Horizon 2020 GRACE project:
Integrated oil spill response actions and environmental effects

Invitation to the GRACE final conference

Tallinn May 23-24, 2019

BOOK OF OBSTRACTS

TAL TECH

SYKE
Suomen ympäristökeskus

ABSTRACTS

PRESENTATIONS

OIL SPILL RESPONSE PREPAREDNESS IN THE BALTIC SEA - FINNISH POINT OF VIEW

Ismo Siikaluoma

The Finnish Border Guard, SAR and Marine Safety Unit
Ismo.Siikaluoma@raja.fi

The Baltic Sea is a unique sea area where seasonal extremes compose a challenging environment for authorities responsible for oil spill response and preparedness. Maritime traffic in the Baltic Sea is dense and annually about 160 million tons of oil is transported through the Gulf of Finland where crossing traffic creates a clear risk area. If a major accident happens, it has been estimated that two oil tanker cargo tanks could be damaged and leak 30 000 tons of oil to the marine environment. According to simulation models, without any response measures, the oil would reach shoreline in three days - somewhere in the Baltic Sea. Oil response preparedness collaboration in the Baltic Sea has long traditions. Helsinki Commission (HELCOM) convention was signed in 1992 although collaboration started already in 1974. The main task of HELCOM is to protect the marine environment of the Baltic Sea from all sources of pollution. In terms of oil pollution preparedness, thanks to the continuous collaboration and persistent work, the Baltic Sea countries share extensive response equipment capacity of about 70 emergency and sea-going response vessels. There is also a shared oil drift forecasting tool for operational use together with aerial and satellite surveillance capability.

HELCOM Response group has agreed upon general response principles which are documented in Response manuals for oil and HNS response. The Baltic Sea countries have also agreed upon several recommendations for responding to pollution incidents.

In Finland, since January 2019, the Ministry of the Interior has been responsible for the preparedness of oil response. However, the preparedness is based on cross-sectoral collaboration. At the open sea, the Finnish Border Guard (FBG) is responsible to lead response operation while near the shoreline the regional rescue services do this job. Oil response fleet of 18 oil response vessels is owned and operated by the FBG, the Navy, governmental companies as well as salvage companies. The Regional Rescue services operate 150 vessels or boats suitable for oil response operation. Finland has 150 km of oil boom.

The Finnish oil response preparedness has been built based on mechanical oil response. Therefore, the oil response vessels are equipped with in-built oil recovery systems. Oil booms are used for restricting and directing the spreading of the oil. Due to the environmental circumstances, authorities should be able to respond oil in darkness, ice and shallow archipelago waters.

At the moment, written oil response tactics or planning tools for commanding a major oil response operation are missing. OILART-project (2019-2020) will develop a concept for this purpose.

According to the HELCOM recommendations, Finland follows a policy of restricted use of oil dissolving chemicals. There is no capability for in-situ burning of oil. Therefore, Grace Project results will be interesting for future oil response method considerations.

MARINE OIL SPILL RESEARCH IN CANADA: MAJOR PROGRAMS AND PROGRESS

Dr. Feiyue (Fei) Wang

Professor and Canada Research Chair in Arctic Environmental Chemistry, Centre for Earth Observation Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
feiyue.wang@umanitoba.ca

In this presentation I will provide an overview of two major national programs on oil spill research and response in Canada. The first one is the Multi-Partner Research Initiative (MPRI) that is coordinated by the Department of Fisheries and Oceans of Canada. Launched in November 2016, the goal of the MPRI program is to establish an integrated, global research network to advance oil spill research in Canada and enhance Canada's level of preparedness and response capability. The program currently funds more than 30 research projects under six program areas: spill treating agents, in situ burning, oil translocation, decanting and oily waste disposal, natural attenuation and bioremediation, and crosscutting expertise. The focus of these projects is to advance scientific knowledge to address major gaps in oil spill response and remediation strategies. The second program is the Churchill Marine Observatory (CMO) that is led by the University of Manitoba. Currently under construction at the Port of Churchill adjacent to North America's only Arctic deep-water port, CMO will be the first oil spill research facility in the world that is located in the Arctic and dedicated to the study of Arctic oil spills. The core CMO infrastructure is an outdoor Oil-in-Sea-Ice-Mesocosm with two sea ice pools to simultaneously accommodate contaminated and control experiments on various scenarios of oil spills in ice-covered waters. CMO is scheduled to become fully operational in early 2020, and will directly support MPRI and other programs for the study of detection, fate, effects, and mitigation of oil spills in the Arctic Ocean. Some recent research findings from the MPRI and CMO programs will also be highlighted, including new developments in non-targeted screening of oil and oil degradation products by high-resolution mass spectrometry, chemical characterization of the residues from in situ burning of crude oil, and the behavior of crude oil when spilt under sea ice.

TESTS WITH IN SITU BURNING IN NORWAY

Hilde Dolva

Norwegian Coastal Administration, Norway
hilde.dolva@kystverket.no

The presentation will focus on in situ burning (ISB) and tests performed in Norway from 2017-2019, managed and financed by the Norwegian Association for Oil and Gas Producers (NOFO) and the Norwegian Coastal Administration (NCA). ISB is not not yet included in Norway's oil spill contingency, but there are plans to do so. There is a need to increase knowledge; therefore laboratory, small-scale and field tests were performed. The tests focused on ignition, smoke emission, soot, amount of Black Carbon (BC), residue, sinking of residue and burning efficiency (BE).

Knowledge gaps need to be closed before ISB can be included in the contingency of the offshore industry and governmental preparedness. Therefore, the tests performed in the field have been especially needed and useful as input for further description of the operational procedures for ISB. The annual offshore large-scale exercise (Oil – on – water exercise) in the North Sea is very useful for gaining practical experience with the method. ISB may especially be a response tool for ice infested areas in the Artic.

Two experimental ISBs were done in the Oil- on-water excercise in June 2018. In June 2019, five ISBs are planned. The ISB can only be performed under suitable weather conditions. In 2018 a crude oil, Oseberg, and ULSFO (Ultra Low Sulphur Fuel Oil) were successfully burnt. The oils were released and contained in a fire-boom before being ignited by use of a Pyro Drone, approximately 6m^3 were released for each burn.

Drones were used to monitor smoke plumes, emission gases and soot distribution. The smoke emission monitoring indicated that the concentrations of SO_2 and NO_x were low. The CO_2 concentration was in the range of 200-500ppm. The soot particle monitoring indicated that approximately 90% of the particles were smaller than 2,5 µm (PM2,5). The BC was estimated to be 97g/kg burned oil for Oseberg and 107 g/kg for ULSFO. It was a challenge to collect and estimate the BE. Methods for estimation of BE by using chemical analyses of the residue are being developed.

In June 2019, five ISBs are planned, including the following oils: Oseberg crude (twice), Oseberg emulsion, Marin Gas Oil (MGO) and IFO 180.

Operation guidelines/documents must be developed for use of ISB as a response method, based on the practical tests performed in the field and from the small-scale testing.

MULTI-COMPARTMENT MODELLING - A TOOL TO ASSESS EXPOSURE OF DIFFERENT ORGANISM GROUPS TO OIL COMPONENTS

Matthias Grote[1], Artur Radomyski[2]

[1] German Federal Institute for Risk Assessment Transport of Dangerous Goods Department Exposure
[2] Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari, Venice, Italy
matthias.grote@bfr.bund.de

Dispersants can be used as oil spill response for promoting the breakup of oil slicks into small droplets and thus to reduce oil slicks reaching coastal areas, coating birds and other marine and shoreline species. However, the use of dispersants increases the bioavailability of oil components, which may trigger increased toxic effects in exposed organisms. The EU project LIFE-VERMEER aims to integrate different modelling tools for the estimation of physico-chemical properties and the environmental distribution of chemicals in the environment in order to provide simple tool for exposure and risk assessment. In the frame of this project a multi-compartment model of the North Sea ecosystem is being set up aiming to include relevant environmental compartment (sea surface, water body, sediments, organism groups, etc.) and relevant processes (dissolution, adsorption, bioaccumulation, etc.). With this model a case study addressing the exposure of different organism groups and humans will be assessed for different oil spill scenarios with and without dispersant use. The presentation will provide an overview on the approaches, challenges and possible outcomes of this comparative analysis.

SENSORS FOR IN SITU OIL SPILL DETECTION – PROBLEMS AND OUTLOOK

Harri T. Kankaanpää[1], Siim Pärt[2] and Leonie Nüßer[3]

[1] Finnish Environment Institute, SYKE, Finland
[2] Tallinn University of Technology, TalTech, Estonia
[3] RWTH Aachen University, Germany
harri.kankaanpaa@ymparisto.fi

Fossil oils and oil products derived from fossil oils are a threat due to the negative effects they induce to aquatic organisms. Diesel oils commonly used as fuels in marine traffic are one of the most toxic types of oil. The prognosed increase in marine traffic increases the risk of oil spills. Ship exhaust gas emissions also contain same chemicals as the oil products. We need to monitor the recipient areas for these emissions and use a set of smart detection tools for that task. Fluorometric sensors, biotests and traditional benchtop analytical chemistry methods are available technologies for this toolkit.
The usefulness of a detection method is dependent on its quality. The findings made during GRACE show that validated biosensors based on zebrafish embryos can be used to detect effects induced by oil in laboratory conditions. Moreover, FerryBox field instrument bundles containing oil fluorometers and data transmission systems can be reliably built and operated over long periods of time. Oil sensors are useful tools to be used in laboratory tests with low amounts of interferences. They should be useful also in cases of higher concentrations of oil in natural conditions.
There is however considerable room for improvement of the fluorometer detectors in order to produce high-quality information about fossil oils in sea water. Biofouling hampers the use of optical UV-fluorometers and fluorometers produce different concentration scale depending on the brand. The main problem however is the low selectivity of *in situ* fluorometric detection. This is due to the typically low background concentration of oil and presence of interfering colour (e.g. humic substances and algae pigments) and turbidity in sea water. The Baltic Sea background appeared geographically quite constant over time and space, indicating that at least no major along-route oil spills occurred during the FerryBox campaign. The background issue and the actual operational ranges of the fluorometric sensors remain to be examined in the future. The foreseen improvements in oil sensor technology and biosensor technology will likely enhance the quality of detection of oil and its effects in the marine environment.

