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Effects in field-collected organisms

D3.15

WP3: Determination of oil and dispersant impacts on biota using effect-based tools and ecological risk assessment

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Executive Summary

In situ oil burning is a response technique of high potential since it substantially reduces the spilled oil volume and here in particular the low molecular weight components, which are more prone to partitioning into the water phase and thus exert acute toxic effects. Therefore, an experimental pilot-scale oil spill was carried out in an enclosed coastal Arctic site in Greenland with the aim of testing the effectiveness and environmental effects of *in situ* oil burning. The present report deals with the off-shore field experiment, while the results of the on-shore activity are reported in deliverable D3.13. Field-caught and transplanted mussels were used for determining bioaccumulation of hydrocarbons and biological effects (biomarkers) after the burning operations. Due to the extreme conditions of the experimental scenario several logistical obstacles were encountered. Therefore, recommendations for pilot studies in the area are given here based on the experiences. Due to these problems, reliability of some of the obtained data is not fully guaranteed, and the present (preliminary) results have to be interpreted with caution. However, elevated tissue levels of THCs were recorded in cagings at 1 m depth compared to the reference sites but not in mussels deployed deeper from the surface (4 and 8 m). Responses were observed in a number of biomarkers representing oxidative stress, biotransformation and lysosomal responses, some of them most likely connected to the oil experiment.

1. Rationale

Combat of oil spills by alternative response techniques should be performed at water depths and distances to land that will ensure dilution and hence non-toxic effects, as well as avoiding smoke from an *in situ* burn to contaminate inhabited areas or residues to reach the seabed. However, under certain circumstances such as extreme oceanic conditions, including ice infested waters, and especially in sparsely populated areas with difficult logistics, the spilled oil may be contained in a closed water body confined by the coastline for mechanical recovery and *in situ* burning, according, e.g., to the Canadian oil response guidelines (Wegeberg et al., 2017). *In situ* oil burning is considered a response technique of high potential since it substantially reduces the volume of the spilled oil including field experiments with high ice concentration. Furthermore, since the burning largely affects volatile components, in particular the low molecular weight polycyclic aromatic hydrocarbons (PAHs) are reduced, which are more prone to partitioning into the water phase and thus exert acute toxic effects.

In this framework, and in relation with the GRACE WP4 activities, experimental pilot-scale oil spills in an enclosed coastal Arctic site in Greenland were conducted with the aim of testing the effectiveness and environmental effects of different oil response actions, including the use of two different *in situ* oil burning experiments: on-shore and off-shore. In both, field-caught and transplanted mussels were used for determining the bioaccumulation of contaminants and the biological effects produced after the *in situ* oil burning experiences by means of chemical analysis and biomarker approach analysis, respectively. The present report deals only with the data obtained in the off-shore experiment while the on-shore experiment is reported separately in GRACE deliverable 3.13. *(Note: since the rationale and analysis methodologies are practically similar in both studies the reports are partially overlapping in terms of written text).*

2. Offshore *in situ* burning experiment

2.1 Location

The offshore *in situ* burning experiment was carried out in a bay (63º 42.1940 N, 51º 27.7180 W) of the vicinity of Faeringehavn, south of Nuuk, Greenland (Fig. 1. In

addition, an adjacent bay (63º 42.3800 N, 51º27.7180 W) was chosen as reference bay.

