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Water Column Monitoring 2021

Assessing the impacts of Ekofisk and Eldfisk offshore oil and gas installations on the marine environment

Photo: Steven Brooks

Norwegian Institute for Water Research

REPORT

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Summary

The Norwegian offshore Water Column Monitoring (WCM) programme investigated the potential effects of Ekofisk and Eldfisk oil and gas installations on the marine environment. Produced water (PW) impacts were assessed in the water column by deploying monitoring stations containing mussels, passive samplers (PSDs), scallops and scientific equipment downstream of the Ekofisk and Eldfisk installations for 6 weeks at 2 depths between March and May 2021 and compared to two reference stations and a day zero group. Chemical and biological effects were measured and a clear relationship between PAH concentration in mussels and proximity to the Ekofisk installation was shown in the 20 m mussels reaching background concentrations in mussels 4000 m downstream. Only low concentrations were found in the Eldfisk mussels and 40 m mussels and scallops showing low exposure to the PW plume. Biological responses in mussels represented a weak response to low PW exposure. In addition, demersal fish were collected from within the Ekofisk safety zone and three regions of the North Sea (Ekofisk region, Egersundbank, Vikingbank). Integrated chemical and biological effects were measured, a relationship between PAH exposure (liver /metabolites) in dab and CYP1A activity (EROD and CYP1A protein), with higher levels in dab populations living in areas of oil and gas activity. Genotoxicity was also observed in cod and dab from the Ekofisk.

Four keywords	Fire emneord
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2. Biological effects assessment	2. Vurdering av biologiske effekter
3. Bioaccumulation of PAH	3. Bioakkumulering av PAH
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The Norwegian Water Column Monitoring programme 2021

Assessing the impacts of Ekofisk and Eldfisk offshore oil and gas installations on the marine environment

Preface

The Water Column Monitoring (WCM) programme performs investigations into the potential biological effects of offshore oil and gas activity on organisms living within the water column of the Norwegian continental shelf. The organisation, Offshore Norway, consists of the oil and gas producing companies on the Norwegian continental shelf. They are obliged by the Norwegian Environment Agency (Miljødirektoratet) to assess potential impacts of their offshore operations, including discharges of produced water (PW) and waste products from drilling operations.

WCM surveys have been performed at various oil and gas fields over the last 30 years and have evolved from an annual programme, focusing on chemical measurements, to a large integrated chemical and biological effect programme performed every three years. The WCM programme is a collaboration between three research institutes, the Norwegian Research Institute (NORCE), the Institute of Marine Research (IMR) and the Norwegian Institute for Water Research (NIVA) together with the University of Stavanger. Analytical support is provided by Norgenotech AS and the Institute of Experimental Medicine AS, Czech Republic and the Institute of energy technology (IFE).

The field investigation required three research cruises. The first research cruise occurred in week 12 (22nd - 28th March 2021) where monitoring rigs were placed out at the Ekofisk and Eldfisk oil and gas fields as well as two reference locations. A second research cruise in week 18 (3rd - 10th May 2021) retrieved the monitoring rigs from the sea and processed the mussels held on each rig for chemical accumulation and biological effect responses. During this cruise fish were collected within the safety zone of the Ekofisk installation and processed for chemical and biological effect responses. These two cruises were performed on the Esvagt Dee supply vessel where the crew assisted with the deployment and retrieval of the monitoring rigs. A third research cruise on the R/V Johan Hjort from IMR occurred in week 19 (11th - 16th May 2021) where fish were collected by trawl from three regions of the North Sea (Ekofisk region, Egersundbank and Vikingbank), and processed on board the vessel for chemical and biological effects endpoints.

The scientific personnel onboard the Esvagt Dee for the deployment cruise included NIVA personnel Bjørnar Beylich and Steven Brooks as well as NORCE personnel Einar Bye-Ingebrigtsen. Harald Lura (ConocoPhillips) and Rolf Sundt (Equinor) represented the client on board the vessel. Scientific personnel onboard for the retrieval cruise that assisted with the sampling of the mussels and fish 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 onboard Esvagt Dee was provided by Medyan Ghareeb (NIVA), with oceanographic data support provided by Nicholas Roden (NIVA) and Lars Golmen (NIVA).

Scientific personnel on board R/V Johan Hjort for the fish trawling and sample collection included Bjørnar Beylich and Samantha Martins (NIVA), Leon Moodley and Einar Bye-Ingebrigtsen from NORCE, as well as IMR personnel Bjørn Einar Grøsvik (cruise leader) and Guri Nesje, Valentin Geslin from the University of Stavanger and Dag Altin from SINTEF.

Oslo, June 2023

Dr Steven Brooks, Project manager

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Summary

The following study was part of the Norwegian Water Column Monitoring (WCM) programme, which performs investigations into the potential effects of offshore oil and gas activities on the marine environment. The WCM 2021 is an integrated biological effects monitoring programme designed to determine the potential effects of Ekofisk and Eldfisk oil and gas installations on caged bivalves and wild fish populations. Significant discharge volumes of produced water (PW) are continuously released from the Ekofisk and Eldfisk installations following partial cleaning to keep the oil in water concentration below discharge limits. The impacts of the PW were assessed in the water column by deploying monitoring stations containing mussels, passive samplers, scallops and scientific equipment (current meters, chlorophyll a, turbidity, temperature, salinity sensors) down-stream (southwest) of the Ekofisk and Eldfisk installations for 6 weeks between March and May 2021. Eight monitoring stations were used with increasing distance southwest of the Ekofisk installation, up to a distance of 4000 m, whilst 3 monitoring stations were placed at 500, 1000 and 2000 m southwest of the Eldfisk installation. These were compared to two reference stations approximately 50 - 80 km east of Ekofisk in an area less impacted by oil and gas activity as well as a day zero (T0) group that represent the mussels prior to deployment. For the Ekofisk and reference stations, mussels and passive sampling devices (PSDs) were held at two depths (18 - 20 m and 40 - 45 m), whilst only one depth (18 - 20 m) was monitored at Eldfisk. Scallops were used as a trial species and used at two Ekofisk and two reference stations as the 40 - 45 m depth only.

Chemical (PAH-NPD, metals) and biological effects (condition index (CI), stress on stress (SoS), micronuclei (MN), lysosomal membrane stability (LMS), histology) were measured in mussels and scallops (except SoS and histology), whilst chemicals (total hydrocarbons, PAH-NPD, alkylphenols, naphthenic acids) were measured in PSDs. A clear relationship between PAH concentrations in mussel tissue and proximity to the Ekofisk installation was measured in the 20 m mussels reaching background concentrations in mussels 4000 m downstream. In contrast, PAH concentrations in the Eldfisk mussels did not show any relationship between proximity to the installation and PAH concentrations were similar to the Ekofisk mussels between 2000 and 4000 m. Mussels from the 40 m depth, only showed low concentrations of PAH similar to reference values and showed no relationship between proximity to the Ekofisk installation. This was also found in scallops placed at the same depth indicating low exposure to the PW plume. The concentrations of PAH-NPD measured in mussels from the closest stations were around 140 ng/g w.w. and were less than half of the concentration measured in previous WCM surveys at Ekofisk. As for mussels, a gradient in freely dissolved concentrations of selected alkylated PAHs (i.e., C3-naphthalenes, C2-phenanthrenes) measured by PSDs can be observed at 20 m depth with increasing distance from Ekofisk. Elevated concentrations of these alkylated PAHs, known to be present in PW, can also be observed, but to a lesser extent, at 40 m depth when compared with the reference stations. A signal of alkylated PAHs can also be seen for Eldfisk compared to reference stations, albeit without a relationship with increasing distance from the platform.

Biological responses in relation to proximity to the Ekofisk installation were only found for LMS and MN, although these were considered to represent a weak response to low PW exposure. Integration of the biomarker data (IBR/n, calculated from star plots of CI, SoS, LMS, MN, digestive gland, gill and gonad histology) showed a weak trend with higher IBR/n scores (increased biological response) in mussels within 1000 m of the Ekofisk installation compared to 2000 m and 4000 m away.

In addition to the monitoring stations, benthic and demersal fish species were collected from within the Ekofisk safety zone and three regions of the North Sea (Ekofisk region, Egersundbank, Vikingbank). The fish species sampled included: cod (*Gadus morhua*) and dab (*Limanda limanda*) from the Ekofisk

safety zone; dab, haddock (*Melanogrammus aeglefinus*) and whiting (*Merlangius merlangus*) from Ekofisk region; cod, dab, haddock and whiting from Egersundbank and haddock and whiting from Vikingbank. An integrated suite of chemical and biological effects assessments was measured in the fish. Chemical measurements included: PAH liver concentrations; PAH metabolites in the bile; per- and polyfluoroalkyl substances (PFAS) in the blood and ^{226}Radium concentrations in fish bone and fillet. The biological effects included CYP1A enzyme activity by ethoxyresorufin-O-deethylase (EROD), CYP1A protein concentration, CYP1A gene expression, DNA adducts and histology in the liver, DNA strand breaks (comet) in red blood cells, and acetylcholine esterase (AChE) inhibition in the fillet.

PAH liver concentrations were found highest in dab from the Ekofisk safety zone, compared to dab from the Ekofisk region and Egersundbank region. The elevated PAH concentrations found in dab liver from Ekofisk safety zone were higher than those observed in the other fish species, even in cod specimens also collected from within the Ekofisk safety zone. PAH metabolites in bile showed that all fish were exposed to some level of PAH and higher in dab from the Ekofisk region. Increased CYP1A enzyme activity (EROD) and CYP1A protein concentrations were found in dab from both the Ekofisk safety zone and the Ekofisk region compared to Egersundbank. A clear relationship was found between PAH exposure (liver /metabolites) in dab and CYP1A activity, with higher levels in dab populations living in areas of oil and gas activity compared to the reference population. The other fish species (cod, haddock and whiting) overall showed low PAH concentrations in the liver and low CYP1A enzyme activity (EROD) and low CYP1A protein concentrations as a result. Male and female dab showed significant differences in EROD and CYP1A, with higher concentrations in males than females. However, the response profile of EROD and CYP1A, with highest activity in Ekofisk region, lower in Ekofisk and lowest in Egersundbank, was demonstrated in both male and female dab, and male and female EROD and CYP1A data for each location were pooled for the integrated assessment (IBR/n and PCA). DNA strand breaks measured with the comet assay were significantly elevated in cod and dab from the Ekofisk safety zone indicating genotoxicity. DNA adducts in fish liver did not support this and found low levels throughout except for haddock from Egersundbank and Vikingbank.

Overall, the integrated data (IBR/n and PCA) identified dab collected from the Ekofisk safety zone and dab from the Ekofisk region to be distinct from the other populations. Chemical concentrations in PAH liver and PAH metabolites showed that these dab populations were recently exposed to PAH compounds prior to sampling and were accompanied with elevated EROD and CYP1A activity levels. However, despite the measurable PAH exposure and clear cellular responses described, higher level orders of biological response such as histological changes in the tissue of the dab were absent.

Sammendrag

Tittel: Norsk Vannsøyleovervåkingsprogram 2021: Vurdering av hvordan Ekofisk og Eldfisk offshore olje- og gassinstallasjoner påvirker havmiljøet
År: 2022
Forfattere: Steven Brooks, Shaw Bamber, Bjørn Einar Grøsvik, Daniela M. Pampanin, Alessio Gomiero, Samantha Martins, Matteo Vitale
Utgiver: Norsk institutt for vannforskning, ISBN 978-82-577-7614-5

Denne rapporten er en del av norsk vannsøyleovervåkingsprogram (WCM) som utfører undersøkelser av potensielle effekter av offshore olje- og gassaktiviteter på havmiljøet. WCM 2021 er et integrert biologisk effektovervåkingsprogram designet for å bestemme mulige innvirkning av Ekofisk og Eldfisk olje- og gassinstallasjoner. Betydelige utslippsvolumer av produsert vann (PW) slippes kontinuerlig ut fra Ekofisk- og Eldfisk-installasjonene etter delvis rensing for å holde oljekonsentrasjonen i vann under utslippsgrensene. Virkningene av PW ble vurdert i vannsøylen ved å utplassere overvåkingsstasjoner som inneholder blåskjell, passive prøvetakere, kamskjell og vitenskapelig utstyr nedstrøms Ekofisk- og Eldfisk-installasjonene i 6 uker mellom mars og mai 2023. Åtte overvåkingsstasjoner ble brukt med økende avstand sørvest for Ekofisk-installasjonen, opp til en avstand på 4000 m, mens 3 målestasjoner ble plassert 500, 1000 og 2000 m sørvest for Eldfisk-installasjonen. Disse ble sammenlignet med to referansestasjoner ca. 50 - 80 km øst for Ekofisk i et område mindre påvirket av olje- og gassaktivitet, samt en dag null (T0) gruppe som representerer blåskjell før utplassering. For Ekofisk- og referansestasjonene ble det uplassert blåskjell og passive prøvetakingsenheter (PSD) på to dyp (18 - 20 m og 40 - 45 m), mens kun ett dyp (18 - 20 m) ble overvåket ved Eldfisk. Kamskjell ble kun brukt på to Ekofisk- og to referansestasjoner ved 40 - 45 m dyp.

Konsentrasjoner av PAH, NPD og metaller og biologiske effekter (kondisjonsindeks (CI), stress på stress (SoS), mikrokjerner (MN), lysosomal membranstabilitet (LMS) og histologi) ble målt i blåskjell og kamskjell (unntatt SoS og histologi), mens kjemikalier (totalt antall hydrokarboner, PAH-NPD, alkylfenoler, naftensyrer) ble målt i passive prøvetakere. En klar sammenheng mellom PAH-konsentrasjoner i blåskjellvev og nærhet til Ekofisk-installasjonen ble målt i blåskjell ved 20 m dyp. Bakgrunnskonsentrasjoner ble målt i blåskjell 4000 m nedstrøms. PAH-konsentrasjoner i blåskjell ved Eldfisk viste ingen sammenheng mellom nærhet til installasjonen og PAH-konsentrasjoner. Blåskjell ved Ekofisk fra 40 m dyp, viste kun lave konsentrasjoner av PAH som tilsvarte bakgrunnsverdier og viste ingen sammenheng med nærhet til Ekofisk-installasjonen. Dette ble også funnet i kamskjell plassert på samme dyp, noe som indikerer lav eksponering for PW-strømmen. Konsentrasjonene av PAH-NPD målt i blåskjell fra de nærmeste stasjonene var rundt 140 ng/g w.w. Dette er mindre enn halvparten av konsentrasjonen som er målt i tidligere WCM-undersøkelser på Ekofisk. I likhet med blåskjell, kan en gradient i fritt oppløst konsentrasjon av utvalgte alkylerte PAH (f.eks. C3-naftalener) målt med PSD observeres på 20 m dyp med økende avstand fra Ekofisk. Forhøyede konsentrasjoner av disse alkylerte PAH-ene, kjent for å være i PW, kan også observeres på 40 m dyp sammenlignet med referansestasjonene. Et signal av alkylerte PAH kan også sees for Eldfisk, dog uten sammenheng med økende avstand fra plattformen.

Biologiske responser på Ekofisk-installasjonen ble kun funnet for LMS og MN på de nærmeste stasjonene, vurdert til å representere responser på lav PW-eksponering. Integrering av biomarkørdataene (IBR/n) viste en svak trend med høyere IBR/n-skår (økt biologisk respons) i blåskjell innenfor 1000 m fra Ekofisk-installasjonen sammenlignet med blåskjell 2000 m og 4000 m unna.

I tillegg til overvåkingsstasjonene ble bunnfiskarter samlet inn fra Ekofisk sikkerhetssone og tre regioner i Nordsjøen (Ekofiskregionen, Egersundbanken, Vikingbanken). Fiskeartene som ble prøvetatt

inkluderte: torsk (*Gadus morhua*) og sandflyndre (*Limanda limanda*) fra sikkerhetssonen Ekofisk; sandflyndre, hyse (*Melanogrammus aeglefinu*s) og hvitting (*Merlangius merlangus*) fra Ekofisk-regionen; torsk, sandflyndre, hyse og hvitting fra Egersundbanken og hyse og hvitting fra Vikingbanken. Et sett av kjemiske analyser og biologiske effektparametere ble målt i fisken. Kjemiske målinger inkluderte: PAH-leverkonsentrasjoner; PAH-metabolitter i galle; per- og polyfluoralkylstoffer (PFAS) i blod og ^{226}Radiumkonsentrasjoner i fiskebein og muskel. De biologiske effektparameterene inkluderte CYP1A-enzymaktivitet ved ethoxyresorufin-O-deethylase (EROD), CYP1A-proteinkonsentrasjon, CYP1A-genekspresjon, DNA-addukter og histologi i leveren, DNA-trådbrudd (komet) i røde blodceller og hemming av acetylkolinesterase (AChE) i muskel.

Høyest PAH-leverkonsentrasjoner ble funnet i lever fra sandflyndre fra sikkerhetssonen på Ekofisk, sammenlignet med lever fra sandflyndre fra Ekofiskregionen og Egersundbanken. De forhøyete PAH-konsentrasjonene som ble funnet i sandflyndrelever fra sikkerhetssonen på Ekofisk var høyere enn de som ble observert i de andre fiskeartene, selv i torskeleverprøver som også ble samlet inn fra Ekofisk-sikkerhetssonen. PAH-metabolitter i galle viste at all fisk ble eksponert for et visst nivå av PAH. Nivåene var også høyere i sandflyndre fra Ekofisk-regionen. Økt CYP1A enzymaktivitet (EROD) og CYP1A proteinkonsentrasjoner ble funnet i sandflyndre både fra Ekofisk sikkerhetssone og Ekofisk regionen sammenlignet med Egersundbanken. Det ble funnet en klar sammenheng mellom PAH-eksponering (lever/metabolitter) i sandflyndre - og CYP1A-aktivitet, med høyere nivåer i sandflyndrepopulasjoner som lever i områder med olje- og gassaktivitet sammenlignet med referansepopulasjonen. De andre fiskeartene (torsk, hyse og hvitting) viste totalt sett lave PAH-konsentrasjoner i leveren og lav CYP1A-enzymaktivitet (EROD) og lave CYP1A-proteinkonsentrasjoner som resultat. Sandflyndre av begge kjønn viste signifikante forskjeller i EROD og CYP1A, med høyere konsentrasjoner hos hannfisk enn hos hunnfisk. Imidlertid ble responsprofilen til EROD og CYP1A, med høyest aktivitet i Ekofisk-regionen, lavere i Ekofisk og lavest i Egersundbank, demonstrert i både hann- og hunnsandflyndre, og EROD og CYP1A data for begge kjønn fra hvert område ble slått sammen for den integrerte vurderingen (IBR/n og PCA). DNA-trådbrudd målt med kometanalyse var signifikant forhøyet i torsk og sandflyndre fra Ekofisk sikkerhetssone, noe som indikerer gentoksisitet. DNA-addukter i fiskelever gav ikke samme mønster og det ble funnet gjennomgående lave nivåer av DNA addukter bortsett fra i hyse fra Egersundbanken og Vikingbanken.

Samlet sett viste de integrerte dataene (IBR/n og PCA) at sandflyndre samlet inn fra Ekofisk-sikkerhetssonen og fra Ekofisk-regionen var forskjellig fra de andre populasjonene. Kjemiske konsentrasjoner av PAH nivåer i lever og av PAH-metabolitter i galle viste at disse sandflyndrepopulasjonene nylig ble eksponert for PAH-forbindelser og forhøyede EROD aktiviteter og CYP1A-nivåer ble målt. Til tross for den målbare PAH-eksponeringen og klare cellulære responser, ble det ikke observert responser som representerer høyere nivåer av biologiske responser, slik som histologiske vevsendringer.

1 Introduction

1.1 General purpose of the study

The Norwegian water column monitoring (WCM) programme is designed to investigate the potential environmental impacts of offshore oil and gas activity on the marine ecosystem. The two major environmental challenges from offshore oil and gas activities are oil contaminated cutting piles produced during drilling operations that are present on the sea floor, and large volumes of production water or produced water (PW), which are discharged to sea following treatment (i.e., oil/water separation). Discharge of oil contaminated cutting piles were prohibited in 1993 due to impact on bottom fauna diversity (Bakke et al., 2013). Regulations controlling the use of hazardous chemicals are in place during drilling operations, although contaminated drill cuttings may still pose a threat to the ecosystem. In addition, regulations on the maximum concentrations of oil in water in the discharged PW are in place at 30 mg/L. The main purpose of the regular WCM survey is to ensure that these discharge regulations set by the Norwegian Environment Agency are sufficient in preventing harm to organisms living within the vicinity of offshore oil and gas installations, and in regions where exploration operations are carried out.

Oil and Gas companies operating in Norwegian waters are required to perform environmental monitoring in order to provide national authorities with information on the potential impacts of their offshore activities, which can be used to update regulations and improve environmental protection. Offshore Norway is a consortium of oil and gas companies that operate in Norwegian waters and are responsible for financing the study. The WCM programme has evolved since its inception in the later 1990s and currently exists as an extensive biological effect monitoring programme, which is performed every 3 years, with research and development activities to support the monitoring programme in the interim years. The programme design was based on the latest Norwegian guidelines (M-408/2020) and was approved by the Norwegian Environment Agency.

1.2 Brief history of the Water Column Monitoring programme

The WCM programme started in the mid-1990s due to concerns of increasing volumes of PW that were discharged into the sea from offshore oil and gas platforms. The early programmes were mostly composed of chemical measurements accumulating over time in passive samplers and/ or mussel cages positioned in the vicinity to the PW discharge. The inclusion of biological effect parameters was strongly influenced by the 2001 and 2002 BECPELAG workshop (Hylland et al., 2006). Biological effects were considered to be the best tools for the assessment of the impacts of contaminants from oil and gas activities. The use of caged mussels, and sometime caged fish, has been a popular approach, which has generally reported elevated concentrations of PAH compounds and some lower-level health effects in mussels within 500 to 1000 m from platforms (Pampanin et al., 2019; Brooks et al., 2011a; Hylland et al., 2008). Typically, PAH concentrations have been found to reduce with distance from the platform and approach offshore reference concentrations approximately 2000 m away.

For the WCM surveys of 2013 and 2014, there was a move away from using caged mussels to solely focus on wild fish populations that live within the 500 m safety zone of offshore installations (Brooks et al., 2013; Brooks et al., 2014). In these studies, demersal fish living on or from the seafloor, which were thought to migrate less than pelagic species, were the main target group and the focus was expanded to include sediment related contamination such as drill cutting piles and leakages from well

deposits on the sea floor. Statistically significant levels of genotoxic and neurotoxic responses were found in fish caught near offshore installations compared to those from references locations and were followed up in the future WCM programmes.

In 2017, the WCM programme merged with the Norwegian Condition Monitoring programme, which was a study that focussed on the general health of fish populations in regions of the Norwegian continental shelf (Pampanin et al., 2019). The now larger WCM programme changed from a mostly annual field study to a large field investigation performed every 3 years, looking at near field and wider impacts of oil and gas activities to the marine ecosystem. The current programme that was postponed by one year due to the COVID pandemic was performed in 2021. This is only the second time that the larger WCM format has been used and has been applied to investigate the impacts of Ekofisk and to a slightly lesser extent Eldfisk. The combination of mussel cages near the Ekofisk and Eldfisk installations as well as wild fish populations at Ekofisk and three regions of the North Sea have been used for this purpose.

1.3 Ekofisk and Eldfisk fields

Ekofisk was the first oil field discovered in the Norwegian sector of the North Sea back in 1969. Production began in 1971 with oil initially being loaded into tankers for distribution, before a concrete storage tank was built in 1973. Since then the field has been developed with many facilities and pipelines; from 1975 an oil pipeline was established to Teesside UK, and an additional pipeline has been transporting gas to Emden, Germany since 1977.

The Ekofisk reservoir produces oil from naturally fractured chalk of the early Palaeocene age. The rock is highly porous and has a low permeability. The main reservoir lies approximately 3000 m deep and with a column height of 300 m. Water injection to maintain well pressure started in 1987 and has been continuously used to maintain well pressure and keep the level of oil and gas production high. Some of the main challenges for production at Ekofisk are in identifying the oil pockets in the increasingly waterflooded reservoir, as well as the safe handling of the increasingly large volumes of PW that need to be treated before being discharged to sea. The annual discharge of PW from Norwegian offshore oil and gas activities has been at approximately 120 million m^3 per year over the last 3 years (https://www.norskpetroleum.no/miljo-og-teknologi/utslipp-til-sjo/).

At Ekofisk, the PW discharge has steadily increased from around 2 million m^3 per year in 1999 to 12.5 million m^3 per year in 2012 and has remained relatively stable until now (Figure 1). Based on these figures, Ekofisk PW makes up approximately 10% of the total PW discharge to sea on the Norwegian continental shelf.

Eldfisk was first discovered in 1970, although production only started in 1979. Since 1997, water has been injected into the well in order to create pressure for removal. The Eldfisk well, being only 10 km south of Ekofisk has many similarities to its neighbour, it lies at a depth of 2700 to 2900 m below the seafloor and the reservoir rock is composed of highly porous and low permeable chalk stone from the late Cretaceous early Palaeocene (https://www.norskpetroleum.no/fakta/felt/eldfisk/). Compared to the Ekofisk complex, the discharge of PW from the Eldfisk installation is small with an annual discharge volume of approximately 1.8 to 2.0 million m^3 per year, around 6 to 7-fold less than that released from Ekofisk.

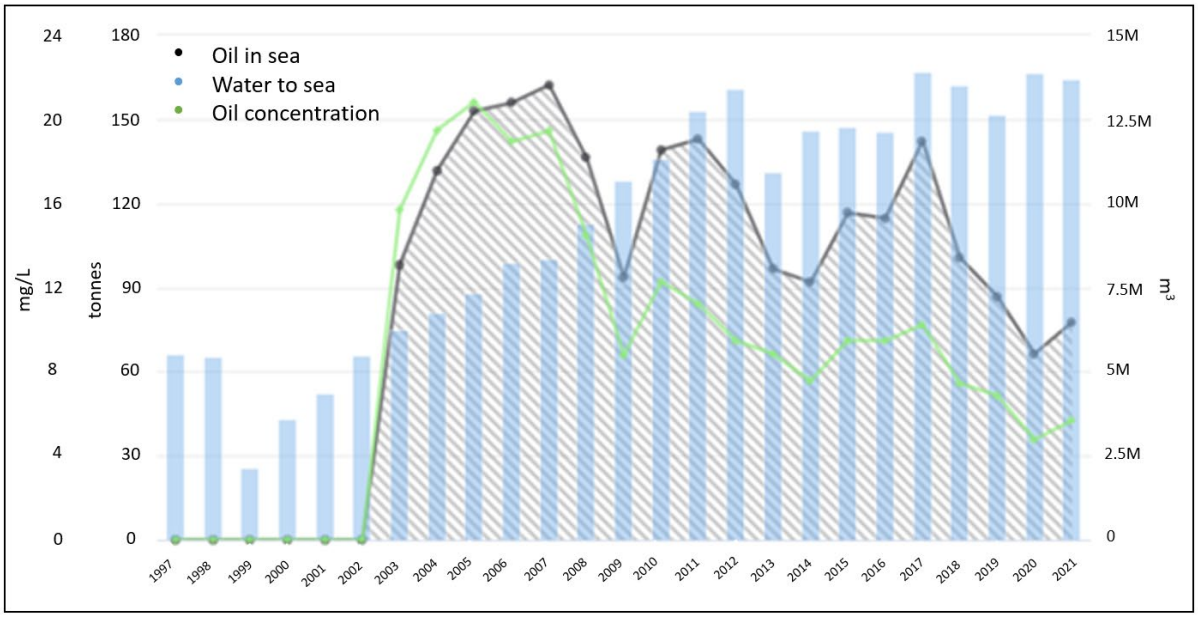


Figure 1. Information on the annual discharge of produced water from the Ekofisk complex, including the mean oil in water concentration and the quantity of oil discharge to sea. (Gingstad et al. 2022).

1.4 Objectives

The main objective of the 2021 WCM programme was to determine the impact of Ekofisk and Eldfisk installations on the marine environment. This was achieved by addressing the following objectives:

- To determine the effects of the PW discharges from Ekofisk and Eldfisk on pelagic species using chemical bioaccumulation and biological effects markers in caged mussels placed for 6 weeks at known distances downstream from both Ekofisk and Eldfisk installations.

- To determine the effects of PW and considering sediment sources of contamination around Ekofisk (i.e., drill cuttings, leaking well deposits, settled PW particulates) by measuring chemical exposure and biological effects markers in wild benthic and demersal fish species.

- To compare the health status of wild fish populations caught from within the 500 m safety zone of the Ekofisk installation to fish caught from three separate regions of the North Sea (i.e., Ekofisk region, Egersundbank and Vikingbank).

2 Methods

2.1 Monitoring programme design

Although the main focus of this study was to assess the impact of PW on organisms within the water column, effects on sediment dwelling species were also considered. The WCM programme used field transplanted mussels to determine the chemical bioaccumulation and biological effects on organisms following a 6-week exposure to the Ekofisk and Eldfisk PW plumes. Two independent locations approximately 50 and 80 km away from the Ekofisk complex were used as references (Figure 2). In addition, wild fish were collected within the 500 m safety zone of the Ekofisk complex as well as from three regions within the North Sea (Ekofisk region, Egersundbank and Vikingbank) to measure chemical and biological effects.

2.1.1 Monitoring stations

The monitoring stations, holding mussels, passive samplers and oceanographic instruments were positioned on a gradient southwest of the Ekofisk complex. Station locations followed the main tidal current direction at Ekofisk, which is known to strongly influence the direction and fate of the discharged PW plume. The DREAM model was used to predict the plume direction as well as the potential risk of exposure to the plume with depth. Based on this information mussel and passive samplers were placed at Ekofisk at two depths, 18-20 m and 40-45 m. The reference stations also contained mussels and passive samplers at the same two depths for comparison. For the Eldfisk monitoring stations only the impact on one depth (18-20 m) was assessed.

2.1.2 Research cruises

Three separate research cruises were required to perform the different operations of the monitoring programme. Detailed information on the three research cruises is provided in the cruise report (Appendix A).

The first cruise, referred to as the deployment cruise, took place in week 12 (22nd - 28th March 2021). The Esvagt Dee supply vessel was used to deploy monitoring rigs (Figure 3) containing mussels, scallops, passive samplers and oceanographic instrumentation at specific locations near to the Ekofisk (Figure 4) and Eldfisk (Figure 5) offshore installations as well as two reference locations.

The second cruise was also with the Esvagt Dee supply vessel and took place 6-weeks after the first cruise in week 18 (3rd - 10th May 2021). During the second cruise the monitoring rigs were successfully retrieved using acoustic releases. Mussels and scallops were processed immediately onboard the vessel and a maximum of 4 hours after collection. Rod and line fishing within the Ekofisk safety zone also took place during this cruise. The captured fish were held alive in large aquaria with flowing seawater prior to processing. All fish were processed within 6 hours of capture.

The third cruise was on board R/V Johan Hjort, which took place in week 19 (10th - 15th May 2021). Fish trawling activities occurred at three regions. Fish were sampled and processed on board the vessel within a few hours of collection.

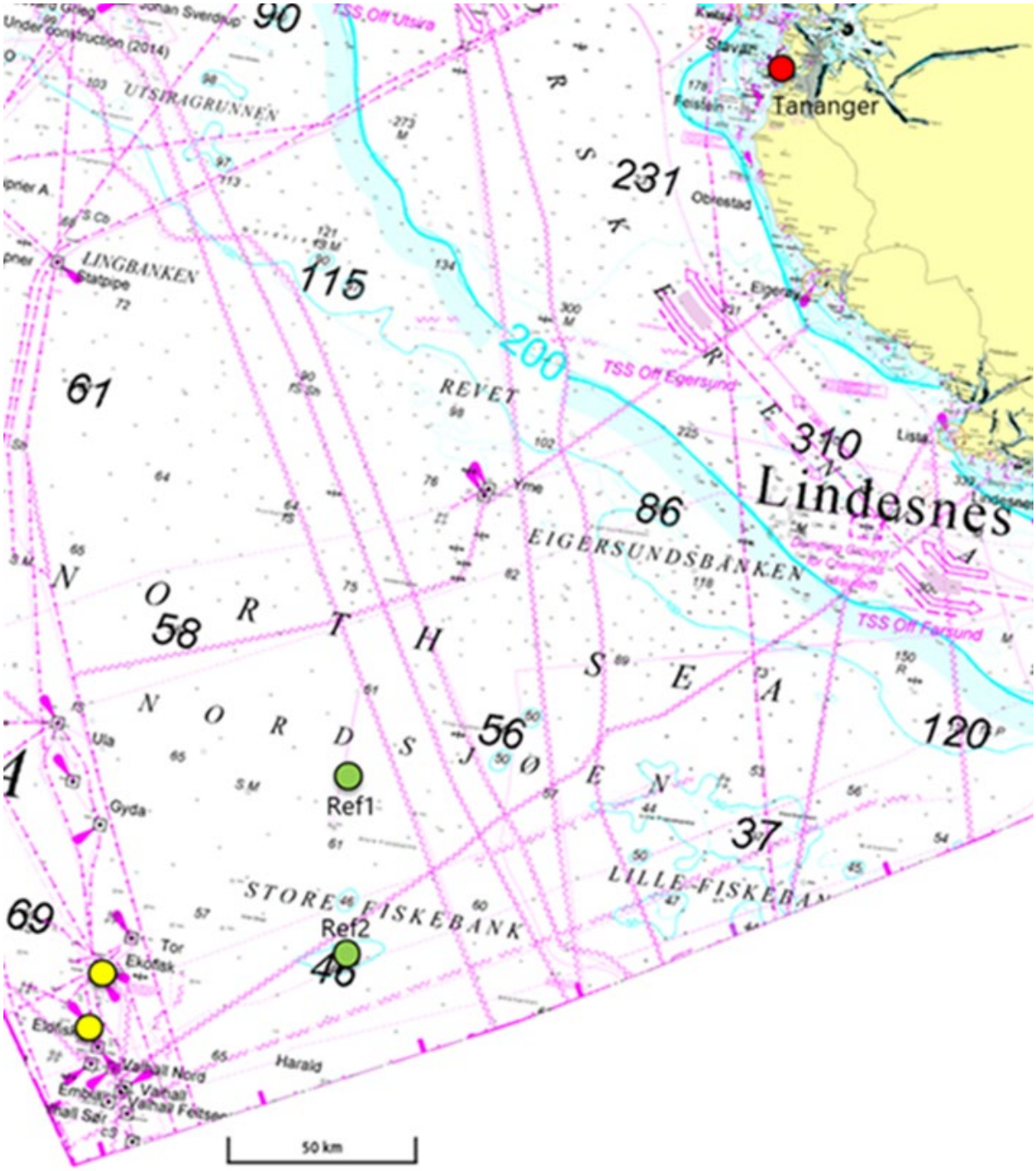


Figure 2. The Norwegian southern sector of the North Sea, highlighting the location of the Ekofisk and Eldfisk complexes (yellow circles) as well as the two reference stations (green circles) and the ConocoPhillips Sea Base port in Tananger (red circle). (Source: Norgeskart.no). Ref 1: 57° 5.6 N 4° 10 E; Ref 2: 56° 39.9 N 4° 15 E.

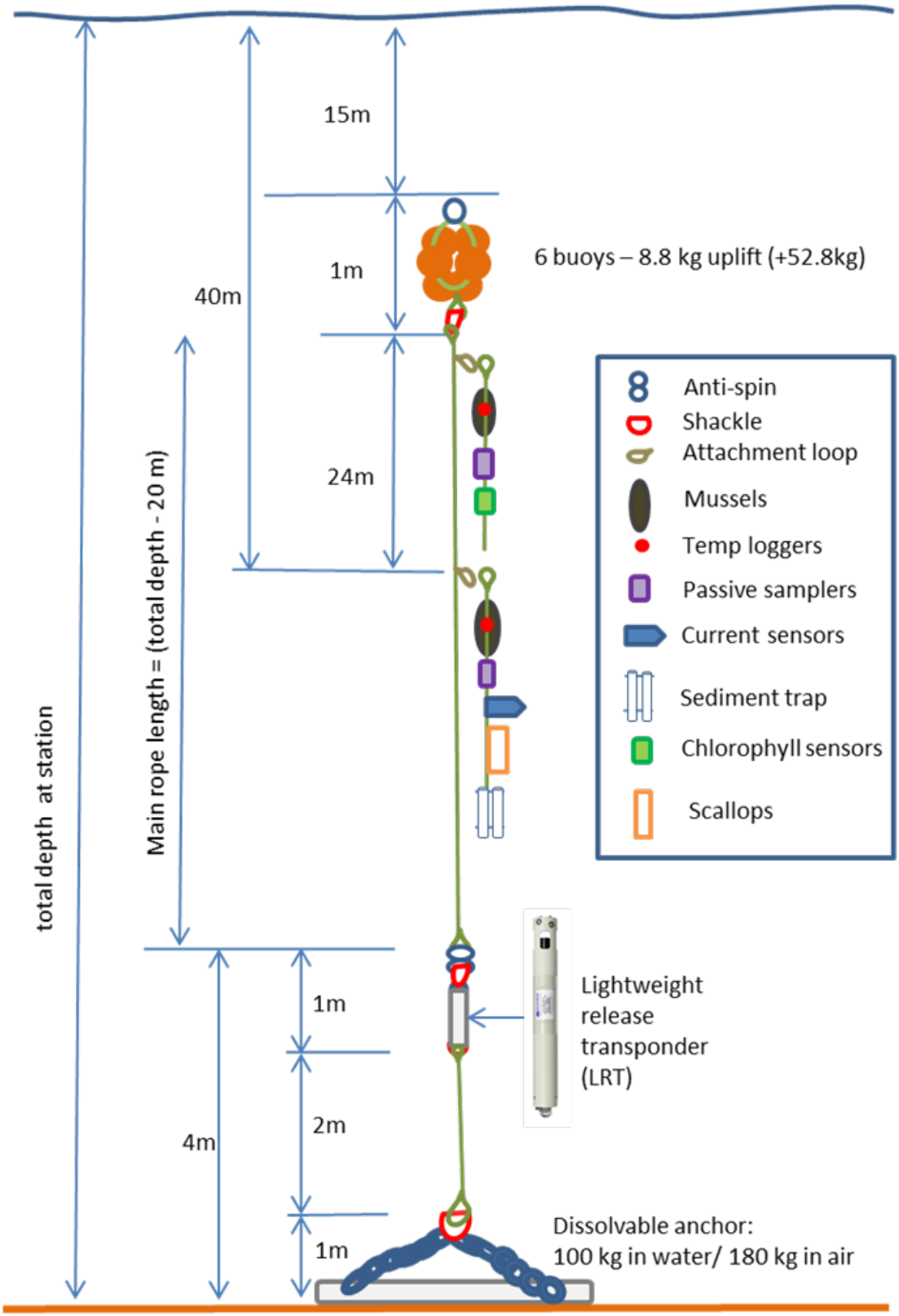
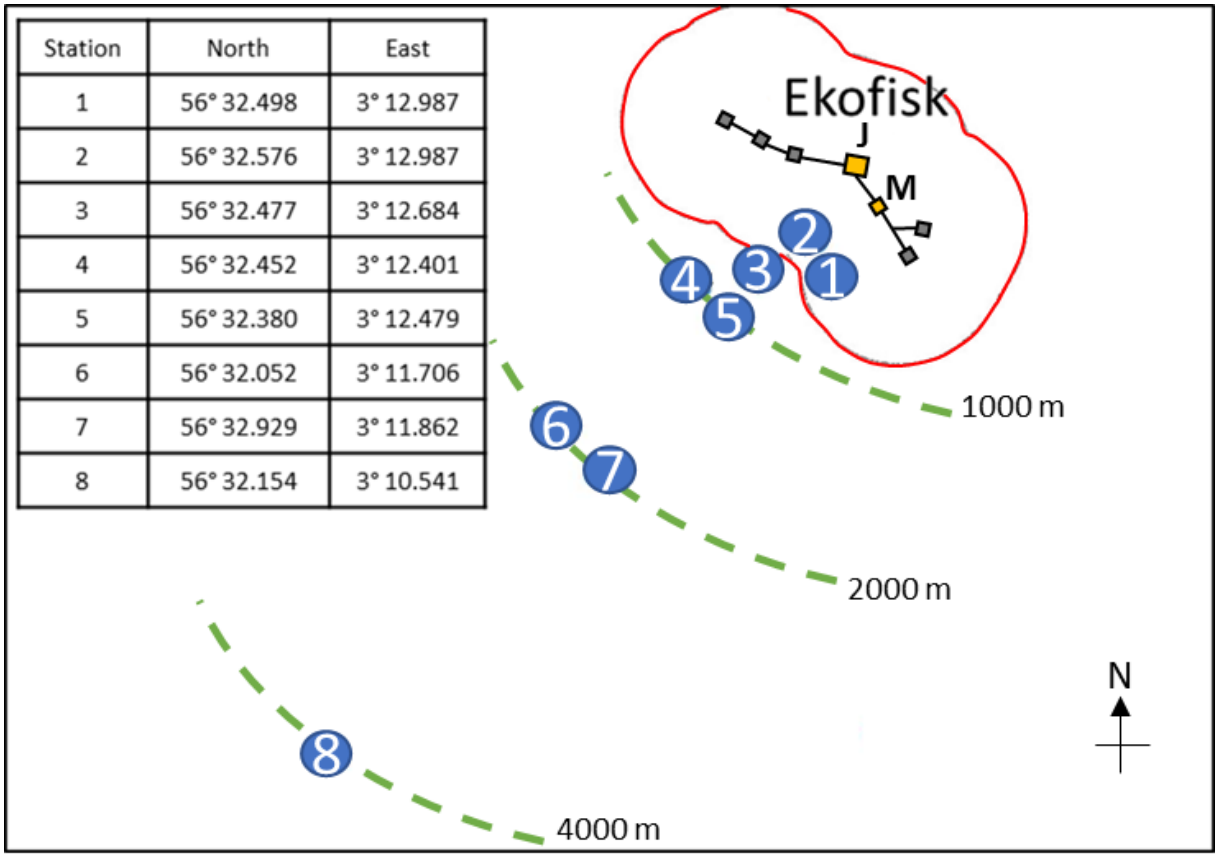


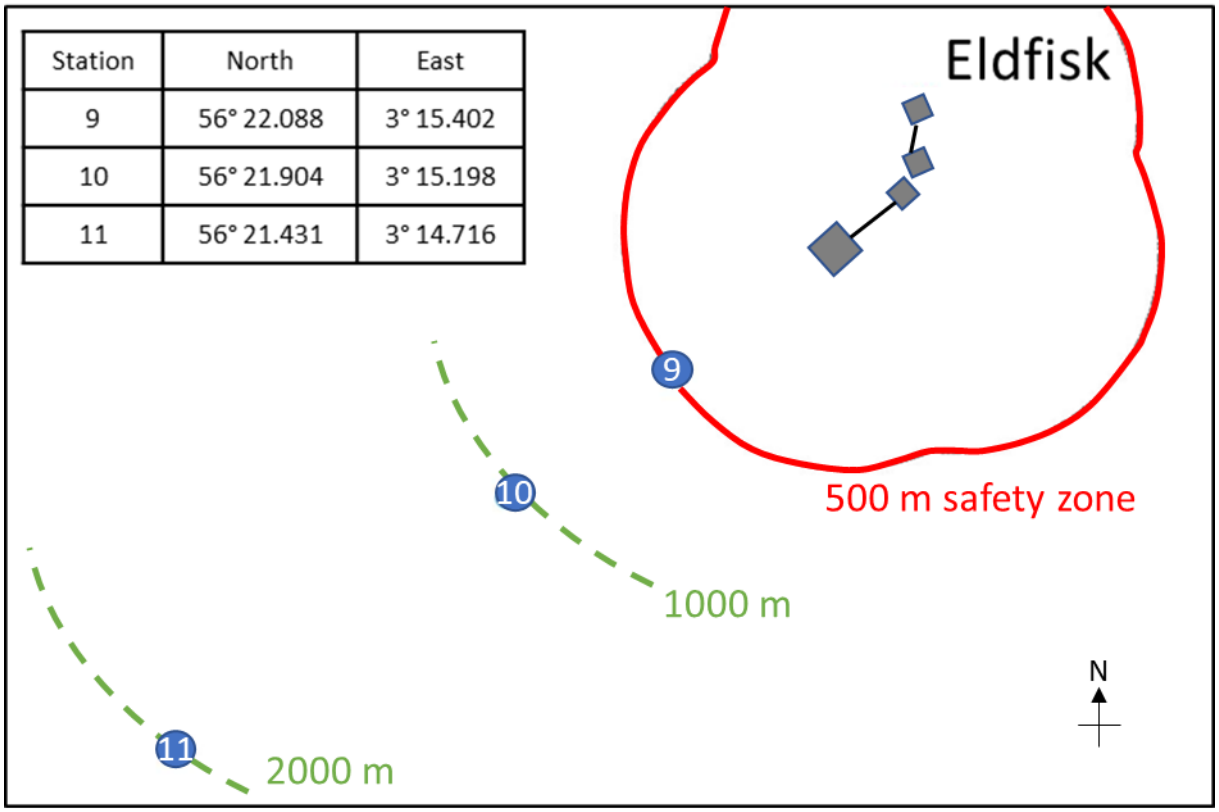
Figure 3. The design of the monitoring rigs, with a dissolvable concrete anchor connected to non-stretchable Dyneema rope to ensure buoys were held 15 m below the sea surface. Equipment ropes connected at approximately 18 and 40 m depths holding a variety of items depending on the station.



Station	North	East
1	56° 32.498	3° 12.987
2	56° 32.576	3° 12.987
3	56° 32.477	3° 12.684
4	56° 32.452	3° 12.401
5	56° 32.380	3° 12.479
6	56° 32.052	3° 11.706
7	56° 32.929	3° 11.862
8	56° 32.154	3° 10.541

St.	Equipment at 20 m	Equipment at 40 m
1	mussels, PSDs	mussels, scallops, PSDs
2	mussels, PSDs	mussels, PSDs, sediment trap
3	mussels, PSDs	mussels, scallops, PSDs, ADCP Current meter
4	mussels, PSDs, CTD with Chl. sensor	mussels, PSDs, CTD with Chl. sensor
5	mussels, PSDs	Mussels, PSDs, sediment trap
6	mussels, PSDs	Mussels, PSDs, sediment trap
7	mussels, PSDs	Mussels, PSDs, ADCP current meter
8	mussels, PSDs	Mussels, PSDs
Ref1	mussels, PSDs Chl. sensor	mussels, scallops, PSDs Chl. Sensor, sediment trap
Ref2	mussels, PSDs	mussels, scallops, PSDs, sediment trap

Figure 4. Approximate positions of the monitoring stations southwest from the Ekofisk complex, including coordinates of each station. Stations 1 and 2 within the 500 m safety zone (red line), with stations 3, 750 m, 4 and 5 (1000 m), 6 and 7 (2000 m) and station 8 (4000 m) from the Ekofisk complex. PW discharged from platforms J and M (yellow boxes). The table indicates the equipment placed on each monitoring rig at each depth. PSD, passive sampling device; ADCP, Aquadopp current profiler; Chl., chlorophyll; CTD, conductivity, temperature, depth.



Station	Equipment at 20 m
9	mussels, PSDs,
10	mussels, PSDs, ADCP current meter at 50 m depth
11	mussels, PSDs,

Figure 5. Approximate positions of the monitoring stations southwest from the Eldfisk complex, including coordinates of each station. Stations 9, 10 and 11 were approximately 500, 1000 and 2000 m downstream of the main tidal driven current. Eldfisk 500 m safety zone (red line). The table indicates the equipment placed on each monitoring rig at each depth. PSD, passive sampling device; ADCP, Aquadopp current profiler.

2.1.3 Source of field transplanted mussels and day zero sampling

Mussels (*Mytilus* sp.) were obtained from the Shellfish hatchery in the outer Trondheimsfjord (Snadder og Snaskum AS), which has been used in previous WCM programmes. Mussels were held at the NORCE laboratory in Stavanger for approximately 2 weeks prior to the deployment cruise, where they were placed in 400 L tanks of clean flowing seawater (8°C) and allowed to depurate. Mussels were fed sparingly (approximately 15 ml Instant Algae, Shellfish diet 1800 was added to each holding tank every 3 to 4 days) to provide a low ration to sustain basic physiology without risking the triggering of spawning.

A subset of mussels was sampled to establish the chemical body burden and health status of the organisms prior to field deployment (i.e., time zero (T0) mussel group). These mussels were sampled for the same parameters as the field exposed mussels, as described in section 2.3. Biological effect measurements included condition index (CI), stress-on-stress (SoS); micronuclei (MN), lysosomal

membrane stability (LMS), and histology and the chemical analyses consisted of the PAH-NPD and metal body burden.

2.1.4 Source of scallops

A few weeks prior to the deployment cruise, scallops (*Pecten maximus*) were collected by diver from waters near Kvitsøy, Rogaland. They were brought back to the NORCE laboratory in Stavanger and held in flowing seawater tanks prior to field deployment. Scallops were fed sparingly to maintain health and reduce the risk of spawning. A subset of scallops was sampled to establish the chemical body burden (PAH-NPD, metals) and biological responses (CI, MN, LMS and histology) prior to field deployment (i.e., time zero (T0) scallop group).

On the morning of the deployment cruise, scallops were moved to large chiller boxes containing fresh seawater and transported onto the vessel. The seawater was exchanged every 6-12 hours, whilst on the vessel in order to maintain optimum health prior to field development. Scallops were used at four stations (Ekofisk station 1 and 3, reference stations 1 and 2), where they were placed in individual compartments of a rigid netted cage (Figure 6).

Figure 6. Cage used for the deployment of the scallops with separate compartments for each individual scallop (n=32).

2.1.5 Oceanographic equipment

Physicochemical properties of the seawater at the Ekofisk, Eldfisk and reference locations were measured with a variety of oceanographic equipment deployed on the monitoring rigs or from the survey vessel during deployment and/or retrieval. Instruments placed on the monitoring rigs included three Aquadopp Current Profilers (ADCPs) placed at monitoring stations 3, 7 and 11. Four CTD (SAIV) instruments equipped with conductivity, temperature, depth, chlorophyll, and turbidity and in some cases oxygen sensors were connected to monitoring Station 4 and reference 1 at both 20 m and 40 m depths throughout the deployment period. Temperature loggers (HOBO onset pendant) were attached to each of the mussel netted socks and monitored temperature for the duration of the mussel exposure. In addition, conductivity, temperature, and depth (CTD) profiles of the water column were taken at the reference stations 1 and 2, as well as Eldfisk and Ekofisk fields during the deployment survey.

2.2 Wild fish populations

2.2.1 Ekofisk safety zone

The fishing activity within the safety zone of the Ekofisk complex was carried out using rod and line fishing from the vessel. The objective was to catch and sample 3 species with 30 individuals for each species. However, from the deployment cruise it was known that dab (*Limanda limanda*) were abundant in the area and cod (*Gadus morhua*) were present. Following two full days of fishing a total of 30 dab and 30 cod were collected and sampled from the safety zone of the Ekofisk complex, detailed information on these 60 fish is shown in Appendix B. No other fish species were caught during the cruise or identified with video surveillance that was performed on the deployment cruise. It was concluded therefore, that only two species of fish were available from the Ekofisk safety zone.