OPERATIONAL OIL SPILL DETECTION AND MONITORING ON FAIRWAY USING FERRYBOX AND SMARTBUOY TECHNOLOGIES

Siim Pärt[1], Tarmo Kõuts[1], Kaimo Vahter[1] and Seppo Virtanen[2]

[1] Tallinn University of Technology, TalTech, Estonia
[2] MeriTaito/SeaHow, Finland
siim.part@taltech.ee

The Baltic Sea, with its high maritime traffic has high probability for oil pollution occurrence. Spatial distribution of detected oil spills show that these are most likely located on major ship routes, which lead to the idea to detect oil spills with FerryBox and SmartBoy systems equipped with UV-fluorometers. The sensors detect the concentration of polycyclic aromatic hydrocarbons (PAHs) which are compounds found in crude oil and oil products.
The FerryBox system developed by the Department of Marine Systems at Taltech is used on board the ferry M/S BALTIC QUEEN, on Tallinn–Stockholm route. For oil detection in the surface layer of the sea an UviLux (Chelsey Inst. Ltd) UV-fluorometer is used and for a two month period also another fluorometer, EnviroFlu (Trios), was installed alongside. In parallel, basic seawater properties are recorded. Parameters are recorded in one minute intervals, giving a spatial resolution of 100-150m. Special web based user interface was built to visualize the real-time data on-line (http://on-line.msi.ttu.ee/GRACEferry).
The SmartBuoy model for oil detection was tested at first with an EnrivoFlu fluorometer and a GSM based data transmission system in winter condition in Port of Neste Porvoo oil refinery. Later a SmartBuoy model with satellite data transmission was developed, tested and deployed to challenging off-shore conditions of the Baltic Sea. The off-shore SmartBuoy buoy was deployed on a junction of the main merchant shipping lanes in the Gulf of Finland. Turner Design C3 fluorometer with three optical sensors was placed inside of the buoy in a vertical monitoring well with open flow through pipe enabling continuous sea water exchange.
Oil detection with the hydrocarbon fluorometers on both systems was successful, although no clear sign of oil contamination leading to oil spill, was detected. Measurement results showed stable and precise operation of both systems. As there are other fluorophores that can also react with the fluorometer (such as CDOM – coloured dissolved organic matter) the absolute values of sensor output are not directly connected to oil concentration in seawater. It could clearly be seen in the case of SmartBoy which also monitored CDOM. Variation of hydrocarbon concentration was clearly caused by river water originated organic carbon compounds, which are detected partly on the same wavelength areas with the hydrocarbon compounds. Correlation between the collected hydrocarbon dataset and the CDOM dataset was very strong, verifying the conclusion that changes in the detected hydrocarbon concentrations were caused by natural background organic carbon variation.
Still we can conclude that both sensor setups could be used as alarms, in case of true oil in water situation, a clear peak should be observed in the hydrocarbon concentration data whereas CDOM values should remain in the background level.

LOCAL SCALE OIL SPILL MODELLING AND RISK ASSESSMENT IN SEASONALLY ICE COVERED SEAS

Urmas Raudsepp[1], Ilja Maljutenko[1], Tarmo Kõuts[1] and Nelly Forsman[2]

[1] Tallinn University of Technology, TalTech, Estonia
[2] SSPA, Sweden
urmas.raudsepp@taltech.ee

Oil spill transport and fate models have been designed for the open sea applications and local scale forecasting, separately. The former means implication of geophysical scale atmospheric and oceanographic models, while the latter one the engineering-type models with strong management component. Usually, the geophysical scale models lack the ability to predict oil spill behaviour at the coastal zone, while engineering and management models lack understanding of the large scale processes. Oil-spill trajectory modelling has been a basic approach for statistical risk analyses. The risk is defined as the product of the probability and the consequence. The aim of the current study is to present coupling of the large scale and local scale approaches with a link to the risk assessment methodology. The modeling system consists of GITM Lagrangian model, GETM three dimensional circulation model and HIRLAM ETA meteorological forecast model. Special emphasis is given to the modelling of oil spill transport in partly ice covered coastal zone and stranding and sedimentation of the oil in small bays with harbors. The model is tested in semi enclosed small bay close to the urban area during the open water and ice conditions. The traffic analysis based on AIS-data and accident statistics for the specific areas, scenarios related to ship cargo and ship bunker are used for quantification of probability of a spill. The highest accident index for the Gulf of Finland is obtained in March, i.e. the presence of sea ice. In general, presented oil spill risk assessment methodology enables to define the areas with the highest risk for oil spill, while oil transport and weathering model enables to calculated oil spill trajectories for environmental vulnerability assessment and for design of response efforts by the EOS (Environment & Oil Spill Response) methodology developed within the GRACE project.

STRATEGIC VIEW ON OIL SPILL RESPONSE SUPPORT USING IN SITU AND REMOTE SENSING DATA TOOLS

Jorma Rytkonen[1], Tarmo Kõuts[2] and Siim Pärt[2]

[1] Finnish Environment Institute, SYKE, Finland
[2] Tallinn University of Technology, TalTech, Estonia
jorma.rytkonen@ymparisto.fi

Successful countermeasures against oil spill require an adequate preparedness, a large toolbox of oil recovery methods, trained personnel and excellent communication and surveillance means. When an accident takes place resulting the oil spill outflow from a ship or from a drilling platform it is the primary importance to have tools to locate the spill, to follow the spill and to have tools to follow effectiveness of the selected countermeasures. The fresh data describing the fate of an oil spill is an evitable knowledge for the on scene commander of the responders, thus to form a situational awareness view over the case.
There are a large set of sensors and tools available for oil spill detection and tracking. The usual way to initiate countermeasures against detected oil spill is based on first alert made by the ship(s) in distress, some other party being close the accident or the third party getting data based on remote controlled means. Typical remote controlled party is a surveillance aircraft or helicopter flying over the oil spill and detecting spill and giving the first warning to the authority. Another option is to get alert by a satellite images, for example as a part of the emsa’s cleanseanet service. After receiving the initial information about the oil spill, the combatting authority needs on-line information about the expected size, position, drift velocity and direction to mobilize the required countermeasures and to have time to localize special sensitive areas to be protected by booms or other relevant ways.
Satellites are currently offering reliable images for oil combating authorities to define if certain sea area is affected by oil spills or not. In operative situation there is a need to get more detailed data, confirmations about the slick size and type, and oil slick spreading phenomenon. In operative situation there is a need to get more detailed data, confirmations about the slick size and type, and oil slick spreading phenomenon, that various surveillance means are in need to receive accurate view enough about the situation.
Briefly basic monitoring tools and their main advantages and/or limitations are discussed. Special focus is directed to the integration of sensors with different platforms in order to have added value for the current data set received from the satellite services to define marine oil slicks.

NEW RESULTS ON BIODEGRADATION OF OIL AND DISPERSED OIL IN COLD MARINE ENVIRONMENT

IMPACT OF DISPERSANTS ON PETROLEUM HYDROCARBON BIODEGRADATION AND DEGRADATION GENE ABUNDANCES

Ossi Tonteri[1], Aura Nousiainen[1], Anna Reunamo[1], Jari Nuutinen[1], Jaak Truu[2] and Kirsten S. Jørgensen[1]

[1] Finnish Environment Institute, SYKE, Finland
[2] Institute of Molecular and Cell Biology, University of Tartu, Estonia
ossi.tonteri@ymparisto.fi

Dispersants are used in oil spill response to enhance oil biodegradation. The effects of dispersant use on the microbial communities at the low temperature in Baltic Sea are unknown. The aim of this study was to investigate the impact of dispersant on the microbial degradation gene abundance and petroleum hydrocarbon biodegradation at low temperature and compare the differences between Baltic Sea and North Atlantic seawater using high and low concentrations of petroleum hydrocarbons.
Microcosm studies were conducted at 5 °C for 12 days using Northern Sea crude oil and dispersant Finasol 51. Water Accommodated Fractions (WAF) and Chemically Enhanced Water Accommodated Fractions (CE-WAF) of crude oil and dilutions of these were used in the experiments. Seawater for experiments was taken from Gulf of Finland (coastal area), the Gulf of Bothnia (open sea) and North Atlantic (coastal area). Oil degradation was assessed by analysing the petroleum hydrocarbons with GC-FID, and biodegradation potential by qPCR targeting alkane and PAH degradation genes.
According to chemical analyses, no degradation was observed using undiluted WAF and CE-WAF. With diluted WAF, highest degradation was 92%, and with diluted CE-WAF 5 %. The results showed higher abundances of alkB and 16SrRNA genes in coastal seawater than in the open sea. Dispersant addition did not seem to enhance biodegradation with the dilutions used at 5°C. Highest oil degradation gene abundances and oil removal was observed in microbes associated with Gulf of Finland coastal seawater and lowest in North Atlantic seawater.

IN SITU ELECTROKINETIC TREATMENT PILOT TEST OF PETROLEUM HYDROCARBON CONTAMINATED MARINE SEDIMENT.

Ossi Tonteri[1], Miikka Tunturi, Emil Vahtera[2], Laura Hoikkala[1], Kaarina Lukkari[1], Miiro Jääskeläinen[3] and Kirsten S. Jørgensen[1]

[1] Finnish Environment Institute, SYKE, Finland
[2] City of Helsinki Environment Centre
[3] Eko Harden Technologies Oy, Helsinki, Finland
ossi.tonteri@ymparisto.fi

The in situ electrokinetic method is a remediation method that has been used successfully in treating petroleum hydrocarbon polluted soils. In this work a pilot-scale test was conducted to assess the effectiveness of the electrokinetic method in treating petroleum hydrocarbon polluted marine
sediments in situ. The pilot-test was carried out in Töölönlahti Bay in Helsinki, Finland. The sediments in
Töölönlahti Bay have been polluted by industrial and municipal wastewaters and contain elevated concentrations of petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs).
Experiment area consisted from three replicate treatment plots and three replicate control plots.
Treatment plots consisted of floating anchored platforms that had the remediation equipment. Each platform had electrical control box connected to 16 electrodes, which were installed 1.5 m deep in the sediment creating a 5 x 5 m grid. The pilot-scale in situ treatment was conducted from August 2017 to August 2018, with treatment time of 10-11 months for each plot. The effectiveness of the remediation process was assessed by sampling the sediment close to the plots and measuring sediment hydrocarbon fractions and PAH compounds in the sediment in treatment and control plots. In addition, microbiological activity and community composition (DAPI staining, qPCR and 16S rRNA sequencing).
Petroleum hydrocarbon concentrations (fractions C_{10}-C_{40}) varied between 500-2500 mg/kg (dw) and PAH16 sum between 5-14 mg/kg (dw) (unnormalised values) during the experiment in all experiment plots. Some treatment plots showed decrease in petroleum hydrocarbon concentrations in sampling 2, but concentrations increased in both control and treatment plots in sampling 3. Likelihood Ratio Tests of the Linear Mixed Effects model indicated some reduction in C_{10}-C_{20} and PAH concentrations in treatment plots, but variation is too high to make clear conclusions.

BIODEGRADATION OF TROLL B CRUDE OIL IN SEAWATER AND ICE/WATER INTERFACE OF THE LAB-FORMED SEA ICE

Dang, Nga P[1], M. O' Sadnick[1], C. Petrich [1], J. Truu[2], K. Oopkaup[2], M. Truu[2]

[1] Northern Research Institute Narvik, N- 8504 Narvik, Norway
[2] Institute of Ecology and Earth Sciences, University of Tartu, 5101Tartu, Estonia
nga.dang@norut.no

Biodegradation of petroleum hydrocarbon has been studied intensively with seawater from the temperate and cold marine environment. But as the oil exploration is moving northward into ice-cover areas, spilled oil can get under sea ice or encapsulate into the growing sea ice. Knowledge about the extent of hydrocarbon biodegradation processes in the sea ice, at ice and water interface, and the involved microorganisms are currently very limited. In this study, we performed a biodegradation experiment in microcosms with unpolluted seawater from the Arctic, and with lab-formed sea ice using both the Arctic and sub-arctic seawater. Oil analyses with GC-FID and GC-MS were performed to follow the changes of hydrocarbon compounds in the experiments. Shotgun metagenomic sequencing and/or 16S amplicon sequencing was applied to investigate the effect of oil on microbial community structure during the ice formation process. The study confirmed that biodegradation occurred in seawater at 4 °C as well at -2 °C. Bacteria belonging to phylum *Bacteroides* were dominant in the seawater microcosms which was contaminated with the oil at 4 °C. But whether biodegradation occurred in the sea ice is still a question. No significant loss of hydrocarbons was seen for an oil lens which was encapsulated in the lab-formed sea ice over 6 months at -10 °C. In another experiment with 2 ppm of dispersed oil, high depletion of n-alkanes from C_5-C_{15}, BTEX, and 2-ring PAHs was identified in the oil-contaminated ice. Quantification of genes related to hydrocarbon-degradation and analyses of the microbial community in the oil-contaminated ice will be performed to identify the contribution of sea ice microorganisms in hydrocarbon transformation processes in the ice.

