PROCEEDINGS OF THE NORWEGIAN ENVIRONMENTAL TOXICOLOGY SYMPOSIUM, SVALBARD, 2018

March 14-16, 2018 in Longyearbyen, Svalbard

Editors: Courtney A. Waugh, Helena Reinhard, Bjørn Munro Jenssen

Abstract Book

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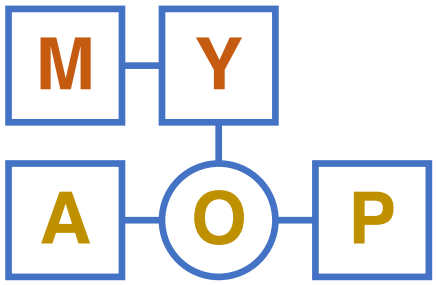

PhD Course on
Adverse Outcome Pathways in environmental toxicology:
practical applications, methods and challenges

SAVE THE DATE
September 24-28, 2018
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Conference Programme

Day 1: Wednesday 14th March

14:00-19:00 **Registration**

14:30-15:00 **Welcome to NETS2018 in Longyearbyen, Svalbard**
Towards a Solution to Pollution in a Warming Arctic

15:00-15:30 **Opening invited plenary Robert Letcher (Environment Canada)**
New Chemicals of Emerging Arctic Concern (CAECs): Degradation and other processes define exposure, trends and effects in Arctic biota/wildlife

Session 1: **Trends and effects of legacy and emerging pollutants:** Bjørn Munro Jenssen, Katrine Borgå

15:30-15:45 **Anna Lippold:** Temporal trends of persistent organic pollutants in Svalbard polar bears (*Ursus maritimus*) in relation to climate – associated changes in feeding habits
15:45-16:00 **Megan Lee:** The impact of heavy metals and organochlorines on pathogen prevalence in an Arctic seabird
16:00-16:15 **Anna Nikulina:** Atmospheric aerosol chemistry and trace gases in Barentsburg, Western Spitzbergen

16:15-16:45 Afternoon tea/coffee break

16:45-17:00 **Mari Løseth:** Corticosterone and thyroid hormone status in white-tailed eagle nestlings in relation to organohalogenated contaminants
17:00-17:15 **Randi Grønnestad:** Occurrence and levels of perfluoroalkyl substances (PFASs) in bank voles (*Myodes glareolus*) from a hot-spot area in Trondheim, Norway
17:15-17:30 **Thea Bechshoft:** Ecological and physiological variables in polar bear toxicology research: a systematic review
17:30-17:45 **Nathalie Briels:** Early-life effects of PFOS and its fluorinated alternative in the domestic chicken
17:45-18:00 **Vidar Torget:** High levels of contaminants in eagle owl liver

18:00-20:00 Poster Session and Ice Breaker Dinner in Canteen

Day 2: Thursday 15th March

Session 2: **Industrial discharges in the environment:** Marianne Frantzen, Jasmine Nahrgang, Helena Reinardy

08:15-08:45 **Invited plenary: Jasmine Nahrgang:** An elevated risk for Arctic organisms from petroleum compounds?
08:45-09:00 **Bjørn Henrik Hansen**: Adhesion of dispersed crude oil droplets onto eggs of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) and associated toxicity to developing fish larvae
09:00-09:15 **John Incardona:** Persistent derangement of larval-juvenile lipid metabolism and growth following transient embryonic oil exposure in Polar cod
09:15-09:30 **Ireen Vieweg:** The effects of chronic crude oil exposure on the biological fitness of polar cod (*Boreogadus saida*)

09:30-10:00 Morning tea/coffee break

10:00-10:15 **Ida Beathe Øverjordet:** Toxicokinetics in Arctic copepods
10:15-10:30 **Live-Guri Faksness:** Should current environmental assessment methods for produced water be revised? The influence of chemical composition on produced water toxicity
10:30-10:45 **Helena Reinardy:** Impacts of fine particle mine tailings on early life stages of cod
10:45-11:00 **Anastasia Georgantzopoulou**: Effects of effluent and sludge containing Ag and TiO2 nanoparticles transformed through lab-scale wastewater treatment processes

11:00-11:30 Morning tea/coffee break

Session 3: **Mixture Toxicology and Multiple stressors**: Veerle Jaspers, Courtney Waugh, and Christian Sonne

11:30-11:45 **Erik Muller:** Lethal and sublethal impacts of copper nano-pesticides on the energy budgets of starving estuarine amphipods

11:45-12:00 **Chisato Kataoka:** Silver nanocolloid increases pathogenic infection risk following disruption of gut microbiota and immune system in medaka fish

12:00-12:15 **Li Xie:** Multiple stressor effects of ionizing (γ) radiation and non-ionising (UV) radiation in duckweed (*Lemna minor*)

12:15-12:30 **Malin Celander:** Increased sensitivity to benzopyrene exposures in fish cell-line when co-exposed to pharmaceuticals

12:30-12:45 **Kareem Eldin Mohammed Ahmed:** revealing the effects of defined persistent organic pollutant mixtures using H295R steroidogenic assay: an LC-MS/MS approach

12:45-13:00 **Vidar Berg:** The use of mixtures of pollutants for toxicity testing. Experiences from past studies and plans for future testing.

13:00-14:00 Lunch

Session 4: **Ecotoxicology and systems toxicology (dCod 1.0):** Anders Goksøyr and Iurgi Imanol Salaverria-Zabalegui

14:00-14:15 **Anders Goksøyr:** dCod 1.0: decoding the systems toxicology of Atlantic cod (*Gadus morhua*) – an introduction

14:15-14:130 **Odd André Karlsen:** The nuclear receptor complement of Atlantic cod (*Gadus morhua*) as potential targets for endocrine disrupting compounds.

14:30-14:45 **Libe Aranguren-Abadía:** Characterization of the aryl hydrocarbon receptors Ahr1a and Ahr2a in Atlantic cod (*Gadus morhua*).

14:45-15:00 **Fekadu Yadetie:** Transcriptome responses in Atlantic cod (*Gadus morhua*) precision-cut liver slices exposed to benzo[a]pyrene and 17α-ethynylestradiol.

15:00-15:15 Afternoon tea/coffee break

15:15-15:30 **Roger Lille-Langøy:** Sequence variations in pxr (nr1i2) from zebrafish (*Danio rerio*) strains affect nuclear receptor function.

15:30-15:45 **Karina Dale:** Ecotoxicological responses in Atlantic cod (*Gadus morhua*) after caging at a capped waste disposal site in Kollevåg, Western Norway.

15:45-16:00 **Zhanna Tairova:** Adverse health effects related to chemical exposure in Atlantic cod (*Gadus morhua*) from Norwegian fjords.

16:00-16:15 **Ketil Hylland:** What can 'omics tell us?

16:15-16:45 Afternoon tea/coffee break

Session 5: **Plastic debris – pathways and impacts in arctic and boreal systems:** Session chairs: Claudia Halsband, Dorte Herzke, Andy Booth, and Martin Wagner

16:45-17:15 **Invited Plenary: Professor Shoshaku Kashiwada**
Globally distributed plastic debris and environment-dependent toxicity

17:15-17:30 **Marte Haave:** Microplastic occurrence and distribution from discharge points to deep basins in an urban model fjord

17:30-17:45 **Tânia Gomes:** Understanding the distribution and impact of micro- and nano-plastics in Norway

17:45-18:00 **Claudia Halsband:** Is crumb rubber a source for pollutants and harmful effects in the marine environment

18:00-18:15 **Iurgi Salaberria:** Benchmarking the uptake and excretion dynamics of microplastics in the boreal marine copepod *Calanus finmarchicus*

18:15-18:30 **Dorte Herzke**: Video connection with IMDC6 Conference, San Diego

18:30-19:30 Poster session and mixer

20:00 Conference dinner

Day 3: Friday 16th March

Session 6: **Environmental toxicology in Svalbard:** Session chairs: Heli Routti and Sophie Bourgeon

09:00-09:30 **Plenary: Heli Routti:** Exposure and effects of pollutants in mammalian predators from Svalbard
09:30-09:45 **Katharina Lühmann:** Activation of the thyroid receptor of fin and blue whales by environmental pollutants
09:45-10:00 **Silje Strand Lundgren:** Immunomodulation by pollutants and metals in seabirds breeding on Svalbard
10:00-10:15 **Roger Lille-Langøy:** Environmental contaminants modulate the transcriptional activity of polar bear and human peroxisome proliferator-activator receptor alpha

10:15-10:30 **Morning tea/coffee break**

10:30-11:00 **Closing invited plenary** **Katrine Borgå:** AnthroTox: Combining natural and social sciences to understand and manage global anthropogenic toxicants

11.00-11:30 Closing remarks and student awards

11.30-12.30 Buffet Lunch in Canteen

Opening Plenary

New Chemicals of Emerging Arctic Concern (CAECs): Degradation and Other Processes Define Exposure, Trends and Effects in Arctic Biota/Wildlife

Robert J. Letcher

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Keywords: New Chemicals; Emerging Contaminants; Degradation and effects; Arctic biota
Session: Legacy and Emerging Pollutants
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Legacy persistent organic pollutants (POPs), and emerging chemicals, are in the Arctic via marine and atmospheric transport. Legacy POPs are generally lipophilic and environmentally stable in the shorter term, and are found at biomagnified and elevated concentrations in the tissues of wildlife. The same cannot be assumed for new POPs (or their precursors) as they are not necessarily lipophilic or chemically stable in the environment. Abiotic and biotic degradation processes on POPs have been emphasized in the recent AMAP (2018) report on Chemicals of Emerging Arctic Concern (CEACs). High priority CEACs include per-/polyfluoroalkyl substances, phthalates, short-chain chlorinated paraffins, siloxanes, pharmaceuticals and personal care products, polychlorinated naphthalenes, current use pesticides, butyltins, polycyclic aromatic hydrocarbons, halogenated natural products, microplastics, and organophosphate ester (OPE) flame retardants (FRs) and plasticizers.

Mammals and fish have occupy different positions in Arctic food webs, and climate-induced changes are occurring at an accelerated rate. Such changes affect Arctic food web composition and structure. Combined with the varying stability of CEACs, the result are changes in the exposure, fate, bioaccumulation and biomagnification as well as effects of CEACs/POPs in biota. The CEACs classified as OPEs have been used globally in modest amounts as far back as the 1960s, but phase-outs and regulation of polybrominated diphenyl ether (PBDE) FRs has equated to increased production and usage of OPEs in the last 15 years. Long-range transport of OPEs occurs based on concentrations reported in Arctic air (particle) samples. Atmospheric OPE concentrations in the Arctic are orders of magnitude greater than for FRs and PBDEs. Local OPE (and other CEAC) releases in Arctic communities may possibly lead to elevated exposures in local marine biota. Relative to Arctic air much lower OPE concentrations have been reported in biota. Degradation can also complicate the source/dietary connection to spatial or temporal trends of POPs. Based on the AMAP CEAC report, the present study provides an overview of new POPs/CEACs in the Arctic and in relation to legacy POPs, and using OPEs and selected mammal and seabird species as examples, to illustrate the importance of degradation and transformation processes on fate, persistence and bioaccumulation, and what little is known about the effects of CEACs in Arctic wildlife.

Acknowledgement: The author thanks the many individuals involved in the AMAP CEAC report (e.g. Derek Muir, Cindy de Wit, Katrin Vorkamp, Simon Wilson and Jennifer Balmer), Also, the numerous individuals involved or collaborating in and/or leading the (Canadian and International) Arctic research presented including researchers (e.g. Rune Dietz, Christian Sonne, Bjorn Jenssen, Markus Dyck, Adam Morris, Magali Houde, and Thea Bechschoft) and students, as well as national, regional and territorial governments and departments, hunters, Hunters Trappers Organizations, and participating Arctic communities. Financial support for this presentation is acknowledged from the Northern Contaminants Program (Indigenous and Northern Affairs Canada) and Environment and Climate Change Canada.

Session 1
Trends and Effects of Legacy and Emerging Pollutants

Session Chairs: Bjørn Munro Jensen and Katrine Borgå

Talks

Anna Leopold: Temporal trends of persistent organic pollutants in Svalbard polar bears *(Ursus maritimus)* in relation to climate – associated changes in feeding habits

Megan Lee: The impact of heavy metals and organochlorines on pathogen prevalence in an Arctic seabird

Anna Nikulina: Atmospheric aerosol chemistry and trace gases in Barentsburg, Western Spitzbergen

Mari Løseth: Corticosterone and thyroid hormone status in white-tailed eagle nestlings in relation to organohalogenated contaminants

Randi Grønnestad: Occurrence and levels of perfluoroalkyl substances (PFASs) in bank voles *(Myodes glareolus)* from a hot-spot area in Trondheim, Norway

Thea Bechshoft: Ecological and physiological variables in polar bear toxicology research: a systematic review

Nathalie Briels: Early-life effects of PFOS and its fluorinated alternative in the domestic chicken

Vidar Torget: High levels of contaminants in eagle owl liver

Posters

Anna Nikulina: Mercury in Benthic Organisms and Their Habitat in Grønfjorden, West Spitsbergen, in Early Springtime

Jose Maria Castaño Ortiz: Per- and polyfluoroalkyl substances (PFASs) in plasma and feathers of Northern Goshawks *(Accipiter gentilis)* nestlings from Norway and Spain

Katrin Hoydal: Mercury trends in Faroese biota

Bjørn Henrik Hansen: Embryonic exposure and effects of quinolines on early life stages of Atlantic haddock *(Melanogrammus aeglefinus)*

Temporal trends of persistent organic pollutants in Svalbard polar bears (*Ursus maritimus*) in relation to climate – associated changes in feeding habits

Anna Lippold[1,2], Sophie Bourgeon[1], Jon Aars[2], Magnus Andersen[2], Anuschka Polder[3], Vidar Torget[3], Jan Ludvig Lyche[3], Jenny Bytingsvik[4], Bjørn Munro Jenssen[5], Andrew Derocher[6], Jeffrey Welker[7], and Heli Routti[2]

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Keywords: organochlorine pesticides, polybrominated diphenyl ethers polychlorinated biphenyls
Session: Legacy and emerging pollutants (trends and effects)
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Persistent organic pollutants (POPs) reach the Arctic ecosystems from lower latitudes mostly via air and ocean currents. They biomagnify through Arctic food webs and reach considerably high concentrations in top predators such as polar bears (*Ursus maritimus*). Although many of these compounds have been banned or restricted for decades, concentrations in Arctic biota still remain high. Temporal trend studies in Arctic biota help assess the effectivness of bans and restrictions, however, trends of POP concentrations in biota are affected by various factors, including dietary source and climate change. Because of retreating sea ice polar bears can be forced to feed at lower trophic levels or consider terrestrial food sources, potentially leading to a decreased uptake of contaminants.

We monitored plasma concentrations of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs) over a 20 year time span (1997-2017; n=306 samples) in female polar bears from Svalbard, Norway. We examined temporal trends in relation to climate – associated changes in feeding habits by using stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) from red blood cells as feeding proxies. BDE-153 and β-HCH concentrations were stable over the time of our study period, whereas concentrations of PCBs, DDE, HCB, oxychlordane and BDE-47 declined. The decline of DDE, HCB and oxychlorane leveled off around 2010. Interestingly, PCB concentrations were stable in recent years when controlled only for changes in winter diet. Declining levels of $\delta^{13}C$ suggest a shift towards more terrestrial prey, with a steeper, more pronounced trend for the last five years. Our findings suggest that a climate-related diet shift leads to lower PCB exposure in polar bears, whereas OCP and PBDE exposure is mainly affected by emissions.

Acknowledgement: The study was funded by the Norwegian Polar Institute, the Ministry of Climate and Environment, the Norwegian Environment Agency and the Research Council of Norway. Funding for fieldwork was provided by the Center for Ice, Climate and Environment (ICE) at the Norwegian Polar Institute and the World Wildlife Fund (WWF).

The Impact of Heavy Metals and Organochlorines on Pathogen Prevalence in an Arctic Seabird

Megan Lee[1], Veerle Jaspers[1], Geir Wing Gabrielsen[2], Jose Castaño-Ortiz[1], Syverin Lierhagen[1], & Courtney A. Waugh[1]

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Keywords: immunomodulation, polychlorinated biphenyls, disease, Arctic
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Mounting evidence suggests that climate change is already altering patterns of disease in northern ecosystems. As warming continues, emergent pathogens may pose new threats to sensitive Arctic species, already under pressure from climate-driven ecological shifts and additional anthropogenic challenges.

One such additional challenge is exposure to immunomodulatory contaminants. Synergistic effects between new disease threats and pollution-linked immune dysfunction may have significant negative impacts on Arctic avian species in the near future. Characterizing this relationship is necessary to develop a more complete understanding of how climate change will impact Arctic birds.

In this study, black-legged kittiwakes *(Rissa tridactyla)* (n=37) from Kongsfjorden, Svalbard, were used as a model species. Levels of heavy metals and selected organochlorine pollutants (polybrominated diphenyl ethers, chlordanes, hexachlorohexanes, higher polychrorinated biphenyl congeners) were measured in blood samples from two different years (2015 and 2017). The prevalence of avian influenza was assessed using an enzyme-linked immunosorbant assay (ELISA).

Arsenic, selenium, and mercury were found to have a significant correlation with the prevalence of avian influenza, suggesting that these pollutants may be the dominant driver of immunomodulation in this host-pathogen system.

Acknowledgement: The authors thank Silje S. Lundgren, Solveig Nilsen, Saga Svavarsdóttir, Delphin Ruche, Phil Bertrand, and Maite Cerezo Araujo for their help in the field and the Svalbard Science Forum (SSF) and the Norwegian Research Council for funding.

Atmospheric aerosol chemistry and trace gases in Barentsburg, Western Spitsbergen

Anna Nikulina[1], Lyudmila Golobokova[2], Igor Lyakushin[1] and Olga Sidorova[1]

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Keywords: aerosol, trace gases, Barentsburg, monitoring
Session: Legacy and Emerging Pollutants / Industrial discharges in the environment
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The distribution of aerosol is monitored in Barentsburg for the last few years to estimate the long-range transport influence. Here, to examine the local effects, we review the data on aerosol chemical properties 2016-2017 together with data obtained from automatic air quality control stations put into service at the beginning of 2017. The stations equipped with analysers by Environnement S.A (France) control the gaseous composition (nitrogen, sulphur, carbon oxides, ozone and particulate matter together with weather parameters) of the near-surface atmospheric layer in Barentsburg settlement itself and up on the mountain near the settlement. The atmospheric aerosol is sampled according to guidelines for international monitoring networks EANET and EMEP.

In aerosol, the maximal concentration of sodium and chlorine ions of marine origin was measured in fall-winter period (September-March). The continental influence reflected in increased content of sulphate and calcium ions was detected mostly in winter-spring time (November-May), ammonium content increased in spring (April-May). The air mass circulation analysis confirms the different prevailing sources throughout the year. On average total content of salts in aerosol grows in dark period of the year.

The gaseous composition of the near-surface layer depends mostly on air mass circulation for both conservative and anthropogenic gases. Thus, preliminary analysis of ground level ozone content showed the synchronous daily variations at both stations. On the large scale, the negative relation of ozone content to atmospheric pressure is perhaps connected to intense horizontal and vertical wind-driven transport. The increase of anthropogenic gases as nitrogen and sulphur oxides coincides with enhanced levels of ozone. Barentsburg power plant and to less extent transport and coal mining activity are the local sources of oxides as well as hydrogen sulphide and particulate matter. The landform peculiarities and frequent temperature inversions significantly influence the distribution of anthropogenic components. Thus, the concentration of sulphur oxide is often higher at the station more distant from the power plant and do not well correlate to the data of another station.

Further analysis of incoming data from newly established analysers will help to discern the trends of annual variability of conservative gases and the distribution features of anthropogenic gases influencing the local population and environment.

Acknowledgement: The project was performed under the Interdisciplinary integration project of RAS no 0345-2018-0002 (AAAA-A17-117122190017-8) and Inter-institutional program for research and monitoring on Spitsbergen Archipelago in 2017.

Corticosterone and thyroid hormone status in White-tailed eagle nestlings in relation to organohalogenated contaminants

Mari E. Løseth[1], Grethe S. Eggen[1], Nathalie Briels[1], Torgeir Nygård[2], Trond V. Johnsen[3], Jan Ove Bustnes[3], Dorte Herzke[4], Giulia Poma[5], Govindan Malarvannan[5], Adrian Covaci[5], Bjørn M. Jenssen[1] and Veerle L.B. Jaspers[1]

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Keywords: thyroid hormones, birds, OHCs, endocrine disruption
Session: Mixture Toxicology and Multiple stressors
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White-tailed eagles (WTE; *Haliaeetus albicilla*) can accumulate a wide range of organohalogenated contaminants (OHCs), due to their apex trophic position. Their diet consists mainly of fish and seabirds, thus long food chains with a high potential for biomagnification of OHCs. Concentrations of per- and polyfluoroalkyl substances (PFASs) have recently been shown to exceed those of other legacy OHCs in WTEs and required closer attention. Several of these compounds are shown to interfere with endocrine systems in birds and potentially cause detrimental health effects. The thyroid system is important for birds' thermoregulation, metabolism, growth and development. Assuring appropriate concentrations and actions of the two major thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) is therefore especially important in nestlings. Chronically elevated stress levels may also affect similar endpoints as the thyroid system, such as growth and immunomodulation. The aim of the present study was to investigate, for the first time, the corticosterone and thyroid hormone levels of nestling WTEs in relation to their plasma concentrations of OHCs. To estimate the baseline stress levels, corticosterone hormones were extracted from feathers ($cort_f$), as levels in blood are highly variable while $cort_f$ provides an integration of the stress status over the period of feather growth. OHCs and thyroid hormones (THs) were analyzed in plasma. Samples were obtained from 70 nestlings from two archipelagos in Norway, Smøla (n=35) and Steigen (n=35), in the summer of 2015 and 2016. Overall, 14 polychlorinated biphenyls (PCBs), 7 organochlorinated pesticides (OCPs), 5 polybrominated diphenyl ethers (PBDEs) and 8 PFASs were quantified in over 50 % of the plasma samples at each location and each year. Significant temporal and spatial differences for $cort_f$, THs and OHCs were detected. Our preliminary analyses show declining concentrations of total T_4 and $cort_f$ with increased Σ_8PFASs concentrations (r_P=-0.3, p<0.01). We also found declining trends of both total T_3 and free T_3 with increased concentrations of Σ_{14}PCBs and Σ_7OCPs (r_P=-0.3, p<0.02). Although these correlations are rather weak, their significance suggests interactions between endocrine systems and OHCs in nestling WTEs.

