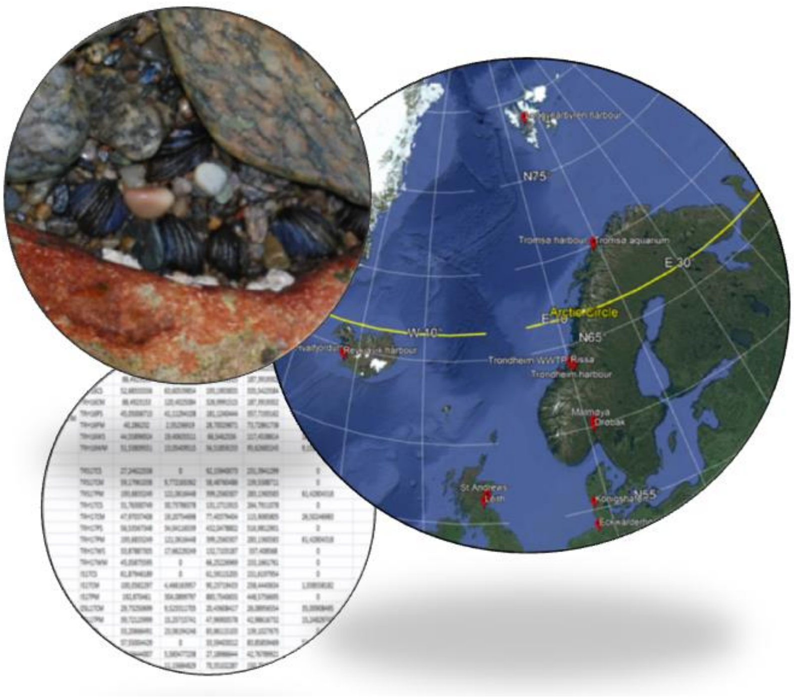
GRACE grant no
679266

Report on baselines and variability on latitudinal variability in biomarker in mussels from the northern Atlantic, and associated database

D3.8

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



Prepared under contract from the European Commission
Contract n° 679266
Research and Innovation Action
Innovation and Networks Executive Agency
Horizon 2020 BG-2014-2015/BG2015-2

Project acronym:	GRACE
Project full title:	Integrated oil spill response actions and environmental effects
Start of the project:	01 March 2016
Duration:	42 months
Project coordinator:	Finnish Environment Institute (SYKE)
Project website	http://www.grace-oil-project.eu

Deliverable title:	Report on baselines and variability on latitudinal variability in biomarker in mussels from the northern Atlantic, and associated database
Deliverable n°:	D3.8
Nature of the deliverable:	Report
Dissemination level:	Public

WP responsible:	WP3
Lead beneficiary:	UPV/EHU

Due date of deliverable:	28/02/2019
Actual submission date:	28/02/2019

Deliverable status:

Version	Status	Date	Author	Approved by
1.0	draft	22/02/2019	Xabier Lekube, Denis Benito, Urtzi Izagirre and Ionan Marigómez (UPV/EHU)	WP members 26/02/2019
2.0	final	28/02/2019	Xabier Lekube, Denis Benito, Urtzi Izagirre and Ionan Marigómez (UPV/EHU)	Steering group 28/02/2019

Table of contents

Executive Summary

The combination of biomarker approach and chemical analysis has been used for monitoring disturbance in marine ecosystem health status after oil spill events. The establishment of baseline values and the range of natural variability for biomarkers, physiological condition and chemical tissue concentrations in mussels at regional scale are essential to identify the adverse biological effects produced by oil spills and oil spill responses on the basis of a mussel watch approach. Likewise, confounding factors related to environmental, climatic and biological conditions varying with latitude can interfere with the interpretation of the results of the monitoring. Based on two monitoring campaigns this report deals with the first approach to establish baselines and natural (latitudinal) variability in biomarkers and physiological conditions in mussels *Mytilus* spp. collected along the northern Atlantic and in the Arctic Ocean; in addition, data to contribute to establish putative background tissue levels of polycyclic aromatic compounds (PAHs) are provided.

