POP-cocktails: Hangover threats for seabirds?

The response of three seabird species to exposure to persistent organic pollutants in the Barents Sea

Kjetil Sagerup

A dissertation for the degree of Philosophiae Doctor

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Preface

When looking back I realise that a Ph.D project is everything but a stand-alone project. A complex network of people from different scientific institutions has been involved in the processes that result in this thesis. I owe thanks to many co-workers and colleagues through years of studying and work since I started out on my master thesis. I offer extend many thanks to Rob Barrett at Tromsø University Museum and Geir Wing Gabrielsen at Norwegian Polar Institute, my two supervisors who always had an open door, guided me through the scientific landscape and dragged me out of ditches, no matter how deep they were. I will also thank my third and formal supervisor Nigel Gilles Yoccoz at Institute of Biology at University of Tromsø who taught me the R language, greatly increased my statistical understanding and significantly improved the statistics in our manuscripts.

This thesis was made possible through a collaboration between Tromsø University Museum and Norwegian Polar Institute. It was financed by the University of Tromsø with a significant contribution from the Norwegian Polar Institute. The Norwegian School of Veterinary Science contributed as a scientific partner and financed large parts of the analysis. Several other financial sources have contributed and are acknowledged in the papers.

I would like to thank Janneche Utne Skaare (National Veterinary Institute, Oslo) and Anuschka Polder (Environmental Toxicology lab at the Norwegian School of Veterinary Science, Oslo) who taught me analytical toxicology and participated as co-authors of the papers; Hans Jørgen Larsen, Grethe Marie Johansen and Tine Borgen (immune lab at the Norwegian School of Veterinary Science, Oslo) for introducing me to immunological laboratory work, John Beck Jensen (Tromsø University Museum) for teaching me molecular DNA techniques, Kjetil Aasbakk (Section for Arctic Veterinary Medicine, Norwegian School of Veterinary Science, Tromsø) for rescuing the last part of this thesis by letting me do the speculative ecological shortcuts of immunology, despite of his disagreement to the method and Terje D. Josefsen (Norwegian Veterinary Institute, Tromsø) for performing the autopsies of the dead glaucous gulls.

A special thank to Jan Ove Bustnes, Sveinn Are Hanssen and Børge Moe (Norwegian Institute for Nature Research, Tromsø) who let med participate in the International Polar Year project BIRD HEALTH. The four field trips to Ny-Ålesund studying black-legged kittiwakes and common eiders were really valuable scientific experiences and vacations from the struggling with this thesis.

Thanks to my post-graduate colleagues and the staff at Tromsø University Museum for a great time staying at the Museum. Thanks to Anne Helene Tandberg and Trond Elling Barstad for scientific discussion and to Jan Magne Gjerde for bringing new insight of magnificent rock art into each coffee-break. I will certainly never forget the 5000-year old bear hunt rock art story from Kanozero, Kola Peninsula; it is simply a great told story.

Last but not least, I would like to thank my family for their constant support, particularly my two children Marie and Henrik and my wife Kristin, who has been very patient, supportive and very comprehensible despite my numerous stay in field, and for the long evenings and weekends when I stayed at the office instead of being with you.

Summary

The overall aim of the work for this thesis was to study immunological effects of pollutants in seabirds from the Barents Sea. Glaucous gull (*Larus hyperboreus*), black-legged kittiwake (*Rissa tridactyla*) and Atlantic puffin (*Fratercula arctica*) were chosen as study objects. The apex predatory glaucous gull have high levels of persistent organic pollutants (POPs), while the fish eating black-legged kittiwake and Atlantic puffin are located lower in the food-chain and have lower levels of POPs.

The pristine Barents Sea area should be clean of pollution, but prevailing air, water and ice drifts transport pollutants from areas of dense human settlement, agriculture and industry into the Arctic. Legacy organic pollutants such as PCBs and OCPs are found at substantial levels in Barents Seas' seabirds. The glaucous gull, the black-legged kittiwake and Atlantic puffin are therefore exposed to a cocktail of different contaminants, leading to an almost infinite number of possible additive, synergistic and antagonistic effect combinations.

To address the question whether POP levels found in the Barents Sea induces immune alteration, field studies of the three species and a laboratory study of glaucous gull chicks were carried out. Studies of the birds are presented in the five papers, the first of breeding glaucous gulls, the second of glaucous gull chicks, the third of non-breeding glaucous gulls, the fourth of breeding black-legged kittiwakes and Atlantic puffins, while the last paper investigated dead adult glaucous gulls collected in the breeding season. In breeding glaucous gulls, positive correlations between levels of intestinal nematode and organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) were observed. The intestinal parasite intensities could be seen as an end result of the immune system's battle against the establishment and survival of the parasites. An increased infection with increased organochlorine (OC) levels might in this relationship be explained by immune suppression activity of OCs.

The influence of the Barents Sea's POP-cocktails on the developing immune system was studied on laboratory-raised glaucous gull chicks. The polluted group that simulated "naturally" polluted glaucous gull chicks responded less well to an influenza vaccine and had lower levels of circulating immunoglobulin-G (IgG) and IgM than the control group.

Arctic species are dependent on lipids as storage and insulation, and seabirds go through large annual lipid cycles. As a consequence, contaminants are released into the blood and the concentrations of lipid-soluble contaminants increase when seabirds utilize their lipid storage. If the annual cycle of contaminant release occurs around levels where effects are initiated, the seabird may end up entering and exiting “windows” of effects. As a follow-up of the breeding glaucous gulls’ pollution and parasite study, an investigation of the intestinal macro-parasites and POPs were carried out after the breeding season in adult birds sampled near Barentsburg in August. These gulls were in good body condition and had lower levels of POPs than the breeding gulls. No correlations between parasite intensity and POP levels were found.

The hypothesis of a POP-induced immune suppression was also investigated in the less polluted black-legged kittiwake and Atlantic puffin. The predictions of an altered level of circulating IgG with OCP, PCB or PBDE levels were tested. The results show no indication of correlations between the IgG and pollutant levels.

The absolute endpoint result of adverse effects from pollutions is death. Autopsies and POP analyses of dead glaucous gulls collected from Bjørnøya were performed in an attempt to establish the causes of death and to evaluate any contribution of POPs. Brain and liver levels of POPs were compared with a comparable sample collected in 1989. The levels of ΣOCP_3 and ΣPCB_{18} were significantly reduced in liver, but not in the brain. The liver levels thereby confirm the general downward trend of legacy OC levels, whereas the brain levels strengthen the theory that the additional stress provided from elevated pollutant levels could be deadly. The POP-cocktail thereby became too strong for these glaucous gulls.

Papers included in the thesis

I. Sagerup, K., Henriksen, E.O., Skorping, A. Skaare, J.U., Gabrielsen G.W. 2000. Intensity of parasitic nematodes is associated with organochlorine levels in the glaucous gull (*Larus hyperboreus*).
Journal of Applied Ecology 37:1-9.

II. Sagerup, K., Larsen, H.J.S., Skaare, J.U., Johansen, G.M., Gabrielsen, G.W. 2009. The toxic effects of multiple persistent organic pollutant exposures on the post-hatch immunity maturation of glaucous gulls.
Journal of Toxicology and Environmental Health - Part A, 72: 1–14, DOI: 10.1080/15287390902959516.

III. Sagerup, K., Savinov, V., Savinova, T., Kuklin, V.V., Muir, D.C.G., Gabrielsen, G.W. 2009 Persistent organic pollutants, heavy metals and parasites in the glaucous gull (*Larus hyperboreus*) on Spitsbergen.
Environmental Pollution, DOI:10.1016/j.envpol.2009.03.031.

IV. Sagerup, K., Åsbakk, K., Polder A., Skaare, J.U., Gabrielsen, G.W., Barrett, R.T. Serum immunoglobulins in black-legged kittiwake and Atlantic puffin using anti-chicken antibodies as an immunological biomarker: POP effects absence.
Manuscript.

V. Sagerup, K., Helgason, L.B., Polder, A., Strøm, H., Josefsen, T.D., Skaare, J.U., Gabrielsen. G.W. Persistent organic pollutants and mercury in dead and dying glaucous gulls (*Larus hyperboreus*) from Bjørnøya (Svalbard).
Manuscript.

Contents

Introduction

The Barents Sea

The Barents Sea covers 1.4 million km^2 and is a shelf sea with an average depth of 230 m (Sakshaug, 1992) within the coasts of mainland Norway, mainland Russia and the islands of Novaya Semlya, Franz Josef Land and Svalbard (Figure 1). It is dominated by cold water, -1.9º C to 6º C and varying light conditions due to it geographical position and partial ice cover. Large parts of the northern and eastern Barents Sea are covered with ice in winter. Two main water currents carry warm water into the Barents Sea. The Norwegian coastal current along the Norwegian and Russian Kola coast and the Norwegian Atlantic current that divides into one stream south and one small stream north of Bjørnøya and continues west of Spitsbergen. Cold water currents flow into the Barents Sea mainly between Novaya Semlya and Franz Josef Land, and between Franz Josef Land and Svalbard. The oceanic Polar Front is established where the warm north and east-flowing Atlantic water meets the cold south and west-flowing Arctic water. An inflow of nutrient-rich and salty Atlantic water, along with the vertical mixing of water and continuous daylight between April and August, produces a characteristic spring bloom.