Aknowledgements: The authors thank the Norwegian Research Council and NTNU for funding the NewRaptor project (230465/F20). We also thank P. Marcinekova, J. Flo, I. Eulaers, A. Gylseth and E.L. Dahl for assisting field-and lab work.

Occurrence and levels of Perfluoroalkyl substances (PFASs) in bank voles *(Myodes glareolus)* from a hot-spot area in Trondheim, Norway.

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Key words: PFASs, rodents, ski wax
Session: Legacy and emerging pollutants
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Perfluoroalkyl substances (PFASs) are surface-active compounds that are applied in a wide range of consumer products, including ski wax and other winter sport products. Granåsen Toppidrettssenter (Trondheim) is a popular sports arena for professional and non-professional skiers, and we therefore suspect it to be a hot-spot area for PFASs. A study by NILU/NINA (2015) reported high PFAS levels in earthworms in skiing areas in Oslo, compared to a reference site. Although relatively few samples were used for this report, these results gave reason for concern, and follow-up studies were recommended. We chose free ranging rodents as a model organism because they are easy to handle, and represent a higher trophic level than earthworms. Our hypothesis was that rodents in hot-spot areas with skiing activities will be more exposed to PFASs, compared to an area with low to no skiing activities.

Bank voles *(Myodes glareolus)* were sampled in June and July 2017 in Granåsen, Trondheim (hot-spot area) and around Jonsvatnet, Trondheim (reference site) using cage traps. Permissions to collect animals were given by Miljødirektoratet and Mattilsynet. Liver samples (n = 21 and 31 at Granåsen and reference site, respectively) were analysed for 9 perfluoroalkyl carboxylates (PFCAs), 3 perfluoroalkyl sulfonates (PFSAs) and 5 perfluoroalkyl sulphonamide- (PASF) based substances. The PFSA levels were significantly higher in bank voles from Granåsen, compared to the reference site. PASF-based substances were below the limit of detection (LOD) in both areas. For the PFCAs, the levels were in general higher in bank voles from Granåsen than the reference site. However, for the short chained PFCAs (PFHxA; C4 and PFHpA; C6) the levels were significantly higher in the reference site than the hot-spot area. This may reflect a local source of short chained PFCAs in the reference area. PFOS was the compound measured at the highest concentrations at both locations. The generally higher PFAS levels in bank voles in an area with high skiing activities, compared to a reference site, indicates that skiing activities are a significant PFAS source of concern for animals living in such environments.

Acknowledgement: We thank the staff at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences (NMBU) for assistance with the contaminant analyses**.** The study is financed by the Faculty of Natural Sciences, NTNU.

Ecological and physiological variables in polar bear toxicology research: a systematic review

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Keywords: contaminants, ecology, polar bear, toxicology
Session: Ecotoxicology and systems toxicology
Corresponding author: Thea Bechshoft (thbe@bios.au.dk)

Ecotoxicology evolved as a scientific field as awareness of the unintended effects of anthropogenic pollutants in biota increased. Polar bears (Ursus maritimus) are often the focus of Arctic contaminant exposure studies because they are apex predators with high contaminant loads. While early studies focused on describing and quantifying pollutants, present-day polar bear toxicological papers often incorporate ecological variables.

This systematic literature review investigates the ecological and physiological variables that have been integrated in such studies. The systematic literature search resulted in 207 papers, published between 1970 and 2016. Representation of each of the 19 polar bear subpopulations varied from 0 to 72 papers; East Greenland, Barents Sea, Southern Beaufort Sea, and Lancaster Sound had the most published research, with over 30 papers each. Samples were collected between 1881 and 2015, primarily from harvested bears (66%); most from the 1990s and 2000s. Adipose tissue, liver, and blood were the most common tissues examined, and mean number of bears analyzed per paper was 76 (range 1–691). Papers investigating temporal trends did so using a mean sample of 61 bears over a 6-year period.The frequency with which ecological and physiological variables were integrated into toxicological papers varied. Age and (or) sex was the only ecological variable(s) considered in 51% of papers. Further, a total of 37% of the papers included in the review investigated physiological effects in relation to contaminant concentrations. Of the papers, 98% dealt with contaminant exposure at the individual level, leaving population level effects largely unstudied. Solitary subadult and adult polar bears were included in 57% and 79% of the papers, respectively. Younger bears were included in fewer papers: yearlings in 20% and cubs-of-the-year in 13%. Only 12% of the papers examined reproduction relative to contaminants. Finally, body condition was included in 26% of the research papers, whereas variables related to polar bear diet were included in ≤9%.

Based on our findings, we suggest future polar bear toxicology studies increase sample sizes, include more ecological variables, increase studies on family groups, and increase the applicability of studies to management and conservation by examining pollution effects on reproduction and survival.

Acknowledgement: ArcticNet, Canadian Association of Zoos and Aquariums, Canadian Wildlife Federation, Environment and Climate Change Canada, Hauser Bears, Natural Sciences and Engineering Research Council of Canada, Polar Bears International, Quark Expeditions, the Villum Foundation, and World Wildlife Fund Canada are acknowledged for their support. R. Togunov is acknowledged for valuable discussions.

Early-life effects of PFOS and its fluorinated alternative in the domestic chicken

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Keywords: PFOS, alternatives, chicken, development
Session: Mixture toxicology and multiple stressors
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After consistently being shown to be persistent, bioaccumulative and toxic, the production and use of perfluorooctane sulfonate (PFOS) was restricted in 2009 by the Stockholm Convention. In electroplating industry however, PFOS is still used as a safety measure to protect workers from toxic metal aerosols. Therefore, electroplating is currently exempt from this restriction, but the demand for alternatives is rising. One of those alternatives, the chlorinated polyfluorinated ether sulfonate F-53B, has been used for decades in the electroplating industry in China. Its use might expand to other regions, as well as to other industries that are currently using PFOS. F-53B has already been detected in Chinese surface water and fish tissues and its similar chemical structure predicts toxic effects and bioaccumulation potential resembling those of PFOS. Until now, toxicity data on F-53B are very scarce and lacking for birds. Therefore, this study aimed to investigate the toxicity of F-53B in developing birds relative to, and in combination with, PFOS. Using a full factorial study design, 160 chicken eggs (*Gallus gallus domesticus*) were injected in the yolk sac with PFOS, F-53B or a mixture of both compounds. Eggs were exposed to environmentally relevant doses of 150 and 1500 ng/g egg or injected with a vehicle control. After 21 days of incubation, chicks were euthanized and their liver was dissected for chemical analysis, gene expression analysis and enzyme assays of oxidative stress-related enzymes. Pipping and hatching success were calculated and hepatosomatic index (HSI) and birth mass were used as general health indicators. The heart rate of the embryos was also monitored regularly during incubation. A decrease in heart rate one day before hatching was observed for all treatments, with a significant decrease of 14 % by PFOS, at both low and high dose. No effect of the treatment was apparent on pipping nor hatching success, but chicks were born with a reduced body mass when exposed to PFOS. F-53B on the other hand, significantly increased the HSI of the chicks, which could indicate a detoxification response. Preliminary results also show an effect of F-53B on glutathione peroxidase (GPx) and of PFOS and F-53B on glutathione reductase (GR). The results of this study highlight the necessity of assessing the toxicity of F-53B, as this compound could potentially be as harmful and bioaccumulative as PFOS, and prone to become equally globally distributed.

Acknowledgements: The authors would like to thank the Norwegian Research Council and NTNU for funding this research that is part of the NewRaptor project (230465/F20). We would also like to thank A. Ullah, C.A. Waugh, I.B. Kroglund, G.S. Eggen and L. Hanssen for the help during the sampling and analysis. B.M. Jenssen and A. Arukwe are acknowledged for their co-supervision of MSc student A. Ullah.

High levels of contaminants in eagle owl liver

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Keywords: eagle owl, organohalogenated contaminants, Norway
Session: Legacy and emerging pollutants
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Introduction and aims: The Eurasian eagle owl is Norway's largest owl, and the population has declined since the end of the 19th century, probably because of anthropogenic activity. The eagle owl is now listed as endangered by Norwegian authorities, and an action plan has been developed, which identify measures for the management of Norwegian eagle owls. Environmental contaminants are considered as one of several factors threatening the population and one of the action requested is to assess the type and levels of contaminants in the Norwegian eagle owl population. This study is a collaboration with the National Veterinary Institute (VI) and supported by the county general in Nordland county. The study aims to map levels of environmental contaminants in eagle owl across Norway.

Materials and methods: 64 birds have been opportunistically collected between 1998 and 2014, geographically distributed throughout Norway as far north as the Helgeland coast. The majority of the samples were sent to VI by authorised taxidermists. The samples comprised of both male and female, juvenile and adult birds. An autopsy has been performed by VI on the carcasses to determine the cause of death, and liver samples collected. Analysis of organohalogenated contaminants were performed on the liver samples by liquid-liquid extraction, acid fat clean-up step and detection by GS-MS. Compound groups analysed were dichlorodiphenyltrichloroethane, its isomers and metabolites (DDTs), polychlorinated biphenyls (PCBs), polybrominated biphenyl ethers (PBDEs), other brominated flame retardants (BFRs), hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), Mirex and chlordanes. Heavy metals were analysed by ICP-MS and rodenticides were analysed by LC-MS.

Results: In general, the results show high concentrations of OHCs in eagle owls. Certain individuals have very high concentrations of several contaminants, and these individuals contribute to the high mean values. Median values are also regarded as high, indicating that a large amount of the birds have generally high concentrations of contaminants. ∑PCB and ∑DDT were higher in concentrations compared to the other OHCs. Of PCBs, PCB-118, -138 and 153 were the most predominant. *p,p'*-DDE was the main contributing compound to the ∑DDT. ∑BDE was the third most predominant contaminant group. The most contaminated birds, with few exceptions, comes from costal municipalities, and suggests that these birds have prayed in the marine food chain. Preliminary statistics show a downward trend for almost all contaminant groups, but the decrease seems not to be significant.

Also included in the overall project is analyses of rodenticides and a selection heavy metals (mercury, lead, cadmium and arsenic). In general, levels of heavy metals were low. One individual had lead concentrations above 1 µg/g, indicating a toxic load, but below levels for toxic poisoning. Rodenticides were detected in 78% of the birds. It was not detected in the two northern most counties. Positive detections were relatively evenly distributed among the 78%. It is uncertain if the detected levels have caused clinical effects or death.

Conclusion/further perspectives: Analyses show very high concentrations of environmental contaminants such as PCBs DDTs and PBDEs in several individuals of eagle owls. In general, a large part of the analysed samples shows high concentrations. Preliminary analysis suggests that highly contaminated birds are connected to the marine food chain. A new set of eagle owl liver will be analysed to extend the time span and strengthen the n geographically. Statistics will be performed on the data to investigate a time trend and to look for differences between geographical regions.

Acknowledgement: The authors thank Fylkesmannen i Nordland for the funding for this project. We also thank the additional persons at VI and NMBU involved in the work with these samples.

Mercury in Benthic Organisms and Their Habitat in Grønfjorden, West Spitsbergen, in Early Springtime

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Keywords: mercury, sediments, benthos, Spitsbergen
Session: Legacy and Emerging Pollutants / Svalbard Toxicology
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Total mercury concentration in benthic organisms and their habitat were obtained for the first time in Grønfjorden (Western Spitsbergen) in vicinity of Barentsburg settlement. The sampling campaign was carried out in early spring 2016. The samples of marine near-bottom water and surface sediments were taken simultaniously with gathering benthic organisms by dredge at five stations along the fjord. The total mercury measurements were performed on the atomic absorber with Zeeman background correction RA-915M by "Lumex" (Russia) at the Analytical laboratory in Barentsburg and for water samples at RPA "Typhoon" in Saint-Petersburg.

In marine water mercury content did not exceed the detection limit of 10 ng/L. The sediments contained from 7.1 to 42.3 ng/g d. w. mercury. The distribution of mercury between stations showed the increasing content in the inner fjord sediments implying that the main source of mercury is rather river runoff bringing high amounts of fine sedimetns then surface runoff and waste dicharges of Barentsburg.

Total mercury concentration in the benthic organisms generally increased toward the inner fjord too and was higher near the mouth of the Grøndalen River. Between the species mercury content was significantly depended on their trophic level and was the highest in the detritophage mollusks *Thyasira gouldi* (up to 91.3 ng/g w.w.), *Cardium* Sp. (12.7), and *Macoma calcarea* (26.2), the specialized predatory sea snail *Cryptonatica affinis* (53.2). The high concentration of mercury of 65.7 ng/g w.w. was found also in muscles of cod *Gadus morhua*, which is a benthophage and secondary predator although not native specie in Gronfjorden.

However a certain difference is observed between stations and benthic species the total mercury concentration in the benthic organisms and their habitat in Grønfjorden was generally relatively low comparing to Barentsb Sea areas and close to the background one.

In July 2017 benthic organims and sediments were sampled again within RV *Dalniye Zelenci* criuse in Isfjorden area and occasionally analysed for total mercury content. Preliminary results show highest mercury content in surface sediments of central Isfjorden comparing to Billefjorden and Gronfjorden and accumulation of total mercury in detritophages and sediment-feeders of Isfjorden as well.

Acknowledgement: The authors thank RPA "Typhoon" and acknowledge the support of projects of the Murmansk Marine Biological Institute KSC RAS (01201366845; 01201366849; AAAA-A17-117052310083-5; AAAA-A17-117100570010-9). The analytical work was performed in frame of Inter-institutional program for research and monitoring on Spitsbergen Archipelago in 2016 and 2017.

Per- and polyfluoroalkyl substances (PFASs) in plasma and feathers of Northern Goshawks (*Accipiter gentilis*) nestlings from Norway and Spain

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Keywords: PFASs, biomonitoring, goshawk, birds of prey
Session: Legacy and emerging pollutants (trends and effects)
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Per- and polyfluoroalkyl substances (PFASs) have been globally detected in marine and terrestrial ecosystems. The accumulation of PFASs in upper trophic levels underlines potential adverse effects in birds of prey, which are powerful sentinels for environmental monitoring. The occurrence and levels of PFASs were analysed in plasma and body feathers from nestlings of a terrestrial raptor, the Northern Goshawk (*Accipiter gentilis*), in nests from northern Norway (Troms) (*n*=12), central Norway (Trøndelag) (*n*=20) and southern Spain (Murcia) (*n*=7). We aimed to address possible explanatory factors (biological, ecological, spatial) that drive intraspecific variation in exposure, and further investigate the potential of feathers as non-invasive biomonitoring tools for PFASs.

This study confirms that goshawk nestlings are exposed to a wide range of PFASs, including perfluoroalkyl sulfonates (PFSAs) like PFOS (0.5 - 23.5 ng/mL plasma, <0.2 - 9.4 ng/g feathers) and perfluoalkyl carboxylates (PFCAs) like PFUnDA (0.4 - 2.8 ng/mL plasma, <0.5 - 2.5 ng/g feathers). PFOS dominated the PFAS profiles in plasma of Norwegian nestlings (59% in Troms, 50% in Trøndelag and 20% in Murcia), whereas long-chain PFCAs contributed more to the overall burden in Spanish goshawks (71% in Murcia, 33% in Troms and 42% in Trøndelag). This may result from differences in dietary input and/or local sources of PFASs. None of the investigated individuals approached or exceeded reference toxicity values for PFOS in serum of predatory birds (1700 ng/mL). This would indicate that current PFOS levels do not pose a health risk for individuals, although the potential effects of long-term exposure and accumulation of complex mixtures of PFASs should not be overlooked. In addition, correlative analyses showed positive associations between plasma and feather concentrations of PFOS, PFUnDA, PFTrDA and ∑PFASs ($0.356 < r_S < 0.631$, $p<0.05$), although not very strong.

Acknowledgement: The authors thank J.E Martínez, M. León-Ortega, M.P. Aldeguer, T.V. Johnsen, S. Randulff and S. Lundgren for their collaboration in the .field. This study was part of the project NEWRAPTOR (Project number: 230465/F20) funded by the Norwegian Research Council and NTNU.

Mercury trends in Faroese biota

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Keywords: Mercury, trend analysis, Faroe Islands
Session: Legacy and emerging pollutants (trends and effects)
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Mercury as an important global contaminant has been analysed in marine fish produce from the Faroe Islands intended for export since the late 1970ies, and as part of the Arctic Monitoring and Assessment programme (AMAP) since 1997. Combining the mercury analyses done either as part of the monitoring or research programs, provides an opportunity to analyse temporal trends of mercury in different species from the Faroe Islands. The mercury time trend was analysed in pilot whale muscle, cod muscle, black guillemot liver and eggs, and arctic char muscle. The trend analyses showed a significant increasing trend in pilot whale muscle and black guillemot eggs and liver, whereas a significant non-linear trend was found in cod and Arctic char muscle. Previously a significant decreasing trend has been reported in Faroese cod, whereas a significant increasing trend has been reported in Faroese Arctic char. In the present model, the cod mercury concentrations were significantly decreasing from 1979 to 1995, and significantly increasing since 1996. In the Arctic char, the significant increase seems to have stopped and the mercury concentrations are now at the same level as 15 years ago.

Several factors can influence the interpretation of long-term mercury trends, and changes in mean yearly sample age and the dietary trophic level can be important factors leading to changes in the mercury exposure, since they influence the degree of bioaccumulation and biomagnification. Changes in trophic level can be analysed by measurement of stable isotopes of nitrogen (δ15N) and carbon (δ13C), and stable isotopes have been analysed in the biota of the Faroe Islands from 2002. Although some positive correlations were found between mercury concentrations and stable nitrogen isotope (d15N), the influence of the d15N to the mercury concentration was small, with r^2 in the range 5%, and changes in dietary trophic level as reflected by stable isotope analyses could not explain the mercury trends. Differences between the lengths of the analysed fish could be an influencing factor in the mercury trend in arctic char, since the mean lengths for the latest samplings were lower than the previous years, but differences in length could not explain the mercury trend in cod.

Acknowledgement: The work was supported by the Danish Ministry of the Environment as part of the Dancea program – Danish Cooperation for Environment in the Arctic

Embryonic exposure and effects of quinolines on early life stages of Atlantic haddock (*Melanogrammus aeglefinus*)

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Keywords: Fish development, benzoquinoline, mixture toxicity,
Session: Legacy and emerging pollutants (trends and effects)
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It is well known that exposure of early life stages (ELS) of fish to oil components cause cardiotoxic effects and developmental deformations. Such effects have been shown after exposure to low levels of dispersed oil exposure, after exposure to water accommodated fractions of oil, produced water and following exposure to single oil components. A number of single oil components have been tested, and for the ones tested, PAHs in particular, very high and environmentally irrelevant concentrations are needed to provoke these effects. Effects of this nature have also been shown in the field after oil spills, so it is vital to determine how these effects occur so that they can be included in environmental risk and damage assessment processes. This demands a determination of which component(s) are responsible. In the present study, we assess the potential for a relatively unexplored group of petrogenic components, the nitrogen heterocyclic aromatic quinolines, to cause these effects. Experiments using single benzoquinoline as well as mixtures were performed. Preliminary data state that the single component even at high concentration (10 mg/L) is insufficient to cause developmental effects on haddock ELS, however, exposure to mixtures resulted in larvae deformations, suggesting that the quinolines is a relevant group to study in the future to further understand how oil can cause cardiotoxicity and deformations in fish ELS.

Acknowledgement: The authors thank the Research Council of Norway for funding the EGGTOX project.

Session 2
Industrial Discharges in the Environment

Session Chairs: Marianne Frantzen, Jasmine Nahrgang, Helena Reinhardy

Talks

Invited Plenary – Jasmine Nahrgang: An elevated risk for Arctic organisms from petroleum compounds?

Bjørn Henrik Hansen: Adhesion of dispersed crude oil droplets onto eggs of cod *(Gadus morhua)* and haddock *(Melanogrammus aeglefinus)* and associated toxicity to developing fish larvae

John Incardona: Persistent derangement of larval-juvenile lipid metabolism and growth following transient embryonic oil exposure in Polar cod

Ireen Vieweg: The effects of chronic crude oil exposure on the biological fitness of polar cod *(Boreogadus saida)*

Ida Beathe Øverjordet: Toxicokinetics in Arctic copepods

Live-Guri Faksness: Should current environmental assessment methods for produced water be revised? The influence of chemical composition on produced water toxicity

Helena Reinardy: Impacts of fine particle mine tailings on early life stages of cod

Anastasia Georgantzopoulou: Effects of effluent and sludge containing Ag and TiO2 nanoparticles transformed through lab-scale wastewater treatment processes

Posters

Ditte Secher Paludan: Transcriptional study on endocrine disruption in female polar cod following a prolonged recovery to short-term exposure to oil spill response residues

Elise Skottene: Oil exposure biomarkers and lipid metabolism in *Calanus finmarchicus*

Bjørn Henrik Hansen: PW-Exposed: Produced water fractionation and advanced chemical and toxicological characterization using sensitive life stages of marine fish species

Frederike Keitel-Gröner: Oil spill in the arctic: are we confident about the use of dispersant to combat oil?

Tomasz Ciesielski: Biomarkers of oxidative-stress in Calanus finmarchicus exposed to WAF from a naphthenic North Sea crude oil

Pål A. Olsvik: DiTail Marine disposal of mine tailings: Impacts on pelagic ecosystem components in Norwegian fjords

Julia Farkas: Diurnal patterns and removal of selected elements in two Norwegian wastewater treatment plants with significant industrial loading

Tânia Gomes: Uptake, elimination and toxicity of depleted uranium (DU) in the freshwater crustacean *Daphnia magna*

Niels Borup Svendsen: Environmental Consulting in the Arctic

Invited Plenary

An elevated risk for Arctic organisms from petroleum compounds?

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In an era of abrupt climate change and receding sea ice, anthropogenic activities are expected to increase significantly in the Arctic as new resources and opportunities are made available. Oil and gas exploration in seasonally ice covered regions, as well as maritime shipping represent important risks of petroleum release in this pristine environment, facing political, socio-economic and technological challenges. The sensitivity of Arctic marine ecosystems to the release of petroleum compounds is thus becoming an increasing concern.

The close relationship of Arctic organisms with their Atlantic and Pacific counterparts suggests that they exhibit very similar response mechanisms to deal with anthropogenic stressors such as pollutants. However, Arctic organisms are adapted to particular environmental conditions such as a high seasonality in light, constant low temperatures and the presence of ice. It is therefore a relevant question to ask whether Arctic organisms are more sensitive than organisms from other regions of the world. This is a vast and multifaceted question that demands to consider several aspects including the environment considered, time and location and the species, their ecological role in the ecosystem and their physiology.