2.2.2 Fish trawling in the North Sea regions

The initial objective of the fish trawling cruise was to collect fish from two regions of the North Sea, Egersundbank and the Ekofisk region (Figure 7). However, in addition to these two regions, fish from Vikingbank were also collected, the specific numbers are summarised in Table 1 and information on these fish can be found in Appendix B. Since cod and dab were collected from the Ekofisk safety zone, these were the target fish from the three regions. However, cod were only found at Egersundbank, whilst dab were found at both Ekofisk region and Egersundbank. In addition, haddock (*Melanogrammus aeglefinus*) and whiting (*Merlangius merlangus*) were caught and sampled from the regions of Ekofisk region, Egersundbank and Vikingbank. These fish species have been assessed in previous WCM programmes and were important for comparison. The grey gurnard (*Eutrigla gurnardus*) was a new species to the WCM programme and was only found at one location. Therefore, priority was given to cod, dab, haddock, and whiting, while the grey gurnard specimens were not used in further chemical or biological effects assessment.

Table 1. The number of fish species caught from the different locations. Ekofisk safety zone fish were caught on the retrieval cruise in week 18, whilst the fish from the three regions of the North Sea, Ekofisk, Egersundbank and Vikingbank were caught on the fish trawling cruise during week 19.

Fish species English/ Norwegian (species name)	Ekofisk safety zone	Ekofisk region	Egersundbank	Vikingbank
cod / torsk (*Gadus morhua*)	30		17	
dab / sandflyndre (*Limanda limanda*)	30	30	30	
haddock / hyse (*Melanogrammus aeglefinus*)		30	30	30
whiting / hvitting (*Merlangius merlangus*)		15	15	8
grey gurnard/ knurr (*Eutrigla gurnardus*)		18		

N.B., As Grey gurnard individuals were only caught from one location and were excluded from further assessments.

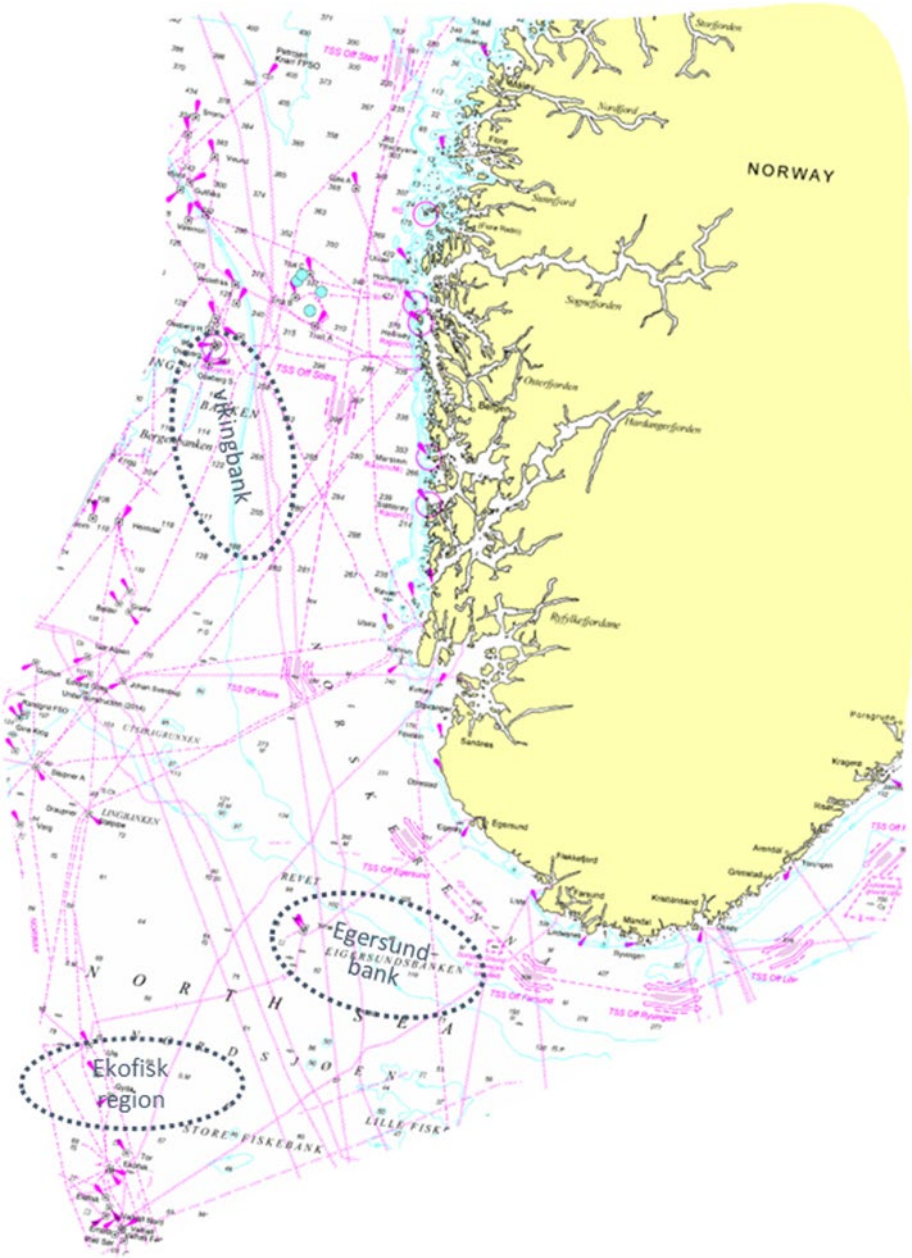


Figure 7. Approximate locations of the three regions of the Norwegian sector of the North Sea where wild fish populations were collected by trawling. Higher oil and gas activities in the Ekofisk region and Vikingbank compared to the Egersundbank (Source: Norgeskart.no).

2.3 Collection of biological tissue, passive samplers and sediment traps

Two 20-foot shipping containers were fixed to the main deck of the Esvagt Dee, one of the containers was used for equipment and sample storage (-80°C and -20°C freezers), whilst the other was used as the main laboratory. All equipment necessary for the collection and storage of the biological material was brought to the vessel and set up within the container.

2.3.1 Mussel sampling

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 either the man-overboard boat or from the main vessel with the help or a grappling hook thrown from the side of the vessel. All monitoring rigs were successfully retrieved using this method.

Mussels collected from each of monitoring rigs were processed within 2 hours as shown in Figure 8. Netted bags were opened using scissors, and the organisms were gently separated if required, by cutting the byssal threads. Mussels were briefly cleaned of external fouling by rubbing the external surface of the mussels gently. Fifteen mussels were randomly selected, placed in a labelled plastic bag and frozen at -20°C for the condition index (CI). A further 12 mussels were selected for the stress on stress (SoS) assay, these were placed in separate compartments of a plastic egg cartoon, which was then placed in polystyrene fish crate containing ice packs and temperature loggers to ensure the temperature remained stable during the assessment. On board the vessel, mussels were assessed daily for mortality. At the end of the cruise crates were transported to the laboratory and placed in incubators at 10 ± 2°C where the SoS assessment was continued for a total of 21 days.

Approximately 300 µl of haemolymph was removed from the adductor mussel of 15 mussels with a syringe and needle (bore size 0.6 mm) containing 300 µl of PBS with 10 mM EDTA. A 100 µl sub sample of the haemolymph PBS suspension was placed into both sides of a cytofunnel (ThermoFisher) and centrifuged at 800 rpm for 2 min directly onto a glass cytoslide. Slides were fixed in methanol for 15 min, dried and stored in boxes at room temperature until the micronucleus (MN) assessment. These same mussels were also used for the collection of histological samples. The whole soft tissue of the mussel was removed carefully from its shell, a cross section of the mussel was taken as shown in the inserted photo of Figure 8, in order to obtain a section of the digestive gland and gonad together. The cross section was carefully positioned in a labelled cassette and placed in Davidson's fixative.

An additional 15 mussels were used for gill histology, where both sections of the gill were removed and carefully positioned on foam inserts within a histological cassette and placed in Davidson's fixative. The digestive gland was also removed from these mussels, placed in a labelled cryovial and snap frozen in liquid nitrogen. These digestive gland samples were stored at -80°C for lysosomal membrane stability (LMS) assessment. Finally, 25 individuals were selected for chemical analyses (PAH-NPD and metals). Following the cutting of the adductor muscle and opening of the shell, mussels were allowed to drain off their excess liquid for a few minutes. The soft tissue of the mussel was removed from the shell and five mussels were each placed into five clean heated treated glass jars.

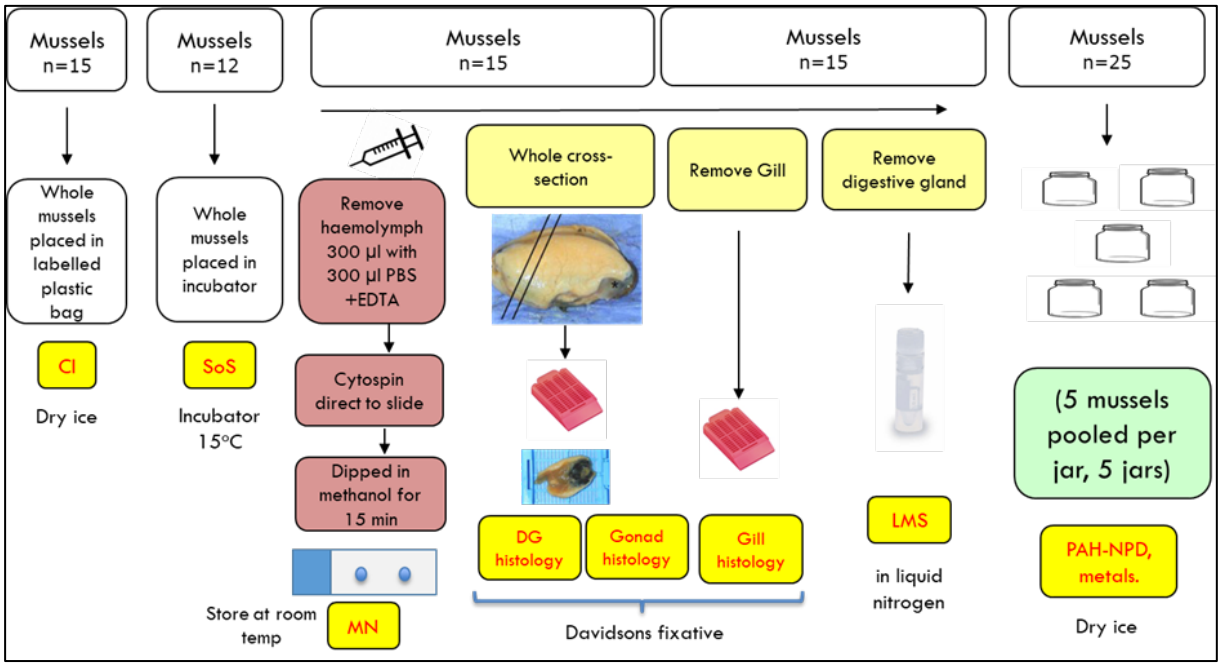


Figure 8. Collection of biological tissue for the different chemical and biological effects assessments for the mussel, (Mytilus sp.). Condition index (CI), SoS (stress on stress), micronucleus (MN) assay, digestive gland (DG), lysosomal membrane stability (LMS), polycyclic aromatic hydrocarbons (PAH) naphthalenes, phenanthrenes and dibenzothiophenes (NPD), phosphate buffer solution (PBS), Ethylenediaminetetraacetic acid (EDTA).

2.3.2 Scallop sampling

Scallops were retrieved from the four monitoring rigs. Cages were opened, and scallops removed and carefully placed in cooler boxes prior to tissue collection. Scallops were processed within 2-4 h after collection as described in Figure 9. Ten scallops were placed in a labelled plastic bag and frozen at -20°C for CI. Haemolymph was removed from the adductor muscle of a further ten scallops and prepared for the MN assay as described earlier for the mussel (section 2.3.1). In these same scallops, digestive gland, gill and gonad were removed, placed in histological cassettes and fixed in Davidson's fixative. A section of the digestive gland was removed, placed in a cryovial and snap frozen in liquid nitrogen, stored at -80°C for the LMS assessment.

Finally, 10 scallops were selected for chemical assessment (PAH-NPD and metals). Scallops were opened and allowed to drain off their excess liquid. The whole soft tissue was removed and placed into a clean heated treated glass jar. Five replicate jars were produced with 2 scallops per jar.

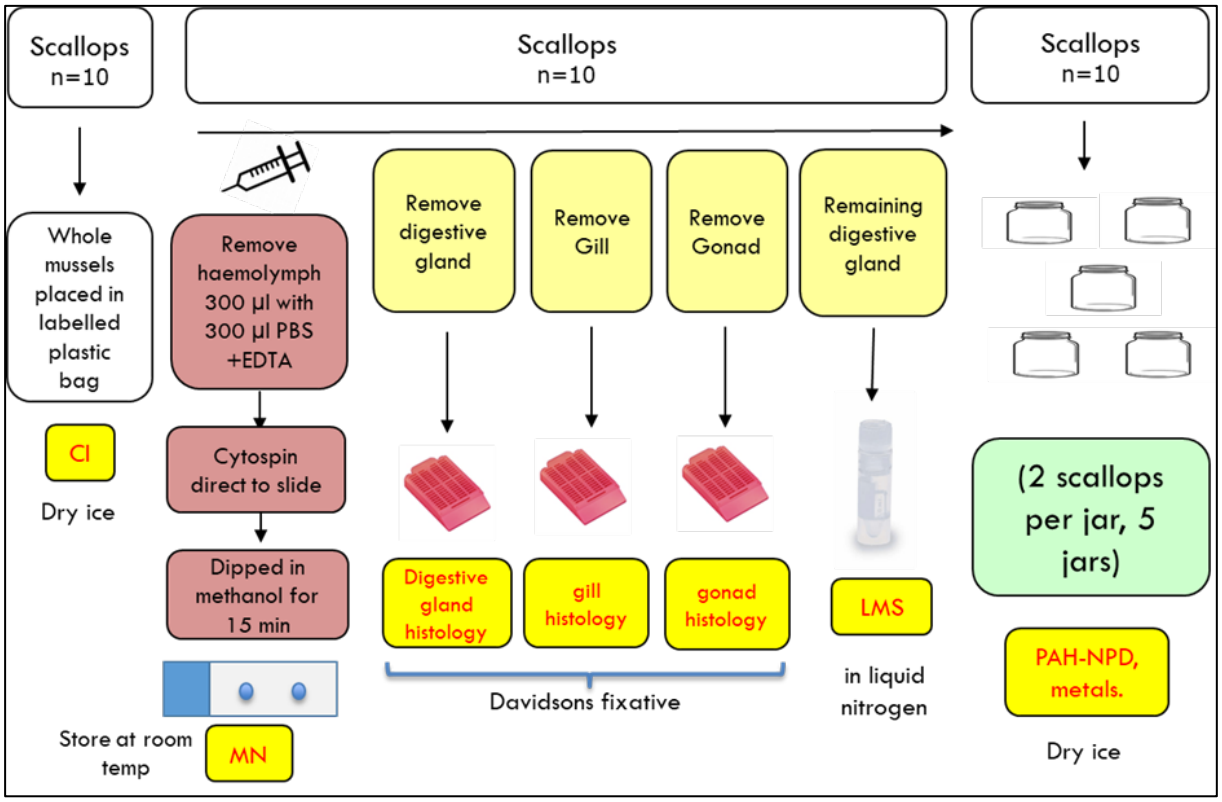


Figure 9. Collection of biological tissue for the different chemical and biological effects assessments for the scallop (Pecten maximus). Condition index (CI), micronuclei (MN), Lysosomal membrane stability (LMS), polycyclic aromatic hydrocarbons (PAH) naphthalenes, phenanthrenes and dibenzothiophenes (NPD), phosphate buffer solution (PBS), Ethylenediaminetetraacetic acid (EDTA).

2.3.3 Fish sampling

The collection of the biological samples for chemical and biological effect assessment in fish are summarised in Figure 10. A blood sample was firstly taken from the caudal vein of each individual, using a heparinised syringe (gauge 1 mm). A 10 µl sample of the blood was taken for the comet assay, whilst the remaining sample was used for chemical analysis of per-fluorinated aromatic substances (PFAS). The fish was then killed with a blow to the head and weight and length measurements taken. This fish was opened for organ dissection and sex identification. The bile was removed from the gall bladder, to avoid potential contamination from spillage, placed in a cryovial and stored at -20°C for PAH metabolite analysis.

The whole liver was dissected from the fish, weighed and divided into sections for the different analyses, with the intension to use the same area of the liver for each analysis. Liver samples snap frozen in liquid nitrogen and stored at -80°C were analysed for ethoxyresorufin-O-deethylase (EROD) to determine cytochrome P450 1A (CYP1A) enzyme activity, CYP1A protein concentration, and the induction of CYP1A mRNA gene expression (qPCR), as well as DNA adducts. Liver fixed in formalin was used for histology, whilst liver frozen and stored at -20°C was used to measure PAH-NPD concentrations.

Gonads were removed and weighed to calculate the gonadosomatic index (GSI). The intestine was removed and a small section of the fore and hind intestine placed in separate cryovials. These were snap frozen in liquid nitrogen for analysis of DNA adducts. From the dorsal surface of the fish a section

of skin was removed, and the underlying muscle fillet was sampled, snap frozen in liquid nitrogen and stored at -80°C for acetylcholine esterase (AChE) inhibition analysis. Finally, otoliths were removed from the cranial cavity of each sampled fish, placed in a paper envelope for age determination.

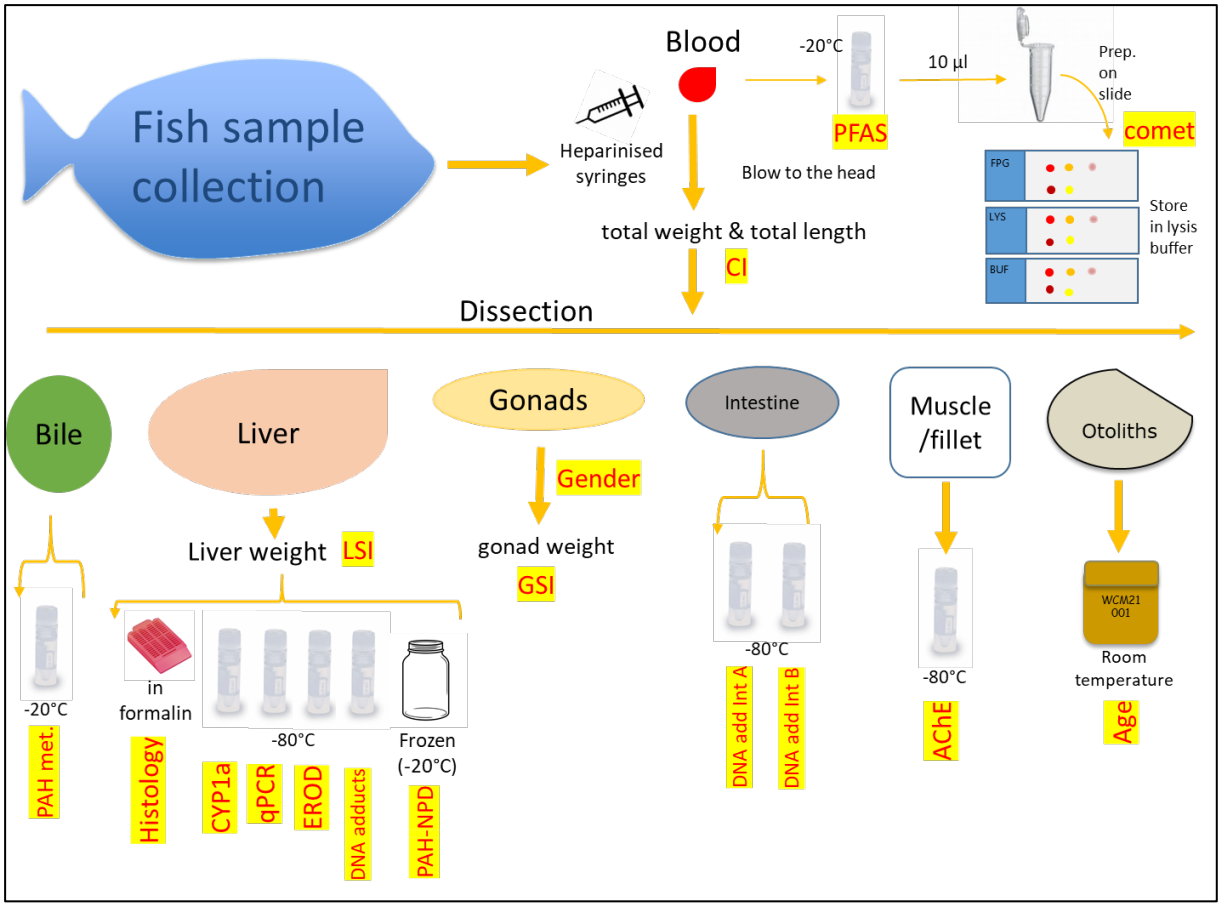


Figure 10. Collection of biological tissue for chemical and biological effects assessment in the fish species collected from the Ekofisk complex safety zone and the regional areas. Condition index (CI), perfluorinated aromatic substances (PFAS), polycyclic aromatic hydrocarbon metabolites (PAH met.), cytochrome P450 1a (CYP1a), quantitative polymerase chain reaction (qPCR), ethoxyresorufin-O-deethylase (EROD), acetylcholine esterase (AChE), liver somatic index (LSI), gonadosomatic index (GSI).

2.3.4 Deployment and retrieval of passive samplers

Passive samplers in the form of polar organic integrated samplers (POCIS) and silicone rubber were deployed at all stations and depths as the mussels. Three silicone strips wrapped around three separate purpose-built metal frames (spider holder) and housed inside a stainless-steel canister. A POCIS holder containing three POCIS samplers were also placed into the same stainless-steel cage. At four of the stations (stations 1, 6, 9 and reference 1), semipermeable membrane devices (SPMDs) were deployed. The silicon membranes were used to measure total hydrocarbon concentration (THC), PAH-NPD and alkyl phenol (AP) concentrations. PAH-NPD were also measured in the SPMDs for comparison with the PAH concentrations from the silicone rubber membranes. The POCIS samplers were used to sample naphthenic acid (NA).

2.3.5 Sediment traps

Sediment traps were placed on five monitoring rigs at an approximate depth of 45 m, these included the two reference stations and Ekofisk stations 2, 5 and 6. Sediment traps consisted of two cylindrical plastic acrylic tubes with a diameter of 10 cm. They were retrieved after approximately 6 weeks and successfully brought to the deck of the vessel without spillage of material. Sediment traps were secured upright and allowed to settle whilst on deck. After 6 ± 2 h most of the overlying water was siphoned off to leave the sediment and a small volume of overlying water. The sediment trap material was transferred into a clean labelled glass bottle. Further reduction in volume of overlying water was made after further settlement of sediment in the glass bottle, and finally stored at -20°C until required.

2.4 Chemical accumulation in passive samplers

Three types of passive sampling devices were used for this study. Silicone rubber samplers were used for the sampling and determination of freely dissolved concentrations of PAHs, alkylated PAHs, THC and APs, while an adapted version of the POCIS targeted the sampling of naphthenic acids. SPMDs were also deployed in parallel to silicone rubber samplers at selected sampling sites to provide a comparison with PS data obtained previously.

2.4.1 Preparation of PSDs for field deployment

Silicone rubber

SSP-M823 silicone rubber (polydimethylsiloxane), produced by Specialty Silicone Products Inc. (USA) and purchased from Shielding Solution Ltd (UK) was used for this work. One single batch of passive samplers were prepared for this study. Samplers were cut into strips measuring 100 cm-long by 2.5-cm wide and with a thickness of 250 µm (nominal mass of polymer of 7 g with a density of 1.2 g/ cm^3) to fit into deployment canisters and spider holders. Samplers were washed in a laboratory dishwasher prior to Soxhlet extraction with ethyl acetate to remove impurities and silicone oligomers. Silicone rubber strips were air-dried prior to further Soxhlet extraction with methanol. This step ensured the samplers were clean and ready for the spiking of performance reference compounds (PRCs) using a co-solvent method with water and methanol. PRCs are isotopically non-naturally occurring, sometimes labelled analogues (deuterated PAHs and non-occurring PCBs) of chemicals of interest, that dissipate from the samplers during sampler exposure in water. PRC release from the samplers during exposure allows us to estimate exchange kinetics during deployment *in-situ*. PRC spiking involved adding known amounts of deuterated PAHs and non-occurring PCBs to a batch of silicone rubber membranes soaking in methanol. The methanol solution was regularly supplemented with ultrapure water over time forcing their distribution to the silicone rubber. This procedure results in homogenous concentrations of PRCs in the silicone rubber samplers. Upon PRC spiking completed, all samplers were placed together in two sealed, large and solvent-rinsed metal jars. Containers were kept at -20°C until exposure. For most exposures one sample was formed from one strip corresponding to 500 cm^2 of sampling surface and a nominal sampler mass of 7 g. Individual metal containers were brought on-site during sampler retrieval to store them separately upon collection.

SPMDs

SPMDs were purchased from Exposmeter AB (Sweden). These were of standard size, 92 cm long, 2.5 cm wide lay-flat polyethylene tubing filled with triolein lipids. These were kept a -20°C until deployment. Performance reference compounds spiked in these samplers and analysed here consisted of deuterated PAHs.

POCIS
The design of the sampler for NA accumulation was based on the standard POCIS device (Harman et al., 2012). In the present design, a receiving phase composed of HLB sorbent incorporated in a 47 mm solid phase extraction disc, was placed in-between two layers of stainless-steel mesh with a 5 µm pore size. The passive sampling unit was held together with standard POCIS metal washers and bolts that were first dish-washed prior to further methanol rinsing. Horizon HLB discs were purchased from Biotage (Sweden). Methanol was flushed through the disc for cleaning and conditioning. Stainless steel mesh was cut to the appropriate size with a metal punch. Slices of stainless-steel mesh were placed in acetone and then methanol for cleaning. Once assembled samplers were kept in clean aluminium foil until deployment. Immediately prior to deployment, the samplers were conditioned by flushing the sampler's receiving phase and mesh with methanol and then ultrapure water. Samplers remained soaked with ultrapure water until deployment in canisters together with silicone rubber samplers.

2.4.2 Sampler retrieval, extraction and clean-up

Silicone
After deployment, samplers were retrieved, and their surface washed on-site using seawater. The surface of the samplers was further cleaned with ultrapure water and dried with a clean tissue when back in the laboratory. All samplers, including field blanks, were extracted for 24 hours by soaking with n-pentane (2 × 250 ml) in glass jars. Internal standards (for PAHs, APs and PCB PRCs) were added to the extraction jar during the first step of the extraction. The two volumes of pentane were joined and reduced to 2 ml with a gentle flow of nitrogen. In most cases, extracts were split into two fractions by volume. One fraction for the quantification of PAHs, alkylated PAHs, PAH- and PCB- PRCs and alkylphenols, the other fraction for the analysis of THC content. The fraction for THC analysis was analysed without further clean up, the other fraction received a general clean-up using gel permeation chromatography (GPC, with dichloromethane and ethyl acetate as mobile phase). This post-GPC sample was reduced in volume (~ 200 µl) under a gentle stream of nitrogen gas and analysed for PAHs/PRCs, PCB-PRCs without further treatment. For the analysis of APs a derivatisation step was necessary. Briefly, approx. 50 % of the post-GPC extract was derivatised with BSTFA + TMCS (99:1) at 60 °C for 3 hours. After derivatisation, the extract was diluted with cyclohexane and reduced in volume (~ 200 µl) under a gentle stream of nitrogen gas to remove traces of the derivatisation agent and analysed for alkylphenols. The fraction (extract was in cyclohexane: ethyl acetate 80:20 and diluted with 0.5 ml isooctane) for THC analysis represented 45 % of the total extract.

POCIS extraction
Upon retrieval POCIS samplers were kept at 4°C until extraction. Samplers were dismantled with the help of spanners. The HLB discs were collected, washed with miliQ water (2% methanol) and placed in falcon tubes. Samples were then frozen and placed in a freeze dryer to remove residual water. Samplers were then soaked in 25 ml of methanol and sonicated for 10 minutes to elute the contaminants accumulated in the discs. Recovery standards (tamoxifen-d5, benzoylecgonine-d3, 3,4-methylenedioxymethamphetamine-d5, sulfamethoxazole-d4 and diazepam-d5, 20 µl [1 µg/ ml] spiked per HLB disk) were added to the discs during the first extraction step. The methanol was recovered, evaporated, and reconstituted in Eppendorf vials in 1 ml. Extracts were centrifuged for 5 minutes and supernatant was transferred into an LC vial for analysis.

2.4.3 Analysis of total hydrocarbons

THC analysis was conducted with an Agilent 7890A gas chromatography (GC) unit linked to a flame ionisation detector (FID). The injection volume was 1 µl with a run time of 22.8 min in splitless mode with gas flow of 2.5 ml/ min. Initial oven temperature was 55°C and then increasing to 325°C at a rate

of 25°C per min. It was then held at this temperature for 10 min. The separation column was a Varian CP sil 8 CB (25 m x 320 µm x 0.25 µm). The FID detector was run at 300°C with a H_2 flow of 30 ml/ min and air flow of 300 ml/ min. An external 5 point-calibration curve was prepared using EDC 95/11 base oil (focusing on C11-C35 hydrocarbons).

2.4.4 PAH-NPD in passive samplers

Analysis for PAH/PAH-PRCs was performed on an Agilent 7890A GC coupled to an Agilent 5975c inert XL EI/CI quadrupole mass spectrometer operated in single-ion monitoring mode (SIM) with electron impact ionisation (70 eV). Analyte separation was on a DB-5MS column (30 m, 0.25 mm inside diameter and 0.25 µm film thickness; Agilent JW Scientific) with a 1 µl pulsed split-less injection (pulse pressure 25 psi for 0.5 min and injector temperature of 280°C). Helium was used as carrier gas with flow set to 1.2 ml/ min. The oven temperature program for the GC consisted of a step at 60°C (held for 2 min) before an increase to 250°C (at the rate of 7°C/ min) and a final increase to 310°C (at the rate of 15°C/ min, held for 5 min). Temperatures for the ion source, quadrupole, and transfer line were 230, 150 and 280°C, respectively. Quantification was performed using the relative response of surrogate internal standards and 7-point calibration curves. Deviation (<20%) of the qualifier ion response relative to that of the quantifier ion was used for identification. Internal standards were naphthalene-d_8, biphenyl-d_{10}, acenaphthylene-d_8, dibenzothiophene-d_8, pyrene-d_{10}, benz[a]anthracene-d_{12}, and perylene-d_{12}.

Analysis for PCB-PRCs and OCs was performed on an Agilent 7890B GC coupled to an Agilent 7010B Triple Quad mass spectrometer operated in multiple reaction mode (MRM) with electron impact ionisation (70 eV). Analyte separation was on a HP-5MS UI column (2x 15 m, 0.25 mm inside diameter and 0.25 µm film thickness; Agilent JW Scientific) with a 1 µL pulsed split-less injection (pulse pressure 25 psi for 0.6 min and injector temperature of 280°C). Helium was used as carrier gas with flow set to 1 ml/ min. The oven temperature program for the GC consisted of a step at 60°C (held for 1 min) before an increase to 120°C (at the rate of 40°C/ min), followed by an increase to 280°C (at the rate of 5°C/ min) and a final increase to 300°C (at the rate of 10°C/ min, held for 3 min). Temperatures for the ion source, quadrupole, and transfer line were 280, 150 and 280°C, respectively. Quantification was performed using the relative response of surrogate internal standards and 7-point calibration curves. Deviation (<20%) of the qualifier ion response relative to that of the quantifier ion was used for identification. Internal standards were CB30, CB53, and CB204 for PCBs/OCs.

2.4.5 Alkylphenols in passive samplers

Analysis for APs was performed on an Agilent 7890A GC coupled to an Agilent 5975c inert XL EI/CI quadrupole mass spectrometer operated in single-ion monitoring mode (SIM) with electron impact ionisation (70 eV). Analyte separation was on a DB-5MS column (30 m, 0.25 mm inside diameter and 0.25 µm film thickness; Agilent JW Scientific) with a 1 µl pulsed split-less injection (pulse pressure 25 psi for 0.5 min and injector temperature of 280°C). Helium was used as carrier gas with flow set to 1.2 ml /min. The oven temperature program for the GC consisted of a step at 60°C (held for 2 min) before an increase to 250°C (at the rate of 7°C/ min) and a final increase to 325°C (at the rate of 15°C/ min, held for 5 min). Temperatures for the ion source, quadrupole, and transfer line were 230, 150 and 280°C, respectively. Quantification was performed using the relative response of surrogate internal standards and 6-point calibration curves. Deviation (<20%) of the qualifier ion response relative to that of the quantifier ion was used for identification. Internal standards were p-cresol-d_8, 3,5-dimethyl-

2,4,6-d_3-phenol, 4-n-propyl-d_{12}-phenol, 2,3,5,6-d_4-4-tert-butyl-d_9-phenol, 4-n-pentyl-d_{11}-phenol, 4-n-octyl-d_{17}-phenol and n-nonyl-d_4-phenol.

2.4.6 Naphthenic acids in passive samplers

The chromatography column, stationary phase, mobile phases, and acquisition method were selected in accordance with a pre-established screening method. A Waters Acquity UPLC system coupled to Xevo G2-S Q-TOF mass spectrometer (MS) (Waters, Milford, MA USA) was used for this work. Chromatographic separation was carried out using an Acquity UPLC HSS C18 column (2.1 x 150 mm, particle size 1.8 µm) (Waters, Milford, MA, USA). Gradient elution was performed at a constant flow of 0.4 ml/ min using 5 mM ammonium formate, pH 3.0 (solvent A) and acetonitrile with 0.1% formic acid (solvent B). The gradient elution starts with 87% A and then increasing B to 95% in 15 minutes: Solvent A, held for 0.5 min; 0.5-10 linear rate to 50% B, 10-10.75 linear rate to 95% B, held for 0.5 min; reconditioning with a linear rate to 87% A, 12.50-15 min. The analytical column and the guard column were kept at 50°C and the sampler manager at 5°C. MS was used in positive and negative ESI mode for acquisition using MSe, allowing both precursor and product ion data to be simultaneously acquired during a single run. The variability in the signal intensity obtained for the recovery standards (tamoxifen-d5, benzoylecgonine-d3, 3,4-methylenedioxymethamphetamine-d5, sulfamethoxazole-d4 and diazepam-d5) added to the extracts during sample preparation was under 20%.

2.4.7 Estimation of freely dissolved concentrations from silicone rubber samplers

Data treatment was the same for all silicone rubber samplers. No corrections for water temperature or salinity were applied to polymer-water partition coefficients, K_{sw} for PRCs or chemicals of interest. Sampling rates, R_s (L/day) were estimated for each silicone rubber at each site by applying the non-linear least square (NLS) method to the PRC dissipation data using the methodology presented by Booij and Smedes (2010). This procedure assumes that the fraction (*f*) of PRC remaining in the sampler after exposure (N_t/N_0), is a continuous function of the sampling rate:

$$f = \frac{N_t}{N_0} = exp\left(-\frac{R_s t}{mK_{sw}}\right) \qquad \text{Eq. 1}$$

The model to estimate R_s from K_{sw} was that given in Rusina et al. (2010) for AlteSil SR but it is also applicable to SSP silicone rubber, since it relies on the assumption that the uptake in the samplers for most hydrophobic substances is under water boundary layer-control. This means the R_s is proportional to the mass transfer coefficient in the boundary layer. The model is based on the optimisation (from the NLS above) of an exposure specific β_{sil} factor ($L^{1.08}$ $kg^{0.08}$ d^{-1}) and known K_{sw} (L/ kg):

$$R_s = \beta_{sil} K_{sw}^{-0.08} \qquad \text{Eq. 2}$$

The complete equation, taking into account linear, equilibrium and partially equilibrated conditions, was used to estimate dissolved concentrations of HCB, PCB, and selected substances of interest:

$$C_w = \frac{n_{acc}}{K_{sw} m (1 - e^{\frac{-R_s t}{K_{sw} m}})} \qquad \text{Eq. 3}$$

with C_w being the freely dissolved concentration (ng/ L), n_{acc} the mass of chemical accumulated in the sampler during exposure, *m* the mass of the SR passive sampler and K_{sw} the SSP-water partition coefficient measured for. Values of K_{sw} values for SSP silicone rubber were from Smedes (2019) for

PAHs and PRCs. For alkylphenols, these were derived from experiments conducted as part of the WCM R&D project. For THC (C_{12}-C_{35}), values of K_{sw} are not available. A range of $logK_{sw}$ values was estimated from $logK_{ow}$ values for this range of hydrocarbons (6.01-11.68). We use the $logK_{sw}$-$logK_{ow}$ regression to infer $logK_{sw}$ values for THC (4.8-9.0).

For SPMDs, a similar modelling procedure was used according to the model by Booij et al. (2003) and SPMD-water partition coefficients from Smedes (2019).

2.4.8 Silicone rubber and POCIS sampling rates

Sampling rates (R_s) for silicone rubber passive samplers are reported in Figure 11. A good agreement between triplicate samplers can be seen in these boxplots. However, some differences were observed between the different sampling stations. Most notably, Rs values are lowest for the reference stations 1 and 2 (both depths). They tend to be highest at the stations closest to Ekofisk platform (10-13 L/d) decreasing to very stable values in the range of 6-9 L/d further away.

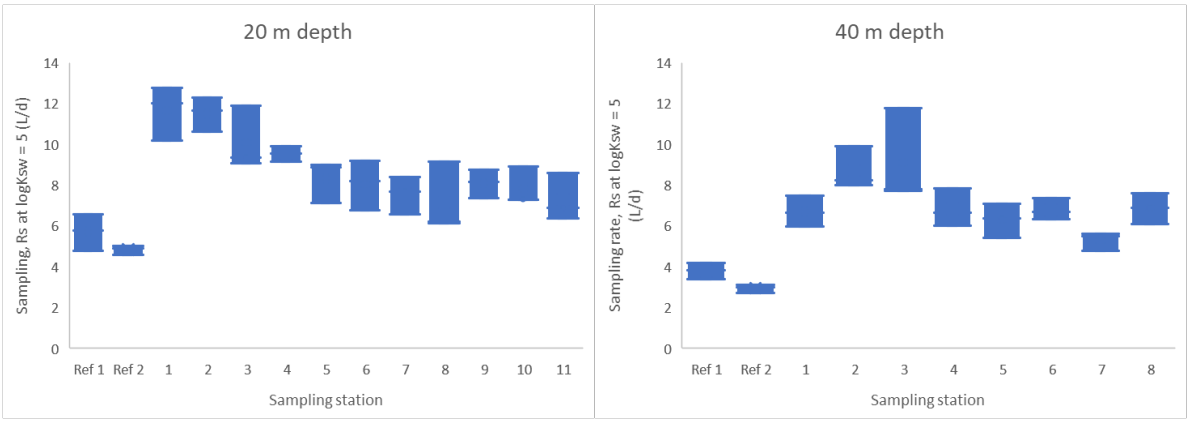


Figure 11. Sampling rates for triplicate silicone rubber passive samplers deployed at each sampling station. Sampling rates, Rs expressed in L/d were calculated from the performance reference compounds dissipation and the non-linear least square method and are given here for a model compound with a silicone-water partition coefficient, $logK_{sw}$ = 5.

No PRCs can be used with the POCIS passive sampler since exchange of chemicals between the HLB disc (receiving phase of the sampler) and water is not isotropic, i.e. the dissipation of PRC does not mimic the uptake of chemicals into the sampler. Contaminant uptake into the POCIS sampler configuration developed for this work (5 µm stainless steel mesh and a Horizon HLB SPE disc) is expected to be relatively insensitive to water turbulence, resulting in similar sampling rates for different sampling station irrespective of difference in water turbulences. Calibration work is being reported as part of the R&D project. The sampling rate (Rs) for POCIS can be expressed with the following equation:

$$\frac{1}{R_s} = \frac{1}{R_{s,max}} + \frac{1}{Ak_w}$$

where Rs,max is the maximum sampling rate that can be achieved, i.e. when water turbulences are highest and mass transfer resistance in the water boundary layer at the surface of the sampler is minimal (Glanzmann et al., 2022). The mass transfer coefficient k_w for the boundary layer at the surface of the POCIS sampler maybe extrapolated from that estimated for SR and PRC dissipation. The average of k_w values for all sites and all silicone samplers was 2.3 +/- 0.7 (30 %) µm/s. In case the mass transfer resistance on the sampler's side is negligible, this is equivalent to an overall mean sampling rate of 0.81 L/d. This means the lower the $R_{s,max}$ value is in comparison with this value, the more uniform

sampling rates will be across all sampling sites. Often reported POCIS sampling rates are in the range of 0.3 L/d or below. With an Rs,max set to 0.3 L/d, most individual sampling rates are within 20% of the average sampling rate of 0.21 L/d that combines mass transfer resistance in the sampler and the boundary layer. The lower the $R_{s,max}$ value, the closer the actual sampling rate will be to the $R_{s,max}$ value. Our R&D work is currently helping to gauge this $R_{s,max}$ value for naphthenic acids. In this study, 8-12 L of seawater are expected to have been sampled by each POCIS passive sampler.

2.4.9 Data processing for naphthenic acids

All the chromatograms were converted to open MS format mzXML using ProteoWizard software package. After the conversion, the files were read into julia programming environment. For the detection of NAs, we followed the method established by Samanipour et al. (2020). In short, the extracted ion chromatogram (XIC) of 180 NAs were generated and then integrated. Signals larger than 300 counts were considered analytical signals and considered for integration. The integrated XIC resulted in a report containing the peak intensity, peak area, and the retention time of the peak apex. These peak lists then were aligned based on the retention time and the reported m/z values with a tolerance of 0.01 Da for mass and 0.2 minutes for the retention time. It should be noted that, given that the NA signals do not follow a regular gaussian peak shape, we used a larger retention window to avoid any issues with the signal alignment. After the alignment, a noise removal was performed, where the signal of NAs that were present only in one out of three replicates was set to zero. After the noise removal, we averaged the peak areas of each NA in the samples using the replicates. These averaged areas were utilized for the interpretation.

2.4.10 Comparison C_w estimated from silicone rubber and SPMDs

Semipermeable membrane devices were deployed alongside silicone rubber samplers at 4 different sites (Ref 1, site 6, 8 and 9). The model from Booij et al. (2003) was used to estimate freely dissolved concentrations. Blank SPMDs exhibited levels for certain compounds that prevented us from using them reliably. The only compound that the silicone rubber-SPMD comparison was possible for was fluoranthene. The SPMD/silicone rubber ratio of estimated C_w for fluoranthene were, 1.05, 1.32, 1.19, and 1.12 for Ref 1, Sites 6, 8 and 9 indicating a similar performance of the two types of samplers.

2.5 Chemical measurements in mussels and scallops

2.5.1 PAH-NPD in mussels and scallops

Internal standards (naphthalene d8, biphenyl d10, acenaphthene d8, phenanthrene d10, anthracene d10, pyrene d10, chrysene d12 and perylene d12) were added to a 5 g 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 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 concentrations in mussels and scallops

Samples were homogenized, lyophilized, weighed and measured in duplicates. Levels are reported in wet weight based on dry weight from lyophilization. Levels of metals in soft tissue of mussels and scallops were determined by inductive coupled plasma mass spectrometer (ICPMS) after decomposition in microwave oven as described by Julshamn et al. (2007). The method is accredited according to NS-EN-ISO 17025 for the elements arsenic, cadmium, copper, zinc, mercury, selenium and lead. This method also determines the unaccredited elements silver, iron, cobalt, manganese, vanadium, molybdenum, chromium, nickel.

2.6 Biological effects assessment in mussels and scallops

Biological effect methods described below were applied to mussels. For the scallops, 4 monitoring stations plus a day zero group (T0) were also measured for the same biological endpoints as the mussel, except for stress on stress and speciation.

2.6.1 Mussel speciation

Mussel speciation was determined in gill tissue based on the method of Inoue et al. (1995). Total DNA was extracted from 20-40 mg of sample from frozen mussels using DNAzol reagent (Invitrogen, Madison, Wisconsin, USA), following the manufacturer's recommended protocol. The tissue was homogenised in 1 ml DNAzol using Precellys 24 bead mill (Bertin, Montigny-le-Bretonneux, France), using ceramic CK14 beads at 5000 rpm for 10 sec. Cell debris were then removed by centrifugation at 10,000 g for 10 min (4°C), before DNA was precipitated from the supernatant by addition of 500 µl of 100% ethanol. Following two wash steps with 75% ethanol, the DNA was pelleted by centrifugation at 4,000 g for 2 min, air dried and dissolved in 8 mM NaOH. The resulting DNA was quantified, and quality controlled on a nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). All samples had optical density (OD) 260/280 values greater than 1.8, indicating pure DNA. For species identification, the polymerase chain reaction (PCR) was used to amplify a specific 180 base pair (bp) segment for *Mytilus edulis*, 168 bp segment for *M. trossulus* or 126 bp segment for *M. galloprovincialis*, as described by Inoue et al. (1995). The 50 µl PCR reactions contained 10 µl of DNA template, 300 µM forward and reverse primers, VWR 2x Taq mastermix (VWR, Radnor, Pennsylvania, USA), and were subjected to a 5 min pre-heating stage at 95°C followed by 35 cycles of 30 sec at 95°C, 30 sec at 55°C, 30 sec at 72°C, and final extension step of 10 min at 72°C. One µl of the PCR product was loaded onto a DNA 1000 chip (Agilent technologies, Santa Clara, California, USA) and run in a Bioanalyzer instrument (Agilent technologies, Santa Clara, California, USA) for visualisation of amplicon size.

2.6.2 Condition index

The 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 (Davenport and Chen, 1987; Pampanin et al., 2005). The dry weight values were recorded after oven drying the shell and the soft tissue separately at 90°C for 48 h.

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

2.6.3 Stress on stress

The SoS assessment was measured in twelve mussels from each group at 11 ± 1°C. The mussels were checked every 24 ± 4 h and mortalities were recorded, with dead animals removed. Mussels were considered deceased if their shells were gaping and showed no sign of movement after gentle tapping of their shells (Pampanin et al., 2005).

2.6.4 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 microscope slides (room temperature). 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 minutes 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 the 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 minutes 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 minutes 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.

2.6.5 Micronuclei formation

Slides prepared using the Cytospin 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 (Bolognesi and Fenech, 2012). 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 was determined microscopically (×100 objective using an Olympus IX71) on a minimum of 2000 cells per exposure group. MN were scored in agranular cells with intact cellular and nuclear membranes when: 1) the nucleus and the MN had a common cytoplasm; 2) colour intensity and texture of MN were similar to the nucleus; 3) the size of the MN was equal or smaller than 1/3 of the nucleus; and 4) MN were apparent as spherical structures with a sharp contour.

Due to the lack of work reporting haemocyte appearance in the studied species of scallops, studies with other species were used to guide visualization and scoring (de la Ballina et al., 2022; Estrada et al., 2013; Davies and Vethaak, 2012).

2.6.6 Histology

The dissection of mussels was performed immediately on board and samples stored in fixative for analysis. Tissue processing was performed overnight using automated Tissue Processor (Leica, Shandon Excelsior). During this process, samples were dehydrated through 100% alcohol and a clearing agent xylene. Tissue samples were then embedded with melted 60°C paraffin. Paraffin embedded tissue samples were cut by semi-automated Microtome (Leica). Paraffin sections measuring 3 µm were mounted onto Superfrost Plus slides (Menzel, Braunschweig) and dried overnight at 37°C. Sections were deparaffinised, rehydrated and haematoxylin and eosin. The staining was performed using Ventana H600 automated stain (Roche). The work from dehydrated to staining was performed at the Department of Pathology, Stavanger University Hospital, Norway. The tissues were examined for health parameters related to physiological conditions, inflammatory and non-specific pathologies.

In the digestive gland, epithelial degeneration (scored as presence 1 or absence 0), granulocytoma (scored as 0 – absent; 1 – sporadic; 2 – multiple / widespread), haemocytic infiltration (scored as the granulcytoma), and the presence (1)/ absence (0) of parasites were recorded.

In gonads, adipogranular tissue index (ADG) (scored as 0 – absent, no ADG cells apparent within vesicular connective tissue; 1 – presence, although ADG cells can be seen, they appear to be scarce; 2 – scattered, ADG cells appear scattered throughout mantle tissue; 3 – frequent, there is a marked increase in the abundance of ADG cells. Some areas may not appear to show absolute consistency; 4 – abundant, ADG cells can be seen to constitute the majority of connective tissue volume), apoptosis (scored as presence 1 or absence 0), atresia (scored as apoptosis), granulocytoma (scored as 0 – absent; 1 – sporadic; 2 – multiple / widespread), haemocyte infiltration (scored as granulocytomas), and the presence (1) / absence (0) of parasites were assessed. In addition, the gonad developmental stage was evaluated as 1 – early gametogenesis; 2 – advanced gametogenesis; 3 – mature gonad; 4 – spawning gonad.

In gill tissue, filament fusion, epithelial lifting, haemocyte infiltration, hyperplasia, hypertrophy and presence of parasites were recorded as presence (1) or absence (0). 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 to avoid bias. A QA/QC analysis was carried out in collaboration with IMR.

2.7 Chemical measurements in fish

2.7.1 PAH concentrations in fish liver

Homogenised tissue was extracted by saponification with 0.5N alcoholic KOH for 2 h, followed by liquid/liquid extraction with hexane. Extracts were volume reduced and cleaned on silica/alumina column using Powerprep© automated clean up system prior to injection on an Agilent N-5975 GC/MS (EI) in SIM mode. The GC/MS system was equipped with a HP-6890 GC, a 30 m x 0.25 mm, 0.25 µm DB-17 ms capillary column from Agilent. Other conditions were: injector temperature 300°C; column temperature, 50°C for 2 min, 50-110°C at 10°C/min, 110-290°C at 6°C/min, 21 min at final temperature, carrier gas He at 36 cm/s. Samples were injected by auto sampler, 1 µl splitless injection.

The method is validated to analyse PAH compounds in concentration of 0.2 ng/g. Levels of detection (LOD) are defined as LOD: Y = YB + 3SDB, and levels of quantification (LOQ) is LOQ= Y = YB + 10SDB, where YB is the response of blank sample signal and SDB is the standard deviation of the blank samples.

2.7.2 PAH metabolites

Fish bile samples were prepared for the analysis of PAH metabolites as described by Jonsson et al. (2003; 2004). Briefly, 25 µl of bile was weighed accurately into a micro centrifuge tube. Internal standards (2,6-dibromophenol, 3-fluorophenanthrene and 1-fluoropyrene) and β-glucuronidase (3000 units) in sodium acetate buffer (0.4 M, pH 5) were added and incubated at 40°C for 2 h. The OH-PAHs were extracted with ethylacetate (4 x 0.5 ml), the combined extract dried with anhydrous sodium sulphate and concentrated to 0.5 ml. Trimethylsilyl (TMS) ethers of OH-PAHs were prepared by adding 0.2 ml BSTFA and incubated at 60°C for 2 h. The GC-MS performance standard triphenylamine (TPA), was added before the prepared samples were transferred to capped glass vials. The TMS ethers of OH-PAHs (TMS-OH-PAHs) in fish bile samples were analysed by a GC-MS system consisting of a HP5890 series II Gas chromatograph, Shimudadzu QP2010 GCMS. Helium was used as carrier gas and the applied column was CP-Sil 8 CB-MS, 50 m x 0.25 mm and 0.25 µm film thickness (Varian). Samples and calibration standards (1 µl) were injected on a split / splitless injector with splitless mode on for one minute. The injector, transfer-line and ion source temperatures were 250°C, 300°C and 240°C, respectively. The GC oven temperature programme was set at 80°C to 120°C at 15°C/ min, 120°C to 300°C at 6°C/ min and then 300°C for 30 min. Mass spectra were obtained at 70 eV in selected ion mode (SIM). Based on the fragmentation pattern of non-alkylated TMS-O-PAHs (Jonsson et al., 2003), the molecular ions were selected for determination of both alkylated and non-alkylated TMS-O-PAHs.