MARINE MICROBIAL COMMUNITY TAXONOMIC AND FUNCTIONAL STRUCTURE AND ITS ASSOCIATION WITH OIL EXPOSURE AND OIL BIODEGRADATION ACTIVITY

Jaak Truu[1], Kristjan Oopkaup[1], Marika Truu[1], Nga Phuong Dang[2], Ossi Tonteri[3], Anna Reunamo[3], Kirsten S. Jørgensen[3]

[1]Institute of Molecular and Cell Biology, University of Tartu, Estonia
[2] Northern Research Institute Narvik, Narvik, Norway
[3]Finnish Environment Institute, Helsinki, Finland
jaak.truu@ut.ee

Advanced DNA sequencing technologies are coupled with the computational challenges to deliver the most relevant biological interpretation of the microbial data collected during the GRACE project. In order to maximize output from microbial data sets obtained during GRACE project the integrative knowledge discovery from multiple omics sources is applied. This analysis is based on the integration of high-throughput sequencing datasets (amplicon based and shotgun metagenomes) obtained during GRACE project and relevant public domain data. To analyse and integrate these large 'omics' data sets novel bioinformatics approaches including metagenomic binning for recovery of population genomes of oil-degrading microbial taxa and machine learning methods are applied. Obtained 'omics' data sets are compared to those found previously in the marine hydrocarbon plumes in order to look for similarities and differences in microbial community composition and metabolic processes in oil-contaminated seawater and sediments due to environmental constraints, remediation strategies and ecosystem type. Information about microbial community taxonomic composition and metabolic markers will be related to kinetic parameters of oil biodegradation and oil remediation strategies using different modelling approaches.

FIELD STUDIES AND GREENLAND IN SITU BURNING

Denis Benito[1], Xabier Lekube[1], Urtzi Izagirre[1], Dennis Bilbao[2], Aino Ahvo[3], Kari K. Lehtonen[3], Nestor Etxebarria[2], Beñat Zaldibar[1], Manu Soto[1] and Ionan Marigómez[1].

[1]CBET Research Group. Department of Zoology and Animal Cell Biology, Faculty of Science and Technology; Research Centre for Experimental Marine Biology and Biotechnology PIE, University of the Basque Country UPV/EHU, Sarriena z/g, Leioa, Basque Country, Spain
[2]IBEA Res Grp, Analytical Chemistry Dept. (Science and Technology Fac.), Univ Basque Country (UPV/EHU), PO Box 644, E-48080 Bilbao, Basque Country, Spain
[3] Finnish Environment Institute, Marine Research Centre, Agnes Sjöbergin katu 2, FI-00790, Helsinki, Finland
denis.benito@ehu.eus

GRACE is a European project with the objective of increasing the knowledge of the oil spills and the oil spill response actions and their environmental effects. Within this framework, the third work-package is involved in the assessment of the biological effects of oil spills and the response methods in the biota from the North Atlantic Ocean and Baltic Sea, which are characterized by extreme environmental conditions. One of the approaches used for this purpose is the biomarker approach in mussels (*Mytilus spp*.), however, biomarkers are sensitive to environmental stressors apart from chemical contaminants, such as temperature, salinity and dissolved oxygen levels. Hence, the objective of the field studies planned in GRACE project is to shed some light on the biomarker baseline levels and responsiveness in the North Atlantic Ocean (changes along a latitudinal sampling and baseline levels in pristine and polluted sites) and Baltic Sea (changes along three different seasons in two subregions). In order to achieve this objective a battery of selected biomarkers including biochemical, cellular and tissue-level biomarkers was applied, and the chemical burden of mussels' soft tissues was determined. The preliminary results of the North Atlantic Ocean study showed clear differences in some biomarker responses among pristine sampling sites that are probably related to environmental factors that change in relation to latitude. In addition, the selected battery of biomarkers was proved to be useful in order to discriminate chemically impacted sites from the pristine ones. The field study conducted in the Baltic Sea showed clear differences in the effect of season-related environmental factors in biomarker responsiveness and reference levels in the mussel populations from each subregion. Apart from the mentioned field studies two *in situ* burning experiments were performed in Greenland with the aim of testing the effectiveness and environmental effects of different oil response actions, the first one being an on-shore experiment and the second one an off-shore burning experiment. The objective of work-package 3 was to assess the impact of these experiments in caged mussels using the battery of biomarkers mentioned before. Overall, both experiments rendered inconclusive results related to the suboptimal processing of the samples and the effect that the caging design could have provoked on mussels.

STUDIES ON THE ADVERSE OUTCOME PATHWAYS RELATED TO OIL SPILLS AND OIL SPILL MITIGATION

Aino Ahvo[1], Sarah Johann[2], Xabier Lekube[3], Tomasz Ciesielski[4], Denis Benito[3], Kari Lehtonen[1], Thomas Benjamin Seiler[2], Ionan Marigomez[3]

[1] Finnish Environment Institute, Marine Research Centre, Helsinki, Finland
[2] RWTH Aachen University, Aachen Biology and Biotechnology, Institute for Environmental Research, Aachen, Germany
[3] University of the Basque Country UPV/EHU, CBET Research Group, Department of Zoology and Animal Cell Biology, Faculty of Science and Technology; Research Centre for Experimental Marine Biology and Biotechnology PIE, Leioa, Basque Country, Spain
[4] Norwegian University of Science and Technology (NTNU), Department of Biology, Trondheim, Norway
aino.ahvo@ymparisto.fi

Adverse Outcome Links and the Adverse Outcome Pathway are constructed models and concepts that portray the steps between a molecular initiating event and a resulting adverse outcome at a higher biological level. Scientific studies aim to build linkages between effects at different biological levels from the molecular and genetic to behavioural, individual and even population level. Different environmental factors can also affect the mechanism of action and the Adverse Outcome Pathway in the organisms.

In the Work Package 3 of the GRACE project we aimed to increase the knowledge of the Adverse Outcome Links and Pathways of oil and oil spill mitigation in several key species: zebrafish (*Danio rerio)*, blue mussel (*Mytilus edulis* and *Mytilus trossulus)*, copepods (*Calanus finmarchicus* and *Limnocalanus macrurus)*, stickleback (*Gasterosteus aculeatus)* and marine medaka (*Oryzias melastigma)*.

In zebrafish, the effects of oil and dispersed oil were studied in the embryo and the adult fish, as well as cell lines. The different biological levels studied included gene expression, biomolecular, cellular, histological, morphological and behavioural responses. Results show strong upregulation in the xenobiotic response mechanisms by dispersed oil and changes in eye development and swimming behavior.

In the blue mussel, the Adverse Outcome Links of oil and dispersed oil were studied at the gene expression, biochemical, histochemical, histological and reproduction levels. We also studied the effect of different environments by conducting studies in different temperatures corresponding to different latitudes in the Northern Atlantic Ocean, and different salinities and temperatures, corresponding to different latitudes and seasons in the Baltic Sea. The effect of oil and dispersed oil could be seen through reactive oxygen species formation and cellular membrane destabilization, as well as reduced growth. Environmental factors were found to affect these pathways.

Gene expression, enzymatic activities and metabolism was studied in the copepods *C. finmarchicus* and *L. macrurus*. Results indicate oxidative stress as a major contributor in the adverse outcome pathway for these species.

In studies with the stickleback, no effects were found at the whole organism level, but analysis is still ongoing in the molecular, biochemical and histological levels. The adverse outcome pathways will also be studied in the marine medaka.

In the GRACE project he Adverse Outcome Links and Pathways of oil exposure have been studied in well known model species as well as key species of the marine ecosystem. Gathering the results from all these species and biological levels will bring valuable information and new insights to the toxicity mechanisms of oil and dispersed oil that will be useful in future risk assessment of oil spills and oil spill mitigation.

AN EFFECT-BASED TOOLBOX FOR THE RAPID AND COST-EFFECTIVE INVESTIGATION AND FINGERPRINTING OF OIL CONTAMINATION

Sarah Johann[1], Mira Goßen[1], Henner Hollert[1], Leonie Nüßer[1], Richard Ottermanns[1], Xabier Lecube[2], Ionan Marigómez[2], Laura de Miguel Jiménez[3], Alberto Katsumiti[2], Aino Ahvo[3], Kari Lehtonen[3], Tomasz Maciej Ciesielski[4], Björn Munro Jensen[4] and Thomas-Benjamin Seiler[1]

[1] RWTH Aachen University, Aachen, Germany
[2] University of the Basque Country (UPV/EHU), Leioa, Spain
[3] Finnish Environment Institute, SYKE, Finland
[4] Department of Biology, Faculty of Natural Sciences at The Norwegian University of Science and Technology, Norway
sarah.johann@rwth-aachen.de

Chemical analysis is often described as the primary method to assess the hazard potential of environmental samples by identifying chemical compounds. Verifying a complex sample presupposes knowledge about the existence of the included compounds and their toxicity. Bioassays are cost and time effective tools that do not require *a priori* information on the identity and physical-chemical properties of a complex environmental sample. Depending on the organizational level the bioassays represent they furthermore provide mechanistic insight into the mode of action of the observed toxicity. A set of methods standardized in DIN EN ISO norms or OECD guidelines that are included in the risk assessment of chemicals or environmental samples were adapted to oil toxicity testing. Besides *in vitro* cell-based methods, also whole-organism tests with laboratory model and study region-relevant species were used to characterize crude oil toxicity. As each oil is a unique and complex sample the resulting toxicity to the exposed biota could also deviate for different oil types. Thus, it is possible to define unique toxicity profiles. The talk will give an overview of biological effect data that were produced by four project partners working in WP3 of the GRACE consortium and investigating the biological effects of different oil types on different biological, organizational levels. Based on statistical evaluation combined with expert knowledge on biological effects, a useful set of bioassays for oil contamination assessment will be suggested. Additionally, limitations and data gaps will be summarized. The talk provides an overview on the state of the art for the toxicity profiling of crude oil contamination in order to initiate further discussions in the GRACE consortium.

BURNING OIL ON ICE

Christian Petrich[1] , Janne Fritt-Rasmussen[2] and Kim Gustavson[2]

[1]NORUT Northern Research Institute, Norway
[2]Department of Bioscience, Aarhus University, Denmark
christian.petrich@norut.no

Oil spills in ice-covered waters pose unique challenges to response activities. In situ burning is a potential response method that has shown promising efficiency in earlier trials. An element of in situ burning in ice is the feedback between the flame of a burn on oil-infiltrated sea ice and the melting ice beneath. A series of experiments were performed in a crude oil pool of initially 0.2 or 0.3 m in diameter on a 1 m^2 freshwater ice block. The oil pools were ignited and the development of the flame, ice temperatures, and ablation rates was monitored. A simple pond spread model was used to derive the burn rates of the spreading pond and burn rates were found mostly around 0.9 mm/min. The burning efficiencies were below 65% and reflect the increase of the pool during burning in addition to the relatively thin initial oil pools. These experiments provide a starting point to address the feedback effect of pond spread and ablation on burns on an oil-infested sea ice.

