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Biological effects monitoring of a thermomechanical cleaned cuttings discharge from the Johan Sverdrup installation

Norwegian Institute for Water Research

REPORT

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Summary

The following study describes an integrated biological effects monitoring programme using field transplanted mussels to determine the potential effects of thermomechanical cleaned cuttings (TCC) discharged from the Johan Sverdrup installation in the North Sea. Chemical body burden (PAHs, metals) and a suite of biological effects markers were measured in mussels positioned at strategic locations in the Johan Sverdrup field for 6-7 weeks and compared to two reference locations and a day zero (T0) group. The biomarkers measured in the mussels included: condition index (CI); stress on stress (SoS); micronuclei (MN); lysosomal membrane stability (LMS); metallothionein (MT) and gill and digestive gland histology. Based on oceanographic parameters, the DREAM model was employed to predict, and then later confirm, the direction of the TCC plume during the discharge period. Exposure to the TCC was limited to a 3-day window at the end of the mussel exposure but this was considered representative of the sporadic nature of the TCC discharge. PAH body burden in mussels was low in all mussel groups positioned at the Johan Sverdrup installation, although slightly above the reference and day zero mussel groups. Metal concentrations were either on or below the lower limit of the Norwegian classification scale for metal concentrations in mussel tissue indicating insignificant risk. Overall, the biomarker responses were considered low and did not differentiate significantly between the mussel groups. The Principal Component Analysis (PCA) showed no clear association between the chemical and biological responses in mussels and proximity to the Johan Sverdrup installation. The short duration of exposure to the TCC discharge may be partly responsible for the lack of chemical accumulation and biological response observed.

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Biological effects monitoring of a thermomechanical cleaned cuttings discharge from the Johan Sverdrup installation

Preface

The report was commissioned by Equinor AS to collect oceanographic, chemical and biological effects monitoring data during the release of thermomechanical cleaned cuttings (TCC) into the North Sea from the Johan Sverdrup installation. The data will be used to fulfil the requirements set by the Norwegian environment agency and determine the potential impact of discharged cuttings to animals in the water column.

The project is a collaboration between the Norwegian Research Institute (NORCE) and the Norwegian Institute for Water Research (NIVA). The field investigation required two research cruises, the first in week 12 (March 22nd-28th) where monitoring rigs were placed out North and South of the Johan Sverdrup installation and a second research cruise in week 18 (May 3rd-10th) that retrieved the monitoring rigs from the sea and processed the mussels held on each rig for chemical accumulation and biological effects responses. Both cruises were performed on the Esvagt Dee supply vessel where the crew assisted with the deployment and retrieval of the monitoring rigs. The same research cruises were also used for the Water Column Monitoring programme, which took place at the Ekofisk installation.

The scientific personnel onboard the Esvagt Dee for the deployment cruise that assisted with the Johan Sverdrup fieldwork included NIVA personnel Bjørnar Beylich and Steven Brooks as well as NORCE personnel Einar Bye-Ingebrigtsen. Rolf Sundt was the Equinor representative on board the vessel. Scientific personnel onboard for the retrieval cruise that assisted with the sampling of the mussels included Bjørnar Beylich, Samantha Martins and Steven Brooks from NIVA, Alessio Gomiero and Elin Austerheim from NORCE, as well as IMR personnel Guri Nesje and Aasim Ali, and Valentin Geslin from University of Stavanger. Technical assistance with the oceanographic instrumentation was provided by Medyan Ghareeb, with oceanographic data support provided by Nicholas Roden and Lars Golmen. The Equinor representative was Lars Petter Myhre.

Chemical and biological effects analysis was divided evenly between NIVA and NORCE. The report was written by Steven Brooks, Shaw Bamber and Alessio Gomiero.

Oslo, August 2022

Steven Brooks, 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 effects of thermomechanical cleaned cuttings (TCC) discharged from the Johan Sverdrup installation in the North Sea. Chemical body burden (PAHs, metals) and a suite of biological effects markers were measured in mussels positioned at strategic locations and at two depths in the Johan Sverdrup field for a period of 6-7 weeks. These responses were compared to two reference locations and a day zero (T0) group. The biomarkers measured in the mussels included: condition index (CI); stress on stress (SoS); micronuclei (MN); lysosomal membrane stability (LMS); metallothionein (MT) and gill and digestive gland histology. Supporting oceanographic parameters such as current direction and speed, temperature, salinity, dissolved oxygen, chlorophyll and turbidity of the seawater were measured either continuously or at specific time points, during the exposure.

Based on oceanographic parameters, the particle DREAM model was employed to predict, and then later confirm, the direction of the TCC plume during the discharge period. This confirmed the suitability of the mussel locations and the depth of the mussels with respect to the TCC discharge. However, despite this, the exposure to the TCC was limited to a 3-day window when the TCC was discharged at the end of the 6-7-week mussel exposure. Based on the TCC discharge between Jan 2020 and May 2021, a 3-4-day average TCC discharge window was observed. Therefore, the exposure duration was considered typical of the sporadic nature of the discharge.

PAH body burden in mussels were low in all mussel groups positioned at the Johan Sverdrup installation, although slightly above the reference and day zero mussel groups. Metal concentrations were either on or below the lower limit of the Norwegian classification scale for metal concentrations in mussel tissue indicating insignificant risk. Overall, the biomarker responses were considered low and 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 Johan Sverdrup TCC discharge. The Principal Component Analysis (PCA) showed a clear differentiation among transplanted mussels at Johan Sverdrup and the reference groups. However, no clear association was found between the chemical and biological responses in mussels and proximity to the Johan Sverdrup installation. The short duration of exposure to the TCC discharge may be partly responsible for the lack of chemical accumulation and biological response observed.

Sammendrag

Tittel: Biologisk effektovervåking av et termomekanisk renset borkaks (TCC)-utslipp fra Johan Sverdrup-installasjonen
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Den følgende studien beskriver et integrert biologisk effektovervåkingsprogram som bruker feltutplasserte blåskjell for å bestemme potensielle effekter av termomekanisk renset borkaks (TCC) som slippes ut fra Johan Sverdrup-installasjonen i Nordsjøen. Kjemisk kroppsbelastning (PAH, metaller) og en rekke biologiske effektmarkører ble målt i blåskjell som var plassert på strategiske steder og på to dyp i Johan Sverdrup-feltet i en periode på 6-7 uker. Responsen hos disse blåskjellene ble sammenlignet med skjell fra to referansesteder og en dag null (T0) gruppe. Biomarkørene målt i blåskjellene inkluderte: tilstandsindeks (CI); stress på stress (SoS); mikronukleus (MN); lysosomal membranstabilitet (LMS); metallothionein (MT) og gjelle- og fordøyelseskjertelhistologi. Supplerende oseanografiske parametere som strømretning og hastighet, temperatur, saltholdighet, oppløst oksygen, klorofyll og turbiditet i sjøvannet ble målt enten kontinuerlig eller på bestemte tidspunkter under eksponeringen.

Partikkel-DREAM-modellen ble basert på oseanografiske parametere og brukt for å forutsi, og deretter bekrefte, retningen til TCC-røykskyen i løpet av utslippsperioden. Dette bekreftet egnetheten til blåskjell lokalitetene og dybden med hensyn til TCC-utslippet. Eksponeringen for TCC var begrenset til et 3-dagers vindu når TCC utslippet fant sted ved slutten av den 6-7 ukers utsettingsperioden. Basert på TCC-utslippet mellom januar 2020 og mai 2021 ble det observert et 3-4-dagers gjennomsnittlig TCC-utslippsvindu. Derfor ble det vurdert at eksponeringstiden var representativ for slike naturlig sporadiske utslipp.

PAH-kroppsbelastningen i blåskjell var lav i alle gruppene plassert ved Johan Sverdrup-installasjonen, men litt over referanse- og dag null gruppene. Metallkonsentrasjoner var enten på eller under nedre grense av den norske klassifiseringsskalaen for metallkonsentrasjoner i blåskjellvev som indikerer ubetydelig opptak. Samlet sett ble biomarkørresponsene ansett som lave og skilte ikke signifikant mellom blåskjellgruppene. På grunn av den lave responsen til biomarkørene var IBR/n like lav, og basert på biomarkørene som ble målt, så det ut til å være minimal påvirkning på den generelle helsetilstanden til blåskjell fra alle stasjoner, inkludert de nærmest Johan Sverdrup TCC-utslippet. Principal Component Analysis (PCA) viste en klar differensiering mellom utplasserte blåskjell hos Johan Sverdrup og referansegruppene. Det ble imidlertid ikke funnet noen klar sammenheng mellom den kjemiske og biologiske responsen i blåskjell og avstand til Johan Sverdrup-installasjonen. Den korte varigheten av eksponering for TCC-utslippet delvis forklare observasjon av mangelen på kjemisk akkumulering og biologisk respons.

1 Introduction

The following study describes a monitoring programme using field transplanted mussels to determine the potential biological effects of a thermomechanical cleaned cuttings (TCC) discharge from the Johan Sverdrup installation. The Johan Sverdrup installation is licenced to Equinor AS, who have the responsibility to ensure that the TCC discharge is not causing detrimental harm to marine life within the seawater recipient. The data will be used to fulfil the requirements set by the Norwegian environment agency and determine the potential impact of TCC to animals in the water column.

1.1 Johan Sverdrup field

The Johan Sverdrup field is approximately 200 km^2 in area and is located 58.8° North in the North Sea. The estimated amount of recoverable petroleum resource is thought to be around 2.7 billion barrels of oil, consisting of 97 % oil and 3% gas. The water depth at Johan Sverdrup is 110 to 120 m. The reservoir is at a depth of 1900 m and contains sandstone from the Jurassic period. Three subsea water injection installations for maintaining reservoir pressure are also present within the field. The production started in October 2019 and the life of the installation is expected to be up to 50 years.

The Johan Sverdrup installation consists of four platforms: 1) a process platform, 2) a drilling platform, 3) a riser platform and 4) an accommodation platform (Figure 1). There is no produced water discharge at Johan Sverdrup. The main discharge is from the intermittent discharge of cuttings following treatment by thermomechanical cuttings cleaner (TCC).

Figure 1. The Johan Sverdrup complex comprising from left to right of the accommodation platform, the process platform, the drilling platform and the riser platform. (Source: Steven Brooks, NIVA).

1.2 Thermomechanical cleaned cuttings

The TCC is a patented technology that recovers oil and its components from oil contaminated drill cuttings through a process of thermal phase separation. Through this process the base oil used in drilling is recovered for potential reuse and the cleaned cuttings are discharged to sea at a concentration of <0.05% oil, eliminating the need to transport the cuttings onshore for treatment and disposal.

1.2.1 How the TCC works

The drill cuttings are fed into a process mill, which grinds the stone and generates friction heat resulting in the flash evaporation of oil and water. From the process mill, approximately 90% of the solids are discharged. The cuttings are transported through cooling conveyors to reduce the temperature to 100℃, the dried cuttings are rehydrated into a slurry, to reduce dust, and discharged to sea. The vapours of oil and water from the process mill flow to the condenser module, where the fine particles still present in the vapour stream are removed in the oil scrubber. The temperature of the vapour stream is reduced to 105-110℃ to condense the oil but not the water, the temperature is reduced further to condense the steam to water. Any carry-over of oil in the condensed steam is removed further within an oil-water separating unit. The discharged treated cuttings should contain less than 0.05% oil.

With the absence of a produced water discharge at Johan Sverdrup the main potential environmental threat is from the TCC discharge. In addition to the physical particle effects of high turbidity created from the release of the treated cuttings, chemical contaminants include naturally occurring metals present in the seabed and drilling rock as well the remaining oil content of the cuttings. At Johan Sverdrup, the TCC process mill and infrastructure were located on the drilling platform (Figure 1 & Figure 3), where a discharge pipe (40 cm diameter) released the TCC slurry at a depth of 17 m. The predicted temperature of the TCC on discharge is estimated at approximately 25°C, with a salinity of around or marginally above 35‰.

1.3 Biological effects measurements

This study focusses on the seawater recipient around the Johan Sverdrup installation and the potential impact of the TCC effluent discharge on pelagic organisms. Mussels are used as a proxy species representing the pelagic compartment and have been previously used to assess the impacts of chemicals and particle discharges from offshore oil and gas installations (e.g. Pampanin *et al*., 2019; Brooks *et al*., 2011a; Brooks *et al*., 2009). Mussels are used widely in biological effects monitoring programmes (reviewed in Beyer et 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. Threshold values are available for the bioaccumulation of numerous chemicals in marine mussels (Ruus et al., 2021). Furthermore, 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 into context to other similar studies. A brief description of the biological effects measurements used in this study are provided.

Condition index (CI) is a whole organism response that provides a simple measure of the general health status of the mussel. The CI is influenced by physiological activity such as growth, reproduction,

secretion, etc., under environmental conditions. It can also be affected by biotic and abiotic factors that cause physiological stress on the organism.

Stress on stress (SoS) is used as a simple and low-cost whole organism response measurement of mussel physiology that can be induced by contaminant exposure, providing a measure of the mussel's overall fitness. The ability of mussels to keep their shells closed and resist desiccation is dependent on the amount of energy (adenosine triphosphate, ATP) available to fuel their adductor muscle and provides a measure of the overall fitness (De Zwaan and Mathiew, 1992). The method works on the premise that if mussels are using metabolic energy on detoxification processes, less energy will be available for other physiological processes, such as maintaining shell closure.

Micronuclei (MN) is regarded as an important tool for biological effects monitoring of DNA damage and is one of the most widely used biomarkers of genotoxicity. Micronuclei themselves are chromatin-containing structures that are surrounded by a membrane and have no detectable link to the cell nucleus. They have been found to occur through either chromosomal breakage or mis-segregation during mitosis due to cellular spindle malfunction or damage to the centromeres (Fenech, 2000). The frequency of MN has been found to increase with exposure to complex mixtures of pollutants (e.g. Baršienė et al. 2006).

Lysosomal membrane stability (LMS) is a widely used in biological effects studies and is a good diagnostic biomarker of individual health status (e.g. Moore, 1990). Studies have shown a reduction of LMS in mussels from contaminated urban and industrial areas when compared to individuals from reference locations.

Metallothionein (MT) is a metal binding protein found involved in metal sequestration for cellular process and detoxification. The increase in MT concentrations has been found to be most associated with Zn, Cu and Cd, although is often used as a biomarker of exposure to metal contamination in general (Hylland et al., 2009). In mussels, two isoforms of MT have been highlighted (MT-10 and MT-20), with MT-20 increasing much more than MT10 under metal stress, which may suggest that this isoform is involved in detoxification processes (Aceto et al., 2011).

Mussel **histopathology** involves the measurement of structural changes in target tissues, such as digestive gland and gills, which can be used to provide an indication of environmental stress (Bignell et al., 2012). Histopathology has been used to investigate changes related to contaminant exposure in mussels (e.g. Auffret, 1988; Marigómez et al., 2006).

1.4 Objective

The main objective of the project was to determine the potential biological effects of exposure to TCC discharge in the receiving waters of Johan Sverdrup installation. Chemical accumulation and biological effects measurements in field exposed mussels were used to assess the potential impacts of the TCC in the seawater recipient.

2 Methods

2.1 Mussel source population and day 0 sampling

Mussels were obtained from a mussel hatchery (Snadder og Snaskum AS) located north of Trondheim. The mussels were delivered to the NORCE laboratory approximately 2 weeks prior to the field deployment. Mussels were placed in flow-through seawater systems at ambient temperature and fed sparingly on Instant Algae ® TW1800 diet to reduce the early onset of reproductive maturation. Mussels were placed in purpose built netted socks, with approximately 100 mussels per sock.

2.2 Offshore monitoring

The monitoring of the Johan Sverdrup installation required a deployment cruise where monitoring rigs were placed out at strategic locations near the installation and two reference locations, as well as a retrieval cruise where the monitoring rigs were collected after 6-7 weeks. The cruise time was combined with the Norwegian Water Column Monitoring (WCM) programme and the two reference locations initially designed for the WCM were also used for comparison in this monitoring programme.

The deployment cruise was performed with the assistance of the boat and crew of the Esvagt Dee, which departed from the ConocoPhillips coastal depot in Tananger on Monday 22nd March 2021 and returned on Sunday 28th March 2021. The monitoring rigs (Figure 2) were deployed at strategic locations around the Johan Sverdrup installation (Figure 3). The monitoring rigs included mussels that were held at two depths (16-20 m and 40-45 m) and oceanographic instruments. The mussels and instrumentation were used to monitor the potential impact of the cutting discharge from Johan Sverdrup during a 6-7-week field exposure.

To reduce the chance of collision with passing vessels, the top of each monitoring rig was held at a depth of 15 m. Equipment ropes were attached to the main rope immediately below the top buoys at an approximate depth of 16 m, and lower down the main rope at a depth of 40 m. This enabled two depths for the placement of the equipment and mussels. The specific items that were placed on each monitoring rig at Johan Sverdrup are summarised in Table 1. Dissolvable concrete anchors (dissolvability in seawater was expected to be several months to years) were used to secure the monitoring rigs to the seafloor and the uplift from the buoys was designed to hold the monitoring rigs vertically in the water column. An acoustic release in the form of a lightweight release transponder (LRT, Sonardyne) was used on each rig, positioned just above the concrete anchor and used in the rig retrieval.

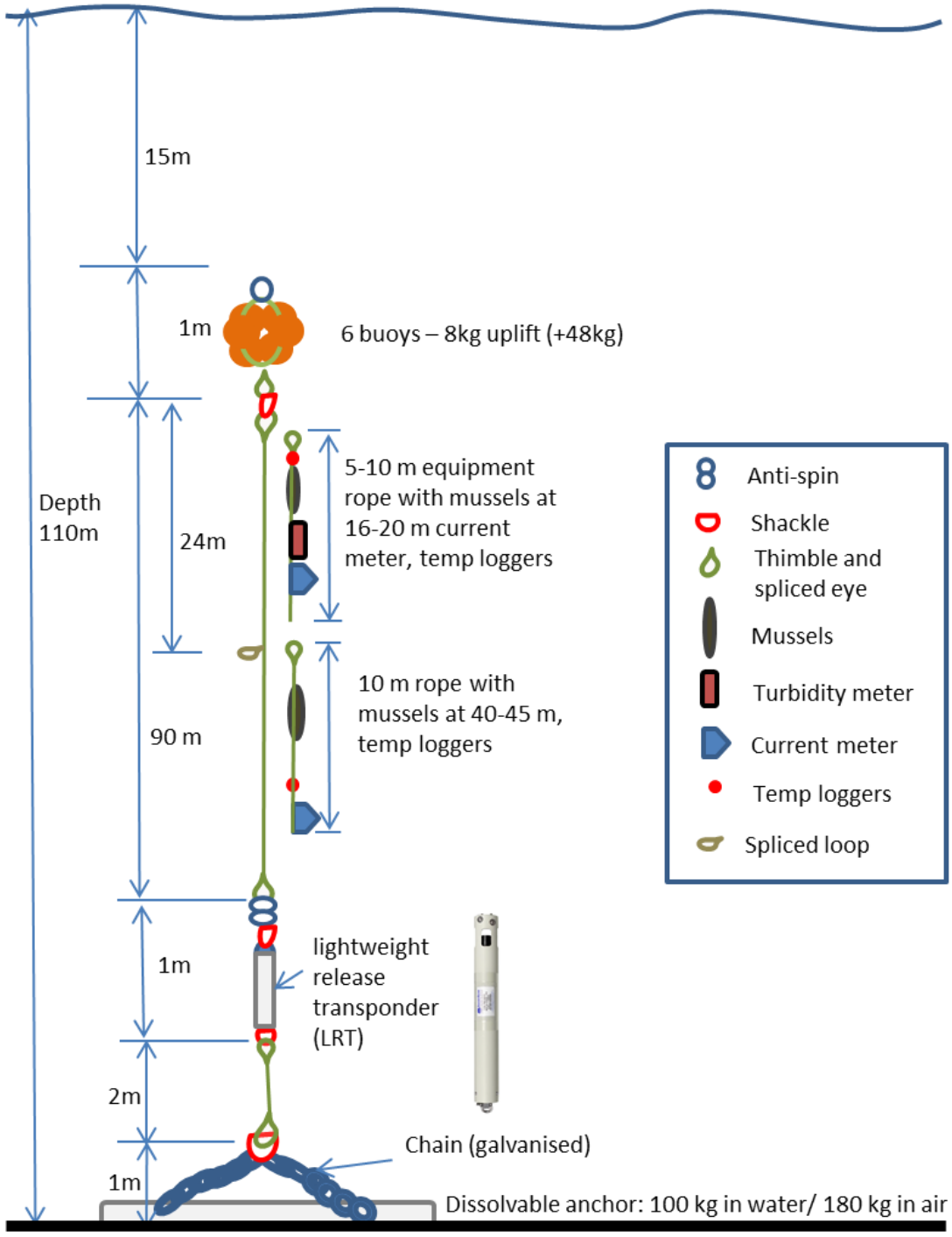


Figure 2. The design of the monitoring rig used to hold mussels and oceanographic equipment at known depths in the water column.

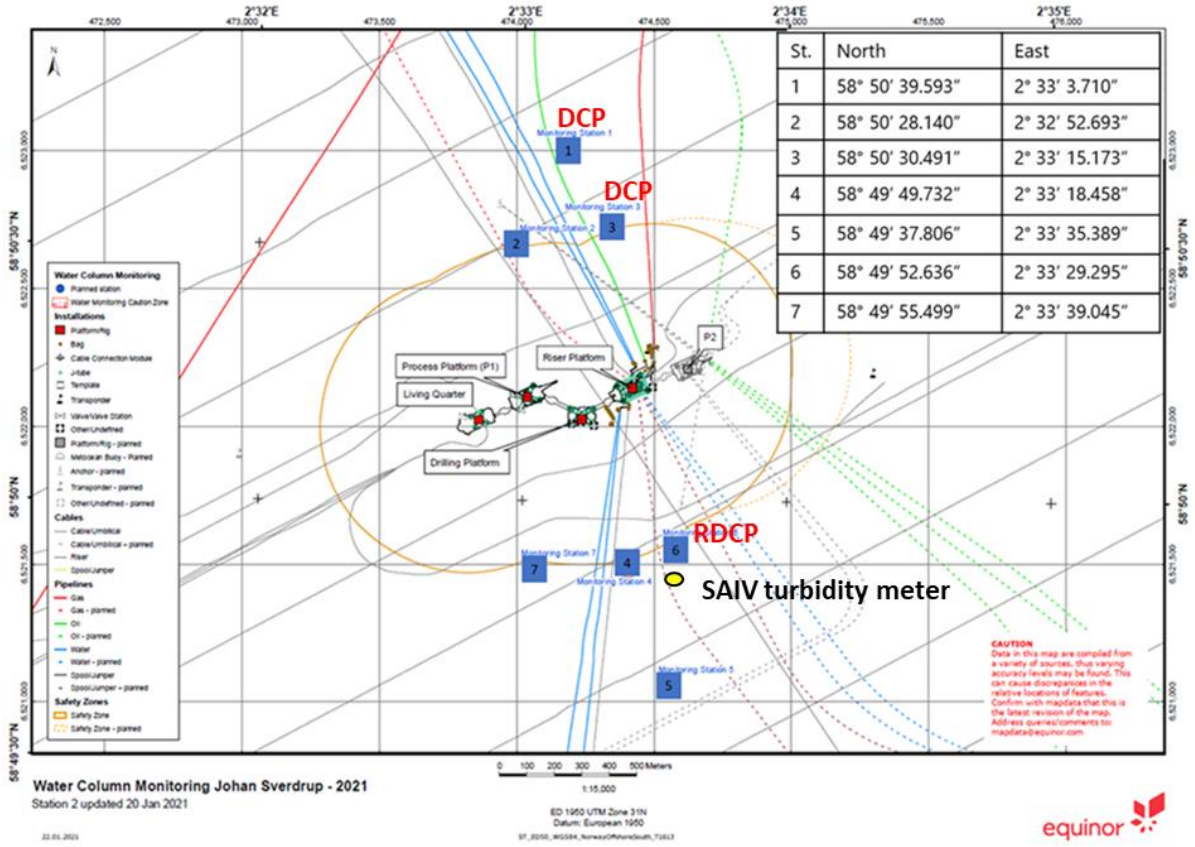


Figure 3. Approximate location of the seven monitoring rigs (blue squares) at the Johan Sverdrup installation. Two stations positioned on the 500 m safety zone north (St2 and St3) and three on the south (St4, St6 and St7) of the installation, with St1 and St5 approximately 1000 m north and south respectively. (Image provided by Equinor). The TCC was discharged from the drilling platform at a depth of 17 m. An additional monitoring rig was placed approximately 50 m south of St 6 for one week at the end of the exposure (yellow circle), with a SAIV turbidity meter at a depth of 22 m. DCP, Doppler current profiler; RDCP, recording doppler current profiler.

Table 1. Information on the deployment time, depth and equipment used on the monitoring rigs at the two reference stations and at the Johan Sverdrup field. Two depths were used at each monitoring station denoted as top rope and bottom rope. LRT, Lightweight release transponders; DCP, Doppler current profiler; RDCP, recording doppler current profiler.

