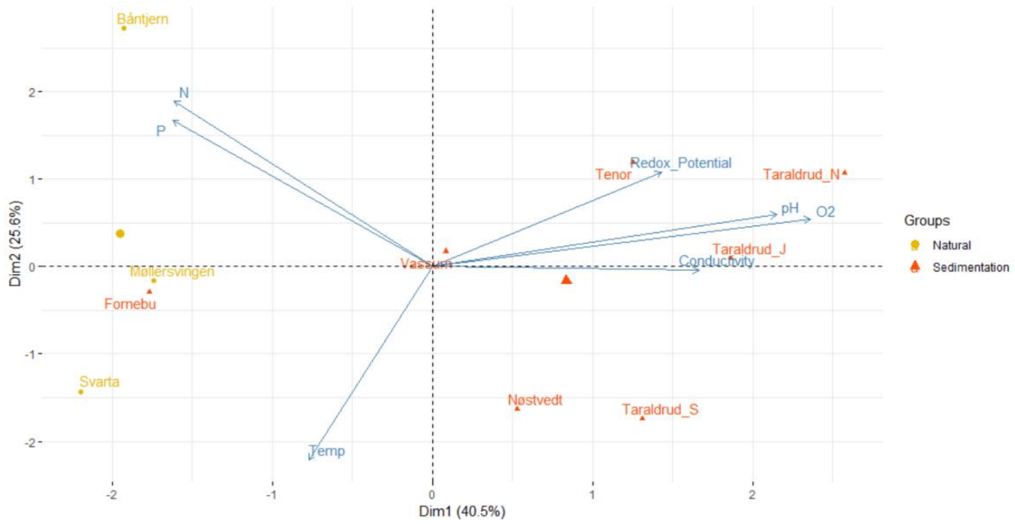


Figure 20 – PCA biplot. All variables, with the exception of pH, were log transformed. Dim1 represents PCA axis 1(x-axis), and Dim2 represents PCA 2 (y-axis). The first and second dimensions captured approximately 66% of the total variation. Components with largest variation are represented by longer arrows. The angle formed by the arrows indicate correlation. The narrower the angle, the more correlated the variables forming the angle are. Data were centered and scaled. Largest triangle and circle are the centroids of the sedimentation and natural clusters, respectively.

3.2 Sediment

3.2.1 PAHs

Environmental Quality Standards (EQS)

PAH-16 were classified according to the EQS set by the Norwegian Committee of Directorates for the Water Framework Directive (Committee of Directorates for the Water Framework Directive, 2018). Classes range from I to V, according to the maximum concentrations determined for each class. Values are based on laboratory tests, risk assessments and reports on acute and chronic toxicity on organisms (Norwegian Environmental Agency, 2016). A short description of the classes and results are presented in *Table 7*.

The highest levels of PAHs were detected in the samples from Taraldrud North, South, Fornebu, and Vassum. They had the most number of PAHs classified within classes III and IV (*Figure 21*). Sediment containing PAHs in class III are identified as being of moderate standards, and class IV are defined as poor by the Norwegian Environmental Agency. PAHs with the lowest quality standards were benzo(*ghi*)perylene, benzo(*b*,*j*)fluoranthene, indeno(*1*,*2*,*3*-*cd*)pyrene, anthracene, and pyrene. No PAHs were classified in class V (possible acute effects).

Table 7 – Concentration of PAHs (µg/kg, dry weight) detected in sediment samples. Classification according to Norwegian Environment Agency (Norwegian Environmental Agency, 2016). The sum of PAH-16 is also given (in white). Values in grey: EQS not provided. Values marked as *: classification may change if value is below stated results.

Class I – Background levels; Class II – Good – No toxic effects; Class III – Moderate – Possible chronic effects; Class IV – Poor – Possible acute effects; Class V – Very poor – Considerable toxic effects

PAHs (µg/kg)	Nøstvedt	Taraldrud Junction	Fornebu	Taraldrud Nord	Tenor	Vassum	Taraldrud South	Båntjern (Ref)	Møllesvingen (Ref)	Svarta (Ref)
Naphthalene	<15	53	69	84	<10	47	54	<20	<10	<10
Fluorene	18	41	83	67	<7	64	32	29	23	<1
Phenanthrene	69	213	270	512	13	283	185	95	80	2.8
Chrysene	62	73	215	215	17	118	176	22	79	3
Fluoranthene	107	221	392	622	22	415	386	333	161	5.3
Pyrene	214	468	659	1190	30	825	641	130	108	4
Benzo(a)anthracene	21	39	117	98	6.8	67	113	13	50	2
Benzo(b,j)fluoranthene	88	142	351	281	30	221	306	42	166	5.5
Benzo(k)fluoranthene	18	33	95	67	7.7	54	97	7.0	50	2
Benzo(a)pyrene	40	73	149	128	13	108	164	17	61	2.4
Indeno(1,2,3-cd)pyrene	35	54	133	117	13	78	134	<20	80	2.6
Dibenz(ac/ah)anthracene	<15*	<30*	<50*	<50*	<10	<40*	44	<8	17	<1
Benzo(ghi)perylene	117	211	230	342	24	235	262	<20	44	<1
Anthracene	12	16	51	47	2.2	41	40	11	19	<1
Acenaphthylene	<15	12	26	36	<10	<15	22	<20	<10	<5
Acenaphthene	<15	<15	21	29	<6	<40	<25	<10	6.8	<6
ΣPAH16	770	1598	2886	3861	200	2603	2670	747	955	25
NAPH1	19	64	100	87	13	45	51	<20	<10	<10
NAPH2	386	368	581	295	207	287	284	106	39	<60
NAPH3	1660	2520	2830	1340	761	1010	905	<100	57	<100
NAPH4	332	1020	810	428	113	353	248	<70	<10	NA
FLO1	50	231	269	211	<10	184	109	<20	21	NA
FLO2	90	624	470	454	<15	478	268	<30	27	NA
FLO3	<190	1410	802	<1100	<40	<980	511	<160	<60	NA
PHEN1	67	428	366	480	<11	425	219	46	40	<5
PHEN2	356	1610	1370	1900	<40	1570	213	<60	113	<5
PHEN3	234	839	902	1030	NA	1050	674	<60	65	<5
PHEN4	<140	<300	335	250	<20	390	490	<30	17.5	NA
CHRY1	309	521	858	1090	83	993	749	<40	80	NA
CHRY2	857	1280	1780	2490	171	2610	1740	<50	<50	NA
DIB	<4	17	20	33	<2	19	19	33	6.3	<1
DIB1	16	88	119	120	<6	101	67	18	12	<10
DIB2	142	501	718	785	<20	690	493	32	55	<10

DIB3	372	996	1340	1590	<60	1550	886	<60	103	<10
BeP	125	213	368	406	40	345	331	20	72	2.6
PER	23	<40	54	65	11	74	80	NA	15	<5

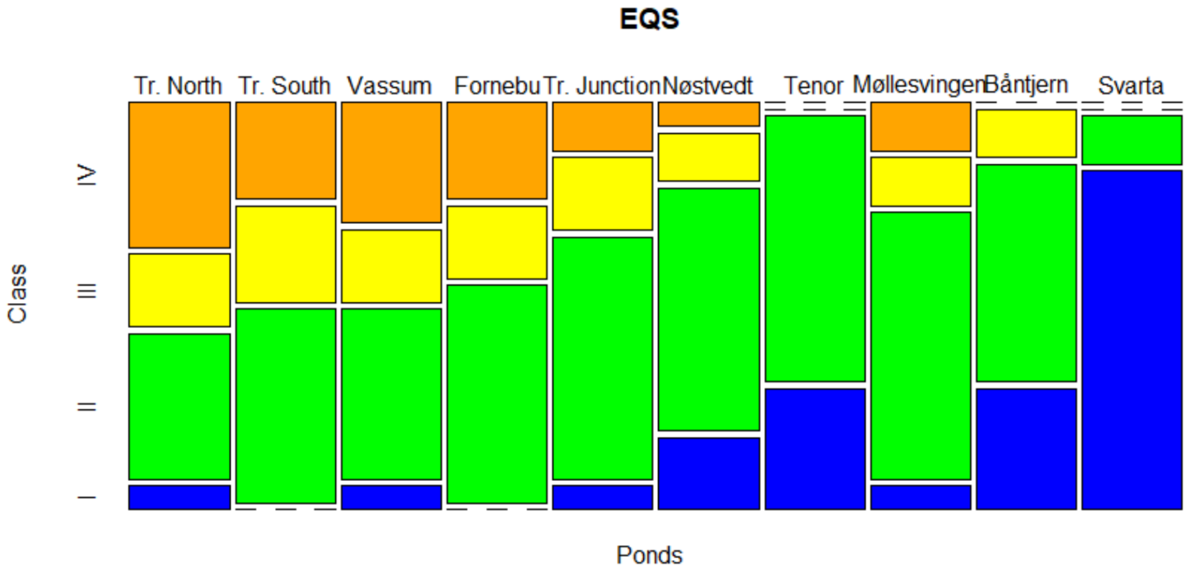


Figure 21 – Mosaic plot of the PAH EQS in ponds. Each bar represents one pond. Classes are represented horizontally. Classes I, II, III, and IV are represented respectively in blue, green, yellow, and orange. Flat dashed lines denote that no PAHs were included in that class.

Parental and Alkylated Concentrations

A significant difference in the concentration of PAH-16 in sedimentation and natural ponds was observed (Welch Two Sample t-test, p= 0.025). Levels of PAHs were in general higher in sedimentation ponds. The exceptions were Tenor and Nøstvedt, where levels were relatively low, even lower than in some of the reference ponds. The highest concentrations of PAHs were detected in Taraldrud North. Addition of alkylated PAHs led, however, to substantial changes in the results. All sedimentation ponds had higher concentrations of PAHs compared to natural ponds once alkylates were included. There was also a significant difference in the sum of PAH-16 and their alkylated forms in sedimentation and natural ponds (Welch Two Sample t-test, p= 0.002).The former EQS classification for PAH-16 in marine sediment, described by Bakke et al. (2009), was used to give a better picture of the difference in PAH concentration once alkylated forms were added (Figure 22). Concentrations detected below LOQ were set as ½ LOQ as explained in section 2.6. Unavailable PAH values were set as zero. Several PAHs changed classes by the current EQS classification, with many changing from class II to class IV (*Table 8*).

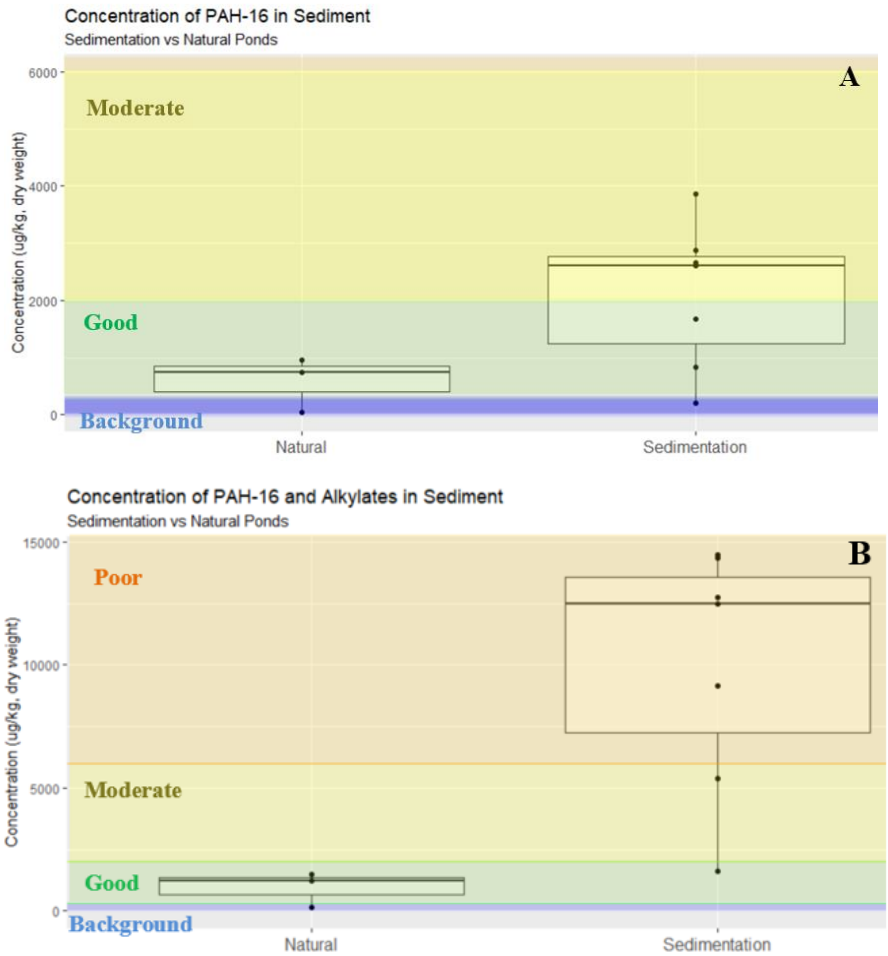


Figure 22 – Concentrations of PAHs in sediment. **A** - Concentration of PAH-16 only. **B** - Concentration of PAH-16 and alkylates. Box represents the 2nd and 3rd quartiles. Horizontal lines represent the median. Whiskers represent the lower (1st) and upper (4th) quartiles. Each data point represents a pond. Natural, n=3. Sedimentation, n=7. Blue area: Class I. Green area: Class II, Yellow area: Class III. Orange area. Class IV. Classes were determined according with the former values in the Norwegian system for classification of organic contaminants in marine sediments (Bakke et al., 2009).

Table 8 - EQS based on Norwegian criteria. Sum of PAHs include their alkylated and non-alkylated forms. Detection of alkylated PAHs in Svarta were below LOQ, and classification may change depending on the true values.

PAHs	NAPH	ΣNAPH	FLO	ΣFLO	PHEN	ΣPHEN	CHRY	ΣCHRY
Nøstvedt	<15	2405	18	254	69	797	62	1166
Taraldrud Junction	53	4025	41	2306	213	3240	73	1801
Fornebu	69	4391	83	1624	270	3242	215	2853
Taraldrud North	84	2234	67	1282	512	4171	215	3795
Tenor	<10	1098	<8	36	13	48	17	270
Vassum	47	1742	64	1216	283	3718	118	3722
Taraldrud South	54	1542	32	920	185	1781	176	2665
Båntjernveien (Ref)	<20	211	29	134	95	216	22	67
Møllesvingen (Ref)	<15	111	23	100	80	316	79	184
Svarta	<10	<90	<1	NA	2.45	<31	1	NA

Relationship between PAH-16 and Average Daily Traffic (AADT)

Linear regression indicated a significant relationship between the sum of PAHs and Annual Average Daily Traffic (AADT). The model includes sedimentation ponds only. The R^2 can be interpreted as 61% of the response (Sum of PAHs) can be explained by AADT (p = 0.03 (*Figure 23*)). The result must, however, be interpreted with caution because Tenor's contribution to the model is an extreme influential outlier. Linear relationship including pond size, and pond size + AADT were also tested, revealing no significant relationship (PAHs ~ pond size, p= 0.66. PAH ~ AADT + pond size; p-value = 0.13).

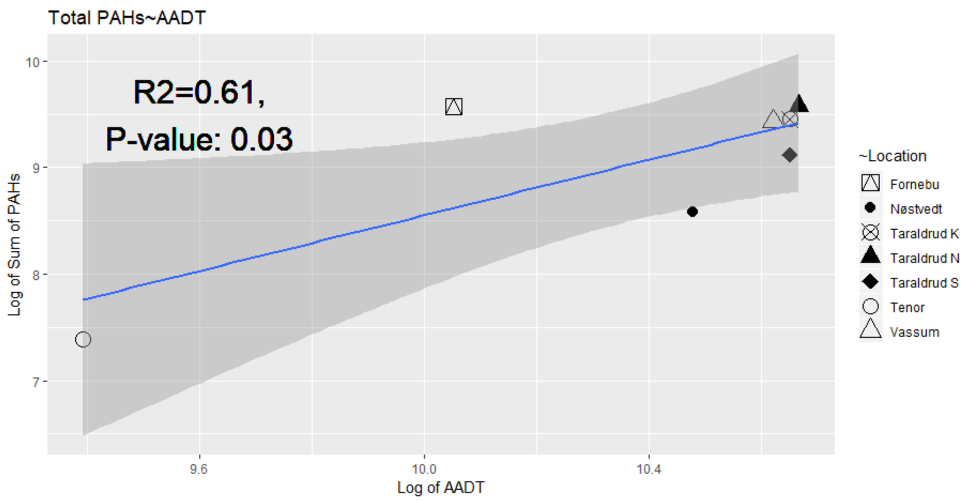


Figure 23 - Linear model. PAH16 as response variable. Annual Average Daily Traffic (AADT) explanatory variable. Confidence band in dark grey. The width of the band increases with an increase in the margin of error.

3.2.2 Potential sources of PAHs

Distribution patterns

The patterns in the proportion of selected PAHs indicate a dominance of alkylated PAHs compared to parental PAHs in the sediment samples (Figure 24). Sedimentation ponds presented very similar distribution patterns. Natural ponds, on the other hand, displayed more distinct patterns in comparison to sedimentation ponds. NAPH3 dominated amongst all NAPH forms in sedimentation ponds, whereas NAPH2 dominated in Båntjern. FLO formed a left skewed pattern in all sedimentation ponds, but not in the natural ponds. From all phenanthrene species, PHEN2 dominated in all ponds. The exception was Båntjern, where the parental PHEN dominated. DIB formed a left-skewed pattern in all sedimentation ponds and in Møllesvingen. Both natural ponds formed very different CHRY patterns compared to the sedimentation ponds.

