UNIVERSITY OF TROMSØ UiT

FACULTY OF BIOSCIENCES, FISHERIES AND ECONOMICS
DEPARTMENT OF ARCTIC AND MARINE BIOLOGY

The effects of persistent organic pollutants (POPs) on thyroid hormones and metabolic rate in exposed herring gull (*Larus argentatus*) chicks

Anne Sveistrup

BIO-3930 Master`s thesis in Biology

May 2010

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Preface

In a state of "master fog" where I hardly remember my own name, I am ready to write my thank-you-list for all the help I have received with my master project and thesis. Some of you have been to greater help than you know.

First I want to thank my two supervisors Geir Wing Gabrielsen and Erling Nordøy for good feedback on my writing and for having patience with a master student where nothing goes as planned.

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Tromsø, May 2010

Anne Sveistrup

Abstract

The main objectives of the present study were to investigate how plasma concentrations of POPs can affect thyroid hormone levels and basal metabolic rate (BMR) in herring gull (*Larus argentatus*) chicks. Thyroid hormones are essential for development and growth and are the major controllers of metabolic rate in birds and mammals. High concentrations of persistent organic pollutants (POPs) may alter the thyroid hormone homeostasis and subsequently the BMR. As a result, the capacity for adaptive thermogenesis, especially under stressful conditions like harsh climate conditions and limited food resources, may be limited. Captured herring gull chicks were raised and exposed to a natural mixture of POPs in cod liver oil. In the second part of the study, the herring gull chicks were starved to mobilize stored fat reserves and increase the POP concentration in blood plasma. Plasma samples were analysed for polychlorinated biphenyls (PCBs), pesticides, polybrominated diphenyl ethers (PBDEs) and metabolites. Additionally, total (T) and free (F) plasma levels of thyroxin (T4) and triiodothyronine (T3) were analysed, and BMR measured using open circuit calorimetry. The results of the present study were decreased T3 and FT4 levels and negative associations were between TT3, TT4/FT4 and POP concentrations in exposed-starved herring gull chicks. Positive correlations were observed between FT4/FT3 ratio and POP concentrations, also in exposed starved chicks. No difference in thyroid hormone levels between exposed and control chicks were observed. A positive correlation between FT4-, and a negative correlation between TT4/FT4 and BMR was measured.

Abbreviation list

AMAP	Arctic Monitoring and Assessment Programme
BFR	brominated flame retardants
BMR	basal metabolic rate
$CaCO_3$	calcium carbonate
CHL	chlordane
CYP	cytochrome P450
DCM	dichlomethane
DDT	dichloro-diphenyl-trichloroethane
EI	electron impact ionization
ESI^-	electrospray ionisation mode
FC	free cholesterol
FT3	free triiodothyronine
FT4	free thyroxine (tetraiodothyronine)
GC- MS	gas chromatograph mass spectrometry
HPT-axis	hypothalamic-pituitary-thyroid axis
HRMS	high resolution mass spectrometry
LCT	lower critical temperature
LOD	limit of detection
MeO-	methoxy-
$MeSO_{2-}$	methylsulphone-
NCI	negative chemical ionization
ng	nanogram
NILU	Norweigan Institute for Air Reasearch
NIST	National Institute of Standards & Technology
NSB	non specific binding
NTNU	Norwegian University of Science and Technology
OH-	hydroxyl-
p	p-value
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyls
PCR	polymerase chain reaction
pg	picogram
POP	persistant organic pollutants
PL	phospholipids
QQ-plots	quantile-quantile plots
QStd	quantification standard
RPM	revolutions per minute
RIA	radioimmunoassay
RQ	respiratory quotient
RRF	relative response factor
SD	standard deviation
SIM	single ion monitoring
SRM	standard reference material
STPD	standardized temperature and pressure dry
T3	triiodothyronine
T4	thyroxine (tetraiodothyronine)
TBG	thyroxine binding globulin
TC	total cholesterol

TG	triglycerides
TL	total amounts of lipids
TRH	thyrotropin-releasing hormone
TSH	thyroid stimulating hormone (thyrotropin)
TT3	total triiodothyronine
TT4	total thyroxine (tetraiodothyronine)
TTR	transthyretin
UCT	upper critical temperature
UDP-GT	uridine diphosphate glucuronosyl transferase
W-test	Shapiro Wilk`s W test
w.w.	wet weight

Contents

1 Introduction

1.1 Persistent organic pollutants (POPs) in the Arctic

Despite few local sources of persistent organic pollutants (POPs) in the Arctic, high levels have been found in animals throughout the region (2004). In regards to the European Arctic most of the pollutants originate in the industrialised areas in Europe. The POPs are transported north, primarily by atmospheric air masses, but also via ocean currents and river runoff (De Wit et al., 2004). The degree of POPs following the long-range transport depends on the physiochemical properties, like the semi-volatility and vapour pressure of the different compounds (Walker, 1996). After reaching the Arctic, the POPs are taken up via diffusion over gills and gastrointestinal tract of lower aquatic organisms in the marine food web (De Wit et al., 2004). Due to their lipophilic characteristics, POPs are accumulated in organisms and the concentrations magnified many times on its way from the lower trophic levels to the top predators in the food chain (Borgå et al., 2001, Hop et al., 2002, Barrie et al., 1992). The highest POP levels in the Arctic have been found in top predators such as herring gulls (*Larus argentatus*) (Helgason et al., 2008) glaucous gulls (*Larus hyperboreus*) (Bustnes et al., 2001, Verreault et al., 2007a), ivory gulls (*Pagophila eburnea*) (Miljeteig et al., 2009), arctic fox (*Alopex lagopus*) (Wang-Andersen et al., 1993) and polar bears (*Ursus maritimus*) (Sandala et al., 2004).

POPs have shown a variety of adverse effects in birds and mammals, including effects on, reproduction and development, behavior, immune system, hormonal and vitamin homeostasis, basal metabolic rate (BMR), biotransformation enzyme induction, and mutagenic and carcinogenic effects (De Wit et al., 2004, Gabrielsen, 2007, Letcher et al., 2009). Murvoll (1999) found a negative correlation between ∑PCB concentration in yolk, and egg volume, yolk mass and hatchling mass in Shag (*Phalacrocorax aristotelis*) from the central Norwegian coast. Immunological effects have been reported in glaucous gulls, where positive relationships were found between persistent PCB congeners and the intestinal parasite (nematodes) load (Sagerup et al., 2000). From a number of studies on nesting glaucous gulls on Bear Island, correlations have been reported between plasma POP concentrations and circulating hormone levels (Verreault et al., 2004, 2006, 2007a). Verreault et al. (2004) reported significantly lower T4 (thyroxin) levels in male glaucous gulls from a high contaminated colony compared to a less contaminated colony. A follow up study on

associations between plasma POP concentrations, thyroid hormone levels and BMR was conducted, and negative associations were reported between plasma POP-levels and BMR (Verreault et al., 2007a).

Many arctic animals, also seabirds, go through periods of fat accumulation followed by periods of fasting. In most seabird species the body mass are highest in winter and lowest in summer during the chick rearing period (Gabrielsen, 2009). Many of the POPs are lipophilic and therefore stored in fat. As the fat is utilized, the POPs are redistributed in the organism and concentrations in the remaining fat increases (De Wit et al., 2004, Walker, 1996).

Detoxification of lipophilic xenobiotics like POPs is processed by hepatic biotransformation enzymes in biotransformation pathways, phase I and II. Phase I include oxidation, hydrolysis, hydration or reduction reactions, and leads to metabolites containing hydroxyl groups. The microsomal monooxygenases are the most versatile of these enzymes. The xenobiotic is then conjugated in Phase II, which leads to more polar and water soluble compounds. The uridine diphosphate glucuronosyltransferase (UDP-GT) is an enzyme in phase II reactions (Walker, 1996).

1.2 Thyroid hormones and effects from POPs

Thyroid hormones exert a wide range of physiological and biochemical effects on an organism. These effects can be categorized into 1) development; growth and maturation, and 2) effects on metabolism (McNabb, 1992).

Thyroid hormone levels are controlled through the hypothalamic-pituitary-thyroid (HPT) axis where the hypothalamus exerts the main control of synthesis and secretion of thyroid hormones (McNabb, 2003, Boron and Boulpaep, 2005). Its secretion of thyrotropin-releasing hormone (TRH) regulates thyroid-stimulating hormone (TSH) secretion from the pituitary, which again controls synthesis and secretion of thyroid hormones from the thyroid gland. Thyroid hormones are synthesized on the protein thyroglobulin in the thyroid gland, where monoiodo- and diiodotyrosines, catalyzed by thyroid peroxidase, are coupled to form triiodotyhyronine (T3) and thyroxin (T4) (McNabb, 1992, 2000). T3 and T4 are released into the circulation where it acts via negative feedback on the hypothalamus and pituitary, which leads to down-regulation of TRH and TSH secretion (Boron and Boulpaep, 2005). T4 is released from the thyroids at a much higher proportion than the biologically active hormone

T3, 95 and 5% respectively (Ucan-Marin et al., 2009). In the blood, three main types of carrier proteins are responsible for binding 99% of the thyroid hormones; thyroxin- binding globulin (TBG), albumin and transthyretin (TTR), where albumin and TTR are the major transport proteins in birds (McNabb, 2000). At the target organ T4 are converted to T3 by 5'/3'-deiodinases (McNabb, 1992).

POPs like PCBs, brominated flame retardants (BFR), dioxins and furans have been reported to cause hypothyroidism (reduced levels of circulating thyroid hormones) in exposed animals (Boas et al., 2006). A variety of mechanisms can lead to disruption of thyroid homeostasis, as different chemicals can interfere at various levels of the HPT axis and thyroid hormone metabolism:

1) POPs and their metabolites compete for binding sites on carrier proteins.

Several PCBs, PBDEs, pesticides and especially their hydroxylated metabolites have a structural resemblance to thyroid hormones (Boas et al., 2006, Van den Berg et al., 1991). Many of them have been reported to competitively displace T4 from human TTR (Ucan-Marin et al., 2009). TTR in gulls has shown a greater affinity to T3 relative to T4, which makes T4 more susceptible to competitive binding (Ucan-Marin et al., 2009). As a result T4 is displaced from carrier proteins and free hormone rapidly enters tissues where it is metabolised and excreted (Brouwer et al., 1990). As a consequence the total thyroid hormone level could decrease and less T4 reaches the target tissues where it can be deiodinised to T3 (Ucan-Marin et al., 2009).

2) Increased thyroid hormone metabolism and excretion as a result of POPs interference with deiodinase activity and biotransformation pathways.

The thyroid deiodinase activity is susceptible to interference from POPs. PCB congeners have been shown to both inhibit and increase the enzyme activity dependent in the deiodinase isozyme studied (Brouwer et al., 1998).
As previously mentioned displacement of thyroid hormones from carrier proteins may lead to enhanced hormone metabolism and excretion (Purkey et al., 2004, Brouwer et al., 1998, Meerts et al., 2000). Additional to the resulting excess of FT4, many organochlorines have been shown to induce hepatic biotransformation enzymes involved in chemical detoxification. One of these enzymes, the phase II enzyme, UDP-GT, increases T4 turnover

by conjugation of T4 to T4-glucuronide which are readily excreted in bile (McNabb, 2003, Van Birgelen et al., 1995). POPs may also result in a change in thyroid hormone metabolism by interference with thyroid hormone sulfotransferases (Brouwer et al., 1998).
3) Interference with thyroid gland function and morphology.

The thyroid gland itself may be object to histological change due to lowered circulating thyroid hormone levels, or by POPs direct influence on the gland (Brouwer et al., 1998, Moccia et al., 1986, Jefferies and French, 1971).

Natural fluctuations in thyroid hormone levels

The thyroid hormone levels in birds show a natural diurnal pattern. The plasma T4 concentration rising and reaching the maximum level at night (dark period) while T3 concentrations rise and are highest during the day (light period) (McNabb, 2000). Also environmental factors, like cold exposure has been shown to influence thyroid hormone levels. Cold temperatures lead to a rise in T3 levels while warm temperatures seem to decrease T3 levels. The influence of temperature on plasma T4 levels are opposite to those on T3 (McNabb, 2000). Another condition that is common for arctic birds is fasting (Gabrielsen, 2009). In chicken, fasting has led to elevated plasma T4 and decreased T3 concentrations (Decuypere et al., 2005), lowered T3 levels has also been reported for herring gulls (Totzke et al., 1999).

The thyroid hormones play a key role in the metabolic events involved in thermogenesis, and the maintenance of a high and constant body temperature in a homeotherm animal (McNabb, 1992). This is of high importance for a mammal or a bird living in the cold environments of the Arctic (Gabrielsen, 2009). The thyroid hormones' role in regulating metabolism has been indicated by reduced basal metabolic rate (BMR) in hypothyroid state and elevated BMR in hyperthyroid state (McNabb, 1992).

1.3 Basal metabolic rate (BMR)

Basal metabolism is defined as the rate of energy used by a post absorptive bird at rest within the thermoneutral zone (Blem, 2000), and is a function of body mass (Kleiber, 1975, Gabrielsen, 1994). An often used method for measuring an animal's metabolic rate is the open circuit calorimetry (Ellis and Gabrielsen, 2002). The method is based on the volume of oxygen consumed by an animal inside a metabolic chamber where the air is pumped through at a known rate (Withers, 1977, Furness and Monaghan, 1987). The metabil rate differs between the active (day) and inactive (night) phases of the daily cycle (Blem, 2000), and depends on temperature, photoperiod, body mass, plumage isolation capacity, time of year, activity, growing (body or new feathers), or whether the animal is digesting food or not (Furness and Monaghan, 1987). Basal metabolism, like thyroid hormones, shows a diurnal rhythm, with a higher level during the day in diurnal birds (Furness and Monaghan, 1987).

BMR of Charadriiformes (gulls and auks) varies with latitude. High latitude charadriiform seabirds appear to have a BMR up to twice as high as those of tropical charadriiform seabirds of the same body mass (Furness and Monaghan, 1987, Ellis and Gabrielsen, 2002).

The BMR is always measured within the bird's thermoneutral zone, which is the temperature range an endothermic animal can tolerate without any change in its metabolic rate (Lawrence, 2000, Ellis and Gabrielsen, 2002). The thermoneutral zone lies between the lower critical temperature (LCT) and upper critical temperature (UCT). LCT for the herring gull lies between 5°C and 10°C (Lustick et al., 1978, Ellis and Gabrielsen, 2002). While the upper critical temperature is dependent on surface temperature where the bird is standing since the feet in a seabird are important for thermoregulation and heat loss (Baudinette et al., 1976, Lustick et al., 1978, Steen and Steen, 1965). When experimentally determining the UCT, panting is used as an indicator that the UCT is reached (Lustick et al., 1978).

Fasting in birds

Birds deprived of food first absorb and metabolise food in their digestive tract before becoming postabsorptive. During the postabsorptive phase glycogen stores in muscle and liver are utilized for energy, followed by gluconeogenesis from protein and fatty acid oxidation (Boron and Boulpaep, 2005). The respiratory quotient (RQ) drops as the substrate

metabolised changes from carbohydrate to lipid (Blem, 2000). Fasting can be separated into three periods (Le Maho, 1983). Period I is indicated by a rapid decrease in body mass. The period is short and mobilization of lipids starts. In period II the fuel metabolized is mainly fat. RQ in this period drops, and the weight loss is slow and stable. Period III starts when fat reserves are depleted and metabolism shifts over to protein catabolism. RQ increases and weight is lost rapidly (Le Maho, 1983, Ellis and Gabrielsen, 2002).