The input of nutrients to the Barents Sea makes it a productive sea area (Sakshaug et al., 1994). The ice edge zone is also particularly productive due to phytoplankton blooms in nutrient-rich melt water that do not easily mix vertically with nutrient poor waters. The main zooplankton comprises the copepod *Calanus finmarchicus* and krill *Thysanoessa sp*. south of the Polar Front, and *C. glacialis* north of the Polar Front (Loeng and Drinkwater, 2007). Zooplankton is important for the energy transfer from primary producers to higher levels of the food web. Several large and commercially important fish stocks inhabit the Barents Sea. The Atlantic cod (*Gadus morhua*), arctic haddock (*Melanogrammus aeglefinus*), Norwegian spring-spawning herring (*Clupea harengus*), capelin (*Mallotus villosus*) and polar cod (*Boreogadus saida*) are important commercial fish stocks and prey for each other and for seabirds and marine mammals (Sakshaug, 1992).

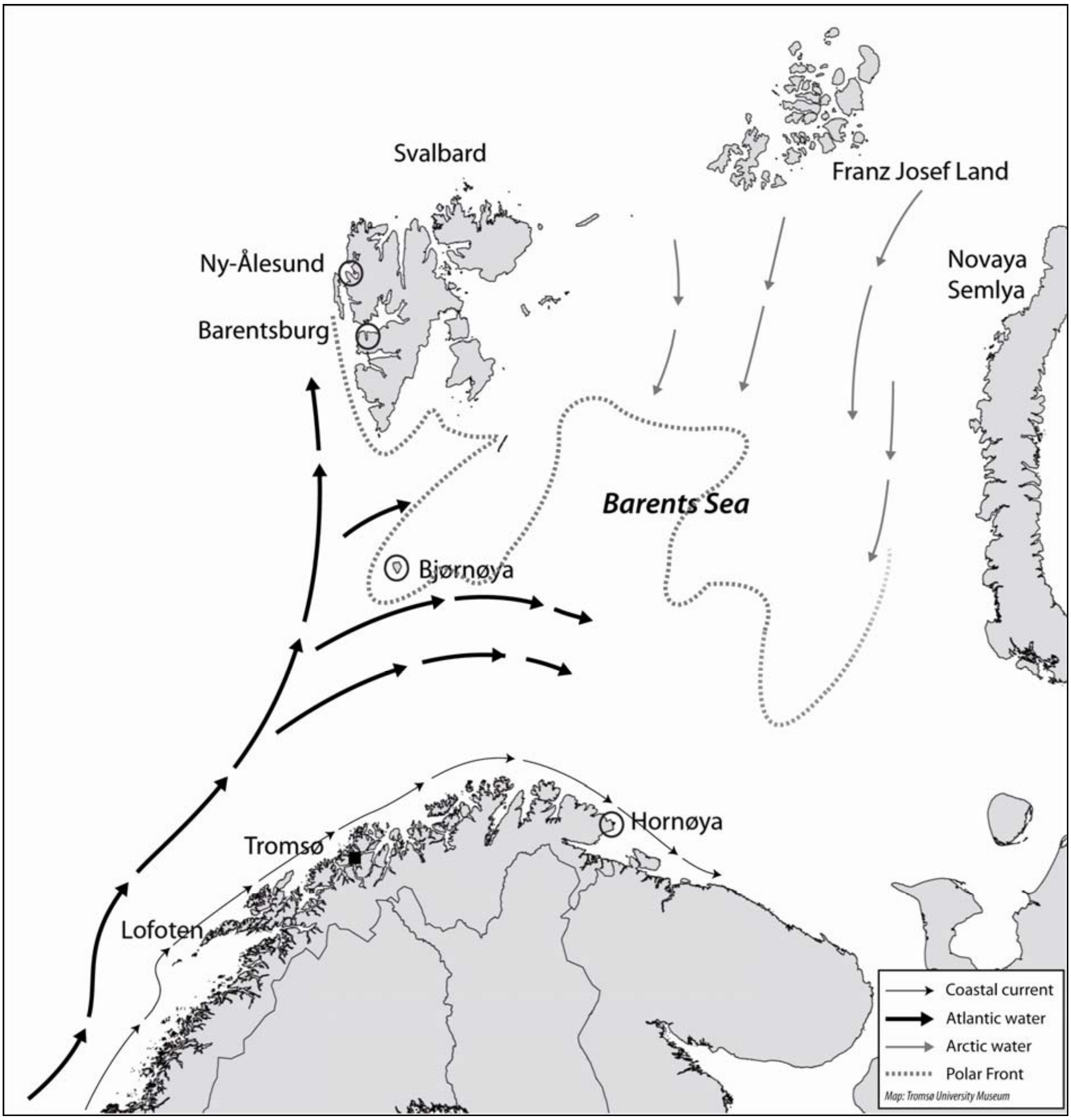

Figure 1: Study area of western Spitsbergen, Bjørnøya and Hornøya. The study areas are marked with open circles. Ocean currents and Polar Front after Sakshaug (1992). (Map by Jan Magne Gjerde and graphics by Ernst A. Høgtun)

Seabirds in the Barents Sea

Seabirds are an important element of the Barents Sea marine ecosystem. Many are true marine birds which only visit the land to breed. Compared to many terrestrial birds, seabirds are long-lived, lay small clutches of eggs, have delayed maturity and have a high annual survival rate. Their life-history strategy makes the seabird populations especially vulnerable to factors that affect adult survival.

The number of seabirds in the Barents Sea is estimated to be about 16 − 20 million breeding and non-breeding individuals but varies considerably according to seasonal movement of birds (Anker-Nilssen et al., 2000; Barrett et al., 2002; Gabrielsen, in press). The Brünnich's guillemot (*Uria lomvia*) predominates (34%), followed by the Atlantic puffin (*Fratercula arctica*) (21%), little auk (*Alle alle*) (13%, but highly variable population estimates), black-legged kittiwake (*Rissa tridactyla*) (12%) and northern fulmar (*Fulmarus glacialis*) (12%) (Barrett et al., 2002; Gabrielsen, in press). The largest breeding colonies are found along the coast of Svalbard, on Bjørnøya and along the coast of northern Norway, the Kola Peninsula, Novaya Zemlya and Franz Josef Land (Anker-Nilssen et al., 2000). Brünnich's guillemots breed mainly in the north and east, little auks in the north and Atlantic puffins and common guillemots (*U. aalge*) in the south and west, while the black-legged kittiwakes are more evenly distributed throughout the Barents Sea.

The most important prey species for cliff breeding seabirds are capelin, sand eels (*Ammodytes* sp.), polar cod, young year-classes of herring, gadids and periodically crustaceans (Anker-Nilssen et al., 2000; Barrett, 2007; Eilertsen et al., 2008). Large gulls (*Larus* sp.) eat, in addition to fish and crustacean, offal and discards from the fishing industry, other seabird eggs and chicks and scavenge cadavers or rubbish dumps when available (Anker-Nilssen et al., 2000; Bustnes et al., 2000). The capelin stock crash in 1985/1986 resulted in a breeding population decline of common guillemot of about 85% at Bjørnøya, Syltefjord and Hornøya in eastern Norway and at Kharlov at Kola Peninsula (Krasnov and Barrett, 1995; Mehlum and Bakken, 1994). Black-legged kittiwakes and Atlantic puffins also show long- and short-term changes in breeding populations that might be related to access to prey (Barrett et al., 2006).

Pollution in the Barents Sea

The levels of persistent organic pollutants (POP) are usually highest near the sources (Risebrough et al., 1968), but pollutants can undergo long-range transport from areas of dense human settlement, agriculture and industry to the Arctic by prevailing air, water and ice movements (Ballschmiter, 1992; Iwata et al., 1993; Oehme, 1991). Local sources of POPs in the Arctic are minimal and have little influence on the residue levels in the marine biota (AMAP, 2004). Figure 6.6 from AMAP (1998) (Figure 2) illustrates the main sources and wind directions of different airborne pollutants reaching three different air-measurement stations in Arctic. By using meteorological measurements for back-tracking air currents, it is possible to simulate that Ny-Ålesund, Svalbard receive HCH- and PCB-elevated air from Europe and Asia/Russia, chlordane-elevated air from US/Canada and clean air from the Arctic Ocean (Figure 2).

The central explanations for global distribution of POPs are fractionation and cold condensation. Global fractionation occurs when mixtures of pollutants with both low and high volatility are transported from their source. The composition of the mixture gradually changes towards more volatile elements with increased distance from the source (Wania and Mackay, 1993). Cold condensation refers simply to the condensation of a pollutant transported northwards. When temperatures drop, the thermodynamic property of a given compound turns ideal for condensation onto soil, water, aerosol particles, snow or ice (Wania and Mackay, 1993).