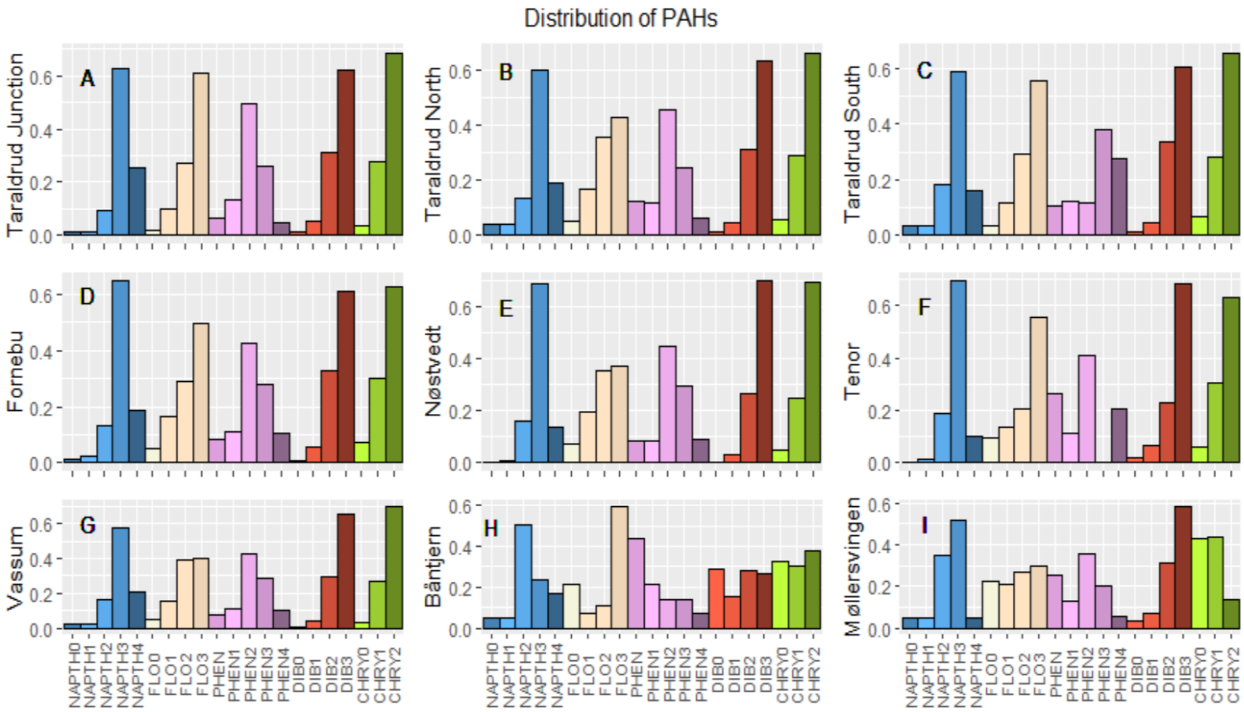


Figure 24 – Bar plot shows the proportion of selected parental PAHs and their alkylated forms. Blue – NAPH and alkylates C1 to C4. Beige – FLO and alkylates C1 to C3. Purple – PHEN and alkylates C1 to C4. Red- DIB and alkylates C1 to C3. Green - CHRY and alkylates C1 and C2.

Source ratios

Most ratios suggest that PAHs in sedimentation ponds are primarily from petrogenic sources, whereas they are mainly pyrogenic in natural ponds (Figure 30). A binomial test revealed an 83% probability of sedimentation pond PAHs being mainly petrogenic (p-value = 0.0001, 95% confidence interval: 0.66 - 0.93), and a 100% probability of reference pond PAHs being mainly pyrogenic (p-value = 0.0005, 95% confidence interval: 0.74 -1). A description of each ratio is presented in section 2.5. Due to the low quantification of most PAHs in Svarta, the pond was only included in the ratios of the isomers.

All the results are individually presented below.

Phenanthrene/Anthracene, Fluoranthene/Pyrene ratios

PHEN/ANTH results indicate that PAHs in all ponds are mainly from pyrogenic origin, with Taraldrud North and Junction being the only two exceptions (*Figure 25*). FLUORA/PYR results suggest that all sedimentation PAHs are from petrogenic origin, and pyrogenic in reference ponds (*Figure 26*).

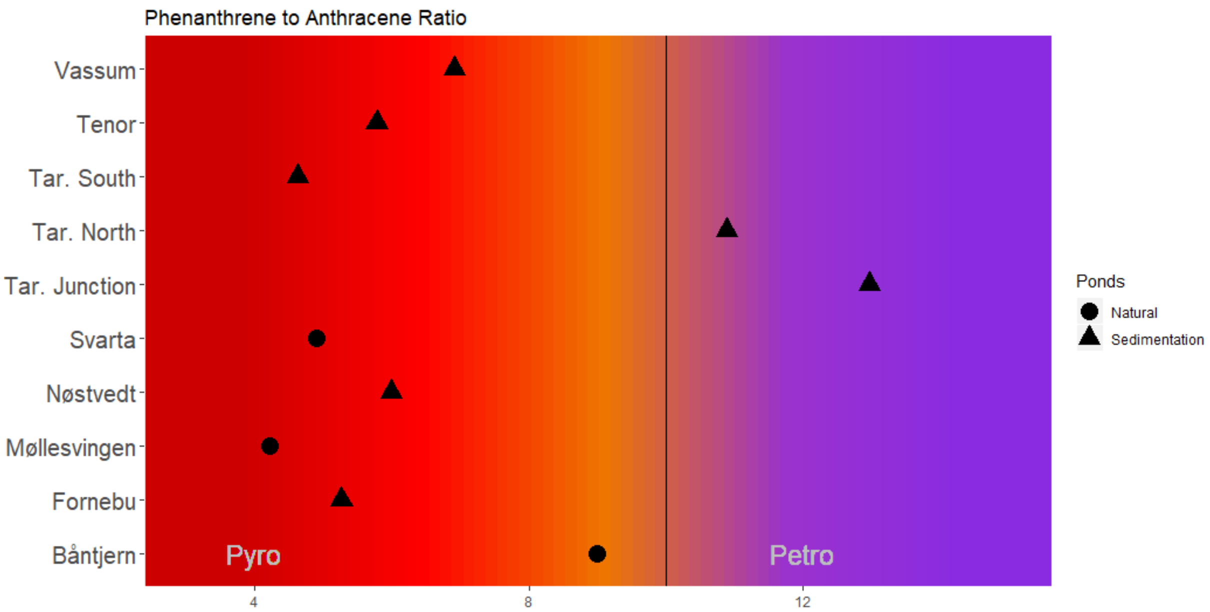


Figure 25 – PHEN/ANTH ratio. Values below 10 indicate dominance of pyrogenic PAHs (red area). Values above 10 indicate petrogenic dominance (purple area).

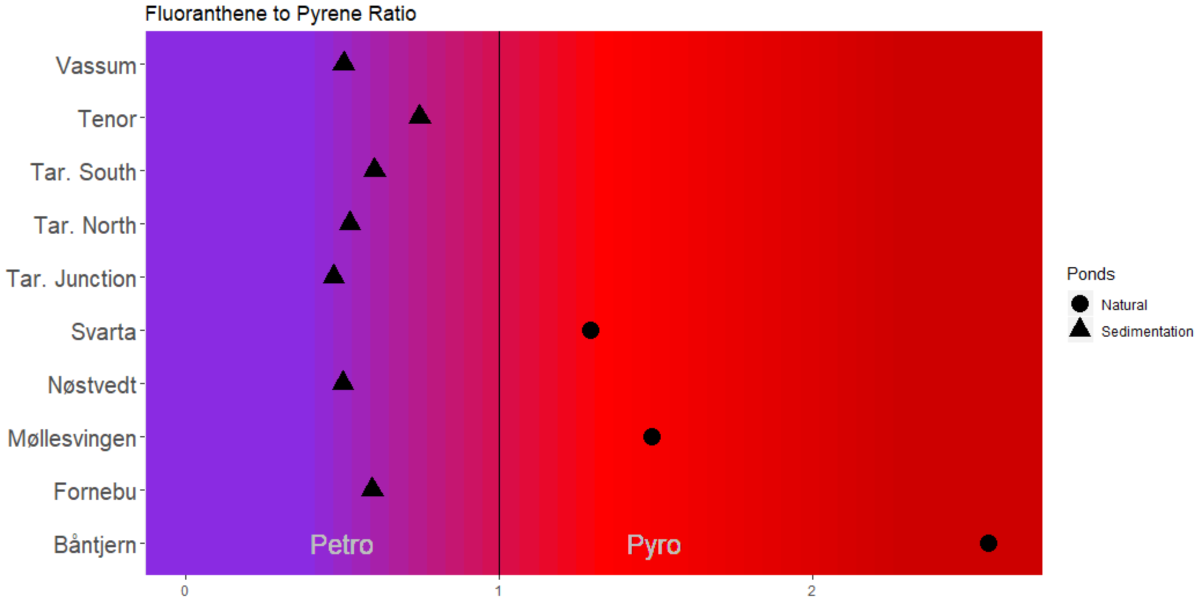


Figure 26- FLUORA/PYR ratios. Values below 1 indicate dominance of petrogenic PAH (in purple). Values above 1 indicate pyrogenic dominance (in red).

Low/high molecular weight ratio (LWM/HMW)

LMW/HMW ratio results were the most distinct from all source-indicator ratios. The results suggested that PAHs in all locations are mainly from pyrogenic origin (Figure 27).

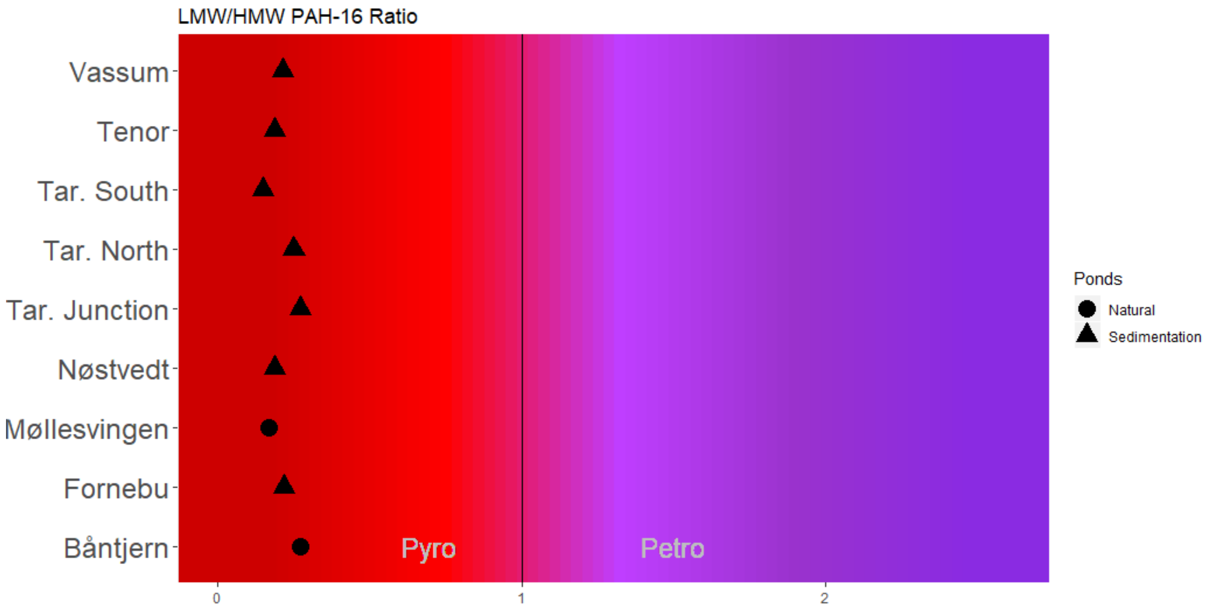


Figure 27 - LMW/HMW. Values below one indicate dominance of pyrogenic PAH (in red). Values above one indicate petrogenic dominance (in purple).

Pyrogenic Index (PI) and Fossil Fuel Pollution Index (FFPI)

Unlike the previous ratios, PI and FFPI use alkylated PAHs in their ratios. Both results indicate that PAHs in all sedimentation ponds are mainly petrogenic, and reference ponds are mainly pyrogenic.

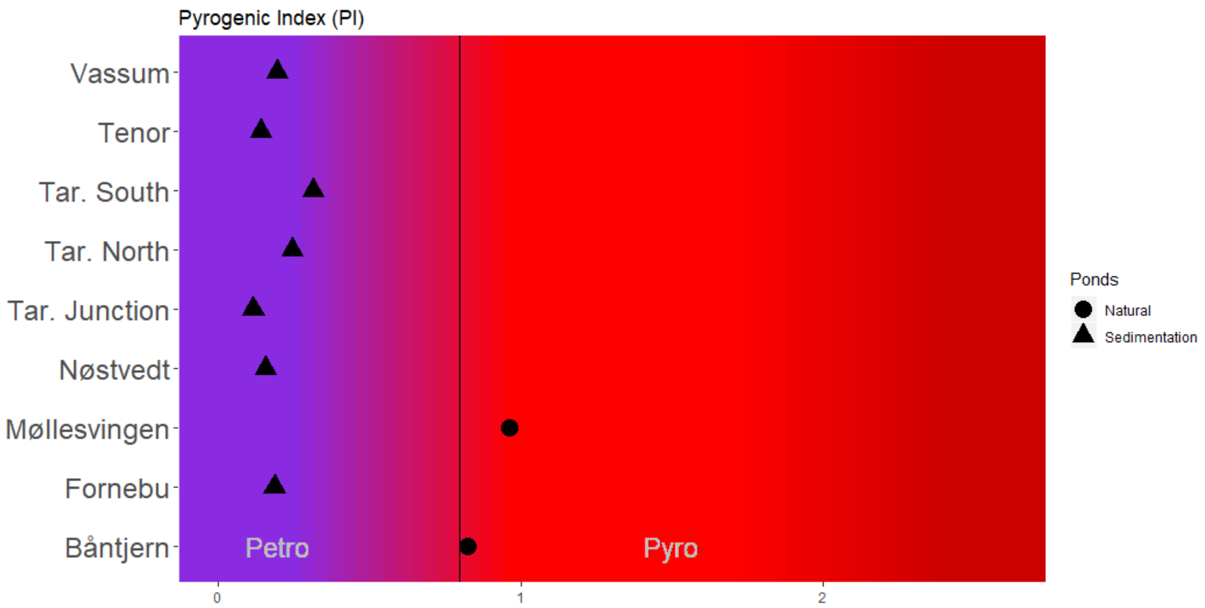


Figure 28 - Pyrogenic Index. Values above 0.8 are considered of pyrogenic origin (in red), and below of petrogenic origin (in purple).

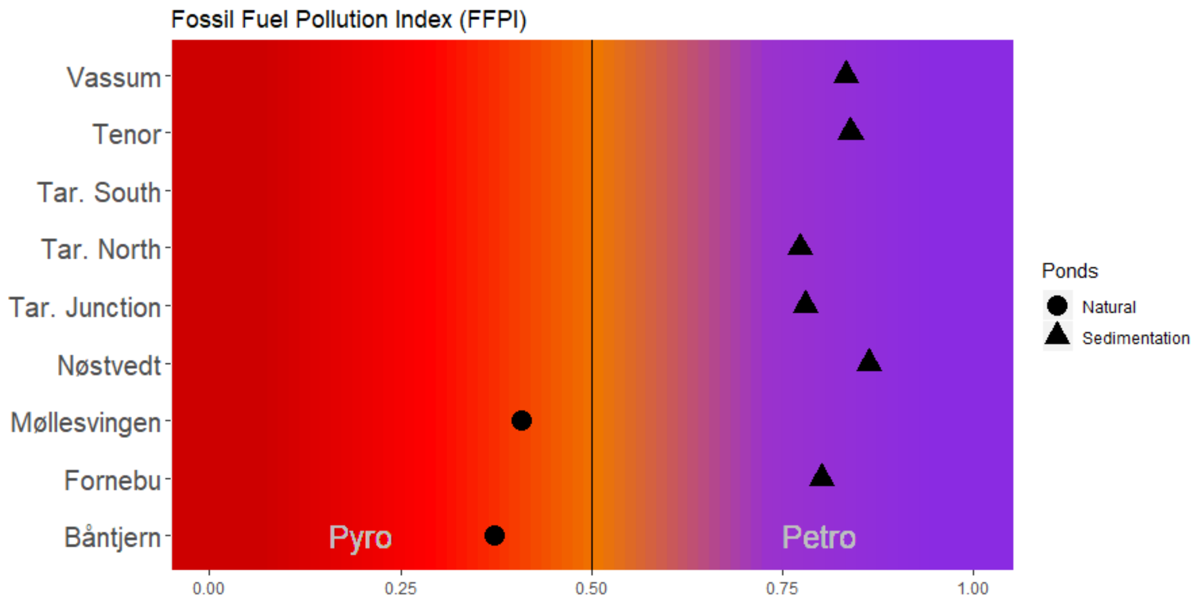


Figure 29 - Fossil Fuel Pollution Index. Ratio above 0.5 are considered from petrogenic origin (in purple). Ratio above 0.5 are considered from pyrogenic origin (in red).

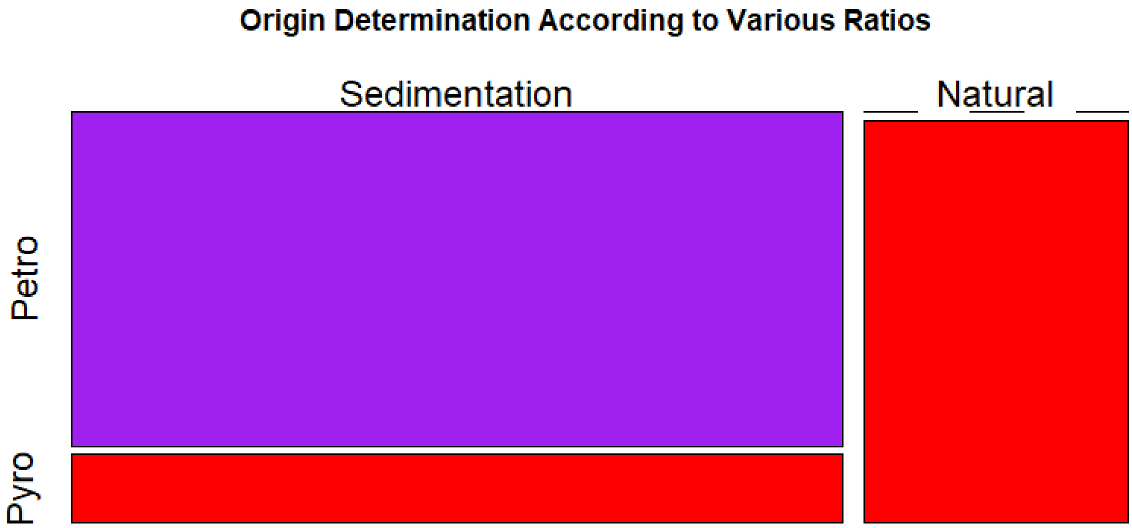


Figure 30 – Mosaic plot of results of all ratios. Purple tiles represent results suggesting petrogenic origin, and red tiles represent pyrogenic origin. Width of the tiles are equivalent to the number of data points (ratios x ponds). Sedimentation ponds: n=35 (5 ratios of 7 ponds). Natural Ponds = n=12 (5 ratios of 2 ponds + 2 ratios of 1 pond (Svarta). Dashed line – no petrogenic results in natural ponds.

3.2.3 BFRs

Levels of the PBDEs and HBCD were classified (Table 9) according to the environmental quality standard set by the Norwegian Committee of Directorates for WFD as described in section 3.2.1. BFRs in all ponds were categorized as class II (no toxic effects). Class I does not apply for BFRs. There are no specific classification values for individual PBDEs, and therefore the sum of the congeners detected was used for the classification.

Table 9 – EQS according to the Norwegian Committee of Directorates for WFD. All values in µg/kg (dry weight.). Classification and maximum concentrations for each class are provided.

