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Genotoxic Effects in relation to Polycyclic Aromatic Hydrocarbons in Blood from Female Common Eiders (*Somateria mollissima*) in Arctic Environments

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*Photo on the front cover was takes Geir Wing Gabrielsen (Norwegian Polar Institute)

ABSTRACT

Increasing intensity of shipping, off-shore oil exploitation and cruise ship tourism are expected to enhance the background levels of polycyclic aromatic hydrocarbons (PAHs) in Arctic areas. Studies on possible relationships between this group of genotoxicants and genotoxic damage on the biota living in these areas are thus important. Most previous studies on genotoxic effects of PAHs have focused on the 16 parent compounds listed by United States Environmental Protection Agency (US-EPA). However, research has found alkylated PAHs to induce toxic effects in the biota. The common eider population at Svalbard has been suggested to be threatened by this contamination. Common eiders are low trophic level birds, and thus they are expected to have low biomagnification of PAHs. The breeding habits of these birds also make them prone to increasing levels of PAHs during the fasting period.

In the present study, whole blood from incubating common eider females was analysed for correlation between genotoxic damage and concentrations of parent PAHs and alkylated PAHs in Tromsø and remote Arctic areas. The genotoxic damage was measured as frequency of DNA double-strand breaks (DSB) and mean molecular length (MML) of the resulting fragments. Sampling was done at three locations in Kongsfjorden (Mietheholmen, Storholmen and Breholmen) and one near Tromsø (Grindøya).

The results showed similar blood levels of PAHs at all four locations. The levels of PAHs were generally low, below detection limit for all types of PAHs for 64 % of the birds. The birds at Grindøya had significantly higher frequency of DNA-DSBs compared to the birds at Breholmen in Kongsfjorden. No significant differences in DNA-FTM were found between the other locations.

A significant positive relationship between DNA-FTM and PAH in blood collected from individuals at Grindøya was found, even in the low concentrations in the present study. The alkylated PAHs were found to be the major contributor to this relationship, with a significant correlation to DNA-FTM. Parent PAHs, however, showed no correlation with DNA damage. This highlights the importance of studies of all types of PAHs when investigating possible genotoxic effects.

In Kongsfjorden, no significant correlation between DNA-FTM and types or sum PAH in blood was found. The low significance levels indicate that individual variation may be important in the Kongsfjorden location. The individuals were suggested to be influenced by differences in

sampling time during the incubation period, exposure to other contaminants, external conditions or biological factors, such as feeding habits. There may also be a closer coupling between the common eiders and their environment at Grindøya than in Kongsfjorden. Thus, a positive correlation between genotoxicity measured as frequency of DNA-DSB and different types and concentrations of PAHs cannot be dismissed in Kongsfjorden.

Further studies are recommended to include knowledge and observations on the start of incubation, for more deliberate choices of sampling times. Additionally, further studies on genotoxicity of PAH should also include PAH metabolites to review the full genotoxic damage induced by PAHs.

SAMMENDRAG

Økende intensitet av shipping, off-shore oljeutvinning og cruiseskipturisme forventes å øke bakgrunnsnivåene av polysykliske aromatiske hydrokarboner (PAHer) i arktiske områder. Studier på mulige sammenhenger mellom denne gruppen av gentoksikanter og gentoksisk skade på biota i disse områdene er derfor viktig. Tidligere studier på gentoksiske effekter av PAHer har i hovedsak fokusert på de 16 stamforbindelsene oppført av United States Environmental Protection Agency (US-EPA). Dette til tross for at forskning har vist at alkylerte PAHer kan indusere toksiske effekter i biota. Denne forurensningen har blitt foreslått å true ærfuglbestanden på Svalbard. Ærfugl har korte næringskjeder, og er derfor forventet å være lite utsatt for biomagnifisering av PAHer. Disse fuglene er også utsatt for økende nivåer av PAHer gjennom inkuberingsperioden, grunnet fasting.

I dette studiet ble blod fra rugende ærfuglhunner analysert for sammenheng mellom gentoksisk skade og konsentrasjoner av stamPAHer og alkylerte PAHer i Tromsø og avsidesliggende arktiske områder. Den gentoksiske skaden ble målt som frekvensen av DNA dobbelt-tråd brudd (DSB) og gjennomsnittlig molekylær lengde (MML) av de resulterende fragmentene. Prøvetaking ble gjennomført på tre lokaliteter i Kongsfjorden (Mietheholmen, Storholmen og Breholmen) og en nær Tromsø (Grindøya).

Resultatene viste tilsvarende konsentrasjoner av PAHer i blod fra alle de fire lokalitetene. Nivåene av PAH var generelt lave, og under deteksjonsgrensen for alle typer PAHer for 64% av fuglene. Fuglene på Grindøya hadde signifikant høyere frekvens av DNA-DSB i forhold til fuglene på Breholmen i Kongsfjorden. Ingen signifikante forskjeller i DNA-FTM ble funnet mellom de andre lokalitetene.

En signifikant positiv sammenheng ble funnet mellom DNA-FTM og PAH-konsentrasjon i blod fra individer på Grindøya, selv i lave konsentrasjoner i denne studien. De alkylerte PAHene ble funnet å være den viktigste bidragsyteren til denne sammenhengen, med en signifikant korrelasjon til DNA-FTM. StamPAHer viste imidlertid ingen sammenheng med DNA-skade. Dette understreker viktigheten av å undersøke alle typer PAHer i studier på mulige gentoksiske effekter.

I Kongsfjorden ble det ikke funnet noen signifikant korrelasjon mellom DNA-FTM og typer eller sum PAH. De lave signifikansnivåene tyder på at individuell variasjon muligens kan være viktig i Kongsfjorden. Individene ble foreslått til å være påvirket av forskjeller i prøvetakingstidspunktet i løpet av inkubasjonstiden, eksponering for andre forurensninger, ytre

forhold eller biologiske faktorer, som for eksempel spisevaner. Det kan også være en tettere kopling mellom ærfugl og deres omgivelser på Grindøya enn i Kongsfjorden. Dermed kan en positiv korrelasjon mellom gentoksisitet målt som frekvensen av DNA-DSB og forskjellige typer og konsentrasjoner av PAHer ikke fraskrives i Kongsfjorden.

Videre studier er anbefalt å inkludere kunnskap og observasjoner om starttidspunktet av inkubasjonen, for mer bevisste valg av prøvetakingstider. I tillegg bør videre studier på gentoksisitet av PAH også inkludere PAH-metabolitter for mer presise målinger av den mulige gentoksisiteten indusert av PAHer.

ABBREVIATIONS

B[a]P	Benzo[a]pyrene
BM	Body mass
BPDE	Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide
C1-BT, C2-BT, C3-BT, C4-BT	Benzothiophenes with one, two, three or four alkyl groups
C1-Ch, C2-Ch, C3-Ch	Chrysenes with one, two or three alkyl groups
C1-DBT, C2-DBT, C3-DBT	Dibenzothiophene with one, two or three alkyl groups
C1-F, C2-F, C3-F	Fluorene with one, two or three alkyl groups
C1-N, C2-N, C3-N, C4-N	Naphthalene with one, two, three or four alkyl groups
C1-P, C2-P, C3-P, C4-P	Phenanthrenes with one, two, three or four alkyl groups
C1-Py, C2-Py	Pyrenes with one or two alkyl groups
CYP450	Cytochrome P450 oxidase system
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DSB	Double-strand break
DNA-FTM	DNA fraction, of total DNA, that migrated
EDTA	Ethylenediaminetetraacetic acid
Kbp	Kilo base pair
K_{ow}	Octanol water partition coefficient
LMPA	Low melting-point preparative agarose
MML	Median molecular length
PAH	Polycyclic aromatic hydrocarbon
RF	Relative front
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulfate
TBE	Tris-borate-EDTA
TE	Tris EDTA
US EPA	United States Environmental Protection Agency
UV	Ultraviolet radiation

CONTENT

1 INTRODUCTION

The intensity of shipping, off-shore oil exploitation and cruise ship tourism is predicted to increase in the Arctic due to climate change and subsequent retraction of ice cover (AMAP, 2007; Peters et al., 2011). This will inevitably lead to elevated background levels of polycyclic aromatic hydrocarbons (PAHs), as well as to increased risks of oil spill accidents and platform blow-outs causing acute PAH contamination. The increase in background levels of PAHs may have long-term genotoxic effects on the biota living in the exposed area. Extensive research has been performed to identify background levels and effects of PAHs in arctic areas, but most of these studies focuses on the parent compounds, and are often experimental exposure studies. Thus studies of environmentally relevant pollution, which includes analyses of alkylated PAH homologues are needed to identify the genotoxic effects of PAHs in this northern environment. Marine seabirds are relevant species for these studies of genotoxic effect of PAHs and to my knowledge; they have not been investigated for effects of PAHs in the Arctic.

1.1 POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons are natural and anthropogenic chemicals that consist of at least two hydrocarbon rings fused together (Newman, 2010). This large group of organic chemicals has a large size range from light two ringed structures (e.g. naphthalene) to heavier and more complex structures (e.g. coronene) (Newman, 2010).

1.1.1 Sources

PAHs are emitted into the environment through both anthropogenic and natural sources. In Norway the main sources of PAH emission are of anthropogenic origin, and include wood burning (during winter) and the aluminium industry (Miljødirektoratet, 2015). After an increased focus on the toxicity of these chemicals during the 1990's, the level of anthropogenic PAH emission has been reduced with more than 60 %, primarily due to more restrictive regulations (Miljødirektoratet, 2015). Over the last years the emission level of PAH has stabilized (Miljødirektoratet, 2015). In Svalbard, anthropogenic sources of PAHs include local emissions, mainly from coalmines and coal combustion, and long-range transported PAH from other parts of the world (Boitsov and Klungsøyr, 2015; Rose et al., 2004). PAHs from coalmines are released into the air and surrounding environment through binding to the coal dust released under the extraction and transport of coal, and through combustion processes (Boitsov and Klungsøyr, 2015). Water contamination of PAHs in Svalbard is mostly a result of surface

deposition from air, erosion of underwater fossil substance formations, long-range transport via water currents or municipal or coalmine wastewater runoff (Boitsov and Klungsøyr, 2015).

Natural sources of PAHs include non-anthropogenic combustions, such as forest fires, or leakage or erosion of coal or oil reservoirs. Due to the low temperatures and arctic climate, natural combustion processes do not pose an issue in Svalbard. However, long-range transported PAHs from Russia or southern Europe may have forest fires as a source. The sediments around Svalbard have been found to have elevated levels of natural PAHs compared to other areas in the Barents Sea (Boitsov and Klungsøyr, 2015; Dahle et al., 2006;2007). This is considered to be due to local sources of natural oil leakage, erosion and weathering of natural coal in the bedrock beneath Svalbard (Dahle et al., 2006). In addition to erosion of underwater coal formation, leakage from oil reservoir may contribute to the environmental exposure of PAHs (Dahle et al., 2007). These sources may also be induced by increasing anthropogenic petroleum industries (Dahle et al., 2007).

1.1.2 Transport and distribution of PAH

PAHs are today one of the most ubiquitous groups of chemical contaminants (Muñoz and Albores, 2011). This is due to their many natural and anthropogenic sources, and the semi-volatile nature of large amounts of these compounds (Skupinska et al., 2004). The semi-volatile nature make PAHs available for to long-range atmospheric and water current transport, which may lead to accumulation of these compounds in remote areas, such as the Arctic (Reiten, 2012; Skupinska et al., 2004). In general, lighter PAHs in gaseous form constitute a larger faction of the airborne PAHs than the heavier particle bound PAHs (Skupinska et al., 2004). The heavier PAH are also less volatile and bind stronger in the organic fraction of sediments due to higher lipophilicity (Ewers, 2009). Even with the long-range transport the amount of PAH pollution in Svalbard is still classified at the lowest level of pollution by the Norwegian pollution authority (SFT) (Reiten, 2012), compared to the levels found on the Norwegian mainland, especially in city harbours with high anthropogenic activity (Pedersen et al., 2015). Still, some locally high levels of especially alkylated PAH has been found in the sediments in Kongsfjorden (Granberg, unpublished).

Due to the distribution of natural and anthropogenic sources, and the general trend that the contamination levels are higher closer to the source there are differences in the concentration of PAHs in the arctic areas (Skupinska et al., 2004). Studies conducted by Dahle et al. (2006; 2007) have shown that the distribution of PAH varies within the Barents Sea, as well as between

different regions in Svalbard. Additionally, this study showed differences in PAH composition and sources between locations. In Svalbard high levels of PAHs have been found in sediments, and source has been defined as the erosion of underwater fossil substances (Boitsov and Klungsøyr, 2014; Dahle et al., 2006). Furthermore, the main contamination load was found in the fine-grained fractions of the sediments (Dahle et al., 2007). This as in grain size affect the sediments bioavailability of organic compounds due to the larger surface area compared to size in the smaller grain sized sediments (Dahle et al., 2007). A positive correlation has also been found between the PAH size and binding affinity in the organic fractions of the sediments (Lindgren et al., 2014).

1.1.3 Properties of different groups of PAH

PAHs can be divided into at least two groups; parent PAH and alkylated PAH (Figure 1), that both include a wide variety of compounds.

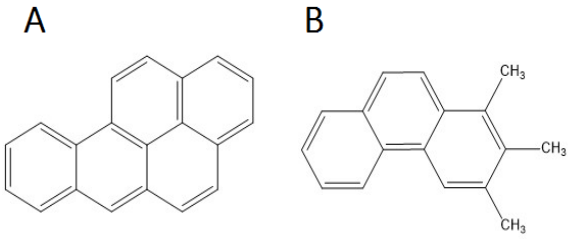

Figure 1. Examples of molecules from the two groups of PAH; A) Parent compound benzo[a]pyrene, and B) Alkylated PAH homologue C3-phenanthrene.

Parent PAHs mostly consist only of unsubstituted fused rings (Figure 1A), and are of relatively low abundance compared to the alkylated PAH homologues depending on the source. Almost all field studies performed on the concentrations and effects of PAHs only report on the 16 parent PAHs (PAH16) certified by United States Environmental Protection Agency (US-EPA). The US-EPA list include naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene and benzo[ghi]perylene (Keith, 2015). In further discussion, these US-EPA listed compounds are referred to as parent PAHs. The parent PAHs have been used by the Ministry of Climate and Environment (Norway) as a parameter for describing the environmental conditions based on PAH pollution from combustion sources such as car and shipping traffic, industry and burning of wood or coal (Klif, 2011). PAH pollution is divided into five classes based on the pollution level, where class I and II are no to low polluted areas, and III, IV and V are defined as different

degrees of polluted areas (Klif, 2011). The PAHs found in the Barents sea around Svalbard fall under class II, which is defined as low parent PAH pollution and low environmental risk (Klif, 2011). For comparison, the areas of Tromsøsundet, Tromsø, were characterised as class III for PAH16 before the massive cleaning of the harbour from 2010 to 2012 (Miljødirektoratet, 2013; Pettersen, 2009).

In addition to the low level of combustion-produced parent PAH found in Svalbard, high levels of naphthalene, phenanthrene and dibenzothiophene (NPD) have been observed (Boitsov and Klungsøyr, 2014). These represent the sum of small PAH, only two or three rings, that mostly have non-heated fossil substances as a source (Boitsov and Klungsøyr, 2014). This NPD group include naphthalene, phenanthrene, dibenzothiophene and their alkylated homologues (Boitsov and Klungsøyr, 2014).

Alkylated PAH homologues differ from the parent PAHs in that they have chemical groups, alkyl groups, added to the structure (Newman, 2010) (Figure 1B). The alkylated PAH homologues have been found to constitute a larger fraction of total PAH in crude oil, produced water and natural coal (Danion et al., 2011; Laumann et al., 2011; Sundt et al., 2011). They also occur more frequently than their parent homologues in contaminated sediments, waters and oil exposed organisms (Granberg, unpublished; Miles et al., 2007; Pampanin and Sydnes, 2013; Stange and Klungsoyr, 1997). Despite the larger abundance of alkylated PAH in the environment, they have been much less studied than their parent compounds. Alkylation of PAH under formation is inversely proportional to the formation temperature, meaning that the degree of alkylation of PAH decreases with higher temperatures under formation (Blais et al., 2015). A large amount of highly alkylated PAHs may thus indicate a non-combustion source, while low alkylation may indicate a high temperature source (Blais et al., 2015).

By chemical analysis, a determination of which PAHs that are present in a certain environment can be established, and possible sources can be identified by looking at the different PAH ratios (Blais et al., 2015).

1.1.4 Uptake and biotransformation of PAHs

The uptake and biotransformation of PAHs depend on the bioavailability of the PAHs and the complexity of the absorbing organism (Lehman-McKeeman, 2013). PAHs are highly lipophilic and thus the PAHs deposited to the surface waters tend to bind to particles and sink to the bottom, where they bind in the sediments (Neff, 2002). The uptake of PAHs in birds is mainly through diet, and thus their feed and feeding habits affect the contamination levels of the birds.

Bottom feeders, including bivalve filter feeders such as mussels, are especially exposed to PAHs in sediments (Boehm et al., 1982; Granberg and Selck 2007). Filter feeders obtain food by filtering particles from the water (Boehm et al., 1982). These marine invertebrates are considered to accumulate both parent and alkylated PAHs, and their toxic metabolites due to poor elimination capacities (Granberg and Selck 2007; Driscoll and McElroy, 1996). Because of their poor elimination capacity, many molluscs (e.g. mussels) reflect the contamination level of the environment (D'Adamo et al., 1997). For higher-level organisms, parent PAHs are not considered to biomagnify (Broman et al., 1990; Neff, 1985), which is attributed to the fact that they are able to metabolize PAHs to more hydrophilic and assumingly more excretable forms. For more efficient excretion, birds have a higher number of biotransformation enzymes and mechanisms that produce more hydrophilic substances that are more easily excreted (Broman et al., 1990; Camus et al., 2003). The highest biotransformation of PAH is conducted in the liver, which is the organ with the highest concentrations of biotransformation enzymes, such as CYP450 and epoxide hydrolase (Parkinson et al., 2013). Other mechanisms are also involved in the biotransformation of parent PAH to their possibly toxic metabolites (Parkinson et al., 2013).