2.7.3 PFAS in fish blood

A suite of 36 PFAS were measured in fish blood based on the method from Verreault et al. (2005). Internal standards were added to 0.5 mL of sample and extracted twice with acetonitrile using an ultrasonic bath. The extract was mixed with ammonium acetate buffer, acetic acid and EnviCarb and then filtered (0.45 µm) before analysed by LC/MS-QToF (ESI negative mode).

The PFAS measured in fish blood included the following compounds:

perfluoro-n-pentanoic acid (PFPA)
perfluoron-hexanoic acid (PFHxA)
perfluoro-n-heptanoic acid (PFHpA)
perfluoro-n-octanoic acid (PFOA)
perfluoro-n-nonanoic acid (PFNA)
perfluoro-n-decanoic acid (PFDA)
perfluoro-n-undecanoic acid (PFUDA)
perfluoro-n-dodecanoic acid (PFDoDA)
perfluoro-n-tridecanoic acid (PFTrDA)
perfluoron-tetradecanoic acid (PFTeDA)
Perfluoro pentadecanoic acid (PFPeDA)
perfluoro-n-hexadecanoic acid (PFHxDA)
perfluoro-1-butanesulfonate (PFBS)
Perfluoro pentansulfonate (PFPeS)
perfluoro-1-hexanesulfonate (PFHxS)
Perfluoro heptanesulfonate (PFHpS)
perfluoro-1-octanesulfonate (PFOS)
Branched isomer perfluoro-1-octanesulfonate (br-PFOS)
Perfluoro nonanesulfonate (PFNS)
perfluoro-1-decanesulfonate (PFDS)
perfluoro-1-dodecansulfonate (PFDoS)
perfluoro-1-octanesulfonamide (PFOSA)
N-methylperfluoro-1-octanesulfonamide (Me-PFOSA)
Nethylperfluoro-1-octanesulfonamide (et-PFOSA)
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol (me-PFOSE)
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (et-PFOSE)
Perfluoroktansulfonamide-HAc (FOSAA)
N-methylperfluoro octanesulfonamidoacetic acid (me-FOSAA)
N-ethylperfluoro octanesulfonamidoacetic acid (et-FOSAA)
4:2 Fluorotelomer sulfonates (4:2 FTS)
1H,2H-perfluorooctane sulfonate (6:2); (6:2FTS)
8:2 Fluorotelomer sulfonates (8:2 FTS)
10:2 Fluorotelomer sulfonates (10:2 FTS)
12:2 Fluorotelomer sulfonates (12:2 FTS)
Perfluoro butanesulfonamide (PFBSA)
Perfluoro hexanesulfonamide (PFHxSA)

2.7.4 ^{226}Radium in fish bone and fillet

The natural radionuclide ^{226}Radium (Ra) was analysed in pooled fillet and bone samples of dab from three locations, Ekofisk, Ekofisk region and Egersundbank. Samples were prepared at the NIVA laboratory and transported on ice to the Institute of energy technology (IFE) for analysis.

Pooled fish samples of fillet and bone were dried to constant weight, followed by dry ashing and then milled to a fine powder. Aliquots of the samples were then digested under high pressure and temperature in a closed microwave system after addition of ^{133}Barium (Ba) as a yield monitor. Sulphate precipitations were used to isolate Ra and Ba from other alpha-emitter and the activity of ^{226}Ra was determined by alpha spectrometry.

2.8 Biological effects assessment in fish

2.8.1 General fish parameters (CI, LSI, GSI, Age)

With the aid of a measuring board and a digital fish scale (Berkley® model BTDFS50-1) the length and total weight of each fish was measured on board the survey vessel. Mature fish were sexed by visual examination of their gonad. During the fish dissection, the entire gonad and the liver were removed and weighed on a motion compensated balance (Marel M2000 series). The collected length and weight data were used to calculate general health parameters.

The CI of each cod was determined by the ratio between total weight and the cube of the fork length of the fish.

$$CI = [\text{weight (g)}/\text{Length (cm)}^3)] \times 100$$

The liver somatic index (LSI, liver index) reflects the animal nourishment status. LSI at 0-sampling (Pre exposure) and at the end of the exposure was calculated as:

$$LSI = [\text{liver weight (g)} / \text{fish weight (g)}] \times 100$$

The gonadosomatic index (GSI, gonad index) reflects the animals' reproductive status. GSI at the end of the exposure was calculated as:

$$GSI = [\text{gonad weight (g)} / \text{fish weight (g)}] \times 100$$

To determine the age of each individual fish, otoliths of cod, haddock and whiting were snapped cleanly through the nucleus and examined under the microscope using illuminated side light to increase the contrast between the annuli (Mjanger et al., 2017). Otoliths of dab were put in water and read whole under the microscope. This is the same procedure as used for reading dab otoliths at Iceland.

2.8.2 Ethoxyresorufin-O-deethylase (EROD)

The EROD activity was measured in the microsomal fraction of fish livers based on the method of Burke and Mayer (1974), modified to a fluorescence plate reader by Eggens and Galgani (1992). The microsomal fraction was prepared on ice with pre-cooled equipment and solutions. Cryo-preserved liver samples were homogenized in a potassium phosphate buffer (0.1 M, pH 7.8) containing KCl (0.15 M), dithiothreitol (DTT) (1 mM), and glycerol (5% v/v), using a Potter-Elvehjem Teflon–glass homogenizer. The homogenate was centrifuged (10,000 g; 30 min, 4°C) before the supernatant was re-centrifuged (50,000 g; 120 min, 4°C). The microsomal fraction was obtained by resuspending the resulting pellet in potassium phosphate buffer (0.1 M, pH 7.8) containing KCl (0.15 M), DTT (1 mM),

EDTA (1 mM), and glycerol (20% v/v). Microsome samples were diluted to ~1.5 mg/ mL in buffer and pipetted (50 µl) in 6 technical replicates onto a 96-well microplate. Pre-prepared resorufin standards (duplicates) were then added to subsequent wells. Quenching was performed on each sample in three of the six technical replicates by adding 10 µl of 0.32 µM resorufin standard. Reaction mixture (200 µl, containing 0.1 M potassium phosphate buffer, pH 8, and 3 µM 7 ethoxyresorufin) was added to the sample wells, before NADPH solution (2.4 mM in final well volume of 275 and 285 µl, respectively) was added to initiate the reaction. Transformation of 7-ethoxyresorufin to resorufin was read in 8 steps on the plate reader. Excitation was at 530 nm and fluorescence emission was measured at 590 nm. EROD activity values were normalized to the protein content in the microsomal fraction and expressed as pmol/ min/ mg microsomal protein. Protein concentrations were determined according to Lowry et al. (1951), adapted to measurement by plate reader. The protein standard was bovine gamma globulin.

2.8.3 CYP1A levels with ELISA

Fish livers were homogenized, and microsomal fractions prepared as described for EROD analysis. The protein concentration was determined by Lowry Protein Assay as used for the EROD assay.

Levels of CYP1A in fish liver were detected by indirect enzyme-linked immunosorbent assay (ELISA) as described in Nilsen et al. (1998). A 1 µg total protein sample was added per well, with 4 replicates per sample, divided between two microplates. For measurements of CYP1A in cod liver, monoclonal mouse anti-cod CYP1A (NP-7, Biosense, Norway) diluted 1:1000 was used. For CYP1A measurements in haddock, whiting and dab, the monoclonal mouse anti-fish CYP1A (C10-7, Biosense, Norway), diluted 1:500 or polyclonal rabbit anti-fish CYP1A (CP-226, Biosense, Norway), diluted 1:500 was selected. For secondary antibodies the polyclonal goat anti-mouse or polyclonal goat anti rabbit (DacoCytomation, Denmark), diluted 1:2000 was employed. Microplates were incubated with tetramethylbenzidine (TMB) substrate for 22.5 minutes before addition of 0.5 M H_2SO_4 and absorbance read at 450 nm.

2.8.4 qPCR: AH receptor/CYP1a1 gene

The gene expression was performed with qPCR analysis (Lacroix et al., 2014).

RNA isolation

The total RNA was extracted from cod liver tissue with Promega Reliaprep simply RNA HT 384, art nr X9601(Nerliens) on a Biomek 4000 Laboratory Automated Workstation (Beckman Coulter) according to the manufacturer's instructions and quantitated using a NanoDrop™-1000 spectrophotometer (Thermo Scientific). The RNA samples were normalised to the concentration of 100 ng/ µl using the Biomek 4000 Laboratory Automated Workstation (Beckman Coulter). Reverse transcription was carried out using SuperScript® VILO™ cDNA Synthesis kit, art nr 11754050 (Life technologies) according to the manufacturer's instructions, and the total RNA input was 500 ng in each reaction in a total volume of 10 µl.

Quantitative real-time RT-qPCR

PCR primers used to quantify the selected genes in cod and haddock were designed based on genome sequence for the respective species. The qPCR assay was run using Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Life Technologies AS) according to the manufacturer's instructions, with 2 µL of cDNA diluted 1 to 10 in a reaction mix containing 400 nM of forward primer, 400 nM of reverse primer in a total volume of 7 µl on a 384 well-plate. For the qPCR assay, amplification and fluorescence detection were performed by a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems) for 40 cycles. Quality controls including "no template controls" (ntc) and "no amplification controls" (nac) were run for quality assessment for each PCR assay.

Mean normalized expression (MNE) of the target genes was determined using a normalization factor based upon EEF1A, UBA52 and RPL37 for cod, calculated by the QuantStudio™ Design and Analysis Software. Selected gene transcripts were: CYP1A, AHR2 and AHRR

2.8.5 Comet assessment

Microscope slides were prepared on board the survey vessel with blood samples taken from the caudal vein of the freshly caught fish. A 10 µl volume of the blood was diluted 1000-fold in ice cold PBS buffer. A 15 µl volume of this diluted blood/PBS solution was added to 85 µl of warmed low melting point agarose. Then 7 µl of this agarose/blood sample was placed on an agarose pre-coated slide as a small spot and allowed to adhere. Each fish sample had one spot on 6 pre-coated slides, 2 slides were labelled as LYS, 2 as FPG and 2 as BUF, with the label relating to the treatment of the slides once back in the laboratory. The slides were kept in cool (4°C) lysis buffer, whilst stored on the survey vessel and transported back to the laboratory in Oslo. The slides were processed by NorGenoTech AS (Oslo) within 10 days of being sampled.

A high-through put method as described in Brunborg et al. (2014) was followed. Briefly, cells embedded in agarose on a microscope slide were lysed, leaving the DNA as a nucleoid, attached to the nuclear matrix. After brief incubation in alkali, gels were electrophoresed at high pH. The DNA becomes attracted to the anode and moves only when breaks are present. After neutralisation and staining, the nucleoids (visualised by fluorescence microscopy) resemble comets; the relative intensity of the comet tail reflects the frequency of DNA strand breaks (SBs). Base alterations (e.g. oxidation) were measured by digesting nucleoids with lesion-specific enzymes; formamidopyrimidine DNA glycosylase (FPG), to detect 8-oxoguanine and other purine oxidation products.

To increase the number of samples that could be handled simultaneously, the high throughput version of the comet assay with 12 mini gels on one microscope slide coated with polycarbonate film substrate was adopted. The test was performed using three different treatments: 1) Lysis only (to measure SBs. Slides labelled as LYS); 2) Incubation with FPG buffer after lysis (slides labelled as BUF); and 3) Incubation with FPG after lysis (to measure oxidised guanine, oxidised bases, slides labelled as FPG). Results expressed as % DNA in tail (median of, in general, 50 comets per sample). % DNA in tail is linearly related to break frequency over the range of damage levels expected. Net FPG-sensitive sites are calculated as the difference between scores for 3 and 2.

2.8.6 DNA adducts with 32P-postlabelling

The ^{32}P-postlabelling protocol is summarised in Figure 12. Carcinogen-modified DNA is digested enzymatically to deoxyribonucleoside 3´-monophosphates with endonuclease (micrococcal nuclease) and exonuclease (spleen phosphodiesterase). In order to increase the sensitivity of the method the enhancement procedure is used to enrich adducts. This procedure uses an enzymatic post-incubation of DNA digests with nuclease P1 (from *Penicillium citrinum*). Nuclease P1 dephosphorylates deoxyribonucleoside 3´-monophosphates of normal nucleotides only to deoxyribonucleosides but not of adducted nucleotides. Deoxyribonucleosides do not serve as substrates of T4-polynucleotide kinase for the transfer of [^{32}P] phosphate from [ɤ-^{32}P]ATP.

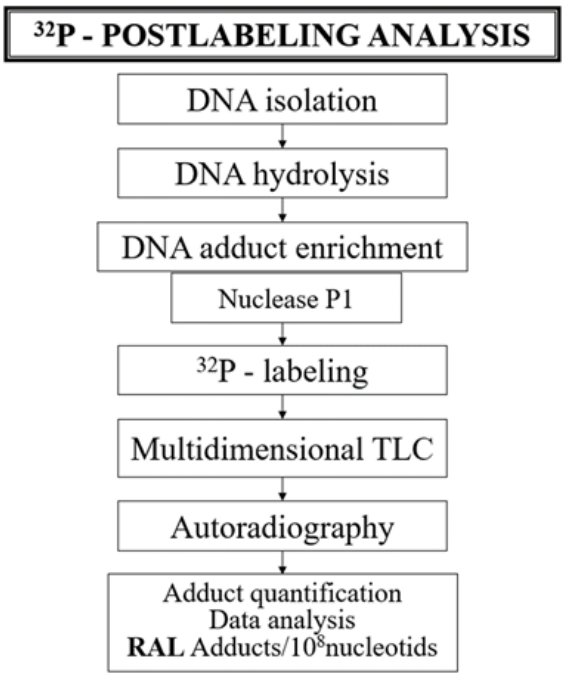


Figure 12. The main steps of the ^{32}P-post labelling protocol for quantification of DNA adducts

Then DNA hydrolysates are converted to 5´-32P-labelled 3´,5´-bisphosphates by incubation with [ɤ-^{32}P]ATP in the presence of carrier ATP and T4-polynucleotide kinase at pH 9.0. This alkaline pH is used in order to minimize the 3´-phosphatase activity of the polynucleotide kinase. ^{32}P-labelled adducts are separated and resolved from the excess of labelled non-modified nucleotides in two dimensions by multidirectional anion-exchange thin layer chromatography (TLC) on polyethyleneimine (PEI) cellulose plates. During the first elution (D1 directions) with aqueous electrolyte labelled unmodified nucleotides and [^{32}P] phosphate is removed from the origin for subsequent resolution using different solvent systems (D3, D4 directions). Location of the adducts is carried out by screen enhanced autoradiography and visualized as dark distinct spots on X-ray films. These areas are then excised for quantitation by liquid scintillation. Adduct levels are calculated as relative adduct labelling (RAL) values, which represent the ratio of count rates of adducted nucleotides over count rates of total (adducted and normal) nucleotides. Utilizing the standard protocol, DNA adducts present at levels of 0.1 adduct in 10^8 normal nucleotides can be detected.

2.8.7 Acetylcholine esterase (AChE) inhibition

The method from Bocquené and Galgani (1998), adapted from Ellman et al. (1961), to determine the AChE activity in biota tissue was followed with minor modifications to assess the activity in extracts from fish fillet samples. Fish samples were homogenized in a potassium phosphate buffer (0.02 M, pH 7) using a Precellys tissue homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). The resulting homogenate was centrifuged at 10,000 g for 20 min at 4°C and the supernatant used for protein determination and AChE analysis. Experiments were performed in 96-well microplates (Sarstedt, Nürnbecht, Germany) and every sample was run in quadruplicate. For this analysis, 220 – 260 µl of 0.02 M phosphate buffer (at pH 7) was mixed with 20 µl of 7.89 mM DTNB and 10 – 50 µl of supernatant. After 5 min of incubation at room temperature, 10 µl of 78.9 mM ATC were added to start the reaction. The enzyme activity was then followed by an absorbance plate reader at 405 nm at room temperature for a total of 10 min (VersaMax microplate reader from Molecular Devices, California, USA).

The enzyme activity was followed by the production of the yellow coloured 5-thio-2-nitrobenzoic acid (TNB) anion. The production of TNB is based on coupling of the following reactions:

Acetylthiocholine (ATC) → thiocholine + acetate
Thiocholine + dithiobisnitrobenzoate (DTNB) → 5-thio-2-nitrobenzoic acid (TNB)

The conversion of DTNB to TNB can be used as a measure of the hydrolysis of ATC into thiocholine. ATC is produced from hydrolysation of the neurotransmitter acetylcholine by AChE. AChE inhibitors will induce a decrease in the production of ATC and therefore a decrease in the production of TNB will be observed.

The change in absorbance per min was used to calculate the AChE activity: AChE activity (µmol ATC/ min / mg protein)= [ΔA × Volt × 1000] ε × light path × Vols × [protein], where ΔA = change in absorbance (OD) per min at 405 nm, corrected for spontaneous hydrolysis, Volt = total assay volume (0.300 mL), ε = extinction coefficient of TNB (1.36 x 104 M/cm), light path = microplate well depth (1 cm), Vols = sample volume (in ml), and [protein] = concentration of protein in the enzymatic extract (mg/ml). Total protein concentrations were determined according to Lowry et al. (1951), adapted to measurement by plate reader, using Immunoglobulin G (IgG) as protein standard.

2.8.8 Fish liver histology

Samples were processed as described in section 2.6.6. Liver tissues were examined for fibrosis, granulocytoma, hematocyte infiltration, necrosis, steatosis, all scored as 0 – absent; 1 – sporadic; 2 – multiple/ widespread. Melano–macrophage aggregates (MMA) were reported as 0 – absent; 1 – area affected (1 – 2 cases); 2 – some areas affected / more than 2 in a sample; 3 – distributed through the entire sampled tissue; while the hepatocyte vacuolization was scored 0-4, according to Hylland et al. (2005). Finally, the presence (1) or absence (0) of parasites were reported.

2.9 Sediment traps

2.9.1 Preparation sediment extracts

Sediment trap material to be analysed for PAHs and used in *in-vitro* bioassay exposures were airdried before 10 g were extracted by accelerated solvent extraction (ASE™, Dionex™ 300, ThermoFisher™). This was performed in two cycles with the following conditions; 100°C, 1500 psi, 60 % “flush volume”, and a solvent of 1:1 hexane: dichloromethane. The 35 ml ASE cells were filled with a mix of sediment and 10 %-deactivated alumina and refilled with Diatomaceous Earth. Sediment extracts were evaporated to a volume of 0.5 ml and active copper was added over night to remove sulphur residues. Extracts were purified and fractionated with hexane and a 1:1 hexane: dichloromethane solution by PowerPrep (Fluid Management Systems, Inc.) using 7.5 g silica columns. Fractions were pooled, and further evaporated until only a small amount of liquid was present, before being dissolved in either DMSO for cell-based assays, or in isooctane for analyses of PAH. The sediment extracts reconstituted in DMSO were equivalent to 12 g dry weight sediment (eQsed)/ml, which was further serially diluted in DMSO to the desired concentrations used for cell-based assays.

2.9.2 PAH-NPD analysis

Chemical analyses of PAHs were performed according to the European quality assurance (QA) standard NS-EN ISO/IEC-17,025 using a GC HP-6890 coupled to an Agilent N-5975 mass spectrometry (MS) (EI-SIM) instrument as described by Boitsov et al. (2020).

2.9.3 *In vitro* bioassay with cell lines

Rainbow trout gill-Waterloo1 (RTgill-W1) cell line derived from *Oncorhynchus mykiss* was used for testing toxicity of the sediment extracts. RTgill-W1 cells, stored in liquid nitrogen, were transferred to a cell cultivating flask, containing Leibovitz's L-15 Medium (L-15), with FBS (10%) and Pen/Strep (1%). Flasks at 90% of confluence were used to seed 96-well cell culture microplates for the sediment extract exposure. Exposure medium was prepared on the day and serially diluted to 5, 10, 20, 40, 60, and 120 mg eQsed/ml. The cell line approach and methods used are described further in Goksøyr et al. (2021).

Reactive oxygen species

The production of reactive oxygen species (ROS) in RTgill-W1 gill cells exposed to sediment extracts was determined by measuring the fluorescence of the oxidized probe (2'7' -dichlorodihydrofluorescein diacetate, Invitrogen) at 485/528 nm as described in (Blanco et al., 2018). The exposure to H_2O_2 was used as a positive control of cellular ROS production. Five replicates were prepared for each concentration of the sediment extract.

Luciferase reporter gene assay

The luciferase reporter gene assay was essentially performed as described in (Lille-Langøy et al., 2021). COS-7 cells were seeded onto 96 well plates (5 000 cells/well) in Dulbecco's modified Eagle's medium (DMEM) with phenol red, supplemented with 10% fetal bovine serum (FBS), 4 mM L- glutamate, 1 mM sodium pyruvate, and 100 U/mL penicillin− streptomycin (Merck KGaA, Darmstadt, Germany), and cultivated at 37 °C with 5% CO2 for 24 h. Cells were transiently co-transfected with a eukaryotic expression plasmid (pcDNA3.1/Zeo(+), 31 ng/well), the pcDNA3.1/Zeo(+)-based plasmids encoding gmAhr2a (3 ng/well) and gmArnt1 (6 ng/well), a luciferase reporter plasmid (pGudLuc6.1, 30 ng/well), and a β- galactosidase normalization plasmid (pCMV-βGAL, 30 ng/ well),45,46 using a Mirus TransIT LT-1 transfection reagent according to the recommendations of the supplier. COS-7 cells were seeded and cultivated in DMEM (4500 mg/L glucose), supplemented with 10% FBS, 1 mM sodium pyruvate, and 4 mM L-glutamine. In the exposure medium, FBS was substituted with 10% charcoal-stripped FBS (VWR International, Radnor). The sediment extracts solved in DMSO were diluted 1:200 in exposure medium, resulting in exposure media with 1× test compound and 0.5% DMSO. The highest concentration exposure media were serially diluted five times (1:2 for the first dilution, then 1:5) in exposure medium supplemented with 0.5% DMSO. Following transfection, cells were incubated in exposure media for 24 h. Reporter gene assays were repeated at least three times and with three technical replicates per exposure. TCDD (30 pM to 100 nM) was used as a known agonist in each experiment. Absorbance and luminescence measurements were performed on a Hidex Sense plate reader (Kem-En-Tek, Sweden). Absorbance measurements to quantify β- galactosidase activity were performed at 420 nm wavelength.

Recorded luminescence was normalized for variation in transfection efficiencies using β- galactosidase enzyme activity. Dose-response curves were calculated by nonlinear regression (3 parameters, GraphPad Prism ver. 9) of fold changes in luciferase activity measured at different concentrations of test compounds relative to solvent control in three independent experiments.

Ethoxyresorufin-O-deethylase activity

The EROD assay was performed as reported in Fernandes et al. (2014), with some modifications (Blanco et al., 2018). β-naphthoflavone (bNF, Alfa Aesar) was used as a positive control for induction of EROD activity. The formation of the fluorescent resorufin was recorded at 537/583 nm, and the Bradford method was used to determine the total cellular protein content in the samples. Results were expressed as pmol of resorufin formed per minute per milligram of protein (pmol/min/mg protein). Five replicates were prepared for each concentration of the sediment extract.

2.10 Integrated data assessment

2.10.1 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 (n) of biomarkers in the data set (i.e., IBR/n) (Broeg and Lehtonen, 2006). The method is based on the relative differences between the biomarkers in each given data set and the IBR value can be calculated by summing-up triangular star plot areas (a simple multivariate graphic method) for each two neighbouring biomarkers in each data set. The positioning of the biomarkers around the star plot can influence the IBR/n, therefore the biomarkers were placed based on their similarity in either cellular and/or physiological function as recommended (Broeg and Lehtonen, 2006). The higher the IBR/n value is indicative of an increase biological response and potential adverse effects.

The IBR procedure follows five simple steps: 1) calculate the mean and standard deviation for each sample group; (2) standardise the data for each sample group: xi'=(xi-x)/s; where, xi'=standardised value of the biomarker; xi=mean value of a biomarker from each sample; x=general mean value of xi calculated from all compared sample groups; s=standard deviation of xi calculated from all samples; (3) add the standardised value obtained for each sample group to the absolute standardised value of the minimum value in the data set (i.e. yi=xi'+|xmin'|); (4) calculate the Star Plot triangular areas by multiplication of the obtained standardised value of each biomarker (yi) with the value of the next standardised biomarker value (yi+1), dividing each calculation by 2 (Ai=(yi x yi+1)/2); and (5) calculate the IBR/n index, which is the summing-up of all the Star Plot triangular areas (IBR=ΣAi) divide by the number of biomarkers in the calculation. In the present study CI, SoS, LMS, MN and histology (digestive gland, gill and gonad) were selected for the IBR calculation in the mussels, with the inverse values of CI, SoS, LMS used, since a decrease in these endpoints was reflective of an adverse impact. For scallops, CI, LMS, MN and histology (digestive gland, gill and gonad) were selected for the IBR calculation. In fish, AChE, EROD, CYP1A, DNA adducts, PAH liver, and liver histology biomarkers were selected for the IBR/n calculation. The inverse values for AChE were used since a reduction in activity was indicative of an adverse effect.

2.10.2 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 and fish. The level of significance was set to p=0.05.

2.11 Statistical treatment

Statistical differences between the groups of biological data were assessed with analysis of variance (ANOVA). Homogeneity of variance for the different groups was checked using the Levene's test and data were log transformed when necessary to obtain homogeneity. Where homogeneity was not achieved, the non-parametric Kruskal-Wallis test was performed on untransformed data. Where homogeneity of variance was achieved a Tukey post-hoc test was performed to compare significant differences between the groups with a level of significance set at p=0.05.

Additionally, the non-parametric Wilcoxon test was used to determine significant differences in LT_{50} values (SoS test) from the mussel stations compared to reference stations including the two depths.

3 Results

3.1 Oceanographic measurements

3.1.1 Current measurements

Current measurements presented as current rows from station 3, approximately 750 m southwest of the Ekofisk complex are presented in Figure 13. The ADCP, positioned at a depth of approximately 40 m and looking up towards the surface, was able to provide current speed and direction at different depths in the water column between 15 and 38 m for the duration of the mussel exposure. As expected, the current direction in the Ekofisk area was tidally dominated and changed equally between the northeast and the southwest directions every 6 hours. The mean current speed of 0.12 m/s and the current directions were almost identical at each depth.

A second ADCP positioned at station 7, 2000 m southwest of the Ekofisk complex was also able to collect current speed and direction data at different depths for the duration of the mussel exposure (Figure 14). This ADCP, was positioned at a depth of approximately 45 m and was orientated to scan upwards in the water column towards the surface and provided current speed and direction at different depths between 5 and 45 m for the duration of the mussel exposure. As described for station 3, the current direction was tidally dominated and changed equally between the northeast and the southwest directions every 6 hours. The mean current speed of 0.12 m/s and the current directions were almost identical at each depth.

A third ADCP positioned at station 10, 1000 m southwest of the Eldfisk complex provided current speed and direction data at different depths for the duration of the mussel exposure (Figure 15). This ADCP was positioned at a deeper depth of approximately 65 m and was orientated to scan upwards in the water column towards the surface and provided current speed and direction at different depths between 16 and 64 m for the duration of the mussel exposure. As described for station 3 and 7, the current direction at station 10 was tidally dominated and changed equally between the northeast and the southwest directions every 6 hours. The mean current speed of 0.13 to 0.15 m/s and the current directions were almost identical at each depth.

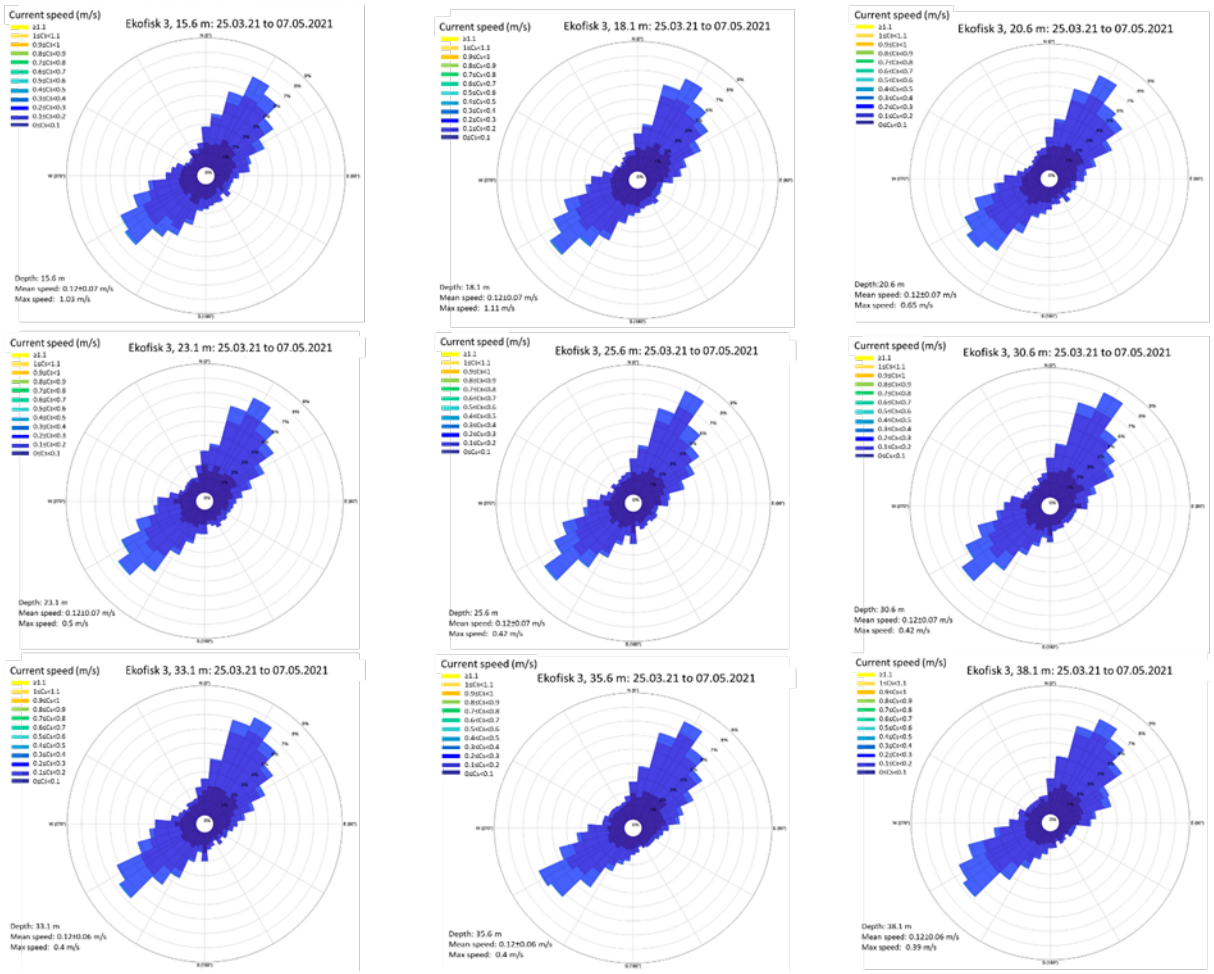


Figure 13. Current rose measured with a Nortek Aquadopp current profiler (ADCP) at Ekofisk station 3, at selected depths between 25th March and 7th May 2021.

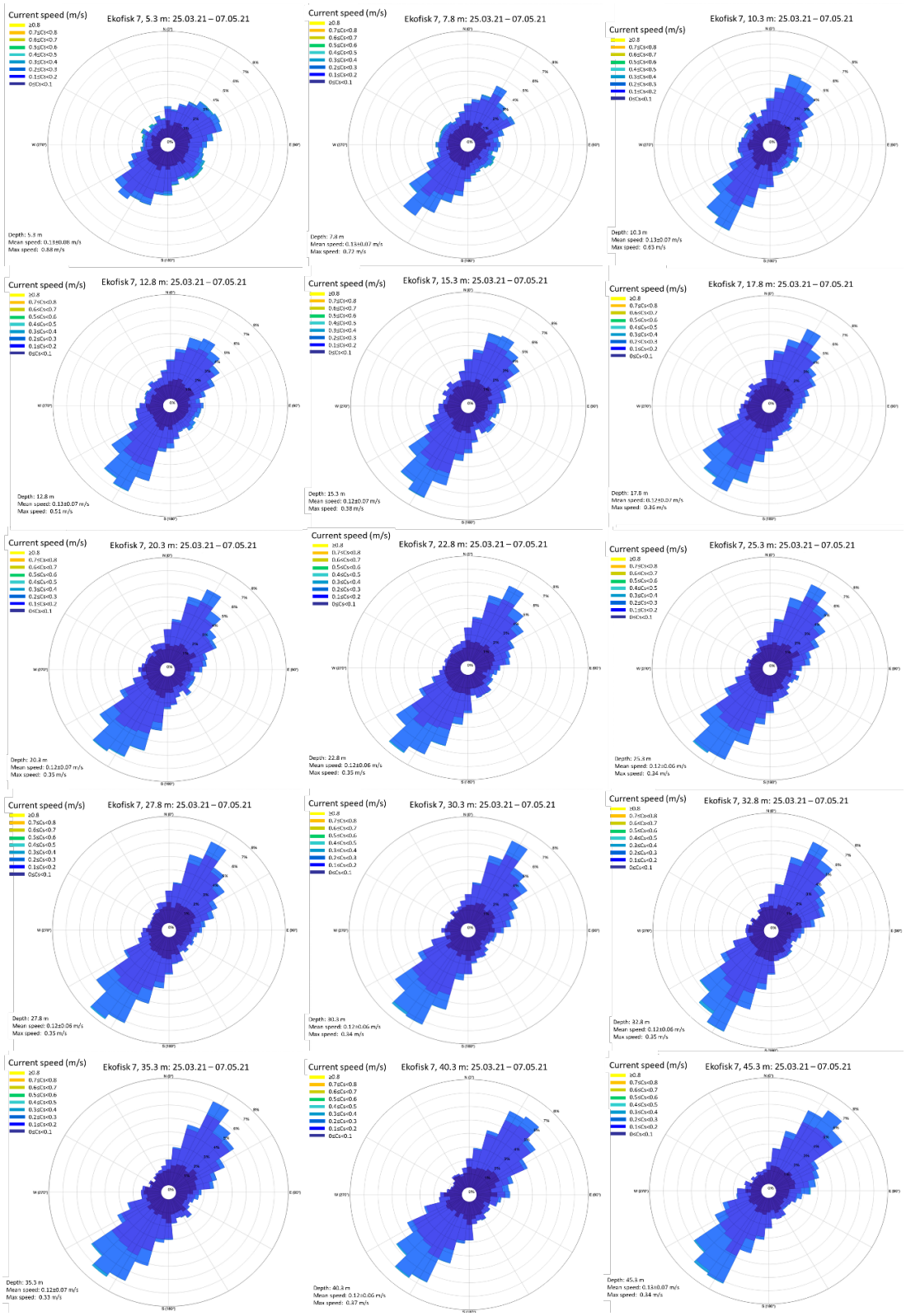


Figure 14. Current rose measured with a Nortek Aquadopp current profiler (ADCP) at Ekofisk station 7, at selected depths between 25th March and 7th May 2021.

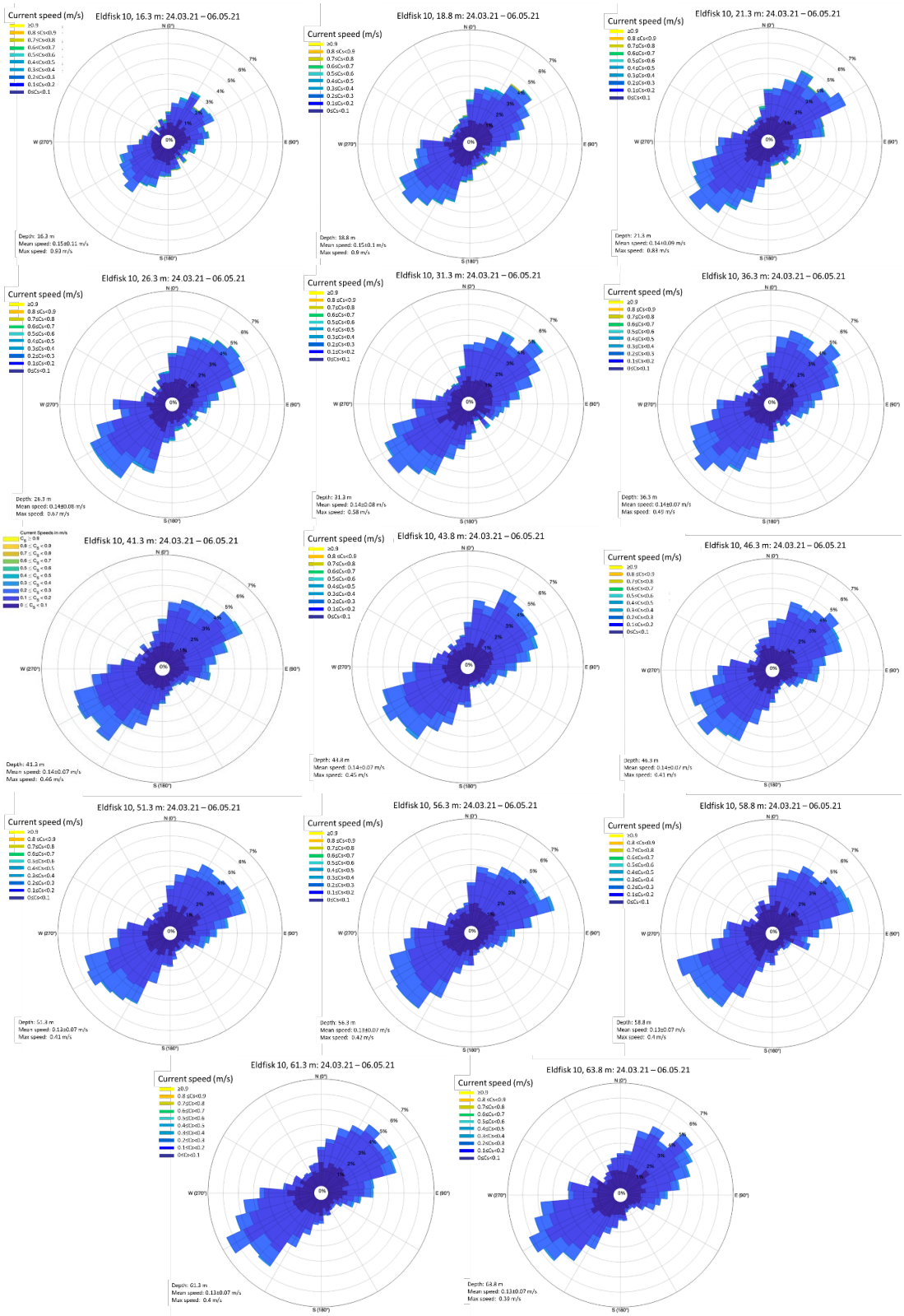


Figure 15. Current rose measured with a Nortek ADCP at Eldfisk station 10, 1000 m southwest of the Eldfisk platform, at defined depths between 16 and 64 m, between 24th March and 6th May 2021.

3.1.2 Seawater profiles during the deployment and retrieval of the monitoring rigs

Physicochemical data including temperature, salinity, dissolved oxygen, chlorophyll and turbidity were collected by vertical profiles during the deployment of the monitoring rigs at the two reference locations (Figure 16). A thermocline was not present, with stable seawater temperatures of 5.4 and 5.6°C throughout the water column at Ref 1 and Ref 2 respectively. For salinity, a weak halocline was present around 50-60 m for Ref 1 and 40 m for Ref 2, although the degree of temperature fluctuation was less than 0.2°C. Similar dissolved oxygen concentrations between 10.4 and 10.7 mg/l were recorded at both reference stations, which were both stable with depth. At Ref 1, the chlorophyll concentration between 1 and 2 µg/l in the top 50 m increased above three with depth. This was tracked by the turbidity profile at Ref 1. For Ref 2, higher chlorophyll values were recorded between 3 and 4 µg/l for the top 40 m. Although the turbidity profile at Ref 2 was similar to the turbidity profile at Ref 1, it did not track the chlorophyll profile at Ref 2.

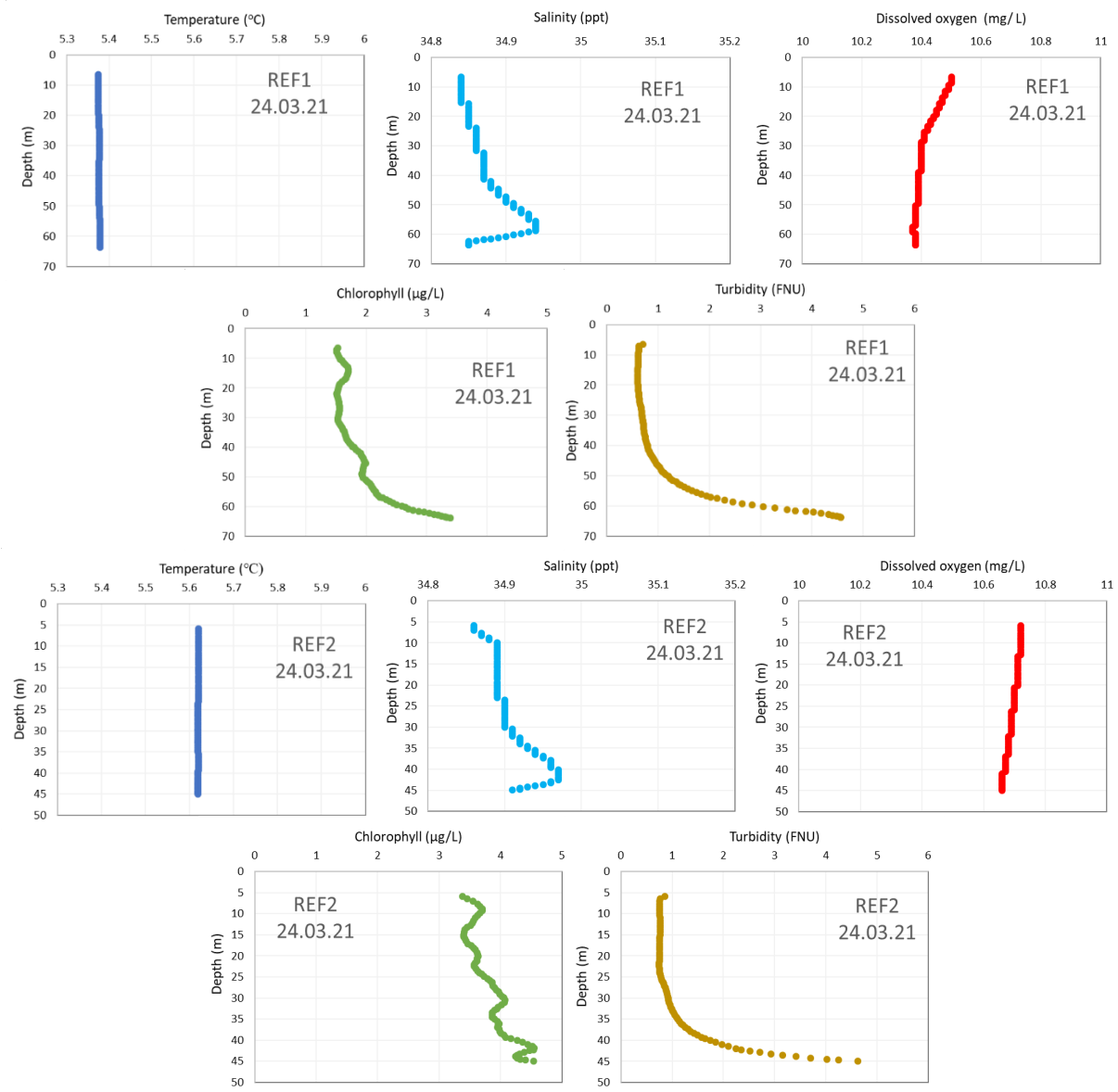


Figure 16. Physicochemical profiles of the water column taken with a YSI EXO2 at reference stations 1 and 2 during the deployment of the monitoring rigs (24th March 2021).

Physicochemical data were also collected by vertical profiles during the deployment at monitoring stations 7 and 11 (Figure 17). Seawater temperatures were relatively stable with depth between 5.7 and 5.9°C at stations 7 and 11, respectively. For salinity, a weak halocline was present around 50 m for Station 7 and 40 m for Station 11, although the degree of temperature fluctuation was less than 0.2°C. Similar and stable dissolved oxygen concentrations of 10.2 ± 0.1 mg/L were recorded with depth at Station 7 and 11. The chlorophyll concentrations were around 1 µg/L in the top 60 m for both stations. The turbidity profile at Station 7 tracked the chlorophyll profile.

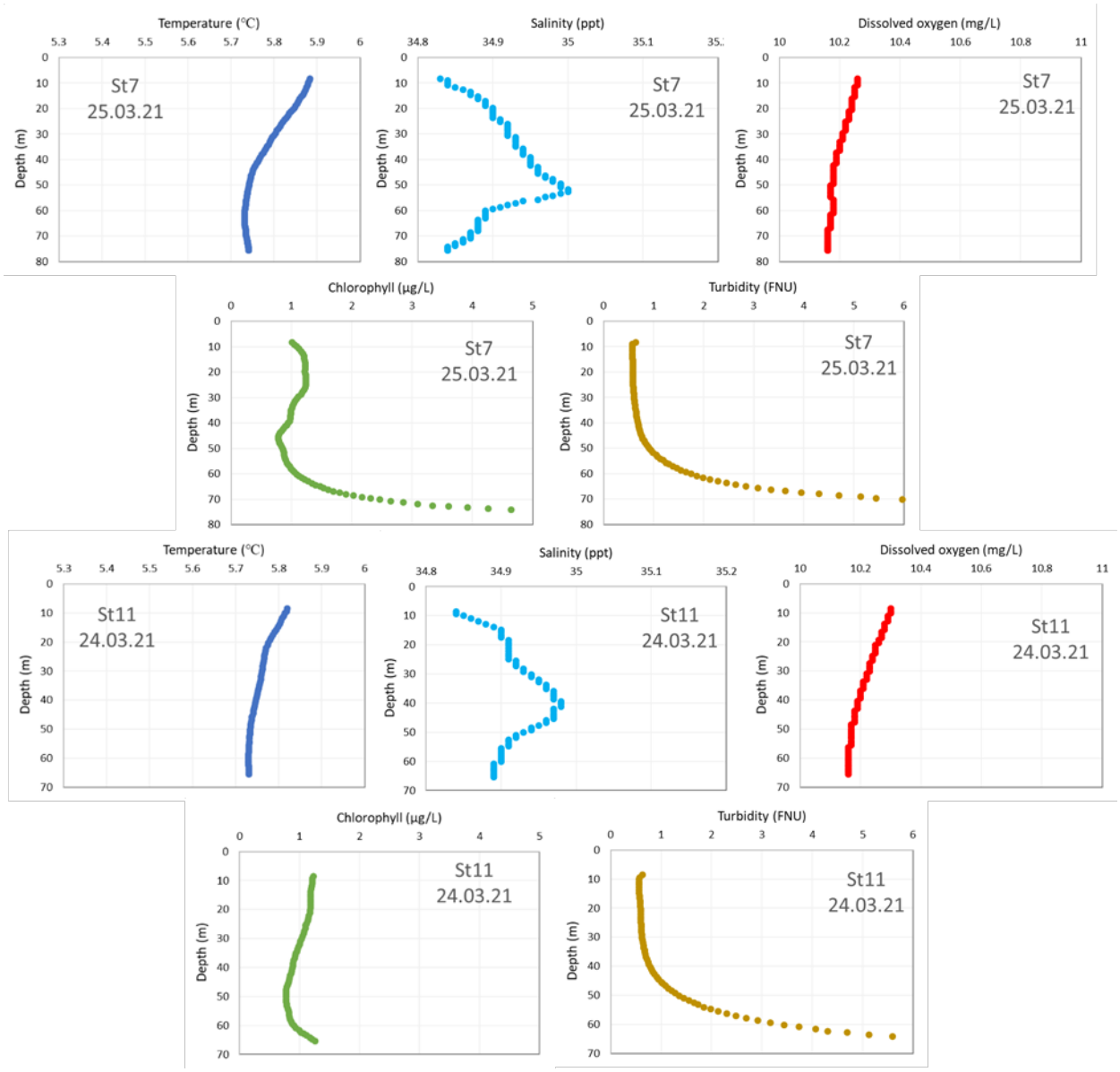


Figure 17. Physicochemical profiles taken with a YSI EXO2 at Ekofisk 7, 2000 m southwest of the platform, and Eldfisk 11, 2000 m SW of the Eldfisk platform, during the deployment of the monitoring rigs (24th March 2021).

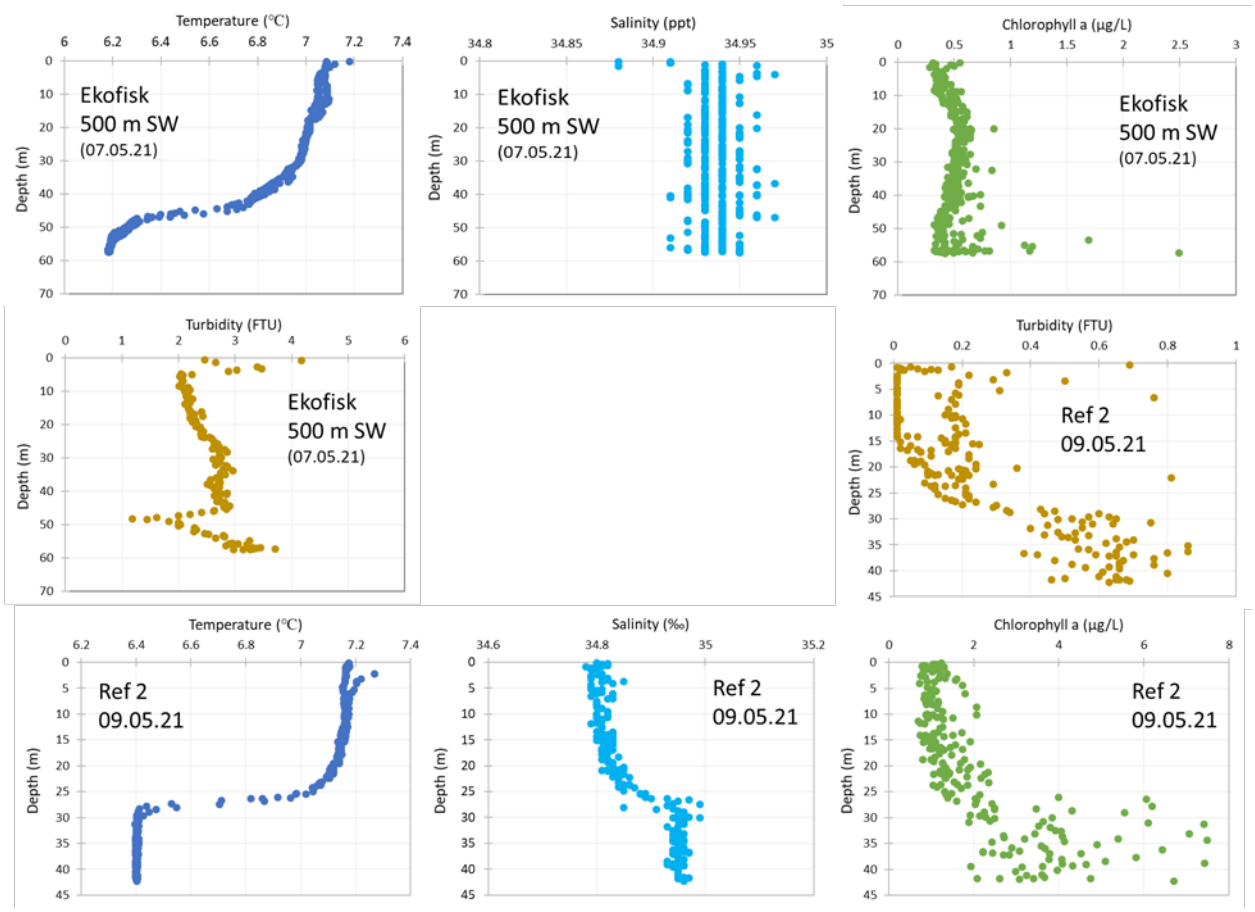


Figure 18. Physicochemical profiles taken with a SAIV SD204 at Ekofisk approximately 500 m southwest of the platform, and Reference 2, during the retrieval of the monitoring rigs (7th and 9th May 2021).

