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# **Reproductive Disorders in Baltic Vertebrate Wildlife (BALTREP 2011)**

*What is the status of, and the threats to reproductive health in Baltic region  
wildlife?*

Proceedings from an international conference in Uppsala, Sweden  
December 7-8, 2011

Cecilia Berg, Katrin Lundstedt-Enkel, Jonas Malmsten & Sara Persson (editors)

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## Program for BALTREP 2011

7 - 8 December 2011,

Venue: the Ekman lecture hall, Norbyvägen 14, Evolutionary Biology Centre (EBC), Uppsala University, Sweden

<b>7<sup>th</sup> December</b>		
<b>Session I. Chair: Cecilia Berg and Sara Persson</b>		
08.00-09.00	<b>Registration, coffee and sandwich</b>	
09.00-09.15	Welcome	Cecilia Berg <i>Organizing committee</i>
09.15-10.00	Contaminants in context with other stressors: effects on wildlife reproduction and sustainability	Val Beasley <i>Key note speaker</i>
<b>10.00-10.30</b>	<b>Coffee, poster session</b>	
10.30-11.00	Integrated monitoring indicates impaired fish health in Swedish coastal waters	Lars Förlin <i>Invited speaker</i>
11.00-11.30	Title to be announced	Claes Andrén <i>Invited speaker</i>
11.30-11.45	Why is the European Environment Agency interested in reproductive disturbances in Baltic wildlife?	Peter Pärt
11.45-12.00	Discussion	
<b>12.00-13.15</b>	<b>Lunch at the EBC restaurant (included in fee)</b>	
<b>Session II. Chair: Leif Norrgren and Bodil Ström Holst</b>		
13.15-13.45	Weak recruitment in Baltic fish populations - is there a problem?	Jens Olsson <i>Invited speaker</i>
13.45-14.00	Can female herring ( <i>Clupea harengus</i> ) contaminant concentration influence the fish reproduction?	Katrin Lundstedt-Enkel
<b>14.00-15.00</b>	<b>Coffee, poster session</b>	
15.00-15.30	Endocrine and reproductive effects of environmental hazardous substances	Jan L. Lyche <i>Invited speaker</i>
15.30-15.45	Polychlorinated biphenyl concentrations in wild mink ( <i>Neovison vison</i> ) from four different areas in Sweden	Sara Persson
15.45-16.15	Discussion	
<b>19.00 -</b>	<b>Social activity; GALA DINNER at Norrlands nation! (Included in fee)</b>  <b>Norrlands Nation</b> is located in down town Uppsala on the west side of the river Fyrisån at Västra Ågatan 14 (see map).	

<b>8<sup>th</sup> December</b>		
<b>Session III. Chair: Katrin Lundstedt-Enkel and Jonas Malmsten</b>		
09.00-09.30	Anomalies of the reproductive system in the St. Lawrence Beluga whales: Three decades of pathology data	Stephane Lair <i>Invited speaker</i>
09.30-10.00	Reproduction in Baltic seals	Britt-Marie Bäcklin <i>Invited speaker</i>
<b>10.00-10.30</b>	<b><i>Coffee, poster session</i></b>	
10.30-10.45	Müllerian Duct Dysgenesis - a cause for female reproductive disorders in wildlife?	Cecilia Berg
10.45-11.00	High frequency of Müllerian duct cysts on the <i>vas deferens</i> in Swedish wild otters ( <i>Lutra lutra</i> )	Anna Roos / Erik Ågren
11.00-11.30	Reproductive impairments and contaminants in white-tailed sea eagles in Sweden, 1965-2010	Björn Helander <i>Invited speaker</i>
11.30-11.45	Discussion	
<b>11.45-13.15</b>	<b><i>Lunch at the EBC restaurant (included in fee)</i></b>	
<b>Session IV. Chair: Leif Norrgren and Peter Pärt</b>		
13.15-13.45	Population trends of Swedish coastal breeding birds	Martin Green <i>Invited speaker</i>
13.45-14.15	Thiamine deficiency in the ecosystem	Lennart Balk <i>Invited speaker</i>
<b>14.15-15.00</b>	<b><i>Coffee, poster session</i></b>	
15.00-15.15	Embryotoxicity of perfluorinated compounds in birds: Is peroxisome-proliferator activated receptor alpha involved?	Björn Brunström
15.15-15.30	Studies on reproduction in moose ( <i>Alces alces</i> ) in southern Sweden	Jonas Malmsten
15.30-15.50	Discussion regarding all presentations	
15.50-16.00	Concluding remarks	Cecilia Berg <i>Organizing committee</i>

## Foreword

The conference “Reproductive Disorders in Baltic Vertebrate Wildlife (BALTREP 2011) - *What is the status of, and the threats to reproductive health in Baltic region wildlife?*” is arranged by CRU, Centre for Reproductive Biology in Uppsala, December 7- 8, 2011, Uppsala. CRU is a multidisciplinary network of about a hundred scientists working in the field of animal and human reproduction at the Swedish University of Agricultural Sciences (SLU) and Uppsala University (UU).

Reproductive disorders in wildlife and humans are matters of great world-wide concern. The Baltic region has a history of several incidents of disturbed reproduction in wildlife. Sterile female seals and eggshell thinning in fish-eating birds in the Baltic region are classical examples of pollutant-induced reproductive disturbances. BALTREP 2011 aims to gather current knowledge of reproductive health in Baltic wildlife including all vertebrate groups (fish, amphibians, reptiles, birds, and mammals) and to identify data gaps and research needs.

The conference will address the following questions: What is the status of the reproductive health in the Baltic terrestrial and aquatic vertebrate fauna today? What are the threats to the reproductive health in the Baltic region? How should we monitor reproductive health? What biomarkers for reproductive disorders/health are there for the various animal groups?

