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ESPIAL

Determining the effects of the discharge effluent from the Karmøy aluminium smelter using an integrated biological effects approach

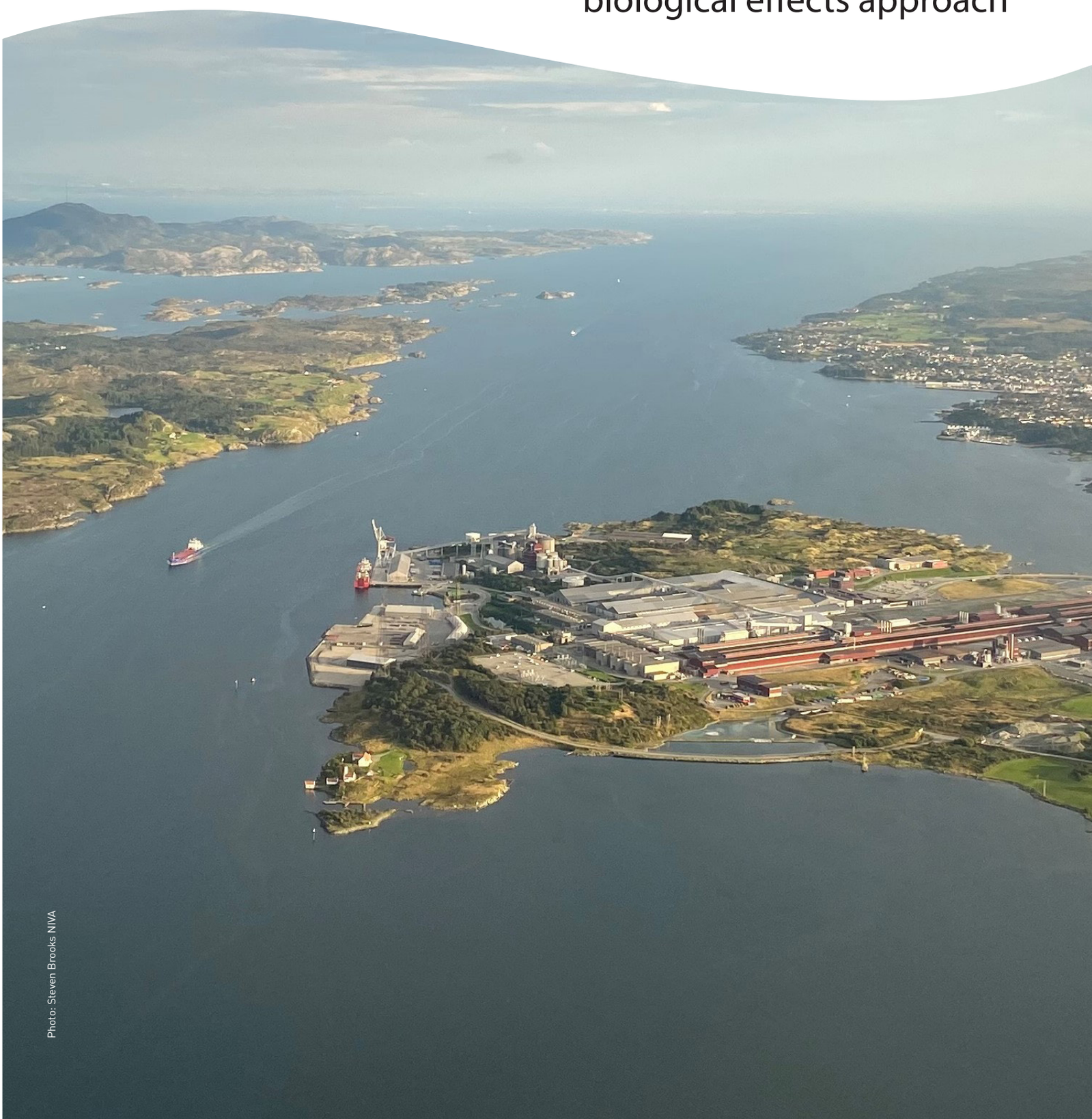

Photo: Steven Brooks NIVA

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Summary

The report describes a monitoring programme using field transplanted mussels to determine the potential biological effects of the effluent discharge from the Karmøy aluminium smelter operated by Hydro Aluminium in Norway. Chemical body burden (54 PAH and metal concentrations) and a suite of biological effects markers were measured in mussels positioned at known distances and at two depths from the aluminium smelters discharge for a period of 8 weeks. In addition, chemical concentrations of the sediment were also measured within the fjord to evaluate the impact of the aluminium smelter on the local marine environment. The highest PAH and metal contamination of the sediment was observed at station 4, a sheltered lagoon 1-1.5 km south of the smelter (Nordalsvågen), and also elevated at station 3, 500 m south of the smelter. PAH contamination in mussels at the closest stations to the aluminium smelter reflected the PAH profiles of the sediment concentrations at each station. This was not true for metals, which were relatively low in all mussels. The PAH concentration in mussels 2 m above the seafloor (bottom), were higher than mussels 5 m from the surface (top/surface) for three out of the four stations. Despite the accumulation of PAH in the mussels, the five biomarker responses were low and overall did not differentiate significantly between the mussel groups. The Principal Component Analysis (PCA) found no clear association between the impact of PAHs and metals with the biological responses in mussels, even in mussels placed closest to the aluminium smelter.

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1. Aluminium smelter	1. Smelteverk av aluminium
2. Effluent	2. Avløp
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ESPIAL

Determining the effects of the discharge effluent from the Karmøy aluminium smelter using an integrated biological effects approach

Preface

The Work described in this report is part of the ESPIAL project. ESPIAL (=Ensuring the Environmental Sustainability of production of PrImary ALuminium) is a multidisciplinary study on the local environmental effects around aluminium smelters in the Nordic countries. The study is initiated and sponsored by "Aluminiumsindustriens Miljøsekretariat" (AMS), and is building on a similar, but more extensive study of the Norwegian smelters from 1991-94, called the "Effect Study". ESPIAL involves new field studies conducted in 2018 – 2020 on the marine environment, air quality, effects on vegetation and on wildlife, in addition to a review of studies conducted through the period after the Effect Study on these subjects. The report at hand is a contribution to ESPIAL and was designed to determine if the discharge water from the aluminium smelter at Karmøy has the potential to cause harm to marine life within the seawater recipient. Mussels were placed within the fjord for 8-weeks, at two locations north and one location south of the aluminium smelter, as well as a reference location approximately 8 km south. Mussels were deployed at two depths, close to surface and 2 m from the bottom with the help of the boat and crew (Frode Ydstebø) of Kvitsøy Sjøtjenester AS. The deployment of the mussels was assisted by Lars Dahle Øverby from the University of Oslo and Dr Steven Brooks from NIVA. Processing of the mussels and laboratory analyses were performed by NIVA Scientists, Dr Tânia Gomes, Maria Elisabetta Michelangeli, Dr Karina Petersen and Dr Steven Brooks. The report was written by Dr Steven Brooks and Dr Tânia Gomes. The project was managed by Dr Ailbhe Macken.

Oslo, September 2022

Dr Ailbhe Macken (Project Manager)

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Summary

The following study describes an integrated biological effects monitoring programme using field transplanted mussels to determine the potential biological effects of the effluent discharge from the Karmøy aluminium smelter operated by Hydro AS in Norway. Chemical body burden (54 PAH and metal concentrations) and a suite of biological effects markers were measured in mussels positioned at known distances and at two depths from the aluminium smelters discharge for a period of 8 weeks. The biomarkers used included: condition index (CI); stress on stress (SoS); neutral red retention (NRR); micronuclei formation (MN); acetylcholine esterase (AChE) inhibition and lipid peroxidation (LPO). In addition, chemical concentrations of the sediment were also measured within the fjord to evaluate the impact of the aluminium smelter on the local marine environment.

The highest PAH and metal contamination of the sediment was observed at station 4, a sheltered lagoon 1-1.5 km south of the smelter (Nordalsvågen), and also elevated at station 3, 500m south of the smelter. Sediment at station 3 has been previously reported to contain historical PAH and metal concentrations, which had derived from the smelter when contaminant discharges were higher. It was unclear whether the PAH and metal contamination at station 4 originated from historical discharges from the smelter or if other industries were also responsible. Sediment concentrations of several PAHs correlated negatively with distance to the southern outlet of the smelter (i.e. lower concentrations observed further from the outlet). However, the number of stations investigated (n=7) were few.

PAH contamination in mussels at the closest stations to the aluminium smelter reflected the PAH profiles of the sediment concentrations at each station. This was not true for metals, which were relatively low in all mussels. The PAH concentration in mussels 2 m above the seafloor (bottom), were higher than mussels 5 m from the surface (top/surface) for three out of the four stations. Highest PAH concentrations in mussels were from station 3 bottom reflecting the higher PAH sediment concentrations at this station.

Despite the accumulation of PAH in the tissues of the mussels, the five biomarker responses were low and overall did not differentiate significantly between the mussel groups. Due to the low response of the biomarkers the IBR/n was equally low and based on the biomarkers measured, there appeared to be minimal impact on the overall health status of the mussels from all stations, including those closest to the aluminium smelter. The Principal Component Analysis (PCA) showed a clear differentiation

among transplanted mussels, highlighting mussels from stations 1 top and bottom and stations 2 and 3 bottom as those with higher chemical body burden. No clear association was found between the impact of PAH's and metals with the biological responses in mussels, even in mussels placed closest to the aluminium shelter.

Sammendrag

Tittel: Determining the effects of the discharge effluent from the Karmøy aluminium smelter using an integrated biological effects approach
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Denne rapporten beskriver et integrert overvåkingsprogram med fokus på biologiske effekter. Det ble benyttet transplanterte/utplasserte blåskjell for å bestemme potensielle biologiske effekter fra utslipp til vann fra Karmøy aluminiumsverk (Hydro AS). Konsentrasjoner av 54 PAH-forbindelser og 11 metaller ble bestemt i blåskjell og sedimenter, og flere biologiske effektmarkører ble målt i blåskjell som ble utplassert i en periode på 8 uker i ulike avstander til smelteverket. Blåskjellene ble plassert i 2 dybder (nær overflaten og nær bunnen). De biologiske effektmarkørene var følgende: kondisjonsindeks (CI), stress på stress (SoS), lysosomal membranstabilitet (NRR), mikrokjernedannelse (mikronuklei; MN) acetylcholinesterase inhibering (AChE) og inhibering av lipid peroksidase (LPO).

De høyeste sedimentkonsentrasjonene av PAH ble funnet ved stasjon 4, en beskyttet vik (Nordalsvågen) 1-1,5 km sør for smelteverket. Det er uklart om de høye PAH-konsentrasjonene ved stasjon 4 stammer fra historiske utslipp fra smelteverket, eller om det kan være andre kilder. Høyere konsentrasjoner ble også funnet ved stasjon 3, 500 m sør for smelteverket. Sedimentene ved stasjon 3 har tidligere blitt rapportert å inneholde historiske forurensninger av PAH og metaller fra smelteverket når utslippene var høyere. Sedimentkonsentrasjonene av flere PAH-forbindelser korrelerte negativt med distansen til det sørlige bassenget ved smelteverket (dvs. lavere konsentrasjoner ble funnet lenger vekk fra dette bassenget). Imidlertid var antall stasjoner for få til å knytte noen konklusjoner til funnet.

PAH-profilene i blåskjell var ganske likt det som ble påvist i sediment ved hver stasjon. Imidlertid gjaldt dette ikke metaller som hadde relativt lave konsentrasjoner i blåskjellene. Generelt var konsentrasjonene av PAH fra blåskjell som var plassert nær bunnen (2 m over bunnen) høyere enn de som var plassert nær overflaten (5 m under overflaten). De høyeste konsentrasjonene ble funnet i blåskjell utplassert ved stasjon 3 ved bunnen, noe som gjenspeilet de høye sedimentkonsentrasjonene som ble funnet ved denne stasjonen.

Selv om blåskjellene akkumulerte PAH, var responsen fra de 5 undersøkte biomarkørene generelt lave, og var ikke signifikant forskjellige mellom de undersøkte stasjonene. På grunn av lav respons i biomarkørene var den integrerte biomarkørresponsen tilsvarende lav. Basert på de målte biomarkørene var det liten påvirkning av den generelle helsen til blåskjellene ved alle stasjoner, inkludert de nærmest aluminiumsverket. Prinsipalkomponentanalysen (PCA) viste en differensiering mellom de utplasserte blåskjellene, der blåskjell fra stasjon 1 (både fra overflaten og bunnen) og stasjon 2 og 3 (bunnen) var de med høyest innhold av akkumulerte stoffer. Ingen klar sammenheng mellom konsentrasjoner av PAH/metaller og biologiske effekter ble funnet, heller ikke i blåskjellene plassert nærmest aluminiumsverket.

1 Introduction

The following study describes an integrated biological effects monitoring programme using field transplanted mussels to determine the potential biological effects of the effluent discharge from the Karmøy aluminium smelter. The aluminium smelter, operated by Hydro AS, is located on the east coast of Karmøy near Haugesund, Norway and discharges waste under licence into the nearby fjord. The facility produces primary aluminum through electrolysis of alumina and has an annual production of 270 000 tons per year (web link[1]). Prior to 2009, the production of aluminium was based on Søderberg technology, which resulted in relatively high levels of PAH contamination to both air and water. However, since 2009 the Søderberg production line was closed and Pre-bake technology was adopted. This has led to a significant reduction in the emissions of PAH compounds to the environment from the effluent. Despite the reduction in PAH in the effluent, historical sediments in the sedimentation ponds are thought to potentially contribute to the contaminant flux to the nearby fjord.

1.1 Effluent discharge

The discharge water from the smelter, mainly effluents from seawater scrubbers, whose main purpose is to remove sulpher dioxide from the pot-gases , is released into the settlement ponds north of the facility (Figure 1). Previously, when the Søderberg production lines were in operation, the discharge from these were directed to the southern pond. The berm of the northern settlement pond is permeable and enables the diluted effluent to disperse into the surrounding fjord recipient where it is diluted further.

The current discharge permit, which was last amended in December 2019, applies to contaminant released from chemical, electrolytic and metallurgic processes at the smelter. The release of contaminants from the smelter have been recorded annually and are reported at norskeutslipp (web link[2]), a summary is shown in Table 1. In addition to the yearly discharge limits of the different chemicals, the maximum concentration of oil in water within the discharge effluent is set at 20 mg/L.

Other than chemicals within the effluent, pH, temperature and oxygen concentrations have been considered to be important factors with regards to the potential impacts on the surrounding marine environment (Schaanning et al. 2014). Of the three factors, and based on the available knowledge of

Web link[1] https://www.hydro.com/no-NO/om-hydro/hydro-worldwide/europe/norway/karmoy/karmoy-primary-production/
Web link[2] https://www.norskeutslipp.no/en/Miscellaneous/Company/?CompanyID=5121

the effects on marine organisms, the reduction in pH was thought to be the more important of these factors that requires at least a 25 times dilution before ill effects are not seen. Furthermore, the combined effects of chemicals and acidification may be important with respect to bioavailability and increasing the potential impact on marine life.

Figure 1. Map of the aluminium smelter highlighting the locations of the settlement ponds north and south of the plant (source: pictures taken from google maps).

Table 1. Yearly discharge of selected chemicals within the water effluent from the Karmøy aluminium smelter (2019-2015), (kg/ year, * tons/ year). Source: www.norskeutslipp.no. (nr not reported, na not available).

Substance	Permit	2020	2019	2018	2017	2016	2015
Arsenic (As)	21	8.2	4.4	6.6	3.9	13.0	5.4
Cadmium (Cd)	1	0.7	0.3	0.6	0.1	0.3	0.1
Chromium (Cr)	13	0.7	0.5	0.8	0.3	6.6	4.7
Copper (Cu)	na	15.5	20.4	31.4	7.4	20.7	89.1
Mercrury (Hg)	0.05	0.01	0.00	0.01	0.00	0.04	0.01
Molybdenum (Mo)	na	1.2	0.8	1.1	0.8	0.9	0.8
Nickel (Ni)	740	159.5	83.5	130.8	79.7	175.5	139.2
Lead (Pb)	38	10.5	6.3	10.8	2.6	7.9	9.9
Vanadium (V)	na	14.8	6.1	9.1	7.8	4.4	5.6
Zinc (Zn)	na	7.5	15.7	27.7	5.2	102.4	42.6
PAH16	na	nr	nr	nr	nr	nr	35.6
Naphthalene	na	nr	nr	nr	nr	nr	9.5
Benzi [ghi] perylene (BGHIP)	na	nr	nr	nr	nr	nr	0.1
Suspended solids *	na	230.3	77.5	75.4	57.6	82.0	82.5

1.2 Biological effects measurements

This investigation focusses on the water column and the potential effects of chemicals from the aluminium smelter on pelagic organisms. Although mussels have been used previously to assess contaminant bioaccumulation in the recipient waters around Karmøy (Øxnevad & Hjermann, 2020), this is the first time that the biological effects in mussels exposed within the water column at Karmøy have been measured. Mussels are used widely in biological effects monitoring programmes (reviewed in Beyer at al., 2017), they are sessile and easily transplanted into different environments, they can filter large volumes of seawater, bioaccumulate contaminants from the filtered seawater, and have a wide range of sensitive validated biological effects methods that can be easily measured. Many of the biological effects measurements have internationally recognised background and environmental assessment criteria (BAC and EAC, Davies and Vethaak, 2012), which enables the biological response(s) to be placed in to context to other similar studies. A brief description of the biological effects measurements used are provided.

Condition index is used to provide a simple measure of organism health status, including the physiological activity such as growth, reproduction, secretion, etc., under environmental conditions. Condition index is a general health parameter that can be influenced by both biotic and abiotic factors that can cause physiological stress on the organism.

Stress on stress (SoS), is a simple and low-cost whole organism response that can show pollutant induced alterations in mussel physiology. The ability of mussels to keep their shells closed and resist air exposure is related to the amount of energy (adenosine triphosphate, ATP) available to fuel their adductor muscle (De Zwaan and Mathiew, 1992). If metabolic energy is spent on detoxification processes in mussels exposed to contaminants, less energy is available for other physiological processes.

The **neutral red retention** (NRR) assay is a method to detect the resilience of lysosome membranes in mussel haemocytes and provides a measure of the functional integrity of cells. The membrane integrity of lysosomes has been found to be affected by a range of environmental stressors, including metals and organic chemicals (Lowe et al. 1995).

Micronuclei (MN) are chromatin-containing structures that are surrounded by a membrane and have no detectable link to the cell nucleus. The MN test provides an indication of chromosomal damage, and has been found to show a time-integrated response to complex mixtures of pollutants (Baršienė et al. 2006). The frequency of MN is regarded as an important tool for *in situ* monitoring of DNA damage.

The **acetylcholine esterase** (AChE) assay has been used to assess the neurotoxicity of environmental samples and exposure to neurotoxic compounds. AChE is an essential enzyme involved in neurotransmission. An inhibition of AChE causes various effects on the central nervous system. Different types of compounds have been described as AChE inhibitors, such as organophosphorus and carbamate pesticides and PAHs.

Lipid peroxidation (LPO) is characterized by the oxidative deterioration of polyunsaturated fatty acids present in cellular membranes, which can alter membrane fluidity and permeability. The formation of lipid peroxides is characterized by the presence of by-products as malondialdehyde (MDA) and

hydroxyalkenals, that have been routinely measured in bivalve species to reflect contaminant-induced oxidative damage.