Class I – Background levels PBDEs : 0 HBCD: 0	Class II – Good No toxic effects PBDEs : 310 HBCD: 172 (limit for freshwater)	Class III- Moderate Possible chronic effects PBDEs : 790 HBCD: 229 (limit for freshwater)	Class IV – Bad Possible Acute effects PBDEs : 1580 HBCD: 2382	Class V – Very bad Considerable toxic effects PBDEs: >1580 HBCD: >2382

	BDE-28	BDE-47	BDE-100	BDE-99	BDE-154	BDE-153	ΣPBDEs	HBCD
LOQ	**0.20**	**0.05-0.1**	**0.05-0.1**	**0.10**	**0.03-0.10**	**0.03-0.10**	**-**	**0.15**
Fornebu	LOQ	0.62	0.16	0.50	0.13	0.16	1.57	0.77
Taraldrud N	LOQ	0.73	0.28	1.82	0.30	0.68	3.81	2.45
Taraldrud K	LOQ	2.4	0.77	5.0	0.84	1.2	10.20	2.45
Taraldrud S	LOQ	0.56	0.17	0.60	0.13	0.18	1.64	0.84
Nøstvedt	LOQ	0.26	0.06	0.32	0.04	0.06	0.74	0.52
Vassum	LOQ	0.77	0.16	1.02	0.14	LOQ	2.09	5.29
Tenor	LOQ	0.10	LOQ	0.21	LOQ	0.03	0.34	0.52
Båntjern	LOQ	0.05	LOQ	LOQ	LOQ	LOQ	0.05	0.97
Møllesvingen	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	0.00	1.42
Svarta	NA	NA	NA	NA	NA	NA	NA	NA

Concentrations of ΣPBDEs and HBCD were compared between sedimentation and natural ponds (*Figure 31* and *Figure 32*). ΣPBDE includes the six priority PAHs according to the Norwegian Environmental Agency (Norwegian Environmental Agency, 2016): BDE-28, -47,-99,-100,-153, and -154. Values below LOQ were set as 0. BFR results were not available for Svarta, and thus this pond is not included.

Taraldrud Junction had the highest levels of ΣPBDE (10 µg/kg), followed by Taraldrud North (4 µg/kg). No PBDEs were detected above LOQ in Møllesvingen, and only BDE-47 was detected in Båntjern (0.05 µg/kg). Vassum had the highest level of HBCD (5 µg/kg), with Nøstvedt and Tenor having the lowest at 0.5 µg/kg. The data were log transformed (log (x+1)), and statistical tests were performed. The results revealed a significant difference in concentration of ΣPBDEs (p-value = 0.006) with higher levels detected in the sedimentation ponds, but no significant difference in HBCD between sedimentation and natural ponds (p-value = 0.4).

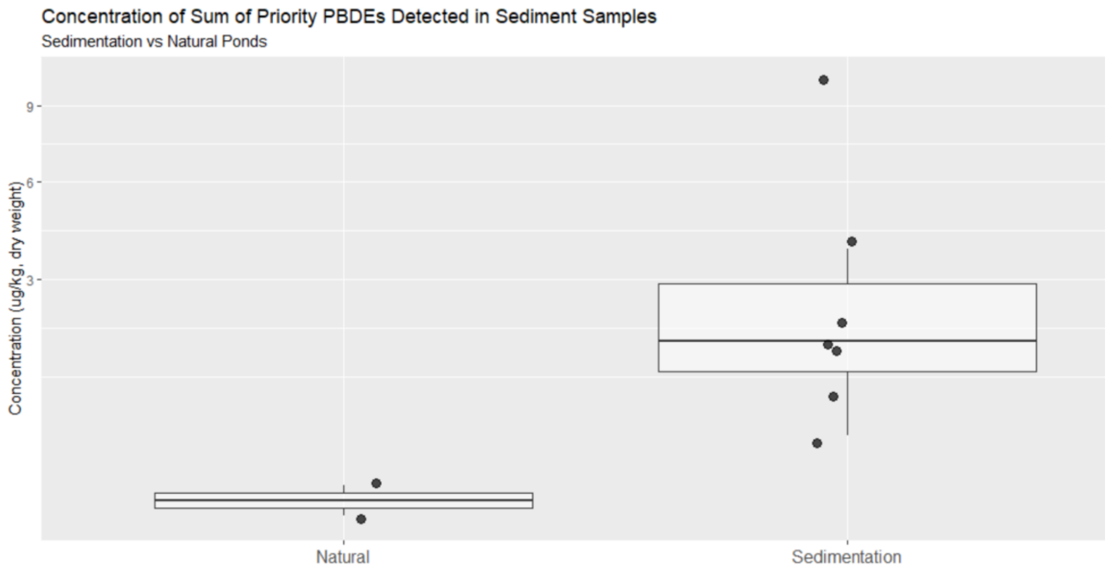


Figure 31 – Boxplot concentration of ΣPBDEs detected in sedimentation and natural ponds. Box represents the second and third quartiles. Horizontal lines represent the median. Whiskers represent the lower (first) and upper (fourth) quartiles. Points outside the whiskers and box are outliers. Natural: n=2, Sedimentation; n=7.

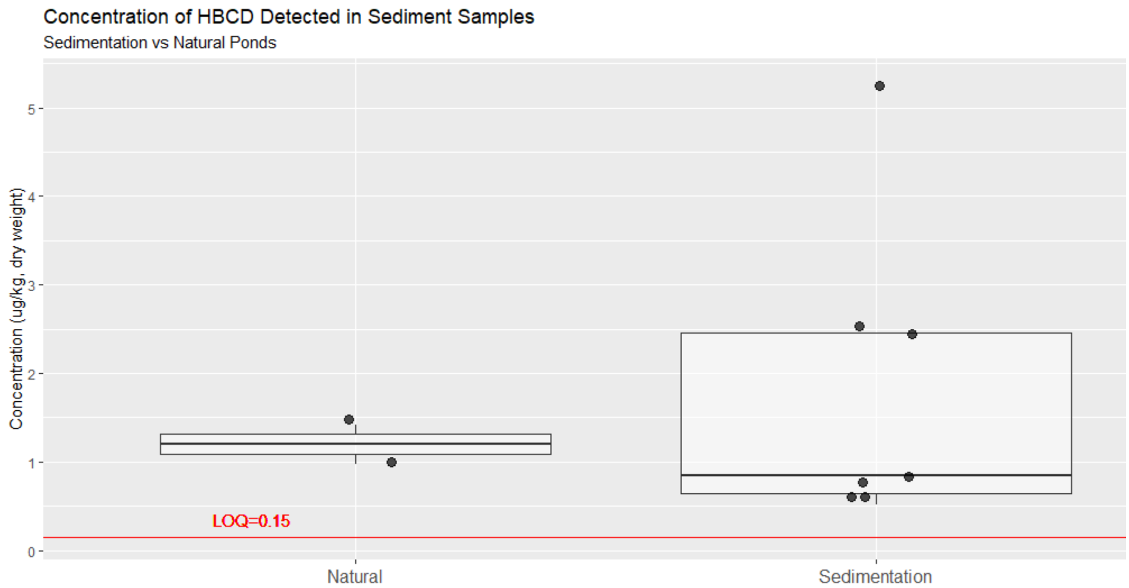


Figure 32 – Concentration of HBCD in sediment samples. Red line determines LOD (0.15). Box represents the second and third quartiles. Horizontal lines represent the median. Whiskers represent the lower (first) and upper (fourth) quartiles. Points outside the whiskers and box are outliers. Natural: n=2, Sedimentation; n=7.

3.3 Dragonfly Larvae

3.3.1 PAH metabolites

Dragonfly larvae hemolymph was analyzed. Some ponds had two samples for quality assurance purposes (Fornebu, Taraldrud North, Nøstvedt, and Møllesvingen). The values are presented in the appendix E2. 1-OH-PYR was detected in only five out of the ten ponds. Båntjern was the only natural pond where the metabolite was detected (Figure 33). Vassum had the highest detected concentration (1.8 ng/ g), and Nøstvedt had the lowest (0.65 ng/g). The statistical test showed no significant difference between ponds (Wilcoxon rank sum test, p=0.4). The statistical results are, however, based on very few detected samples. In addition, Båntjern is an influential outlier. Results therefore must be interpreted with caution.

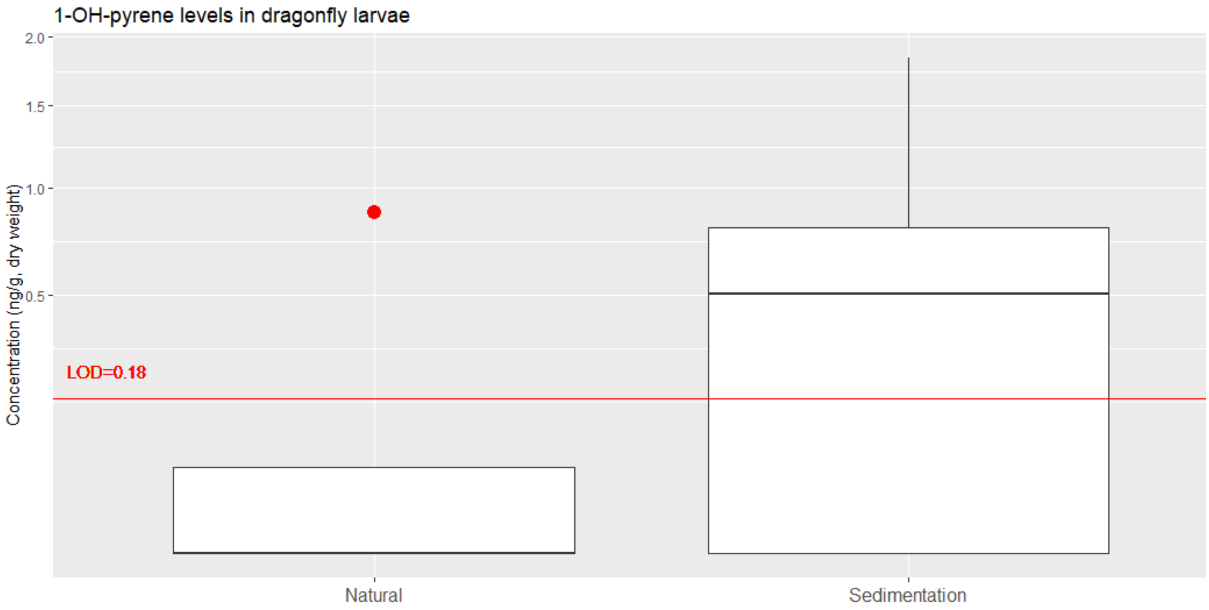


Figure 33 – Box plot of the levels of 1-OH-pyrene detected in dragonfly larvae. Box represents the 2nd and 3rd quartiles. Black horizontal lines represent the median. Whiskers represent the lower (1st) and upper (4th) quartiles. Red point represents Båntjern (outlier). Natural: n=4, Sedimentation; n=10. Red line defines the limit of detection. Values below LOD were set to 0.

3.3.2 PAHs

Concentrations of PAHs in tissues, exuvia and whole dragonfly larvae were determined in order to investigate whether bioaccumulation is a concern. The sum of PAHs were constituted of the PAHs ACE, ACY, PHEN, FLO, and PYR. These compounds were detected in at least 80% of all samples, and for that reason they were the only ones used in the following analyses. Small larvae were not found in Møllesvingen.

The highest concentrations of total PAHs in both *Big* and *Small* larvae groups were detected in Tenor (379 and 248 ng/g respectively). The lowest were detected in Svarta (111 and 28 ng/g respectively). All results are given in appendix E5.

A comparison between the concentrations of PAHs in small and big dragonfly larvae from sedimentation and natural ponds did not reveal a significant difference between groups (Welch Two Sample t-test, p-value = 0.2, *Figure 34*). Big larvae from Båntjern had relatively high concentrations of phenanthrene, fluoranthene and pyrene in their tissues compared to the equivalent in the other natural pond samples (*Table 10*).

Table 10 - Concentrations of selected PAHs in dragonfly tissue from natural ponds. Concentrations are given in ng/g.

	PHEN	FLUORA	PYR
Båntjern	57	35	49
Møllesvingen	20	5.3	18
Svarta	26	6.3	12

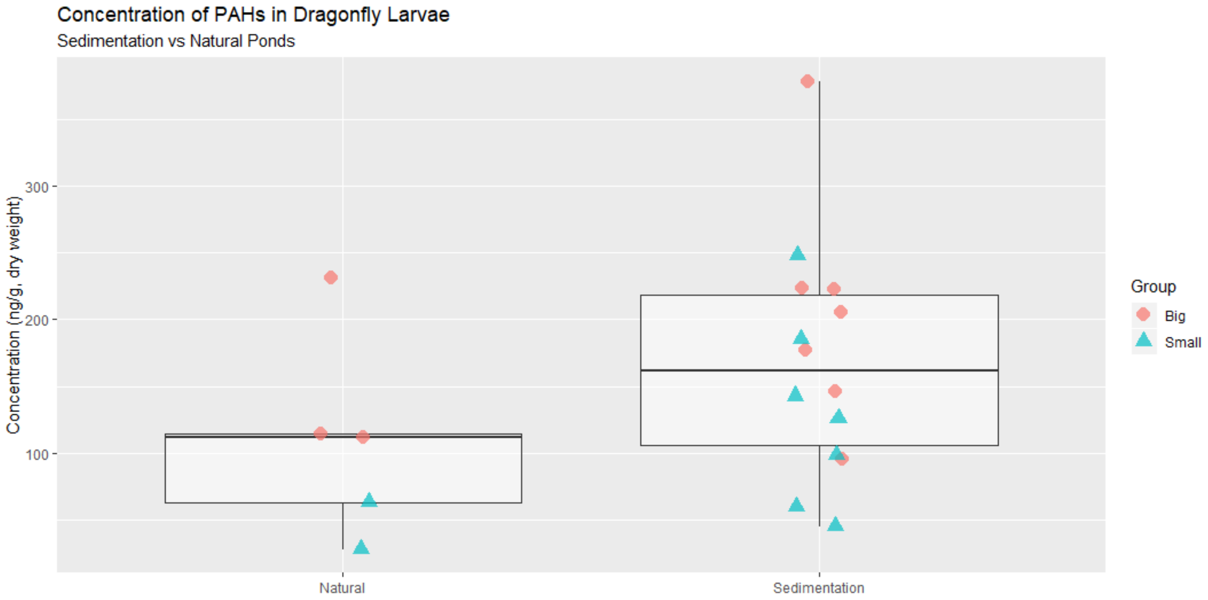


Figure 34 – Boxplot of the log of the concentration of PAHs in sediment and natural ponds. Box represents the 2nd and 3rd quartiles. Black horizontal lines represent the median. Whiskers represent the lower (1st) and upper (4th) quartiles. Points outside the whiskers and box are outliers. Natural: n=5, Sedimentation; n=14

A comparison between PAH concentrations in small and big larvae, independent of location, showed a significant difference in the total concentration of PAHs between groups (Welch Two Sample t-test, p=0.02), and a higher concentration of PAHs in the *Big* group (Figure 35). There was also a significant difference between exuvia and tissues sampled from the same larvae (Welch Two Sample t-test, p<0.001, *Figure 36*).

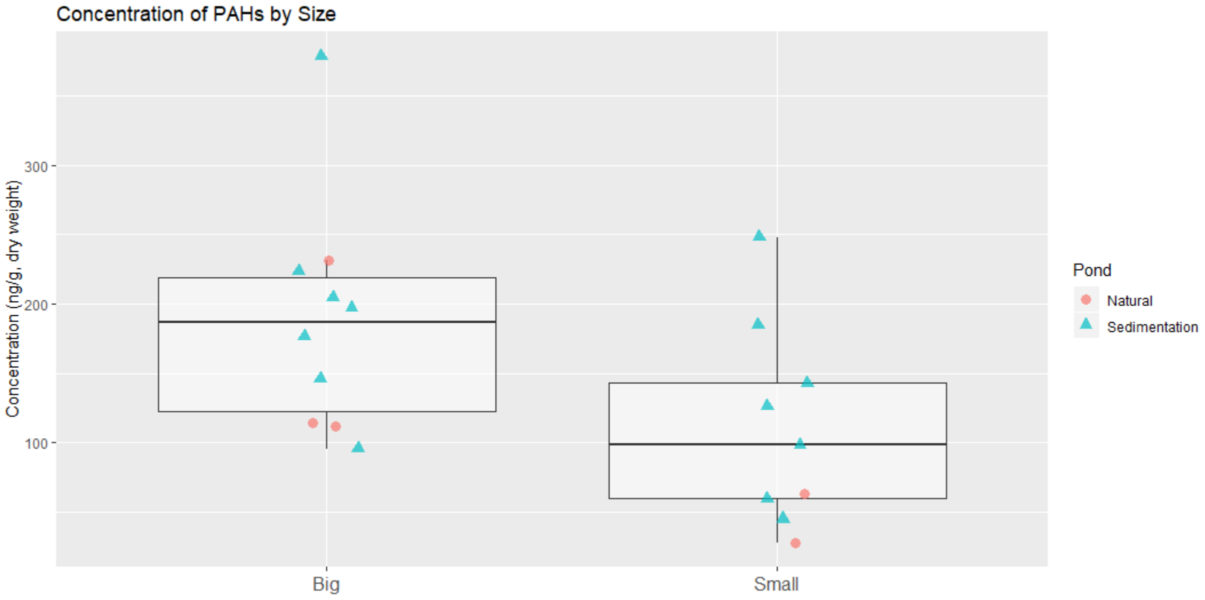


Figure 35 – Boxplot of the log of concentration of PAHs in big and small larvae. Big is the sum of the concentration detected in exuvia and tissues of big larvae. Box represents the second and third quartiles. Horizontal lines represent the median. Whiskers represent the lower (first) and upper (fourth) quartiles. Points outside the whiskers and box are outliers. Big, n= 10. Small, n = 9

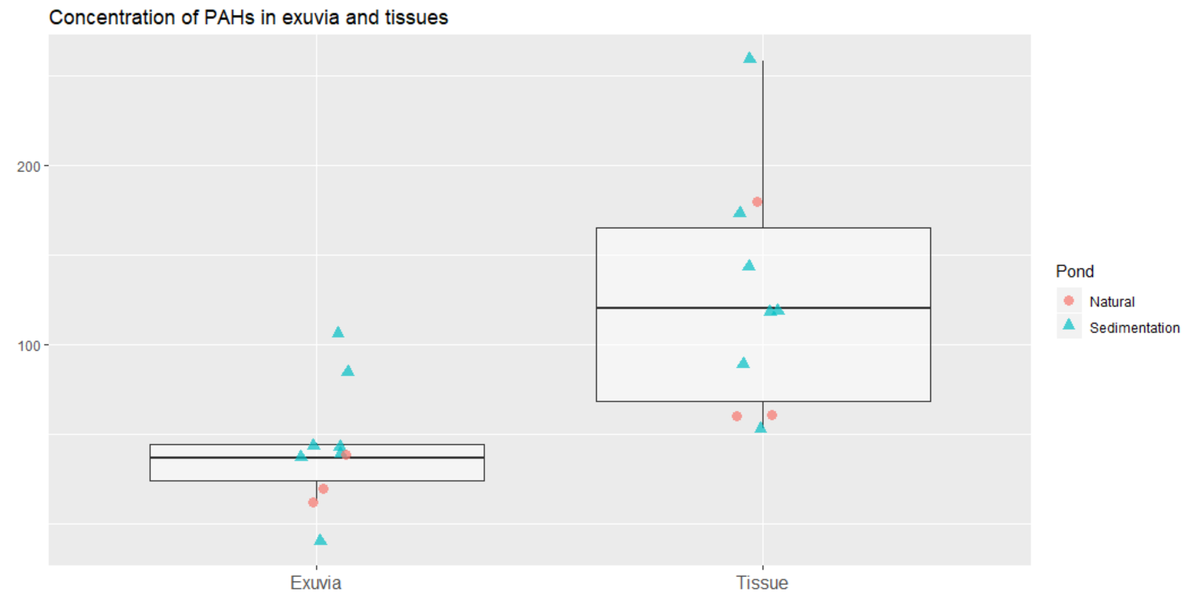


Figure 36 - Boxplot of concentration of PAHs in exuvia and tissues of big larvae. Box represents the 2nd and 3rd quartiles. Black horizontal lines represent the median. Whiskers represent the lower (1st) and upper (4th) quartiles. Points outside the whiskers and box are outliers. Exuvia: n=10, tissue; n=10

A principal component analysis of the concentration of 1-OH-pyrene, PAHs in sediment and dragonfly larvae, and length and weight of the larvae was performed. Only data from group *Big* was used. PAHs pyrene, phenanthrene, and fluoranthene were used for the analysis. Acenaphthylene and acenaphthene were not used due to the low levels quantified in the sediment samples. Approximately 67% of the total variation was captured by the first two axes.