Abbreviations:

Vv_{LYS}: volume density of lysosomes in digestive cells ($\mu m^3/\mu m^3$)
LP: Labilization Period of the lysosomal membrane in digestive cells (min)
Vv_{NL}: volume density of neutral lipids in digestive gland (µm3/µm3)
Vv_{LPF}: volume density of lipofuscins in digestive gland (µm3/µm3)
Vv_{BAS}: volume density of basophilic cels in digestive gland epithelium (µm3/µm3)
MLR/MET: mean luminal radius to mean epithelial thickness (µm/µm)
CTD: connective tissue to digestive diverticula ratio
FCI: flesh condition index
ADG Index: adipogranular cell index

1. Rationale

After an oil spill, biomonitoring for assessing disturbance in marine ecosystem health status can be performed by recording biomarkers and chemical tissue burdens in combination using mussels as sentinel organisms (e.g. Prestige oil spill; Garmendia et al., 2011; Marigómez et al., 2013). However, confounding factors related to environmental, climatic and biological conditions varying with latitude (photoperiod, SST, salinity and nutrients), or the life-cycle, the lifespan and the metabolism of the biota – age, reproductive condition, growth – can interfere with the interpretation of the biomonitoring data, as they can evoke responses in biomarkers and condition parameters that are not related to the presence of chemical pollutants (Garmendia et al., 2010; Izagirre et al., 2014). In order to efficiently implement this kind of biomonitoring programmes, baselines and the natural variability range must be identified at regional scale for biomarkers, condition parameters and chemicals' tissue burdens. Thus, adverse outcome links can be properly identified to establish cause-effects relationships and to obtain reliable estimates of the impact and the needs for intervention after an oil spill.

This report constitutes a pioneering first approach to identify putative baselines and natural (latitudinal) variability in biomarkers and condition parameters in mussels *Mytilus* spp. collected along the northern Atlantic and in the Arctic Ocean. Likewise, new data are reported to contribute to identify regionally-relevant baseline values of chemical's tissue burdens in mussels (more details on the studied region is already detailed in Deliverable 3.3).

2. Sampling of mussels for the establishment of baselines

Blue mussels are distributed all along the northern Atlantic Ocean and also in the Artic Ocean, the northernmost mussels being found in Svalbard. *Mytilus edulis* is the most abundant mussel species found in subartic and Artic areas; however the presence of other species such as *Mytilus trossulus*, *Mytilus galloprovincialis* and hybrids has also been reported (Mathiesen et al., 2016). It is conceivable that, though they are closely related, these species can present differences in their biological responsiveness against environmental factors and pollutants; however, for practical reasons in a first approach (a sufficient data set to decide in this respect will be available only after years of duely targeted research) we have considered those dissimilitarities to be of minor importance in comparison with the differences and gradients in natural factors at regional scale. Nevertheles, efforts have been already addressed to shed some light over this issue, and the different mussel species collected in this field study are being currently characterised in a parallel investigation carried out at PiE-UPV/EHU in collaboration with the University of Stirling.