1.4 The study species

The herring gull (*Larus argentatus*) belongs to the order Charadriiformes, has a circumpolar distribution and consists of several subspecies (Cramp, 1977). Its breeding area reaches from 30- 70°N, and is located mainly at the coast, but the gull can also be found breeding far inland (Cramp, 1977). The herring gull is found along the Norwegian coast all year round, and is the second most abundant species after black-legged kittiwake (*Rissa tridactyla),* with 30.500 pairs counted in Troms and Finnmark in 2006 (Anker-Nilssen et al., 2007). Typical characters of the genus Larus, like a powerful and hooked bill, large head, long, broad wings and relatively short legs, are common (Cramp, 1977). The herring gull feeds on a great variety of food items such as molluscs, crabs, fish, other birds, carrion and offal. The feeding strategy varies from opportunistic to specialized (Davis, 1975). The food is obtained in many different ways including scavenging, predation, piracy and shallow diving. Adult birds often tend to be more specialized than juveniles (Davis, 1975).

The herring gull is one of the species that feeds at higher trophic levels. For that reason, concentrations of POPs such as polychlorinated biphenyls (PCB), brominated flame retardants (BFR), different pesticides like dichloro-diphenyl-trichloroethane (DDT) and chlordanes (CHL) have been reported at much higher concentrations than for seabirds at lower trophic levels. POP levels registered in herring gulls both from Canada and the coast of Northern Norway are mainly from analyses of eggs. The highest contaminant levels have been recorded in herring gull eggs from The Great Lakes in Canada (Norstrom et al., 2002, Norstrom and Hebert, 2006), some of them at ten times higher levels than reported from herring gull eggs from the coast of Northern Norway by Helgason et al. (2008, 2009), and Barrett(1996). PCB levels are showing a decreasing trend (Pekarik and Weseloh, 1998), while new classes of POPs like PBDEs are showing a rising trend (Norstrom et al., 2002).

1.5 Objectives of the study

The main objective of the present study was to investigate how plasma concentration of POPs affected thyroid hormone levels and BMR in exposed herring gull chicks.

The herring gull was chosen as a study species due to its position at the top of the food chain. The species is also relatively easy to keep in captivity.

Captured herring gull chicks were exposed to a natural mixture of POPs. Plasma concentrations of POPs, thyroid hormone levels and BMR were compared with results from non-exposed herring gull chicks.

The second part of the study included a period of starving to induce fat mobilization and thereby increase the POP levels in plasma.

Hypotheses:

i) Exposed herring gull chicks were expected to show decreased or altered thyroid hormone levels and BMR compared to non-exposed chicks.

ii) Exposed and starved herring gull chicks were expected to show a larger decrease in thyroid hormone levels than non-exposed, starved chicks.

2 Materials and Methods

2.1 Experimental design

2.1.1 Capture

A total of 40 herring gull (*Larus argentatus*) chicks, age 3-5 days (Risch and Rohwer, 2000) were captured from their nests at Værholmen (69°64'N,17°98'E), Store Kalholmen and Lille Kalholmen (69°62'N,17°99'E) in Troms county, 25th and 29th of June 2008. The experimental study was carried out in Kårvika, Ringvassøya (69°86'N, 18°92'E) where the herring gull chicks were kept in captivity for 66-72 days.

Permission for the capture of 40 herring gull chicks was given by the Directorate for Nature Management, and the experiment approved by The National Animal Research Authority**.**

2.1.2 Captive herring gull chicks

The age difference within the herring gull chick groups was approximately one week. The chicks were visually inspected and placed in two groups according to their body size. One group consisted of experimental chicks (exposed), the remaining chicks comprised the control group (non-exposed). This was done instead of a complete random allocation since the sample size was small and a majority of big chicks in one of the groups could have biased the sample. The two groups were kept in two outdoor aviaries (pens) where the chicks could move around freely. Each aviary (pen), approximately 6 m^2, was sheltered from wind and rain and equipped with heat lamps and water. The aviaries were cleaned twice a day. The chicks were fed block frozen, air thawed herring (*Clupea harengus)* ad libitum. One vitamin tablet was given each chick every fourth day (Fish eater tablets, Mazuri Zoo Foods, England) (Appendix 4). The body mass of the herring gull chicks was recorded every second day to control that their rate of growth was similar to that of wild herring gull chicks (Dunn and Brisbin, 1980). On day 24, the experimental groups were given 2x4 ml distilled cod liver oil (Möller's, Axellus AS, Oslo) for two days. Thereafter they received 2x6 ml oil daily for 38 days. The oil was given orally via an esophageal tube. The distilled oil contained a natural but concentrated mixture of organic pollutants. Fish and oil was analyzed for contaminant levels at the Norwegian Institute of Air Research (NILU) (Tromsø, Norway). Results will be presented in Helgason (unpublished data). For comparison of contaminant levels in herring gull plasma to the levels in the oil, previous oil analysis result from 2007 (Norwegian School

of Veterinary Science, Oslo, Norway), is submitted in this thesis (Appendix 6). The control chicks were given the same volume of cleaned cod liver oil as the exposed (Möller's, Axellus AS, Oslo, Norway). The total amount of oil given to each chick was 472 ml.

From a total of 40 herring gull chicks, three were removed and killed before the end of the experiment due to injuries (2) and loss of appetite (1). At the time of the first blood sampling, day 66, the exposed and control group consisted of 18 and 19 birds, respectively.

2.2 Basal metabolic rate (BMR) measurements.

Seabirds show an inverse relationship between size and lower critical temperature (LCT). LCT also depends on latitude, with a lower LCT at higher latitudes (Ellis and Gabrielsen, 2002). The relationship is given in equation 1

LCT = 43.15 – 6.58 log mass – 0.26 latitude (Ellis and Gabrielsen, 2002) (Equation 1)

LCT is given in degrees Celsius, mass in g, and latitude in degrees north. By using this equation, the birds in the present study, with an average body mass of 940g had an LCT of 5.6°C.

The respiratory quotient (RQ) gives a ratio of how much CO_2 that is produced pr O_2 consumed, and changes according to what substrate that is metabolised. The RQ for a post-absorptive bird has been measured to 0.71. This gives a caloric equivalenct of 19.8 kJ/L O_2 (Kleiber, 1975, Ellis and Gabrielsen, 2002, Verreault et al., 2004, Gabrielsen et al., 1988). A carbohydrate diet yields an RQ of 1.0, lipids an RQ of 0.71.

Measurements of BMR were obtained from 7 exposed and 10 control herring gull chicks. Initially, 11 exposed and 12 control chicks were measured. However, due to loss of blood samples for thyroid hormone analysis and one very stressed bird in the metabolic chamber, the number of chicks was reduced in this experiment. Analysis of thyroid hormones and organic contaminants were obtained from 12 exposed and 18 control birds.

Open circuit indirect calorimetry was used for measurements of BMR. The experiment was performed from 2 to 14 days before blood sampling. Only post-absorptive chicks were used in the BMR measurements. The herring gull chicks were fasted for at least five hours before the experiment (Ellis and Gabrielsen, 2002). The temperature in the metabolic chamber was

in the range of 13 - 20°C, which is within the thermoneutral zone of gull species (Gabrielsen et al., 1988). Approximately 50% of the experiments were carried out at night in the dark. The birds did not show any visible signs of stress during the metabolic experiment.

A metabolic chamber (55cm x25cm x30cm ~42L) made of veneer/fibreglass with a plexiglass cover was placed in a ventilated room where the temperature was close to outdoor temperature (10-20°C). The herring gull chicks were weighed before they were placed inside the metabolic chamber. Each chick spent 4-5 hours in the chamber. Both oxygen consumption and carbon dioxide production was measured during the metabolic experiment. Metabolic data used were from a calm period of 10 minutes, usually after a couple of hours of acclimatisation in the chamber, where the O_2 consumption and CO_2 production was stabile. For approximately 1/3 of the herring gull chicks the CO_2 measurements deviated from the parallel O_2 measurements. For that reason I have avoided using the CO_2 levels in calculations for any of the birds, and instead assumed an RQ of 71, representative for a post-absorptive bird.

Before the first BMR measurements the gas analysers were calibrated with nitrogen gas containing 95% N_2 and 5% CO_2 (Kleiber, 1975). This was done to set the O_2 level to zero, and CO_2 level to 5%. Ambient air was used to set the CO_2 level to 0.033% and O_2 level to 20.95%. The instruments were also calibrated with ambient air before each BMR measurement. All results are given at STPD (Standard conditions for Temperature and Pressure Dry) (Ruch et al., 1973).

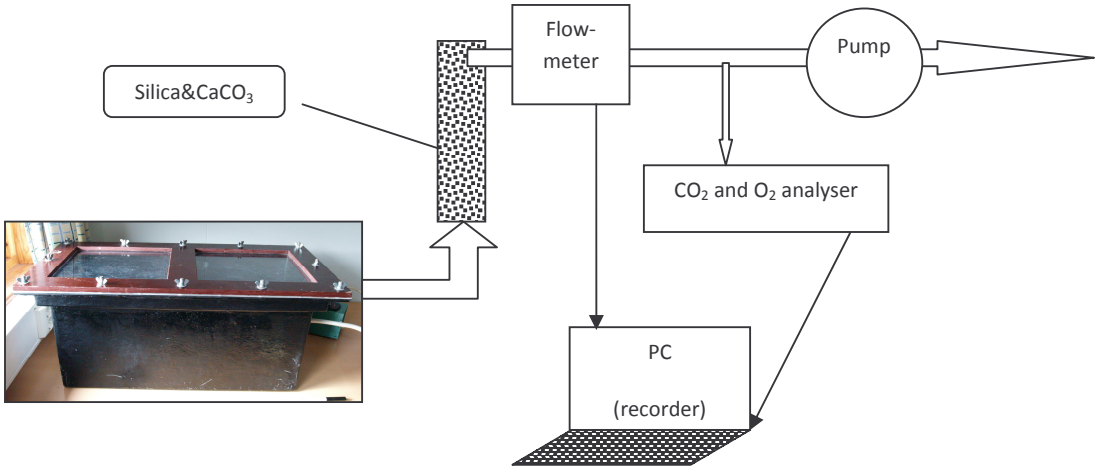

Figure 1 Experiment setup for BMR measurements of herring gull (*Larus argentatus*) chicks.

Ambient air was pumped through the chamber at a rate of 3-5 L/min, and the air was adjusted to keep the CO_2 content in the chamber between 0.03-1.0% and O_2 content above 20%. The air flow was measured with a flowmeter (Bronkhorst Hi-Tec, F-111) connected to a readout (Bronkhorst High-tech B.V, Model 5611-EA). A Singer DTN-325 volumeter was used to calibrate the flowmeter. The air was dried with a mixture of silica and calcium carbonate ($CaCO_3$) together with a MLA0110 In-line polypropylene filter, before the O_2 and CO_2 content in outlet air was measured with a gas analyser (ADInstruments ML206 Gas Analyzer connected to ADInstruments Powerlab 4/25).

2.3 Blood sampling and biometric measurements

At day 66 of the experiment a total of 9 experimental and 9 control birds were anaesthetised with Isofluran (Isoba® Vet, Schering-Plough Ltd.Uxbridge, England) before a blood sample (~13ml) was taken from the brachial vein, near the humeral-radial-ulnar joint, using a 15ml heparinised syringe. The chicks were then killed by a heavy blow to the head followed by decapitation. For some of the smaller herring gull chicks there were problems collecting enough blood from the vein, so additional blood were taken directly from the heart after death. Biometrical measures were recorded from each herring gull chick; wing length (±0.1cm), bill+head (±0.01mm), tarsus (±0.01mm), total body length (±1cm) and body mass (±10g). The blood samples were kept in 15ml vials on ice for up to 8 hours before they were centrifuged at 5000rpm for 7 minutes. (Labofuge Heraeus Christ, Houm AS, Oslo). Blood plasma (~3ml) were transferred to a cryogenic tube and kept at -80°C for thyroid hormone analyses, and to a 4ml glass vial, kept at -20°C, for contaminant analyses.

Starvation

The food supply for the remaining herring gull chicks (9 exposed and 10 control) were reduced to less than 10% for the last 7 days of the experiment. They were then sampled like the first group. The limitation of food supply during the last part of the experiment, instead of total starving, was done to simulate natural conditions with periods of reduced food supply at parts of the year. For simplicity, the semi-starved chicks are from now named starved chicks.

2.4 Analyses of data

2.4.1 Sex determination

Sex determination of the herring gull chicks was performed at The Norwegian University of Science and Technology (NTNU) (Trondheim, Norway). Herring gull liver was used as a substrate for the analysis of gender. The method is described by Griffiths et al (1998). In short the method is based on a Polymerase Chain Reaction (PCR) where a gene is amplified and separated with gel electrophoresis. Female birds have two different chromosomes (ZW) and male birds only one (ZZ). The gene is found in both chromosomes but the length of the sequence differs between them. Gel electrophoresis results in two bands for female birds and only one band for male.

2.4.2 Chemical analyses

The chemical analyses of organic contaminants were performed at the laboratories of the Norwegian Institute of Air Research (NILU) (Tromsø, Norway). Instrumental analyses for the brominated compounds were performed at NILUs laboratories at Kjeller, Norway.

Principles

The plasma samples were extracted using solid phase extraction. They were separated into three fractions and lipids were removed on florisil columns. The first fraction contained neutral compounds, and the other two, metabolites. The metabolites were going through several steps of purification, including clean-up on acidic silica columns. One µl of the sample was injected into a gas chromatograph (GC) connected to a mass spectrometer (MS), where the compounds were detected. The GC-column consists of a mobile phase (helium gas) and a stationary phase (the walls of the column). The injected sample, which condenses on the stationary phase, is slowly warmed up, and the different compounds evaporate, dependent on their affinity to the stationary phase and their boiling points. They are then carried by the mobile phase through the GC column, ionised into fragments and detected in the MS. The compounds are identified from their retention time through the GC column and the mass of their ion fragments in the MS.

Procedures

Two grams of plasma was weighed and spiked with internal standards for PCBs, pesticides, PBDEs and metabolites. Two ml of formic acid and two ml of deionised water was added to the samples, and left overnight for the plasma proteins to degrade. The samples were extracted using solid phase extraction with Waters Oasis® HLB Extraction 3cc (60mg) Cartridges (Waters Co., USA) in a Rapid Trace SPE Workstation (Caliper Life Sciences, Inc.). The cartridges were conditioned with methanol, dichloromethane and 1% HCl, then samples were added on top and cartridges dried with N_2. Dichloromethane was used as an eluent for the extraction. A volume of ~1ml hexane was added to each eluate and evaporated to ~0,5ml in a Rapid Vap Vacuum Evaporation System and prepared for further clean-up on florisil columns.