2 Methods

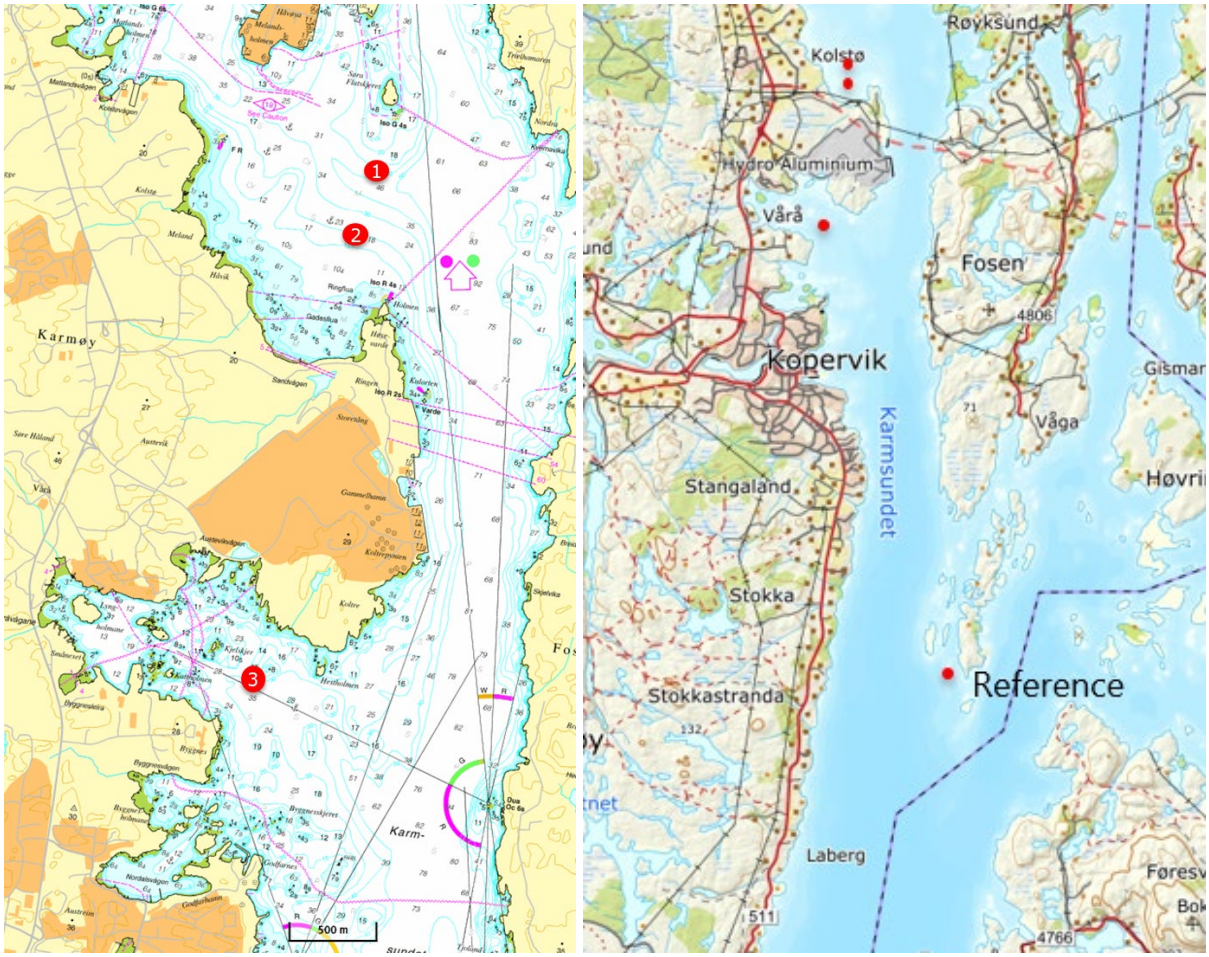
2.1 Deployment and retrieval of field mussels

The mussels were collected from a local population at Buøyflæet, Kvitsøy (59° 02.689 N 5°24.334 E) and positioned at three stations near the aluminium smelter (Figure 2). Two stations were placed north of the smelter whilst one station was placed to the south. A further station approximately 8 km south of the smelter was used as a reference location. At each station, mussels were positioned at two depths: 1-2 m from the seafloor and approximately 5 m from the surface. Approximately 100 mussels were used at each station and depth. Mussels were deployed on Wednesday 19th August 2020 and were retrieved after 8 weeks.

The moorings, standing vertically in the water column, consisted of a concrete anchor, rope and 8 kg buoys (Figure 3). The buoys were placed above the mussels to ensure stability in the water column, a surface marker buoy with light was used to minimise the likelihood of collision with boating traffic. The equipment for the mussel rigs, the mussels and their deployment in the fjord were provided and performed by Kvitsøy Sjøtjenester AS, with assistance from NIVA personnel.

Mussels were retrieved by Kvitsøy Sjøtjenester AS and transported on ice to the NIVA laboratory in Oslo by overnight courier. To ensure the mussels were processed on the day of arrival at the laboratory, only one station, containing mussels at two depths were collect per day, with 4 stations collected over 4 days between 12th-15th October 2020. No mussel mortalities were observed during inspection.

All mussels used in the study were measured and were of a similar size (shell length 59.2 ± 6.6 mm, mean ± standard deviation).



Mussel Station	Coordinates N	Coordinates E	Depth (approx.)
1	59°19.767	5°18.933	40-50 m
2	59°19.583	5°18.783	15-20 m
3	59°18.183	5°18.700	30-35 m
reference	59°14.888	5°21.559	40-50 m

Figure 2. Location of the mussel stations north and south of the Karmøy aluminium smelter and the reference location approximately 8 km south including co-ordinates and approximate depth.

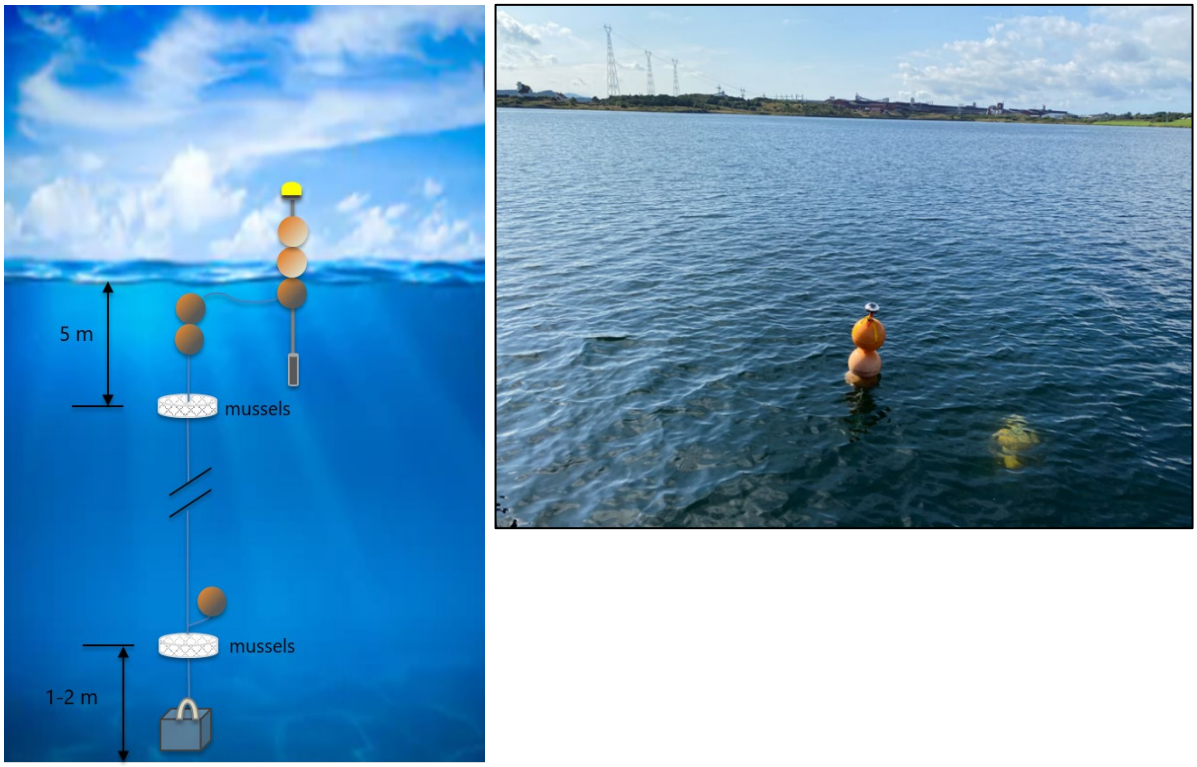


Figure 3. The design of the mussel rig with mussels held within the water column approximately 2 m from the seafloor and 5m from the surface (left image), and mussel rig recently deployed in the field at station 1 (right image).

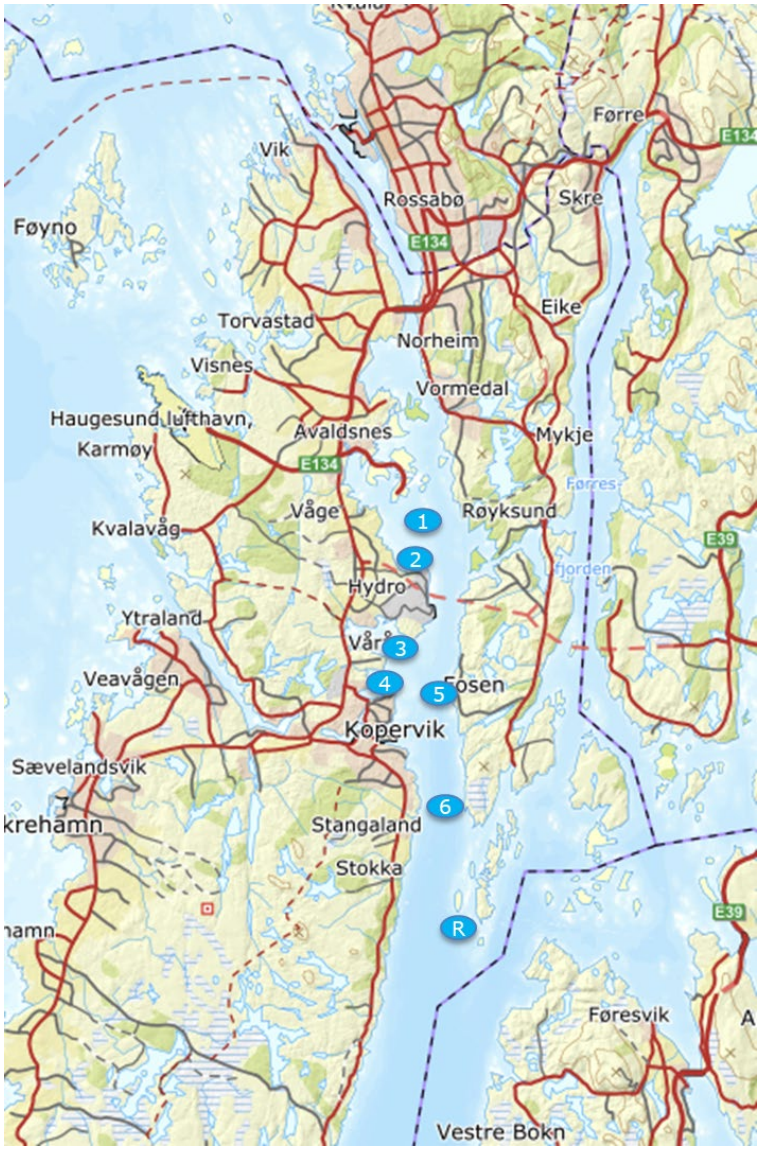
2.2 CTD profiles

Profiles of the physicochemical properties of the seawater at the four mussel stations were measured on the day the mussels were deployed (19.08.2020). A CTD unit (SAIV SD208) equipped with turbidity and oxygen sensors were lowered to the sea floor and returned to the surface. The physicochemical parameters were measured at 2 second intervals.

2.3 Sediment sampling

Sediment samples were collected following the successful deployment of the mussel stations. Sediment samples were collected with a Van Veen grab, where the top 2 cm of sediment was sampled and placed in heat treated glass jars for chemical analysis. Duplicate samples were collected from seven stations including the 4 mussel stations and 3 additional locations in the fjord (Figure 4).

Sediment from two separate grabs were used at the reference station, whilst the sediment from one grab, split into two glass jars, were used for the other 6 stations.



Sediment station	Coordinates N	Coordinates E
1	59°19.767	5°18.933
2	59°19.583	5°18.783
3	59°18.183	5°18.700
4	59 17.683	5 18.317
5	59°17.900	5°19.917
6	59°16.233	5°20.483
Reference (R)	59°14.888	5°21.559

Figure 4. Location of the sediment sites north and south of the Karmøy aluminium smelter including a reference site located approximately 8 km south. Specific coordinates of the stations included.

2.4 Analysis of mussel samples

Length measurements were taken from all mussels sampled. Haemolymph was taken from 10 mussels for micronuclei (MN) assessment. In the same individuals, gill and digestive gland were removed and snap frozen in liquid nitrogen then stored at -80°C until used for measurements of lipid peroxidation

(LPO). Haemolymph was taken from another 15 mussels for neutral red retention (NRR). The gills of these mussels were removed and snap frozen in liquid nitrogen then stored at -80°C until used for acetylcholine esterase (AChE) inhibition. Additional mussels were used for stress on stress (SoS) and for condition index (CI). Furthermore, three replicates of five mussels were pooled for chemical analysis. Details of each biological effects measurement and chemical analyses are provided below.

A sub-sample of mussels from the same population as those transplanted into the fjord were brought back to the NIVA laboratory on ice and processed for chemical and biological effects endpoints. These mussels were referred to as the Day 0 group, since they reflect the condition of the mussels at the start of the field exposure. The Day 0 mussels were sampled on the day after the field mussels were placed in the fjord.

2.4.1 Tissue chemistry

Mussels were opened by cutting through their posterior adductor muscle with a sterile scalpel, excess water was drained, and the soft tissue removed and placed in a high temperature treated (550°C) glass container.

Metal analysis: Metal concentrations (As, Cd, Cr, Cu, Pb, Mn, Hg, Mo, Ni, V, Zn) were determined in homogenised whole soft tissue samples using inductively coupled plasma-mass spectrometer (ICP-MS, Perkin-Elmer Sciex ELAN 6000).

PAH analysis: For each exposure group, triplicate samples of five mussels per sample were collected for analysis of 54 PAH concentrations. Gas chromatography coupled to mass spectrometry (GC/MS) was employed for the PAH analysis. For details about the chemical analyses see Grung et al. (2020).

2.4.2 Condition index

The condition index (CI) was measured in fifteen mussels from each group by determining the ratio of the dry weight of the soft tissue divided by the valve dry weight multiplied by 100 (Moschino and Marin, 2006; Orban et al., 2002). The dry weight values were recorded after oven drying the shell and the soft tissue at 80°C for 24 h.

$$CI = \left(\frac{soft\ tissue\ dry\ weight\ (g)}{shell\ dry\ weight\ (g)}\right) x\ 100$$

2.4.3 Stress on stress

The stress on stress (SoS) assessment was measured with fifteen mussels from each group. Mussels were placed in a humid chamber at 15 ± 0.5°C with a 16 h: 8 h light dark cycle. The mussels were checked every 24 ± 4 h and mortalities were recorded and removed from the incubator. Mussels were considered deceased if their shells were gaping and showed no sign of movement after gentle tapping on their shells.

2.4.4 Neutral red retention

Lysosomal stability was measured in mussel haemocytes using the neutral red retention (NRR) procedure adapted from Lowe and Pipe (1994). Approximately 0.1 ml of haemolymph was removed from the adductor muscle of the mussel with a syringe containing approximately 0.1 ml of filtered (0.2 µm) seawater. The haemolymph/saline solution was placed in a microcentrifuge tube, from which a 40 µl sample was removed and pipetted onto the centre of a microscope slide. The slide was left in a dark humid chamber for 15 min to allow the cells to adhere to the slide. Excess liquid was removed from the slide after this time and 40 µl of neutral red solution added. The neutral red solution was taken up inside the haemocytes and stored within the lysosome of the mussel. The ability of the lysosome to retain the neutral red solution was checked every 15 min by light microscopy (x400 magnification). The test was terminated and the time recorded when greater than 50% of the haemocytes leaked the neutral red dye out of the lysosome into the cytosol.

2.4.5 Micronuclei formation

Approximately 0.1 ml of haemolymph was removed from the posterior adductor muscle of the mussel with a syringe and needle (0.6 ml) containing 0.1 ml of PBS buffer (100 mM PBS, 10 mM EDTA). The haemolymph and PBS buffer mixed solution was transferred onto a cytoslide by centrifugation at 800 rpm for 2 minutes using a Cytospin 4 centrifuge (Thermo Scientific). Cytoslides were then placed in a humid chamber for 15 min to enable the haemocytes to adhere. The adhered haemocytes were fixed with 1% glutaraldehyde in 100 mM PBS for 5 min, rinsed in PBS buffer and left to air-dry in the dark overnight. Slides were stained with 1 µg/ ml bisbenzimide 33258 (Hoechst) solution for 5 min, rinsed with distilled water and mounted in glycerol McIlvaine buffer (1:1). The frequency of MN was measured on coded slides without knowledge of the exposure status of the samples to eliminate bias. The frequency of micronuclei in haemocytes was determined microscopically (×100 objective) on a minimum of 2000 cells per exposure group. Micronuclei were scored in cells with intact cellular and nuclear membranes when: 1) nucleus and micronuclei have a common cytoplasm, 2) colour intensity

and texture of micronuclei is similar to the nucleus, 3) the size of the micronuclei is equal or smaller than 1/3 of the nucleus, 4) MN are apparent as spherical structures with a sharp contour.

Partly due to cell clustering, the staining with bisbenzimide 33258 (Hoechst) solution proved problematic when identifying the outline of the cells and in many cases, it was not possible to score a sufficient number of cells, i.e., 2000 cells per group. An attempt was made to re-stain the slides from groups with insufficient numbers of counted cells using the Giemsa solution, a non-fluorescent dye that allows an easier visualization of the cell outline. Slides from selected groups were de-stained by washing with PBS buffer (100 mM PBS, 10 mM EDTA), air-dried and re-stained with Giemsa 3% in Sørensen buffer (pH 6.8) for 5 minutes at room temperature. Slides were then rinsed in washing solution (Sørensen buffer, pH 6.8) and left to air-dry in the dark overnight. Slides were mounted in a Eukitt mounting solution and reassessed microscopically (×100 objective).

2.4.6 Acetylcholine esterase inhibition

Acetylcholine esterase (AChE) activity was determined in the gills of fifteen mussels. Gills were homogenized on ice in five volumes of Tris–HCl buffer (100 mM, pH 8.0) containing 10% Triton and the resulting homogenate was centrifuged at 12,000 g for 30 minutes at 4°C. Measurements of AChE activity were performed following the method described by Bocquené and Galgani (1998). This method is based on the coupled enzyme reaction of acetylthiocholine (ATC) as the specific substrate for AChE and 5,50-dithio-bis-2-nitrobenzoate as an indicator for the enzyme reaction at 405 nm using a molar extinction coefficient of 13.6 mM/cm. AChE activity was expressed in nmol of ATC per min per mg of total protein.

2.4.7 Lipid peroxidation

Lipid peroxidation (LPO) was evaluated by determining malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE), both by-products of polyunsaturated fatty acid peroxidation, following the method described by Erdelmeier et al. (1998). Briefly, the gills of 10 mussels were homogenized in 3 volumes of 0.02 M Tris-HCl containing 0.5 M BHT (pH 7.4) at 4ºC. The resulting homogenate was centrifuged at 15,000 g for 20 minutes at 4ºC and the supernatant used for total protein determination and LPO analysis. LPO analysis was based on the reaction of two moles of N-methyl-2-phenylindole (3:1 mixture of acetonitrile/methanol), a chromogenic reagent, with one mole of either MDA or 4-HNE under acidic conditions (methanesulfonic acid) at 45°C for 60 min to yield a stable chromophore. Malondialdehyde

bis-(1,1,3,3-tetrametoxypropane) was used as a standard at a maximal absorbance of 586 nm. LPO levels were expressed as nmol MDA + 4-HNE per gram of total protein.

2.4.8 Total protein concentration

Total protein concentration was measured in the cytosolic fractions of the gill samples used for AChE activity and LPO levels according to the Lowry method (Lowry, 1951) using Bovine Immunoglobulin G (IgG) as a standard.

2.5 Statistical analysis

Statistical analyses and graphs for all individual biomarkers (except SoS) were performed using Statistica 14 (Dell). All data were tested for normality and homogeneity of variance with a Levene's test. Since homogeneity of variance was achieved, significant differences between groups were detected using one-way analysis of variance (ANOVA) and the Tukey post-hoc test, with the level of significance set at p=0.05.

The statistical software used for preparing figures and statistical chemical analysis of PACs in biota and sediment was JMP (16.0.0). The distance to each outlet was calculated as a straight line, and no influence of current has been estimated. Also, the minimum distance to any outlet was calculated. The concentration of each PAC was then correlated with the distance (in km) after both values were log 10-transformed. If the concentration of the PAC was below the LOQ, half the LOQ was used instead.

2.5.1 Integrated assessment

The Integrative Biological Response (IBR) index was developed to systematically combine a suite of biomarker responses in order to provide a holistic evaluation of organism health status following chemical exposure (Beliaeff and Burgeot, 2002). The IBR/n, which accounts for the number of biomarkers in the data set, was used to integrate the biomarker data (Broeg and Lehtonen, 2006). In the present study CI, SoS, NRR, MN, AChE and LPO were selected for the IBR calculation. The inverse values of CI, SoS, NRR, and AChE were used since a decrease was reflective of an adverse impact. The IBR index was calculated by summing-up triangular star plot areas for each two neighbouring biomarkers in a data set.

2.5.2 Principle component analysis (PCA)

A Principal component analysis (PCA) was performed using XLStat2021® (Addinsoft, Paris, France) to highlight the main variables responsible for the variance of data obtained for all groups. A Pearson's correlation analysis was also performed to evaluate the strength of association between chemical body burden and biological responses of mussels. The level of significance was set to p=0.05.