The organizing committee consists of Cecilia Berg and Katrin Lundstedt-Enkel, Department of Environmental Toxicology, Uppsala University, Jonas Malmsten and Sara Persson, Department of Clinical Sciences, SLU, and Leif Norrgren, Department of Biomedical Sciences and Veterinary Public Health, SLU.

Financial support for the conference is provided by the CRU and the Swedish research council Formas.

We hope that you will find opportunities for learning and networking and wish you a warm welcome!

Cecilia Berg

On behalf of the organizing committee

# Map



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## Oral presentation

### Contaminants in context with other stressors on wildlife reproduction and sustainability

Val Richard Beasley

Department of Comparative Biosciences  
College of Veterinary Medicine  
University of Illinois at Urbana-Champaign

#### **Introduction:**

Reproductive toxicology of wildlife will be viewed in relation to overall health, other environmental stressors, and responsibilities of the scientific community.

#### **Aim:**

To consider well-studied toxicants and less understood yet ubiquitous contaminants that undermine wildlife reproduction.

#### **Materials and Methods:**

Examples will be drawn from the literature.

#### **Results:**

Chlorinated, brominated, and fluorinated organics are important reproductive toxicants. Restriction of organochlorine insecticides and polychlorinated biphenyls and environmental detoxification have enabled partial recoveries of wildlife communities. However, polybrominated diphenyl ethers and their impacts are increasing in some environments. Recalcitrant and developmentally important perfluorinated compounds are increasing in parts of the world. Other toxicants that may be extremely important in regard to reproduction include mercury and petroleum. Nitrate seems to be a direct-acting reproductive toxicant, and a cause of hypoxia, which disrupts endocrine physiology. The triazine herbicide, atrazine, causes demasculinization of a wide array of species. Most notably in the case of DDT and atrazine, investigators who reported reproductive effects of toxicants in wildlife have been derided by end users and industry-associated scientists. Such conflicts can be resolved through additional research.

#### **Discussion:**

The complexity of exposures and the diversity of species, strains, and environments can make replication of findings from wildlife toxicology studies a challenge. Regardless, repeated findings of adverse reproductive effects from commonly encountered exposures should prompt stewardship action.

#### **Conclusions:**

Opportunities abound for investigation, as well as engagement of citizens, governments, non-governmental organizations, and businesses to catalyze more informed choices regarding chemicals to be allowed to enter the bodies of wildlife.



# **Integrated monitoring indicates impaired fish health in Swedish coastal waters**

Lars Förlin

Department of Zoology, University of Gothenburg, Box 463, S405 30 Gothenburg, Sweden.

Modern society utilises more man-made chemicals than ever before and hundreds of new chemicals are entering the market with the aquatic environment ending up as the ultimate sink for many hazardous substances. It is therefore a continuous need to monitor biological effects caused by hazardous chemicals in the aquatic environment. Here an overview of fish monitoring work especially in Sweden will be given and a few case studies presented.

The health status of two sentinel fish species perch (*P fluviatilis*) and the viviparous eelpout (*Z. viviparus*) have been regularly studied in monitoring programmes in Swedish coastal sites for many years. Perch belongs to the dominating fish species in Baltic archipelagos, while the viviparous eelpout is relative common at the open coasts of the Baltic up to the Northern Quark and in the Kattegatt and Skagerrak coastal waters. The stationary behaviour makes both species well-suited for environmental monitoring and ecotoxicological research. In reference coastal sites, currently four sites, with no or small local point sources of contaminants fish health has been assessed to study emerging impacts of pollutants or other stressors. In these sites the fish health studies together with analytical chemistry work to measure anthropogenic chemicals and fish ecology studies, form an integrated fish monitoring program supported by the Swedish EPA.

The fish health is assessed by a battery of biological endpoints (biomarkers) including for example biomarkers measuring for detoxifications defences such as CYP1A/EROD, endocrine disruption such as vitellogenin, oxidative stress including antioxidant defences system and oxidative damages, blood chemistry, haematology and genotoxicity, and also studies of reproductive success.

More than 20 years of studies in Swedish reference coastal sites seem to indicate that the fish are generally in good health. However, clear time trends for example ca. 30% reduction of relative gonad sizes in female perch and marked increases of the activity of the detoxification enzyme EROD in both perch and eelpout provide “early warnings” of environmental changes over time. The results seem also to indicate small changes in antioxidant defence and immune defence systems of the fish. The increasing time trends of EROD activities seem to indicate that fish are exposed to hazardous substances like polycyclic aromatic hydrocarbons (PAH) and/or other planar compounds i.e. dioxin-like compounds. The reduction in the relative ovary size of the female perch seems to be paralleled by a reduction of number of eggs in the mature females. So far there are however no clear evidence for reduced or impaired perch populations in these reference sites. It will also be reported that the analyses of eelpout reproductive success seem to indicate small increase of abnormal larvae development over time in the reference coastal sites. Comparison will be made to polluted coastal sites where relative large numbers of dead and malformed eelpout larvae have been reported.