The distribution of airborne PCB congeners and HCB along a latitudinal transect from the south of the United Kingdom to Northern Norway showed that the ΣPCB levels decreased and the relative contribution of volatile compounds, such as the HCB and low-chlorinated PCB increased with latitude (Meijer et al., 2003). This is further reflected in tissues of fish and seals showing that the levels of HCH and HCB are evenly distributed or increase northwards, while those of PCBs, DDTs and chlordanes decrease with latitude, reviewed by Wania and Mackay (1993). The levels and distribution in seabirds is not that easy to compare due to the different species' foraging strategies, their mobility and migration. For example, the glaucous gull (*Larus hyperboreus*) has higher levels of most organochlorines (OCs) than the great black-backed gull (*L. marinus*) at the Norwegian coast (Steffen et al., 2006).

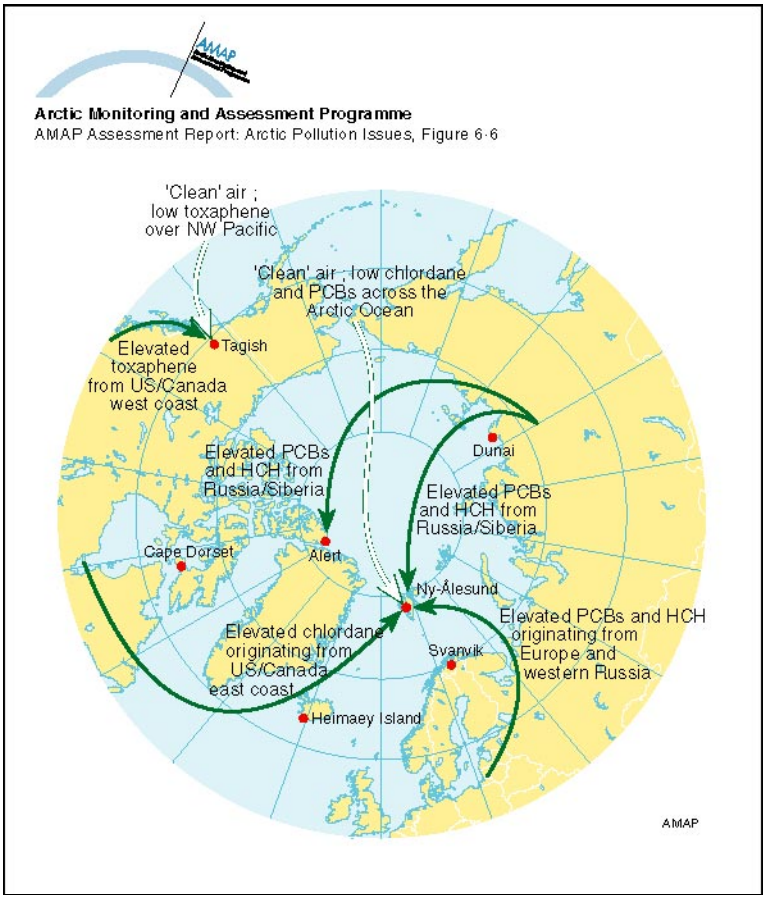

Figure 2: Illustration of the air transport of POPs to the Arctic. Figure 6·6 "Source regions for HCH, chlordane, toxaphene, and PCBs in Arctic air based on 5-day back-trajectories for elevated air concentrations at Tagish, Alert, and Ny-Ålesund" from AMAP (1998). (AMAP Sources: Oehme, M., Haugen J. E. and Schlabach M., 1995. Ambient levels of persistent organochlorine in spring 1992 at Spitzbergen and the Norwegian mainland: Comparison with 1984 results and quality control measures. Science of the Total Environment 160/161: 139-152 and Barrie et al. unpubl. data.)

Steffen et al. (2006) suggest that the glaucous gull has a higher proportion of seabird eggs and chicks in the diet, thereby foraging at a higher trophic level than the great black-backed gull. The levels of *p,p′*-DDE and ΣPCB in common guillemot eggs are, however, about 18 and 4 times higher, respectively, in the Baltic Sea than at Hornøya south in the Barents Sea (Helgason et al., 2008; Lundstedt-Enkel et al., 2006). The Barents Sea is at some distance from the pollutants source and the air, fish, seabird and seal examples above shows that it is a rather clean sea, with a few exceptions exemplified with the top-predatory glaucous gull.

The common organic pollutants of PCBs and OCPs are associated with dissolved organic carbon in water and are bioaccumulated in zooplankton with increasing efficiency according to the pollutants' octanol-water partitioning coefficient (K_{ow}) (Borgå et al., 2005). The K_{ow} is the ratio of solubility in octanol and water, respectively. A high K_{ow} indicates a hydrophobic fat-soluble compound (Hawker and Connell, 1988). It is further well known that the persistent organic pollutants tend to be more concentrated up in the food chain (Borgå et al., 2001; Jensen et al., 1969). The levels could be up to 100-fold higher in predators than in the prey, depending on the ability of the compound to accumulate and the predators' ability to metabolise the compound. In general, selective elimination of POPs is higher among homoeothermic predators than among their poikilothermic prey, and this usually reflects the abiotic composition of POPs (Borgå et al., 2004). In addition, the body size, age, sex, reproduction, migration, feeding ecology, biotransformation and seasonal changes in habitat will all influence the levels of POPs presented in an organism (Bustnes et al., 2003; Henriksen et al., 1998).

Arctic seabirds are thought to be particularly vulnerable to contaminant exposure because they go through large seasonal lipid cycles resulting in possible peak exposures of contaminants during periods of emaciation. Arctic species are dependent on lipids as storage and insulation and the lipid dynamics within the organism thereby influence the levels of pollutants in blood and tissues. Since a central factor in toxicology and ecotoxicology is at what quantitative level a chemical initiates an effect (Walker et al., 2006), concentrations of pollutants provide a basis for judging toxicological effects. When stored lipids are used, the concentration of contaminants increases in the blood of a seabird and might result in a physiological imbalance, increased energy expenditure and a deterioration of health. This annual lipid dynamics complicate the study of effects of pollution as the seabirds possibly pass in and out of "windows" where POP levels are high enough to cause effects.

The glaucous gull and other seabirds such as the black-legged kittiwake and Atlantic puffin are exposed to a cocktail of different contaminants, leading to an almost infinitive number of possible additive, synergistic and antagonistic effect combinations. It is therefore of concern that new chemicals are now entering the biota accumulate and contribute further negatively to the health of seabirds and other top predators.

The immune system

In broad terms, infectious organisms are parasitic life forms living in or on a host from which they obtain their nutrients and in which they reproduce. The hosts' defences against infection include barrier mechanisms and cell-mediated and antigen-specific immune responses. Skin, and for birds, feathers are parts of the barrier that, together with mechanical removal of ecto-parasites, protect the host from many infections. Any infections that break through the barrier become a task for the immune system. The immune system distinguishes between self and non-self origin and responds against non-self to prevent an establishment and/or elimination of an infection (Abbas and Lichtman, 2003).

The classical division of the immune system is into an unspecific innate immunity and a specific adaptive immunity. The division is related to function, but the two parts are an integrated system. The innate immunity consists of cellular and biochemical defence mechanisms that reacts quickly against microbes but responds in essentially the same way to repeated infections. The components of the innate immunity are the barriers such as the epithelial surfaces, phagocytic cells and regulating proteins. The adaptive immunity differs in its high specificity for distinct molecules and an ability to "remember" and thereby respond more vigorously to repeated infections. The components of the adaptive immunity are lymphocytes and their products. The lymphocytes produce antibodies that structurally recognize foreign substances (antigens). Antibodies are produced by B lymphocytes (B-cell) and recognize and neutralize the target microbe for elimination. After an elimination of an infection, a small pool of B-cells still contains the specific antibodies and constitutes the memory. The second respond to an infection or a vaccine is quicker and involves a higher amount of antibodies than the first (Figure 3). The innate immunity co-operates with the adaptive immunity through phagocytic cells and regulatory proteins.

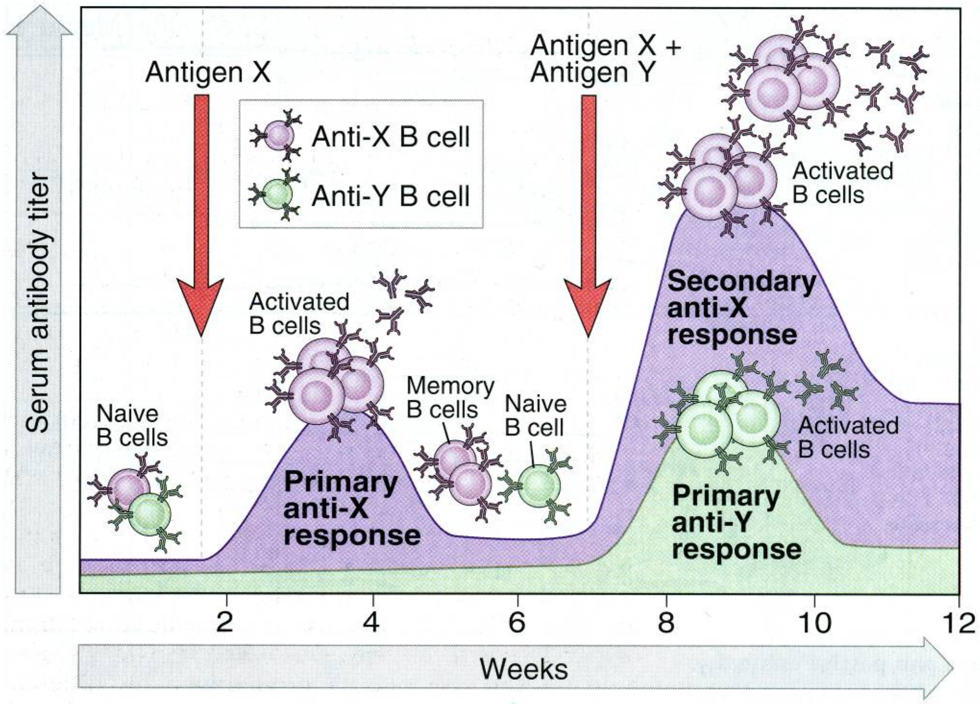

Figure 3: Figure 1.4 from Abbas and Lichtman (2003). Specificity, memory and self-limitation of immune responses.