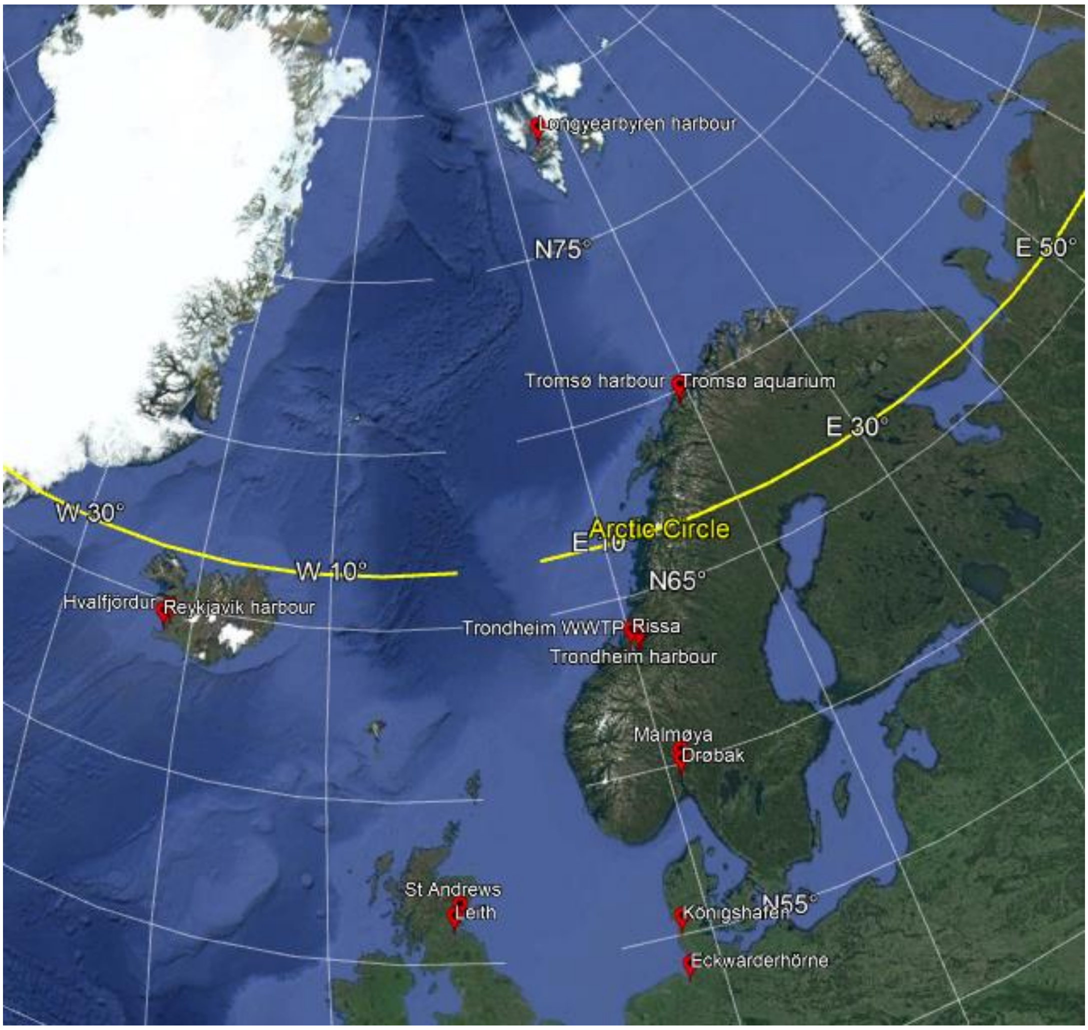


Figure 1. Map of the Arctic Ocean and the northern Atlantic Ocean indicating in red color the sampling sites showing a latitudinal transect. Map: Google Earth.

Table 1. Details of mussel sampling locations, sampling dates and type of mussels collected at each place.

Country	Location	Latitude	Longitud	Date	Mussel type
Norway	Svalbard (Longyearbyen)	78.236081	15.606482	21/09/2017	Subtidal
	Tromsø (port)	69.654177	18.968459	**20/10/2016** 18/09/2017	Intertidal
	Tromsø (acuarium)	69.642089	18.94639	**20/10/2016** 18/09/2017	Intertidal
	Trondheim (port)	63.442692	10.425494	**17/10/2016** 20/09/2017	Intertidal
	Trondheim (WWTP)	63.444867	10.341331	**19/10/2016** 21/09/2017	Intertidal
	Rissa	63.561753	9.899776	**18/10/2016** 21/09/2017	Intertidal
	Oslo (Malmøya)	59.873896	10.757743	15/09/2017	Subtidal
	Oslo (Drøbak)	59.615889	10.652007	18/09/2017	Intertidal
Iceland	Havfjördur	64.358154	-21.486458	07/09/2017	Intertidal
	Reykjavik (port)	64.155605	-21.939218	08/09/2017	Intertidal
Scotland	St. Andrews	56.333602	-2.776173	21/09/2017	Intertidal
	Leith	55.977359	-3.140404	20/09/2017	Intertidal
Germany	Königshafen	55.042374	8.449102	10/10/2017	Intertidal
	Eckwarderhörne	53.520301	8.231816	11/10/2017	Intertidal

Mussels were collected following a latitudinal transect along the northern Atlantic Ocean (Figure 1; Table 1). In addition, it was decided to add a sampling locality in Svalbard because at present the northernmost populations of mussles are seemingly located in this archipelago (Berge et al., 2005). Except in Svalbard, mussels at each sampling locality were collected in at least one a priori non-impacted and one a priori impacted site, in order to investigate also variability in the responsiveness of biomarkers againts environmental impact.