Claes Andrén

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## **Why is the European Environment Agency interested in reproductive disturbances in Baltic wildlife?**

Peter Pärt

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The European Environment Agency (EEA) is an agency of the European Union with the mission to provide sound, independent information on the environment. EEA's flagship product is the State of the Environment in Europe Report (SOER) published every five years, aimed at providing information on the state of, trends in and prospects for Europe's environment, including causes, impacts and potential responses. This report is a major information source for those involved in developing, adopting, implementing and evaluating environmental policy, and also the general public. The latest SOER was published 2010. EEA is also producing targeted assessments and specialized reports on different aspects of environmental quality. Observations of reproductive disturbances in different groups of vertebrates represent a clear sign of a serious imbalance in the ecosystem. It is one of the few concrete examples of the multicausality of environmental impacts including both contaminants and large-scale ecological changes. There is a need to report on this to a wider audience and to raise the awareness in the political circles. EEA is currently preparing several reports addressing environmental issues in marine and coastal environments including climate change and pollution impacts. In this context we hope to use information from this workshop in our future reporting and to spread the information in wider circles. For more information on the work of EEA web pages at: [www.eea.europa.eu](http://www.eea.europa.eu)



## **Weak recruitment in Baltic fish populations - is there a problem?**

Jens Olsson and Magnus Appelberg

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The reproductive output of a species serves as the basis for population growth. In fish, however, there is generally a rather weak stock-recruit relationship, especially at higher population densities. Here we review the current knowledge on influential factors for the recruitment of off shore species in the Baltic Sea such as cod and herring. We focus on the recent findings suggesting weak recruitment of coastal predatory fish as perch and pike. The most recent findings and the current state-of-the-art concerning the influence of resource availability, competition, predation and abiotic limitations on the recruitment success are presented. We also discuss the current recruitment status of perch and pike along the Swedish Baltic coast.



# Can female herring (*Clupea harengus*) contaminant concentrations influence the fish reproduction?

Katrin Lundstedt-Enkel<sup>1,2</sup>

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<sup>2</sup> Swedish museum of Natural history, Stockholm, Sweden.

## Introduction

Herring (*Clupea harengus*) is the most dominant commercial fish species in the Baltic Sea as well as being an important food source for several predators in the marine environment. It is heavily contaminated and human consumption of Baltic Sea herring is restricted to 2-3 times a year *via* recommendations by the Swedish Food Administration.

## Aim

To study the relationships between the multivariate pattern of contaminants and the fish reproductive stage.

## Material and Methods

The reproductive phase, *i.e.* state of gonadal maturity, was determined (using a five-point scale; 1 - 5) by ocular inspection of the gonads through a small incision on the fish abdomen. The fish to sample for further chemical analysis was selected using multivariate design from almost 400 individuals with determined biology. Concentrations of organochlorines and brominated flame retardants were determined in female herring (n = 60) of varying age (2-13 years), weight (25-200 g), and body length (16-29 cm) caught at three locations in the Swedish part of the Baltic Proper.

## Results

In an orthogonal partial least squares regression (OPLS) with the reproductive phase as Y and contaminant concentrations (wet weight, ww) as X ( $R^2X= 0.76$ ;  $R^2Y= 0.07$ ;  $Q^2= 0.05$ ) loading plot it can be seen that all contaminant concentrations are positively correlated with herring reproductive phase, though the model is weak.

## Discussion and Conclusions

All contaminant concentrations (ww) are positively correlated with female herring reproductive phase, lipid content and condition factor. Suggesting either 1) that the mixture of the environmental chemicals contribute to the hormonal (estrogenic) influence and advances oocytes maturation and lipid deposition or 2) that the fatty fish have eaten more thus ingesting more contaminants and reach maturation earlier than lean fish with lower contaminant concentrations. Most interestingly, if the modelling is done with concentrations in lipid weight (lw) instead of wet weight the correlations are almost reversed, *i.e.* the fish with the higher contaminant concentrations (lw) of a majority of the contaminants are the leaner with smaller ovaries. This underscores the importance of how the contaminant concentrations are denoted. I recommend using ww to negate the “lipid dilution effect”.



# Endocrine and reproductive effects of environmental hazardous substances

Jan L. Lyche<sup>1\*</sup>, Vidar Berg<sup>1</sup>, Elisabeth Lie<sup>1</sup>, Rasoul Nourizadeh-Lillabadi<sup>1</sup>, Adam Zerihun<sup>2</sup>, Duncan J. Colquhoun<sup>2</sup>, Camilla Karlsson<sup>1</sup>, Irma Oskam<sup>3</sup>, Janneche Utne Skåre<sup>1,2</sup>, Peter Alestrøm<sup>1</sup>, Erik Ropstad<sup>1</sup>

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## Introduction

A variety of environmental pollutants have the potential to disrupt the endocrine systems of animals thereby inducing developmental and functional disorders in endocrine tissues and biological systems regulated by hormones. Adverse effects on reproduction, immune functions, behavior and endocrine signaling are reported in fish and mammals exposed to environmental persistent organic pollutants (POPs).

## Aim

To investigate potential adverse effects of POPs in aquatic and terrestrial environments.

## Material and Methods

Toxic potency of individual chemicals and natural mixtures of POPs were investigated in experimental models and field studies. Natural mixtures used were extracted from fish liver (fish liver oil).

## Results

Exposure to PCB 153 and PCB 126 during gestation and lactation with levels comparable to those detected in polar bears (*Ursus maritimus*) at Svalbard affected endocrine and immune functions in goats. Delayed puberty and testicular abnormalities were also documented.

Increased prevalence of tuberculosis and diffuse pathology were observed in burbot (*Lota lota*) from Lake Mjøsa (polluted) but not in those from Lake Losna (background). Mixtures extracted from burbot from both lakes caused precocious puberty, skewed sex ratios and reduced hatching rates in zebrafish (*Danio rerio*). Histopathology resembling the lesions observed in burbot from Lake Mjøsa was observed in zebrafish exposed to the Mjøsa mixture.

## Conclusion

Similar effects on endocrine and immune functions were documented in free ranging polar bears and in the goat model. Furthermore, the adverse effects observed in wild fish (burbot) and model fish (zebrafish) exposed to the same mixture of POPs raise the question whether environmental POPs represents a health risk to wildlife.



# Polychlorinated biphenyl concentrations in wild mink (*Neovison vison*) from four different areas in Sweden

Sara Persson<sup>1</sup>, Björn Brunström<sup>2</sup>, Anna Rotander<sup>3</sup>, Bert van Bavel<sup>3</sup>, Britt-Marie Bäcklin<sup>4</sup>, Hans Kindahl<sup>1</sup>, and Ulf Magnusson<sup>1</sup>

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## Introduction

The mink is an excellent sentinel species in monitoring of PCB. It is a top predator that largely feeds on aquatic prey and its reproductive system is known to be sensitive to PCB.