I will attempt to discuss these aspects and present some of my recent research on an Arctic key species, the polar cod *Boreogadus saida*. I will highlight current challenges within Arctic marine petroleum ecotoxicology and gaps of knowledge.

Adhesion of dispersed crude oil droplets onto eggs of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) and associated toxicity to developing fish larvae

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Keywords: Petroleum, chorion, fish development, cardiac toxicity
Session: Industrial Discharges in the Environment
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The decreasing sea ice coverage in the Arctic opens new opportunities for industrial activities, such as shipping, mining and oil exploration. These activities introduce increased risks of accidental spills of oil through e.g. blow-outs and spills from boat accidents. During acute crude oil spills into the marine environment, a large fraction of petrogenic components will be contained in micron-sized oil droplets. The formation of dispersed oil droplets occurs naturally as a result of wave action and turbulence in the water. In some cases, the application of chemical dispersants is used to facilitate the dispersion of the oil in the water column.

Recently, it has been shown that adhesion of dispersed crude oil droplets to the chorion of developing fish eggs may enable transfer of heavy oil components to the embryo. However, little is known about the adhesion properties of oil onto egg chorions and how this is affected by chemical dispersants. Knowledge on the oil-chorion interactions is of high importance to enable better prediction of the environmental consequences of oil spills. Toxicity is generally believed to be associated with the dissolved fraction of oil components and not particulate oil droplets, however, if adhesion of oil droplets to chorions facilitate transfer of crude oil components to the embryos, there is reason to believe this will facilitate toxicity as well.

In the present work, we studied oil-chorion interactions by exposing eggs of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) to either chemically or mechanically dispersed crude oil. Oil adhesion was studied using fluorescence microscopy and GC/MS analyses to quantify PAH uptake. In addition, we studied the contribution of oil droplets to dispersion toxicity by exposing cod eggs to un-filtered (dispersions containing droplets) and filtered (water soluble fractions) dispersions in a flow-through system. Acute as well as chronic toxicity endpoints were included in the assessment.

While we observed that relatively few oil droplets adhered onto the chorion of eggs from both species, approximately 30% more petrogenic compounds still accumulated in haddock eggs compared to cod eggs. In addition, for both species, mechanically dispersed crude oil had a higher affinity to eggs than chemically dispersed crude oil droplets. Oil droplets appear to contribute to toxicity, including acute mortality, condition of hatched larvae and development of craniofacial and jaw development.

Acknowledgement: The authors thank the Research Council of Norway for financing the EGGTOX project.

Persistent derangement of larval-juvenile lipid metabolism and growth following transient embryonic oil exposure in Polar cod

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Keywords: oils spills, PAHs, sublethal effects, delayed mortality
Session: Industrial discharges in the environment
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Studies following the Exxon Valdez oil spill in Alaska showed that exposure to low levels of crude oil during embryogenesis reduced later growth in surviving juvenile fish, but the underlying mechanisms are unknown. For high latitude gadid species, especially Polar cod (*Boreogadus saida*), population recruitment depends on maximizing growth and energy allocation in the form of lipids during the first spring and summer after hatch. Transcriptome sequencing in embryos of the gadid Atlantic haddock (*Melanogrammus aeglefinus*) suggested early disruption of lipid metabolism occurred secondary to oil-induced cardiocirculatory impairment, potentially leading to persistent metabolic defects and poor growth after hatch. Here we tested this hypothesis in Polar cod embryos from a captive broodstock. Embryos were exposed to physically dispersed microdroplets of Alaskan oil for 3 days during cardiac organogenesis, followed by a 4-day washout period and transfer to clean water. Total polycyclic aromatic hydrocarbon concentrations were 0.9 ± 0.3, 3.3 ± 0.4, and 15 ± 5 µg/L in water (droplet-associated plus dissolved) and 63 ± 11, 505 ± 105, and 1170 ± 170 ng/g wet weight in embryos, respectively. Embryos were assessed for oil-induced cardiotoxicity immediately after exposure (28 dpf) and at hatch (42 dpf). Biometrics and lipid composition were determined at several points up to ~150 days post-hatch. For the 15 µg/L and 3.3 µg/L exposure concentrations, either all or most of the hatched larvae quickly succumbed to severe craniofacial malformations. Large numbers of grossly normal larvae from the 0.9 µg/L exposure grew through metamorphosis, despite having measureable cardiac defects at hatch. However, these fish showed significant growth impairment. Initially indistinguishable in embryos, there was a dose-dependent increase in triacylglycerols (TAG) and free fatty acids (FFA) in yolk sac larvae that persisted through first feeding. In surviving juveniles (0.9 µg/L exposure), TAG levels were reduced after metamorphosis relative to controls. These findings suggest that storage and fuel lipids (TAG and FFA) are underutilized or inappropriately synthesized (or both) in first-feeding larvae, while these lipids are burned excessively in juveniles rather than accumulating for over-wintering. This in turn suggests a model in which embryonic oil exposure leads to an irreversible lipid-bioenergetic deficit as a mechanism underlying poor growth and, consequently, reduced recruitment.

The effects of chronic crude oil exposure on the biological fitness of polar cod (*Boreogadus saida*)

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Keywords: Arctic, petroleum discharge, polar cod, specific growth rate
Session: Industrial discharges in the environment
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Climate models predict extended periods with sea ice-free Arctic waters during the next decades, which is going to allow more shipping activity and an easier access to petroleum resources in the Arctic. Increased industrial activity raise concern about the biological effects of accidental petroleum release on key species of the Arctic marine ecosystem, such as polar cod (*Boreogadus saida*). In the present study, adult polar cod were dietarily exposed to four environmentally relevant crude oil doses (0, 20, 100 and 200 µg crude oil· g food^{-1}) over a 7-month period, matching the reproductive development of the fish. Fish survival, changes of body indices and somatic growth were monitored on a monthly basis. The crude oil treatments had no significant effects on the survival of the fish, while the presence of parasites almost doubled mortality. Neither crude oil treatment nor parasite presence had significant effects on the hepatosomatic index of the fish and the gonadosomatic index was also not affected by the treatments. Surprisingly, the lowest crude oil dose increased polar cod growth during the experiment by 0.05 %·day^{-1}, while higher doses had no effect on growth. The results of the present experiment showed that the chronic exposure of polar cod to environmentally relevant crude oil doses induced only few significant effects at the organismal level. This could imply that this Arctic fish species is relatively robust to the dietary exposure with petroleum compounds at such low exposure levels.

Toxicokinetics in Arctic copepods

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Keywords: oil components; produced water; PAH; Modelling
Session: Industrial Discharges in the Environment
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Understanding the toxicokinetics of petrogenic oil components in key species in Arctic marine food webs is becoming increasingly important due to higher potential for accidental oil spills and discharges of produced water in the Arctic. Environmental risk assessment (ERA) tools for such events may include predictions of body residues of oil components in biological compartments in scenario modelling, which gives indications of toxic effects and trophic transfer potentials. The *Calanus* species (Crustacea) dominates the zooplankton in Arctic and sub-Arctic pelagic food webs. They build up large lipid stores during their late developmental stages, making them important energy resources in Arctic food webs. Their high lipid content enhances the bioaccumulation potential of organic contaminants during exposure to oil components in the water column. Hence, high consumption of lipid-rich *Calanus* may contribute substantially to the accumulation of organic contaminants in organisms at higher trophic levels, making the *Calanus* sp. good candidates for environmental monitoring.

We have performed several controlled toxicokinetics experiments with Arctic *Calanus* sp., from which models describing the uptake and depuration kinetics were developed. Models fitted to the experimental data provided estimates of bioconcentration factors (BCFs) and elimination rates to be used in scenario modelling. Parameter estimation for all components in oil and produced water is not possible, so the parameters need to be linked to chemical descriptors in quantitative structure activity relationships (QSAR). The most commonly used chemical descriptor is the octanol-water partitioning coefficient (K_{ow}). We present results from the experimental work, compare parameter estimates, and evaluate K_{ow} as QSAR predictor in Arctic copepods.

Should current environmental assessment methods for produced water be revised? The influence of chemical composition on produced water toxicity

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Keywords: Produced water, toxicity, polar fraction, EIF
Session: Industrial discharges in the environment
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Produced water (PW) from offshore oil production platforms represents the largest discharge of effluent into the offshore environment and is known to contain a complex mixture of dissolved organic compounds and the residues of added production chemicals. The compounds routinely monitored in PW include oil-in-water, BTEX, PAHs (including alkylated naphthalenes, phenanthrenes and dibenzothiophenes), phenol and alkylated phenols. These compounds and the added chemicals are used to calculate the environmental impact factor (EIF), which accounts for both PW composition and release rate, and is used to identify which oil-deriving compounds or production chemicals contribute most to the environmental risk. However, there is a concern from the Norwegian Environment Agency (NEA) that the current input to the EIF calculations may not include all of the relevant compounds responsible for observed toxicity. In the current study, PW was collected from five oil platforms on the Norwegian continental shelf representing fields of different operational ages and crudes with different physical and chemical properties. PW samples were subjected to extraction followed by isolation of non-polar and polar fractions using solid phase extraction recovering 80% of the total GC amenable material. A thorough chemical characterization of the fractions and total extracts was performed, but here only the results from GC and GCMS analysis, including BTEX, PAHs and phenols, are discussed. The total PW extract, the polar and the non-polar fractions were subject to acute toxicity tests using *Acartia tonsa* nauplii. The LC_{50} values for the total PW extracts ranged between 0.05 and 1.77 ppm (based on total GC amenable fraction analysis). For three of the PW, the observed toxicity was mainly attributed to the polar fraction. Interestingly, the toxicity of the last two PW was mainly attributed to the non-polar fraction. This study demonstrates that PW toxicity may be associated with compounds that are currently poorly characterized and suggests that in many cases PW toxicity is not directly correlated with the GC quantifiable compounds used for regulating discharges today. NEA and the oil industry are now investigating the whole effluent toxicity (WET) of PW. Our study will provide valuable input to this work. Further studies should be conducted with a wider array of PWs from a range of sources to determine if alternative methods of characterization are needed for regulation of PW discharges.

Acknowledgement: The authors thank the Research Council of Norway for financing the project (RESOLVE, project no. 243720/E40), and Statoil and Shell for providing produced waters.

Impacts of fine particle mine tailings on early life stages of cod

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Keywords: Mine tailings, cod, early life stages, epigenetics
Session: Industrial discharges into the environment
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Mineral industries produce millions of tons of mine tailings waste and, in Norway, one disposal option is to deposit tailings waste through coastal submarine discharges. Mine tailings slurry include a fraction of fine particles <100 µm and can have elevated levels of metals from incomplete ore mineral recovery. Prolonged suspension of small particles in the water column may bring them into contact with locally spawned pelagic fish eggs, including commercially valuable Atlantic cod. The objectives of this study were to conduct two exposure experiments of newly-fertilised cod eggs to suspended mine tailings particles (and Magnafloc flocculant) from the proposed Nussir ASA copper mine in Finnmark, Norway. Cod eggs were exposed to concentrations up to and beyond permit disposal limits of suspended mine tailings particles (3 mg/l) in flow-through aquaria (Experiment 1: 0-3 mg/l; Experiment 2: 0-10 mg/l). Particle concentrations and dissolved metals characterised the exposure parameters, and a suite of biological endpoints evaluated effects of exposure on survival, development, egg bouyancy, hatching, larval behaviour, and gene expression changes in toxicity markers and epigenetic regulation. There was no adverse effect of mine tailings exposure on embryo survival; however, a slight but significant elevation in larval mortality after hatching suggests a protective effect of the chorion on the embryos and increased stress when in direct contact with particles after hatching. Egg bouyancy was reduced, likely due to adhered particles on the chorion surface. There were no differences with treatment in timing of hatching, cardiac activity, and embryo and larvae morphometrics. There was a treatment-dependent up-regulation of selected stress marker genes (*hsp*, *cyp1c1*) but no indication of activation of metallothioniens (*mt* gene) or markers for genotoxicity (*gadd45* and *rad51*). Gene markers for DNA and histone methyltransferases did show treatment-related up-regulation; however, the long-term implications of changes to epigenetic control of DNA in response to environmental stressors such as mine tailings is still unknown. Studies of the effects of fine-fraction mine tailings waste are limited and more studies on the long-term impacts are necessary to guide best practice management of future mine tailings disposal.

Acknowledgement: The authors thank: all the staff from the Akvaplan-niva FISK station at Kraknes; Randi Olsen and Augusta Hlin Aspar for SEM and histological analyses, UiT; technical help from Paul Dubourg; Anna Vader and Stuart Thomson from UNIS.

Effects of effluent and sludge containing Ag and TiO_2 nanoparticles transformed through lab-scale wastewater treatment processes

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Keywords: Nanomaterials; transformation; wastewater treatment processes; ecotoxicology
Session: Industrial discharges in the environment
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The majority of nanomaterials (NMs) used in industrial and commercial applications are likely to enter the wastewater stream and reach wastewater treatment plants (WWTPs). In Oslo, Norway, the WWTPs receive both municipal and industrial wastewater. The treated effluents are discharged to aquatic recipients and the stabilised sludge is applied on agricultural land, however, the transformation of the particles and the potential hazard they pose in these compartments are poorly understood. The overall goal of this study was to elucidate the behavior of Ag and TiO_2 NPs during biological wastewater treatment, and investigate the subsequent effects of transformed particles present in the effluent and sludge relative to their pristine counterparts. A laboratory-scale wastewater treatment system was established and combined with a battery of ecotoxicological assays and characterization techniques. The system was based on activated sludge treatment with a pre-denitrification system and fed with synthetic wastewater spiked daily with 10 µg Ag NPs/L (PVP coated, 25 nm, nanoComposix) and 100 µg TiO_2 NPs/L (5 nm, NM-101, JRC) over a period of 5 weeks. Samples from all reactors, including the effluent, were collected weekly and analyzed by sequential filtration and inductively coupled plasma mass spectrometry (ICP-MS) to determine the NP fractionation and partitioning. Transmission electron microscopy and single particle ICP-MS (sp-ICP-MS) were performed on selected samples. The effects of transformed particles present in the effluents were assessed using a battery of bioassays including freshwater and marine algae (growth inhibition, reactive oxygen species -ROS- formation), crustaceans and *in vitro* models of relevance for NP toxicity assessment (RTgill-W1 cell line, metabolic activity, epithelial integrity, ROS formation, gene expression). The effects of the aged particles through biosolids application were evaluated using coelomocytes, primary cells involved in immune defense mechanisms, isolated from the exposed earthworms *Eisenia fetida*. The observed effects were organism-dependent, with bottom feeding organisms and algae being more sensitive. The *in vitro* models offered a useful tool for the assessment of environmental samples. Through a relevant exposure scenario, this study adds useful pieces to our still fragmentary understanding of the environmental fate of weathered NPs.

Acknowledgement: The authors thank Pawel Krzeminski and Adam Lillicrap (Norwegian Institute for Water Research, Oslo, Norway) for the useful insights and discussions. The authors gratefully acknowledge Maria Hultman (Norwegian Institute for Water Research, Oslo, Norway) for her valuable advice and help with the qPCR analysis and Marianne S. Kjos (SINTEF, Norway) for the ICP-MS analysis. The authors express special thanks to the operators of the Bekkelaget WWTP, Oslo, Norway. The work reported here has been undertaken as part of the Research Council of Norway (RCN) funded projects NanoWASTE "Investigating the fate of nanomaterials in waste water treatment plants; removal, release and subsequent impacts" (Grant Agreement number 238972/O70) and NanoCharm (ProjectNr: 221391/RI). The authors wish to thank the RCN for their financial support.

Transcriptional study on endocrine disruption in female polar cod following a prolonged recovery to short-term exposure to oil spill response residues

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Keywords: Oil spill response residues; reproductive toxicity; gene expression; polar cod
Session: Industrial discharges in the environment
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With the petroleum industry moving north and closer to the ice edge, there is an enhanced risk of key species in the Arctic foodweb such as polar cod, *Boreogadus saida*, being exposed to an eventual oil spill. Different response actions may be used to combat oil spills at sea, (e.g. *in situ* burning of oil and chemical dispersion of oil) including no action (i.e. natural (mechanical) dispersion of oil).

This study analysed tissue samples (brains, gonads, and livers) from sexually mature females collected from a previous experiment that exposed maturing polar cod to burned oil residue (BO), chemically dispersed oil (CDO), and mechanically dispersed oil (MDO), followed by seven months growth in clean conditions concurrent with reproductive development. Extensive gene expression (quantitative reverse transcription PCR) analyses investigated endocrine disruption by a suite of genes involved in enzymatic and hormonal control of the hypothalamic-pituitary-gonadal axis. There was significant downregulation in expression of follicle stimulating and luteinising hormone genes (*fsh* and *lh*, respectively) in the brains, and a non-significant trend in expression of the *20-β-hsd* gene in the gonads of female fish exposed to BO residues, compared to unexposed control fish. The hydroxysteroid dehydrogenase (*20-β-HSD*) enzyme is responsible for synthesis of the maturation inducing hormone (MIH) in the Δ4 pathway of the steroidogenesis, triggered by lutenising hormone (LH). Though *fsh* was downregulated, no patterns were seen for genes enconding for enzymes involved in oocyte growth (e.g. aromatase). There were slight changes in expression levels of the gonadotrophic releasing hormone gene in brains after exposure to MDO treatment, but overall, no significantly altered expression of oil toxicity gene markers in livers (*vtg* and *cyp1a*) in any of the treatments.

Results obtained from this study suggest that female polar cod exposed to BO residues are more sensitive toward altered transcription than females exposed to CDO or MDO. The results will be compared against water chemistry data from the exposures and histological analysis of ovarian tissue from the exposed females for a wider understanding of the effects of oil spill response actions on sexual maturation in female polar cod.

Acknowledgement: The authors thank Anna Vader and Martin Hansen for their contribution to this project as well as Stuart Thomson for technical assistance.

Oil exposure biomarkers and lipid metabolism in *Calanus finmarchicus*

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Keywords: copepods, lipid metabolism, oil exposure, biomarkers
Session: Industrial Discharges in the Environment
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As oil production in the Atlantic Ocean continues to develop towards northern regions, it becomes imperative to evaluate the biological impacts of discharges from the production. Copepods have been used as test species for evaluation of the toxicity of various chemicals (e.g. 1, 2). Most oil components are lipid soluble, and may accumulate in the oil sac of copepods. Crustaceans preferentially catabolize lipids during fasting (3), which can affect the bioavailability of the contaminants. Contaminants can also directly or indirectly influence lipid homeostasis (4, 5). Here, we present preliminary data from two of our studies where *C. finmarchicus* have been exposed to two different solutions (one water accommodated fraction: WAF, and one water soluble fraction: WSF) of petrogenic oil from the North Sea.

In the first study, copepodites in diapause were collected from the Trondheimsfjord and brought into the laboratory where they were exposed to a WSF for 4 days. They were sampled at several time points as they progressed towards the adult stage over the course of 20 days, while only relying upon endogenous energy sources. RNA-sequencing results from this study indicate that oil exposure during diapause termination can influence development of *C. finmarchicus* copepodites, and that the copepodites "pause" lipid catabolism during exposure, before increasing the catabolism during recovery. 26 genes were commonly upregulated in the exposed group at all time points compared to control at time 0. Three of these genes (a thioredoxin, a transcriptional repressor and a small ubiquitin modifier) are potential useful biomarkers for exposure, and will be investigated further.

In the our second experiment, adult females of *C. finmarchicus* were exposed to a WAF of North Sea crude oil for 4 days and sampled each day. qPCR results show that gene expression of TP-β, an enzyme involved in the β-oxidation of long-chain fatty acids, was downregulated in both control and exposed groups after 48 hours, and remained downregulated until the end of the study. This suggests that the sudden stop in food supply, which usually occurs at the beginning of exposure studies, alters the lipid utilization in the copepods. This can affect the bioavailability of stored lipid soluble contaminants. This will be further assessed by analyses of more genes related to lipid catabolism.

Acknowledgements: The authors thank the Department of Biology for grantning Ms. Skottene the stipend for travelling to WHOI, SINTEF for chemical analysis of the exposure mediums, Mari-Ann Østensen, Øystein Leknes, Siv Anina Etter, Christoffer H. Hilde, Justyna Swiezak, Signe Dille Løvmo, Iurgi Salaberria, Kjersti R. Dahl, Hanny Rivera, Keshuai Li, Torfinn Sparstad, Yang Jin, and Roger Normann. The study was conducted as part of the GRACE project (Integrated Oil Spill Response Actions and Environmental Effects), which is supported by the European Union Programme (H2020-BG-2015-2) under grant agreement no. 679266.

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PW-Exposed:
Produced water fractionation and advanced chemical and toxicological characterization using sensitive life stages of marine fish species

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Keywords: Produced water emissions; Oil & Gas industry; In vitro toxicity; fish embryo toxicity,
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Produced water (PW) from the oil and gas industry represents the largest direct discharge of effluent into the marine environment worldwide. Approximately 130 million m^3 PW is released on the Norwegian continental shelf annually from offshore production platforms. Organic compounds (mostly hydrocarbons), metals, radioisotopes, production chemicals, solids and dissolved gases comprise the major contents of PW. Regulatory authorities classify substances discharged to the marine environment according to environmental risk, with specific focus on persistence, bioaccumulation and toxicity, and by defining 'predicted no effect concentrations' (PNEC) for several predefined PW components, primarily resolved oil components, particulate oil and production chemicals. However, the presence of unknown components with uncharacterized and potential long-term toxicity are not in any way regulated by the authorities. The PW-Exposed project addresses the urgent need to provide a detailed understanding of the composition and toxicological impacts of PW. By utilizing a combination of sophisticated fractionation approaches and cutting-edge high-resolution chromatography and mass spectrometry, we will provide a thorough chemical characterization of PW. PW fractions will be tested utilizing an expanded in vitro toxicity screening toolbox. This will specify which component groups are the main drivers for PW toxicity and their potential involvement in specific and important biological processes. This approach will prioritize and select the most potent toxic fractions for final toxicological investigations using embryos of pelagic and benthic fish species. These tests will validate the contribution from toxicity-driving component groups at the organism level. By directly engaging with key stakeholders such as oil companies and the Norwegian Environment Agency, the data generated in the project will be used as a basis for improving current PW risk assessment approaches.