It also has to be mentioned that mussels were collected in 2016, only in Tromsø and Trondheim as a pilot experience (more detailed in Deliverable 3.3), and in 2017 in all the sampling sites shown in Table 1. In addition, farmed mussels from a depuration facility located in Rissa were collected in 2016 (Figure 2).

Figure 2. Mussels from the depuration facility at Rissa.

Mussels of two different sizes (small: 2-3 cm; large: 3,5-4,5 cm) were collected in each sampling point. All the mussels were intertidal except the ones from Svalbard and Malmøya, which were subtidal.

3. Preliminary results

3.1. Background level for biomarkers

Mean values of the measured biomarkers of the mussels collected during 2016 and 2017 are shown in Table 2 and Table 3, respectively. Latitudinal differences in mussels are observed in critical parameters such as gamete development, which is important for interpretation of biomarkers, because the gender and the reproductive condition can be major confounding factors. This fact must be taken into consideration when designing a biomonitoring programme at regional scale in the study area, as well as for inter-site comparison of biomonitoring data.

Table 2. Mean values of selected biomarkers (1) and condition parameters recorded in mussels collected in 2016. S=small, L=large.

Location/Year		Size	Vv_{LYS} (µm³/µm³)	LP (min)	Vv_{NL} (µm³/µm³)	Vv_{LPF} (µm³/µm³)	Vv_{BAS} (µm³/µm³)	MLR/MET (µm/µm)	CTD	FCI	Sex Ratio (M/F)	Gonad Index	ADG Index
Tromsø 2016	**Aquarium**	**S**	0.006	14.75	0.21	0.06	0.15	0.21	0.56	37.36	0.80	3.75	2.17
		L	0.008	21.25	0.17	0.06	0.15	0.22	0.50	51.08	0.73	3.89	2.16
	Port	**S**	0.005	10.50	0.10	0.10	0.21	0.21	0.57	47.27	1.00	3.37	0.95
		L	0.007	9.40	0.09	0.09	0.16	0.19	0.57	43.85	1.25	3.56	1.89
Trondheim 2016	**Rissa**	**S**	-	-	-	-	0.14	0.15	0.29	60.55	1.11	1.61	2.76
		L	0.007	17.00	0.09	0.12	0.13	0.15	0.35	67.59	0.64	1.61	2.61
	Port	**S**	0.003	7.60	0.02	0.11	0.23	0.20	0.56	66.76	2.00	1.50	0.84
		L	0.003	8.00	0.03	0.10	-	-	-	-	-	-	-
	WWTP*	**S**	0.004	12.00	0.06	0.10	0.14	0.18	0.51	34.15	1.00	3.10	2.50
		L	0.004	13.00	0.05	0.13	0.17	0.14	0.51	33.13	1.00	3.40	2.56
	Farm	**L**	0.020	17.00	0.07	0.12	0.11	0.17	0.41	65.30	0.58	1.77	3.00
Regional values		**max**	0.020	21.250	0.210	0.130	0.230	0.220	0.570	67.590	2.000	3.890	3.000
		mean	**0.007**	**13.050**	**0.089**	**0.099**	**0.159**	**0.182**	**0.483**	**50.704**	**1.011**	**2.756**	**2.144**
		min	0.003	7.600	0.020	0.060	0.110	0.140	0.290	33.130	0.580	1.500	0.840

*WWTP: Waste Water Treatment Plant; (1) Biochemical biomarkers and data of additional localities (investigated beyond the initial GRACE Plan in order to provide an added value to this study; and for which additional financiation external to GRACE was achieved; mainly UPV/EHU's own resources)

Table 3. Mean values of selected biomarkers (1) and condition parameters recorded in mussels collected in 2017. S=small, L=large.