## Aim

The aim of the study was to investigate the exposure of free-ranging mink to PCBs in different areas in Sweden and to assess the risk of reproductive toxicity.

## Material and Methods

101 male mink were sampled from four different areas in Sweden; the Koster Islands in Skagerrak (K;  $n=26$ ), the Märsta inland region (M;  $n=25$ ), the Gävle Baltic Coast (G;  $n=25$ ), and the inland of Northern Sweden (N;  $n=25$ ). PCBs were analyzed in subcutaneous fat using GC/MS after sample extraction and clean up. Data was analyzed by least square mean comparisons and a multiple regression model with area, age (0, 1 or  $\geq 2$  years), season (autumn, winter, spring) and body condition (g subcutaneous fat/kg body weight) as independent variables.

## Results

The least square mean  $\sum$ PCB was significantly higher in the G area close to the Baltic Coast (10.0  $\mu\text{g/g}$  lipid weight,  $p<0.001$  –  $p=0.0021$ ) than in the K, M and N areas (3.2, 3.1 and 1.5  $\mu\text{g/g}$  lw). The overall arithmetic mean of all mink was 7.3  $\mu\text{g/g}$ , ranging from 0.1 to 55.6  $\mu\text{g/g}$  lw.

## Discussion and Conclusions

The mean concentrations in this study were similar to levels found in Swedish mink in the 1990s although levels of PCBs generally are decreasing. Up to 30% of the mink in this study showed higher concentrations than those associated with reproductive toxicity in experimental studies.



## **Anomalies of the reproductive system in St. Lawrence Beluga whales (*Delphinapterus leucas*): Three decades of pathology data**

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Post-mortem examinations of beluga whales have been used as a tool to indirectly assess health of the St. Lawrence Estuary beluga whale population. Between 1983 and 2011 a total of 470 beluga carcasses from that population were reported stranded (=beach-cast) or drifting. A total of 213 of these carcasses, including 87 mature females, were collected and transported to the pathology laboratory for a post-mortem examination. Standard necropsies were conducted on each carcass using an evolving but relatively uniform protocol.

Anomalies of the reproductive system have been occasionally observed in this population over the years. These anomalies include two cases of intersexes and ten cases of reproductive organ cancers (five mammary glands cancers, three ovarian tumours, one urethral carcinoma and one uterine adenocarcinoma). A dozen cases of neonatal mortality, which were believed to be a result of mismothering or calf weakness, have been observed since 1983. This figure is most likely a gross underestimate of this phenomenon; a large proportion of beluga calves that die are probably never found due to their colour and small size.

From 1983 to 2009, fatal dystocia was only occasionally diagnosed, with 4 cases observed over a 27-year period, for a yearly average of 0.15 cases per year or 5.3% of the mature females examined. Interestingly, five cases of dystocia were diagnosed during the last two years, for a yearly average of 2.5 cases representing 41.7% of the mature females examined in 2010 and 2011. Concurrently, only two live newborn calves were observed in the beluga herd during the summer of 2011. This exceptionally low apparent calf production or survival, and the unusual occurrence of dystocia, suggest that this population has recently suffered from reproductive impairment. The cause of the apparent cluster of dystocia cases is currently under investigation. Potential identified risk factors include increasing contamination with endocrine disrupting xenobiotics such as polybrominated diphenyl ethers, and increased exposure to saxitoxins produced by harmful algal blooms. Ongoing monitoring of calf production and of mortality causes should enable us to determine if this high occurrence of reproductive impairments is a transient phenomenon or a new significant issue for the conservation of this population.



## **Reproduction in Baltic seals**

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The number of Baltic grey and ringed seals decreased during the 1900s to only a few thousands in the late 1980s. In the 1970s and 1980s uterine obstructions, probably caused by foetal death, resulted in sterility and were found in 7 years or older Baltic grey and ringed seals (42 and 50% respectively of the investigated adult females). The last cases in Sweden were observed in 1993 in grey seals and in 2009 in ringed seals. Animals showing uterine occlusions were born between 1946 and 1984. The Baltic grey seal population has increased by 8% yearly since the 1990s, while the ringed seals only have increased by 4,5 % yearly during the same period. Pregnancy rate has increased in examined grey seals from 9 % in 1977-1986 to 60 % in 1987-1996 and in 1997-2006 to 76 %. Uterine leiomyomas have only been observed in grey seals. Grey seals showing uterine leiomyomas were born between 1944 and 1991. Several studies have indicated PCB as a cause of these reproductive lesions. Concentrations in sediment and guillemot eggs showed that in 1985, exposure to PCB were similar to that in 1945 with a peak in late 1970s. The median PCB concentration in juvenile grey seals in the 1980s was 64 mg/kg lipid weight and in ringed seals 17,5 mg/kg lipid weight. As regards foetal death and uterine occlusions, ringed seals may therefore be more sensitive to PCB than grey seals and that could be one of the reasons for the lower reproductive rate.



# Müllerian Duct Dysgenesis - a cause for female reproductive disorders in wildlife?

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## Introduction

The Müllerian ducts are common precursors of the female reproductive tract present in early life stages of most vertebrates. In mammals they develop into oviducts, uterus, and vagina. In birds, reptiles and frogs they develop into oviducts. We hypothesised that exposure to endocrine disrupting chemicals during Müllerian duct differentiation impairs reproductive function in female birds and frogs.

**Aim:** to characterise effects of early life exposure to endocrine disrupting environmental contaminants on female reproductive organ development and fertility in frogs and birds.