Oil spill in the arctic: are we confident about the use of dispersant to combat oil?

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Keywords: oil spill, chemical dispersant, *Pandalus borealis*, larval stages
Session: Industrial Discharges in the Environment
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The Arctic is under increasing environmental pressures from industrial development such as oil and gas exploration and shipping routes for goods transport, both representing potential hazards for accidental oil discharges. Oil spill preparedness and responses need to be strengthened. Oil spill responses involve trade-offs, including the usage of dispersants to combat and mitigate the potential impacts. Scientific knowledge is needed to support decision-making based on a Net Environmental Benefits Analysis (NEBA). This is especially demanded for key species of the northern latitudes, which represent a valuable resource for the local communities.

There is insufficient knowledge of the consequences of dispersant use onto Arctic species life stages and particularly larval stages, generally considered less robust. Planktonic shrimp larvae (*Pandalus borealis*) are relevant because of their distribution, both offshore and along the entire Norwegian coastline and up to Svalbard (in the North Atlantic and in the Barents Sea). *P. borealis* is a key species in these ecosystems and an economically (and culturally) important species.

A series of experimental studies with the planktonic larvae of *P. borealis* exposed to oil (O) and chemically dispersed oil (OD) were conducted to provide new toxicity data relevant to NEBA. An exposure system with high-energy mixing of O and OD was started at day 0 (oil added 10g in 150L seawater, dispersant added 4% of oil added, nominal oil concentration ~ 67 mg l^{-1}). The water from this system was used at day 1 to prepare O and OD solutions with a total hydrocarbon content (THC) in the range 1% (100 times dilution), 10% (10 times dilution) and 100% (no dilution). Treatments with dispersant only (D) in the same dilution range were also prepared in addition to a control (C) group. Shrimp larvae (n=10, stage I, 3 replicate bottles) were added to the treatment solutions day 1 and feeding and mortality were studied after 24h incubation and following 24h and 98h recovery.

Overall, the results from this series of experiments showed that OD was significantly more toxic to planktonic crustaceans than O. These results claim for a careful consideration of the use of dispersants when larval stages are present as the consequences for valuable marine resource populations of the Arctic ecosystem are uncertain.

Acknowledgement: The authors thank the Research Council of Norway (RCN #228107), industries and Norwegian authorities for financial support of this study, which is part of the Environmental Risk Management module of the Research Centre for Arctic Petroleum Exploration (ARCEx).

Biomarkers of oxidative-stress in *Calanus finmarchicus* exposed to WAF from a naphthenic North Sea crude oil

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Keywords: copepods, biomarkers, crude oil exposure, oxidative stress
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Calanus finmarchicus is an ecologically important species in the North, Norwegian and Barents Seas, periodically constituting up to 90% of the standing stock of zooplankton. Due to continued development of areas for oil and gas production, there are environmental discharges occurring both continuously and accidentally in these areas. Existence of reliable biomarkers for oil exposure can be fundamental for monitoring programs and decision-making processes in case of environmental discharges and oil spills. To assess the potential negative effects of oil exposure on keystone component of marine ecosystems, *C. finmarchicus* was exposed to water accommodated fraction (WAF) of naphthenic North Sea crude oil. Adult non-ovulating females from the continuous lab culture at SINTEF/NTNU Sealab, were exposed without feeding to sub-lethal concentration of WAF in seawater and collected at 5 different time points 0, 24, 48, 72 and 96 hours. Several oxidative stress biomarkers were tested with gene expression (qPCR), enzymatic activity analyses and lipid peroxidation assay for MDA (malondialdehyde) concentrations. Enzymatic assays demonstrated induction of GST (glutathione S-transferases) and increase of GSH (gluthatione) and MDA levels at each time point sampled. However, qPCR results showed both up- and downregulation of GST at 48 and 72 hours and downregulation of SOD (superoxide dismutase) at 72 hours. Our study indicates that GST enzymatic activity, GSH and MDA can be applied as a biomarkers of crude oil exposure in *C. finmarchicus*.

Acknowledgement: The authors thank Ewa Cichosz, Clara P. Igisch and Ane Haarr for help with experimental work. The study was financed by the GRACE project (Integrated Oil Spill Response Action and Environmental Effect), which is supported by the EU Program Horizon 2020 (H2020-bg-2015-2) under the grant agreement no. 679266.

DiTail
Marine disposal of mine tailings: Impacts on pelagic ecosystem components in Norwegian fjords

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Keywords: Submarine tailing disposal; Fjord ecosystems; Pelagic species; Modelling
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The rapidly increasing demand for mineral resources is driving expanding mining activities worldwide. Mining generates extensive quantities of tailings that need to be disposed of. In some countries, including Norway, tailings are disposed in the sea, despite a current lack of knowledge on environmental implications. During submarine tailing disposal large quantities of fine inorganic particles, associated metals and chemical additives are introduced into the marine environment, where they can spread in the water column and thus cause significant stress for exposed organisms. Exposure to mine tailings, especially to inorganic particles, does not necessarily cause acute toxicity, but can potentially cause reduced food intake and impaired physiological functioning. This can lead to reduced growth and development, which has severe consequences especially for early life stages of animals, e.g. fish larvae. In the DiTail project, we will investigate impacts of mine tailings and mine tailing components on the planktonic copepod species *Calanus finmarchicus*, and early life stages of Atlantic cod (*Gadus morhua*). We will gain an in-depth understanding on tailing effects from a molecular to individual level, applying state of the art molecular techniques, measuring tailored physiological endpoints, and integrating these responses into an energy-budget framework for the individual organism. Through integration of effects data into tailing transport models that will be developed within this project, we will assess the exposure of individuals, which in turn will give valuable information about effects on local populations in terms of impacts on recruitment. The data and models generated in this project will substantially support risk assessment of mine tailing disposal in marine environments.

Acknowledgement: This project is funded by The Research Council of Norway (RCN, project number 281093) and the Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway.

Diurnal patterns and removal of selected elements in two Norwegian wastewater treatment plants with significant industrial loading

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Keywords: elements in wastewater, removal efficiency, diurnal variations
Session: Industrial Discharges in the Environment
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Wastewater treatment plants (WWTP) that collect industrial and household discharges can be a significant source of pollutants to the environment. In this study, we investigated influent patterns and removal of selected elements in two full-scale WWTPs, Ladehammeren (LARA) and Høvringen (HØRA) in Trondheim, Norway. Both WWTPs have significant industrial loading contributions (up to 40% in LARA). The WWTP employ preliminary and primary treatment steps, including chemically aided flocculation, and discharge directly into Trondheimsfjord. In a 7 day sampling campaign, 24 h composite samples of influent and effluent wastewater as well as sludge samples were taken to determine influent concentrations and removal of selected elements including Al, P, S, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Ba, Gd and Pd. To study release patterns and gain information about potential sources such as industrial discharges, we determined and modelled diurnal variations of elemental concentrations in 8 h composite samples covering morning, evening and night discharges.

Element concentrations in 24 h composite influent samples were highest for S>P and Al and lowest for Cd<Gd<As and Cr. Concentrations of Mn, Fe, Ni, Ba, Gd and Cd were higher in HØRA compared to LARA, with Fe loadings being approximately double. Removal efficiencies varied between the analysed elements, and were highest for Al (86%), P (74%) and Cu (57%) in LARA. In contrast, removal rates were below <50% for P, Cu and S in HØRA. In LARA, concentrations of Fe, Ni and S were significantly higher in the treated effluent compared to the raw influent, deriving from the use of inorganic flocculant. This was also reflected in Fe and S concentrations in treated sludge.

Element concentrations in 8 h composite samples mostly followed general diurnal discharge patterns, with higher concentrations in mornings and evenings and lower concentrations at night. Concentrations of several elements correlated well with total suspended solid concentrations (TSS), with the strongest correlations observed for P, Fe and Al (R2>0.8 in LARA, R2>0.7 HØRA). Correlations of TSS with Cu, Cr, As, Cd, and Pb were more pronounced in HØRA than in LARA, which can potentially be attributed to the higher industrial loading contributions in LARA. Enrichment factors were high for P>Cu>Zn>Cd>As, and were above 10 for Cr and Ni in biosolids, indicating anthropogenic sources for these elements. Several elements also occurred as nano- and micron-sized particles.

Uptake, elimination and toxicity of depleted uranium (DU) in the freshwater crustacean *Daphnia magna*

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Keywords: Depleted uranium, accumulation, elimination, *Daphnia magna*
Session: Industrial discharges in the environment
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Uranium is naturally found in freshwater at concentrations ranging from < 6 µg/L to 2000 µg/L in the vicinity of uraniferous sites. Knowledge on uranium toxicity in non-human biota is still scarce, as well as its bioaccumulation in aquatic food webs. Nonetheless, even though uranium has no metabolic role, its uptake and accumulation in aquatic organisms can lead to both chemical and radiological effects. With this in mind, this study aimed to improve the understanding on the uptake and elimination of depleted uranium (DU) in the freshwater crustacean *Daphnia magna*. The acute toxicity of depleted uranium in *D. magna* was evaluated by determining the 24 hrs and 48 hrs LC_{50} of DU at concentrations ranging from 0.03 to 300 mg/L at pH 7.0 and temperature 20 ± 1ºC in moderately hard EPA water. In addition, *D. magna* was exposed to waterborne uranium at the non-lethal concentrations of 0.05 mg/L and 0.5 mg/L for a period of 96 hours followed by a 72 hours depuration period also at constant pH in the syntetic water. Total concentrations of DU were determined in the whole body and haemolymph of exposed daphnids at different time points (6, 10, 24, 48, 72 and 96 hours) using ICP-MS of digested samples throughout the exposure and depuration periods. The estimated LC_{50} to produce 50% mortality of daphnids to DU at pH 7.23-8.31 was 8.6 mg/L and 4.5 mg/L for 24 and 48 hrs respectively. Bioaccumulation results showed a time and concentration dependent uptake of DU in the whole body of daphnids after 96 hrs of exposure. However, while for the highest concentration of 0.5 mg/L DU a steady state seems to be reached after 12 hours, at the lower concentration of 0.05 mg/L a continuous accumulation of DU was achieved with time. On the other hand, DU concentrations in the whole body of daphnids decreased with time during the depuration period. Haemolymph results also showed a time and concentration dependent accumulation of DU, even though accumulation was lower than that found for the whole body. As for the elimination period, a significant reduction of DU in haemolymph was detected in the first 6 hours, followed by a steady state in the remaining depuration period. The findings obtained in this study provided further insight into both the accumulation/elimination kinetics of DU and acute toxicity in *D.magna* and highlighted the need of carrying out additional experiments to assess the potential for bioaccumulation at different aquatic trophic levels.

Acknowledgement: This work was supported by the Research Council of Norway through its Centres of Excellence funding scheme, project number 223268/F50.

Environmental Consulting in the Arctic

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Keywords: Nutrient runoff, explosives, landfill leachates, microbial remediation
Session: Industrial Discharges in the Environment

In the Arctic, NIRAS are specialists within hydropower and energy, infrastructure, permafrost engineering, harbours and airports, environmental engineering, planning and sector analysis. Furthermore, for the past 20 years, NIRAS has conducted environmental impact assessments (EIA) and strategic environmental assessments (SEA) for a wide range of projects.

As the Arctic environment is particularly vulnerable to pollution, every aspect of e.g. infrastructure and hydropower projects must be investigated in detail to identify, reduce, and prevent potential negative environmental effects. Most recently, NIRAS assessed the potential environmental impacts associated with establishing and operating a new airport in Qeqortoq and expanding airports in Nuuk and Ilulissat (all in Greenland). Results showed that the estimated c. 7.000 tons of explosives required to remove 14,9 million m^3 of solid rock will release approximately 56 tons of nitrogen oxides (NO_x) into the environment. To avoid eutrophication of the pristine environment, several mitigating measures have been proposed by NIRAS, such as pre-blast precautions and deposition of nutrient-contaminated snow in areas where eutrophication is less likely.

In northeast Greenland, NIRAS has investigated possible marine and terrestrial pollution from landfill leachates. NIRAS collected sediment samples, liver samples from shorthorn sculpin (*Myoxocephalus scorpius)* and polychaetes samples in order to estimate levels of heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins. Elevated levels of several heavy metals and PCBs were found in sediments in the coastal zone close to the landfill (25 m). Liver samples from sculpins caught close the landfill showed higher PCB concentrations compared to those caught 3,7 km north of the landfill. These results suggest a possible local, bioaccumulation of PCBs in sculpin liver. Further, NIRAS's findings underpin the necessity for remediation of abandoned landfills in the Arctic.

In general, remediation of oil polluted grounds in the Arctic is difficult. NIRAS has investigated the use of landfarming of oil polluted grounds to stimulate evaporation of oil and growth of oil decomposing bacteria in the Arctic. With a demonstrable annual reduction in oil levels of 26% and a five year reduction of 82%, treated grounds show significantly lower oil levels compared to untreated grounds.

Acknowledgments: Fieldwork was conducted in Greenlandic military territory owned by the Danish Defence Department in collaboration with the Danish Ministry of Defence Estate Agency.

Session 3
Mixture Toxicology and Multiple Stressors

Session Chairs: Veerle Jaspers, Christian Sonne and Courtney Waugh

Talks

Erik Muller: Lethal and sublethal impacts of copper nano-pesticides on the energy budgets of starving estuarine amphipods

Chisato Kataoka: Silver nanocolloid increases pathogenic infection risk following disruption of gut microbiota and immune system in medaka fish

Li Xie: Multiple stressor effects of ionizing (γ) radiation and non-ionising (UV) radiation in duckweed *(Lemna minor)*

Malin Celander: Increased sensitivity to benzopyrene exposures in fish cell-line when co-exposed to pharmaceuticals

Kareem Eldin Mohammed Ahmed: Revealing the effects of defined persistent organic pollutant mixtures using H295R steroidogenic assay: an LC-MS/MS approach

Vidar Berg: The use of mixtures of pollutants for toxicity testing. Experiences from past studies and plans for future testing

Posters

Alexander Badry: Effect of environmental pollutants on virus activated innate immune system signalling pathways

Jose Maria Castaño Ortiz: Effects of perfluorooctane sulfonate (PFOS) on the antiviral immune response using an in vitro model

Julia Farkas: Effects of engineered silver nanoparticles and the water soluble fraction of crude oil on the marine copepod Calanus finmarchicus

Lethal and sublethal impacts of copper nano-pesticides on the energy budgets of starving estuarine amphipods

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Keywords: Nanotoxicology; DEBtox
Session: Mixture Toxicology and Multiple Stressors
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Estuaries are major recipients of run-off pesticides from agricultural and urban origin, including nano-based copper formulations. Advantages of nano-based copper formulations over ionic froms include better application control and slower release of copper, but the size and shape of nano formulation change the environmental behavior and toxicity profile of copper. The aim of this research is to assess the impact of those modern pesticides, in particular CuPRO, and Kocide, on an estuarine non-target species, the amphipod *Leptocheirus plumulosus.* The assessments are based on dynamic energy budgets. With this approach, due to its process oriented structure, toxicity assessment statistics are independent of exposure time and of choice of sublethal endpoints. Moreover, with this approach, the impacts of multiple stressors can be delineated, in this case those due to copper exposure and those due to food stress. Copper speciation was rapid, with little change observed in dissolved and aggregated copper after 1 day. The copper accumulation profile did not depend on the form in which copper was administered, but increased with dose. The impact of copper on respiration showed a regular dose-response pattern with little difference among copper formulations; the no-effect body burden and the body burden at which the respiration rate doubled was estimated from pooled data at 149 and 303 µg Cu/ g DW, respectively. Similarly, survival declined in a normal dose-response manner with no difference among formulations. The no-effect body burden and LC_{50} at day 7 were estimated at 188 and 291 µg Cu/ g DW. Sublethal impacts were more pronounced than lethal ones, especially before day 7, indicating that cannibalism has an mitigating impact on the decline of the total amount of biomass in the container. We conclude that the toxicity profiles of nano-copper and ionic copper are similar for this amphipod, which indicates that this species, being a detritus feeder, readily ingests nanoparticles.

Silver nanocolloid increases pathogenic infection risk following disruption of gut microbiota and immune system in medaka fish

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Keywords: silver nanocolloid, medaka, gut microbiota, infection
Session: Mixture Toxicology and Multiple stressors
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Immune homeostasis is achieved and maintained due to extensive interplay between gut microbiota and host mucosal immune system. Dysbiosis, which is unbalance of gut microbiota, have been noted for a large spectrum of human/vertebrates diseases. This study tested hypothesis that *"pollutant chemicals which have antibacterial activity may effect on immune system via gut-microbiota disruption"*. Silver nanocolloid (SNC) is an emarged nanoparticle due to its antibioticterial activity and used globally. Hence, to test this hypothesis, SNC was chosen as a chemical model.

Adult medaka (5 months old), which is commonly used as a typical freshwater fish model in toxicology and ecotoxicology, were employed in this study. Medaka exposed to SNC were subjected to Single Particle ICP-MS (SP-ICPMS) analysis to see SNC distribution in medaka body, to histlogical analysis of gut, and to metagenome analysis of gut microbiota. In addition, short chain fatty acid (acetic acid, propionic acid and butyric acid), which are known to activate host immune system and produced by gut microbiota, were measured quantitatively using LC-MS/MS. Also, expression of immune related genes (*NFκB p105*, *NFκB p100* and *TNFα*) in medaka exposed to SNC were measured by qRT-PCR. Furthermore, SNC-exposed medaka was tested bacterial challenge using *Edwardsiella tarda*. SP-ICP-MS analysis revealed large amounts of SNC (>90.0%) were accumulated in gut. Histological analysis showed number of exfoliated cell and vacuolated cell in SNC exposed medaka trends upward compared with control. In addition, number of clump trends downward by SNC exposure. These data indicates SNC accumulated in gut and then damaged gut tissue/microbiota. Metagenome analysis showed SNC changed gut microbiota in medaka; ratios of Proteobacteria phylum decreased and Fusobacteria phylum were increased. LC-MS/MS analysis indicated SNC decreased concentrations of acetic acid and butyric acid in gut with SNC concentration dependent manner. Furthermore, bacterial challenge demonstrated increase of pathogenic infection by SNC exposure. Through this study, SNC exposure declined immune system *via* disruptions of gut-microbiota and supression of immune relative genes in medaka; hence pathogenic infection increased. Imbalance of the symbiosis by SNC would facilitate bacterial infection to medaka.

Acknowledgement: The authors thank Professor Yasushi Okinaka (Hiroshima University, Japan) for his gift of *Edwardsiella tarda* and his scientific advice on the conduct of this project. This project was supported by research grants from the Kurita Water and Environment Foundation (to CK), and the Inoue Enryo Memorial Foundation for Promoting Science from Toyo University (to CK), and by two Grant-in-Aids for Challenging Exploratory Research (award 17K20047 to SK) and for the Strategic Research Base Project for Private Universities (award S1411016 to SK) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Multiple stressor effects of ionising (γ) radiation and non-ionising (UV) radiation in duckweed (*Lemna minor*)

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Keyword: Gamma radaition, UV radaition, combine effects, Lemna minor
Session: Mixture Toxicology and Multiple stressors
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In nature environment, aquatic biota is facing to the ionizing radiation emitted from natural occurring radionuclides where phenomenal of effects may also be enhanced by radiation from human activity such as nuclear power plants accident, nuclear medicine and weapon tests. Among different ionizing radiation types, the toxicity of high dose gamma (γ) radiation is frequently studied in different aquatic organisms such as mammals, fish, crustaceans, higher plants, and algae. However, there is still lack of knowledge about the toxic effects of low dose ionizing radiation and how other stressors such as ultraviolet radiation (UVR) cause multiple stressor effects in aquatic organisms. The objective of the present work was to study the combined effect of low-dose γ-radiation (13.2, 20.3 and 47.1 mGy/h) and UVR (UVB 0.5 w/m2) in the aquatic plant duckweed (*Lemna minor*) using a combination of genomic, functional and adverse toxicity endpoints. The results indicate that single γ-radiation reduced *L. minor* reproductive rate at a high dose (47.1 mGy/h, 7.9 Gy) after 7 days' exposure. At the cellular level, γ-radiation inhibited photosystem II (PS II) maximal efficiency (F_v/F_m) and oxidative phosphorylation (OXPHOS) and enhanced the non-photochemical quenching (NPQ), light-saturated PS II operating efficiency (F_v'/F_m'), electron transport rate (ETR) and reactive oxygen species (ROS) formation. Single UVR caused similar effects as IR and additionally induced morphological change (size and colony disconnection) in the plant. When exposed in combination, enhanced reproductive inhibition, OXPHOS reduction, PSII inhibition, NPQ and ROS formation were observed for the high γ-radiation dose (47.1 mGy/h). Antagonistic effects on F_v'/F_m', pigments content, photochemical quenching (qP) and ROS formation were observed at low to intermediate γ-radiation doses (13.2 and 20.3 mGy/h). Mechanistic studies to elucidate the mode of action (MOA) are will be used to identify the most relevant toxic pathways being perturbed by the single and multiple stressors tested. Combine study with radiation and chemicals are currently on going.

Acknowledgements: This work was supported by the Research Council of Norway through its Centres of Excellence funding scheme, project number 223268/F50

Increased sensitivity to benspyrene exposures in a fish cell-line when co-exposed to pharmaceuticals

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Mixture effects, PAH, CYP1A biomarker, fish
Session: Mixture Toxicology and Multiple Stressors
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The marine and other aquatic environments are continously polluted with mixtures of pollulants, including polyaromatic hydrocarbons (PAHs) and pharmaceuticals. Many fish populations reside and reproduce in areas that are exposed to chemical mixtures. Mixture toxicity is complex as it involves different mechanisms. Mixture toxicity can be a result of toxicodymamic interactions between different classes of chemicals based on their mode-of-actions (MOAs). Mixture toxicity can also be a result of toxicokinetic interactions between different chemicals that share common pathways for their elimination, independent of their MOAs. This study focuses on mixture effects between the PAH and arylhydrocarbon receptor agonist benspyrene and different classes of pharmaceuticals; the antimycotic clotrimazole (CLO), the glucocorticoid receptor agonist dexamethasone (DEX) and the macrolide antibiotic rifampicin (RIF). The PLHC-1 cell line was used as a fish liver model. The CYP1A biomarker response (EROD activity) was used to assess sensitivity to exposure to benspyrene alone and when mixed with a pharmaceutical for 24 and 48h. Cells were exposed to 5 or 10 nM benspyrene alone or mixed with either 5 or 10 µM CLO, DEX or RIF. Benspyrene was 3 orders of magnitude more potent than CLO to elicit the same CYP1A response. Exposures to DEX or RIF alone had no significant effect on the CYP1A biomarker. Adding the efflux-inhibitor RIF had no significant effect on the CYP1A biomarker. Cells exposed to benspyrene mixed with DEX had slightly higher CYP1A activities compared to cells that were exposed to benspyrene alone. The mechanism behind this synergistic mixture effect is likely a result of a stimulating crosstalk between the arylhydrocarbon and the glucocorticoid receptors. Cells exposed to benspyrene mixed with CLO displayed one order of magnitude higher CYP1A activities compared to cells that were exposed to benspyrene alone. The mechanism behind this synergistic mixture effect was probably caused by a toxicokinetic interaction between CLO and benspyrene. Thus, CLO inhibits the CYP1A enzyme which can prolong the halflife of the more potent CYP1A inducer benspyrene, as CYP1A enzymes are needed for benspyrene clearance. The study shows that toxicokinetic interactions on the chemical elimination pathway, either via a stimulating receptor crosstalk or via inhibition of CYP1A ezymes, can result in increased sensitivity to PAH exposure.