The PCA did not express a clear distinction between the types of ponds. This is due to the relatively large values of 1-OH-pyrene and PAH in larvae in the samples from Båntjern, positioning this natural pond on the right side of the x-axis. These values were especially low in Svarta and Møllesvingen, and they lay on the left side of the x-axis. Taraldrud junction shared similar results with Møllesvingen. Vassum lies on the far bottom right corner due to its particularly low values for weight, combined with high vales for 1-OH-pyrene, and PAH in larvae and sediment. Taraldrud North, on the other hand, stands alone on the far top right due to the particularly large values for size and weight of the larvae combined with high concentration of PAHs in the sediment. The other ponds share similarities, and thus all variables have values close to center of the PCA.

A positive correlation between PAH concentration in larvae (parental PAHs and 1-OH-pyrene) and sediment, and larval length is observed. On the other hand, the PCA captured a negative correlation between larval weight and concentration of PAHs in larvae.

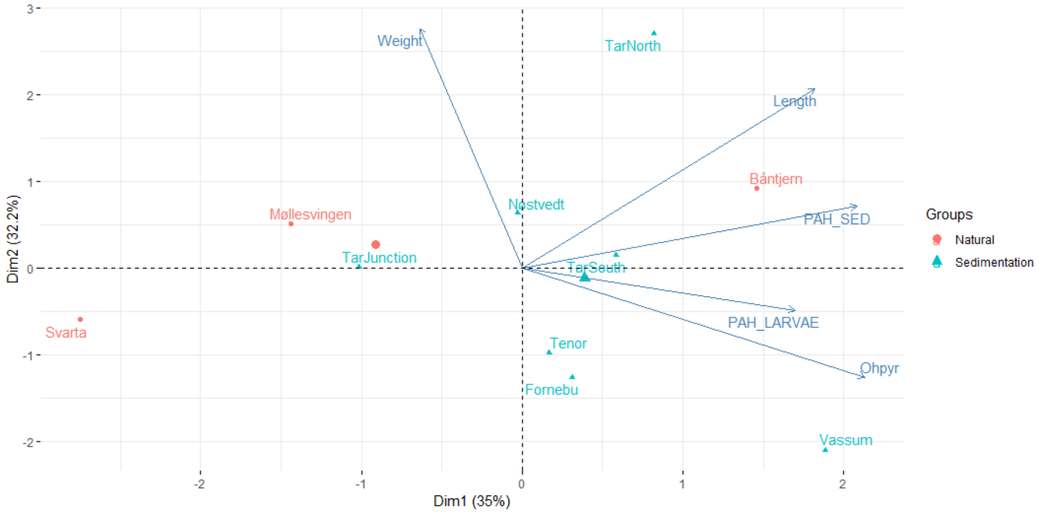


Figure 37 - PCA biplot. Dim1 represents PCA 1(x-axis), and Dim2 represents PCA 2 (y-axis). Components with largest variation are represented by longer arrows. The angles between arrows indicate the level of correlation between variables. The narrower the angle, the more correlated are the variables. Largest triangle and circle are the centroids of the sedimentation and natural clusters, respectively.

3.3.3 BFRs

BFRs were detected at very low concentrations in the larvae, with most sample results below the level of detection. Qualitative analyses in the dragonfly samples were performed using the results of detected PBDEs, and HBCD. (*Figure 38*) The specific concentrations are listed in appendix E3. BFRs were detected in more samples in the group *Small* than group *Big*, but a 2-sample binomial test for equality of proportions revealed no significant difference in the probability of detecting BFRs in the two groups (Pearson's chi-squared test statistic, p-value =1). BFRs were detected in more tissue samples than exuvia, but with no significant difference (p-value = 0.58).

The same binomial test revealed a significant difference in the probability of detecting BFRs in larvae from sedimentation ponds and natural ponds, with an expected probability of 45% of detecting BFRs in larvae living in sedimentation ponds, and a 25% probability of detecting in larvae living in natural ponds (p-value = 0.02).

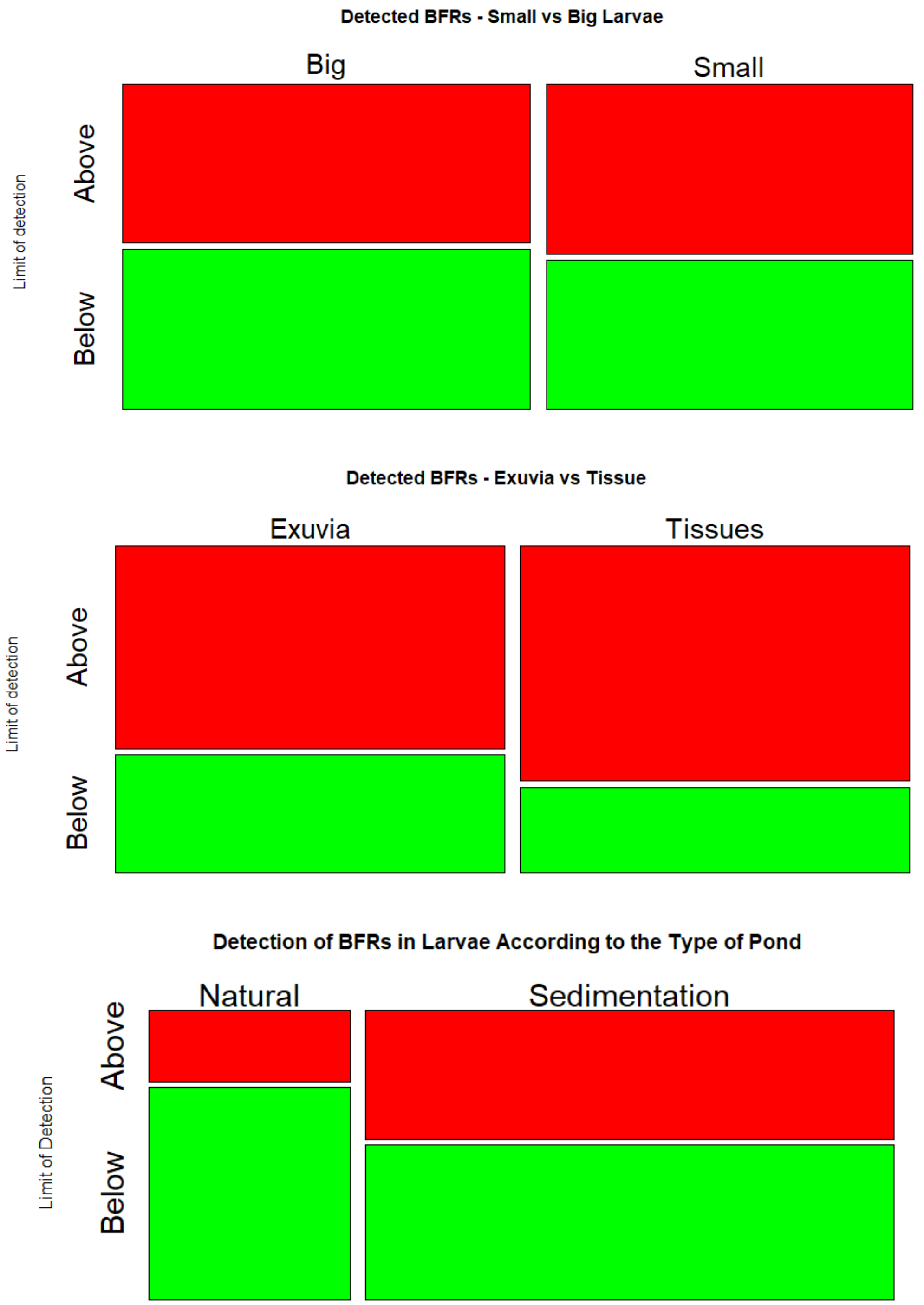


Figure 38 - Mosaic plot. Green tiles represent values below LOD. Red tiles represent values above LOD. Width of the tiles represent the number of data points in each group. “Big” contains values from ten ponds. “Small” contain values of 9 ponds. Small larvae were not found in Møllesvingen. “Natural” contains values from three natural ponds.”Sedimentation” contains values from seven sedimentation ponds.

4 Discussion

4.1 Water

Principal component analysis showed that the water characteristics were more similar within sedimentation ponds. Fornebu was the only exception, being more similar to the natural ponds.

Båntjern had the highest concentrations of nitrogen and phosphorous of all ponds. These results might be associated with the use of fertilizers in a vegetable garden located near the pond. Conductivity was much higher in sedimentation ponds (above 1200 µS/cm) compared to natural ponds (maximum 200 µS/cm). Conductivity is a measure of the water's power to pass electrical current, and increases with an increase of dissolved substances in water (Skarbøvik et al., 2016). The measure can be used as an indicator of water quality (EPA, 2018). Conductivity in highway runoff increases substantially when road salts are present (Hallberg et al., 2007), and can be used as a proxy for road salt contamination (Meland et al., 2010b). High levels of salts have also been shown to increase the bioavailability of trace metals (Heier et al., 2013). Furthermore, some studies showed a positive relationship between conductivity and dissolved organic carbons (Monteiro et al., 2014; Sobek et al., 2007). Therefore, high conductivity in sedimentation ponds is a good indicator of high levels of contaminants in water.

4.2 Sediment

4.2.1 PAHs

The results showed a significant difference in concentrations of PAH-16 in sediment, with higher levels being detected in the sedimentation ponds. The results support part of the hypothesis 1, that contaminant levels in sediment are higher in sedimentation ponds than in natural ponds. Consequently, the biota living in these ponds are exposed to higher levels of traffic-related contaminants than biota living in the ponds that are not directly affected by highway runoff. Previous studies have shown that traffic-related contaminants can lead to negative effects in aquatic organisms such as fish (Grung et al., 2016; Meland et al., 2010b), and amphibians (Brand et al., 2010). Therefore, these sedimentation ponds might not be suitable habitats.

Tenor and Nøstvedt samples contained the lowest concentrations of PAHs amongst all of the sedimentation ponds. The results from Tenor were exceptionally low, even lower than in one of the natural ponds (Båntjern). Tenor receives runoff from an area with a relatively low AADT, which perhaps explains the low levels of traffic-related contaminants. Regression analysis revealed a relationship between the PAH concentrations, and AADT. The results, however, were not reliable because Tenor was a highly influential observation within very few data points (appendix F). Another possible reason for such relatively low levels of PAHs might be that these ponds are not functioning properly, and hence, they may not be receiving the runoff or retaining contaminants as predicted. Unpublished documents obtained from the NPRA database suggested including both Tenor and Nøstvedt in the National Transport Plan's priority list for maintenance of sedimentation ponds in 2015 and 2017, respectively (Norwegian Public Roads Administration, 2014, 2016b). According to the documents, Nøstvedt's forebay has a leakage. No recent maintenance seemed to have been done by the time field work for this study took place, and the volume of water seemed quite low in Nøstvedt's forebay. It is therefore likely that Nøstvedt and Tenor are in need of maintenance. Further examination of these ponds should be considered to investigate their functionality.

Many of the sediment samples were classified to be of moderate or poor quality, according to the EQS set by the Norwegian Environmental Agency (2016). Exposure to moderate levels of PAHs (class III) might cause negative effects in organisms over time. Exposure to concentrations determined as poor (class IV) might cause negative effects in organisms even during a short exposure time (Norwegian Environmental Agency, 2016).

Moreover, priority PAHs include parent PAHs only. Andersson and Achten (2015) argue that the advantages of using a limited list as a proxy for these compounds are the decrease in analytical complexity, reduced costs, and better comparability between analyses performed worldwide. In this study, however, PAHs were detected at much higher concentrations than their non-alkylated forms. Several studies expressed the effects of alkylated PAHs in aquatic organisms, such as growth inhibition, malformation , reduction of survival rates (Vignet et al., 2014a) and behavioral disruption (Vignet et al., 2014b). A toxicity test performed by Turcotte et al. (2011) revealed that alkylated forms of phenanthrene were more toxic than its parental form, and that toxicity increased with increase of alkyl substituents.

The addition of alkylated forms to the total sum of PAHs detected caused significant changes in EQS classification. Several PAHs changed their classification from class II to class IV. As discussed previously, PAHs from petrogenic origin often contain high levels of alkylated PAHs in relation to their parental forms. Therefore, analysis of only the parent forms might potentially ignore much higher concentrations of the total PAHs in many situations, thus distorting the true environmental status, and the potential threat from these contaminants.

4.2.2 PAH source apportionment

Various fingerprint methods were used to estimate the source of origin of PAHs in sediment samples. Distribution of the proportion of PAHs showed similar patterns in sedimentation ponds, differing from the natural ponds. Three out of the five ratios indicate that PAHs in all sedimentation ponds are mainly from petrogenic origin. All ratios indicate that natural ponds dominated by pyrogenic PAHs. The results are in line with hypothesis 2 - PAHs from sedimentation ponds might come from similar sources, and PAHs detected in natural ponds might come from other sources.

PHEN/ANTH ratios suggest a pyrogenic dominance in five sedimentation ponds. Interpretation of PHEN/ANTH ratios, however, vary in literature, going as low as >5 for petrogenic sources (Neff et al., 2004). Only Taraldrud South and Møllesvingen would have remained classified as pyrogenic if such a ratio had been adopted. The ratios for PHEN/ANTH and FLUORA/PYR were similar to those identified as road dust by Kose et al. (2007). In the same study, road dust was determined to be a mixture of petrogenic and pyrogenic PAHs, but dominated mainly by tire and asphalt wear-off.

LMW/HMW ratios were quite low, an indication that all ponds were dominated by pyrogenic PAHs. Low levels of LMW PAHs in sediment do not mean necessarily that the levels are low in the whole ecosystem. LMW PAHs have a lower Kow, and are possibly more mobile. Moreover, petrogenic PAH complexes are dominated especially by PAHs containing alkyl substituents (Neff et al., 2004). The results would have changed significantly if alkylated species were included in the ratio.

The importance of accounting for PAH alkylates was once again demonstrated when determining PAH apportionment. Several indices can be used to characterize the nature of

PAHs (Stogiannidis & Laane, 2015), but many of them need alkylated data, including PI and FFPI included in this study (Boehm & Farrington, 1984; Z. Wang et al., 1999).

PAH mixtures change over time and under certain conditions. For example, in a study aimed on characterizing PAHs in lubricating oil, J. Wang et al. (1999) observed that new lubricating oils have fewer PAHs, but that the number of PAHs and their concentrations increased with driving distance. Wang et al. (1999) proposed the PI after a study in which it was observed that most two- and three-ring PAHs and their alkylated homologues are destroyed when diesel is burned. Thus, PAH source characterization is not a simple task. Nevertheless, different ratios of various PAHs can give clues to their source, and this information can be a powerful tool in targeting the main point sources. It is important to keep in mind, however, that since PAHs are ubiquitous compounds, PAHs detected in road runoff are not exclusively from traffic.

4.2.3 BFRs

There was a significant difference in concentrations of BFRs in the ponds, with higher levels detected in the sedimentation ponds. The results are in line with part of hypothesis 1, that contaminant levels are higher in sediment from sedimentation ponds than from natural ponds. Tenor and Nøstvedt were exceptions. BFR levels in these ponds were lower than in the natural ponds Båntjern and Møllesvingen (no results were available for Svarta). Potential reasons for the low levels of contaminants in Tenor and Nøstvedt were discussed in section 4.2.

Levels of BFRs in all sedimentation ponds were categorized as class II (good) according to the EQS set by the Norwegian Environmental Agency. Nevertheless, 71% of the total sum of BFRs were detected at Taraldrud Junction (33.5%), Vassum (20%) and Taraldrud North (17.5%). Taraldrud Junction is approximately 1 km away from many businesses, including a recycling station (appendix G). Taraldrud North is only 1 km from Taraldrud Junction. There is therefore a possibility that BFRs detected in these locations did not come from traffic, but from urban pollution. Vassum receives tunnel wash runoff from three different tunnels, and sources of BFRs could be from building materials such as foam used for insulation.

Overall, HBCD was detected at higher concentrations than individual PBDEs. This is possibly because HBCD has been included in the list of priority substances just recently, in the 2nd

semester of 2018 (Committee of Directorates for the Water Framework Directive, 2018). Hence, HBCD has been in use more recently than PBDEs. Levels of HBCD were higher in Båntjern and Møllesvingen than in Fornebu, Taraldrud South, Nøstvedt and Tenor. Both Båntjern and Møllesvingen are surrounded by houses. Leaching from home products such as textiles and electronics might explain the relatively high levels of HBCD compared to in some sedimentation ponds. In addition, these two ponds were restored between 2004 and 2005 (Strand, 2006). Leaching from material used during the restoration process might also be a source of HBCD.

4.3 Dragonfly Larvae

4.3.1 PAH Concentration in Larvae

A statistical test indicated no significant difference in the concentration of PAHs in dragonfly larvae according to pond type. Hence, the results do not support the part of hypothesis 1 that contaminant levels are higher in dragonfly larvae from sedimentation ponds than from natural ponds. Nevertheless, Båntjern and Tenor had especially high concentrations of PAH within their groups, and were detected as outliers (appendix F). With data of only 10 ponds, and two of them being outliers, the results might be unreliable.

PAHs were detected in exuvia samples, indicating that dragonfly larvae do eliminate PAHs through molting. The results partially support hypothesis 4, that dragonfly larvae accumulate organic contaminants in the exuvia. Comparison of the larvae samples independent of location shows, however, a significant difference in the concentration of PAHs in the exuvia and tissue of big larvae, with higher concentrations being detected in tissue. Hence, despite the larvae’s capacity to eliminate PAHs through molting, the level of elimination is likely not enough to avoid bioaccumulation. Therefore, the findings do not support the second part of hypothesis 4, that exuvia depuration significantly reduce bioaccumulation in tissues. Concentrations of PAHs in *Big* and *Small* were also significantly different, and higher concentrations were detected in the larger larvae. Thus, the results are not in line with hypothesis 5, that concentrations of PAHs in earlier and later instars are equal. These results, therefore, indicate bioaccumulation. A more insightful answer would perhaps have been accomplished if the biota-sediment accumulation factor (BSAF) had been calculated. BSAF is a ratio used to determine bioaccumulation of sediment-associated compounds into biota (Burkhard, 2009). To calculate BSAF, it would have been necessary to normalize the contaminant concentrations to the organic carbon content in sediment, and the lipid content in the larvae. However, quantification of the contaminants would not have been possible if fat extraction had been chosen due to the small amount of material. Thus it was not possible to calculate BSAF.