## Material and Methods

Tadpoles of the model frog, *Xenopus tropicalis*, were exposed to the estrogen ethynylestradiol (EE2), or the progestin levonorgestrel (LNG). Embryos of the domestic chicken were exposed to EE2 or the pesticide *o,p'*-DDT. Reproductive organs and fertility were assessed when the animals reached sexual maturity.

## Results

In frogs, developmental EE<sub>2</sub> exposure caused oviductal agenesis (absence of oviducts) in the phenotypic females rendering them sterile. LNG exposure resulted in severely impaired ovary and oviduct development, and sterility in female frogs.

In female birds, embryonic exposure to EE2 or *o,p'*-DDT resulted in the production of eggs with thinner shells compared with controls.

## Discussion and Conclusions

We show that the Müllerian duct differentiation is a sensitive target for several environmental pollutants in birds and frogs. Our findings support the hypothesis that female reproductive disorders and infertility in wildlife may reflect developmental impacts induced by endocrine disrupting chemicals during early life stages. In conclusion, exposure to endocrine disruptors during early life stages can permanently impair female reproductive organ development and fertility.



# High frequency of müllerian duct cysts on the vas deferens in swedish wild otters (*lutra lutra*)

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## Introduction

It is believed that PCB was responsible for the dramatic decline of otters (*Lutra lutra*) that occurred after the 1950s in Sweden. So far, few pathological effects on otters that can be correlated to contaminants have been reported.

## Aim

The aim was to investigate the frequency of cysts found on the *vas deferens* in wild otters, and to elucidate possible trends.

## Material and Methods

Between 2005 and 2010, we investigated the reproductive organs from 135 dead male otters for the presence of cysts on the *vas deferens*. The number of cysts on each *vas deferens* was recorded and cyst size was measured. A subset of cysts were fixed in formalin and processed for light microscopy.

## Results

At least one cyst was seen in 70% of the male otters. The number of cysts varied from zero to more than ten, and could be either unilateral or bilateral. The cysts measured from one to ten mm in length and contained a clear watery fluid. At microscopy the cysts were adjacent to, but not connected with the *vas deferens*. The cyst internal side was lined with a single layer of cuboidal or flattened epithelial cells.

## Discussion and Conclusions

These cysts are suggested to be vestigial remnants from the fetal female Müllerian ducts that normally regress in males under the influence of anti-Müllerian hormone during embryogenesis. The high frequency and number of cysts could indicate fetal exposure to elevated concentrations of estrogen-like compounds, but this needs to be studied further.



# Reproductive impairments and contaminants in white-tailed sea eagles in Sweden, 1965-2010

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## Introduction

Poor breeding success among free-ranging white-tailed sea eagles in Sweden was observed already in the 1950s. Annual monitoring of sea eagle reproduction started in 1965 and was adopted into the national monitoring programme under the EPA in 1989.

## Aim

To study reproductive parameters in a strongly exposed top predator in aquatic food webs as an indicator of effects from contaminants.

## Material and Methods

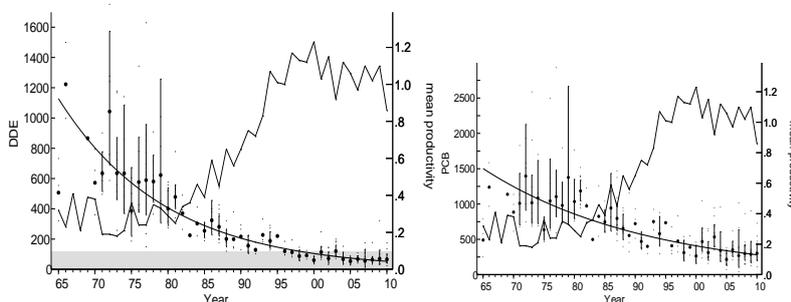
Occupied nests were checked for reproductive output (0/1/2/3 young) in coastal and inland populations. Percent productive nests (breeding success), number of young per productive nest (brood size) and number of young per occupied nest (productivity) were recorded. Dead eggs and shells were saved, and nestlings were ringed and sampled (feathers, blood). Chemical analyses of organochlorine (OC) contaminants were performed at the ITM, Stockholm University.

## Results

Reproduction in the Baltic population 1965-1982 was reduced by 80 % compared to before 1954. Strong relationships show between residue concentrations of DDE and PCB and productivity. DDE and PCB decreased after banning in the 1970s, but reproduction did not start to improve until the mid-1980s. As concentrations decreased in the eggs, young females began to reproduce well whereas old females, with a previously much higher exposure to OCs, remained unproductive. A reference population in Lapland had 5-10 times lower OC concentrations and reproduced normally over the study period.

## Discussion and Conclusions

DDE concentrations are now below estimated reproductive effect levels, but some effects from PCB may still show (embryotox, deformities). The massive impact from DDT-PCB on eagle reproduction is now almost gone. However, a local population (south Bothnian Sea) still shows significantly more dead eggs and fewer young per successful nest, implying an impact from other contaminants.



*DDE & PCB (ppm in egg lipids) and productivity of sea eagles, Swedish Baltic coast.*



# **Population trends of Swedish coastal breeding birds**

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## Thiamine deficiency in the ecosystem

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Thiamine, vitamin B<sub>1</sub>, is a water-soluble vitamin that is essential for vertebrates. Inside the cell, thiamine is phosphorylated to thiamine diphosphate, which functions as a cofactor for at least 5 life sustaining enzymes in the cellular metabolism. Previous studies have shown that sub-lethal thiamine deficiency in vertebrates may give rise to a large number of health effects, such as reduced feeding, memory and learning disturbances, immune suppression, and damage to the blood-brain barrier. Relationships have also been demonstrated between thiamine deficiency and altered lipid metabolism and neurodegenerative diseases.