Monitoring station	Time of deployment (DD.MM.YY 00:00)	Depth (m)	LRT Code	Temp logger code		ITEMS on the equipment ropes	
				Top rope	Bottom rope	Top rope	Bottom rope
1	23.03.2021 08:15	115	304.3	9700545	9700551	Mussels, temp, DCP Seaguard current	Mussels, temp
2	23.03.2021 09:45	115	314.3	9712569	10388129	Mussels, temp	Mussels, temp
3	23.03.2021 10:40	115	302.3	1156580	9700548	Mussels, temp, DCP Seaguard current	Mussels, temp
4	23.03.2021 11:20	114-115	303.3	1156573	1156574	Mussels, temp	Mussels, temp
5	23.03.2021 15:30	115	319.3	9712551	20809685	Mussels, temp	Mussels, temp
6	23.03.2021 12:40	115	316.3	9700541	20809687	Mussels, temp	Mussels, temp, Aanderaa RDCP current
7	23.03.2021 13:30	115	300.3	9700542	9712554	Mussels, temp	Mussels, temp
Ref 1	24.03.2021 04:00	65	313.3	20809689	9712579	Mussels, temp, SAIV CTD, chlorophyll, turbidity	Mussels, temp
Ref 2	24.03.2021 09:00	48	306.3	10388127	9744732	Mussels, temp	Mussels, temp

2.3 Oceanographic instrumentation

Current meters were placed on three of the monitoring rigs at Johan Sverdrup. Two Seaguard DCPs (acoustic doppler current profiler, Aanderaa) that were also equipped with salinity, temperature and turbidity sensors, were placed on monitoring rigs 1 and 3. An Andeera RDCP (recording doppler current profiler) was placed on monitoring rig 6.

In addition, an SAIV CTD profiler equipped with a turbidity sensor was deployed on a fixed mooring approximately 50 m south of monitoring station 6 for one week at the end of the 6-7-week exposure. This was used to provide additional information on the potential turbidity plume from the discharged cuttings.

2.4 Mussel sampling

The monitoring rigs were retrieved by sending a specific release code from a command deck unit to the LRT fitted to the bottom of the monitoring rigs. Once the LRT was released, the concrete mooring detached, and the monitoring rig floated slowly to the surface where it was retrieved with the man-overboard boat. All monitoring rigs were successfully retrieved using this method.

The processing of the mussels to obtain the various tissues needed for analysis was performed on board the vessels within 2 hours of collection and typically within 30 min. A summary of the tissue collection is illustrated in Figure 4.

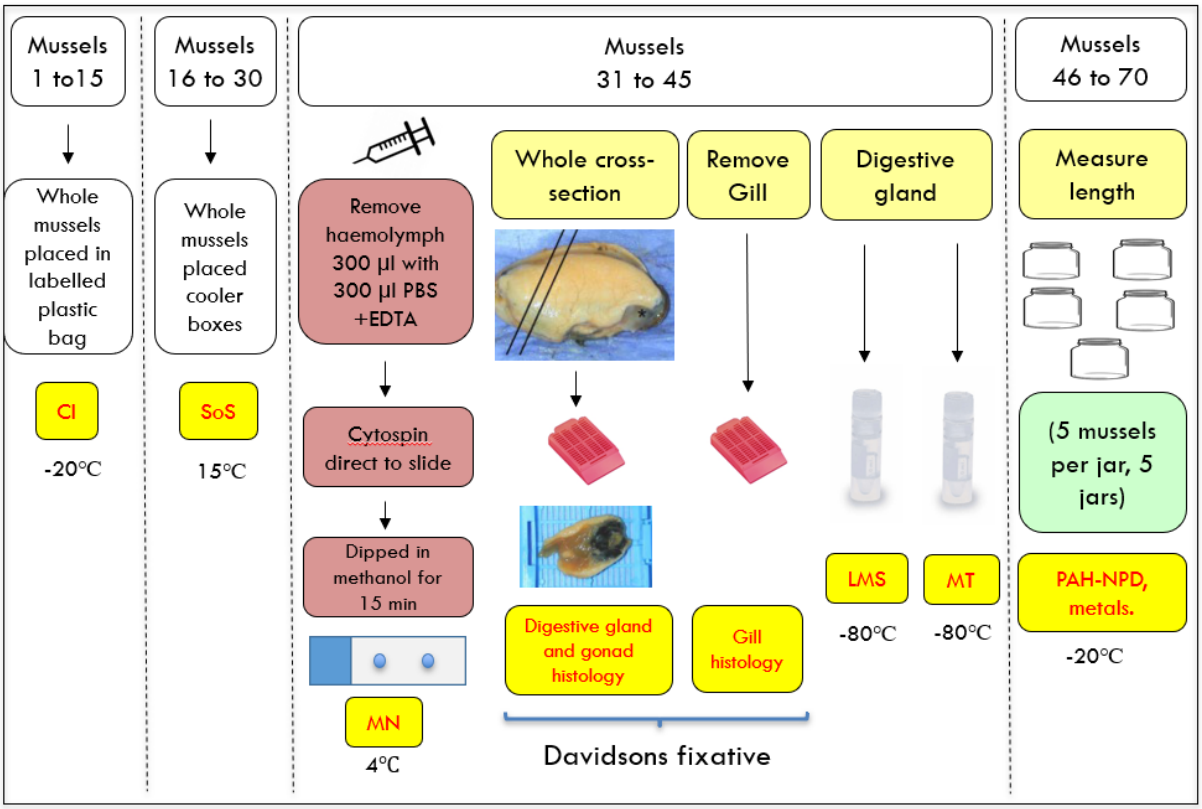


Figure 4. Mussel tissue collection and storage. Condition index (CI), stress on stress (SoS), micronuclei (MN), lysosomal membrane stability (LMS), metallothionein (MT), polycyclic aromatic hydrocarbons (PAH), naphthalenes, phenanthrenes, dibenzothiophenes (NPD).

2.5 Chemical analysis in mussels

2.5.1 PAH analysis

Internal standards (naphthalene d8, biphenyl d10, acenaphthene d8, phenanthrene d10, anthracene d10, pyrene d10, chrysene d12 and perylene d12) were added to a 5g sub-sample of the mussel homogenate before extraction by saponification. Analytes were extracted twice with 40 mL cyclohexane and dried over sodium sulphate. The extracts were reduced by a gentle stream of nitrogen and cleaned by size exclusion chromatography. Samples were analysed by gas chromatography mass spectrometry (GC-MS) with the MS detector operating in selected ion monitoring (SIM) mode. The GC was equipped with a 30 m column with a stationary phase of 5% phenyl polysiloxane (0.25 mm i.d. and 0.25 µm film thickness), and the injector operated in 'splitless' mode. The initial column temperature was 60°C, which after two minutes was raised stepwise to 310°C. The carrier gas was helium and the column flow rate was 1.2 mL/ min. Quantification of individual components was performed by using the internal standard method. The alkylated homologues were quantified by baseline integration of the established chromatographic pattern and the response factors were assumed equal within each group of homologues.

2.5.2 Metal analysis

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

2.6 Biological effects assessment in mussels

2.6.1 Food availability of field exposed mussels

Visualisation of faeces after a depuration period of approximately 8 ± 2 h was used as a simple qualitative method to assess the availability of food for the field transplanted mussels.

Following the retrieval, five individual mussels from each monitoring station including both depths were randomly selected, cleaned of external fouling and rinsed in clean seawater. The cleaned mussels were placed in glass jars containing approximately 200 ml of clean seawater. Mussels were kept in a cool dark environment for 8 ± 2 h in order to provide sufficient time for the mussels to excrete their gut content as faeces. Following this depuration phase, photographic images were taken of the jars to provide a qualitative measure of the presence of faeces excreted by the mussels.

2.6.2 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 shell 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.

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

2.6.3 Stress on stress

The stress on stress (SoS) assessment was measured in twelve mussels from each group. Immediately upon retrieval the mussels were each put into an individual section of a labelled plastic egg carton which in turn was placed within a polystyrene cool box fitted with a lid (5 cartons per box), together with a temperature logger. The mussels were checked every 24 ± 4 h and mortalities were recorded, with dead animals removed. On return to shore the mussels were transferred within their cool boxes to a controlled climate room with temperature set at 11°C for the remainder of the assessment. Mussels were considered deceased if their shells were gaping and showed no sign of movement after gentle tapping of their shells.

2.6.4 Micronuclei formation

Haemolymph is a major component of the soft tissue in mussels, in which different populations of circulating haemocytes play important roles in transport and digestion of nutrients, and in processes such as detoxification. Haemolymph was selected as the tissue for the micronuclei (MN) analysis. The MN scoring was restricted to the haemocyte`s agranular cells, which are reported to be more sensitive for use as a biomarker than other cell types (Bolognesi and Fenech 2012).

Approximately 0.3 ml of haemolymph was removed from the posterior adductor muscle of the mussel with a syringe and needle (0.6 ml bore size) containing 0.3 ml of PBS buffer (100 mM PBS, 10 mM EDTA). The haemolymph and PBS buffer were mixed in an Eppendorf and 250 µl of the mixture was aliquoted into both sides of a double cytofunnel. The mixture was transferred to a glass cytoslide by centrifugation at 800 rpm for 2 minutes using a Cytospin 4 centrifuge (Thermo Scientific). After centrifugation, the cytoslides were placed flat on the bench at room temperature for 15 min to enable the haemocytes to adhere. The adhered haemocytes were fixed with methanol for 10 min and air-dried for a further 10 minutes. The slides were stored in slide boxes at 4 °C until further preparation and analysis.

Slides were stained following Bolognesi and Fenech (2012). Slides were stained in a Giemsa 3% solution in Sørensen buffer (pH 6.8) for 5 minutes at room temperature, then rinsed in washing solution (Sørensen buffer, pH 6.8) two times and air-dried in the dark overnight. To eliminate bias, the frequency of MN was measured on coded slides without prior knowledge of the exposure status of the samples. The frequency of MN in haemocytes was determined microscopically (×100 objective) on a minimum of 2000 cells per exposure group. Micronuclei were scored in agranular cells with intact cellular and nuclear membranes when: 1) the nucleus and the MN have a common cytoplasm; 2) colour intensity and texture of MN are similar to the nucleus; 3) the size of the MN is equal or smaller than 1/3 of the nucleus; and 4) MN are apparent as spherical structures with a sharp contour.

2.6.5 Lysosomal membrane stability

The LMS analysis was performed according to Moore (1976). After dissection of the mussels (15 mussels per station), digestive glands were removed immediately and placed into cryovial tubes and kept frozen at -80°C until analysis. Five digestive glands were attached by glue to an aluminium chuck. Ten slices from each chuck were cut in 8 µm thick sections in a Cryostat machine (with cabinet temperature at -30°C with its knife cooled at -25°C). Sections were then transferred to warm microscope slides (room temperature), which were pre-labelled in time series 0, 3, 6, 10, 20, 30, 40, and 50 min. The microscope slides were stored again in the freezer at -40°C prior to LMS analysis. The determination of LMS was based on the time of acid labilization treatment required to produce the maximum staining intensity according to UNEP/RAMOGE (1999), after demonstration of

hexosaminidase (Hex) activity in digestive cell lysosomes. Serial cryostat sections were exposed to acid labilization in intervals of 0, 3, 6, 10, 20, 30, 40, and 50 min in citrate buffer in a shaking bath at 37°C, to find out the range of pre-treatment time needed to complementary labilize the lysosomes. After each time interval was reached, all slides were removed from citrate buffer.

Sections were incubated in a medium prepared using 7 g low viscosity polypeptide in 100 ml citrate buffer (pH=4.5) and 40 mg naphthol AS BI N-acetylβ-D-glucosamidase in 5 ml dimethyl sulfoxide (DMSO). Sections were incubated for 20 min in a shaking bath at 37°C for demonstration of Hex activity. Sections were then washed in 3 % NaCl for 2 min at room temperature, before embedding in the reaction medium (0.2 g of the diazonium salt Fast Violet B in 200 ml of phosphate buffer) for 10 min in the dark at room temperature. The visualization of the enzyme-substrate complex was achieved by a post-coupling reaction. Finally, sections were rinsed three times with distilled water, left to dry out at room temperature, and then mounted with mounting medium (glycerate gelatin). Stained slides were viewed under an optical microscope dividing each section into four approximately equal areas for statistical interpretation. Lysosomes appear reddish-purple due to the reactivity of the substrate with N-acetyl-β-hexosaminidase.

The average labilization period for each digestive gland corresponded to the average incubation time in the acid buffer that produced the maximum staining reactivity. A mean value was then derived for each station. The image analysis was performed by comparing intensity of staining using an image processing software called ImageJ.

2.6.6 Metallothionein

Metallothionein content was evaluated in digestive gland homogenates according to Viarengo et al. (1997). After an acidic ethanol/ chloroform cytosolic fractionation of the tissue homogenate, MT concentration was quantified by evaluating the sulfhydryl residue content by spectrophotometric method, using Ellman's reagent (5,5-dithiobis 2-nitrobenzoic acid). Metallothionein content was spectrophotometrically determined using DTNB reaction. A standard curve was built using glutathione (GSH), where the absorbance at 412 nm is a function of GSH concentration (nmol/ml). The results were expressed as lg/g of tissue. Protein concentrations were measured according to Bradford (1976), using bovine serum albumin (BSA) as standard.

2.6.7 Mussel histology

The dissection of mussels was performed immediately on board. All analysed tissue (gill and digestive glands) were dissected, placed in pre-labelled histology cassettes and stored in histological fixative (Baker's calcium-solution: 4% formaldehyde, 1% $CaCl_2$, 2.5% NaCl) for later wax sectioning. Tissue samples were no thicker than 1 cm to ensure proper fixation, but long and wide enough to represent the different areas of the tissue. Samples were then stored at 4°C until embedding. A 4 mm cross-section of each mussel, including digestive diverticulum, gills and mantle were dehydrated in alcohols. The tissues were cleared in HistoChoice (Sigma H2779) and embedded in paraffin. Histological sections (5 µm) were cut using a microtome Leica RM2125 RTS, mounted on slides, dried at 37°C for 24 hours and stained with haematoxylin and eosin. The tissues were examined for health parameters related to physiological conditions, inflammatory and non-specific pathologies. The DG tubules atrophy was recorded using a scoring index ranging from 0 to 3 (Brooks et al., 2009). Digestive glands were examined for vacuole degeneration, haemolytic infiltration while gills were assessed for cilia erosion cilia fusion and lumen enlargement. Each alteration was scored according to its severity and frequency (0 = absence of alteration, 1 = ≤ 10 % of the histological section showed the alteration, 2 = between

10% and 50% of the histological section showed the alteration, 3 = between 50% and 100% of the histological section showed the alteration (Sensini et al. 2008). All micrographs were captured using an AxioCam MRc5(Zeiss) digital camera mounted on a Zeiss Axioplan 2 light microscope (Göttingen, Germany). The slides were analysed blind.

2.7 Integrated biological response index

The Integrative Biological Response (IBR) index was developed as an assessment tool that combines the measured biomarker responses in order to provide a holistic evaluation of organism health status (Beliaeff and Burgeot, 2002). The IBR was further developed to include the number of biomarkers in the data set (i.e. IBR/n) (Broeg and Lehtonen, 2006). In the present study CI, SoS, MN, LMS, and MT were selected for the IBR calculation. The inverse values of CI, SoS, LMS 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.

2.8 Principle component analysis

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 Discharged cleaned cuttings

The amounts of cleaned cuttings discharged into the seawater recipient following TCC treatment are shown in Table 2 and Figure 5. The period of cutting discharge was limited to 3 days towards the end of the 6-7-week mussel exposure, occurring on the 2nd to the 4th May 2021. Approximately 331 tonnes of cleaned cuttings were discharged over the three days. The discharged TCC slurry had an approx. temperature of 25°C and approx. salinity of 35‰.

Table 2. The total amount of solids following TCC treatment that were discharged during the mussel deployment. GC/FID - Gas chromatography with flame ionization detection

Date	Time			Discharge tailings (tonnes)	Water amount (tonnes)	GC/FID oil conc. wt%
	from	to	h			
02.05.2021	09.00	18.00	9	43.06	430	0.019
02.05.2021	18.00	21.00	3	17.02	170	0.033
03.05.2021	21.00	00.00	4	18.00	180	0.031
03.05.2021	00.00	03.00	3	16.50	165	0.045
03.05.2021	03.00	06.00	3	17.60	176	0.049
03.05.2021	06.00	09.00	3	18.47	185	0.015
03.05.2021	09.00	12.00	3	17.27	173	0.015
03.05.2021	12.00	15.00	3	19.12	191	0.035
03.05.2021	15.00	18.00	3	18.78	188	0.013
03.05.2021	18.00	21.00	3	19.30	193	0.051
04.05.2021	21.00	00.00	4	18.43	184	0.031
04.05.2021	00.00	03.00	3	17.88	179	0.022
04.05.2021	03.00	06.00	3	17.80	178	0.099
04.05.2021	06.00	09.00	3	19.07	191	0.029
04.05.2021	09.00	12.00	3	18.16	182	0.057
04.05.2021	12.00	15.00	3	16.18	162	0.055
04.05.2021	15.00	18.00	3	18.39	184	0.054
		Sum		331.03		
		Average				0.038

The metal concentrations and the particle size distribution of the TCC discharge taken on a separate occasion were used as a representation of the expected concentrations during the mussel deployment (Table 3 and Table 4). Based on the Norwegian classification scheme on the condition of sediments in relation to contaminant concentrations, the metals Hg, As, Pb, Cd, Cu, Cr, Ni and Zn have classifications available (M-608, 2016, revised 30.10.2020). With exception to Ni, which was classified as Class III moderate, all other metals were classified as good (Class II) or background (Class I).

The particle size distribution of the TCC plume showed that the largest size fraction was in the 2-3 µm category. Almost 90% of the TCC particles were below 100 µm in size, whilst the final 10% were below 600 µm.

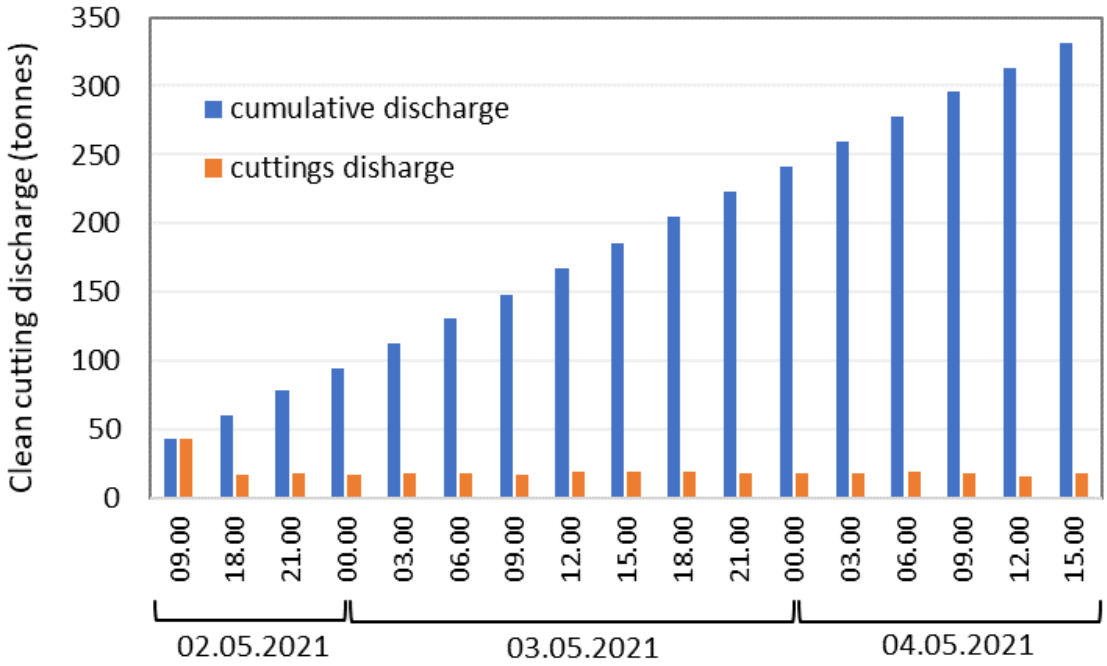


Figure 5. Tonnes of cleaned cuttings discharged to sea between the 2nd and 4th May 2021. These were the only cuttings discharged during the mussel deployment.

Table 3. Measured concentrations of metals and particle size distribution of the TCC that was discharged from the Johan Sverdrup installation on the 26.11.2021 (not during in the exposure period).

Metal	Hg	Al	As	Pb	Fe	Cd	Cu	Co	Cr	Mg	Mn	Ni	Zn
mg/kg TS	<0.03	15000	15	24	3000	0.82	47	14	37	5500	1100	44	110

Table 4. Particle size distribution of the TCC discharge plume

Size interval (µm)	Fraction (%)	Cumulative fraction (%)		Size interval (µm)	Fraction (%)	Cumulative fraction (%)
1<>2	6.55	6.55		30<>40	3.60	81.00
2<>3	10.40	16.95		40<>60	4.30	85.30
3<>4	8.64	25.59		60<>80	2.31	87.61
4<>5	8.71	34.30		80<>100	1.92	89.53
5<>6	7.93	42.23		100<>150	2.35	91.88
6<>8	6.89	49.13		150<>200	1.54	93.42
8<>10	6.02	55.15		200<>250	1.77	95.19
10<>14	5.51	60.66		250<>300	0.97	96.16
14<>18	5.22	65.88		300<>400	1.88	98.04
18<>25	7.28	73.16		400<>500	1.39	99.43
25<>30	4.24	77.40		500<>600	0.57	100.00

3.1.1 Modelling of the TCC discharge plume

Modelling of the TCC discharge following the monitoring programme was performed in order to confirm the expected plume direction and suitability of exposure to the monitoring stations. For detailed information on the modelling of the TCC discharge plume, please see separate Sintef report (Nepstad and Ditlevsen, 2022). Inputs into the model included information on the TCC slurry discharge, such as temperature (25°C), particle diameter (1 µm to 1 mm), variable release rate between 2nd and 4th May 2021, and depth of discharge outlet (17 m), as well as the properties of the seawater recipient, temperature, salinity, turbidity, ocean currents from monitoring stations 1, 3 and 6. In addition to wind strength and direction.

A snapshot of the model at one time point is shown below (Figure 6). The top inserted panels show the distribution of the plume with depth, with the diamonds denoting the position of the mussels. The main plume slurry (90%) sank within meters of the discharge outlet and became trapped in a thermocline at around 50 m. Based on the model, 10% of the fine material was expected to escape from the main body of the slurry, this material remained at the approximate depth of the discharge (17 m), a similar depth as the top mussel group. The bottom inserted panel shows the expected concentration of TCC over time with the peaks indicating episodic exposure. Overall, the plume model confirmed the suitability of the monitoring stations in capturing the potential impact of the TCC plume.

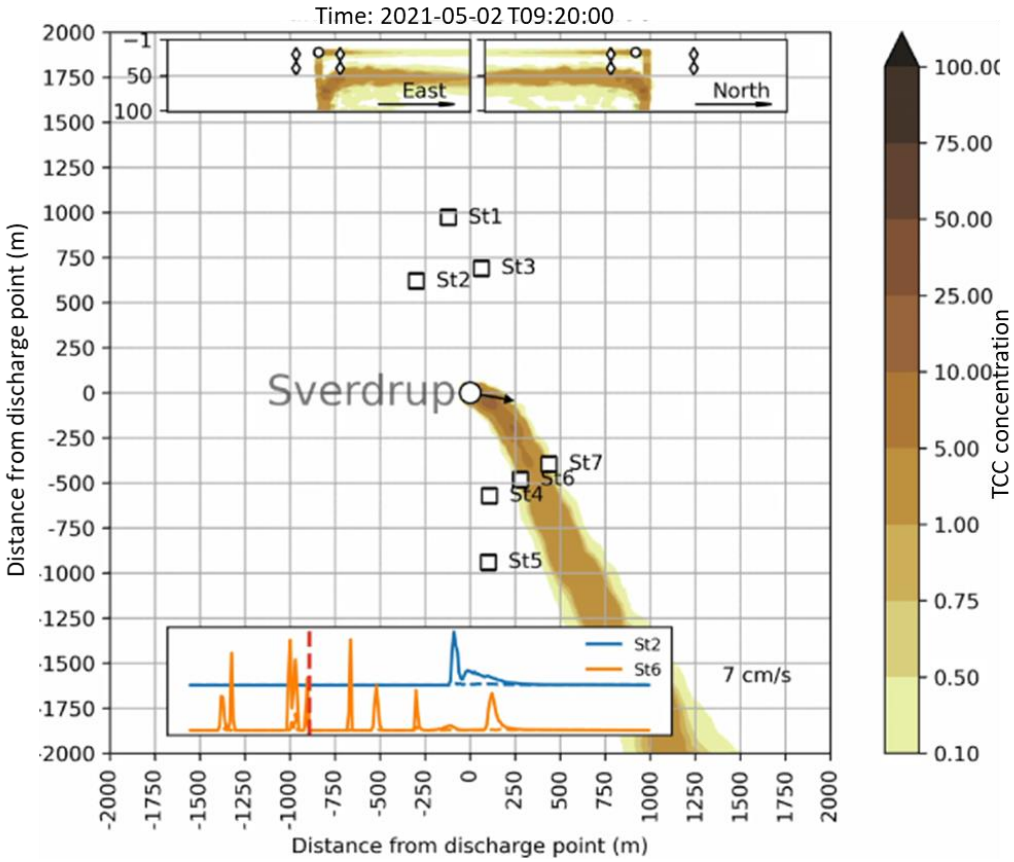


Figure 6. Snapshot image of the dynamic model of the TCC discharge from the Johan Sverdrup installation. Squares indicate the positions of the monitoring stations. Top inserts show the concentration of the TCC plume with depth, with diamonds indicating the monitoring rigs. The bottom insert indicates the expected concentration of TCC at monitoring stations 2 and 6 with episodic peaks.

The TCC deposition on the seafloor was also modelled and provides an indication of the expected area and extent of the distribution of the TCC (Figure 7). Since the dense TCC slurry was expected to sink almost immediately from the 17 m outlet, most of the TCC is distributed within 200 m north and south of the installation, influenced partly by the tidal current direction.