The most insidious effect of POPs on the immune system is to decrease an organism's resistance to infection or cancer. Immunosuppressive effects of OCs can be measured as reduced antibody production when exposed to a foreign antigen or a vaccine, changes in B- and T-cell populations, decreased delayed-type hypersensitivity, decreased resistance to infections, and decreased natural killer cell activity (Tryphonas, 1994; Wong et al., 1992). All of these have been used as biomarkers for immunosuppressive effects in the laboratory as well as in free-living animals.

The immune system is therefore of major importance for the host health by decreasing the impact of infectious organism. The prevention and control of infections do, however, require resources that could otherwise have been used in other life functions (Sheldon and Verhulst, 1996). Life-history theory states that individuals must share resources among competing life history traits (Stearns, 1992). Assuming limited resources, individuals who allocate their resources to a single life function, such as reproduction, heat production or growth, could decrease other life functions such as the immune system (Sheldon and Verhulst, 1996). For example, blue tits (*Parus caeruleus*) who were subjected to cold stress responded less well to a diphtheria-tetanus vaccine than the control group (Svensson et al., 1998). This vaccine experiment showed that cold stress could lower the immune system's ability to fight infections. In a field study of breeding glaucous gulls, Bustnes et al. (2004) found that females with high levels of hexachlorobenzene (HCB) and oxychlordane had a significantly poorer response to a diphtheria-toxoid vaccine than females with lower levels of HCB and oxychlordane. This indicated that the female glaucous gulls with the highest levels of HCB and oxychlordane either had fewer resources with which to build antibodies against the vaccine, or that the pollutants directly suppressed the immune system. Bustnes et al. (2006a) found further that treatment with an anti-helminth drug remove the lowered nesting success effect that un-treated glaucous gull males shows with increasing levels of OCs. This indicated that both helminths and OC levels have negative influences on the birds nesting success and that a reduction of one of the stressors removed the effect. The female had lower levels of OCs and either OC levels or helminth treatment was found to influence fitness. These examples show that an additional stress factor, such as POPs, on top of the natural stress, could have detrimental effects. It further suggests that groups of birds could be inside (male glaucous gulls) or outside (female glaucous gulls) a “window” of effects. Since the immune system seems to be a sensitive target for the toxic effects of organic pollutants, are the immune related effects of long-term influence of POPs were in the main focus in the present thesis.

Aims of this thesis

The overall aim of my work was to study immunological effects of pollutants in free-living seabirds from the Barents Sea using the glaucous gull, black-legged kittiwake and Atlantic puffin as model systems.

In paper I we hypothesized that a possible negative effect of POPs on the immune system would be an increase in levels of intestinal macro-parasites. We used the most polluted seabird in the Arctic, the adult glaucous gull, and addressed correlations between parasite intensities and OCP and PCB concentrations.

To evaluate further whether the existing POP-cocktail in the Arctic influenced the immune system, we set up a laboratory study using glaucous gull chicks (paper II). By using herring gull (*Larus argentatus*) and great black-backed gull eggs, we simulated a “natural” POP source, the chemical composition of which is quite different from that of the technical mixtures used in most laboratory effect studies. We investigated whether this POP composition at levels previously found in glaucous gull chicks from Bjørnøya influenced the developing immune system of glaucous gull chicks.

To further study the POPs' influence on intestinal macro-parasite fauna, an immune system endpoint result, adult glaucous gulls were sampled after the breeding season (paper III). As the energy-demanding breeding season was over, we expected the gulls to be in better body condition and thereby have lower POP levels than gulls used in paper I. The same hypothesis and prediction as for paper I were used.

The black-legged kittiwake and the Atlantic puffin are two common seabird species in the Barents Sea and both species have suffered from reduced breeding success and the numbers of black-legged kittiwakes are declining rapidly. Although their POP residue levels are much lower than in glaucous gulls, we hypothesized that POPs also influenced their immune system and tested the prediction that OCPs, PCBs or PBDEs covariate with circulating levels of IgG (paper IV).

The absolute endpoint result of any adverse effect from pollutions is death (paper V) and dead glaucous gulls were collected from Bjørnøya in three breeding seasons (2003-2005). Autopsies and POP analyses were performed in an attempt to establish the causes of death and to evaluate any contribution of POPs.

Methods

Study areas

The studies for paper I and V was performed on Bjørnøya (74º 21′ N, 19º 05′ E) in the western Barents Sea (Figure 1). Bjørnøya is an important breeding location as it is the only island close to the Polar Front between the coast of Norway and Spitsbergen in the Barents Sea. Approximately half a million seabird pairs breed at the southern part of the island (Strøm, 2006a), with the main species being common guillemot, Brünnich's guillemot, black-legged kittiwake and glaucous gull (Anker-Nilssen et al., 2000). It hosts the densest colonies of glaucous gulls in Europe. The gulls collected for the study in paper I were further used in studies of stable isotopes (Sagerup et al., 2002), microsomal 7-ethoxyresorufin O-deethylase (EROD) activity and hepatic vitamin A stores (Henriksen et al., 2000).

The glaucous gulls chicks for paper II were hatched in an incubator at the Norwegian Polar Institute's Research Station in Ny-Ålesund, Svalbard (78º 55′ N, 12º 30′ E) (Figure 1). The eggs were collected along the Nordenskiöld land at the west coast of Spitsbergen. These chicks were further used to study POPs effects on chromosome aberrations and DNA strand breaks and adduct formation (Krøkje et al., 2006; Østby et al., 2005).

The glaucous gulls for paper III were collected near Barentsburg (78º 05′ N, 14º 20′ E) at Svalbard (Figure 1).

In paper IV, we used black-legged kittiwakes and Atlantic puffins from Hornøya (70º 22′ N, 31º 10′ E) from the southwestern Barents Sea (Figure 1). Hornøya is a small island at the eastern point of the Norwegian coast. It has a high diversity of breeding species. Most abundant are the black-legged kittiwake, Atlantic puffin, common and Brünnich's guillemot, shag (*Phalacrocorax aristotelis*), herring gull and great black-backed gull. A non-destructive blood sampling of black-legged kittiwakes and Atlantic puffins was carried out over three breeding seasons.

Study species

Glaucous gull *Larus hyperboreus*

The glaucous gull (paper I, II, II and V) is a large gull, slightly smaller than the great black-backed gull, but larger than the herring gull. Its body length is 65-78 cm (wing length 41-51 cm) and it weight 1200-2100 g. The sexes are alike, but the male is on average larger (Picture plate 1). It obtains the adult plumage colours and starts breeding at five years of age. The breeding population is estimated to be 7000 − 17 000 breeding pairs in the northern and eastern Barents Sea (Anker-Nilssen et al., 2000). The glaucous gull does not breed in the southern part of the Barents Sea. The glaucous gull has a high-arctic circumpolar distribution and the total world population is probably over 100 000 pairs (del Hoyo et al., 1996). Little is known about the population trend, but on Bjørnøya the population has suddenly decreased dramatically (by 65%) between 1986 and 2008 (Strøm, 2006; Strøm pers com).

The glaucous gull breeds usually as single pairs, or in small colonies of 5-15 pairs. In a few places breeding colonies can host over 100 pairs. On Bjørnøya, several large colonies of 50-100 pairs exist. It lays 1-3 eggs, most often three and both parents incubate the eggs for 27-28 days.

The glaucous gull is the only common avian predator in the Arctic. It is a generalist predator feeding on a wide variety of fish, molluscs, crustaceans, eggs, chicks and adults of other seabirds, carrion, refuse and small mammals (if present) (Barry and Barry, 1990; Bustnes et al., 2000; Lydersen et al., 1989). The glaucous gulls accumulate high levels of PCBs (Table 1) since they feed high in the food chain and have low capacity for metabolizing OCs.

Black-legged kittiwake *Rissa tridactyla*

The black-legged kittiwake (paper IV) is a medium size gull. The length is about 41 cm (wing length 30-34 cm) and its weight ranges from 300-500 g (Picture plate 1). The male is slightly larger than the female and data from paper IV shows that 82% of the Hornøya black-legged kittiwakes could have be sexed according to the total head length alone. The black-legged kittiwake is the most numerous species of gull in the word with approximately 6-8 million breeding pairs (Lloyd et al., 1991). Its habitat is mostly oceanic with a circumpolar distribution in both the arctic and boreal zones of the northern hemisphere. The Barents Sea population is estimated to 560 000 pairs (Gabrielsen, in press). More than half of the Barents

Sea population breeds along the north east coast of Norway and Kola Peninsula. The Norwegian population has decreased by as much as 50 to 75% (between 1980 and 2005) in four annually monitored colonies in the Barents Sea area (Barrett et al., 2006). A decreased hatching success (Barrett, 2007) and a rapid decline in breeding numbers had brought the black-legged kittiwake into the "Norwegian red list - 2006" of species of concern where it is defined as "vulnerable".