3 Results

3.1 Physicochemical properties of the fjord recipient.

A series of CTD profiles were taken at each of the mussel stations on the day of the deployment (Figure 5 and Figure 6). Physicochemical conditions of the seawater were found to differ with depth and the mussels placed at 5 m from the surface were likely to experience different oxygen, temperature and salinity conditions compared to mussels located 2 m from the seafloor. At station 1, oxygen concentrations of 8 mg/L and 7.25 mg/L were recorded at the surface and at the seafloor respectively, with lowest concentrations of 6.2 mg/L at a depth of 30 m. Temperature and salinity differences between 18 and 12°C and 30 and 33 ppt were evident for surface and bottom waters respectively. Turbidity measurements were more comparable with depth with most values falling between 0.5 and 1 FTU.

Despite station 2 being shallower than station 1 (18 m compared to 44 m) large differences in oxygen, temperature and salinity were also found between the top and bottom waters. Oxygen, temperature, and salinity of the waters between top and bottom mussels were approximately 8.2 and 7.2 mg/L, 17 and 14.5°C and 30 ppt and 31.5 ppt, respectively. In contrast, turbidity remained relatively stable with depth at approximately 0.6 FTU.

Station 3, on the south of the Aluminium smelter, had similar oxygen, temperature and salinity profiles as that described for station 1. Top and bottom mussels were exposed to oxygen, temperature, salinity conditions of 8.3 and 7.2 mg/L, 18 and 12°C, and 29.5 and 33 ppt respectively. The turbidity profile at station 3 was clearly different to the other stations, with higher turbidity in deeper water. Turbidity remained stable at around 0.5 FTU from surface to 30 m, before increasing to a maximum of 2.3 FTU close to the seafloor.

The reference station, approximately 8 km south of the smelter, had similar oxygen, temperature and salinity profiles to the other stations. Mussels at the surface (5m deep) were exposed to higher oxygen and temperature and lower salinities than those mussels located 2 m above the seafloor. Top and bottom mussels were exposed to oxygen, temperature, salinity conditions of 8.3 and 7.4 mg/L, 18.5 and 14°C, and 28.5 and 32.5 ppt respectively. The turbidity was lower at the reference station than all other stations, with <0.5 FTU at the surface to 20 m and <0.3 FTU below 25 m.

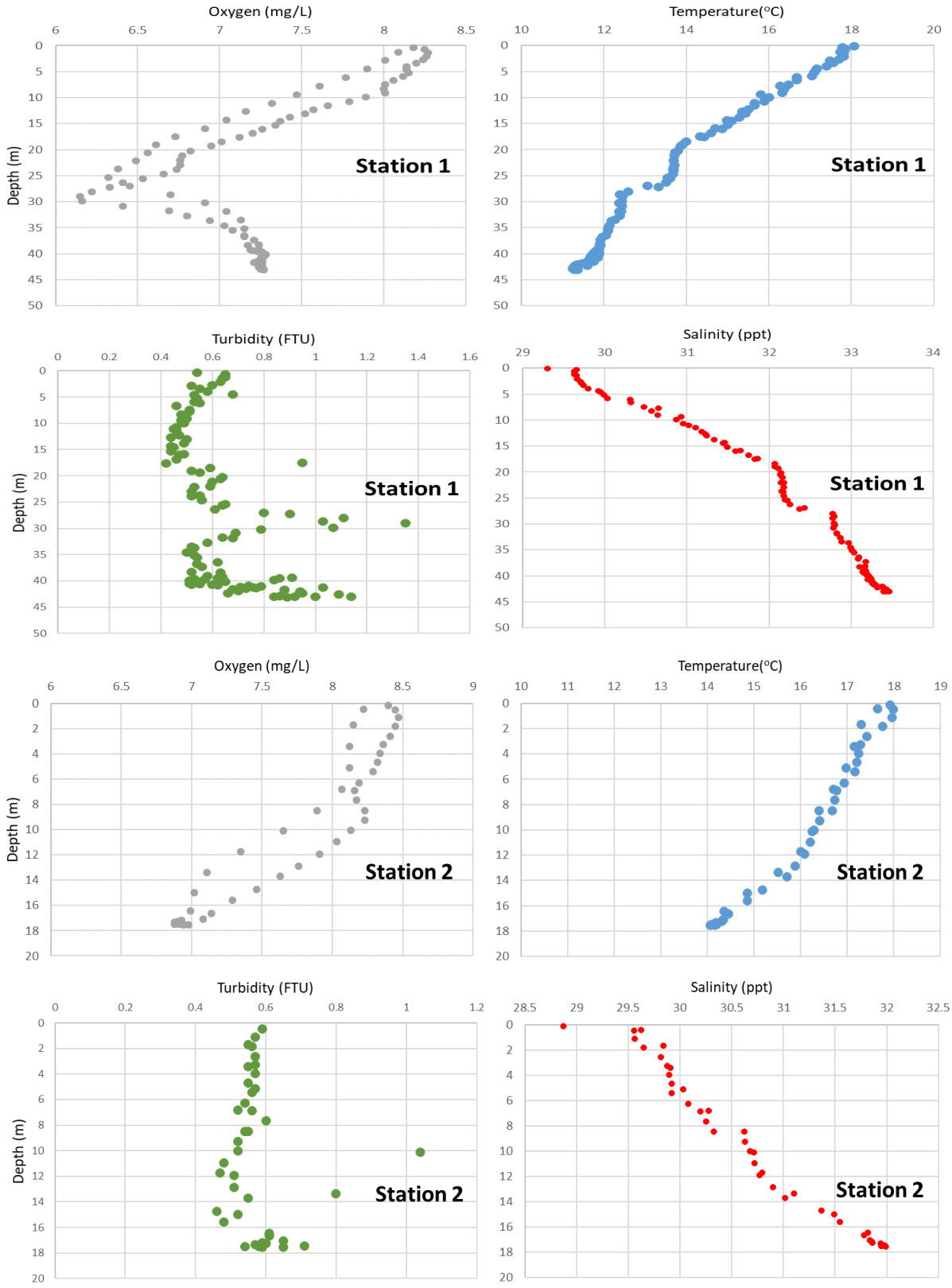


Figure 5. Depth profiles of physicochemical properties of the seawater at mussel stations 1 and 2 including oxygen concentration, temperature, turbidity and salinity. Profiles taken on the day of the mussel deployment (19.08.2020, station 1 at 14:33 and station 2 at 14:41).

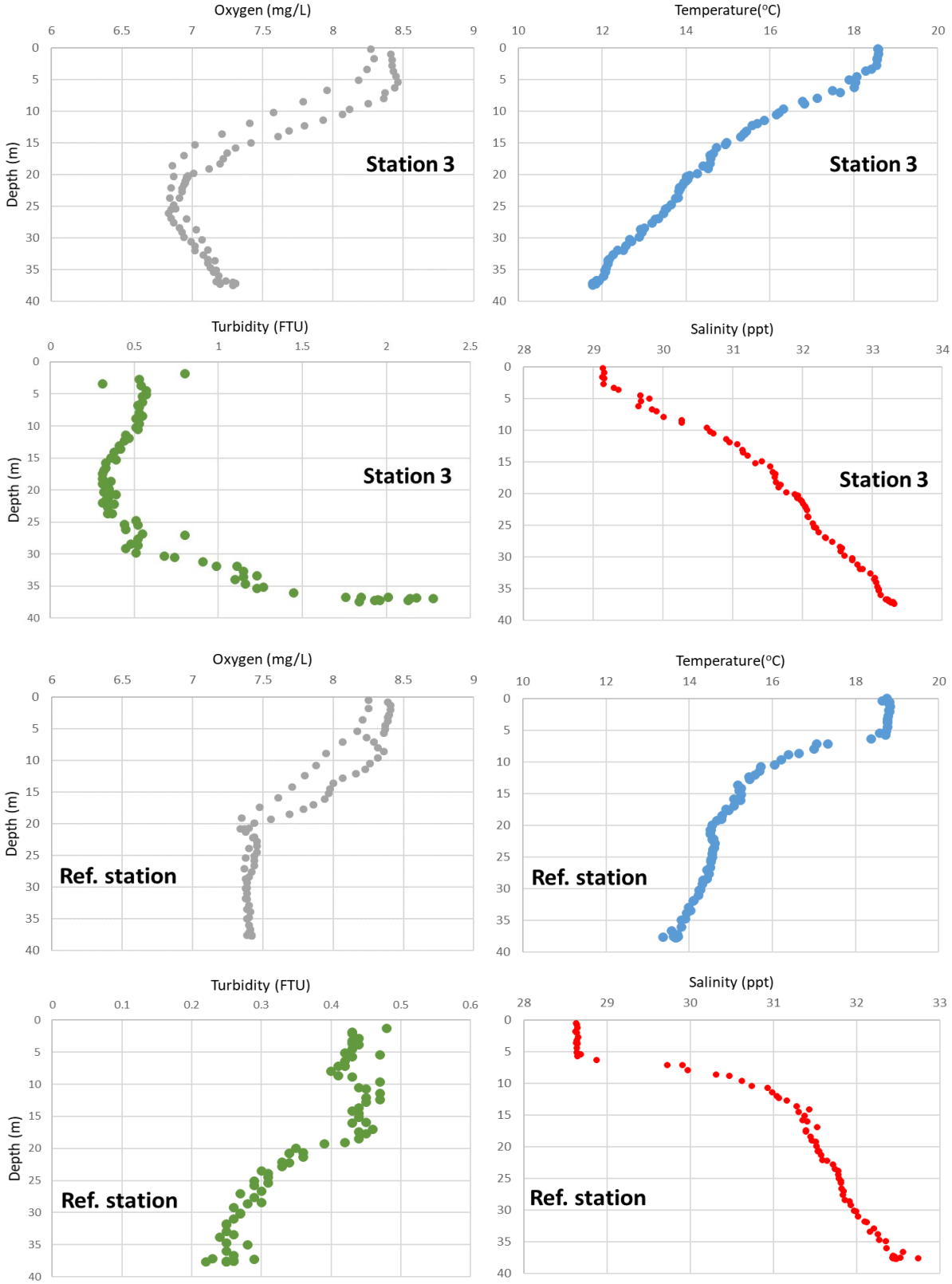


Figure 6. Depth profiles of physicochemical properties of the seawater at mussel station 3 and the reference station, including oxygen concentration, temperature, turbidity and salinity. Profiles taken on the day of the mussel deployment (19.08.2020, station 3 at 14:50 and reference station at 15:07).

3.2 Chemical concentrations

3.2.1 Mussel metals

The metal concentrations measured in the whole mussel homogenates of day zero and field transplanted mussels are shown in Table 2. For the 11 metals measured, there were no obvious differences between metal concentration and proximity to the aluminium smelter. The surface mussels at group 1 had slightly higher concentrations of Pb (0.23 mg/kg), Cu (1.4 mg/kg) and Zn (17 mg/kg) compared to the other mussel groups. Since only one pooled sample of mussels was measured, statistical comparisons were not possible, although considering the closeness of the data, differences between mussel groups were thought to be absent.

Table 2. Metal concentrations (mg/kg) in whole mussel homogenates from the day zero mussels (T0) and the field transplanted mussels 5 m below the surface (surface) and 2 m above the sea floor (bottom).

Group	depth	Hg	As	Pb	Cd	Cu	Cr	Ni	Zn	Mn	Mo	V
T0		0.011	2.5	0.11	0.15	0.70	0.07	0.10	12.00	0.80	0.10	<0.2
1	surface	0.015	3.8	0.23	0.14	1.40	0.13	0.20	17.00	1.00	0.10	0.30
1	bottom	0.013	3.4	0.16	0.18	0.8	0.18	0.2	14	0.9	0.1	<0.2
2	surface	0.015	3.2	0.14	0.12	1	0.08	<0,1	11	0.9	<0.1	<0.2
2	bottom	0.014	3.3	0.16	0.13	0.8	0.13	0.1	15	0.8	0.1	<0.2
3	surface	0.015	3.0	0.14	0.13	1	0.09	0.1	12	0.9	0.1	0.2
3	bottom	0.014	3.2	0.15	0.15	0.8	0.14	0.2	15	0.8	0.1	<0.2
ref	surface	0.015	3.3	0.12	0.14	0.9	0.09	0.1	12	1.1	0.1	0.3
ref	bottom	0.018	2.7	0.14	0.16	0.7	0.11	0.2	9.9	0.9	0.1	<0.2

3.2.2 Mussel PAH

The concentration of PAH16 in mussels from the day zero group (Time 0) and in mussels located at specific locations from the aluminium smelter are shown (Figure 7). Lowest concentrations of PAH16 were found in mussels from the T0 group, followed by the reference location (R). Highest concentrations of PAH16 were found in the field transplanted mussels at stations 1, 2 and 3, with highest concentrations measured in mussels 2 m above the seafloor at station 3 located south of the smelter. Clear differences between surface and bottom mussels were found at station 3 and to a lesser degree at station 1 and station 2. In all cases, PAH concentrations were higher in mussels from the bottom group (2 m above the seafloor) compared to those placed 5 m below the surface.

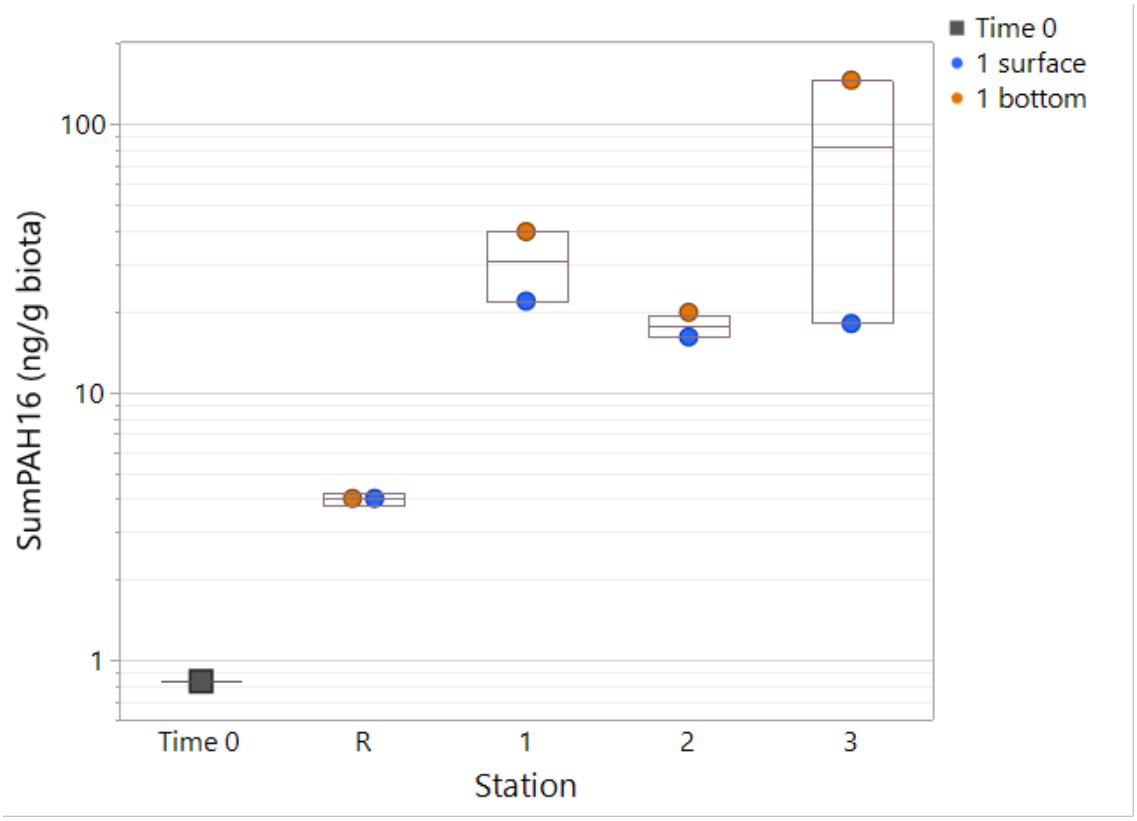


Figure 7. Sum of PAH16 (log scale) in mussel whole mussel homogenate sampled from the different groups. Time 0 represents mussels sampled from the donor population that were not placed out in the fjord. All other groups were placed in the fjord for 8 weeks at approximately 5 m below the surface (surface) or 2 m from the sea floor (bottom).

The sum of all PAH measured in mussels from the different groups showed a very similar profile to that described for PAH16 (Figure 8). Highest concentrations of all measured PAH were found in the mussels positioned at stations 1, 2 and 3, with highest concentrations in the mussels positioned 2 m above the sea floor. This difference in depth was most pronounced at station 3, with an approximate 10-fold higher concentration at in mussels 2 m from the sea floor.

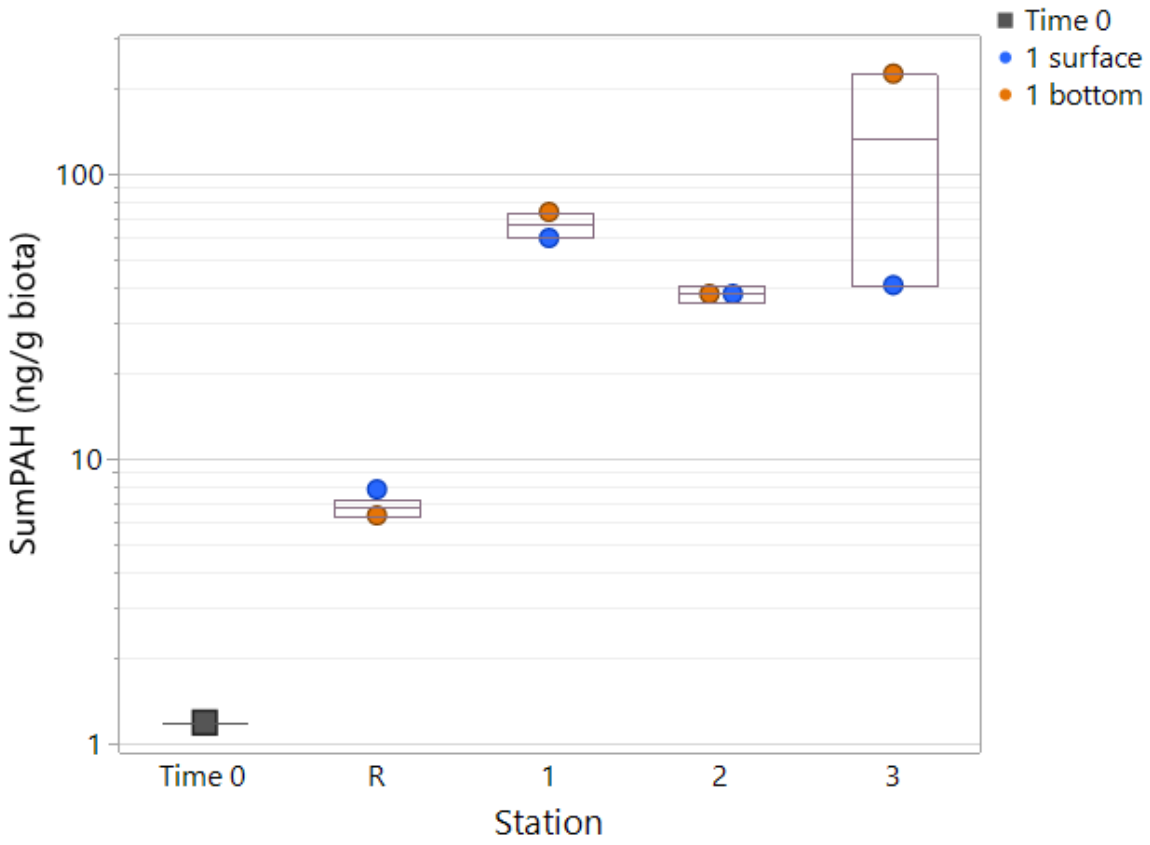


Figure 8. Sum of all measured PAH (log scale) in mussel whole mussel homogenate sampled from the different groups. Time 0 represents mussels sampled from the donor population that were not placed out in the fjord. All other groups were placed in the fjord for 8 weeks at approximately 5 m below the surface (surface) or 2 m from the sea floor (bottom).