Thiamine deficiency was recently demonstrated in several species of wild birds that are dying in large numbers from a paralytic disease in the Baltic Sea area. The egg, liver, and brain had reduced thiamine concentrations, and the liver and brain had reduced activities of the thiamine-dependent enzymes. In the liver and brain, there were also elevated proportions of apoenzyme, *i.e.* enzyme without the thiamine diphosphate cofactor. Moreover, paralyzed individuals were successfully remedied by thiamine treatment. Excess mortality and breeding failure were linked to a thiamine deficiency syndrome, which most probably has contributed significantly to declines in many bird populations during the last decades. Both adults and juveniles are subject to excess mortality, and breeding failure is caused by several factors, such as reduced number of eggs, reduced chick survival, and altered behavior, including rudimentary nest building, reduced aggression rates (attack diving), and low noise level in the colonies.

The thiamine deficiency may be induced either by a causative agent(s) acting directly on the affected individual, and/or by insufficient transfer of thiamine between the trophic levels in the food web. Classical persistent organic pollutants are not primarily suspected, since the affected species occupy a wide range of ecological niches and positions in the food web. The authors stress that they are open to the possibility that other wildlife, beside fish and birds, may suffer from thiamine deficiency as well.



# **Embryotoxicity of perfluorinated compounds in birds: Is peroxisome-proliferator activated receptor alpha involved?**

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## **Introduction**

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are ubiquitous environmental contaminants found in wildlife in the Baltic Sea region. These compounds are known to interact with peroxisome-proliferator activated receptors (PPARs) in mammals.

## **Aim**

We tested the hypothesis that PFOS and PFOA can activate transcription of genes that are regulated by PPAR $\alpha$  in bird embryos and that PPAR activation may be involved in embryotoxicity.

## **Material and Methods**

Chicken embryos were exposed to PFOS, PFOA, or the potent PPAR $\alpha$  agonist GW7647 via injection into eggs incubated for 15 days. Samples of embryonic livers and kidneys were taken 72 hours after injection. Transcript levels for PPARs and for a number of genes that are known to be regulated by PPAR $\alpha$  were analyzed using qPCR.

## **Results**

The highest doses of PFOS and PFOA tested caused mortality while no mortality was found in embryos treated with GW7647. PPAR mRNA was expressed in liver and kidney, and several PPAR $\alpha$ -regulated genes were activated by treatment with GW7647. Only transcript from one PPAR $\alpha$ -regulated gene was induced after treatment with PFOA whereas PFOS did not cause any significant effect. PFOS concentrations in liver from the treated chicken embryos were within an order of magnitude higher compared with levels found in livers of guillemot (*Uria aalge*) chicks from the Baltic whereas PFOA concentrations were well above those found in avian wildlife.

## **Discussion and conclusions**

Our results show that PPARs are expressed in chicken embryos and can be activated by PPAR $\alpha$  agonists. However, the results do not support that the embryo lethality of PFOS and PFOA in birds are mediated via activation of PPAR $\alpha$ .



# Studies on reproduction in moose (*Alces alces*) in southern Sweden

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## Introduction

In 2006, hunters from Öland in the Baltic sea reported lower than expected calf/cow ratio in the moose population. In order to elucidate underlying factors, a research project was initiated in 2007.

## Aims

The aim of this study is to characterize female and male moose reproduction parameters, and to compare findings on the island population with populations in the mainland of Sweden.

## Materials and Methods

From 2007-2010, investigations of moose reproductive organs have been performed during opening week of moose hunting on Öland on the mainland (southern Sweden). Several different samples were collected, including blood, spleen, and male and female reproductive organs. In field laboratories, reproductive organs were macroscopically investigated (females: ovarian activity, uterus - embryo presence/quality. Males: testes size, semen collected, fixated for later analyses.

## Results

Sample size amounts to 350 moose from four different mainland moose populations, and the island population. On Öland, a majority of female moose were pregnant, but loss of embryos and unfertilized *ova* (based on number of corpora lutea) has been observed in 25% of investigated females. Similar findings have been noted in mainland moose, but in lower numbers. A lower calf/cow ratio was observed on Öland (0.4) compared to the mainland (>1.0).

## Discussion

Embryonic mortalities have not been described in cervids previously. Observed calf/cow ratio, in combination with field laboratory findings indicate that a relatively high proportion of calves are lost at pregnancy, peri-natally or during early calthood on Öland. Tick-borne disease cannot be ruled out as a cause.



## Posters

### Monitoring of amphibian tadpoles in Sweden

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#### Introduction

There is a global problem of decreases and extinctions of amphibian populations. Also in Sweden, several amphibian species are considered as endangered and therefore, the inclusion of this animal class into environmental assessment is important. Moreover, measurements of various parameters in metamorphosing frog tadpoles can be used for monitoring of thyroid disrupting effects caused by chemical exposure since metamorphosis to a great extent is controlled by thyroxin.

#### Aim

The aim with this project is to examine if effects of thyroid disrupting chemicals are detected in tadpoles in Swedish waters and to monitor health and distribution aspects in Swedish tadpoles.

#### Material and Methods

Potential reproduction sites for moor frog (*Rana arvalis*), common frog (*R. temporaria*) and common toad (*Bufo bufo*) were examined in June and July 2010 and 2011. Up to 10 tadpoles were collected from each site. Morphological, histological and biochemical measurements connected to thyroid disruption were made. Samples for determination of species, chytrid fungus infection and biochemical measurements were taken. Water samples were measured regarding pH, conductivity, oxygen and nitrogen and frozen for later chemical analysis. Sampling was also performed on unexposed tadpoles grown in the laboratory from collected eggs of common frog and moor frog. Further, laboratory exposure of tadpoles to the thyroid disruptor propylthiouracil (PTU) were performed and compared with results from studies on *Xenopus*.