The black-legged kittiwake breeds on steep cliffs in colonies which vary from a few birds to tens of thousands along the coast. The black-legged kittiwake starts breeding when at least four years old (Wooller and Coulson, 1977). It lays 1-3 eggs, most often two. Both parents incubate the eggs for about 27 days. It is a surface feeder eating 80% (estimated mass %) small fishes and 20% crustaceans (Barrett et al., 2002). In the Barents Sea area capelin, polar cod, herring, euphausiids and amphipods are common components of their diet (Barrett, 2007; Lydersen et al., 1989). The levels of POPs are generally much lower (11% blood level) than those of glaucous gulls (Table 1).

Atlantic Puffin *Fratercula arctica*

The Atlantic puffin (paper IV) is a small auk. Its body length is about 30 cm (wing length 16-20 cm) and it weights 400-600 g. It is a characteristic bird often called "sea parrot" because of the colourful broad bill (Picture plate 1). The Atlantic puffin breeds on both sides of the North Atlantic. The word population is estimated to be 2.5-5 million breeding pairs (Gaston and Jones, 1998). Of these, about 900 000 pairs breed in the Barents Sea area (Anker-Nilssen et al., 2000; Barrett et al., 2006). Less than 5000 pairs breed along the Kola Peninsula, less than 100 pairs on Novaya Zemlya and none on Franz Josef Land (Anker-Nilssen et al., 2000). The Svalbard population is estimated to about 10 000 pairs included Bjørnøya (Anker-Nilssen et al., 2000). The Barents Sea population is stable or slightly increasing, while within the Norwegian Sea which includes the large Røst population in Lofoten the population is declining (Barrett et al., 2006; Durant et al., 2006). The Atlantic puffin is also a species of concern and defined as "vulnerable" in the "Norwegian red list - 2006".

The Atlantic puffin breeds in burrows of 1-4 m long, depending on the soil condition. It lays only one egg which both parents incubate for 39-45 days. The Atlantic puffin starts breeding at five years of age (Harris and Osborn, 1981).

The Atlantic puffin is a diver which eats mainly fish. Common food items in the Barents Sea are capelin, sand lance, herring and gadids (Atlantic cod and saithe) (Eilertsen et al., 2008). The PCB levels are similar, or slightly lower than in the black-legged kittiwakes and only about 5% of than concentration in glaucous gull blood (Table 1).

Picture plate 1: Next page. Picture of glaucous gull (upper), black-legged kittiwake (middle) and Atlantic puffin (lower). Photo by Kjetil Sagerup (gg) and Rob Barrett (blk, Ap).

Table 1: The ΣPCB (ng/g lipid wt. (weight)) levels of Barents Sea glaucous gull, black-legged kittiwake and Atlantic puffin. The mean are rounded and the standard deviation is not present for simplifying of the table. Some of the levels are recalculated from the published mean wet wt. level after the equation: lipid wt. = (wet wt.*100) / lipid %.

Year (month)	# PCB	n	Egg	Liver	Blood	Study area	Reference
Glaucous gull							
1990 (July)	10	13		498 000		Svalbard	(Daelemans et al., 1992)
1992/1993 (June)	21	5	25 400			Svalbard	(Barrett et al., 1996)
1996 (July)	9	40		75 300		Bjørnøya	(Henriksen et al., 2000)
2001 (August)	32	20		27 900		Barentsburg	paper III
2002/2004 (July)	41	30	11 800			Bjørnøya	(Verreault et al., 2005)
1997 – 2006 [a]	6	487			59 000	Bjørnøya	(Verreault et al., in prep)
2006 (June) [b]	58	31	18 700			Bjørnøya	(Verboven et al., 2008)
Black-legged kittiwakes							
1992 (July)	10	5		11 200		Kola	(Savinov et al. 2007)
1992 (June)	21	9		28 700		Hornøya	(Henriksen et al., 1996)
1992/1993 (June)	21	22	7000			Hornøya	(Barrett et al., 1996)
2002 (July)	10	10		7000		Kola	(Savinov et al., 2007)
2003 (June)	20	5	7300			Hornøya	(Helgason et al., 2008)
2003-2005 (June) [c]	9	97			6300	Hornøya	paper IV
Atlantic puffin							
1982 (July)	Arochlor	12		57 500		Hornøya	(Ingebrigtsen et al., 1984)
1992 (July)	10	7		24 500		Kola	(Savinov et al., 2007)
1992/1993 (June)	21	22	12 000			Hornøya	(Barrett et al., 1996)
2002 (July)	10	6		12 300		Kola	(Savinov et al., 2007)
2003 (June)	20	5	4600			Hornøya	(Helgason et al., 2008)
2003-2005 (June) [c]	9	91			3200	Hornøya	paper IV

[a] Mean of both sexes from the 10 years 1997 to 2006. Calculated from a graph.
[b] Egg yolk. Different levels from "bird-cliff" colony (22 900 ng/g lipid wt., Glupen) and "sea-level" colonies (15 200 ng/g lipid wt., Kapp Harry and Teltvika). This is a good example of the difficulties to compare levels. The majority of lipids, and thereby lipid soluble PCBs, are contained in the yolk. The PCB levels in these eggs are probably not different from the whole egg homogenate from 2002/2004.
[c] Mean of both sexes despite significant differences between the sexes (paper IV).

Laboratory methods

The laboratory methods are described in detail in each of the papers. Table 2 gives an overview of the different analytical methods used in this thesis.

Table 2: Laboratory methods used to analyse tissue and blood for the present thesis.

	Laboratory	Method	Accreditation	Paper
OCP, PCB, PBDE, HBCD	Env. Tox., NVH Oslo, Norway	GC, GC-MS, LC-MS-MS	NS-EN ISO/IEC 17025 (TEST 137). Int. inter-calibration	I, II, IV, V
OCP, PCB	Thypoon, Obninsk, Russia	GC	Nasjonal acr. Int. inter-calibration	III
PBDE, HBCD, Toxaphene	NWRI, Burlington, Canada	GC-MS	Int. inter-calibration	III
HM	Dep. Env. Monit. Pol., Obninsk, Russia	AAS	Certif. ref. mat. DORM-1, DOLT-1	III
Hg	NVI, Oslo, Norway	AAS	NS-EN ISO/IEC 17025, Int. inter-calibration	V
Antibody	Immune, NVH, Oslo, Norway	Virus neutralisation, passive haemagglutination		II
Antibody	Arctic vet., NVH, Tromsø, Norway	ELISA		IV
IgG	Immune, NVH, Oslo, Norway	Mancini		II
IgG	Arctic vet., NVH, Tromsø, Norway	ELISA		IV
IgM	Immune, NVH, Oslo, Norway	Mancini		II
Lymphocyte proliferation	Immune, NVH, Oslo, Norway	LPT (Stimu., incub., count.)		II
Parasite	UiT, Tromsø, Norway. White Sea Biol. S., St. Petersburg, M. Mar. Biol. Inst., Murmansk, Russia	Counting, species identification		I, III, V
Autopsy	NVI, Tromsø, Norway	Standard		V
Sexing	UiT, TMU, Tromsø, Norway. M. Mar. Biol. Inst., Murmansk, Russia	Gonad inspection in field or lab		I, II, III, V
Sexing	UiT, TMU, Tromsø, Norway	PCR		IV, V

General discussion

Levels of effects

Biological effects can be measured at different stages of biological organization, from the molecular to ecosystem level. The molecular level responds quickly to a perturbation, while the ecosystem level responses are slower at the same time as the ecological relevance increases, illustrated in figure 06-56 from Arctic monitoring and assessment program (AMAP, 1998) (Figure 3).