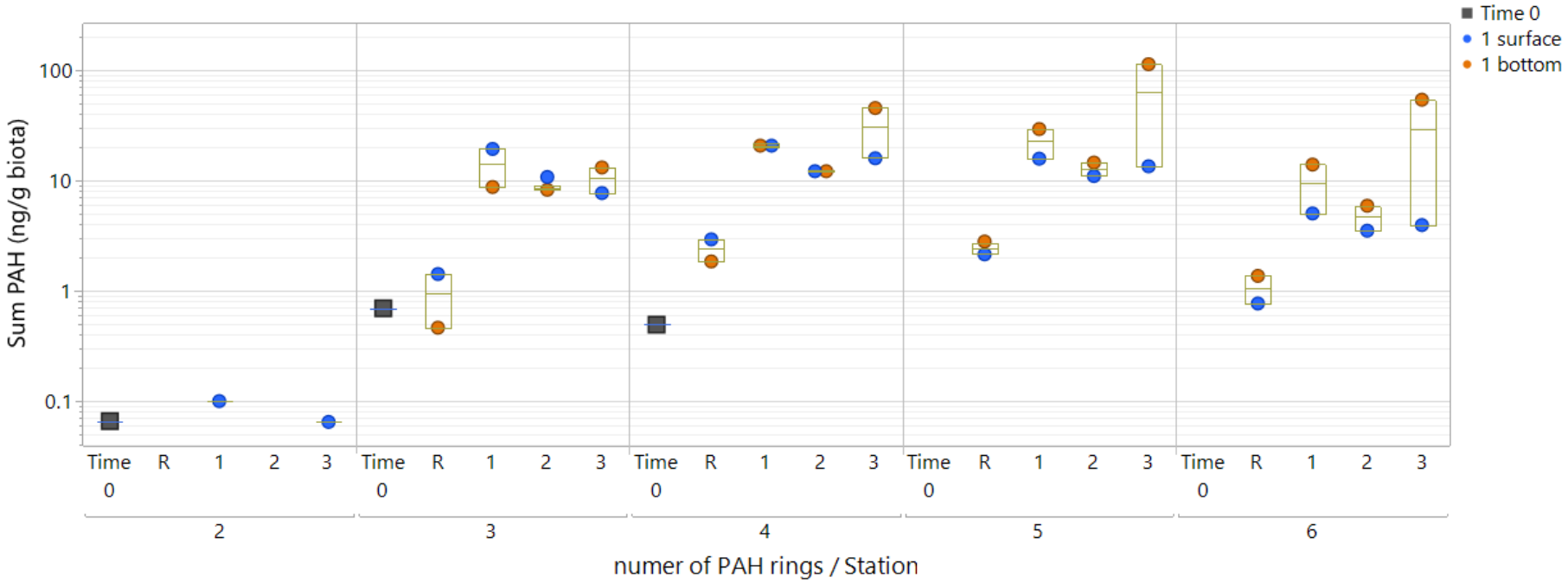


Figure 9. Sum of all measured PAH (log scale) in mussels from the different stations. Mussels separated into 2, 3, 4, 5 and 6 ring PAH.

The PAH concentrations in mussel tissue with respect to the number of aromatic rings (2 to 6) are shown in Figure 9. In general, a larger number of aromatic rings indicates a heavier PAH. The data shows that 3-5 ring PAH concentrations were more dominant at stations 1 to 3, with slightly lower concentrations of 6-ring PAH and almost absent concentrations of 2-ring PAH. No 5 ring or 6 ring PAHs were detected in mussels from the T0 population. These mussels were not deployed in the fjord but were collected approximately 20 km away from the smelter.

3.2.3 Sediment PAH

Sediment PAH concentrations from the seven stations are presented in Figure 10. Since only one sample was analysed, statistical comparisons between the different stations was not possible. However, station 4 had dramatically higher concentrations of PAH compared to the other stations, with over 3 times higher than the next highest at station 3. These PAH were mostly composed of 5 ring PAH, followed by 4 and 6 ring PAH (Figure 11). Despite the differences in overall concentrations, the composition of different number of ringed PAH were very similar at all stations. The individual components of the PAH are shown in appendices.

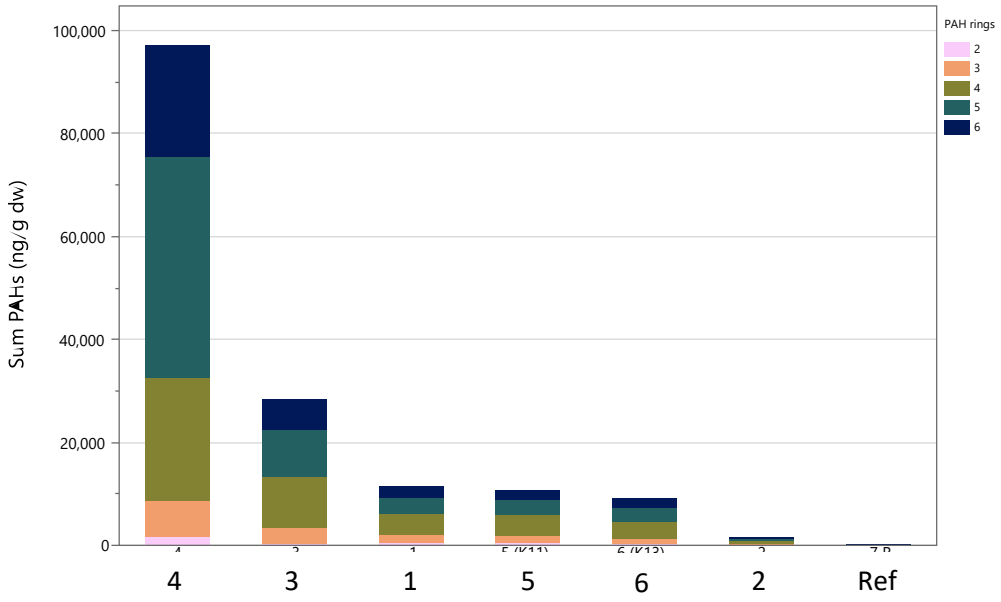


Figure 10. Sum of measured PAH in sediment from the different stations separated into 2, 3, 4, 5 and 6 PAH. Stations ranked in the order of concentration of sum PAH. Stations 1, 2, 3 and ref, correspond to the mussel stations of the same name. Single sediment samples analysed for a total of 52 PAH including alkylated naphthalene, phenanthrene, dibenzothiophene, chrysene, fluorene and pyrene.

For the stations near the smelter, highest concentrations of PAH were measured in the sediment south of the smelter at station 3. Station 2, the closest station north of the smelter had low concentrations of sediment PAH, almost comparable to the lowest concentrations measured at the reference station.

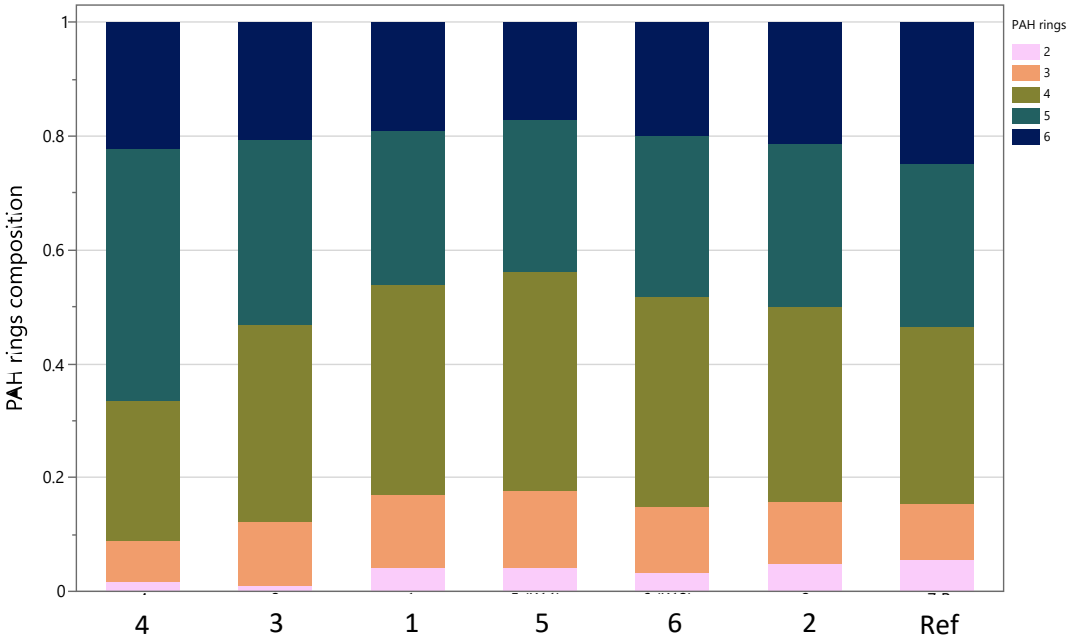


Figure 11. Proportional composition of the 2, 3, 4,5 and 6 ring PAH from the different sediment stations.

Sediment samples were collected from the same locations as the mussel stations (1, 2, 3 and reference). The PAH profiles of the sediment and the mussels at both depths are shown for comparison in Figure 12 for stations 1 and 2 and Figure 13 for stations 3 and reference.

Although much higher concentrations are typically observed in the sediment compartment, the mussel PAH profiles of the surface and bottom mussels are similar to the profiles of the respective sediment. There were no obvious differences between the PAH concentration and profile for the two depths for both stations 1 and 2.

The sediment PAH at station 3 was much higher than the other stations where mussels were deployed. Although similar PAH profiles, the PAH concentration in mussels from the bottom were markedly higher than those from the surface. This may suggest some interaction between the PAH of the contaminated sediment and the overlying mussels at station 3. At the reference station, the sediment PAH concentrations were at the lowest sediment concentrations. Here the mussel PAH body burden was equally low in both surface and bottom mussels and suggest minimum contribution of PAH from

the sediment to the bottom mussels at the reference station. In contrast, the Time 0 mussels showed a very different PAH profile to the field transplanted mussels and the sediment. Only a few smaller ringed PAH were detected with the absence of 5 and 6 ring PAH.

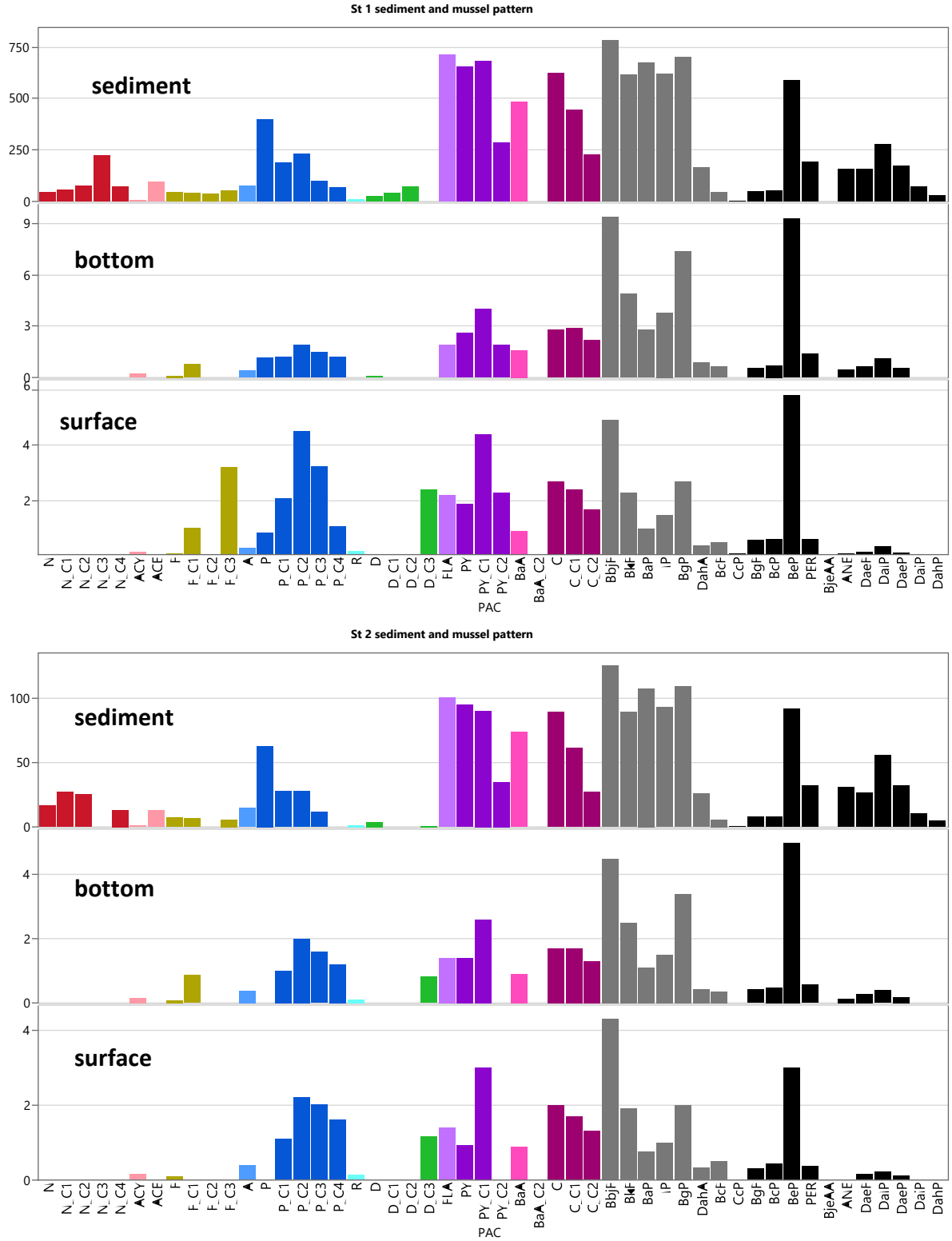


Figure 12 . Comparison of the PAH profiles of the sediment to those of the overlying mussels 5 m below the surface (surface) and mussels 2 m from the seafloor (bottom) at station 1 and 2 north of the smelter (ng/g dw sediment and ng/g ww mussels).

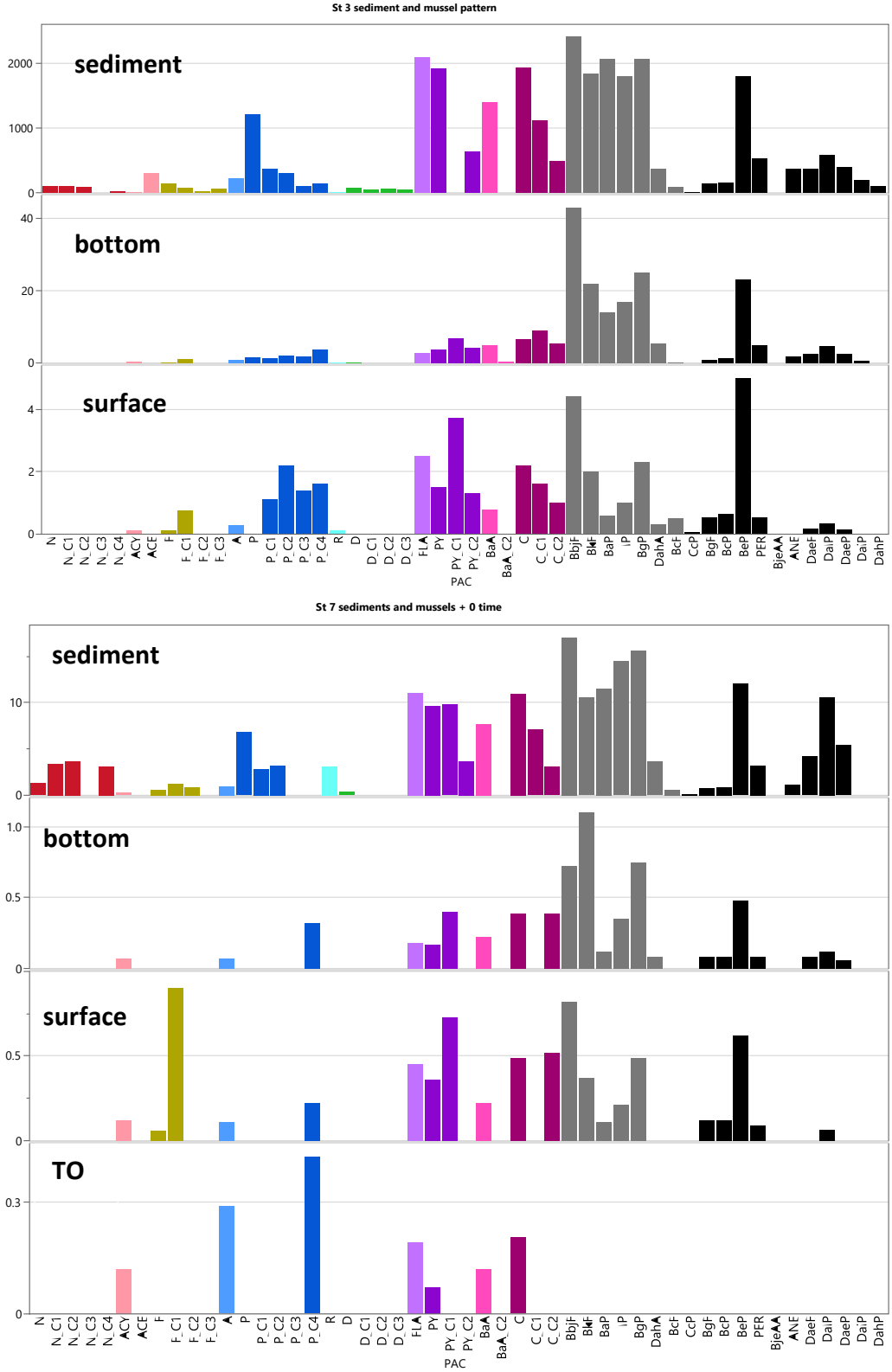


Figure 13 Comparison of the PAH profiles of the sediment to those of the overlying mussels 5 m below the surface (Mussel surface) and mussels 2 m from the seafloor (Mussels bottom) at station 3 south of the smelter and reference location (station 7). PAH concentrations in the time 0 mussels also included for comparison (ng/g dw sediment and ng/g ww mussels).

3.2.3.1 Polyaromatic Compounds (PACs) Correlation with distance

The correlation with distance to the smelter was calculated, and selected examples are shown in Figure 14. Overview of all correlations with distance are given in the appendix, and the details for all correlations can be seen in Table 3. There were 5 PACs that correlated significantly with the distance to the southern outlet; ANE (anthranthene), A (anthracene), N (naphthalene), PER (perylene) and BaA (benzo(a)anthracene). Two of these PACs are small ring PACs (two and tree rings), whereas the other are heavy ring PACs. There were also 16 PACs that did not correlate with distance to the southern outlet ($p>0.2$). The majority of these PACs were alkylated PACs, and also many of those with smaller rings, indicating a petroleum source. In contrast, one of these PACs (R, retene) is an indicator of wood burning.

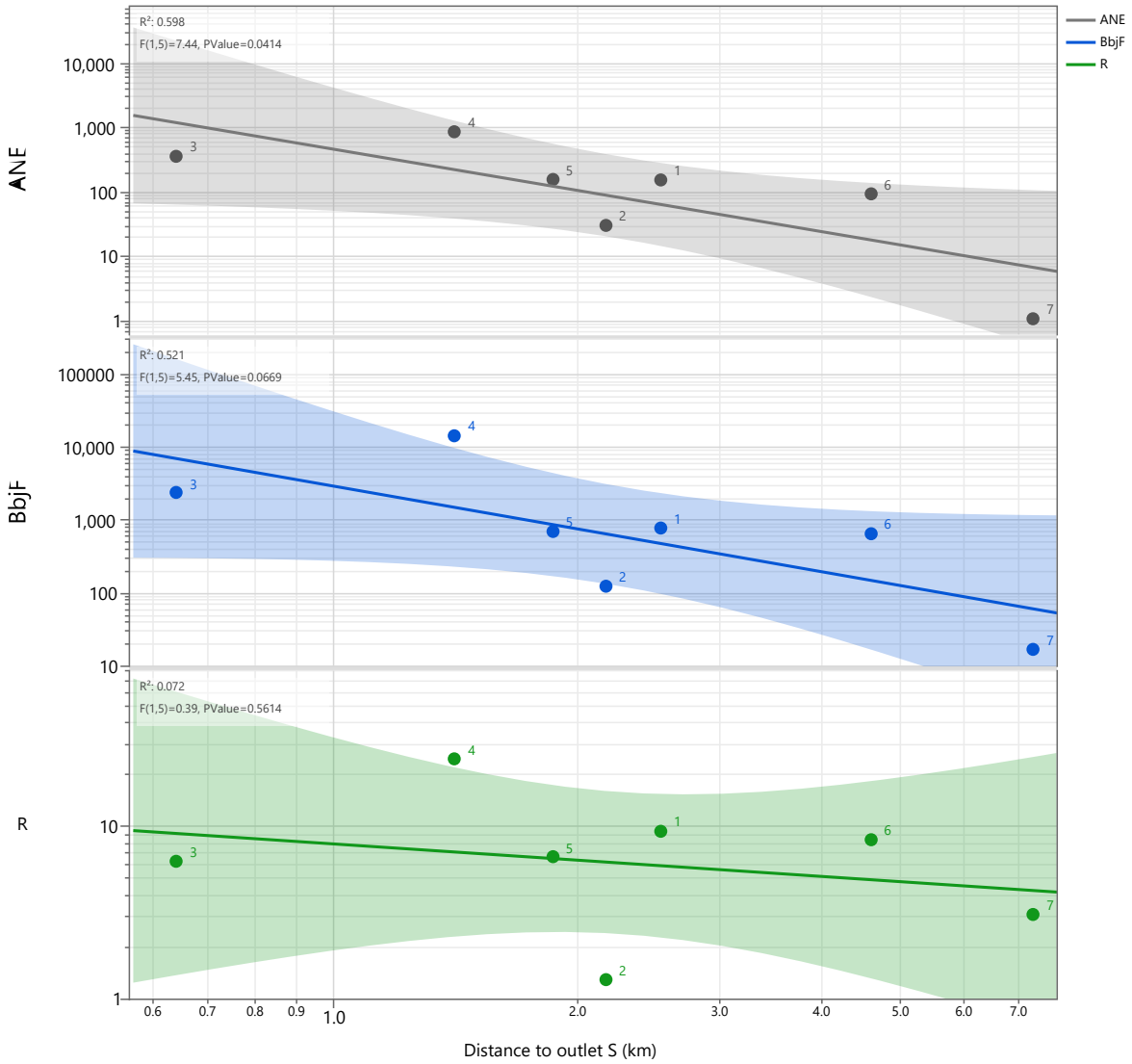


Figure 14 Correlation with distance of selected PACs to the southern outlet of the smelter. Three PAH (ANE=anthranthene, BbjF=benzo(b,j)fluroanten, R=retene) are shown here, with the correlation coefficient and fitness.