Nevertheless, principal component analysis using variables from larvae in the group *Big* detected a positive correlation between PAH concentrations in sediment and larvae. That can be interpreted as that PAHs become more bioavailable when the contaminant burden in the environment increases. PAH concentrations also had a positive correlation with the length of the larvae. Hence, higher levels of PAHs were detected in larger larvae. The result are in line with the significantly higher levels of the contaminants detected in the big larvae compared to the small larvae. A negative correlation between weight and concentrations of PAHs was, however, detected. This is likely a reflection of growth dilution, a phenomenon in which contaminant concentrations in biota decrease due to increase in biomass (Peace et al., 2016). A larva that is ready to metamorphose is practically an adult individual inside the exuvia. Such a significant increase in body mass in the later instars is therefore expected.

Virtually all 5- and 6-ring PAHs in dragonfly larvae were detected below quantification levels. The only 4-ring PAHs quantified were those with Kow < 5.5. Those are pyrene and anthracene. Thus, the less hydrophobic PAHs might be more bioavailable than the HMW species. Particles smaller than 62 µm tend to remain suspended in the water column (Bilotta & Brazier, 2008). Surface area is inversely proportional to particle size. As a result, smaller particles have a higher contaminant-carrying capacity than larger particles (Bilotta & Brazier, 2008; Zhang et al., 2015). In an experiment in which the distribution of PAHs from road runoff was quantified in particulate fractions, Nielsen et al. (2015) detected a significant amount of low- and middle weight PAHs in the colloidal and dissolved fractions in traffic runoff.

Pyrene, fluoranthene, and phenanthrene were observed to be the most toxic PAHs during a toxicity test performed on freshwater amphipods exposed to highway runoff (Boxall & Maltby, 1997). Carls et al. (2008) demonstrated the toxic effects of dissolved petrogenic PAHs by exposing fish embryos indirectly to oil droplets that were kept isolated by using an agarose barrier. Thus, the effects of PAHs in dragonfly larvae, as well as an analysis of PAHs in water and in suspended particles to investigate the bioavailability of these organic compounds, should be considered in futures studies.

4.3.2 PAH Metabolites

Levels of 1-OH-PHEN, 1-OH-PYR and 3-OH-BaP were screened in dragonfly larvae haemolymph. Only 1-OH-PYR was detected, at very low levels, and only in larvae from five ponds, including one natural pond (Båntjern). Therefore, it is not clear whether dragonfly larvae metabolize PAHs. It seems clear however that if dragonflies do metabolize PAHs, then the efficiency is limited. Hence, metabolites are not effective biomarkers for PAH exposure in dragonfly larvae. The results, therefore, appear to be in contrast with the hypothesis 3 that dragonflies metabolize PAH, and metabolites can be used as biomarkers to study PAH exposure in dragonflies.

As hemimetabolous species, dragonflies go through a major transition during metamorphosis. While their larval stage specializes in growth, their adult life is specialized for dispersal and reproduction (Stoks & Córdoba-Aguilar, 2012). In addition, the aquatic and terrestrial life stages are associated with completely different habitats, lifestyle, and appearance. With so many drastic changes happening in such a short period of time, it is not unreasonable to suggest that PAH metabolism occurs only during certain larval stages. Energy might be allocated for preparation of metamorphosis, and these changes in the energy budget might be followed by a suspension or cessation of metabolism of xenobiotics during the last larval stages. Metabolite analysis at different life stages would be needed to support this hypothesis.

Another hypothesis is that the low levels of 1-OH-pyrene detected are a result of trophic transfer. Previous studies observed the uptake of PAH metabolites through diet (Beach & Hellou, 2011; V. Carrasco Navarro et al., 2012a; V. Carrasco Navarro et al., 2012b; McElroy et al., 1991). Phase I metabolites such as 1-OH-pyrene may remain longer in the prey’s body due to invertebrates’ ineffective metabolic processes. In addition, invertebrates excrete compounds slower than vertebrates due to the lack of kidneys (Lyytikäinen et al., 2007). Thus, metabolic PAHs may remain in invertebrate prey long enough to be transferred to predators. Macroinvertebrates biotransform PAHs at different efficiency levels (V. Carrasco Navarro, 2013; Rust et al., 2004; Stroomberg et al., 2004; Van Brummelen et al., 1996). A better understanding of the diet of the larvae in these ponds could perhaps explain why 1-OH-pyrene were detected in some samples, but not in others. An investigation of the potential prey available in these ponds, and their metabolic rates would be beneficial. The potential trophic transfer is of especial concern due to the toxic nature of PAH metabolites, and hence the need for further investigation.

4.3.3 BFR Concentration in Larvae

BFRs were detected in more samples in the *Small* group than in the *Big* group. A binomial test, however, revealed no significant difference between the two groups. Hence, the probability of detecting BFRs in small and big larvae were the same. There was no significant difference between the levels of BFRs detected in exuvia and tissues either. The results indicate that dragonflies eliminate BFRs, and are therefore in line with hypothesis 5. The levels of detection of the contaminants were so low in the exuvia that it is unlikely that elimination through molting is significant. Díaz-Jaramillo et al. (2016) detected OH-PBDEs in the feces and organs of polychaetes and crabs. Thus it is possible that dragonflies can metabolize certain BFRs. In the same study, significant amounts of BDE-47 were detected in the feces of polychaetes. BFRs are highly hydrophobic, and they should not be easily bioavailable. It is therefore possible that little bioavailability, in combination with growth dilution, are key factors for why bioaccumulation in dragonfly larvae was not observed.

4.4 Sedimentation Ponds as a Nature-Based Solution

Biodiversity was clearly observed during field work in all ponds containing well-established vegetation. A large variety of macroinvertebrates and amphibians, including damselflies, water beetles, and tadpoles were spotted. Newts, including the species *Triturus cristatus,* listed in the Norwegian list for threatened species (Artsdatabanken, 2015) were also spotted in several ponds, including ponds not used in this study.

Dragonfly larvae were easily found, several exuvia were found on the surrounding vegetation, and many adult individuals were spotted flying above the sedimentation ponds, indicating a successful completion of life cycle. Dragonfly larvae are faced with reduced predation-induced stress in most sedimentation ponds since they are often free of fish. For instance, Knight, McCoy, Chase, McCoy, & Holt (2005) observed a lower abundance of larvae and adult individuals in and around natural ponds with fish compared to natural ponds without fish populations. Hence, it can be the case that a reduction of predation stress may be beneficial enough such that even with an increase in chemical stressors, sedimentation ponds are still a source habitat for tolerant dragonfly species.

Sedimentation ponds are, however, home to many other organisms. In several studies, the negative effects of exposure to highway runoff were observed in biota (Grung et al., 2016; Johansen, 2013; Meland et al., 2010b). Nevertheless, a reduction in predation and competition against less tolerant species may also mean that sedimentation ponds are, overall, good habitats despite being polluted. One way to reduce the exposure of organisms to high levels of contaminants, whilst maintaining their ecosystem services, could be closing off just the forebay. This is the area of the sedimentation ponds where the largest fraction of particle-bound contaminants settle. The new cleaning strategy for future sedimentation ponds includes the removal of contaminants adhered to the smallest particle fractions by infiltration. This strategy is a good step towards protecting receiving natural bodies from water-soluble and less hydrophobic contaminants, but it does not protect the organisms living in these ponds, nor solve the issue in ponds that have already been built.

Sedimentation ponds may also become ecological traps – a habitat that is selected by organisms despite not being able to sustain population growth (Battin, 2004). In 2012, over 400 tadpoles were found dead in Vassum after a discharge of tunnel wash (Johansen, 2013). The event led to a change in regulation, and now new treatment systems for tunnel wash runoff are built as closed solutions (Norwegian Public Roads Administration, 2016a). Newly built open sedimentation ponds may also become ecological traps. For instance, a dragonfly larva was spotted in a new sedimentation pond during fieldwork in Østfold, Norway. Vegetation around the pond has not been established yet, and thus it is unlikely that the larva will be able to conclude its final molt. The ecological role of sedimentation ponds must be considered during the design and construction process to enhance their capacity as suitable habitats. For that, it is important to better understand the factors driving certain species to migrate to these artificial water bodies. In addition, monitoring and proper maintenance of ponds are essential to protect their biodiversity.

5 Conclusion

Overall, sediment from sedimentation ponds had higher levels of contaminants than from natural ponds. Adding the alkylated forms to the total concentration of PAHs also led to significant differences in EQS. There is no reason to believe that alkylated PAHs are not toxic, and their levels must be considered in future studies and monitoring.

Source apportionment suggested that petrogenic PAHs dominate in sedimentation ponds, and PAHs are mainly pyrogenic in natural ponds. A better understanding of the origin of PAHs in road runoff can help reduce the problem by tackling its source.

Statistical tests did not indicate a significant difference in concentrations of PAHs in larvae from sedimentation and natural ponds. The results, however were strongly driven by the relatively high concentrations of PAHs in larvae from Båntjern. That does not mean, necessarily, that this natural pond is more polluted than the sedimentation ponds, but it is likely that some PAHs are more bioavailable. Further studies should investigate more ponds, giving especial attention to the water chemistry, and suspended particles.

1-OH-pyrene was detected at very low concentrations, and only in some samples. Therefore, it is not clear whether dragonfly metabolize PAHs at very low levels or that the metabolite detected is a product of trophic transfer. In either case, the results indicate that the use of metabolites as a biomarker for PAH exposure is not an efficient method in dragonfly larvae. Possible trophic transfer of metabolites represent the transfer of genotoxic substances. It is, therefore, crucial to understand the fate of PAH metabolites in invertebrates.

PAH results show a significant difference between concentrations in the exuvia, tissue and larvae analyzed as a whole. The findings indicate that accumulation in the exuvia is not enough to avoid bioaccumulation of PAHs. Thus, dragonfly larvae are potential vectors of these contaminants to the terrestrial environment.

In addition to their contribution to biodiversity in highly urbanized areas, sedimentation ponds are oases for research into the effects and fate of contaminants in the environment. It is crucial, however, that they are designed, maintained and managed correctly in order to remain as an environmental solution instead of an environmental burden.

6 Further Perspectives

PAHs were detected in relatively low concentrations in two sedimentation ponds that have been stated as in need of maintenance. It is crucial that all sedimentation ponds, are designed, monitored, and maintained accordingly in order to protect their biodiversity.

This study showed that determining the concentration of alkylated PAHs gives a more realistic picture of the status of sedimentation ponds receiving highway runoff. In addition, these results help identify the possible sources of contamination. It is therefore crucial to include alkylated PAHs in future studies. The concentration of PAHs in suspended particles, and dissolved in water, should not be ignored.

One interesting result was that the levels of BFRs were higher in ponds that were likely to receive urban runoff. A further comparison of the profiles of persistent pollutants in urban and highway runoff recipients would help in determining the impact caused by different anthropogenic activities.

Further investigation of the origin of PAH metabolites in dragonfly larvae would help clarifying the results of this study. An experimental study looking at the trophic transfer of PAH metabolites in dragonfly larvae in different instars would be complementary to this thesis. It would also be beneficial to look at the effects of contaminants related to highway runoff in dragonfly larvae, and the levels of contamination in adult specimens.

7 References

Aas, E., Baussant, T., Balk, L., Liewenborg, B., & Andersen, O. K. (2000). PAH Metabolites in Bile, Cytochrome P4501A and DNA Adducts as Environmental Risk Parameters for Chronic Oil Exposure: A Laboratory Experiment with Atlantic Cod. *Aquatic Toxicology, 51*(2), 241-258. doi:https://doi.org/10.1016/S0166-445X(00)00108-9

Agency for Toxic Substances and Disease Registry. (1995). Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). from U.S. Department of Health and Human Services, Public Health Service. https://www.atsdr.cdc.gov/toxprofiles/tp69.pdf

Agency for Toxic Substances and Disease Registry. (2017). Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs). from U.S. Department of Health and Human Services,, Public Health Service https://www.atsdr.cdc.gov/toxprofiles/tp207-c4.pdf

Andersson, J., Mácsik, J., van der Nat, D., Norström, A., Albinsson, M., Åkerman, S., . . . Jönsson, R. (2018). *Reducing Highway Runoff Pollution (REHIRUP). Sustainable Design and Maintenance of Stormwater Treatment Facilities*. Retrieved from Sweden: https://trafikverket.ineko.se/se/reducing-highway-runoff-pollution-sustainable-design-and-maintenance-of-stormwater-treatment-facilities

Andersson, J. T., & Achten, C. (2015). Time to Say Goodbye to the 16 EPA PAHs? Toward an Up-to-Date Use of PACs for Environmental Purposes. *Polycyclic Aromatic Compounds, 35*(2-4), 330-354. doi:10.1080/10406638.2014.991042

Antonova, O., Toncheva, D., & Grigorov, E. (2015). Bladder Cancer Risk from the Perspective of Genetic Polymorphisms in the Carcinogen Metabolizing Enzymes. *J buon, 20*(6), 1397-1406.

Artsdatabanken. (2015). Rødliste for Arter (In Norwegian). https://www.artsdatabanken.no/Rodliste

Askew, R. (2004). *The Dragonflies of Europe* (2 ed.): Apollo Books.

Åstebøl, S. O., & Hvitved-Jacobsen, T. (2014). Håndbok 295 - Vannbeskyttelse i Vegplanlegging og Vegbygging (*In Norwegian*). In K. W. Kronvall & S. Meland (Eds.), (pp. 90). Norway: Statens vegvesen.

Auckland Regional Council. (2003). TP10 - Design Guideline Manual Stormwater Treatment Devices; Chapter 5 - Pond Design, Construction, and Maintenance: Auckland Regional Council.

Bækken, T., Avolio, C. M., Veidel, A., & Willberg, M. (2005). *Forurensningsreduserende Effekt av Nygård Rense- og Fordrøyningsbasseng for Vegavrenning, samt Vegavrenning til Kjeksrudbekkene og Brekkebekken (In Norwegian)*. Retrieved from Oslo, Norway:

Bakke, T., Källqvist, T., Ruus, A., Breedveld, G. D., & Hylland, K. (2009). Development of Sediment Quality Criteria in Norway. *Journal of Soils and Sediments, 10*(2), 172-178. doi:10.1007/s11368-009-0173-y

Battin, J. (2004). When Good Animals Love Bad Habitats: Ecological Traps and the Conservation of Animal Populations. *Conservation Biology, 18*(6), 1482-1491. doi:doi:10.1111/j.1523-1739.2004.00417.x

Beach, D. G., & Hellou, J. (2011). Bioaccumulation and Biotransformation of 1-Hydroxypyrene by the Marine Whelk *Neptunea lyrata*. *International Journal of*

Environmental Analytical Chemistry, 91(13), 1227-1243. doi:10.1080/03067310903524830

Bilotta, G. S., & Brazier, R. E. (2008). Understanding the Influence of Suspended Solids on Water Quality and Aquatic Biota. *Water Research, 42*(12), 2849-2861. doi:https://doi.org/10.1016/j.watres.2008.03.018

Bjørseth, A., & Ramdahl, T. (1983). *Handbook of Polycyclic Aromatic Hydrocarbons : 1* (Vol. 1). New York: Marcel Dekker.

Boehm, P. D., & Farrington, J. W. (1984). Aspects of the Polycyclic Aromatic Hydrocarbon Geochemistry of Recent Sediments in the Georges Bank Region. *Environmental Science & Technology, 18*(11), 840-845. doi:10.1021/es00129a007

Boivin, P., Saadé, M., Pfeiffer, H. R., Hammecker, C., & Degoumois, Y. (2008). Depuration of Highway Runoff Water into Grass-Covered Embankments. *Environmental Technology, 29*(6), 709-720. doi:10.1080/09593330801986972

Bowling, J. W., Leversee, G. J., Landrum, P. F., & Giesy, J. P. (1983). Acute Mortality of Anthracene-Contaminated Fish Exposed to Sunlight. *Aquatic Toxicology, 3*(1), 79-90. doi:https://doi.org/10.1016/0166-445X(83)90008-5

Boxall, A. B. A., & Maltby, L. (1997). The Effects of Motorway Runoff on Freshwater Ecosystems: 3. Toxicant Confirmation. *Archives of Environmental Contamination and Toxicology, 33*(1), 9-16. doi:10.1007/s002449900216

Brand, A. B., Snodgrass, J. W., Gallagher, M. T., Casey, R. E., & Van Meter, R. (2010). Lethal and Sublethal Effects of Embryonic and Larval Exposure of Hyla versicolor to Stormwater Pond Sediments. *Archives of Environmental Contamination and Toxicology, 58*(2), 325-331. doi:10.1007/s00244-009-9373-0

Brittain, J. E., Saltveit, S. J., Bremnes, T., Pavels, H., Løfall, B. P., & Nilssen, J. P. (2017). *Biodiversity in Wet Sedimentation Ponds Constructed for Receiving Road Runoff*. Retrieved from https://www.vegvesen.no/fag/publikasjoner/Publikasjoner/Statens+vegvesens+rapporter/_attachment/1833907

Brooks, S., & Cham, S. (2009). Module 8: Advanced Identification of Dragonflies and Damselflies (Odonata): Larvae and Exuviae *The Identification of Freshwater Invertebrates to Species Level: a Distance-learning Course* (pp. 23): Freshwater Biological Association.

Brown, J. N., & Peake, B. M. (2005). Sources of Heavy Metals and Polycyclic Aromatic Hydrocarbons in Urban Stormwater Runoff. *Science of The Total Environment, 359*(1), 145-155. doi:https://doi.org/10.1016/j.scitotenv.2005.05.016

Buckland-Nicks, A., Hillier, K. N., Avery, T. S., & O'Driscoll, N. J. (2014). Mercury bioaccumulation in dragonflies (Odonata: Anisoptera): Examination of life stages and body regions. *Environmental Toxicology and Chemistry, 33*(9), 2047-2054. doi:doi:10.1002/etc.2653

Burgess, R. M. (2009). Evaluating Ecological Risk to Invertebrate Receptors from PAHs in Sediments at Hazardous Waste Sites (Final Report). Cincinnati, OH: U.S. Environmental Protection Agency, Ecological Risk Assessment Support Center.

Burkhard, L. (2009). Estimation of Biota Sediment Accumulation Factor (BSAF) from Paired Observations of Chemical Concentrations in Biota and Sediment. Cincinnati, OH: U.S. Environmental Protection Agency, Ecological Risk Assessment Support Center.

Canadian Council of Ministers of the Environment. (1999). *Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Polycyclic Aromatic Hydrocarbons (PAHs)*. Winnipeg: Canadian Council of Ministers of the Environment Retrieved from http://ceqg-rcqe.ccme.ca/download/en/243.