Acknowledgement: The authors thank University of Gothenburg, the funding agencies FORMAS (project number 942-2015-605) Sweden and the dCod and Digital Life Norway (project number 248840) Research Council of Norway.

Revealing the effects of defined persistent organic pollutant mixtures using H295R steroidogenic assay: an LC-MS/MS approach

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Keywords: H295R cell line, steroidogenesis, LC-MS/MS, endocrine disruption
Session: Mixture Toxicology and Multiple stressors
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The presence of environmental pollutants in our ecosystem may impose harmful health effects to wildlife and humans. Several of these toxic chemicals have a potential to interfere with the endocrine system. In our daily life, we are exposed to several of these endocrine disrupting chemicals at the same time. The aim of this work was to assess exposure effects of several environmentally relevant mixtures of these chemicals on steroidogenesis, using the H295R cell line model in combination with liquid chromatography tandem mass spectrometry (LC-MS/MS). By using this method, we could simultaneously analyse 19 of the steroids in the steroid biosynthesis pathway, providing a deeper insight into how the steroidogenesis is effected by pollutants. Our results showed a noticeable down-regulation in steroid production, when cells were exposed to the highest concentration of a mixture of brominated and fluorinated compounds. However, up-regulation was observed with estrone under the same condition, as well as with some other steroids when cells were exposed to the fluorinated mixture, and the mixture of chlorinated and fluorinated compounds. Other mixtures resulted in only slight deviations from the control. The method has the capability to be used as a high-throughput screening tool for endocrine disrupting chemicals.

Acknowledgment: This work is partially funded by Stress-POP project (project number: 213076) and dCod 1.0 project (project no. 248840) funded by the Norwegian Research Council. The Hormone laboratory at Haukeland University Hospital, Bergen is acknowledged for providing instruments and materials for this work. Also, Roger Lille-Langøy (staff engineer) is acknowledged for his helpful discussions and Silje Larsen (master student) for her contribution with cell culturing.

The use of mixtures of pollutants for toxicity testing. Experiences from past studies and plans for future testing.

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Keywords: Mixed pollution
Session Session: Marine Pollution 1
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In general, the history of toxicity testing has been dominated by assessment of single chemicals. This is not surprising, considering the need to assess the risk of exposure from chemicals released from specific activities. Normally in wild life populations and humans, a variety of chemicals can be detected, and consequently, data on occurrence and effects of mixtures of chemicals is needed. With the exception of dioxins and dioxin-like PCBs, the toxicological research has so far been devoted primarily to the study of single agents with little focus on combined effects of multiple chemicals. A key issue is whether combined chronic low-dose exposure to mixed stressors produce adverse health effects through additive or synergistic mechanisms when individual agents are present at levels that do not induce observable effects. For endocrine disrupters, there is unified agreement about additive effects of compounds in a mixture with similar modes of action. Thus, effects of exposure to mixtures may be expected even at doses well below no-observed adverse-effect levels (NOAELs) for the individual components.

At the Laboratory of Environmental Toxicology (MT-lab), Norwegian University of Life Sciences (NMBU), a variety of methods for toxicity testing have been used. Single chemicals have been used for exposure of model animals and *in vitro* studies, and in numerous studies, mixtures have been extracted from wild animals. These studies include steroidogenic effects in leydig cells, and steroid secretion in ovarian follicular cells (both pigs), following exposure with extracts from cod (*Gadus morhua*) liver oil. Also exposure to cod liver oil extracts influenced the steroidogenesis in the human H295R adrenocortical cell line. In a three generation exposure study, zebrafish (*Danio rerio*) was exposed to extracts from the highly exposed Lake Mjøsa burbot (*Lota lota*). This exposure enabled the study of population dynamic parameters such as growth, sex ratio, onset of puberty and follicle maturation, but also genome-wide transcription profiling. The zebrafish embryo model has been used at MT-lab as a inexpensive fast model for developmental and survival endpoints as well as genom transcription.

During the future work we intend to extract mixtures from different cod populations and test for possible differences in potency for toxic effects in different cod populations from Norwegian waters. It is intended to use in vitro models as well as the zebrafish model.

Acknowledgement: Acknowledgement: This study is part of dCod 1.0 (Project no. 248840), which is linked to Center for Digital Life Norway (DLN), financed by the Research Council of Norway (NFR).

Effect of environmental pollutants on virus activated innate immune system signalling pathways

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Keywords: Immunotoxicology, gallid herpresvirus, polychlorinated biphenyls
Session: Mixture Toxicology and Multiple Stressors
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Environmental pollutants like polychlorinated biphenyls (PCBs) are known to exert a high immunomodulating potential and their exposure in combination with virus infections has shown to result in increased host mortality. The immune system of host organisms is a yet overlooked part in the assessment of different severities during viral infections which cannot be explained by host genetics or virulence of the infecting strain alone. Especially, microRNA (miRNA) signalling pathways have shown to be modulated during viral infections which is linked to various adverse effects including cancer. In combination with pro- and anti-inflammatory cytokines, miRNAs like miR-155 represent an important line of defence against virus infections. The current in vitro study was performed using chicken embryo fibroblasts (CEFs) which were first exposed to a PCB mixture (Aroclor1260) before being stimulated with a synthetic RNA virus (poly(I:C)) or infected with the lymphoma-causing Gallid Herpes Virus 2 (GaHV-2). The expression pattern of miR-155, different pro-inflammatory cytokines (TNF-α and IL-8), transcription factor NF-κB1, as well as IL-4 as anti-inflammatory cytokine was investigated 8h, 12h or 18h after virus inoculation using quantitative real-time polymerase chain reaction (qPCR). The study demonstrated an Aroclor1260 mediated downregulation of miR-155 expression in poly(I:C) treated cells up to 12h whereas GaHV-2 infected cells showed an increased miR-155 expression after 12h in combination with Aroclor1260. Furthermore, Aroclor1260 increased the expression of pro-inflammatory cytokines after 8h in poly(I:C) treated cells whereas GaHV-2 infected cells were unaffected. After 12h, most of the investigated cytokine genes were downregulated independent of Aroclor1260 exposure in poly(I:C) treated as well as GaHV-2 infected CEFs. The observation was assumed to be linked to a lack of additional danger signals provided by poly(I:C), downregulation measures by GaHV-2 and the fact that fibroblasts were assumed to represent a rather early source of pro-inflammatory signals during viral infections. After 18h, upregulation measures seem to be induced again which might be due to recovery processes and the lack of regulatory signals provided by other cell types. Taken together, the current study provides evidence that PCBs can modulate highly regulated innate immune system signalling pathways in poly(I:C) treated as well as GaHV-2 infected CEFs.

Acknowledgement: The authors thank Jose Castaño-Ortiz for the assistance during the laboratory work as well as Prof. Bernd Sures and Nathalie Briels for giving critical advice.

Effects of perfluorooctane sulfonate (PFOS) on the antiviral immune response using an *in vitro* model

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Keywords: perfluorooctane sulfonate, immunotoxicology, microRNA, gallid herpesvirus
Session: Mixture Toxicology and Multiple stressors
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Modulation of the immune function is a major toxicological concern associated with per- and polyfluoroalkyl substances (PFASs), and a link between exposure and prevalence of infectious diseases has been established in humans and wildlife. It has recently been suggested that the expression of microRNA-155 (miR-155) and related genes constitute an important innate host response against viral infections, since deregulation of this pathway is linked to increased host susceptibility and influences disease outcomes. In this study, perfluorooctane sulfonate (PFOS)-mediated changes in immune gene expression were investigated *in vitro* using a virus-exposed host, namely primary chicken embryo fibroblasts (CEFs). Briefly, CEFs were experimentally exposed to perfluorooctane sulfonate (PFOS) (22.2 ppm) before stimulation with either Gallid herpesvirus-2 (GaHV-2) or a RNA viral analogue (Poly (I:C)). We harvested CEFs at different post-infection time points (6h, 12h, 18h and 24h) to address time-dependent changes in immune gene expression (miR-155, NF-kB, TNF-α, IL-8 and IL-4), using quantitative real-time polymerase chain reaction (qPCR) analysis.

In response to *in vitro* exposure to PFOS, this study detected a trend towards downregulation of the baseline expression of cytokines (IL-8 and IL-4) and the transcription factor NF-kB in CEFs (p-values<0.16). Nonetheless, the expression of our target genes was not altered following combined exposure to PFOS and stimulation with viruses. This may relate to the fact that GaHV-2 and Poly (I:C) did not stimulate target gene expression in the first place (without PFOS) at the investigated time points, which was an assumption to study modulation of the viral response by PFOS. Implementing earlier harvesting time points could provide a better insight into induction of pro-inflammatory signals by fibroblasts, as they may trigger danger cues at very early stages during viral infections. Therefore, although this study failed to identify modulation of the viral response after exposure to PFOS, we provide further mechanistic evidence that this compound could interfere with the baseline expression of important immune signalling components.

Acknowledgement: The authors thank Alexander M Badry for assistance in the laboratory and NTNU for funding support through Dr. Waugh's postdoctoral fellowship.

Effects of engineered silver nanoparticles and the water soluble fraction of crude oil on the marine copepod *Calanus finmarchicus*

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Keywords: Engineered nanoparticles, oil, marine copepods, oxidative stress
Session: Mixture Toxicity and Multiple Stressors
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Nanotechnology is increasingly used in the oil and gas industry, with applications in drilling and hydraulic fracturing fluids, enhanced oil recovery, heavy oil viscosity reduction and hydrocarbon detection. Engineered nanoparticles (ENPs) can thus potentially reach the environment together with oil components. In this study we investigated the acute toxicity of polyvinylpyrrolidone (PVP) coated silver nanoparticles (AgNPs) and ionic silver (Ag+) on the marine copepod *Calanus finmarchicus*, which is one of key species of the North Atlantic food web. Further, we evaluated oxidative stress responses on gene expression- and enzymatic level after exposure to AgNPs (two concentrations, low: NPL, high: NPH), Ag+, and the water-accommodated fraction (WAF) of crude oil as single components and the respective mixtures of AgNPs or Ag+ with WAF. Results show that Ag+ was approximately 3 times more toxic in acute tests than AgNPs, with 96 h LC50 concentrations of 403 µg L-1 and 147 µg L-1 for AgNPs and Ag+, respectively. Ag uptake was similar between AgNP and Ag+ exposures, and was not impacted by the presence of WAF. However, AgNPs were found to be more associated with animal's surfaces in the presence of WAF. Silver nanoparticle exposure caused an increase in gene expression of SOD, GST, HSP90 and CYP330A1, indicating oxidative stress responses in exposed animals. Alterations of enzymatic activities were generally low, with significant effects seen only on SOD activity in NPL exposures, while NPL and NPH caused increased activities of GST. Combined AgNPs+WSF exposures caused only slightly altered responses in SOD, GST and CYP330A1 gene expression compared to the single exposures of either NPs or WSF, showing that interactive effects of AgNPs and WAF were relatively limited within the applied experimental conditions.

Acknowledgement: The authors thank the Research Council of Norway; projectnumber: 216464/E40

Session 4
Ecotoxicology and Systems Toxicology-dCod1.0

Session Chairs: Anders Goksøyr and Iurgi Imanol Salaverria-Zabalegui

Talks

Anders Goksøyr: dCod 1.0: decoding the systems toxicology of Atlantic cod *(Gadus morhua)* - an introduction

Odd André Karlsen: The nuclear receptor complement of Atlantic cod *(Gadus morhua)* as potential targets for endocrine disrupting compounds

Libe Aranguren-Abadía: Characterization of the aryl hydrocarbon receptors Ahr1a and Ahr2a in Atlantic cod (*Gadus morhua)*

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Sofie Söderström: Effects of perfluorinated compounds on Atlantic cod *(Gadus morhua)* PPARαa and PPARαb nuclear receptors

dCod 1.0: decoding the systems toxicology of Atlantic cod (*Gadus morhua*)

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Keywords: ecotoxicology; systems biology; fish; toxicogenomics
Session: Marine Pollution 1: *Ecotoxicology and systems toxicology (including dCod 1.0)*
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The goal of the dCod 1.0-project is to combine competencies in environmental toxicology, biology, bioinformatics and mathematics across the traditional department boundaries, to create a deeper understanding of the Atlantic cod's adaptations and reactions to stressors in the environment. Building on thorough studies and mapping of the cod genome and long research traditions on the physiology, toxicology and reproduction biology of cod, the dCod project will expand our knowledge with genomics based methods, where studies of how the cod genome responds under different environmental conditions will be investigated.

dCod 1.0, in synergy with iCod 2.0, aim to generate large amounts of experimental data using *in vitro*, *ex vivo*, aquaria and field studies, to be the basis of mathematical models that can describe responses based on different scenarios. Overall, the ambition is to create a tool for environmental monitoring and risk assessment that can be used in assessing the impacts of for example the oil industry, aquaculture, sewage discharge into harbours and industrial discharge into Norwegian fjords. In this presentation we will highlight the experiences during the first period involving transdisciplinary activities, establishing a shared language, and the responsible research and innovation (RRI) aspects of the project.

the dCod 1.0 project is funded under the Digital Life Norway initiative of the BIOTEK 2021 program, and the iCod 2.0 project under the ECOSYSTEM program of the Research Council of Norway (project no. 248840 & 244564).

The nuclear receptor complement of Atlantic cod (*Gadus morhua*) as potential targets for endocrine disrupting compounds

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Keywords: reporter gene assays, perfluorinated compounds, peroxisome proliferator-activated receptor
Session: Ecotoxicology and systems toxicology (including dCod 1.0)
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Nuclear receptors are members of a large superfamily of ligand-activated transcription factors that are involved in the regulation of essential physiological processes, such as embryonic development, differentiation, maturation and reproduction, as well as metabolic homeostasis. However, it has been shown that nuclear receptors can be molecular targets for environmental pollutants that are mimicing the structure of their endogenous ligands. Such compounds can act as agonists or antagonists, and disrupt crucial and fine-tuned cellular signaling pathways. Among the most described interactions between nuclear receptors and endocrine disruptors, is the activation of the estrogen receptor by ethynylestradiol in teleosts, which eventually may cause feminization of male fish and loss of their ability to reproduce. We have recently mapped the full complement of nuclear receptors in the Atlantic cod genome (72 NRs distributed into 8 subfamilies), and we have established luciferase-based reporter gene assays for a selected set of these genes, including the androgen receptor (AR), estrogen receptor (ERα), peroxisome proliferator-activated receptors (PPARαa, αb, β/δ, γ), vitamin D receptors (VDRα, β), as well as the bHLH-PAS aryl hydrocarbon receptors (AHR1a, 2a). Notably, the pregnane X receptor (PXR) gene, which is a highly important xenosensors in most fish and other vertebrates, is not present in the Atlantic cod genome (Eide et al., submitted). The reporter gene assays are used as screening tools with a structurally diverse library of both emerging and legacy pollutants, as well as mixtures of pollutants, for identifying compounds that can act as endocrine disruptors. One of our recent findings is the selective agonistic activation of PPARαb by the perfluorinated carboxylic acids, PFOA and PFNA. Interestingly, the sulfonated perfluorinated compound, PFOS, did not act agonistic, nor antagonistic, on neither PPARαa or PPARαb. However, co-exposures of PFOS together with the PPARα model-agonist, WY-14643, showed potentiating effects of PFOS for both PPARα subtypes. These results may suggest an allosteric binding of PFOS that putatively stabilizes the activated conformation of the PPARα receptors and facilitates recruitment of cofactors needed for initiating transcription of the reporter gene.

Acknowledgement: This work is supported by the projects iCod2.0 (244564) and dCod1.0 (248840) funded by the Research Council of Norway.

Characterization of the aryl hydrocarbon receptors Ahr1a and Ahr2a in Atlantic cod (*Gadus morhua*)

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Keywords: Ahr, Arnt, Atlantic cod
Session: Ecotoxicology and systems toxicology (including dCod 1.0)
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The aryl hydrocarbon receptor (AHR) is a transcription factor that regulates the expression of important enzymes involved in the biotransformation of xenobiotics. The main groups of pollutants involved in AHR activation are polycyclic aromatic hydrocarbons (PAHs), dioxins, and co-planar polychlorinated biphenyls (PCBs). Atlantic cod (*Gadus morhua*) is important to North Atlantic fisheries industry and is commonly used as an indicator species for marine pollution. Recently, the cod genome was sequenced and phylogenetic analyses revealed two Ahr-encoding genes, *ahr1a* and *ahr2a*. In order to expand our understanding on how Atlantic cod cope with environmental stressors, increased knowledge of Ahr-mediated xenobiotic responses is important. The cod *ahr1a* and *ahr2a* were therefore cloned and used in *in vitro* luciferase reporter gene assays, together with the Atlantic cod aryl hydrocarbon receptor nuclear translocator (Arnt). To compare the transcriptional activity of the cod Ahrs, four model AHR agonists, including 6-formylindolo [3,2-b] carbazole (FICZ), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), β-naphthoflavone (BNF), 3,3´,4,4´,5-polychlorinated biphenyl (PCB 126), and benzo[a]pyrene (B[a]P) were used as agonists in the assays. Tissue specific expression of *ahr1a*, *ahr2a, arnt1 and arnt2* were determined by quantitative real time PCR (qPCR) analyses in 12 different tissues. Furthermore, velocity sedimentation assays were performed to assess the ligand binding affinity of ^{3}H[TCDD] or ^{3}H[BNF] to cod Ahr1a and Ahr2a. The receptor with the highest binding affinity to ^{3}H[TCDD] and highest activation for the majority of the agonist compounds tested was Ahr1a, whereas Ahr2a was more strongly expressed in all the tissues sampled. The endogenous ligand, FICZ, was the most potent compound for both receptors in the luciferase gene reporter assays. Interestingly, TCDD and PCB-126 produced different activation profiles for Ahr1a and Ahr2a, where Ahr1a was activated at lower concentrations and had a higher activation response for both compounds. In conclusion, an Arnt-based luciferase gene reporter system has been established for Atlantic cod and the different activation and tissue specific expression indicate a different functional specialization of the Ahrs. Whereas Ahr2 forms have been shown to be involved in mediating toxicity in most fish species, these results suggest a possible role of Ahr1a in mediating toxicity in Atlantic cod.

Acknowledgement: This work has been funded by the Ocean Outlook fellowship 2017, a collaboration between Woods Hole Oceanographic Institution and Bergen Marine Research Cluster and the Research Council of Norway projects iCod2.0 (244564) and dCod1.0 (248840).

Transcriptome responses in Atlantic cod (*Gadus morhua*) precision-cut liver slices exposed to benzo[a]pyrene and 17α-ethynylestradiol

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Keywords: *ex vivo*, estrogens, anti-estrogens, AHR, ER
Session: Ecotoxicolology and systems toxicology
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Environmental chemicals that activate the aryl hydrocarbon receptor (AHR) and the estrogen receptor (ER) pathways are among pollutants of major concern. The aim of this study is to map transcriptome responses in Atlantic cod (*Gadus morhua*) precision-cut liver slices (PCLS) exposed to two model compounds benzo[a]pyrene (BaP) and 17α-ethynylestradiol (EE2) that activate the AHR and ER pathways, respectively.

We exposed *ex vivo* liver slice cultures to BaP and EE2, alone or as binary mixtures for 48 hours, and performed in-depth RNA-seq based transcriptome mapping followed by systematic bioinformatics analyses. Liver slices from each fish (total n = 8) were used in seven treatment groups consisting of DMSO (control), BaP (10 nM and 1 µM), EE2 (10 nM and 1 µM), equimolar mixtures of BaP and EE2 (10 nM and 1 µM). In total, 47 RNA samples were sequenced (approximately 50 million, 75 bp paired-end reads per sample) using Illumina HiSeq 4000.

The exposure of liver slices in culture to BaP and EE2 resulted in significant differential expression (mostly up-regulation) of several genes. Strong up-regulation of genes in the AHR pathway such as genes coding for the cytochrome P450 1a (CYP1A) enzyme and the AHR repressor (ahrrb) was observed in BaP treated liver slices. EE2 treatment of liver slices resulted in up-regulation of higher number genes including genes coding for precursors of vitellogenin (VTG) and eggshell zona pellucida (ZP) proteins. The deep sequencing has also enabled us to identify some genes previously not shown to be induced by estrogens. Pathway enrichment and network analysis of the differentially expressed genes showed that the AHR and ER pathways are among the top affected by BaP and EE2 treatments, respectively. Anti-estrogenic effects of the AHR ligand BaP was also observed, as evidenced by lower number of EE2 responsive genes differentially expressed in the mixture treated groups compared to EE2 alone.

The results give further mechanistic insights into the AHR and ER pathways and environmental compounds that activate them. The transcriptome analysis also demonstrates the usefulness of PCLS culture for omics studies.

Acknowledgement: The study was supported by the dCod 1.0 (248840) and the iCod 2.0 (244564) grants from the Research Council of Norway.