Table 3 Overview of correlation with distance (to outlet S) for different PACs. The PACs shown in Figure 14 are indicated with blue coloured cells

PAC	Slope	p	Significant
ANE	-2.12295	0.0414	Yes (<0.05)
A	-1.97458	0.0478	
N	-1.59419	0.0482	
PER	-1.85388	0.0483	
BaA	-1.88571	0.0489	
F	-1.97044	0.0511	Maybe (<0.1)
FLA	-1.90223	0.0517	
P	-1.88228	0.052	
PY	-1.96243	0.0526	
BaP	-1.95366	0.053	
D	-1.89656	0.0532	
C	-1.92533	0.0536	
ACE	-1.92584	0.0538	

BcP	-1.90394	0.0539	
BgF	-1.9105	0.054	
P_C1	-1.7316	0.054	
DaeP	-1.6042	0.0546	
C_C1	-1.86345	0.0549	
F_C1	-1.46809	0.057	
PY_C2	-1.86981	0.0573	
N_C1	-1.19713	0.0589	
C_C2	-1.85553	0.0593	
DaeF	-1.67425	0.0617	
BkF	-1.97107	0.0618	
DalP	-1.55391	0.0638	
BgP	-1.86709	0.0642	
BcF	-1.74506	0.0642	
BeP	-1.96072	0.065	
P_C2	-1.64117	0.066	
IP	-1.83327	0.0662	
BbjF	-1.94901	0.0669	
P_C3	-1.67014	0.074	
DahA	-1.78925	0.0764	
F_C3	-1.46433	0.0782	
PY_C1	-2.07471	0.2394	Probably not
BaP_C1	-1.11798	0.1177	
N_C2	-1.31263	0.1283	
ACY	-1.2342	0.1347	
CcP	-1.40045	0.1381	
F_C2	-1.26769	0.1465	
D_C1	-1.29665	0.1482	
D_C2	-1.06904	0.1581	
CA_C1	-0.9668	0.2246	
D_C3	-1.56385	0.185	
N_C4	-0.78707	0.1855	
P_C4	-0.79552	0.3494	
DahP	-0.92701	0.3394	
DaiP	-0.90077	0.3661	
R	-0.31273	0.5614	
N_C3	-0.29663	0.6027	

3.2.1 Sediment metals

A suite of 11 metals were measured in single sediment samples from the 7 stations (Figure 15), with stations 1-3 representing the same location as the mussel groups 1-3 and sediment station 7 representing the mussel reference group. By far the most contaminated station was station 4, with elevated concentrations of Hg (0.487 mg/kg), As (24 mg/kg), Pb (130 mg/kg), Cd (4.3 mg/kg), Cu (120 mg/kg), Cr (63 mg/kg), Mo (43 mg/kg), Ni (76 mg/kg), Zn (390 mg/kg) and V (120 mg/kg). Of the eleven metals measured only Mn was not elevated at station 4 above the other stations. For the other stations, elevated concentrations of As (17 mg/kg) and an increase in Cu (29 mg/kg) were found at station 1, north of the smelter. However, at the closest station north of the smelter (station 2), low concentrations were measured for all metals, similar to that found at station 7, which corresponded to the mussel reference location.

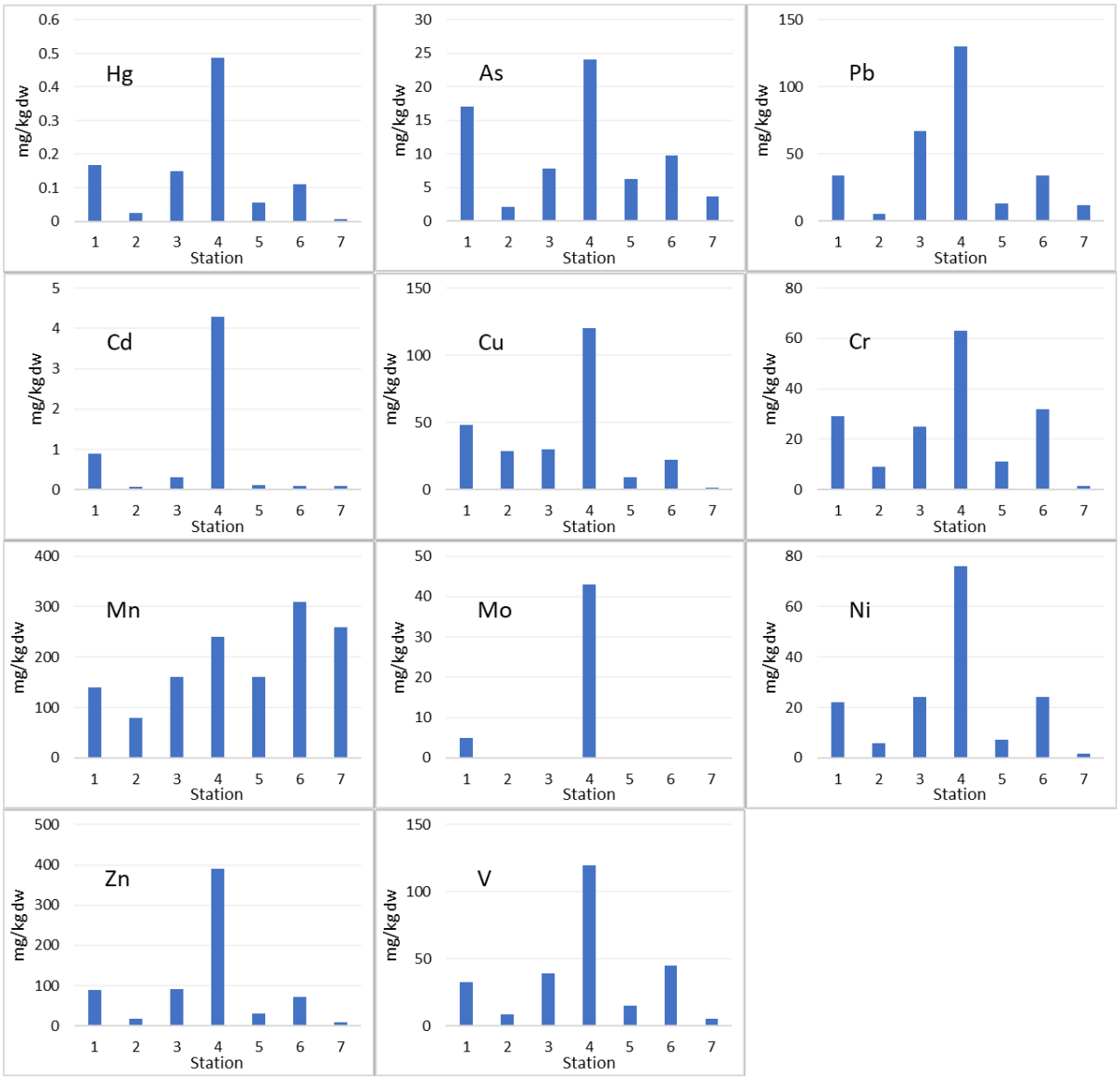


Figure 15. Metal concentrations measured in single sediment samples from the different stations (mg/kg dw).

3.3 Biomarker responses in mussels

3.3.1 Condition index

The condition index calculated by dividing the dry weight of the mussel soft tissue by the dry weight of the shell multiplied by 100, is shown in Figure 16. Significant differences in condition index were found between the mussel groups (ANOVA, Tukey, p>0.05). The lowest condition index was found in mussels from station 1T and 3B.

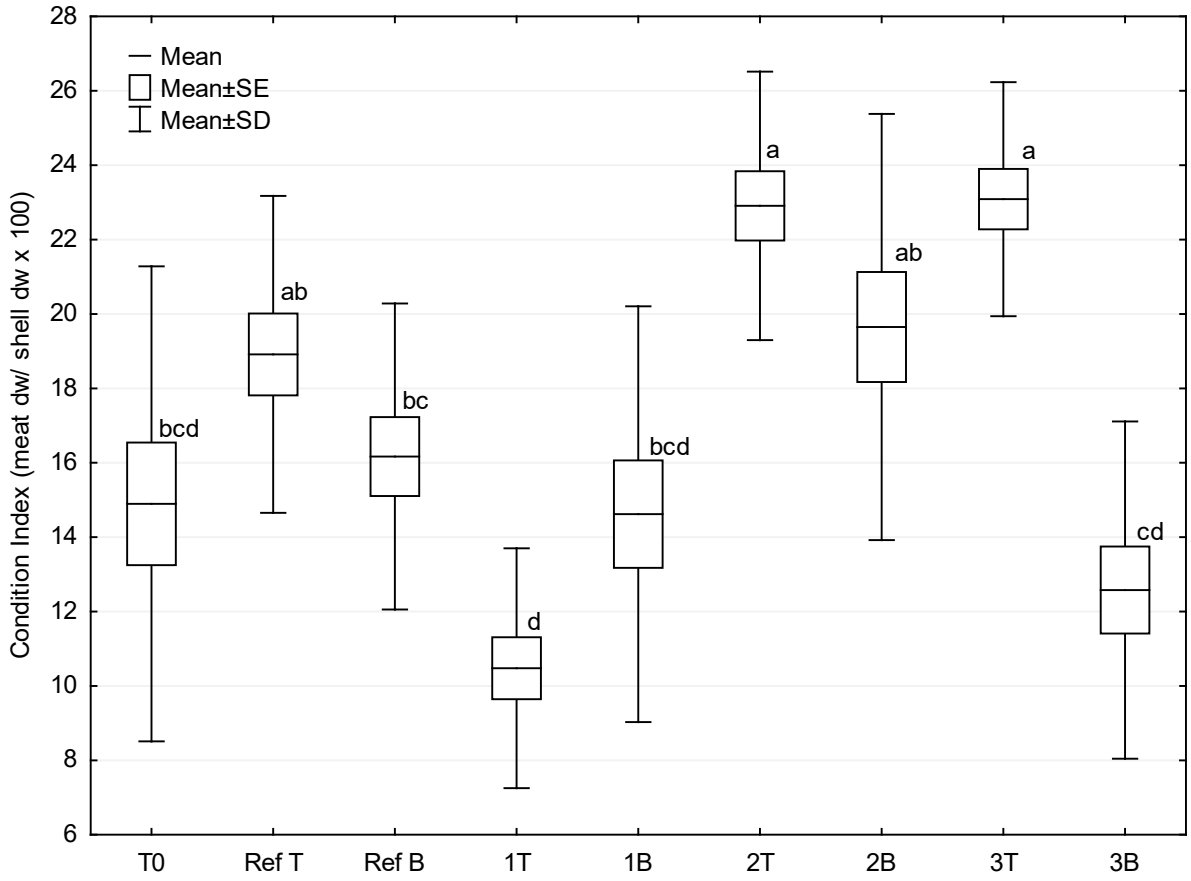


Figure 16. Condition index measured in mussels from the pre-transplanted population (T0) and in mussels held in the water column approximately 5 m below the surface (Top, T) or 2 m from the seafloor (bottom, B) at the four mussel stations (1, 2, 3 and ref) for an 8 week period. Letters denote significant difference between the groups (ANOVA, Tukey, p<005, n=15).

3.3.2 Stress on stress

The stress on stress test, measures the duration of time that a mussel can survive out of water and provides an indication of the general fitness of the mussel. The survival curves for the different mussel groups are shown in Figure 17. The calculated LT_{50} values, which is the time needed to cause 50% mortality of the population, were found between the range 5.6 and 9.8 day. The lowest LT_{50} values

were found in mussels from 2T, the closest station North of the smelter. Lower values of 7.2 and 6.6 days were also reported for station 1 top and bottom mussels respectively, as well as the day zero (T0) mussels at 6.1 days.

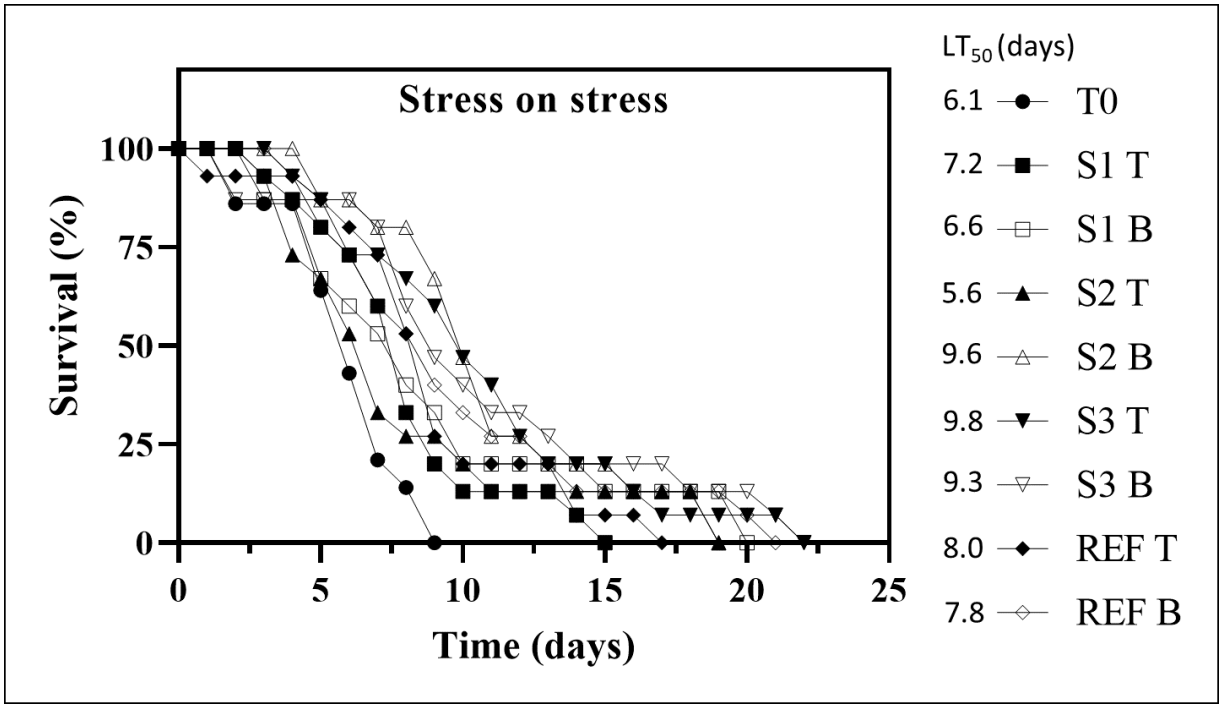


Figure 17. Stress on stress survival curves measured in mussels from the pre-transplanted population (T0) and in mussels held in the water column approximately 5 m below the surface (Top, T) or 2 m from the seafloor (bottom, B) at the four mussel stations (1, 2, 3 and ref) for an 8 week period. The lethal time corresponding to 50% mortality in the population (LT_{50}) are listed on the right side of the figure for each group (n=15).

3.3.3 Neutral red retention

The NRR times for the different mussel groups are shown in Figure 18. The mean NRR values ranged between 70 and 96 min with the higher NRR value indicating a better overall fitness of the mussel. Despite a slight lowering of the NRR in mussels from station 2 top and bottom, no significant differences between the groups were found (ANOVA, Tukey, $p<0.05$).

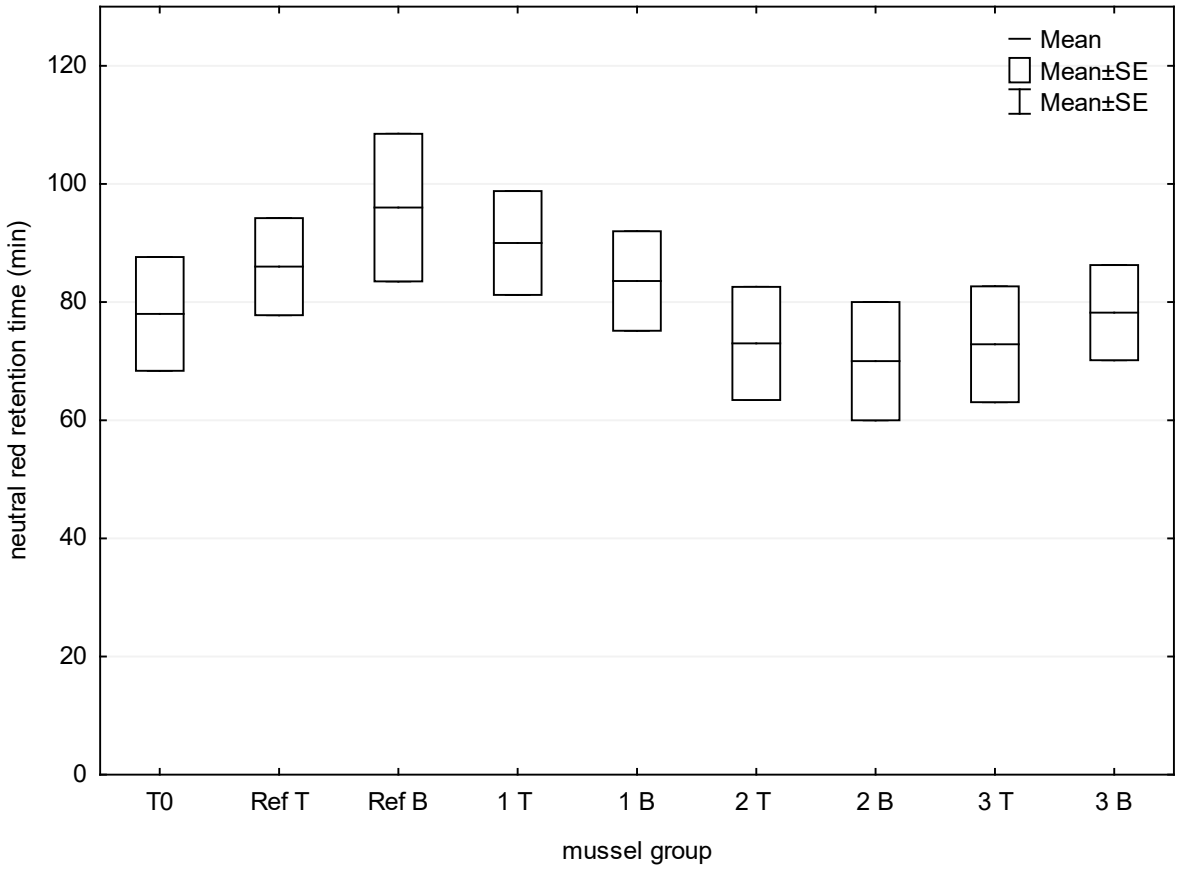


Figure 18. Neutral red retention times in the lysosomes of mussel haemocytes. Measured in mussels from the pre-transplanted population (T0) and in mussels held in the water column approximately 5 m below the surface (Top, T) or 2 m from the seafloor (bottom, B) at the four mussel stations (1, 2, 3, ref) for an 8 week period. No significant differences between the groups (ANOVA, Tukey $p<0.05$, n=15).

3.3.4 Micronuclei formation

The micronuclei slides were initially stained with Hoechst solution that is a blue fluorescent dye and enables nuclei and micronuclei to be detected with fluorescence microscopy. However, partly due to cell clustering, this staining method proved problematic when identifying the outline of the cells and in many cases prevented a sufficient number of cells to be assessed for some groups. Consequently, slides from groups that had insufficient numbers of counted cells were re-stained with Giemsa solution and reassessed. However, an extremely high frequency of micronuclei was observed when slides were re-stained with Giemsa, which was thought to be an artefact of the re-stain (Figure 19). For this reason, the micronuclei frequency was not used in the IBR/n calculation or the PCA assessment.