Physicochemical data were collected by vertical profiles during the retrieval of the monitoring rigs in May from within the Ekofisk 500 m safety zone, near stations 1 and 2, and at Ref 2 (Figure 18). Within the 500m safety zone a thermocline was present around 40-45m. Seawater temperatures above and below the thermocline were around 7°C and 6.2°C. The salinity profile remained stable with depth between 34.9 and 34.95 ppt. Chlorophyll was also stable with depth with chlorophyll concentrations around 0.5 µg/L. The turbidity was mostly stable at 2 FTU between 5 and 45 m, before a sharp reduction to 1 FTU at 50 m.

At Ref 2, a thermocline was present at a depth of 25 m changing from 7.2 to 6.4°C. For salinity, a weak halocline was present at a depth of 25 m at Ref 2, although the degree of fluctuation was less than 0.2°C. The chlorophyll concentrations were around 1 to 2 µg/L in the top 25 m, above the thermocline and halocline, increasing above this value at deeper depths. The turbidity profile at Ref 2 appeared to track the chlorophyll profile.

3.1.3 Seawater properties at selected monitoring rigs during the exposure period

The physicochemical properties of the seawater at Ref 1 for the duration of the mussel exposure at the approximate depth of the 20 m mussels were recorded (Figure 19). Temperature increased over the 6-week exposure duration from 5.5°C on the 24th March to 7°C on the 5th May. Two large step increases in temperature occurred on the 9th-10th April and the 21st-22nd April. Salinity showed a slight increase from 34.8 at the start rising to 35.1 in the middle of the exposure before varying within this

range at the end of the exposure in May. Chlorophyll was abundant at this 22 m depth throughout the exposure, although higher chlorophyll concentrations were measured in late March/ beginning of April and again in mid-April. Most turbidity measurements were below 1 FTU, except for peaks between the 5th-7th April and again 3rd May, which may be related to storm events.

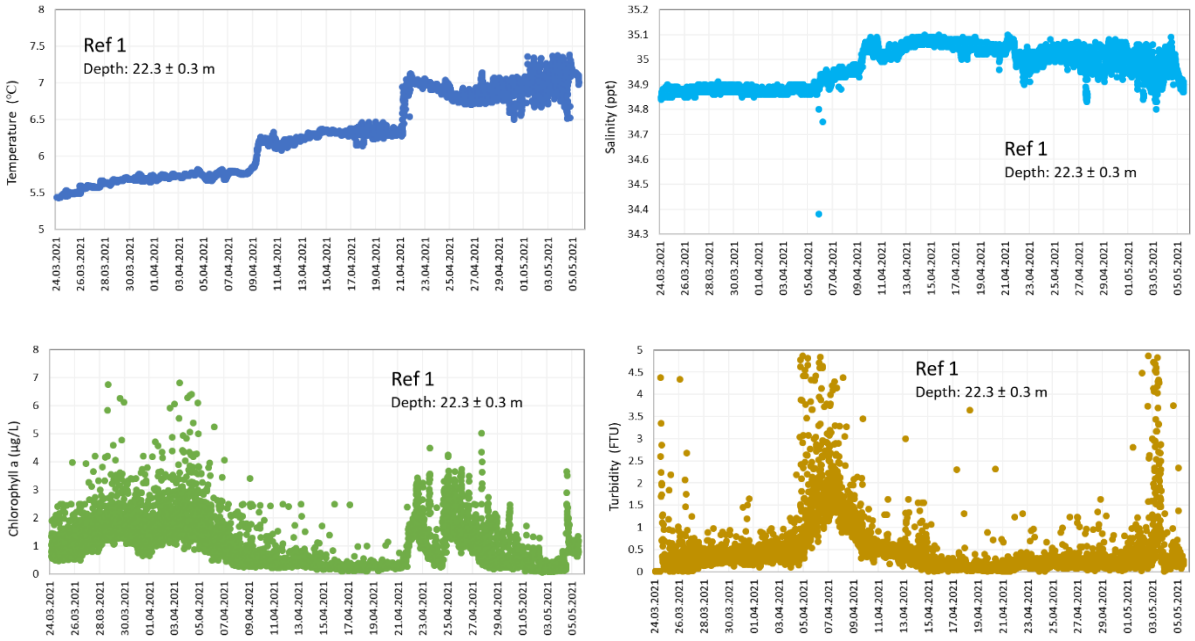


Figure 19. Physicochemical parameters of the seawater measured on the monitoring rig reference 1 at a fixed depth of 22.3 ± 0.3 m during the exposure period (24th March to 5th May).

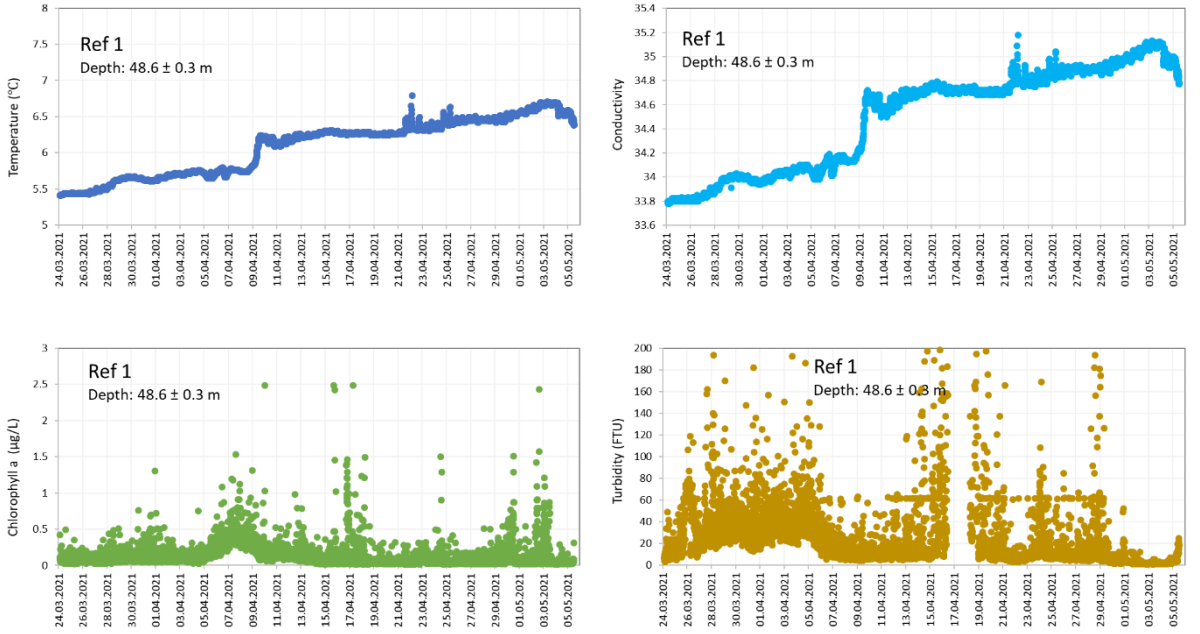


Figure 20. Physicochemical parameters of the seawater measured on monitoring rig reference 1 at a fixed depth of 48.6 ± 0.3 m during the deployment period (24th March to 5th May).

Physicochemical properties were also taken of the seawater at Ref 1 at the deep depth of 49 m (Figure 20). The temperature increased over the 6-week exposure duration from 5.5°C on the 24th March to 6.5°C on the 5th May. This final temperature at the end of the exposure in May was 0.5°C colder than the temperature measured at a depth of 22 m on the same monitoring rig. Salinity changes at 49 m, showed a larger increase from 33.8 at the start rising to 35 at the end of the exposure in May. The majority of chlorophyll measurements were below 0.5 µg/l during the exposure with slight elevations in chlorophyll over 1 µg/l at various points throughout the exposure. The turbidity measurements were high and variable throughout the exposure and would suggest a possible interference with the turbidity sensor.

Physicochemical properties of the seawater at Station 4, 1000 m southwest of the Ekofisk platform at a fixed depth of 20 m were recorded (Figure 21). Temperature increased over the 6-week exposure duration from 6°C on the 24th March to 7°C on the 5th May. Salinity remained stable between 34.8 and 35.0. Chlorophyll was abundant at this 20 m depth throughout the exposure, although higher chlorophyll concentrations were measured in late March/ beginning of April and again in mid-April and was similar to chlorophyll measurements at Ref 1 at 22 m.

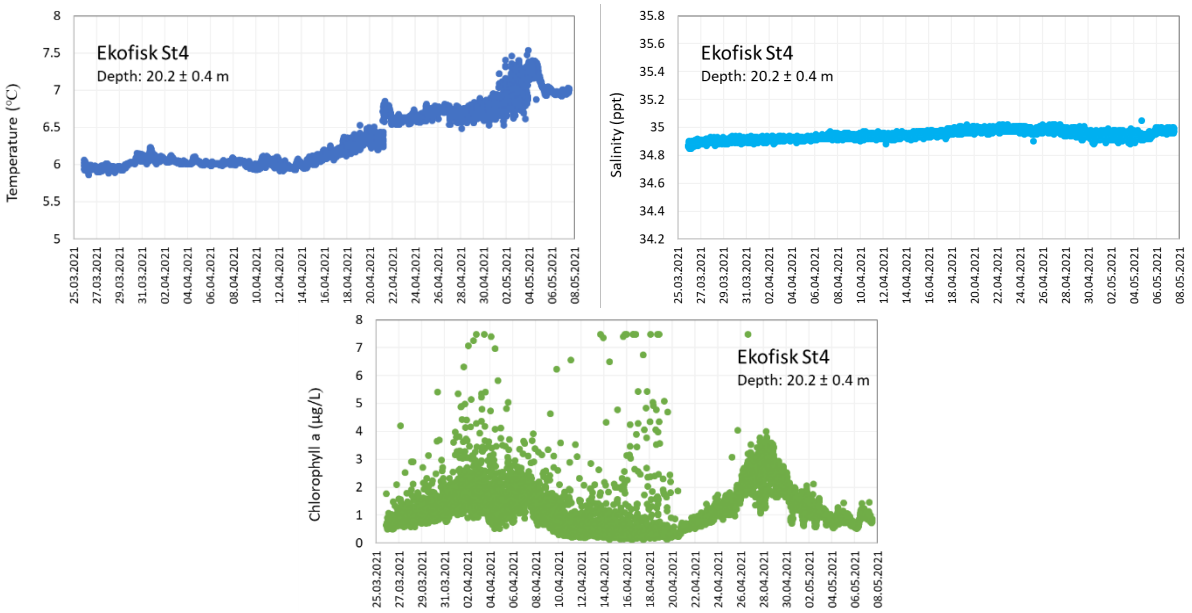


Figure 21. Physicochemical parameters of the seawater measured on the monitoring rig station 4, 1000 m southwest of the Ekofisk platform at a fixed depth of 20.2 ± 0.4 m during the exposure period (25th March to 8th May).

Physicochemical properties were also taken of the seawater at Station 4 at the deep depth of 44 m (Figure 22). Temperature increased over the 6-week exposure duration from 6°C at the start to 7°C towards the end, similar to that seen at the shallower depth of 20 m at the same station. Salinity remained relatively stable at 35.1 ppt for the 6-week exposure. The chlorophyll measurements were mostly below 2.5 µg/l during the exposure with slight elevations in chlorophyll above 3 µg/l at various points throughout the exposure. This was similar to chlorophyll measurements at the same depth for Ref 1. The turbidity measurements were mostly below 1 FTU, although a peak in turbidity around the 6th-7th April was recorded. The date agrees with a similar increase in turbidity at Ref 1, 20 m and may confirm a period of bad weather.

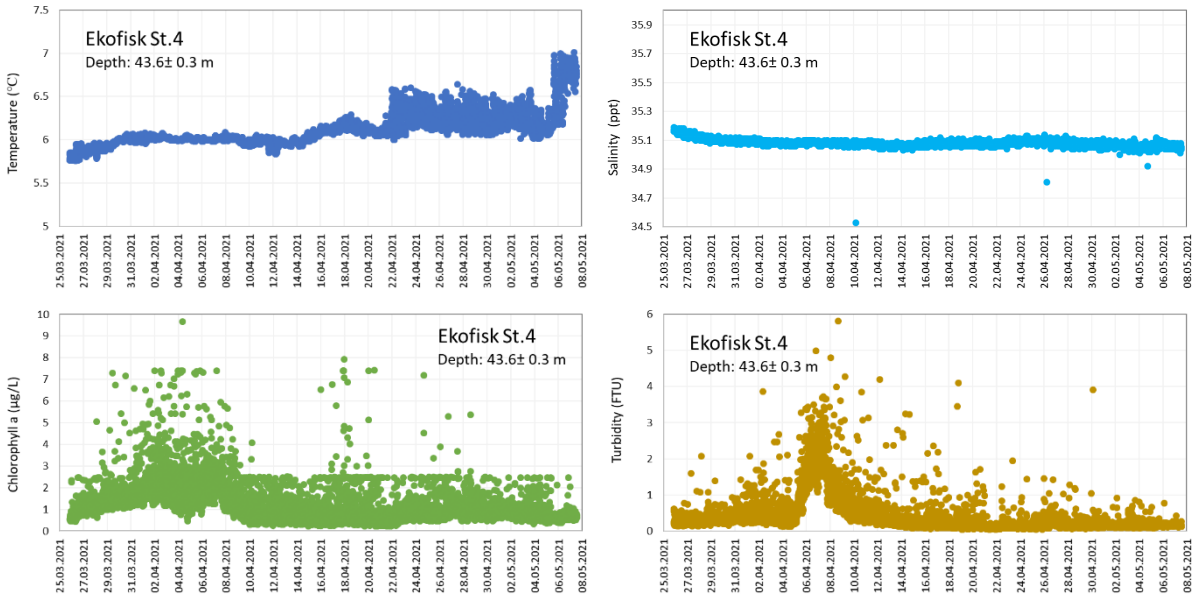


Figure 22. Physicochemical parameters of the seawater measured on the monitoring rig station 4, 1000 m southwest of the Ekofisk platform at a fixed depth of 43.6 ± 0.3 m during the exposure period (25th March to 8th May).

*Table 2. Minimum and maximum water temperatures recorded with data loggers (Onset Hobo Pendant UA-002-64) placed on the mussel cages during the 6-week deployment. Temperature measurements taken every 10 min. * indicates either lost or damaged logger that could not be read.*

Station	20 m depth		40 m depth	
	Min temp (°C)	Max temp (°C)	Min temp (°C)	max temp (°C)
R1	5.55	7.68	5.66	7.18
1	6.17	7.88	*	*
2	6.06	7.88	*	*
3	6.17	7.98	6.06	6.98
4	6.06	7.68	*	*
5	6.06	7.68	5.86	6.67
6	6.06	7.88	5.96	6.98
7	6.06	7.58	5.76	6.57
8	6.17	7.78	5.96	7.08
9	5.96	7.58		
10	*	*		
11	6.17	7.68		

Temperatures recorded with the data loggers that were placed on the mussel cages, reflect the actual temperatures the mussels would have experienced over the six weeks at the different stations including the two depths of 20 and 40 m (Table 2). The values include minimum and maximum temperatures over the 6-week exposure. At all stations the temperature was found to increase during the exposure with colder temperatures in March at the start of the exposure. The temperature ranges agree with the other temperature sensors placed at selected stations, with most temperatures at 20 m depth around 6°C at the start of the exposure increasing to around 8°C at the end. Overall, the deep waters at all stations were consistently colder at all stations.

3.2 Produced water discharge at Ekofisk and Eldfisk

3.2.1 Properties of the produced water

The temperature of the PW at the outlet of Ekofisk and Eldfisk is approximately 70°C with a salinity of 40-45‰. This is in contrast to the receiving seawater, which during the mussel deployment had an approximate average temperature of 7 ± 2°C and salinity of 35‰.

The discharge of PW from Ekofisk was from both the Ekofisk 2/4 J and Ekofisk 2/4 M platforms. The Ekofisk 2/4 J platform is characterised as a processing and transportation platform, that processes oil from four fields in the Ekofisk area including Eldfisk and Embla. The C-tour PW cleaning system is located on the 2/4 J platform, this has been established since 2006, where a combination of natural gas liquids and hydro cyclones have helped to reduce the oil in water concentration of the PW before discharging to sea. The PW from 2/4 J is discharged at a depth of 49 m. The Ekofisk 2/4 M platform is a combined production and process platform. The 2/4 M platform does not have a C-tour system but does use a similar technology of hydro cyclones to clean the PW before discharging. The PW from 2/4 M is discharged from six underwater outlets that are between 20 m and 40 m deep. In contrast, the PW discharge from Eldfisk is from just one location at a depth between 15 and 20 m.

The Norwegian authorities require the oil in water concentration of the PW from offshore installations to not exceed monthly average values of 30 mg/l. The daily average oil in water concentrations for PW discharged from the Ekofisk 2/4 J and 2/4 M and Eldfisk platforms for the duration of the mussel exposures are shown in Figure 23. Overall, the oil in water concentration was marginally lower from the 2/4 J and Eldfisk with most daily average values below 3 mg/l. Exceptional spikes of around 10 mg/l and 20 mg/l on 4th and 9th April for 2/4 J and peaks of 14 mg/l and 26 mg/l on the 11th and 15th April for Eldfisk were observed. The daily average oil in water concentration from the 2/4 M was typically below 5 mg/l, with several small spikes between 10 and 15 mg/l during the exposure. Overall, the daily average oil in water concentration was below the regulation limit.

The daily volume of effluent from 2/4 J was higher than the effluent volume discharged from 2/4 M. The 2/4 J discharge was between 20 000 and 25 000 m^3 per day, except for 22nd April when it reduced to 10 000 m^3/day. The 2/4 M discharge remained below 20 000, reducing to 7 000 on the 22nd April. For Eldfisk, the daily volume of PW effluent was consistently lower than that of either of the Ekofisk outlets. For most of the exposure duration the PW effluent volume from Eldfisk was around 7000 m^3/day, although this reduced to around 1000 m^3 between the 10th and 17th April.

Despite a few episodic spikes, the actual amount of oil in water discharged daily from the 2/4 J and 2/4 M where quite similar between 50-200 kg/day for most of the exposure duration. In contrast, the actual amount of oil in water discharged daily from Eldfisk was around 30 kg/day and remained consistent throughout the monitoring period.

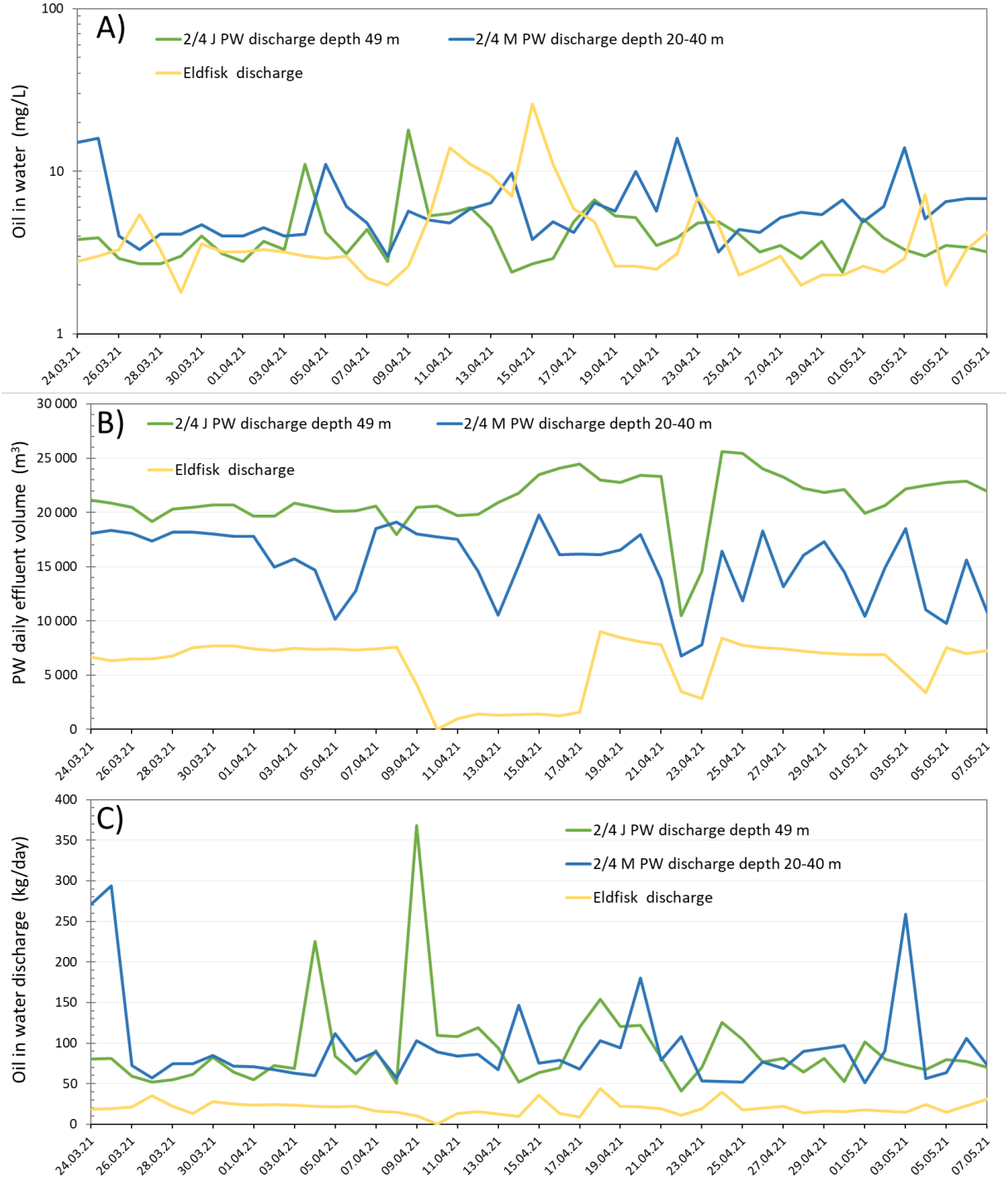


Figure 23. Information on the produced water discharged from the Eldfisk and Ekofisk platforms 2/4 J and 2/4 M for the duration of the mussel exposure. A) Oil in water daily average concentrations (mg/l), B) the volume of effluent discharged (m^3) and C) the daily amount of oil in water discharge (kg/day).

3.2.2 Direction of the PW discharge plume

Produced water from the Ekofisk complex is released into the sea at different depths from the Ekofisk M and the Ekofisk J platforms. The dominant tidal forces in the southwest and northeast directions are the main influences on the direction of the PW plume. DREAM simulations of the Ekofisk PW plume

have been performed with information collected during the mussel exposure, including current direction and properties of the PW and seawater recipient (Figure 24).

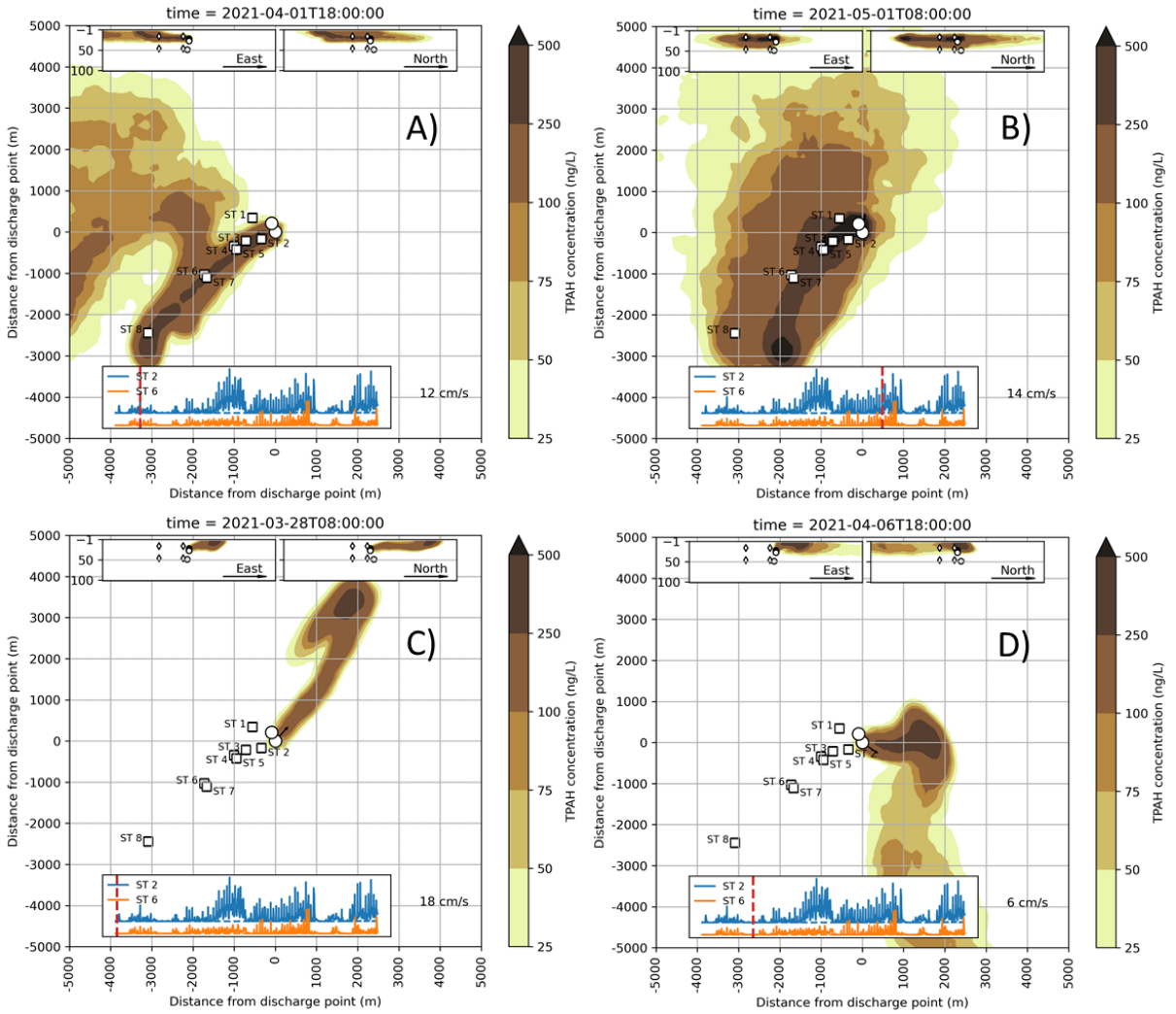


Figure 24. Snapshot images of the dynamic model of the produced water (PW) discharge from the Ekofisk 2/4 J and 2/4 M platforms (DREAM). Top inserts in all 4 figures show the concentration of the PW plume with depth, with the diamonds denoting position of Station 2 and Station 6 at depths 20 and 40-45 m. Bottom inserts show the expected pulse exposure of mussels at Station 2 and Station 6 to the PW plume.

The model shows the predicted concentration of PAH in the seawater recipient, whilst the tidal cycle was in the southwest direction (Figure 24 A and B). Here, the monitoring stations appeared ideally positioned for optimal exposure to the PW plume. The model also revealed that mussels at 20 m were much more exposed to the PW plume than the deeper mussels at 40-45 m. Both Station 2 and Station 6 were shown to have an episodic exposure to the PW plume most likely due to the tidal dominated current at Ekofisk. Higher exposure concentrations were modelled for Station 2 due to the closer proximity to the source of the discharge (~200 m) compared to Station 6 (~2000 m). Figure 24 C and D show the expected PW plume direction with a tidal current in the northeast direction and when the tide is changing at slack water. In these examples the mussels are not exposed directly to the plume.

3.3 Chemical accumulation in passive samplers

3.3.1 Total hydrocarbon concentrations

The accumulations of THC in silicone membranes placed out at the different monitoring stations and at the two different depths for 6 weeks were calculated into actual dissolved concentrations in water (Figure 25). Median THC concentrations were found highest at the reference stations R1 at 40 m and 20 m depth followed by R2 at 40 m, between 1 and 2 µg/l. All monitoring stations at Ekofisk (1-8) or Eldfisk (9-11) had median THC concentrations between 0.2 and 0.9 µg/l with no obvious relationship between THC concentration and proximity to the Ekofisk or Eldfisk discharge outlets.

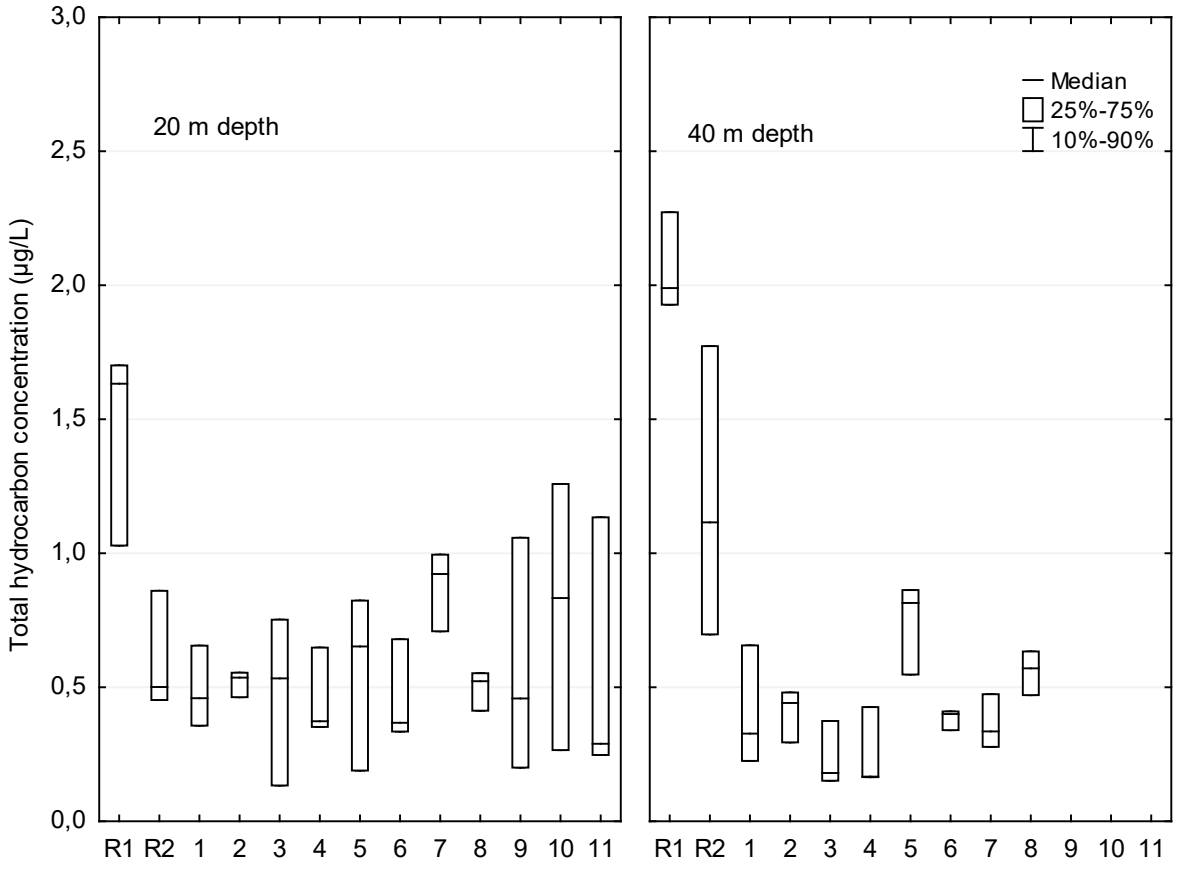


Figure 25. Total hydrocarbon (THC) concentrations of the seawater calculated from silicone extracts positioned at the different monitoring stations at Ekofisk (1-8) and Eldfisk (9-11) compared to the reference location (R1 and R2), including depth (20 m and 40 m) where applicable. No significant difference between groups (Kruskal Wallis, p>0.05, n=3).

3.3.2 PAH-NPD concentrations

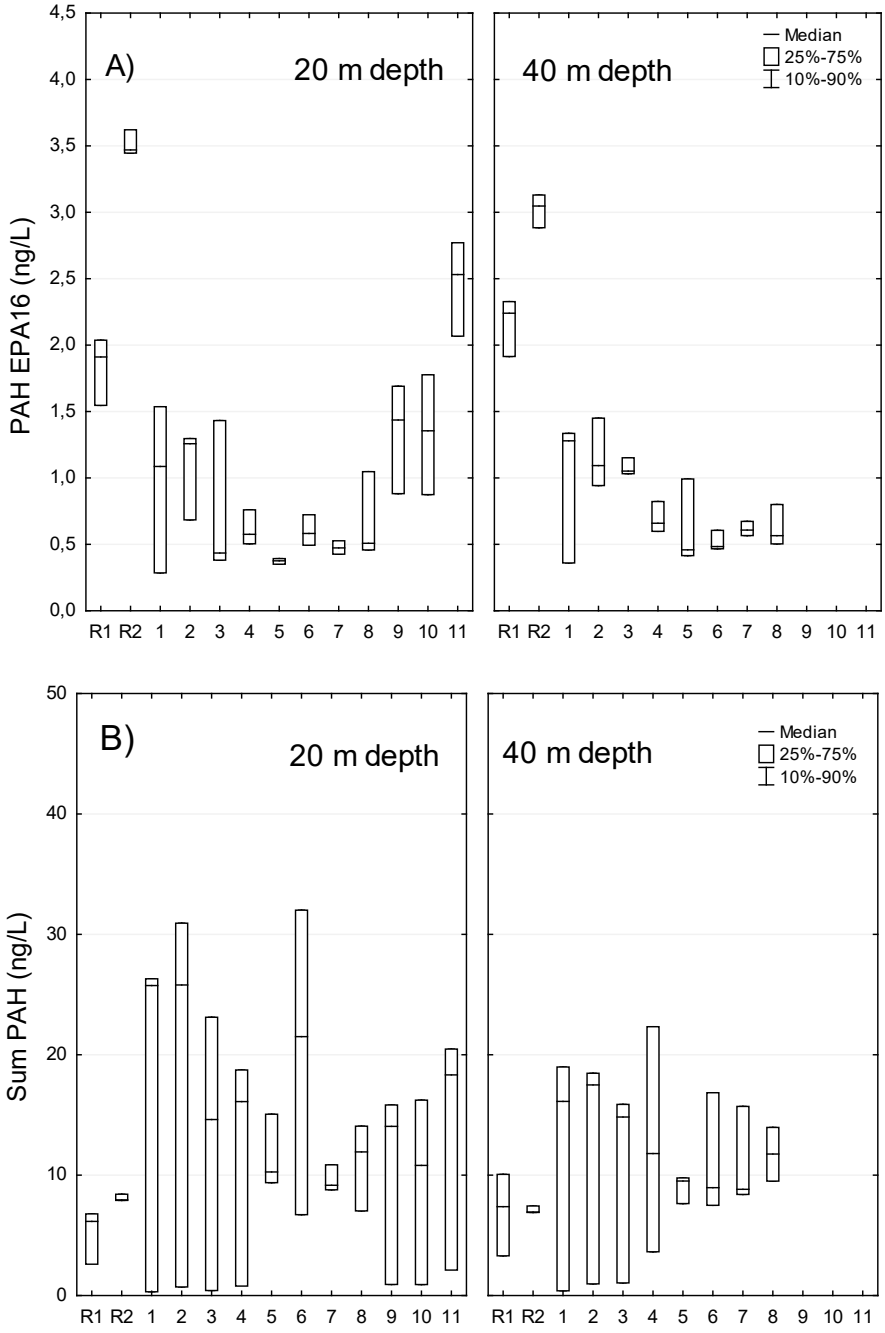


Figure 26. Sum PAH EPA16 (A) and Sum PAH (B) concentrations of the sea water calculated from silicone extracts positioned at the different monitoring stations at Ekofisk (1-8) and Eldfisk (9-11) compared to the reference (R1 and R2), including depth (20 m and 40 m) where applicable. Only measured values above limits of detection (LOD). No significant difference between groups (Kruskal Wallis, p>0.05, n=3)

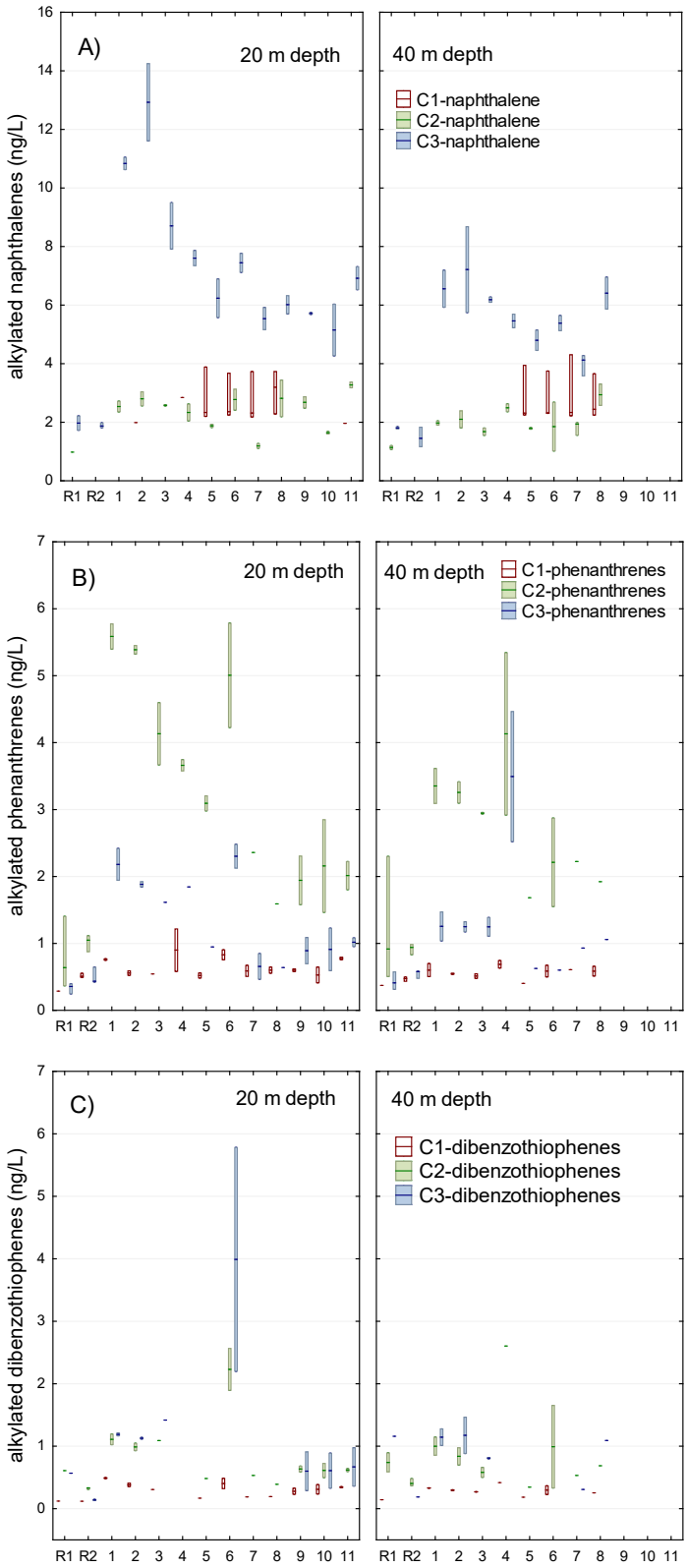


Figure 27. The freely dissolved concentration of alkylated naphthalenes (A), phenanthrenes (B) and dibenzothiophenes (C) in sea water, calculated from silicone extracts from the monitoring stations at Ekofisk (1-8), Eldfisk (9-11) and reference (R1-R2), including depth (20 m and 40 m) where applicable. (median, quartiles, 10/90 percentiles). Only measured values above limits of detection (LOD).

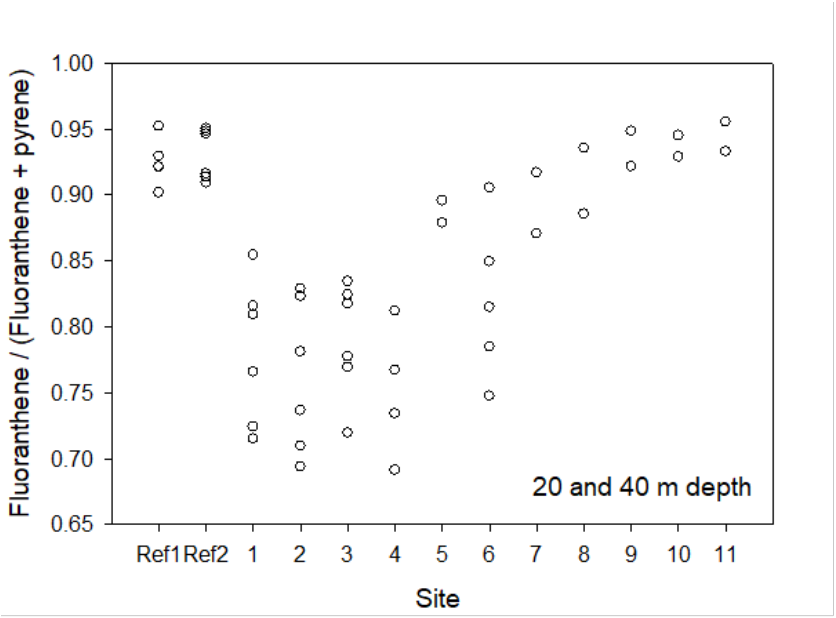


Figure 28. PAH diagnostic ratio based on freely dissolved concentrations of fluoranthene and pyrene measured at each sampling locations. Each dot represents an individual measurement (from one silicone membrane). When one of the two compounds was below LOQ, data were not included.

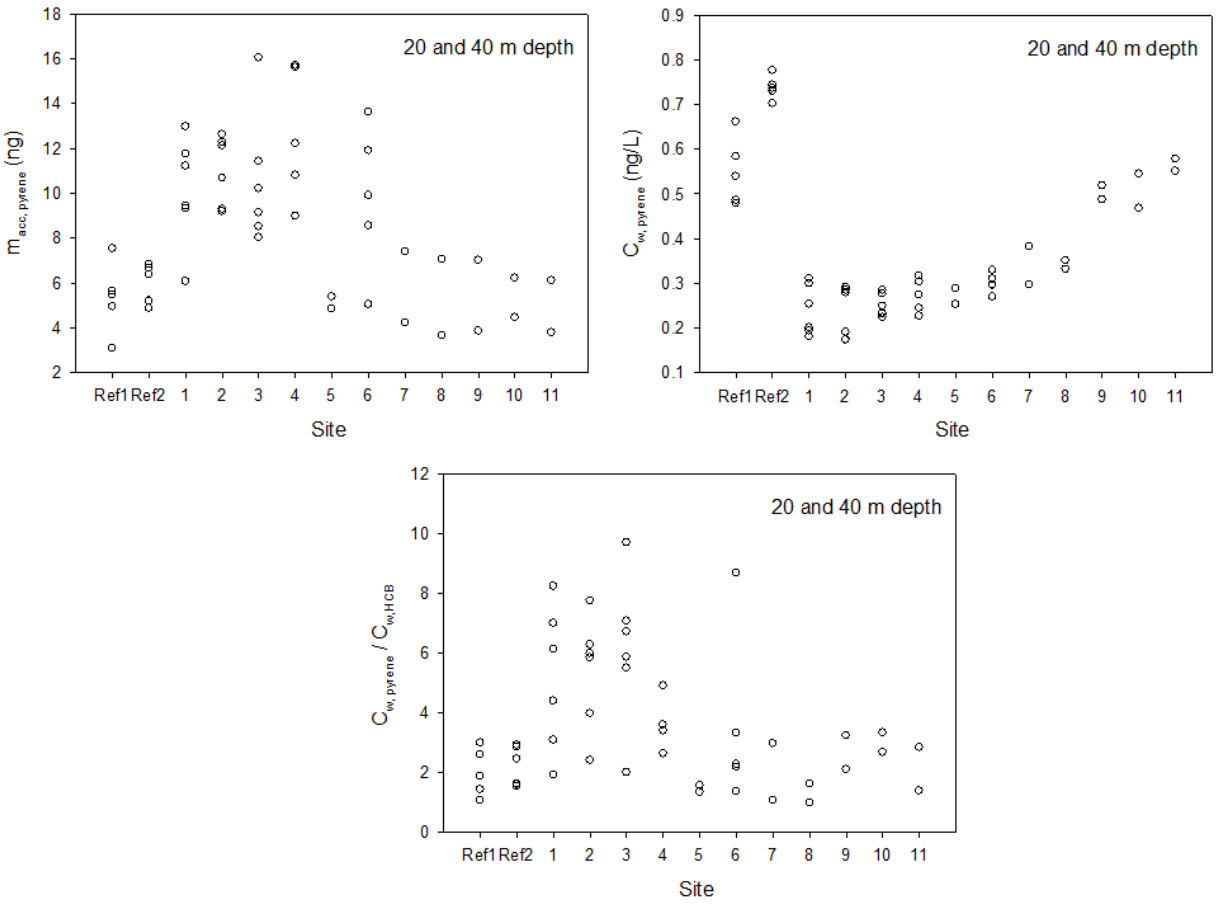


Figure 29. Pyrene masses accumulated in the silicone rubber samplers (m_{acc} in ng), estimated freely dissolved water concentrations (C_w in ng/L) and ratio of C_w for pyrene over that of hexachlorobenzene (HCB).

The accumulations of PAH in silicone membranes placed out at the different monitoring stations and at the two different depths for 6 weeks were calculated into actual freely dissolved water concentrations of PAH16 and Sum PAH (Figure 26). Median PAH16 concentrations were found highest at the reference stations R2 at 40 m and 20 m, followed by R1 at 20 and 40 m, between 2.7 and 4.3 ng/L. This concentration range (for US EPA PAHs) was in line with ranges reported by Harman et al. (2009a) using SPMD passive samplers. All monitoring stations at Ekofisk (1-8) or Eldfisk (9-11) had median PAH16 concentrations between 1.6 and 2.4 µg/L (except Station 11 at 3.5 ng/L), with no obvious relationship between PAH16 concentration and proximity to the Ekofisk or Eldfisk discharge outlets and no obvious differences seen between the two depths.

For the Sum PAH there were no significant differences between the concentrations of the different monitoring stations or between the different depth, with no clear relationship between Sum PAH and proximity to either the Ekofisk or Eldfisk discharge.

The concentration of alkylated naphthalene, phenanthrene and dibenzothiophenes are presented for the different monitoring stations (Figure 27). For the alkylated naphthalene C1 to C3, the C3-naphthalene appeared to show clear differences in concentration with highest values found in the closest stations to the Ekofisk platform. Slightly elevated concentrations of C2 naphthalenes. Clearer concentration profile can be observed for C3 naphthalenes. Estimated concentrations at 20 m depth closest to Ekofisk in the range of 10-12 ng/L are in line with previously reported concentrations of 10-21 ng/L (Harman et al., 2009a; Harman et al., 2010). This was more pronounced at 20 m but also present to a lesser extent at 40 m. A similar concentration gradient and at similar levels to previously measured away from the Ekofisk platform were found for C2-phenanthrene at both 20 m and 40 m. C2 and C3 dibenzothiophenes were in a concentration range of 0.4 to 1.2 ng/L and were similar to those found with SPMDs and reported in 2009 (Harman et al 2009a).

Sometimes, PAH diagnostic ratios are used to differentiate sources of contamination for PAHs (e.g. petroleum vs pyrogenic etc). Since fluoranthene and pyrene were the most often detected PAHs with silicone rubber samplers, the fluoranthene over fluoranthene + pyrene ratio was used and plotted on Figure 28. An interesting pattern can be seen where the PAH ratio is consistently in the range 0.90 - 0.95 for the reference and Eldfisk sampling locations. However, this ratio drops to 0.65 - 0.85 for Stations 1-4. This pattern is similar for masses accumulated (data not shown) and for calculated freely dissolved concentrations. These ratios would tend to indicate input of PAHs from distinct sources for Stations 1-4 and the other sites. Here, difference in pyrene concentrations at the different sites appear to be responsible for the variation in PAH diagnostic ratio (Figure 29). Differences in masses of pyrene accumulated in SR can be seen at the different sites, but these are not apparent when converted to freely dissolved concentrations. When normalised or benchmarked to the freely dissolved concentration of hexachlorobenzene (HCB), a globally distributed persistent organic chemical (Allan et al., 2021). HCB is not expected to be present in PW and concentrations in seawater on a regional scale are likely to be very uniform. This benchmarking alleviates possible uncertainties with the estimation of sampling rates and freely dissolved concentrations. All lines of evidence here indicate an increase in pyrene concentration at Sites 1-4

3.3.3 Alkylphenol concentrations

The quantifiable concentrations of APs above analytical detection limits in the silicone membrane extracts from the monitoring stations 5, 6, 7, and 8 are presented (Table 3). Of the twenty targeted APs, only 10 were detected in any of the silicone extracts from the monitoring stations. These 10 APs were not detected in all monitoring stations. At the reference stations, detections were only of p-cresol, 4n-butylphenol and 4 tert-octylphenol in one sampler only. Most of the detected APs were from

Ekofisk station 5 to 8. The most consistently detected AP was 4-n-hexylphenol with concentrations in the range of 3 - 4 ng/L. Other detected APs included 4-n-heptylphenol, 4-n-octyl phenol, 4-n-nonylphenol with estimated concentrations around or below ng/L level.

Table 3. Alkylphenol concentrations above the limit of quantification (LOQ) in silicon membrane passive samplers from the monitoring stations at Ekofisk (1-8), Eldfisk (9-11) and reference (R1 and R2) including 20 m (t) and 40 m depths (b) as applicable. Single values represent concentration from one PSD sampler, whilst mean and relative percentage difference (RPD%) provided in brackets when concentration quantified above LOQ in 2 PSD membranes. No alkylated phenols were quantified in all three membranes from the same station.