MECHANICAL REMOVAL OF OIL UNDER ICE - CONCEPTS AND EQUIPMENT DEVELOPMENT

Rune Högström

Lamor, Finland
rune.hogstrom@lamor.com

Along with the new commercial routes in the Arctic Sea and increased oil exploration in the Arctic region comes also the risk of potential oil spills in the area. Oil spill response possibilities as well as currently available technologies are much more limited in Arctic areas versus oil spill response in open water environment and thus development is required. The research project was started by understanding the environmental conditions, and a set of scenarios were determined. Based on these criteria, requirements and limitations were defined. Two main recovery methods and three different ways to move the oil recovery unit were identified. After planning, design and testing, a unit was developed that was proven to be able to recover the oil form under the ice. These identified methods were tested and based on the outcome, one prime solution was seen to be the most effective of these. This was a unit using an inclined rubber scraper in combination with an increased water flow in the vicinity of the under-ice surface combined with an integrated preliminary oil water separation method and additionally an oil suction method from above the ice surface. The water content in the recovered oil could be significantly reduced by using the integrated separation. The unit was designed to be modular and to fit on existing ROVs utilizing them for the movement and observation.

IN SITU BURNING AND EFFECTS FROM OIL SPILLS ON ARCTIC SHORELINES

Kim Gustavson, Susse Wegeberg and Janne Fritt-Rasmussen

Department of Bioscience, Aarhus University, Denmark
kig@bios.au.dk

This presentation introduces and gives an overview of different projects conducted as part of the EU Horizon 2020 research project GRACE (Integrated oil spill response actions and environmental effects). The main objective of the projects was to improve the knowledge base for combating oil spills in Arctic waters. Hence, the projects focus on developing, comparing and evaluating the effectiveness and environmental effects of different oil spill response methods in an Arctic climate. The paper will include results of experimental work performed in Greenland coastal waters in the following subprojects:

- In situ burning field trials in Greenland coastal waters 2017. Two burns were completed; offshore and near the coast. Focus will be on environmental impacts from the burns and lessons learned with respect to increased usability of in situ burning.
- Studies on natural removal of stranded oil on rocky coasts in Greenland. Focus will be on the effects of oil smothering and removal of oil from rocky coast with respects to oil types, degradation by sunlight and wave wash.
- Future work as a follow up on the GRACE project. Introduction of work that are planned to be completed in Northeast Greenland summer 2019

ENVIRONMENT & OIL SPILL RESPONSE (EOS) - AN ANALYTIC TOOL FOR ENVIRONMENTAL ASSESSMENTS TO SUPPORT OIL SPILL RESPONSE PLANNING. I & II

Susse Wegeberg, Janne Fritt-Rasmussen and Kim Gustavson

Aarhus University, Denmark
sw@bios.au.dk

Here we present the EOS tool; Environment & Oil Spill Response (EOS) - an analytic tool for environmental assessments to support oil spill response planning. The EOS tool is based on oil spill scenarios and published as well as expert knowledge on the environment in the sea area in question. The overall aims of the analysis are to identify the most environmental beneficial methods for combating an oil spill in a specific sea area; location or region. Pros and cons of the major available oil spill response techniques are evaluated; mechanical recovery, chemical dispersion of oil and in situ burning , but also doing nothing, and leave the oil to be natural dispersed and degraded, may be an (only) option.
The EOS tool can hence be used for evaluation of inclusion of mechanical recovery, in situ burning and chemical dispersants in national oil spill contingency plans by a general assessment for particular areas / regions of environmental pros and cons of a particular oil spill response method. The pros and cons are evaluated for the different marine spatial compartments; sea surface, water column, seabed, and coast, and for four seasons.
EOS constitutes a decision-making tool on a scientific and operational basis that synthesizes available relevant knowledge and advance the qualified framework on which a national oil spill strategy can be based. In addition, the results obtained through the EOS tool can, e.g., be used for establishment of cross-border and trans-boundary co-operation and agreements.
The process in the EOS tool is based on a number of Excel sheets, with links to descriptive boxes, for gathering data, calculations and scores. The data and scores are finally used in the decision trees for each of the oil spill response methods and for each season.
Several concepts, methodologies or tools are developed, which are used for supporting and optimising oil spill response and preparedness. However, the EOS tool is considered to be novel and unique with respect to 1) including simple and transparent calculations, scoring and decision tree systems; 2) being generic and not restricted to a specific activity or specific seas; 3) not linked to a particular oil spill simulation model, although we recommend using oil spill simulation models which may provide relatively detailed data (e.g., Seatrack Web); and 4) the EOS tool being open source.

OPERATIONAL ADD-ONS TO THE EOS TOOL

Bjørn Forsman and Nelly Forsman

SSPA, Sweden
bjorn.forsman@sspa.se

Work package 5 develops and launches a strategic Environment & Oil Spill Response (EOS) tool for decision-making, to design an appropriate and fast national oil spill response strategy combining the right mix of interventions (e.g., mechanical recovery, in situ burning, chemical dispersants and/or bioremediation). The matrices outlined and presented in deliverable D5.4 shall serve as a compliment to the EOS tool to answer the question “Can we?” when evaluating different oil spill response methods.

The operational requirements included for assessment are categorized as operational window (time window, weather window and ice conditions) and resource logistics (equipment, personnel and transport). Matrix Z1 is a generic compilation of basic operational requirements for each response method. The operational probability, i.e. the probability that the specific method can be used for a specific oil spill scenario, is assessed in Matrix Z2. The operational probability is expected to give guidance in selection of response method, where an OSR method with high operational probability is more likely to be efficient for a specific oil spill scenario.

The oil in ice code developed within Work Package 4, serves as an add-on to the EOS tool, in order to facilitate communication, planning and efficient operations. Several parameters have an impact on oil in ice and the subsequent handling of a spill. Based on the extent of impact the following five characteristic ice and oil parameters and classes are selected to be included in the oil in ice code: Ice type, Sea ice concentration, Temperature, Ice dynamic and Oil classification. These parameters, and their interrelation, affect the choice of oil recovery method. By using the oil in ice Code, a uniform communication and description of the oil spill situation is enabled, and will facilitate the selection of appropriate response strategy.

INDUSTRY EFFORTS IN ARCTIC SPILL RESPONSE TECHNOLOGY DEVELOPMENT

Mathijs Smit

Chair Environmental Effects Technical Working Group, Arctic Response Technology JIP, International Association of Oil and Gas Producers (IOGP)
mathijs.smit@shell.com

The prevention of spills is the top priority of oil and gas operators where ever they operate in the world. However, having a robust and credible oil spill response plan and capability, to mitigate impacts and protect sensitive resources is essential for safe and responsible drilling, especially under harsh conditions in remote regions like the Arctic. The Arctic Response Technology Joint Industry Program (ART-JIP) was conducted over the course of a five-year period from 2012-2017. This industry research program, coordinated by the International Association of Oil and Gas Producers (IOGP) focused on priority areas where new research and technology development had the best chance of significantly advancing in the near future, the capability to respond to spills in the presence of ice as well as in open water and represents a significant achievement in the field of Arctic oil spill response research. Topics were chosen to encompass all the key elements of an integrated offshore response system: In Situ Burning, Dispersants, Remote Sensing, Environmental Effects, Trajectory Modelling, and Mechanical Recovery. The program was initiated by nine oil and gas companies (BP, Chevron, ConocoPhillips, Eni, ExxonMobil, North Caspian Operating Company, Shell, Statoil (now Equinor) and Total) and the work executed by leading scientific, engineering, and consulting firms across the globe.

The research consolidated a vast amount of existing knowledge in these six key areas to provide a robust and more accessible baseline for future regulators, users and industry representatives concerned with assessing, approving, planning, executing and providing oversight to ensure safe Arctic drilling and production programs in the future. The scientific research added a significant new knowledge base to the existing peer-reviewed literature on oil spill impacts, herders and burning, dispersants, remote sensing and trajectory modelling. With this new information, these tools can more confidently take their place as response strategies alongside traditional methods such as mechanical recovery. As a result of past efforts and now the ART JIP, a range of operationally proven tools is available to suit specific regional environments, seasons, drilling and production programs.

The ART JIP set out to leave a lasting imprint by fostering the acceptance of new oil spill response strategies, facilitating the understanding of environmental choices associated with the different response tools and conducting significant new research that builds upon the decades of prior work. A fundamental objective of the ART JIP was to make all results from the research effort publicly available. The results, findings, and strategic implications have been extensively documented and the results can be found on the ART JIP's legacy website (www.arcticresponsetechnology.org/), conference proceedings, and journals.

USING NEBA AND ENVIRONMENTAL RISK METHODS FOR OIL SPILL RESPONSE PLANNING AND PREPAREDNESS

Richard J Wenning and Michael Bock

Ramboll US, USA
rjwenning@ramboll.com

The priorities for oil spill response (OSR) are to protect people, prevent or mitigate environmental damages, and minimize the long-term impact. Several science-based OSR preparedness and assessment tools have evolved in different regions of the world and collectively under the framework of net environmental benefits analysis (NEBA). The collection of tropic, temperate and polar -specific assessment tools have two objectives. First, predict the behavior of spilled oil and associated ecological consequences in offshore, nearshore and shoreline environments. And second, examine the potential for different spill response technologies to mitigate the ecological consequences. This latter application may be the most important function of NEBA tools.

The environmental assessment paradigm developed in the GRACE program, the Environment & Oil Spill Response (EOS) tool, builds upon studies of oil behavior and ecological responses with the aim to support both local-scale and transboundary OSR planning and preparedness. Its application to offshore western Greenland and the Baltic Sea identifies OSR options that will best mitigate the consequences of spilled oil and reduce environmental impacts. EOS is an important addition to the global suite of NEBA tools.

This presentation highlights the importance of EOS and related NEBA tools for region-specific ecological and environmental assessments relevant to oil transportation and exploration. The common assessment framework and shared ecological, environmental and oil chemistry attributes of different assessment models are discussed. Continuous study of OSR science and technology information is the best approach for improving the predictive ability of OSR assessment models. Research after GRACE should be encouraged to continue building knowledge about western Greenland, the Baltic Sea and other marine environments

POSTERS

OIL SPILL RISK ASSESSMENT METHODOLOGY FOR ICE COVERED WATERS AND ARCTIC CONDITIONS

Nelly Forsman and Björn Forsman

SSPA Sweden AB
nelly.forsman@sspa.se

A structured overall spill risk assessment model for oil spills in Arctic and sub-arctic conditions is developed to be used in combination with the SNEBA and its add-ons to identify response capacity needs, priority areas, and localization of resources.
The presented spill risk assessment methodology is based on well-established principles and a large number ofstudies and similar projects have been reviewed and subject for exchange of information. The presented spill risk assessment method is applied for two trial sites; one in Disko Bay in west Greenland and one south of Helsinki in the Gulf of Finland. A set of Arctic factors is introduced in the method to take into account risk influence imposed by the presence of sea ice and other characteristic Arctic conditions.
AIS data and empirical accident data were used to derive a monthly accident index for the trial sites and seasonal variation of the index was analysed. The Gulf of Finland demonstrate a correlation between increased accident probability and the presence of ice. The Disko Bay do not demonstrate corresponding correlation.
The accident index derived for the specific trial site in the Helsinki area is essentially the same as corresponding index calculated for the entire Gulf of Finland area. The consequence component of the spill risk is quantified by a calculated spill volume in m^3 for each specific identified accidental event and each identified dimensioning ship category. Associated probability and consequence figures are presented and compared in risk matrices to facilitate identification and prioritization of critical spill risk events. For the Disko Bay case, accidents (grounding, foundering, or ice damage) with a product/chemical tanker is clearly indicated as a high risk event in terms of spill risk. For the Gulf of Finland area, accidents with a crude oil carrier indicates the highest risk in the matrix.