1.1.5 PAH toxicity

The ubiquitous distribution and high environmental concentrations and persistence of PAHs, makes the genotoxicity of these chemicals of special concern. Early studies on the toxicity of PAHs were mainly revolved around the parent compounds, which were mostly found not to bioaccumulate and biomagnify in the food web (D'Adamo et al., 1997; Neff, 1985). Even though most studies have focused on the parent compounds, many alkylated homologues have been found to be more persistent and tend to bioaccumulate and biomagnify to a greater degree in the food chain (Harris et al., 2011; Irwin et al., 1997). Recent studies (Harris et al., 2011; Lindgren et al., 2014; Sundt et al., 2011) have also attributed many of the genotoxic effects of PAHs to alkylated, rather than parent PAHs in a wide range of organism including those of meiofauna and the microbial benthic communities, mussels and sea otters.

1.2 GENETIC TOXICITY

Genetic toxicology is defined as the field of toxicology that assesses the effect of chemical and physical agents on DNA (Figure 2) and on the genetic processes of living cells (Preston and Hoffmann, 2013). This field of research measures genotoxic endpoints including DNA strand

breaks, sister chromatid exchange, unscheduled DNA synthesis and DNA adducts (Figure 2). Studies measuring genotoxic endpoint as results of chemical pollutants in avian species are few. One of the earliest studies on this subject was carried out by Dubois et al. (1995), who showed genotoxic effects in avian cell cultures exposed to polychlorinated biphenyls. Later field experiments have measured genotoxic effects of environmentally relevant concentrations of PCB through measurement of induction in amount of DNA double strand breaks (DSB) (Krøkje et al., 2006) and DNA-adduct (Østby et al., 2005). In both studies, the high trophic level bird, glaucous gull (*Larus hyperboreus*) was used. Furthermore, the genotoxicity of PAHs and organochlorines on chromosomes have been studied in the low trophic level common eider (*Somateria mollissima)* by Matson et al. (2004), and Fenstad et al. (2014) have studied the level of DNA-DSB in relation to a mixture of POPs in common eider.

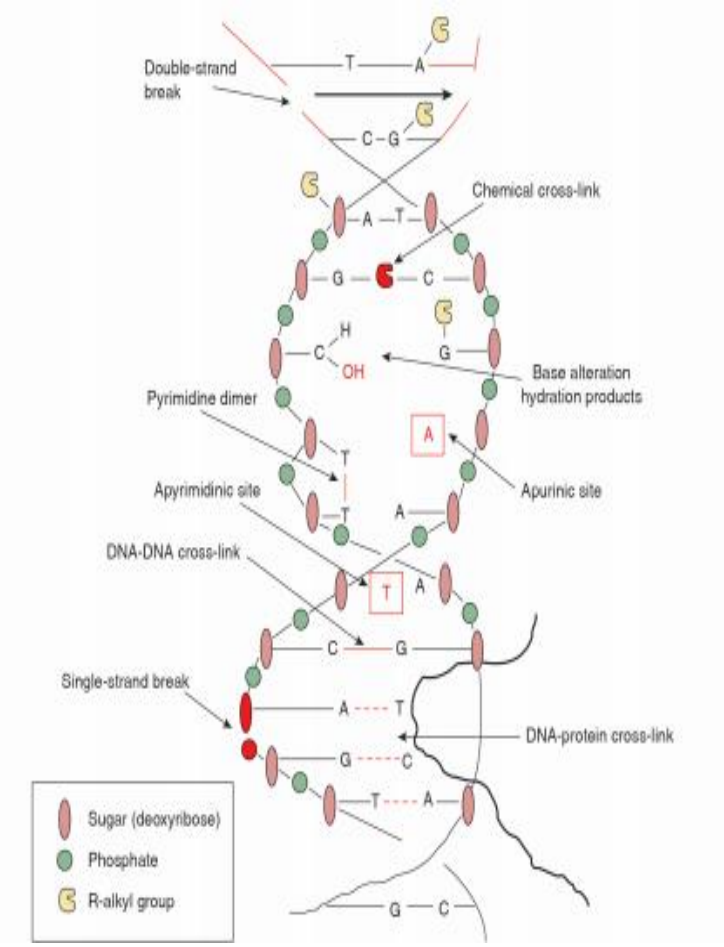

Figure 2. Illustration of different types of DNA lesions (Klaunig, 2013)

1.2.1 DNA Double strand break as a genotoxic endpoint

The loss or addition of bases are common events in the cell and are normally rapidly repaired by the cells repair mechanisms (Alberts et al., 2014). DNA strand breaks can occur if a loss or addition of a base is not correctly repaired. Errors in the cells repair system can happen if a chemical agent alter the DNA molecule before the base is replaced (Krøkje et al., 2006; Pfeiffer, 1998). DNA breaks can be single-stranded or double-stranded (Preston and Hoffmann, 2013). The DNA-DSB is fatal because they obstruct replication and transcription, and the continuity of the DNA template, which can result in apoptosis, mutations, chromosomal rearrangement

(Thacker, 1986; Jackson, 1999) and carcinogenesis (Jeggo, 1998; Kanaar et al., 1998, Pfeiffer, 1998).

The study of DNA-DSB gives a valid indication of genotoxic damage since the formation of DNA- DSB in most cases is a toxic endpoint of chemically induced lesions in DNA. The DNA DSB can occur directly or be produced during replication or repair as a result of other DNA lesions (Figure 2). The determination of DNA-DSB has proven as a successful indication of genotoxic damage. A main advantage of measuring genotoxicity through the frequency of DNA-DSB is the small amount of blood needed (Fenstad et al., 2014; Krøkje et al., 2006; Theodorakis et al., 1994). In addition to the ethical advantages of this method, it is possible to sample the same birds over time, as done by Fenstad et al. (2014). Furthermore, reliable results from very small amount of blood (µl), means that the sampling can be conducted without sacrificing the animals (Krøkje et al., 2006).

1.2.2 Genetic toxicity of PAHs

Many studies have been conducted on the toxicity of PAHs, and they have identified a wide range of PAHs and their metabolites as toxic to both aquatic and terrestrial biota through inducing DNA damage. The PAH induced DNA-damage occurs through DNA-adduct formation and increasing formation of reactive oxygen species (ROS) (Muñoz and Albores, 2011), both of which can result in DNA strand breaks. A study conducted by Newman (2010) has shown a difference in the toxicity of PAHs based on the molecular size. Lighter PAHs have in most cases been shown to have higher acute toxicity, but the heavier PAHs have shown more long-term effects, such as carcinogenicity (Newman, 2010).

Several PAHs, such as benzo[a]pyrene (B[a]P), have been classified as possible human carcinogens by the International Agency for Research on Cancer (IARC), and others have been suggested to increase birth defects and reproductive complications in animals (ATSDR, 1995). The carcinogenicity of PAHs are mainly induced by PAH metabolites forming DNA-adducts at locations critical to regulation of cell differentiation or growth (Gehle, 2011). A PAH metabolite often used an example, due to its high toxicity and carcinogenic properties are benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), which derives from biotransformation of B[a]P (Figure 3) (Muñoz and Albores, 2011). This epoxide is highly reactive and with its planar structure it is exceptional in forming adducts with DNA that, if not repaired, can lead to DNA strand breaks (Ewers, 2009; Preston and Hoffmann, 2013). Other, less toxic metabolites of B[a]P can be formed instead of BPDE, when oxidation occur on carbon atom located further

from the bay region (Figure 3), and thus easier hydrolysed further to less genotoxic compounds (Muñoz and Albores, 2011). The bay region of B[a]P prevents binding of catalysing enzymes due to steric hindrance in the molecule, which makes it hard to detoxify the compound through hydrolysis. As such the genetic toxicity of the different PAHs largely differs, but the metabolites of PAHs are generally seen as more genotoxic than their parent compounds.

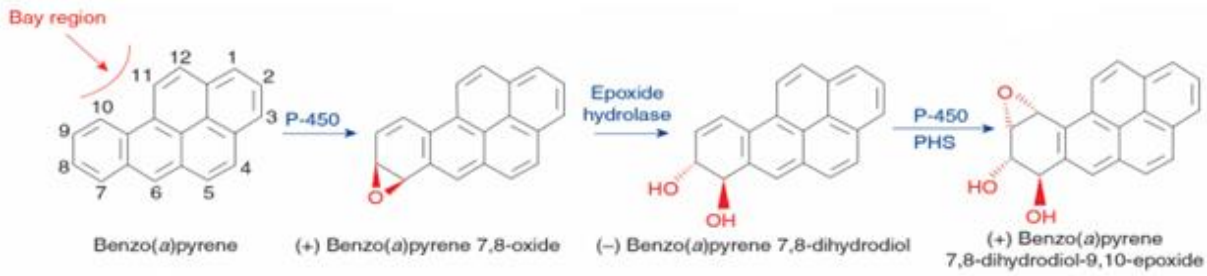

Figure 3. Biotransformation of benzo[a]pyrene to the genotoxic compound benzo[a]pyrene 7,8-dihydrodiol-9,10-epoxide (Klaunig, 2013).

1.2.3 Evolutionary toxicity

Evolutionary toxicology, also known as genetic ecotoxicology, has been defined as "the study of damages inflicted by contaminants to the genetic material of wild populations of plants and animals" (Bonisoli-Alquati, 2014). By expanding the view to include both genetic and population effects of chemical contaminants, a better picture of the long-term effects of pollution can be assessed.

Effects of genotoxic compounds represent a major challenge because the damage can be transmitted to the offspring (DeRosa et al., 1998). Such effects could cause altered dynamics at population and community level, which potentially can have great ecological impact (Belfiore and Anderson, 2001; Bickham, 2011; Bickham et al., 2000; Wurgler and Kramers, 1992). An increase in the amount of DNA-DSB can lead to a reduction in population size due to decreased reproduction success of exposed individuals. This will cause a reduction in genetic diversity and allele frequency of the population that may again lead to reduced population size (Bickham, 2011). Such a decrease may result in a bottleneck event where the resulting population has much lower genetic variation (Belfiore and Anderson, 2001; Bickham, 2011; Bickham et al., 2000), and thus are more sensitive to other stress induced by changing climate (Bickham et al., 2000) or increasing contamination levels (Bickham, 2011).

1.3 STUDY SPECIES - COMMON EIDER (*SOMATERIA MOLLISSIMA*)

1.3.1 Distribution of common eiders

The common eider (*Somateria mollissima)* breeds in the circumpolar zone on islands and along the coast of Northern Europe including Svalbard, Northern America and Siberia (BirdLife International, 2016). The overall European population, which constitutes over 60 % of the global population, is estimated to include between 791,000 and 955,000 pairs (Birdlife International, 2015). The common eiders are mainly resident on small islands for predator protection, with rocky coast where there are large amounts of molluscs for prey (BirdLife International, 2016).

The nominate race *borealis* that nests on islands in Svalbard experienced a population decrease in the 1900s due to egg and dawn harvesting, but has been stable after the founding of bird sanctuaries in 1973 (Bakken et al., 2003). Other anthropogenic factors including overharvesting of aquatic resources, pollution, disturbance and hunting have also shown to have negative effects on the population size (BirdLife International, 2016). The common eider has not shown the same decrease in these areas (Strøm and Descamps, 2016), with the exception of a steep population decrease in 2013, which differed from the trend observed over other years (Moe et al., 2016).

Compared to common eiders living on the main land and further south, the common eiders living in Svalbard are exposed to a harsh environment, and are due to this exposed to a larger amount of stress, which has been shown to make these birds and populations more sensitive to the added effects of pollutions (Fenstad et al., 2014).

1.3.2 Diet

The common eider is part of the marine food chain and feeds at a low tropic level on benthic invertebrates (Dahl et al., 2003). In northern Norway, common eiders primarily feed on blue mussel (*Mytilus edulis*) and roe of lumpfish (*Cyclopterus lumpus)* but occasionally also on other mollusc such as horse mussel *(Modiolus modiolus), Modiolaria discors* and ocean quahog (*Arctica islandica*) (Bustnes and Erikstad, 1988; Dahl et al., 2003; Ydenberg and Guillemette, 1991). However, on Svalbard, where blue mussels are absent, the eider ducks feed on a variety of benthic invertebrates, e.g. amphipods, gastropods and polychaetes (Lydersen et al., 1989). It forages close to the shoreline between rocks and dives down to 15 meters (Frimer, 1995). The simple bottom feeding prey of the common eiders are of low trophic levels in the food chain compared to prey of other marine birds, that often include fish of higher trophic level. By

feeding on low throphic level orgamisms the biomagnification of chemicals are assumed to be of lower consern in this bird compared to organisms higher in the foodchain. As the prey of common eiders normally are simple organisms without a well developed biotransformation, these organisms have low activation rate of chemicals through biotransformation (Broman et al., 1990). For the common eider this means high consumption of parent compounds compared to other marine bird feeding higher in the food chain (Broman et al., 1990). Marine birds feeding on small animals such as the blue mussel need to consume a large amount of feed. The high consumption of low trophic level organisms in the common eider leads to accumulation of parent PAHs in the fatty tissues of the bird (Bustnes, 2013).

1.3.3 Breeding

The common eiders typically breed on islands to avoid predators. In sub-arctic and arctic areas such as the northern Norway and Svalbard the predator absence depends on the ice cover between the mainland and the islands (Lehikoinen et al., 2006; Mehlum, 2012). Sea ice makes it possible for predators such as the arctic fox to get from the mainland out to the islands and predate on eggs and nestlings (Bakken et al., 2003). On the northern Norwegian mainland, this is typically in late April and May, and in Svalbard the sea ice typically melts from late May to late June (Mehlum, 1991). The common eider starts breeding when the nests have been free from ice several weeks (Mehlum, 1991). They breed for 24-26 days, starting from the day the first egg is laid. Common eider female ducks do not leave the nest for the entire breeding season, and survives on build up fat storage. During this fasting period, the female common eider may have a potential loss of 30-45% of its body mass (Fenstad et al., 2014). This reduction in body mass causes redistribution of lipophilic contaminants to the circulatory system (Fenstad et al., 2014). Consequently, the increased amount of contaminants in the blood can lead to adverse effects on the integrity of DNA.

The harsh breeding conditions in Svalbard cause the female common eiders in this area to have smaller clutch sizes compared to common eiders on the Norwegian mainland (Bakken et al., 2003), due to the mother's condition in this extreme environment. After hatching, the female can swim up to 20 km together with her chicks from the breeding ground to the feeding ground (Bustnes, 1996; Gauthier and Bédard, 1976). In the fall and wintertime, the birds located at Grindøya commonly stay inside an area of 20 km from the breeding ground (Erikstad et al., 2010), while common eider birds breeding in Kongsfjorden migrate to Iceland or the coastal areas of Norway, typically around Lofoten (Bakken et al., 2003; Hanssen et al, 2016).

1.4 GENOTOXIC EFFECTS OF PAHS IN COMMON EIDERS

Avian species are ideal bioindicators for studies on genotoxic effects of chemical contaminants, due to the diversity of their ecological niches, their ubiquity across environments, their conspicuousness, abundance and approachability, their well-known life histories and the availability of historical data series (Bonisoli-Alquati, 2014). The main feature of common eiders compared to other studied seabirds is their feeding and breeding habits. Common eiders feed lower in the food chain, with main food sources including bivalves and molluscs in norther Norway, and amphipods and polychaetes in Svalbard (Michael et al., 1997; Lydersen et al.,1989), as seen in section 1.3.2. These simple organisms without a well-developed biotransformation system are expected to have less biotransformed compounds than those that consume already biotransformed compounds in addition to its own biotransformation. (Granberg and Selck 2007; Driscoll and McElroy, 1996; Neff, 1985). The Svalbard eiders feeds on a wider variety of species at more different locations compared to Grindøya. Thus, the Svalbard eiders are expected to have higher individual differences in PAH contamination compared to the more stationary eiders at Grindøya, which mainly feed on the same species, the blue mussel.

Common eider females are expected to have higher concentrations of contamination in blood due to the extra stress induced by the fasting period (Fenstad et al., 2014). PAHs are known as genotoxicants in most animals, and potential human carcinogens (ATSDR, 1995; Newman, 2010). Therefore, it is important to study whether the concentration of the different types of PAHs in the Arctic is high enough to produce genotoxic damage in the biota in these areas. Previous studies on the genotoxic effects of organic pollutions in avian species are few, and the extrapolation of effects suggested for other mammalian species may not apply to avian species (Krøkje et al., 2006).

1.5 AIM OF THE STUDY

The aim of the present study was to investigate the relationship between genotoxic damage and concentrations of parent PAH, alkylated PAH homologous and total sum PAH in whole blood from common eider (*Somateria mollissima*), hereafter eider, at three locations in Kongsfjorden, Svalbard and one at Grindøya, Tromsø.

2 MATERIALS AND METHODS

2.1 SAMPLING AND SAMPLING LOCATION

2.1.1 Sampling method

Whole blood samples were collected from female eider ducks during the breeding season in May and June of 2014. All samples were collected by Kjetil Sagerup (Akvaplan-Norwegian Institute for or Water Research (NIVA)) and Sveinn Are Hanssen (Norwegian Institute for Nature Research (NINA)) in accordance with the method described by Fenstad et al. (2014). A fishing rod with a nylon snare at the end was used to capture the incubating birds, and the clutch size was observed. Body mass was determined by weighing (Pesola Medio-Line 42500, Ecotone-Poland), and the wing length (mm) was measured as the distance from the carpal joint to the tip of the longest primary when the wing is flattened, which was measured using a ruler with a stop. Blood (5-8 ml) was sampled from the jugular vein, alternatively the brachial vein, using a heparinized syringe, and transferred to Eppendorf tubes (1.5 ml) for later DNA analysis. Blood samples from Tromsø (Grindøya) were collected on May the 26th and June the 2nd in 2014, about three weeks prior to the sample collection on Svalbard (June 19th-25th). The whole blood samples were transported to Tromsø at the end of field season. The blood samples for DNA analysis were later transported to NTNU. The blood was kept on – 80 ℃ until analysis.

2.1.2 Sampling location

Whole blood samples were collected from twelve birds at Grindøya, Tromsø, and ten to eleven birds from each of three locations with increasing distance from the town Ny-Ålesund to the fjord mouth of Kongsfjorden, (Svalbard), i.e. Mietheholmen (n=10), Storholmen (n=11) and Breholmen (n=10). An overview of the sample locations in Kongsfjorden is given in Figure 4.

The population in Svalbard has been monitored by the Norwegian Polar Institute since 1981, and the total breeding pairs is estimated to 13.500-27.500 (Prestrud and Mehlum, 1991; Sander et al., 2006). The population size in Kongsfjorden is estimated to constitute 3000-3500 breeding pairs (Strøm and Descamps, 2016). The population on the small island of Grindøya (0.65 km^2) located outside the coast of Tromsø had an estimated population size from 200 to

500 breeding pairs in the period from 1987 to 2007 (Descamps et al., 2010), and about 150 pairs in 2009 (Erikstad et al., 2010).