Carls, M. G., Holland, L., Larsen, M., Collier, T. K., Scholz, N. L., & Incardona, J. P. (2008). Fish embryos are damaged by dissolved PAHs, not oil particles. *Aquat Toxicol, 88*(2), 121-127. doi:10.1016/j.aquatox.2008.03.014

Carrasco Navarro, V. (2013). *PAHs: Comparative Biotransformation and Trophic Transfer of Their Metabolites in the Aquatic Environment : Fate of Polycyclic Aromatic Hydrocarbons in Aquati Experiments.* (PhD), University of Eastern Finland, Finland. Retrieved from http://epublications.uef.fi/pub/urn_isbn_978-952-61-1138-4/urn_isbn_978-952-61-1138-4.pdf

Carrasco Navarro, V., Leppänen, M. T., Honkanen, J. O., & Kukkonen, J. V. K. (2012a). Trophic Transfer of Pyrene Metabolites and Nonextractable Fraction from Oligochaete (*Lumbriculus variegatus*) to Juvenile Brown Trout (*Salmo trutta*). *Chemosphere, 88*(1), 55-61. doi:https://doi.org/10.1016/j.chemosphere.2012.02.060

Carrasco Navarro, V., Leppänen, M. T., Kukkonen, J. V. K., & Godoy Olmos, S. (2012b). Trophic Transfer of Pyrene Metabolites between Aquatic Invertebrates. *Environmental Pollution, 173*, 61-67. doi:https://doi.org/10.1016/j.envpol.2012.09.023

Céréghino, R., Biggs, J., Oertli, B., & Declerck, S. (2008). The Ecology of European Ponds: Defining the Characteristics of a Neglected Freshwater Habitat. *Hydrobiologia, 597*(1), 1-6. doi:10.1007/s10750-007-9225-8

Clément, B., Cauzzi, N., Godde, M., Crozet, K., & Chevron, N. (2005). Pyrene Toxicity to Aquatic Pelagic and Benthic Organisms in Single-Species and Microcosm Tests. *Polycyclic Aromatic Compounds, 25*(3), 271-298. doi:10.1080/10406630591007260

Clevenot, L., Carré, C., & Pech, P. (2018). A Review of the Factors That Determine Whether Stormwater Ponds Are Ecological Traps And/or High-Quality Breeding Sites for Amphibians. *Frontiers in Ecology and Evolution, 6*(40). doi:10.3389/fevo.2018.00040

Combes, S. A., Rundle, D. E., Iwasaki, J. M., & Crall, J. D. (2012). Linking Biomechanics and Ecology Through Predator–prey Interactions: Flight Performance of Dragonflies and their Prey. *The Journal of Experimental Biology, 215*(6), 903-913. doi:10.1242/jeb.059394

Committee of Directorates for the Water Framework Directive. (2018). *Veileder 2:2018 Klassifisering av Miljøtilstand i Vann - Økologisk og Kjemisk Klassifiseringssystem for Kystvann, Grunnvann, Innsjøer og Elver (In Norwegian)*. Norway Retrieved from http://www.vannportalen.no/globalassets/nasjonalt/dokumenter/veiledere-direktoratsgruppa/klassifiseringsveileder_print_02.2018.pdf.

Corbet, P. S. (1999). *Dragonflies : Behaviour and Ecology of Odonata*. Colchester: Harley Books.

Crumrine, P. W. (2010). Size-structured cannibalism between Top Predators Promotes the Survival of Intermediate Predators in an Intraguild Predation System. *Journal of the North American Benthological Society, 29*(2), 636-646. doi:10.1899/09-006.1

D'Adamo, R., Pelosi, S., Trotta, P., & Sansone, G. (1997). Bioaccumulation and Biomagnification of Polycyclic Aromatic Hydrocarbons in Aquatic Organisms. *Marine Chemistry, 56*(1), 45-49. doi:https://doi.org/10.1016/S0304-4203(96)00042-4

Davies, B., Biggs, J., Williams, P., Whitfield, M., Nicolet, P., Sear, D., . . . Maund, S. (2008). Comparative Biodiversity of Aquatic Habitats in the European Agricultural Landscape. *Agriculture, Ecosystems & Environment, 125*(1), 1-8. doi:https://doi.org/10.1016/j.agee.2007.10.006

De Luca, G., Furesi, A., Leardi, R., Micera, G., Panzanelli, A., Costantina Piu, P., & Sanna, G. (2003). Polycyclic Aromatic Hydrocarbons Assessment in the Sediments of the Porto Torres Harbor (Northern Sardinia, Italy). *Marine Chemistry, 86*(1), 15-32. doi:https://doi.org/10.1016/j.marchem.2003.11.001

De Luca, G., Furesi, A., Micera, G., Panzanelli, A., Piu, P. C., Pilo, M. I., . . . Sanna, G. (2005). Nature, Distribution and Origin of Polycyclic Aromatic Hydrocarbons (PAHs) in the Sediments of Olbia Harbor (Northern Sardinia, Italy). *Marine Pollution Bulletin, 50*(11), 1223-1232. doi:https://doi.org/10.1016/j.marpolbul.2005.04.021

de Wit, C. A. (2002). An Overview of Brominated Flame Retardants in the Environment. *Chemosphere, 46*(5), 583-624. doi:http://dx.doi.org/10.1016/S0045-6535(01)00225-9

Díaz-Jaramillo, M., Miglioranza, K. S. B., Gonzalez, M., Barón, E., Monserrat, J. M., Eljarrat, E., & Barceló, D. (2016). Uptake, Metabolism and Sub-lethal Effects of BDE-47 in two Estuarine Invertebrates with Different Trophic Positions. *Environmental Pollution, 213*, 608-617. doi:https://doi.org/10.1016/j.envpol.2016.03.009

Dodder, N. G., Strandberg, B., & Hites, R. A. (2002). Concentrations and Spatial Variations of Polybrominated Diphenyl Ethers and Several Organochlorine Compounds in Fishes from the Northeastern United States. *Environmental Science & Technology, 36*(2), 146-151. doi:10.1021/es010947g

Dreij, K. (2005). *Carcinogenic Polycyclic Aromatic Hydrocarbons Theoretical, Molecular, in Vitro and Cellular Characterization of Biotransformation and DNA Damage.* (Doctoral), Karolinska Institutet, Stockholm. Retrieved from https://openarchive.ki.se/xmlui/bitstream/handle/10616/38551/thesis.pdf?sequence=1&isAllowed=y

Dybwad, I. M. (2015). *Effects of Tunnel Wash Water on Biomarkers in Three-Spined Stickleback (Gasterosteus aculeatus) and Brown Trout (Salmo trutta): A Lab and Field Study.* (Master thesis), University of Oslo, Oslo, Norway. Retrieved from http://urn.nb.no/URN:NBN:no-49917

EPA, U. S. E. P. A. (2018). National Aquatic Resource Surveys - Indicators: Conductivity. Retrieved from https://www.epa.gov/national-aquatic-resource-surveys/indicators-conductivity

European Commission. (2008, 08.06.2016). Priority Substances and Certain Other Pollutants according to Annex II of Directive 2008/105/EC. Retrieved from http://ec.europa.eu/environment/water/water-framework/priority_substances.htm

Commission Regulation (EC) No 552/2009 of 22 June 2009 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annex XVII (Text with EEA relevance), (2009).

European Commission. (2015). *Towards an EU Research and Innovation Policy Agenda for Nature-Based Solutions & Re-Naturing Cities* Retrieved from Luxembourg: http://ec.europa.eu/newsroom/horizon2020/document.cfm?doc_id=10195

European Commission. (2016, 08.06). WFD: Timetable for Implementation. Retrieved from http://ec.europa.eu/environment/water/water-framework/info/timetable_en.htm

Ewa, B., & Danuta, M.-Š. (2017). Polycyclic Aromatic Hydrocarbons and PAH-Related DNA Adducts. *Journal of Applied Genetics, 58*(3), 321-330. doi:10.1007/s13353-016-0380-3

Ferreras-Romero, M., Márquez-Rodríguez, J., & Ruiz-García, A. (2009). Implications of Anthropogenic Disturbance Factors on the Odonata Assemblage in a Mediterranean Fluvial System. *International Journal of Odonatology, 12*(2), 413-428. doi:10.1080/13887890.2009.9748354

Fletcher, T. D., Shuster, W., F. Hunt, W., Ashley, R., Butler, D., Arthur, S., . . . Viklander, M. (2014). SUDS, LID, BMPs, WSUD and More – The E#volution and Application of Terminology Surrounding Urban Drainage. *Urban Water Journal, 12*(7), 525-542. doi:10.1080/1573062X.2014.916314

Folkeson, L. (2009). Sources and Fate of Water Contaminants in Roads. In A. Dawson (Ed.), *Water in Road Structures. Geotechnical, Geological and Earthquake Engineering* (Vol. 5). Dordrecht: Springer.
Giessing, A. M. B., Mayer, L. M., & Forbes, T. L. (2003). Synchronous Fluorescence Spectrometry of 1-hydroxypyrene: a Rapid Screening Method for Identification of PAH Exposure in Tissue from Marine Polychaetes. *Marine Environmental Research, 56*(5), 599-615. doi:https://doi.org/10.1016/S0141-1136(03)00045-X
Goretti, E., Ceccagnoli, D., La Porta, G., & Di Giovanni, M. V. (2001). Larval Development of *Aeshna cyanea* (Müller, 1764) (Odonata: *Aeshnidae*) in Central Italy. *Hydrobiologia, 457*(1), 149-154. doi:10.1023/A:1012227400613
Gorga, M., Martínez, E., Ginebreda, A., Eljarrat, E., & Barceló, D. (2013). Determination of PBDEs, HBB, PBEB, DBDPE, HBCD, TBBPA and Related Compounds in Sewage Sludge from Catalonia (Spain). *Science of The Total Environment, 444*, 51-59. doi:https://doi.org/10.1016/j.scitotenv.2012.11.066
Grung, M., Holth, T. F., Jacobsen, M. R., & Hylland, K. (2009). Polycyclic Aromatic Hydrocarbon (PAH) Metabolites in Atlantic Cod Exposed via Water or Diet to a Synthetic Produced Water. *Journal of Toxicology and Environmental Health, Part A, 72*(3-4), 254-265. doi:10.1080/15287390802539210
Grung, M., Petersen, K., Fjeld, E., Allan, I., Christensen, J. H., Malmqvist, L. M. V., . . . Ranneklev, S. (2016). PAH Related Effects on Fish in Sedimentation Ponds for Road Runoff and Potential Transfer of PAHs from Sediment to Biota. *Science of The Total Environment, 566–567*, 1309-1317. doi:http://dx.doi.org/10.1016/j.scitotenv.2016.05.191
Grung, M., Ranneklev, S., Meland, S., Fjeld, E., Kringstad, A., Rundberget, J. T., . . . Christensen, J. H. (2018). Sedimentation Ponds: Hotspots for Urban Contaminants.
Guerra, P., Alaee, M., Eljarrat, E., & Barceló, D. (2011). Introduction to Brominated Flame Retardants: Commercially Products, Applications, and Physicochemical Properties. In E. Eljarrat & D. Barceló (Eds.), *Brominated Flame Retardants* (Vol. 16). Spain: Springer, Berlin, Heidelberg.
Hallberg, M., Renman, G., & Lundbom, T. (2007). Seasonal Variations of Ten Metals in Highway Runoff and their Partition between Dissolved and Particulate Matter. *Water, Air, and Soil Pollution, 181*(1), 183-191. doi:10.1007/s11270-006-9289-5
Heier, L. S., Nyheim, M. T., Skipperud, L., & Meland, S. (2013). *Mobility and Uptake of Antimony, Cadmium and Cobalt in Dragonfly Larvae (Odonata, Anisoptera) as a Function of Road Salt Concentrations—a Tracer Experiment*, Dordrecht.
Jansson, B., Asplund, L., & Olsson, M. (1987). Brominated Flame Retardants — Ubiquitous Environmental Pollutants? *Chemosphere, 16*(10), 2343-2349. doi:https://doi.org/10.1016/0045-6535(87)90291-8
Johansen, S. L. (2013). *Element Accumulation and Levels of Four Biomarkers in Common Frog (Rana temporaria) Tadpoles in Two Sedimentation Ponds and a Naturally Occurring Pond.* (Master's), Norwegian University of Life Sciences, Ås, Norway. Retrieved from https://brage.bibsys.no/xmlui/handle/11250/189585
Kelly, B. C., Ikonomou, M. G., Blair, J. D., & Gobas, F. A. P. C. (2008). Bioaccumulation Behaviour of Polybrominated Diphenyl Ethers (PBDEs) in a Canadian Arctic Marine Food Web. *Science of The Total Environment, 401*(1–3), 60-72. doi:http://dx.doi.org/10.1016/j.scitotenv.2008.03.045
Knight, T. M., McCoy, M. W., Chase, J. M., McCoy, K. A., & Holt, R. D. (2005). Trophic Cascades across Ecosystems. *Nature, 437*, 880. doi:10.1038
Kose, T., Yamamoto, T., Anegawa, A., Mohri, S., & Ono, Y. (2007). Source Analysis for Polycyclic Aromatic Hydrocarbon in Road Dust and Urban Runoff Using Marker

Compounds. *Desalination, 226*(1), 151-159. doi:https://doi.org/10.1016/j.desal.2007.01.239
Law, R. J., & Biscaya, J. L. (1994). Polycyclic Aromatic Hydrocarbons (PAH)—Problems and Progress in Sampling, Analysis and Interpretation. *Marine Pollution Bulletin, 29*(4), 235-241. doi:https://doi.org/10.1016/0025-326X(94)90415-4
Le Viol, I., Mocq, J., Julliard, R., & Kerbiriou, C. (2009). The Contribution of Motorway Stormwater Retention Ponds to the Biodiversity of Aquatic Macroinvertebrates. *Biological Conservation, 142*(12), 3163-3171. doi:https://doi.org/10.1016/j.biocon.2009.08.018
Lerda, D. (2011). *Polycyclic Aromatic Hydrocarbons (PAHs) Factsheet*. Belgium: European Union Retrieved from https://ec.europa.eu/jrc/sites/jrcsh/files/Factsheet%20PAH_0.pdf.
Livingstone, D. R. (1998). The Fate of Organic Xenobiotics in Aquatic Ecosystems: Quantitative and Qualitative Differences in Biotransformation by Invertebrates and Fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 120*(1), 43-49. doi:https://doi.org/10.1016/S1095-6433(98)10008-9
Lyytikäinen, M., Pehkonen, S., Akkanen, J., Leppänen, M., & Kukkonen, J. V. K. (2007). Bioaccumulation and Biotransformation of Polycyclic Aromatic Hydrocarbons During Sediment Tests with Oligochaetes (*Lumbriculus variegatus*). *Environmental Toxicology and Chemistry, 26*(12), 2660-2666. doi:doi:10.1897/01-171.1
Mangani, G., Berloni, A., Bellucci, F., Tatàno, F., & Maione, M. (2005). Evaluation of the Pollutant Content in Road Runoff First Flush Waters. *Water, Air, and Soil Pollution, 160*(1), 213-228. doi:10.1007/s11270-005-2887-9
Marvanova, S., Vondracek, J., Penccikova, K., Trilecova, L., Krcmarr, P., Topinka, J., . . . Machala, M. (2008). Toxic Effects of Methylated Benz[a]Anthracenes in Liver Cells. *Chem Res Toxicol, 21*(2), 503-512. doi:10.1021/tx700305x
Mayer, P., & Holmstrup, M. (2008). Passive Dosing of Soil Invertebrates with Polycyclic Aromatic Hydrocarbons: Limited Chemical Activity Explains Toxicity Cutoff. *Environmental Science & Technology, 42*(19), 7516-7521. doi:10.1021/es801689y
McElroy, A. E., Cahill, J. M., Sisson, J. D., & Kleinow, K. M. (1991). Relative Bioavailability and DNA Adduct Formation of Benzo[a]pyrene and Metabolites in the Diet of the Winter Flounder. *Comp Biochem Physiol C, 100*(1-2), 29-32.
Meland, S. (2010). *Ecotoxicological Effects of Highway and Tunnel Wash Water Runoff.* (Doctoral thesis), Norwegian University of Life Sciences, Ås, Norway. Retrieved from http://hdl.handle.net/11250/2431910
Meland, S. (2015). *Management of Contaminated Runoff Water - a Review*. Paper presented at the Teknologidagene 2015, Trondheim, Norway. http://www.vegvesen.no/_attachment/1026395/binary/1060097?fast_title=01.+NORWAT+%E2%80%93+status+og+vannh%C3%A5ndtering+i+Europa.pdf
Meland, S. (2016b). *Management of Contaminated Runoff Water: Current Practice and Future Research Needs*. Paper presented at the Conference of European Directors of Roads, Belgium.
Meland, S. (2018). *Identification of Species - UrbanCOMET*. Unpublished.
Meland, S., Borgstrøm, R., Heier, L. S., Rosseland, B. O., Lindholm, O., & Salbu, B. (2010a). Chemical and Ecological Effects of Contaminated Tunnel Wash Water Runoff to a Small Norwegian Stream. *Science of The Total Environment, 408*(19), 4107-4117. doi:https://doi.org/10.1016/j.scitotenv.2010.05.034
Meland, S., Ranneklev, S. B., & Hertel-Aas, T. (2016a). Forslag til Nye Retningslinjer for Rensing Avveiavrenning og Tunnelvaskevann (*in Norwegian*). *Vann, 3*.

Meland, S., Salbu, B., & Rosseland, B. O. (2010b). Ecotoxicological Impact of Highway Runoff Using Brown Trout (*Salmo trutta*) as an Indicator Model. *Journal of Environmental Monitoring, 12*(3), 654-664. doi:10.1039/B919420G

Monteiro, M. T. F., Oliveira, S. M., Luizão, F. J., Cândido, L. A., Ishida, F. Y., & Tomasella, J. (2014). Dissolved organic carbon concentration and its relationship to electrical conductivity in the waters of a stream in a forested Amazonian blackwater catchment. *Plant Ecology & Diversity, 7*(1-2), 205-213. doi:10.1080/17550874.2013.820223

Moser, B. K., & Stevens, G. R. (1992). Homogeneity of Variance in the Two-Sample Means Test. *The American Statistician, 46*(1), 19-21. doi:10.2307/2684403

National Center for Biotechnology Information. Tert-butyl Methyl Ether. *PubChem Compound Database; CID=15413*. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/15413

NCBI, N. C. f. B. I. PubChem Compound Database. Retrieved 10.09.2018, from National Center for Biotechnology Information https://pubchem.ncbi.nlm.nih.gov

Neff, J. M., Stout, S. A., & Gunster, D. G. (2004). Ecological Risk Assessment of Polycyclic Aromatic Hydrocarbons in Sediments: Identifying Sources and Ecological Hazard. *Integrated Environmental Assessment and Management, 1*(1), 22-33. doi:doi:10.1897/IEAM_2004a-016.1

Nelson, D. R., & Strobel, H. W. (1987). Evolution of Cytochrome P-450 Proteins. *Mol Biol Evol, 4*(6), 572-593. doi:10.1093/oxfordjournals.molbev.a040471

Newsted, J. L., & Giesy, J. P. (1987). Predictive Models for Photoinduced Acute Toxicity of Polycyclic Aromatic Hydrocarbons to *Daphnia magna*, Strauss (Cladocera, Crustacea). *Environmental Toxicology and Chemistry, 6*(6), 445-461. doi:doi:10.1002/etc.5620060605

Ngabe, B., Bidleman, T. F., & Scott, G. I. (2000). Polycyclic aromatic Hydrocarbons in Storm Runoff from Urban and Coastal South Carolina. *Science of The Total Environment, 255*(1), 1-9. doi:https://doi.org/10.1016/S0048-9697(00)00422-8

Nielsen, K., Kalmykova, Y., Strömvall, A.-M., Baun, A., & Eriksson, E. (2015). Particle phase distribution of polycyclic aromatic hydrocarbons in stormwater — Using humic acid and iron nano-sized colloids as test particles. *Science of The Total Environment, 532*, 103-111. doi:https://doi.org/10.1016/j.scitotenv.2015.05.093

Norwegian Biodiversity Information Center (in Norwegian). (2013). Øyenstikkere Odonata. from Naturhistorisk museum, Universitetet i Oslo, Norsk institutt for naturforskning, NIBIO Norsk institutt for bioøkonomi https://www.artsdatabanken.no/Pages/133473

Norwegian Environmental Agency. (2016). *Quality Standards for Water, Sediment and Biota (In Norwegian)* (M-608). Retrieved from Norway: http://www.miljodirektoratet.no/Documents/publikasjoner/M608/M608.pdf

Norwegian Ministry of Climate and Environment. (2011). *Europaparlaments- og Rådsdirektiv 2000/60/EF av 23. Oktober 2000 om Fastsettelse av en Ramme for Fellesskapstiltak på Området Vannpolitikk (In Norwegian)*. Norway Retrieved from https://lovdata.no/static/NLX3/32000l0060.pdf.