ZEBRAFISH EMBRYO BEHAVIOUR-TRIGGERED BIOSENSOR SYSTEM FOR OIL SPILL MONITORING AND DETECTION DIRECTLY IN FLOW-THROUGH SYSTEM

Leonie Nuesser[1], Eric M. Wielhouwer[2], Christian Neuser[1], Tarmo Kõuts[3], Henner Hollert[1] and Thomas-Benjamin Seiler[1]

[1] Institute of Environmental Research, RWTH Aachen University, Aachen, Germany
[2] Syntecnos Screening Technologies BV, Rotterdam, Netherlands
[3] Tallinn University of Technology, Tallinn, Estonia
leonie.nuesser@bio5.rwth-aachen.de

Next to motoring water with traditional sensors the concept of biological early warning systems (BEWS) based on the continuous tracking of organisms' physiological reaction that can indicate the presence of toxic substances, has been established for water monitoring all over the world. In response to a decrease in water quality caused by the presence of toxic chemicals or a change in physico-chemical parameters the organisms react with a distinctive and quantifiable change in their behavior. We suggest a system based on hatched zebrafish embryo swimming activity as an alternative to BEWS utilizing adult fish. The use of fish early developmental stages in BEWS holds certain advantages. Replacing larger scale systems required for adult fish leads to a smaller footprint of the complete system which allows to increase the number of individuals used for monitoring. As behavioral data is characterized by high inter individual variance this increases the statistical robustness of the data output and thereby allows to detect changes in behavior and hence, the underlying chemical contamination more sensitively. Furthermore, the miniaturization of the system allows to include an internal control, that is housed under the exact same conditions as the organisms used for monitoring. The system would allow the data output of both control and exposed organisms to be compared in real-time and be used to set a dynamic threshold that triggers the alarm. In the instance that an external stimulus induces behavioral reactions of the system this will also be reflected by the control group and will thereby aid to minimize false positive alarms. In order to utilize fish embryos in a BEWS, the organisms need to be exposed in an appropriate system under flow-through conditions. Here we present a BioWell Desktop Screener System (BWDS) with incorporated customized flow-through well plate on a microtiter scale, designed for the utilization in the BEWS. The introduced biowellplate (BWP) was assembled in a customized housing case that can hold all parts necessary for the operation of the BWP and evaluation of the test organisms. First validation experiments showed that the system is suitable for the prolonged incubation of zebrafish embryos under flow-through conditions. The assembly was tight and performed well within the given parameters. Zebrafish embryo behavior appears to be unaffected by the tested flow rate, hence the prototype is suitable for utilization as part of the proposed BEWS. The findings from the current work demonstrate that a BEWS based on the behavioral responses of zebrafish embryos can be a suitable addition to the real-time monitoring of water resources.

USE OF DIFFERENT AUTONOMOUS PLATFORMS FOR OIL SPILL DETECTION AND MONITORING

Siim Pärt

Tallinn University of Technology, Department of Marine Systems, Akadeemia 15A, EE-12618 Tallinn, Estonia
siim.part@taltech.ee

In case of an oil spill, a quick detection of the pollution is needed. After the detection, decision makers are in need of in-situ information in order make most optimal and best decision in particular conditions and minimize the environmental damage. Real-time environmental data as well as oil slick observations are crucial if a spill trajectory forecast is to be accurate. Also, recorded data can be assimilated into dynamic models to improve the accuracy of the model predictions. There are many novel platforms available for oil spill detection and tracking such as FerryBoxes, Smartbuoys, UAVs, drifter buoys and gliders.
FerryBoxes and Smartbuoys covering areas with high marine traffic allow detection of small spills, which stay undetected with conventional remote sensing methods, but are most numerous and detectable only with in situ measurements.
Unmanned Aerial Vehicles (UAVs) have the potential to deliver information quickly and economically for areas of difficult access and have the potential to fill an important gap in surveillance capability. UAVs cover wide range of scales of applications for oil spill response and can be matched to operational requirements, helping to form a hierarchy of observation scales. Monitoring of oil spills using UAVs includes mapping of the spread, relative and absolute oil layer thickness, as well as classification of the type of oil. UAVs can also be used to monitor localized oil pollution such as oil leakage from shipwrecks.
Drifter buoys have a long history of use for purposes ranging from mapping large-scale ocean currents to following oil spills to aiding search and rescue operations. A drifter buoy can be used to track oil spills during response operations providing the response teams with real-time, accurate information related to speed, position, direction and etc. of the pollutant. There are several oil tracking buoys specially designed for oil spill response in mind.
Gliders have matured to standard oceanographic instruments in the world ocean within last two decades or so. Available remote-sensing techniques are efficient and well developed for on water oil, but less useful for underwater releases before surfacing. Gliders provide a useful platform for determining the spatial distribution of an oil spill and its temporal evolution, underwater.

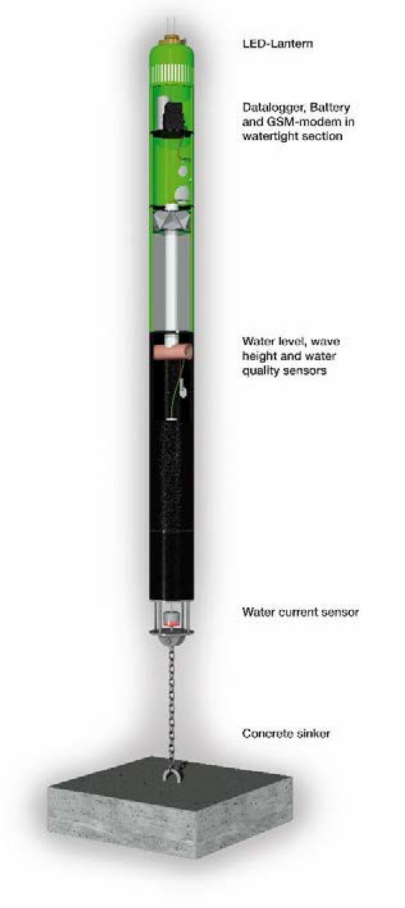
SMART BUOY TECHNOLOGY FOR REMOTE OIL SPILL MONITORING

Seppo Virtanen, Meritaito Ltd (SeaHow), Finland

Meritaito Ltd (SeaHow), Finland
seppo.virtanen@meritaito.fi

Smart Buoy concept by Meritaito (SeaHow)
The Finnish company Meritaito Ltd manufactures polyethylene navigation buoys. The company has developed Smart Buoy which combines regular navigation buoy with modern monitoring sensors and remote communication technology, enabling remote on-line monitoring stations in off shore locations.

Description of the test Set Up
In the Grace-project the new Smart Buoy structure for oil detection with satellite data transmission was developed, tested and deployed to challenging off-shore conditions of the Baltic Sea for the autumn period in 2018. In addition to test the monitoring technology, goal was to detect potential illegal oil effluents originated from vessel traffic navigating on the merchant shipping lanes of the Gulf of Finland.



The Smart Buoy with the integrated Oil Spill sensor technology performed successfully in challenging off-shore conditions. As monitoring result, no clear sign of oil contamination was detected. The monitored variation in the collected hydrocarbon concentration was caused by river water originated organic carbon. Nevertheless, as the sensor technology was capable to detect oil contamination in the laboratory experiment, true oil contamination can be detected also in off-shore conditions.

Results

The SmartBuoy platform with the integrated sensor technology was successful monitoring method in challenging off-shore conditions. The buoy tolerated several storm events with significant wave heights taller than surface parts of the buoy. Also satellite data transmission was successful excluding the strongest storm periods when the buoy was partly submerged by breaking waves. After the storm periods the buoy worked normally again.

Oil detection with the hydrocarbon fluorometer was successful, although no clear sign of oil contamination was detected. Collected hydrocarbon concentration varied during the monitoring period, but variation was identical with variation of collected colored organic carbon (CDOM) concentration. In case of true oil in water situation, a clear peak would be observed in the hydrocarbon concentration data whereas CDOM values should remain in the background level.

IN SITU ELECTROKINETIC TREATMENT PILOT TEST OF PETROLEUM HYDROCARBON CONTAMINATED MARINE SEDIMENT

Ossi Tonteri[1], Miikka Tunturi[2], Emil Vahtera[3], Laura Hoikkala[1], Kaarina Lukkari[1], Miiro Jääskeläinen[4] and Kirsten S. Jørgensen[1]

[1]Finnish Environment Institute, Marine Research Centre, P.O.Box 140, 00251 Helsinki, Finland
[2]Lamor Corporation, Rihkamatori 2, 06100 Porvoo, Finland
[3]City of Helsinki, Urban Environment Division, Environmental Protection, Viikinkaari 2a, 00790 Helsinki, Finland
[4]Eko Harden Technologies Oy, Huopalahdentie 24, 00350 Helsinki, Finland
ossi.tonteri@ymparisto.fi

Introduction: The *in situ* electrokinetic method is a remediation method that has been used successfully in treating petroleum hydrocarbon polluted soils. The method uses electric current to remove contaminants and is based on various electro-chemical processes e.g. electro-osmosis, electrophoresis and electromigration. In this work a pilot-scale test was conducted to assess the effectiveness of the electrokinetic method in treating petroleum hydrocarbon polluted marine sediments *in situ*. The pilot-test was carried out in Töölönlahti Bay in Helsinki, Finland. The sediments in Töölönlahti Bay have been polluted by industrial and municipal wastewaters and contain elevated concentrations of petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs).

Fig. 1: Sediment sampling conducted close to the platform connected to the remediation equipment.

Methods: The pilot-scale *in situ* treatment was tested using three replicate treatment plots and three replicate control plots. Treatment plots consisted of floating anchored platforms connected to the remediation equipment (Fig. 1). On each platform there was a control box that controls 16 electrodes, which were installed 2 m deep in the sediment, creating a 5 x 5 grid. Constant electrical current of total maximum 800 W was applied to each treatment area. The effectiveness of the remediation process was assessed by sampling the sediment in the treatment and control plots. Chemical analyses included petroleum hydrocarbons (fractions C10-C21 and >C21-C40) and PAH compounds. In addition, effects of the treatment on microbiological activity and community composition were analysed with DAPI staining, quantification of petroleum hydrocarbon degradation genes by qPCR and 16S rRNA sequencing for identification of microbial community composition.

Results: Preliminary results show that petroleum hydrocarbon concentrations (fractions C10-C40) varied between 500-2500 mg/kg (dw) in all of the experiment plots and control areas.

Some degradation of petroleum hydrocarbons C10-C40 could be observed temporarily in area 2 and most clearly in area 3, but this has not been confirmed as statistical analysis is not yet finished. In experiment plot 1, there was so far no clear trend of reduction in C10-C40 petroleum hydrocarbons in sediment. PAH sum concentrations were between 5-14 mg/kg (dw). Further analysis of data will be conducted using a linear mixed effects model taking into account between and within plot variation and dependence of temporal replicate results in measured parameters.

Discussion: So far it is difficult to make clear conclusions on the effects of the treatment, due to large variation in the sediment between the test plots. The results show so far that electrokinetic treatment may enhance degradation of petroleum hydrocarbons in one of the plots, but not in the other two plots.

Acknowledgements: This research was a part of the GRACE project (http://www.grace-oil-project.eu/) and has received funding from the EU's Horizon 2020 research and innovation programme (grant agreement No 679266). The pilot-test was done in collaboration with a project funded by Tekes (the Finnish Funding Agency for Technology and Innovation).