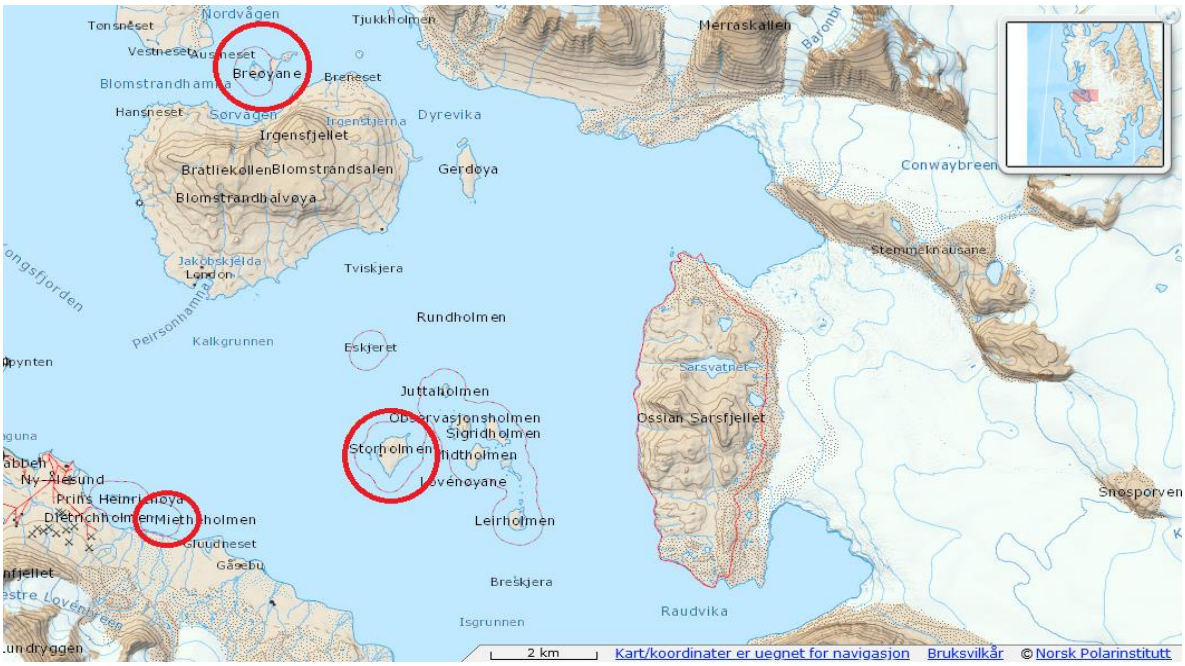

Figure 4. Sample locations in Kongsfjorden, Svalbard; Storholmen, Mietheholmen and Breholmen (TopoSvalbard, Norwegian Polar Institute, 2016).

2.2 CHEMICAL ANALYSIS

The chemical analysis of the eider blood for quantification of parent PAHs and alkylated PAHs homologues was conducted at the University of Copenhagen, Department of Plant and Environmental Sciences. The following PAHs were analysed for in the whole blood samples: naphthalene, acenaphthylene, acenaphthene, fluorene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno(1,2,3-c,d)pyrene, dibenzo[ah]anthracene, benzo[ghi]perylene, alkylated naphthalene's (C1-N, C2-N, C3-N and C4-N), alkylated dibenzothiophene (C1-DBT, C2-DBT and C3-DBT), alkylated flourenes (C1-F, C2-F and C3-F), alkylated pyrenes (C1-Py and C2-Py), alkylated phenanthrenes (C1-P, C2-P, C3-P and C4-P), alkylated chrysenes (C1-Ch, C2-Ch and C3-Ch) and alkylated benzothiophenes (C1-BT, C2-BT, C3-BT and C4-BT). In further discussions, these compounds make up the term total PAHs.

2.3 ANALYSIS OF DNA STRAND BREAKS

The analyses of DNA-DSB were performed by gel electrophoresis at the Department of Biology at NTNU. The principle behind gel electrophoresis is the use of an electric currant to separate molecules with the same charge (such as DNA with its negative change) by size. The process makes use of a porous gel, often made out of the polysaccharide agarose that are ideal for molecule sizes in the range between 100bp and 25kbp (Sambrook and Russell, 2001). The pores in the gel are filled with a running buffer for the DNA to wriggle through, and the DNA is located in wells at the anode side when the current is turned on. DNA occurs in a clustered supercoiled "ball" in the well. With large amount of fragmentation (DNA-DSB) this "ball" of DNA become more relaxed, leading to the release of more fragments into the gel, that due to its negative charge travels towards the cathode (Collins et al., 2008). This means that the amount of DNA migrated into the gel relative to the total amount of DNA in the in the well can be used as a measure for the amount of DNA-DSB in the DNA sample, and that the length of the migrating fragments is negatively correlating to the amount of DNA-DSB (Fenstad et al., 2014).

The plug preparation and agarose gel electrophoresis were performed on the eider whole blood as described by Fenstad et al. (2014). The method used was first developed for use on fish samples by Theodorakis et.al. (1994), and has since been modified for use in the study of genotoxicity in birds, such as the glaucous gull (Krøkje et al., 2006) and common eider (Fenstad et al., 2014). The method of measuring DNA-DSB was used as it includes a non-invasive sampling method.

2.3.1 Chemicals and Equipment

The chemicals and equipment used under plug preparation, agarose gel electrophoresis and detection of DNA-DSB are listed in Table 1.

2.3.2 Agarose plug preparation

Plugs were prepared as described by Theodorakis et al. (1994), to reduce the mechanistic damages to DNA inflicted during handling. The blood from eiders (10 µl) was diluted in TE-buffer (500ul, Tris base (10mM), EDTA (1mM), pH = 8) at 37 ̊C. Low-melting point agarose (LMPA, 1%, 37 ̊C) was then added in a 1:1 ratio to the TE-buffer and mixed, before the samples were centrifuged (8000x) for a few seconds to homogenize the sample and to avoid adding coagulated blood into the plugs. Plugs (50µl) were cast from the supernatant into plug moulds

Table 1. Chemicals and equipment used in the analysis of DNA-DSB.

Chemicals	***Producer***	***Product number***
Activated charcoal	Sigma-Aldrich	C3014-2
Agarose	Sigma	A9539
Boric acid (H3BO3)	Sigma	B7901
Ethyl bromide	Bio-Rad	161-0433
Ethylenediaminetatraacetic acid (EDTA)	Bio-Rad	161-0729
HindIII-digested lambda DNA	Fermentas	#SM0101
Low melting Agarose	Bio-Rad	162-0019
Lambda DNA	Fermentas	#SM0231
Loading Dye (6x)	Fermentas	#R0611
NaCl	Sigma	S3014
Proteinkinase K	Sigma-Aldrich	P2308
Sodium dodecyl sulphate (SDS)	Bio-Rad	161-0301
Trizma base	Sigma	T6066
Equipment	***Producer***	***Product number***
Centrifuge	Heraeus - Biofuge Fresco	75005521
Heating-block	Techne	
Electrophoresis equipment	Bio-Rad	
Filters	Whatman	10 311 851
Gel Doc 2000	Bio-Rad	
Indicator tape	Steam	
Metal spatles		
Microwave	Electrolux	
Pipettes 2ul, 20ul, 200ul, 500ul	Gilson – Pipetman	
Pipette tips		
20 ul	Molecular BioProducts	
200 ul	Sarstedt	70.760.502
500 ul	Sarstedt	70.762.100
Plastic tubes		
0.5 ml	Sarstedt	72.699
1.5 ml	Simport	T330-5N
Plug molds	Bio-Rad	#170-3713
Rotamax 120 orbital shaker	Heidolph	82004-958

and solidified at 4℃ for 1 hour. Lastly, the plugs were added to 550µl of lysis buffer (100 mM NaCl, 10 mM Tris, 25 mM EDTA, 0.5% SDS, pH 8, with proteinase K at 1 mg ml^{-1}) and incubated for 16 hours at 55℃ to lyse the cells and release the DNA in the plug.

2.3.3 Agarose gel electrophoresis

After the incubation, plugs were chilled at 4℃ from 4 to 6 hours before they were loaded into wells of a 0.6 % agarose gel. Two gels were run simultaneously. Three plugs from each individual bird were loaded into each gel, together with three standard ladders (lambda+HindIIIdigested lambda ladder, Appendix 1), as illustrated in Figure 5. LMPA (1%) was used to seal the wells. Tris-borate-EDTA (TBE)-buffer (90 m*M* Tris base, 90 m*M* boric acid, 2 m*M* EDTA, pH 8) was used both in making the gel and as a running buffer. The electrophoresis was run at 2.3 V cm^{-1} for 14 hours, before the gel was stained with ethidium bromide solution (one drop in 500 ml) for 2 hours. The gels were washed with tap water for 20 minutes on an orbital shaker to remove all access ethidium bromide not bound to DNA. Finally, the gel was imaged based on the UV- intensity (Bio-Rad Gel Doc 2000).

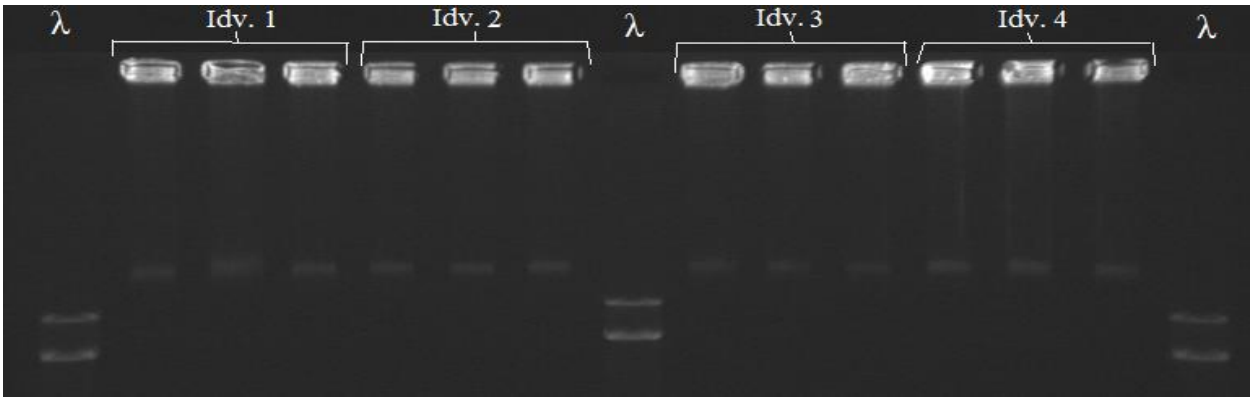

Figure 5. An example of the gel setup, including the three plugs from each of four different individuals (idv.), together with the three lanes with whole lambda + HindIII lambda digest ladder (Thermo Fisher). Imaged using the GelDoc2000.

2.4 STATISTICAL ANALYSIS

2.4.1 DNA damage analysis

2.4.1.1 Data extraction

The Bio-Rad image analysis software, Quality One, was used to capture the image produced by the Gel Doc system. Three DNA staining intensity curves were made for each individual plug (resulting in nine DNA staining intensity curves per individual) (Figure 5), to include potential

horizontal differences. As a relative measure indicating the frequency of DNA-DSB, extracted gel image data and DNA staining intensity curves were used in calculations for determining the fraction of total DNA loaded in the well that were released into the gel by electrophoresis (DNA-FTM, Fenstad et al., 2014), and the mean molecular length (MML) of the migrated fragments.

2.4.1.2 DNA migration (DNA-FTM)

The relative amount of DNA in the gel (both in well and migrated fragments) was measured by calculation of the area under the two peaks of the DNA staining intensity curve. The percentage of total DNA that had migrated as fragments into the gel (DNA-FTM) was calculated as a measure of the relative genotoxic damage in the form of DNA-DSB (Fenstad et al., 2014), using the equations:

Total DNA = A1 + A2

DNA-FTM = A2/Total DNA x 100 %,

Where A1 is the DNA left in the well, and is represented by the area under the graph of the first peaks (Figure 6). A2 is the DNA that migrated into the gel, represented by the area under the intensity curves of the second peaks (Figure 6).

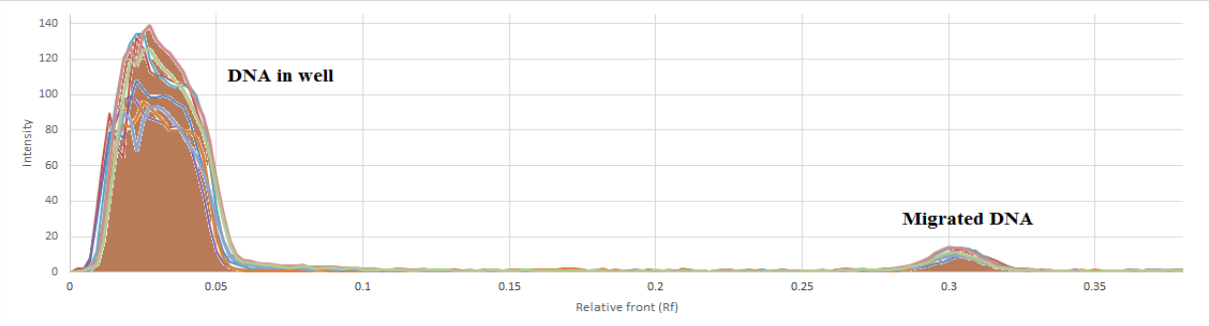

Figure 6. Plot of the intensity of the fluorescence against the migration distance of DNA in the gel. The areas under the intensity curves (marked in brown) were used in the measurement of DNA-FTM by comparing the two peaks and the MML by finding the corresponding rf-value of the 50 % of the intensity sum of the second peak.

2.4.1.3 Mean molecular length (MML)

As a secondary measure of genotoxic damage, the mean molecular length of the migrated DNA fragments were calculated from the extracted gel image data. Based on the gel image data, the rf-value corresponding to 50 % of the area under the second peak of the DNA staining intensity curve (Figure 7A) was calculated. This rf-value was compared with a standard curve obtained from the whole lambda + HindIII digested lambda fragments ladder run in the same gel (Figure

7B). The standard curve was derived from the known relationship between rf-values and length of DNA (Figure 7B). The MML value was expressed in kilo base pairs (kbp). Due to the low range of the standard DNA ladder, an extra point of 100 kbp was added to the standard curve to make it possible to quantify the MML over molecular length of the lambda-HindIII digested lambda standards.

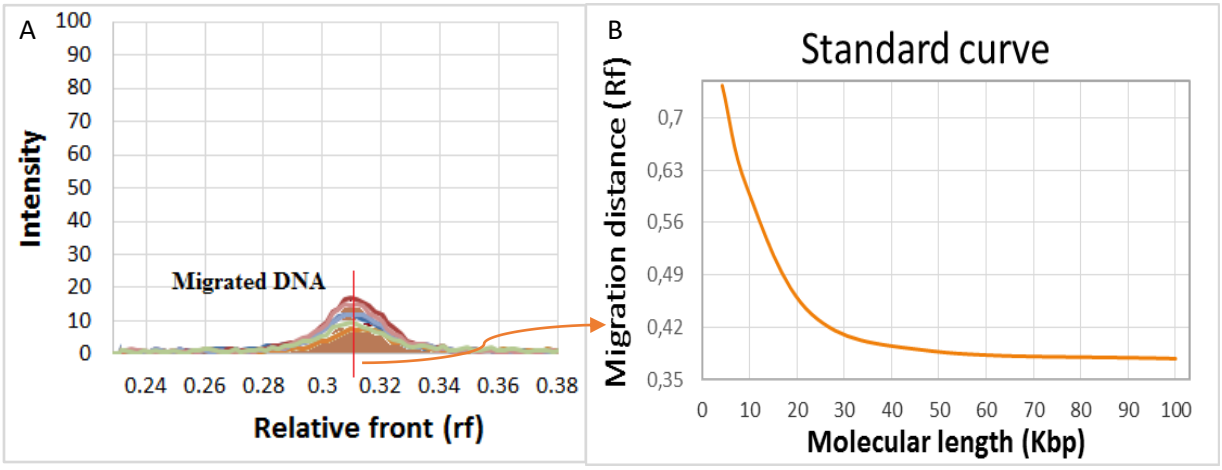

Figure 7. Illustration of the A) DNA staining intensity curve and B) the standard curve obtained from the whole lambda + HindIII digested lambda fragments involved in the calculations of the MML. Including an arrow symbolizing the relationship between the two curves.

2.4.2 Calculations and Statistical analysis

For the chemical analysis, the average blank was subtracted from the samples and average values for each location were calculated.

Statistical significant differences between birds from the different locations were analyzed using the parametric one-way ANOVA test and Scheffe simultaneous confidence intervals. The probability level determining significance was $P<0.05$.

3 RESULTS

3.1 BODY MASS AND CLUTCH SIZE

The average clutch size and body mass (BM) of the breeding female eiders from which the whole blood samples were collected in the present study are presented in Table 2. For individual biometric data, see Appendix 2.

Table 2. Average values for clutch size and body mass (BM), including the range of the samples and sample size (N), for the breeding eiders from three different islands in Kongsfjorden, Svalbard; Mietheholmen, Storholmen and Breholmen, and a population from Grindøya, Tromsø.

Location	***N***	***Average Clutch size (range)***	***Average BM (range)***
Mietheholmen (Svalbard)	10	1.80 (1-3)	1963 (1750 - 2320)
Storholmen (Svalbard)	11	2.91 (1-4)	1671 (1400 - 1950)
Breholmen (Svalbard)	10	3.90 (2-5)	1681 (1430 - 2060)
Grindøya (Tromsø)	12	4.25 (3-6)	2110 (1890 - 2430)

The measured body mass and clutch sizes from the different locations showed that the birds from Grindøya (Tromsø) had slightly larger body mass and larger clutch sizes compared to the eiders from the three locations in Kongsfjorden (Table 2). Statistically significant differences were found both in in body mass and clutch size between the different locations. The body mass of the eiders from Grindøya was found to be statistically significant ($p = 0.018$) higher than the body mass of the birds sampled from Storholmen and Breholmen. In addition and although not statistically significant, eiders from Grindøya had larger body mass than the ones from Mietheholmen (Table 2). The body mass of the birds from Mietheholmen was significantly larger than the body mass of the birds from the two other locations in Kongsfjorden; Storholmen and Breholmen.