Cartridges were packed with 1g of deactivated florisil, and the compounds were separated into three fractions in the Rapid Trace SPE Workstation. Fraction one was eluted with DCM:hexane (1:3) and contained the neutral compounds like PCBs, PBDEs, DDTs and other chlorinated pesticides. Fraction two, eluted with acetone:hexane (1:9) contained $MeSO_2$-compounds, and fraction three, eluted with methanol:DCM (1:4), contained OH-compounds. The first fraction was evaporated to 0.2ml in the Rapid Vap Vacuum Evaporation System, added to a GC-vial, further evaporated to ~30µl under a gentle flow of nitrogen, and 10µl recovery standard was added. The second and third fractions were taken through another step of clean-up on acidic silica, where 0.7g acidic silica (25% H_2SO_4 mixed with activated silica) was packed in cartridges for the Rapid Trace SPE Workstation with 0.2g activated silica on top. The third fraction was taken through a derivatization step with diazomethane as a derivatization agent, where a methyl group replaced the hydrogen atom on the hydroxyl groups of the compound, before the acidic silica clean-up.

2.4.3 Lipid determination

Enzymatic lipid determination was done to determine the content and classes of lipids in blood plasma. Measurements of total cholesterol (TC), free cholesterol (FC), triglycerides (TG) and phospholipids (PL) was used to calculate the total lipid amount (TL) (Akins et al., 1989).

$$TL = 1.677\ (TC-FC) + FC + TG + PL \qquad \text{(Equation 2)}$$

2.4.4 Instrumental analyses

The analysis of the chlorinated and OH-MeOBDE compounds was performed by gas chromatography (GC) on an Agilent 7890A gas chromatograph (GC) equipped with an Agilent 7683B automatic injector and an Agilent 5975C mass spectrometer (MS) (Agilent, Folsom, CA, US). The GC was fitted with a 30 m DB-5 MS column (5% phenyl-methylpolysiloxane; 0.25 mm i.d., 0.25 mm film thickness) from J&W Scientific (CA, US). Splitless injection of 1 μL aliquot of the sample extract and helium as a carrier gas at a constant flow of 1.5mL/min, was used. Temperature program was as followed: initial temperature 70°C (2 min), 15°C/min to 180°C, 5°C/min to 280°C (5 min). The MS were running in the electron impact mode (EI) for the analysis of PCBs and DDTs and negative ion chemical ionisation mode (NCI) for chlordanes and metabolites. Methane was used in as reaction gas in NCI mode. The MS was operated in single ion monitoring mode (SIM).

The instrumental analyses for brominated compounds are described by Mariussen et al. (2008). It was performed with a high resolution gas chromatograph (HP6890-GC) (Agilent, Folsom, CA, US) connected to a VG AutoSpec high resolution MS (HRMS) operating in EI mode. The GC was fitted with a Zebron fused silica capillary column (ZB-1, 15 m, 0.25 mm id, 0.1 μm film thickness. Samples were injected with a splitless injection, injection temperature 300°C, and the temperature programme was: 88°C for 2.5 min, 13.2°C/min to 220°C, then 7°C/min to 320°C with a holding time of 5 min.

Calculations and quality control

A quantification standard (Q-std) with a known concentration of ^{13}C and ^{12}C were analyzed with the samples. A relative response factor (RRF) was calculated for the ratio between ^{13}C and ^{12}C (Equation 3).

$$RRF = \frac{Area\ 12C\ Qstd \times Amount\ 13C\ Qstd}{Area\ 13C\ Qstd \times Amount\ 12C\ Qstd} \quad \text{(Equation 3)}$$

RRF was then used to calculate the amount of ^{12}C in the samples from known amount of ^{13}C in samples (^{13}C from internal standard added to the samples) (Equation 4).

$$Amount\ 12C\ sample = \frac{Area\ 12C\ sample \times Amount\ 13C\ sample}{RRF \times Area\ 13C\ sample} \quad \text{(Equation 4)}$$

Recoveries were estimated from the calculated amount of ^{13}C in the samples divided by the amount of ^{13}C added each sample in the internal standard in the start of the sample processing (Equation 5).

$$Recovery(\%) = \frac{Amount\ 13C\ caluculated}{Amount\ 13C\ added} \times 100 \qquad \text{(Equation 5)}$$

The laboratory is attending an interlaboratory comparison, ring test, organised by the Arctic Monitoring Assessment Program (AMAP) three times each year, with uncertainties of ±20%. To check for interferences, one blank containing deionised water was prepared for each 10 samples. Standard reference material (NIST 1589a, human serum) was prepared for each batch (30 samples). The blanks and standard reference materials were treated like the plasma samples. Reference samples met the laboratories established criteria of less than 20% deviation from target concentration. The limit of detection (LOD) for instrumental analysis was set to three times blank values if contaminant was detected in the blanks. Otherwise the LOD was set to three times the noise level of chromatograms.
The quantification standard analysed with the samples also served as a control for checking the repeatability of the instrumental analysis.

2.4.5 Thyroid hormone analyses

The thyroid hormone analyses were performed at the Norwegian University of Science and Technology (NTNU) (Trondheim, Norway). The method described below is developed for human plasma but is also validated for other mammalian and bird species (Verreault et al., 2004).

A commercially available solid phase radioimmunoassay based on antibody coated tubes and human serum calibrators, were used to determine the concentrations of free (F) and total (T) thyroxine (T4) and triiodothyronine (T3) in plasma. The antibodies were highly specific for T4 and T3 dependent on the kit. The Radioimmunoassay (RIAs) kits used were: TT4, FT4, TT3, FT3 (Coat-A-Count®, Diagnostic Products Corporation Inc., LA, US)

A ^{125}I labelled thyroid hormone analogue (tracer) competed with sample hormone for antibody sites on the wall of the coated tubes for a fixed time (one to three hours dependent on the kit). A blocking agent was preventing the tracer from binding to thyroid hormone-binding proteins in plasma. The more hormone in the sample added, the less ^{125}I- labelled hormone would bind to the antibodies on the tube walls.

Procedures

Uncoated polypropylene tubes for total (T) tracer activity and non-specific-binding (NSB) were prepared in duplicates. Human serum calibrators with increasing concentrations of thyroid hormone levels (from A to F/G dependent on the kit) were added to antibody-coated tubes, and were used to make a logarithmic scaled calibration curve for quantification of thyroid hormones in herring gull plasma. Aliquots of plasma samples were added to marked antibody-coated tubes, 25µl, 50µl, 100µl and 100µl for TT4, FT4, TT3 and FT3, respectively. One ml of the radioactively labelled tracer where added, samples vortexed and incubated in 37°C water bath for 1-3 hours, dependent on the kit. All tubes except the one for total tracer activity were decanted and shaken hard to remove all unbound tracer, before the radioactivity in tubes were counted in a Packard gamma counter (Cobra II Auto-gamma series, Packard BioScience Company, Dowers Grove, IL, US). The number of counts recorded, were inversely proportional to native thyroid hormones from the samples bound to the antibodies on the walls of the tubes.

Quality control of thyroid hormone analyses

The concentrations to set the calibration curves for the different kits were 0–309nmol/L (TT4), 0–128.7pmol/L (FT4), 0–9.22nmol/L (TT3) and 0–65pmol/L (FT3).

The detection limits for the calibration curves were 1.8039nmol/L (TT4), 0.0431pmol/L (FT4), 0.0089nmol/L (TT3) and 0.0162pmol/L (FT3). None of the samples hormone levels were measured outside the limits of the calibration curve.

To test for repeatability, three parallels were analysed for TT4 and FT4, two parallels for TT3 and FT3. If the counts of the parallels deviated more than 15 percents, new samples were prepared and analysed again.

2.4.6 Statistical analysis

Exploratory statistics were done using Microsoft Excel 2007, while R 2.8.1 was used conducting the inferential statistics.

For calculations of mean, standard deviation and median, presented in tables, only values above the detection limit were used. Contaminants detected in less than 70% of the samples in all of the experimental groups, were excluded from statistical analyses. Concentrations below the detection limit for contaminants detected in more than 70% of the samples were given values to half the detection limit to avoid missing values in the data set. No individuals were excluded due to deviating results in the analyses (outliers), as individuals with deviating results may contain important information in biologically effect studies.

Concentrations of contaminants are presented and analysed in wet weight concentrations since wet weight have been shown to be more relevant for exploring biological effects (Henriksen et al. 1996).

Shapiro Wilk's W test and quantile-quantile plots (QQ-plots) were used to check for normality of the samples. Due to a small sample size and a dataset that was not normally distributed, non-parametrical test were used for further analyses. Mann-Whitney U (Wilcoxon test) was used to test for differences between the exposed and control herring gull groups. Students –T test was used to control for the results and gave a similar answer as the Mann-Whitney U test (not reported). A Spearman rank order correlation test was used to check for associations between variables. The significant threshold was set to $p<0.05$ for all statistical analyses.

3 Results

3.1 Biometrical variables

The female and male herring gull chicks used in this study were devided into four groups; exposed, non-starved and starved, and control non-starved and starved. The distribution of chicks in each group is presented in table 1. Biometrical variables are presented in fig 1.

Table 1 Distribution of female and male herring gull (*Larus argentatus*) chicks in the experimental groups; exposed-, non-starved and starved, control-, non-starved and starved.

	Experimental groups			
	Non-starved		Starved	
	♀	♂	♀	♂
Exposed (n=12)	2	4	4	2
Control (n=18)	7	2	3	6

There was no significant difference in the arithmetic mean of body mass from chicks between the exposed and control non-starved groups. However, a trend was observed between the two starved groups, where chicks from the exposed-starved group had a lower body mass than the control-starved ($W = 11$, $p = 0.06$). A difference in body mass of herring gull chicks was also seen between females and males in the non-starved groups combined ($W = 0$, p-value = 0.0004), and in the starved groups combined ($W = 0$, $p = 0.0003$), with 22% heavier body mass in male chicks in the non-starved and 26% heavier body mass in males in the starved group.

The mean body mass before starvation in the two starved groups, were 839g and 945g for exposed-starved and control-starved, respectively. The weight loss for those two groups were not significantly different ($W = 19$, $p = 0.37$). The reason for the larger body mass in exposed non-starved and control starved, may be that those groups contained a majority of male herring gull chicks, while exposed starved and control non-starved consisted mainly of female chicks.

In the exposed groups the body mass of herring gull chicks was 21% larger in the non-starved group compared to the starved group ($W = 32$, $p = 0.03$). No difference in body mass was observed for the control groups. The reason for this difference may be due to a higher body

mass in the control-starved group at the beginning of starvation compared to that of the control group.

The average weight loss in herring gull chicks in the starvation experiment was 11.7% (range 8-13%) for the exposed group, and 11% (range 5-14%) in the control group. The uneven distribution of males and females in the groups seem to be the most important factor explaining the great variation in body mass in the experimental groups.

Table 2 Biological variables in exposed non-starved, exposed-starved, control non-starved and control-starved groups of herring gull (*Larus argentatus*) chicks. Body mass (±10g), total body (±1cm), wing (±0.1cm), bill+head (±0.01mm), and tarsus (±0.01mm).

	Exposed non-starved (n=6)			Exposed-starved (n=6)		
Biomet. measures	Mean ± SD	Range	Median	Mean ± SD	Range	Median
Body mass (g)	900 ± 93	770 - 1032	908	742 ± 72	666 - 832	716
Total body (mm)	578 ± 23	550 - 600	588	575 ± 29	535 - 610	573
Wing (mm)	677 ± 26.6	645 - 710	673	671 ± 25	645 - 710	664
Bill + head (mm)	127 ± 6.5	116 - 133	130	120 ± 5.0	113 - 126	119
Tarsus (mm)	76.8 ± 2.6	73.1 - 79.4	77.2	75.3 ± 2.7	73 - 79	74.0

	Control non-starved (n=9)			Control-starved (n=9)		
Biomet. measures	Mean ± SD	Range	Median	Mean ± SD	Range	Median
Body mass (g)	831 ± 112	714 - 992	800	841 ± 106	665 - 992	857
Total body (mm)	558 ± 34	510 - 610	555	601 ± 43	540 - 670	605
Wing (mm)	659 ± 35.6	595 - 715	660	690 ± 26.9	645 - 730	691
Bill + Head (mm)	126 ± 8.4	116 - 138	125	123 ± 6	113 - 130	126
Tarsus (mm)	74.1 ± 3.6	69.5 - 81.1	72.6	76.6 ± 4.4	69.5 - 83	77.3

In the control groups, a larger total body length was measured for the chicks in the starved group compared to the non-starved, this may also be an indicator of sex distribution ($W = 18$, $p = 0.05$). Lipid percentage in plasma was significantly higher in the control non-starved compared to the control-starved ($W = 9$, $p = 0.04$) (appendix 1).

No significant difference was found between any other biometric parameters.

3.2 Persistent organic pollutant (POP) levels

All POP concentrations are presented in ng/g wet weight. Only the ∑ of contaminant groups are reported. Congener concentrations will be reported in Helgason (unpublished results). PCB concentrations are given as total ∑, and are also divided into metabolic groups based on persistency (Borgå et al., 2005b, Boon et al., 1997). The range of PCB concentrations in the four groups were 19-195 ng/g for the exposed, non-starved group, 313-1258 ng/g for the exposed-starved group, 2-14 ng/g for the control, non-starved group and 4-27 ng/g in the control-starved group. Of all contaminants quantified, ∑PCB accounted for on average 63%, 70%, 63% and 64% in the exposed, exposed-starved, control and control-starved groups, respectively. ∑DDT was the second most abundant contaminant group with 22%, 19%, 23% and 24%. All concentration levels are given in appendix 1. Table 3 shows the relative concentrations of PCBs categorized into metabolic group I-V.

Table 3 Percent distribution of PCB congeners in the five metabolic groups in exposed, exposed-starved, control and control-starved herring gull chicks.

Exposure	PCB gr I %	PCB gr II %	PCB gr III %	PCB gr IV %	PCB gr V %
Exposed	42,4	41,0	14,2	1,8	1,0
Exposed-starved	38,7	46,6	13,7	0,9	0,5
Control	40,4	38,2	16,4	3,9	1,8
Control-starved	42,3	37,8	15,9	3,5	1,4

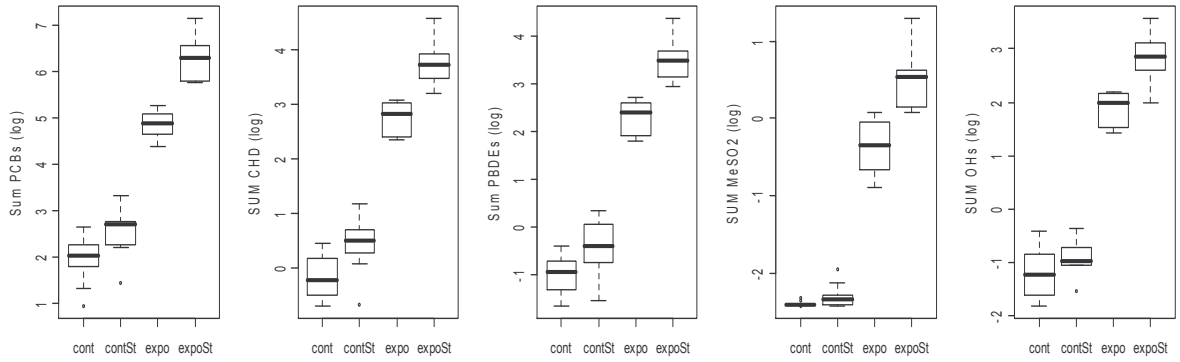

Figure 2 Box plots of plasma concentrations (log) (ng/g wet weight) of A: Sum PCBs, B: Sum CHD, C: Sum PBDEs, D: Sum MeSO2 compounds E: Sum OH- compounds in the exposed (expo, n=6), exposed-starved (expoSt, n=6), control (cont, n=9) and control-starved (contest, n=9) herring gull (*Larus hyperboreus*) chicks.