IMPACT OF DISPERSANTS ON PETROLEUM HYDROCARBON BIODEGRADATION AND DEGRADATION GENE ABUNDANCES IN THE BALTIC SEA.

Ossi Tonteri[1], Aura Nousiainen[1], Anna Reunamo[1], Jari Nuutinen[1], Jaak Truu[2] and Kirsten S. Jørgensen[1]

[1] Finnish Environment Institute, SYKE, Finland
[2] Institute of Molecular and Cell Biology, University of Tartu, Estonia
ossi.tonteri@ymparisto.fi

Dispersants are used in oil spill response to enhance oil biodegradation. The effects of dispersant use on the microbial communities at the low temperature, brackish Baltic Sea are unknown. The aim of this study was to investigate the impact of dispersant on the microbial degradation gene abundance and petroleum hydrocarbon biodegradation at low temperature, using Baltic Sea water.
Microcosm studies were conducted at 5 °C for 12 days using Northern Sea crude oil and dispersant Finasol 51. Water Accommodated Fractions (WAF) and Chemically Enhanced Water Accommodated Fractions (CE-WAF) of crude oil and dilutions of these were used in the experiments. Seawater for experiments was taken from Gulf of Finland (coastal area) and the Gulf of Bothnia (open sea). Oil degradation was assessed by analysing the petroleum hydrocarbons with GC-FID, and biodegradation potential by qPCR targeting PAH-degradation genes.
According to chemical analyses, no degradation was observed using undiluted WAF and CE-WAF. With diluted WAF, highest degradation was 92%, and with diluted CE-WAF 5 %.
The results showed higher abundances of alkB and 16SrRNA genes in coastal seawater than in the open sea. Degradation gene copy numbers increased towards the end in all experiments. The copies of alkB genes were in general more than 10^3 times higher than PAH degradation genes, indicating a higher immediate potential for alkane degradation.
Dispersant addition did not seem to enhance biodegradation with the dilutions used. Highest oil degradation gene abundances and oil removal was observed in microbes associated with coastal seawater.

APPLICATION OF GENOME-RESOLVED METAGENOMICS FOR STUDY OF OIL-DEGRADING SEA-ICE MICROBIAL COMMUNITY

Angela Peeb[1], Jaak Truu[1], Kristjan Oopkaup[1], Marika Truu[1], Nga Phuong Dang[2]

[1]Institute of Molecular and Cell Biology, University of Tartu, Estonia
[2] Northern Research Institute Narvik, Narvik, Norway

There is evidence of oil biodegradation occurring at subzero temperatures but to what extent and which microorganisms are involved and how they contribute to the oil biodegradation process in the sea ice in the Arctic is still not well known. The goals of this study were to assess oil-degrading microbial community structure and metabolic properties as well as to characterize metagenome-assembled microbial genomes recovered from sea-ice mesocosms. The experiment was performed in 120L mesocosms filled with the sea water. Crude oil was injected under the ice and ice with entrapped oil was kept at -14 °C for 3 months prior to melting for DNA isolation and subsequent shotgun metagenomic sequencing. Microbial community phylogenetic and functional diversity was assessed using a combination of different bioinformatics tools. Draft metagenome-assembled genomes (MAGs) were assembled from environmental DNA. Sea-ice with oil bacterial community was dominated by Pelagibacteriales, Rhodobacteriales and Altermonadales. Ten and three MAGs were recovered from control and oil-treated mesocosms, respectively. MAGs from oil treated mesocosms belonged to the genera Glaciecola and Ascidiaceihabitans. These MAGs possessed pathways for degradation of aromatic compounds, alkanes and synthesis of biosurfactants. Additional analysis revealed that two oil-degrading bacterial lineages, Roseobacter and Colwellia were abundant in obtained metagenomes but were not recovered during the binning process.

CELL AND TISSUE-LEVEL BIOMARKERS IN ATLANTIC MUSSELS (*MYTILUS EDULIS*) EXPOSED TO CRUDE OIL AND OIL+DISPERSANT WAFS AT VARIOUS LOW TEMPERATURES

J. Aguirre-Rubí, D. Benito, L. De Miguel, U. Izagirre, X. Lekube, M. Soto, I. Marigomez

Department of Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (Plentzia Marine Station, PiE-UPV/EHU), University of the Basque Country, Bilbao and Areatza z/g, (48620) Plentzia, Bizkaia, Spain.
ionan.marigomez@ehu.eus

Nowadays, the yet large global demand of oil maintains a high risk of environmental disasters such as oil spills. Polar and Subpolar region, little is to known about the behaviour and toxicity of the oil for native fauna inhabiting these cold latitudes. This study was aimed to determine how cell and tissue-level biomarkers respond in Atlantic mussels (*Mytilus edulis*) exposed to crude oil and oil+dispersant WAFs at various low temperatures. Farmed mussels were imported from Trondheim (Norway) in October 2016 to experimental facilities of Plentzia Marine Station (PiE-UPV/EHU) in the Basque Country. Mussels 3.5-4.5 cm length (210 per tank) were placed in 3 thermally controlled exposure chambers into replicated 40 L tanks and a progressive acclimatization was carried out to 3 different temperatures (5, 10 and 15 °C) for 5 weeks. Four experimental groups were established, including one control group, two oil WAF exposure groups (WAF5%, WAF25%) and one oil+dispersant exposure group (WAF5%+D) with their respective replicates. Experimental samplings were performed at days 0; 11; and 26, after which a 7 d recovery period was allowed (day 33). For histological examination 10 mussels per experimental replicate were carefully dissected and central cross-sectioned slices (5 mm thick, including digestive gland, gonad (mantle) and additional tissues were fixed in formalin fixative for 24h and paraffin embedded. Additionally 10 mussels were dissected to obtain the digestive gland and it was frozen in liquid nitrogen for histochemical analysis. A battery of biomarkers at different level of complexity were measured: (a) Tissue level biomarkers[1] (Volume Density of Basophilic cells, VvBAS); (b) Cell level biomarkers[2,3] (Lysosomal Membrane Stability, LMS; Lysosomal Structural Changes, LSC; Lipofuscin, VvLF and Neutral Lipid Accumulation, (VvNL). Vv_{BAS} was the most sensitive biomarker measured at 5 ºC and 15 ºC after 11 d exposure and less markedly at 26 d. WAF5%+D was the treatment with lowest Vv_{BAS} values. Besides, significant differences were obtained between treatments depending on the exposure time. A similar response profile, increase at day 11 with less effects at day 26, was observed in Vv_{LPF} but in this case the effect was similar at the three studied temperatures. Likewise, an increasing trend in Vv_{LPF} was envisaged: WAF5%>WAF5%+D>WAF25%. In contrast, this trends were not observed in Vv_{NL}. Vv_L values also varied with temperature, WAF type and exposure time in paralel with Vv_{LPF}. Small sized lysosomes (median values 3.8-6.5 $\mu m^2/\mu m^3$) were observed along the experiment. The most clear changes in lysosome membrane stability were observed at days 26 and 33 at 10 ºC. The Lysosomal Response Index (LRI)[3] indicated a lower level of general stress at 5 ºC than at the other temperatures at day 26, and a higher toxicity in presence of the dispersant than in the oil WAF exposure groups. Environmental temperature is a critical factor that largely affects the responsiveness of biomarkers at tissue and cellular level measured in mussels *Mytilus edulis* exposed to environmental contaminants. Tissue and cellular biomarkers were enough sensitive to distinguish at short and long-term the effect of contaminant independent of dose and temperature.

Keywords: Biomarkers, *Mytilus edulis*, crude oil and oil+dispersant

Acknowledgements - Thanks to Total Spain for kindly providing the dispersant and staff at Driftslaboratoriet Mongstad, Equinor (former statoil) for supplying the sample of crude oil used in the experiments.

CAN ZEBRAFISH LARVAE BE USED AS A BIOSENSOR FOR CONTAMINANT DETECTION IN THE BALTIC SEA?

Sarah Johann, Leonie Nüßer, Henner Hollert & Thomas-Benjamin Seiler

Institute for Environmental Research (Biology V), RWTH Aachen University, DE-52074 Aachen, Germany
sarah.johann@bio5.rwth-aachen.de

The zebrafish (*Danio rerio*) has been established as a popular model in a variety of scientific fields like biomedical research and (eco)toxicology. In the context of toxicity assessment, changes of motoric behavior in zebrafish larvae is emerging as a new sensitive endpoint to detect low concentrated contaminants. The Baltic Sea is a relatively shallow brackish sea which has become a sink for a large portion of organic pollutants, as it is characterized by a restrict water exchange with the global oceans [1,2]. Contaminations can be river-borne, atmospheric or of local character mostly caused by oil spillage. Within the new EU-funded project "GRACE" we focus on oil spill monitoring, (bio)degradation and impacts on biota in the Baltic Sea and the North Atlantic caused by acute and diffuse oil spill events.
To adapt zebrafish embryo bioassays to the specific parameters of the Baltic Sea thresholds for salinity tolerance of zebrafish larvae were determined. First, normal development and survival was determined in the prolonged fish embryo toxicity assay (FET). Secondly, behavioral responses of 4 dpf larvae exposed to different salinities up to 0,8 % were determined using the light/dark transition test.
To test for the ability of the larvae to react to sudden contamination events, the larvae were exposed to ethanol in a low concentration range combined with different salinities and immediately monitored for avoidance behavior. The tracking device DanioVision (Noldus, Wageningen, The Netherlands) and the software EthoVision (Noldus) were used to detect the locomotor activity of zebrafish larvae for 95 min in response to light stimuli in a light-dark transition test. Ethanol was selected as an established model compound affecting zebrafish behavior to ensure whether the larvae react successfully under the influence of salinities relevant in the Baltic Sea.

TOXICITY ASSESSMENT OF A NAPHTENIC NORTH SEA CRUDE OIL USING MULTI-LEVEL ENDPOINTS IN ZEBRAFISH EARLY LIVE STAGES

Sarah Johann, Leonie Nüßer, Henner Hollert & Thomas-Benjamin Seiler

Institute of for Environmental Research, RWTH Aachen University, DE-52074 Aachen, Germany
sarah.johann@bio5.rwth-aachen.de

Steadily increasing oil consumption necessitates the development of risk assessment strategies covering future concepts for oil spill response. The EU-funded project GRACE focuses on diffuse oil spills in cold climate and ice-infested areas of the Baltic Sea and the Northern Atlantic Ocean. Crude oil is one of the most complex sample types for ecotoxicological investigations as it consists of thousands of compounds with widely varying physical-chemical properties. Hence, several common methods and tools had to be modified to gain a representative and reproducible picture of the toxicity. In this study, a regional representative naphtenic North Sea crude oil was used to evaluate oil spill and response impacts on biota by means of *in-vitro* effect-based tools.

The acute toxicity of water-accommodated fractions (WAFs) on zebrafish (*D. rerio*) embryos and larvae was investigated using optimized exposure scenarios. Furthermore, the swimming behavior of larvae at the age of 96 hours post fertilization was evaluated in a light dark transition test to assess avoidance and neurotoxic effects. Focusing on more mechanism-specific toxicity, the oxidative stress response and genotoxic effects via the induction of micronuclei were investigated using zebrafish larvae or a zebrafish liver cell line (ZFL).

Further experiments in the project examined ecologically relevant target species at a regional scale (bivalves, crustacean) and additional sensitive biomarker endpoints. Based on the toxicity data of model and regional organisms, species-specific direct links between molecular events and effects on the organismic level (adverse outcome links (AOL)) will be established to contribute to the whole project’s challenge of oil pollution assessment by identifying novel relevant and sensitive biomarkers.