Norwegian Public Roads Administration. (2010). *Sedimentation Ponds and Ditches in Region East - Summary Report 2009 (In Norwegian)*. Retrieved from Unpublished:

Norwegian Public Roads Administration. (2014). *Prioriteringsliste 2015* Unpublished.

Norwegian Public Roads Administration. (2016a). Håndbok N500 - Vegtunneler *(In Norwegian)*: Statens vegvesen.

Norwegian Public Roads Administration. (2016b). Prioriteringsliste 2017 Unpublished.

Norwegian Public Roads Administration. (2018). Håndbok N200 - Vegbygging *(In Norwegian)*: Statens vegvesen.

Paus, K. A. H., Åstebøl, S. O., Robbe, S., Ulland, V., & Lausund, E. (2013). *Tilstanden til Rensebassenger i Norge (in Norwegian)*. Retrieved from

Peace, A., Poteat, M. D., & Wang, H. (2016). Somatic Growth Dilution of a Toxicant in a Predator–Prey Model Under Stoichiometric Constraints. *Journal of Theoretical Biology, 407*, 198-211. doi:https://doi.org/10.1016/j.jtbi.2016.07.036

Penning, T. M. (1993). Dihydrodiol Dehydrogenase and its Role in Polycyclic Aromatic Hydrocarbon Metabolism. *Chemico-Biological Interactions, 89*(1), 1-34. doi:https://doi.org/10.1016/0009-2797(93)03203-7

Piha, H. E., Dulio, V., & Hanke, G. (2010). *River Basin-Specific Pollutants - Identification and Monitoring*. Retrieved from http://publications.jrc.ec.europa.eu/repository/bitstream/JRC61421/henna.piha.pdf

Rust, A. J., Burgess, R. M., Brownawell, B. J., & McElroy, A. E. (2004). Relationship Between Metabolism and Bioaccumulation of Benzo[α]pyrene in Benthic Invertebrates. *Environmental Toxicology and Chemistry, 23*(11), 2587-2593. doi:doi:10.1897/03-354

Ruus, A., Schaanning, M., Øxnevad, S., & Hylland, K. (2005). Experimental Results on Bioaccumulation of Metals and Organic Contaminants from Marine Sediments. *Aquatic Toxicology, 72*(3), 273-292. doi:https://doi.org/10.1016/j.aquatox.2005.01.004

Ruxton, G. D. (2006). The Unequal Variance T-test is an Underused Alternative to Student's T-test and the Mann–Whitney U Test. *Behavioral Ecology, 17*(4), 688-690. doi:10.1093/beheco/ark016

Samways, M. J., & Steytler, N. S. (1996). Dragonfly (Odonata) distribution patterns in urban and forest landscapes, and recommendations for riparian management. *Biological Conservation, 78*(3), 279-288. doi:https://doi.org/10.1016/S0006-3207(96)00032-8

Shaw, G. R., & Connell, D. W. (2001). DNA Adducts as a Biomarker of Polycyclic Aromatic Hydrocarbon Exposure in Aquatic Organisms: Relationship to Carcinogenicity. *Biomarkers, 6*(1), 64-71. doi:10.1080/135475001452805

Skov, H., Hansen, A. B., Lorenzen, G., Andersen, H. V., Løfstrøm, P., & Christensen, C. S. (2001). Benzene Exposure and the Effect of Traffic Pollution in Copenhagen, Denmark. *Atmospheric Environment, 35*(14), 2463-2471. doi:https://doi.org/10.1016/S1352-2310(00)00460-X

Sobek, S., Tranvik, L. J., Prairie, Y. T., Kortelainen, P., & Cole, J. J. (2007). Patterns and regulation of dissolved organic carbon: An analysis of 7,500 widely distributed lakes. *Limnology and Oceanography, 52*(3), 1208-1219. doi:doi:10.4319/lo.2007.52.3.1208

Spehar, R. L., Poucher, S., Brooke, L. T., Hansen, D. J., Champlin, D., & Cox, D. A. (1999). *Comparative Toxicity of Fluoranthene to Freshwater and Saltwater Species Under Fluorescent and Ultraviolet Light* (Vol. 37): Springer-Verlag.

Srogi, K. (2007). Monitoring of Environmental Exposure to Polycyclic Aromatic Hydrocarbons: a Review. *Environmental Chemistry Letters, 5*(4), 169-195. doi:10.1007/s10311-007-0095-0

Statistics Norway. (2017a, 22 mars 2018). Registered Vehicles (in Norwegian). Retrieved from https://www.ssb.no/transport-og-reiseliv/statistikker/bilreg/aar

Statistics Norway. (2017b). 11842: County Roads. Tunnels, Bridges and Road Safety (C) 2015 - 2017. Retrieved from https://www.ssb.no/en/statbank/table/11842

Stegeman, J. J., & Lech, J. J. (1991). Cytochrome P-450 Monooxygenase Systems in Aquatic Species: Carcinogen Metabolism and Biomarkers for Carcinogen and Pollutant Exposure. *Environmental Health Perspectives, 90*, 101-109.

Stockholm Convention. All POPs Listed in the Stockholm Convention. Retrieved from http://chm.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx

Stogiannidis, E., & Laane, R. (2015). Source Characterization of Polycyclic Aromatic Hydrocarbons by Using Their Molecular Indices: An Overview of Possibilities. In D. M. Whitacre (Ed.), *Reviews of Environmental Contamination and Toxicology* (pp. 49-133). Cham: Springer International Publishing.

Stoks, R., & Córdoba-Aguilar, A. (2012). Evolutionary Ecology of Odonata: A Complex Life Cycle Perspective. *Annual Review of Entomology, 57*(1), 249-265. doi:10.1146/annurev-ento-120710-100557

Stout, S. A., Uhler, A. D., & Emsbo-Mattingly, S. D. (2004). Comparative Evaluation of Background Anthropogenic Hydrocarbons in Surficial Sediments from Nine Urban Waterways. *Environmental Science & Technology, 38*(11), 2987-2994. doi:10.1021/es040327q

Strand, L. Å. (2006). *Vurdering av Effekt av Restaureringstiltak i Fem Dammer og Undersøkelse Av Nyanlagte Dammer i Oslos Byggesone (In Norwegian)*. Oslo, Norway: Oslo Kommune

Strand, L. Å. (2008). *Registrering av Amfibier i Oslo Kommune i 2008 (In Norwegian)*. Retrieved from http://www.naturarv.no/getfile.php/773806.813.dxcxtyqpwv/NorskNaturarvOslodammer08-reduced.pdf

Streets, S. S., Henderson, S. A., Stoner, A. D., Carlson, D. L., Simcik, M. F., & Swackhamer, D. L. (2006). Partitioning and Bioaccumulation of PBDEs and PCBs in Lake Michigan. *Environmental Science & Technology, 40*(23), 7263-7269. doi:10.1021/es061337p

Stroomberg, G. J., Zappey, H., Steen, R. J. C. A., van Gestel, C. A. M., Ariese, F., Velthorst, N. H., & van Straalen, N. M. (2004). PAH Biotransformation in Terrestrial Invertebrates—a New Phase II Metabolite in Isopods and Springtails. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 138*(2), 129-137. doi:https://doi.org/10.1016/j.cca.2004.06.004

Sun, Z., Brittain, J. E., Sokolova, E., Thygesen, H., Saltveit, S. J., Rauch, S., & Meland, S. (2018). Aquatic Biodiversity in Sedimentation Ponds Receiving Road Runoff - What are the Key Drivers? *Sci Total Environ, 610-611*, 1527-1535. doi:10.1016/j.scitotenv.2017.06.080

Sundt, R. C., Ruus, A., Jonsson, H., Skarphéðinsdóttir, H., Meier, S., Grung, M., . . . Pampanin, D. M. (2011). Biomarker Responses in Atlantic Cod (*Gadus morhua*) Exposed to Produced Water from a North Sea Oil Field: Laboratory and Field Assessments. *Marine Pollution Bulletin, 64*(1), 144-152. doi:https://doi.org/10.1016/j.marpolbul.2011.10.005

Takeuchi, I., Miyoshi, N., Mizukawa, K., Takada, H., Ikemoto, T., Omori, K., & Tsuchiya, K. (2009). Biomagnification Profiles of Polycyclic Aromatic Hydrocarbons, Alkylphenols and Polychlorinated Biphenyls in Tokyo Bay Elucidated by Delta13C and Delta15N Isotope Ratios as Guides to Trophic Web Structure. *Marine Pollution Bulletin, 58*(5), 663-671. doi:10.1016/j.marpolbul.2008.12.022

Turcotte, D., Akhtar, P., Bowerman, M., Kiparissis, Y., Brown, R. S., & Hodson, P. V. (2011). Measuring the Toxicity of Alkyl-Phenanthrenes to Early Life Stages of Medaka (*Oryzias latipes*) Using Partition-Controlled Delivery. *Environ. Toxicol. Chem., 30*(2), 487-495. doi:10.1002/etc.404

Van Brummelen, T. C., Verweij, R. A., Wedzinga, S. A., & Van Gestel, C. A. M. (1996). Polycyclic Aromatic Hydrocarbons in Earthworms and Isopods from Contaminated Forest Soils. *Chemosphere, 32*(2), 315-341. doi:https://doi.org/10.1016/0045-6535(95)00340-1

Van Praet, N., Covaci, A., Teuchies, J., De Bruyn, L., Van Gossum, H., Stoks, R., & Bervoets, L. (2012). Levels of Persistent Organic Pollutants in Larvae of The Damselfly *Ischnura elegans* (Odonata, Coenagrionidae) from Different Ponds in Flanders, Belgium. *Science of The Total Environment, 423*, 162-167. doi:http://dx.doi.org/10.1016/j.scitotenv.2012.02.045

Vignet, C., Le Menach, K., Lyphout, L., Guionnet, T., Frère, L., Leguay, D., . . . Bégout, M.-L. (2014b). Chronic Dietary Exposure to Pyrolytic and Petrogenic Mixtures of PAHs Causes Physiological Disruption in Zebrafish—part II: behavior. *Environmental Science and Pollution Research, 21*(24), 13818-13832. doi:10.1007/s11356-014-2762-6

Vignet, C., Le Menach, K., Mazurais, D., Lucas, J., Perrichon, P., Le Bihanic, F., . . . Cousin, X. (2014a). Chronic Dietary Exposure to Pyrolytic and Petrogenic Mixtures of PAHs Causes Physiological Disruption in Zebrafish - Part I: Survival and Growth. *Environmental Science and Pollution Research, 21*(24), 13804-13817. doi:10.1007/s11356-014-2629-x

Villalobos-Jiménez, G., Dunn, A., & Hassall, C. (2016). Dragonflies and damselflies (Odonata) in urban ecosystems: A review. *EJE, 113*(1), 217-232.

Wan, Y., Jin, X., Hu, J., & Jin, F. (2007). Trophic Dilution of Polycyclic Aromatic Hydrocarbons (PAHs) in a Marine Food Web from Bohai Bay, North China. *Environmental Science & Technology, 41*(9), 3109-3114. doi:10.1021/es062594x

Wang, J., Jia, C. R., Wong, C. K., & Wong, P. K. (1999). Characterization of Polycyclic Aromatic Hydrocarbons Created in Lubricating Oils. *Water, Air, and Soil Pollution, 120*(3), 381-396. doi:10.1023/a:1005251618062

Wang, Z., Fingas, M., Shu, Y. Y., Sigouin, L., Landriault, M., Lambert, P., . . . Mullin, J. (1999). Quantitative Characterization of PAHs in Burn Residue and Soot Samples and Differentiation of Pyrogenic PAHs from Petrogenic PAHs−The 1994 Mobile Burn Study. *Environmental Science & Technology, 33*(18), 3100-3109. doi:10.1021/es990031y

Wang, Z., & Fingas, M. F. (1997). Development of Oil Hydrocarbon Fingerprinting and Identification Techniques. *Marine Pollution Bulletin, 47*(9), 423-452. doi:https://doi.org/10.1016/S0025-326X(03)00215-7

Wang, Z., Yang, C., Brown, C., Hollebone, B., & Landriault, M. (2008). A Case Study: Distinguishing Pyrogenic Hydrocarbons From Petrogenic Hydrocarbons. *International Oil Spill Conference Proceedings, 2008*(1), 311-320. doi:10.7901/2169-3358-2008-1-311

Webster, L., Tronczynski, J., Korytar, P., Booij, K., and Law, R. (2010). *Determination of parent and alkylated polycyclic aromatic hydrocarbons (PAHs) in biota and sediment*. Retrieved from Danmark: http://www.ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20%28TIMES%29/times45/091214-TIMES%2045%20PAHs-FINAL2.pdf

Whyte, J. J., Jung, R. E., Schmitt, C. J., & Tillitt, D. E. (2000). Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Crit Rev Toxicol, 30*(4), 347-570. doi:10.1080/10408440091159239

Wong, T. H. F., Breenm, P. F., & L G Somes, N. (1999, 22-26 February). *Ponds vs Wetlands - Performance Considerations in Stormwater Quality Management.* Paper presented at the 1st South Pacific Conference on Comprehensive Stormwater and Aquatic Ecosystem Management, Auckland, New Zealand.

Wood, M., Beresford, N., & Copplestone, D. (2011). Limit of Detection Values in Data Analysis: Do They Matter? *Radioprotection, 46*(6), 85-90. doi:10.1051/radiopro/20116728s

Zedler, J. B., & Kercher, S. (2005). WETLAND RESOURCES: Status, Trends, Ecosystem Services, and Restorability. *Annual Review of Environment and Resources, 30*(1), 39-74. doi:10.1146/annurev.energy.30.050504.144248

Zhang, X., Xia, X., Li, H., Zhu, B., & Dong, J. (2015). Bioavailability of Pyrene Associated with Suspended Sediment of Different Grain Sizes to Daphnia magna as Investigated by Passive Dosing Devices. *Environmental Science & Technology, 49*(16), 10127-10135. doi:10.1021/acs.est.5b02045

8 Appendices

Appendix A – List of Materials Used

A1. Material Used in Fieldwork

Material
Glass jars
Gloves
Waders
Net
Marker
Disinfectant
Cooler with ice
Tape to mark jars
Aluminum foil
Van Veen grab

A1. Chemicals used for PAH metabolite analysis

Chemical	Producer
Triphenylamine (I.S.)	Sigma Aldrich/Fluka
β-glucoronidase/aryl sulfatase[10] from *Helix pomatia*	Merck Millipore/Sigma Aldrich
Methanol	VWR/Sigma A.
2-OH-naphthalene	NA
1-OH-fenantrene	NA
1-OH-pyrene	NA
3-OH-benzo[a]pyrene	NA
Acetonitrile (ACN)	VWR/Sigma A.
Ethanol	NA

10 Stabilized aqueous solution for biochemistry. Merck: E.C. 3.2.1.31 + E.C.3.1.6.1.

A2. Chemicals used for PAH and BFR extraction

Chemical
NIST®SRM® 2974a - Organics in Freeze-Dried Mussel Tissue (*Mytilus edulis*)
NIST®SRM® 1944 - New York/New Jersey waterway sediment
Cyclohexane
Dichloromethane
Internal Standards for PAHs and BFRs
Ethyl acetate (LS-MS graded) – For PAH extraction only
Sulfuric acid – For BFR extraction only

Appendix B – Instruments setup

B1. GPC

System	Agilent Technologies, Wilmington, DE, USA
Column	PLgel 10um, 100A (pore size), 300x7.5mm
Fraction collected	4.8 – 11.3 µL/min
Temperature	50°C
Mobil phase	ethyl acetate: cyclohexane 80:20
Flow	2 mL/min

B2. PAHs - Gas chromatography/Mass spectrometry

System	GC 6890 MSD 5973 MS column: DB-5ms (ID 0.25 mm, length 30 m, film 0.25 µm Agilent Technologies, Wilmington, DE, USA
Mode	Splitless
Gas flow	1.2 mL/min
Temperature	60 °C - Hold time 2 min. 7 °C per min. to 250 °C 15 °C per min to 310 Hold time 6min Injector: 300°C Transfer line: 290°C MS source: 230°C

B3. PAH metabolites – High Performance Liquid Chromatography

System	Waters 2695 Separations Module 2475 fluorescence detector C18 column with precolumn, type Vydac 201TP5415 (5µm particle size, 4,6x250mm)
Fluorescence quantification	Naphthalene (ex/em, 325/358 nm) Phenanthrene after 8.5 min (ex/em, 251/364 nm) alternatively: 256/380 Pyrene after 13.5 min(ex/em, 246/384 nm) alternatively: 346/384 B[a]P after 22,5 min (ex/em, 380/430 nm) ISD triphenylamine (ex/em, 300/360 nm)
Gradient	Mobile phase A: 40:60 v/v acetonitrile: ammonium acetate buffer 0.05M, pH 4.1 Mobile phase B: 100% acetonitrile - flow 1 mL/min
Others	Reservoir degasses continuously. Injection volume: 25 µL, column temperature: 30°C.