Sequence variations in *pxr* (*nr1i2*) from zebrafish (*Danio rerio*) strains affect nuclear receptor function

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Keywords: allelic variation, pregnane X receptor, biotransformation
Session: Ecotoxicology and systems toxicology (including dCod 1.0)
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Regulators of biotransformation are of particular interest in pharmacology and toxicology, determining in part the metabolism, disposition and toxicity of chemicals. The nuclear receptor NR1I2 (pregnane X receptor, PXR) is a prominent xenosensors that regulates expression of biotransformation enzymes governing elimination of many exogenous as well as endogenous compounds. Zebrafish (*Danio rerio*) has only one gene locus for pxr, but variants have been identified in zebrafish, but the extent and significance of these variants is unknown. We hypothesize that sequence variation occurring in the Pxr gene of zebrafish may affect the action of many chemicals in this species, a key model organism in various fields of research, including environmental toxicology. Here, we examine variation in Pxr sequences from four different strains of zebrafish and assess the responses of each Pxr to clotrimazole and butyl-4-aminobenzoate. Pxr variants differed in their ability to bind these structurally diverse ligands and to regulate reporter gene expression, *in vitro*. We infer that the observed sequence variations in zebrafish Pxrs likely affect the response to putative Pxr agonists and cause strain-specific biotransformation of xenobiotics in zebrafish. Thus, the choice of zebrafish strain could affect the outcome of downstream toxicological studies.

Acknowledgement: This work was supported by the Norwegian Research Council Programs Environment 2015 Project 181888 (AG) and BIOTEK2021 Project 248840 (AG), The Bergen Research Foundation (LMM), and the Boston University Superfund Research Program NIH 5P42ES007381 (JJS and JVG) and NIH 5U41HG003345 (JVG).

Ecotoxicologial responses in Atlantic cod (*Gadus morhua*) after caging at a capped waste disposal site in Kollevåg, Western Norway

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Keywords: Ecotoxicology, Atlantic cod, biomarkers, caging
Session: Marine Pollution 1 (dCod)
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The bay of Kollevåg in Askøy outside of Bergen was utilized as a waste disposal site from 1930 to 1975. In the following years, the garbage site was capped by sand and stone and reopened as a recreational area in 1983. However, despite additional measures to cover the waste, the surrounding sediment and biota are polluted. In this project, we wanted to investigate the possible effects of released contaminants using caged Atlantic cod (*Gadus morhua*). Juvenile cod were caged at three different locations in the Kollevåg area and at a reference location for six weeks (August - October 2016). During the sampling campaign, more than 1000 samples were collected from 82 fish. Sediment and tissue samples (bile and liver) were subjected to chemical analyses, which detected a gradient of exposure to contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) among the four locations. Furthermore, liver somatic index (LSI) and k-factor were significantly decreased in fish from the innermost station compared to the reference station. However, the degree of oxidative stress measured using the thiobarbituric acid reactive substances (TBARS) assay, did not reflect the burden of contaminants. Established biomarkers, such as vitellogenin, heat shock protein 70 (Hsp70), metallothionein (MT), and biotransformation enzymes (Cyp1a, Cyp3a, Gst, Cat), were quantified in several tissues. Surprisingly, whereas the enzyme activities of Cat and Gst were significantly reduced in the caged fish in the two innermost stations of Kollevåg, levels of Cyp1a and Vtg do not seem to be affected. Transcriptomics and proteomics analyses will be used to reveal more details on mechanisms behind the observed changes, so far reflecting a moderately polluted situation.

Acknowledgement: The Kollevåg study is part of dCod 1.0 (Project no. 248840), a large project linked to Center for Digital Life Norway (DLN), financed by the Research Council of Norway (NFR). This work is also supported by Bergen municipality and by the iCod 2.0 project (Project no. 244564) funded by NFR.

Adverse health effects related to chemical exposure in Atlantic cod (*Gadus morhua*) from Norwegian fjords

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Keywords: Atlantic cod, biological effects, chemical exposure
Session: Marine Pollution 1 (dCod)
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Introduction and aims: Environmental pollutants and climate change may both have an impact on the health and well-being of Atlantic cod found along the Norwegian coastline. The present study is a part of the dCod 1.0 project on the systems toxicology of Atlantic cod, which aims to create tools for environmental monitoring and risk assessment by combining environmental toxicology, biology, bioinformatics and mathematics. This study assessed potential adverse health effects related to chemical exposure in Atlantic cod from known polluted Norwegian fjords by linking exposure levels, pathological and physiological phenotypes, biomarkers and topological data analysis.

Materials and methods: Atlantic cod was caught by trawling from the inner (polluted) and outer (reference) Oslofjord (OF) in November 2016, and from Frierfjord (FF) (polluted) and outer OF in March 2017. Blood and organs were sampled for chemical analysis and assessment of biological effects, including biliary polycyclic aromatic hydrocarbons (PAH) metabolites (HPLC/F), heavy metals and persistent organic pollutants (POPs) (GC/MS) and perfluorinated compounds (LC/MS-MS) in liver, DNA-damage in white blood cells (Comet assay), histopathology and plasma levels of steroid hormones, enzymes, proteins and lipids.

Results: In general, the levels of PAH metabolites, POPs and heavy metals were higher in the inner OF compared to the outer OF. The median liver lipid contents in fish from the inner OF, outer OF and FF were 57%, 48% and 15%, respectively. Cod from the outer OF had significantly higher plasma levels of proteins (albumin, total protein) and lipids (triglycerides, free fatty acids) than the inner OF, suggesting alterations in liver physiology, such as lipid metabolism. Furthermore, histopathological findings were more prevalent in the outer OF compared to inner OF, with inflammation, granulomas and/or steatosis in 60% of the cod livers from outer OF compared to 20% in the inner OF. Analyses of cod from FF are ongoing and will be included at the conference.

Conclusion: This study suggests that despite the higher levels of chemical exposure in the inner OF and FF, unknown environmental factors may affect cod health in the outer OF. Genomics and proteomics analyses will be performed to relate the phenotypic findings to genotype.

Acknowledgement: dCod 1.0 (Project no. 248840) is associated with the Center for Digital Life Norway (DLN) initiative of the BIOTEK 2021 program, and the iCod 2.0 project under the ECOSYSTEM program of the Research Council of Norway (project no. 244564).

What can 'omics tell us?

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Keywords: ecotoxicology; fish; toxicogenomics; metabolomics, proteomics
Session: Marine Pollution 1: *Ecotoxicology and systems toxicology (including dCod 1.0)*
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Biological sciences are currently undergoing a silent revolution: over the past decade we have become able to quantify the majority (or so we believe) of the gene transcripts, proteins and small molecules in cells and tissues. These analyses generate thousands and millions of data points and provide a snapshot of the state of a cell, frozen in time. The reason we want this information in ecotoxicology is not only to understand the inner workings of cells, but to be able to project and predict the consequences of external stressors on cellular health and eventually health of the individual. We are all aware of studies wherein exposure to different concentrations of the same substance yields entirely different transcript profiles, not to mention what happens when we mix substances. The situation is somewhat akin to photographs of a football match taken minutes apart – although the players are the same, absolute positions and their influence on the game change continuously.

Thus, we wish to predict processes from a snapshot, sometimes a series of snapshots. Although containing thousands of data points, it is still the situation at one point in time. The assumption is clearly that the presence of a toxicant within a cell will elicit persistent processes relating to e.g. biotransformation, lipid metabolism or changes in the cytoskeleton that will be evident in the transcriptome and/or proteome. There are a couple of strategies by which to address the inherent variability in the system: one is to link transcripts, proteins and metabolites to strengthen putative association between exposure and specific responses, the second to allocate responses to defined pathways, as is already being done, but which should be adapted to the species under study to a larger extent than being done at the moment. Although we may miss components present at low concentrations in our analyses, some of which may have important roles, available omics profiles have indicated that toxicant stress to cells affect processes such as e.g. the cell cycle, biotransformation, lipid metabolism, immune responses, cellular signalling, the cytoskeleton and energy turnover, all of which appears likely from results from other analyses. The ability to recognise and eventually quantify the magnitude of changes to specific processes is a promising path towards future integration of omics data with physiological and histological changes in the organism. We still face many challenges to grasp how toxicants impacts cells, in understanding the modulating effects of e.g. miRNAs, the dynamics of cellular signalling and radical generation in cells, as well as critical aspects of cell physiology such as membrane transport and DNA fragmentation. The most promising way forward at the moment is to combine omics profiles with cellular and organismal physiology, histological changes in tissues and modulation of individual behaviour.

The aim of this presentation is to review the limitations and possibilities of data generated through the use of omics technologies and how they can be integrated with our understanding of physiological processes within the organism. The concepts will be discussed in the context of the systems toxicology approach used within the dCod 1.0 project.

The dCod 1.0 project is funded under the Digital Life Norway initiative of the BIOTEK 2021 program of the Research Council of Norway (project no. 248840).

Cognitive tests within toxicology: suitable indicators of neurotoxic effects of environmental pollutants in Atlantic cod (*Gadus morhua*)?

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Keywords: cognition, toxicology, Atlantic cod
Session: Marine Pollution 1 (dCod)
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The ability to exhibit flexibility in behavioural patterns, particularly in a changing environment, requires fish to possess the capacity to learn, remember and update information. Cognition is the mental process of acquiring knowledge, memory, evaluation and comprehension. Reduced cognitive abilities may reduce an animal's overall fitness, through alterations in reproductive capacity, behaviour and the ability to cope with a changing environment, such as climate change.

In classical conditioning, the animal learns to associate a neutral stimulus (conditioned stimulus, CS) with an event that elicits a response without training (unconditioned stimulus, US). When an association is established, the CS elicits a response similar to the response elicited by the US. To learn an association between stimuli separated by a temporal gap (trace conditioning) is dependent on advanced brain functions. It is well known that fish, including Atlantic cod, can easily be conditioned to associate a CS with a US. However, whether trace conditioning is influenced by exposure to environmental pollutants in Atlantic cod is not previously studied.

The aims of the study are to investigate if associative learning is a suitable indicator of neurotoxic effects of environmental pollutants in Atlantic cod, and if effects on this type of cognitive process are associated with changes in neurotransmitter levels in the cod brain.

Juvenile Atlantic cod (N=96) was injected intraperitoneally with chlorpyrifos and/or cypermetrin. We tested a simple conditioning task where the fish came to a feeding ring in response to a light cue to receive food. To test if the fish learned that light (CS) is associated with feeding (US), we recorded the latency time from applying light (for 5 s) until the fish respond to this light by swimming to surface within the ring, before feed was introduced 20 s after the light has been switched off. Appetite was also included as an endpoint. Acetylcholinesterase inhibition and levels of monoamines (dopamine, norepinephrine, epinephrine, histamine, serotonin) were measured in the brains. Analyses are ongoing and results will be presented at the conference.

Acknowledgement: This study is part of dCod 1.0 (Project no. 248840), which is linked to Center for Digital Life Norway (DLN), financed by the Research Council of Norway (NFR).

Establishing A Cell-Free Method for Xenobiotic Detection Using Ligand Activated Receptors from Atlantic Cod (*Gadus morhua*)

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Keywords: Ecotoxicology, xenosensors, vitamin D receptor, AlphaLISA
Session: Ecotoxicology and systems toxicology (including dCod 1.0)
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We are developing receptor-ligand based xenosensors for use in detector systems for contaminant monitoring. This is a new method to study the interaction between ligands and receptors based on a modified version of AlphaLISA. It is a bead-based, cell-free technique, based on stable, recombinant expression of receptor protein constructs.

The coastal and more stationary Atlantic cod (*Gadus morhua*) is becoming more frequently used as a model species, for environmental monitoring in the North Atlantic Ocean. Coastal ecosystems are affected by pollution from both land and sea; fertilizers, sewage disposal, oil, and industrial chemicals will all eventually end up in the ocean. The chemical defensome is a collection of genes that participates in responses to environmental stressors, and includes the cytochromes P450 (CYPs). The CYPs are a large superfamily of proteins that are important to metabolize toxic compounds. The transcription of many CYPs is regulated by certain ligand-activated receptors, denoted as xenosensors. Xenosensors contains a ligand-binding domain (LBD) and a DNA-binding domain (DBD). As a proof of concept of the cell-free assay we are focusing on the Vitamin D receptors (VDR) in cod. VDR is part of the NR1I-subgroup of the nuclear receptors, which also includes the important xenobiotic sensor, pregnane X receptor (PXR). VDR is naturally activated by vitamin D3 or calcitriol and controls the transcription of genes that have important roles in regulating the calcium and phosphate homeostasis in vertebrates. Two isoforms of VDR are present in Atlantic cod; VDRα and VDRβ. Other roles of the VDRs, apart from regulating the synthesis of calcitriol, is not known, but due to the lack of PXR in cod VDR may have adopted a broader functional role. VDR in cod seems to be expressed in most tissues, which is in contrast to PXR that normally is highly expressed in the tissues that accumulate and degrade chemical compounds, such as the liver and intestine.

Calcitriol was found to activate both VDRα and β in Atlantic cod using a luciferase gene-reporter assay. Ligand activation assays were also performed with bile acid derivatives, and various exogenous compounds, including environmental contaminants. However, none of these compounds were found to activate VDR. Here, cloning and recombinant expression of cod VDRs for use in AlphaLISA is described.

Acknowledgement: The project is part of the dCod 1.0 project, funded by the Research Council of Norway (248840), linked to Center for Digital Life Norway.

Modulation of Steroidogenesis in Female Juvenile Atlantic Cod (*Gadus morhua*) exposed to Polycyclic Aromatic Hydrocarbons (PAHs) and Perfluoroalkyl substances (PFASs)

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Keywords: fish; ecotoxicology; ovarian steroidogenesis
Session: Ecotoxicology and systems toxicology (including dCod 1.0)
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Polycyclic aromatic hydrocarbons (PAHs) and perfluoroalkyl substances (PFASs) have been associated with adverse health effects in several model organisms. They are very persistent in aquatic environments and bioaccumulate in tissues, causing reproductive and developmental toxicity in organisms. Thus, the present study has investigated gonadal and interrenal steroidogenic responses after exposure to different doses of PAHs and PFASs in female cod. Benzo[a]pyrene, dibenzothiophene, fluorene, naphthalene, phenanthrene and pyrene were used to prepare a PAH mixture, while perfluorononanoic- (PFNA), perfluorooctanoic (PFOA), perfluorooctanesulfonic (PFOS) and perfluorotridecanoic acid (PFTrA) were used to prepare PAH and PFAS mixture respectively, that were injected intraperitoneally to female cod. Fish were exposed twice to either low (1x) or high dose (20x) of each compound mixture, whereas the control group was exposed to solvent vehicle (1:1 of oil and PBS). The expression pattern of steroidogenic enzyme genes including, *StAR*, *P450scc*, *3β-HSD*, *cyp17*, *cyp19a*, *11β-hsd*, *17β-hsd*, and *20β-hsd* were analysed in the ovary and head kidney using real-time PCR. Plasma concentration of 17β-estradiol (E2) and testosterone (T) were determined using enzyme immunoassay (EIA). In general, both mixtures produce an apparent concentration-specific change in the expression patterns of steroidogenic enzyme genes. Ovarian tissue showed decreases in the expression of StAR, 3β-hsd, cyp17 and cyp19a and increases in P450scc and 17β-hsd mRNA, whereas 11β-hsd was not affected by either mixture. Contrary to the gonad, head kidney responded differently, showing that PAHs increased the expression of StAR, which was not affected by PFASs. Both mixtures caused an increase- and decrease of interrenal 3β-hsd and cyp17, respectively. Furthermore, results of RNA sequencing supported our hypothesis that ovarian steroidogenesis is one among several cellular pathways that were affected by PAH exposure. Overall, the present findings suggest potential consequences on fish reproductive success and survival because of the significant role played by hormones in fish metabolism, adaptation and response to stress, reproduction and sexual maturation.

The dCod 1.0 project is funded under the Digital Life Norway initiative of the BIOTEK 2021 program of the Research Council of Norway (project no. 248840).

Effects of perfluorinated compounds on Atlantic cod (*Gadus morhua*) PPARαa and PPARαb nuclear receptors

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Keywords: environmental toxicology, peroxisome proliferator-activated receptor alpha (PPARα) , perfluorinated compounds (PFCs), luciferase reporter gene assay (LRA)
Session: Ecotoxicology and systems toxicology
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Atlantic cod (*Gadus morhua*) resides in habitats near offshore oil platforms, petroleum recovery facilities, as well as coastal industries and municipal wastewater treatment plants, thus it must cope with both legacy and emerging environmental contaminants. Considering the number of anthropogenic chemicals present in the environment, our knowledge is still scarce regarding how contaminants, alone or in mixtures, affect the biology of cod. To address potential effects on its energy homeostasis, we focus on the peroxisome proliferator-activated receptors (PPARs), which are ligand-activated transcription factors that regulate, inter alia, the expression of genes involved in the lipid- and glucose metabolism. Atlantic cod has four expressed PPAR-subtypes (PPARαa, PPARαb, PPARβ/δ, and PPARγ). In this study, we focus on characterizing and comparing PPARαa and PPARαb in regard to their activation profiles. *In vitro* screening of ten perfluorinated compounds (PFCs) ability to activate cod PPARαa- and PPARαb ligand binding domain-constructs was carried out with a COS-7 cell-based luciferase reporter gene assay (LRA). Of the three perfluorinated sulfonic acids (PFSAs) tested, only PFHxS (C6-S) activated the PPARαb construct, while none of the PFSAs activated the PPARαa construct. When screening the seven perfluorinated carboxylic acids (PFCAs), only the PPARαb construct was activated. Notably, the potency of the PFCAs increased with increasing carbon number (C) from C4 to C8, thereafter the activation decreased with carbon numbers larger than C9. Exposure to a binary mixture of PFOS and PFOA was tested on both cod PPARα constructs, where the mix elicited a higher activation of the PPARαb construct compared to the single compound exposures. The binary mixture had no effect on the PPARαa construct. When the agonist, WY14643, was exposed in a binary mixture together with either PFOS or PFOA, the activation potency of the mixture was generally higher than when WY14643 was acting alone, this was true for both the PPARαa- and PPARαa construct. From this study, we can conclude that the activation potential of the PFCAs was higher compared to their PFSA counterparts when used in single compound exposures, as well that the activation potency depends on the number of carbons in the PFC backbone. Thus, exposure of Atlantic cod to PFCs with 6 to 9 carbons, could potentially modulate the lipid- and glucose metabolism through directly interfering with at least one PPARα-subtype.

Acknowledgement: The project is funded by the Research Council of Norway grant iCod 2.0 (project no. 244564) and dCod 1.0 (project no. 248840).

Session 5
Plastic debris - pathways and impacts in Arctic and boreal systems

Session Chairs: Claudia Halsband, Dorte Herzke, Andy Booth, and Martin Wagner

Talks

Invited Plenary Professor Shoshaku Kashiwada: Globally distributed plastic debris and environment-dependent toxicity

Marte Haave: Microplastic occurrence and distribution from discharge points to deep basins in an urban model fjord

Tânia Gomes: Understanding the distribution and impact of micro- and nano-plastics in Norway

Claudia Halsband: Is crumb rubber a source for pollutants and harmful effects in the marine environment

Iurgi Salaberria: Benchmarking the uptake and excretion dynamics of microplastics in the boreal marine copepod *Calanus finmarchicus*

Posters

Andy Booth: Marine Microplastic: Realistic test materials for laboratory studies of ecosystem impacts

Andy Booth: Exploring the effects of nylon microplastic on the development and energy reserves in coldwater copepods

Isabel Sofía Abihssira García: Potential impact of microplastics on the environmental footprint of Atlantic salmon farming

Monica Sanden: Surveillance of microplastics (MPs) in seafood – ongoing activity and future perspectives at Institute of Marine Research (IMR)

Invited Plenary

Globally Distributed Plastic Debris and Environment-Dependent Toxicity

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Keywords: nanotoxicology, plastic debris, salinity, temperature
Session: Marine Pollution 2
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Plastic debris in ocean was firstly reported in 1988 papar published by NOAA of the United States. In the paper, it was named for “Great Pacific Garbage Patch”, but actually it is more akin to a diffuse soup of plastic floating, and not visible even no islands of trash in ocean. In the North Pacific Ocean, plastic trashes released from coastal area of the North Pacific Ocean, inclusing the USA, Japan, Korea, China, Taiwan, Phillippine, Indonesia and other countries, are drawn into the North Pacific Gyre. Plastic debris are able to be degraded to small size by UV radiation and other environmental factors; however they will remain as environmentally persistence pollutants. Continuous drawn of plastic debris into the Gyre will lead to concentrate of marine debris, and then the Great Pacific garbage patch forms graduatelly. Not only North Pacific Gyre, there are South Pacific Gyre, North Atlantic Gyre, South Atlantic Gyre, and Indian Ocean Gyre; hence, the garbage patch formations should be occurred globally. In the case, plastic debris would be broken down into various sizes (milli-, micro- or nano-meter size), with leaching potentially toxic chemicals (BPA, PCBs and others) and derivatives of polystyrene. They are concerned about biological effects in marine ecosystem. In addition, it is reported that plastic debris can absorb organic pollutants (PCBs, PAHs and others) from seawater.

Biological effects of plastic debris will be expected into broad ranges, from simple biological inhibitions (*e.g.* clogs of digestive tracts or ghost-fishing) to more complicated ecological toxicology (nanotoxicology-type research). Polluted area are located from high latitude to low latitude, means different salinity and temperature. My research group investigates fish toxicity of nano-sized metals and polystyrene, and salinity-dependen and temperature-dependent toxicity. We thought ecotoxicity of plastic debris would be size-matter toxicology and nanotoxicology is applicable to explore ecotoxicity of plastic debris. Through my talk, I would like to share our activities including our research hypothesis and supporting data obtained.

Acknowledgement: Professor Kashiwada expresses thanks to Dr (c). Chisato Kataoka and Dr. Hisato Takeuchi to give technical helps in preparation for this presentation. This project was supported by two Grant-in-Aids for Challenging Exploratory Research (award 17K20047 to SK) and for the Strategic Research Base Project for Private Universities (award S1411016 to SK) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.
Max 2500 characters, including spaces.

Microplastic occurrence and distribution from discharge points to deep basins in an urban model fjord

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Keywords: Microplastics, Sediment, Quantification, FTIR
Session: Marine Plastics
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Increased production, use and release of plastics since mass-production started in the 1950's has led to omnipresence of microplastic particles (<5 mm) in areas far from human activities, and the ocean floor is a likely recipient. It is expected that urban harbors are highly contaminated by microplastics from anthropogenic activities, wastewater, run-off and maritime traffic, and that the highest concentrations of microplastics will be found in urban areas. Environmental impact assessments should follow knowledge about relevant environmental levels of microplastics and effects-studies of relevant exposure scenarios. Laboratory studies have shown uptake and affected energy balance and reproduction in molluscs and lugworms after high exposure to microplastic particles, but realistic levels of microplastics in the marine environment are largely unknown. Uni Research has done marine monitoring since the 70's in the fjords around Bergen. The in-depth knowledge about abiotic and biotic factors in the fjord system makes it an ideal area for investigating levels of urban microplastics in marine recipients. This study used sediment from discharge points to deep sedimentation sites in the Byfjorden in Bergen, and extracted microplastic particles by density separation with Zinc-Chloride in a Microplastic Sediment Separator. Polymers and particles (>10µm) were identified using Fourier Transform Infrared Spectroscopy at the Biologische Anstalt Helgoland of the Alfred Wegener Institute.