Effect of exposure

When comparing the non-starved groups, the levels for all compounds were significantly higher in the exposed compared to the control group ($W > 54$, $p \leq 0.0004$). The MeO-compound showed the same, but less significant ($W = 54$, $p = 0.001$). The difference in plasma concentrations between the starved groups showed that the exposed had significantly higher levels for PCB, PCB gr. I-III, V, OH-compounds, DDT and CHL ($W > 52$, $p \leq 0.0004$), and for PCB gr. IV, MeO-, $MeSO_2$-, PBDE ($W > 48$, $p \leq 0.002$) than the control group.

Effect of starving

Generally, the exposed groups showed a more significant difference between the starved and non-starved, than did the control groups. The only exception was for DDT where the difference between the control groups was more significant.

For the exposed groups, the PCB, PCB group I-III, CHDs and PBDEs ($W = 0$, $p \leq 0.002$), $MeSO_2$- ($W = 0$, $p = 0.004$) DDT, OH-compounds, ($W = 3$, $p \leq 0.02$), PCB group V ($W = 0$, $p = 0.05$), concentrations were significantly higher in the starved , compared to the non-starved group. For the control groups, concentrations were significantly higher for DDT ($W = 11$, $p = 0.008$), PCB, CHD ($W = 15$, $p = 0.02$), PCB group I-III, V, and PBDE ($W \geq 15$, $p = 0.05$) in the exposed compared to the control group.

3.3 Thyroid hormone levels

Thyroid hormone levels and ratios between the hormones in herring gull plasma are given in appendix 2. Levels and ratios of thyroid hormone in exposed and control groups are presented in figure 3 and 4.

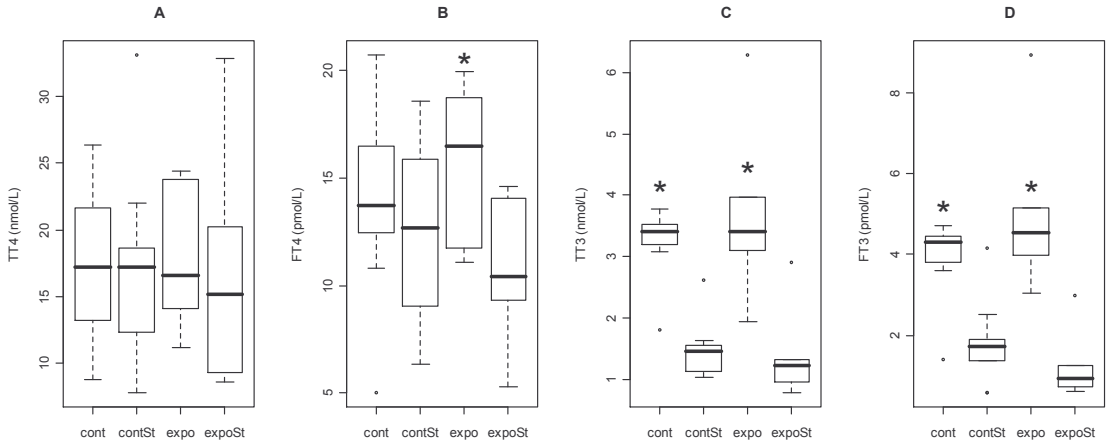

Figure 3 Box plots of thyroid hormone levels, with significantly different means marked with [*]; TT4 (nmol/L) (A), FT4 (pmol/L) [*] expo-expoSt (p = 0.03)(B), TT3 (nmol/L) [*] expo-expoSt (p=0.004), cont-contSt (p = 0.00008) (C) , and FT3 (pmol/L)[*] expo-expoSt (p=0.002), cont-contSt (p= 0.004)in plasma for exposed, non-starved (expo, n=6), exposed-starved (expoSt, n=6), control, non-starved (cont, n=9) and control-starved (contSt, n=9) herring gull (*Larus argentatus*) chicks.

Effect of exposure and starving

There was no significant difference in TT4 levels between any of the four groups. For FT4 the exposed-starved group had on average 32% lower plasma levels than the exposed non-starved group (W = 32, p = 0.03). The FT4 level difference for the control groups was not significant (W = 49, p = 0.49), and was on average 13% lower in the control-starved group. TT3 and FT3 levels were significantly lower in the starved groups compared to the non-starved (fig 3). TT3 levels were 62% and 54% lower in the exposed-starved (W = 35, p = 0.004) and control-starved (W = 80, p = 8.227e-05) groups, respectively. While FT3 levels were 75% lower in the exposed-starved (W = 36, p = 0.002) and 55% lower in the control-starved group (W = 72, p = 0.004), compared to the corresponding non-starved groups.

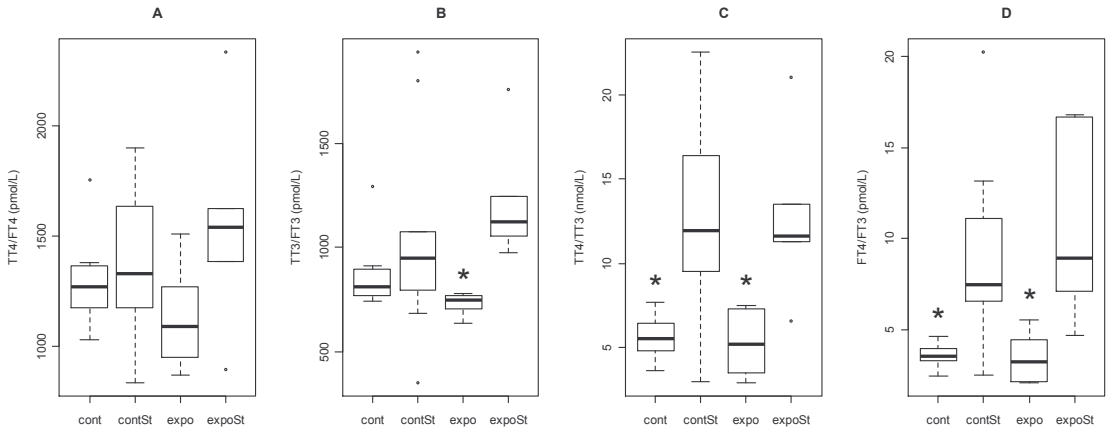

Figure 4 Box plots of thyroid hormone ratios, A:TT4/FT4, B: TT3/FT3, C: TT4/TT3, D: FT4/FT3 in plasma for exposed (expo, n=6), exposed-starved (expoSt, n=6), control (cont, n=9) and control-starved (contSt, n=9) herring gull (*Larus hyperboreus*) chicks.

No significant difference was observed for the TT4/FT4 ratio, but a trend was seen for the exposed non-starved and exposed-starved group with a higher ratio in the exposed-starved group ($W = 7$, $p = 0.09$). For the non-starved groups, TT3/FT3 ratio was significantly lower in the exposed compared to the control group ($W = 9$, $p = 0.036$), and higher in the exposed-starved compared to exposed non-starved ($W = 0$, $p = 0.002$). For TT4/TT3 and FT4/FT3 ratios, only the starving seemed to have had an effect on the ratios. For the exposed-starved TT4/TT3 ratios was significantly higher ($W = 3$, $p = 0.01$) than for the exposed non-starved group. Control-starved ratios were significantly higher ($W = 9$, $p = 0.004$) compared to the ratios of the control non-starved group. For FT4/FT3 ratio, both exposed-starved ($W = 1$, $p = 0.004$) and control-starved ($W = 10$, $p = 0.006$) had a higher ratio compared to the corresponding non-starved groups.

3.4 Correlations between POP levels and thyroid hormone levels and ratios

Correlations found between thyroid hormone levels in plasma and contaminant concentrations are shown in table 4. No correlation was found between TT4 and any of the summarized contaminants. A positive correlation was found between FT4 and PCB gr. V in the exposed-starved group (Spearman R; r = 0.94, p = 0.02) (not shown in table). Negative correlations were found for TT3 and PCB, PCB group I, III, CHL and PBDE in the exposed-starved group (Spearman R; r ≤ -0.88, p = 0.03).

Table 4 Relationships between thyroid hormone levels, thyroid hormone ratios and POPs (Spearman R). Significant relationships (p ≤ 0.05) are marked with [*], nearly significant relationships (p ≤ 0.1) are marked with [§].

	TT3	TT4/FT4		FT4/FT3
∑	Expo-starved	Expo-starved	Cont-starved	Expo - starved
PCBs (23)	-0.88*	-0.94 *	0.7 *	0.83 §
PCB I (7)	-0.89 *	-0.94 *	0.48	0.83 §
PCB II (6)	-0.6	-0.77§	0.7 *	0.6 §
PCB III (6)	-0.88 *	-0.94 *	0.4	0.83 §
PCB IV (5)	-0.66	-0.77§	0.6 §	0.89 *
PCB V (1)	-0.31	-0.37	0.52	0.54
OHs (10)	-0.82 §	-0.94 *	0.68	0.89 *
MeO (1)	0.54	0.42	0.25	-0.31
MeSO2s (12)	-0.82 §	-0.94 *	0.42	0.89 *
CHL (4)	-0.94 *	-1 *	0.42	0.94 *
DDT (3)	-0.82 §	-0.94 *	0.48	0.89 *
PBDE (10)	-0.88 *	-0.94 *	0.61§	0.83 §

For the thyroid hormone ratios negative correlations were seen between TT4/FT4 and PCB, PCB group I, III, OH-, $MeSo_2$-, CHL, DDT, and PBDE in the exposed-starved group (Spearman R; r ≤ -0.84, p = 0.02), and positive correlations between TT4/FT4 and PCB and PCB group II in the control-starved group (Spearman R; r ≤ -0.7, p = 0.04). FT4/FT3 showed positive correlations to PCB group IV, OH-, MeSO2-, CHL and DDT in exposed starved group (Spearman R; r ≤ -0.89, p = 0.03).

Figure 5 and 6 shows the strongest relationships between POPs and thyroid hormone ratios.

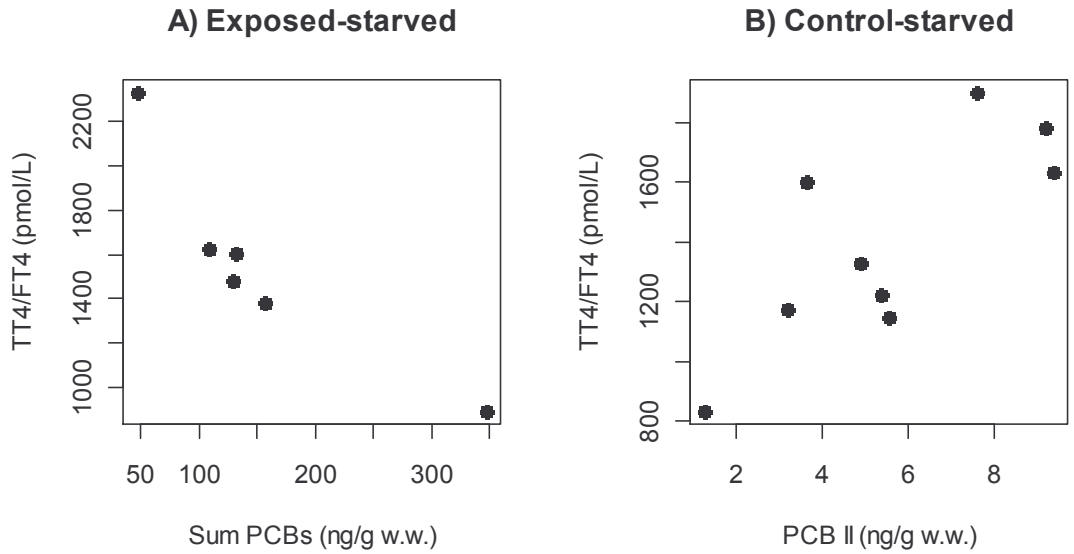

Figure 5 Scatterplot of A: ∑PCB (ng/g wet weight) and TT4/FT4 (pmol/L) in exposed-starved (Spearman R; r = -0.94, p= 0.02) (n=6) and B: PCB group II (ng/g wet weight) and TT4/FT4 (pmol/L) control starved herring gull (*Larus argentatus)* chicks (Spearman R; r = 0.7, p= 0.04) (n=9).

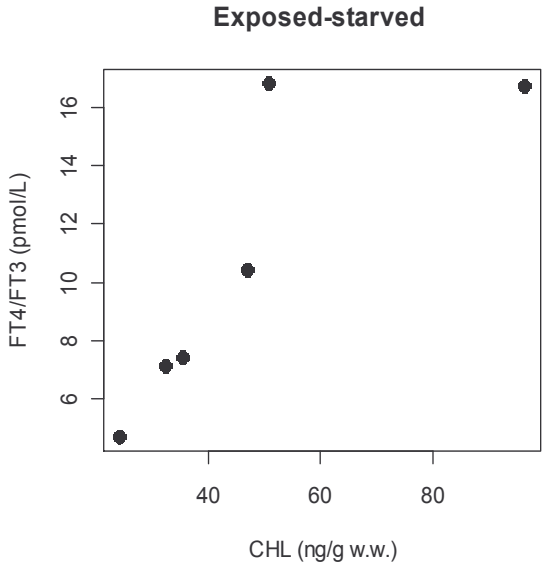

Figure 6 Scatterplot of A: CHL (ng/g wet weight) and FT4/FT3 (pmol/L) in exposed-starved herring gull (*Larus argentatus)* chicks (Spearman R; r = -0.94, p= 0.02) (n=6).

3.5 Basal metabolic rate (BMR) and thyroid hormones.

To control for possible effects of body mass in BMR comparisons, BMR was calculated on a mass-spesific basis (ml O_2/g·h). Males had significantly longer body length (W = 0.5, p-value = 0.001), negatively correlated body length and BMR (Spearman R; r = -0.59, p-value = 0.01). Males were 16% heavier than females (W = 2, p-value = 0.002), yet no significant difference in non-mass-specific (ml O_2/h) or mass-specific (ml O_2/g·h) BMR was seen between the sexes.

The metabolic measurements were carried out 2-14 days before the blood samples for thyroid hormone analyses were taken. These measurements include only two groups of herring gull chicks, one exposed and one control group. The BMR measurements were done before the 7 days of starving. Thyroid hormone blood samples, on the other hand, were taken after starvation for some of the birds, and therefore the two groups in this part of the study contains TH levels for both starved and non-starved chicks. A total of 57% of the exposed herring gull chicks (2♀, 2♂) were starved before blood sampling, whereas 70% in the control group (2♀, 5♂). Since starving has been shown to have a great influence of thyroid hormone concentrations in blood, especially for T3, starving may explain some of the lower levels in the control group (Decuypere et al., 2005).

BMR, body mass at the time of BMR measurements, POP concentrations and thyroid hormone levels and ratios is given in table 7. Distribution of females and males in the exposed and control group and time of day when measurements have been performed is given in Table 6.

Table 6 Distribution of females and males in the exposed and control groups, and time of day when BMR was measured.

	Measuring BMR			
	Day		**Night**	
	♀	♂	♀	♂
Exposed (n=7)	0	4	2	1
Control (n=10)	2	3	2	3

Table 7 Body mass, basal metabolic rate (BMR), plasma concentration of thyroid hormones, total (T) (nmol/L) and free (F) (pmol/L) T4 and T3, and thyroid hormone ratios TT4/FT4, TT3/FT3, TT4/TT3, FT4/FT3 in exposed (n=7) and control (n=10) group of herring gull (*Larus argentatus*).