The clutch sizes found in the present study (average from 1.80 to 4.25 eggs) were in the lower range compared to the normal clutch size for eiders (4-6 eggs) (Strøm and Descamps, 2016). At Grindøya, the clutch size (3-6 eggs) was within the normal range, and was significantly larger ($p<0.0005$) than the clutch size at Storholmen (1-4 eggs) and Mietheholmen (1-3 eggs). The average clutch size for eiders from all three locations in Svalbard was lower than the normal range. Among the locations in Kongsfjorden, Breholmen had the largest clutch sizes (avg. 3.90), but not the heaviest individuals. The birds sampled from Mietheholmen were observed to have

higher average body mass (1960 g) compared to those from Breholmen (1681 g) and Storholmen (1670.91 g), but a smaller clutch size (Figure 8). Individuals sampled from Storholmen had the lowest average body mass (1670.91 g) out of the four locations and the lightest individuals over all (1400 g). Still these birds had slightly larger clutch sizes than the birds at Mietheholmen. The birds at Mietheholmen had the largest body mass of the Svalbard locations. In two locations (Storholmen and Breholmen), eight individuals had body mass below 1600 g (Figure 8). No correlation was found between body mass and clutch size in the present study, neither as a total, nor for each of the four studied locations (Figure 8).

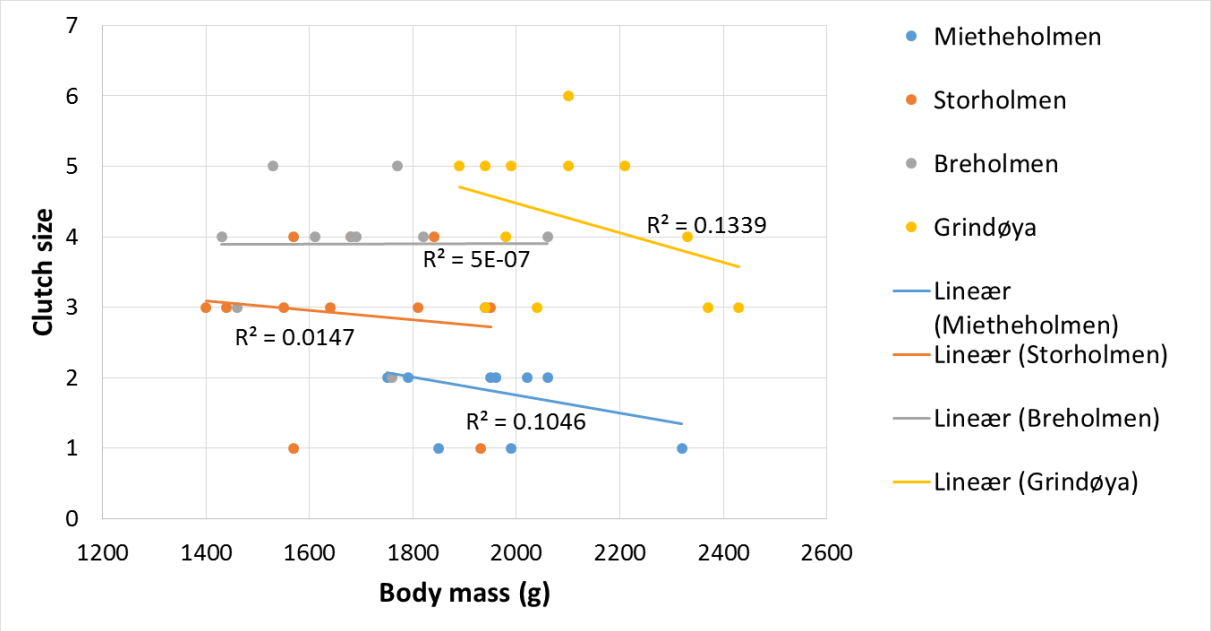

Figure 8. Correlations between clutch size and body mass for common eiders located at Mietheholmen (n=10), Storholmen (n=11), Breholmen (n=10) and Grindøya (n=12).

3.2 PAH COMPOSITION AND CONCENTRATION IN WHOLE BLOOD

The total concentration of PAHs showed great variation between individuals ranging from very low and below LOD (64% of the individuals) to concentrations of several hundred ng g whole blood $^{-1}$. All concentrations above zero after the subtraction of the average blank were included in the present study to be able to evaluate the very low concentration levels. Both parent PAHs and alkylated PAH homologues were found at all four locations: Mietheholmen, Storholmen, Breholmen and Grindøya. The average concentrations of the 42 different PAHs analyzed for during chemical analysis, are presented in Figure 9. For more details on individual levels, see Appendix 3.

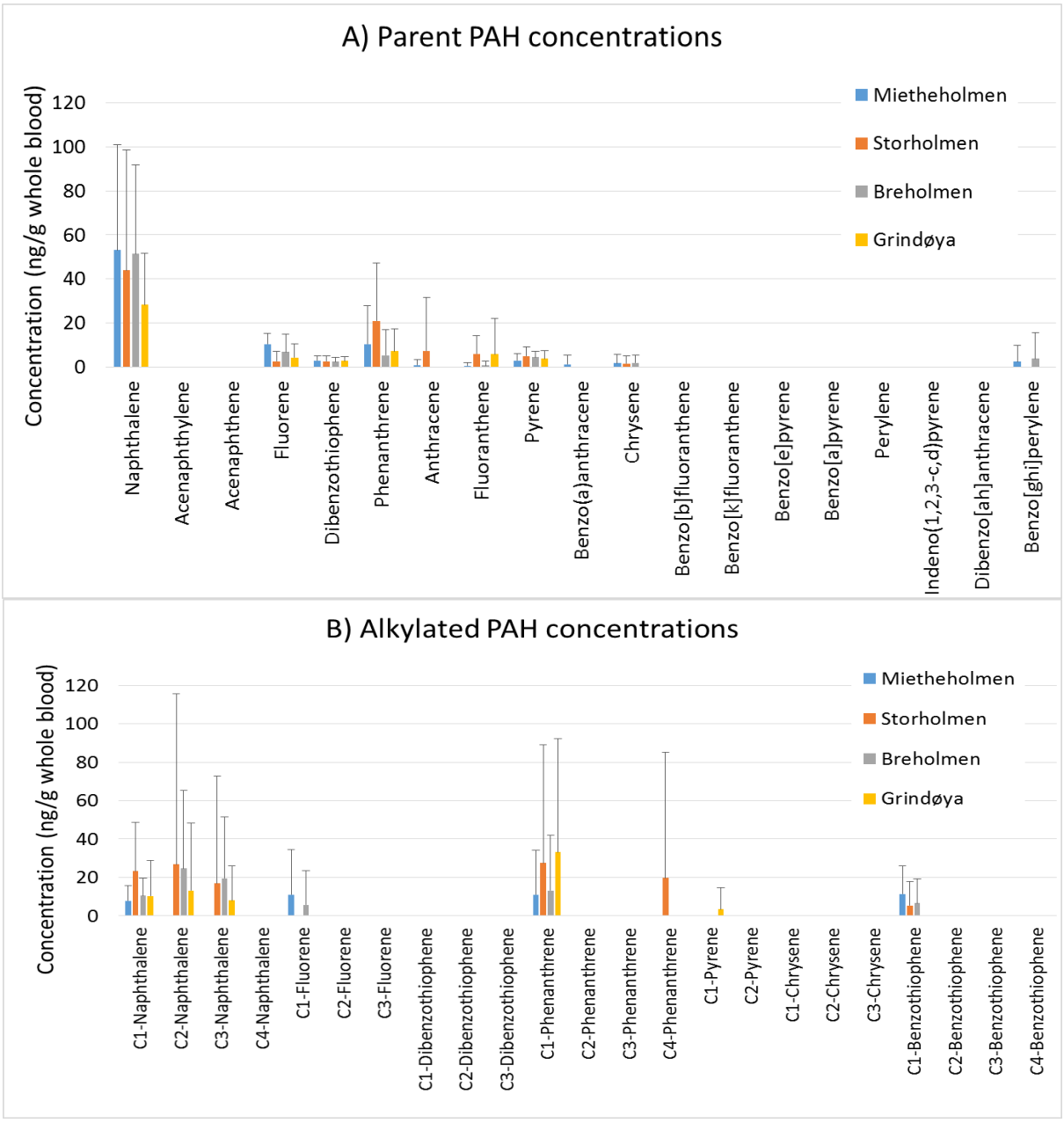

Figure 9. The average concentration (ng g whole blood^{-1}) of A) parent PAHs and B) alkylated PAHs in the 42 analysed PAHs in blood of common eider sampled at the four locations: Miethelholmen (n=10), Storholmen (n=11), Breholmen (n=10) and Grindøya (n=12).

Seven out of 18 parent compounds and 16 out of the 28 alkylated PAHs were found in the eider blood. All alkylated PAHs were of low alkylation, with the exception of C4-phenanthrene (Figure 9 B). C4-phenanthrene was only found in one (S7) out of forty-two samples.

The result of the chemical analysis showed large variation in all PAH concentrations within the same locations. All locations included individuals with few or non-detected PAHs, and

individuals with a high number of different PAHs. The concentrations of the different PAHs were also found to highly vary between individuals, as seen from the high error bars of Figure 9. The highest individual concentration (267 ng g whole blood^{-1}) was found for the second-degree alkylation of naphthalene in sample S7 from Storholmen (Figure 9 B). This sample was the only one from Storholmen containing C2-phenanthrene.

Naphthalene and alkylated naphthalene were present in high concentrations in the eider blood from all locations, though the birds from Grindøya had slightly lower average levels than those found in Svalbard. The highest average level of naphthalene was found in the eider blood from Mietheholmen. The samples from this location had the lowest average concentrations of alkylated naphthalene. Blood collected from Mietheholmen was found to have the highest level of different parent PAHs (10 out of 16), while blood from Storholmen had slightly higher level of different alkylated PAHs.

No significant difference was found between locations for the alkylated PAH (p=0.471), parent PAH (p= 0.937) or total PAH (p= 0.684) concentrations in the eider blood from Kongsfjorden (Svalbard) and Grindøya (Figure 10).

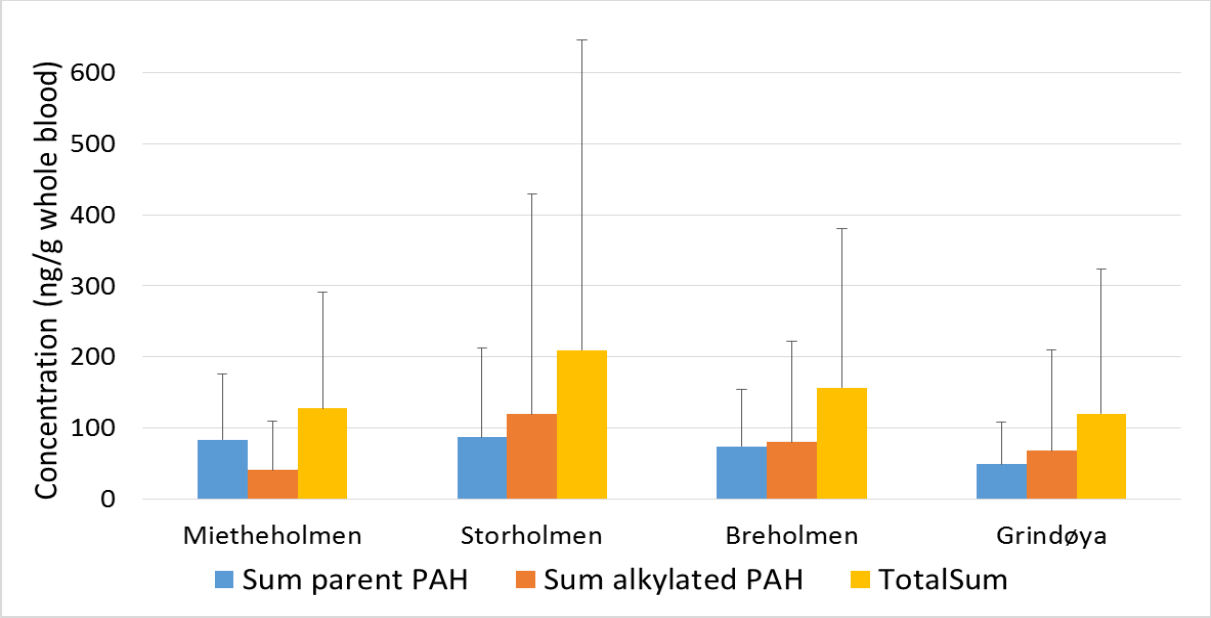

Figure 10. The sum concentration of parent PAH and alkylated PAH, together with total sum of PAH found in the whole blood samples collected from eiders at the four different location; Mietheholmen (n=10), Storholmen (n=11), Breholmen (n=10) and Grindøya (n=12).

The level of total sum PAH (Figure 10) was rather similar in the eiders from Mietheholmen, Breholmen and Grindøya, while the eiders from Storholmen showed a higher total concentration. Of the total sum PAH, alkylated PAHs showed equal or higher contribution than

parent PAHs in birds from Storholmen, Breholmen and Grindøya (Figure 10). On the contrary, the eiders from Mietheholmen were found to have higher concentrations of parent compounds compared to alkylated PAHs (Figure 10).

3.3 GENOTOXIC DAMAGE – DOUBLE STRAND BREAK ANALYSIS

The genotoxic damage is presented as DNA-FTM and MML, and the resulting average from the four locations is presented in Table 3. For more details on the different DNA-FTM and MML for each individual, see Appendix 4 and 5.

Table 3. Calculated average DNA-FTM (%) and MML (kbp) in the whole blood samples from eiders collected at Mietheholmen, Storholmen, Breholmen and Grindøya, including sample size (N) and range.

	N	***Avg. DNA-FTM (%)***	***Range***	***Avg. MML (kbp)***	***Range***
Mietheholmen	10	8.26	4.94 - 19.15	336.84	183.33 – 481.17
Storholmen	11	8.48	4.74 - 18.67	371.97	283.33 – 498.17
Breholmen	10	5.60	4.1 – 6.66	389.14	263.22 – 522.61
Grindøya	12	10.54	5.57 - 21.47	362.30	143.28 – 504.17

3.3.1 DNA-FTM

The degree of DNA-FTM in the eider blood cells was found to increase in the following order: Breholmen < Mietheholmen < Storholmen < Grindøya (Table 3). Eiders from Breholmen had both the lowest average (5.60 %) and the lowest individual level (4.1 %). This was also the most remote location in Kongsfjorden. The highest average DNA-FTM (10.54 %) was found in the Grindøya population, which also had the highest individual level (21.47 %). Eiders from Mietheholmen and Storholmen had quite similar levels of DNA-FTM, with averages between 8 and 9 %. A statistically significant difference ($p=0.041$) was found between the location with the lowest average DNA-FTM (Breholmen) and the highest average DNA-FTM (Grindøya). No statistical significant difference was found between any of the other locations.

A large variation in individual DNA-FTM levels was found within Mietheholmen, Storholmen and Grindøya, with the highest variation in the Grindøya population (Figure 11A). Only low variation was found among the birds sampled at Breholmen. Figure 11 shows the variation in DNA-FTM and MML between individual eiders within each site.

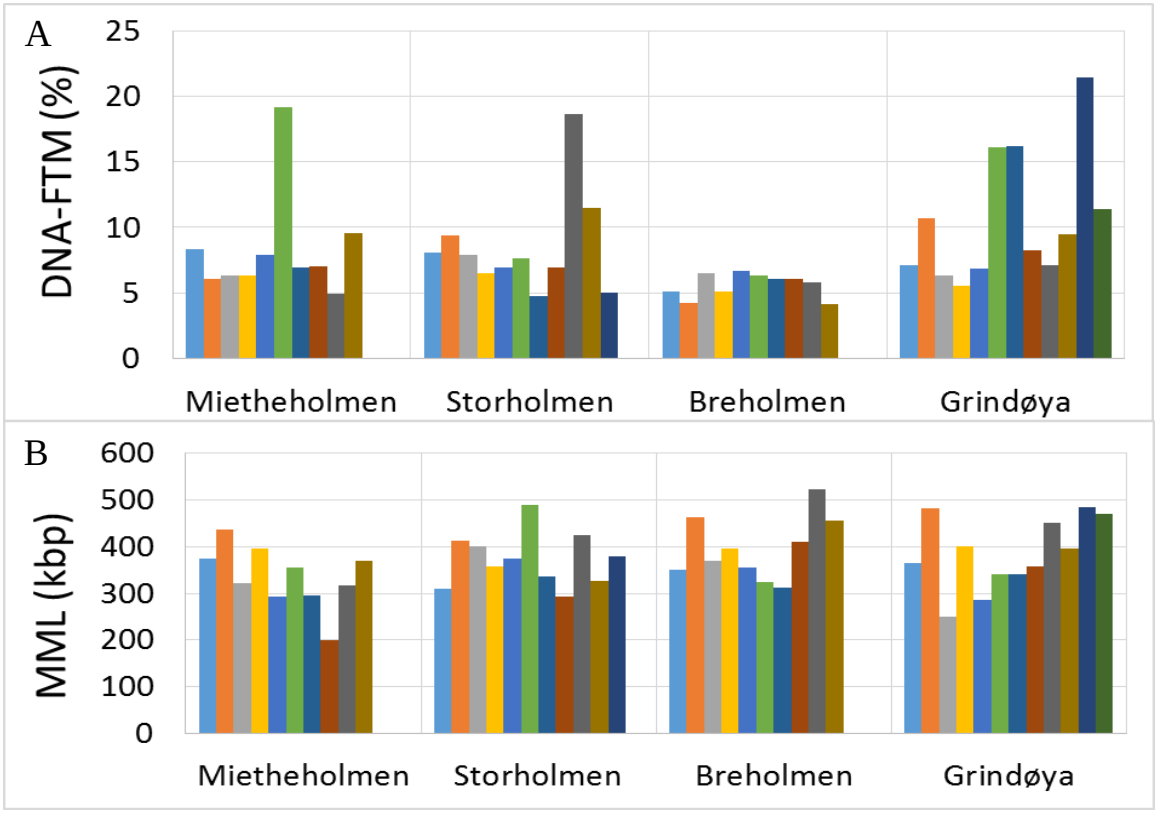

Figure 11. Calculated A) DNA-FTM (%) and B) MML (kbp) in whole blood collected from eiders at Mietheholmen (n=10), Storholmen (n=11), Breholmen (n=10) and Grindøya (n=12).