The number of plastic particles was ~200 000 kg-1 dry sediment at the discharge site. Distribution and deposition at deep stations (~320 m) followed expected patterns. The number of particles varied from 12 000 to 71 000 kg-1 dry sediment. The study demonstrates the capability to isolate and identify microplastics >10 µm from sediment, and to differentiate polymers, size distribution and total numbers of microplastic particles. The microplastic particles were from assumed land based sources (packaging) as well as marine sources (rope and boat paints). This study is the first to report concentrations of identified plastic particles down to sizes below the limit of visual identification from a Norwegian fjord. The results gives a baseline for comparison of concentrations of microplastics of different size classes among sites. The results can also be used as a reference when determining high and low contamination thresholds for environmental management guidelines for microplastics.

Acknowledgement: The authors thank RFFVest for funding through pre-project RFFV#258890, and project owner Bergen Kommune Vann-og Avløpsetaten (Anne Cornell) for the good collaboration. Uni Research Computing (Helge Avlesen) and Christian Michelsen Research (Benny Svardal) took part in the project.

Understanding the distribution and impact of micro- and nano-plastics in Norway

Tânia Gomes, Inger Lise Nerland Bråte, Rachel Hurley, Anastasia Georgantzopoulou, David P. Eidsvoll, Bjørnar Beylich, Marianne Olsen, Bert van Bavel and Amy Lusher

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Keywords: plastic, monitoring, distribution, effects
Session: Marine plastics
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Method development and standardization has been at the forefront of microplastics (MPs) research. Developing methods to investigate the presence of MPs in the freshwater environment began slightly later than those for the marine environment; however, we are closer to standardized methods having learnt from the challenges encountered in the marine realm. Research at NIVA has covered a broad spectrum of environmental matrixes including drinking water, sewage systems, freshwater and marine sediment, water, and biota. Here we present the highlights from our MPs monitoring and exposure studies at NIVA. NIVA's research aim is twofold, firstly to understand the distribution of small plastics in the environment and secondly to understand their ecotoxicological effects. In this presentation, several case studies regarding both the distribution and the biological effects of MPs and nanoplastics (NPLs) will be discussed. Potential source and vectors for MPs input to Norwegian ecosystems have been investigated. Sludge samples from eight wastewater treatment plants across Norway contained MPs consisting of beads (38%), fragments (32%), fibres (28%) and glitter (2%). Monitoring studies have been conducted in rivers, fjords and coastal sediments revealing the presence of MPs of different shapes, sizes, and polymer form showing MPs contamination from different sources. Mussels have been utilised as bioindicators along the coast of Norway. An average of 1.5 MPs per individual was reported with highest levels of MPs in the northern most stations in the Barents Sea, as well as within the Oslofjord. Further research has investigated the uptake and the biological effects of environmentally relevant MPs exposure in mussels. *Mytilus galloprovincialis* ingested more polyethylene from toothpaste when it was weathered prior to exposure. This ingestion led to tissue alteration in the gill, gonad, mantle and digestive gland. Globally MPs exposure studies have emphasised the adverse effects of MPs exposure although research suggests that NPLs will have far reaching consequences at the cellular and molecular level. For this reason, NIVA is currently researching the impact of different-sized plastics in aquatic organisms, and their potential transfer along the aquatic food chain. NIVA will continue these monitoring programs with an aim to understand the spatial and temporal changes in MPs and NPLs abundance and toxicity to a range of aquatic organisms.

Acknowledgement: The authors thank Nina Buenaventura and Karine Iversen for their assistance with laboratory analysis. Calin Constantin Steindal (Museum of Cultural History, UiO) for assistance with chemical analysis of plastics. The research work presented here has been funded by JPI-Oceans BASEMAN and JPI-Water IMPASSE projects, Norwegian Research Council, Norwegian Environmental Agency, Nordic Council of Ministers and internal funding at NIVA.

Is crumb rubber a source for pollutants and harmful effects in the marine environment?

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Keywords: crumb rubber; ecotoxicity; leaching; copepods
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In Norwegian coastal communities, rubber microplastic granules (≤ 5 mm in size) derived from discarded vehicle tires are used in large quantities on outdoor synthetic turf sports pitches. Through transport by waste water effluents and terrestrial runoff, these rubber particles are considered a significant source of MPs to the marine ecosystem. In the here presented interdisciplinary project we study the composition, degradation and environmental impacts of these rubber granules from locations in northern Norway and Svalbard. Their persistence and residence time in the Arctic marine environment is unknown. These rubber particles pose a potential health risk for arctic wild life through direct ingestion, especially at the base of the marine food chain, but may also provide an exposure route for toxic additive chemicals present in tires to marine organisms. Furthermore, the rubber particles may act as a vector for other persistent organic and heavy metal pollutants already present in the marine environment. Arctic marine environments present special abiotic conditions for the degradation of these particles, with cold water temperatures and long periods with unlimited sunlight. During a 12 months period, rubber crumbs were placed out in the ocean in stainless steel containers and sub-sampled continuously for the measurement of persistent organic pollutants, metals and additives. Hydrophobic persistent organic pollutants such as PAHs, PCBs, DDTs, bisphenols, as well as metals were measured to establish the adsorption and leaching kinetics in seawater under in situ conditions. Samples were extracted using ultrasound and nonpolar solvents, followed by GPC and SPE clean up. Chemical analyses using pyroGC/MS, GC/MS/MS and LC/HRMS were done in the laboratories of NILU, Tromsø and SINTEF, Trondheim. Exposure experiments with rubber leachate were also conducted and high mortality rates were found for different marine zooplankton species.

Acknowledgement: The authors thank FRAM Centre for providing funding for the MARS project, and Otto Storan for help with the experiments.

Benchmarking the uptake and excretion dynamics of microplastics in the boreal marine copepod *Calanus finmarchicus*

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Keywords: microplastics, copepod, uptake, excretion
Session: Marine Pollution 2: Plastic debris – pathways and impacts in arctic and boreal systems
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The marine copepod *Calanus finmarchicus* is a filter feeder and a keystone species of the Northern Atlantic that, alongside other crustaceans, is known to ingest microplastic (MP). Potential effects of MP ingestion include obstruction, abrasion, lower energy budget due to reduced food intake, and toxicity related to the leakage of chemicals from microplastics into the organism. The magnitude of all these effects is largely determined by microplastic uptake and excretion rates, and particularly by retention or accumulation times inside an organism. However quantitative uptake and excretion data are currently unavailable. In the current study, non-ovulating female *C. finmarchicus* were exposed 0-96 h to a non-restrictive concentration (750 MP particles/mL) of a comprehensively characterized 10 μm spherical MP of general purpose polystyrene in the presence of microalgae (7500 cells/mL). Excretion dynamics were studied over 1-72 h after a 24 h exposure to MP in either the presence or absence of microalgae. Custom made transparent plankton wheels immersed in a water bath were used to expose *C. finmarchicus* in a semi-static setup to keep MP and microalgal particles in suspension and to maintain constant temperature. MP counts were performed in triplicate in exposure media, copepods and their fecal pellets as well as on container walls for a total of 16 sampling time points. Findings show unconventional uptake kinetics for MP in *C. finmarchicus* when compared to dissolved organic chemicals. Excretion of MP was rapid although there was indication that retention may occur. Excretion dynamics were affected by the presence of microalgae. The current study was designed as a benchmarking exercise with commercial microbeads of uniform size. The findings can therefore not be generalized for MPs of other shapes, compositions, conditions, densities and sizes, but should rather be considered as a reference for future studies using MP with more environmentally relevant characteristics and concentrations.

Acknowledgement: The authors thank the Norwegian Research Council for providing funding for the PLASTOX project (grant award 257479). This project has received funding under the framework of JPI Oceans.

Marine Microplastic: Realistic test materials for laboratory studies of ecosystem impacts

Andy M. Booth[1], Susanne Kühn[2], Albert van Oyen[3], André Meijboom[2], and Jan Andries van Franeker[2]

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Session: Marine Pollution 2, Plastic debris – pathways and impacts in arctic and boreal systems
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Reported studies investigating the possible effects of plastic litter on marine biota have almost exclusively utilised pristine plastic materials that are homogeneous in polymer type, size, shape and chemical composition. This is particularly the case for microplastics (marine litter < 5mm), as collecting samples of such material from the marine environment in quantities sufficient for use in laboratory impacts studies is simply not feasible. Crucially, weathered plastics collected from the marine environment show considerable physical and chemical differences to pristine and post-production consumer plastics. In the current study, we describe the preparation and characterisation of a more environmentally realistic marine litter-derived microplastic reference material (≤3 mm) for use in fate and effects studies. Weathered marine plastic litter (351 items) was collected from the coast of the island of Texel (The Netherlands) and carefully identified and categorised (fibre-based, packaging, foam, plastic boxes and jerry cans, bottles, gloves and miscellaneous plastic materials). Ropes, nets and sheeting comprised ~70% of the collected material, which contained 9 different polymer types. The macroplastic material was sub-sampled and subjected to a cryo-milling and sieving process to create the microplastic reference material.

To confirm that the original macroplastic polymer distribution was mirrored in the generated microplastic sample, it was subjected to ATR-FTIR and differential scanning calorimetry analysis. Particle size distribution (PSD) of the microplastic, measured using laser diffraction and sieving, showed that 68% (by mass) of the particles were in the range between 0.5 and 2.0 mm. Particle number increased with decreasing particle size fraction. Scanning electron microscopy revealed a wide range of particle sizes and shapes reflecting the properties of the different polymers. ICP-MS and ICP-OES analyses revealed the presence of a broad range of metals and other elements (e.g. Al, Cr, Fe, Mg, Pb, S and Zn) associated with the final sample. Many of these represent common inorganic plastic additives used as colourants, fillers and stabilisers. The additive organic chemical profile of the microplastic mixture was also determined by GC-MS analysis following extraction by ethyl acetate and ultrasonication. A broad range of plasticisers, stabilisers, antioxidants and flame retardants were identified.

Acknowledgement: The authors thank the Norwegian Research Council and the Netherlands Organisation for Scientific research for providing funding for the PLASTOX project. This project has received funding under the framework of JPI Oceans.

Exploring the effects of nylon microplastic on the development and energy reserves in coldwater copepods

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Keywords: microplastic; copepods; development; energetics
Session: Marine Pollution 2, Plastic debris – pathways and impacts in arctic and boreal systems
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Microplastic debris is a pervasive and widespread pollutant that poses a risk to aquatic biota and healthy marine ecosystems. Copepods are an abundant and ecologically important class of zooplankton, common to marine ecosystems across the globe. Field studies and laboratory exposures have identified that copepods readily consume microplastic particulates. In the copepod *Calanus helgolandicus*, prolonged exposure to polystyrene microbeads resulted in significant reductions in feeding, egg size, hatching success and survival. We hypothesise exposure to microplastics reduces feeding in copepods, resulting in energetic shortfalls for which lipids can act as a proxy. The coldwater copepod *Calanus finmarchicus* is a keystone species, common to the North Atlantic. During maturation, these copepods rapidly build-up their wax-ester store (oil sac); this lipid reserve is essential to the copepod's buoyancy regulation and energetic budget when overwintering, and is of high nutritional value to predators. Following a 48-hour acclimation period, juvenile *C. finmarchicus* were incubated in natural seawater containing a mixed assemblage of cultured algae (control), with the addition of either nylon granules (10-30 µm) or fibres (10x30 µm) at a concentration of 100 microplastics mL^{-1}. Algal ingestion rates and developmental stage were monitored daily, while prosome length, oil sac size and lipid profiles were assessed following the six-day experiment. No significant differences in growth, sex-ratios or oil-sac size were identified, however we observed juvenile copepods moulted into adults significantly earlier (ANOVA, $P<0.05$) when exposed to microplastic. We discuss the impact microplastic exposure can have on feeding and energetics of animals, in relation to the individual and marine food webs as a whole.

Acknowledgement: Rachel Coppock is funded through a UK Natural Environment Research Council GW4+ PhD studentship (NE/L002434/1). Matthew Cole acknowledges funding from the Natural Environment Research Council discovery grant (NE/L007010). The authors also thank the Norwegian Research Council for providing funding for the PLASTOX project (257479/E40). This work has received funding under the framework of JPI Oceans. We acknowledge Iurgi Salaverria and Anders Olsen for arranging for the experimental work to take place at NTNU SeaLab.

Potential impact of microplastics on the environmental footprint of Atlantic salmon farming

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Keywords: Aquaculture; Microplastics, Contaminants, Environmental impact
Session: Marine Pollution 2 (Plastic debris – pathways and impacts in arctic and boreal systems)
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The exponential increase of microplastics in the marine environment is a global problem that may have serious implications for fisheries and seafood safety. One of the greatest threats associated is the potential of plastic to transport and increase the persistence and bioavailability of contaminants like persistent organic pollutants (POPs) or heavy metals. Environmental pollutants have different sorption affinity for each kind of synthetic polymer depending on their physiochemical properties. Aquaculture feed might contain low levels of POPs originating mainly from fish oils, heavy metals originating mainly from fish meal, and pesticides originating from agricultural ingredients. In areas with intensive aquaculture, waste food and fecal matter from fish farms facilities might represent a local source of these pollutants in the marine environments. In addition, erosive wear of pneumatic feeding tubes might represent a source of microplastics around fish farms. Commercially, Atlantic salmon farming is today one of the economically most important industries in Norway and aquaculture is one of the fastest growing food production sectors in the world. The environmental footprint of the industry should therefore be kept as low as possible. With this background, and due to the lack of knowledge about the occurrence and implications of microplastics in aquaculture, this project aims to increase the understanding of the potential role of microplastics on the dispersion of contaminants associated with fish farms, in order to ensure the sustainability of marine aquaculture. To address it, we plan to cage a set of the most environmentally relevant virgin plastic polymers as well as blue mussels next to Atlantic salmon farms, and determine the chemical composition of pollutants adsorbed by the polymers and accumulated in the mussels after a period in the sea, as well as the degree of microplastic ingestion in the mussels. To determine the mechanistic effect of pollutants sorbed to the microplastic particles, follow-up laboratory exposure experiments will be conducted with blue mussels and fish larvae.

Acknowledgement: This project is funded by the Faculty of Biosciences and Aquaculture of Nord University (Bodø) as well as supported by the International Research Institute of Stavanger (IRIS) and the Institute of Marine Research of Bergen.

Surveillance of microplastics (MPs) in seafood – ongoing activity and future perspectives at Institute of Marine Research (IMR).

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Keywords: Instrumentation, Microplastics (MPs), Seafood safety, Quantification,

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The accumulation of plastics in the oceans has gained increased attention the last years and when exposed to various environmental stressors, microplastics (MPs) are formed (defined as plastic particles < 5 mm). Degraded secondary MPs together with primary MPs from consumer products, are now found in increasing amounts in our marine environment. Recent publications indicated that size matters and plastic particles below 20 µm sizes could enter internal organs of marine organisms. A fundamental issue precluding surveillance of MPs in seafood is the lack of standard operation protocols (SOP) for MPs sampling and detection. Institute of Marine Research (IMR) participates in a European network to evaluate all approaches from sampling to identification of MPs (BASEMAN JPI-Oceans; http://jpi-oceans.eu/baseman). Within the BASEMAN framework, IMR organize an inter-laboratory method performance trial for MPs determination. IMR is now constructing a new laboratory dedicated for MPs analysis (air sluice doors, filtered, overpressure air) holding a wet laboratory. Our aim is to combine two detection methodologies: Automated µFTIR (Fourier transform infrared microscopy) with focal plane array to provide images for MP particles above 2.5 – 10 µm, and pyrolysis Gas Chromatography/Mass Spectrometer (GC/MS) for the quantification of plastic polymers. For both methodologies a procedural optimization and testing is required, before they are ready for surveillance of Norwegian seafood. Our aim is to quantify MPs in seafood with the determination of polymer type and additives present. Filter feeders, marine organisms eaten whole (anchovies and prawns) and bottom feeding fish such as halibut and flounder and crab, are our initial focus. At present, no results are available. The project will be presented with focus on ongoing activity and future perspectives at Institute of Marine Research (IMR).

Session 6
Environmental toxicology in Svalbard

Session Chairs: Heli Routti and Sophie Bourgeon

Talks

Invited Plenary Heli Routti: Exposure and effects of pollutants in mammalian predators from Svalbard

Katharina Lühmann: Activation of the thyroid receptor of fin and blue whales by environmental pollutants

Silje Strand Lundgren: Immunomodulation by pollutants and metals in seabirds breeding on Svalbard

Roger Lille-Langøy: Environmental contaminants modulate the transcriptional activity of polar bear and human peroxisome proliferator-activator receptor alpha

Posters

Emily Hill: Exposure of the Common Eider *(Somateria Mollissima)* to Toxic Elements in Relation to Migration Strategy and Wintering Area

Gro Dehli Villanger: Time trends in perfluoroalkyl substances in white whales *(Delphinapterus leucas)* from Svalbard

Julia Farkas: Seasonal variation in concentrations of metallothionein and Cd, Zn and Cu in tissues of polar fox and rock ptarmigan from Svalbard

Torunn Slettemark Hovden: Effects of per- and polyfluoroalkyl substances (PFASs) on thyroid hormone status in Svalbard glaucous gulls

Tatiana Drotikova: Source identification of individual soot agglomerates in Arctic air by transmission electron microscopy

Åse-Karen Mortensen: Trace elements and thyroid hormones in glaucous gull

Tomasz Ciesielski: Are snow buntings *(Plectrophenax nivalis)* in Svalbard affected by local anthropogenic pollution?

Invited Plenary

Exposure and effects of pollutants in mammalian predators from Svalbard

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Keywords: polar bear, arctic fox, PFAS, POP
Session: Svalbard Toxicology
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Top-predators inhabiting Svalbard area are exposed to high concentrations of pollutants because the input of pollutants in the area is largely affected by winds and ocean currents from Europe and North-America. During recent years ecotoxicological studies on mammalian predators from Svalbard have focused on polar bears, walruses and arctic foxes. These studies show a general decline of lipophilic legacy persistent organic pollutants (POPs). Among perfluoroalkyl substances (PFAS), decline in PFOS since early 2000s has levelled of in 2009-2010, while several perfluoroalkyl carboxylates are increasing. As body condition has a large influence on tissue concentrations of lipophilic POPs, rapidly declining Arctic sea ice is related to increased concentrations of lipophilic pollutants in polar bears through changes in body condition. Increasing PFAS concentrations with increased availability of sea ice habitat is likely related to feeding habits, or, to efficient uptake of PFAS by ice-associated food webs. Correlative field studies using transcript levels of genes, hormone concentrations, and metabolomics as endpoints, as well, in vitro studies on polar bear receptors and adipose tissue-driven stem cells indicate that contaminants can disrupt lipid metabolism in polar bears. Furthermore, the effects in free-ranging polar bears were more pronounced when sea ice conditions were stressful. Correlative studies on walruses using hormone concentrations, transcript level of genes and pathogen exposure as endpoints indicate that the species is moderately affected by pollutants.

Acknowledgement: The author thanks numerous collaborators involved in the studies, in particular Sabrina Tartu. The studies have been financed by the Norwegian Research Council, Norwegian Polar Institute, the Ministry of Climate and Environment, the Norwegian Environment Agency, Fram Centre Incentive Funding, Fram Centre Hazardous Substances Program and the University of Bergen.

Activation of the thyroid receptor of fin and blue whales by environmental pollutants

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Keywords: baleen whales, endocrine disruption, thyroid hormone receptor, in-vitro
Session: Environmental toxicology in Svalbard
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Arctic wildlife is impacted by multiple stressors, including exposure to high levels of pollutants. Baleen whales, such as fin and blue whales (*Balaenoptera physalus* and *B. musculus,* respectively), feeding in the Arctic Ocean are exposed to a variety of persistent organic pollutants (POPs). Since pollutants accumulate in blubber, this tissue can be used as an indicator for POP levels in whales. Although several studies have measured pollutant patterns in fin and blue whale blubber, we lack knowledge concerning the effects of pollutants at a mechanistic level. One critical, yet rarely studied in wildlife, mode of action of POPs, is endocrine disruption. POPs have been shown to affect the function of the thyroid hormone receptor, which is involved in a number of vital processes such as development and metabolism.

As part of the project "Giants of the ocean – affected by anthropogenic pollutants?", activation of the thyroid receptor beta (TRB) by environmentally relevant pollutants was studied. POP concentrations (ng/g lipid weight) were determined in blubber biopsies from fin and blue whale close to Svalbard, Norway. The following values are average POP concentrations, and in parenthesis the dominant compounds of the sums: 3.91 for Mirex, 13.9 for Σ_4PBDE (BDE-47), 15.7 for Σ_3HCHs (β-HCH), 62.0 for HCB, 124.5 for Σ_5CHLs (*trans*-Nonachlor), 186.1 for Σ_4Toxaphenes (#50), 205.6 for Σ_{10}PCBs (PCB-153), and 329.2 for Σ_6DDT (*o,p'*-DDD). Pollutants were classified as environmentally relevant, if they exceeded the concentration of 50 nM in the blubber of whales biopsies. The ligand-binding domain of the TRB from fin whales was cloned, sequenced and used in an GAL4-UAS based *in vitro* luciferase reporter assay to study transcriptional activity of TRB when exposed to an endogenous ligand (T3) and 11 legacy POPs abundant in whale blubber. The *in vitro* assays showed that PCB-153 and *op'*DDT are weak agonists of the TRB.

Additional studies will be needed that focus on the effects of mixtures of POPs on the thyroid receptor to develop more realistic exposure scenarios.

Acknowledgement: The authors thank the Fram Centre Hazardous Substances Flagship, Norwegian Polar Institute, and the Norwegian Research Council ICE whales grant (No. 244488/E10 to KM.K) for funding this project.