Akylated phenol (ng/L)	R1		R2		1		2		3		4		5	
	t	b	t	b	t	b	t	b	t	b	t	b	t	b
o-cresol														
m-cresol														
p-cresol	83147							89966						
2,4-dimethyl +3,5-dimethyl phenol														
4-ethylphenol														
4-n-propylphenol														218
2,4,6-trimetyl phenol														
4-tert-butylphenol														
4-isopropyl-3-methylphenol														
2-tert-butyl-4-methylphenol														
4-tert-butyl-2-methylphenol					26									
2,5-diisopropylphenol														
4-n-butyl phenol	53.7	42.8			44.5									
4-pentylphenol														
4-n-hexyl phenol													4.0 (6.4)	
4-tert-octylphenol				2.1										
BHT (4,6-di-tert-butyl-2-methylphenol)														
n-heptylphenol													0.7 (1)	
4-n-octyl phenol														
4-n-nonylphenol													0.03	

Akylated phenol (ng/L)	6		7		8		9	10	11
	t	b	t	b	t	b	t	t	t
o-cresol									
m-cresol									
p-cresol			66830						
2,4-dimethyl +3,5-dimethyl phenol									
4-ethylphenol									
4-n-propylphenol	1063				133				
2,4,6-trimetyl phenol									
4-tert-butylphenol									
4-isopropyl-3-methylphenol									
2-tert-butyl-4-methylphenol						137	50		
4-tert-butyl-2-methylphenol			24			39			
2,5-diisopropylphenol									
4-n-butyl phenol						63	42		47
4-pentylphenol									
4-n-hexyl phenol	4	3.2 (25)	3.5 (33)	3.5 (11.8)	3.5 (69)	4.3 (24)			
4-tert-octylphenol									
BHT (4,6-di-tert-butyl-2-methylphenol)									
n-heptylphenol	1.7 (125)			0.6	0.7				
4-n-octyl phenol	1.3		0.2		0.2 (72)	0.1 (25)			
4-n-nonylphenol	0.09 (39)	0.05 (15)							

A slightly higher number of APs were detected in samplers deployed at 20 m depth compared with those exposed at 40 m below the surface. APs were not detected at station 3 or 4 and only 1 or 2 APs were detected above limits of quantification (LOQ) at the closest stations 1 and 2. The AP concentrations showed no relationship between concentration and proximity to the PW discharge at Ekofisk or Eldfisk.

Silicone rubber passive sampling is optimum for the more hydrophobic chemicals. Because of the low silicone-water partition coefficients for the cresols and other short chain alkylated phenols, quantification limits are relatively high for these compounds. In addition, concentrations of these chemicals in silicone will reach equilibrium rapidly and concentrations estimated reflecting the last days of exposure only.

3.3.4 Naphthenic acid concentrations

The number of NA isomers detected in the adapted POCIS samplers for the different monitoring stations are presented in Figure 30. At 20 m depth, the average detection frequency of the NAs was 12 isomer groups per sampling location, whilst an average of 20 NAs were detected in all the stations at the 40 m depth. At 20 m, the highest detection frequency was observed at Station 3 and 11 with 23 and 24 NA isomers respectively. These stations were located 750 m southwest of Ekofisk and 2000 m southwest of Eldfisk installations respectively. At the depth of 40 m, however, the highest NA detection frequency was associated with the reference station (R2). Similar observations were seen for the distribution of PAHs and other analysed target analytes.

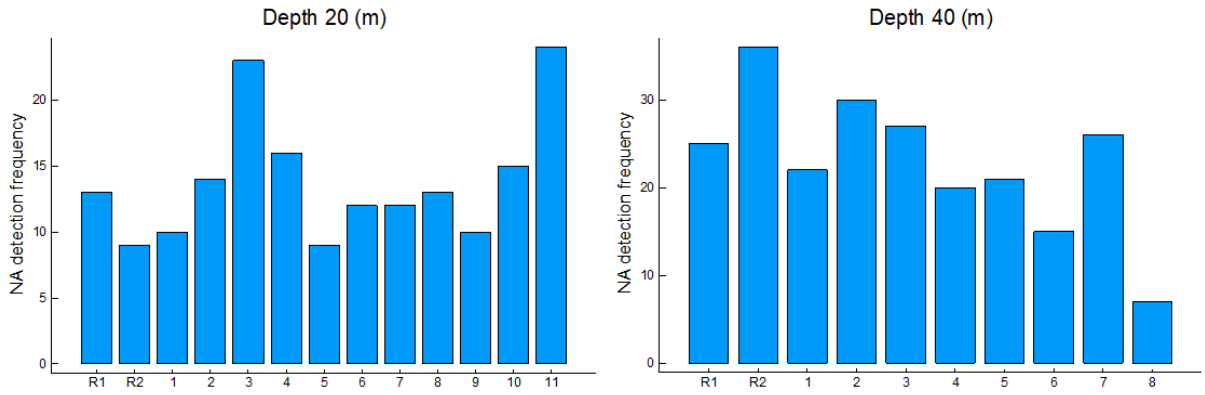


Figure 30. The number of naphthenic acid (NA) isomers detected in the passive samplers from the Ekofisk (1-8), Eldfisk (9-11) and reference (R1 and R2) monitoring stations. Note PSDs placed at 20 and 40 m depth at Ekofisk and reference, whilst only at 20 m depth for Eldfisk.

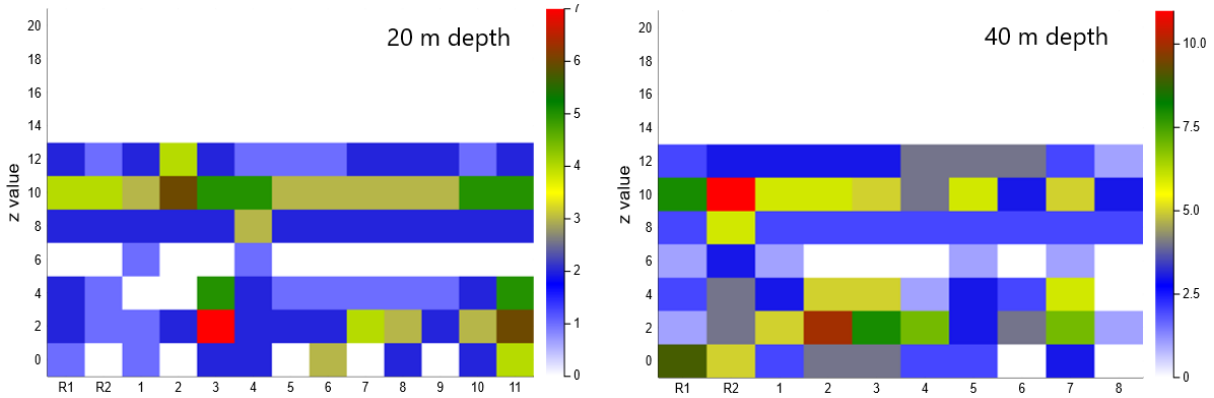


Figure 31. The distribution of naphthenic acids (NAs) in passive samplers based on the z value from the Ekofisk (1-8) and Eldfisk (9-11) and reference (R1 and R2) monitoring stations. PSDs placed at 20 m and 40 m depth at Ekofisk and reference, whilst only at 20 m depth for Eldfisk.

The distribution of NAs at each monitoring station based on the z value, which provides information on the number of aromatic rings (z/2 = number of rings) of each naphthenic acid isomer are shown in Figure 31. Station number 3 at 20 m depth had the largest detection frequency for single ring NAs. At the same depth, all the sampling locations showed similar detection frequencies for the 5-ring NAs. These 5-ring NAs have been previously associated with the acidic fraction of the crude oil. Overall, the isomer groups at 20 m depth showed a homogenous distribution of NAs in terms of the number of rings. At 40 m depth, the 5-ring and single ring NAs showed to have the highest detection frequency. At this depth the reference locations showed a high detection frequency of the naturally occurring aliphatic NAs.

When looking at the number of carbons from the detected NA isomers, the numbers ranged from 10 to 35 carbons (Figure 32). The largest density of NAs for both depths were observed for n values of carbon between 15 and 25, which agrees with previous studies that have measured NAs in produced water from Norwegian oil fields (Samanipour et al., 2020; Samanipour et al., 2018). In terms of number of carbons, the 40 m sites showed higher levels of homogeneity in the distribution of NAs. However, large NAs, indicated by the higher number of carbons, were consistently detected in the Ekofisk and Eldfisk stations compared with the reference groups. Detailed results for each site and each depth are shown in Figure 33.

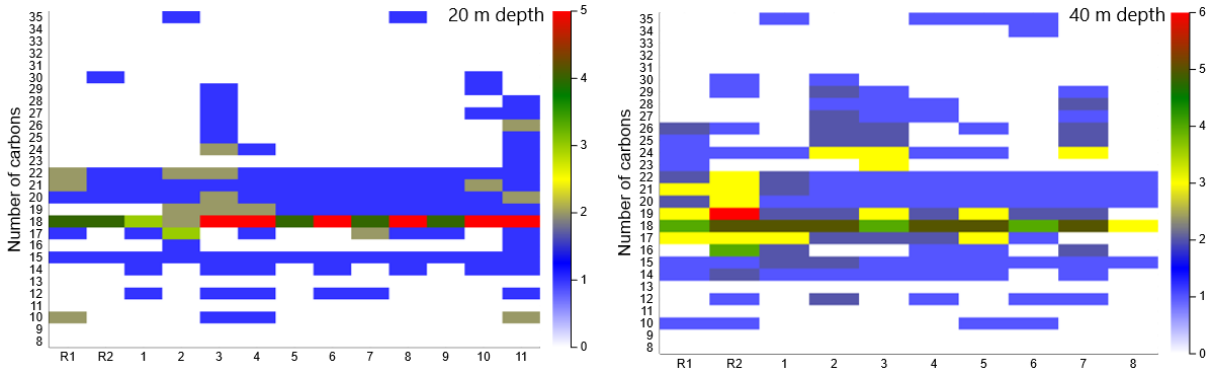
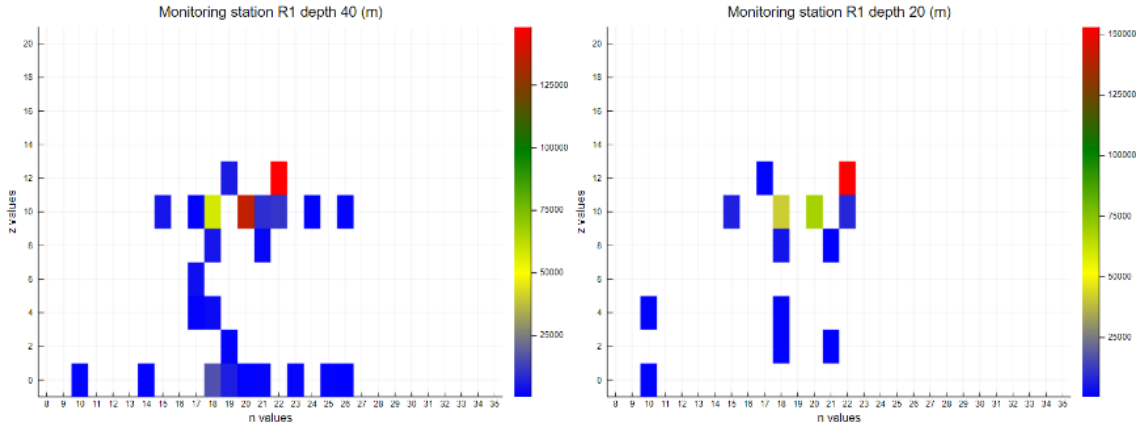
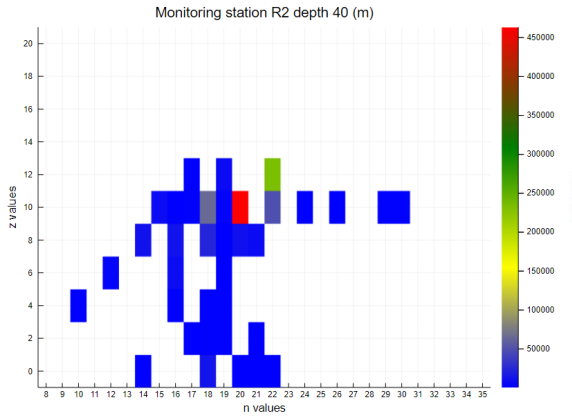
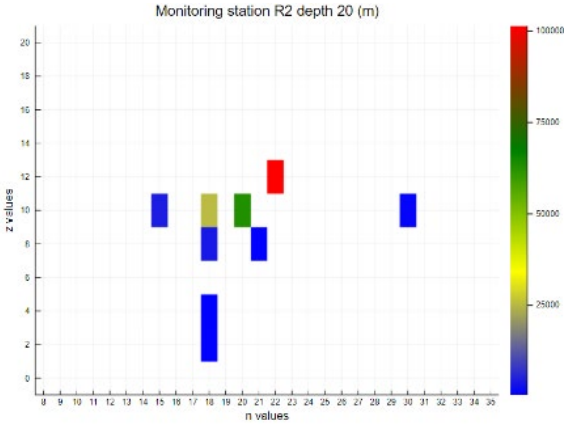


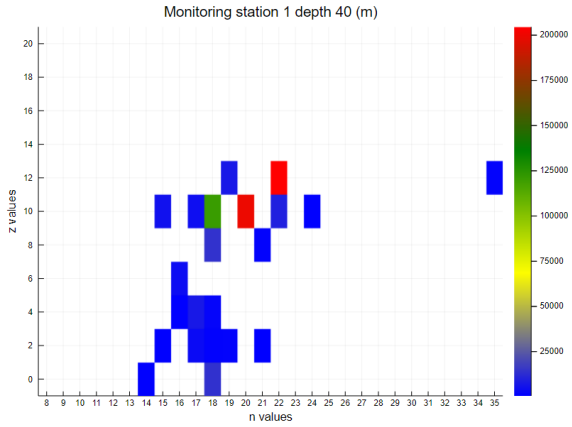
Figure 32. The distribution of naphthenic acids (NAs) in passive samplers based on the number of carbons from the Ekofisk (1-8) and Eldfisk (9-11) and reference (R1 and R2) monitoring stations. PSDs placed at 20 m and 40 m depth at Ekofisk and reference, whilst only at 20 m depth for Eldfisk.

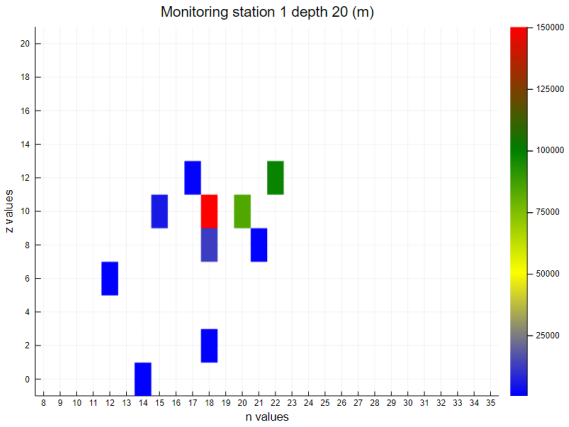

Monitoring station R1 depth 40 (m)
z values
n values

Monitoring station R1 depth 20 (m)
z values
n values


Monitoring station R2 depth 40 (m)
z values
n values


Monitoring station R2 depth 20 (m)
z values
n values


Monitoring station 1 depth 40 (m)
z values
n values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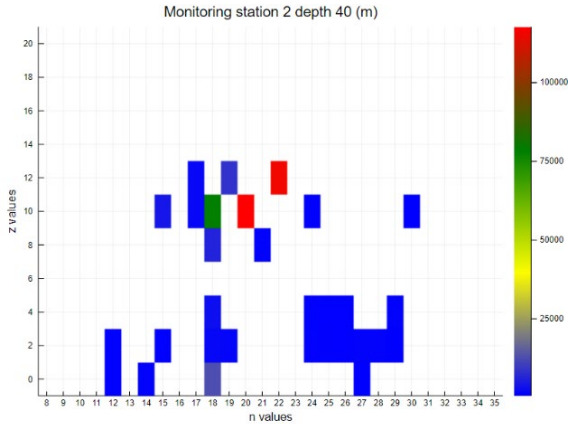
z values
n values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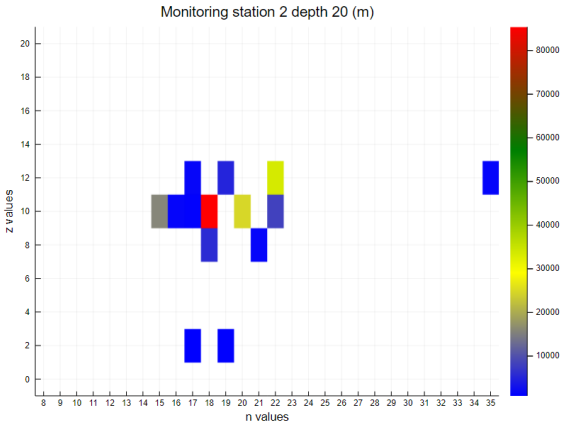
z values
n values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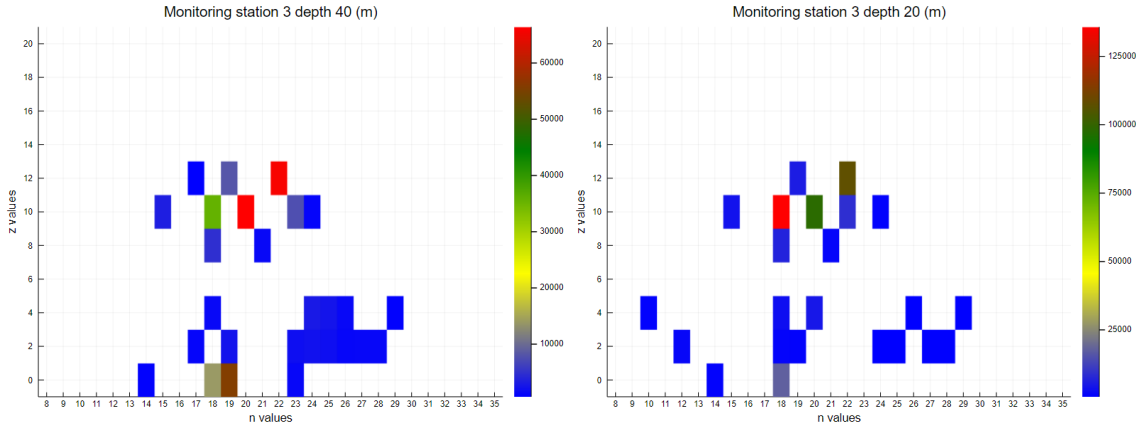
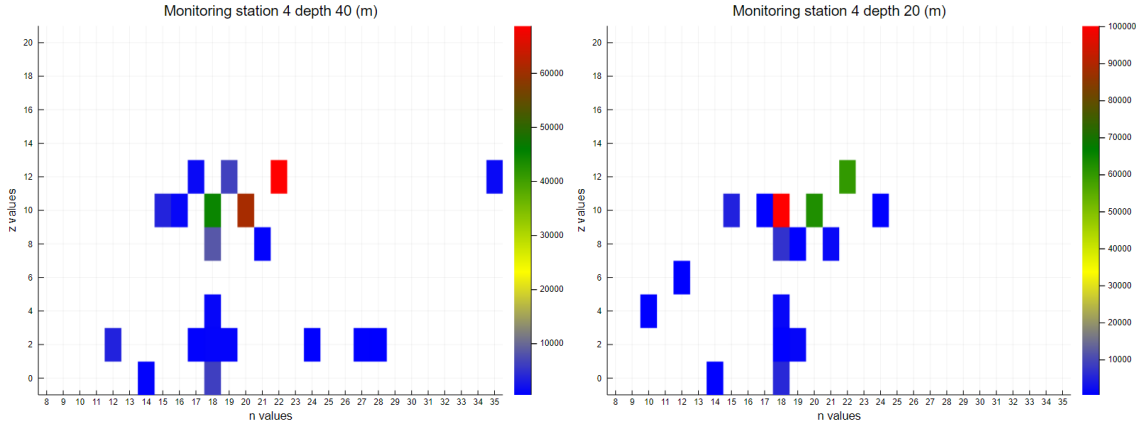
z values
n values


Monitoring station 3 depth 40 (m)
z values
n values

Monitoring station 3 depth 20 (m)
z values
n values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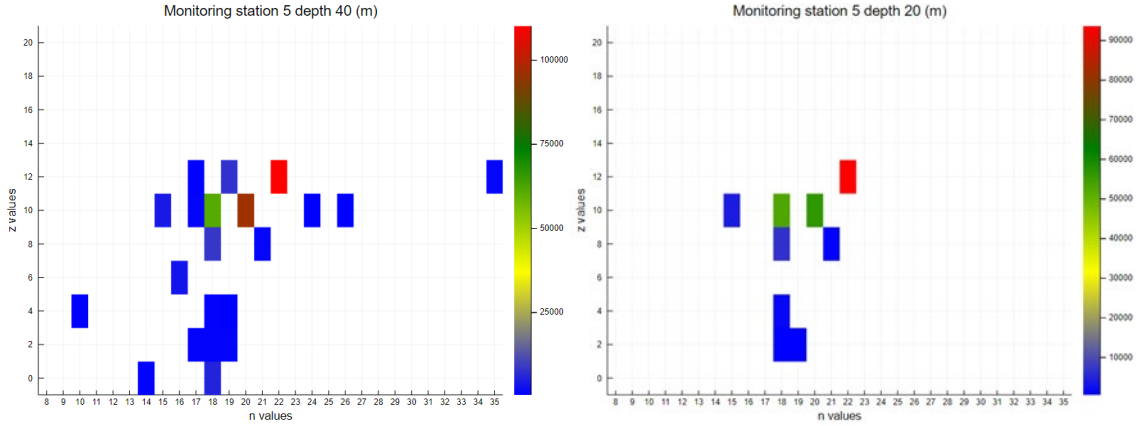
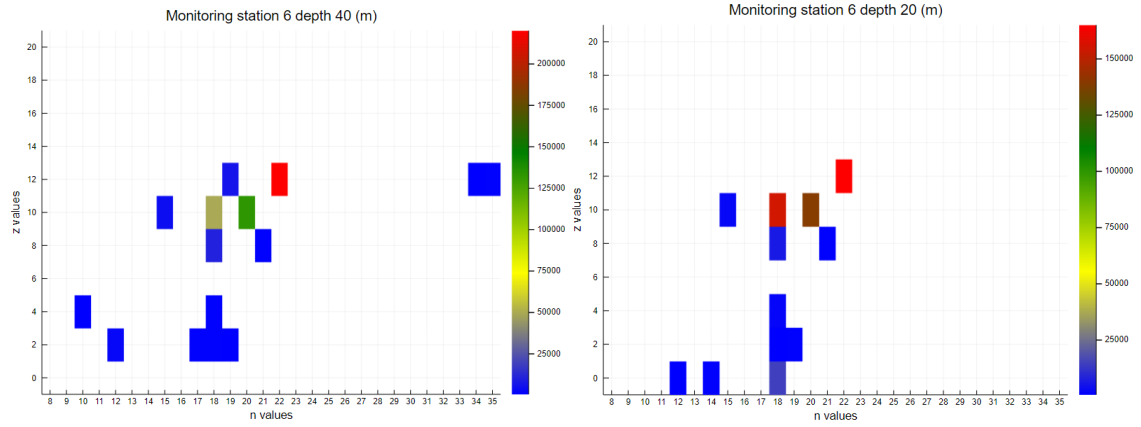
z values
n values

Monitoring station 4 depth 20 (m)
z values
n values


Monitoring station 5 depth 40 (m)
z values
n values

Monitoring station 5 depth 20 (m)
z values
n values


Monitoring station 6 depth 40 (m)
z values
n values

Monitoring station 6 depth 20 (m)
z values
n values

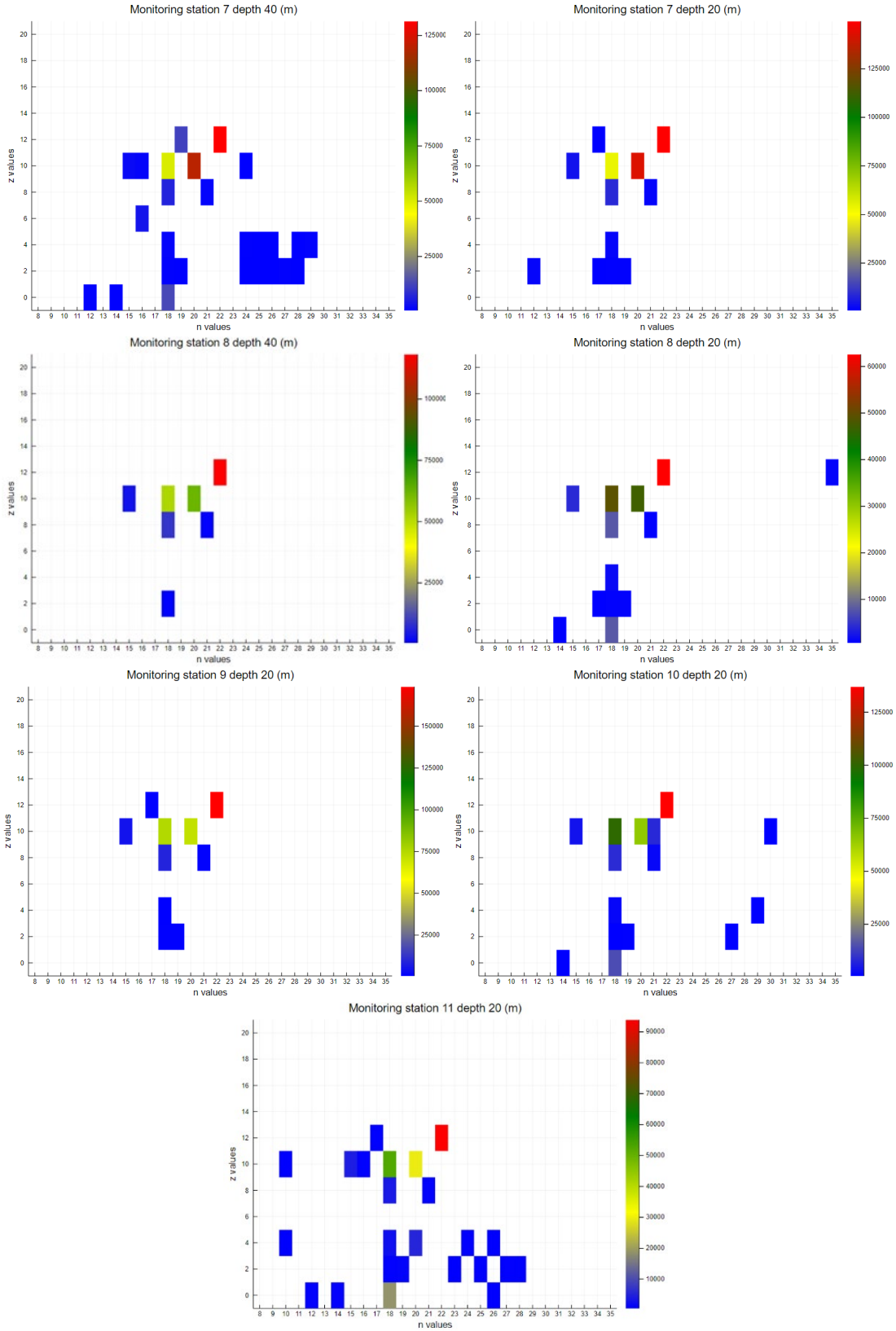


Figure 33. The distribution of naphthenic acids (NAs) in passive samplers based on the number of carbons (n) and ring (z/2) from the Ekofisk (1-8) and Eldfisk (9-11) and reference (R1 and R2) monitoring stations. PSDs placed at 20 m and 40 m depth at Ekofisk and reference, whilst only at 20 m depth for Eldfisk.

3.4 Mussels

3.4.1 Chemical bioaccumulation in mussels

3.4.1.1 PAH-NPD in mussels

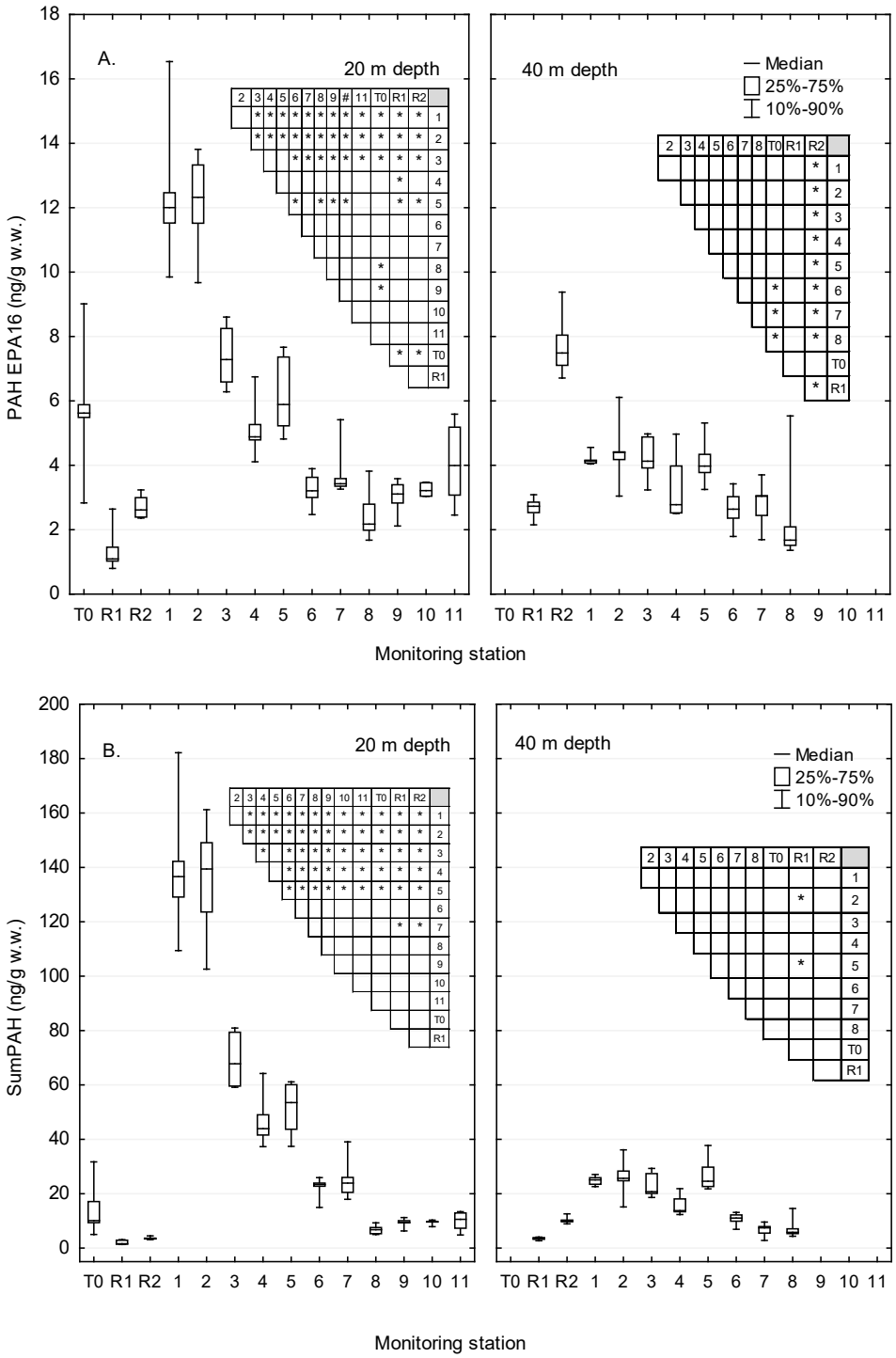


*Figure 34. A) EPA PAH16 and B) Sum of PAH concentrations in mussels from the monitoring stations around the Ekofisk (1-8) and Eldfisk (9-11) installations compared to the reference stations and day zero (T0) group. Mussels held at depths of 20 m (left graph) and 40 m (right graph). Inserted tables show * significant differences between groups (ANOVA, Tukey p<0.05).*

The bioaccumulation of PAH in mussels from the day zero group and all stations are shown as both the sum of PAH EPA16 and the sum of all PAH concentrations (Figure 34). PAH 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 additional compounds, dibenzothiophene, naphthalenes C1 to C4, phenanthrenes/ anthracenes C1 to C4, dibenzothiophenes C1 to C3. For mussels at 20 m depth, highest concentrations of PAH16 were measured in mussels from the closest stations (Station 1 and 2) to the PW discharge outlets. PAH16 concentration in mussels from station 1 and 2 were approximately 12 ng/g w.w. These values were significantly higher than all the other mussel groups at the 20 m depth (ANOVA, Tukey, $p<0.05$). A very similar and more pronounced difference was observed for the Sum PAH, which also includes the alkylated NPD compounds associated with PW effluents. Sum PAH concentrations of 140 ng/g (w.w.) were found in the tissue of mussels from the two closest stations to the Ekofisk discharge outlets. These values were significantly higher than the Sum PAH concentrations measured in all other mussel groups from 20 m (ANOVA, Tukey $p<0.05$). Elevated concentrations of Sum PAH were also measured in mussels from Station 3, 4 and 5, around 750 to 1000 m from Ekofisk. These values were significantly higher than Sum PAH measured in mussels further away at 2000 m (Station 6 and 7) and 4000 m (Station 8) from Ekofisk as well as the day zero (T0) and reference mussels. The Eldfisk mussel stations (Station 9-11) had low concentrations of Sum PAH, which were similar to that measured in the reference mussels.

In mussels from the 40 m depth, only low concentrations of PAH16 and Sum PAH were measured. The highest PAH16 concentration in the 40 m mussels was from the reference group (R2), which was significantly higher than all other mussel groups at 40 m (ANOVA, Tukey $p<0.05$). No significant difference was found between Ekofisk stations 1 to 8 at 40 m for PAH16 or SumPAH. However, for the SumPAH, R1 was significantly lower than that measured in mussels at Stations 2 and 5 (ANOVA, Tukey $p<0.05$).

3.4.1.2 Metals in mussels

A selection of 15 metals were analysed in the soft tissue of mussels from the different stations including silver (Ag), arsenic (As), cadmium (Cd), cobolt (Co), chromium (Cr) copper (Cu), Iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), vanadium (V) and zinc (Zn) (Table 4). There were no clear relationships between metal concentration and proximity to the either the Ekofisk or Eldfisk PW discharge outlets. Furthermore, there were no clear differences between any of the metal concentrations and mussel depth.

Table 4. Metal concentrations in mussels from the stations as Ekofisk (1-8) and reference station 1 at 20 m and 40 m, as well as Eldfisk at 20 m. Fifteen metals include silver (Ag), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), vanadium (V), zinc (Zn). (mg/kg w.w.)

	Station	Ag		As		Cd		Co		Cr		Cu		Fe		Hg	
	depth	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
REF	R1 20 m	0.012		1.40		0.26		0.065		0.075		1.10		14.0		0.012	
REF	R1 40 m	0.013		1.20		0.17		0.052		0.150		1.00		28.0		0.016	
EKOFISK	St1 40 m	0.013	0.001	1.40	0.28	0.19	0.01	0.053	0.006	0.107	0.033	1.94	1.50	21.5	0.71	0.015	0.001
EKOFISK	St2 20 m	0.015	0.001	1.40	0.14	0.18	0.01	0.055	0.007	0.093	0.004	0.91	0.10	18.5	2.12	0.015	0.001
EKOFISK	St2 40 m	0.017	0.003	1.35	0.07	0.19	0.01	0.051	0.005	0.090	0.014	0.99	0.02	23.0	1.41	0.018	0.001
EKOFISK	St3 20 m	0.011		1.30		0.15		0.045		0.095		0.80		19.0		0.016	
EKOFISK	St3 40 m	0.014	0.003	1.30	0.00	0.17	0.01	0.047	0.004	0.081	0.004	1.00	0.01	17.0	1.41	0.015	0.001
EKOFISK	St4 20 m	0.016		1.40		0.17		0.058		0.093		1.10		22.0		0.015	
EKOFISK	St4 40 m	0.012		1.30		0.18		0.049		0.076		0.91		18.0		0.015	
EKOFISK	St5 40 m	0.018	0.004	1.70	0.00	0.20	0.01	0.056	0.006	0.140	0.024	2.83	0.95	20.5	1.73	0.013	0.001
EKOFISK	St6 20 m	0.017	0.002	1.53	0.12	0.19	0.02	0.057	0.005	0.124	0.048	1.92	0.92	18.2	2.04	0.013	0.002
EKOFISK	St6 40 m	0.012	0.003	1.56	0.22	0.18	0.01	0.049	0.003	0.123	0.030	1.97	0.78	18.4	1.51	0.013	0.001
EKOFISK	St7 20 m	0.014	0.002	1.62	0.08	0.19	0.01	0.060	0.006	0.124	0.058	2.18	0.97	17.6	1.82	0.013	0.001
EKOFISK	St7 40 m	0.014	0.003	1.56	0.17	0.18	0.00	0.048	0.004	0.104	0.021	1.81	0.56	19.4	1.34	0.014	0.002
EKOFISK	St8 20 m	0.017	0.002	1.74	0.05	0.22	0.02	0.062	0.004	0.160	0.031	2.10	0.63	21.4	1.95	0.016	0.001
EKOFISK	St8 40 m	0.012	0.001	1.78	0.15	0.19	0.02	0.051	0.004	0.148	0.017	2.50	0.49	17.6	1.14	0.016	0.001
ELDFISK	St9 20 m	0.014	0.006	1.60	0.42	0.19	0.05	0.055	0.013	0.107	0.033	1.55	1.07	22.0	1.41	0.015	0.001
ELDFISK	St10 20 m	0.022		1.40		0.18		0.050		0.078		1.10		20.0		0.015	
ELDFISK	St11 20 m	0.015	0.003	1.70	0.22	0.18	0.03	0.052	0.011	0.118	0.059	1.73	0.53	19.0	3.16	0.013	0.001

	Station	Mn		Mo		Ni		Pb		Se		V		Zn	
	depth	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
REF	R1 20 m	0.50		3.00		0.12		0.19		0.61		0.23		11.0	
REF	R1 40 m	0.51		2.10		0.17		0.25		0.49		0.11		17.0	
EKOFISK	St1 40 m	0.70	0.07	5.55	0.07	0.14	0.035	0.24	0.03	0.61	0.07	0.28	0.01	15.0	1.41
EKOFISK	St2 20 m	0.63	0.01	1.20	0.14	0.10	0.021	0.21	0.00	0.56	0.04	0.21	0.01	12.0	0.00
EKOFISK	St2 40 m	0.62	0.09	5.95	1.20	0.11	0.000	0.25	0.03	0.57	0.01	0.32	0.02	14.5	0.71
EKOFISK	St3 20 m	0.52		7.40		0.10		0.24		0.47		0.28		11.0	
EKOFISK	St3 40 m	0.60	0.10	3.42	3.51	0.11	0.007	0.22	0.06	0.53	0.11	0.26	0.11	11.8	3.11
EKOFISK	St4 20 m	0.58		1.10		0.07		0.21		0.54		0.19		15.0	
EKOFISK	St4 40 m	0.49		6.30		0.10		0.20		0.52		0.25		20.0	
EKOFISK	St5 40 m	0.89	0.07	6.45	0.13	0.19	0.099	0.23	0.01	0.80	0.06	0.27	0.02	14.5	1.73
EKOFISK	St6 20 m	0.69	0.11	0.97	0.09	0.08	0.012	0.16	0.01	0.69	0.10	0.16	0.01	11.8	1.17
EKOFISK	St6 40 m	0.73	0.15	4.50	0.60	0.11	0.010	0.21	0.02	0.67	0.10	0.22	0.02	12.9	1.07
EKOFISK	St7 20 m	0.78	0.13	1.08	0.04	0.09	0.008	0.19	0.02	0.73	0.09	0.17	0.01	14.0	1.41
EKOFISK	St7 40 m	0.76	0.20	8.30	0.88	0.13	0.015	0.22	0.02	0.66	0.12	0.20	0.02	12.6	1.52
EKOFISK	St8 20 m	0.76	0.06	0.83	0.05	0.12	0.010	0.17	0.01	0.71	0.02	0.15	0.03	14.0	1.41
EKOFISK	St8 40 m	0.86	0.15	2.88	0.08	0.16	0.087	0.23	0.01	0.76	0.06	0.18	0.01	14.2	1.30
ELDFISK	St9 20 m	0.80	0.42	0.80	0.06	0.09	0.000	0.20	0.01	0.66	0.25	0.21	0.03	13.0	1.41
ELDFISK	St10 20 m	0.71		0.93		0.09		0.22		0.67		0.24		17.0	
ELDFISK	St11 20 m	0.84	0.17	0.53	0.07	0.10	0.026	0.16	0.03	0.66	0.12	0.15	0.02	13.0	0.82

3.4.2 Biological effects in mussels

3.4.2.1 Mussel speciation

Mussels were identified from a sub-sample of 110 randomly selected individuals that were taken from the same population as used in the field deployment (Table 5). Of the 110 individuals sampled in 2021, 90% were identified as *M. edulis*, with the remaining 10% identified as hybrids of either *M. edulis*/ *trossulus* (8%) or *M. trossulus*/ *galloprovincialis* (2%). The dominance of *M. edulis* in the population was similar to previous WCM campaigns where the same source population in the Trondheim region was used. For example, *M. edulis* made up 82% of the sampled population in 2017 and 88% of the population in 2012. The *M. edulis*/ *trossulus* hybrid was identified in 8% of the population in 2021, which was also similar to that found in 2017 and 2012. Since it has been shown in controlled laboratory studies that differences in chemical bioaccumulation and biological effects responses do occur between *Mytilus* species (Brooks et al., 2015a), it was important to establish the proportion of *M. edulis* in the population. The high proportion of *M. edulis* in the population would suggest that species differences are unlikely to impact the chemical bioaccumulation and/or biological effects responses in the present study.

Table 5. Identification of the species of mussels including hybrids used in the WCM programme in the present study of 2021 and the previous two WCM programmes when mussels were used. Values represent the percentage of individuals.

Mytilus species / hybrid	2021	2017	2012
	n = 110	n = 96	n = 66
M. edulis	90	82	88
M. trossulus			1
M. galloprovincialis			1
M. edulis/ trossulus hybrid	8	11	8
M. edulis/ galloprovincialis hybrid		5	2
M. trossulus/ galloprovincialis hybrid	2	2	

3.4.2.2 Condition index (CI)

The CI is presented for all monitoring stations at Ekofisk (1-8) and Eldfisk (9-11) as well as the two reference stations and the day zero (T0) group in Figure 35. A significantly lower CI was found in T0 mussels with a median value of 8.2, which was significantly lower than all mussel groups held at 20 m and half of the mussel groups at 40 m. All field exposed mussels had a median CI between 9.3 and 17.9. Highest CI values were found in mussels from Eldfisk, with highest median CI of 17.9 in station 11 mussels, 2000 m southwest of the Eldfisk installation. The CI from station 11 was significantly higher than many of the Ekofisk stations and both reference stations at the 20 m depth. Overall, CI values were marginally higher in the 20 m mussels compared to the 40 m mussels at both Ekofisk and the reference stations.

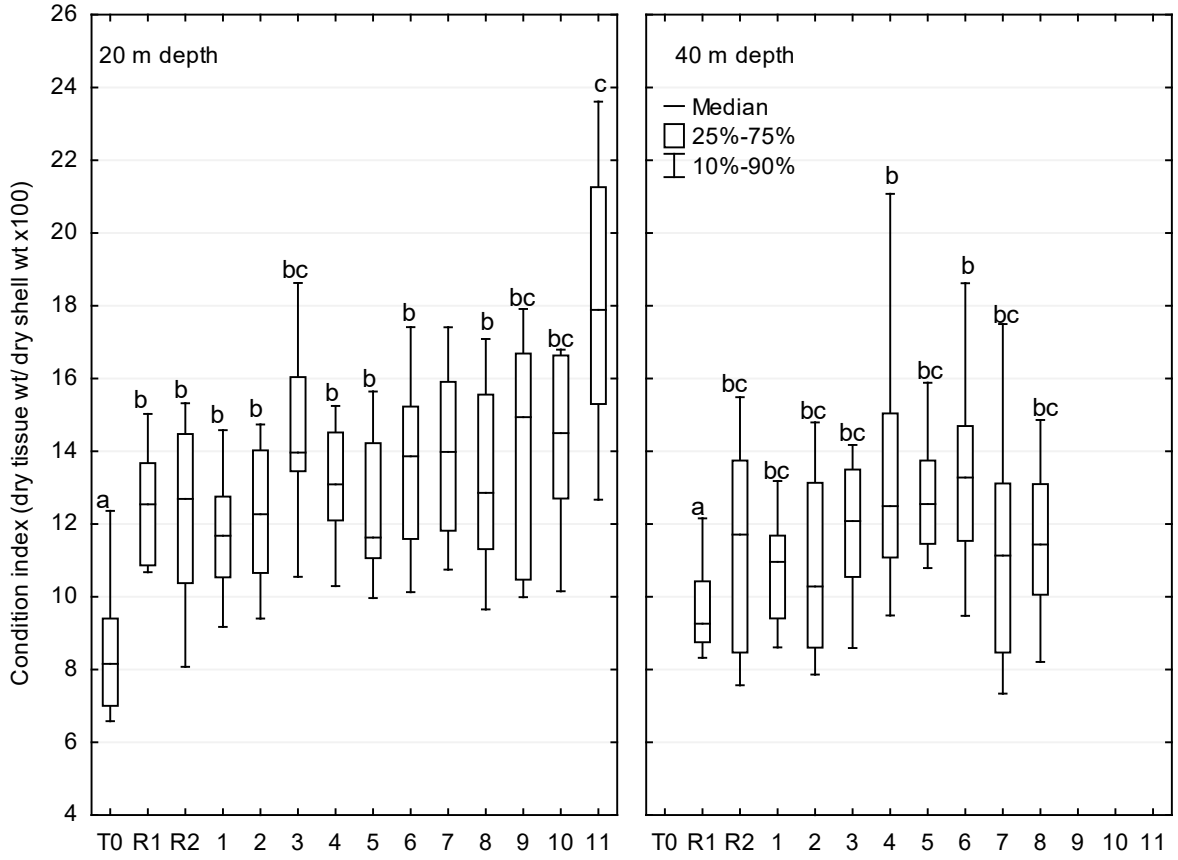


Figure 35. Condition index in mussels from the monitoring stations at Ekofisk (1-8), Eldfisk (9-11) and reference (R1-R2), including depth (20 m and 40 m) (median ± quartiles (box) and 10/90 percentiles (outer line). Different letters denote significant differences for the same depth (ANOVA, Tukey p<0.05, n=15).

3.4.2.3 Stress on stress (SoS)

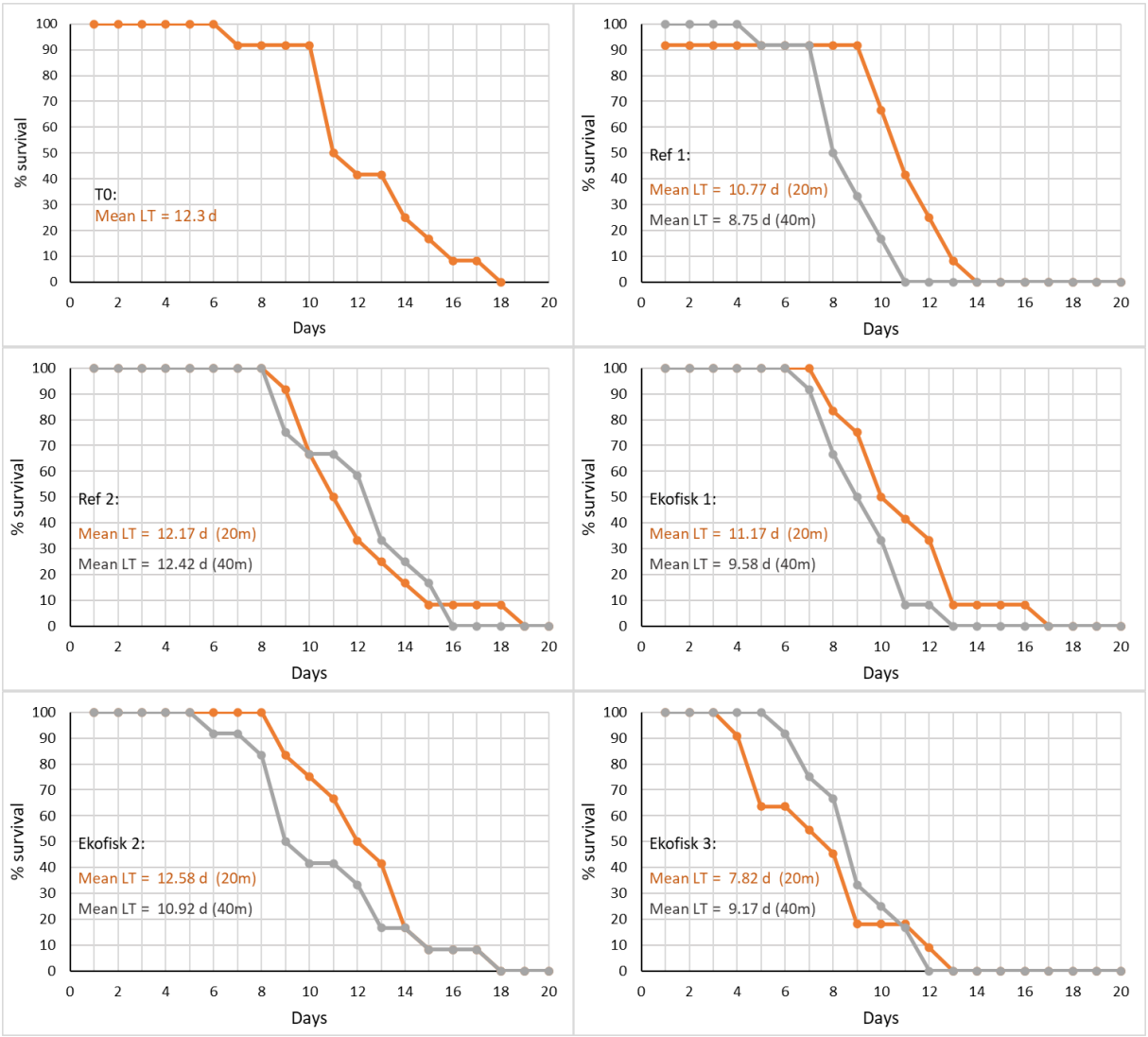
Data gathered from the SoS test were processed to provide percentage survival data used to generate plots (Figure 36), which were then further refined to give time to event data to enable statistical analyses.

Time to event (death) data were generated from individuals within each test group from which the mean and median lethal time (LT) were derived. Mean LT data was then used to produce confidence limits at the 95% level. These values are presented in Table 6. LT data from individuals within sites were compared against that obtained from a reference site using the non-parametric Wilcoxon test to determine if any significant differences were present. In addition, the Wilcoxon test was used to compare the two different depths from each deployment site.

ICES assessment criteria have provisional values reported for this assay for *Mytilus* spp. The background assessment criteria (BAC) is 10 days, with environmental assessment criteria (EAC) set at 5 days (Davies and Vethaak, 2012). Therefore, organisms are considered healthy if the mean LT is more than 10 days, are considered stressed but compensating if the value is between 5 and 10 days and finally, they are considered severally stressed if the mean LT value is less than 5 days. In the present study 8 groups recorded mean lethal times below 10. These were the 40 m deployments at R1 and

Ekofisk stations 1, 3, 4, 5, 6, and 7, and one group, Ekofisk 3 at the 20 m depth. None of the sites produced a value below the ICES EAC of 5 days (Table 6).
Time to death data from all sites were compared against values obtained from the reference sites at the relevant depth of deployment using the Wilcoxon test. Ekofisk 3 was the only site that showed a significant difference at 20 m depth compared to the reference. At 40 m, Ekofisk stations 1, 3, 4, 5 and 7 were shown to be significantly lower than their corresponding reference group. Furthermore, R1 at 40 m depth was significantly lower than R2 at the same depth.