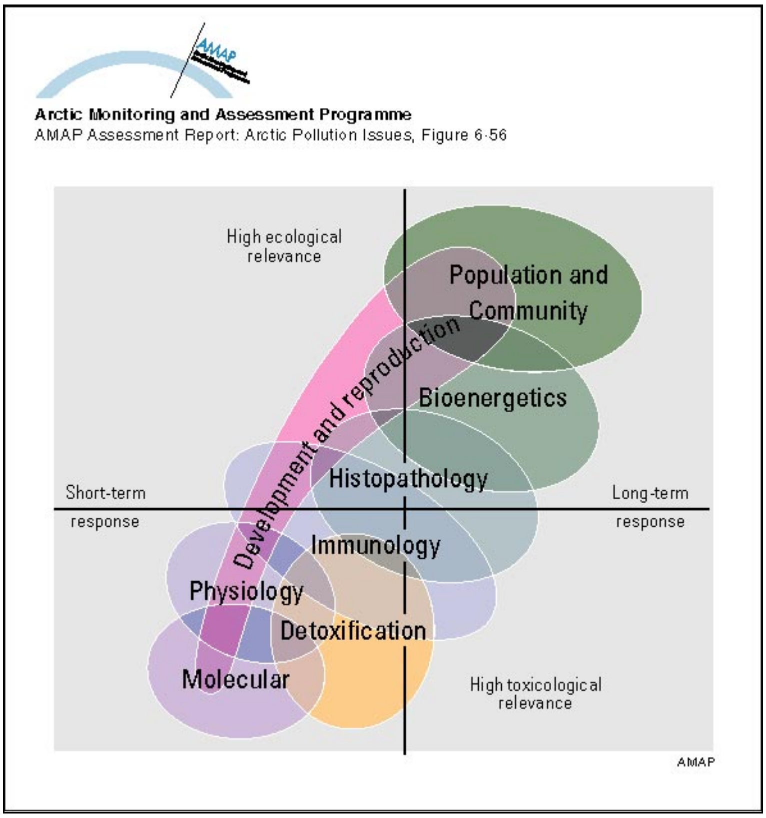

Figure 3: Figure 06-56 from Arctic monitoring and assessment program (AMAP, 1998) after Peakall (1992). Time scale and relevance of toxicological effects on different biological levels.

A xenobiotic is defined as a chemical compound that is foreign to an organism (Appendix 1). This definition is broader than, and includes, the contaminants. From a historical point of view, natural xenobiotics have existed from early history of the earth. The detoxication mechanisms in animals have therefore not evolved as a response to resent anthropogenic contaminants, but probably as protection against toxic xenobiotics produced by plants (Walker et al., 2006). All xenobiotics included in this thesis are man-made, but it is important to keep in mind that contaminants will be subjected to the same detoxification processes as natural xenobiotics.

A pesticide is constructed for a specific effect and is used outdoors on agricultural crops. Industrial products are often released to the environment through leakages and improper waste management. The early insecticide DDT was heavily used from 1940 to 1972. It is rather stable in the environment, but its metabolite DDE is even more stable and dominates in biological samples. In adult glaucous gulls (paper III), ΣDDT consist of about 3% DDT, 94% DDE and 3% other DDT isomers. In comparison, the original insecticide constitutes about 77% DDT and less than 8% DDE. Similar changes are found for other pesticides and industrial products. The solubility (K_{ow}), bioavailability and stability against biological degradation of any compound determine its composition up through the food chain. Congeners with low chlorination and vicinal hydrogen atoms in *meta* and *para* position are typically reduced whereas the more stable high-chlorinated, non vicinal hydrogenated compounds are more persistent. Each organism will excrete, metabolise or store a compound. As a result, accumulation in organisms and biomagnification in food-chains are typical features of persistent congeners. The characteristics of a technical mixture therefore weaken upwards through the food-chain.

A general principle in toxicology and ecotoxicology is that there is a relationship between the quantity (dose) of a chemical an organism is exposed to and the degree of consequence (response) (Walker et al., 2006). This dose-response relationship provides a basis for an assessment of effect. According to this principle, at least two possible effect options may arise: The effect of POPs gradually increases as levels increase, or a threshold level needs to be crossed before an effect is initiated.

The observed levels of pollutants could be compared to established threshold levels for earlier observed effects. Levels for no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effects level (LOAEL) threshold are established for birds and mammals (AMAP, 2004). The threshold levels are normally based on ΣPCB or summarized toxic equivalent (ΣTEQ) values. The TEQ value is calculated from individual toxic equivalent factor (TEF) for each dioxin-like pollutant, weighted against the most toxic congener

2,3,7,8-TCDD (tetrachlorodibenzo-p-dioxin). The TEF values for birds are based on studies in domestic chickens. Few levels of effects for birds are established. The birds' LOAELs are related to reproductive effects in black-crowned night herons (*Nycticorax nycticorax*), Forster's terns (*Sterna fosteri*), common terns (*S. hirundo*), double-crested cormorants (*Phalacrocorax auritus*) and herring gulls. These reproductive effects comprised reduced reproduction and hatching success, chick deformity and egg mortality and occurs at LOAEL 3500 to 22 000 ng ΣPCB/g wet wt., or ΣTEQ 0.2 to 2.2 ng/g wet wt. (AMAP, 2004, and references therein).

The wildlife in northern ecosystems are experiencing increasing stress from climate change and exposure to POPs, which include the legacy OCPs and PCBs, trace elements and an increasing complexity of new organic contaminants (Boonstra, 2004; Muir and Howard, 2006). Adverse biological effects mediated by chronic contaminant exposure have been suggested as potential stressors in glaucous gulls (Gabrielsen, 2007), ivory gulls (*Pagophila eburnea*) (Miljeteig et al., 2007), and in great and lesser black-backed gulls (*Larus fuscus*) (Bustnes et al., 2006b, 2008).

Paper I

Forty adult glaucous gulls were sampled in the breeding season to test the hypothesis of an OCs suppression of the immune system. The prediction of an increased intensity of parasites with increasing OC levels was tested. In the glaucous gull, the intensity of intestinal parasitic nematodes was positively correlated with concentration of OCPs and PCBs. Three of the four nematode species found have intermediate hosts of fishes, amphipods and squids (Anderson, 1992), while the lifecycle of the fourth is unknown. The glaucous gull food of fish and shore items contains lower levels of POPs than seabird eggs and chicks. As a result, food preference for fish results in lower POP residue levels than a preference for seabird eggs and chicks (Bustnes et al., 2000). The higher nematode intensities in the birds with the highest OCP and PCB levels was therefore not related to food specialisation as seabird eggs and chicks are not intermediate hosts for nematode larval stages.

After paper I was published, Sures and Knopf (2004) found that European eels (*Anguilla anguilla*) infected with nematodes produce antibodies to the nematodes, but when the eels were also dosed with PCB-126 no antibodies to the nematode were produced. This study supports the results from paper I. The PCBs can suppress the immune system. Since antibodies against nematodes might be reduced or totally depressed, the nematodes can more readily establish themselves and their survival can be higher, resulting in the correlations described.

Paper II

The laboratory study of glaucous gull chicks was set up to study whether the developing immune system became adversely affected by environmentally relevant POP levels. The POP source of herring- and great black-backed gull eggs was chosen since the congener composition in the eggs is different to that of technical mixtures. In addition to the congeners measured in the food, the fed gull eggs contained brominated flame retardants (Helgason et al., in press), probably toxaphenes (paper III) and different metabolites (Verboven et al., 2008). The control group was given hen eggs, which were almost completely free for contaminants.

Through this setup, the experimental group received OCP and PCB residue levels similar to those of free-living glaucous gulls from Bjørnøya (Henriksen, 1999; Table 6 paper II). Poorer response to the foreign antigen of influenza virus (EIV) and lower circulating levels of IgG and IgM in blood were found in the experimental group. These results corroborate experimental studies of harbour seals (*Phoca vitulina*) (De Swart et al., 1996), European eels (Sures and Knopf, 2004), domestic goat kids (Lyche et al., 2006) and domestic chicken (Halouzka et al., 1994) that all found suppressed antibody response. The adaptive immunity seems therefore to be adversely influenced by POPs.

The ability of lymphocytes to proliferate *in vitro* was tested with mitogens and antigens. Further were lymphocytes stimulated with bout mitogen (Con A) and four different PCB congeners (99, 156, 153 or 126) to study whether the lymphocytes responded differently to non-dioxin-like di-*ortho* PCBs (99, 153) and dioxin-like non-*ortho* PCB (126) or mono-*ortho* PCB (156). The results from these tests were not clear. There was a tendency for the blood and spleen lymphocytes to proliferate better (higher Δ cpm) in the experimental than the control group, but only a few were statistically significant due to high variation (Table 4 paper II). The different PCBs did not produce different *in vitro* responses, indicating that either the PCBs did not stimulate or inhibit the *in vitro* lymphocyte proliferation, or that the dioxin-like non-*ortho* Cl-substituted PCB-126 and mono-*ortho* Cl-substituted PCB-156 reacted similarly as did the non-dioxin-like di-*ortho* Cl-substituted PCB-99, 153.

It is difficult to explain why the proliferation was highest in the POP-exposed group. Lymphocyte proliferation is an important process for clonal expansion in response to antigens. A higher exposure of environmental microbes in this group might explain the result. The unclear results corroborate, however, results from two experiments exposing chickens to PCB-126, one produced a non-significant increased response to PHA, while the other experiment found a significant decreased response (Lavoie and Grasman, 2007). The lymphocyte proliferation seems therefore to be a highly variable immunological endpoint for POP-effect studies.

Paper III

Since we assumed that the breeding season is the most energy demanding time of the year for an adult seabird, we wanted to test the prediction of increased parasite intensity with POPs in non-breeding glaucous gulls. We therefore sampled 20 adult glaucous gulls in August to survey the parasites and make an extended POP analysis. In these glaucous gulls there were no relationships between levels of parasites and POPs. There was, however, a positive correlation between cestode intensity and selenium (Se) and one positive correlation between acanthocephalan intensity and the heavy metal mercury (Hg). The levels of the heavy metals and selenium were however low and correlations due to immune alteration were therefore not expected. We concluded that these two correlations either arose as a coincidence or as a relationship between the elements' concentrations and larval intensity in the food items. The result did therefore not corroborate paper I where nematode intensity positive correlate to the OC levels. The non-breeding birds were in good body conditions and the mean PCB level was only 37% of that measured in breeding gulls from Bjørnøya (paper I). The August sampled glaucous gulls may therefore bee outside the "window" of effect, suggesting that the within-variation of POP levels did not alter the immune response to the intestinal helminths.