Immunomodulation by pollutants and metals in seabirds breeding on Svalbard

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The Arctic has been shown to be a sink for various global pollutants, such as pesticides, industrial chemicals, metals and combustion by-products due to long-range transport from lower latitudes (AMAP, 2017). Climate change can further alter the transportation and distribution of these compounds in the Arctic (Ma et al., 2016). The Arctic is warming at a much faster pace compared to other regions of the globe, and the Barents area is considered a "hot-spot" even within the Arctic context (AMAP, 2017). Many of the chemicals that reach the Arctic have shown to have an immunomodulatory effect on various wildlife (e.g. Feng et al., 2016), which could increase their susceptibility to infectious diseases. Despite wildlife being highly exposed, the documentation on effects of pollutants on the immune system in Arctic species is sparse. This project investigated immunomodulation by various contaminants (e.g. polychlorinated biphenyls, organochlorinated pesticides and brominated diphenyl ethers) and metals (e.g. Hg, Cd and Pb) on a seabird species breeding in Kongsfjorden, the black-legged kittiwake (*Rissa tridactyla*).

Environmental contaminants modulate the transcriptional activity of polar bear and human peroxisome proliferator-activated receptor alpha

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Keywords: lipid metabolism, PPARA, perfluoralkyl substances, polychlorinated biphenyls
Session: Environmental toxicology in Svalbard
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The polar bear (*Ursus maritimus*) is an apex predator of arctic marine ecosystems that uses sea ice as a platform to hunt. During the spring polar bears feed excessively and accumulate energy, while they during periods of absence of sea ice go through extensive fasting periods. During fasting, polar bears depend almost entirely on energy stored in white adipose tissue and on mechanisms that promote release of energy stored as fat. Peroxisome proliferator-activated receptor alfa (PPARA/NR1C1) is a ligand activated nuclear receptor protein that is highly expressed in tissues with high fatty acid catabolism such as the liver. It is a key regulator of lipid metabolism, including β-oxidation, uptake and transport. Synthetic chemicals, in particular perfluoroalkyl substances, have been shown to induce transcriptional activity of mammalian PPARAs. Here, we cloned PPARA from East Greenland polar bear liver tissues and studied *in vitro* transactivation of human and polar bear peroxisome proliferator-activated receptor alfa (hPPARA) by environmental contaminants, including perfluoroalkyl substanses (PFASs), brominated flame retardants (BFRs), ochlorinated pesticides, and polychlorinated biphenyls (PCBs), using a luciferase reporter assay. In addition, we predicted ligand binding potential of the contaminants using protein-ligand docking and scoring methods. An amino acid sequence alignment showed that six hinge and ligand-binding domain amino acids have been substituted in polar bear PPARA compared to human PPARA. Perfluorocarboxylic acids (PFCAs) and perfluorosulfonic acids (PFSAs) induced the transcriptional activity of both human and polar bear PPARA *in vitro*. The major PFCAs in polar bears and humans, PFNA and PFOA, respectively, increased polar bear PPARA-mediated luciferase activity to a level comparable to that of the potent PPARA agonist WY-14643 (appr. 8-fold, 25 µM). Several BFRs were weak agonists of human and polar bear PPARA. In single compound exposures, the PCBs tested did not have agonistic effects, or only weak agonistic effects. In contrast, a technical mixture of PCBs (Arochlor 1254) strongly induced the transcriptional activity of polar bear (appr. 22-fold) and human (appr. 8-fold) PPARA. Our findings demonstrate that the transcriptional activivity of human and polar bear PPARA can be modified by environmental pollutants at environmentally relevant concentrations. The results indicate that environmental pollutants may affect lipid catabolism in both humans and polar bears.

Acknowledgement: This study was funded by the Fram Centre Hazardous Substances Program, the University of Bergen, and the Norwegian Polar Institute. Protein-ligand docking and scoring was performed by Lisa Bjørnsdatter Helgason. The Danish Cooperation for Environment in the Arctic (DANCEA) and Arctic Research Centre are acknowledged for financial support to collect field samples.

Exposure of the Common Eider (*Somateria mollissima*) to Toxic Elements in Relation to Migration Strategy and Wintering Area

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Keywords: Common eider, Eggs, Toxic elements, Migration
Session: Svalbard Toxicology
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The Arctic is a known sink for many anthropogenic pollutants due to the process of long range transport and the cold-condensation effect at high latitudes. Toxic elements such as mercury (Hg), lead (Pb) and arsenic (As), are naturally occurring in the environment, but human activity has led to their redistribution, resulting in the accumulation of high concentrations in certain areas. These elements are bioavailable to a range of organisms and exposure can increase formation of reactive oxygen species (ROS) in the body, potentially leading to cellular injury and genotoxic effects in Arctic biota.

The common eider (*Somateria mollissima*) is a long-lived sea duck. Benthic sediments, upon which eiders feed, are a reservoir for toxic elements, and concentrations typically exceed those which are deemed safe. During spring, female eiders consume large quantities of benthic prey, including bivalves, gastropods and sea urchins. Eiders are capital breeders and need to build up body reserves for producing and laying eggs and surviving the subsequent incubation fast. While Svalbard breeding eiders are migratory and spend the winter at the coasts of North Norway or Iceland, eiders breeding in Iceland and North Norway are sedentary. This variability in migration strategy and habitat use is likely to give rise to varying exposure to toxic trace elements.

During the summers of 2010, 2011, 2012 and 2017, eider eggs were collected from Svalbard breeding females and migration strategy was determined by use of geolocators. In 2017, eggs were also collected from sedentary female eiders breeding in the two wintering areas, Iceland and North Norway. Eggs were analysed for a suite of elements (ca. 60) and concentrations were related to migration strategy and year of sampling. We expect eggs to predominantly reflect exposure to elements in prey during the weeks of egg-production, but also exposure during winter, as long-term exposure may contribute to body burden of some elements. With our study design we therefore aim to relate habitat use and migration strategies to concentrations of both toxic and non-toxic elements in eider eggs. This will have implications for the integrated management of arctic breeding eiders with regard to their wintering and breeding areas.

Acknowledgement: The authors thank the Norwegian Polar Institute for the use of the Sverdrup station in Ny Ålesund and Kings Bay for the provision of logistical support and accommodation. Permissions for fieldwork and instrumentation were granted by the Governor of Svalbard, Norwegian Animal Research Authority and Norwegian Food Safety Authority.

Time trends in perfluoroalkyl substances in white whales (*Delphinapterus leucas*) from Svalbard

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Keywords: time trend, Svalbard, marine mammals, perfluoroalkyl substanses
Session: Environmental Toxicology in Svalbard
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Perfluoroalkyl substances (PFASs) are a group of persistent contaminants found in high concentrations in human and wildlife blood worldwide, including in Arctic wildlife. This is a serious concern given their toxic properties. Phase-outs of some of the most toxic substances in this chemical group have been ongoing since 2000, resulting in temporal shifts in PFAS levels and patterns, including changes in some marine mammal populations in Svalbard, Norway. However, no studies have yet reported levels of PFASs in white whales (*Delphinapterus leucas*) from this area. The objective of the present study was to investigate present levels and patterns of PFAS in white whales from Svalbard and compare them to levels found in white whales sampled 15 years ago.

Plasma collected from live-captured white whales from two time periods were included in this study; 9 animals were sampled recently (August 2013 and 2014) and 11 were sampled 1.5 decades ago (July to October, 1996-2001). Analyses of the plasma included 19 different PFASs, using high performance liquid chromatography/tandem mass spectrometry. The 11 PFAS detected included 7 C_9-C_{14} perfluorinated carboxylic acids (PFCAs) and 3 C_6-C_8 sulfonic acids (PFSAs) as well as perfluorooctanesulfamide (PFOSA).

Recent levels of the most dominant compound in white whales, perfluorooctane sulfonate (PFOS; median = 22.2 ng/ml) were relatively low, close to an order of magnitude lower than what is reported in polar bears (*Ursus maritimus*) from Svalbard, and about half the level reported for seals from this area. R*ecent* (2013-2014) levels of C_7-C_8 PFSAs, C_{13}-C_{14} PFCAs, Σ_3PFSA and Σ_{11}PFAS were significantly lower than in the *past* (1996-2001), while some individual C_9-C_{12} PFCAs and Σ_7PFCA were significantly higher in the *recent* group of white whales. In addition, the contribution of individual PFASs to the total PFAS load in white whales changed between the two sampling periods: PFOS dropped from 75 to 50 %, perfluoroundecanoate (PFUnDA) increased from 9 to 17%, perfluorononanoate (PFNA) increased from 1 to 4 %, and perfluorododecanoate (PFDoDA) increased from 1 to 3 %, while the contribution of PFOSA remained relatively unchanged. These results follow the same basic trends that have been reported in other studies. The most dramatic change has been the decline of PFOS levels since the end of the 1990s, corresponding to production phase-out of PFOS and related compounds around 2000 and a global restriction in 2009.

Seasonal variation in concentrations of metallothionein and Cd, Zn and Cu in tissues of polar fox and rock ptarmigan from Svalbard

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Keywords: fox, ptarmigan, metallothionein, Svalbard
Session: Svalbard toxicology
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The high Arctic undergoes extreme seasonal changes in climate and biological activities. Many terrestrial animals are thus building up large fat reserves to have sufficient energy to survive the winter months. The consumption of body fat can be accompanied by utilisation of proteins, potentially including metallothioneins. In this study we investigated concentrations of metallothionein, Cd, Zn, and Cu in heart, kidney and liver of polar fox (*Vulpes lagopus*) and the Svalbard rock ptarmigan (*Lagopus muta hyperborea*) in spring and autum. Heart, liver and kidneys were sampled and transported on ice, and subsequently kept frozen at -25°C or -80 °C until analyses. Metals were analysed in digested organs with AAS. Metallotionein was quantified in tissues using modified Cd-chelex and tetrathiomolybdate chelex assays.

Our results show that there were seasonal differences in element concentrations. In polar fox, Cu was higher in kidney ($p=0.004$) and liver ($p=0.028$) in spring compared to autum, with Zn being higher ($p=0.01$) in spring in the heart only. Cd concentrations were low in heart in both seasons (<0.1 µg/g tissue; ww), and were slightly higher in kidney and liver. In Svalbard rock ptarmigan Cu was higher in kidney in spring ($p=0.014$). In liver, however, concentrations were significantly increased in autumn ($p=0.001$), potentially deriving from dietary Cu intake. Zinc and Cd were higher in kidney and liver in spring ($p<0.05$). Metallothionein concentrations were highest in liver in foxes, and in kidney in ptarmigans. In fox, MT was correlated with Cu in kidney in spring animals, and in liver in autum animals. Similarly, MT and Zn concentrations were positively related in kidneys and livers in foxes. In ptarmigans, MT concentrations in kidney were strongly correlated with Cd. Element concentrations were higher in spring compared to autum in most tissues of both species. In contrast, no clear trend could be determined for MT concentrations, which were only significantly higher in kidneys in fox in autumn, but higher in kidneys and liver in ptarmigans in spring. This is likely due to interspecies differences, both related to element intake via diet, but also physiological differences related to protein utilisation during starvation. Further investigations will include multivariate analyses to study relations between MT and elements.

Effects of per- and polyfluoroalkyl substances (PFASs) on thyroid hormone status in Svalbard glaucous gulls

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Keywords: glaucous gull, PFAS, endocrine disruption, thyroid hormones
Session: Poster, Environmental toxicology in Svalbard
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The aim of this project is to investigate concentrations of per- and polyfluoroalkyl substances (PFASs) in relation to thyroid disruptive effects in glaucous gulls (*Larus hyperboreus*). Liver concentrations of PFASs and plasma concentration of thyroid hormones (TH) and thyroid stimulating hormone (TSH) were analysed in 15 glaucous gulls from Adventfjorden and Sassendalen, Svalbard, Norway. Moreover, histopathologic changes in thyroid glands were examined. Multiple correlation analysis will be applied to assess to possible relationships between contaminants and response variables.

Although the use of many persistent organic pollutants (POPs) are banned or restricted by the Stockholm Convention (1) and their concentrations in the Arctic environment are generally decreasing, concentrations of some POPs are still very high. For some Arctic top predators the concentrations of some chemicals are above threshold levels for reproductive and health effects (2, 3, 4). The glaucous gull is among the Arctic top predators with the highest concentrations of POPs (3, 4). Both legacy POPs and novel anthropogenic chemicals, like PFASs, have been reported. Perfluorooctane sulfonate (PFOS), a PFAS which is now restricted and listed in annex B in the Stockholm Convention (1), has a high biomagnification potential in the Arctic marine food web (5).

Several anthropogenic organic pollutants affect TH regulation in animals. THs regulate growth, differentiation, development, metabolism, thermogenesis, motor function, and cognitive abilities, and are involved in reproductive function in all vertebrates. Exposure to TH disrupting compounds may therefore have serious effects in wildlife populations (6). In rats, PFASs are among the contaminants that cause depressed levels of TH (7). Thyroid disruption may occur at several levels in the organism. Ren et al. (2016) reported that PFOS and perfluorooctanoic acid (PFOA) can displace TH from the TH transport protein transthyretin in humans (8). Thus, in glaucous gulls, TH displacement from transport proteins may occur due to the current exposure levels of PFOS and PFOA. Linking molecular mechanisms to potential harmful effects on higher organismal level is, however, challenging due to the complex interactive cellular and physiological processes, and the impact of multiple other stressors (9). Information on possible relationships between hepatic concentrations of PFASs, hormone concentrations and thyroid histopathology will be presented.

Acknowledgement: The authors thank Martin Hartmann Munck for assistence in the field and UNIS for access to field equipment and laboratories. The project is funded by Polarforskningsprogrammet, The Research Council of Norway (RCN), project 268419 and an Arctic Field Grant fellowship from RCN/Svalbard Science Forum, project 269970. Permision for sampling was provided by Sysselmannen/The Governor of Svalbard.

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Source identification of individual soot agglomerates in Arctic air by transmission electron microscopy

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Keywords: Soot; Source appointment; Coal burning; Arctic aerosol
Session: Svalbard toxicology
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Soot originates from incomplete combustion and is an important constituent of the atmospheric aerosol due to its strong light absorption efficiency. Soot (black carbon) influences the Arctic climate via four different mechanisms: (a) direct forcing by absorption of solar radiation, (b) reduction of albedo after deposition on snow, (c) indirect and semidirect forcing by changing properties of clouds, and (d) radiative forcing outside the Arctic leading to changes in energy transport by the atmosphere and oceans.

Traditionally, the contribution of different sources to black carbon in the Arctic was determined either based on emission inventories or based on chemical tracers measured at the receptor. In the latter case, results are usually obtained from bulk chemical analysis. In contrast, a single particle approach for source identification is elaborated in the present study.

Individual soot agglomerates collected at four different locations on the Arctic archipelago Svalbard (Norway) were characterised by transmission electron microscopy and energy-dispersive X-ray microanalysis. For source identification of the ambient soot agglomerates, samples from different local sources (coal burning power plants in Longyearbyen and Barentsburg, diesel and oil burning for power generation in Sveagruva and Ny Ålesund, cruise ship) as well as from other sources which may contribute to Arctic soot concentrations (biomass burning, aircraft emissions, diesel engines) were investigated.

Diameter and graphene sheet separation distance of soot primary particles were found to be highly variable within each source and are not suited for source identification. In contrast, concentrations of the minor elements Si, P, K, Ca and Fe showed significant differences which can be used for source attribution. The presence/absence of externally mixed particle groups (fly ashes, tar balls, mercury particles) gives additional hints about the soot sources.

Biomass/wood burning, ship emissions and coal burning in Barentsburg can be excluded as major source for ambient soot at Svalbard. The coal power plant in Longyearbyen is most likely a major source of soot in the settlement of Longyearbyen but does not contribute significantly to soot collected at the Global Atmosphere Watch station Zeppelin Mountain near Ny Ålesund. The most probable soot sources at Svalbard are aircraft emissions and diesel exhaust as well as long range transport of coal burning emissions.

Acknowledgement: The authors thank the Svalbard Environmental Protection Fund (Svabard Miljøvernfond, AtmoPart project) and the Deutsche Forschungsgemeinschaft for financial supports (DFG; grant KA 2280/2).

Trace elements and thyroid hormones in glaucous gull

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Keywords: Elements, Arctic, top predator, thyroid hormones
Session: Poster, Svalbard Toxicology
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The aim of this study was to investigate the concentration of elements in different tissues; plasma, liver, kidney, muscle and feathers, from 15 individuals of glaucous gull (*Larus hyperboreus*) captured in Sassendalen and Adventalen on Svalbard 2017. The levels of thyroid hormone (TH) and thyroid stimulating hormone (TSH) was measured to investigate possible associations with the concentration of trace elements. Previous studies on glaucous gull have focused on the association between THs and organohalogenated contaminants (OHCs) but the association with trace elements as not yet been investigated in glaucous gull and neither has the levels of TSH. Several trace elements has been detected in the Arctic, and some are increasing such ad mercury (Hg) that is ten times higher in top predators now than in pre-industrial times. Some trace elements can accumulate and lead to harmful effects in organisms. Thus, they can contribute to the total toxicity potential in these gulls that are already exposed to high concentrations of organic pollutants in the harsh Arctic environment.

The THs regulate several important functions such as growth, development, thermogenesis and reproduction. Therefore chemicals disrupting the TH homeostasis are a great concern in ecotoxicology. Previously trace elements such as cadmium (Cd), selenium (Se) and Hg have been found to affect the TH system. It has been shown that Cd can accumulate in the thyroid gland and cause changes to the follicle cells indirectly through oxidative stress. In addition Cd has been associated with changed TH and TSH levels. Hg and the molar ratio between Hg and Se has been associated with decreased T3 levels in brown trout (*Salmo trutta*) from Mjøsa. This observation was explained by detoxification of Hg via formation of Se-Hg complexes that affect the bioavailability of both Hg and Se. However, less bioavailability of Se can disrupt the function of Se-dependent enzymes such as deiodinase. Knowledge on the relationship between trace elements and TH system in Arctic top predators is scarce and need to be investigated further. This study will results on concentrations and distribution of trace elements in different tissues of glaucous gull and their association with TH and TSH.

Acknowledgement: The authors thank for the access to field equipment and laboratories at UNIS and for funding by the Research Council of Norway (project 268419) and AFG from Svalbard Science Forum. Permissions for sampling were given by Sysselmannen.

Are snow buntings (*Plectrophenax nivalis*) in Svalbard affected by local anthropogenic pollution?

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Keywords: snow bunting, feathers, chemical elements, Svalbard
Session: Environmental toxicology in Svalbard
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Local urban pollution issues are of increasing concern in the Arctic. Longyearbyen is the largest settlement in Svalbard, Norway, and Adventdalen, including Longyearbyen, is an area with an extensive history of coal mining. In addition, emissions of potentially toxic elements to the local environment may occur from the coal fired power plant and urban related activities in Longyearbyen. In the present study, the snow bunting (*Plectrophenax nivalis*), which is the only passerine that regularly breed in the high Arctic environment of Svalbard, was applied as an indicator species to study accumulation of urban derived pollution in the Adventdalen area. The main objective was to assess how accumulation of elements varies with sex and age in snow buntings, and to which extent the concentrations are associated with potential local pollution originating from mining activity, emissions from the coal-fired power plant and other urban activities in Longyearbyen, Svalbard. Thus, feathers were collected from adult-nestling pairs of snow buntings. Significant differences in feather concentrations of elements were found between adult and nestling snow buntings in Longyearbyen and Adventdalen. Nestlings were characterized by having significantly higher concentrations of the essential elements Na and Zn, and the non-essential elements Hg and Al than the adults adults (Kruskall-Wallis test, $p<0.05$). On the other hand, concentrations of Ba, Cd, Cu, Mn, Ni, Pb, Sn and V were significantly higher in the adults as compared to in the nestlings. There were no sex-difference in the feather-concentrations in the adult birds in any of the areas. The concentration pattern of 'geogenic' rare earth elements (REE), Mn and Cr in adult bird feathers indicates the possibility that external contamination of the feathers may 'mask' the actual internal burdens of elements. The results indicate elevated concentrations of some elements associated with urban activity. The higher concentrations of Hg in the nestlings as compared to the adults may be of concern.

Acknowledgement: The study was funded by VINTERSPURV project 'Reproductive biology of the snow bunting (*Plectrophenax nivalis*)' funded by the Svalbard Evironmental Protection Fund. The authors thank Morten Ingebrigtsen, Julie Huseklepp Tunli and Gina Beitveit for help in field work.

Closing Plenary

AnthroTox: Combining natural and social sciences to understand and manage global anthropogenic toxicants

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Pollution by the anthropogenic toxicants persistent organic pollutants (POPs) is a cumulative global environmental problem. POPs travel long distances, passively with atmospheric and sea currents, and actively with global trade in industrial products and waste. They accumulate in the food chain, negatively affecting animal and human life, including reproduction, immune function, and carcinogenesis. From their origin as part of industrial production, to their release into the environment, e.g. through waste recycling and disposal, these substances engage with society.

While essential to modern day life, toxicants induce negative effects and cause concerns, and call for regulation, which in turn involves different, often conflicting, societal and political interests. To understand how environmental and social processes and their relationships dictate flows and impacts of anthropogenic toxicants, the environmental chemists, ecotoxicologists and social scientists in the research platform AnthroTOX aims at understanding relationships between distribution and ecosystem exposures of anthropogenic toxicants, and chemical management strategies (waste handling and regulatory efforts). We focus in this initial phase on organic contaminants which may be released and associated with electrical and electronic equipment (ePOPs); regulated and non-regulated flame retardants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDE) and selected new halogenated flameretardants (HFRs), respectively.

Most of the proposed fieldwork will be in the tropics, which for different, climatic, social and political-economic reasons attract large amounts of these hazardous substances, but for which as yet little data exists. We will study Tanzania comparing an urban site Dar es Salaam with a remote site Zanzibar, to assess spatial and temporal trends in contaminant exposure, accumulation in the marine food web, and social processes related to the release and spread of toxicants, and activities leading to exposure and harmful effects, as well as local and international risk-containing and regulation efforts. AnthroTOX supports 6 PhDs with the following projects:

a) Identify sources affecting spatial and temporal trends of regulated and emerging ePOPs in the physical environment of urban and rural locations.
b) Examine local practices related to e-waste processing and deposition, and linked risk enhancing and reducing practices related to ePOPs.
c) Quantify bioaccumulation of regulated and emerging ePOPs with particular emphasis on marine biota and water.
d) Study fishermen's, consumers' and other stakeholders' knowledge and practices related to environmental pollutants and marine products.
e) Study e-waste exports, specifically between Norway and Africa, tracing specific products, investigating global networks and concerns.
f) International policy and regulation, and the related use of indices and standardised metrics, and translation of scientific knowledge.