Location/Year		Size	Vv_{LYS} ($\mu m^3/\mu m^3$)	LP (min)	Vv_{NL} ($\mu m^3/\mu m^3$)	Vv_{LPF} ($\mu m^3/\mu m^3$)	Vv_{BAS} ($\mu m^3/\mu m^3$)	MLR/MET ($\mu m/\mu m$)	CTD	FCI	Sex ratio (M/F)	Gonad Index	ADG Index
Svalbard	**Longyear-byren**	**S**	-	-	-	-	0.15	0.21	0.38	-	1.22	3.53	1.93
		L	-	-	-	-	0.15	0.25	0.44	65.90	1.86	5.00	1.27
Tromsø 2017	**Aquarium**	**S**	0.004	16.00	0.26	0.07	0.12	0.27	0.36	43.17	0.64	2.61	2.53
		L	0.004	18.75	0.09	0.09	0.14	0.24	0.47	36.01	0.73	2.76	1.00
	Port	**S**	0.005	13.00	0.13	0.07	0.15	0.27	0.49	50.84	1.80	1.94	1.58
Trondheim 2017	**Rissa**	**S**	0.005	16.25	0.26	0.16	0.11	0.14	0.30	73.30	1.60	1.66	2.40
		L	0.002	19.38	0.14	0.11	0.09	0.16	0.37	83.98	0.80	2.73	3.25
	Port	**S**	0.003	13.00	0.05	0.12	0.13	0.26	0.50	78.98	1.33	1.19	1.64
		L	0.003	9.25	0.05	0.08	0.12	0.35	0.38	84.33	1.80	1.58	3.11
	WWTP*	**S**	0.004	17.50	0.14	0.09	0.14	0.25	0.44	40.90	0.55	1.43	1.88
		L	0.005	14.06	0.09	0.08	0.13	0.24	0.52	39.92	0.73	1.60	2.39
Regional values		**max**	0.005	19.38	0.260	0.160	0.150	0.350	0.520	84.330	1.860	5.000	3.250
		mean	**0.004**	**15.000**	**0.130**	**0.096**	**0.130**	**0.241**	**0.429**	**58.369**	**1.163**	**2.383**	**2.053**
		min	0.002	9.25	0.050	0.070	0.090	0.140	0.300	36.010	0.550	1.190	1.000

*WWTP: Waste Water Treatment Plant; (1) Biochemical biomarkers and data of additional localities (investigated beyond the initial GRACE Plan in order to provide an added value to this study; and for which additional financiation external to GRACE was achieved; mainly UPV/EHU's own resources)

In the studied localities, some biomarkers were clearly different between the polluted and reference sites. Thus, lysosomal biomarkers were seemingly the most sensitive endpoints and were able to discriminate between impacted and non impacted sites in all the latitudes studied (Note: this comparison was not made in Svalbard). In contrast, latitudinal differences in biomarker values between reference sites were not relevant even though it cannot be disregarded that different *Mytilus* spp species were seemingly at different localities (unpublished preliminary results; ongoing research). On the other hand, the Condition index was clearly lower in Tromsø than in Trondheim and in the WWTP site than in the other sites in the latter locality.

3.2. Background levels of PAHs

Mean concentrations of 16 PAHs measured in mussels collected during 2016 and 2017 are detailed in Table 4 and Table 5, respectively. Differences in the load of PAHs in mussels of different size/age were only observed in some localities. Overall, total PAHs are high for the expected polluted sites and always higher than the reference ones for most of the localities and years. However, differences between both years are also observed for some localities showing a changing environment.

4. Concluding remarks

Differences in total PAHs are high for the expected polluted sites and always higher than the reference ones for most of the localities and years although a few samples are still to be analysed. Nevertheless, for a better understanding of the contamination levels and its relation with the biomarkers observed, other chemical analysis such as metals and PCB are needed. Those measurements are in progress as a parallel study carried out by PiE-UPV/EHU in order to expand the regional analysis of contaminants beyond the PAHs originally scheduled within the frame of the GRACE Project.

Biomarkers were responsive to environmental impact and were clearly different between the sites *a priori* identified as impacted and reference, which was in agreement with the results of PAHs tissue levels in mussels. The results obtained are valuable to establish baselines (background levels and natural – latitudinal – variability) for biomarkers, condition parameters and PAH tissue levels in sentinel mussels from Svalbard to Scotland-Germany, which will be helpful to carry out monitoring of the biological effects of contaminants at regional scale in the the northern Atlantic and in the Arctic Ocean. Additional biomarkers (biochemical) now are being analysed and will be available soon. Complementary research activities are ongoing to include other potential confounding factors (species, presence of other stress sources such as other chemicals and emerging contaminants, etc.) in the framework of this monitoring design.