When 20 and 40 m depths were compared within each station, Ekofisk stations 4, 7 and 8 showed a significant reduction at the 40 m depth. The three Eldfisk stations had mussels deployed at 20 m depth only and none of these sites were significantly different from the reference stations at that depth.



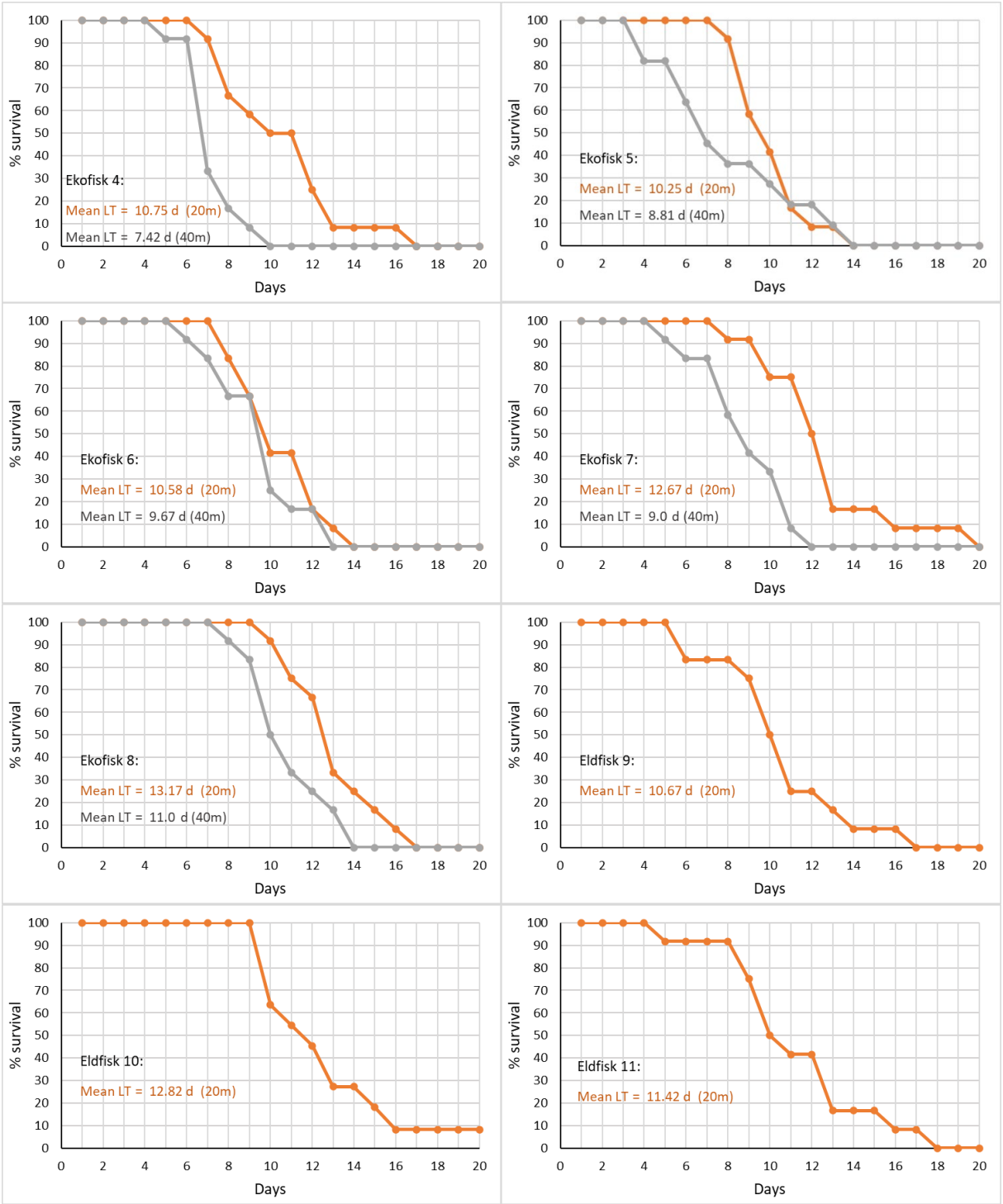


Figure 36. Stress on stress curves for mussels collected from the day zero group (T0) and monitoring stations at Ekofisk (1-8), Eldfisk (9-11) and reference (R1-R2), including depth (20 m and 40 m).

Table 6. Collated stress on stress data including mean and median lethal times (LT) for each mussel group, together with 95 % confidence limits.

Monitoring station	Mean LT (days)	Standard error	95% Confidence limits		Median LT (days)
			Lower	Upper	
T0	12.33	2.53	10.899	13.767	11.5
Ref 1 (20 m)	10.77	3.19	8.963	12.576	11
Ref 1 (40 m)	8.75	1.66	7.812	9.688	8.5
Ref 2 (20 m)	12.17	2.79	10.588	13.746	11.5
Ref 2 (40 m)	12.42	2.64	10.921	13.913	13
Ekofisk 1 (20 m)	11.17	2.59	9.702	12.631	10.5
Ekofisk 1 (40 m)	9.58	1.73	8.605	10.562	9.5
Ekofisk 2 (20 m)	12.58	2.64	11.087	14.079	12.5
Ekofisk 2 (40 m)	10.92	3.37	9.010	12.823	9.5
Ekofisk 3 (20 m)	7.82	2.96	6.069	9.568	8
Ekofisk 3 (40 m)	9.17	2.08	7.989	10.344	9
Ekofisk 4 (20 m)	10.75	2.93	9.094	12.406	11
Ekofisk 4 (40 m)	7.42	1.24	6.715	8.118	7
Ekofisk 5 (20 m)	10.25	1.66	9.312	11.188	10
Ekofisk 5 (40 m)	8.818	3.4	6.172	10.191	7
Ekofisk 6 (20 m)	10.58	1.98	9.466	11.701	10
Ekofisk 6 (40 m)	9.67	2.15	8.452	10.881	10
Ekofisk 7 (20 m)	12.67	3.06	10.938	14.395	12.5
Ekofisk 7 (40 m)	9.00	2.13	7.794	10.206	9
Ekofisk 8 (20 m)	13.17	2.08	11.989	14.344	13
Ekofisk 8 (40 m)	11.00	1.91	9.921	12.079	10.5
Eldfisk 9 (20 m)	10.67	3.08	8.921	12.412	10.5
Eldfisk 10 (20 m)	12.82	3.43	10.791	14.845	12
Eldfisk 11 (20 m)	11.42	3.45	9.465	13.369	10.5

3.4.2.4 Lysosomal membrane stability (LMS)

The LMS results are shown in Figure 37. The lowest labilisation times, below 20 min, were recorded in mussels from the T0 group as well as monitoring stations 2 and 3 at 20 m and station 2 a 40 m. Lower labilisation times were also found in mussels from station 1, 20 m and station 3, 40 m, just above 20 min. All other mussel groups recorded mean labilisation times above 32 min. The mussels from Eldfisk, had some of the highest labilisation times around 40 min.

ICES assessment criteria have been established in *Mytilus* sp. with a BAC and EAC of 20 and 10 min respectively (Davies and Vethaak, 2012). With this in mind, none of the mussel groups had LMS values below the EAC, although four groups (T0, 2 and 3 at 20 m, and 2 at 40 m) were below the BAC value indicating a mild stress response but compensating.

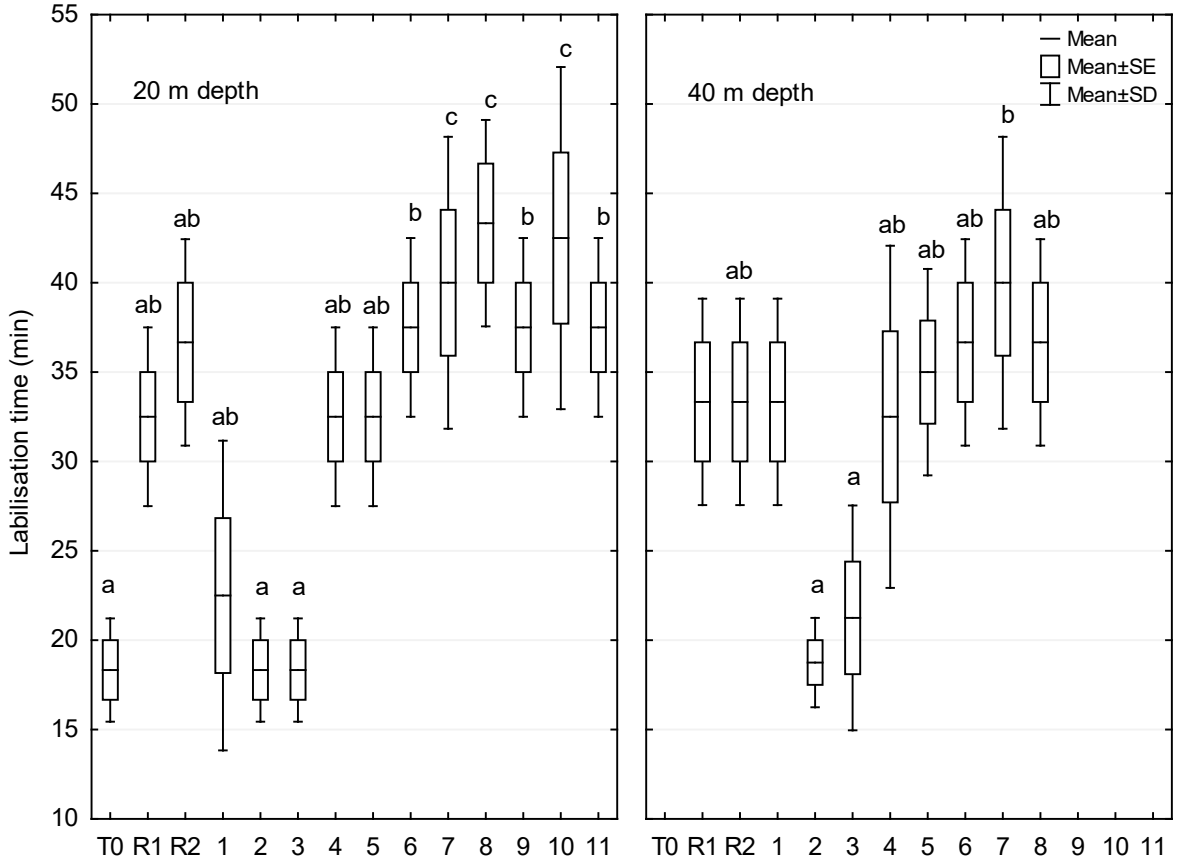


Figure 37. Lysosomal membrane stability of digestive gland cells of mussels collected from the monitoring stations at Ekofisk (1-8), Eldfisk (9-11) and reference (R1-R2), including depth (20 m and 40 m) (mean ± standard error (SE) (box) and standard deviation (SD) (outer line). Different letters denote significant differences for the same depth (ANOVA, Tukey p<0.05, n=15).

3.4.2.5 Micronuclei (MN) formation

MN frequency in mussel haemocytes from day zero (T0) and field exposed groups are shown in Figure 38. Lowest frequencies of MN were recorded in the T0 group, followed by the reference stations 1 and 2 at 40 m and R2 at 20 m. These values were below the ICES background assessment criteria (BAC) of 2.5 MN/ 1000 cells and indicative of background reference responses (Vethaak and Davies, 2012). All mussels at Ekofisk and Eldfisk had MN frequencies above the BAC, with mean values ranging between 3 and 8.7 MN per 1000 cells. Due to the large variation within groups only the highest value at station 7 at 40 m was significantly different from the T0 and R2 40 m groups (ANOVA, Tukey, p<0.05). All other field exposed groups including the different depths were statistically similar to each other.

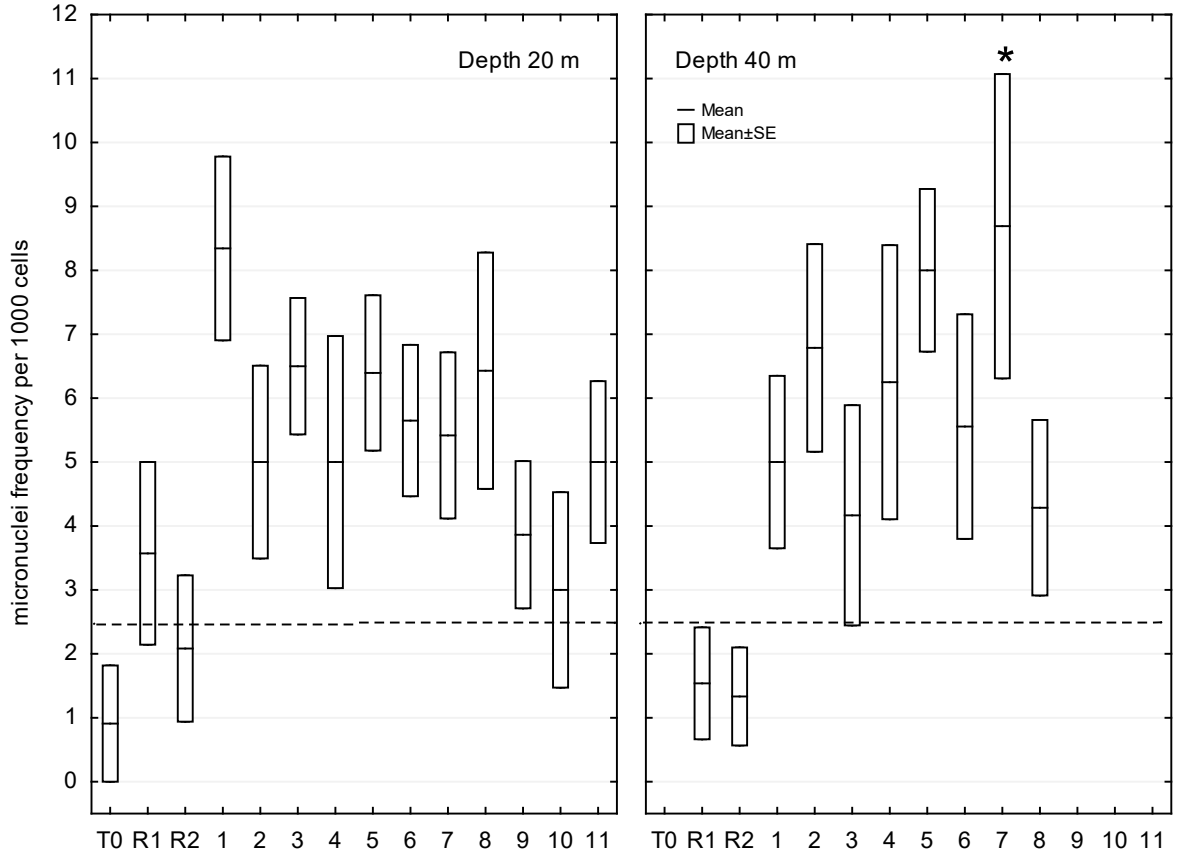


Figure 38. Micronuclei frequency per 1000 cells in mussel haemocytes from the different groups. Asterisks (*) denote statistically significant difference from T0 for the R2 40m mussels (ANOVA, Tukey, p<0.05, n=15). Dotted line denotes the ICES BAC value of 2.5 MN/ 1000 cells.

3.4.2.6 Mussel histology

Mussel digestive gland histology

Five histological features of the digestive gland are presented including, epithelial degeneration, granulocytomas, haemocytic infiltration, and the presence/ absence of parasites (Figure 39). Epithelial degeneration is a disintegration and subsequent absence of digestive and basophilic cells within digestive epithelia and it is a sign of general stress. This lesion was measured as absent or present (0 or 1) and showed lowest levels in the day zero (T0) and reference mussels. Higher occurrence of this histological feature was observed in field exposed mussels although no clear relationship to the discharge outlet at Ekofisk or Eldfisk was observed and no significant differences between the mussel stations were found (Kruskal Wallis, $p>0.05$). The presence of granulocytomas represents an inflammatory condition characterized by clusters of haemocytes or disintegration and sloughing of tissue and it is a sign of general stress. It was recorded on a severity scale of 0 to 2. The mean values ranged between 0 and 0.75 with no significant difference between mussels from the different stations (Kruskal Wallis, $p>0.05$), and there was no clear relationship with proximity to the Ekofisk or Eldfisk discharge outlet. Similarly, for the stress response of haemocytic infiltration there was no significant differences between mussels from the different stations and no clear relationship between response and proximity to the discharge outlets of Ekofisk or Eldfisk. The presence of haemocytes in the digestive gland represents an inflammatory condition and it is characterized by infiltration of haemocytes in localized areas as a sign of general stress. Parasites of the digestive gland were equally present

amongst all mussel groups and no differences were found in proximity to the installations or between 20 m and 40 m mussels.

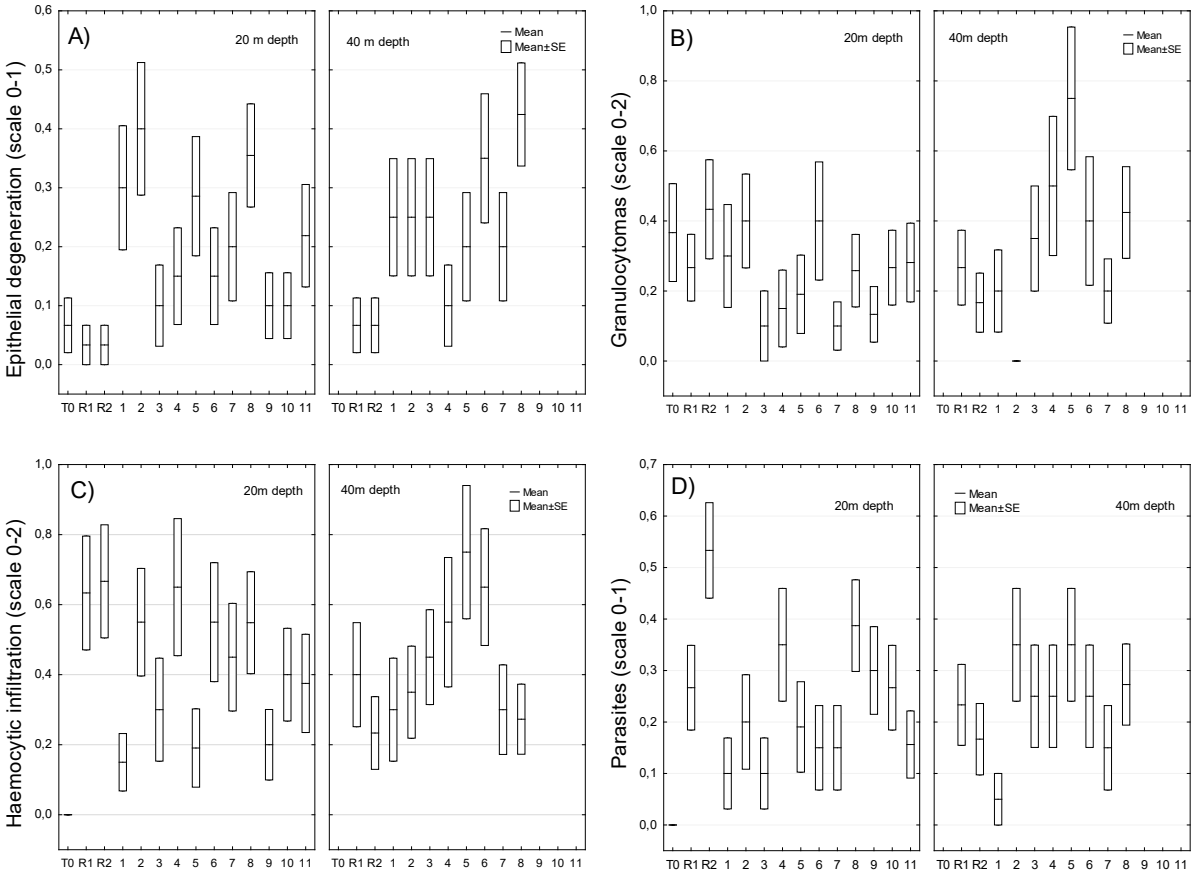


Figure 39. Digestive gland histology in mussels from the monitoring stations around the Ekofisk (1-8) and Eldfisk (9-11) installations compared to the reference stations (R1, R2) and day zero (T0) group. Mussels held at depths of 20 m (left graph) and 40 m (right graph). Digestive gland histological endpoints include A) epithelial degeneration, B) granulocytomas, C) haemocytic infiltration, and D) parasites. No significant differences between groups for each histological feature (Kruskal Wallis p>0.05, n=20-30).

Mussel gonad histology

A total of six histological features of the gonad are presented, these include adipogranular tissue (ADG), apoptosis, atresia, granulocytomas, haemocytic infiltration and parasites (Figure 40). Differences in ADP tissue in the gonad sections were observed with significantly lower ADP in T0 mussels compared to Station 1 and 2 at 20 m and R1 at 40 m (ANOVA, Tukey, $p<0.05$). Significant differences were also observed in gonadal stage where significantly higher stages were found in T0 and Station 11 mussels compared to R1 and Station 2, and between Station 6 and Station 2 (Kruskal Wallis, $p<0.05$). For all the other gonad histology markers, no significant differences between the groups were found.

Gonadal stages of the mussels from the different groups are presented in Figure 41. Although some earlier developmental stages were present in some stations, the dominant gonadal stage in all stations were 3 and 4, indicating a well-developed gonad ready for a mid-May spawning.

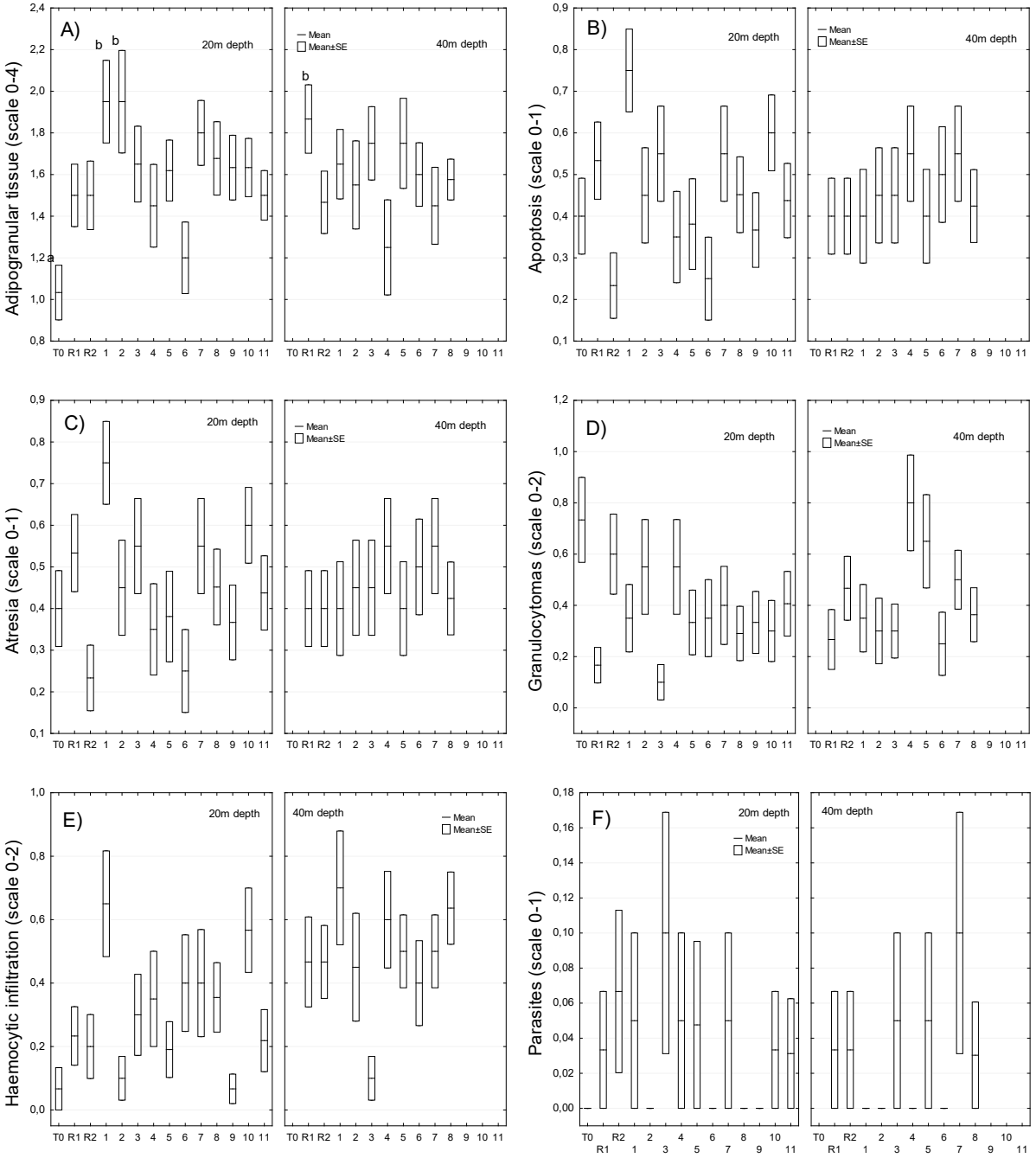


Figure 40. Gonad histology in mussels from the monitoring stations at Ekofisk (1-8) and Eldfisk (9-11) compared to reference (R1, R2) and day zero (T0) groups. Mussels held at 20 and 40 m. Gonad histological endpoints include A) adipogranular tissue, B) apoptosis, C) atresia, D) granulocytomas, E) haemocytic infiltration, F) parasites. Different letters denote significant differences between groups (ANOVA, Tukey for figure A, Kruskal-Wallis for all other figures, p<0.05, n=20-30).

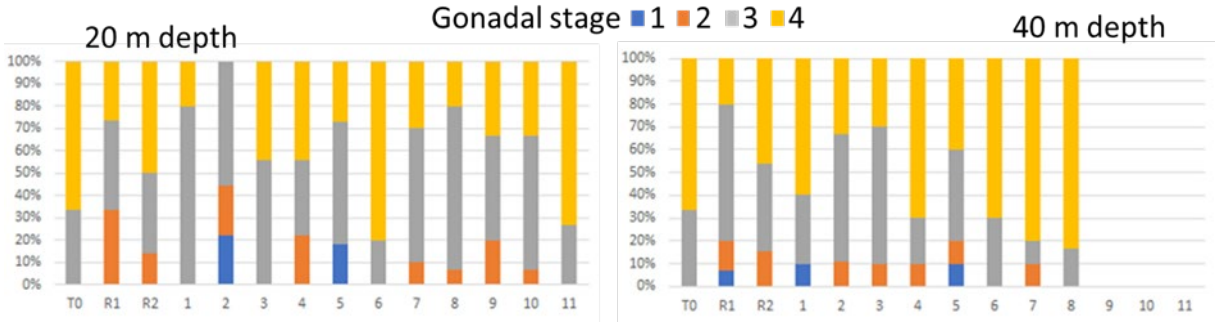


Figure 41. Gonadal stage in mussels from the monitoring stations at Ekofisk (1-8) and Eldfisk (9-11) compared to reference (R1, R2) and day zero (T0) groups.

Gill histology

Histological endpoints were measured in mussel gill tissue sections from the different field exposed and day zero (T0) groups. A total of six histological features of the gill are presented, these include: filament fusion, epithelial lifting, haemocytic infiltration, hyperplasia, hypertrophy, and parasites (Figure 42). There were no significant differences between mussel from the different stations for any of the gill histological endpoints measured (Kruskal Wallis, $p>0.05$). There was also no clear relationship between increased gill histological endpoint and proximity to the Ekofisk or Eldfisk discharge outlet. In order to provide a more sensitive evaluation of the gill lesions, an automated scoring system is under development, where the presence of the lesions will be quantified.

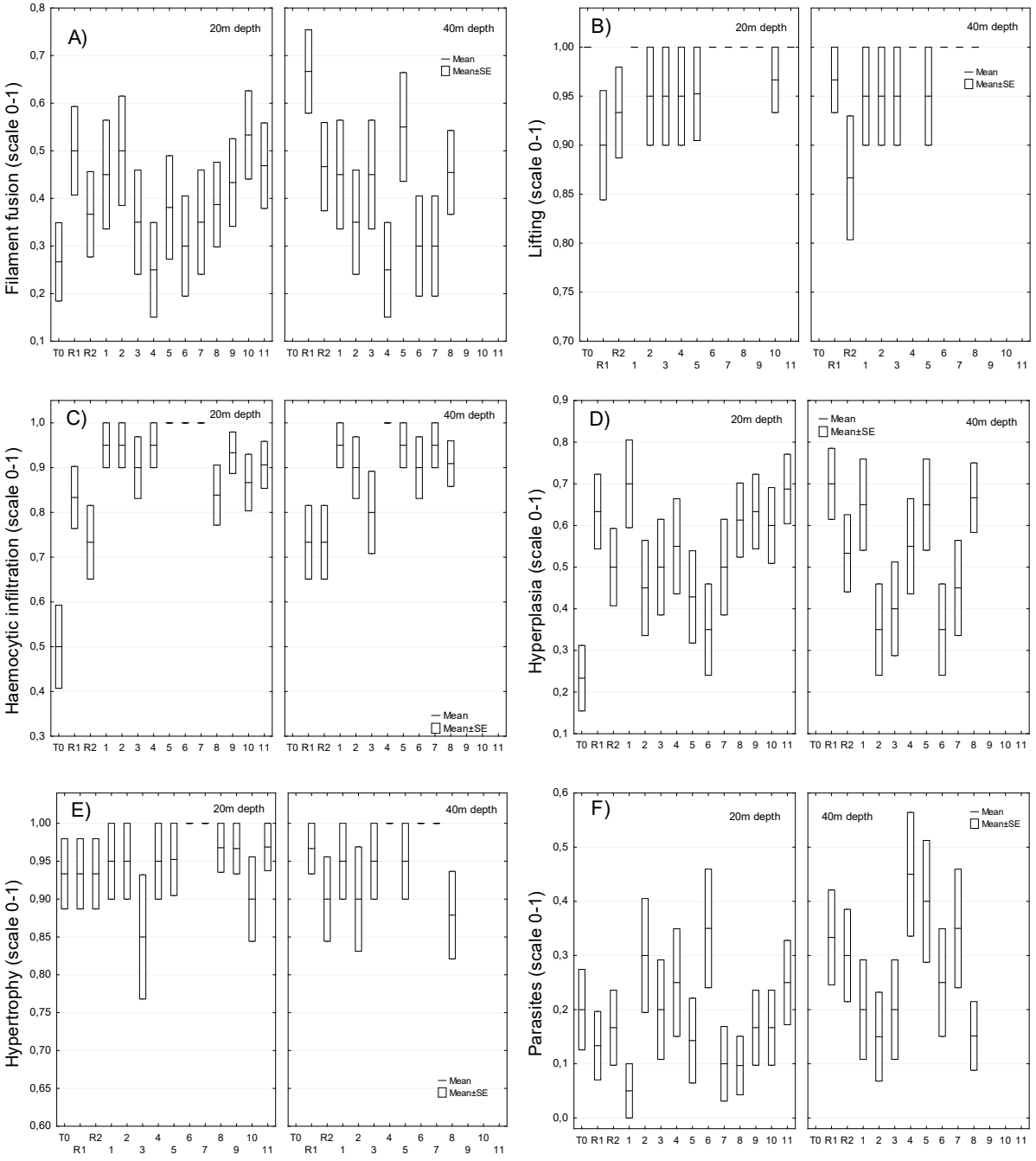


Figure 42. Gill histology in mussels from the monitoring stations at Ekofisk (1-8) and Eldfisk (9-11) compared to reference (R1, R2) and day zero (T0) groups. Mussels held at 20 and 40 m. Gill histological endpoints include A) filament fusion, B) lifting, C) haemocytic infiltration, D) hyperplasia, E) hypertrophy and F) parasites.

3.5 Scallops

3.5.1 Chemical bioaccumulation in scallops

3.5.1.1 PAH-NPD concentrations in scallops

The bioaccumulation of PAH in scallops from the day zero (T0) group, the reference stations (R1 and R2) and two selected Ekofisk stations (1 and 3) are shown as the sum of PAH EPA16, Sum NPD and Sum PAH (Figure 43). Unlike the mussels, scallops were only placed at 40-45 m depth.

The median PAH EPA16 concentrations in scallops for the five groups ranged between 14 and 22 ng/g (w.w.) with no significant difference between the groups. The Sum of all PAH was very similar to the PAH EPA16 in all groups and indicates a small contribution of alkylated PAH (i.e. NPD) towards the total bioaccumulated PAH in the scallop tissue. The NPD concentrations were low in all groups with median concentrations less than 5 ng/g (w.w.). However, the Ekofisk stations 1 and 3 did show a small increase in NPD values compared to the reference groups indicating some exposure to the plume, although this did not result in a statistically significant difference.

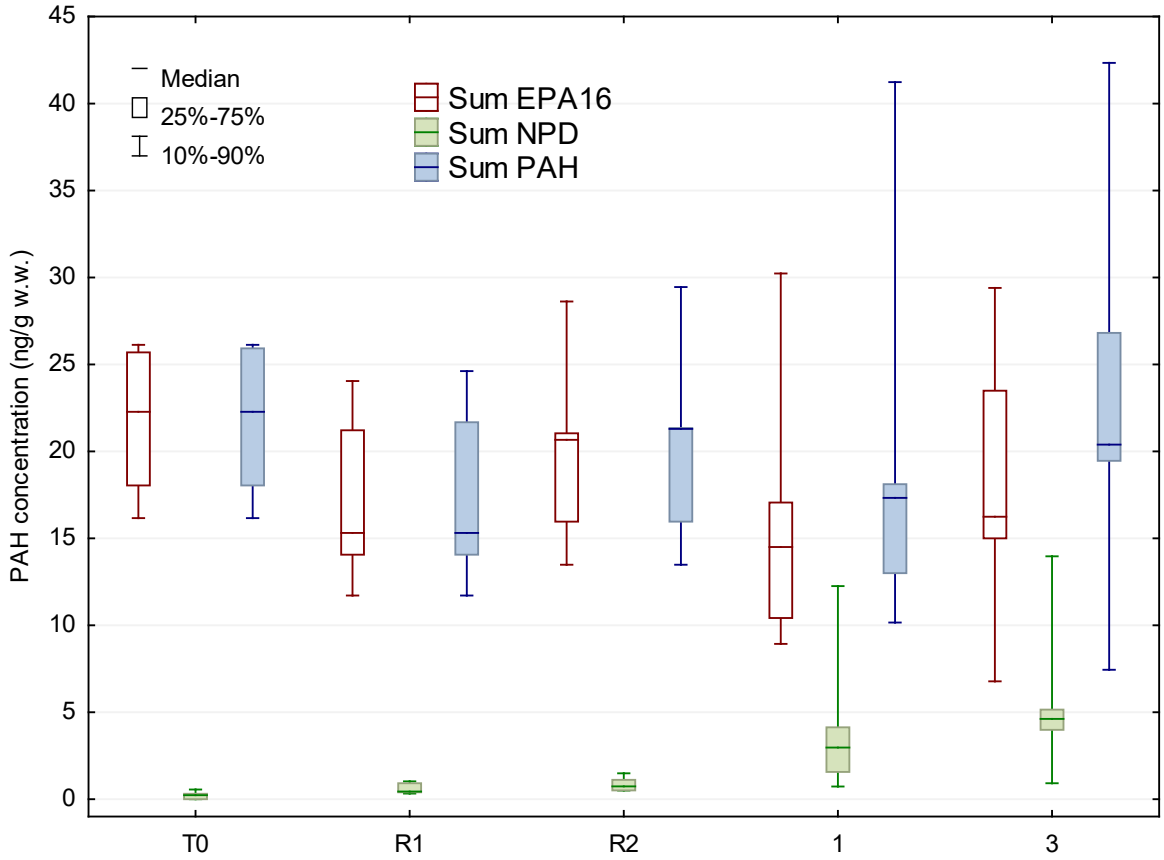


Figure 43. Sum of EPA PAH16, NPD and PAH concentrations in scallops from the two selected monitoring stations at Ekofisk (1, 3) compared to the reference stations (R1, R2) and day zero (T0) group. Scallops held at a depth of approximately 40 m. No statistical differences between groups for Sum PAH EPA16, Sum NPD or Sum PAH (ANOVA, Tukey, p>0.05).

3.5.1.2 Metal concentrations in scallops

Metals from the soft tissue of scallops were analysed from four of the deployed rigs (R1, R2, 1 and 3) in addition to the day zero (T0) group. Metals analysed included Silver (Ag), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), vanadium (V) and zinc (Zn) (Table 7).

Some significant differences in metal concentrations between the groups were found (ANOVA, Tukey $p<0.05$). These include, significantly lower As concentrations in R2 scallops compared to T0, and significantly lower Fe concentrations in Station 3 compared to T0. In addition, Hg concentration in Station 3 scallops were significantly lower than the T0 group. For Mo, R2 and stations 1 and 3 were significantly higher than the T0 group. Overall, there was no increase in the concentration of metals in scallops from the groups closest to the Ekofisk platform (Station 1 and 3) compared to the reference and T0 scallops.

*Table 7. Metal concentrations in soft tissue of scallops from the different stations. Mean ± standard deviation, n = 5 (mg/kg w.w.), * indicate significant difference from T0 (ANOVA, Tukey p<0.05).*

Station	Ag	As	Cd	Co	Cr	Cu	Fe
T0	0.09±0.08	2.90±0.39	5.06±1.03	0.06±0.01	0.18±0.11	1.33±0.84	57.8±10.1
R1	0.06±0.11	2.44±0.36	3.66±2.37	0.07±0.02	0.11±0.04	1.79±1.64	47.4±23.2
R2	0.04±0.02	1.86±0.13*	3.82±1.83	0.06±0.02	0.15±0.05	2.75±1.97	41.0±19.1
1	0.02±0.01	2.58±0.40	3.94±1.46	0.07±0.02	0.13±0.03	2.84±2.90	36.0±30.5
3	0.02±0.01	2.54±0.47	4.06±2.98	0.06±0.01	0.12±0.05	2.27±1.67	18.2±5.9*

Station	Hg	Mn	Mo	Ni	Pb	Se	V	Zn
T0	0.018±0.001	7.78±3.91	0.22±0.06	0.18±0.11	0.15±0.04	0.49±0.07	0.55±0.05	22.6±5.18
R1	0.016±0.002	19.7±10.7	1.66±0.56	0.19±0.17	0.24±0.05	0.52±0.10	0.39±0.08	27.4±8.1
R2	0.014±0.003*	10.3±8.69	5.28±1.18*	0.43±0.33	0.25±0.24	0.54±0.05	0.49±0.15	24.2±5.5
1	0.015±0.002	9.70±8.88	2.08±1.03*	0.42±0.56	0.25±0.24	0.53±0.20	0.43±0.12	23.2±9.3
3	0.017±0.003	8.67±8.22	2.30±1.04*	0.25±0.23	0.16±0.08	0.59±0.15	0.41±0.24	28.6±4.9

3.5.2 Biological effects in scallops

3.5.2.1 Condition Index (CI)

The CI values are presented for two monitoring stations at Ekofisk (1 and 3), the two reference stations and the day zero (T0) group in Figure 44. A significantly lower CI was found in T0 scallops compared to R2 and Station 3. All field exposed scallops, from the reference and Ekofisk stations, had very similar median CI, between 9 and 10.

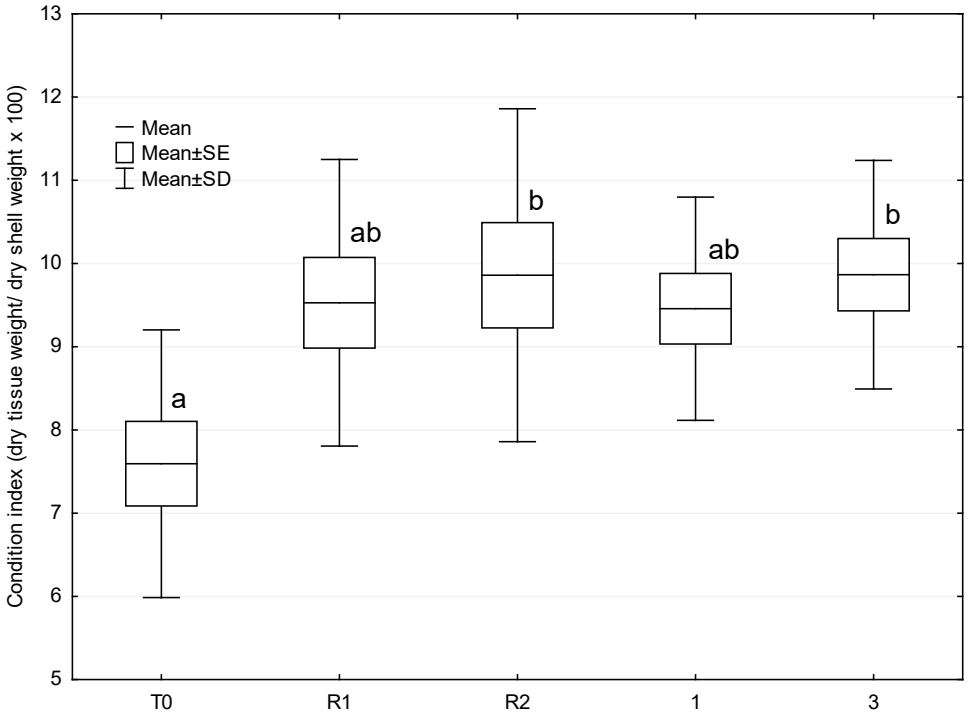


Figure 44. Condition index in scallops from the two selected monitoring stations at Ekofisk (Station 1 and 3) compared to the reference stations (R1, R2) and day zero (T0) group. Different letters denote statistical differences between groups (ANOVA, Tukey, p<0.05).

3.5.2.2 Lysosomal membrane stability (LMS)

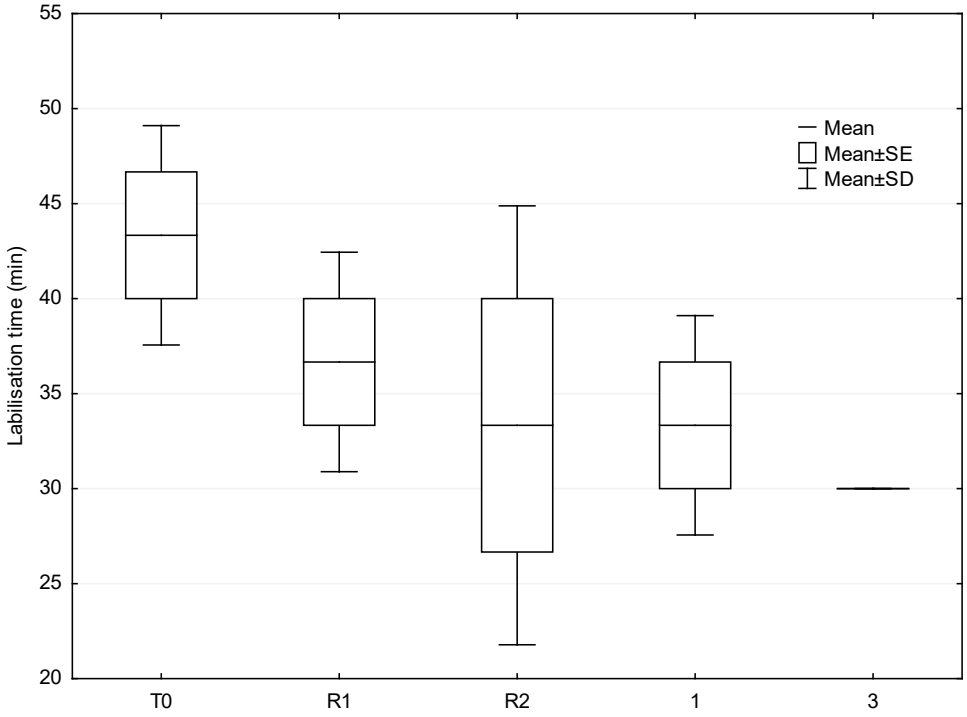


Figure 45. Lysosomal membrane stability in scallops from the two selected monitoring stations at Ekofisk (Station 1 and 3) compared to the reference stations (R1, R2) and day zero (T0) group. No significant difference between groups. (Kruskal Wallis, p>0.05, n=10).

The LMS results are presented for scallops collected from two monitoring stations at Ekofisk (1 and 3) as well as the two reference stations and the day zero (T0) group in Figure 45. Mean labilisation times ranged from 30 min at Station 3 to 43 min for the T0 group. There was no significant difference between labilisation times for the five groups of scallops (Kruskal Wallis, p>0.05).

3.5.2.3 Micronuclei formation

The frequency of MN in the agranular cells of scallop haemocytes are presented for the two monitoring stations at Ekofisk (1 and 3) as well as the two reference stations and the day zero (T0) group (Figure 46). Highest MN frequencies of 7.5 per 1000 cells were found in scallops from one of the reference stations (R2). This was significantly higher than the MN in scallops from the closest Ekofisk station (Station 1, 2 MN/ 1000 cells, ANOVA, Tukey, p<0.05).

ICES assessment criteria for MN are not available for scallops. However, in a related species, the islandic clam (*Chlamys islandica*) a BAC of 4.5 MN per 1000 cells has been proposed (Davies and Vethaak 2012). Based on this value, R2 and Station 3 are the only two scallop groups to be above this benchmark value. Overall. there was no clear relationship between increased MN frequency in scallops and proximity to the Ekofisk discharge outlet.

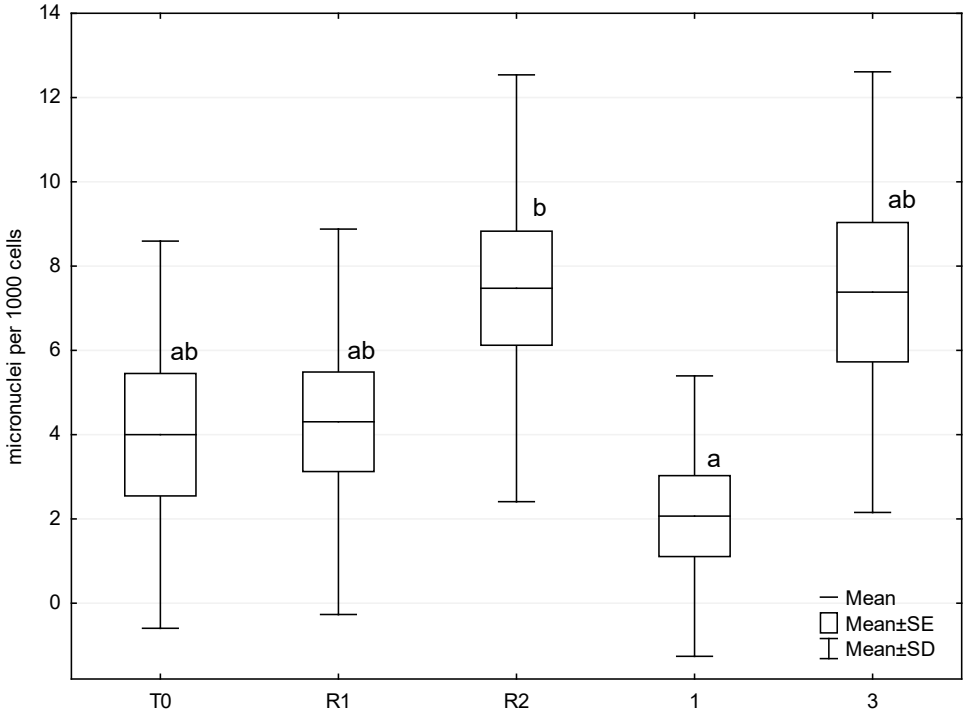


Figure 46. Micronuclei formation in scallops from the two selected monitoring stations at Ekofisk (Station 1, Station 3) compared to the reference stations (R1, R2) and day zero (T0) group. Scallops held at a depth of approximately 40 m. Different letters denote statistical differences between groups (ANOVA, Tukey, p<0.05, n=10-15).

3.5.2.4 Histology

Histological evaluations were carried out in scallops for the first time in the WCM programme. Due to the absence of previous reference material from the WCM surveys, the lack of data regarding the use of these parameters as biomarkers in this species, these results are considered preliminary and not ready for being introduced as biomarkers for the WCM2021.

3.6 Fish

3.6.1 Chemical concentrations in fish

3.6.1.1 PAH-NPD in fish liver

Sum PAH concentrations from the liver of fish collected from the Ekofisk safety zone and the three regions of the North Sea are presented in Figure 47. Median concentrations were highest in dab from the Ekofisk safety zone at 70 ng/g (w.w.), which was significantly higher than dab from Egersundbank (ANOVA, Tukey p<0.05). Median Sum PAH concentrations were also elevated in cod from the Ekofisk safety zone compared to cod from the Egersundbank, as well as whiting from Vikingbank, although no significantly differences were found. Haddock and whiting showed no significant differences between Sum PAH concentrations.

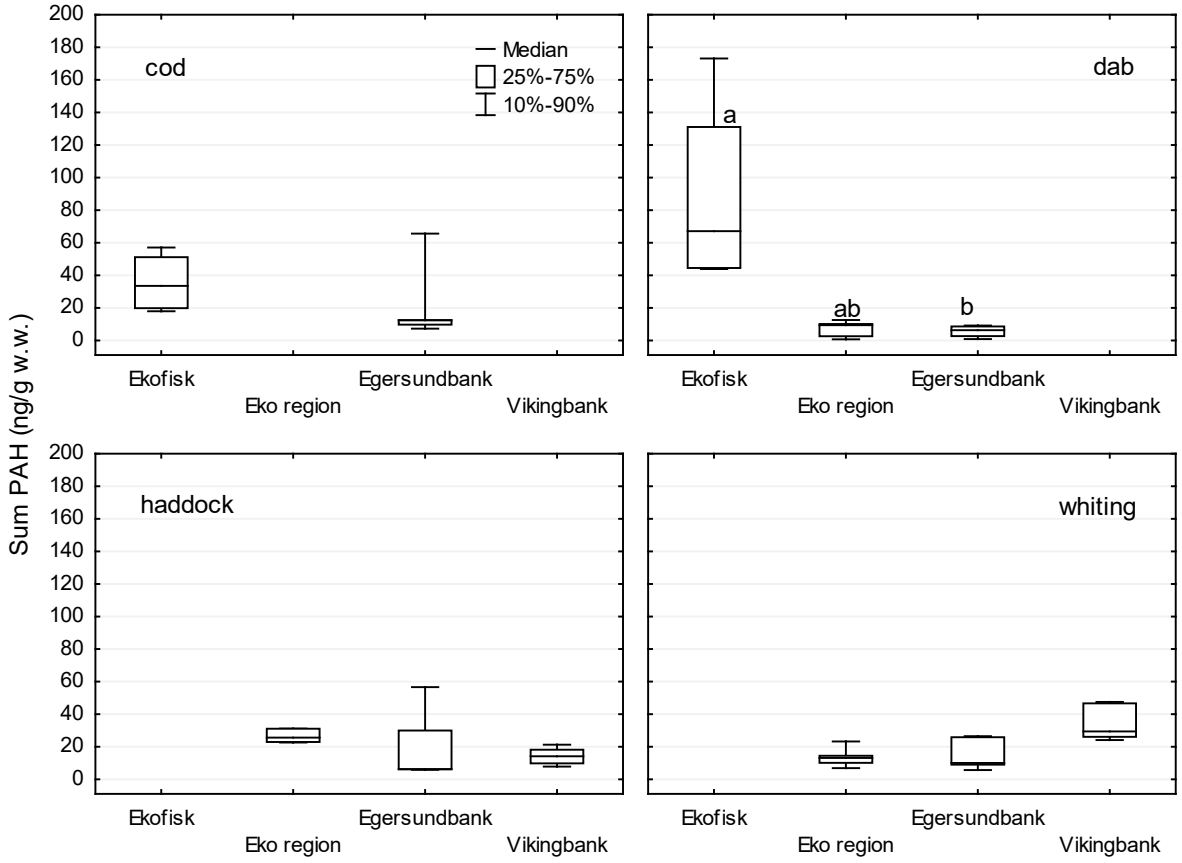


Figure 47. Sum PAH (49 compounds) in fish livers from the four fish species at the different locations (median, quartiles (box), 10/90 percentiles). Different letters denote significant differences between locations for each fish species (ANOVA, Tukey, p<0.05, n=5).