3.3.2 MML

The average MML was found to be highest in the blood cells from eiders collected at Breholmen, with a level of 389.14 kbp. The blood cells from eiders collected at Mietheholmen had the lowest level, amounting to 336.84 kbp. These average MML of the migrated fragments did not follow the same order as the average DNA-FTM (Figure 11 A and B). Neither did the MML vary as much as the DNA-FTMs between locations (Figure 11 A and B). Still the highest range in MML was found in the Grindøya population, similar to the highest range in DNA-FTM.

The individual levels of MML (lengths below 200 kbp) were found to be shortest at Grindøya and Mietheholmen. These were also the two locations with the highest individual levels of DNA-FTM (Table 3). Breholmen showed a high invers relationship between MML and DNA-FTM, while the other three locations showed a small increase in MML with increasing DNA-FTM.

3.4 CORRELATIONS BETWEEN PAH CONCENTRATIONS AND TYPES, BODY MASS AND DNA DAMAGE

3.4.1 PAH concentrations and body mass

Correlations between body mass and total sum PAH in eider blood samples collected at Mietheholmen, Storholmen, Breholmen and Grindøya are presented in Figure 12. Due to a high level of total PAHs over twice the size of the second highest concentration, one sample (S7) was excluded.

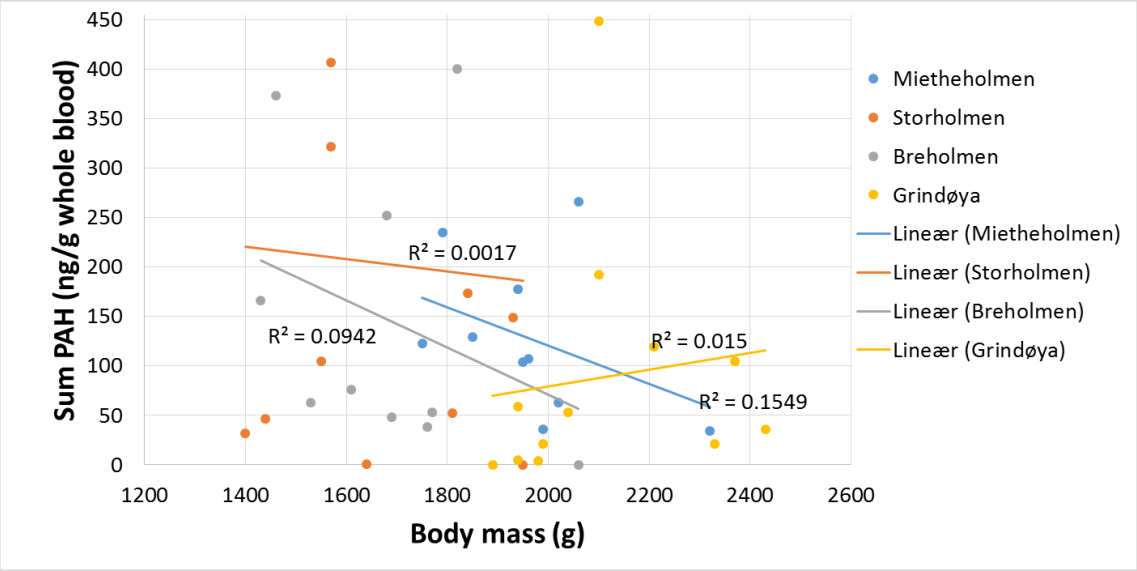

Figure 12. Correlation between total sum PAH concentrations (ng g whole blood^{-1}) and body mass (g) in the common eider populations at Mietheholmen (n=10), Storholmen (n=11), Breholmen (n=10) and Grindøya (n=12). The sample S7 was excluded due to extremely high sum PAHs

No significant correlation was found between body mass and total sum PAH at the four locations investigated in the present study, Mietheholmen (p=0.255), Storholmen (p=0.211), Breholmen (p=0.280) and Grindøya (p=0.966). The non-significant linear relationship between total PAH concentration and body mass showed a very weak negative relationship at Storholmen, while Grindøya showed a very slight positive relationship. In addition, no significant correlation was found between body mass and the sum parent PAH (p=0.78) or alkylated PAH (p=0.39).

3.4.2 PAH concentrations and DNA-FTM

No significant correlation was found between the average level of DNA-FTM and the sum of parent PAH, alkylated PAH and total sum PAH (Figure 13). A significant correlation was neither found between DNA-FTM and the sum of parent PAH (p=0.367), alkylated PAH (p=0.421) and total sum PAH (p= 0.865) on an individual level.

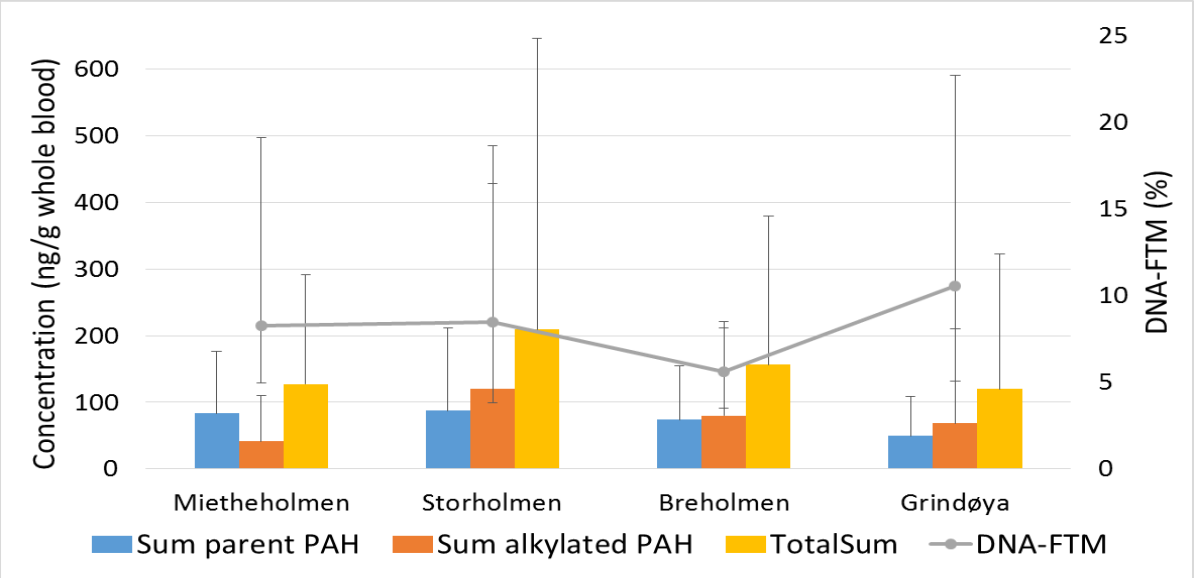

Figure 13. Total sum PAHs, sum of parent PAHs and alkylated PAHs homologues plotted together with the average DNA-FTM found in common eider whole blood from four different arctic locations; Mietheholmen (n=10), Storholmen (n=11), Breholmen (n=10) and Grindøya (n=12).

The sum of all three types of PAHs was found to be highest at Storholmen, but this location did not have the highest DNA-FTM (Figure 13). The low DNA-FTM found at Breholmen compared to Mietheholmen did not correlate with the higher total PAH concentration found at Breholmen. Additionally, Grindøya was found to have the highest levels of DNA-FTM, but not the highest levels of any type PAHs compared to the other locations (Figure 13).

Correlation analysis on individual levels of DNA-FTM and the sum and different types of PAHs are presented in Figure 14. For more compounds, see Appendix 6.

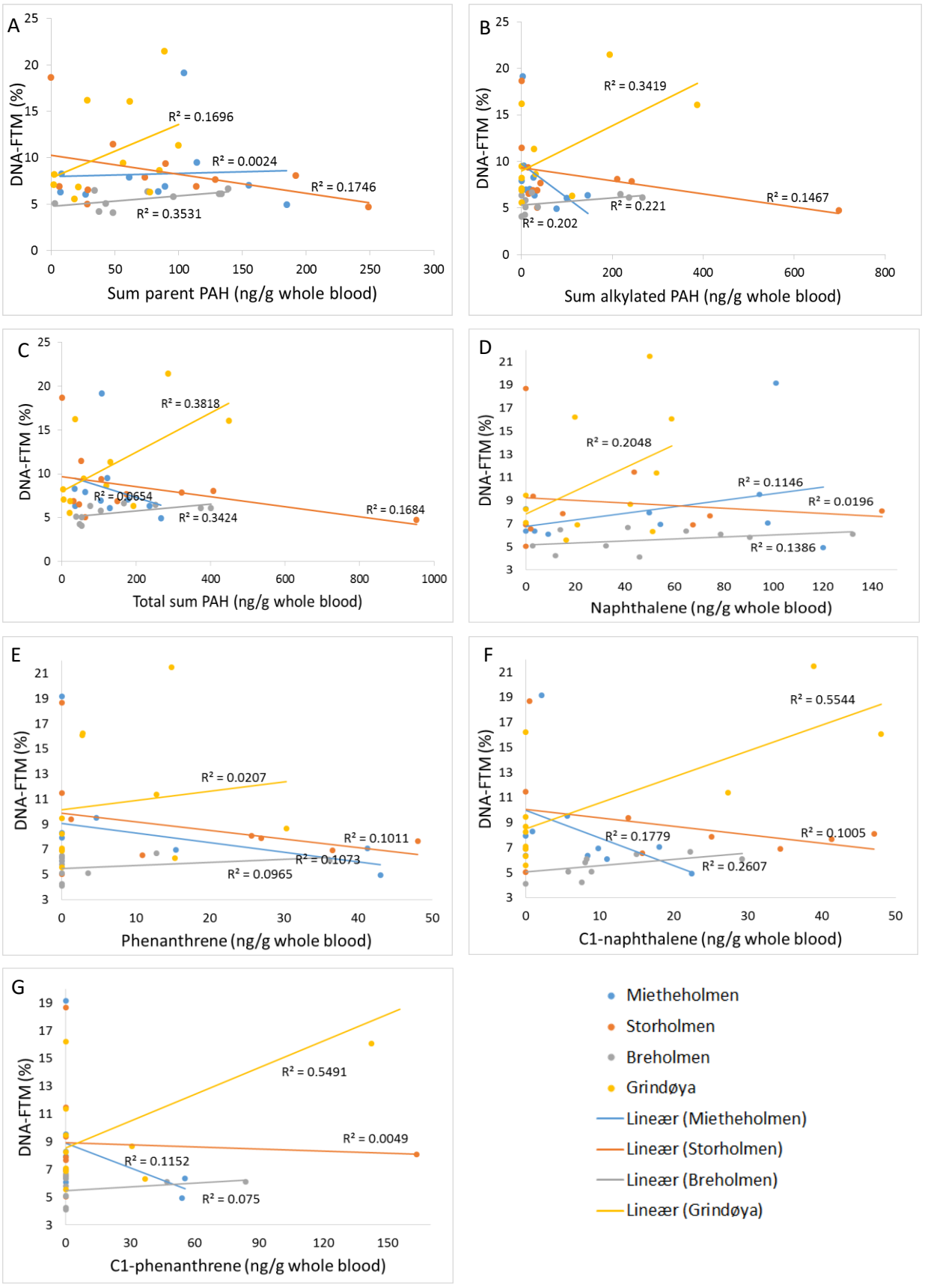

Figure 14. Relationships between DNA-FTM (%) and the concentration (ng g whole blood^{-1}) of A) parent PAH, B) alkylated PAH and C) total sum PAH in the blood collected at Mietheholmen (n=10), Storholmen (n=11), Breholmen (n=10) and Grindøya (n=12). Including D) naphthalene, E) phenanthrene, F) C1-naphthalene and G) C1-phenanthrene. Sample S7 was excluded due to extremely high sum PAHs values.

There was a significant and positive relationship (p=0.04) between Sum PAH and DNA-FTM for eiders at Grindøya (Figure 14 C). The eiders at Grindøya also showed a nearly significant relationship (p=0.06) between alkylated PAHs and DNA-FTM (Figure 14 B). At Breholmen, parent PAH and total sum PAH showed a nearly significant relationship with DNA-DTM (Figure 14 A and C). No significant relationship between the three different PAHs and DNA-FTM was found for Storholmen and Mietheholmen.

On average, alkylated naphthalene and phenanthrene were found in the highest concentrations amongst the alkylated compounds (Figure 13 A). A significant correlation was found between the low alkylated naphthalene and phenanthrene in relation to DNA-FTM (C1-N p= 0.0085, C1-P p= 0.009, Figure 14 F and G). No significant relationship was found between DNA-FTM and parent naphthalene and phenanthrene.

4 DISCUSSION

4.1 BODY MASS AND CLUTCH SIZE

The body mass of the eiders collected in the present study ranged from 1400 g to 2430 g and is within the normal range (1200g – 2800 g) described for eiders in Svalbard (Strøm and Descamps, 2016). The normal range for the worldwide population of eiders has been defined as 1600 g – 2800 g (Barth, 2009). In the present study, all individuals collected from Mietheholmen in Svalbard and Grindøya in Tromsø, were above 1600 g. However, some individuals from Storholmen and Breholmen were below this weight. Accordingly, the eiders in the present study have weights either below or in the lower part of the normal range. A possible explanation for the low body mass may be that the eiders in Svalbard belong to the subspecies *Borealis*, which is considered the smallest of the eider subspecies. This subspecies has been found to have average body mass of ca 1650 g (Baldassarra, 2014). However, the average body mass of 1864 g for the birds in the present study are high compared to the average for the subspecies *Borealis*.

The body mass of eiders vary over time (Fenstad et al., 2014). In 2008 (n=8) and 2009 (n=15), the body mass of female eider ducks at Storholmen was measured by Fenstad et al. (2014) at day 5 and day 20 of incubation. Fenstad et al. (2014) reported average body mass ranging from 1752 g at day 5 to 1348 g at day 20 in 2008, and correspondingly from 1790 g to 1422 g in 2009. The average body mass for the birds collected at Storholmen in the present study (2014) was 1672 g, which is only slightly lower than average for day 5 in Fenstad et al. (2014). The birds in the present study were sampled in June, but the time of sampling in relation to incubation is unknown. Still these relatively high body mass may indicate that the sampling was not conducted at the end of the incubation period. Early sampling of eiders may impose a problem in studies on maximal concentrations of organic contaminants and their genotoxicity in eiders, since the full effect of the fasting period develop late in the incubation.

In the present study, a significant difference ($p = 0.018$) in body mass between birds from Grindøya (Tromsø) and two of the locations in Kongsfjorden (Storholmen and Breholmen) was identified. These differences may possibly be explained by harsher living conditions in Svalbard compared to Grindøya. However, there were no significant differences between the average body mass of the birds collected at Mietheholmen (1963 g) and Grindøya (2110 g). Mietheholmen and Grindøya had significantly higher body mass compared to those found in

the other Svalbard locations (1671 g and 1681 g). This difference in body mass within Kongsfjorden may possibly be explained by variation in the starting time of incubation. All samples from Kongsfjorden were collected within few days. However, data on sampling time in relation to incubation at the four locations was absent in the present study.

The clutch sizes found in Kongsfjorden (on average 1.8 – 3.90 eggs for each location) were in the lower range compared to the normal clutch size for eiders (4-6 eggs) (Strøm and Descamps, 2016). For the Grindøya location, the clutch size (average of 4.25 eggs) was within, but still in the lower range of the normal clutch size (Strøm and Descamps, 2016). The average clutch sizes found at Mietheholmen (1.80) and Storholmen (2.91) were low compared to previous clutch sizes measured at the same locations in the period from 1981 to 2000 (Mehlum, 2012). Mehlum (2012) found average clutch size at Mietheholmen from 2.26 to 5.51 and at Storholmen from 2.76 to 4.13. Breholmen, however, showed high average clutch size of 3.90 eggs in the present study compared to the previously measured range from 2.49 to 3.46 (Mehlum, 2012).

Increasing body mass of eiders is known to positively correlate with clutch size (Eirikstad et al., 1993). This correlation was not found in the eider populations in the present study (Figure 8). A possible explanation for these contradicting results may be that Mietheholmen, which has the lowest average clutch size (1.8 eggs) and high average body mass (1963 g), had a later start of incubation compared to the other locations. Previous studies (Hanssen et al., 2002; Mehlum, 1991; 2012; Lehikoinen et al, 2006) have found late incubation to correlate negatively with clutch size of eider ducks, and as these birds are fasting during incubation, an early sampling time is expected to give higher body mass. These studies have shown that differences in incubation may be related to differences in ice melt, migration and female body conditions (Hanssen et al., 2002; Mehlum, 1991; 2012; Lehikoinen et al, 2006).

4.2 BLOOD CONCENTRATIONS OF PAHS IN FEMALE EIDERS

4.2.1 Total sum PAHs

The great variation in blood PAH levels, with 64% of the birds having very low or absent PAH concentrations, found in the present study may possibly be explained by the time of sampling during the incubation period. This is of special importance for eiders, as the time of sampling has been found to affect the level of organic contaminants in the blood from these birds (Fenstad et al., 2014). During the fasting period levels of organic contaminants, such as PAHs, increase as the birds mobilise part of their fat reserves (Bustnes et al., 2012; Fenstad et al., 2014). Fenstad

et al. (2014) measured the levels of persistent organic pollutions in eider females at day 5 and day 20 of incubation in 2008 and 2009. The levels of these organic contaminants were in both years found to increase during the fasting period, with a significantly increasing level of persistent organic pollution when the birds reached a weight below 1600g (Fenstad et al., 2014). Additionally, the higher metabolic rate in eiders at high latitudes will increase the weight loss of fasting and increase the level of mobilized PAHs during the incubating birds (Bustnes et al., 2012). As such, even though the PAH contamination load in the arctic today is relatively low, an increase in overall concentrations of organic contaminants, such as PAHs, will potentially have larger effects on fasting eiders in this areas compared to more temperate areas. However, the time in incubation of blood sampling in the present study is unknown, and thus it is unknown whether the level of PAHs in the eider blood is at its highest or lowest in the incubation period. Recommendations for further studies on organic contaminant concentrations in eiders is thus to include observations of the start of incubation to obtain more deliberate choice of sampling time. For studies on the maximal exposure level of PAHs in eiders, the samples should be collected after day 20 of incubation (Fenstad et al., 2014).

Other possible additional explanations for the observed low levels of PAHs in blood from the eider ducks may include the sampling in clean environments, low sediment bioavailability of PAHs and the low trophic level of the common eider.