B4. BDE - Gas chromatography/Mass spectrometry

System	GC 6890 MSD 5973 MS column: DB-XLB (30 m x 250 µm x 0.25 µm) Agilent Technologies, Wilmington, DE, USA
Mode	Splitless
Gas flow	1.2 mL/min
Temperature	60 – 310ºC Injector: 300ºC Transfer line: 300ºC MS source: 250ºC

Appendix C – Samples

C.1. List of Dragonfly Larvae Samples

Code	Wet weight	Dry weight
1 - Nøstvedt Tissues	3.5024	0.8551
2- Nøstvedt Exuvia	7.9369	2.3317
3- Nøstvedt Whole animal	1.9625	0.3046
4- Taraldrud North Tissues	2.6537	0.7763
5-Taraldrud North Exuvia	5.7786	1.5912
6-Taraldrud North Whole animal	3.6168	0.6802
7- Taraldrud South Tissues	5.2795	1.2837
8- Taraldrud South Exuvia	5.8522	1.806
9 -Taraldrud South Whole animal	0.8986	0.2142
10 - Taraldrud Junction Tissues	2.0102	0.5317
11- Taraldrud Junction Exuvia	2.2033	0.7247
12- Taraldrud Junction Whole animal	4.3406	0.9584
13- Tenor Tissues	3.4386	0.877
14- Tenor Exuvia	3.6168	1.1413
15- Tenor Whole animal	2.4019	0.5052
16- Fornebu Tissues	2.7658	0.692
17- Fornebu Exuvia	3.8806	1.2151
18- Fornebu Whole animal	0.9413	0.2138
19 - Vassum Tissues	3.593	0.9199
20- Vassum Exuvia	4.0921	1.2855
21- Vassum Whole animal	1.7484	0.352
22 - Båntjern Tissues	4.0018	0.8805
23 – Båntjern Exuvia	4.0239	1.0742
24- Båntjern Whole animal	5.4731	0.9046
25-Svarta Tissues	2.7809	0.6527
26-Svarta Exuvia	3.8864	1.1086
27-Svarta Whole animal	2.0301	1.3145
28- Møllesvingen Tissues	4.0715	1.0368
29- Møllesvingen Exuvia	4.5952	1.3629
30 -SRM	0	1.01
31- Blank 1	0	0
32-Blank 2	0	0

Appendix D – Packages Used with the Statistical Program R-Studio

Packages	Function
Reshape2	Transform wide data (one column for each variable) into long data (all the variables in one column)
Ggplot2	Plot visualization
Factoextra	Simplified extraction and visualization of multivariate data
factormineR	Convert values in a column into row names in an existing data frame, PCA
Car	Statistical tests
Vegan	PCA
Devtools	Access to developing packages
Faraway	Multicollinearity analysis
Vcd	Visualization of categorical data
Ggpubr	Publication of statistical tests in plots
Rcolorbrewer	Color patterns for background

Appendix E – Results

E1- Water Sample Results

Pond	Total Phosporous µg/L	Total Nitrogen µg/L	pH	Conductivity (µS/cm)	Temp (˚C)	O2 (mg/L)	Redox Potential
Fornebu	95	1100	6.8	3338	17.52	2.11	499
Taraldrud North	51	430	7.6	1227	13.86	9.85	807
Taraldrud Junction	29	670	7.4	1253	16.6	9.8	749
Taraldrud South	15	480	7.0	1310	19.7	7.34	757
Nøstvedt	19	450	7.2	1321	17.35	3.45	542.1
Vassum	120	930	7.6	1518	18.74	4.71	561
Tenor	98	850	7.4	1890	15.4	11.98	618
Båntjern	140	2700	6.9	200	13.76	3.29	710
Møllesvingen	120	830	6.8	95	19.37	3.43	755.9
Svarta	80	610	6.9	64	19.06	3.31	422.5

E2 – PAH metabolites

Pond	Haemolymph Weight (g)	I.S. Weight (g)	**Factor ISTD**	1-OH-pyrene	ISTD	**1-OH-pyrene** [11]
Nøstvedt	0.01106	0.008412	14.317	0.39	7500	0.654
Nøstvedt	0.01167	0.00839	13.533	0.464	7500	0.657
Tar North	0.01488	0.00871	11.018	0.072	7500	0.106
Tar North	0.01435	0.00815	10.691	0.066	7500	0.094
Tar South	0.01648	0.00784	8.955	0.706	7500	0.843
Tar Junct.	0.01175	0.00847	13.569	0.081	7500	0.147
Tenor	0.01466	0.0076	9.758	0.036	7500	0.047
Fornebu	0.02075	0.00821	7.448	0.379	7500	0.376
Fornebu	0.01844	0.00776	7.921	0.919	7500	0.971
Vassum	0.01801	0.008034	8.397	1.641	7500	1.837
R-Bån	0.01601	0.00766	9.006	0.727	7500	0.873
R-Svarta	0.02371	0.00802	6.367	0.073	7500	0.062
R -Mølles	0.01391	0.00812	10.988	0.103	7500	0.151
R- Mølles	0.01465	0.00819	10.523	0.035	7500	0.049
Blank	0.015737857	0.00861	10.298	0.093	7500	0.128
Blank	0.015737857	0.00737	8.815	0.045	7500	0.053
Ref lav	0.01814	0.00759	7.876	79.607	7500	83.598
Ref høy	0.02095	0.00796	7.152	260.441	7500	248.358

[11] LOD=0.18. Values below LOD are considered noise

E3. Results from BFR analysis in dragonfly larvae[12]

	BFRs	Fornebu	Taraldrud North	Taraldrud Junction	Taraldrud South	Nøstvedt	Vassum	Tenor	Båntjern	Møllesvingen	Svarta
Tissue - BFRs	**BDE-28**	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
	BDE-49	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015
	BDE-47	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015
	BDE-100	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015
	BDE-99	0.03	0.03	0.06	<0,01	<0,01	0.04	0.06	0.09	0.02	0.02
	BDE-126	<0,01	<0,01	<0,01	<0,01	<0,01	0.09	<0,01	<0,01	<0,01	<0,01
	BDE-154	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
	BDE-153	0.02	0.02	0.04	<0,01	<0,01	<0,01	0.04	<0,01	0.02	0.02
	BDE-183	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015
	BDE-196	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03
	HBCD*	0.166	<0.018	<0.017	0.027	<0.013	0.190	<0.011	0.026	<0,005	<0,005
Exuvia - BFRs	**BDE-28**	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
	BDE-49	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015
	BDE-47	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015
	BDE-100	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015
	BDE-99	0.01	0.01	0.02	0.01	<0,01	0.04	0.02	0.02	0.01	<0,01
	BDE-126	<0,01	<0,01	<0,01	<0,01	<0,01	0.02	<0,01	<0,01	<0,01	<0,01
	BDE-154	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
	BDE-153	<0,01	0.01	0.01	<0,01	0.02	<0,01	<0,01	<0,01	<0,01	<0,01
	BDE-183	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015
	BDE-196	<0,03	<0,03	<0,03	<0,03	<0,10	<0,03	<0,03	<0,03	<0,03	<0,03
	HBCD*	0.035	<0.009	<0,005	<0.013	<0.018	0.121	<0.011	<0,005	<0,005	0.641
Whole - BFRs	**BDE-28**	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	NA	<0,01
	BDE-49	0.09	<0,015	<0,015	0.08	0.05	0.03	<0,015	<0,015	NA	<0,015
	BDE-47	<0,015	<0,015	0.02	<0,015	0.02	0.02	0.02	<0,015	NA	<0,015
	BDE-100	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	NA	<0,015
	BDE-99	<0,01	<0,01	0.10	0.01	0.04	0.08	0.06	0.01	NA	<0,01
	BDE-126	<0,01	<0,01	<0,01	<0,01	<0,01	0.02	<0,01	<0,01	NA	<0,01
	BDE-154	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	NA	<0,01
	BDE-153	<0,01	<0,01	0.03	<0,01	<0,01	<0,01	0.01	<0,01	NA	<0,01
	BDE-183	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	<0,015	NA	<0,015
	BDE-196	<0,03	<0,03	0.03	<0,03	<0,03	<0,03	<0,03	<0,03	NA	<0,03
	HBCD*	0.052	<0.013	<0.017	0.022	0.054	0.190	<0,005	0.087	NA	<0.017

[12] Below limit of quantification
Below limit of detection

E4. Results from BFR analysis in Sediment[13]

	BFRs	Fornebu	Taraldrud North	Taraldrud Junction	Taraldrud South	Nøstvedt	Vassum	Tenor	Båntjern	Møllesvingen	Svarta
Sediment	**BDE-28**	<0,2	<0,2	<0,5	<0,2	<0,2	<0,2	<0,2	<0,2	<0,2	NA
	BDE-49	0.32	0.55	0.49	0.25	0.10	0.76	<0,1	0.19	0.23	NA
	BDE-47	0.62	0.73	2.4	0.56	0.26	0.77	0.10	0.05	<0,05	NA
	BDE-100	0.16	0.28	0.77	0.17	0.06	0.16	<0,05	<0,05	<0,05	NA
	BDE-99	0.50	1.82	5.0	0.60	0.32	1.02	0.21	<0,1	<0,1	NA
	BDE-126	<0,1	<0,1	<0,2	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	NA
	BDE-154	0.13	0.30	0.84	0.13	0.04	0.14	<0,03	<0,05	<0,05	NA
	BDE-153	0.16	0.68	1.2	0.18	0.06	<0,05	0.03	<0,03	<0,03	NA
	BDE-183	0.29	0.44	0.29	0.45	0.12	0.27	0.03	0.17	<0,03	NA
	BDE-196	<0,2	<0,2	0.45	0.30	<0,2	<0,2	<0,2	<0,2	<0,2	NA
	HBCD	0.77	2.45	2.45	0.84	0.52	5.29	0.52	0.97	1.42	NA
	ΣPBDEs[14]	1.57	3.81	10.2	1.64	0.74	2.09	0.34	0.05	0	NA

[13] Below LOD

[14] ΣPBDEs includes the 6 priority PBDEs according to the Norwegian Environmental Agency (Norwegian Environmental Agency, 2016); BDE – 28, -47, -99, -100, -153, -154 . Limits below LOD were set as 0.

E5. PAH Results for Biota

	PAHs	Fornebu	Tar. North	Tar. Junction	Tar. South	Nøstvedt	Vassum	Tenor	Båntjern	Møllersvingen	Svarta
Tissue	ACY	34	22.3	13.8	30.5	29.1	40.1	17.7	22.7	14.7	18.1
	ACE	15	15	7.8	17	19	34	7	13	12	8.3
	PHEN	31	36	15	43	24	45	79	57	20	26
	FLUORA	13	11	4.6	10	6.4	16	48	35	5.3	6.3
	PYR	46	33	24	17	13	34	123	49	18	12
Exuvia	ACY	NA	8.3	4.7	22.1	12.2	8.1	5.8	7.5	7.5	6.3
	ACE	NA	4.8	6.8	19	12	5.7	4.2	5.5	5.7	3.5
	PHEN	15	12	11	21	17	17	27	18	11	13
	FLUORA	4.1	3.5	3.2	6.5	4.3	8.8	20	15	2.2	4.9
	PYR	7.4	31	4.7	19	9.2	15	47	8.6	18	13
Whole	ACY	25.7	16.8	43.3	12.4	13	44.5	34.1	8.2	NA	9.6
	ACE	24	12	47	10	23	30	64	7.7	NA	4.2
	PHEN	26	17	30	10	21	17	43	15	NA	5
	FLUORA	14	5.2	7.8	3.6	4.6	9.7	32	11	NA	1.2
	PYR	53	8.6	57	9	37	25	75	21	NA	7.8

E6. Source Apportionment

Location	PHEN/ANTHR	FLUORA/PYR	LMW/ MWW	FFPI	PI
Fornebu	5.2692	0.5954	0.2195291	0.4151	0.1867
Tar. North	10.888	0.5226	0.2514688	0.4038	0.2465
Tar. Junction	12.968	0.4708	0.2728102	0.3994	0.1175
Tar. South	4.6347	0.6026	0.1488679	0.3791	0.3145
Nøstvedt	5.9969	0.5004	0.1881342	0.4437	0.1597
Vassum	6.9167	0.5034	0.2159406	0.4317	0.1968
Tenor	5.7843	0.746	0.1884265	0.434	0.142
Båntjern	8.9994	2.5614	0.2724912	0.2015	0.8254
Møllesvingen	4.2195	1.4867	0.1699968	0.2232	0.9633
Svarta	4.9	1.2923	NA	NA	NA

Appendix F – Cook's Distance

Influential observations by Cooks distance. Red line: cutoff line. Values above the line are 4 times the mean.

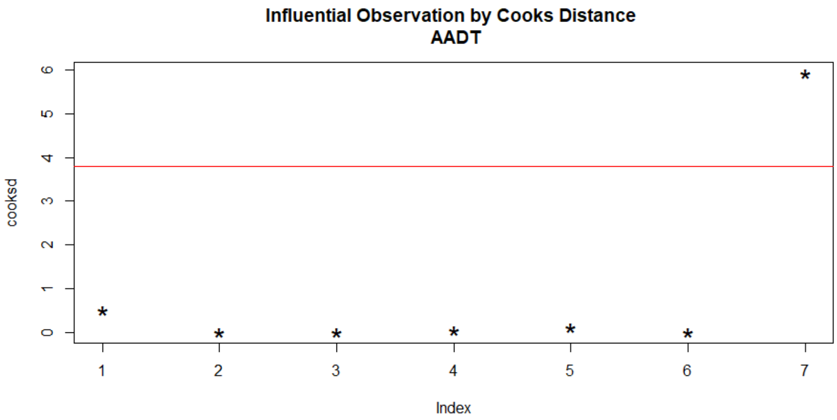


Figure 39 - ΣPAH16~AADT. Point #7: Tenor

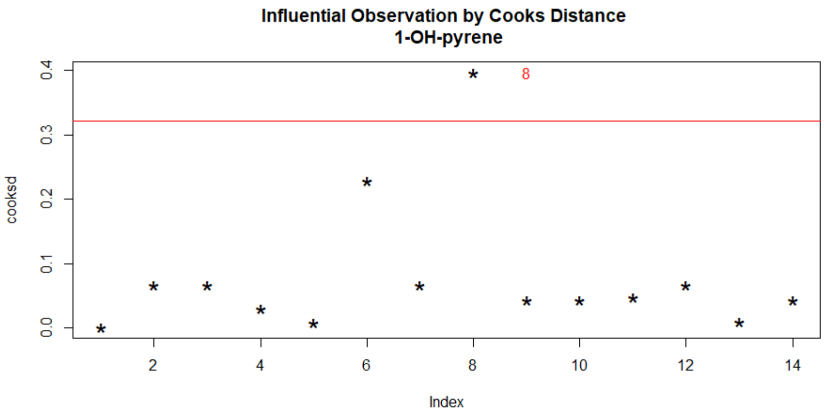


Figure 40 - 1-OH-pyrene. Point #8: Båntjern

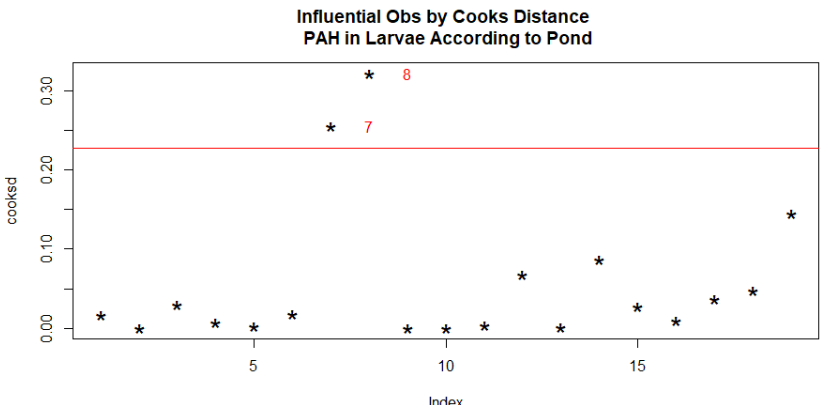


Figure 41 –PAH in larvae according to pond. Point #7: Big larvae, Tenor. Point #8: Big larvae, Båntjern.

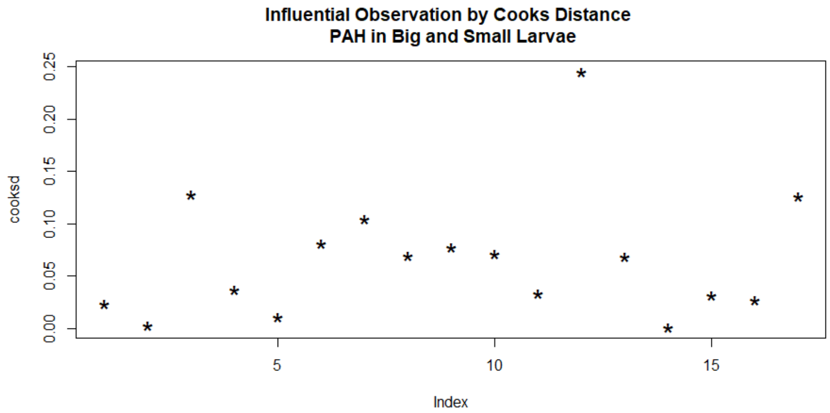
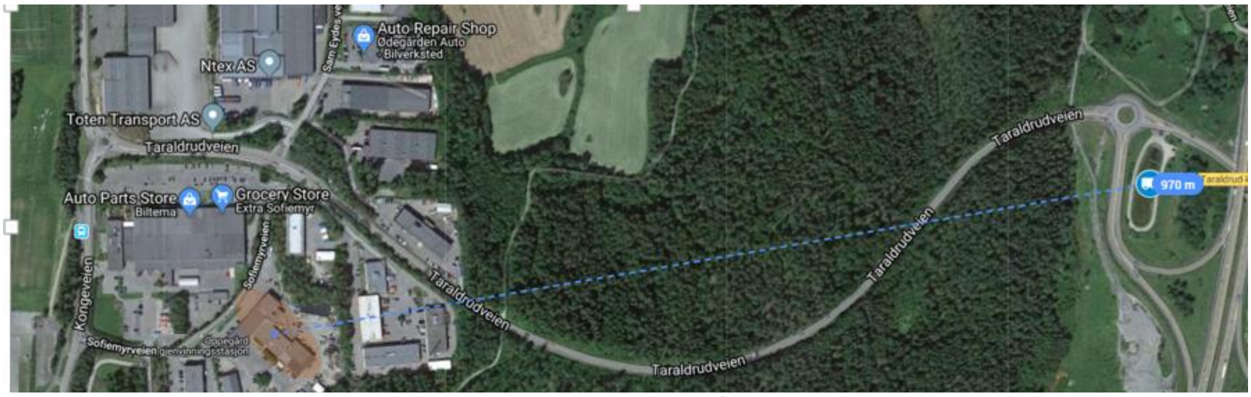


Figure 42 – Concentration of PAHs in big and small larvae. No influential points.

Appendix G – Sedimentation ponds

Sedimentation Pond (Construction year)	County Municipality	Size (m^2)	Recipient	Receives water from
Taraldrud North (2004)	Akershus Ski	780	Snipetjernet	E6 south (Ringnes - Oslo) 26000m2 of road between Taraldsrud and Oslo
Taraldrud Junction (2004)	Akershus Oppegård	1400	Not known	E6 south (Ringnes - Oslo)
Taraldrud South (2004)	Akershus Ski	474	Gjersjøelva/ Grytetjernet	E6 south (Ringnes - Oslo) 54500m2 of road between taralsrud and Oslo
Fornebu (2002)	Akershus Bærum	Forebay 145; main basin 480	Storøykilen nature reserve	Rv 166
Nøstvedt (2009)	Akersus Oppegård	Forebay 40 main basin:340	Gjersjøelva/ Tussetjernet	E6 south (Ringnes - Oslo)
Tenor (Slitu) (2007)	Østfold Eidsberg	Forebay 175; main basing 480	Lekumelva (Glommavassdraget)	E18 (Mormarken -- Sekkelsten)
Vassum (2000)	Akersus Frogn	Forebay 68 Main basinp:363	Årungelva	E6 south (Korsegården-Vassum) Tunnels Nordbytunnelen (3850 m long), Smihagen (950 m) and Vassum (850 m) + overflowed from 17000 m2 of road area



Picture 1 – Map shows the distance between Taraldrud Junction and Oppgård Recycling Station (highlighted). The pond is approximately 1km away from several businesses, and connected by a road.