	Exposed (n=7)			Control (n=10)		
Variables	Mean ± SD	Range	Median	Mean ± SD	Range	Median
Body mass(g)	926 ± 70,6	828 - 1012	910	949 ± 97,3	754 - 1064	998
ml O2/g/h	1 ± 0,15	0,69 - 1,12	1,0	1,11 ± 0,33	0,72 - 1,66	0,98
BMR (J/g/h)	19,9 ± 2,95	13,7 - 22,2	20,7	22,1 ± 6,55	14,2 - 32,9	19,4
Lipid %	1,04 ± 0,23	0,78 - 1,48	1,05	1,22 ± 0,23	0,98 - 1,58	1,18
∑DDTs	81,3 ± 55,2	28,2 - 156	48,3	4,5 ± 2,84	1,06 - 9,54	3,63
∑PCBs	344 ± 251	79 - 705	313	13,2 ± 8,27	2,37 - 27,2	12,7
TT4	21,4 ± 6,2	14,9 - 32,8	20,2	17,9 ± 7,82	7,78 - 33,1	16,0
FT4	14,8 ± 3,95	9,3 - 20	14,6	13,4 ± 4,76	6,35 - 20,7	12,9
TT3	2,78 ± 1,88	0,96 - 6,29	2,91	2,19 ± 1,06	1,03 - 3,77	1,59
FT3	3,45 ± 2,89	0,87 - 8,93	2,98	2,73 ± 1,5	0,57 - 4,72	2,22
TT4/FT4	1,5 ± 0,42	0,99 - 2,33	1,48	1,35 ± 0,33	0,84 - 1,9	1,29
TT3/FT3	0,93 ± 0,19	0,7 - 1,14	0,98	0,92 ± 0,37	0,35 - 1,8	0,91
TT4/TT3	10,6 ± 5,8	2,95 - 21	11,3	9,73 ± 5,69	2,98 - 22,5	8,85
FT4/FT3	7,06 ± 5,09	2,1 - 16,8	4,72	6,18 ± 3,31	2,51 - 13,2	5,58

No significant difference in thyroid hormone levels or ratios were observed between the exposed and control group.

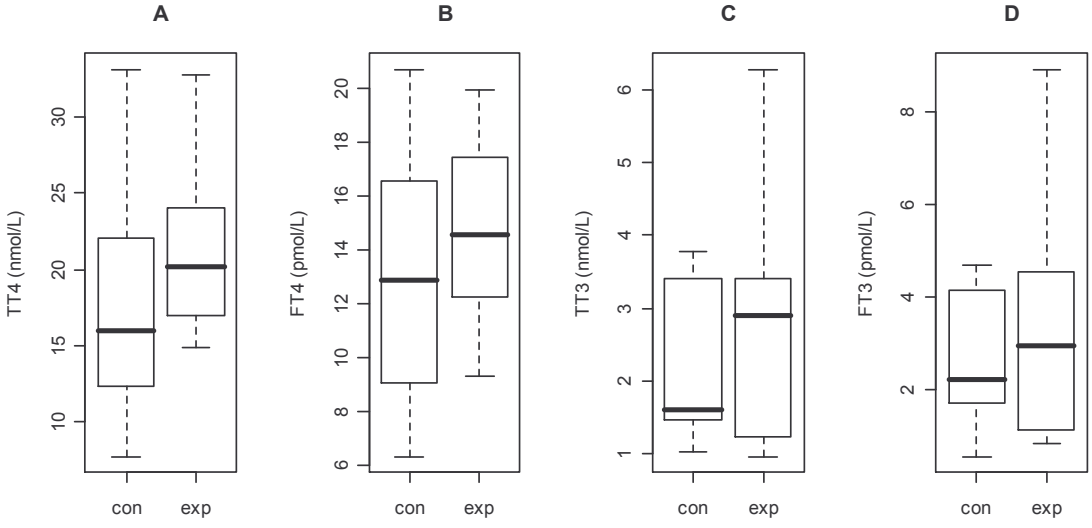

Figure 6 Thyroid hormone levels in exposed (exp) and control (con) group of herring gull (*Larus argentatus*) chicks. Total thyroid hormone levels (TT4, TT3) given in nmol/L, free thyroid hormone levels (FT4, FT3) in pmol/L. Exposed group; n=7, (2 ♀, 5♂ males). Control group; n=10,(4 ♀, 6 ♂).

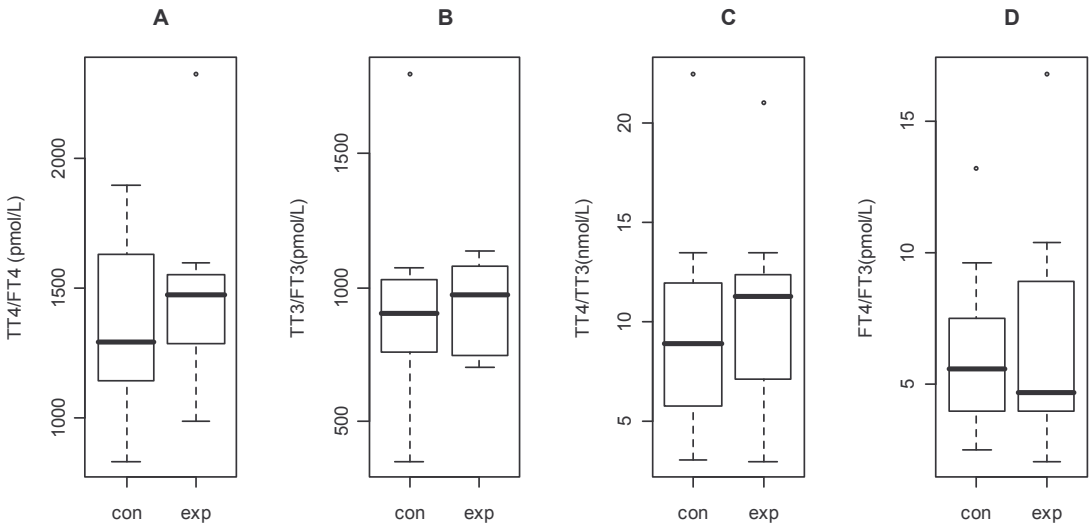

Figure 7 Thyroid hormone ratios in exposed (exp) and control (con) group of herring gull (*Larus argentatus*) chicks. A: TT4/FT4 (pmol/L), B: TT3/FT3 (pmol/L, C: TT4/TT3 (nmol/L and D: FT4/FT3 (pmol/L).

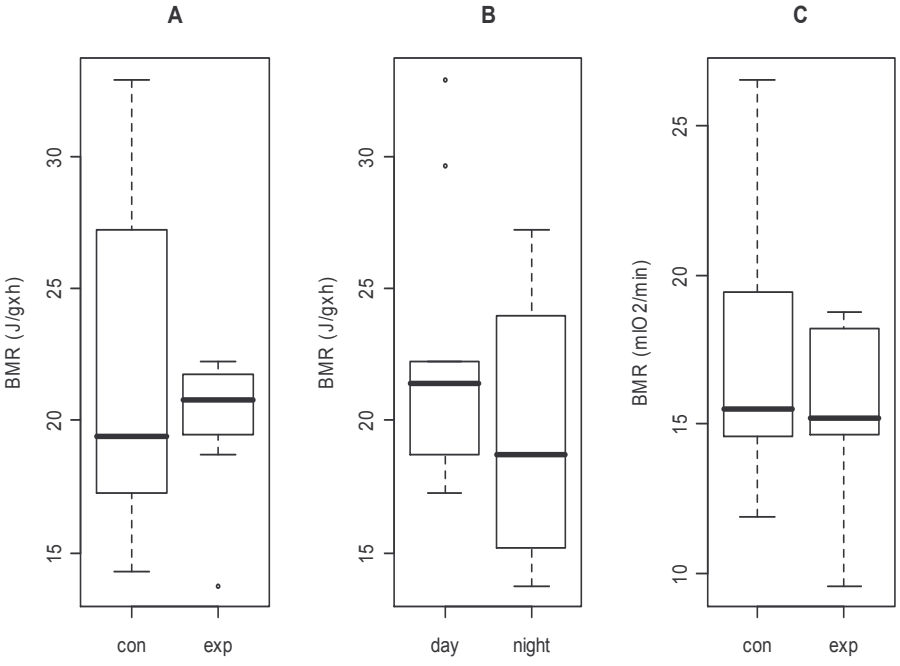

Figure 8 The range of BMR for exposed (exp) (n=7) and control (con) (n=10) herring gull (*Larus argentatus*) chicks. A: BMR on a mass specific basis (J/g·h) in exposed (exp) (n=7) and control (con) (n= 10) group, B: BMR (J/g·h) of chicks measured in the day (n=9) and the night (n=8), C: Non-mass specific BMR (ml O2/min) in exposed and control group.

3.6 Correlations between BMR and thyroid hormone levels and ratios

A positive correlation was seen between BMR and FT4 in the exposed group (Spearman R; r = 0.82, p = 0.03), and also between BMR and FT4 for male chicks in the exposed group (Spearman R; r= 1, p = 0.017) (n=5). A trend was observed between BMR and TT4 for female chicks in the control group (Spearman R; r= 1, p = 0.08) (n=4). For thyroid hormone ratios a negative correlation was seen between BMR and TT4/FT4 for exposed chicks (Spearman R; r= -0.82, p = 0.03) (n=7).

A negative correlation between total body length and BMR was observed for all chicks (Spearman R; r= -0.59, p = 0.01) (n=17) (figure 10) No correlation was observed for BMR and any HOC concentration in the herring gull chicks.

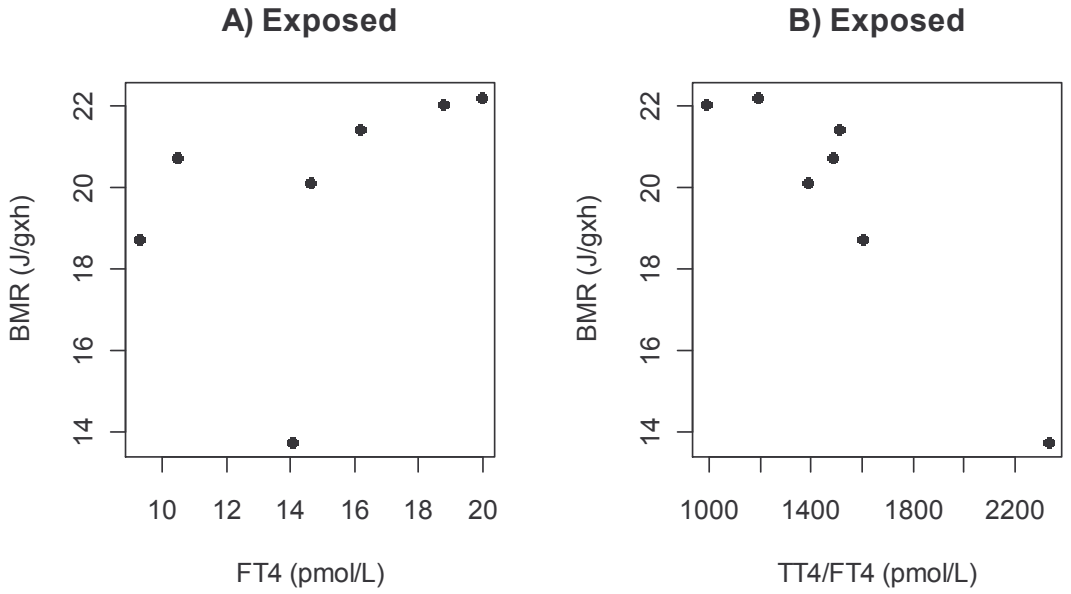

Figure 9 Scatter plots of relationships between BMR and A: FT4 (pmol/L) in exposed (Spearman R; r =0.82, p-value = 0.03) (n=7) and B: TT4/FT4 (pmol/L) in exposed (Spearman R; r= -0.82, p = 0.03) (n=7) herring gull (*Larus argentatus*) chicks.

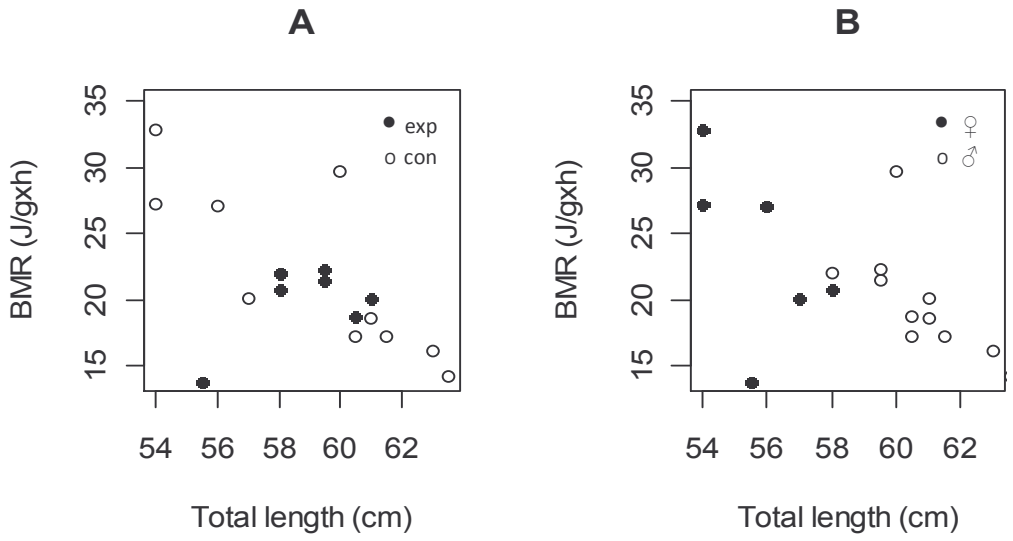

Figure 10 Scatter plots of correlations between BMR (J/g·h) and totallength (cm) shown for A: exposed (exp) and control (con), and for B: females (♀) and males (♂) (B) in herring gull chicks (Spearman R; r = -0.59, p = 0.01).

4 DISCUSSION

4.1 Persistent Organic Pollutants in gulls

The POP concentrations from the exposed, non-starved and starved herring gull chicks in this study were comparable with levels registered in herring gull eggs at the coast of Northern Norway (Helgason et al., 2008, Helgason et al., 2009, Barrett et al., 1996), the Faroes (Bourne, 1972) and the Canadian Great Lakes (Norstrom et al., 2002, Norstrom and Hebert, 2006). The POP concentrations in plasma samples of the present herring gull chicks were also in the range of those reported from adult glaucous gull plasma by Verreault et al. (2004) and Bustnes (2001, 2002).

Verreault (2007b) reported ∑PCB plasma levels in adult glaucous gulls (*Larus hyperboreus*) from Ny Ålesund, Svalbard, on average three times higher than that of the exposed-starved herring gull chicks in the present study (45congeners against the 23 measured in the present study). ∑PBDE in glaucous gulls from from Ny Ålesund was on average 40% higher than in the herring gull chicks from the present study. PBDE levels in the present exposed non-starved chicks were in the range of those reported in eggs from herring gull in de Wit et al. (2006) from the coast of northern Norway, but seven times lower than the levels measured in herring gull eggs from the Canadian Great Lakes (Norstrom et al., 2002). Therefore, based on the levels of POPs in the chicks and based on the levels for effects, especially in the exposed-starved group, one would expect to observe effects on thyroid hormone levels and the BMR.