The Norwegian pollution authorities have classified Svalbard as an area with low PAH pollution and low environmental risk (class II) (Klif, 2011; Reiten, 2012). This supports the low level of PAH contamination in the present study. However, differences between the four locations did not seem to correlate with the remoteness (Figure 10). Firstly, Grindøya was not found to have higher total blood levels of PAHs than the three Svalbard locations. This, even as Grindøya is located close to the city of Tromsø with a population of 72 000 (Statistics Norway, 2015). Secondly, the location closest to Ny-Ålesund, Mietheholmen, did not have the largest PAH concentrations of the Kongsfjorden samples. The blood levels of total PAHs in the Mietheholmen birds were similar to the most remote location (Breholmen). This, in addition to the higher level at Storholmen, may indicate that the main load of PAHs at the studied locations did not originate from the higher number of local pollution sources associated with towns, but rather from few local sources including weathering of natural coal or mining activity. The local sources also contribute to high levels of PAH in certain areas, possibly explaining the presence of high levels of several PAHs in some individuals, and the absence in others based on the different feeding grounds of the eider ducks.

Furthermore, the sediment bound PAHs have been found to have low bioavailability in the European arctic due to the low temperatures (Camus et al., 2002). This may affect the amount of PAHs taken up in the food chain. However, avian species living at higher latitudes also have been found to be more sensitive to biomagnified and bioaccumulated organic contaminants with toxic metabolites, as they have increased metabolic rates to maintain body temperature (Bustnes et al., 2012). Additionally, differences in bioavailability between sediments at the feedings ground may also possibly explain the individual variation in PAH concentrations within and between locations.

Finally, a possible explanation could be found in the food chain length. There is a short food chain from sestons to molluscs, such as mussels, to the eider ducks (Dahl et al., 2003). This makes these birds less subjected to biomagnification of organic contaminants, such as PAHs, compared to other fish eating seabirds (Harris et al., 2011; Irwin et al., 1997). Levels of several PAHs have been found at higher concentrations in omnivores compared to mulluscivores in the Russian arctic, with naphthalene having the largest difference in average concentration of 27.25 ng g ww^{-1} versus 18.25 ng g ww^{-1} (AMAP, 2004). In Kongsfjorden, a significantly lower concentration of several selected persistent organic pollutions have been found in eiders compared to the higher trophic level seabird, Brünnich's guillemot (*Uria lomvia*) (Murvoll et al., 2007). It is however important to bear in mind that PAHs are less persistent than the selected persistent organic pollutants investigated by Murvoll et al. (2007), and thus a less distinct difference due to food web length could be expected. Additionally, the inequality in organic contaminants concentration in bird species may also be explained by differences in intrinsic physiology and toxicokinetics between species (Murvoll et al., 2007).

Food chain length may also possibly contribute to explain the relatively high levels of total PAHs in the birds collected at Storholmen compared to the other locations. In Kongsfjorden, eiders feed on a variety of different invertebrates (Lydersen et al., 1989), which may be of slightly different trophic levels. A study conducted by Boehm et al. (1982) found differences between deposit-feeding (*Macoma balthica*) and suspension-feeding (*Mytilus edulis*) bivalves, where the deposit-feeding reflected the sediment concentration, while the suspension-feeders reflected the PAH concentrations and types in the water column. Differences in choice of food, position in the food chain and species differences in metabolic rates may possibly affect the concentration of all types of PAHs and other organic contamination within and between locations (Herzke et al., 2009).

4.2.2 Parent PAH

Similar to the total sum of PAH discussed above, the levels of parent PAHs were expected to be higher at Grindøya (Tromsø) compared to Kongsfjorden. The city traffic, industry and wood burning are known sources of parent PAHs (Norwegian Environment Agency, 2015), which would suggest higher levels of this type of PAH in the eiders from Grindøya compared to the eiders located in Svalbard. Similarly, low concentrations of parent PAHs were expected at the most remote location, Breholmen. However, similar levels of sum parent PAH were found at the four locations.

The similarities in parent PAH concentrations between locations may possibly be explained by the nature of the individual parent compounds found in the present study. This as the most abundant parent PAHs (naphthalene, phenanthrene and dibenzothiophene) are light PAHs of only two or three rings that often originate from petrogenic sources, including fossil substances or oil spill (Boitsov and Klungsøyr, 2014). The absence of heavier PAH compounds may possibly be explained by a lower bioavailability due to molecule properties including lipophilicity, structure and size. Negative correlations have previously been found between the molecular size and lipophilicity (K_{ow}) and contaminant concentrations in marine organisms (Baumard et al., 1998; Neff, 2002). This corresponds to the presence of only smaller size PAHs with low log K_{ow} found in the present study.

The absence of heavier PAHs may also possibly be explained by the low bioavailability of the heavier PAHs in the sediments (Camus et al., 2002; Lindgren et al., 2014). In an experimental study conducted in Isfjorden (Svalbard) exposing sediments to oil, heavier PAHs in pray of eiders, the bivalve *M. truncate,* were not found, even though sediment samples showed the presence of these PAHs (Camus et al., 2003). The high amount of lighter PAHs compared to heavier PAHs may also possibly be affected by lighter PAH compounds composing a larger fraction of the airborne long range transported PAHs that are deposited in Kongsfjorden and around Grindøya (Ewers, 2009; Muñoz and Albores, 2011).

4.2.3 Alkylated PAH

Alkylated PAHs were found in similar or slightly higher concentrations than parent compounds at all locations, except Mietheholmen (Figure 10). This is in line with previous studies (Miles et al., 2007; Pampanin and Sydnes, 2013; Stange and Klungsoyr, 1997), which have shown alkylated PAHs to occur more frequently than their parent homologues in contaminated

sediment, waters and oil exposed organisms. Moreover, a high alkylated PAH:parent PAH ratio was expected in the eider blood samples due to the elevated levels of alkylated PAHs in Svalbard sediments (Granberg, unpublished). Furthermore, the dominance of alkylated PAHs in the PAH signature of the sediments of Kongsfjorden, suggests petrogenic rather than pyrogenic sources of PAH emission (Granberg, unpublished).

No significant difference was found between Grindøya and the three Kongsfjorden locations (Figure 10). The similar levels between Grindøya and Kongsfjorden were unexpected, since the sediment PAH fingerprint has been found to differ (Granberg, unpublished). The difference in main sources of PAH between Grindøya and Tromsø affects the ratio between different types of PAHs in the sediments, with a high alkylated PAH:parent PAH ratio in Kongsfjorden and a low ratio off shore of Grindøya. The high ratio of alkylated PAH:parent PAH in the blood collected from the eider ducks located at Grindøya may possibly be explained by the slower elimination rate and higher persistence of alkylated PAHs compared to parent PAHs in biota (Harris et al., 2011).

The deviating low level of alkylated PAHs compared to parent PAHs found in the blood collected at Mietheholmen was unexpected. This as sediment samples from locations of the coast of the same islands in Kongsfjorden in the same year (2014) showed higher levels of alkylated PAHs (almost 3000 μg kg DW^{-1}) at Mietheholmen compared to the other Kongsfjorden locations (Granberg et al, unpublished). A possible explanation for these contradicting results may be difference in sediment types between locations, resulting in different bioavailability (Lindgren et al., 2014). Still sediments with lower bioavailability would not change the ratio, only the concentrations and size of the compounds entering the food chain. A combination of low sediment bioavailability of PAHs, in addition to a local source of parent PAHs close to the feeding grounds of the Mietheholmen populations may possibly explain the low ratio of alkylated PAH:parent PAH at Mietheholmen.

The difference in the PAH signature between the closely located Storholmen and Mietheholmen may also possibly be due to differences in migratory feeding grounds. Eiders in Svalbard are known to migrate to Iceland or along the coast of norther Norway (Hanssen et al., 2016). The exact migration location for the individual birds in the present study is unknown. However, differences in pollution level at the areas the birds migrate to, may have affected the blood concentrations of PAHs in the eider ducks. Other possible explanation may be linked to the high individual variation, which will be further discussed in section 4.4.2, or to an adaptive

response of increasing biotransformation activity due to long-time exposures to the parent PAHs (Parkinson et al., 2013).

The low level of alkylation found the present study, with only one PAH found with more than two alkyl groups (C3-naphthalene), may possibly be explained by the higher lipophilicity of the more alkylated compounds (Irwin et al. 1997). These results in lower bioavailability due to a stronger binding to organic matter in the sediments, and thus less of these compounds are entering the food chain (Baumard et al., 1999). Then again, a higher lipophilicity will also induce the accumulation of an absorbed chemical in the mussel and eider tissue (Baumard et al., 1999; Irwin et al. 1997). At Mietheholmen no compounds were found with more than one alkyl group. This may indicate sources of combustion for the alkylated PAHs in the Mietheholmen samples.

The greater concentrations of alkylated PAHs compared to parent compound found in the eider ducks from three out of four locations in the present study, indicate that the parent PAHs do not accurately describe PAH concentrations or diversity in the studied areas. Additional support for this statement is the similar sediment distribution (Granberg, unpublished) and the higher persistence of alkylated compared to parent PAHs in biota (Harris et al., 2011).

4.3 GENOTOXIC EFFECTS

4.3.1 Level of genotoxic damage

4.3.1.1 DNA-FTM

The fraction of total DNA that migrated in the whole blood samples in the present study ranged from about 4 % to 22 %. These levels are low compared to the DNA-FTMs found at day 5 (16.7 % – 37.8 %) and day 20 (28.7 - 60.2%) of incubation over two years (2008 and 2009) in a study of eiders by Fenstad et al. (2014). The mentioned study showed variation in DNA-FTMs with both year and time of sampling in relation to incubation. The sampling time during the birds incubation in the present study is unknown, but previus discussion (5.1 and 5.2) have suggested that the sampling was done early in the incubation period. Early in the incubation period, there is a low fat-mobilisation due to fasting and the stress burden of incubation is low. The early sampling time may therefore have resulted in lower levels of contaminants in the blood stream and as a result caused lower damage (Strøm and Descamps, 2016). However, the average DNA-FTM found at day 5 by Fenstad et al. (2014) was still higher (37.4 % in 2008 and 16.7 % in

2009) than what was found in the present study (5.6-10.56 %). This could possibly be explained by annual differences, as seen in Fenstad et al. (2014) between 2008 (37.4 %) and 2009 (16.7 %). It should be taken into account that the birds studied by Fenstad et al. (2014) were collected from Storholmen, which also was found to have the highest DNA-FTM (8.47 %) out of the Kongsfjorden locations in the present study.

Another explanation for low levels of DNA DSBs in the eiders may be the short food chain of the eider ducks (Dahl et al., 2003). By feeding low in the food chain, eiders are expected to consume food with lower levels of persistent organic contaminants and less biotransformed compounds compared to other higher trophic level seabird (Broman et al., 1990). This is due to the biomagnifying properties of these chemicals, and the lower biotransformation rate in invertebrates compared to vertebrates (Camus et al., 2003).

The different time of sampling between Grindøya, Tromsø (late May), and the samples from Svalbard (late June) could have affected the amount of time the birds had been lying on the eggs and hence the PAH concentrations in their blood. However, the difference is probably small, since the ice melts earlier in Tromsø than in Svalbard, and thus the breeding starts earlier at Grindøya compared to Svalbard, and the three weeks later sampling is assumed not to affect the percentage of breaks largely.

4.3.1.2 MML

The calculated mean molecular length of the migrated DNA was found to be significanly higher in the present study (336.84 - 389.14 kbp) compared to those found in eiders in 2008 and 2009 (67.6 and 55.2 kbp) by Fenstad et al. (2014). As the MML previously has been found to be inversily proportional to DNA-FTM (Fenstad et al., 2014), this result supports the previously found low level of DNA-FTM. A possible explanation for the lower levels genotoxic damage found at the four locations in the present study compared to the results found at Storholmen by Fenstad et al. (2014), may be a difference in sampling time affecting the MML as described for DNA-FTM comparing the two studies.

4.3.2 Differences in genotoxic effect between locations

One possible explanation for the difference in genotoxic effect between locations may be the differences in anthropogenic activity. Grindøya is, located close to the city of Tromsø (population of 72 000) and the birds living here are thus expected to be exposed to larger amount of contamination (not just PAHs) than the birds living in Kongsfjorden in Svalbard. Breholmen

had the lowest percentage of DNA fragmentation, which was expected from the location of this island. Breholmen is located the furthest away from Ny-Ålesund and are the most remote location, and closest to the mouth of Kongsfjorden. The statistically significant difference in DNA-FTM between Breholmen and Grindøya may thus possibly be explained by the remoteness of the population location. This theory is partly supported by the two other locations, since Mietheholmen and Storholmen are located close to each other and have the most similar values of DNA-FTM, 8.36 and 8.48, respectively. Another possible explanation for the significantly higher DNA-FTM at Grindøya may also be an unknown local source of DNA-DSB-inducing contaminants.

No significant difference was found in DNA-FTM within Kongsfjorden, which may be due to the fairly closely located islands and feeding habits of Svalbard eiders. Especially, the eiders incubation on Mietheholmen and Storholmen may have feed at the same feeding grounds before incubation. Additionally, bird from the same location may have feed in different location and on different species (Lydersen et al., 1989), thus increasing individual variation within each location. This as the amount of contamination varies within Kongsfjorden (Dahle et al., 2006; Granberg, unpublished), and thus the level of contamination in the feed of the eider, and the eider itself will vary.

The low range and little variance in DNA-FTM within the population at Breholmen may possibly be representing the background level of DNA-FTM. DNA-DSBs occur naturally as a result of disrepair of the thousands of abasic sites that occur per cell per day as a result of both exogenic and endogenic factors (Fenstad et al., 2014; Krøkje et al., 2006). DNA integrity can be influenced naturally by heat energy, and oxidative stress has also been found to directly cause DNA-DSB (Klaunig, 2013). However, the background level of DNA-FTM in eiders in unpolluted areas is as far as I know, unknown. Additionally, the background level of DNA-DSB may differ between species and cell types, due to differences in excision repair activity, metabolic activity or anti-oxidant concentration (Lee and Steinert, 2003).

4.4 CORRELATIONS BETWEEN PAH CONCENTRATIONS, BODY MASS AND DNA DAMAGE

4.4.1 PAH concentrations and body mass

The present study showed no correlation between PAH concentrations and body mass. Fenstad et al. (2014) has shown levels of organic contaminants to increase in female eiders during the

fasting period, with a significantly increasing level of persistent organic pollution when the birds reached a weight below 1600g (Fenstad et al., 2014). Due to the similar, though weaker persistence of PAHs compared to most polychlorinated biphenyls studies by Fenstad et al. (2014), some increase during the fasting period are expected. However, an increase in contaminant concentration was not found to correlate with lower body mass in the present study. This may be related to the relatively high overall body mass in the present study compared to the study by Fenstad et al. (2014), which again may be related to the early sampling time.

Furthermore, Fenstad at al. (2014) measured a decrease in body mass of the same individuals from day 5 to day 20 during the incubation period. In the present study, samples were collected only once, and differences between individuals compared. This difference in measuring may have affected the results, as the low body mass are not necessarily due to fasting, but may also be affected by individual variation. Factors affecting individual variation will be further discussed in section 4.4.2.

4.4.2 PAH concentrations and genotoxic damage

The relatively high average DNA-FTM found at Grindøya compared to the three locations in Kongsfjorden was not correlated to the sum PAH concentrations in the different locations (Figure 13). However, on an individual level, Grindøya showed a significant positive relationship between DNA-FTM and total PAH concentrations and a nearly significant correlation to alkylated PAHs (Figure 14 C). Fenstad et al. (2014), Krøkje et al. (2006) and Østby et al. (2005) have found strong correlation between other organic contamination concentrations and genotoxicity in avian species. In eiders living elsewhere (Baltic Sea), high levels of PAH in the environment have also been found as a likely causes for the high levels of genotoxic effects on chromosomes in eiders (Matson et al., 2004). The level of PAH contamination found in the Baltic Sea was, however, much higher than the levels found at the four locations in the present study. Still, the positive relationship found at the Grindøya location supports these previous findings. Additionally, the stronger correlation found between DNA-FTM and the relative amount of alkylated PAHs compared to the parent PAH (Figure 14 A and B), also show the importance of studies on all types of PAHs when investigating possible genotoxic effects.

In Kongsfjorden, no significant relationship was found between DNA-FTM and neither type, nor total sum PAH. This may be due to other stress factors than PAH contamination inflicting

the frequency of DNA-DSB, or possibly the high individual variation in contamination levels in individual in Kongsfjorden.

4.4.2.1 Factors affecting the individual variation

The high individual variation in genotoxicity as well as both type and sum PAH found in the present study may possibly be related to several factors.

First, differences in sampling time in relation to incubation may cause individual variation. All samples in Kongsfjorden were collected within a few days (Appendix 2), without considering the length of incubation for each individual. Not all female eider ducks starts incubation at the same day, and therefore the time of sampling in relation to incubation may vary within and between locations (Hanssen et al., 2002; Mehlum, 1991; 2012). The day of incubation has been found to positively correlate to organic contaminant concentrations in female eider ducks (Fenstad et al., 2014). Differences in PAH concentrations and possibly genotoxicity may hence be expected. The average body mass and clutch size measurements support these differences in collection time, since they indicated a general trend that birds from Mietheholmen started incubation later than the birds from the other locations (Section 4.1). Studies (Hanssen et al., 2002; Mehlum, 1991) have shown that the time the bird start incubation to negatively correlate with clutch size. The low average body mass and high clutch size found at Breholmen, may also indicate that eiders at Breholmen generally started incubation earlier than birds breeding at the other locations in Kongsfjorden. This again may possibly explain the nearly significant correlation between DNA-FTM and total sum and parent PAH found for birds at Breholmen, but not for the other locations in Svalbard.