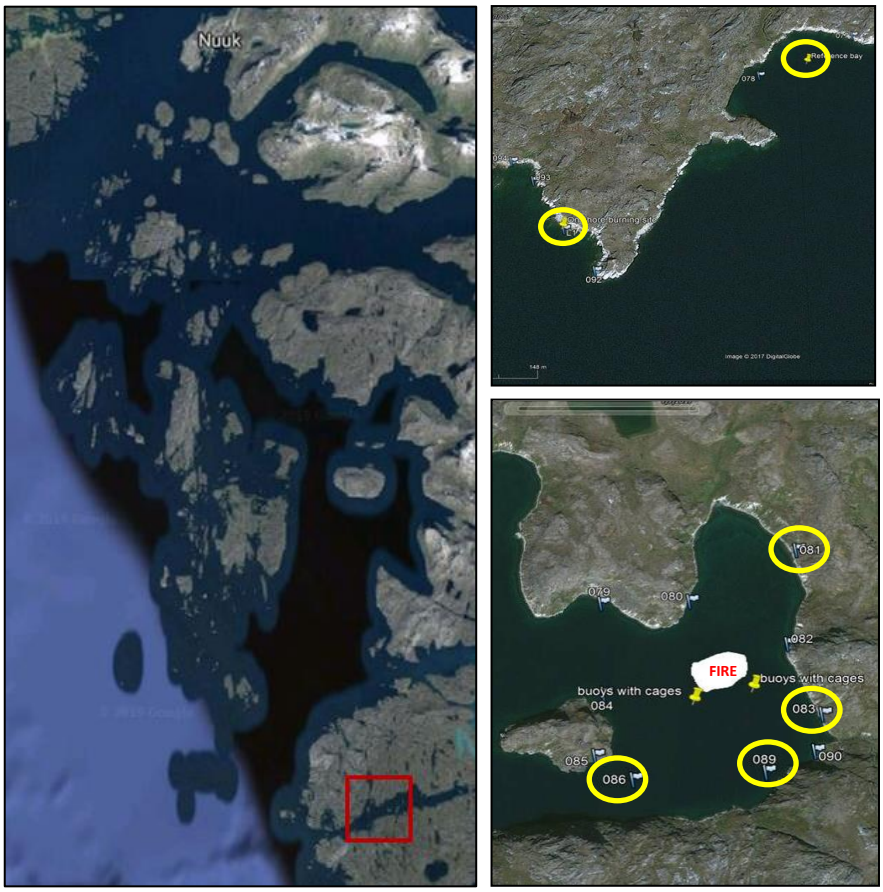

Fig. 1. Left: general map of the southwestern coast of Greenland near Nuuk, showing the study region (square). Top right: Area of the offshore burning experiment (left corner) and the sampling sites in the reference bay (right corner). Low right: area of the burning experiment showing the locations of mussel cages close to the fire and mussel sampling sites in the shoreline.

2.2 Experimental set-up

The on-shore *in situ* oil burning took place on the 2nd of July 2017. The mussel cages were placed just inside the area of the burn. Mussels were collected also around the bay at more distant locations from the burn. Approximately 1000 L of IFO180 (5 bbls of 200 L each) were released into the pyroboom in the bay and towed by two vessels (*see cover photo*). The caged mussels were collected three days later and taken to Nuuk for storage. The mussels were transported in air (dry box) at ambient temperature. The transport lasted 3-4 hours and once in Nuuk the mussels were placed *in toto* (no dissection) in a -80 ºC freezer for storage. A part of the collected mussels was

transported to AU (Denmark) for chemical analysis, and the rest was left in Nuuk for several weeks before they were transported to UPV/EHU and then further on to SYKE for the biomarker analyses.

2.3 Sample preparation and transportation

Working in Greenland involves several logistical limitations. The lack of dry ice and liquid nitrogen was a major obstacle for the transportation of samples at the required temperature (at least -80 ºC to secure fully reliable biomarker analysis). All specialized logistic companies contacted referred to the difficulties of collecting the samples and transporting them under the required conditions, mostly due to the lack of partner companies in the area and the limited flight connections with Nuuk. Thus, the only feasible way was to use a dry shipper for sample transport. However, dry shippers have very limited amounts of space. Consequently, a careful selection of samples to be transported was needed. For more details on the transportation see D3.13.

Table 1. Samples available from the offshore *in situ* oil burning experiment for the biomarker analyses. *n* = number of samples.

Reference area		**Offshore *in situ* burning area**	
	n		*n*
Cage A		**Station 088 (fire)**	
1 m depth	10	1 m depth	9
4 m depth	9	4 m depth	10
		8 m depth	8
Cage B		**Shore**	
1 m depth	10	081	21
4 m depth	8	083	20
		089	9
		086	20

Mussel dissection was carried out with special care avoiding breaking the cold chain in order to maximize sample availability and quality. During the dissection a general occurrence of thick layers of ice was observed inside the mussel. For the digestive gland, a tissue core (small biopsy: approx. 8-12 mm^3) was obtained from each single mussel in order to prepare a set of tissue arrays to analyse lysosomal biomarkers

(histochemistry). The remaining part of the digestive gland was divided in two portions, one excised for oxidative stress biomarkers (to SYKE), and the second left inside a cross-section of the mussel containing different organs (mantle, gills and foot tissue) for the analysis of tissue level biomarkers and histological (e.g., gonad) and histopathological examination (essentially, gills and the digestive tract) (UPV/EHU). A portion of the gills was also left out of this cross-section and processed and transported for oxidative stress biomarker analysis at SYKE. The digestive gland and gill samples dissected for SYKE were transported to Finland in a dryshipper.