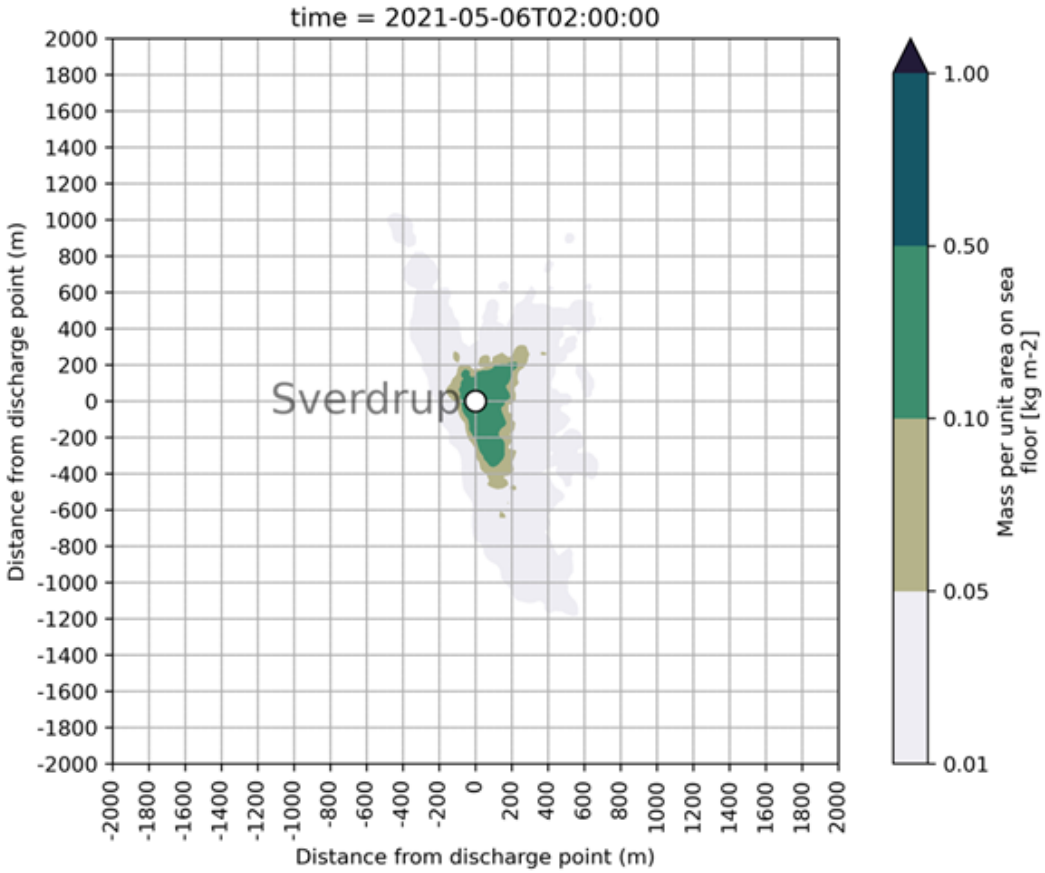


Figure 7. Model of the deposition of the TCC discharge from the Johan Sverdrup installation

3.2 Oceanographic measurements

Turbidity measurements were recorded for the duration of the exposure at stations 1 and 3 as well as the reference station 1 (Figure 8). Background scatter was apparent in all turbidity data but particularly at station 1. Turbidity was mostly below 1 FTU for all stations, however an increase in turbidity was recorded in all three stations around the 7th April, which may be due to adverse weather conditions during this time. For the Johan Sverdrup stations a slight elevation in turbidity was seen around the 12th April at both stations. At station 3, episodic increases in turbidity on the 28th April 3rd and 8th May were seen, unsure whether these increases are due to the TCC discharge or from sensor interference, bearing in mind the TCC was only discharged between the 2nd and 4th May. A large increase in turbidity was also found on the 3rd May at the reference station for reasons not entirely understood.

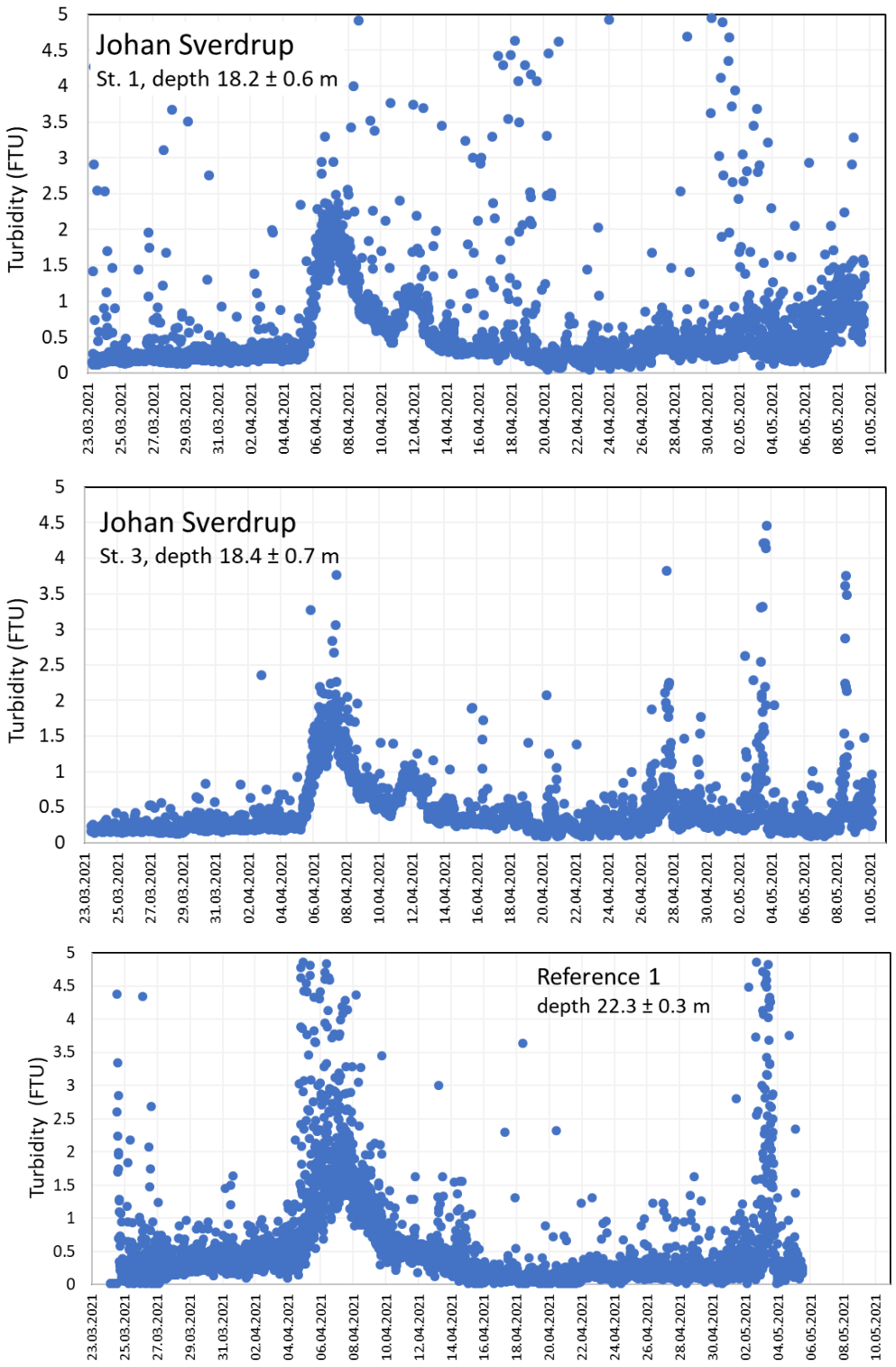


Figure 8. Turbidity measurements recorded at 20-minute intervals during an approximate 6-7-week deployment from stations 1 (top) and 3 (middle) and the reference location (bottom). Depth of the sensors at each location are represented as mean ± standard deviation for the entire duration.

Temperature measurements were also recorded from the same devices as turbidity at stations 1 and 3 and the reference station 1 (Figure 9). Almost identical temperature profiles over time were found for the two Johan Sverdrup stations, at a depth of 18 m, starting at 7.3℃ at the beginning of the exposure, dropping to 6.9℃ on the 12th April before slowly increasing to around 7.6℃ at the end of the exposure. In contrast, the reference station 1, started with lower temperatures of 5.5℃ slowly rising to around 7℃ at the end of the exposure. The reference station 1 was approximately 150 km south of the Johan Sverdrup complex and experienced colder water bodies.

An additional monitoring station approximately 50 m south of station 6 was used to monitor turbidity, temperature and salinity for the final week of the exposure (Figure 10). Turbidity measurements at a depth of 22 m mostly remained below 1 FTU between the 3rd and the 10th May. However, small episodic increases in turbidity were shown on the 4th May and again on the 8th May. This station was south-south east of the Johan Sverdrup complex. The temperature at the end of the exposure was between 7.2 and 7.8℃ and supports the temperature data from the other stations previously mentioned. The salinity at this station remained stable at 35 ppt.

Vertical profiles of the water column for salinity, temperature, chlorophyll a and turbidity were taken on the retrieval of the monitoring rigs at reference station 2 and Johan Sverdrup stations 1 (Figure 11) and stations 4 and 6 (Figure 12). Although salinity remained relatively stable with depth a thermocline was evident at 30 m for the reference station and around 50 m for stations 1, 4 and 6. Chlorophyll a was present at all depths and in most part the turbidity appeared to pattern the chlorophyll a measurement.

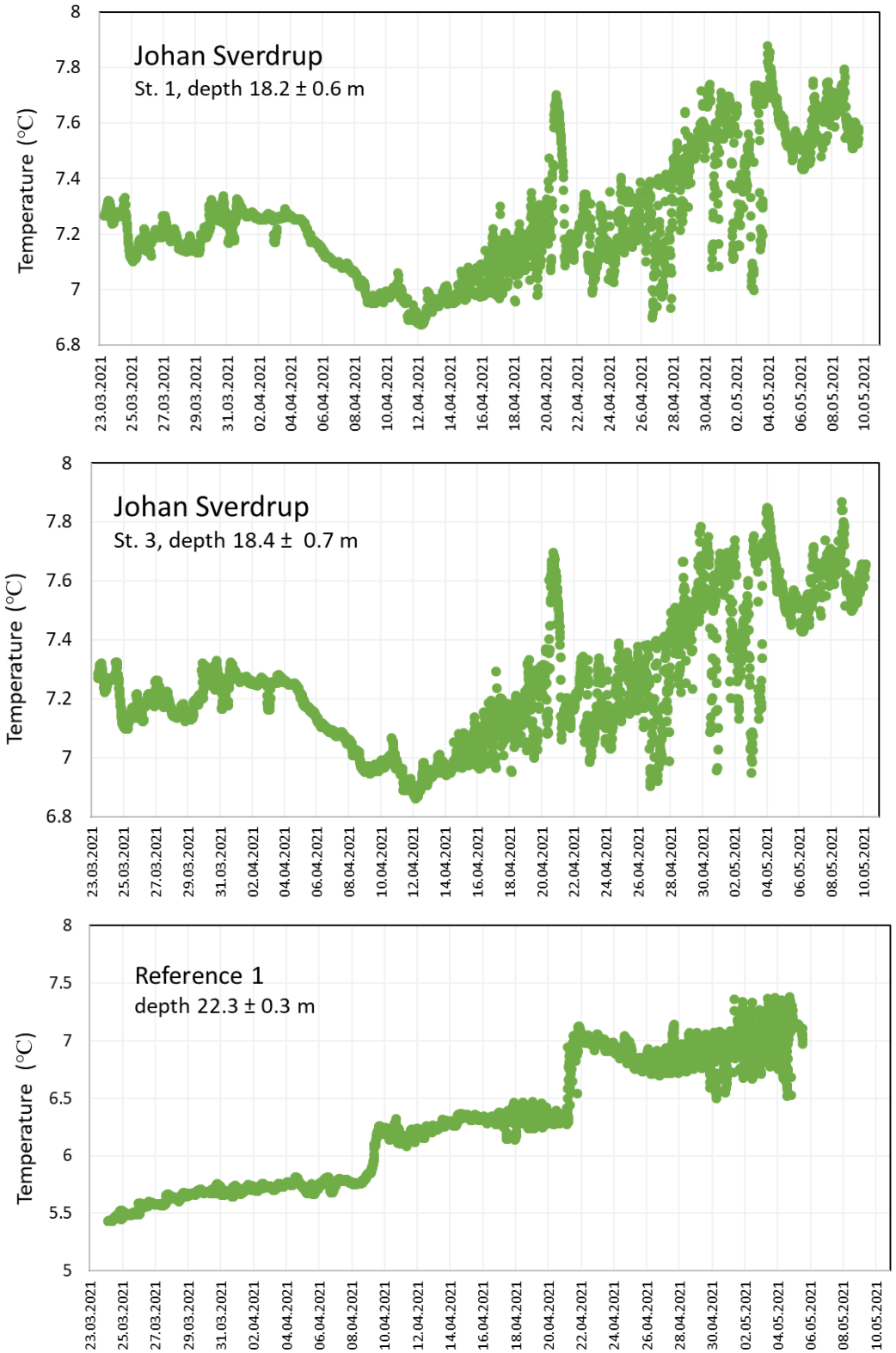


Figure 9. Temperature recorded at 20-minute intervals during an approximate 6-week deployment from stations 1 (top) and 3 (middle) and the reference location (bottom). Depth of the sensors at each location are represented as mean ± standard deviation for the entire duration.

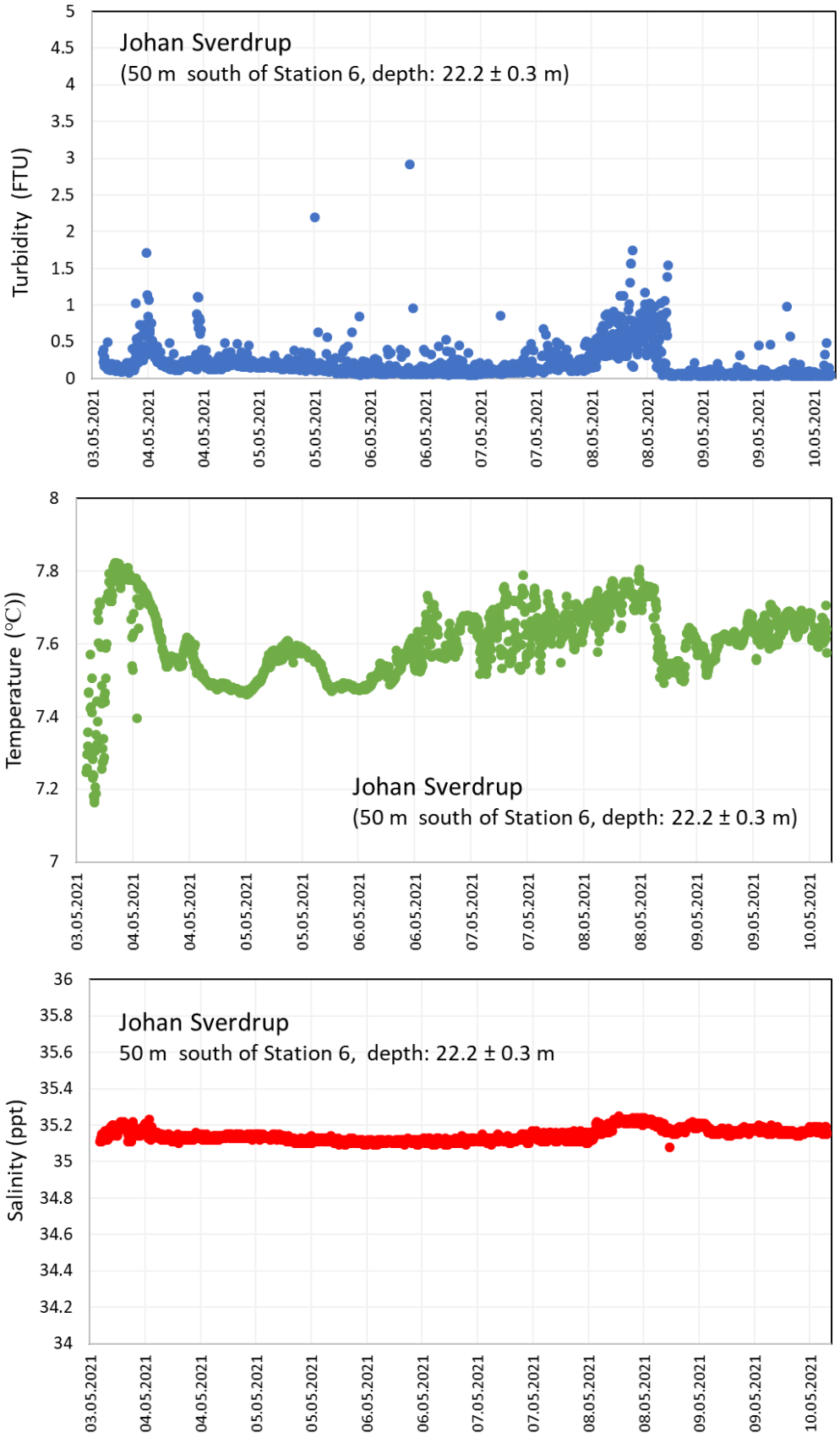


Figure 10. Turbidity, temperature and salinity measured on a fixed mooring approximately 50 m south of station 6 at a mean depth of 22.2 ± 0.3 m (mean ± SD) for seven days (03.05.2021 to 10.05.2021).

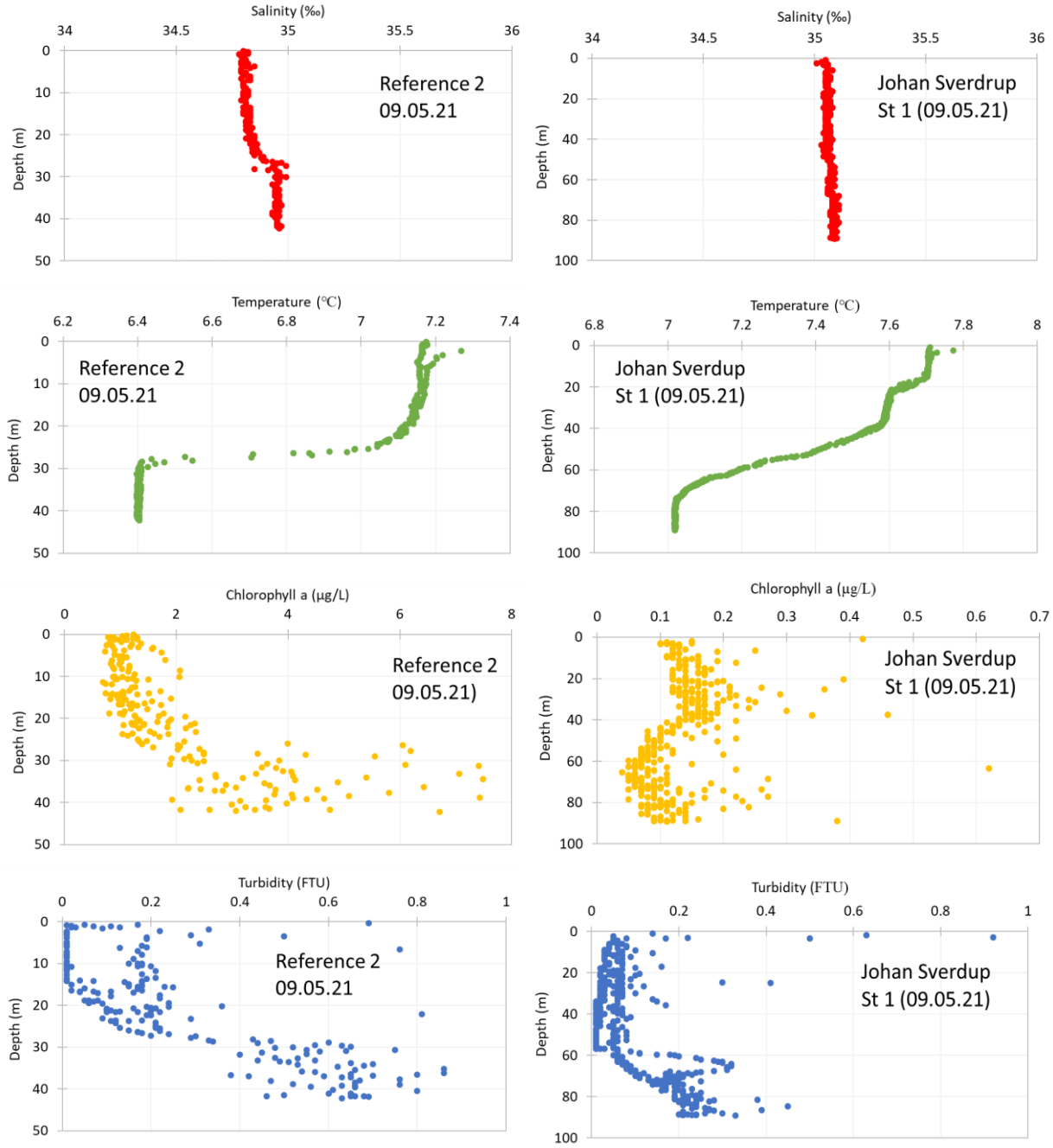


Figure 11. Vertical profiles of seawater parameters taken at Reference 2 and Johan Sverdrup monitoring station 1 during the retrieval of the monitoring rigs. Values were recorded on a SAIV deployed on a rope from the main vessel.

Temperature loggers were attached to the netted bags holding the mussels at all stations, and for both depths, for the duration of the exposure (Figure 13). The reference stations for both top and bottom mussels recorded similar temperatures starting at around 5.5°C in late March increasing to around 7°C and 7.5°C for bottom and top mussels respectively, at the end of the exposure in early May. In contrast, temperatures recorded at the Johan Sverdrup stations all started around 7.5°C and remained relatively stable throughout the exposure duration. The top mussels at Johan Sverdrup were slightly warmer, approaching 8°C compared to the bottom mussels at the end of the exposure in May. Overall the temperature loggers supported the temperature measurements from the current meters (Figure 9).

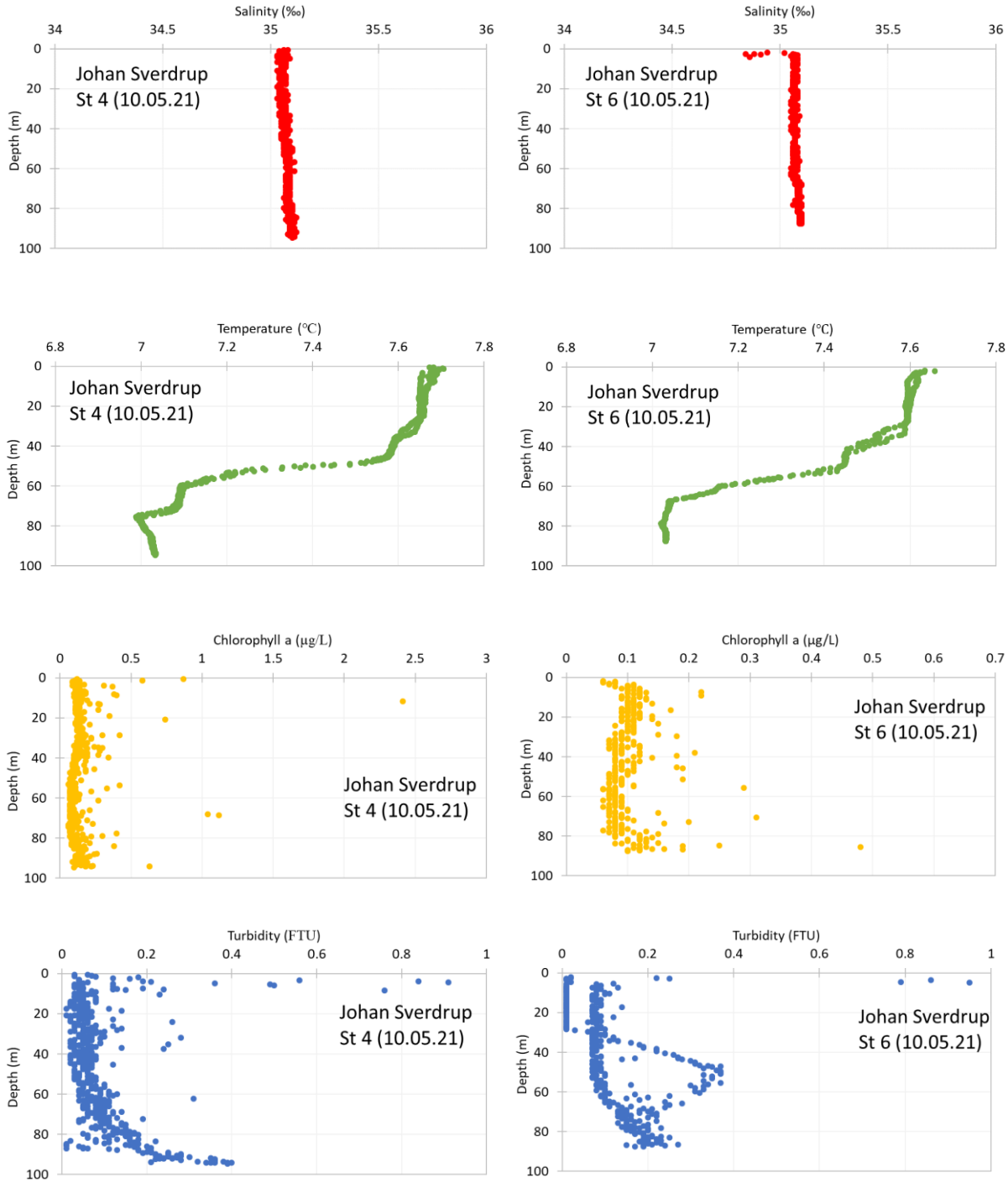
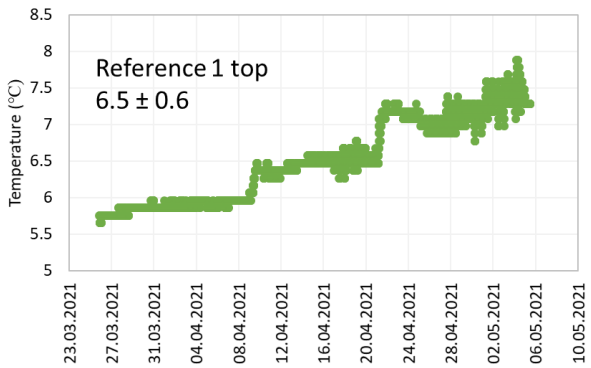
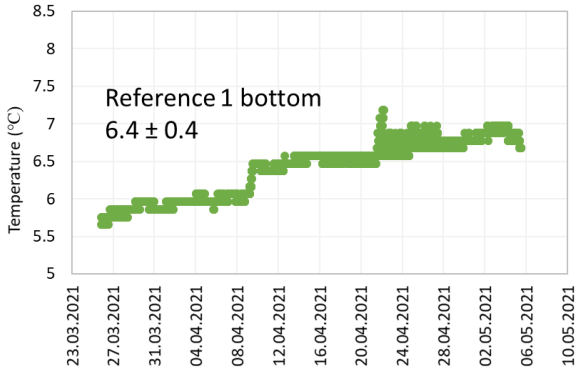
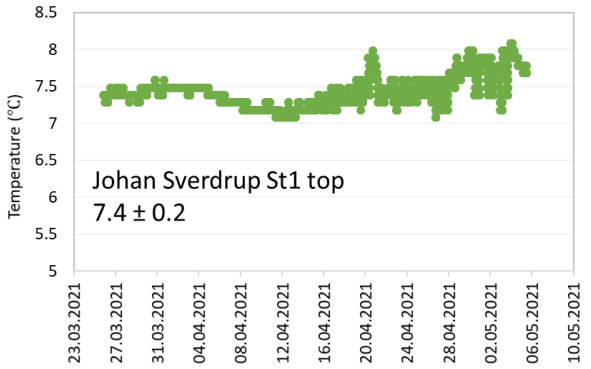
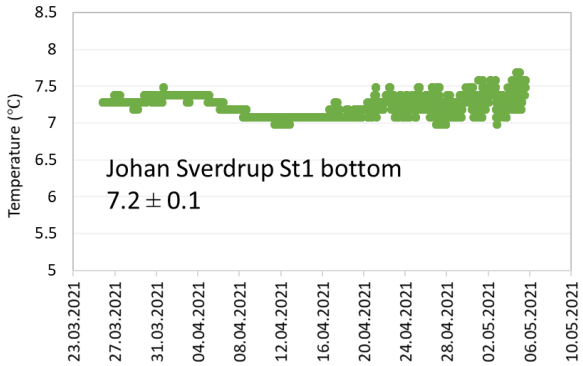


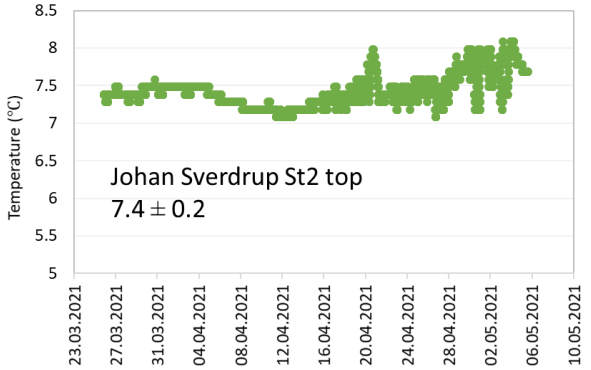
Figure 12. Vertical profiles of seawater parameters taken at Johan Sverdrup monitoring stations 4 and 6 during the retrieval of the monitoring rigs. Values recorded with a SAIV deployed on a rope from the main vessel.