OIL SPILL AND RESPONSE IMPACTS ON BIOTA IN COLD CLIMATES – EFFECT-BASED TOOLS AND ECOLOGICAL RISK ASSESSMENT

T.-B. Seiler[1], S. Johann[1], L. Nüßer[1], K. Lehtonen[2], A. Ahvo[2], R. Turja[2], A. Reunamo[2], J. Nuutinen[3], I. Marigómez[4], M. Soto[4], N. Etxebarria[4], U. Izagirre[4], A. Orbea[4], X. Lekube[4], E. Gil-Uriarte[4], A.J. Olsen[5], B.M. Jenssen[5], I. Salaberria[5], T.M. Ciesielski[5], D. Altin[6], T. Kõuts[7], S. Pärt[7], M. Duchemin[8], K. Jörgensen[2] & H. Hollert[1]

[1] Institute for Environmental Research (Biology V), RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany
[2] Finnish Environment Institute, Marine Research Center, Hakuninmaanti 6, 00430 Helsinki, Finland
[3] Finnish Environment Institute, Laboratory Center, Hakuninmaantie 6, Finland
[4] Plentzia Marine Station (PiE-UPV/EHU), Univ. Basque Country, Areatza w/n, Plentzia, Basque Country (Spain)
[5] Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway
[6] BioTrix, Finn Bergs Veg 3, 7022 Trondheim, Norway
[7] Tallinn University of Technology, Marine System Institute, Akadeemia tee 15a, Estonia
[8] DM Conseil, 7, Allee du Bois Rond 69360 Serewin du Rhone, France
seiler@bio5.rwth-aachen.de

The EU H2020-funded project GRACE addresses diffuse oil spills in cold climate and ice-infested areas of the Baltic Sea and the northern Atlantic Ocean. Within a consortium of comprehensive expertise, the overall objectives are the improvement of marine oil spill detection, monitoring and oil spill response technologies. The work package introduced here (WP3) focuses on the particular environmental impacts of oil spills and response actions (in collaboration with WP4) on biota in these extreme environmental conditions. This is achieved by examining ecologically relevant target species at a regional scale (bivalves, crustacean, fish) and the zebrafish as a well-characterized model organism in ecotoxicology. Regional specimen were sampled from representative locations in the Baltic Sea and northern Atlantic. Bivalves were furthermore sampled with respect to seasonal and longitudinal variation to establish biomarker baselines. Selected model oil types and commonly used dispersants as representatives for the study region and samples from oil biodegradation and remediation experiments (provided by WP2) were investigated using bioassay batteries with sensitive biomarker endpoints. Based on the toxicity data species-specific direct links between molecular events and effects on an organism level (adverse outcome links; AOL) for the test organisms are established. This approach is a useful tool to develop risk assessment strategies covering future concepts for oil spill response. Using the PETROTOX model and a data gap analysis, threshold values are derived and trophic levels as well as test species revealed that are currently missing from the available regulations. This fed into the experimental planning of the other tasks in the WP. Together with WP1, zebrafish-based on-line oil detection biosensors are developed and analysed, which serve for background information to biodetection. The whole GRACE project contributes to the challenge of the prediction, the measurement and the assessment of the evolution of oil pollution.

INTERSPECIES COMPARISON OF LYSOSOMAL RESPONSES OF MUSSEL EXPOSED TO WATER ACCOMMODATED FRACTION (WAF) OF OIL AND DISPERSED OIL

V. de Konick, D. Benito, U. Izagirre, X. Lekube, M. Soto and I. Marigómez

CBET Research Group, Department of Zoology and Cell Biology, Faculty of Science and Technology and Research Center for Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country, Plentzia, Basque Country (Spain)

As a part of the Integrated oil spill response actions and environmental effects (GRACE) project, the impacts of oil and oil-dispersants on biota using biomarkers are studied to gain sound science-based knowledge about the effects of oil contamination in cold climates such as Norwegian Sea and the Baltic Sea. Mytilus species are important sentinel organisms in marine systems and are widely distributed along European coastal areas. Their sedentary state makes them excellent for studying the pollution status for a given area. Along the GRACE regions different mussel species could be found which may show different responses to pollutants. In the present study, three Mytilus (*Mytilus trossulus, M. edulis,* and *M. galloprovincialis)* species cohabitants in the studying area were exposed to WAF of 25% crude oil (Naphthenic North Atlantic) and 5% WAF oil-dispersant mixture (Finasol OSR 52). In addition, a fourth mussel species, *Xenostrobus securis* was also employed in the experiment. This species is an invasive mussel in Basque estuaries but it may well occur in other regions including the cold ones. For this reason, their responsiveness to WAF exposures were also investigated like in the other 3 mussel species. Differences in lysosomal responses to pollutants after 3 and 7 days were measured. The values of the control mussels were totally different depending on the species for all the analysed biomarkers. A clear lysosomal membrane destabilization could be observed for all the species. However, the responses were different among the species according to the exposure time. Both types of WAF treatments caused different alterations in the structure of lysosomes and in the amount of neutral lipid and lipofuscins depending on the time and species. This study shows that the four species had a different responsiveness to pollution which could be a confounding factor for the environmental health status assessment after an oil spill and oil spill responses.

BIOMARKER AND METABOLOM RESPONSES IN *CALANUS FINMARCHICUS* EXPOSED TO THE WATER ACCOMMODATED FRACTION OF NORTH SEA CRUDE OIL

Tomasz Maciej Ciesielski[1], Sofia Soloperto[1], Ewa Agnieszka Cichosz[1], Dag Altin[2], Anna Hallmann[3], Elise Skottene[1], Trond Størseth[4], Bjørn Henrik Hansen[4], Bjørn Munro Jenssen[1]

[1]Norwegian University of Science and Technology, NTNU NO-7491, Trondheim, Norway
[2]BioTrix, Trondheim, Norway
[3]Department of Pharmaceutical Biochemistry, Medical University of Gdańsk, Gdańsk, Poland
[4]SINTEF Ocean AS, Environment and New Resources, Trondheim, Norway
tomasz.ciesielski@bio.ntnu.no

Calanus finmarchicus is an ecologically important species in the North-, Norwegian- and Barents-Seas. Due to continued development of areas for oil and gas production, as well as increased shipping, there are both continuous and accidental environmental discharges of petroleum oil in these regions. Existence of reliable biomarkers of oil exposure is fundamental for monitoring programs and decision-making processes in case of environmental oil discharges or oil spills. Therefore, the present study aimed at investigating toxic responses and validating oxidative stress, lipid peroxidation and protein damage biomarkers in *Calanus finmarchicus* exposed to a water accommodated fraction (WAF) of a naphthenic crude oil from the North Sea (NS). In addition, effects of oil exposure on the metabolome profile was investigated.
Non-ovulating adult females (n=5580) of *C. finmarchicus* from a continuous lab culture at NTNU Sealab were used in exposure studies with WAF of a naphthenic NS crude oil. Individuals were exposed, without feeding, in 5L glass bottles (n=36) at 10 °C to a sub-lethal concentration of WAF corresponding to the 50% of the 96h LC_{50} and were sampled at 5 different time points: 0, 24, 48, 72 and 96 hours. Standard analyses for chemical composition of crude oil and WAF were performed and biomarkers of oxidative stress, lipid peroxidation and protein damage were determined in control and exposed *C. finmarchicus*. Morover, untargeted metabolomics using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy technique was applied for studing effects of exposure on metabolome profile.
Significant induction of glutathione S-transferases (GST) and increase of MDA and GSH concentrations in the exposed group, suggests that lipid peroxidation is a main toxic endpoint in *C. finmarchicus* exposed to WAF of the naphthenic NS crude oil. On the other hand, gene expression results showed inconsistent responses with both up- and downregulation of GST-2 at 48 and 72 hours and downregulation of superoxide dismutase (SOD) at 72 hours. A total of 27 metabolites were quantified with the ^{1}H-NMR spectroscopy technique. The concentrations of some metabolites, such as proline, were significantly lower in the WAF exposed animals than in the control animals. Similary, the organic acid malonate concentration decreased during the experiment in control animals, but increased in the WAF exposed animals. These changes could be explained by the combined effects of oil and starvation.
GST enzymatic activity and MDA concentrations appear to be reliable biomarkers of oxidative stress in *C. finmarchicus* exposed to a WAF of a naphthenic NS oil and lipid peroxidation is

suggested as major toxic endpoint. Metabolome changes suggest that exposure to NS oil WAF might impair energy balance in *C. finmarchicus* and malonate concentration seems to be a promising biomarker of oil exposure.

Acknowledgement: The work was funded through European Union's Horizon 2020 GRACE project (grant agreement No 679266).

COMPARATIVE TEMPERATURE-DEPENDANT TOXICITY OF LIGHT OIL, INTERMEDIATE OIL AND DIESEL OIL WAFS ALONE AND MIXED WITH DISPERSANT: SEA URCHIN BIOASSAYS

L. de Miguel-Jiménez[1], D. Bilbao[2], N. Etxebarria[2], X. Lekube[1], U. Izagirre[1] and I. Marigómez[1]

[1]CBET Research Group, Department of Zoology and Cell Biology; [2]Department of Analytical Chemistry, Faculty of Science and Technology and Research Center for Marine Biology and Biotechnology (PiE-UPV/EHU),University of the Basque Country, Basque County (Spain)

Shipping and oil platforms in the North Atlantic and Baltic Sea have been growing during the last years and thus, the risk of oil spills is gaining concern. Different factors such as the type of spilled oil, weather conditions and water temperature can modify the potential toxicity of spill products; So dispersants do, as well. Moreover, dispersants can be toxic themselves and make oil chemicals more available to biota, and though they can contribute to reduce the residence time of the oil in the environment (depending on factors such as the environmental temperature and the oil type) they also cause the concentration of hydrocarbons in water to rise, resulting in enhanced toxicity. Presently, the influence of Finasol OSR52 dispersant on the toxicity of the water accommodated fraction of three oil types, Naphthenic North Atlantic (NNA WAF), Intermediate Fuel Oil 180 (IFO180 WAF) and Marine Diesel Oil (MDO WAF) for sea urchin larvae and embryos (*Paracentrotus lividus*) was compared after preparing WAF (oil and oil+Dispersant) at different seawater temperatures (5, 10, 15, 20 and 25°C). Different PAH levels and profiles were recorded in WAF depending on the oil type, the temperature and the presence of dispersant. Developmental alterations and larvae abnormalities were determined, and catalase enzyme activity was analysed in larvae as oxidative stress biomarker. Overall, toxicity was lower for oil WAF than for oil+D WAF at all the studied temperatures. Thus, the presence of dispersant seemingly increased oil toxicity; however, the degree of effect varied with the oil type and the temperature at which WAF was produced.