Tabla 4. Mean concentrations of measured PAH compounds in ng/g d.w. mussel samples collected in 2016. Phe=phenanthrene, Ant=anthracene, Flr=fluoranthene, Pyr=pyrene, BaFlu=benzo[a]fluorene, BbFlu= benzo[b]fluorine, BaAnt=benzo[a]anthracene, Chy=chrysene, BbFlr=benzo[b]fluoranthene, BkFlr=benzo[k]fluoranthene, BaPyr=benzo[a]pyrene, BePyr=benzo[e]pyrene, IPyr=Indeno[1,2,3-cd]pyrene, DBahAnt=Dibenz[ah]anthracene, BghiPer=benzo[ghi]perylene, DBaePyr=Dibenzo[ae]pyrene. S=small, L=large.

Location/Year 2016		Size	Phe	Ant	Flr	Pyr	BaFlu	BbFlu	BaAnt	Chy	BbFlr	BkFlr	BaPyr	BePyr	IPyr	DBahAnt	BghiPer	DBaePyr	Σ_{16}PAH
Trondheim	Rissa	S	52.69	67.18	193.19	335.54	32.07	26.21	68.34	54.69	54.66	13.82	0.00	26.28	0.00	0.00	0.00	0.00	924.66
		L	62.95	47.12	164.68	324.58	44.19	25.35	0.00	10.90	0.00	0.00	17.91	0.00	0.00	0.00	0.00	0.00	697.69
	Port	S	45.05	41.24	181.12	357.72	56.56	23.90	56.56	67.88	91.29	16.35	71.81	35.70	22.55	0.00	0.00	0.00	1067.74
	WWTP*	S	44.56	12.98	66.55	117.45	10.96	6.16	0.00	14.85	22.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	296.11
		L	51.54	13.17	56.52	95.63	9.15	7.36	0.00	12.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	245.74
	Farm	L	42.42	0.00	28.70	0.00	1.23	0.03	0.00	0.00	0.00	0.00	3.26	5.62	8.24	0.00	9.93	0.00	72.39
Tromsø	Aquarium	S	48.70	4.94	60.68	153.98	0.00	5.85	0.00	14.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	288.74
		L	88.49	134.11	327.00	187.39	44.19	25.35	38.11	105.25	0.00	0.00	54.27	0.00	0.00	0.00	0.00	0.00	1004.17

*WWTP: Waste Water Treatment Plant

Tabla 5. Mean concentrations of measured PAH compounds in ng/g d.w. mussel samples collected in 2017. Phe=phenanthrene, Ant=anthracene, Flr=fluoranthene, Pyr=pyrene, BaFlu=benzo[a]fluorene, BbFlu= benzo[b]fluorine, BaAnt=benzo[a]anthracene, Chy=chrysene, BbFlr=benzo[b]fluoranthene, BkFlr=benzo[k]fluoranthene, BaPyr=benzo[a]pyrene, BePyr=benzo[e]pyrene, IPyr=Indeno[1,2,3-cd]pyrene, DBahAnt=Dibenz[ah]anthracene, BghiPer=benzo[ghi]perylene, DBaePyr=Dibenzo[ae]pyrene. S=small, L=large.