Reference 1 top
6.5 ± 0.6
Temperature (°C)
8.5
8
7.5
7
6.5
6
5.5
5
23.03.2021
27.03.2021
31.03.2021
04.04.2021
08.04.2021
12.04.2021
16.04.2021
20.04.2021
24.04.2021
28.04.2021
02.05.2021
06.05.2021
10.05.2021


Reference 1 bottom
6.4 ± 0.4
Temperature (°C)
8.5
8
7.5
7
6.5
6
5.5
5
23.03.2021
27.03.2021
31.03.2021
04.04.2021
08.04.2021
12.04.2021
16.04.2021
20.04.2021
24.04.2021
28.04.2021
02.05.2021
06.05.2021
10.05.2021


Johan Sverdrup St1 top
7.4 ± 0.2
Temperature (°C)
8.5
8
7.5
7
6.5
6
5.5
5
23.03.2021
27.03.2021
31.03.2021
04.04.2021
08.04.2021
12.04.2021
16.04.2021
20.04.2021
24.04.2021
28.04.2021
02.05.2021
06.05.2021
10.05.2021


Johan Sverdrup St1 bottom
7.2 ± 0.1
Temperature (°C)
8.5
8
7.5
7
6.5
6
5.5
5
23.03.2021
27.03.2021
31.03.2021
04.04.2021
08.04.2021
12.04.2021
16.04.2021
20.04.2021
24.04.2021
28.04.2021
02.05.2021
06.05.2021
10.05.2021


Johan Sverdrup St2 top
7.4 ± 0.2
Temperature (°C)
8.5
8
7.5
7
6.5
6
5.5
5
23.03.2021
27.03.2021
31.03.2021
04.04.2021
08.04.2021
12.04.2021
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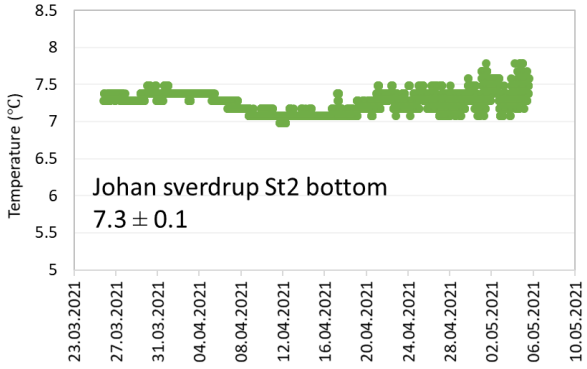
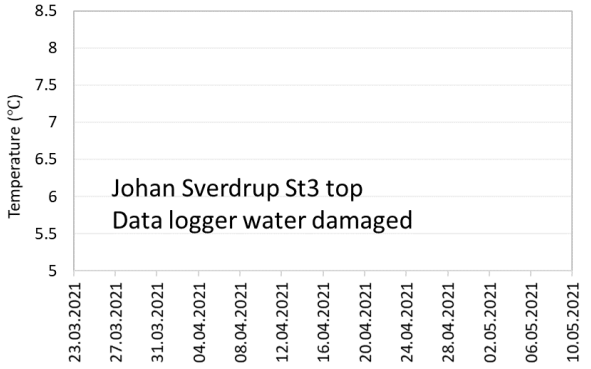
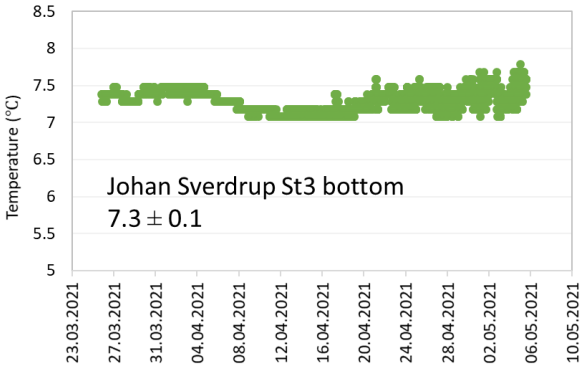
02.05.2021
06.05.2021
10.05.2021


Johan sverdrup St2 bottom
7.3 ± 0.1
Temperature (°C)
8.5
8
7.5
7
6.5
6
5.5
5
23.03.2021
27.03.2021
31.03.2021
04.04.2021
08.04.2021
12.04.2021
16.04.2021
20.04.2021
24.04.2021
28.04.2021
02.05.2021
06.05.2021
10.05.2021


Johan Sverdrup St3 top
Data logger water damaged
Temperature (°C)
8.5
8
7.5
7
6.5
6
5.5
5
23.03.2021
27.03.2021
31.03.2021
04.04.2021
08.04.2021
12.04.2021
16.04.2021
20.04.2021
24.04.2021
28.04.2021
02.05.2021
06.05.2021
10.05.2021


Johan Sverdrup St3 bottom
7.3 ± 0.1
Temperature (°C)
8.5
8
7.5
7
6.5
6
5.5
5
23.03.2021
27.03.2021
31.03.2021
04.04.2021
08.04.2021
12.04.2021
16.04.2021
20.04.2021
24.04.2021
28.04.2021
02.05.2021
06.05.2021
10.05.2021

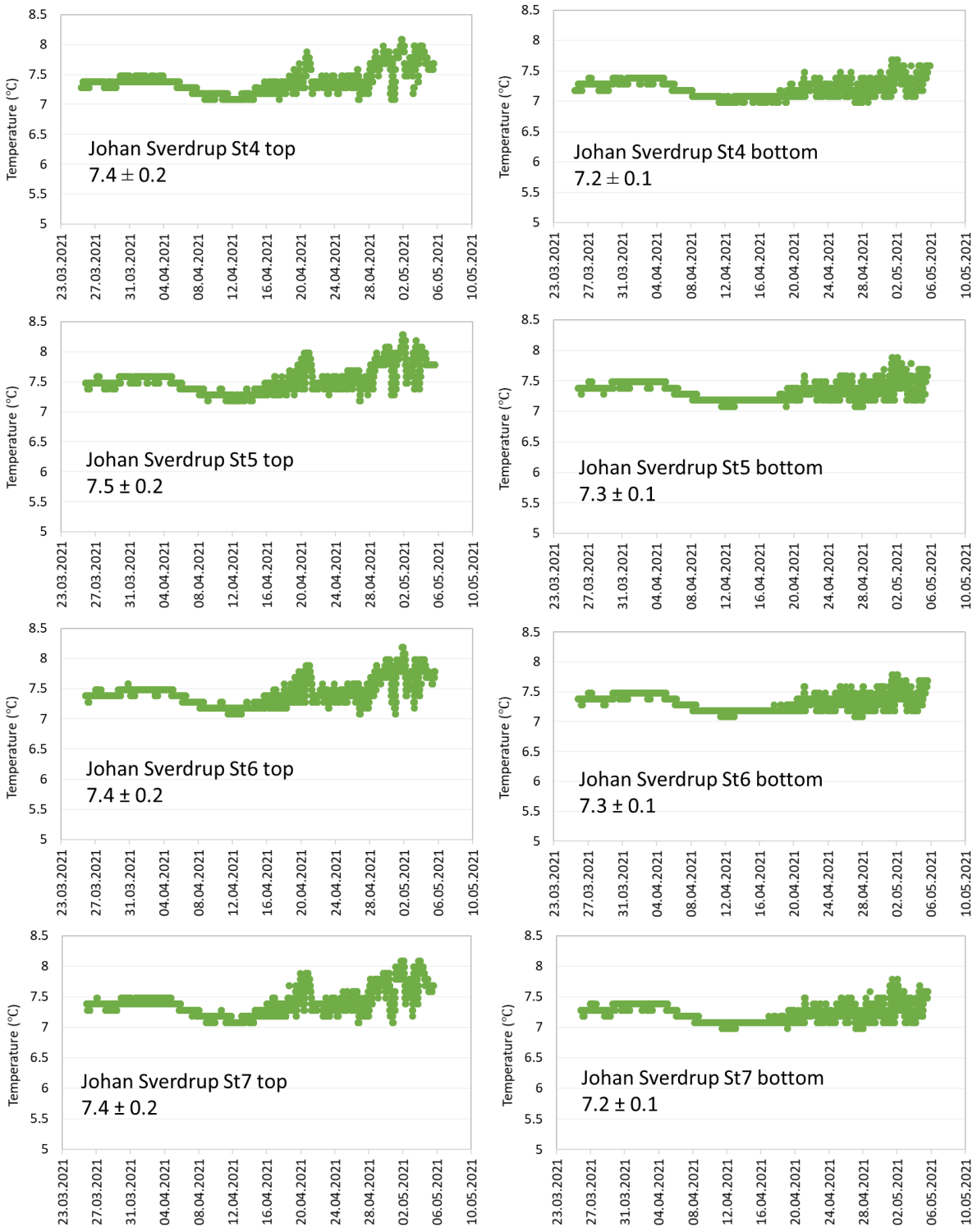


Figure 13. Temperature measured at 10 min intervals with sensors placed in the mussel cages during the field deployment at all monitoring stations. Top and bottom indicates an approximate depth of 18 -20 m and 40-42 m respectively. Values on graphs show mean ± standard deviation.

3.2.1 Current measurements

Current measurements presented as current rows and progressive vectors are presented for stations 1, 3 and 6 (Figure 14 to Figure 17). The DCP instruments located at stations 1 and 3 provide current data at the depth of the sensor (i.e. 18 m), whilst the RDCP can provide data at different depths. For station 1 and station 3, the dominant direction of the current was south-south-east at a bearing of 150°-160°. Almost identical current rose, and progressive vectors were shown for stations 1 and 3.

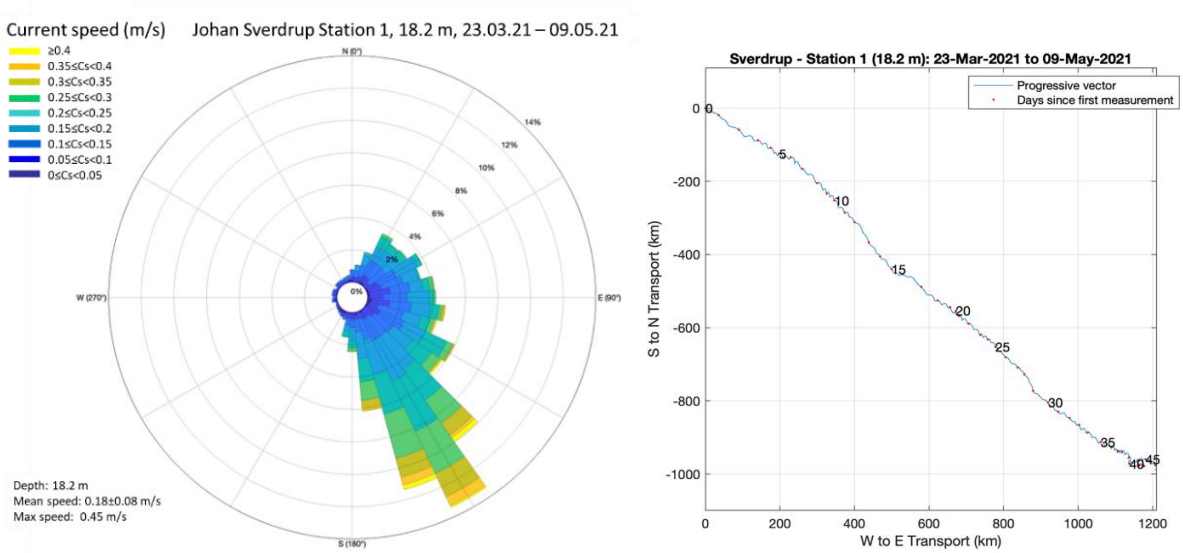


Figure 14. Current rose and progressive vectors measured with the Seaguard RCM single-point current meter deployed at Johan Sverdrup Station 1 (18.2m depth) for the duration of the mussel field deployment between 23rd March and 9th May 2021.

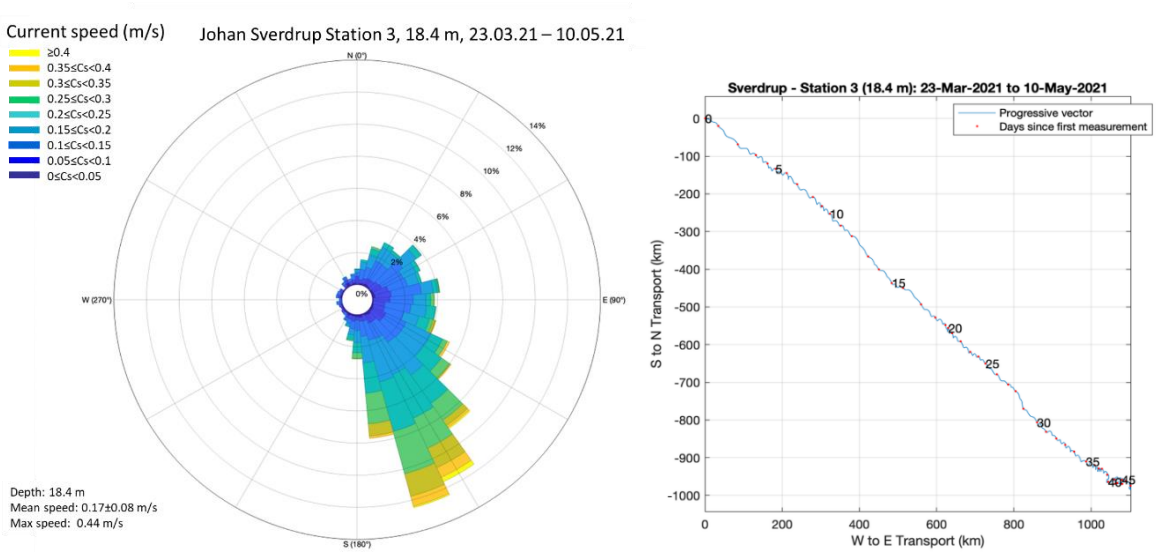


Figure 15. Current rose and progressive vectors measured with a Seaguard RCM single-point current meter deployed at Johan Sverdrup Station 3 (18.4 m depth) for the duration of the mussel field deployment between 23rd March 2021 and 9th May 2021.

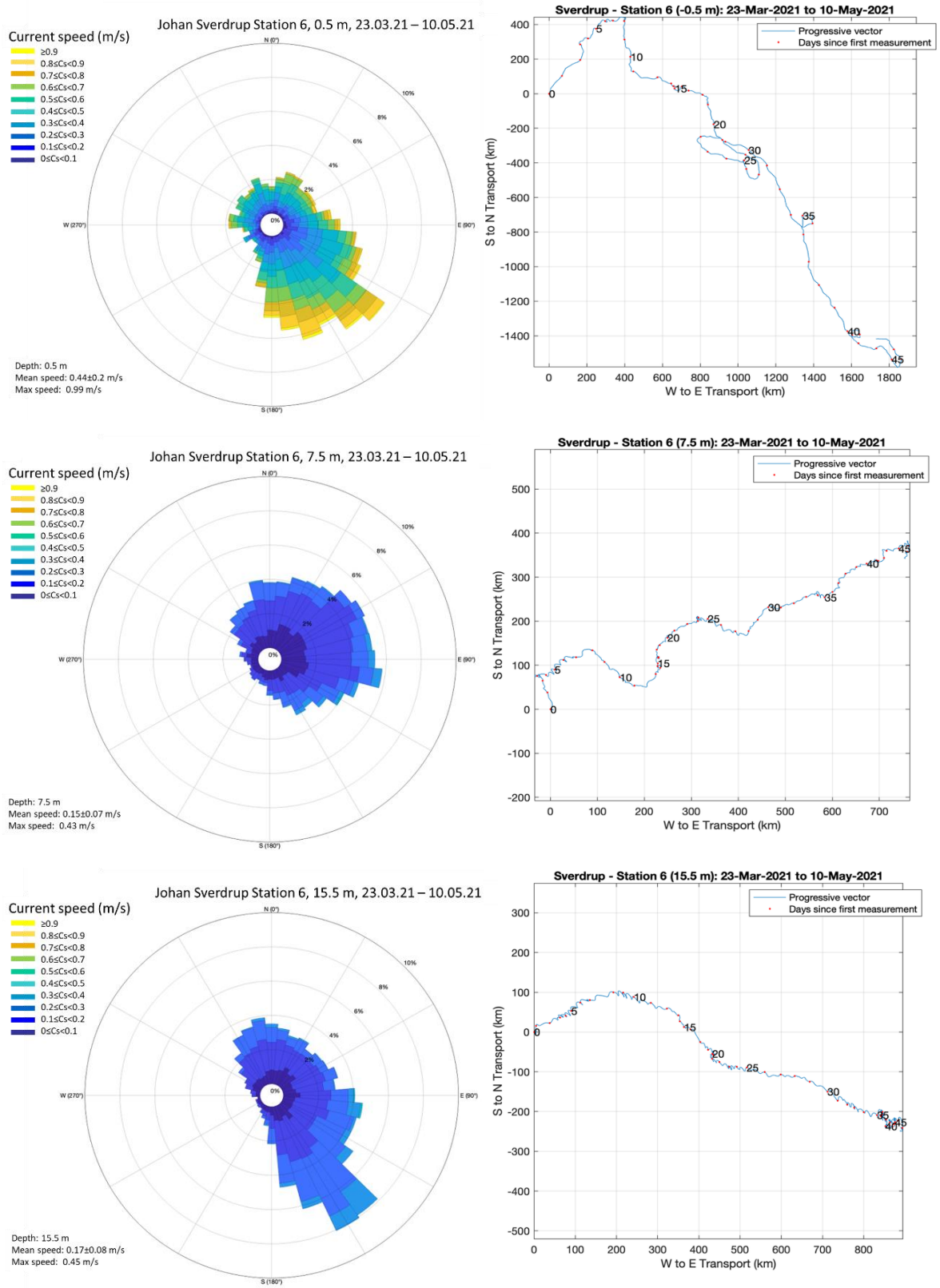


Figure 16. Current rose and progressive vectors measured with an RDCP current profiler deployed at station 6 for the duration of the mussel field deployment. Selected depths include surface waters (0.5 m) and 7.5 and 15.5 m depth.

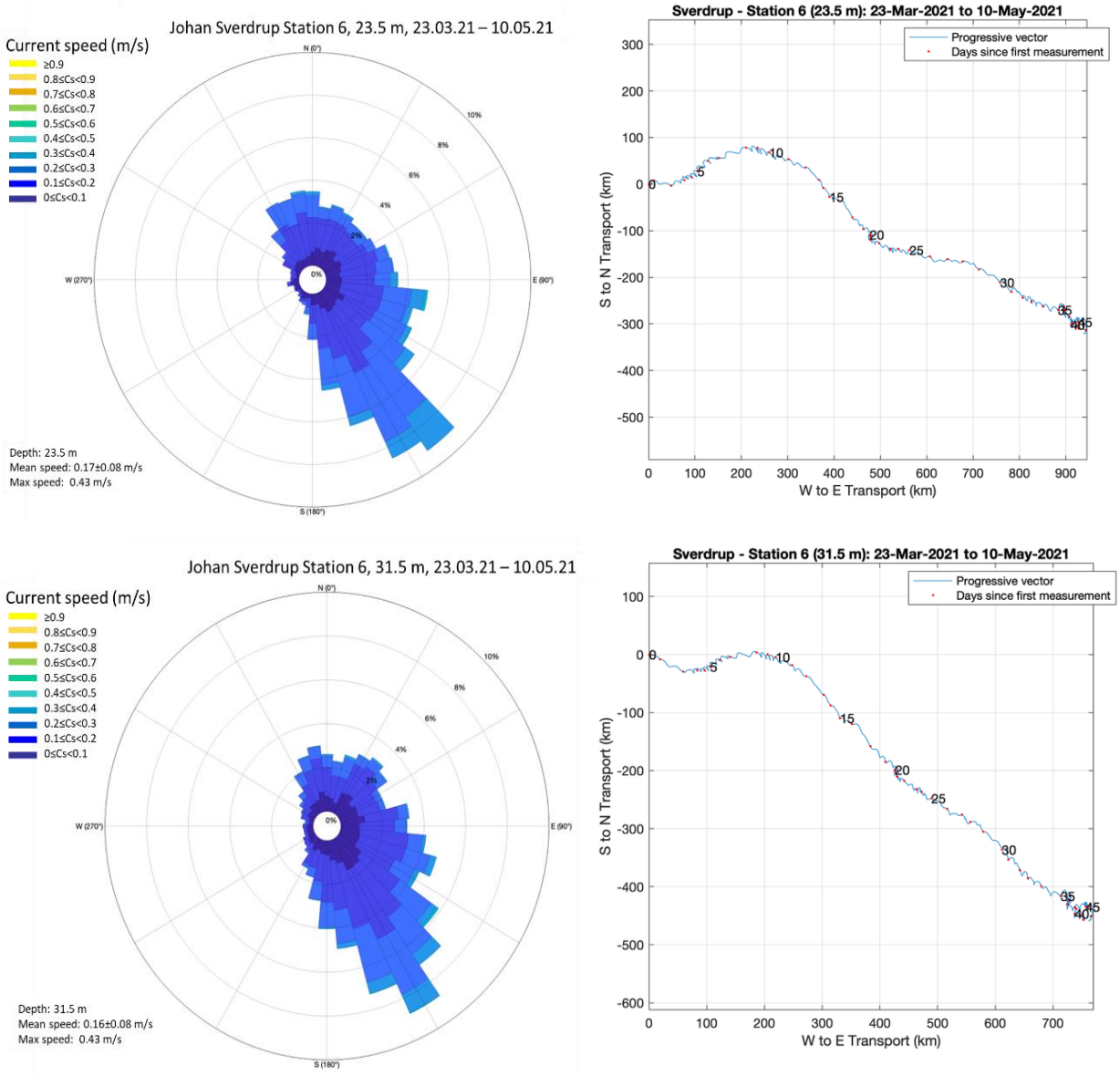


Figure 17. Current rose and progressive vectors measured with a recording doppler current profiler (RDCP) deployed at station 6 for the duration of the mussel field deployment. Selected depths at 23.5 (top row) and 31.5 m (bottom row).

The RDCP at station 6 shows current data at five different depths (0.5, 7.5, 15.5, 23.5, 31.5m). Similar current rose profiles were shown at depths 15.5m and below, with the dominant current in the south-south-east direction as shown for station 1 and 3. The surface waters at station 6 were also in the south east direction at slightly higher current speeds. However, in contrast to the other depths the current direction at 7.5 m was weakly in the north-east direction.