The second factor explaining individual variation is differences in feed and feeding habits, as this may possibly affect the concentration and type of PAH absorbed in the individual bird (Boehm et al., 1982). As eiders are benthivores, feeding on invertebrates, the birds in the present study are expected to feed on bottom organisms at the coastline of the island they are habituating (Bustnes and Erikstad, 1988; Dahl et al., 2003; Ydenberg and Guillemette, 1991). Still, individual differences in preferable feeding places and prays may cause differences in levels of contamination in the different individuals. This is of special importance in the birds located in Kongsfjorden, as the food selection on Svalbard is sparse causing these eider ducks to feed on a variety of different bivalves compared to the birds feeding at the mussel rich coast at Grindøya (Erikstad et al., 2010; Lydersen et al., 1989). Additionally, the low food recourses cause birds from the same locations to feed at a wide variety of different locations (Lydersen et al., 1989),

which may be more or less contaminated. This leads to higher individual differences in contamination level, which may have affected the overall relationship between genotoxicity and the type, sum and individual average level of PAH contamination for the birds. At Grindøya, however the birds are expected to feed on the high levels of similarly contaminated blue mussels located at the seafloor around Grindøya (Erikstad et al., 2010). In addition, as these birds overwinters in areas close to the breeding location (Descamps et al., 2010; Erikstad et al., 2010), the level of all types of PAH exposure are expected to be more constant, which may explain the stronger correlation found at this location. On the contrary, eiders breeding in Kongsfjorden migrate longer distances (Iceland and northern Norway) (Hanssen et al., 2016), resulting in an even larger variation in contamination exposure.

Other biological factors, which may affect individual variation, include differences in age and overall body conditions. The age may affect the level of DNA-FTM because many PAHs have been found to accumulate in eiders over time, which results in higher concentrations of these lipophilic pollutions in older individuals (Granberg and Selck, 2007; Harris et al., 2011). It should be considered that all individuals are incubating females and are therefore sexually mature individuals. Thus, all individuals are older than 2-4 year (Strøm and Descamps., 2016). As eiders are known to live for 20-25 years (Follestad and Lorentsen, 2016), the difference in age is possibly large. Information about the age of the individuals in the present study are unknown, since the birds populating Svalbard are ring marked in adult age. The low weight of some birds may thus be due to low age, rather than fasting during incubation as described by Fenstad et al. (2014). Together with the early sampling previously discussed for both PAH concentration and genotoxic damage, young age may thus contribute to lower contamination due to less bioaccumulation and fat reservoirs.

The overall conditions of the birds due factors including, sickness and fat percentage may also affect the PAH concentrations and genotoxic damage in the blood samples. This as weather and sickness may induce the stress to the individuals, leaving less energy for DNA repair (Lehikoinen et al., 2006). Harsh weather conditions and induced stress have been shown to affect the biotransformation rate in birds (Bustnes et al., 2012), which may affect the frequency of DNA-DSB. Diseases can also effect a whole population, and thus make specific populations more vulnerable to other DNA-FTM inducing factors. Fat percentage in the birds plays a role for the concentration of lipophilic contaminants that can be stored in the bird and released in the blood by mobilization of fat during fasting (Fenstad et al., 2014). This weight loss by reduction in fat reservoirs release lipophilic contaminants, such as PAHs, into the blood, which

are expected to lead to higher DNA-FTM, as shown for other biomagnifying contaminants by Fenstad et al. (2014). Additionally, other conditional factors including heat energy and oxidative stress may have affected the level of genotoxicity in the female eider ducks (Fenstad et al., 2014).

Individual differences in toxicokinetic factors such as absorption and biotransformation of the genotoxic compounds, DNA repair rates, cell turnover, and adduct stability may affect the level of PAH and genotoxicity (Krøkje et al., 2006; Parkinson et al., 2013). The high PAH turnover rate in vertebrates, which may be higher than for several persistent organic pollutions found to induce genotoxic effects in avian species, may also explain the lack of correlation between DNA-FTM and PAH concentrations in Kongsfjorden. Additionally, abiotic factors including total contamination load due to larger or higher number of sources in certain areas, and differences in sediment bioavailability may, as previously discussed for the total level of PAH contamination (Section 4.2.1), also affect the individual variation.

To summarize, the many factors possibly affecting the level of genotoxicity and PAH concentrations in individual birds may have affected the average PAH and DNA-FTM, and possibly the relationship between type and total sum PAH and DNA-FTM. Based on this individual variation, and the positive correlation found at Grindøya, a possible relationship between PAH concentrations and genotoxic damage can thus not be dismissed in Kongsfjorden. This as the individual variation in these factors within the sampled groups lowers the reproducibility and interpretation of the present study. The variation induced by these factors could have been less important with a larger sample size. Additionally, variations in external factors, including weather condition, ice melt, predators, breeding and random coincidences, can also affect the reproducibility of the results of this field study.

4.4.2.2 Exposure to individual contaminants

The concentrations of individual PAH compounds present in the collected blood samples may possibly influence the level of DNA-FTM. As previously discussed, only lighter PAHs (naphthalene, phenanthrene, dibenzothiophene, fluorine, pyrene and chrysene) were found in the eider blood samples. This may possibly explain the low genotoxicity, as the heavier compounds of four to six aromatic rings, such as benzo[a]pyrene, have been found to inflict the most long-term toxic effect on embryos, young and adult birds (Albers, 2006). The higher lipophilicity with larger molecular size significantly increase the toxicity of the PAHs (Sverdrup et al., 2002). The higher toxicity of these heavier PAHs may be linked to them being stronger

aryl hydrocarbon receptor agonists with the most genotoxic metabolites (Lee et al., 2015; Muñoz and Albores, 2011; Newman, 2010).

The parent compound naphthalene was found in the highest concentrations at all the studied locations, except Grindøya (Figure 13). The lack of correlation between DNA-FTM and naphthalene concentrations indicate that naphthalene does not strongly inflict genotoxic damage. This is supported by a review study conducted by Schreiner (2003), who showed no genotoxic effects of naphthalene. Still, naphthalene has been found to have several other toxic effects including cytotoxicity in a wide range of different organism (Schreiner, 2003).

However, for the low alkylated naphthalene (C1-naphthalene), a significant positive correlation to DNA-FTM was found at Grindøya. Additionally, the alkylated PAH, C1-phenanthrene, also showed a significant positive relationship to DNA-FTM in blood collected at Grindøya. This alkylated PAH was found in higher concentrations in the location with the highest level of DNA-FTM compared to the other locations (Figure 13). A new study conducted by Mu et al. (2016) has found some alkylated phenanthrenes to cause embryotoxicity to the fish species, marine medaka trough cytochrome P450 metabolism. Effects of C1-phenathene on cytochrome P450 metabolism may also possibly affect the level of genotoxicity. However, Mu et al. (2016) studied toxicity in fish, which may not be transmittable to avian species, such as the eider. This due to species differences in sensitivity to the different PAHs, which may be a result of differences in the binding affinity to the aryl hydrocarbon receptor (Lee et al., 2015).

In summary, the positive relationship found between DNA-FTM and the two alkylated compounds present in the largest concentrations, and the lack of correlation for their parent compounds indicates that sum parent PAH is not a good proxy for genotoxicity in eiders from Grindøya, and that alkylated PAHs should be included in further studies on the genotoxic effects of PAH. Still, more specific PAH analyses is needed to fully investigate the relationship between genotoxic damage and PAH types and concentrations.

4.5 EVOLUTIONARY TOXICOLOGY EFFECTS IN THE POPULATIONS OF EIDERS IN KONGSFJORDEN

The eider population in Kongsfjorden (Svalbard) has not increased as expected after the founding of the bird sanctuaries in 1973 (Bakken et al., 2003). Increasing temperatures and earlier ice melt (Hanssen et al., 2013) were also expected to cause increase in the population. It is therefore of interest to look for possible explanations for this population stability. In the

present study, genotoxicity in relation to PAH type and concentrations were investigated as a possible factor affecting the population. However, based on the low PAH concentrations and lack of correlation to genotoxic damage found for the three sampled locations in Kongsfjorden, genotoxicity caused by total sum PAH was not seen as the main reason for the lack of population increase. Moreover, neither alkylated PAHs, nor parent PAHs individually were found to affect the DNA-FTM.

Results found in the highly contaminated Baltic Sea suggested genotoxic effects in eiders to be related to PAH concentrations (Matson et al., 2004). With time, increasing PAH contamination in Kongsfjorden, due to increasing number and size of anthropogenic sources may affect the relationship between genotoxicity and PAH concentrations. Additionally, the possibility of higher levels of genotoxic compounds in eiders at the end of the incubation period, due to fasting, may possibly affect the survival of the chicks. However, the time at which the female birds in the present study have been sampled in relation to incubation are unknown.

The egg and hatchling survival have previously been suggested as a possible explanation for the population stability (Hagen et al., 2005). A study on crude oil exposure by Grau et al. (1977) showed both parent PAHs and their metabolites to strongly reduce hatchability of bird eggs. A newer review study on the breeding success of eiders in a previous PAH pollution fjord, Ranfjorden (in the north of Helgeland) showed that PAHs might affect the rate of embryo mortality and survival of the transition from egg to breathing in air (Bustnes, 2013). Bird embryos are especially sensitive to petroleum products, such as PAHs (Hoffman et al., 2002). Mixtures of PAHs have been found to increase the embryo mortality of eiders at concentrations of 2 mg kg egg^{-1} (Brunström et al., 1990). In the present study, an average total PAH concentration of 76.15 ng g whole blood^{-1} was found in the incubating female eiders. This indicates a low level of maternal transfer of PAH from mother to her eggs. Additionally, other factors, for example egg predators (Hanssen et al., 2013) may contribute to the lack of increasing levels of eiders in Kongsfjorden.

4.6 METHOD ADVANTAGES AND POSSIBLE PROBLEMS

The main advantage of the present method are the non-invasive sampling. Using only small amounts of blood to measure exposure and genotoxic effects of chemical pollutions it is highly preferable compared to measurements in organs that require scarifying the animal. The liver is originally thought to have the highest concentrations of organic pollutions, such as PAH, and

the liver is also where most of the biotransformation of PAHs takes place (Parkinson et al., 2013). However, a study on glaucous gulls conducted by Henriksen et al. (1998) found a correlation between the levels of organic contamination in blood and liver. According to these results, blood samples can be used to indicate liver tissue concentrations of these types of contaminants in glaucous gulls and possibly other avian species (Henriksen et al., 1998b). Still, it should be considered that even though a correlation was found, higher levels of contamination could be expected in the liver of the studied eider compared to the sampled whole blood.

In addition, this method has opened the possibility of measuring the genotoxic effects of the same individual more than once, which give better results for studies on differences in genotoxicity over time as seen by Fenstad et al. (2014).

One of the main problems of the present study is the fact that the time of sampling in relation to the incubation period are unknown. However, from the previous discussion, the time of sampling was suggested to be early in the incubation period. Observations of the incubation start are thus suggested for further studies on genotoxic effects and analysis of persistent organic pollutions, so that well informed decisions can be made regarding the sampling time.

The use of frequency of DNA-DSB as a biomarker for genotoxic effects of contaminant exposure has been proven successful by Fenstad et al. (2014), Krøkje et al. (2006) and Theodorakis et al. (1994). To be defined as good biomarkers for genotoxic effects caused by chemical contamination, the genotoxic effect (DNA-FTM) must be specific to the studied contamination. However, PAHs are not the only inducer of the frequency of DNA-DSB. Other factors or chemicals may also induce the frequency of these DNA-damages (Bonisoli-Alquati, 2014). DNA-DSB are naturally occurring in all organisms, but the rate of breaks and repairs varies with individual factors, such as biotransformation rate, feeding preferences and the general health of the individuals. The measured effect can be a result of interactions, either chemical or biological, affecting the genotoxic effect (e.g. synergistic or antagonistic effects). Thus, suggestions for further studies would be to investigate further the interactions between biological factors (e.g. time of incubation) and chemical contaminants.

5 CONCLUSION

In the present study, a significantly positive relationship between DNA-FTM and total sum PAH was found in blood collected from common eiders at Grindøya. The results also showed that alkylated PAHs, rather than parent PAHs were the main cause of the significant correlation. These results indicate that alkylated PAHs affect the frequency of DNA-DSB in common eiders, even in the low concentrations found in the present study. The stronger correlation found at Grindøya between DNA-FTM and alkylated PAHs compared to the parent PAH, also shows the importance of studying all types of PAHs when investigating possible genotoxic effects.

In Kongsfjorden, no significant correlation between DNA-FTM and types or sum PAH was found. However, Breholmen showed nearly significant correlation between DNA-FTM and both parent and sum PAH. The low significance levels found in the present study suggest that individual variation are important in the Kongsfjorden location. The individuals were suggested to be influenced by different sampling time, exposure to other contaminants external conditions or biological factors such as feeding habits. There may also be a closer coupling between the common eiders and their environment at Grindøya than in Kongsfjorden. Thus, a positive correlation between genotoxicity measured as frequency of DNA-DSB and different types and concentrations of PAH cannot be dismissed in Kongsfjorden.

Further studies on organic contamination concentrations and chemically induced genotoxic damage in incubating female common eiders, are recommended to include knowledge and observations of the start of incubation, for more deliberate choices of sampling times. Additionally, further studies on genotoxicity of PAHs should include PAH metabolites to review the full genotoxic damage induced by PAHs.

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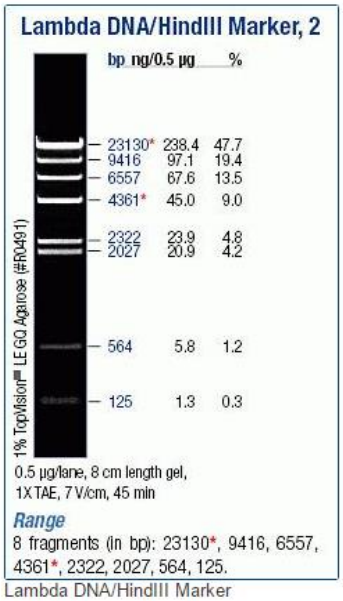
APPENDIX

1. DNA ladder of known fragment sizes. Whole lambda and HindIII lambda digest (Thermo Fisher).



Lambda DNA/HindIII Marker

2. Biometric data collected during sampling of the common eider blood samples at the four different locations. Including date of sampling, ID, body mass (BM), head, wing and tarsus measurements, clutch size and number on ring marking.

Location	***Date***	***ID***	***BM***	***Head***	***Wing***	***Tarsus***	***Clutch size***	***Ring***
Mietheholmen	19.06.2014	M1	2320	120	300	49.7	1	
Mietheholmen	19.06.2014	M2	1850	123.4	305	52.7	1	
Mietheholmen	19.06.2014	M3	1790	109.1	282	48.6	2	
Mietheholmen	19.06.2014	M4	1990	117.5	300	50.8	1	37377
Mietheholmen	19.06.2014	M5	2020	118.6	291	51.6	2	
Mietheholmen	19.06.2014	M6	1960	120.7	295	51.3	2	16319
Mietheholmen	19.06.2014	M7	1950	123.5	298	53.2	2	
Mietheholmen	19.06.2014	M8	1940	116.7	299	51.1	3	23784
Mietheholmen	19.06.2014	M9	2060	119.9	301	50.9	2	
Mietheholmen	19.06.2014	M10	1750	118.5	301	50.4	2	
Storholmen	20.06.2014	S1	1950	119.6	301	55.3	3	47505
Storholmen	20.06.2014	S2	1570	111.7	285	49.4	1	23847
Storholmen	20.06.2014	S3	1550	118.3	290	51	3	22925
Storholmen	20.06.2014	S4	1570	116.2	286	47.7	4	23843
Storholmen	20.06.2014	S5	1440	119.4	290	48.8	3	16419
Storholmen	20.06.2014	S6	1930	117.2	304	51.1	1	47513
Storholmen	20.06.2014	S7	1840	121.3	304	52.2	4	23805
Storholmen	20.06.2014	S8	1680	118.7	304	52.3	4	23716
Storholmen	21.06.2014	S9	1400	117.3	298	50.5	3	23727
Storholmen	21.06.2014	S10	1640	116.1	288	49.4	3	47503
Storholmen	25.06.2014	S11	1810	117.9	297	51.9	3	23841
Breholmen	20.06.2014	B1	1530	117.4	298	51.1	5	
Breholmen	20.06.2014	B2	2060	119.2	294	51.8	4	
Breholmen	20.06.2014	B3	1760	115.4	296	47.6	2	
Breholmen	20.06.2014	B4	1690	115.7	291	51.9	4	
Breholmen	20.06.2014	B5	1680	114.4	288	49.5	4	
Breholmen	20.06.2014	B6	1770	116.6	295	52.8	5	
Breholmen	20.06.2014	B7	1430	119.9	305	52.9	4	
Breholmen	20.06.2014	B8	1610	117.8	296	51.3	4	
Breholmen	20.06.2014	B9	1460	121.8	302	49.9	3	
Breholmen	20.06.2014	B10	1820	117	296	52.9	4	
Grindøya	26.05.2014	1	2370	123.8	301	51.9	3	37461
Grindøya	26.05.2014	2	2040	116.5	294	50.6	3	38917
Grindøya	26.05.2014	3	1890	120.4	298	51.3	5	38929
Grindøya	29.05.2014	4	2210	126.2	300	55.5	5	37462
Grindøya	29.05.2014	5	2100	125.4	303	53.4	5	42520
Grindøya	02.06.2014	6	2330	122.3	298	52	4	37463
Grindøya	02.06.2014	7	1990	125.3	308	53.3	5	30093
Grindøya	02.06.2014	8	2100	125.6	302	53.5	6	42523
Grindøya	02.06.2014	9	2430	120.6	293	51.2	3	37453
Grindøya	02.06.2014	10	1980	122.8	292	50.4	4	37455
Grindøya	02.06.2014	11	1940	126.9	298	52.5	5	37464
Grindøya	02.06.2014	12	1940	123.5	301	52.3	3	38924

3. Overview of the individual levels of PAH contamination present in the common eider blood sample collected at Mietheholmen (M1-12), Storholmen (S1-S11), Breholmen (B1-B10) and Grindøya (1-12).