Lipophilic POPs deposited in e.g adipose tissue is released into the circulation as fat reserves are mobilized during starvation. Consequentially, POP concentrations in plasma increases (Furness and Monaghan, 1987). The POPs are redistributed to well circulated and lipid containing tissues like the liver and brain (Södergren and Ulfstrand, 1972). The expected elevated plasma POP concentrations, as a result of starvation, is shown in the present study where the ∑PCB concentrations in the exposed-starved herring gull chicks were on average 4.5 times higher (range 313-1258 ng/g ww) compared to the exposed non-starved group (range 79-195 ng/g ww). The control-starved group had a ∑PCB level (range 4-27 ng/g ww) twice the level of the control, non-starved group (range 2-14 ng/g ww). ∑DDT, ∑CHL, $\sum MeSO_2$ and ∑OHs showed a similar pattern (appendix 1). The pattern of the present study is ∑PCB > ∑DDT > ∑CHL > ∑PBDE > ∑OH- > $\sum MeSO_2$ - > ∑MeO-, which is similar to

tissue concentration pattern of POPs observed in arctic seabird species (∑PCB > ∑CHL ≈ ∑DDT > ∑PBDE) (Letcher et al., 2009).

The POP group that are found at the highest concentration levels in arctic seabirds are the PCBs. The different congeners which are divided into metabolic groups based on the *ortho*-Cl substitution and the vicinal H-atoms in *meta-para* and *ortho-meta* positions on the biphenyl rings (appendix 3) (Walker, 1990). In short, the most persistent PCB congeners are those found in metabolic group I-III, which are lacking vicinal H atoms in *meta-para* position and are thereby bioaccumulated to a greater extent, while congeners in group IV and V are more easily metabolized (Borgå et al., 2005b). Congeners found in group III, mono-*ortho* congeners (with only one Cl-substitution in the *ortho*-position) have been shown to induce phase I biotransformation enzymes (Borgå et al., 2005b).

Of ∑PCB in this study, metabolic group I and II comprised the highest levels in all 4 herring gull groups; 83 and 85% of ∑PCB in the exposed and exposed-starved group, and 79 and 80 % of ∑PCB in the control and control-starved groups, respectively. The highest proportion of persistent PCBs was found in the exposed-starved group (Walker, 1990). High contaminant levels in this herring gull group may have caused the activation of hepatic biotransformation oxygenases. This may have led to metabolism and elimination of PCBs in the less persistent groups IV and V (McNabb, 2007).

4.2 Thyroid hormones and effects from POPs

In order to induce fat mobilization the herring gull chicks were starved. This resulted in elevated plasma POP levels which also resulted in alterations in plasma thyroid hormone levels. Starvation influences the thyroid hormone levels in birds as in mammals, especially the T3 levels are depressed (McNabb, 2000, Totzke et al., 1999). In literature it is stated that caution must be taken when interpreting effects of POPs on thyroid hormones as a result of starvation. This must be distinguished different from those induced by food deprivation.

Food deprivation is associated with increased plasma T4 and decreased T3 levels, as well as increased plasma free fatty acids in chicken and mammals (Decuypere et al., 2005). The decrease in T3 level is suggested to be the result of a shift in the balance between two different isozymes of deiodinases. The shift involves inhibition of deiodinase type 1 which deiodinates T4 to T3 in the liver, kidney and thyroid gland, and activation of deiodinase type 3, which is responsible for deiodination and thereby degradation of T3 (Decuypere et al., 2005). Another isozyme; deiodinase type 2, is responsible for T4 to T3 deiodination in the pituitary, central nervous system and placenta. The type 2 deiodinase is not affected by low caloric intake. This maintains the local T3 levels responsible for the negative feedback on the pituitary and hypothalamus at normal concentration levels even under food restrictions (Boron and Boulpaep, 2005). The low T3 levels lead to a decline in metabolic rate, and thereby to energy saving for a starving bird.

The thyroid hormone levels in this study were in the range of those of glaucous gulls reported by Verreault et al. (2004, 2007a). A study on blood chemistry changes in fasting herring gulls by Jeffrey et al. (1985) gave T4 levels two times as high as the levels in the present study. The T3 levels however, were in the same range as the current. Adult birds from many species, have a plasma concentration of TT4 in the range 6-19 pmol/ml and a TT3 concentration in the range 0.7-1.5 pmol/ml, according to McNabb (2000). The herring gull chicks from the present study are at the upper end of the TT4 range, while starved chicks are within the TT3 range. The non-starved chicks have TT3 levels twice as high.

No difference was found in the TT4 levels between the experimental and control groups in the present study. From figure 3 a slight trend can be seen for lower TT4 levels in the exposed-starved group (not significant). The exposed-starved herring gull chicks had a significantly lower level of FT4 compared to the exposed-non-starved group (fig 3). In the

two control groups there were no such difference. This might imply that the contaminant levels in the exposed-starved group might have influenced the FT4 levels. TT3 and FT3 levels were not significantly different in the exposed groups compared to control groups. However both the TT3 and FT3 levels were reduced in both starved groups (exposed and control) compared to the non-starved groups. Much of the decrease in T3 may be a result of starvation, which result in a shift in the deiodinase activity as described above by Boron (2005).

There are several levels of the HPT-axis where POPs can interfere and disrupt thyroid hormone homeostasis. Three main areas are 1) transport proteins, 2) induced thyroid hormone metabolism and excretion, and 3) disruption of thyroid gland function and morphology. A possible explanation for the lowered FT4 levels in the exposed-starved group can be due to an increased thyroid hormone metabolism induced by high POP levels. Many organochlorines induce hepatic biotransformation enzymes in phase I and II biotransformation pathways. These are involved in chemical detoxification processes resulting in metabolic products with higher water solubility that make them more easily excreted through bile and urine. POPs have been reported to induce the biotransformation phase II enzyme, UDP-GT, which plays a role in T4 dynamics in mammals. UDP-GT conjugates T4 to form T4-glucuronide which is readily excreted in bile (McNabb, 2003). Reports of induced T4 glucuronidation after exposure to individual PCB congeners has been given for rats (Morse et al., 1993, Van Birgelen et al., 1995). Relatively little is known about UDP-GT activity and hormone binding proteins in birds compared to mammals (McNabb, 2003).

Webb and McNabb (2008) reported increased UDP-GT activity but no significantly decreased T4 levels in Japanese quail after being exposed to Aroclor 1254. In the same study, mice were given the same doses of Aroclor 1254 as the Japanes quail. They showed a higher increase of UDP-GT activity and decreased T4 plasma levels than the quail. Webb and McNabb (2008) and McNabb (2003) suggested that bird's thyroid function could be less vulnerable to PCBs than that in mammals.

Positive correlations were found between FT4/FT3 ratios and OH- and $MeSO_2$ –metabolites, CHLs, DDTs and PCB group IV in the present study (table 4). The alteration of T4/T3 ratio is an indicator of POPs effects on thyroid homeostasis as well as iodine deficiency, since high

concentrations of iodine favours T4 synthesis (Peakall 1992). However, in the marine environment iodine deficiency is not considered a problem since the ocean represents a large reservoir of iodine. Verreault (2004) reported negative correlations between FT4/FT3 and POPs in glaucous gulls. The decline in FT4/FT3 might come from an increased T4 metabolism, and excretion as a consequence of high POP levels, as well as from increased conversion of T4 to T3 (Boas et al., 2006). The result from the present study showing an increased FT4/FT3 ratio in relation to POP concentrations, might be a result from starvation. This is likely because the results are shown in the exposed-starved group, where T3 levels are expected to be low due to the effects of starvation (Decuypere et al., 2005).

POPs competition for binding sites on transport proteins like the TTR and albumin, can potentially displace T4 from TTR and facilitate excretion of FT4. The result is a decrease in circulating TT4 (Lans et al., 1994, Brouwer et al., 1986). PCBs and PBDEs and especially some of the OH-metabolites with a similar molecular structure to T4, has been shown to displace T4 and to a lesser degree T3 from both TTR and albumin in gulls (Ucan-Marin et al., 2009). The displacement may result in less thyroid hormone bound to transport proteins and a total decrease in plasma concentrations of thyroid hormones (Ucan-Marin et al., 2009). An increased conversion of T4 to T3 can also alter the relative concentrations of the thyroid hormones (Boas et al., 2006). This may explain the decreased FT4 levels in exposed-starved herring gull chicks in the present study. Although, the expected increased T3 levels from this activation, was not observed. Most likely due to the effects from starvation resulting in decreased T3 levels (Decuypere et al., 2005).

Negative, positive and no relationships of thyroid hormones have been reported in POPs effects studies of seabirds. Verreault et al. (2004) reported negative correlations between T4 levels, T4/T3 ratios and OCs in male glaucous gulls from Bear Island. Grasman et al. (1996) reported no difference in plasma T4 concentrations in herring gull chicks from Canadian Great Lakes sites with great differences in PHAH (PolyHalogenated Aromatic Hydrocarbons) contamination. Van den Berg et al. (1994) reported reductions in plasma T3 and FT4 in hatchling cormorants (*Phalacrocorax carbo*) from a heavily contaminated colony compared with a less contaminated colony. In the last study PCB concentrations were inversely correlated to thyroid hormone levels. Gould et al. (1999) reported that chicken embryos from eggs injected with Aroclor 1254 and 1242 before incubation had significantly decreased plasma T4 concentrations at day 21 of incubation. McNabb (2003) reported diverging results

from herring gulls from high PCB contaminated sites from the Canadian Great Lakes at different life stages. Pipping embryos showed decreased thyroid gland stores of T4 in 10 of 12 samples, thyroid gland hypertrophy and significantly decreased circulating T4 levels in 42% of the embryos. All herring gull prefledglings sampled showed decreased T4 content in the thyroid gland. Thyroid gland hypertrophy was observed in 67% of the prefledglings. Circulating T4 levels were decreased in only 29% of the samples from the same age class. Adult herring gulls showed thyroid gland hypertrophy with decreased gland T4 content. Plasma concentrations on the other hand did not differ from birds at low PCB contaminated sites. The McNabb (2003) study suggested that hypothyroidism, measured as plasma concentration of T4, is common during development in herring gulls, but it is rare in adult birds. Adult bird's capacity to maintain circulating hormone levels, despite effects from contaminant exposure, seem to be a consequence of the activation of HPT-axis and the extracellular storage of hormone in the thyroid gland. McNabb's (2003) study may also imply that birds in an early stage of life and without a fully developed thyroid function are more vulnerable to the effects of POPs. For prefledglings and adult birds, deficiencies of thyroid hormone stores could be limiting under stressful conditions such as, during molt or adverse weather, where hormone release may be increased (McNabb, 2003).

4.3 Basal metabolic rate (BMR)

True BMR is measured in a post absorptive state at rest in a thermoneutral environment (Boron and Boulpaep, 2005, Blem, 2000). All birds except for one (that was removed from the study) were behaving calmly in the metabolism chamber. The metabolic measurements of herring gull chicks were also done within the thermoneutral zone and in birds in a post-absorptive state. After spending two months in captivity, the chicks were used to being handled by humans, so the BMR measured is considered to be close to basal metabolic levels.

The BMR measurements in the present study were not significantly different in the exposed- and in the control herring gull group. An average of 19.9 J/g/h was recorded for the exposed and 22.1 J/g/h for the control group. Bryant and Furness (1995) measured BMR of herring gulls to 19.3 J/g/h (Ellis and Gabrielsen, 2002). Compared to the calculated BMR obtained when using the allometric equation for seabirds given in Ellis and Gabrielsen (2002), the herring gull chicks of this study have a BMR of 108% of the expected values.

Metabolic rate changes during the day, with higher levels in the day and lower at night. Since the groups were too small, separating them into day and night groups would not have given significant results. Plots were used to look for relationships. A slight trend for lower BMR levels at night were observed, but no significant difference was shown. The variation of the BMR measurements were large, and made it difficult to interpret the results. Another aspect that could have affected the BMR results, is that BMR measurements and blood samples were not obtained at the same day, from practical reasons.

For the birds that were starved before blood sampling, especially T3 levels could have been affected by starvation. The distribution of males and females in the groups were uneven, but since the number of herring gull chicks in the groups was too small, males and females are not analysed separately (see table 6 for sex distribution).

In the present study a positive correlation was seen between FT4 and BMR. A negative correlation was found between TT4/FT4 and BMR in the exposed herring gull chicks (fig 9). There was also a negative correlation between total body length and BMR, which shows a tendency for larger birds to have a lower BMR. No correlations were seen between POPs and BMR, contrary to the findings of Verreault et al. (2007a) who reported a negative correlation between CHLs, PCBs and DDTs and BMR in the glaucous gull in Bear Island. No correlation between thyroid hormones and BMR was found in Verreault et al.'s (2007a) study. The thyroid hormones are considered to exert the major control of BMR, with T3 as the biologically active hormone (McNabb, 2000). A possible explanation for the positive correlation between FT4 and BMR could be that the higher contaminated birds in the group have a lower level of FT4 (if the relationship is the same as that shown in figure 3 for thyroid hormone levels , with lowered T4 levels for exposed-starved chicks and not for control-starved), and from that a lower BMR. This is of course just speculations.

The negative correlation between BMR and Total body length is expected since BMR is a function of body mass and bigger animals have a lower metabolism, also a slightly lower mass specific BMR (Kleiber, 1975).

Experiments performed on birds and mammals so far is limited to chlorinated contaminants so structure-activity (physiochemical properties) relationships for effect on BMR is not yet defined (Verreault et al., 2007a).

4.4 Conclusions and recommendations for further studies

The herring gull chicks in the present study showed a significant decrease in T3 levels. The decrease was observed both in the exposed and in the control chicks. Most likely this can be due to starvation since starving birds in previous studies have shown a decrease in T3 levels in plasma. Additionally, T4 levels were significantly lower in the exposed –starved compared to the corresponding non-starved group. This effect was not observed in the control group, and might be an effect of the high POP levels in the exposed-starved chicks. While negative correlations between TT3 and POP levels might be a result of starvation, the TT4/FT4 ratio that negatively correlates with POP levels in the exposed-starved group, could be an affect of high POP levels interference with thyroid transport proteins, hormone metabolism, or activation or inhibition of other biochemical pathways in the HPT-axis. Even though a difference in BMR in the exposed and control group was not observed, the possibility of an association between POP levels and BMR can't be excluded. The internal variability of the BMR in each herring gull group was large, and combined with a small sample size, this made it difficult to see any clear trends.

The herring gull has, in several studies, shown to be a robust seabird. Despite high POP levels in plasma, adult birds seem to compensate for lowered thyroid content in the thyroid gland by stronger activation of the HPT-axis. Chicks at an early stage of development are more vulnerable to the effects of high POP levels, as also may be the case for adult herring gulls facing harsh conditions such as cold climate and periods of natural fasting. More studies are recommended to increase the knowledge of influence of POPs on the thyroid hormone homeostasis in seabirds. To increase sample size without harming the wild seabird population, taking plasma samples combined with BMR measurements are recommended.

References

AKINS, J., WALDREP, K. & BERNERT JR, J. 1989. The estimation of total serum lipids by a completely enzymatic'summation'method. *Clinica chimica acta; international journal of clinical chemistry,* 184**,** 219-226.