3.2.1.1 Current direction during the discharge of the TCC

As shown in Table 2, the TCC was only discharged towards the end of the mussel exposure from the 2nd May to the 4th May. The current directions during this short time window are shown for stations 1, 3 and 6 (Figure 18). Similar patterns in current rose were found at stations 1 and 3, north of the Johan Sverdrup installation, with the main current directed towards the south-south-east as previously

described when the total duration of the mussel exposure was considered (Figure 14 and Figure 15). Interestingly, the current was directed north-east for some of the time during the TCC discharge event at station 1 and 3. The current speed at these stations during the TCC discharge were slower with a mean speed of 0.13 and 0.12 m/s. South of the platform at station 6, the dominant direction of the current was north and south during the 3-day TCC discharge.

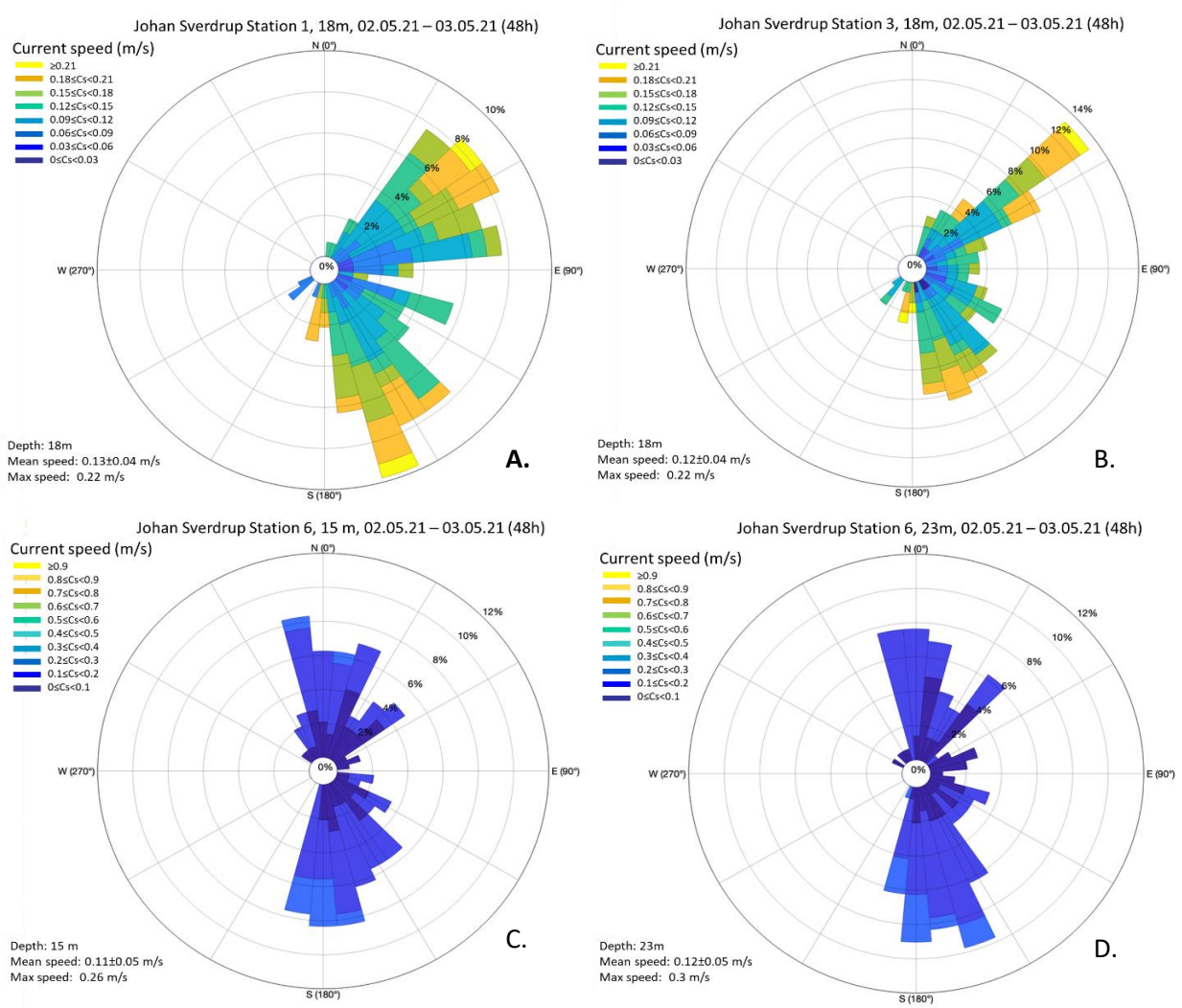


Figure 18. Current rose for the duration of the TCC discharge. A) station 1, depth 18m, B) station 3, 18m depth, C) station 6, 15m depth, and D) station 6, 23m depth.

3.3 Mussel biometry

Length measurements were taken from a sub sample of mussels from the day zero group (T0) and from each monitoring station including both depths (Figure 19). For the field transplanted mussels, median mussel length ranged from 52 to 60 mm with no significant difference among the mussel groups (Kruskal Wallis, $p<0.05$). However, the T0 mussels were noticeably smaller than the field exposed mussel groups with a median length of 48 mm. Due to priority given to packing and sealing of the nets to be deployed, only the smaller mussels from the stock delivered remained for T0

assessment. These smaller mussels did however provide sufficient tissue material to carry out the required assessments.

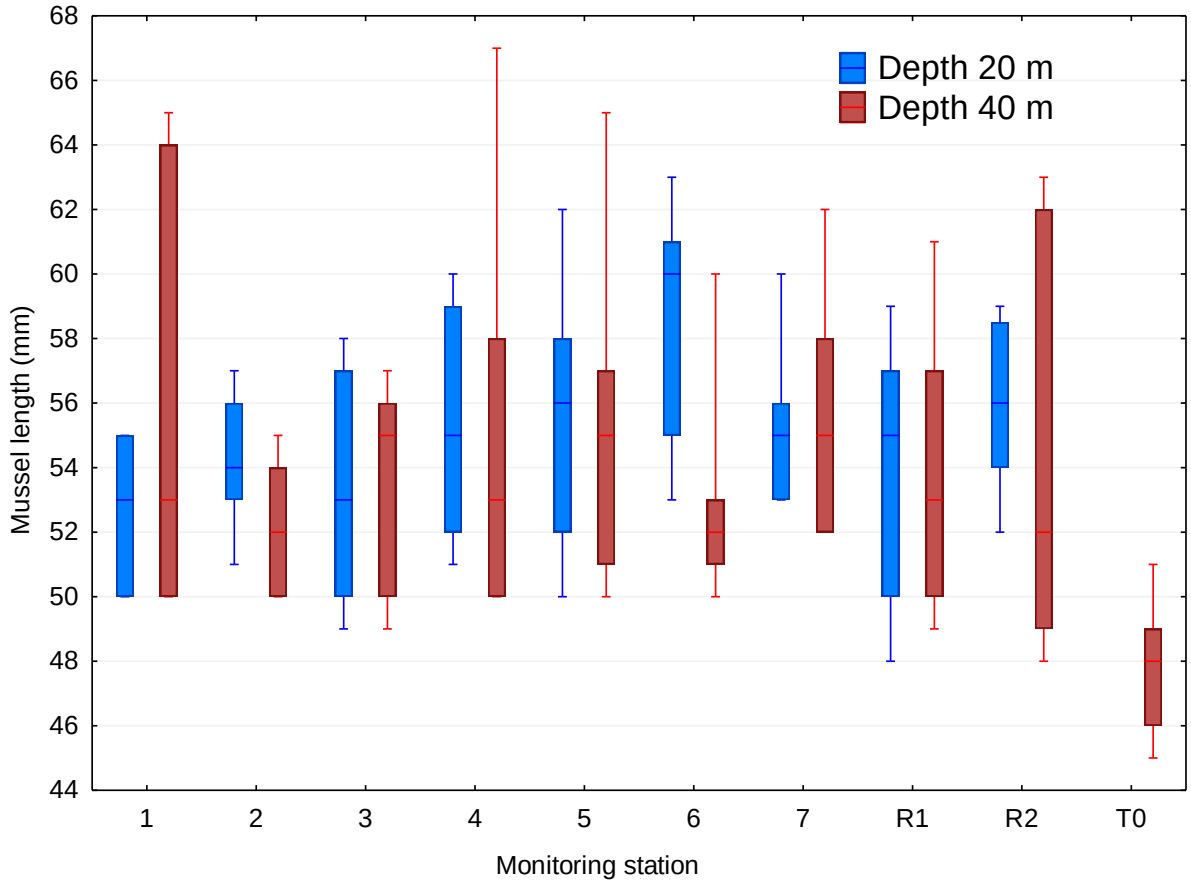


Figure 19. Mussel length measured in a subset of mussels from all field exposed groups and the day zero (T0) group. Reference 1 & 2 (R1 & R2). (median ± quartiles (box) and 10/90 percentiles (outer line, n=15).

3.4 Chemical concentrations in mussels

3.4.1 PAH-NPD

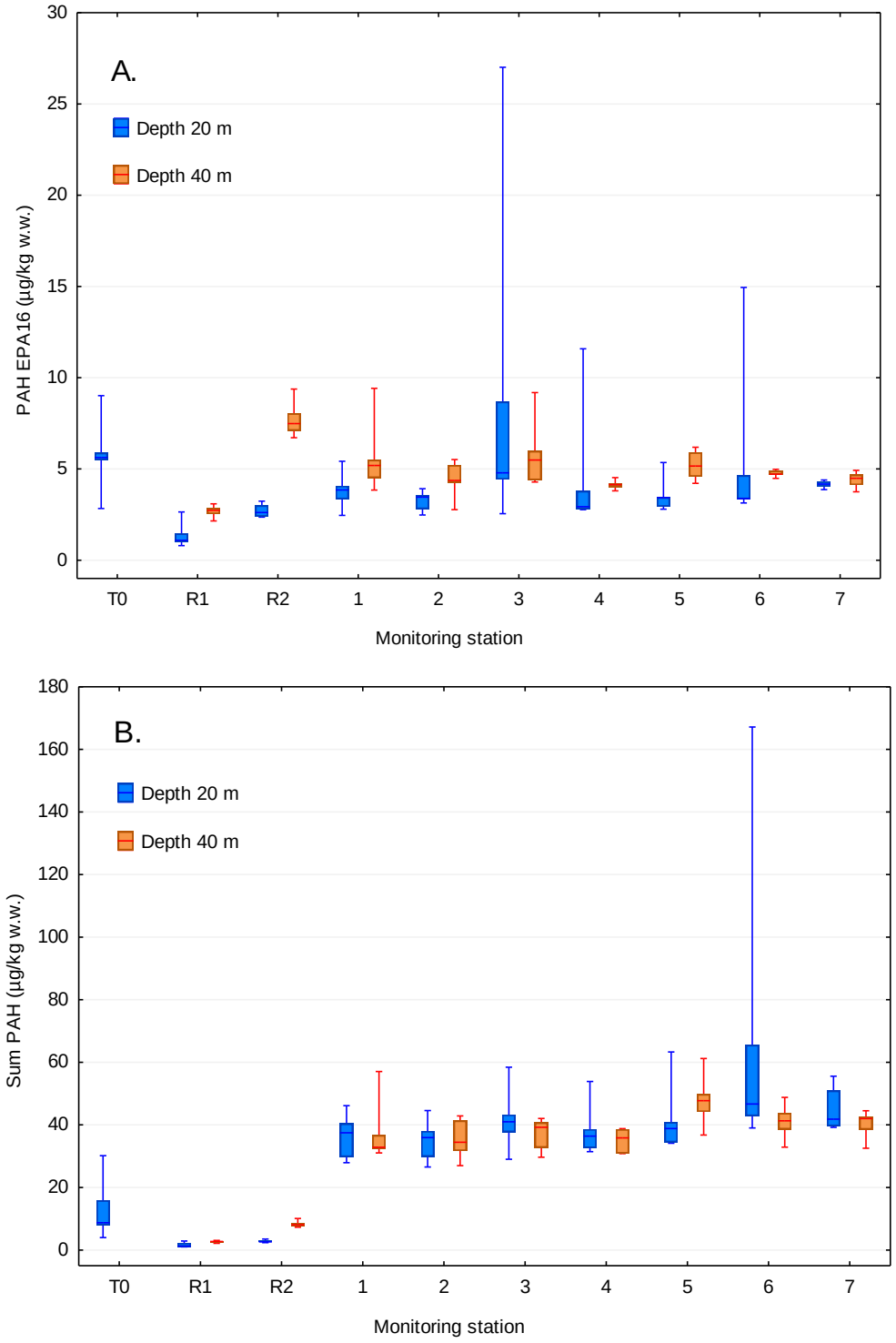


Figure 20. A) EPA PAH16 and B) Sum of PAH concentration in mussels from the monitoring stations around the Johan Sverdrup installation, median, quartiles (box), 10/90 percentiles (bar), n=5.

The bioaccumulation of PAH in mussels from the day zero group and all monitoring stations are shown as both the sum of EPA16 and the sum of all PAH concentrations (Figure 20). EPA16 includes the following compounds, naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene, dibenz(a,h)anthracene. Whilst the sum of PAH included the addition compounds, dibenzothiophene, naphthalenes C1 to C3, phenanthrenes/ anthracenes C1 to C3, dibenzothiophenes C1 to C3.

For EPA16, the median concentrations in all groups were below 8 µg/kg (w.w.). Significant differences were found between PAH EPA16 concentrations at Ref2 40 m and the other reference groups, whilst Ref1 20 was significantly different from T0, st2 40 m, and st5 40 m (Kruskal Wallis, $p<0.05$). Despite these differences there was no obvious increase in PAH EPA16 in the mussels from the Johan Sverdrup field compared to the reference and day zero groups.

For the sum PAH, median concentrations in mussels placed at the Johan Sverdrup field were within a similar range between 35 to 45 µg/kg (w.w.). In contrast sum PAH in mussels from the reference and the day zero (T0) groups were markedly less, below 10 µg/kg (w.w.). Statistically however, only the reference groups R1 20 m, R1 40 m and R2 20 m where significantly different to St5 40 m, St6 20 m and st7 20 m (Kruskal Wallis, $p<0.05$).

In comparison with the sum PAH concentrations reported around offshore oil and gas installations in the North Sea, the concentrations measured in mussels from the Johan Sverdrup field were low. For example, sum PAH of around 400 µg/kg (w.w.) was found in mussels held for 6 weeks 500m from Statfjord A and B platforms and over 100 µg/kg (w.w.) in mussels positioned 10km downstream from Statfjord A (Pampanin et al., 2019).

The NPD compounds, particularly including the alkylated groups C1 to C3 contributed most to the sum PAH concentration. In all cases, the concentration of parent compound was much lower and their respective alkylated groups. The highest concentrations of NPD compounds were measured in the station 6 top mussels as described for the sum PAH. Overall naphthalenes were the most abundant with smaller and relatively equal contributions of phenanthrene and dibenzothiophenes in the mussels from the Johan Sverdrup field (Figure 21 to Figure 23) In contrast, NPD concentrations were almost undetected at the reference and day zero groups.

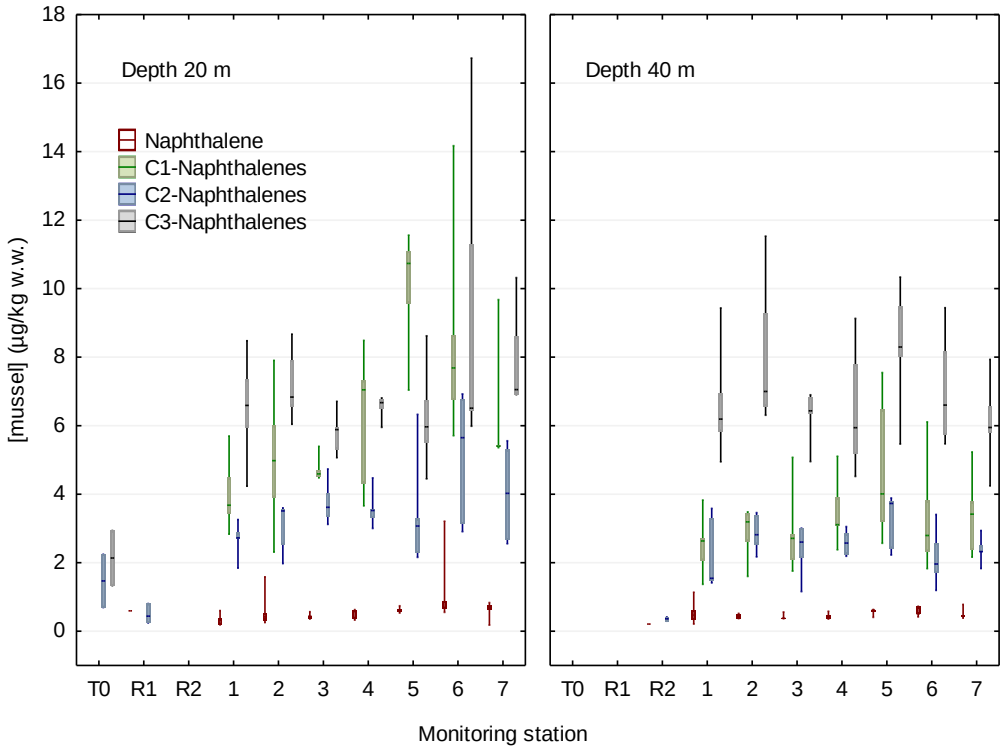


Figure 21. Naphthalene and alkylated naphthalene (C1-C3) concentrations in mussels from the different stations around the Johan Sverdrup installation, median, quartiles (box), 10/90 percentiles (bar), n=5.

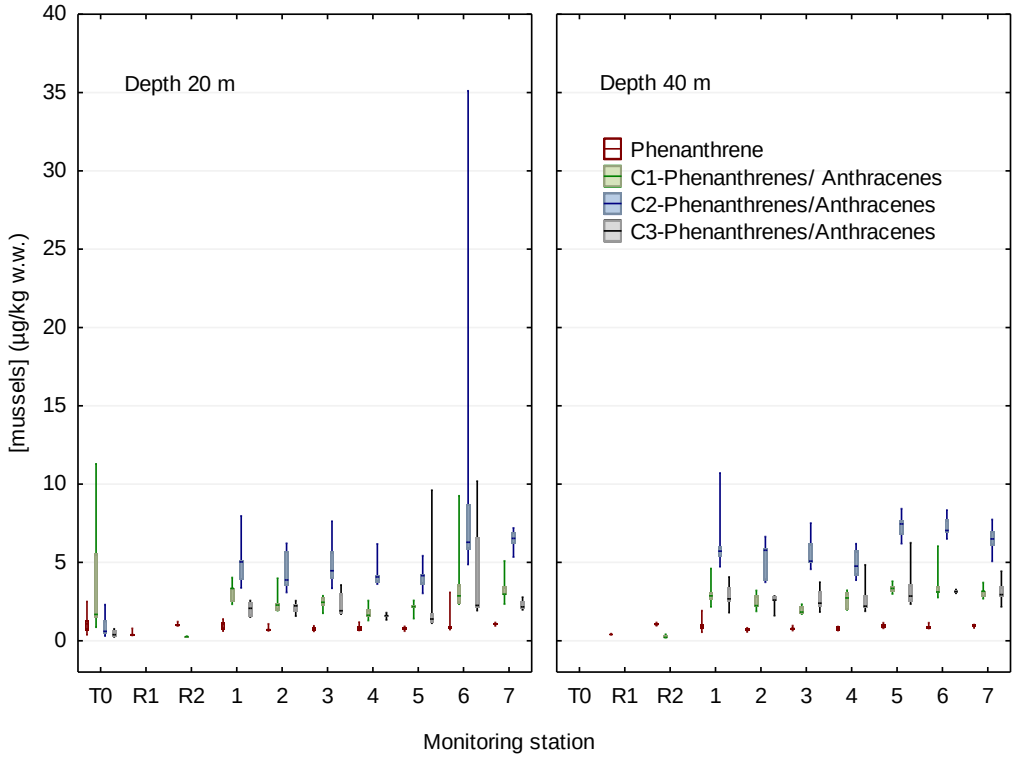


Figure 22. Phenanthrene and alkylated phenanthrene/ anthracene concentrations in mussels from the different stations around the Johan Sverdrup installation, median, quartiles (box), 10/90 percentiles (bar), n=5.

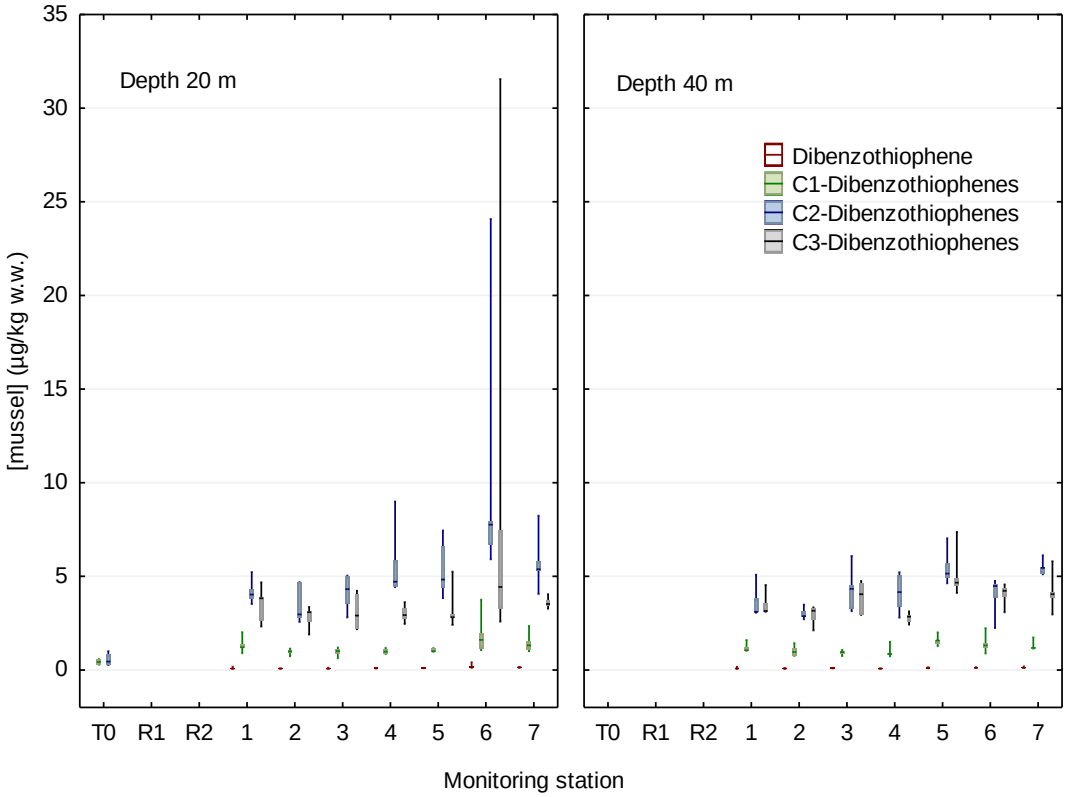
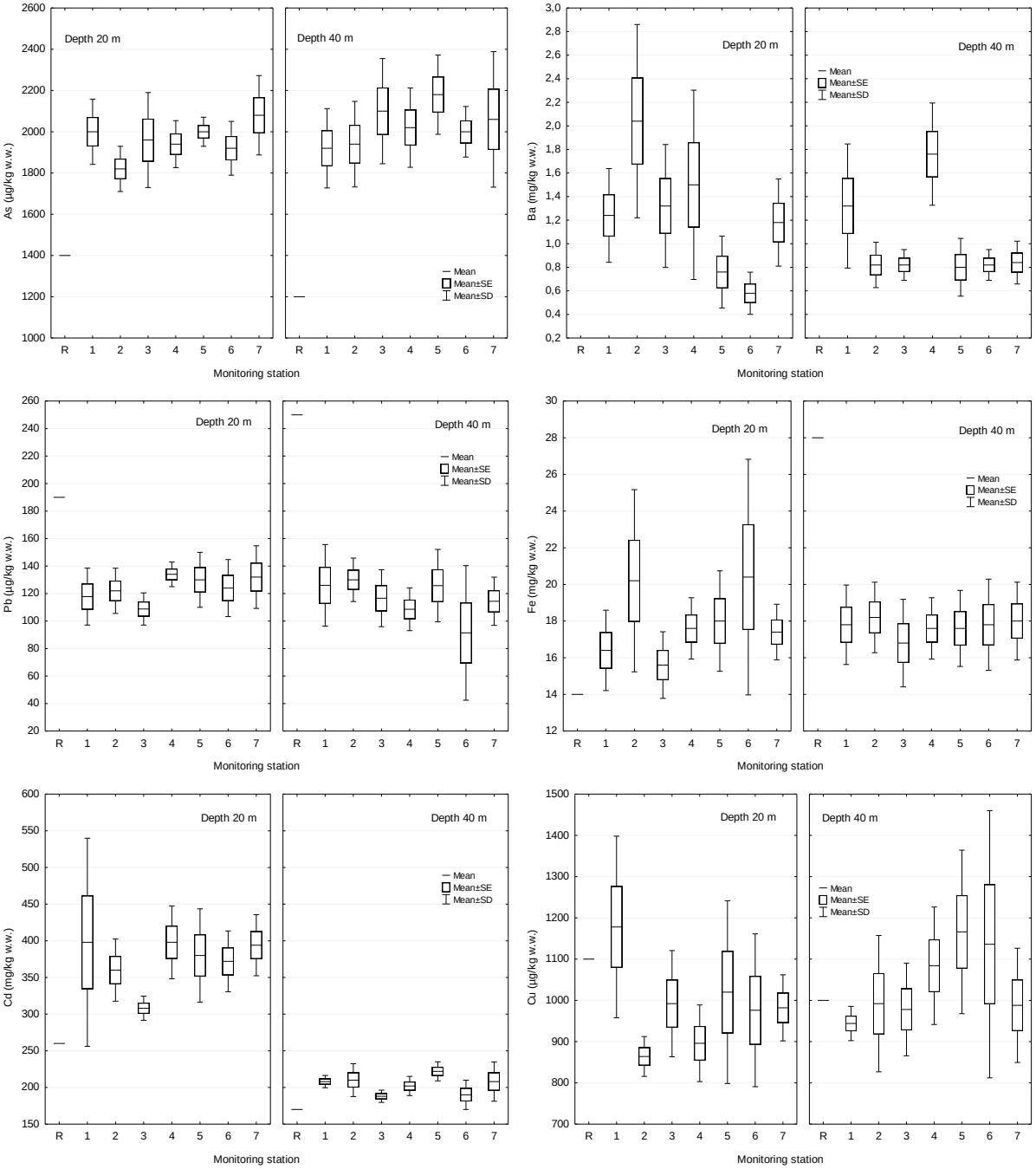


Figure 23. Dibenzothiophene and alkylated dibenzothiophene concentrations in mussels from the different stations around the Johan Sverdrup installation, median, quartiles (box), 10/90 percentiles (bar), n=5.