3. The biomarker battery

The selected biomarkers are commonly employed for biological effect assessment in marine pollution monitoring. Gamete maturation was also used as supporting parameter. The battery of biomarkers included the enzymatic oxidative stress biomarkers catalase (CAT) and glutathione reductase (GR), and the biotransformation enzyme glutathione *S*-transferase (GST) (also related to oxidative stress), lysosomal responses, and tissue-level biomarkers. These oxidative stress biomarkers have been regularly used in biomarker-based pollution impact assessment in the North and Baltic Seas (Brooks et al., 2011; Turja et al., 2013; 2014; Lehtonen et al., 2016). Lysosomal responses to pollutants in mussel digestive cells are widely used as effect biomarkers (Izagirre & Marigómez, 2009; Brooks et al., 2011; Garmendia et al., 2011; Marigómez et al., 2013). Lysosomal enlargement (augmented volume density: Vv_{LYS}) has been reported in response to pollutant exposure and lysosomal membrane destabilization (reduced labilization period: LP) is recommended by OSPAR as a core biomarker for marine pollution monitoring programs. Intracellular accumulation of neutral lipids (augmented volume density; Vv_{NL}) has been related to exposure to various stress sources including, e.g., PAHs and other organic chemicals (Cancio et al., 1999; Marigómez and Baybay-Villacorta, 2003; Marigómez et al., 2013). Likewise, changes in cell type composition in the digestive gland epithelium (e.g., increase in volume density of basophilic cells: Vv_{BAS}), atrophy of the digestive epithelium (augmented lumen-to-epithelium ratio: MLR/MET), inflammatory responses, and loss of digestive gland histological integrity (augmented connective-to-diverticula ratio: CTD) have been reported to occur in response to pollutant exposure (Brooks et al., 2011; Marigómez et al., 2013).

Conclusively, this battery of biomarkers was conceived as a covering and feasible tool (under the particular conditions and restrains of a field experiment in Nuuk) to investigate the impact of *in situ* burning of oil spills in Arctic conditions.

4. Preliminary Results

4. 1. Chemical analysis

Analysis of total hydrocarbons (THC) of mussels caged in the reference bay and after the offshore *in situ* oil burning experiment were performed by AU as a part of WP4 (Table 1). Disregarding a couple of deviations from the pattern the THC levels are systematically higher in mussels caged at the depth of 1 m at the burning location (station 088) compared to both one on-shore sampling site (089) and the reference bay sites. At 4 m and 8 m the THC tissue levels between the stations 088 and 089 are similar.

Taken into account that the experiment was quite short (the mussels were exposed only for three days) the accumulation of THC released from the burning oil has been rapid. Tissue accumulation of PAHs within a few days of oil exposure has been observed also earlier in laboratory studies (e.g., Turja et al., submitted manuscript).

Table 1. Total hydrocarbon (THC) levels with fractionation measured in mussels caged near the off-shore *in situ* burning site (088) at three depths and at a local on-shore sampling site (089), and reference sites at another bay after the experiment.

Station	088	088	088	089	089	089	Mean of 4 reference stations
Depth	1 m	4 m	8 m	1 m	4 m	8 m	
THC µg/g ww							
C5 - C9	58	7	20	10	16	6	26
C10 - C25	162	63	76	97	78	59	83
C26 - C35	466	265	270	346	279	280	309

4. 2. Tissue level biomarkers and histopathology

It was not technically possible to perform a reliable analysis since the integrity of the digestive gland tissue was critically compromised. Thus, general stress biomarkers such as basophilic cell volume density, epithelial thinning and connective to digestive tissue ratio could not be obtained, and the histopathological examination of the digestive gland was unfeasible. Likewise, the histological integrity of the gills and the mantle tissue was affected. These problems were apparently due to the volume changes and further ice crystal formation in the tissues as the mussels were frozen *in toto* with water inside the shell cavity. This way of processing might also have had

consequences for other biomarkers, and therefore their results must also be interpreted with caution.

4. 3. Oxidative stress biomarkers

The activities of CAT, GST and GR showed constantly higher values at higher depths (4 and 8 m) compared to 1 m at the off-shore burning site (station 088) and one on-shore sampling sites (station 089) (Table 2). Thus, comparisons between the sites are most appropriate between the near-surface values which for all the enzymes showed lower values at the burning site station. In regard to GST the levels at station 088 were low throughout the depth range. This type of a bell-shape stress response might be caused by the burning event and subsequent release of chemical residues to the surface layer where the mussels were caged at 1 meters depth. Mussels in the reference bay showed no effect in enzyme activity levels at caging depths of 1 and 4 m. Unlike at station 088 directly affected by the fire, mussels at the shore stations 081 and 086 showed elevated CAT and GST levels in comparison with the reference bay mussels, this being the biomarker response mode common in cases when the stress is not overwhelming the enzymatic defence system's capacity.

However, a biasing factor could be that the intertidal mussels were collected from the reference bay and caged both at the reference and the experimental bays only for one day and therefore were already subjected to circatidal and feeding cyclic rhythms, or could be reacting to an abnormally prolonged immersion time.