	Naphthalene	Fluorene	Dibenzothio	Phenanthrer	Anthracene	Fluoranthen	Pyrene	Benzo(a)an	Chrysene	Benzo[ghi]p	C1-N	C2-N	C3-N	C1-BT	C1-F	C1-P	C4-P	C1-Py
M1	0	8.369	0.237	0	0	0	0	0	0	0	0	0.921	0	0	24.925	0	0	0
M2	9.054	11.759	2.180	0	0	0	0	0	6.443	0	0	10.968	0	0	29.241	59.661	0	0
M3	3.433	8.916	5.639	0	8.31818075	4.42190494	10.806	12.703	11.416	23.813	0	8.367	0	0	30.458	51.263	55.576	0
M4	0	7.272	0	0	0	0	0	0	0	0	0	0	0	0	28.708	0	0	0
M5	49.860	9.891	1.767	0	0	0	1.563	0	0	0	0	0	0	0	0	0	0	0
M6	101.048	0	1.242	0	0	0	2.996	0	0	0	0	2.145	0	0	0	0	0	0
M7	54.318	16.090	5.275	15.436	0	0	3.268	0	0	0	0	9.783	0	0	0	0	0	0
M8	97.800	13.002	4.835	41.261	0	0	2.898	0	0	0	0	18.017	0	0	0	0	0	0
M9	120.212	17.015	5.191	43.030	0	0	4.469	0	0	0	0	22.455	0	0	0	0	54.065	0
M10	94.431	11.677	3.016	4.706	0	0	3.215	0	0	0	0	5.574	0	0	0	0	0	0
S1	143.929	9.667	4.559	25.639	0	5.506	7.186	0	0	0	0	47.095	0	0	0	0	163.480	0
S2	2.856	0	1.533	1.260	80.199	0.690	4.784	0	0	0	0	13.885	0	0	0	0	0	0
S3	14.787	0	6.439	26.955	0	10.536	12.543	0	8.813	0	0	25.147	0	0	0	0	217.118	0
S4	1.866	0	2.527	10.897	0	2.091	5.795	0	7.968	0	0	15.761	0	0	0	0	0	0
S5	67.393	0	0.956	36.566	0	3.810	6.089	0	0	0	0	34.420	0	0	0	0	0	0
S6	74.279	0	3.134	48.051	0	3.670	2.883	0	0	0	0	41.339	0	0	0	0	0	0
S7	134.621	11.852	5.929	80.828	0	11.609	9.983	0	0	0	0	78.686	294.498	185.827	0	0	138.93804	0
S8	0	6.460	0	0	0	0	0	0	0	0	0	0	0	0	25.241	0	0	0
S9	0	0	0	0	0	0	0	0	0	0	0	0.486	0	0	0	0	0	0
S10	43.687	0	3.884	0	0	0	4.661	0	0	0	0	0	0	0	0	0	0	0
S11	0	0	0	0	0	27.206	1.253	0	0	0	0	0	0	0	34.583	0	0	0
B1	2.639	0	0	0	0	0	0.255	0	0	0	0	5.751	0	0	29.592	0	0	0
B2	11.981	11.382	2.898	0	0	0	6.841	0	7.511	0	0	7.554	0	0	0	0	0	0
B3	13.824	11.139	0	0	0	0	5.016	0	4.164	0	0	14.968	94.872	77.798	30.309	0	0	0
B4	32.248	0	1.412	3.566	0	1.369	5.750	0	0	0	0	8.853	0	0	0	0	0	0
B5	41.135	24.438	4.992	12.771	0	5.728	9.405	0	7.942	37.421	0	22.230	0	0	0	0	0	0
B6	64.727	8.666	0.957	0	0	0	2.075	0	0	0	0	0	0	0	0	0	0	0
B7	78.606	11.864	5.552	36.172	0	0	5.009	0	0	0	0	8.253	62.506	55.188	6.775	56.732	47.234	0
B8	132.044	0	1.885	0	0	0	1.363	0	0	0	0	29.264	90.001	62.585	0	0	83.771	0
B9	90.479	0	1.044	0	0	0	5.518	0	0	0	0	8.027	0	0	0	0	0	0
B10	45.953	0	4.396	0	0	0	2.461	0	0	0	0	0	0	0	0	0	0	0
1+2+8	42.174	9.750	3.936	30.319	0	0	2.441	0	0	0	0	0	0	0	0	0	30.929	0
3	51.192	8.349	2.920	15.292	0	0	2.805	0	0	0	0	0	29.097	45.651	0	0	36.861	0
4	16.136	0	2.763	0	0	0	2.217	0	0	0	0	0	0	0	0	0	0	0
5	20.679	0	0	0	0	0	0.677	0	0	0	0	0	0	0	0	0	0	0
6	58.864	0	0	2.771	0	0	0.178	0	0	0	0	48.037	115.700	43.455	0	0	142.456029	0
7	19.745	0	7.724	2.865	0	0	5.773	0	0	0	0	0	0	0	0	0	0	0
8	0	0	1.397	0	0	0	2.359	0	0	0	0	0	0	0	0	0	0	0
9	0	0	3.075	0	0	0	1.975	0	0	0	0	0	0	0	0	0	0	0
10	0	0	2.395	0	0	53.619	2.811	0	0	0	0	0	0	0	0	0	0	0
11	50.0532171	17.2710908	2.329	14.858	0	0.734	5.902	0	0	0	0	38.958	0	0	0	0	155.537	0
12	52.8229574	10.7816477	2.623	12.799	0	9.891	13.616	0	0	0	0	27.356	0	0	0	0	0	0

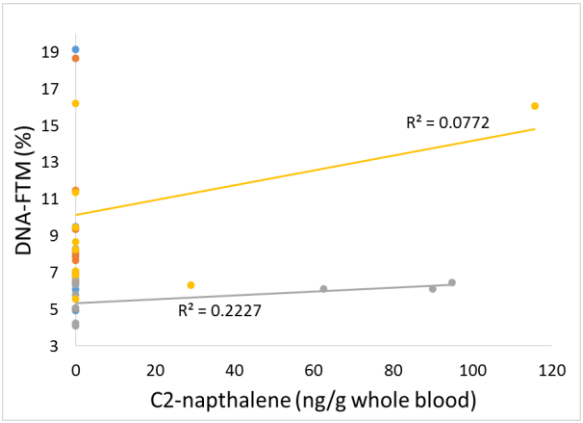
4. Overview of the average DNA-FTM (%) for each gel, sample, sample date and location, including the sample size (n) from each gel. The sample size represent each line drawn trough each lane containing a plug. Meaning thee plugs resulting in a sample size of 9.

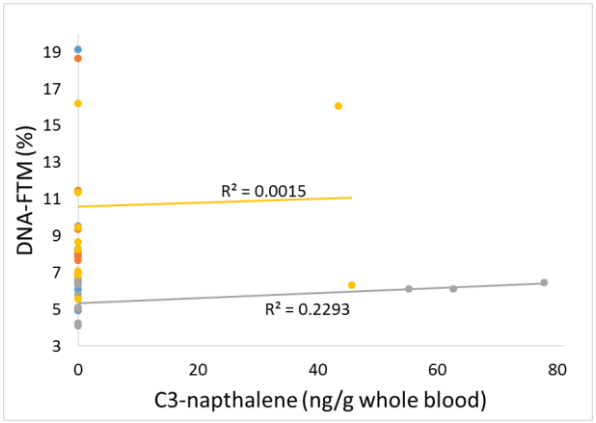
Location	**Date**	**Sample ID**	**Gel 1 (n)**	**Gel 2 (n)**
Mietheholmen	19.06.2014	M1	7.79 (9)	8.79 (9)
Mietheholmen	19.06.2014	M2	5.43 (9)	6.72 (9)
Mietheholmen	19.06.2014	M3	-	6.36 (9)
Mietheholmen	19.06.2014	M4	6.32 (9)	6.36 (9)
Mietheholmen	19.06.2014	M5	8.92 (9)	6.93 (9)
Mietheholmen	19.06.2014	M6	19.15 (9)	-
Mietheholmen	19.06.2014	M7	7.23 (9)	6.65 (6)
Mietheholmen	19.06.2014	M8	6.94 (9)	7.17 (9)
Mietheholmen	19.06.2014	M9	4.94 (9)	-
Mietheholmen	19.06.2014	M10	7.90 (9)	11.13 (1)
Storholmen	20.06.2014	S1	7.47 (9)	8.69 (9)
Storholmen	20.06.2014	S2	8.64 (9)	10.09 (9)
Storholmen	20.06.2014	S3	6.47 (9)	9.29 (9)
Storholmen	20.06.2014	S4	6.78 (9)	6.30 (9)
Storholmen	20.06.2014	S5	7.68 (6)	6.13 (6)
Storholmen	20.06.2014	S6	9.36 (9)	5.96 (9)
Storholmen	20.06.2014	S7	3.84 (6)	5.64 (9)
Storholmen	20.06.2014	S8	6.62 (9)	7.21 (9)
Storholmen	21.06.2014	S9	-	18.67 (9)
Storholmen	21.06.2014	S10	12.31 (9)	10.61 (6)
Storholmen	25.06.2014	S11	4.73 (9)	5.33 (6)
Breholmen	20.06.2014	B1	5.29 (9)	4.89 (9)
Breholmen	20.06.2014	B2	4.98 (8)	3.50 (9)
Breholmen	20.06.2014	B3	4.81 (9)	8.13 (9)
Breholmen	20.06.2014	B4	5.08 (18)	-
Breholmen	20.06.2014	B5	6.75 (9)	6.57 (9)
Breholmen	20.06.2014	B6	6.35 (9)	-
Breholmen	20.06.2014	B7	5.66 (9)	6.54 (9)
Breholmen	20.06.2014	B8	5.66 (9)	6.54 (9)
Breholmen	20.06.2014	B9	5.38 (9)	6.23 (6)
Breholmen	20.06.2014	B10	4.10 (18)	-
Grindøya	26.05.2014	1	7.50 (9)	6.64 (9)
Grindøya	26.05.2014	2	11.81 (9)	9.56 (9)
Grindøya	26.05.2014	3	5.35 (6)	7.27 (9)
Grindøya	29.05.2014	4	6.06 (9)	5.08 (9)
Grindøya	29.05.2014	5	8.65 (6)	5.11 (9)
Grindøya	02.06.2014	6	18.62 (9)	13.51 (9)
Grindøya	02.06.2014	7	22.73 (3)	9.71 (6)
Grindøya	02.06.2014	8	8.35 (9)	8.13 (9)
Grindøya	02.06.2014	9	7.08 (9)	-
Grindøya	02.06.2014	10	10.22 (9)	8.71 (9)
Grindøya	02.06.2014	11	20.33 (6)	22.60 (6)
Grindøya	02.06.2014	12	10.98 (9)	11.76 (9)

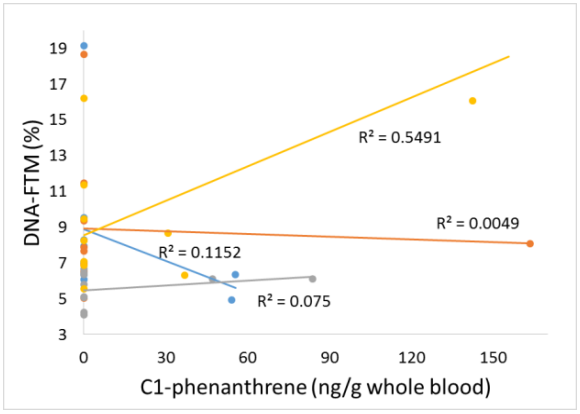
5. Overview of the MML of the whole blood samples collected at the four locations, including date, sample ID and the results from the two gels run simultaneously.

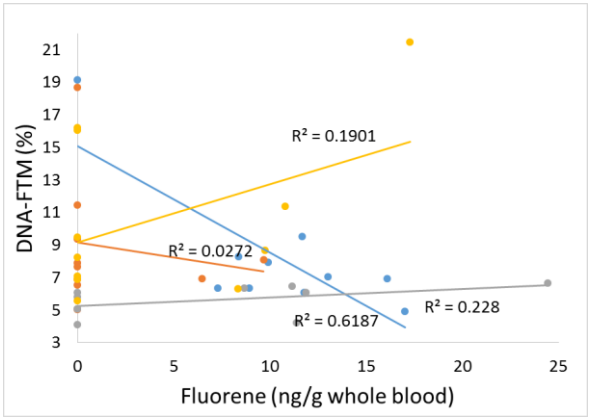
Location	**Date**	**Sample ID**	**Gel 1**	**Gel 2**
Mietheholmen	19.06.2014	M1	381.17	369.56
Mietheholmen	19.06.2014	M2	416.81	454.44
Mietheholmen	19.06.2014	M3		320.67
Mietheholmen	19.06.2014	M4	308.37	481.17
Mietheholmen	19.06.2014	M5	223.26	363.89
Mietheholmen	19.06.2014	M6	326.50	385.11
Mietheholmen	19.06.2014	M7	245.50	347.50
Mietheholmen	19.06.2014	M8	183.33	216.42
Mietheholmen	19.06.2014	M9	376.83	258.96
Mietheholmen	19.06.2014	M10	326.50	414.00
Storholmen	20.06.2014	S1	333.89	283.33
Storholmen	20.06.2014	S2	407.44	419.83
Storholmen	20.06.2014	S3	441.50	357.78
Storholmen	20.06.2014	S4	358.14	
Storholmen	20.06.2014	S5	440.83	306.67
Storholmen	20.06.2014	S6	488.61	
Storholmen	20.06.2014	S7	334.89	
Storholmen	20.06.2014	S8	298.61	288.56
Storholmen	21.06.2014	S9	498.17	350.56
Storholmen	21.06.2014	S10	326.92	
Storholmen	25.06.2014	S11	442.61	317.06
Breholmen	20.06.2014	B1	336.33	362.56
Breholmen	20.06.2014	B2	470.61	456.88
Breholmen	20.06.2014	B3	334.22	404.44
Breholmen	20.06.2014	B4	312.41	480.00
Breholmen	20.06.2014	B5	317.56	390.42
Breholmen	20.06.2014	B6	383.44	263.22
Breholmen	20.06.2014	B7	310.75	314.00
Breholmen	20.06.2014	B8	393.92	428.00
Breholmen	20.06.2014	B9	522.61	
Breholmen	20.06.2014	B10	468.50	443.83
Grindøya	26.05.2014	1	379.83	351.28
Grindøya	26.05.2014	2	504.17	458.19
Grindøya	26.05.2014	3	143.28	354.94
Grindøya	29.05.2014	4	312.37	488.28
Grindøya	29.05.2014	5	252.44	319.16
Grindøya	02.06.2014	6	463.17	216.48
Grindøya	02.06.2014	7	312.67	367.92
Grindøya	02.06.2014	8	283.67	431.75
Grindøya	02.06.2014	9	451.39	
Grindøya	02.06.2014	10	364.67	428.11
Grindøya	02.06.2014	11	497.08	473.67
Grindøya	02.06.2014	12	486.17	453.00

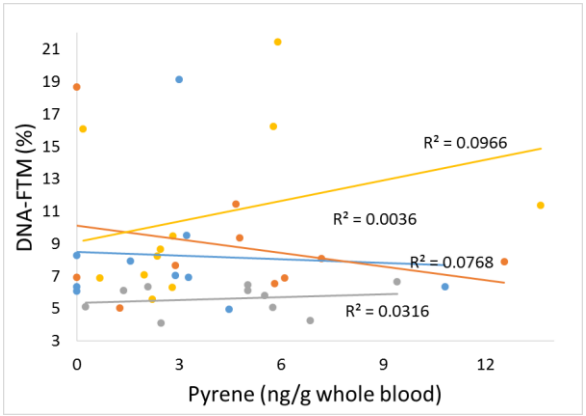
6. Regression analysis between DNA-FTM and individual PAH compounds found to be present in the blood samples collected at Mietheholmen (n=10), Stoholmen (n=11), Breholmen (n=11) and Grindøya (n=12).

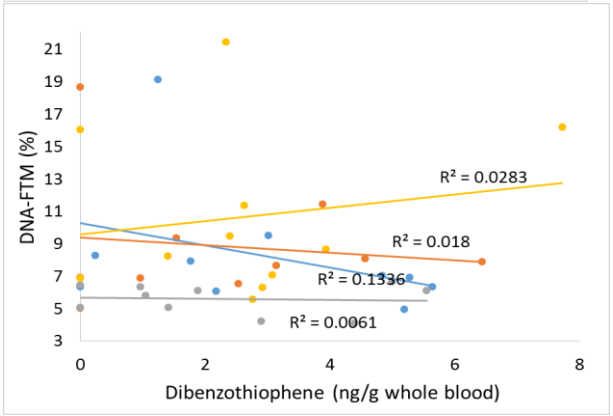


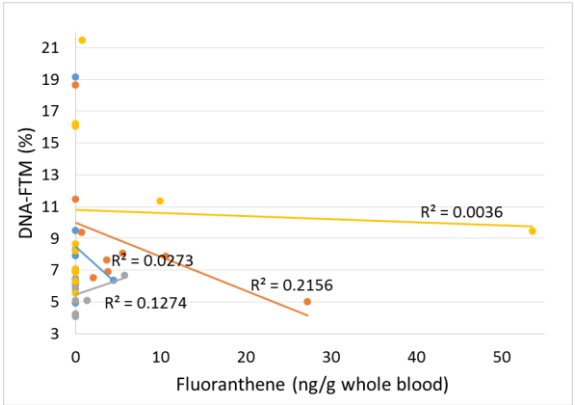


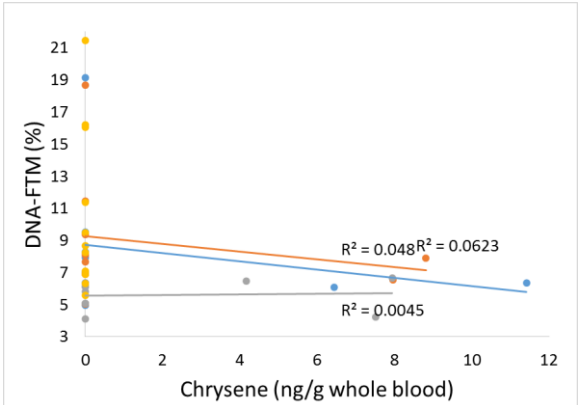


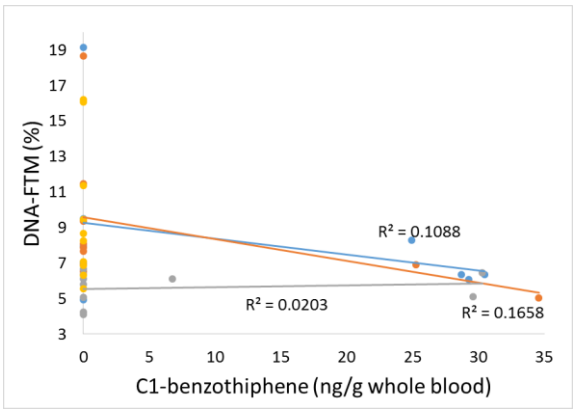











DNA-FTM (%)
19
17
15
13
11
9
7
5
3
0
5
10
15
20
25
30
35
C1-benzothiphene (ng/g whole blood)
R² = 0.1088
R² = 0.0203
R² = 0.1658
Mietheholmen
Storholmen
Breholmen
Grindøya
Lineær (Mietheholmen)
Lineær (Storholmen)
Lineær (Breholmen)
Lineær (Grindøya)