ANKER-NILSSEN, T., BARRETT, R., BUSTNES, J., ERIKSTAD, K., FAUCHALD, P., LORENTSEN, S., STEEN, H., STRØM, H., SYSTAD, G. & TVERAA, T. 2007. SEAPOP studies in the Lofoten and Barents Sea area in 2006. *NINA Report,* 249**,** 68.

BARRETT, R., SKAARE, J. & GABRIELSEN, G. 1996. Recent changes in levels of persistent organochlorines and mercury in eggs of seabirds from the Barents Sea. *Environmental Pollution,* 92**,** 13-18.

BARRIE, L., GREGOR, D., HARGRAVE, B., LAKE, R., MUIR, D., SHEARER, R., TRACEY, B. & BIDLEMAN, T. 1992. Arctic contaminants: sources, occurrence and pathways. *Science of The Total Environment,* 122**,** 1-74.

BAUDINETTE, R., LOVERIDGE, J., WILSON, K., MILLS, C. & SCHMIDT-NIELSEN, K. 1976. Heat loss from feet of herring gulls at rest and during flight. *American Journal of Physiology,* 230**,** 920.

BLEM, C. R. 2000. Energy balance. *In:* WHITTOW, G. (ed.) *Sturkie's avian physiology.* San Diego: Academic Press. 327-341.

BOAS, M., FELDT-RASMUSSEN, U., SKAKKEBÆK, N. E. & MAIN, K. M. 2006. Environmental chemicals and thyroid function. *European Journal of Endocrinology,* 154**,** 599-611.

BOON, J., VAN DER MEER, J., ALLCHIN, C., LAW, R., KLUNGSØYR, J., LEONARDS, P., SPLIID, H., STORR-HANSEN, E., MCKENZIE, C. & WELLS, D. 1997. Concentration-dependent changes of PCB patterns in fish-eating mammals: structural evidence for induction of cytochrome P450. *Archives of Environmental Contamination and Toxicology,* 33**,** 298-311.

BORGÅ, K., GABRIELSEN, G. W. & SKAARE, J. U. 2001. Biomagnification of organochlorines along a Barents Sea food chain. *Environmental Pollution,* 113**,** 187-198.

BORGÅ, K., WOLKERS, H., SKAARE, J., HOP, H., MUIR, D. & GABRIELSEN, G. 2005a. Bioaccumulation of PCBs in Arctic seabirds: influence of dietary exposure and congener biotransformation. *Environmental Pollution,* 134**,** 397-409.

BORGÅ, K., WOLKERS, H., SKAARE, J. U., HOP, H., MUIR, D. C. G. & GABRIELSEN, G. W. 2005b. Bioaccumulation of PCBs in Arctic seabirds: influence of dietary exposure and congener biotransformation. *Environmental Pollution,* 134**,** 397-409.

BORON, W. F. & BOULPAEP, E. L. (eds.) 2005. *Medical physiology: a cellular and molecular approach,* Philadelphia, Pa.: Elsevier Saunders.

BOURNE, J. 1972. Polychlorinated biphenyls in North Atlantic seabirds. *Marine Pollution Bulletin,* 3**,** 171-175.

BROUWER, A., MORSE, D., LANS, M., GERLIENKE SCHUUR, A., MURK, A., KLASSON-WEHLER, E., BERGMAN, A. & VISSER, T. 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicology and industrial health,* 14**,** 59.

BROUWER, A., MURK, A. J. & KOEMAN, J. H. 1990. Biochemical and Physiological Approaches in Ecotoxicology. *Functional Ecology,* 4**,** 275-281.

BROUWER, A., VAN DEN BERG, K., BLANER, W. & GOODMAN, D. 1986. Transthyretin (prealbumin) binding of PCBS, a model for the mechanism of interference with vitamin A and thyroid hormone metabolism. *Chemosphere,* 15.

BUSTNES, J., BAKKEN, V., ERIKSTAD, K., MEHLUM, F. & SKAARE, J. 2001. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. *Journal of Applied Ecology***,** 791-801.

BUSTNES, J., FOLSTAD, I., ERIKSTAD, K., FJELD, M. & SKAARE, J. 2002. Blood concentration of organochlorine pollutants and wing feather asymmetry in glaucous gulls. *Functional Ecology,* 16**,** 617-622.

CRAMP, S. (ed.) 1977. *Handbook of the birds of Europe, the Middle East and NorthAfrica: the birds of the Western Palearctic,* Oxford: Oxford University Press.

DAVIS, J. 1975. Specialization in feeding location by herring gulls. *Journal of Animal Ecology,* 44**,** 795-804.

DE WIT, C., ALAEE, M. & MUIR, D. 2006. Levels and trends of brominated flame retardants in the Arctic. *Chemosphere,* 64**,** 209-233.

DE WIT, C., FISK, A., HOBBS, K., MUIR, D., GABRIELSEN, G., KALLENBORN, R., KRAHN, M., NORSTROM, R. & SKAARE, J. 2004. AMAP Assessment 2002: Persistent organic pollutants in the Arctic. *Arctic Monitoring and Assessment Programme,* Oslo, Norway.

DECUYPERE, E., VAN AS, P., VAN DER GEYTEN, S. & DARRAS, V. 2005. Thyroid hormone availability and activity in avian species: A review. *Domestic Animal Endocrinology,* 29**,** 63-77.

DUNN, E. H. & BRISBIN, I. L., JR. 1980. Age-Specific Changes in the Major Body Components and Caloric Values of Herring Gull Chicks. *The Condor,* 82**,** 398-401.

ELLIS, H. & GABRIELSEN, G. W. 2002. Energetics of Free-Ranging Seabirds. *In:* SCHREIBER, B. (ed.) *Biology of Marine Birds.* 2002 ed.: CRS Press

FURNESS, R. & MONAGHAN, P. 1987. *Seabird ecology*, Chapman and Hall/Methuen, Inc., New York, USA.

GABRIELSEN, G. 1994. *Energy expenditure in Arctic seabirds.* Ph.D. dissertation, University of Tromsø.

GABRIELSEN, G. 2007. Levels and effects of persistent organic pollutants in arctic animals. *In:* ØRBÆK, J. B., KALLENBORN, R., TOMBRE, I., HEGSETH, E. N., FALK-PETERSEN, S. & HOEL, A. H. (eds.) *Arctic Alpine Ecosystems and People in a Changing Environment.*

GABRIELSEN, G. W. 2009. Seabirds in the Barents Sea. *In:* SAKSHAUG, E., JOHNSEN, G. & KOVACS, K. (eds.) *Ecosystem Barents Sea.* Trondheim, Norway: Tapir Academic Press. 415-452

GABRIELSEN, G. W., MEHLUM, F. & KARLSEN, H. E. 1988. Thermoregulation in four species of Arctic seabirds. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology,* 157**,** 703-708.

GOULD, J., COOPER, K. & SCANES, C. 1999. Effects of polychlorinated biphenyls on thyroid hormones and liver type I monodeiodinase in the chick embryo. *Ecotoxicology and environmental safety,* 43**,** 195-203.

GRASMAN, K., FOX, G., SCANLON, P. & LUDWIG, J. 1996. Organochlorine-associated immunosuppression in prefledgling Caspian terns and herring gulls from the Great Lakes: an ecoepidemiological study. *Environmental health perspectives,* 104**,** 829.

GRIFFITHS, R., DOUBLE, M., ORR, K. & DAWSON, R. 1998. A DNA test to sex most birds. *Molecular Ecology,* 7**,** 1071-1075.

HELGASON, L., BARRETT, R., LIE, E., POLDER, A., SKAARE, J. & GABRIELSEN, G. 2008. Levels and temporal trends (1983–2003) of persistent organic pollutants (POPs) and mercury (Hg) in seabird eggs from Northern Norway. *Environmental Pollution,* 155**,** 190-198.

HELGASON, L., POLDER, A., FØREID, S., BÆK, K., LIE, E., GABRIELSEN, G., BARRETT, R. & SKAARE, J. 2009. Levels and temporal trends (1983-2003) of polybrominated diphenyl ethers and hexabromocyclododecanes in seabird eggs from North Norway. *Environmental Toxicology and Chemistry,* 28**,** 1096-1103.

HOP, H., BORGA, K., GABRIELSEN, G. W., KLEIVANE, L. & SKAARE, J. U. 2002. Food Web Magnification of Persistent Organic Pollutants in Poikilotherms and Homeotherms from the Barents Sea. *Environmental Science and Technology,* 36**,** 2589-2597.

JEFFERIES, D. & FRENCH, M. 1971. Hyper-and hypothyroidism in pigeons fed DDT: an explanation for the 'thin eggshell phenomenon'. *Environmental Pollution,* 1**,** 235-242.

JEFFREY, D., PEAKALL, D., MILLER, D. & HERZBERG, G. 1985. Blood chemistry changes in food-deprived herring gulls. *Comparative biochemistry and physiology. A, Comparative physiology,* 81**,** 911.

KLEIBER, M. 1975. *The Fire of Life. An introduction to animal energetics.*, Robert E. Krieger Publishing Company, Huntington, New York, 123-130.

LANS, M., SPIERTZ, C., BROUWER, A. & KOEMAN, J. 1994. Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBs, PCDDs and PCDFs. *European Journal of Pharmacology,* 270**,** 129.

LAWRENCE, E. (ed.) 2000. *Henderson's Dictionary of Biological Terms,* Essex: Pearson Education Limited.

LE MAHO, Y. 1983. Metabolic adaptations to long-term fasting in antarctic penguins and domestic geese. *Journal of Thermal Biology,* 8**,** 91-96.

LETCHER, R. J., BUSTNES, J. O., DIETZ, R., JENSSEN, B. M., JØRGENSEN, E. H., SONNE, C., VERREAULT, J., VIJAYAN, M. M. & GABRIELSEN, G. W. 2009. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Science of The Total Environment,* Article in press.

LUSTICK, S., BATTERSBY, B. & KELTY, M. 1978. Behavioral Thermoregulation: Orientation Toward the Sun in Herring Gulls. *Science,* 200**,** 81-83.

MARIUSSEN, E., FJELD, E., BREIVIK, K., STEINNES, E., BORGEN, A., KJELLBERG, G. & SCHLABACH, M. 2008. Elevated levels of polybrominated diphenyl ethers (PBDEs) in fish from Lake Mjøsa, Norway. *Science of the Total Environment,* 390**,** 132-141.

MCNABB, F. 2007. The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. *Critical reviews in toxicology,* 37**,** 163-193.

MCNABB, F. M. A. 1992. *Thyroid hormones*, Englewood Cliffs, N.J: Prentice Hall.

MCNABB, F. M. A. 2000. Thyroids. *In:* Whitthow, G.C. (ed.) *Sturkie's Avian Physiology.* Fifth ed. San Diego, London, Boston, New York, Sydney, Tokyo, Toronto: Academic Press. 461-472.

MCNABB, F. M. A., FOX, G.A., 2003. Avian thyroid development in chemically contaminated environments: is there evidence of alterations in thyroid function and development? *Evolution & Development,* 5**,** 76-82.

MEERTS, I., VAN ZANDEN, J., LUIJKS, E., VAN LEEUWEN-BOL, I., MARSH, G., JAKOBSSON, E., BERGMAN, A. & BROUWER, A. 2000. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicological Sciences,* 56**,** 95.

MILJETEIG, C., STRØM, H., GAVRILO, M., VOLKOV, A., JENSSEN, B. & GABRIELSEN, G. 2009. High levels of contaminants in ivory gull *Pagophila eburnea* eggs from the Russian and Norwegian Arctic. *Environmental science & technology,* 43**,** 5521.

MOCCIA, R., FOX, G. & BRITTON, A. 1986. A quantitative assessment of thyroid histopathology of herring gulls (*Larus argentatus*) from the Great Lakes and a hypothesis on the causal role of environmental contaminants. *Journal of Wildlife Diseases,* 22**,** 60.

MORSE, D. C., GROEN, D., VEERMAN, M., VANAMERONGEN, C. J., KOETER, H. B. W. M., VANPROOIJE, A. E. S., VISSER, T. J., KOEMAN, J. H. & BROUWER, A. 1993. Interference of Polychlorinated Biphenyls in Hepatic and Brain Thyroid Hormone Metabolism in Fetal and Neonatal Rats. *Toxicology and Applied Pharmacology,* 122**,** 27-33.

MURVOLL, K., SKAARE, J., NILSSEN, V., BECH, C., ØSTNES, J. & JENSSEN, B. 1999. Yolk PCB and plasma retinol concentrations in shag (*Phalacrocorax aristotelis)* hatchlings. *Archives of Environmental Contamination and Toxicology,* 36**,** 308-315.

NORSTROM, R. & HEBERT, C. 2006. Comprehensive re-analysis of archived herring gull eggs reconstructs historical temporal trends in chlorinated hydrocarbon contamination in Lake Ontario and Green Bay, Lake Michigan, 1971–1982. *Journal of Environmental Monitoring,* 8**,** 835-847.

NORSTROM, R., SIMON, M., MOISEY, J., WAKEFORD, B. & WESELOH, D. 2002. Geographical Distribution (2000) and Temporal Trends (1981- 2000) of Brominated Diphenyl Ethers in Great Lakes Herring Gull Eggs. *Environmental Scence and Technology,* 36**,** 4783-4789.

PEKARIK, C. & WESELOH, D. 1998. Organochlorine contaminants in herring gull eggs from the Great Lakes, 1974-1995: change point regression analysis and short-term regression. *Environmental Monitoring and Assessment,* 53**,** 77-115.

PURKEY, H., PALANINATHAN, S., KENT, K., SMITH, C., SAFE, S., SACCHETTINI, J. & KELLY, J. 2004. Hydroxylated polychlorinated biphenyls selectively bind transthyretin in blood and inhibit amyloidogenesis: rationalizing rodent PCB toxicity. *Chemistry & biology,* 11**,** 1719-1728.

RISCH, T. S. & ROHWER, F. C. 2000. Effects of parental quality and egg size on growth and survival of herring gull chicks. *Canadian Journal of Zoology,* 78**,** 967.

RUCH, T. C., PATTON, H. D. & HOWELL, W. H. (eds.) 1973. *Howell-Fulton physiology and biophysics. ,* Philadelphia: Saunders.

SAGERUP, K., HENRIKSEN, E., SKORPING, A., SKAARE, J. & GABRIELSEN, G. 2000. Intensity of parasitic nematodes increases with organochlorine levels in the glaucous gull. *Journal of Applied Ecology,* 37**,** 532-539.

SANDALA, G., SONNE-HANSEN, C., DIETZ, R., MUIR, D., VALTERS, K., BENNETT, E., BORN, E. & LETCHER, R. 2004. Hydroxylated and methyl sulfone PCB metabolites in adipose and whole blood of polar bear (*Ursus maritimus*) from East Greenland. *Science of the Total Environment,* 331**,** 125-141.

STEEN, I. & STEEN, J. 1965. The importance of the legs in the thermoregulation of birds. *Acta Physiologica Scandinavica,* 63**,** 285.

SÖDERGREN, A. & ULFSTRAND, S. 1972. DDT and PCB relocate when caged robins use fat reserves. *Ambio,* 1**,** 36-40.

TOTZKE, U., FENSKE, M., HUPPOP, O., RAABE, H. & SCHACH, N. 1999. The Influence of Fasting on Blood and Plasma Composition of Herring Gulls (*Larus argentatus*). *Physiological and biochemical zoology,* 72**,** 426-437.