3.4.2 Metals

Eleven metal concentrations were measured in field transplanted mussels (Figure 24). Provisional reference (PROREF) values for metal concentrations in mussels have been established from the Norwegian coastal monitoring programme (Schøyen et al., 2021). In addition, a recent report has proposed environmental quality standards (EQSs) for chemical contaminants in blue mussel (*Mytilus edulis*) (Ruus et al., 2021, M-1939)

Arsenic concentrations ranged between 1800 and 2200 µg/kg (w.w.) in mussels from the Johan Sverdrup field. These arsenic concentrations were below the PROREF concentrations of 2500 µg/kg w.w. and reflect typical background concentrations in mussel tissue from the Norwegian coast. Interestingly, EQS values for arsenic in mussel tissue have been recently proposed at 2100 µg/kg (w.w.) (Ruus et al., 2021, M-1939), a value like that measured in the tissue from the mussels at Johan Sverdrup. However, the same report questioned the practical applicability of the very low EQS values.


Depth 20 m
Depth 40 m
As (µg/kg w.w.)
Ba (mg/kg w.w.)
Pb (µg/kg w.w.)
Fe (mg/kg w.w.)
Cd (mg/kg w.w.)
Cu (µg/kg w.w.)
Mean
Mean±SE
Mean±SD
R 1 2 3 4 5 6 7
Monitoring station

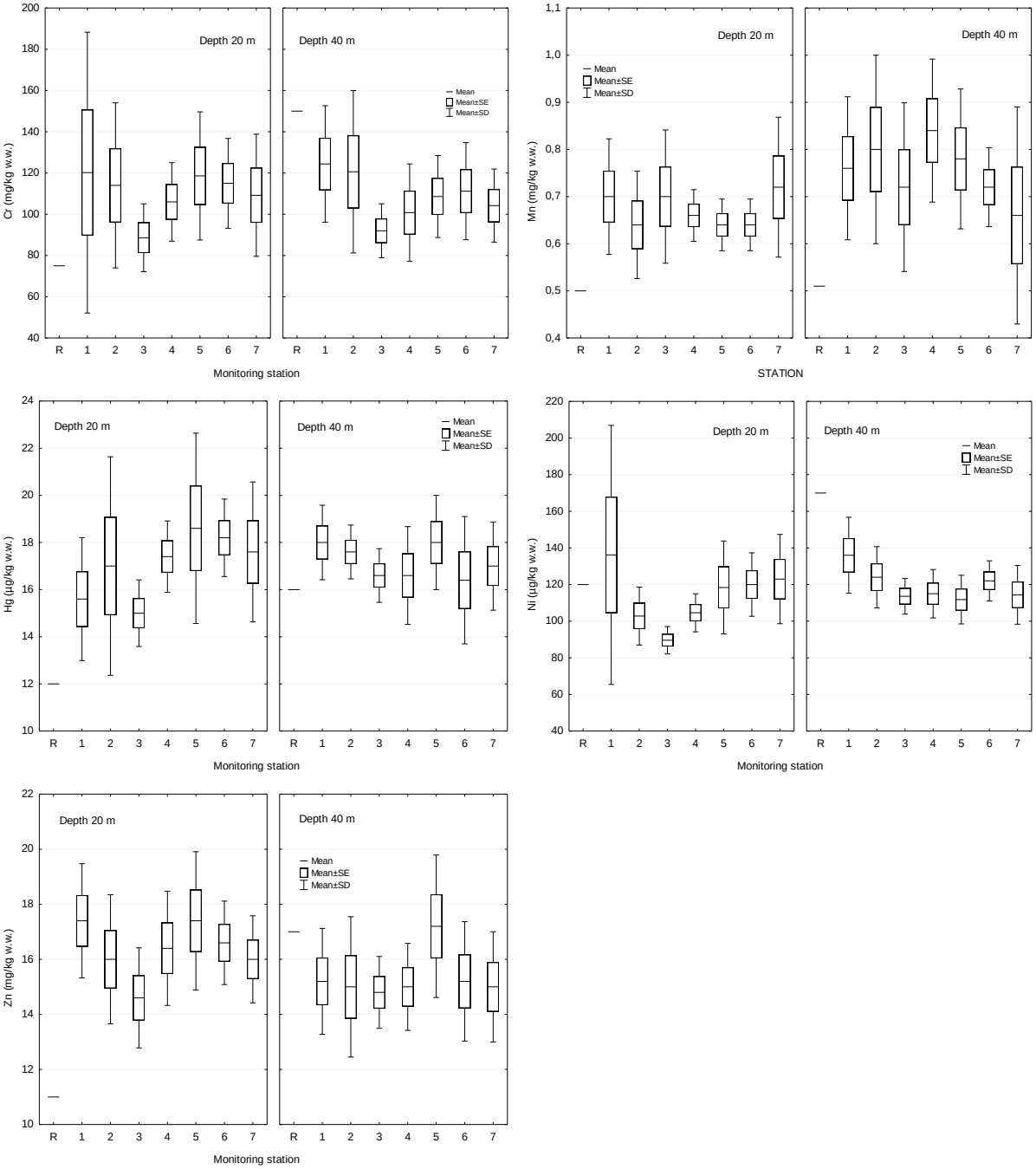


Figure 24. Metal concentrations in the whole mussel homogenates from the seven monitoring stations at Johan Sverdrup for the two depths, top (20 m) and bottom (40 m). values displayed in either µg/kg or mg/kg wet weight (w.w.), n=5.

Median barium concentrations were within a narrow range between 2 and 0.5 mg/kg w.w. Reference values have not been proposed for barium in mussel tissue from the Norwegian coastal monitoring programme. For comparison, maximum barium concentrations in mussel tissue placed in the vicinity of Statfjord A and Gullfaks C for 6 weeks, were 0.65 mg/kg w.w. and 0.48 mg/kg w.w., respectively (Pampanin et al., 2019; Brooks et al., 2011b).

For all metals measured, there were no clear differences between mussels positioned at 20 m depth compared to those measured at 40 m depth. An exception to this was cadmium concentrations. In all Johan Sverdrup stations, cadmium concentrations in mussels from 20 m were approximately double the concentration of mussels from 40 m. Very similar values were found between Johan Sverdrup stations for the same depth, at around 400 µg/kg w.w. in mussels from 20 m and 200 µg/kg w.w. in mussels from 40 m. Suggested PROREF and EQS of 199 and 180 µg/kg w.w. would indicate the 40 m mussels reflected background concentrations, whilst those mussels from the 20 m depth at all stations showed elevated concentrations.

Compared to other offshore oil and gas fields, cadmium concentrations ranged between 1000 and 2000 µg/kg w.w. in mussels deployed for 6 weeks as Statfjord A and reference stations, whilst the day zero group (not placed in the field) ranged between 110 and 150 µg/ kg w.w. (Pampanin et al., 2019). This was also found for the WCM2011, when mussels were placed at Gullfaks C for 6 weeks, whilst their day zero population contained cadmium concentrations around 200 µg/kg w.w. (Brooks et al., 2011b). Therefore, the cadmium concentrations measured in the mussels from 20 m at Johan Sverdrup were slightly elevated but were much less than those measured in mussels from Statfjord A and Gullfaks C fields.

Median chromium concentrations ranged between 90 and 120 µg/kg (w.w.) in mussels from the Johan Sverdrup field, with no significant differences between the mussel groups. These chromium concentrations were below the provisional reference (PROREF) concentrations of 361 µg/kg w.w. and reflect typical background concentrations in mussel tissue from the Norwegian coast. The proposed EQS value for Chromium in mussel tissue is 425 µg/kg (w.w.), a value markedly higher than that measured in the tissue from the mussels at Johan Sverdrup.

Median copper concentrations ranged from 850 to 1200 µg/kg (w.w.) in mussels from the Johan Sverdrup field, with no significant difference between the mussel groups or mussel depths. The copper concentrations measured were below the provisional reference (PROREF) concentrations of 1400 µg/kg w.w. and reflect typical background concentrations in mussel tissue from the Norwegian coast. No additional EQS value has been proposed for copper in mussel tissue.

Median iron concentrations ranged from 15 to 20 mg/kg w.w. with no noticeable difference between mussel stations or between depths. No PROREF or EQS values are suggested for iron in mussel tissue. Compared to other offshore oil and gas fields, iron concentrations ranged between 11 and 50 mg/kg w.w. in mussels deployed for 6 weeks as Statfjord A, with highest concentrations in the day zero group (Pampanin et al., 2019). Whilst mussels placed out at Gullfaks C for 6 weeks measured iron concentrations between 3 and 32 mg/kg w.w., with the day zero group also recording the highest concentrations (Brooks et al., 2011b).

Median lead concentrations ranged from 90 to 110 µg/kg (w.w.) in mussels from the Johan Sverdrup field, with no significant difference among the mussel groups or mussel depths. The lead concentrations measured were below the provisional reference (PROREF) concentrations of 615 µg/kg w.w. and reflect typical background concentrations in mussel tissue from the Norwegian coast. An EQS value of 195 µg/kg w.w. has been proposed for lead in mussel tissue, which is still above the concentration measured in the mussels around Johan Sverdrup.

Median manganese concentrations ranged from 0.65 and 0.85 mg/kg (w.w.) in mussels from the Johan Sverdrup field, with no significant difference between the mussel groups or mussel depths. No PROREF or EQS value have been proposed for manganese in mussel tissue.

Median mercury concentrations ranged from 15 to 18 µg/kg (w.w.) in mussels from the Johan Sverdrup field, with no significant difference between the mussel groups or mussel depths. The mercury concentrations measured were slightly above the PROREF concentrations of 12 µg/kg w.w. and the proposed EQS of 5.7 µg/kg w.w. The authors point out that the proposed EQS value were based partly on the biomagnifying properties of mercury, and that most environmental concentrations will exceed the proposed EQS (Ruus et al., 2021). An EQS value of 20 µg/kg w.w. currently exists, which exceeds the mercury concentrations of the mussels from the Johan Sverdrup field. Compared to other offshore oil and gas fields, mercury concentrations ranged between 7 and 16 µg/kg w.w. in mussels deployed for 6 weeks as Statfjord A and reference stations, whilst the day zero group (not placed in the field) ranged between 11 and 15 µg/kg w.w. (Pampanin et al., 2019). For the WCM2011, mussels placed at Gullfaks C for 6 weeks showed mercury concentrations between 10 and 20 µg/kg w.w. including the day zero group (Brooks et al., 2011b). These studies reflect very similar mercury concentrations to those measured in mussels from Johan Sverdrup field.

Median nickel concentrations ranged between 90 and 135 µg/kg (w.w.) in mussels from the Johan Sverdrup field, with no significant differences among the mussel groups. These nickel concentrations were below the provisional reference (PROREF) concentrations of 290 µg/kg w.w. and reflect typical background concentrations in mussel tissue from the Norwegian coast.

Median zinc concentrations ranged between 14.5 and 17.2 mg/kg w.w. in mussels from the Johan Sverdrup field, with no significant differences among the mussel groups. These zinc concentrations were narrowly below the provisional reference (PROREF) concentrations of 17.7 mg/kg w.w. and reflect typical background concentrations in mussel tissue from the Norwegian coast.

3.5 Biological effects measurements in mussels

3.5.1 Food availability in field exposed mussels

Photographic images were taken of the food excreted by the mussels from a selection of the monitoring stations around the Johan Sverdrup installation. This assessment was performed in mussels from monitoring station 1, 2, 4 and 7 from top (20 m) and bottom (40 m) only. For the assessment of food availability, it was important to differentiate between the appearance of mussel faeces that have gone through the digestive system of the mussel and pseudo-faeces, which are particles that have been sorted by the gill cilia and excreted without being ingested. Clear structural differences exist between faeces, representing digested food, and pseudo-faeces as shown in Figure 25.

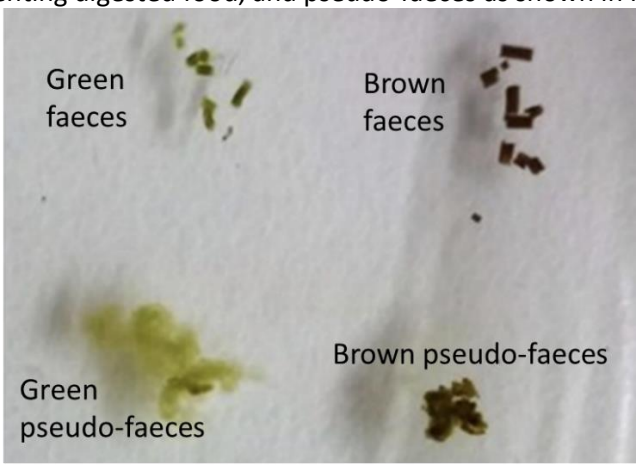


Figure 25. Typical mussel faeces and pseudo-faeces (taken from Galimany et al. 2018).

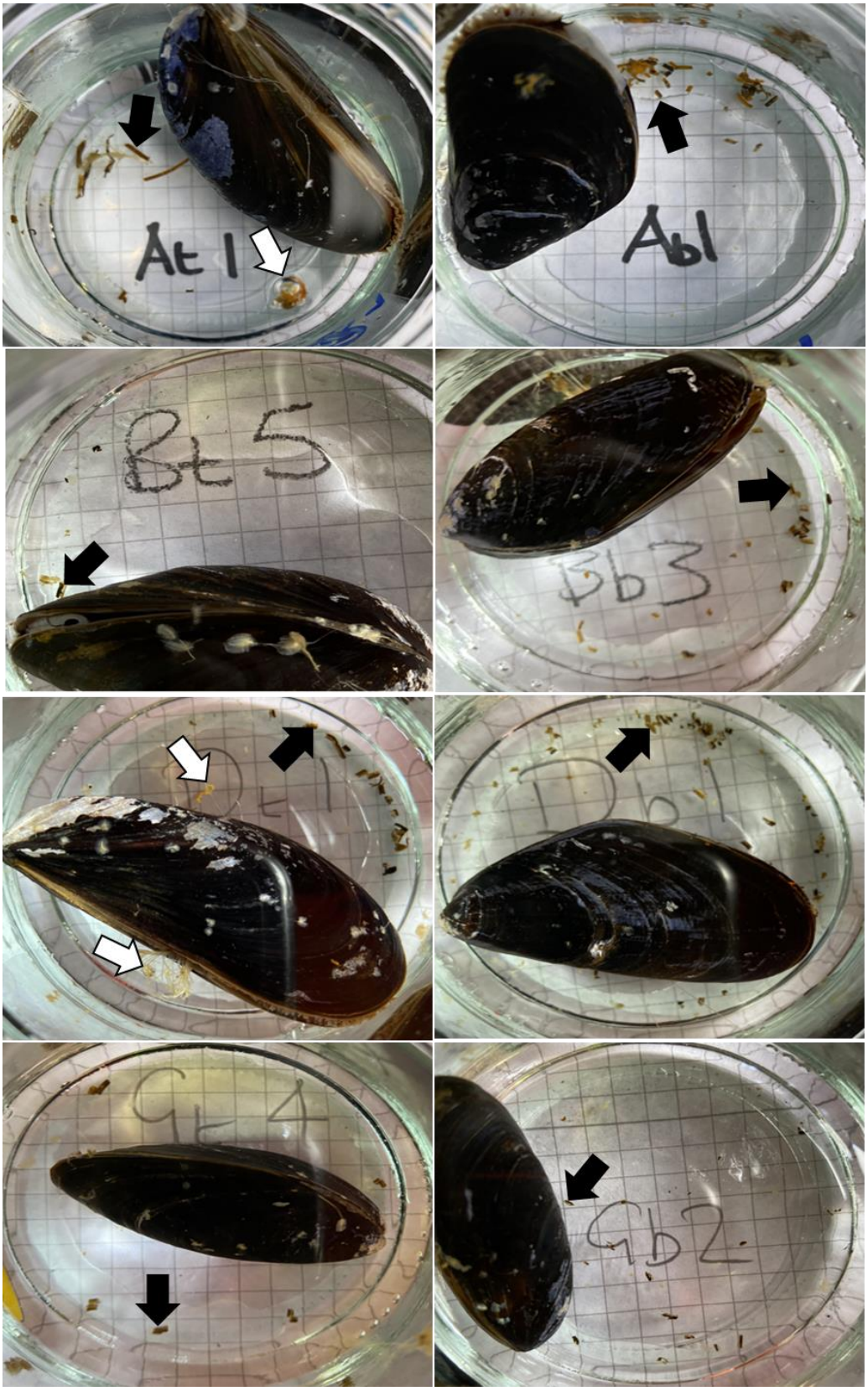


Figure 26. Representative images of the excreted material from the gut of the mussels during a 6-8 h depuration period. Coded monitoring stations 1 (A), 2 (B), 4 (D) and 7 (G), top (t), bottom (b), replicates 1 to 5. Black and white arrows denote examples of faeces and pseudo-faeces respectively.

All field transplanted mussels from the monitoring stations showed some faeces within the depuration period. Representative photos of mussel faeces excreted by individual mussels are shown in Figure 26. There was no apparent difference in the amount of mussel faeces between monitoring stations. There was a tendency for mussels held at the deeper depth of 40 m to have slightly more faeces than those mussels from the 20 m depth. However, no quantitative measurement was taken of the faecal material. Based on these findings, food availability was considered sufficient for the field exposed mussels and should not act as a confounding factor in the overall assessment of mussel health status.

3.5.2 Condition index

The condition index, measured as the dry weight of the mussel soft tissue divided by the dry weight of the shell multiplied by 100 is presented for all monitoring stations (Figure 27). For the monitoring stations at Johan Sverdrup, the condition indices of the mussel groups were comparable with median values ranging between 13 and 17, with 12 of 14 values above 15. In contrast, the reference groups showed slightly lower condition indices with median values ranging between 9 and 13, whilst the day zero (T0) mussels had the lowest median condition index of 8.2.

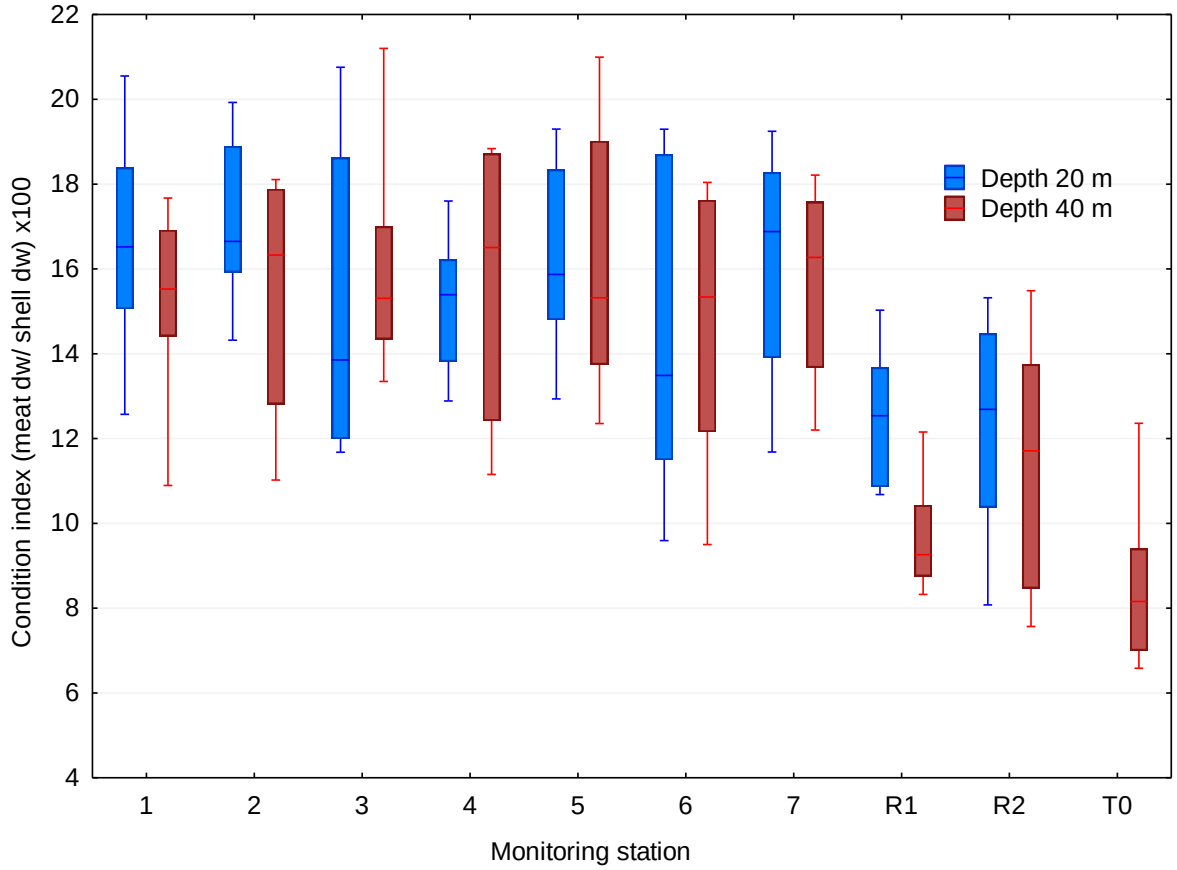


Figure 27. Condition index in mussels from the different monitoring stations at approximate depths of 20 and 40 m (median ± quartiles (box) and 10/90 percentiles (outer line, n=15).

3.5.3 Stress on stress

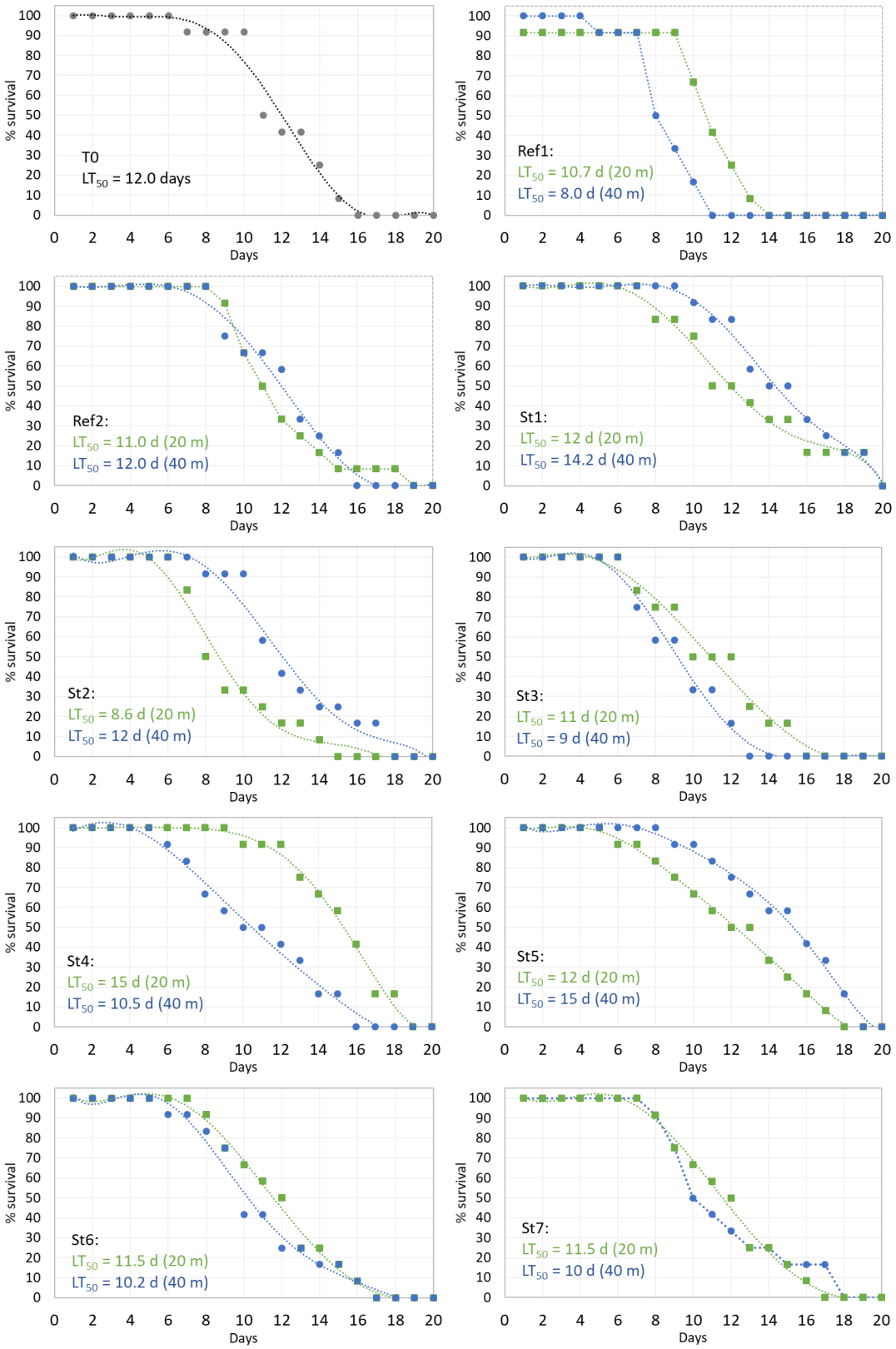


Figure 28. Stress on stress in day zero (T0) and field transplanted mussels around the Johan Sverdrup field and two reference sites in the North Sea.