Table 2. Oxidative stress and biotransformation biomarkers measured after the experiment in mussels caged near the off-shore *in situ* burning site at three depths, at the on-shore sampling sites and at sites at the reference bay. CAT: catalase activity (µmol min^{-1} mg $protein^{-1}$; GST: glutathione *S*-transferase activity (nmol min^{-1} mg $protein^{-1}$); GR: glutathione reductase activity (nmol min^{-1} mg $protein^{-1}$).

	CAT	GST	GR
REF - 1 m	52.6	63.00	18.90
REF - 4 m	55.5	68.90	17.90
088 - 1 m	47.7	41.43	15.28
088 - 4 m	78.5	54.84	20.48
088 - 8 m	75.4	51.11	19.48
081	71.2	82.27	17.92
083	55.9	67.03	14.29
086	65.4	90.53	18.57
089	58.5	61.41	17.05

4. 4. Lysosomal biomarkers

Higher values of VvLYS and SvLYS were observed at the burning site station 088 at 1 m depth and at the on-shore sampling station 086 (Table 3). At the latter also VvNL showed the highest response. Lysosomal structural changes have been reported in response to pollutant exposure and lysosomal membrane destabilization. At the reference stations depth had a marked effect on all biomarker values with higher levels measured in all the parameters at the depth of 1 m compared to 4 m, this being largely the pattern observed at the burning site station 088 as well. As mentioned, the sub-optimal processing of the samples may have influenced some of the lysosomal biomarkers (Blanco-Rayón et al., 2019).

Due to the high variability in the data further analysis need to be conducted before a proper interpretation of the results can be carried out in terms of environmental impact of *in situ* burning and a possible reduction of acute toxicity caused by an off-shore oil spill.

Table 3. Lysosomal biomarkers measured in mussels caged near the off-shore *in situ* burning site at three depths, at the on-shore sampling sites and at sites at the reference bay after the experiment. VvLYS: volume density of digestive cell (DC) lysosomes (μm^3 LYS/μm^3 DC); SvLYS: surface density of DC lysosomes (μm^2 LYS/μm^3 DC); S/VLYS: surface-to-volume ratio of DC lysosomes (μm^2 LYS/μm^3 LYS); NvLYS: numerical density of DC lysosomes (1/μm^3 DC); LP: lysosomal membrane labilization period (min); VvLPF: volume density of digestive cell lipofuscins (μm^3 LPF/μm^3 DC); VvNL: volume density of digestive cell neutral lipids (μm^3 NL/μm^3 DC).

Station	VvLYS	SvLYS	S/VLYS	NvLYS	LP	VvLPF	VvNL
REF - 1 m	0.00077	0.0041	5.59	0.0019	12.2	0.035	0.010
REF - 4 m	0.00028	0.0016	3.00	0.0009	5.0	0.014	0.004
088 - 1 m	0.00089	0.0044	5.10	0.0020	11.9	0.041	0.008
088 - 4 m	0.00057	0.0033	5.99	0.0018	10.0	0.028	0.008
088 - 8 m	0.00066	0.0038	6.09	0.0019	12.5	0.029	0.011
081	0.00030	0.0022	9.60	0.0020	14.8	0.063	0
083	0.00020	0.0016	7.93	0.0014	14.5	0.043	0
086	0.00089	0.0049	5.82	0.0024	ND	0.038	0.030
089	0.00021	0.0016	7.62	0.0014	14.5	0.063	0.008

5. Concluding Remarks

Differences in the tissue levels of THCs were recorded, especially at the depth of 1 m, where an accumulation of hydrocarbons three days post-experiment could be observed. Together with the observed changes in a number of biomarkers evidencing rapidly induced biological effects the overall impact of the *in situ* oil spill burning experiment on the mussels could be established. A more detailed analysis of the data gathered both in the off-shore and on-shore experiments (separate report) will reveal more information on the effects, their connection to the exposure levels and their possible mechanisms.

For future studies, in order to get fully reliable data, it is important to improve logistics in distant locations where scientific infrastructure (e.g., the availability of cold storage facilities and materials for sample transportation) is deficient. Due to the problems encountered the present results have to be interpreted with caution. Tissue-level biomarkers, gills and digestive gland histopathology, and gamete developmental stages could not be properly determined. The biology of the caged mussels needs also to be considered for the experimental design: the use of subtidal mussels for subtidal caging (best option), or extension of the acclimatization for at least beyond 2 weeks (which is not easy from the logistic point of view and most likely not the best solution from the biological point of view, since intertidal and subtidal mussels respond differently), and/or collect in parallel intertidal feral mussels both at the reference and the experimental sites. Samples should also be dissected *in situ* or at least taken to the laboratory under acceptable transport conditions (depending on the endpoint) for further-on processing for either biomarker determination or depuration before chemical analysis. Finally, the best available practices must be secured for safe sample transportation without breaking the cold chain.

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