The frequency of micronuclei in those cells stained with the Hoechst solution were overall low, and except for Ref T, did not exceed the background assessment criteria (BAC) of 2.5 (Davies and Vethaak, 2012). The Ref T mussels had the highest frequency of micronuclei at 5 per 1000 cells.

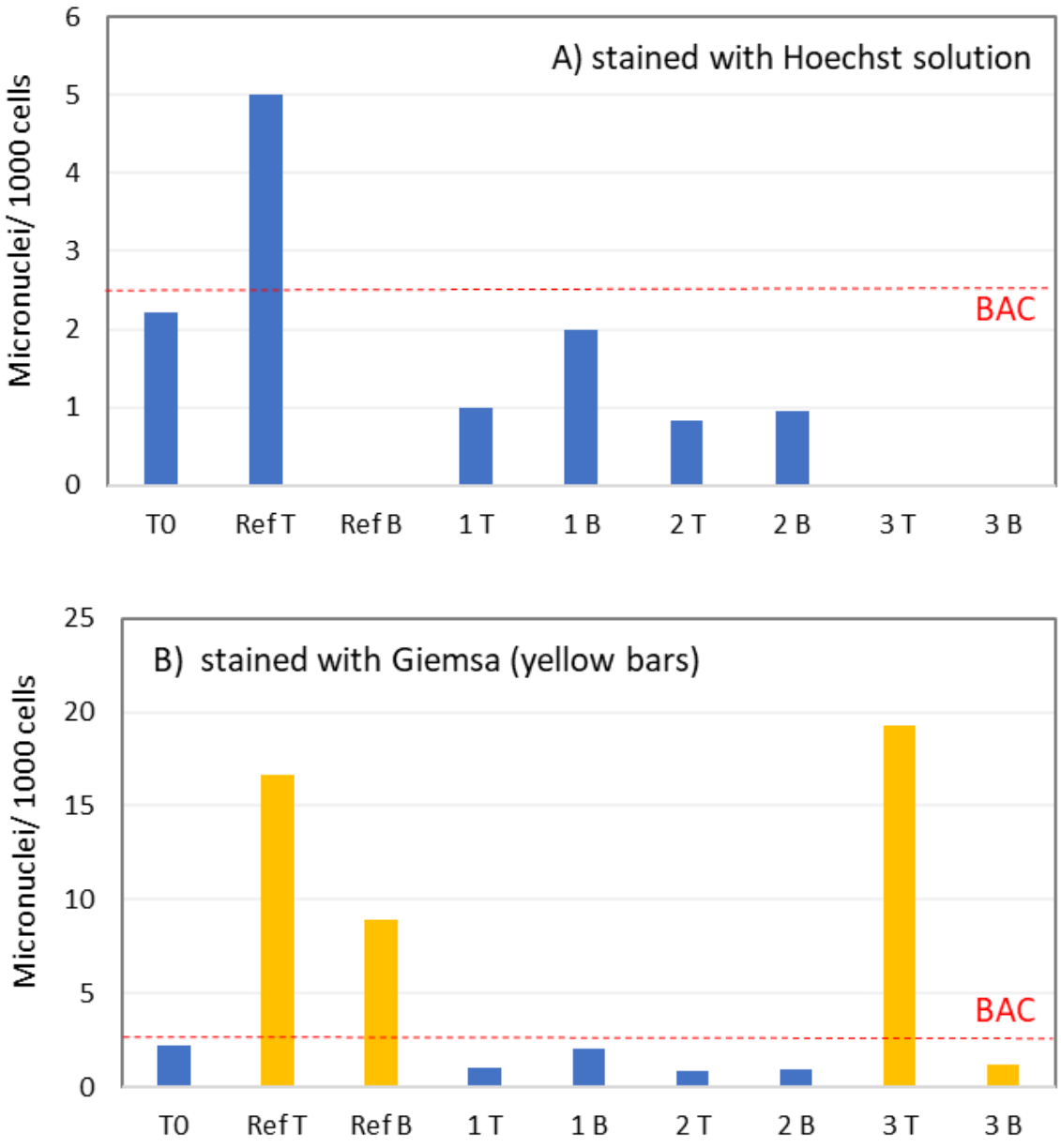


Figure 19. Micronuclei formation in mussel haemocytes from the pre-transplanted population (T0) and in mussels held in the water column approximately 5 m below the surface (Top, T) or 2 m from the seafloor (~~bottom~~Bottom, B) at the four mussel stations (1, 2, 3, ref) for an 8 week period. A) slides stained with Hoechst solution; B) slides re-stained with Giemsa solution (yellow bars).

3.3.5 Acetylcholine esterase inhibition

Inhibition of the enzyme acetylcholine esterase (AChE) is a sensitive biomarker of exposure to neurotoxic compounds. A significant lowering of AChE activity is an indication of exposure. AChE activity in the different mussel groups are shown in Figure 20. Activity levels ranged between 8.4 and 11.3 nmol ATC/ min/ mg protein, with lowest levels at station 2 bottom and highest at day zero (T0). However, there were no significant differences in AChE activity between the mussel groups (ANOVA, Tukey p>0.05).

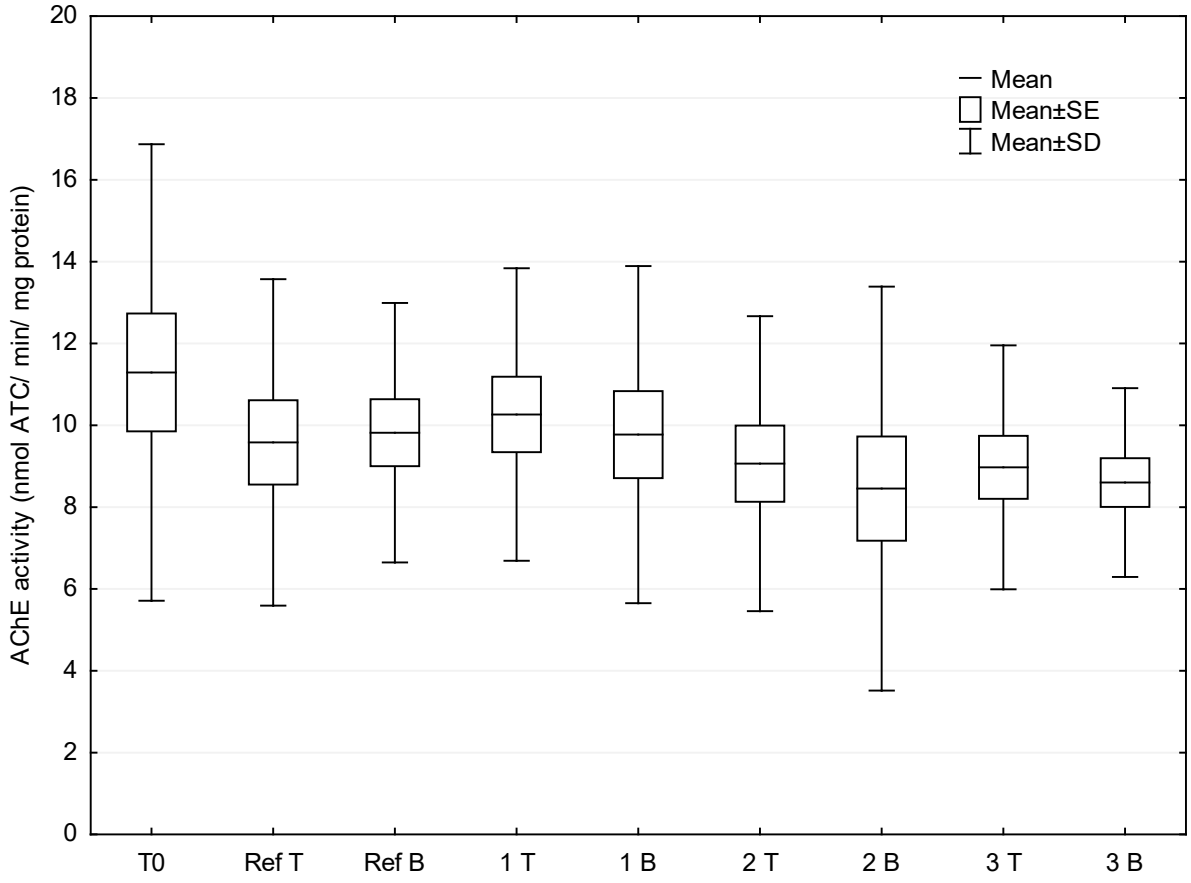


Figure 20. Acetylcholine esterase (AChE) activity in the gill tissue of mussels from the pre-transplanted population (T0) and in mussels held in the water column approximately 5 m below the surface (Top, T) or 2 m from the seafloor (bottom, B) at the four mussel stations (1, 2, 3, ref) for an 8 week period. No significant differences between the groups (ANOVA, Tukey p<0.05, n=15).

3.3.6 Lipid peroxidation

Lipid peroxidation measured as the quantity of MDA and 4-HNE in gill tissue samples of mussels is shown in Figure 21. Significantly higher lipid peroxidation was found in the day zero (T0) group. Lower levels of lipid peroxidation were recorded in mussels from the reference groups, particularly those

from the reference top. For the field transplanted mussels, highest levels of lipid peroxidation were recorded in mussels from station 3B, these were significantly higher than the reference T mussels, but comparable to all other mussel groups (ANOVA, Tukey, p<0.05).

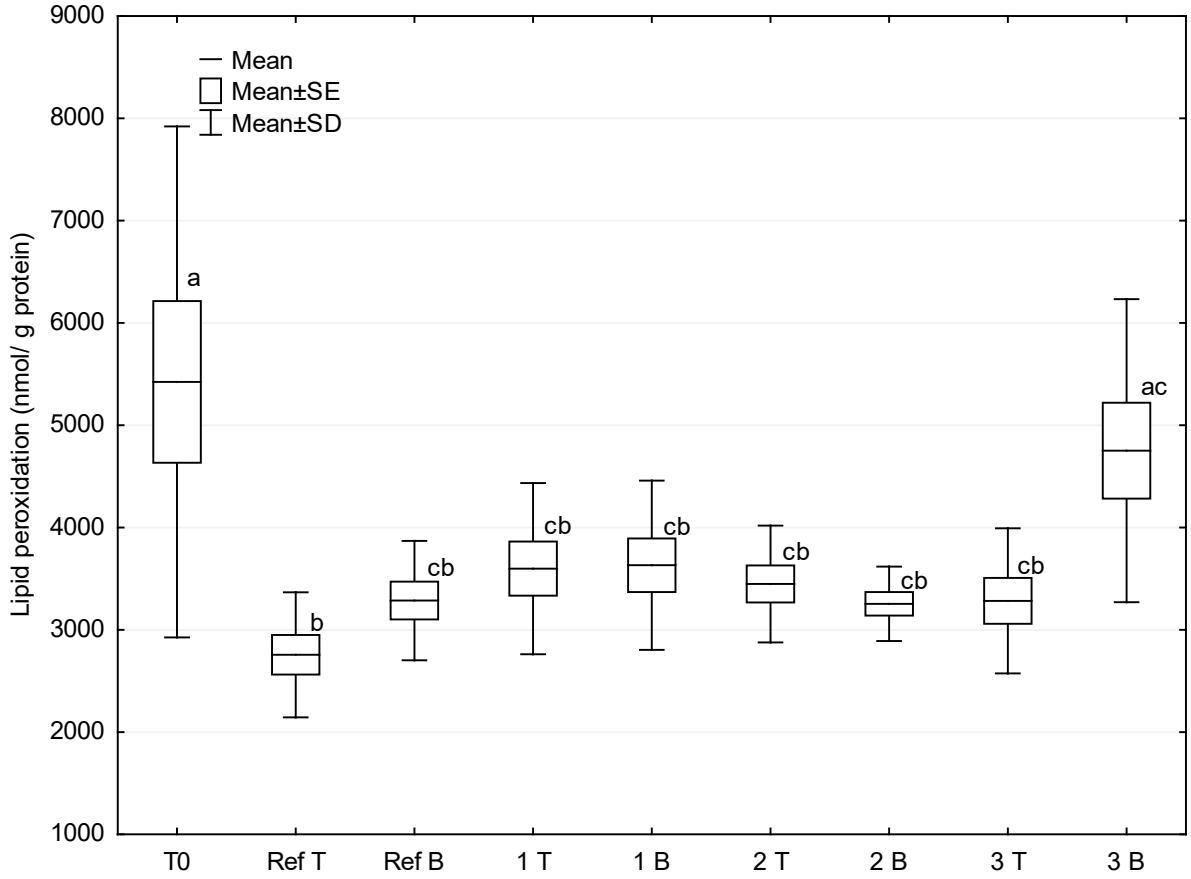


Figure 21. Lipid peroxidation in the gill tissue of mussels from the pre-transplanted population (T0) and in mussels held in the water column approximately 5 m below the surface (Top, T) or 2 m from the seafloor (Bottom, B) at the four mussel stations (1, 2, 3, ref) for an 8 week period. Letters denote significant differences between the groups (ANOVA, Tukey p<0.05, n=10).

3.4 Integrated biological response

The integrated biological response (IBR/n) was used to combine the five individual biomarker results in order to provide an overall assessment of mussel health status from the different groups. Due to the uncertainty of the micronuclei results they were omitted from the IBR/n calculation. The spider plots indicate the contribution of the different biomarkers to the overall IBR/n value (Figure 22). In almost all cases, LPO and AChE contributed most to the IBR/n scores. In contrast, very little contribution to the IBR/n was provided by CI, SoS or NRR. Overall, the IBR/n scores for all groups were

low with the highest IBR/n of 0.79 found in the reference surface mussels (Ref T) with all other groups below 0.2

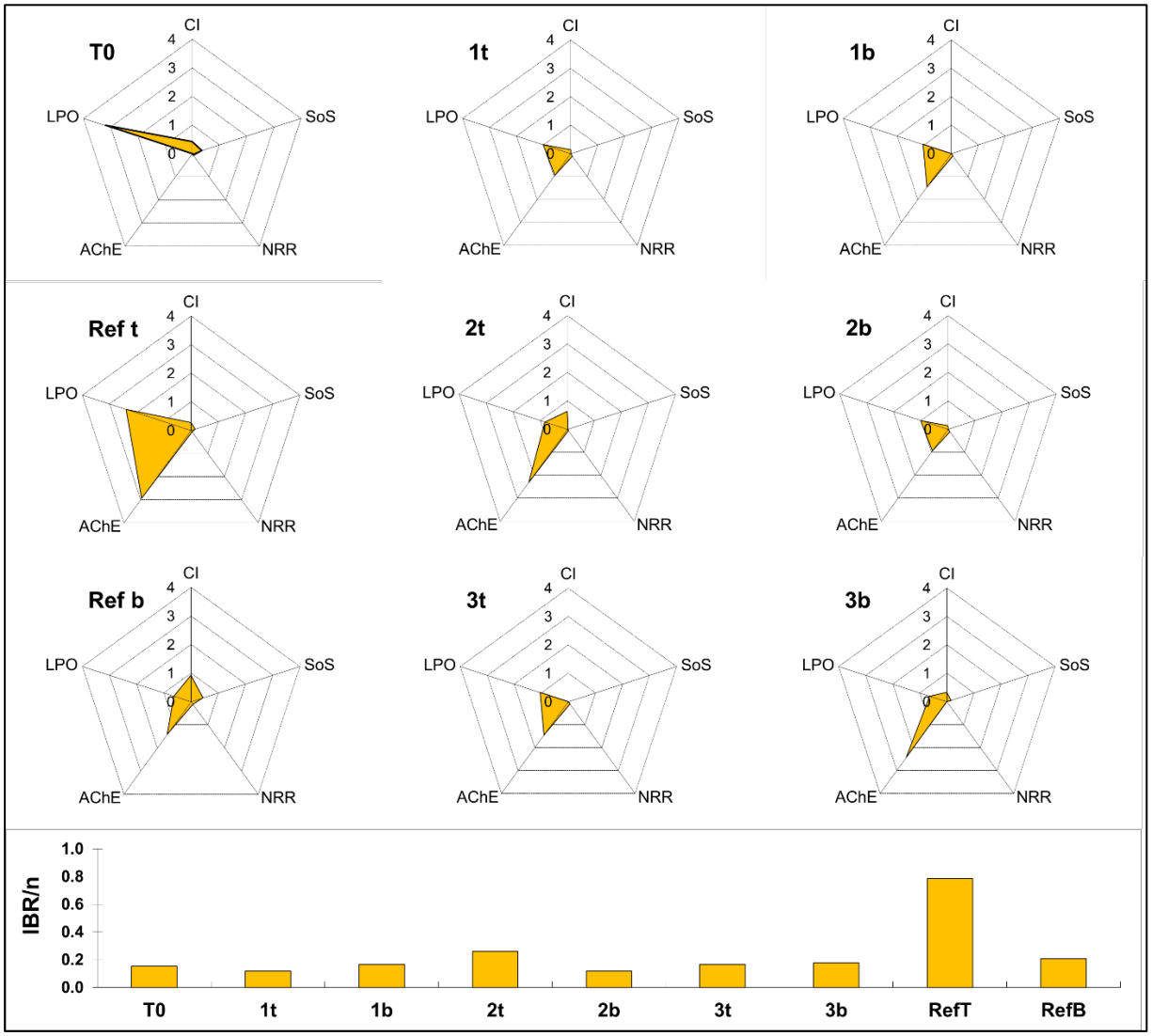


Figure 22. Integrated biological response (IBR/n) calculated from star plots of mean normalised biomarker data in mussels located from the pre-transplanted population (T0) and in mussels held in the water column approximately 5 m below the surface (Top, t) or 2 m from the seafloor (bottom, b) at the four mussel stations (1, 2, 3, Ref) for an 8 week period. Condition index (CI), Stress on stress (SoS), neutral red retention (NRR), acetylcholine esterase (AChE), lipid peroxidation (LPO).

3.5 Principal component analysis

A Principal component analysis (PCA) was used to discriminate the main variables responsible for the variance of chemical body burden and biological effects measured in transplanted mussels. Overall, the PCA showed a clear spatial differentiation between mussel groups, highlighting the different responses obtained in relation to placement in the water column (2 m above the seafloor or 5 m below

the surface), as well as chemical body burden. PC1 accounted for 32.1% of variance and showed a separation between the mussel groups with the highest and lowest PAH's and metal levels. PC2 explained 23.3% of the variance and differentiated the mussel groups with the highest and lowest chemical body burden in relation to depth (bottom and surface). The PCA showed that field transplanted mussels at station 1, 2 and 3 presented the highest concentrations of PAHs and metals, particularly those positioned 2 m above the sea floor (bottom group). Mussels from these stations presented the highest concentrations of PAH 16, PAH 41, total chrysene and pyrene, Cr, Ni, Cd and Mo, associated with stronger responses in SoS and LPO. Surface mussels at station 1 also presented the higher levels of Mn, V, Cu, total dibenzothiophenes, total fluorene, As, Pb and Zn, as well as the highest NRR. As expected, day zero mussels (T0) and mussels positioned at the reference site (bottom and surface) were the less impacted groups, alongside surface mussels from station 2 and 3. These mussel groups had the lowest chemical body burden and presented higher CI and AChE levels, indicative of a good health status.

Only two statistically significant associations were showed by the correlation analysis between the chemical measurements and the biological responses in the mussel groups (Table A1 in Appendix A). A negative correlation between condition index and Ni was detected, as well as a negative correlation between LPO and Hg.