Location/Year 2017		Size	Phe	Ant	Flr	Pyr	BaFlu	BbFlu	BaAnt	Chy	BbFlr	BkFlr	BaPyr	BePyr	IPyr	DBahAnt	BghiPer	DBaePyr	Σ_{16}PAH
Trondheim	Rissa	S	31,76	30,74	131,17	264,79	0,00	0,00	16,36	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	474,83
		L	47,97	19,19	77,40	115,91	26,50	3,40	0.00	0.00	15,80	0,00	0,00	0,00	0.00	0,00	0.00	0,00	306,617
	Port	S	56,54	34,04	432,05	516,98	0,00	0,00	61,13	86,57	60,27	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1247,58
		L	193,68	121,06	399,26	283,14	61,43	25,89	37,88	78,92	63,09	11,27	66,61	0,00	14,56	0,00	29,31	0,00	1386,10
	WWTP*	S	33,88	17,66	132,71	337,41	0,00	0,00	14,58	55,81	28,11	0,00	0,00	0,00	0,00	0,00	0,00	0,00	620,16
		L	45,06	0,00	66,25	153,17	0,00	0,00	0,00	48,83	23,55	0,00	0,00	0,00	0,00	0,00	0,00	0,00	336,85
Tromsø	Aquarium	S	27,25	0,00	92,16	231,39	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	350,80
		L	59,18	9,77	58,49	139,56	0,00	0,00	22,56	2,04	0,00	0,00	14,13	0,00	20,08	0,00	22,41	0,00	348,23
	Port	L	193,68	121,06	399,26	283,14	61,43	25,89	37,88	78,92	63,09	11,27	66,61	0,00	14,56	0,00	29,31	0,00	1386,10
Oslo	Drøvak	L	29,73	9,52	20,44	26,09	35,01	6,43	7,87	2,19	0,00	0,00	11,61	8,79	8,58	0,00	2,19	0,00	168,46
	Malmøya	L	39,72	15,26	47,97	42,99	15,25	5,43	3,38	17,96	0,00	0,00	14,75	0,00	4,10	5,86	5,21	0,00	217,87
Iceland	Hvafjördur	S	61,88	0,00	61,59	151,62	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	275,09
		L	100,06	4,47	90,24	258,44	1,04	0,00	8,70	8,27	0,00	0,94	16,28	8,63	10,70	0,00	60,64	0,00	568,40
	Reykjavik	L	192,87	304,09	883,75	448,58	0,00	0,00	52,63	223,52	98,75	13,46	0,00	0,00	0,00	0,00	0,00	0,00	2217,65
Scotland	Leith	S	33,21	23,06	83,86	139,10	0,00	0,00	0,00	41,98	29,69	0,00	0,00	0,00	0,00	0,00	0,00	0,00	350,90
Germany	Königshafen	S	37,55	0,00	33,59	83,86	53,62	3,21	0.000	0.00	18,08	0,00	0,00	0,00	0.00	0,00	0,00	0,00	229,91
		L	36,11	0,00	27,19	42,77	4,02	2,64	0,00	0,00	34,86	31,04	0,00	0,00	0,00	0,00	0,00	0,00	178,62
	Eckwarderhörne	L	39,11	11,08	70,35	0,00	4,97	3,48	0,00	0.00	74,70	0,00	32,48	0,00	0,00	0,00	0,00	0,00	236,18

*WWTP: Waste Water Treatment Plant

5. Acknowledgements

Thanks are due to Snadder og Snaskum AS in Rissa (Norway) for providing us with farmed mussels and Helena Reynardi and Aleksey Shestov from UNIS, Perrine Geraudie from Akvaplan-niva, Halldór Halldórsson from the University of Iceland, Stefano Carboni from the University of Stirling and Roland Klein from the University of Trier for helping us in the field sampling and logistics.

6. References

Berge J, Johnsen G, Nilsen F, Gulliksen B, Slagstad D. 2005. Ocean temperature oscillations enable reappearance of blue mussels *Mytilus edulis* in Svalbard after a 1000 year absence. Mar Ecol Prog Ser, 303: 167-195.

Garmendia L, Soto M, Cajaraville MP, Marigómez I. 2010. Seasonality in cell and tissue-level biomarkers in *Mytilus galloprovincialis*: relevance for long-term pollution monitoring. Aquat Biol, 9: 203–219.

Garmendia L, Soto M, Vicario U, KimY, Cajaraville MP, Marigómez I. 2011. Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: Tissue-level biomarkers and histopathology. J Environ Monit, 13: 915-932.

Izagirre U, Garmendia L, Soto M, Etxebarria N, Marigómez I. 2014. Health status assessment through an integrative biomarker approach in mussels of different ages with a different history of exposure to the Prestige oil spill. Sci Total Environ, 493: 65-78.

Marigómez I, Garmendia L, Soto M, Orbea A, Izagirre U, Cajaraville MP. 2013. Marine ecosystem health status assessment through integrative biomarker indices: a comparative study after the Prestige-oil spill “Mussel Watch”. Ecotoxicology, 22: 486–505

Mathiesen SS, Thyrring J, Hemmer-Hansen J, Berge J, Sukhotin A, Leopold P, Bekaert M, Sejr MK, Nielsen EE. 2017. Genetic diversity and connectivity within *Mytilus* spp. In the subartic and Artic. Evolutionary Applications, 10:39-55