CELLULAR AND TISSUE-LEVEL BIOMARKERS IN MUSSELS (*MYTILUS EDULIS*) SAMPLED IN TWO DIFFERENT STUDY AREAS IN THE NORTHERN ATLANTIC

D. Benito, U. Izagirre, X. Lekube, I. Marigomez, B Zaldibar and M. Soto

CBET Research Group, Department of Zoology and Cell Biology, Faculty of Science and Technology and Research Center for Marine Biology and Biotechnology (PiE-UPV/EHU),University of the Basque Country, Plentzia, Basque Country (Spain)
denis.benito@ehu.eus

Biomarker approach has been widely used in mussel monitoring programs for several years. However, up to know it has not been commonly used in high latitude study areas. In order to establish reference values of cellular and tissue-level biomarkers in the Northern Atlantic Ocean, mussels of two sizes (small, 2-3 cm; large, 3.5-4.5 cm) from selected polluted (commercial harbor & ports, WWTP dumping area) and reference sites in Tromsø and Trondheim (Norway) were sampled in early autumn of 2016 and late summer 2017. Different tissue-level biomarkers including cell type composition (VvBAS) in digestive gland epithelium, structural changes of digestive alveoli (MLR/MET), relative proportion of digestive and connective tissue (CTD) and histopathological alterations in the digestive gland were measured. In addition, lipofuscin and neutral lipid accumulation, lysosomal membrane stability (LMS) and structural changes in the endo-lysosomal system (LSC) of digestive cells were also determined. Higher VvBAS values were recorded in polluted sites than in mussels from reference sites in both study areas. Moreover, mussels from impacted sites exhibited enhanced atrophy of the digestive alveoli (high MLR/MET values) and retraction of digestive diverticula resulting in apparently higher relative extent of interstitial connective tissue (high CTD ratio). Regarding inflammatory responses, parasitic burden and atresia, higher weighed prevalence values than in the reference site were recorded in the two polluted sites from Trondheim. Differences between the two mussel sizes were recorded in parasitic burden, large mussels exhibiting a higher level of parasitization than small mussels. Lipofuscin accumulation was higher and neutral lipid accumulation lower in the polluted sites than in reference sites in both study areas. Lysosomal biomarkers were different between the two sizes. Overall, all biomarkers respond similarly in both study areas indicating the suitability of the selected biomarkers in order to be applied in the Northern Atlantic Ocean.

TOXICITY OF CRUDE OIL WAF ASSESSED BY BIOASSAYS IN *HEDISTE DIVERSICOLOR*

S. Blanco, L. de Miguel, X. Lekube, I. Marigómez, M. Soto and U. Izagirre

CBET Research Group, Department of Zoology and Cell Biology, Faculty of Science and Technology and Research Center for Marine Biology and Biotechnology (PiE-UPV/EHU),University of the Basque Country, Basque Country (Spain)

An increasing concern on lethal and sublethal effects of crude oil has is occurring in the recent years, due to the huge number of maritime oil platforms, and consequently, oil transportation that largely increases the probability of accidental oil spills in the sea. The impact of crude oil has been typically studied through its water accommodated fraction (WAF) due to the high bioavailability of the released compounds present high bioavailability to marine organisms. Polychaetes are often used as models in ecotoxicology to evaluate the status of marine water and sediments, as they are easy-to-work-with that tend to form the dominant sediment dwelling fauna modifying biological and physicochemical characteristics of sediments. Benthic infaunal invertebrate species, such as *Hediste diversicolor*, can be used to monitor pollution effects due to their sensitivity to changes in sediments. *Hediste diversicolor* possess wandering cells in the celome that play an important role in homeostasis and in immune defense against external stimuli, called coelomocytes. Moreover, the celomic cavity communicates with the external environment in each segment of the animal through which these cells can are in direct contact with toxic substances. Nevertheless, this specie has not been studied as a possible model WAF's toxicity testing, although is potentially affected by oil pollution. Thus, the aim of this work was to test the responsiveness of this species to WAF. Comparisons between toxicity bioassays were performed using different percentages (0, 20, 40, 60, 80 and 100%) of Naphthenic North Atlantic crude oil (NNA WAF) in 1:200 concentration by employing two methods of exposure: using wet filter paper (contact-paper; modified from test 207 in OECD, 1984) and directly immersing polychetes in NNA WAF solutions. The exposure times were 48 and 120 h. After the exposure, three endpoints were measured: the median lethal dose (LD50) and two sublethal effects (changes in coelomocyte viability and lysosomal biomarkers). While contact-paper method apparently did not affect polychaetes, sublethal effects were observed in polychaetes after immersion. The viability of coelomocytes decreased, and they exhibited marked alterations in lysosomal biomarkers. The results indicate that polychaetes can be suitable sentinel species for oil spill assessment, but further studies with more severe conditions and different crude oils are required.

THE USE OF PRECISION CUT LIVER SLICES (PCLS) TO ASSESS THE TOXIC EFFECTS OF OIL WATER ACCOMMODATED FRACTIONS (WAF) IN *SOLEA SENEGALENSIS*

T. Hafez[1], R. Duran[2] and M. Ortiz-Zarragoitia[1]

[1]CBET Research Group, Department of Zoology and Cell Biology, Faculty of Science and Technology and Research Center for Marine Biology and Biotechnology (PiE-UPV/EHU),University of the Basque Country, Plentzia), Basque Country (Spain);
[2]Équipe Environnement et Microbiologie UMR IPREM5254, Université de Pau et des Pays de l'Adour (UPPA), Pau (France)

Precision cut liver slices (PCLS) has been extensively used to study the toxic effects of xenobiotics. PCLS maintains the cellular architecture and cell-to-cell communication, which are lost in hepatocyte cultures and cellular metabolism of the slices closely resembles tissue *in vivo* conditions. PCLS has been extensively applied in mammalian toxicology studies. However, fewer PCLS studies have been done on marine fish. Oil contamination is one of the most concerning threats to marine biota and ecosystems. Spills are combated using cleaning agents or dispersants, but the effect of those chemicals could enhance the toxic effects on exposed organisms. In this work, we develop a PCLS protocol using the liver of the marine fish species *Solea senegalensis*. The liver of adult soles was dissected out, placed in iced cold buffer and cored into 8 mm in diameter pieces. Using a tissue slicer, liver cores were sliced into 300 µm thick slices and quickly transferred into cell media containing 0% (control), 25%, 50%, 75% and 100% of Water Accommodated Fraction (WAF) of Naphthenic North Atlantic crude oil. An additional group was exposed to a combination of WAF plus dispersant (Finasol OSR 52) using the same methods previously mentioned. PCLS were incubated for 48 hours at 15ºC in triplicates. Cytotoxicity was assessed with the lactate dehydrogenase (LDH) assay. In addition, slices were analyzed histologically in order to detect alterations in cellular structure. Transcription levels of genes involved in the metabolism of organic xenobiotics such as cyp1a1 and cyp3a, were assessed. Preliminary results suggest that sole PCLS are stable for 48 h and responded to WAF exposure scenarios in a dose dependent manner.

EFFECTS OF OIL SPILL ON COASTAL SEAWEED IN THE ARCTIC

Susse Wegeberg, Janne Fritt-Rasmussen and Kim Gustavson

Aarhus University, Denmark
sw@bios.au.dk

In case of an acute oil spill response operation, decision making regarding the operational response strategy and prioritizing biology at risk must be resolute. For that a Spill Impact Mitigation Assessment, SIMA, is often performed to achieve the optimal environmental benefit with respect to choice of oil spill combat methodology and biology at risk. To provide data for assessing beaching oil spill impacts in the Arctic areas, the effects of oil smothering of the macroalgae *Fucus distichus*, which is a dominant species in the intertidal zone of the coasts in the Arctic, as well as its self-cleaning potential by wash in sea, were studied. Effects of four different oil types were tested, including crude oil types, bunker oil and marine diesel. Different oil types have varying properties depending on the origin of crude oil and refinery process, and hence may have different effects due to their physical and chemical characterizations. Photosynthetic activity was measured as proxy for effect on growth and the self-cleaning potential was tested by wash in sea for oil smothered tips of *F. distichus* over a period of 2 weeks. The removal of the oils from the seaweed surface was considered as relatively fast (T1/2 ~ 3-4 days). Depending of oil type, the oil inhibited or stimulated photosynthetic activity. Marine diesel inhibited photosynthetic activity, whereas the three other oil types stimulated the activity. Thus, in general, the results indicated 1) that oil smothering was relatively fast washed off in the sea water; 2) that, depending on the oil type, photosynthetic activity were stimulated or inhibited; and 3) that the photosynthetic activity was still affected (stimulated or inhibited) even after 14 days, although oil on the tip surface was completely or almost completely washed off. The studies were funded by the Government of Greenland and the European Commission Horizon 2020 programme.

PLUGGING IN A FUZZY LOGIC MODEL TO AN INTERACTIVE 'ENVIRONMENT & OIL SPILL RESPONSE' TOOL

Madis-Jaak Lilover

Tallinn University of Technology, Department of Marine Systems, Akadeemia 15A, EE-12618 Tallinn, Estonia
madis.lilover@taltech.ee

The ultimate task of the research project 'GRACE' is to develop a strategic tool for choosing oil spill response methods. To fulfil that task an interactive tool 'Environment & Oil Spill Response (EOS)' was created to find the most proper oil spill combat technique which minimises the environmental impact of both oil spill and oil spill response techniques. This evaluation can be completed for all marine spatial compartments (sea surface, water column, seabed, and coast) by assessing the environmental pros and cons of the different oil spill response techniques relying on knowledge matrixes about environmental sensitivities and effects, corresponding scores, and a decision tree technique. Here we present a fuzzy logic model, which can be used instead of or parallel to the decision tree technique to merge expert's opinions per marine spatial compartments. To get input data for a fuzzy logic model a data mapping toolbox is needed to convert 'scores for EOS analyses' into an input data ranks of the fuzzy logic model.

Finally, the model merges experts opinion (scores or ranks) about different compartments into one single score which represents the damage reduction if the single or combined response method is implemented. According to the final score, an action plan is accepted or rejected.

The primal fuzzy logic model presented here has generic membership functions and knowledge rules. In future applying model for specific geographical location and season, those could be accordingly adjusted.

EFFECT OF OIL EXPOSURE ON THE BALTIC SEA MUSSEL *MYTILUS TROSSULUS* MICROBIOME

Anna Reunamo[1], Raisa Turja[1], Aino Ahvo[1], Jari Nuutinen[1], Kari K. Lehtonen[1], Kristjan Oopkaup[1], Jaak Truu[2], Kirsten S. Jørgensen[1]

[1] Finnish Environment Institute, SYKE, Finland
[2] Institute of Molecular and Cell Biology, University of Tartu, Estonia
anna.reunamo@ymparisto.fi

Mytilus spp. are widespread and commonly used marine bioindicator species of environmental contamination. The Baltic Sea blue mussel (*Mytilus trossulus*) is a key species in the low diversity brackish Baltic Sea. Blue mussels filter large amounts of water exposing them to a variety of pollutants. We studied how the *Mytilus* microbiome is affected by exposure to crude oil and a dispersant, and what is the petroleum hydrocarbon degradation potential of this microbiome.
A semi-static exposure experiment was carried out at two different salinities, 5.6 and 15 PSU, at 5°C for 21 days. Artificial sea water and water accommodated fraction of crude oil was used in the experiment. Gill and digestive tract communities were studied separately. DNA was extracted and PAH-RHDα and 16S rDNA genes were quantified with qPCR. Bacterial 16S rRNA genes were sequenced on Illumina MiSeq. Polycyclic aromatic hydrocarbons (PAHs) were analyzed from the mussel tissue with GC-MS.
The use of a dispersant increased the concentration of oil in seawater and thus more PAHs accumulated in mussel tissues. Salinity was the most important factor impacting the microbiome structure, and also gill and digestive tract communities differed from each other. Response to oil addition was dependent on dispersant use, especially in case of digestive tract community at the higher salinity. The abundance of PAH-degradation genes increased in the microbiome of oil-exposed mussels, especially with dispersant addition. Bacterial communities changed during the experiment in control aquariums as well, suggesting an important role of the surrounding environment on the *Mytilus* microbiome.