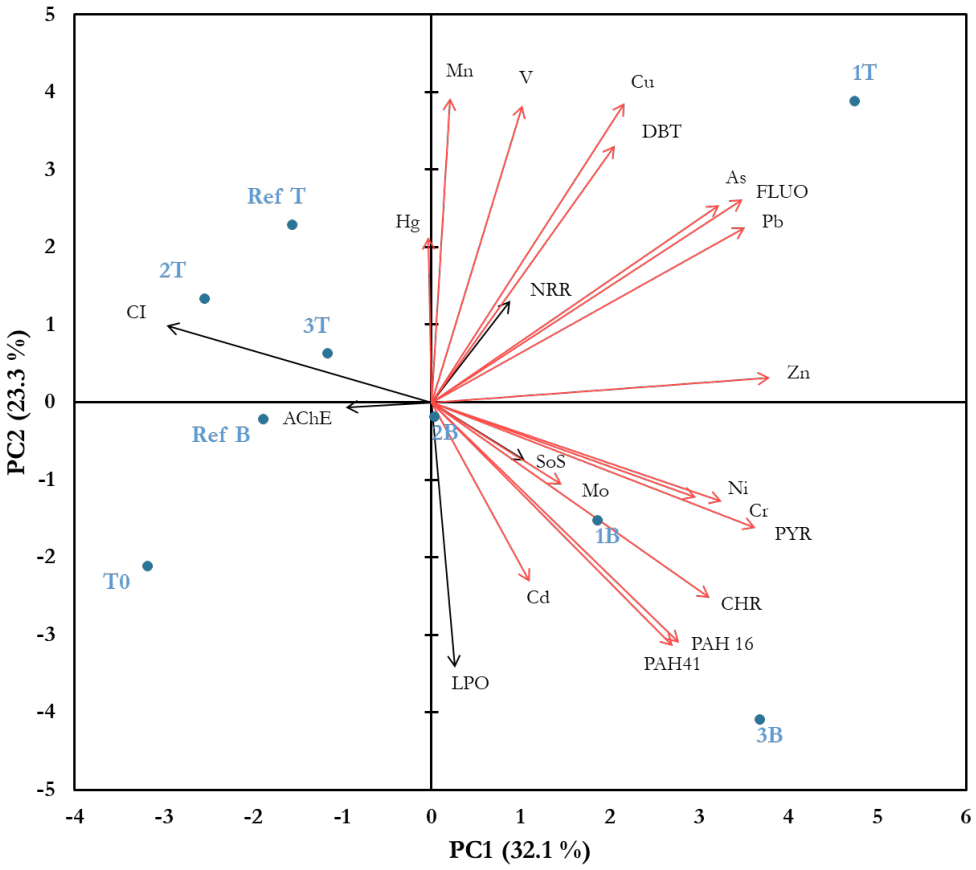


Figure 23. Principal Component Analysis of chemical measurements (red) and biological responses (black) in mussels located from the pre-transplanted population (T0) and in mussels held in the water column approximately 5 m below the surface (Top, T) or 2 m from the seafloor (Bottom, B) at the four mussel stations (1, 2, 3, Ref) for an 8 week period (blue). CI – Condition index; SoS – Stress on stress; NRR – Neutral red retention; AChE – Acetylcholine esterase activity; LPO – Lipid peroxidation; CHR – Total chrysene; DBT – Total Dibenzothiophenes; FLUO – Total fluorene; PAH16 – Sum of PAH16; PAH 41 – Sum of PAH 41; PYR – Sum of pyrene including C_1 and C_2; AS – Arsenic; Cd – Cadmium; Cr – Chromium; Cu – Copper; Pb – Lead; Mn – Manganese; Hg – Mercury; Mo – Molybdenum; Ni – Nickel; V – Vanadium; Zn – Zinc.

4 Discussion

4.1 Contaminant concentrations in sediments

4.1.1 Metals in sediment

Metals concentrations in the sediment samples from the seven stations revealed marked differences between the groups, with elevated concentrations of most metals including Hg, As, Pb, Cd, Cu, Cr, Mo, Ni, Zn and V measured at station 4. The Norwegian environment agencies classification scheme for metal concentrations in sediments, produced in 2016 and revised in 2020, indicate the state of the sediment depending on contaminant concentrations including metals (Table 4). Based on these criteria, the sediment from station 4 was consider class IV (bad) for Cu, whilst As, Cd, Ni and Zn were class III (moderate) and Hg, Pb and Cr class II (good). None of the metals from station 4 that were included in the classification scheme were considered background. Station 4 was a lagoon area south of the aluminium smelter. Due to the location of station 4, it was unclear whether the elevated concentrations had originated directly from the smelter or from other possible sources. For example, Cu is a common active ingredient in antifouling coatings on marine vessels and structures (Thomas & Brooks, 2010) and may have contributed to Cu concentrations in the lagoon. For the other stations, station 1, 3 and 6 had slightly elevated concentrations of some metals although they were either considered background (class I) or good (class II). There appeared no obvious relationship between sediment metal concentration and proximity to the Aluminium smelter.

Table 4. Classification scheme for the sediment condition from background to extreme based on metal concentration (source: M-608/2016) (mg/ kg d.w.).

Metal	Background (I)	Good (II)	Moderate (III)	Bad (IV)	Extreme (V)
As	0-15	15-18	18-71	71--580	>580
Pb	0-25	25-150	150-1480	1480-2000	2000-2500
Cd	0-0.2	0.2-2.5	2.5-16	16-157	>157
Cu	0-20	20-84		84-147	>147
Cr	0-60	60-620	620-6000	6000-15500	15500-25000
Hg	0-0.05	0.05-0.52	0.52-0.75	0.75-1.45	>1.45
Ni	0-30	30-42	42-271	271-533	>533
Zn	0-90	90-139	139-750	750-6690	>6690

4.1.2 PAH in sediment

PAH concentrations in sediment showed marked differences between the groups. As previously described for metals, the sediment from station 4 had the highest concentrations of PAH contamination compared to the other sediment stations. The PAH concentration at station 4 was approximately three times the concentration of the sediment at station 3, which was the next highest. It was unclear whether the elevated concentrations of PAH at station 4 were due to historical contamination from the aluminium smelter, when discharges of PAH were more significant, or from other sources such as road run off, boating activity and/ or other local industries. When examining the alkylated PAHs, very similar profiles were found for alkylated phenanthrenes (C1-C4), dibenzothiophenes (C1-C3), chrysene (C1-C2), fluorene (C1-C3) and pyrene (C1-C3). However, for alkylated naphthalenes, C2-naphthalene at station 4 was approximately 9 times higher than the other sediment stations and resulted in a different PAH profile. This may indicate that the C2 naphthalene at station 4 originated from a different source and other inputs of PAH should be considered.

Station 3, the closest station south of the aluminium smelter, was expected to have elevated concentrations of PAH based on previous surveys that have shown historical PAH contamination. The sum PAH in the sediment at station 3 was comparable with the concentrations measured at the same location in 2008 and 2015 at approximately 20-30 mg/ kg d.w. (Håvardstun, 2016).

At all stations, PAH16 made up between 73 and 80% of the total PAH compounds measured. Of these PAH16, 10 PAHs (phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b,j)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene) contributed towards 92-94% of the total PAH measured. Station 4 had significantly higher concentrations of Benzo(b,j)fluoranthene, contributing to 20% of the total PAH in the sediment sample.

The Norwegian classification scheme for the EPA-PAH16 concentrations in sediment are shown in Table 5. Based on these values for the sum of PAH16, station 7 was background (I) and station 2 was good (II), whilst station 6 was moderate (III). Stations 1, 3 and 5 were consider bad (IV), whilst station 4 was three times over the threshold of 20 000 µg/kg and categorised as extreme (V). When looking at the individual PAH, Benzo(g,h,i)perylene concentrations were considered extreme at stations 3 and 4, whilst anthracene, fluoranthene, chrysene, benzo(b,j)fluoranthene, benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene were considered extreme at station 4 only. These PAH were also reporting bad

(IV) classification in sediment at station 1, 3, 5 and 6. Despite station 2, the closest to the northern discharge pond, having an overall PAH16 classification as good (II), PAH concentrations, indicating bad status (IV), were found for chrysene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene.

Table 5. Classification scheme for the sediment condition from background to extreme based on PAH concentration (source: Miljødirektorat M-608/2016, µg/ kg d.w.)

PAH (µg/kg d.w.)	Background (I)	Good (II)	Moderate (III)	Bad (IV)	Extreme (V)
Naphthalene	0-2	2-27	27-1754	1754	>8769
Acenaphtylene	0-1.6	1.6-33	33-85	85-8500	>8500
Acenaphthene	0-2.4	2.4-96	96-195	195-19500	>19500
Fluorene	0-6.8	6.8-150	150-694	694-34700	>34700
Phenanthrene	0-6.8	6.8-780	780-2500	2500-25000	>25000
Anthracene	0-1.2	1.2-4.8	4.8-30	30-295	>295
Fluoranthene	0-8	8-400		400-2000	>2000
pyrene	0-5.2	5.2-84	84-840	840-8400	>8400
Benzo(a)anthracene	0-3.6	3.6-60	60-501	501-50100	>50100
Chrysene	0-4.4	4.4-280		280-2800	>2800
Benzo(b,j)fluoranthene	0-90	90-140		140-10600	>10600
Benzo(k)fluoranthene	0-90	90-135		135-7400	>7400
Benzo(a)pyrene	0-6	6-183	183-230	230-13100	>13100
Indeno(1,2,3-cd)pyrene	0-20	20-63		63-2300	>2300
Dibenz(a,h)anthracene	0-12	12-27	27-273	273-2730	>2730
Benzo(g,h,i)perylene	0-18	18-84		84-1400	>1400
PAH16	0-300	300-2000	2000-6000	6000-20000	>20000

4.2 Contaminant concentrations in mussel tissue

4.2.1 Metals in mussels

A Norwegian classification scheme has been developed to indicate the level of risk posed by the concentration of metal(s) in mussel tissue. The scheme divides the metals concentrations into five categories of increasing risk from insignificant (I), moderate (II), marked (III), severe (IV) to extreme (V). The concentration of metals measured in mussel tissues in the present study were all below the upper limit of classification I, indicating insignificant. It should be noted that Mo, Mn and V were not included in the classification scheme although concentrations of these metals were below or marginally above their detection limits and were not thought to have presented any degree of concern.

Table 6. The Norwegian classification scheme of the relative risk of metal concentrations in the soft tissue of marine mussels (mg/kg w.w.). Also including the Norwegian Provisional high reference contaminant concentration (PROREF) for those metals and the available Environmental Quality Standard (EQS). 2013/39/EU, M-856/2017.

Metal (mg/ kg w.w.)	Classification (upper limit for class I to IV)					EQS	PROREF
	Insignificant (I)	Moderate (II)	Marked (III)	Severe (IV)	Extreme (V)		
As	10	30	70	140	>140		2.50
Cd	0.4	1	4	8	>8		0.18
Cu	2	6	20	40	>40		1.40
Cr	0.2	1	3	10	>10		0.36
Pb	0.6	3	8	20	>20		0.2
Hg	0.04	0.1	0.3	0.8	>0.8	0.02	0.012
Ni	1	5	10	20	>20		0.29
Zn	40	80	200	500	>500		17.66

Updates to the Norwegian classification scheme are available with data principally generated from the Norwegian annual coastal monitoring programme, which has generated a very large amount of data over the last 20 years. From here, a provisional high reference contaminant concentration (PROREF) for the metals in mussel tissue have been suggested. With exception to Cr, the PROREF concentrations are generally lower than the lowest category of the Norwegian classification scheme and would provide a more conservative estimate of risk. Based on the PROREF values, Pb at station 1 surface,

were marginally above, whilst Cd and Cu were identical to the PROREF value at 1b and 1s respectively. For Hg, all field transplanted mussels had higher concentrations than the PROREF value, but lower than the Environmental Quality standard (EQS) that is only available for Hg (2013/39/EU). Overall, the risk of metal concentration to the mussels was low and was considered of minimal concern.

As a comparison, native mussels were collected from three locations outside from the Karmøy aluminium smelter as part of a monitoring programme in 2019 (Øxnevad & Hjermann, 2020). The native mussels were collected at Høgevarde (just outside the North settling pond), Helgelandsvika (near sediment station 5) and Bygnesvågen (near sediment station 4). Overall, the metal concentrations measured in these native mussels were comparable to the concentrations in mussels transplanted out into the field for 8 weeks in the present study.

4.2.2 PAH in mussels

The PAH concentrations in mussels from the three locations closest to the aluminium smelter were approximately 100-fold and 10-fold higher than the day zero and reference station mussels respectively. This would suggest some relationship between proximity to the aluminium smelter and PAH accumulation in the mussels. Highest concentrations were found in the mussels positioned approximately 2 m above the sediment at station 3. As mentioned previously, this station was the site of historical PAH contamination (Håvardstun, 2016) and may suggest an exposure pathway from the contaminated sediment to the mussels in the water column. It should be noted however, that such historical PAH contamination was likely to have originated from the Søderberg process. It has been demonstrated that PAHs from Søderberg aluminium smelters are much less bioavailable from the sediment than expected, due to the strong adsorption of PAHs to soot particles in the sediment (Ruus et al., 2010; Næs et al., 1998). However, despite this possible trapping of PAHs in sediment, bioaccumulation of PAHs in the mussels from the sediment, particularly for station 3, were observed.

Although station 2 was the closest station north of the smelter, it was station 1 that showed high concentrations of PAH for these two northern groups. Other industries in the area north of the smelter may have contributed to the higher concentrations of PAH in the more northerly station. Overall, for the mussel stations closest to the aluminium smelter, higher concentrations were found in the bottom mussels 2m from the seafloor compared to the mussels 5 m from the surface. This difference was more pronounced at station 3, less pronounced at station 1 and not present at station 2. The accumulation of PAH may be influenced by the physicochemical properties of the seawater, particularly temperature

and salinity but also food availability and particle density in the water column. From the CTD profiles taken during deployment of the mussels, some indication of the mussel's physicochemical environment could be determined. Temperature differences between the surface and bottom mussels were large, particularly for the deeper stations, like station 1 (17℃ surface and 14℃ bottom) and station 3 (18℃ surface and 12℃ bottom). Salinity differences were also apparent with surface waters of 29 and 30‰ and deeper waters closer to 33‰. Although mussels can thrive at these temperatures and salinities, increased metabolism may be expected in warmer waters, which may have some implications towards filtering rates and chemical bioaccumulation. However, it is important to point out that this was only a snapshot of an 8-week exposure period and although stratification of the water column was strong in late August, when the mussels were deployed, stratification would be less in the Autumn when mussels were retrieved. Therefore, it is important not to over interpret the differences observed.

Using the Norwegian classification scheme for PAH16 in mussel tissue, the mussels from Day zero, as well as the reference station, station 2 and station 3 top mussels, had PAH concentrations representative of the lowest classification (< 50 ng/g w.w.) of insignificant (class I). Station 1 mussels were less than 200 (Class II, moderate), whilst the mussels from station 3 bottom were above 200 and considered marked (class III). The more conservative PROREF benchmark of 34 ng/g (w.w.) was exceeded by all mussels closest to the smelter (stations 1-3), which would indicate that these mussels had PAH concentrations higher than typical coastal water background concentrations developed for Norwegian coastal waters.

In relation to the composition of PAH in mussel tissues, the day 0 mussels that were collected from a clean location in Kvitsøy approximately 50 km south of Karmøy not only had low concentrations of PAH, but the PAHs detected were only 2, 3 and 4 ring, with the absent of the heavy 5 and 6 ring PAH. In contrast, five ring PAHs were the more dominant type in the field exposed mussels, and particularly in those closest to the aluminium smelter.

For comparison with other mussel field deployments, PAH concentrations as high as 400 ng/g (w.w) were reported in mussels placed for 6 weeks at 500 m from the Statfjord A platform (Pampanin et al., 2017). These concentrations were almost double of those measured at Karmøy. However, mussels placed 10 km downstream from Statfjord B platform for 6 weeks accumulated 53 ng PAH/g (w.w.), which were similar to the concentrations found in mussels located at stations 1 to 3. When comparing the PAH concentrations in mussels from Karmøy with those from Sunndalsfjord (Brooks et al. 2021).

The highest PAH concentrations measured in mussels approximately 1 km from the Sunndalsfjord aluminium smelter were 8-10 ng/g (w.w.). These concentrations are similar to the reference mussels in the Karmøy study.

Comparison between the PAH concentration profiles of the sediment and the overlying mussels were performed at the 4 stations to see if PAH's in the sediment were potentially contributing to uptake in the mussels. It was clearly shown that day 0 mussels had a very different PAH profile than the field transplanted mussels. There was a good agreement between the PAH profile of the sediment and the PAH profile in the overlying mussels for the four stations. At station 3, south of the smelter and to a lesser extent at station 1 (furthest north), highest concentrations of PAH were in the bottom mussels, although PAH profiles were similar between surface and bottom mussels. It appears that the higher PAH concentrations of sediment at station 3 may be contributing to the higher concentrations of PAH in the bottom mussels from this station. For station 2, little difference in PAH concentrations for surface and bottom mussels were observed, although again PAH profiles were similar between surface and bottom mussels. The shallow depth at station 2 may explain the lack of difference between surface and bottom mussels.

4.3 Biological effects responses

Five biological effects endpoints were measured in the mussels from the day 0 group and the field exposed mussels. Integrated biological effects methods are typically applied to monitoring studies where organisms are exposed to complex mixtures of environmental chemicals. The biological effects methods used encompass whole organism responses as well as genotoxicity, neurotoxicity and oxidative stress. Although the methods used are considered sensitive tools to assess the biological effects of chemical exposure, the responses observed in the present study were overall low and showed no clear biological effect of the aluminium smelter discharge effluent.

Condition index is a whole organism response which can be impacted by chemical exposure but also influenced by reproductive and nutritional status, which in turn is influenced by physicochemical properties of the seawater. It was previously described how the mussels at the different depths were exposed to different temperatures and salinities. The temperature of the surface waters during deployment of the mussels was 5-6℃ warmer than the bottom waters, which over an 8-week exposure may be expected to have an influence on condition index. Comparatively higher condition indices were

observed in surface mussels occupying the warmer waters than the bottom mussels at three of the four stations.

Additional whole organism responses were measured with the stress on stress method. This method provides information on the available energetic status of the mussels and their ability to keep their shell closed and withstand desiccation. The lethal time for 50% of the population to die (LT_{50}) ranged from 5.6 to 9.8 days, with the higher the value the better condition of the mussel. For the field transplanted mussels, lower LT_{50} values were found in mussels located north of the smelter at station 1 and 2, closest to the location of the smelter effluent and may indicate a response. When comparing the LT_{50} values with similar field studies, values between 8 and 12 days were found in mussels transplanted for 6-week into Bøkfjorden in Kirkenes (Brooks et al., 2015), and between 6.5 and 8.2 days in the Sunndalsfjord near Molde (Brooks et al., 2021).

ICES assessment criteria in the form of background and environmental assessment criteria (BAC and EAC) have been developed for stress on stress in mussels and calculated as 10 and 5 days respectively (Davies and Vethaak, 2012). Based on these values, all of the mussels in the present study where below the BAC but above the EAC, which would indicate some minimal impact in all groups.

A measure of cell integrity and fitness can be provided by the NRR assay. However, the results showed no significant differences in NRR between the mussel groups. Based on ICES assessment criteria for NRR with a BAC of 120 min and EAC of 50 min, all mussels in the study had mean NRR values between 50 and 120 min. This indicates that the mussels were stressed but compensating.

The frequency of MN in the mussel haemocytes is a sensitive biomarker of exposure to genotoxic compounds (Bolognesi & Fenech, 2012). The BAC value for MN frequency is currently 2.5 per 1000 cells (Davies and Vethaak, 2012). Based on this value only mussels from the reference surface group exceeded the BAC value. Although problems with scoring the slides prevented reference bottom and station 3 to be properly assessed, the stations north of the smelter had low frequency of MN ranging from 0.8 to 2 micronuclei per 1000 cells. The impact of the effluent discharge in the northern settling pond was considered to have minimal impact on micronuclei formation in this study. When compared to similar studies in Norwegian fjords, micronuclei frequencies as high as 10 per 1000 cells were reported in Frænfjord up to 2 km from the Hustadmarmor mine (Brooks et al., 2018), whilst 4.7

micronuclei per 1000 cells were found in mussels placed in Bøkfjord within 1 km of the Sydvaranger iron ore mine discharge (Brooks et al., 2015).

The exposure to neurotoxic compounds was measured with the AChE inhibition assay. However, as reported for several of the other biomarkers, no significant differences were observed between mussel groups. AChE activity ranged between 8.4 and 11.3 and was comparable with the AChE activity in mussel transplanted for 6 weeks in the Sunndalsfjord (Brooks et al., 2021).

Measurement of lipid peroxidation did show significant differences between the field exposed groups with lowest lipid peroxidation indicating lower oxidative stress in mussels from the reference station and significantly higher oxidative stress in the bottom mussels from station 3. Elevated concentrations of PAH were found in the mussels from station 3, which may be responsible for the higher level of oxidative stress present in these mussels. In comparison to other studies, lipid peroxidation levels around 2200 to 2600 nmol/ g protein were reported in mussels placed for 6 weeks in the Sunndalsfjord (Brooks et al., 2021). These lipid peroxidation levels were considered unresponsive and indicative of a background response and were similar to the lipid peroxidation levels found in the reference mussels in the present study. Unfortunately, ICES assessment criteria have not been established for lipid peroxidation and so comparisons within the wider context cannot be made.

Surprisingly, high but variable levels of lipid peroxidation were found in the day 0 mussels, which were comparable to the levels observed in the highest field exposure group described above. Contaminant concentrations for both PAH and metals were consistently low in these mussels and the reason for these higher measured concentrations from day zero (T0) group is unclear.

4.4 Integrated biological response (IBR)

Combining the biomarker data into an integrated biological response (IBR/n), an overall assessment of the health status of the mussels within each group can be achieved. The star plots highlighted which biomarkers were most responsible for the IBR/n and in almost all cases the oxidative stress marker lipid peroxidation and the neurotoxic biomarker acetylcholine esterase (AChE) were shown to contribute most to the IBR/n score. However, when looking at the individual LPO and AChE responses they did not very clearly differentiate between the mussel groups. This low responsiveness of these biomarkers as well as the other three biomarkers measured, corresponds with the low IBR/n values calculated in all groups. Essentially the biomarkers measured, and the calculated IBR/n values indicate

a very modest biological response in all mussel groups including those placed near to the aluminium smelter.