Sum NPD concentrations in the liver of fish collected from the Ekofisk safety zone and the three regions of the North Sea are presented (Figure 48). The sum NPD concentrations in fish liver showed a similar pattern to the Sum PAH, indicating that the alkylated PAH were mostly responsible for the increased Sum PAH described above. Highest median concentrations of NPD, constituting the alkylated PAH, were found in dab from the Ekofisk safety zone (60 ng/g w.w.). These values were significantly higher than the Sum NPD in dab from the Egersundbank region (ANOVA, Tukey, p<0.05). Median NPD

concentrations were also elevated in cod from the Ekofisk safety zone compared to cod from the Egersundbank, and in whiting from the Vikingbank region.

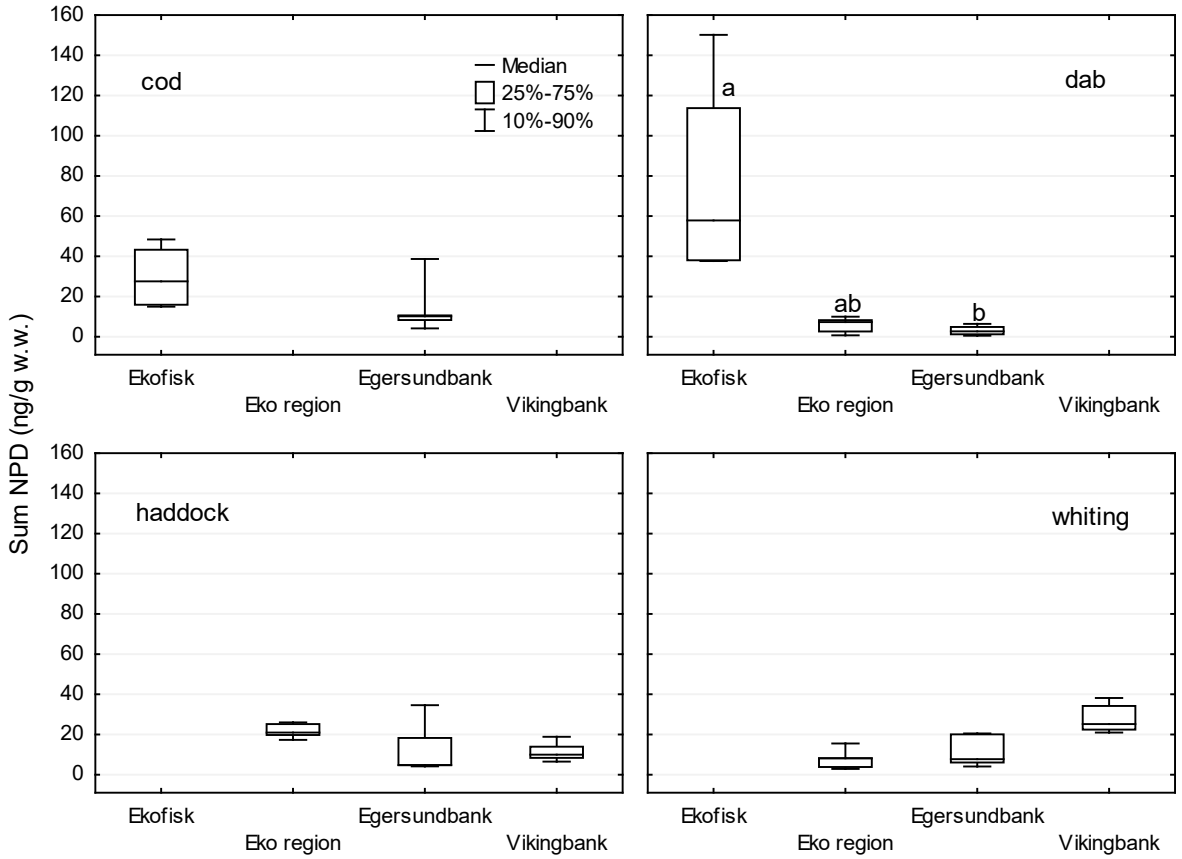


Figure 48. Sum of NPD concentrations in fish livers from the four fish species at the different locations (median, quartiles (box), 10/90 percentiles). Different letters denote significant differences between groups (ANOVA, Tukey, p<0.05, n=5).

3.6.1.2 PAH metabolites in fish bile

PAH metabolites of OH-naphthalene, OH-phenanthrene and OH-pyrene were measured in the bile of the four species from the different locations (Figure 49 and Figure 50). Only PAH metabolites above the LOQ are presented. For OH-naphthalene, all fish were found to have measurable concentrations of at least one metabolite in their bile, indicating exposure to the parent compound. Higher and variable concentrations of 2-OH-naphthalene, C1-OH-naphthalene and C3-OH-naphthalene were measured in dab from the Ekofisk region, whilst OH-phenanthrene and OH-pyrene were not detected in whiting from any region.

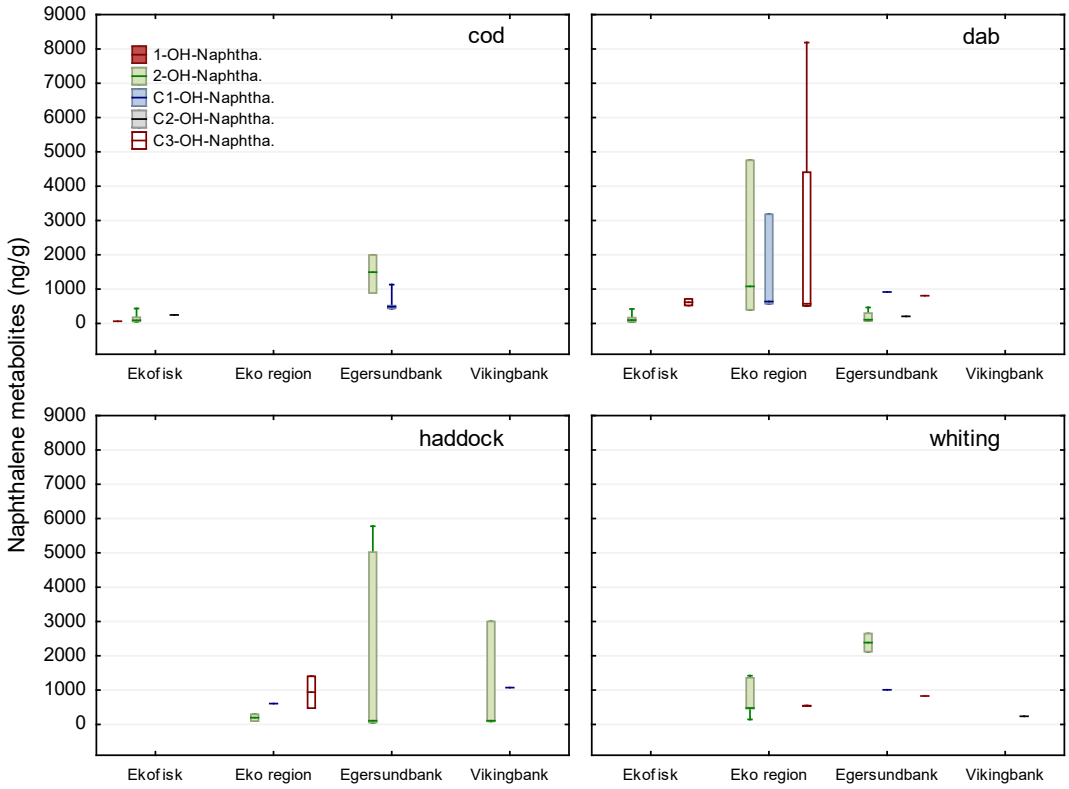


Figure 49. Metabolites of naphthalene in fish bile from the four fish species at the different locations (median, quartiles (box), 10/90 percentiles).

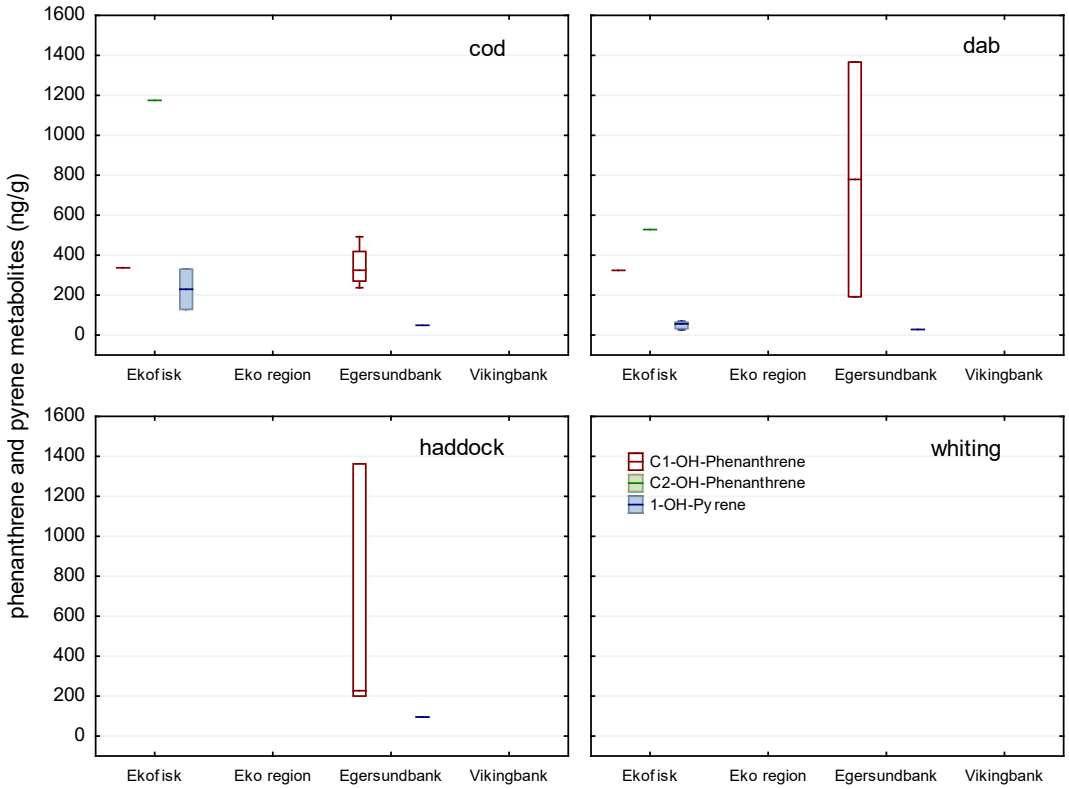


Figure 50. Metabolites of phenanthrene and pyrene in fish bile from the four fish species at the different locations (median, quartiles (box), 10/90 percentiles).

3.6.1.3 PFAS in fish blood

A range of 36 PFAS compounds were measured in the blood of the four fish species collected from the Ekofisk safety zone and three regions of the North Sea (Ekofisk, Egersundbank and Vikingbank). Of the 36 PFAS compounds measured, only 14 were found above analytical detection limits, with 2 of the 14 PFAS chemicals only found in one fish. The twelve PFAS compounds detected in more than one sample included, PFOA, PFNA, PFDA, PFUdA, PFDoDA, PFTrDA, PFBS, PFOS, br-PFOS, PFOSA, PFBSA and PFHxSA. Six of the most common PFAS compounds detected in fish blood are shown (Figure 51).

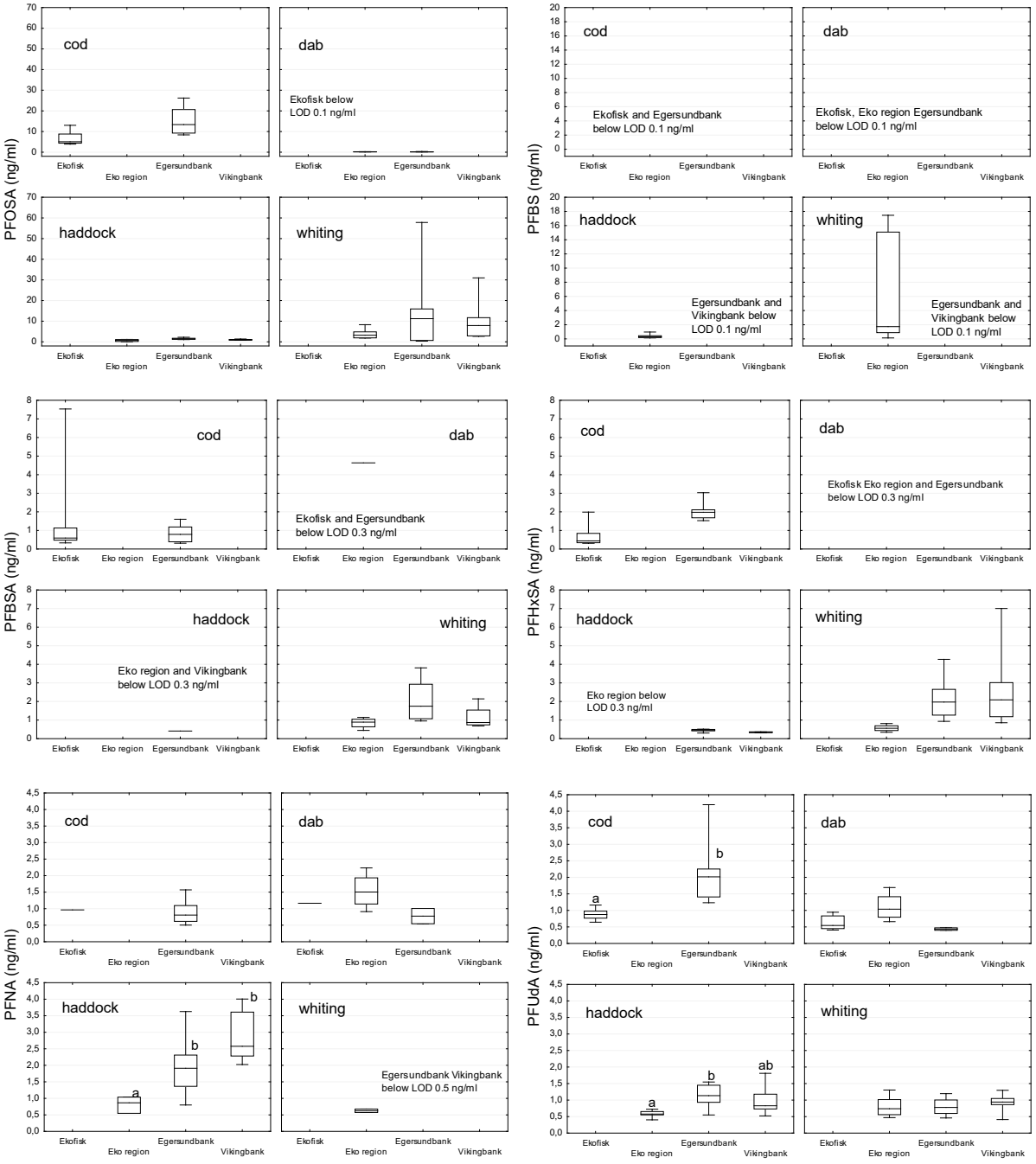


Figure 51. The six most dominant PFAS compounds of the 12 that were measured above detection limits in fish blood collected from the fish species from the different locations. Compounds include PFOSA, PFBS, PFBSA, PFHxSA, PFNA and PFUdA. Median, quartiles, 10/90 percentiles (n =10, except at Vikingbank where n=7). Different letters denote significant differences (ANOVA, Tukey p<0.05).

Overall, PFOSA was the most dominant PFAS compound detected with highest concentrations measured in cod and whiting. In contrast, PFOSA was undetected in most of the dab and haddock samples from all three locations. For cod, PFOSA made up most of the sum PFAS concentration, with similar concentrations in those caught at Ekofisk to those from Egersundbank. For PFOSA there were no significant differences between the locations for each fish species.

Interestingly, PFBS were found in relatively high but also variable concentrations in whiting from the Ekofisk region but were absent in whiting from Egersundbank and Vikingbank. PFBS were also below the LOD (0.1 ng/ml) in all cod and dab locations as well as haddock from Egersundbank and Vikingbank. PFBS was detected marginally above LOD in haddock from the Ekofisk region.

As described for PFOSA, PFBSA was highest in cod and whiting populations, whilst dab and haddock were mostly below LOD (0.3 ng/ml). However, no significant difference in PFBSA were found between the locations for the different fish species. This was also seen for PFHxSA with higher concentrations in cod and whiting and almost absent in dab and haddock.

In contrast to PFOSA, PFBSA and PFHxSA, that were more prevalent in cod and whiting, PFNA concentrations were higher in dab and particularly haddock. Significantly higher PFNA concentrations were measured in haddock from both the Vikingbank and Egersundbank compared to the Ekofisk region.

PFUdA was measured above LOD in all fish and from all locations, with median concentrations ranging between 2 and 0.4 ng/ml. In cod, significantly higher PFUdA concentrations were found in Egersundbank than from within the Ekofisk safety zone. In haddock, significantly higher concentrations of PFUdA were found in Egersundbank fish compared to the Ekofisk region, although both regions were similar to Vikingbank fish.

Overall, the Ekofisk complex did not appear to cause any significant increase in PFAS concentration in fish blood compared to PFAS measured in fish blood from other regions of the North Sea.

3.6.1.4 ^{226}Radium concentration in fish bone and fillet

The concentrations of ^{226}Ra in pooled samples of dab bone and fillet, collected from within the Ekofisk safety zone, the wider Ekofisk region and the Egersundbank are presented in Table 8. ^{226}Ra was only measured above detection limits in two of the ten pooled samples, in dab fillet from the Ekofisk safety zone and in dab bone from the Ekofisk region. These measured concentrations were only marginally above detection limits and represent a low accumulation.

Table 8. The concentration of ^{226}Ra in the bone and fillet of dab collected from within the Ekofisk safety zone and two regions of the North Sea (mBq/kg).

Location	Fish bone	Fish fillet
Ekofisk	≤ 240	**50 ± 40**
	≤ 130	≤ 40
Eko region	≤ 100	≤ 60
	260 ± 220	≤ 60
Egersundbank	≤ 230	≤ 60

3.6.2 Biological effect in fish

3.6.2.1 General fish health status

General supporting parameters were measured in the fish collected from the Ekofisk safety zone and the three regions of the North Sea. These include length, weight, CI, LSI, GSI and age.

The fork length of cod from the Egersundbank region (median 35 cm) were significantly longer than cod collected from the Ekofisk safety zone (median 26 cm, Kruskal Wallis $p<0.05$, Figure 52). Dab from the Ekofisk safety zone (median 26 cm) were significantly longer than dab from Egersundbank (median 22 cm, Kruskal Wallis $p<0.05$). Furthermore, whiting from the Ekofisk region (median 23 cm) where significantly smaller than those from Egersundbank (median 31 cm, Kruskal Wallis $p<0.05$).

Perhaps not too surprising was that the same pattern in the significant differences in fork length between locations for the different fish species was also reflective for fish weight (Figure 53). Significantly heavier cod were sampled from Egersundbank than the Ekofisk safety zone, significantly heavier dab were sampled from the Ekofisk safety zone compared to the Ekofisk regions, whilst significantly lighter whiting were sampled from the Ekofisk region compared to Egersundbank (Kruskal Wallis, $p<0.05$).

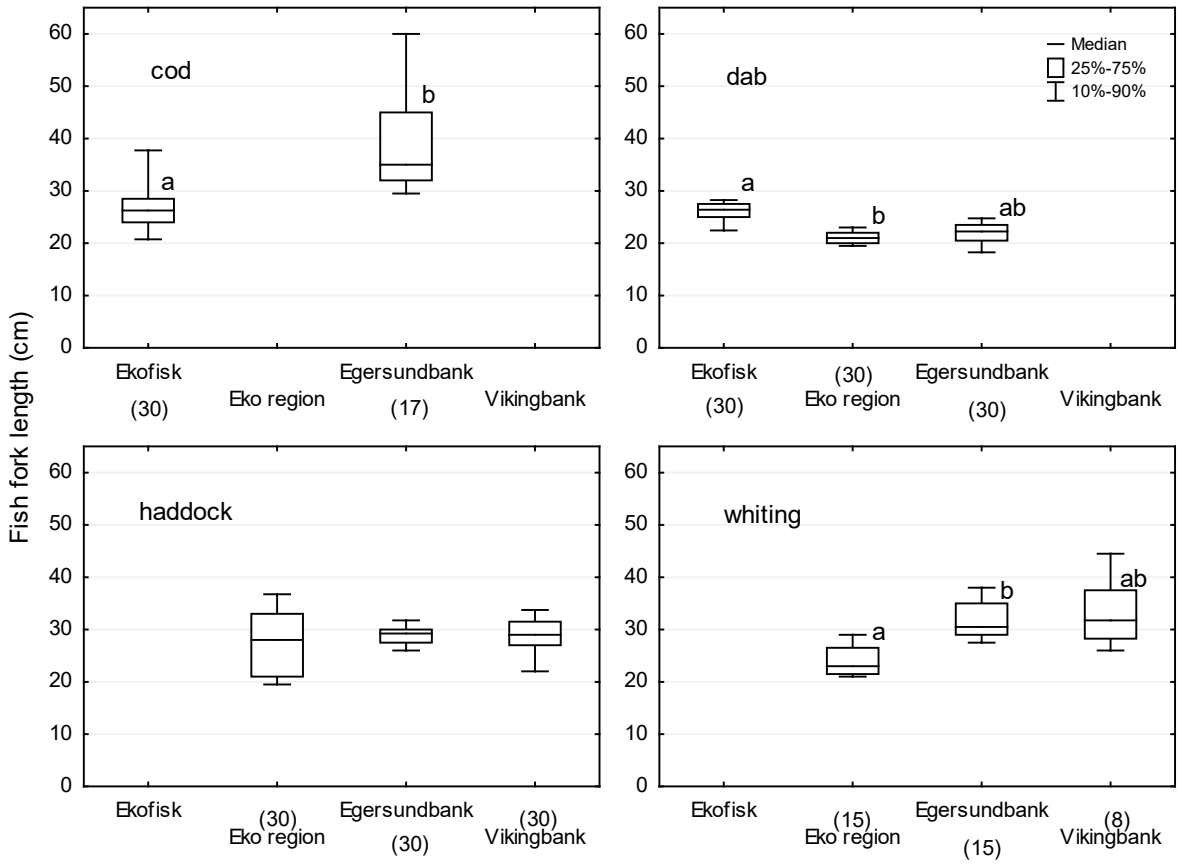


Figure 52. Fork length for all fish species caught from with the Ekofisk safety zone and three regions of the North Sea. Numbers in parenthesis denote replicates. Different letters denote significant differences between groups (Kruskal Wallis, p<0.05).

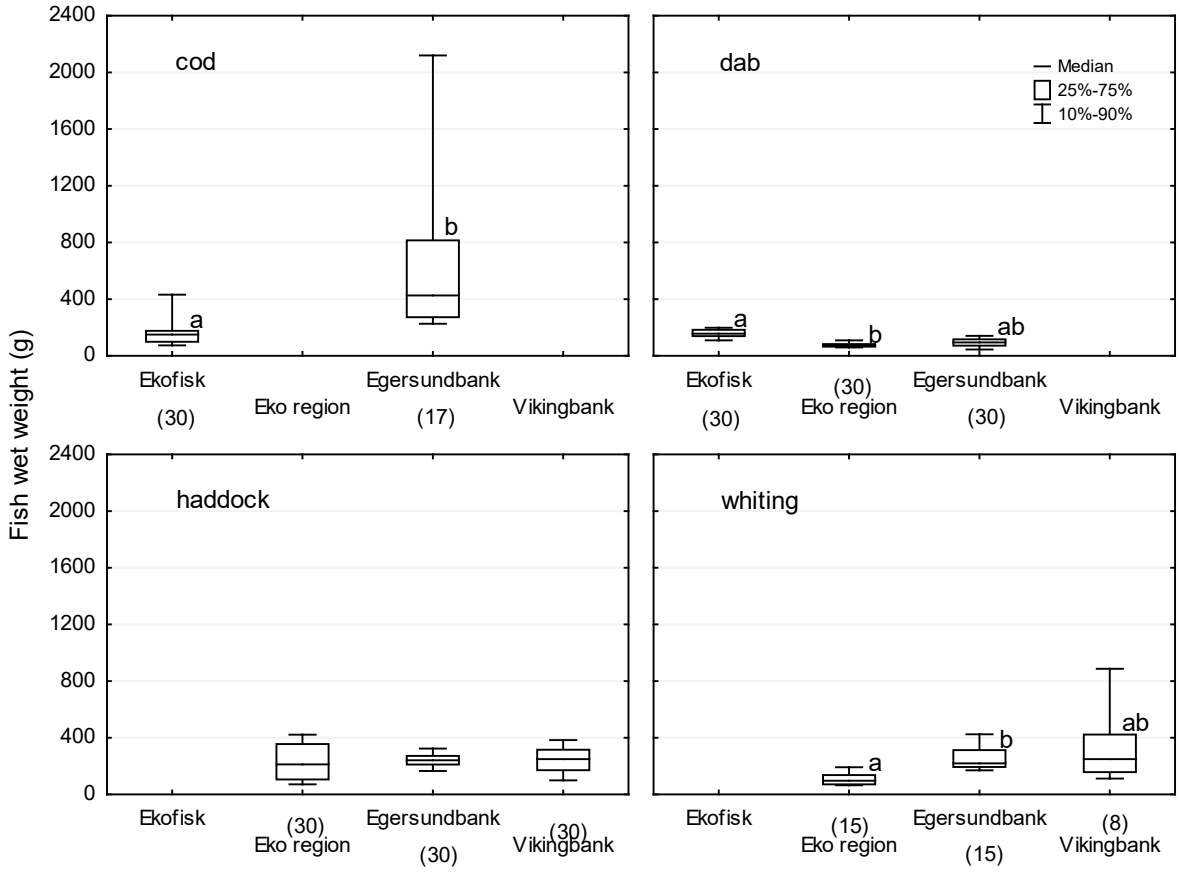


Figure 53. Whole fish wet weight for all species caught from with the Ekofisk safety zone and three regions of the North Sea. Numbers in parenthesis denote replicates. Different letters denote significant differences between groups (Kruskal Wallis, p<0.05).

The CI measured as the wet weight divided the cube of the length, multiplied by 100 are presented for the four fish from the different locations (Figure 54). Despite differences in length and weight of the fish collected, the CI showed no significant differences between the locations for the separate fish species.

The LSI, which can provide a measure of the nutritional status of the fish showed no significant differences between cod from Ekofisk and Egersundbank or in whiting from the three regions (Figure 55). Significant differences in LSI were found for dab and haddock, with a significantly lower LSI in dab from the Ekofisk safety zone compared to the Egersundbank region, and a significantly higher LSI in haddock from the Ekofisk region compared to Egersundbank and Vikingbank regions (Kruskal Wallis, $p<0.05$).

The GSI, which provides a measure of the reproductive stage of the fish, was divided into male and female and compared for each species between locations (Figure 56). The GSI scores showed clear gender differences for dab and whiting, whilst GSI or male and female cod and also male and female haddock were similar. There were no significant differences in GSI for male or female GSI between locations.

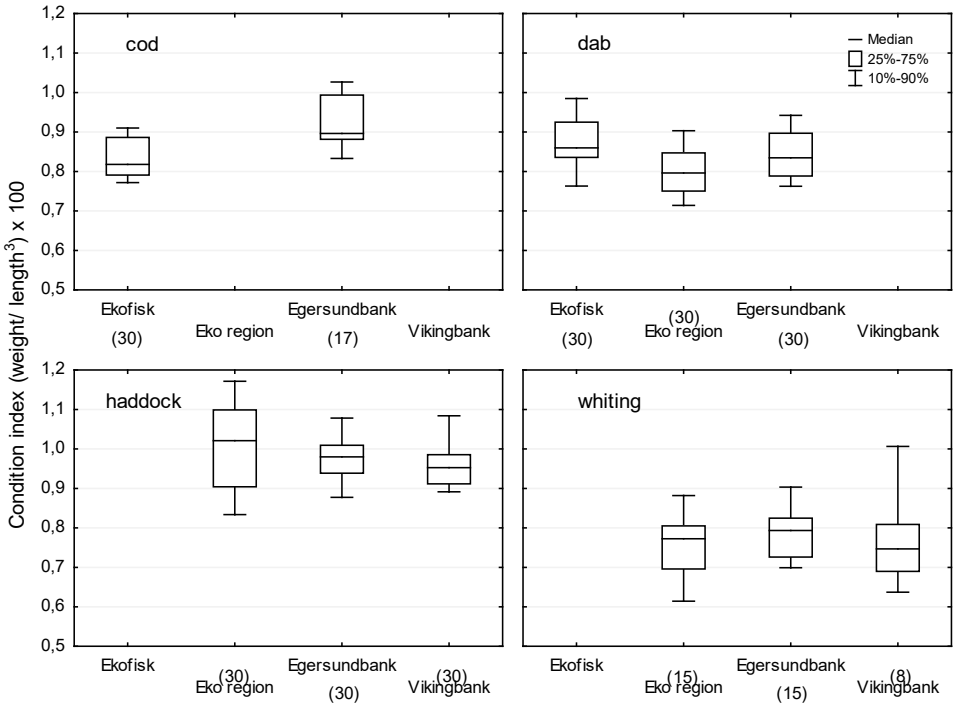


Figure 54. Condition index calculated for all species caught from with the Ekofisk safety zone and three regions of the North Sea. Numbers in parenthesis denote replicates. No significant differences between groups (Kruskal Wallis, p>0.05).

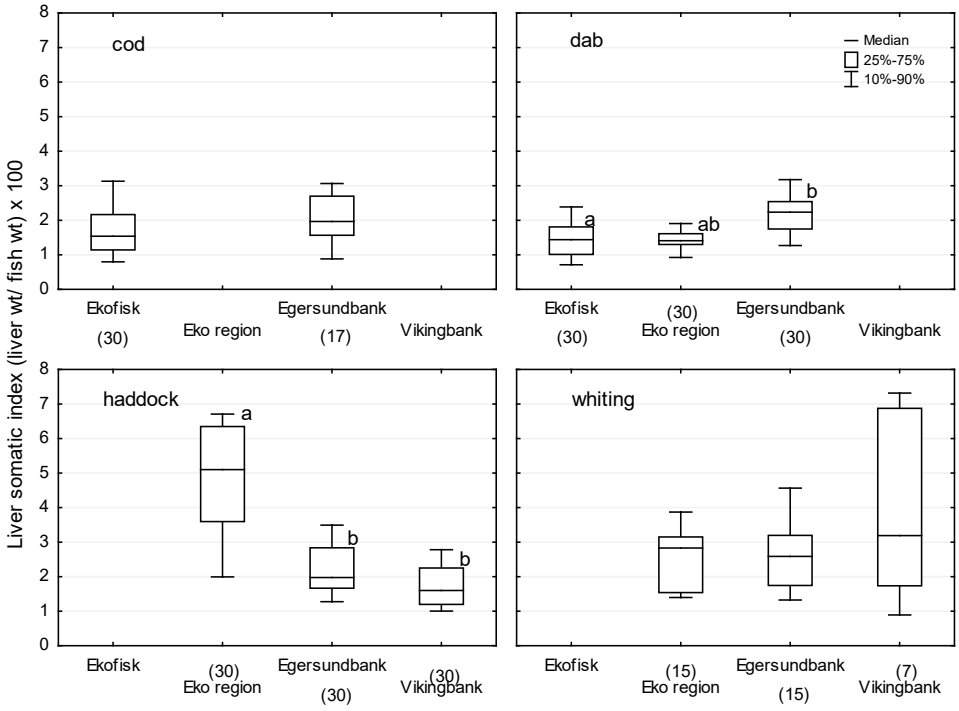


Figure 55. Liver somatic index calculated for all species caught from with the Ekofisk safety zone and three regions of the North Sea. Numbers in parenthesis denote replicates. Different letters denote significant differences between groups (Kruskal Wallis, p<0.05).

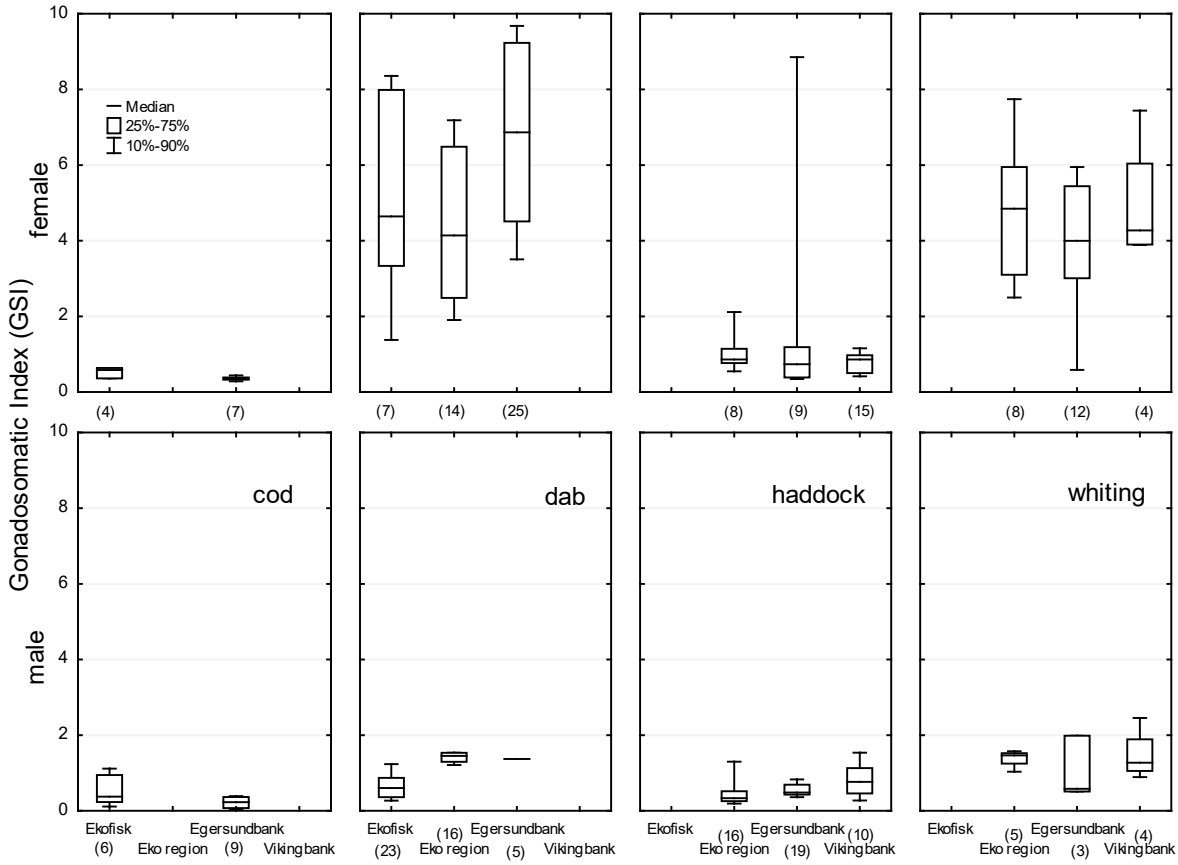


Figure 56. Gonadosomatic index calculated for all species caught from with the Ekofisk safety zone and three regions of the North Sea, separated between sexes. Numbers in parenthesis denote replicates. No significant differences between regions for each fish species and gender (Kruskal Wallis, p>0.05).

The fish age estimated from otolith rings for the four fish species from the different locations is shown (Figure 57). Significantly older cod were collected from Egersundbank (median 2 yrs.) compared to Ekofisk safety zone (median 1 yr.) (ANOVA, Tukey, $p<0.05$). Significantly older dab were found from the Ekofisk safety zone (median 6 yrs.) compared to both Ekofisk and Egersundbank (media 5 yrs.) regions (ANOVA, Tukey, $p<0.05$). Furthermore, significantly younger whiting were collected from the Ekofisk region (median 2 years) compared to both Egersundbank (median 3 yrs.) and Vikingbank (median 4 yrs.).

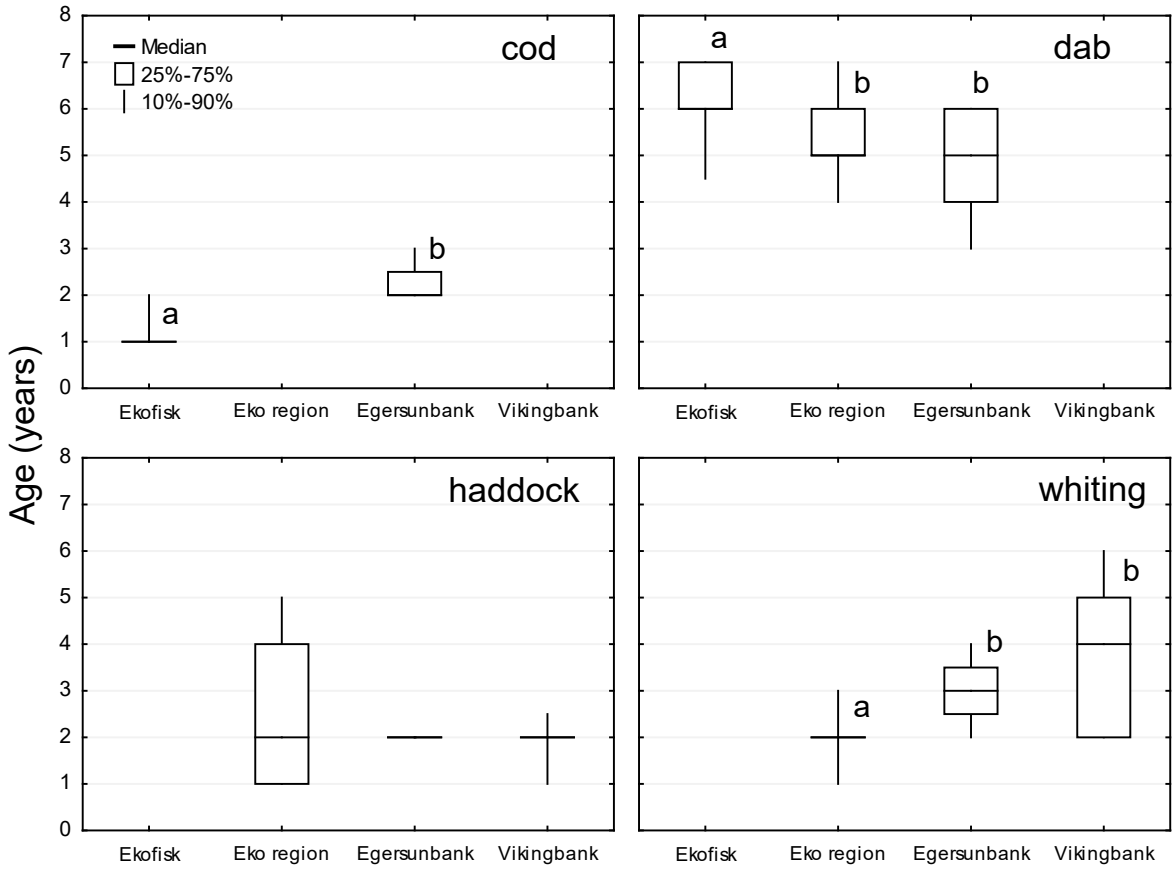


Figure 57. Age determined from otolith rings for all fish species caught from within the Ekofisk safety zone and three regions of the North Sea. Different letters denote significant differences between locations (ANOVA, Tukey, p<0.05 for cod, dab; Kruskal-Wallis, p<0.05 haddock, whiting).

3.6.2.2 Ethoxyresorufin-O-deethylase (EROD) activity

EROD activity was measured in the liver microsomal fraction of fish collected from the Ekofisk safety zone and the three regions of the North Sea (Figure 58). EROD activity was significantly elevated in dab collected from Ekofisk (median 113 pmol/min/mg protein) and the Ekofisk region (median 188 pmol/min/mg protein) compared to Egersundbank (median 26 pmol/min/mg protein) (ANOVA, Tukey, $p<0.05$). In addition, EROD activity in haddock from the Ekofisk region (median 28 pmol/min/mg protein) was significantly lower than haddock from Egersundbank (median 65 pmol/min/mg protein) and Vikingbank (median 71 pmol/min/mg protein) (ANOVA, Tukey $p<0.05$).

ICES assessment criteria are available for cod with a proposed BAC of 145 nmol/min/mg protein (Davies and Vethaak, 2012). EROD activity in cod was well below this threshold value at both Ekofisk (24 pmol/min/mg protein) and Egersundbank (34 pmol/min/mg protein). ICES assessment criteria are also available for dab with a BAC of 680 pmol/min/mg protein for the microsomal fraction (Davies and Vethaak, 2012). Based on this value, all EROD activity in dab from the three locations was well below the proposed ICES BAC value.

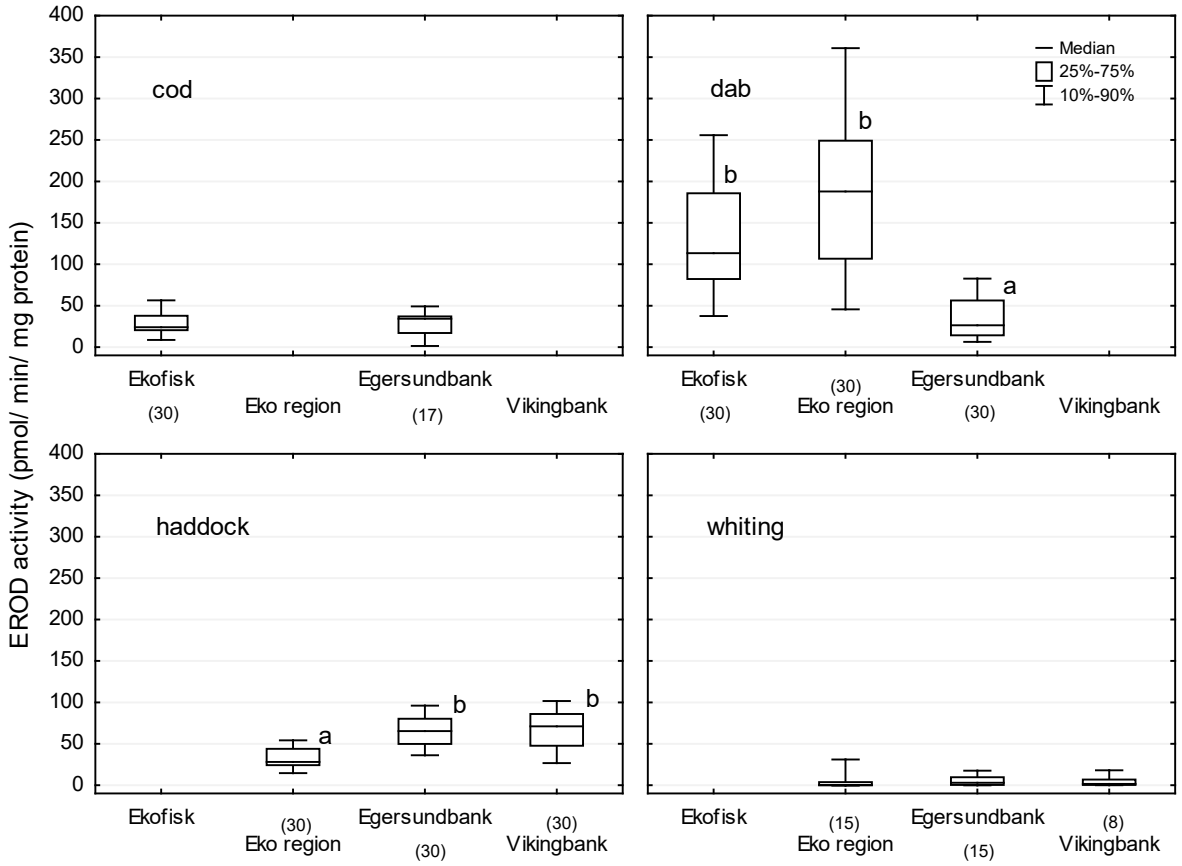


Figure 58. Ethoxyresorufin-O-deethylase (EROD) activity for all fish species caught from within the Ekofisk safety zone and three regions of the North Sea. Numbers in parenthesis denote replicates. Different letters denote significant difference between locations (ANOVA, Tukey, p<0.05).

Differences in EROD activity are known to occur between male and female dab with higher activities measured in males than females (Stagg et al., 1995). The EROD activity for male and female dab from three regions of the North Sea are presented (Figure 59). As anticipated, differences were observed between male and female dab with higher EROD activity in males than females from all locations. However, this was only significantly higher in male dab from Ekofisk and Ekofisk region (ANOVA, Tukey, $p<0.05$) and not Egersundbank. When separating the dab EROD data based on sex, EROD activity from the Ekofisk region was significantly higher than both Egersundbank and Ekofisk in both males and females (ANOVA, Tukey $p<0.05$). Whilst Ekofisk was not significantly elevated above Egersundbank dab (males or females). Separate ICES assessment criteria are available for male and female dab with a BAC of 147 and 178 pmol/min/mg protein respectively. The median EROD activity for male dab at the Ekofisk region (219 pmol/ min/ mg protein) was the only group above the respective ICES BAC value. There were no significant differences in EROD activity in relation to sex for any of the other fish species in this study.

Despite the sex difference in dab, similar profiles of EROD activity were observed, with lowest values at Egersundbank higher at Ekofisk and highest at Ekofisk region in both male and female fish. Due to this, as well as including higher numbers of replicates and avoiding the difficulty of selecting only males or only female dab, the EROD data from both male and female dab were combined when calculating the IBR/n and PCA assessment.

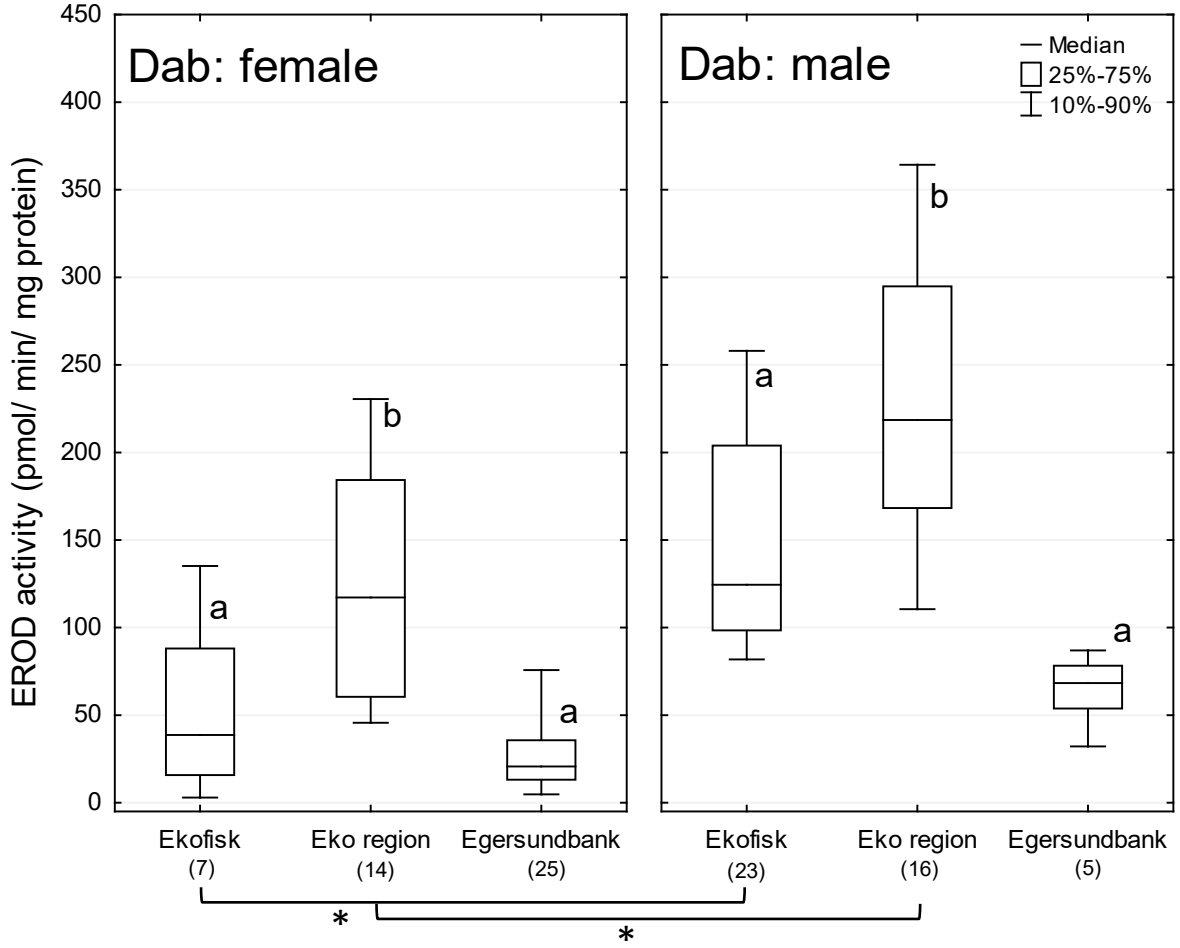


Figure 59. *Ethoxyresorufin-O-deethylase (EROD) activity for male and female dab (L. limanda) caught from within the Ekofisk safety zone and two regions of the North Sea. Numbers in parenthesis denote replicates. Different letters denote significant difference between locations for the same sex, * denotes significant differences between sexes from the same location (ANOVA, Tukey, p<0.05).*

3.6.2.3 CYP1A

ELISA absorbance with fish anti-CYP1A antibodies were performed with the liver microsomal fraction prepared for EROD activity measurements. Three anti-fish CYP1A antibodies were used: NP-7 is a monoclonal antibody and only used with cod, while C10-7 and CP-226 are mono- and polyclonal antibodies and used for the other species. CP-226 was also used with cod for comparison (Figure 60).

For cod, the monoclonal anti-CYP1A antibody (NP-7) demonstrated low levels of absorbance, with cod livers from the Egersundbank showing low, but significantly higher levels than cod liver from the Ekofisk safety zone. When the polyclonal anti-CYP1A antibody (CP-226) was used, higher CYP1A levels were observed but there was no significant difference in CYP1A levels between the two groups (not shown).

For dab, similar response patterns were seen with both anti-fish CYP1A antibodies (C10-7 and CP226). Highest levels were seen in dab liver from Ekofisk region, lower in dab caught within the security zone of Ekofisk, although levels at both stations were significantly different from each other and had significant higher levels compared with dab from the Egersundbank (ANOVA, Tukey, p<0.05).