#### Results, Discussion and Conclusions

In total, 16 tadpole sites were found in 2010 and 18 were found in 2011. No indication of thyroid disruption has so far been detected. *Rana* frogs responded similar as *Xenopus* frogs after exposure to PTU.



# **Pathological evaluation of testicles and spermatic cord in male Swedish brown bear (*Ursus arctos*)**

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## **Introduction**

It is well known that many xenobiotic compounds can act as thereby affecting reproductive health in both humans and animals.

## **Aim**

The aim of the study was to investigate if there are any pathological changes in male gonads of brown bears that could indicate exposure to endocrine disruptors.

## **Material and Methods**

Testicles and spermatic cords from 303 male brown bears (0-23 years) were investigated. The bears were killed during the legal hunt or died of other causes and sent to the National Veterinary Institute for autopsy during the years 2006-2010. In bears from the legal hunt only parts of the spermatic cord were investigated but in a separate study of 23 bears the whole spermatic cord was investigated *in situ*.

## **Results**

The most frequent finding was uni- or bilateral cysts adjacent to vas deferens. Cysts were initially recorded in 14% (41/303) of the bears. In the separate study, where the whole spermatic cord was investigated, cysts were observed in 52.2% (12/23) of the bears. Other pathological findings were unilateral testicular degeneration (2/303), chryptorchidism (2/303), segmental aplasia of the epididymidis (2/303) and interstitial cell tumor (2/303).

## **Discussion and conclusions**

Cysts adjacent to the vas deferens were observed in bears of all ages and are most likely congenital remnants of the Müllerian duct. The cysts might be a result of exposure to estrogenic compounds during fetal life. Similar cysts have been induced in mice by exposure to diethylstilbestrol and have also been described in Swedish otters (*Lutra lutra*). Beside cysts only occasional pathological findings were recorded.



# **AHR2-dependence of effects on the swimbladder and *CYP1* and *COX-2* transcription in PCB126-exposed developing zebrafish**

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2) Biology Department, Woods Hole Oceanographic Institution, MA.

## **Introduction**

PCB126 causes edemas, malformations, hemorrhages, etc. in fish larvae. One of the most sensitive targets for PCB126 in zebrafish (*Danio rerio*) is the developing swimbladder, which fails to inflate due to an unknown mechanism.

## **Aim**

To study roles of aryl hydrocarbon receptor 2 (AHR2), cytochrome P4501 (CYP1), and cyclooxygenase-2 (Cox-2) in the effect of PCB126 on the swimbladder in zebrafish larvae.

## **Material and Methods**

Starting 24h post-fertilization zebrafish embryos were exposed to PCB126. After 24h the embryos were transferred to clean water and held until 4 days post-fertilization, *CYP1A*, *CYP1B1*, *CYP1C1*, *CYP1C2*, *COX-2a* and *COX-2b* mRNA expression was analyzed by quantitative PCR. AHR2 translation was blocked using antisense oligo morpholinos.

## **Results**

PCB126 inhibited swimbladder inflation and induced *CYP1* and *COX-2* mRNA expression in dose-dependent manners. Among larvae exposed to 2nM PCB126 27% showed inhibited swimbladder inflation. However, larvae exhibiting inflated and not inflated swimbladder showed similar *CYP1* and *COX-2* mRNA expression levels. AHR2-morpholino treatment rescued the swimbladder effect and blocked *CYP1A*, *COX-2a*, and *COX-2b* induction by PCB126.

## **Discussion and Conclusions**

These results show that PCB126 blocks swimbladder inflation via an AHR2-mediated mechanism, but do not link the swimbladder effect to *CYP1* or *COX-2* induction. Histological data suggest the toxicity may involve disrupted cell differentiation, and possibly inhibited cell proliferation.



# Seroprevalence of *Neospora caninum* in gray wolves in Scandinavia

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## Introduction

Transmission of the protozoan parasite *Neospora caninum* between wild and domestic animals has gained some interest during recent years. Because of the close relationship between gray wolf (*Canis lupus*) and dog it has been suggested that gray wolf is a definitive host for the parasite.

## Aim

The aim of this study was to estimate the seroprevalence of *N. caninum* in Scandinavian gray wolves and to investigate any geographical patterns of the infection.

## Materials and Methods

The investigation was based on blood samples collected from 109 wolves between 1998 and 2009 within the Scandinavian wolf project Skandulv. They were analysed by *N. caninum* iscom ELISA and those with absorbance values exceeding 0.20 were also analysed by immunoblotting. Samples that were positive in both tests were deemed positive.

## Results

Four (3.7%) wolves were positive at the first sampling. They were all sampled 2005 at different locations, and were both females and males. From one male wolf three samples were collected over a 7-year period. No antibodies were detected at the first sampling in 1998 when he was approximately 8 months old but when he was sampled again 5 and 7 years later the ELISA and immunoblotting were positive.

## Discussion

The results indicate that *N. caninum* infection is present in Scandinavian wolves. It is unclear how the wolves acquired the infection



# Prevalence of antibodies against *Toxoplasma gondii* and *Neospora caninum* in moose (*Alces alces*) and roe deer (*Capreolus capreolus*) in Sweden

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## Introduction

*Toxoplasma gondii* and *Neospora caninum* are two coccidian parasites with a worldwide distribution. *T. gondii* is a common parasitic zoonosis in the world, which can cause disease and mortality in children and immuno-compromised persons. *N. caninum* is an important cause of abortions in cattle. Wildlife has been identified as reservoirs and transmitters for both parasites.

## Aim

The purpose of this study was to investigate the seroprevalences of *T. gondii*, and *N. caninum* in moose, and roe deer in Sweden.

## Materials and Methods

Blood samples were collected from 417 moose during 2000–2005 and from 199 roe deer during 1990–2007. Samples were investigated for presence of antibodies by a *T. gondii* direct agglutination test and a *N. caninum* iscom ELISA. Because the iscom ELISA has not been validated for moose or roe deer, sera that gave a positive result were further investigated by immunoblot analysis to verify presence of antibodies.