We did not correct for multiple comparison in paper III, or in the other papers. The probability of making a Type II error, i.e. accepting the null hypothesis when it is wrong, will increase when making corrections (Nakagawa, 2004; Rothman, 1990). The total number of possible correlations in paper III exceeds 300. A standard Bonferroni correction at $\alpha = 0.05$ and $k = 300$ will change each individual test (Type I error rate of α/k) to $\alpha = 0.00017$. Such strong correction would lead to a small rejection region. The power of each test may be too low to detect important deviation from the null hypothesis being tested (Kleinbaum et al., 1998). In my opinion, it is better to do the tests without correction and biologically evaluate the result. In toxicological studies, multiple comparisons will decrease the probability of finding significant results with detailed research (i.e. measuring more compounds). The most common POPs are in addition, highly correlated. Highly correlated predictors (congeners) behave similarly in statistical tests. It would therefore be more correct to judge highly correlated congeners as a group rather than individual congeners, simply because of their statistical equality.

Paper IV

To study whether seabirds with lower levels of pollution, but still with the same complex POP-cocktail, could also be affected by the pollutants, the black-legged kittiwake and Atlantic puffin were chosen as study objects. As Table 1 indicates, the blood ΣPCB levels of Atlantic puffin and black-legged kittiwake are only 5 to 12% of the blood levels in glaucous gulls. The results showed no indication of OCP, PCB or PBDE levels altering circulating IgG levels in either species. This suggests that the IgG proteins of the adaptive immunity are not affected by such low levels of POPs, or that the total IgG measurement is not a suitable endpoint variable for evaluating perturbation of the avian immune system. The prediction of changed IgG levels with POP levels could have been rejected. However, there are still some uncertainties that should be tested before the prediction or the hypothesis can be rejected. There were large variations in the IgG unit. The mean IgG levels were 164% ± 6 SE (range 18 - 347) and 85% ± 6 SE (rage 36 - 148) for the black-legged kittiwake and the Atlantic puffin, respectively. These ranges could be a result of variable infection levels or that the anti-chicken IgG cross-reacts with another plasma protein that varies among individuals.

The absent association is in contrast to the results from paper II where the POP-exposed group of glaucous gull chicks had significantly lower IgG levels. The mean blood concentrations of ΣPCB_9 in black-legged kittiwakes were 4.7 and 9.6 µg/g lipid wt. for females and males, respectively. The corresponding concentrations were 2.8 and 3.9 µg/g lipid wt. in Atlantic puffins. The mean blood concentration of ΣPCB_9 in the experimental group of glaucous gull chicks was 4.5 µg/g lipid wt. at day 56, the end of the experiment (paper II). However, at 15 days of age, when the immunization started, the chicks had an almost eight times higher level of ΣPCB_9 (34.2 µg/g lipid wt.) concentration. Even though the black-legged kittiwakes had higher levels of ΣPCB_9 than the experimental group of gull chicks at the end of the experiment, the PCB levels had been higher in the latter. The glaucous gull chicks' immune system was furthermore in a developmental state, while the black-legged kittiwake and the Atlantic puffin were adults.

The immunization of the black-legged kittiwakes sampled in 2006 was a pilot project. Fifty black-legged kittiwakes were immunized, 34 were recaptured and 27 could be fully analysed in the immune laboratory. The primary response to tetanus-toxoid immunization was not correlated to POP levels. This is also in contrast to the results of paper II where the experimental group responded less well to EIV immunization. At the same time, the response to EHV, REO and TET immunization did not differ between the groups. Experimental immunization studies have shown a suppressed antibody titer with POP levels, while the directions and strengths of the response in field studies have been variable. The differences between experimental and field studies reflect the problems with confounders as sex, age, size, food, weather conditions, parasites and infections and numerous others. The laboratory experiment can focus one or a few pollutants and have control on the confounders, while the field study needs to take into account that the complex POP-cocktail can initiate additive, synergistic or even antagonistic effects at the same time as the numbers of confounders are high. The solution of the cooperative properties of the mixture of POPs and the confounders was in this study solved by using a large sample size, at least for an ecotoxicology study, by restricting the study to adult birds and by including confounders as sex and size units in the statistical analysis.

The method using immunization should be further tested in new pilot studies. It is critical to use an immunization (vaccine) that both gives a unique new antibody response and that the antibody can easily be detected in the laboratory. For example, our the measurement of antibodies against *Clostridium perfringens* in black-legged kittiwake failed and 3 of 5 rabies-antibody measurements did not pass the quality control due to high variation between replicates. Tests of sheep red blood cells (SRBC) immunization or other vaccines need to be made prior to a full-scale study.

The Atlantic puffin has generally lower levels of POPs and is much harder to study than the black-legged kittiwake. Atlantic puffins breed in burrows, are harder to catch (it is almost impossible if you need to catch a predefine bird) and have a tendency to desert their nest if caught early in the incubation period. As a result, further intensive studies of POP effects on Atlantic puffins are not recommended, whereas monitoring studies of POP levels in eggs or blood are less problematic. Further studies of the adaptive immunity and POP levels are therefore recommended made using black-legged kittiwakes.

The conclusion from the study of black-legged kittiwakes and Atlantic puffins is that further investigations need to be made before the prediction of POP-induced alteration of the immune system can be rejected.

Paper V

The last paper discusses the cause of death of glaucous gulls found during the breeding seasons 2003, 2004 and 2005 on Bjørnøya in the Barents Sea. The dead gulls were collected and brought in for autopsies. Samples from liver and brain were analysed for OCPs, PCBs, PBDEs and Hg. During the fieldwork, some clearly sick birds were also observed. These lost their balance, had bad coordination of head and feet and had bouts of convulsions. As a result, they often deserted their chicks, moved to a nearby freshwater washing place and died within one or two days. These descriptions fit the symptoms of poisoning, and are the same as described for four passerine bird species dying of PCB contamination (Stickel et al., 1984). An obvious question to ask is whether these glaucous gulls died of pollution.

The studied gulls were emaciated. Only 2 of 21 were classified to be in normal condition. All were found dead in late in the incubation period or in the chick-rearing period, which is the most energy demanding period of an adult seabirds' annual cycle (Coulson et al., 1983; Moe et al., 2002). Any emaciation will result in the redistribution of lipid-soluble POPs to organs still containing fats, for example the liver and the brain. The mean liver levels of POPs in the sick and dead glaucous gulls was 10-40 times higher than from randomly-sampled glaucous gulls from the Barents Sea area (Borgå et al., 2001; Haukås et al., 2007; Henriksen et al., 2000). The brain levels of *p,p*'-DDE or PCBs seemed, however, to be at apparently non-lethal levels (Ohlendorf et al., 1981; Sileo et al., 1977; Stickel et al., 1984).

A range of adverse effects has been related to high POP levels in randomly sampled and apparently healthy glaucous gulls, reviewed by Bustnes (2006) and Gabrielsen (2007). A rapid mobilisation of body-lipids at this energy-demanding period results in a sudden increase of circulating pollutants. Bustnes et al. (2001, 2005) found a negative correlation between oxychlordane and PCB concentrations and feeding efficiency. This indicates an additional stress as the glaucous gulls' ability to find food are reduced with increased pollution levels. This relationship alone can accelerate emaciation and thereby increase the concentration of pollutants. It is easy to see that the described effect could lead to a negative spiral. Emaciation leads to higher levels of POPs and higher levels of POPs decrease the gull's ability to forage which again leads to further emaciation. It is not known whether the described spiral effect occurs. If this kind of negative-negative feedback mechanism exists, there might be a threshold level of POPs above which the spiral is impossible to stop, and the only result will be death.

We further compared the liver and brain residue levels with a sample of dead and dying glaucous gulls from 1989 (Gabrielsen et al., 1995). The 2003-2005 liver levels of ΣOCP_3 and ΣPCB_{18} were both 12% of the 1989 levels (paper V). The 2003-2005 and 1989 brain levels of ΣOCP_3 and ΣPCB_{18} were, however, the same. The liver levels thus followed the general decreasing temporal trend of persistent organic chlorinated compounds in arctic seabirds (Braune et al., 2005; Helgason et al., 2008). The brain levels on the other hand, strengthen the theory that POPs contributed to the death of the gulls. It seems that emaciation and redistribution of POPs, continues until the ΣPCB levels in brain reaches 200-300 μg/g lipid wt.

It is natural that old or weak birds die during the incubation and chick-rearing periods. The observed inactivity, poor balance and convulsions suggest, however, an effect of toxins. We therefore conclude that contamination levels add supplementary stress to those glaucous gulls that deplete their body reserves during incubation and chick-rearing. These gulls were definitely inside the "window" of effect and the POP-cocktail became too strong.