Table 5. Summary of stress of stress showing the days corresponding to 50% mortality (LT_{50}) for each mussel groups.

Monitoring station	LT_{50} (d)	
	top (20 m)	bottom (40 m)
T0	12	
Ref 1	10.7	8.0
Ref2	11.0	12.0
St 1	12.0	14.2
St 2	8.6	12.0
St 3	11.0	9.0
St 4	15.0	10.5
St 5	12.0	15.0
St 6	11.5	10.2
St 7	11.5	10.0

The LT_{50} values ranged from 8 to 15 days (Figure 28 and Table 5). ICES assessment criteria exist for stress on stress with a background (BAC) and environmental (EAC) assessment criteria of 10 and 5 days respectively (Davies and Vethaak, 2012). In the present study, only three LT_{50} values were below the BAC including station 2 at 20 m and station 3 at 40 m, as well as reference station 1 at 40 m. In fact, the lowest LT_{50} was measured in mussels from the 40 m reference 1 station. The LT_{50} values of all other mussel groups were either on or above the ICES BAC and indicative of healthy mussels with little impact of the field exposure.

3.5.4 Micronuclei formation

Micronuclei frequency in mussel haemocytes from day 0 and field exposed groups are shown in Figure 29. Baseline frequencies for micronucleated haemocytes are usually between 0.5 and 5.0 MN per 1,000 cells (Bolognesi and Fenech, 2012), and ICES BAC for micronuclei frequency in *Mytilus edulis* have been suggested at 2.5 MN per 1000 cells (Davies and Vethaak, 2012). Taking this into account, the mean micronuclei frequencies were above this BAC in four groups, in station 3 and 4 top mussels and station 1 and 2 bottom mussels. However, mussels from stations 4 top and stations 1 and 2 bottom, were only marginally above the BAC on or less than 3 MN per 1000 cells. Highest MN frequency was found in mussels from station 3 top at 4.5 MN per 1000 cells. Fourteen of the eighteen mussel groups recorded micronuclei frequencies below the BAC value. However, no significant differences between the mussel groups were found (Kruskal-Wallis ANOVA, $p>0.05$).

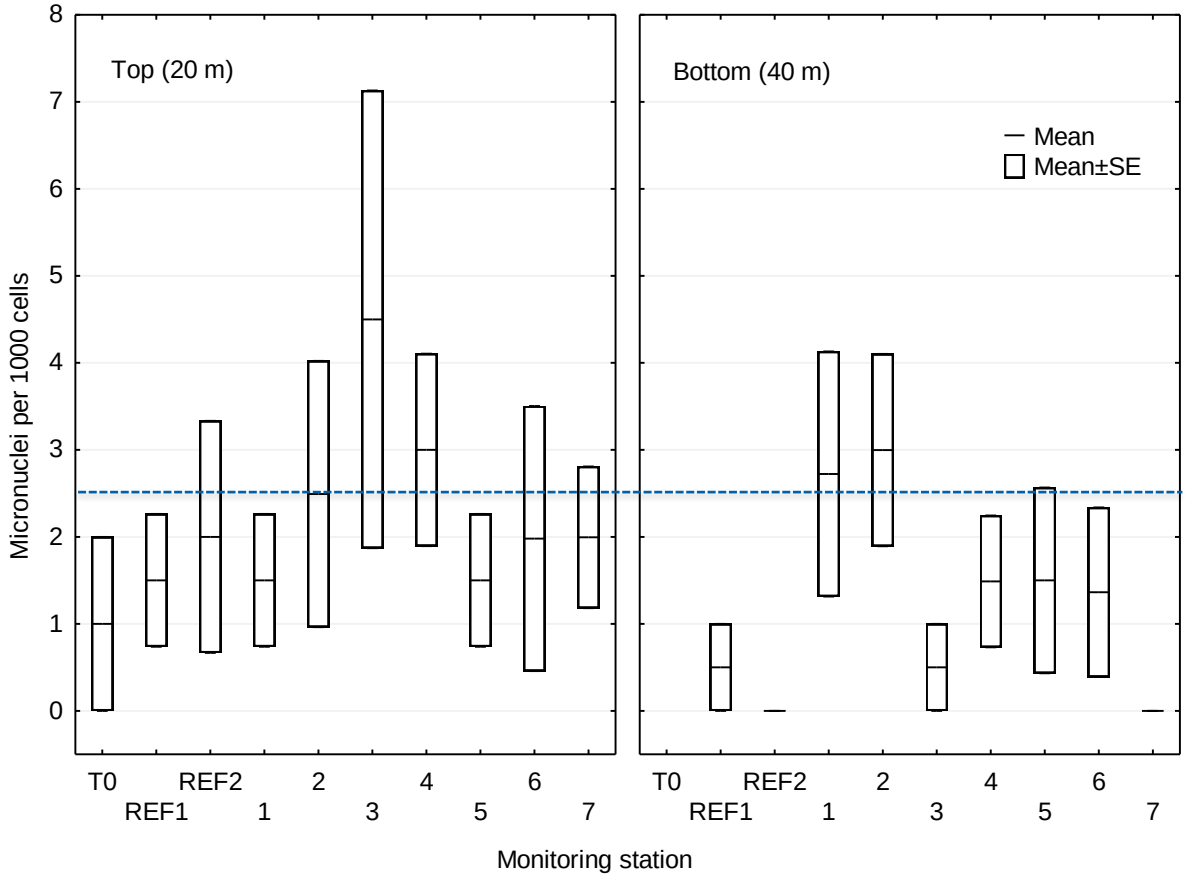


Figure 29. Micronuclei frequency per 100 cells in in day zero (T0) and field transplanted mussels around the Johan Sverdrup field and two reference sites in the North Sea. No significant differences between groups (Kruskal-Wallis ANOVA, p>0.05). Dashed line indicates ICES BAC of 2.5 MN/1000 cells.

3.5.5 Lysosomal membrane stability

LMS results presented as mean labilization time for the different mussel groups are shown (Figure 30). Lowest labilization times were found in day zero (T0) mussels with a mean of approximately 18 min, all field exposed mussels showed a labilization time of greater than 23 min, with some mussels measuring a labialization time of 36 min. Despite these apparent differences, there was no significant difference in LMS labilization time among the groups (ANOVA, Tukey, p>0.05). The ICES BAC for LMS is 20 min (Davies and Vethaak, 2012). All field exposed mussels were above this BAC and were indicative of healthy mussels.

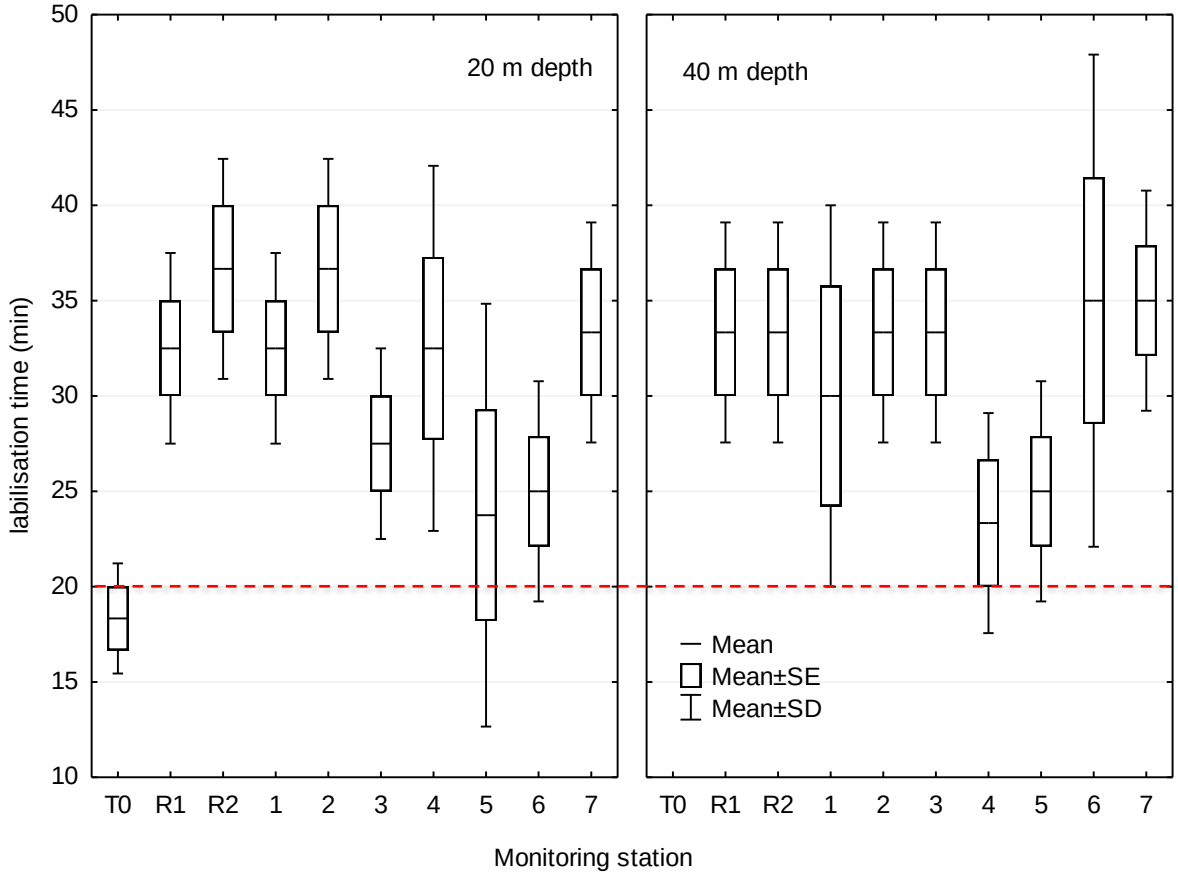


Figure 30. Lysosomal membrane stability (LMS) measured in hepatocytes of mussels from day zero (T0) and field transplanted groups around the Johan Sverdrup field and two reference sites in the North Sea. Field transplanted mussels were deployed at a depth of approximately 20 m and 40 m. No significant differences between groups (ANOVA, Tukey p>0.05). Dashed line indicates ICES BAC of 20 min (note EAC of 10 min).

3.5.6 Metallothionein

Metallothionein concentration in digestive gland tissue of mussels from the different groups are presented (Figure 31). Some variation in MT was found among the mussel groups, with highest concentrations found in mussels from monitoring station 7 at 40 m (mean 162 µg MT/g tissue) and at 20 m (mean 143 µg MT/g tissue), and the lowest at station 5 at 40 m (mean 35 µg MT/g tissue). In general, most other stations reported similar concentrations. The reference groups for both depths were comparable to the groups deployed at the Johan Sverdrup field. An ICES BAC has not been established in mussels for metallothionein using the spectrophotometric method and cannot be used as a threshold.

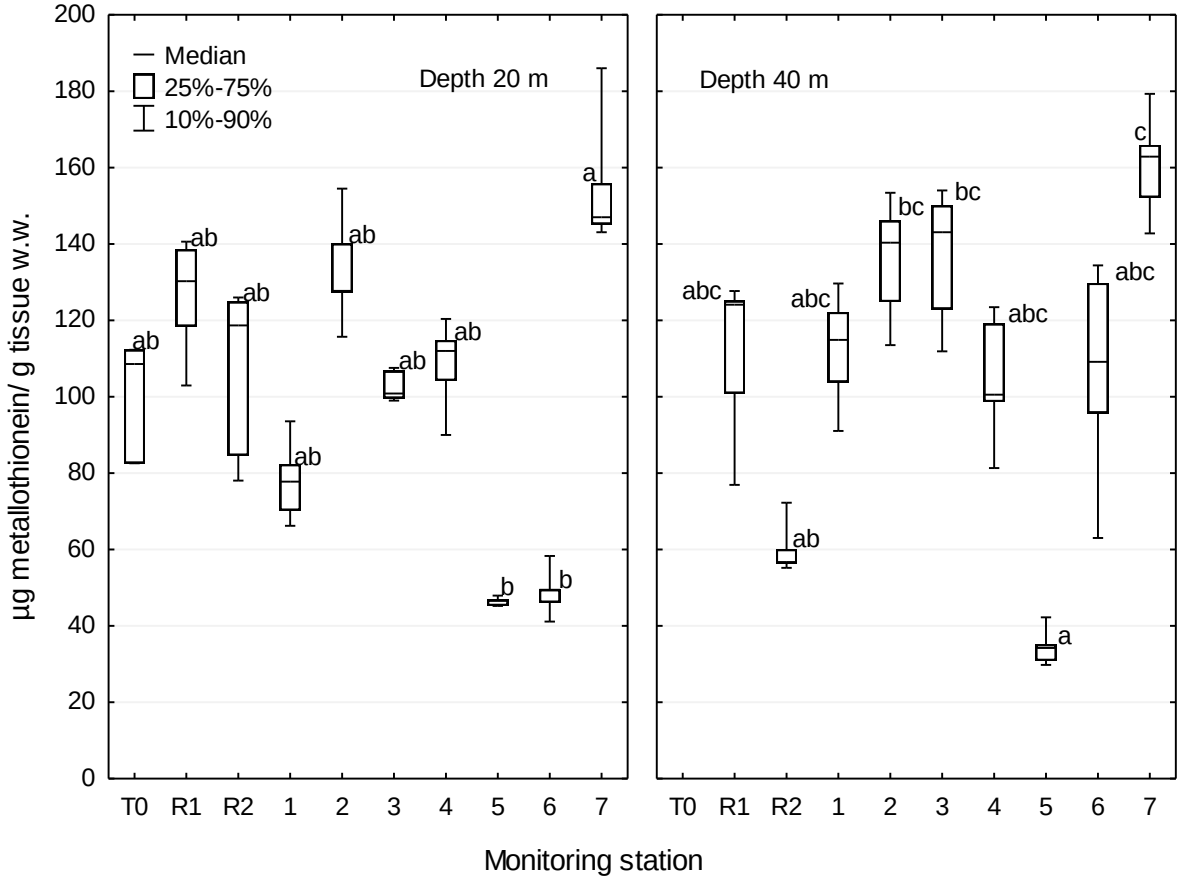


Figure 31. Metallothionein concentration in digestive gland tissue of mussels from the Johan Sverdrup monitoring stations, reference stations and the day zero (T0) groups. Median ± quartiles (Box) 10/90 percentiles (outer line). Kruskal-Wallis $p<0.05$, $n=5$. Letters denote significant similarities between the groups.

3.5.7 Mussel Histology

3.5.7.1 Digestive gland

Histological examination of the three structural features of the digestive gland tissue of the mussels from the Johan Sverdrup monitoring stations are presented (Figure 32). Vacuolar degeneration and tubules atrophy were comparable among the groups. Vacuolar degeneration scored low severity of 0 or I in 50-80% of mussels in the different groups with no obvious differences among them. For tubules atrophy, a low severity score of 0 - I was shown in 80 -100% of the mussels from the different groups, with little difference among them. For necrosis, the highest severity score of III was recorded in mussels from stations 5 and 6 at 20 m depth.

3.5.7.2 Gill histology

Histological examination of the four structural features of the mussel gills from the Johan Sverdrup monitoring stations are presented, with 15 mussels per group (Figure 33). With regards to hypertrophy and hyperplasia of the gill epithelium most groups had a low severity score between 0-I in 50 to 80% of mussels, with no obvious differences among between the groups. Similar low severity scores of 0-I for gill fusion were recorded in all groups, with only station 5 mussels at 20 m and 40 m demonstrating

a severity of II in 20% of individuals. For cilia erosion, station 5 20 m, stood out as the only group to exhibit severity level III, which was found in 20% of the individuals. Otherwise, low severity (0-I) was shown in 50%-80% of individuals for all groups. Finally, gill lumen enlargement in the mussels was comparable between the Johan Sverdrup stations at a low level of severity, an exception to this was station 6 at 20 m that showed 20% exhibiting severity level III.

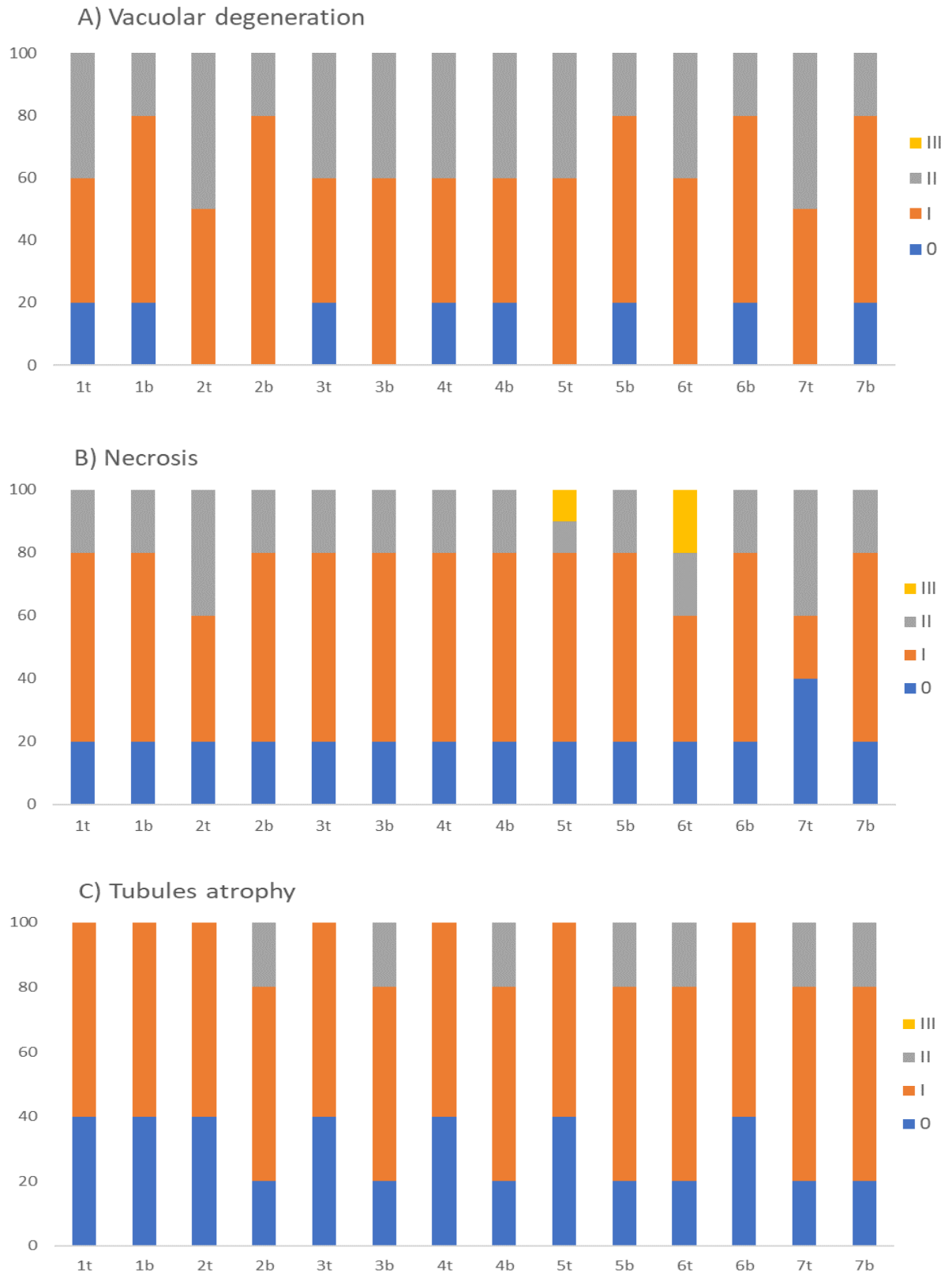


Figure 32. Histological markers in the digestive gland of mussels from the monitoring stations at the Johan Sverdrup field. A) Vacuolar degeneration; B) Necrosis; C) Tubules atrophy. Scale of impact from 0 no impact to III severe impact. 15 mussels per groups

Figure 33. Histological markers in the gill tissue of mussels from the monitoring stations at the Johan Sverdrup field. A) Hypertrophy and hyperplasia of gill epithelium; B) Gill cilia erosion; C) Gill fusion; D) Gill lumen enlargement. Scale of impact from 0 no impact to III severe impact.

3.6 Integrated Biological Response index (IBR/n)

The integrated biological response (IBR/n) was used to combine the individual biomarker results in order to provide an overall assessment of mussel health status from the different groups. The biomarkers included in the IBR/n calculation were CI, SoS, LMS, MN and MT. Histology (digestive gland and gill) was not included in the IBR/n assessment. The spider plots indicate the contribution of the different biomarkers to the overall IBR/n value (Figure 34). The size of the coloured areas on the spider plots is dependent on the relative biomarker responses, which are summarised in the bar chart at the bottom of the figure. Highest IBR/n were found in T0 mussels, closely followed by R1b and 3t. The calculated IBR/n was comparable between the Johan Sverdrup mussels and the reference groups. Although differences in the IBR/n were found between the top and bottom mussels at some of the stations, there was no obvious pattern to show that mussels from either depth were more or less affected than the other. For the day zero (T0) group, the biological responses were markedly different to the field exposure groups, with LMS and CI contributing strongly to the IBR/n score.

3.7 Principle component analysis (PCA)

Principal component analysis (PCA) was used to separate the main variables responsible for the variance of chemical body burden and biological effects measured in field transplanted mussels. Overall, the PCA showed a clear spatial differentiation between mussel groups from the Johan Sverdrup installation and the two reference stations (Figure 35). PC1 accounted for 39.42% of variance and showed a separation between the groups with higher and lower PAH and metals Cd and Mn, Pb and As. PC2 explained 21.91% of the variance. The PCA showed that Johan Sverdrup transplanted mussels had highest concentrations of PAHs and certain metals (Mn, Hg, Cd, As) than the reference groups, associated with stronger responses in CI, SoS and MN.

Several statistically significant associations were shown by the correlation analysis between the chemical measurements and the biological responses in the mussel groups (Table A 1). Positive correlations between condition index and SumPAH, SumNPD, Hg, As and Mn were found, with negative correlations detected between condition index and Pb, Ni. In addition, negatively correlations were found between LMS and Sum PAH, MN an Ni and MT and Zn.

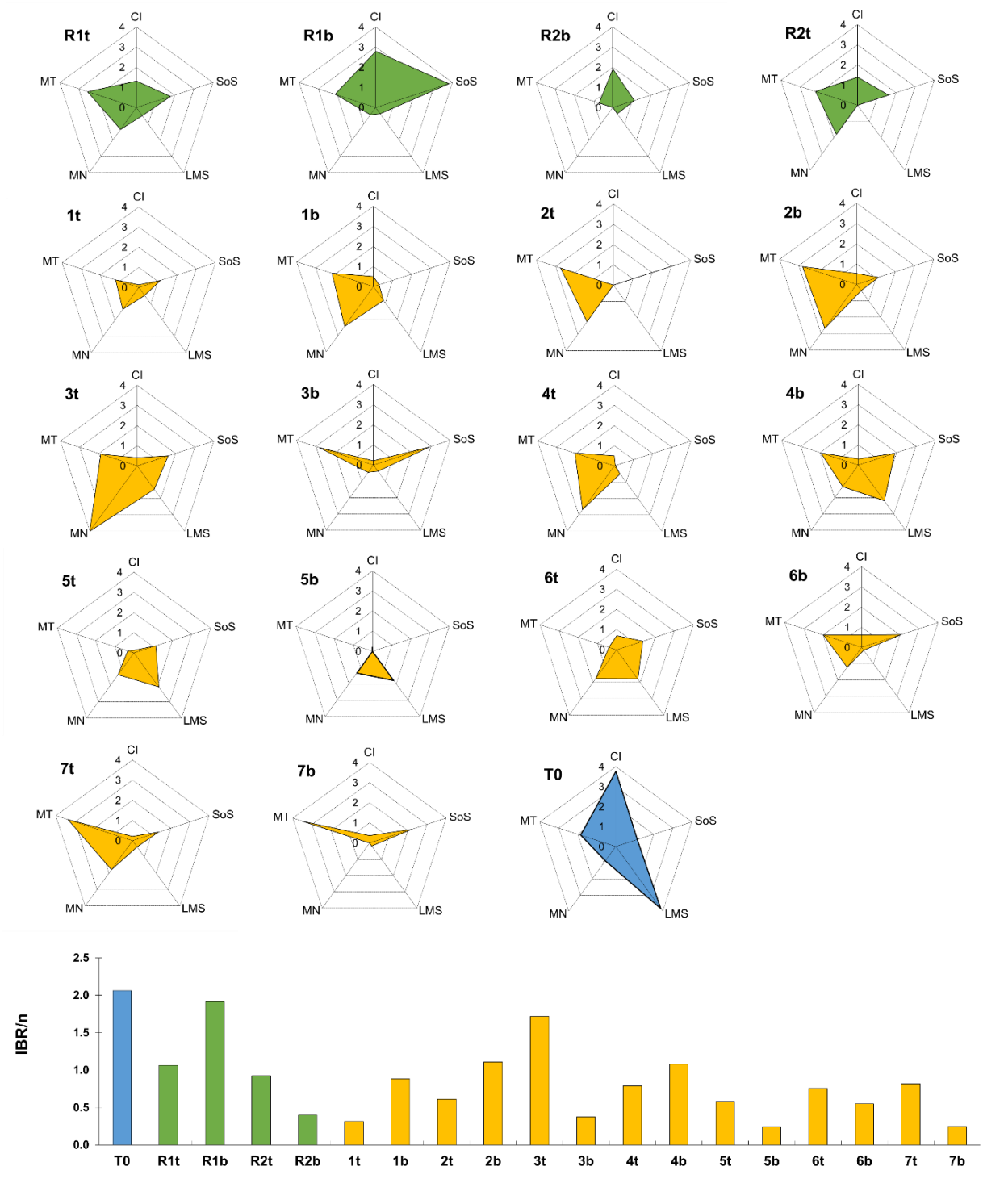


Figure 34. Integrated biological response (IBR/n) calculated from star plots of mean normalised biomarker data in mussels located from the pre-transplanted population (T0) and in mussels held in the water column at approximately 20 m (t) and 40 m (b) the seven Johan Sverdrup stations and the two reference stations (R1 and R2) for a 6-7 week period. Condition index (CI), Stress on stress (SoS), lysosomal membrane stability (LMS), micronuclei (MN), metallothionein (MT).