## Results

Antibodies to *T. gondii* were detected in 85 (20%) and 68 (34%) moose and roe deer sera, respectively. In moose, the seroprevalence was higher in south and central Sweden than in the north, whereas there was no difference between the regions for roe deer. Adult moose and roe deer had higher odds of being seropositive than young animals but there were no difference in seroprevalence between males and females. One roe deer was positive by immunoblotting and was regarded as *N. caninum* positive, whereas all moose sera were negative.

## Discussion

We show that *T. gondii* infection is widely spread in the Swedish moose and roe deer populations. Precautions should therefore be taken when handling internal organs and carcasses of harvested cervids. Proper handling and cooking of game meat also is important to prevent toxoplasmosis in humans.



# Effects of brominated dioxins on Ah-receptor regulated gene expression, EROD activity, and reproductive health in zebrafish (*Danio rerio*)

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## Introduction

High levels of polybrominated dibenzo-*p*-dioxins (PBDDs) have recently been detected in Baltic Sea biota including fish. Due to their similarities to the highly toxic polychlorinated dibenzo-*p*-dioxins (PCDDs), concern has been raised about their potential biological effects.

## Aim

The aim was to investigate if PBDDs affect reproduction and early life-stage development in zebrafish and if PBDDs may act via the aryl hydrocarbon receptor (AHR) pathway.

## Material and Methods

Adult zebrafish were exposed for nine weeks to feed spiked with 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) or to a mixture of PBDDs, which was designed to reflect relative concentrations measured in Baltic Sea biota. Parameters studied were: spawning success, gonad morphology, hepatic vitellogenin gene expression, offspring early life-stage development, hepatic ethoxyresorufin-*O*-deethylase (EROD) activity, and hepatic expression of a number of aryl hydrocarbon receptor (AHR)-regulated genes.

## Results

The brominated dioxins spiked to the feed were detected in female fish and in their eggs; however, only minor effects were seen on early life-stage development. Both the PBDD mixture and TBDD clearly induced AHR-regulated genes and EROD activity. Exposure to TBDD reduced spawning success, altered ovarian morphology and reduced vitellogenin gene expression.

## Discussion

Effects were seen at concentrations higher than those measured in Baltic Sea biota; however, zebrafish have previously shown relatively low sensitivity to AHR agonists. Studies on native fish species are therefore needed to better estimate the risk for fish in the Baltic Sea.

## Conclusion

Overall, our results provide suggestive evidence that PBDDs cause effects on fish reproduction similar to those of PCDDs and interfere with the AHR pathway.



# Exposure to environmental progestin impair egg development and fertility in the frog *Xenopus tropicalis*

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## Introduction

Amphibian populations are declining world-wide and one of the suggested causes is endocrine disruption. Environmental concentrations of pharmaceutical estrogens have been demonstrated to disrupt sexual development and impair fertility in frogs and fish. Recently, the pharmaceutical progestin (synthetic progesterone) levonorgestrel (LNG) was shown to threaten reproduction in female fish.

## Aim

We tested the hypothesis that environmental progestin exposure causes reproductive disorders in amphibians.

## Material and Methods

We characterized effects of LNG on reproductive organ differentiation, oogenesis (egg development) and fertility in *Xenopus tropicalis* (African clawed frog). Following tadpole exposure, developmental effects were determined at metamorphosis (gonadal histology) and at sexual maturity (fertility, gonadal histology). Effects on oogenesis were assessed histologically following both tadpole and adult exposure.

## Results

Developmental LNG exposure resulted in severely impaired egg and oviduct development, and caused sterility in female frogs, whereas no effects on male fertility were detected. The developmental effects of LNG were not detected until the females became sexually mature, 9 months after the exposure was discontinued.

Adult LNG exposure inhibited egg development. The lowest tested concentration, 1.3 ng/L, increased the proportions of previtellogenic oocytes and reduced the proportions of vitellogenic oocytes, indicating inhibited vitellogenesis.

## Discussion and Conclusions

The study shows that 1) progestin concentrations found in the aquatic environment impair oogenesis in frogs, 2) several stages of oogenesis (initiation of meiosis and vitellogenesis) as well as differentiation of the Müllerian ducts (precursors of oviducts) are sensitive targets for progestins. Considering the crucial role of oogenesis in female fertility our results indicate that progestins in the environment may pose a risk to reproduction in wild frog populations.



# **Developmental disorders in eelpout (*Zoarces viviparus*) offspring from Baltic coastal waters**

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## **Introduction**

The eelpout (*Zoarces viviparus*) is in several of the Baltic countries used for environmental monitoring of coastal waters. It is a viviparous species, having internal fertilization and hatching of larvae, and gives birth after 5 months of pregnancy. The reproductive capacity of each individual female, including mortality and prevalence of developmental disorders of the larvae can be studied within each brood. The eelpout is relatively stationary and the health effects can therefore be linked to the local contaminant situation.

## **Aim**

The aim was to examine malformations in offspring for comparison of prevalence and type of malformations in eelpout sampled at different locations along the Baltic Sea coast.

## **Material and Methods**

Eelpout was sampled at different Baltic Sea locations as part of the national monitoring programs. The broods from sampled females were examined and the malformations of larvae were categorized.

## **Results, Discussion and Conclusions**

The prevalence of malformations in offspring from eelpout sampled at sites along the Swedish coast is generally low compared with sites in Denmark and Germany. The most common malformations include spinal axis deformities, cranial deformities, eye defects, and to some extent Siamese twins. Most of the females did not produce any malformed offspring, but in some cases up to 19% of the larvae in the brood was malformed at Swedish sites. The quantification of larval disorders, together with other biomarkers, is an applicable component in environmental monitoring.



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