Concluding remarks

The present thesis summarizes five studies of three different seabird species that all receive a cocktail of POPs through their food. These persistent pollutants have the potential to induce chemically-related stress. Three of the studies found statistically significant results that could be seen as indication of chemically-related stress effects, while two studies did not find any associations related to pollutants.

The laboratory study showed that environmentally comparable levels and composition of POPs resulted in a suppressed immune system. The ability to respond to an immunization (i.e. a simulated infection) was poorer, the levels of IgG and IgM proteins were lower and the *in vitro* lymphocyte proliferation was altered. The experimental groups' blood levels of ΣPCB_9 was 4.5 µg/g lipid wt. at eight weeks of age and were comparable to levels of PCB in glaucous gulls chicks from Bjørnøya. The blood level of ΣPCB_9 was 34.2 µg/g lipid wt. at two weeks of age when the immunization started and the growth dilution had not started. It is difficult to suggest a threshold level of effect in developing glaucous gull chicks since we only had one POP-exposed group, but adverse immune effects is at least induced at LOAEL 34.2 µg/g lipid wt. in two week old glaucous gull chicks.

The two papers addressing adult glaucous gulls tested the same prediction and found that different conclusions were drawn depending on POP levels measured in the liver. The hepatic mean level of ΣPCB_9 was 75.3 µg/g lipid wt. in Bjørnøya glaucous gulls and 27.9 (ΣPCB_{32}) µg/g lipid wt. in the Barentsburg glaucous gulls. Positive correlations between OC levels and nematode infection intensities were found in Bjørnøya gulls, while no such parasite relationships existed for the Barentsburg gulls. These results raise the question whether the glaucous gull living in an environment where some birds accidentally, or by foraging in higher levels of the food chain, move into a pollution state where the levels of pollutants exceed a threshold above which effects are initiated, inside the "window" of effect. It also raises the question whether the annual lipid cycle could change the gull's pollutant levels that it moves above and below the threshold level of effects during a year. Supported by a large number of effect studies of glaucous gulls (Gabrielsen, 2007, and references therein) a LOAEL of 75 µg/g liver lipid wt. is suggested for immune effects in adult glaucous gulls.

The black-legged kittiwake and Atlantic puffin study clearly indicated that the POP levels were either below the threshold level for immune effects, or that the IgG proteins were not a suitable endpoint for evaluating avian immune effects. A third possibility is that the commercial anti-chicken-IgG-kit cross-reacted with other proteins and disturbed the measurements. Further effect studies of the black-legged kittiwakes are suggested.

Dead and dying glaucous gulls from 2003-2005 were compared with dead and dying glaucous gulls from 1989. The liver levels were significantly lower in 2003-2005, while the summarized OCP and PCB levels in the brain were exactly the same in 1989 as in 2003-2005. Some birds were also seen as they got sick. These birds had lower activity levels, poor balance and bouts of tremors all of which fit the description of poisoning. The birds were in poor body condition and 38% were classified as completely or severely emaciated. It seems that emaciation and redistribution of POPs continues until the POP-cocktail in brain gives a hangover that totally immobilizes the gulls and results in death.

Despite that the immune system was suggested as one of the most sensitive targets for the toxic effects of PCBs more than a decade ago (Tryphonas, 1994), there are large gaps in our knowledge of how long-term low level exposure of POPs affects the immune system. Further effect studies of POPs on marine birds should focus on the combined effects of POPs and other anthropogenic or natural stressors. Using laboratory, semi-field and/or field designs, these studies should also aim to identify the effects of single classes of contaminants, including the legacy OCs and more recently identified POP candidates (e.g., brominated flame retardants, perfluorinated alkyl substances and current-use chemicals) and their combined effects.

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Paper I

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Paper II

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Paper III

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Paper IV

Sagerup, K., Åsbakk, K., Polder A., Skaare, J.U., Gabrielsen, G.W., Barrett, R.T.

The effect of persistent organic pollutants (POPs) on the immune system of black-legged kittiwakes and Atlantic puffins in the Barents Sea.

Manuscript.

Paper V

Sagerup, K., Helgason, L.B., Polder, A., Strøm, H., Josefsen, T.D.,

Skaare, J.U., Gabrielsen. G.W.

Persistent organic pollutants and mercury in dead and dying glaucous gulls (*Larus hyperboreus*) from Bjørnøya (Svalbard).

Manuscript.

Appendix 1: Abbreviations and some definitions [1]

^{13}C	$^{13}C/^{12}C$ stable isotope ratio
^{15}N	$^{15}N/^{14}N$ stable isotope ratio (standardized against air)
ΣCHL	Sum of *cis*- and *trans*-chlordane and *cis*- and *trans*-nonachlor (concentrations)
ΣDDT	Sum of DDT, DDD, and DDE (concentrations)
ΣHCH	Sum of (concentrations of) α-, β- and γ-HCH isomers
ΣPCBx	Sum of a number of individual polychlorinated (PCB) congeners. A method of expressing the PCB content of a sample by measuring and summing the quantities of specific PCB congeners. The number and identification of the congeners determined varies from laboratory to laboratory and for different types of samples. (See also Total PCB)
ΣPBDEx	Sum of a number of individual polybrominated diphenyl ether (PBDE) congeners
AMAP	Arctic Monitoring and Assessment Programme (Arctic Council Working Group)
Aroclor	Technical PCB mixtures. Aroclor 1254 denotes 12 carbon atoms and 54 weight % of chlorine. USA and UK.
BDE	Brominated diphenyl ether. Typically used in association with an IUPAC number to identify a particular BDE congener (i.e. BDE-47)
Bioaccumulation	The net accumulation of POPs from all exposure routes, usually expressed as the concentration of a POP in an organism on a lipid weight basis divided by the concentration found in water (truly dissolved) or air (gas phase)
Biomagnification	The increased accumulation of POPs with each trophic level in a food web, expressed as the concentrations in the organisms divided by the concentrations in its food, both on a lipid weight or organic carbon (sediments, soils) basis
BMF	Biomagnification factor
CHL	Chlordane
Clophen	Technical PCB mixtures, Germany company
Con A	Concanavalin A
Congener	An individual PCB or PBDE
Coplanar PCBs	PCB molecules that can take on a planar configuration and that are dioxin-like. These include non-*ortho* and mono-*ortho* PCBs
DDD	1,1-dichloro-2,2-bis (4-chlorophenyl) ethane
DDE	1,1-dichloro-2,2-bis (4-chloro-phenyl) ethylene
DDT	Dichlorodiphenyltrichloroethane (an organochlorine pesticide) 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane
EHV	Herpes virus
EIV	Influenza virus
GC	Gas chromatograph
HBCD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane (organochlorine insecticides; including the γ-HCH isomer, lindane)
Hg	Mercury
IgG	Immunoglobulin G
IgM	Immunoglobulin M

in vitro	Experiments carried out "in glass", i.e. outside of living organisms
in vivo	Experiments carried out within living organisms
IUPAC	International Union of Pure and Applied Chemistry
KLH	Keyhole limpets hemocyanin
Kow	Partitioning coefficient between octanol and water
Lipophilic	Affinity for lipid; lipophilic substances exhibit a preference to accumulate in fat and fatty tissues
Lipid wt.	Lipid weight – basis of determination of concentration. Lipid wt. = (wet wt.*100)/lipid%
LOAEL	Lowest-(observed)-adverse-effect-level
LOEL	Lowest-(observed)-effect-level
LPS	Lipo-poly-saccharine
Mono-*ortho* PCBs	PCB molecules with one chlorine in the *ortho* position; i.e. with 2,3,3',4,4' chlorine substitution (i.e. PCB-118 and 105) (See also coplanar PCBs)
MS	Mass spectrometer
NOAEL	No-(observed)-adverse-effects-level
NOEL	No-(observed)-effects-level
Non-*ortho* PCBs	PCB molecules with no chlorines in the *ortho* position; i.e., non-*ortho* substituted PCBs; PCB congeners with 3,3',4,4' chlorine substitution (i.e. PCB-77, 126 and 169). See also coplanar PCBs
OC	Organochlorine
Parlar	An individual toxaphene congener
PFAs	Perfluorinated acids
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PHA	Phyto-hemagglutinin
Planar PCBs	Collective name for non-*ortho* PCBs and mono-*ortho* PCBs
POP	Persistent organic pollutant
PPD	Purified protein derivative of *Mycobacterium avium* subsp. *paratuberculosis tuberculin*
PWM	Poke weed mitogen
REO	Reovirus
SCCP	Short-chain chlorinated paraffins
Sovol (Sovtol)	Technical PCB mixtures, from former USSR and one USA company
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin, the most toxic dioxin
TEF	Toxic equivalency factor
TEQ	TCDD equivalents
TET	Tetanus toxoid
Total PCB	An (older) method for expressing the PCB content of a sample in which the sample is quantitated against a technical PCB product (such as Aroclor 1254) as the standard. The analysis is carried out using packed column chromatography
Toxaphene	Polychlorobornanes and camphenes
Wet wt.	Wet weight – basis of determination of concentration
Xenobiotic	Chemical compound that is foreign to an organism; normally a synthetic chemical compound

[1] Main part of appendix 1 after Arctic Monitoring and Assessment Program (AMAP, 2004).

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