4.5 Principle component analysis

The integration of chemical and biological responses in transplanted mussels through PCA analysis showed that placement in the water column was one of the most significant factors affecting the spatial responses observed and directly connected with chemical body burden. The PCA showed a clear differentiation between mussel groups placed at the different stations and depths, highlighting mussels from stations 1 top and bottom and stations 2 and 3 bottom as those presenting the highest concentrations of PAHs and metals. However, similar to the assessment provided by the IBR, the PCA did not show any clear impact of PAH's and metals in biomarker responses measured in the mussels, even in those located closer to the aluminium smelter.

5 Conclusion

Sediment chemistry identified significant, and based on the Norwegian classification scheme, often extreme (class V) sediment contamination of metals and PAHs measured at station 4 (Nordalsvågen). This station was within a sheltered lagoon approximately 1 to 1.5 km south of the aluminium smelter. It remains unclear as to whether this contaminated sediment was the result of historical contamination from the aluminium smelter from a time when discharges of PAH and metals were more significant or whether other sources were responsible (e.g. local industry and small boat harbour).

Historical contamination of metals and PAHs were measured in sediments at station 3, approximately 500 m from the aluminium smelter's southern settling pond. Based on the Norwegian classification scheme, PAHs such as benzo(g,h,i)perylene was at concentrations considered extreme (V), whilst anthracene, fluoranthene, chrysene, benzo(b,j)fluoranthene, benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene were classified as bad (IV) at station 1, 3, 5 and 6.

Higher concentrations of PAH were measured in mussels positioned near to the smelter (Stations 1, 2, & 3) compared to the reference and day 0 mussels. The PAH profiles in the mussels patterned the PAH profiles of the sediment at all stations, with highest concentrations of PAH in the bottom mussels at 3

of the 4 stations. Highest concentrations of PAH were found in the bottom mussels from the station 3, the station with the highest sediment PAH where mussels were present.

The day 0 mussels that were not placed out in the fjord had low concentrations of metals and PAH in their tissues. The PAHs in the day 0 mussels were 2, 3, and 4 ring compounds and where absent of 5 and 6 ring PAHs. In contrast, mussels placed within the fjord, and particularly those placed close to the aluminium smelter had higher concentrations of 5 ring PAHs.

Despite the accumulation of PAHs in the tissue of mussels at the closest stations to the aluminium smelter, the five biological effects responses measured were low and did not significantly differentiate between the stations. The IBR/n calculation was used to integrate the biological responses, however due to the low responsiveness of all five biomarkers the IBR/n scores were equally modest and indicated very little impact on the overall health status of the mussels from all stations. The PCA allowed the differentiation between the groups with the highest and lowest chemical body burden, with mussels from stations 1 top and bottom and stations 2 and 3 bottom presenting the highest concentrations of PAHs and metals. However, no clear association was found between PAH's and metals and biological responses recorded in mussels, even in those located closer to the aluminium smelter.

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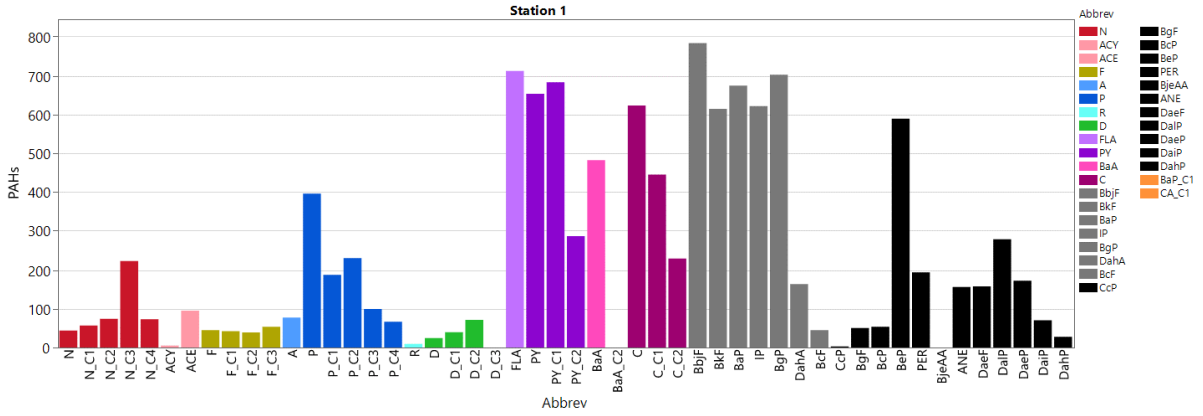
Web links:

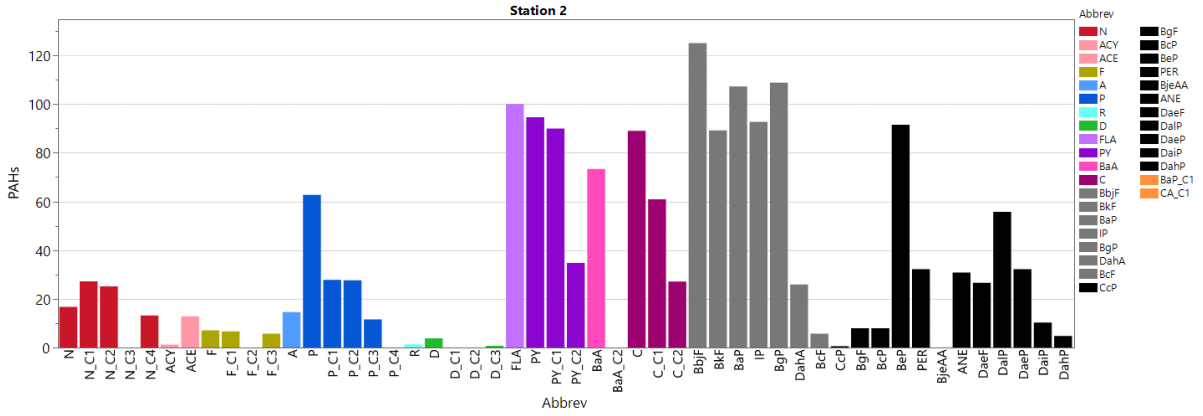
[1] https://www.hydro.com/no-NO/om-hydro/hydro-worldwide/europe/norway/karmoy/karmoy-primary-production/

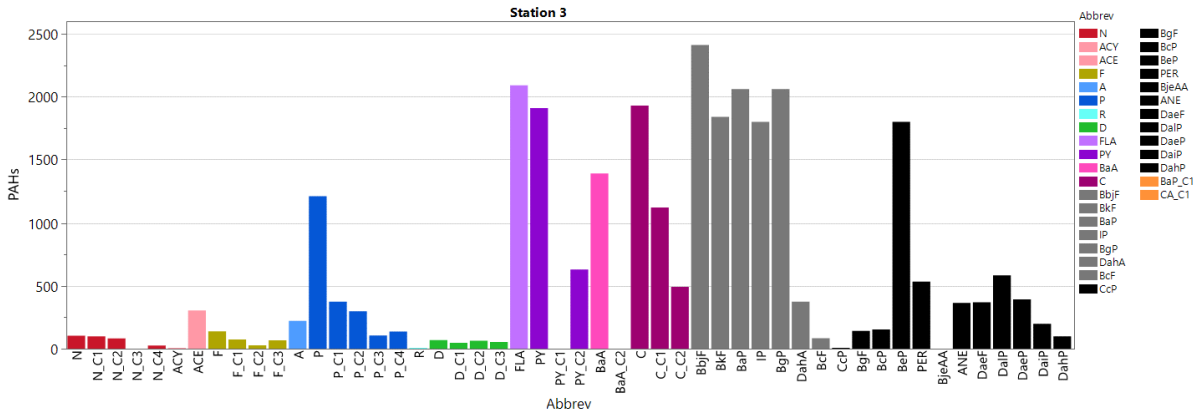
[2] https://www.norskeutslipp.no/en/Miscellaneous/Company/?CompanyID=5121

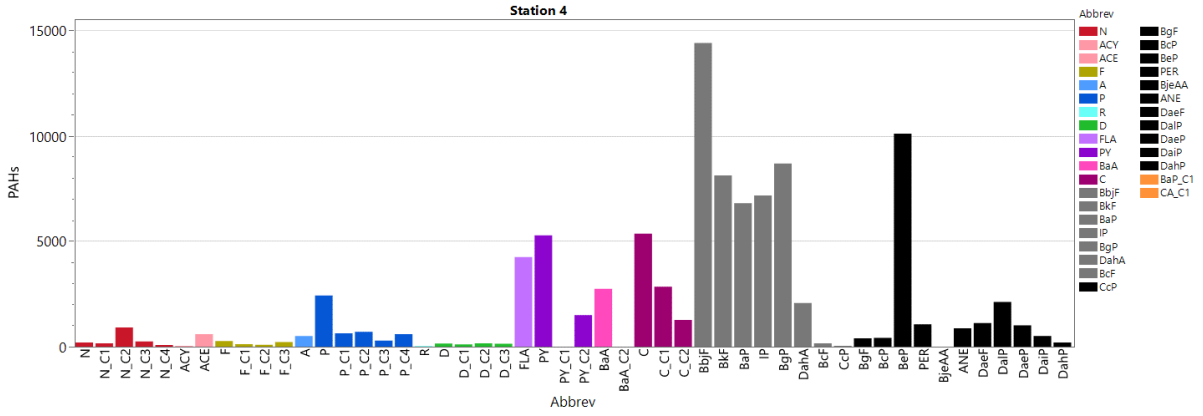
Appendix A.

Sediment PAH (ng/g dw)









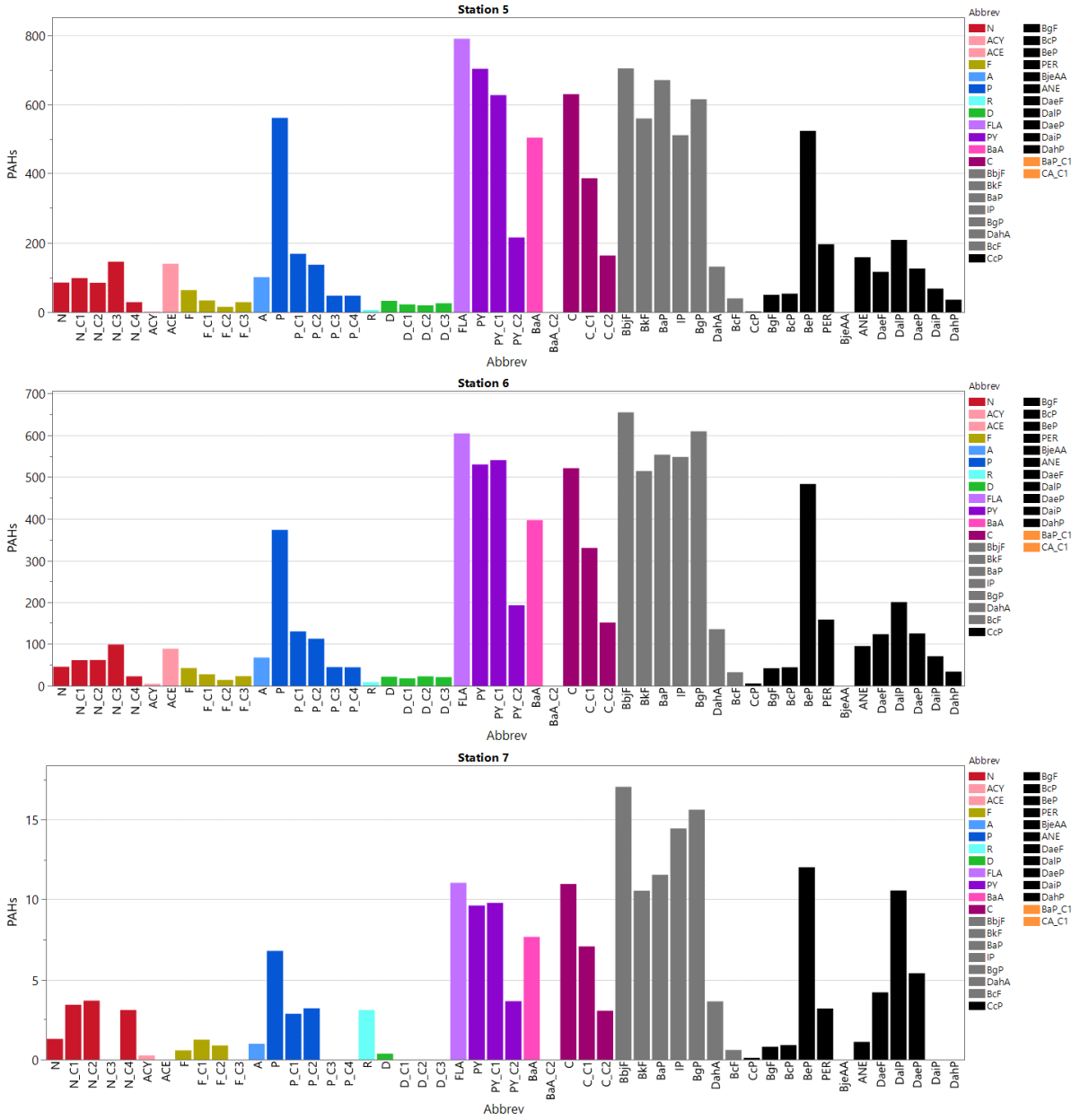

Station 5
Station 6
Station 7
PAHs
Abbrev
N
N_C1
N_C2
N_C3
N_C4
ACY
ACE
F
F_C1
F_C2
F_C3
A
P
P_C1
P_C2
P_C3
P_C4
R
D
D_C1
D_C2
D_C3
FLA
PY
PY_C1
PY_C2
BaA
BaA_C2
C
C_C1
C_C2
BbjF
BkF
BaP
IP
BgP
DahA
BcF
CcP
BgF
BcP
BeP
PER
BjeAA
ANE
DaeF
DaIP
DaeP
DaiP
DahP
BaP_C1
CA_C1

Table A1– *p*-values for the Pearson's correlation of chemical measurements and biological responses in mussels located from the pre-transplanted population (T0) and in mussels held in the water column approximately 2 m below the surface (Top, T) or 5 m from the seafloor (Bottom, B) at the four mussel stations (1, 2, 3, Ref) for an 8 week period (values in bold are different from 0 with a significance level alpha = 0.05). CI – Condition index; SoS – Stress on stress; NRR – Neutral red retention; AChE – Acetylcholine esterase activity; LPO – Lipid peroxidation; CHR – Total chrysene; DBT – Total Dibenzothiophenes; FLUO – Total fluorene; PAH16 – Sum of PAH16; PAH 41 – Sum of PAH 41; PYR – Sum of pyrene including C1 and C2; AS – Arsenic; Cd – Cadmium; Cr – Chromium; Cu – Copper; Pb – Lead; Mn – Manganese; Hg – Mercury; Mo – Molybdenum; Ni – Nickel; V – Vanadium; Zn – Zinc.

Variables	CI	SoS	NRR	AChE	LPO	CHR	DBT	FLUO	PAH 16	PAH 41	PYR	Hg	As	Pb	Cd	Cu	Cr	Ni	Zn	Mn	Mo	V
CI	**1**	0.162	-0.571	-0.320	-0.479	-0.382	-0.255	-0.556	-0.398	-0.401	-0.405	0.190	-0.240	-0.500	-0.601	-0.138	-0.511	**-0.809**	-0.587	0.033	-0.500	-0.04
SoS	0.162	**1**	-0.235	-0.161	-0.250	0.322	-0.237	0.103	0.347	0.351	0.322	0.258	0.074	0.043	-0.200	-0.073	0.164	0.214	0.232	-0.140	0.522	0.178
NRR	-0.571	-0.235	**1**	0.283	-0.168	-0.193	0.079	0.279	-0.152	-0.156	-0.149	0.496	0.055	0.259	0.557	0.106	0.199	0.655	-0.083	0.514	0.338	0.284
AChE	-0.320	-0.161	0.283	**1**	0.522	-0.240	-0.119	0.035	-0.128	-0.145	-0.310	-0.223	-0.460	-0.287	-0.018	-0.018	-0.598	0.035	-0.163	0.121	0.284	0.302
LPO	-0.479	-0.250	-0.168	0.522	**1**	0.323	-0.160	-0.120	0.400	0.392	0.212	**-0.679**	-0.437	-0.188	0.246	-0.297	-0.080	0.143	0.141	-0.646	0.119	-0.45
CHR	-0.382	0.322	-0.193	-0.240	0.323	**1**	0.046	0.281	**0.977**	**0.982**	**0.947**	-0.113	0.369	0.309	0.122	0.081	0.545	0.391	0.548	-0.336	0.042	-0.19
DBT	-0.255	-0.237	0.079	-0.119	-0.160	0.046	**1**	**0.774**	-0.121	-0.109	0.185	0.121	**0.679**	**0.834**	-0.395	**0.847**	0.090	-0.019	0.574	0.234	-0.287	0.361
Fluo	-0.556	0.103	0.279	0.035	-0.120	0.281	**0.774**	**1**	0.148	0.165	0.476	0.061	**0.790**	**0.900**	-0.070	**0.847**	0.365	0.435	**0.816**	0.384	0.267	0.645
PAH EPA-16	-0.398	0.347	-0.152	-0.128	0.400	**0.977**	-0.121	0.148	**1**	**1.000**	**0.874**	-0.126	0.210	0.146	0.190	-0.091	0.485	0.414	0.441	-0.379	0.115	-0.26
PAH41	-0.401	0.351	-0.156	-0.145	0.392	**0.982**	-0.109	0.165	**1.000**	**1**	**0.887**	-0.130	0.229	0.167	0.194	-0.076	0.504	0.422	0.460	-0.377	0.120	-0.25
PYR	-0.405	0.322	-0.149	-0.310	0.212	**0.947**	0.185	0.476	**0.874**	**0.887**	**1**	-0.107	0.533	0.507	0.148	0.295	0.640	0.462	**0.669**	-0.213	0.113	-0.01
Hg	0.190	0.258	0.496	-0.223	**-0.679**	-0.113	0.121	0.061	-0.126	-0.130	-0.107	**1**	0.100	0.194	-0.139	0.183	-0.025	0.157	-0.316	0.399	-0.111	0.217
As	-0.240	0.074	0.055	-0.460	-0.437	0.369	**0.679**	**0.790**	0.210	0.229	0.533	0.100	**1**	**0.787**	-0.112	**0.731**	0.551	0.199	**0.741**	0.455	-0.043	0.490
Pb	-0.500	0.043	0.259	-0.287	-0.188	0.309	**0.834**	**0.900**	0.146	0.167	0.507	0.194	**0.787**	**1**	0.000	**0.770**	0.542	0.464	**0.770**	0.182	0.109	0.347
Cd	-0.601	-0.200	0.557	-0.018	0.246	0.122	-0.395	-0.070	0.190	0.194	0.148	-0.139	-0.112	0.000	**1**	-0.412	0.625	**0.749**	0.042	-0.069	0.506	-0.28
Cu	-0.138	-0.073	0.106	-0.018	-0.297	0.081	**0.847**	**0.847**	-0.091	-0.076	0.295	0.183	**0.731**	**0.770**	-0.412	**1**	0.016	0.000	0.509	0.516	-0.172	**0.715**
Cr	-0.511	0.164	0.199	-0.598	-0.080	0.545	0.090	0.365	0.485	0.504	0.640	-0.025	0.551	0.542	0.625	0.016	**1**	**0.707**	0.607	-0.107	0.357	-0.18
Ni	**-0.809**	0.214	0.655	0.035	0.143	0.391	-0.019	0.435	0.414	0.422	0.462	0.157	0.199	0.464	**0.749**	0.000	**0.707**	**1**	0.435	0.000	**0.707**	0.043
Zn	-0.587	0.232	-0.083	-0.163	0.141	0.548	0.574	**0.816**	0.441	0.460	**0.669**	-0.316	**0.741**	**0.770**	0.042	0.509	0.607	0.435	**1**	-0.055	0.346	0.249
Mn	0.033	-0.140	0.514	0.121	-0.646	-0.336	0.234	0.384	-0.379	-0.377	-0.213	0.399	0.455	0.182	-0.069	0.516	-0.107	0.000	-0.055	**1**	0.000	**0.825**
Mo	-0.500	0.522	0.338	0.284	0.119	0.042	-0.287	0.267	0.115	0.120	0.113	-0.111	-0.043	0.109	0.506	-0.172	0.357	**0.707**	0.346	0.000	**1**	0.244
V	-0.040	0.178	0.284	0.302	-0.448	-0.192	0.361	0.645	-0.258	-0.251	-0.013	0.217	0.490	0.347	-0.281	**0.715**	-0.175	0.043	0.249	**0.825**	0.244	**1**

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