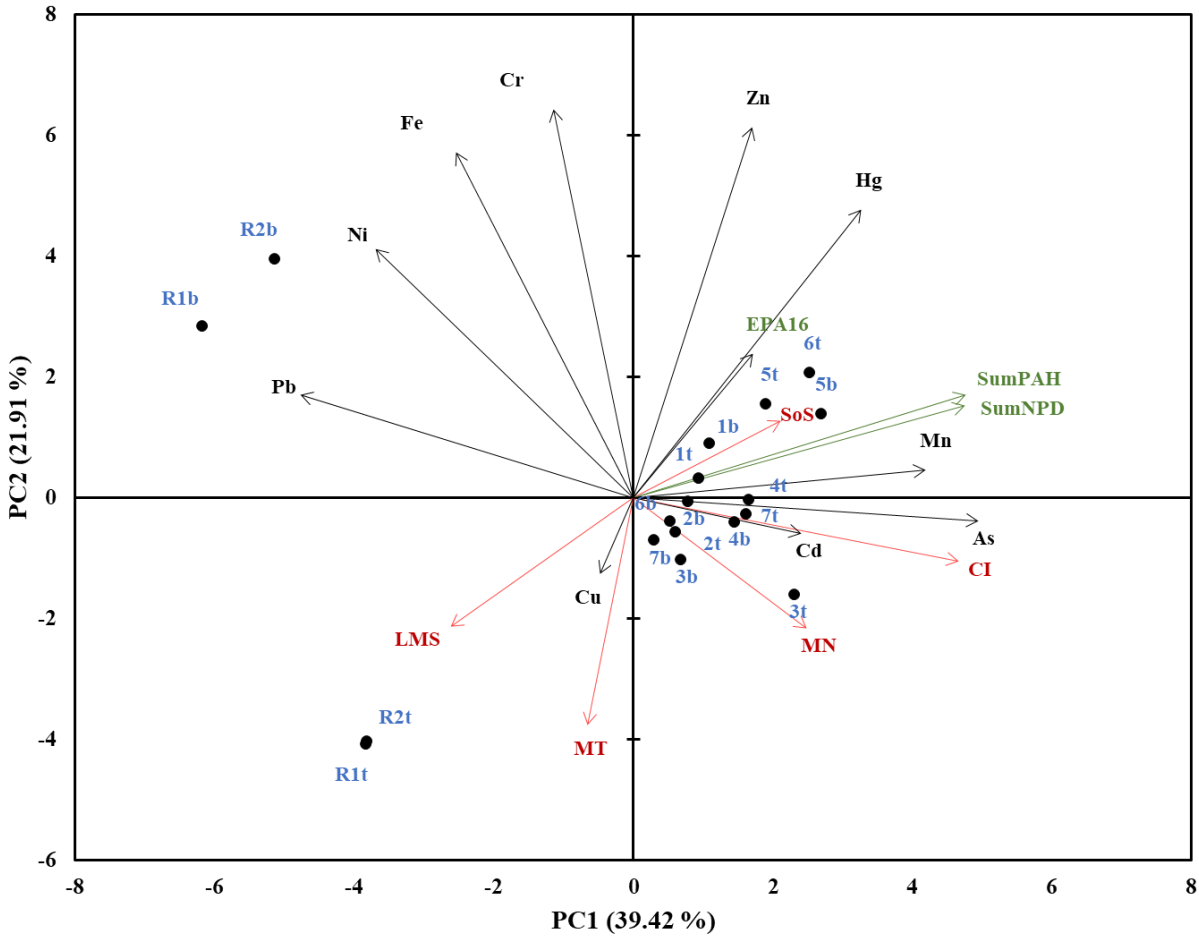


Figure 35. Principle component analysis of chemical measurements (metals- black, PAH - green) and biological responses (red) in mussels held in the water column at approximate depths of 20 m (top , t) and 40 m (bottom, b) at the nine mussel stations (1, 2, 3, 4, 5, 6, 7, R1, R2) for a 6-7 week period (blue). CI – Condition index; SoS – Stress on stress; LMS – Lysosomal membrane stability; MN – micronuclei; MT – metallothionein; EPA16 – Sum of EPA PAH16; Sum PAH – Sum of PAH; SumNPD – sum of naphthalene, phenanthrene and dibenzothiophenes alkylated and parent compounds; AS – Arsenic; Cd – Cadmium; Cr – Chromium; Cu – Copper; Fe – Iron; Pb – Lead; Mn – Manganese; Hg – Mercury; Ni – Nickel; Zn – Zinc.

4 Discussion

4.1 TCC exposure and physicochemical parameters

The monitoring programme was designed to investigate the potential impacts of the TCC discharge effluent on the marine environment, using chemical and biological effects measurements in exposed mussels as a proxy. A typical approach with biological effects monitoring studies using mussels is to measure a suite of chemical and biological responses following an extended exposure duration, such as 4-8 weeks, to the discharged effluent. In the current study, although mussels were placed on the monitoring rigs for a period of 6-7 weeks, the exposure duration to the TCC discharge was limited to only 3 days. Furthermore, the 3-day exposure to the TCC was at the end of the mussel deployment

period. This is likely to have implications on the bioaccumulation of chemicals from the TCC, as well as on the timing of the biological responses in the mussels. However, based on the TCC discharge from the Johan Sverdrup installation between Jan 2020 and May 2021, a 3-4-day average TCC discharge window per month was recorded. Therefore, the exposure duration of 3 days in the present study was representative of the sporadic nature and regularity of the TCC discharge.

4.1.1 Modelling of TCC discharge

A particle DREAM model describing the dispersion of the TCC into the seawater recipient from a depth of 17 m, was applied to see if the position of the monitoring stations and the depth of the mussels were suitable to monitor the TCC plume. The model generates particles at the discharge point, which are transported with the currents and turbulence in the sea. Inputs into the model include information on the TCC slurry discharge, such as temperature (25°C), particle diameter (1 µm to 1 mm), variable release rate between 2nd and 4th May 2021. As well as physical factors such as, wind strength and direction, the depth of discharge outlet (17 m), and the properties of the seawater recipient, temperature, salinity, turbidity, ocean currents from monitoring stations 1, 3 and 6.

Most of the dense TCC slurry was predicted to sink quickly from the 17 m discharge outlet, some particles (10%) were expected to escape from the main slurry stream and distribute at the same depth as the discharge depth. This would result in an expected exposure of the TCC to mussels in the 18-20 m depth group. The model predicted that the due to the presence of a thermocline at 50 m, the particles were likely to be trapped above this depth. As a result, the mussels held at 40-45 m where likely exposed to some elements of this trapped plume.

Based on the model, the locations of the mussels around the Johan Sverdrup installation were thought to be well positioned to optimise the exposure of the mussels to the TCC plume. However, despite being suitably positioned, the actual physical exposure of the mussels to the TCC was expected to be small due to the short 3-day release window and episodic exposure strongly influenced by the tidal forces in the prominently north-south direction during the release period, as confirmed by the current meters on three of the monitoring rigs.

The turbidity sensors placed for the duration of the exposures on the monitoring rigs showed episodic peaks in turbidity during the 6-7-week exposure. These appeared to be indicative of adverse weather conditions since the peaks in turbidity did not coincide with the timing of the TCC discharge window. Some increases in turbidity were observed at Johan Sverdrup during the 2nd-4th May 2021, when the TCC was released, although a peak in turbidity was also reported at the reference station during this time, so it was unclear whether the increase in raised turbidity was a reliable measure.

4.2 Chemical accumulation

With the absence of a produced water discharge at the Johan Sverdrup installation the bioaccumulation of PAH in the field exposed mussels was expected to be low. The sum of PAH concentrations in the mussels from the Johan Sverdrup field did show significantly higher concentrations than the reference mussels. However, there was no pattern with proximity to the Johan Sverdrup installation and all Johan Sverdrup mussel groups showed concentrations that were typical of offshore reference groups. Differences between the reference and Johan Sverdrup mussels in this study were most likely reflecting differences in oceanic water bodies, rather than a point source discharge. However, the slight elevation in PAH in mussels from the Johan Sverdrup field compared to

the reference mussels, may have derived from oil that was not removed from the TCC discharge, creating a small general elevation of PAH in the surround waters compared to the reference areas.

When compared to other offshore monitoring programmes where discharges of produced water from offshore installations in the North Sea have been assessed, the sum PAH values of 35 to 45 µg/kg (w.w.) in mussels from Johan Sverdrup were 10 times less than that measured in mussels held 500 m downstream from the Statfjord A and B for 6 weeks (Pampanin et al., 2019). Further, mussels placed 10 km downstream of these platforms were overall double the sum PAH measured at Johan Sverdrup. Therefore, it was reasonable to conclude that PAH accumulation and affect was not a factor of concern in the present study.

Since the TCC effluent was composed of crushed bedrock, metals naturally present in the rock are potential sources of contamination to the mussels. Furthermore, the metals present in the rock may become transformed as the trapped and complexed metal may become oxidised, influencing metal speciation and bioavailability. It may be assumed that the metal accumulated in the whole mussel tissue of field exposed mussels will reflect the metals that are bioavailable. A government initiated Norwegian classification scheme has been established to indicate the level of potential risk posed by the concentration of metal(s) in mussel tissue. The scheme divides the metals concentrations into five categories of increasing risk including: I – insignificant; II – moderate; III – marked; IV – severe; and V- extreme (Table 6). All measured concentrations of metals in the present study were either on or below the classification I of insignificant. Only Cd showed clear differences between top and bottom mussels with the 20 m mussels measuring Cd concentrations double that of mussels from 40 m. Although all values were below the Norwegian classification scheme, the Cd concentrations in the top mussels were above the proposed EQS of 199 µg/kg (w.w.), which was based on data from the Norwegian coastal monitoring programme (Ruus et al. 2021).

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)					PROREF	Proposed EQS
	Insignificant (I)	Moderate (II)	Marked (III)	Severe (IV)	Extreme (V)		
As	10	25	70	140	>140	2.5	0.21
Cd	0.4	1.8	4	8	>8	0.18	0.199
Cu	2	6	20	40	>40	1.40	-
Cr	0.2	1	3	10	>10	0.36	0.425
Pb	0.6	3	8	20	>20	0.2	0.615
Hg	0.04	0.1	0.3	0.8	>0.8	0.012	0.0057 (0.02*)
Ni	1	5	10	20	>20	0.29	2.322
Zn	40	80	200	500	>500	17.66	-

*existing EQS value

When comparing the measured concentrations of metals in the TCC slurry prior to discharge only concentrations of Ni were found to be slightly elevated with a classification scheme of moderate (Class III). However, this slight elevation in Ni from the TCC did not relate to any increase in Ni concentration in the mussels. Cadmium concentrations in the TCC slurry were also not elevated and could not explain the higher Cd concentrations in the mussels from all the Johan Sverdrup field stations at 20 m, compared to mussels at 40 m.

4.3 Biological responses in mussels

For all field exposed mussel groups, the size range was comparable, with the vast majority of mussels ranging between 50 and 60 mm in length. The day 0 mussels, however, were slightly smaller between 45 and 50 mm. The impacts on chemical bioaccumulation and biological response should be considered.

Food availability was measured in order to confirm that the mussels placed in cages in the North Sea had sufficient food available to sustain life and ideally to provide optimal health. This question was made more important since mussels were placed at two depths of 18-20 m and 40-45 m. Stratification of the water column, with a thermocline, was found at around 50 m, which was below the bottom mussel groups and thought unlikely to interfere with food ability. Chlorophyll sensors were used on profiles taken at the start and end of the exposure, which confirmed the presence of phytoplankton in the water column. In addition, stomach clearance tests confirmed convincingly that both top and bottom mussels from the selected monitoring stations had enough food available and would not contribute as a confounding factor to the biological responses measured.

The condition index is influenced by both reproductive stage and nutritional status. Although no differences in condition index were found between the mussels from the Johan Sverdrup field, condition indices were markedly lower in the day zero (T0) and reference groups. One possible explanation for these differences in condition index was that the mussels in the day zero group were at a lower stage of reproductive development when sampled at day zero (T0) in March. The field transplanted mussels produced a higher condition index as temperatures slowly increased and when sampled in May had a more developed gonad. The difference between the reference and Johan Sverdrup mussel groups may therefore be related to temperature differences between these two areas. The temperature logger data demonstrated that the reference mussels were exposed to colder temperatures between 6 and 6.5℃ for the first few weeks rising to 7 and 7.5℃ in the final few weeks. In contrast, mussel at the Johan Sverdrup field were exposed to warmer and more stable temperatures of around 7.5℃ for the whole 6-7-week exposure duration. The warmer waters were likely to speed up reproductive development and lead to higher condition indices.

Food availability may also have contributed to the higher condition index of the Johan Sverdrup mussels compared to the reference group. Although no quantitative assessment of food availability was performed, the depuration experiment clearly showed that the mussels at Johan Sverdrup were well fed. While the gut clearance experiments were not performed in mussels from the reference stations (due to the use of a chlorophyll sensor), the chlorophyll sensor detected quite low concentrations of phytoplankton.

Seasonal variation in the CI of *M*. *edulis* has been previously reported by Kagley et al. (2003). In the Puget sound on the west coast of the United States, the CI of *M*. *edulis* was found to increase markedly between March and the peak spawning period of May-June. A similar seasonal phenomenon was

observed in *M*. *edulis* sampled from the coast of Scotland (Okumuş and Sterling, 1998), with both examples linking temperature and food availability to the differences in CI observed.

The ability of the mussel to survive out of air provides a simplified indication of the energy available to maintain shell closure and withstand desiccation and in so doing providing a measure of the overall fitness of the mussel. Of the 19 mussel groups, only three groups recorded LT_{50} values below the ICES BAC of 10 days. These values were 8, 8.6 and 9 days and well above the EAC of 5 days. This would indicate that the mussels were overall in good health or a high level of fitness. The lowest LT_{50} value was found at one of the reference stations at a depth of 40 m. There was no obvious relationship between stress on stress measurements and proximity to the Johan Sverdrup installation, and overall the mussels demonstrated LT_{50} values indicative of healthy mussels.

When comparing the LT_{50} values with similar field studies, comparable values between 8 and 13.8 days were found in mussels transplanted for 6 weeks at the Statfjord A and B offshore field (Pampanin et al 2019), and between 8 and 12 days in mussels transplanted for 6-weeks at the coastal fjord of Bøkfjorden in Kirkenes (Brooks et al., 2015).

Micronuclei measured in the haemocytes of mussels is an effective tool for the measurement of genotoxicity. It is widely used, and ICES assessment criteria have been developed with a BAC of 2.5 micronuclei per 1000 cells. Although no significant differences among the mussel groups were found for micronuclei frequency, four groups had mean values marginally above the BAC, with the highest mean value of 4.5 MN/ 1000 cells at station 3, 20 m. Station 3 mussels were placed on the 500 m safety zone north of the installation and based on the information from the TCC plume model may have been partially exposed to the plume during the 3-day discharge window.

For LMS, based on laboratory and field studies, the ICES assessment criteria have been established with 20 min and 10 min indicative of the BAC and the EAC respectively. All field mussels demonstrated an LMS value above the BAC with many over 30 min. These values, markedly above the ICES BAC, are indicative of healthy mussels, showing no detrimental effects of exposure within the Johan Sverdrup field. The day zero (T0) mussels appeared to be different to the field exposed mussels, with a labilization time of 18 min, which is below the BAC value and may indicate a stressed but compensating response. As previously discussed for CI, the lower labilization time may reflect more the time at which the mussels were sampled. The earlier sampling of the mussels in the colder waters of March before the spring conditioning may have played a part in the apparent lower labilization time of this mussel group. Seasonal differences in labilization time of *Mytilus* sp. have been previously reported with longer times found during the spring and autumnal months where mussels develop reproductive conditioning (Kagley et al., 2003).

Although MT showed a degree of variability in response among the monitoring stations, comparable MT activity was found between the mussels from the day zero and two reference stations and those from the Johan Sverdrup field, with no obvious response with distance from the installation. ICES assessment criteria were not currently available for MT in mussels using the spectrophotometric sulfhydryl method (Viarengo et al., 1997). The concentrations of MT in the digestive gland of the mussel in this study were within the range as those reported by Viarengo et al., (1997) as well as those observed in other gas fields (Gomiero et al., 2011; 2015).

Variable levels of alterations in the digestive gland and gill histopathology were observed in bivalves collected during the biomonitoring program. Overall, there were no severe histopathology alterations (score III). In most of the cases mussels showed absent-to-mild changes as necrosis, tubules atrophy, gill fusion, hypertrophy and hyperplasia. Few episodes of severe cilia erosion and lumen enlargement

(score III) were observed in a limited number of sites, respectively. However, the homogeneous distribution of the observed ultrastructural changes across the different sampling sites points out both the lack of severe stress syndrome in the observed mussels as well as the absence of a gradient of stress.

4.3.1 Integrated assessment (IBR/n and PCA)

By 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 obtained. Of the five biomarkers selected in the IBR/n assessment, the star plots highlighted which were the most responsible for the IBR/n score. The day zero group had the highest IBR/n of around 2, however from the star plots it was apparent that CI and LMS were the endpoints most responsible. The lower CI in day zero mussels was thought to be due to the mussels being less reproductively developed in the colder waters of March when sampled compared to the slightly warmer waters and later season when reproductive development was likely to increase when the field deployed mussels were sampled in May. The contribution from LMS to the IBR/n was due to the comparatively lower LMS of the day zero group at 18 min. However, this value was found not to be statistically different to the other mussel groups, and only marginally below the 20 min BAC value indicating background levels of membrane integrity and general health status.

Of the field exposed mussel groups, reference 1 40 m, and station 3 20 m calculated the highest IBR/n scores. The IBR/n score for reference R1b, was mostly from the whole organism responses CI and SoS. As discussed earlier the lower temperatures at the reference station may have contributed to lower CI values in R1b. The other reference stations also recorded lower CI values. The reason for the lower SoS values of the R1b group are not clear, although the LT_{50} of 8 days was only slightly below the 10-day background BAC and above the EAC of 5, indicating some signs of reduced fitness. For all the other mussel groups the IBR/n was on or below 1, with relatively low responses in biological effects. Overall, the biomarkers measured, and the calculated IBR/n values indicated a very modest biological response in all mussel groups including those placed near to the Johan Sverdrup installation.
The integration of chemical and biological responses in transplanted mussels through PCA analysis showed that location 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 Johan Sverdrup field compared to the reference location. However, the PCA did not show any clear impact of PAH's and metals on biomarker responses measured in the mussels, even in those located closer to the Johan Sverdrup installation.

5 Conclusion

- The particle DREAM model was used to place the monitoring rigs in the most suitable position of the TCC discharge and later, based on the collected oceanographic parameters, confirmed the suitability of the monitoring rig locations to measure exposure to the TCC plume.
- The TCC discharge was limited to a 3-day window that was towards the final week of the 6-7-week mussel deployment period.
- Bioaccumulation of PAHs in mussels from around the Johan Sverdrup installation were found to be slightly elevated above the reference mussels. With the absence of a produced water discharge at Johan Sverdrup, the small increase in mussel PAH may have derived from the small amounts of oil that was not removed from the TCC discharge creating a small general elevation in the surrounding waters. Alternatively, the small increase in mussel PAH may be an indication of a general increase of PAH in regions of the North Sea that have oil production facilities. The PAH concentrations measured in the mussels were comparable to that measured in mussels approximately 10 km from a produced water discharge, as seen in previous Water column monitoring surveys.
- The concentration of metals in mussels were on or below the lowest category of Norwegian classification scheme and were representative of an insignificant risk. An interesting difference in metal concentrations observed was the consistently higher Cd concentration between mussels from 20 m compared to 40 m at the Johan Sverdrup field. The reason for this elevated concentration of Cd was not clear but based on the measured concentration of Cd in the TCC, was thought not to be directly due to the TCC discharge.
- The biological effects measurements were overall low and representative of healthy mussels, this was also reflective of the low IBR/n score.
- The PCA found no clear association between the chemical and biological responses in mussels and proximity to the TCC discharge.
- Overall, the biological effects monitoring programme showed no adverse impacts of the TCC discharge on chemical bioaccumulation and biological response in the mussels. However, the TCC discharge was limited to only a 3-day window, and the lack of response in the mussels may reflect this low exposure.
- The 3-day TCC exposure window was representative of the regularity of TCC discharge at Johan Sverdrup.

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Appendix A.

Table A 1. *p*-values for the Pearson's correlation of chemical measurements and biological responses in mussels held in the water column at approximate depths of 20 m and 40 m at nine mussel stations (1, 2, 3, 4, 5, 6, 7, R1, R2) for a 6-7 week period (values in bold are different from 0 with a significance level alpha = 0.05). CI – Condition index; SoS – Stress on stress; LMS – Lysosomal membrane stability; MN – micronuclei; MT – metallothionein; EPA16 – Sum of EPA PAH16; Sum PAH – Sum of PAH; SumNPD – sum of naphthalene, phenanthrene and dibenzothiophenes alkylated and parent compounds; AS – Arsenic; Cd – Cadmium; Cr – Chromium; Cu – Copper; Fe – Iron; Pb – Lead; Mn – Manganese; Hg – Mercury; Ni – Nickel; Zn – Zinc.

Variables	CI	SoS	LMS	MN	MT	SumPAH	EPA16	SumNPD	Hg	As	Pb	Fe	Cd	Cu	Cr	Mn	Ni	Zn
CI	**0**	0.375	0.281	0.195	0.838	**0.001**	0.620	**0.001**	**0.037**	**<0.0001**	**<0.0001**	**0.018**	0.050	0.778	0.255	**0.001**	**0.001**	0.329
SoS	0.375	**0**	0.128	0.169	0.058	0.220	0.320	0.241	0.215	0.226	0.505	0.368	0.346	0.657	0.905	0.209	0.634	0.383
LMS	0.281	0.128	**0**	0.425	**0.004**	**0.041**	0.221	**0.049**	0.111	0.134	0.263	0.753	0.564	0.477	0.989	0.097	0.390	0.193
MN	0.195	0.169	0.425	**0**	0.854	0.226	0.267	0.260	0.939	0.271	0.103	0.065	0.063	0.293	0.212	0.198	**0.012**	0.557
MT	0.838	0.058	**0.004**	0.854	**0**	0.376	0.292	0.417	0.371	0.912	0.668	0.352	0.572	0.141	0.221	0.846	0.485	**0.046**
SumPAH	**0.001**	0.220	**0.041**	0.226	0.376	**0**	0.107	**<0.0001**	**0.000**	**<0.0001**	**<0.0001**	0.385	0.080	0.527	0.998	**0.002**	**0.034**	**0.045**
EPA16	0.620	0.320	0.221	0.267	0.292	0.107	**0**	0.222	0.251	0.357	0.450	0.521	0.696	0.402	0.538	0.260	0.647	0.230
SumNPD	**0.001**	0.241	**0.049**	0.260	0.417	**<0.0001**	0.222	**0**	**0.001**	**<0.0001**	**<0.0001**	0.334	0.061	0.570	0.952	**0.003**	**0.032**	0.054
Hg	**0.037**	0.215	0.111	0.939	0.371	**0.000**	0.251	**0.001**	**0**	**0.008**	0.077	0.245	0.521	0.122	**0.022**	**0.009**	0.876	**<0.0001**
As	**<0.0001**	0.226	0.134	0.271	0.912	**<0.0001**	0.357	**<0.0001**	**0.008**	**0**	**<0.0001**	**0.029**	0.223	0.946	0.339	**<0.0001**	**0.003**	0.239
Pb	**<0.0001**	0.505	0.263	0.103	0.668	**<0.0001**	0.450	**<0.0001**	0.077	**<0.0001**	**0**	**0.006**	0.209	0.948	0.125	**<0.0001**	**0.000**	0.704
Fe	**0.018**	0.368	0.753	0.065	0.352	0.385	0.521	0.334	0.245	**0.029**	**0.006**	**0**	0.215	0.238	**<0.0001**	0.178	**0.000**	**0.014**
Cd	0.050	0.346	0.564	0.063	0.572	0.080	0.696	0.061	0.521	0.223	0.209	0.215	**0**	0.422	0.542	0.973	0.096	0.349
Cu	0.778	0.657	0.477	0.293	0.141	0.527	0.402	0.570	0.122	0.946	0.948	0.238	0.422	**0**	0.402	0.820	0.637	0.527
Cr	0.255	0.905	0.989	0.212	0.221	0.998	0.538	0.952	**0.022**	0.339	0.125	**<0.0001**	0.542	0.402	**0**	0.865	**0.000**	**0.000**
Mn	**0.001**	0.209	0.097	0.198	0.846	**0.002**	0.260	**0.003**	**0.009**	**<0.0001**	**<0.0001**	0.178	0.973	0.820	0.865	**0**	0.080	0.252
Ni	**0.001**	0.634	0.390	**0.012**	0.485	**0.034**	0.647	**0.032**	0.876	**0.003**	**0.000**	**0.000**	0.096	0.637	**0.000**	0.080	**0**	0.276
Zn	0.329	0.383	0.193	0.557	**0.046**	**0.045**	0.230	0.054	**<0.0001**	0.239	0.704	**0.014**	0.349	0.527	**0.000**	0.252	0.276	**0**

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