UCAN-MARIN, F., ARUKWE, A., MORTENSEN, A., GABRIELSEN, G., FOX, G. & LETCHER, R. 2009. Recombinant transthyretin purification and competitive binding with organohalogen compounds in two gull species (*Larus argentatus* and *Larus hyperboreus*). *Toxicological Sciences,* 107**,** 440-450.

VAN BIRGELEN, A., SMIT, E., KAMPEN, I., GROENEVELD, C., FASE, K., VAN DER KOLK, J., POIGER, H., VAN DEN BERG, M., KOEMAN, J. & BROUWER, A. 1995. Subchronic effects of 2, 3, 7, 8-TCDD or PCBs on thyroid hormone metabolism: use in risk assessment. *European Journal of Pharmacology: Environmental Toxicology and Pharmacology,* 293**,** 77-85.

VAN DEN BERG, K. J., VAN RAAIJ, J., BRAGT, P. C. & NOTTEN, W. R. F. 1991. Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels in vivo. *Archives of Toxicology,* 65**,** 15-19.

VAN DEN BERG, M., CRAANE, B., SINNIGE, T., VAN MOURIK, S., DIRKSEN, S., BOUDEWIJN, T., VAN DER GAAG, M., LUTKE-SCHIPHOLT, I., SPENKELINK, B. & BROUWER, A. 1994. Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant (*Phalacrocorax carbo*) after in ovo exposure. *Environmental Toxicology and Chemistry,* 13**,** 803-816.

VERREAULT, J., BECH, C., LETCHER, R. J., ROPSTAD, E., DAHL, E. & GABRIELSEN, G. W. 2007a. Organohalogen contamination in breeding glaucous gulls from the Norwegian Arctic: Associations with basal metabolism and circulating thyroid hormones. *Environmental Pollution,* 145**,** 138-145.

VERREAULT, J., LETCHER, R., ROPSTAD, E., DAHL, E. & GABRIELSEN, G. 2006. Organohalogen contaminants and reproductive hormones in incubating glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. *Environmental Toxicology and Chemistry,* 25**,** 2990-2996.

VERREAULT, J., SHAHMIRI, S., GABRIELSEN, G. & LETCHER, R. 2007b. Organohalogen and metabolically-derived contaminants and associations with whole body constituents in Norwegian Arctic glaucous gulls. *Environment International,* 33**,** 823-830.

VERREAULT, J., SKAARE, J., JENSSEN, B. & GABRIELSEN, G. 2004. Effects of organochlorine contaminants on thyroid hormone levels in Arctic breeding glaucous gulls, *Larus hyperboreus. Environmental health perspectives,* 112**,** 532.

WALKER, C. H. 1990. Persistent Pollutants in Fish-Eating Sea Birds--Bioaccumulation, Metabolism, and Effects. *Aquatic Toxicology,* 17.

WALKER, C. H. 1996. *Principles of ecotoxicology,* London, Taylor & Francis.

WANG-ANDERSEN, G., SKAARE, J., PRESTRUD, P. & STEINNES, E. 1993. Levels and congener pattern of PCBs in Arctic fox, *Alopex lagopus*, in Svalbard. *Environmental pollution(1987),* 82**,** 269-275.

WEBB, C. & MCNABB, F. 2008. Polychlorinated biphenyl effects on avian hepatic enzyme induction and thyroid function. *General and Comparative Endocrinology,* 155**,** 650-657.

WITHERS, P. 1977. Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. *Journal of Applied Physiology,* 42**,** 120.

APPENDIX

APPENDIX 1 Lipid % in plasma and halogenated organic contaminant levels (ng/g wet weight) in plasma of herring gull (*Larus argentatus*) chicks.

	Exposed non-starved (n=6)			Exposed-starved (n=6)			Control non-starved (n=9)			Control-starved (n=9)		
∑POP (nr congeners)	Mean ± SD	Range	Median	Mean ± SD	Range	Median	Mean ± SD	Range	Median	Mean ± SD	Range	Median
Lipid %	1.1 ± 0.09	0.99 - 1.23	1.08	1.01 ± 0.28	0.77 - 1.48	0.92	1.24 ± 0.16	1.07 - 1.58	1.20	1.19 ± 0.21	0.98 - 1.54	1.18
∑PCB (23)	133 ± 40.9	79 - 195	130	611 ± 350	313 - 1258	536	7.46 ± 3.56	2.37 - 14.1	7.45	14.6 ± 7.53	4.05 - 27.2	15.0
∑PCB gr I (7)	56.3 ± 15.8	32.7 - 77.5	56.6	242 ± 163	106 - 558	204	3.07 ± 1.71	0.95 - 6.19	2.92	6.24 ± 3.49	1.87 - 12.1	5.97
∑PCB gr II (6)	54.4 ± 15.4	32.6 - 73.8	54.2	280 ± 145	132 - 522	254	2.85 ± 1.26	0.9 - 4.7	2.75	5.53 ± 2.77	1.26 - 9.4	5.40
∑PCB gr III (6)	18.8 ± 5.37	12.5 - 26.5	18.0	83.7 ± 49.2	43.8 - 174.9	68.7	1.25 ± 0.75	0.4 - 2.79	1.20	2.24 ± 1.11	0.81 - 4.05	1.95
∑PCB gr IV (5)	3.05 ± 5.45	0.63 - 14.2	0.88	5.26 ± 5.32	1.47 - 15.1	3.10	0.24 ± 0.18	0.07 - 0.61	0.19	0.49 ± 0.52	0.08 - 1.74	0.27
∑PCB gr V (1)	1.41 ± 0.93	0.71 - 3.17	1.06	2.34 ± 0.75	1.41 - 3.31	2.30	0.11 ± 0.02	0.08 - 0.14	0.11	0.2 ± 0.09	0.04 - 0.34	0.20
∑OHs (10)	6.86 ± 2.24	4.11 - 9	7.34	19.1 ± 9.69	7.34 - 35.8	17.5	0.3 ± 0.19	0.1 - 0.64	0.26	0.41 ± 0.16	0.16 - 0.68	0.35
MeO (1)	0.14 ± 0.04	0.09 - 0.19	0.14	0.17 ± 0.05	0.09 - 0.23	0.18	0.04 ± 0.02	0.03 - 0.07	0.04	0.05 ± 0.02	0.04 - 0.08	0.04
∑$MeSO_2$ (12)	0.72 ± 0.29	0.35 - 1.07	0.71	1.86 ± 0.94	1.07 - 3.65	1.71	0.01 ± 0.01	0.01 - 0.02	0.01	0.03 ± 0.02	0.01 - 0.07	0.02
∑chlordanes (4)	16.1 ± 4.71	10.4 - 21.5	16.6	47.7 ± 25.7	24.2 - 96.4	41.2	0.93 ± 0.41	0.48 - 1.57	0.81	1.68 ± 0.77	0.5 - 3.26	1.63
∑DDT (3)	45.7 ± 18.8	28.2 - 72.2	41.2	153 ± 102	47.1 - 347	130	2.62 ± 1.04	1.06 - 3.9	2.99	5.18 ± 2.46	2.66 - 9.54	4.22
∑PBDE (10)	10.5 ± 3.69	5.94 - 15.2	10.9	37.9 ± 21.9	18.8 - 79.9	32.80	0.36 ± 0.17	0.12 - 0.63	0.34	0.72 ± 0.44	0.14 - 1.37	0.63

APPENDIX 2 Thyroid hormone levels and ratios in plasma of herring gull (*Larus argentatus*) chicks in the exposed and control groups. TT4, TT3, TT4/TT3 are given in nmol/L, FT4, FT3, TT4/FT4, TT3/FT3 and FT4/FT3 are given in pmol/L.

	Exposed non-starved (n=6)			Exposed-starved (n=6)			Control non-starved (n=9)			Control-starved (n=9)		
	Mean ± SD	Range	Median	Mean ± SD	Range	Median	Mean ± SD	Range	Median	Mean ± SD	Range	Median
TT4	17.8 ± 5.42	11.2 - 24.4	16.6	16.9 ± 8.89	8.64 - 32.8	15.2	17.8 ± 5.57	8.77 - 26.3	17.2	17.2 ± 7.43	7.78 - 33.1	17.2
FT4	15.7 ± 3.62	11.1 - 20	16.5	10.7 ± 3.4	5.32 - 14.6	10.4	14.1 ± 4.75	5.00 - 20.7	13.8	12.4 ± 4.18	6.35 - 18.6	12.7
TT3	3.69 ± 1.45	1.94 - 6.29	3.41	1.4 ± 0.77	0.78 - 2.91	1.23	3.24 ± 0.58	1.81 - 3.77	3.42	1.48 ± 0.47	1.03 - 2.61	1.47
FT3	5.03 ± 2.04	3.04 - 8.93	4.55	1.25 ± 0.88	0.62 - 2.98	0.94	3.92 ± 1.01	1.40 - 4.72	4.31	1.78 ± 1.09	0.57 - 4.16	1.72
TT4/FT4	1130 ± 240	869 - 1510	1089	1553 ± 464	895 - 2330	1540	1297 ± 204	1031 - 1754	1271	1403 ± 345	836 - 1899	1330
TT3/FT3	732 ± 54	639 - 778	749	1213 ± 280	976 - 1754	1122	863 ± 171	741 - 1291	809	1058 ± 507	353 - 1933	950
TT4/TT3	5.29 ± 2.13	2.95 - 7.5	5.23	12.6 ± 4.73	6.6 – 21.0	11.6	5.48 ± 1.31	3.65 - 7.70	5.58	12.6 ± 5.63	2.98 - 22.5	12.0
FT4/FT3	3.45 ± 1.35	2.1 - 5.52	3.22	10.5 ± 5.14	4.72 - 16.8	8.90	3.61 ± 0.73	2.44 - 4.61	3.57	9.09 ± 5.3	2.51 - 20.2	7.49

APPENDIX 3

Detected contaminants:

∑chlordanes: Oxy-chlordan, cis-chlordan, trans-nonachlor, cis-nonachlor

∑MeO: 2-MeOBDE 68

∑PCB: 28, 47/49, 52, 99, 101, 105, 118, 123, 128, 138, 141, 149, 153, 156, 157, 167, 170, 180, 183, 187, 189, 194

PCB grI: 153, 167, 180, 183, 187, 189, 197

PCB grII: 47/49, 99, 128, 138, 170

PCB grIII: 28, 105, 118, 123, 156, 157

PCB grIV: 47/49, 52, 101, 141

PCB grV: 149

∑OH: 4-OH-PCB107, 3-OH-PCB153, 4-OH-PCB146, 3-OH-PCB138, 4-OH-PCB130, 4-OH-PCB163, 4-OH-PCB187, 4-OH-PCB172, 4'-OH-PCB193, 4-OH-BDE49

∑DDT: p,pDDT, p,pDDE, o,pDDE

∑PBDE: 28, 47, 71/49, 66, 99, 100, 119, 153, 154

∑MeSO: 3-MeSOCB49, 4-MeSOCB49, 4-MeSOCB91, 4-MeSOCB101, 3-MeSOCB87,

4-MeSOCB110, 3-MeSOCB149, 4-MeSOCB149, 3-MeSOCB132, 4-MeSOCB132, 4-MeSOCB141,

3-MeSO2DDE

Detected in less than 70% of the individuals in any of the groups of birds.

PCBs:	PBDE-138	5-OH-BDE99
PCB 18	PBDE-183	4-OH-BDE101
PCB 31	**OH-comp.**	**MeSO-PCBs:**
PCB 33	PCP	3MeSOPCB52
PCB 37	4-OH-PCB 120	4MeSOPCB52
O,P-DDT	2-OH-BDE68	3MeSOPCB91
O,P-DDD	6-OH-BDE47/75	3MeSOPCB101
BFRs:	5-OH-BDE47	3MeSOPCB110
TBA	5-OH-BDE100	3MeSOPCB141
PBDE-77	4-OH-BDE103	3MeSOPCB174
PBDE-85	TBBPA	4MeSOPCB174

APPENDIX 4

Mazuri Zoo Foods: Fish Eater Tablets [1]

Fat	%	21.30
Protein	%	16.00
Fibres	%	1.00
Ashes	%	16.80
Starch	%	1.30
Glucids	%	23.10
Total energy	MJ/kg	19.10
Digestable enregy	MJ/kg	15.60
Metabolic energy	MJ/kg	14.10
Linoleic acid	%	0.12
Linolenic acid	%	0.03
Ca	%	0.11
P	%	0.31
Na	%	0.03
Cl	%	1.40
K	%	0.38
Mg	%	0.66
Fe	mg/kg	41.03
Cu	mg/kg	6.15
Mn	mg/kg	1.03
Zn	mg/kg	20.51
Co	µg/kg	41.03
I	µg/kg	2.05
F	mg/kg	1.05
Vit. A		12820512.00
Vit. D3		2564103.00
Vit. E	mg/kg	225641.00
Vit. B1	mg/kg	63815.38
Vit. B2	mg/kg	1548.72
Vit. B6	mg/kg	1543.59

APPENDIX 5. Polychlorinated biphenyl congeners in metabolic groups based on their ortho-Cl substitution and vicinal H atoms in meta-para and ortho-meta positions (Borgå et al., 2005a)

IUPAC[1] no.	Vicinal H atoms		*Ortho*-Cl	Cl-substitutions
	m,p[2]	o,m[2]		
Group I: PCB congeners with no vicinal hydrogen atoms, persistent.				
153	-	-	2	2,2',4,4',5,5'
167	-	-	2	2,3',4,4',5,5'
180	-	-	2	2,2',3,4,4',5,5'
183	-	-	2	2,2',3,4,4',5',6
187	-	-	2	2,2',3,4',5,5',6
189	-	-	2	2,3,3',4,4',5,5'
197	-	-	2	2,2',3,3',4,4',6,6'
Group II: Vicinal H atoms in ortho-meta position, ≥2 Cl-substitutions in ortho-position				
47/49	-/+	+/+	2	2,2',4,4'/2,2',4,5'
99	-	+	2	2,2',4,4',5
128	-	+	2	2,2',3,3',4,4'
138	-	+	2	2,2',3,4,4',5'
170	-	+	2	2,2',3,3',4,4',5
Group III: Vicinal H atoms in *ortho-meta* position, < 2 Cl-substitutions in *ortho*-position				
28	-	+	1	2,4,4'
105	-	+	1	2,3,3',4,4'
118	-	+	1	2,3',4,4',5
123	-	+	1	2,3',4,4',5'
156	-	+	1	2,3,3',4,4',5
157	-	+	1	2,3,3',4,4',5'
Group IV: Vicinal H atoms in *meta-para* position, ≤ 2 Cl-substitutions in *ortho*-position				
47/49	-/+	+/+	2	2,2',4,4'/2,2',4,5'
52	+	-	2	2,2',5,5'
101	+	-	2	2,2',4,5,5'
141	+	-	2	2,2',3,4,5,5'
Group V: Vicinal H atoms in *meta-para* position, > 2 Cl-substitutions in *ortho*-position				
149	+	-		2,2',3,4',5',6

APPENDIX 6

Content of POPs in cod liver oil ng/g ww

Analysed by Norwegian School of Veterinary Science, Oslo, Norway

	Contaminated oil	Cleaned oil
∑ PCBs	23274,5	31,8
∑ DDTs	12190	7
∑ Chlordanes	3071	ND
∑ PBDEs	1167,5	1,8
∑ HCHs	43	ND
HCB	30	ND