For haddock, similar response patterns were seen with both antibodies. Highest levels were seen in haddock liver from the Ekofisk region, although the levels were not statistical different from the Egersundbank or Vikingbank.

In whiting, the two anti-CYP1A antibodies (C10-7 and CP-226) displayed similar response patterns although the levels for CP-226 were approximately 4-fold lower (only C10-7 shown). Highest levels were observed at the Ekofisk region, although the difference between the areas were not significantly different for either antibody (Figure 60).

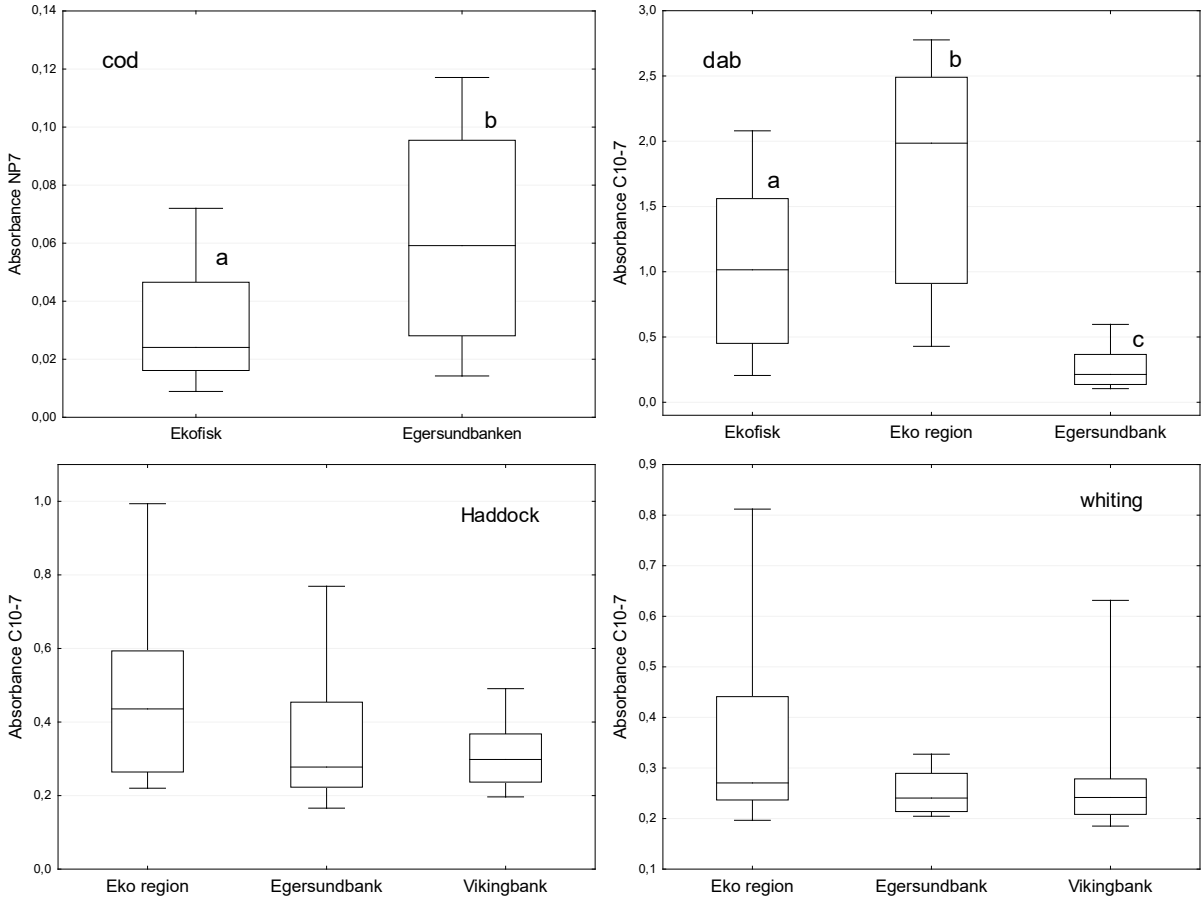


Figure 60. ELISA absorbance (median, quartiles, 10/90 percentiles) with NP-7 monoclonal antibody in cod, and C10-7 monoclonal antibody in dab, haddock and whiting. Different letters denote significant differences between groups (ANOVA, Tukey, p<0.05).

Differences in CYP1A protein concentrations were considered with respect to sex for all species. However, only dab were found to have significant differences in CYP1A protein concentrations between sexes (Figure 61). CYP1A protein concentrations showed relatively higher concentrations in males than females in dab from Ekofisk and Ekofisk region, although no sex differences were observed in the dab from Egersundbank, which was similar to that observed for EROD activity.

As described for EROD, similar profiles of CYP1A protein were observed, with lowest values at Egersundbank followed by Ekofisk and then Ekofisk region in both male and female fish. Due to this, as well as including higher numbers of replicates and avoiding the difficulty of selecting only males or only female dab, the CYP1A protein data from both male and female dab were combined when calculating the IBR/n and PCA assessment.

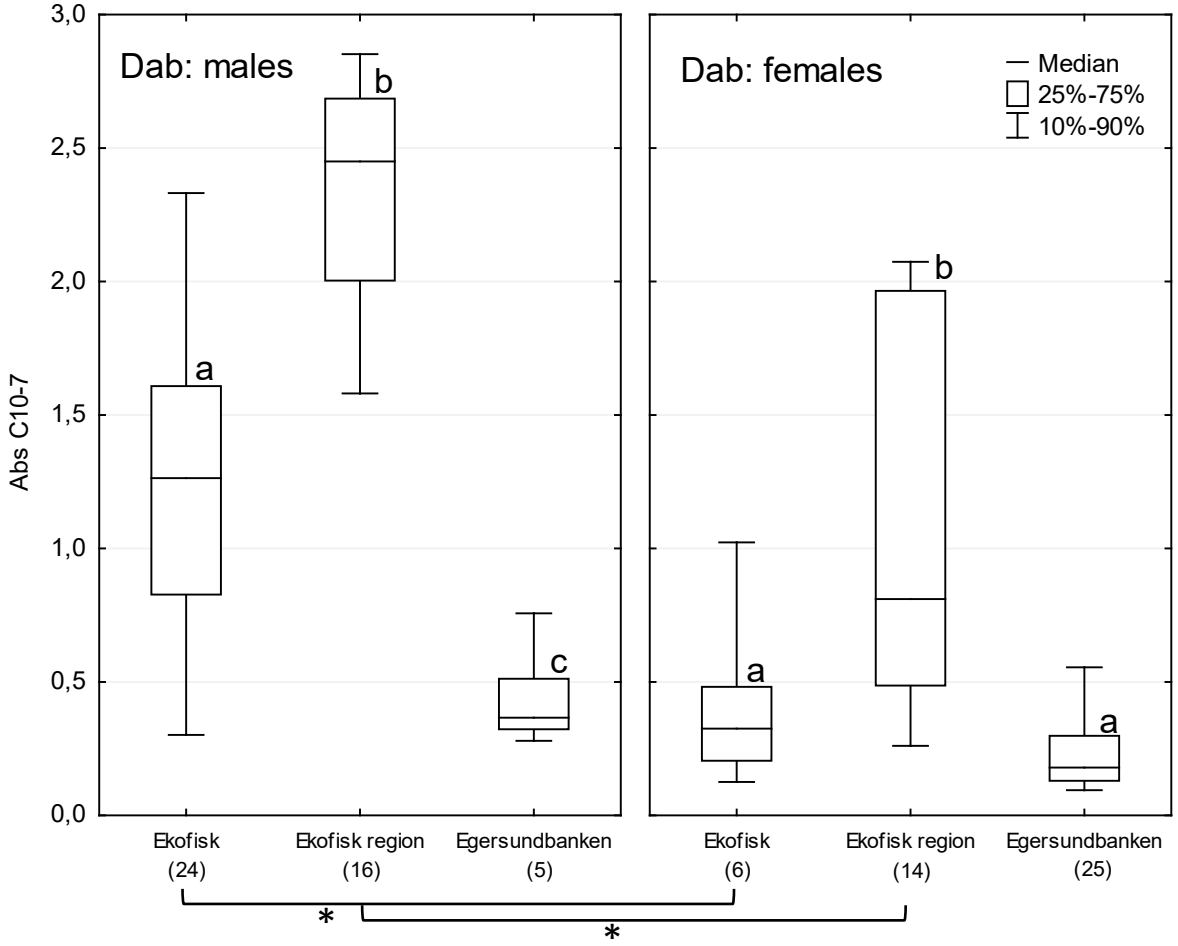


Figure 61. *ELISA absorbance (median, quartiles, 10/90 percentiles) with C10-7 monoclonal antibody in male and female dab. Different letters denote significant differences between groups for the same sex, * denotes significant differences between sexes from the same location (ANOVA, Tukey, p<0.05).*

3.6.2.4 qPCR

The qPCR levels were normalised to reference genes. For cod, 30 individuals were collected at Ekofisk while 17 were caught at Egersundbank. Unfortunately, due to the poor RNA quality in many cod samples from Egersundbank, only 6 individuals had sufficient quality to be compared with CYP1A levels from Ekofisk (Figure 62). No significant differences were found between the Ekofisk and Egersundbank for either AHR2 or CYP1A (ANOVA, Tukey p>0.05).

CYP1A expression in haddock liver demonstrated significantly higher levels from Ekofisk region compared with Egersundbank and Vikingbank (ANOVA, Tukey, p<0.05) (Figure 63). CYP1A expression in haddock was not significantly different between Egersundbank and Vikingbank. The AHRR2 expression in haddock was significantly higher in haddock from the Egersundbank compared to the Ekofisk region and Vikingbank (ANOVA, Tukey p<0.05). In addition, AHRR2 expression in haddock from Vikingbank was significantly higher than the Ekofisk region.

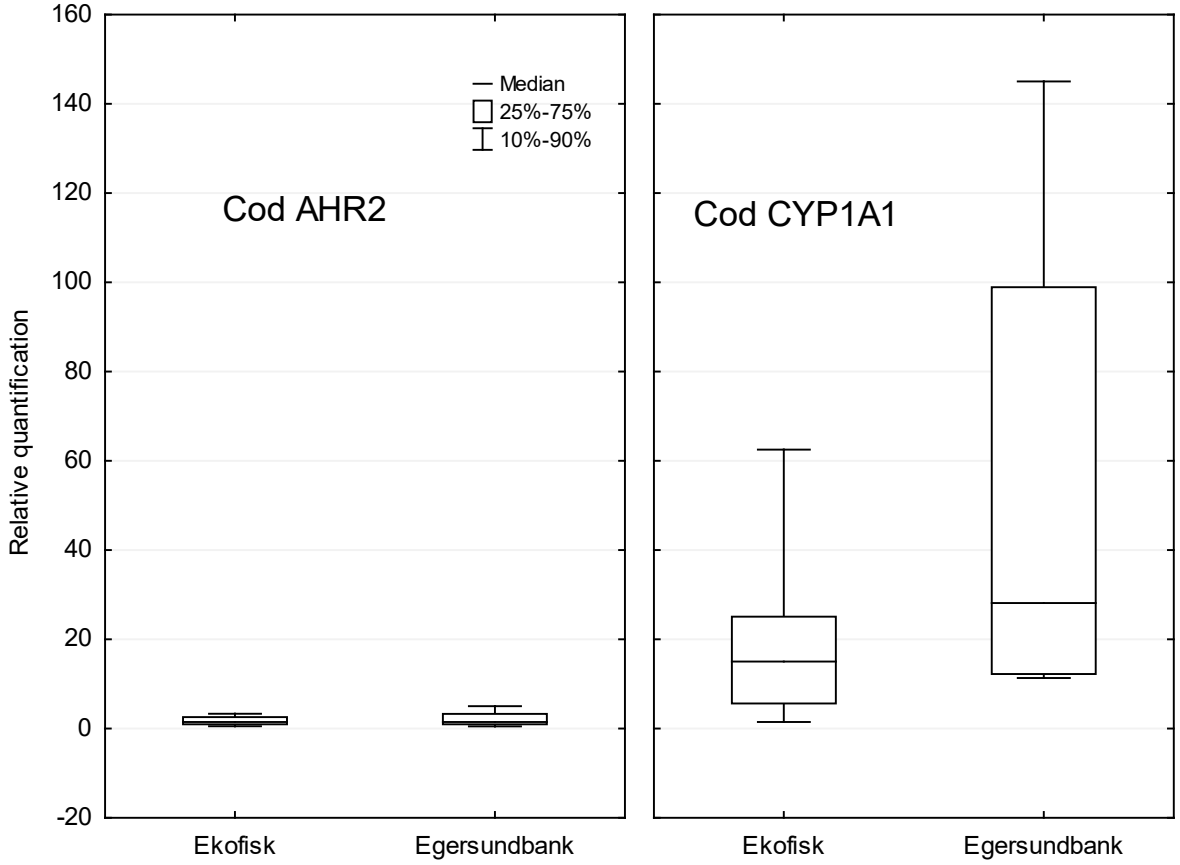


Figure 62. Relative quantification of aryl hydrocarbon receptor, AHR2 and cytochrome P4501A, CYP1A expression in cod liver. n=28 for Ekofisk and 6 for Egersundbank). No significant differences between regions for either AHR2 or CYP1A1 (ANOVA, Tukey, p>0.05).

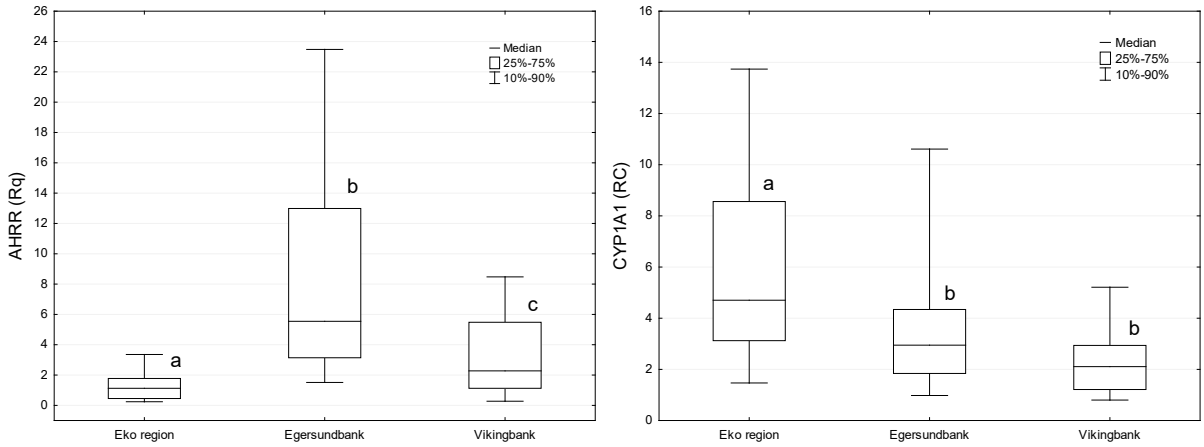


Figure 63. Relative quantification of aryl hydrocarbon receptor repressor (AHRR) (left) and CYP1A (right) expression in haddock liver. Different letters denote significant differences between the groups (ANOVA, Tukey p<0.05, n=28 per region).

3.6.2.5 Comet assessment

The percentage of DNA damage, measured through the comet assay, is presented for the fish collected from the Ekofisk safety zone and the three regions of the North Sea in Figure 64. Significantly larger comet tails, indicating DNA strand breakages, were measured in cod from the Ekofisk safety zone compared to cod from Egersundbank (ANOVA, Tukey, $p<0.05$). Significantly higher percentage comet tails were also found in dab from the Ekofisk safety zone compared to the wider Ekofisk region and Egersundbank (ANOVA, Tukey, $p<0.05$). For haddock and whiting, there were no significant differences in the percentage comet tails between the sampling regions with generally a low level of DNA damage in all groups.

ICES assessment criteria are available for cod and dab with a proposed BAC of 5% comet tail (Davies and Vethaak, 2012). Based on this value, all cod and dab populations had mean % comet tails above the 5% threshold, although only marginally above for dab from the Ekofisk region (8%) and the Egersundbank region (9%).

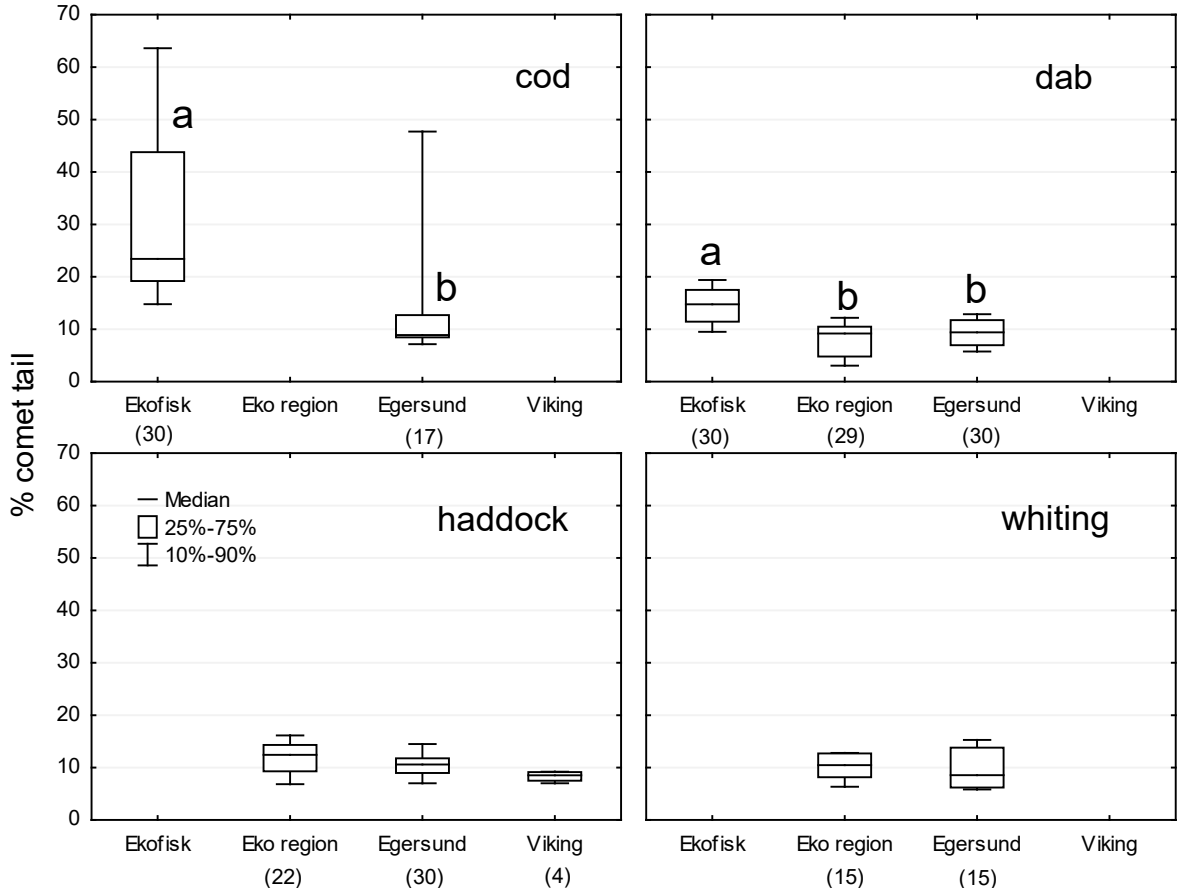


Figure 64. DNA strand breakage in fish blood samples from all fish species caught from within the Ekofisk safety zone and three regions of the North Sea. Different letters denote significant difference between locations (ANOVA, Tukey, p<0.05). Numbers in parenthesis denote replicates.

In addition to DNA strand breakages, the comet assessment can also be used to measure oxidation of DNA base pairs that provides further information on chronic stress responses. The percentage of DNA damage together with the oxidation of base pairs for the fish from the Ekofisk safety zone and the three regions of the North Sea are presented (Figure 65). The highest percentages of DNA damage combined with oxidation of base pairs were found in cod from the Ekofisk safety zone, although

similarly high levels were found in cod from Egersundbank. Significantly increased DNA damage plus oxidation of base pairs were found in dab from the Ekofisk safety zone compared to dab from the Ekofisk region and Egersundbank region (ANOVA, Tukey, p<0.05).

When oxidation of base pairs was added to the DNA damage, significant differences were found between the haddock populations. For example, haddock from Vikingbank had significantly lower percentage of the DNA damage plus oxidation of base pairs compared to both the Ekofisk region and the Egersundbank (ANOVA, Tukey, p<0.05). Whiting showed no significant differences between the sampling locations.

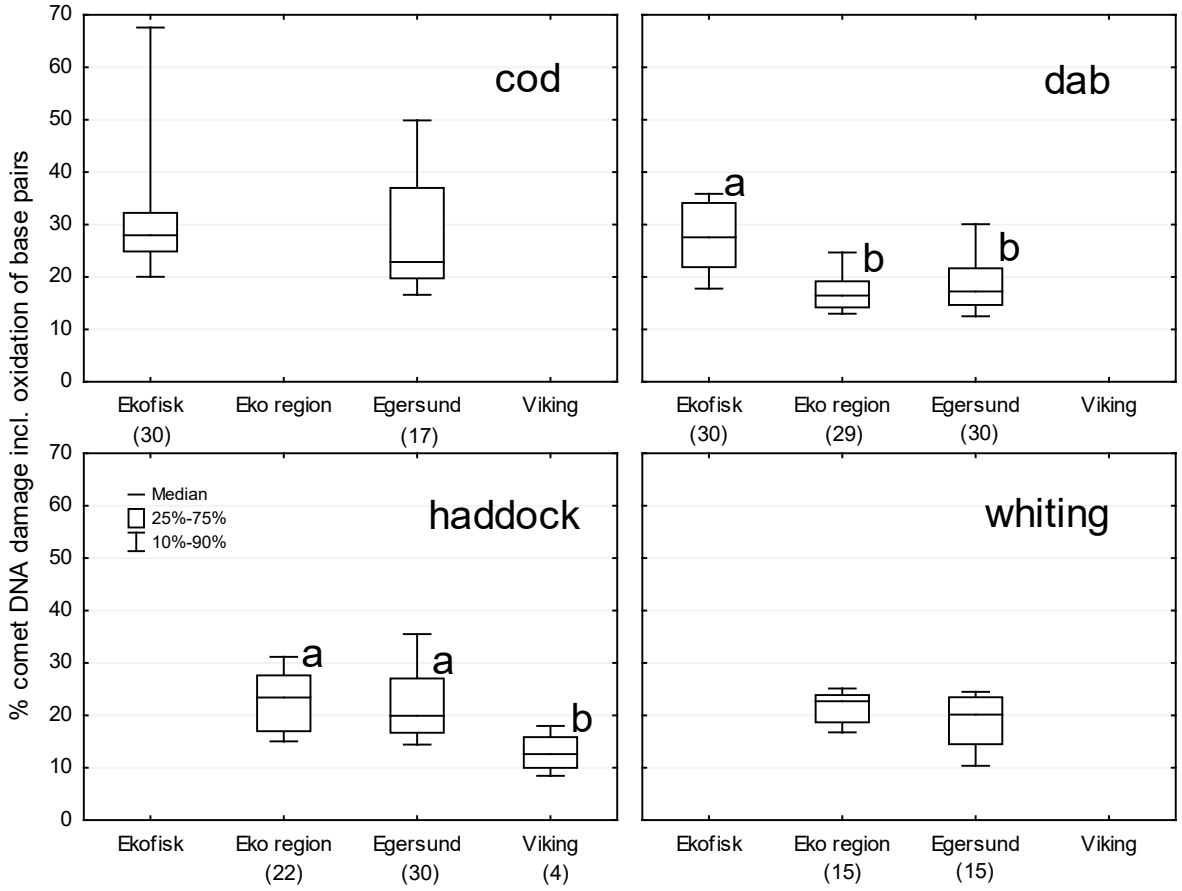


Figure 65. DNA strand breakage and oxidation of base pairs in fish blood samples from all fish species caught from within the Ekofisk safety zone and three regions of the North Sea. Different letters denote significant difference between locations (ANOVA, Tukey, p<0.05). Numbers in parenthesis denote replicates.

3.6.2.6 DNA adducts

DNA adducts measured in the liver of fish from the different locations by the method of ^{32}P post-labelling are presented (Figure 66). Significantly higher DNA adduct levels were found in haddock from the Vikingbank and Egersundbank compared to the Ekofisk region (ANOVA, Tukey, p<0.05).

ICES assessment criteria for DNA adducts are available for cod, dab and haddock with BAC values of 1, 1.6 and 3 respectively, in addition to a common EAC value of 6 nm adducts per mol DNA (Davies and Vethaak, 2012). Based on these values, DNA adducts in cod from both the Ekofisk safety zone and the

Egersundbank locations were below the BAC. Whilst DNA adduct levels in the dab were all marginally above the BAC but below the EAC. The mean DNA adduct levels in haddock were below the BAC in the Ekofisk region population, but above the BAC and below the EAC in haddock from Egersundbank and Vikingbank.

DNA adducts were also measured in the intestine of fish from the different locations (Figure 67). Overall, the DNA adduct levels in fish intestines were generally low in all fish from the different locations with no significant differences between the groups. However, dab from the Egersundbank had a mean adduct level of 10 nm adducts per mol DNA adducts. Due to large variation in DNA adducts in this dab group no significant differences between locations were observed.

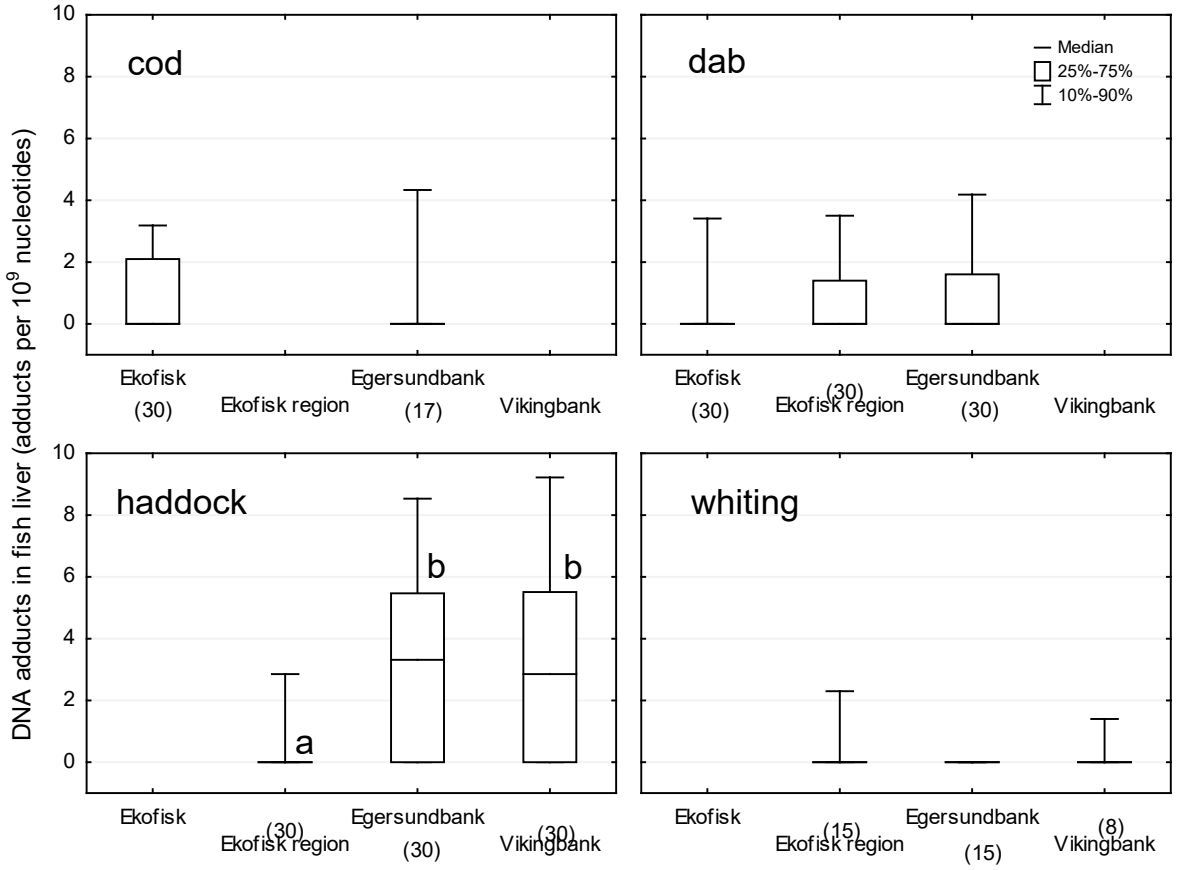


Figure 66. DNA adducts in fish liver samples from all fish species caught from within the Ekofisk safety zone and three regions of the North Sea. Different letters denote significant difference between locations (ANOVA, Tukey, p<0.05). Numbers in parenthesis denote replicates.

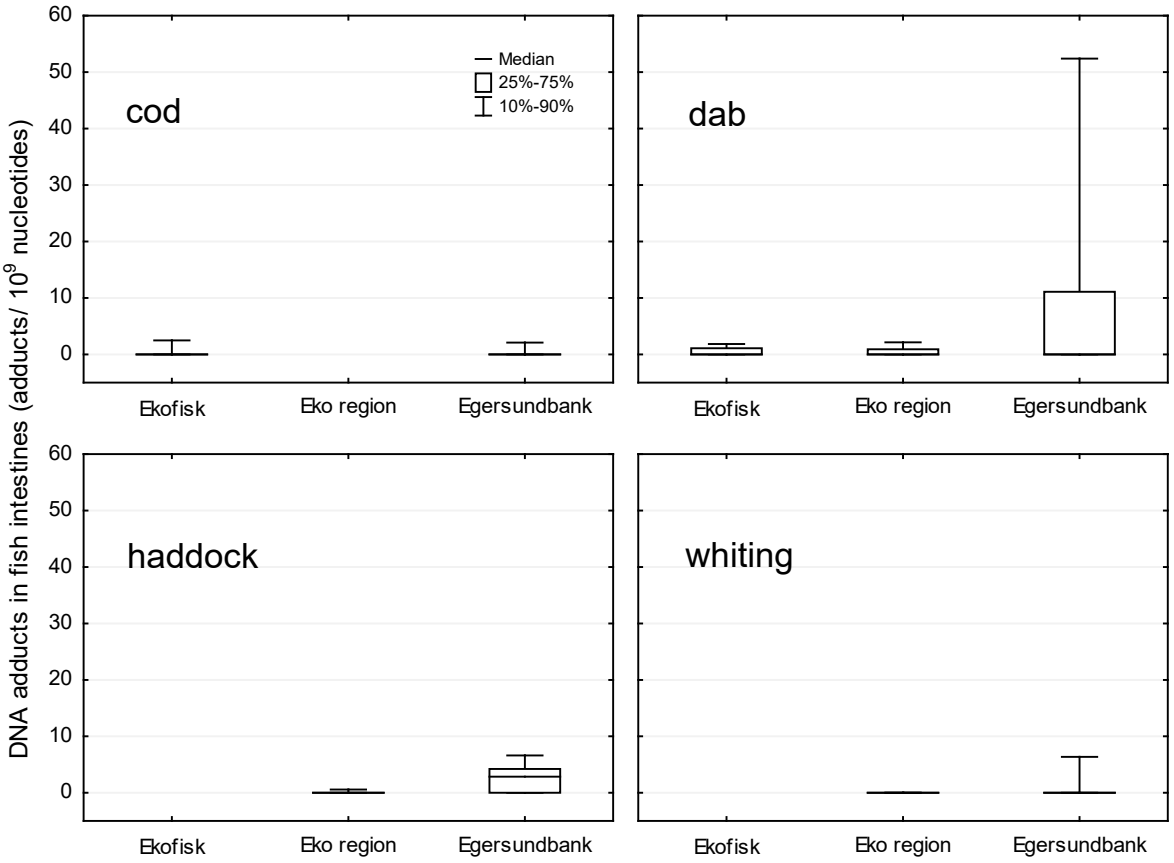


Figure 67. DNA adducts in fish intestine samples from all fish species caught from within the Ekofisk safety zone and two regions of the North Sea. No significant differences between locations (ANOVA, Tukey, p>0.05). Numbers in parenthesis denote replicates.

3.6.2.7 Acetylcholine esterase inhibition

AChE activity in the fillet of fish from the different location is shown in Figure 68. A significant inhibition of AChE activity was observed in cod from the Egersundbank compared to those from the Ekofisk safety zone (ANOVA, Tukey, $p<0.05$). Significant differences in AChE activity were also found in haddock, with significantly lower AChE activity in Vikingbank and Egersundbank compared to the Ekofisk region (ANOVA, Tukey, $p<0.05$). AChE activity in dab and whiting were not significantly different between the three locations they were sampled from. Interestingly, AChE activity in dab was approximately double that of whiting and suggests species differences.

ICES assessment criteria are available for dab with a proposed BAC of 150 nmol ATC/ min/ mg protein and EAC of 105 nmol ATC/ min/ mg protein. It is important to remember that since the test measures the inhibition of the AChE enzyme, the EACs are lower than the respective BACs. In the present study the median AChE activities were in the region of 50 to 60 nmol ATC/ min/ mg protein, approximately 2-fold lower than the proposed ICES EAC. This questions whether the ICES assessment criteria for AChE in dab are suitable as a reference marker in this study.

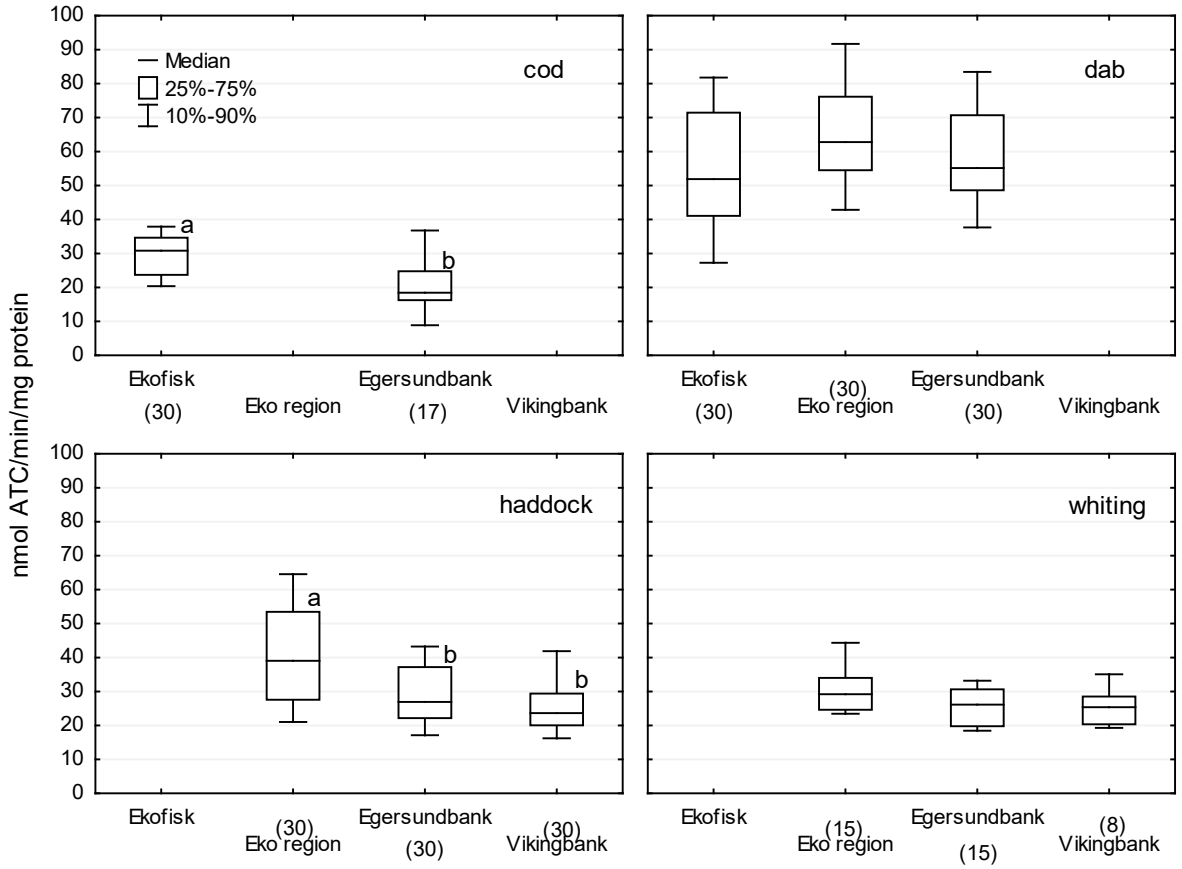


Figure 68. Acetylcholine esterase inhibition for all fish species caught from within the Ekofisk safety zone and three regions of the North Sea. Different letters denote significant difference between locations (ANOVA, Tukey, p<0.05). Numbers in parenthesis denote replicates.

3.6.2.8 Fish liver histology

Histological endpoints were measured in the liver of fish from the Ekofisk safety zone and three regions of the North Sea (Figure 69). Eight histological features of the liver are presented including, fibrosis, granulocytomas, hepatocyte vacuolization, haemocyte infiltration, melanomacrophage aggregates (MMA), necrosis, parasites and steatosis. Comparisons between the groups for each fish species, for the different histological endpoints, showed that only hepatocyte vacuolisation in haddock and whiting were significantly different (Kruskal Wallis, $p<0.05$). Haddock from the Ekofisk region had significantly higher frequencies of hepatocyte vacuolisation compared to haddock from both Egersundbank and Vikingbank. In contrast, whiting from Vikingbank had significantly higher frequencies of hepatocyte vacuolisation compared to whiting from Ekofisk region and Egersundbank.

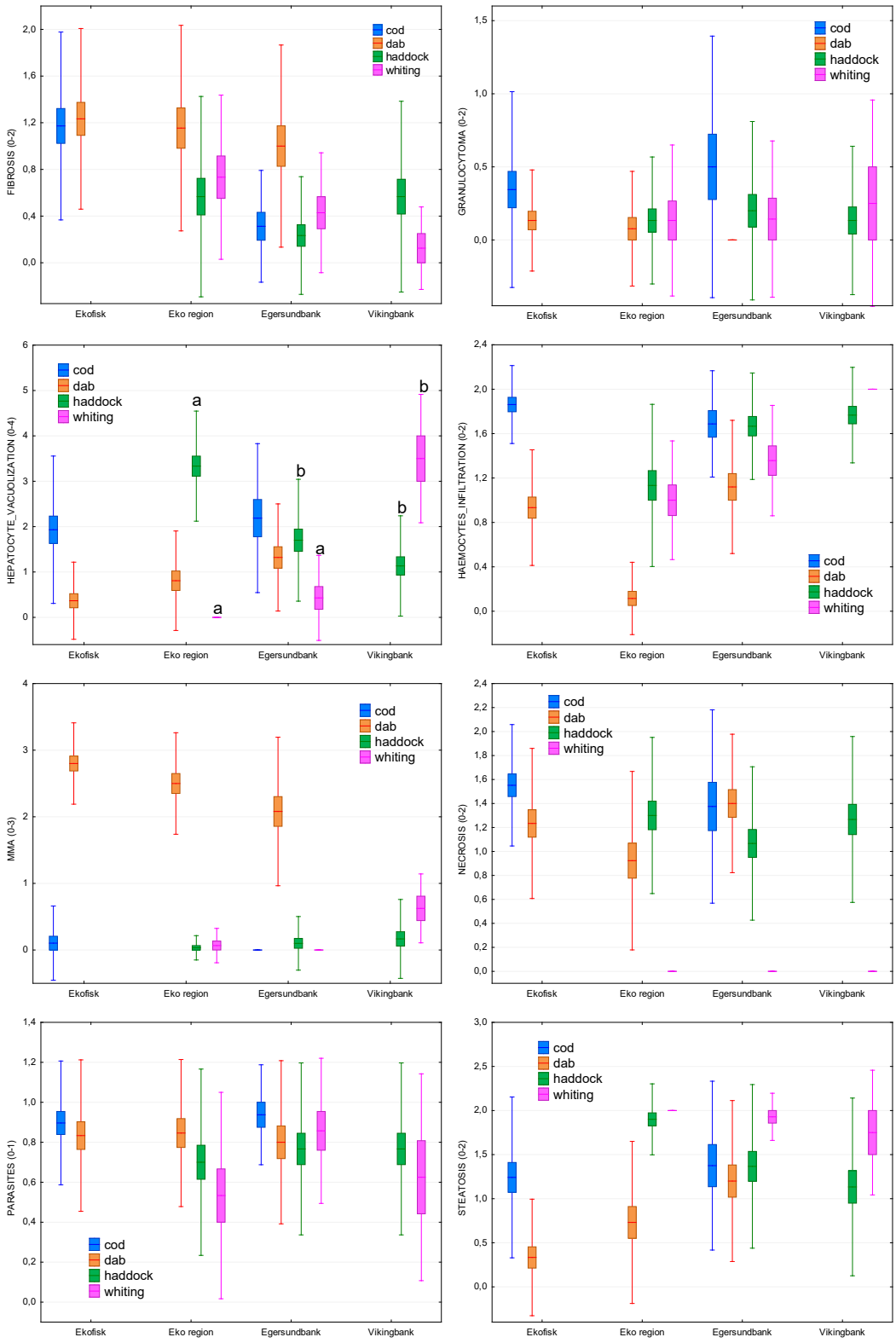


Figure 69. Histological markers in fish liver samples for all fish species caught from within the Ekofisk safety zone and three regions of the North Sea. Different letters denote significant differences between groups within fish species (Kruskal Wallis, p<0.05) (Mean ± SD (outer line), SE (box)).

3.7 Sediment traps

3.7.1 PAH-NPD concentration

The concentration of PAH in the collected sediment trap material from the reference stations (R1 and R2) and three selected Ekofisk stations (2, 5 and 6) are shown as the sum of PAH EPA16, Sum NPD and Sum PAH (Figure 70). The sediment traps were placed at a depth of approximately 40 to 45 m. Two sediment traps were used for PAH analyses at each station and each were analysed resulting in two replicates at each station. Due to the limited replication no statistical analysis could be performed. A third sediment trap per station was extracted for use for *in vitro* assays with no internal PAH standard added.

The median PAH EPA16 concentrations in the sediment trap material for the five groups ranged between 1000 and 1300 ng/g (d.w.). The Sum NPD concentrations were relatively lower than PAH EPA16 in all locations with median concentrations ranging between 500 to 700 ng/g (d.w.). The Sum PAH was obtained by combing PAH16 with Sum NPD.

Overall, the PAH concentrations of the sediment trap material were high. Highest PAH were measured in R2 whilst lowest PAH were measured in R1. The PAH concentration of the three Ekofisk stations were relatively equal and did not appear to be influenced by proximity to the Ekofisk installation.

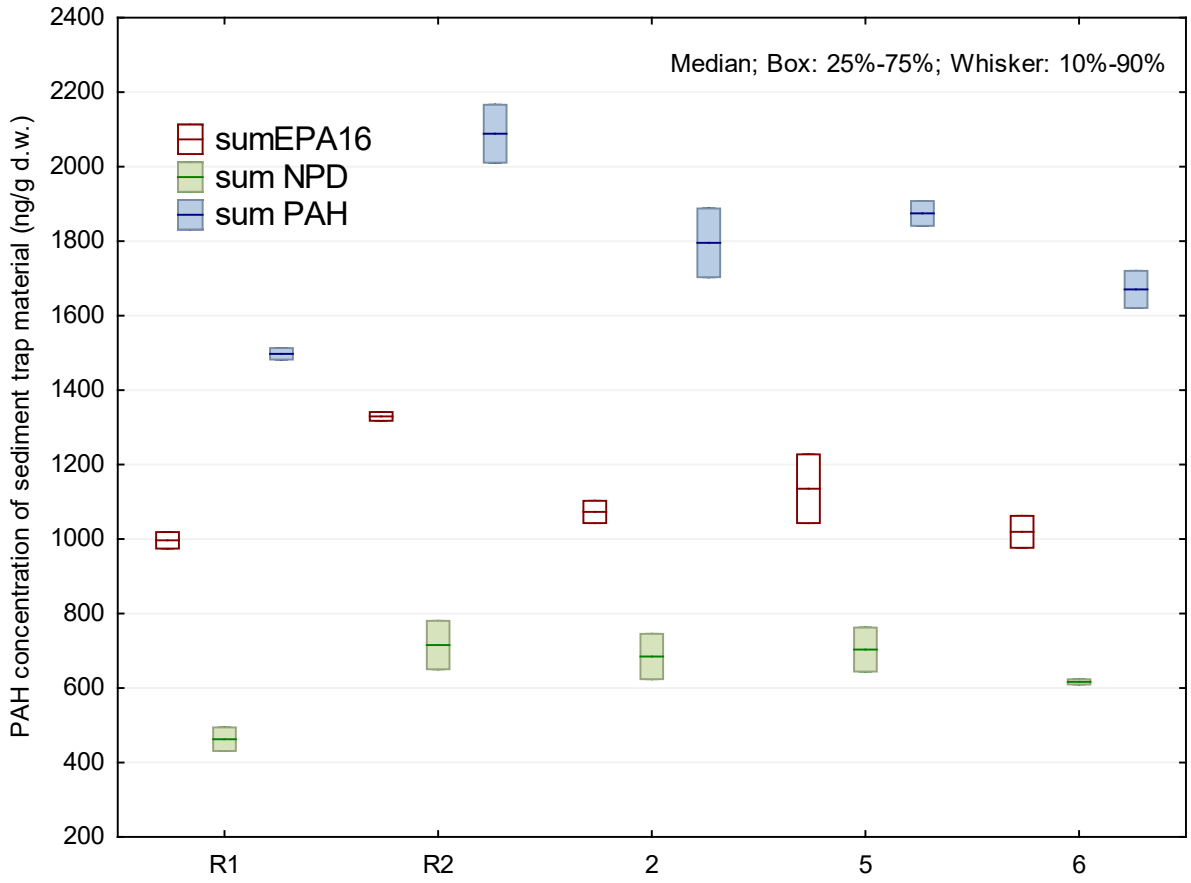


Figure 70. Sum of PAH EPA16, Sum NPD and Sum PAH concentrations in sediment trap extracts collected from the reference stations (R1 and R2) and the three monitoring stations at Ekofisk (2, 5 and 6).

3.7.2 In-vitro bioassay: ROS generation

The generation of reactive oxygen species (ROS) in rainbow trout gill cell lines (RT gill-W1) was determined following 24 hour exposure to diluted sediment extracts from the two reference locations (R1, R2) and the Ekofisk monitoring stations (2, 5 and 6) (Figure 71). Significant generation of ROS above control values were found in sediment trap extracts from R1, Station 5 and 6. For R1, significant ROS generation was only shown at the highest extract concentration of 120 mg eQsed/ml, whilst 20 and 10 mg eQsed/ml was sufficient to cause a significant increase in ROS generation for Station 5 and 6 extracts respectively.

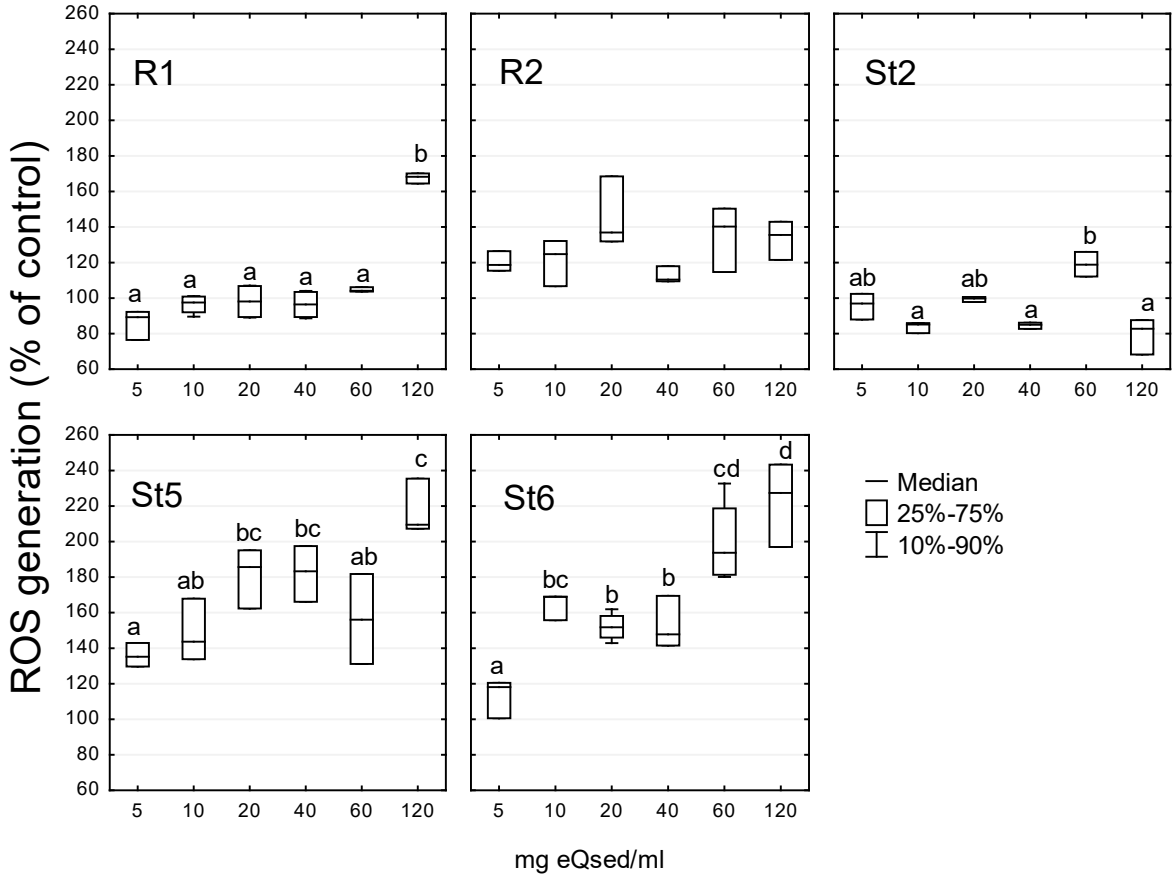


Figure 71. Reactive oxygen species generation in rainbow trout gill-Waterloo1 (RT gill-W1) cell lines exposed for 24 h to sediment extracts collected from sediment traps at the Ekofisk monitoring stations 2, 5 and 6 and reference stations R1 and R2. Different letters denote significant differences between the different eQsed concentrations tested for each respective extract (ANOVA, Tukey, p<0.05).

The activation of the AhR1 receptor used in the luciferase-based report gene assay following exposure to the diluted sediment extracts of the reference stations (R1, R2) and the Ekofisk stations (2, 5, 6) plus a blank are presented (Figure 72). All sediment extracts were found to activate the AhR1 receptor to equal measure with no apparent differences between response.

Induction of EROD activity in rainbow trout gill cell lines (RT gill-W1) was determined following 24-hour exposure to diluted sediment extracts from the two reference locations (R1, R2) and the Ekofisk monitoring stations (2, 5 and 6) (Figure 73). Significant EROD induction above control values were found in all sediment trap extracts. For sediment extracts collected at stations R1, 2 and 6, the lowest extract concentration of 5 mg eQsed/ml was sufficient to cause a significant EROD response above

control levels. For stations R2 and st5, 20 and 10 mg Qsed/ml respectively, caused significant EROD activities above control levels.

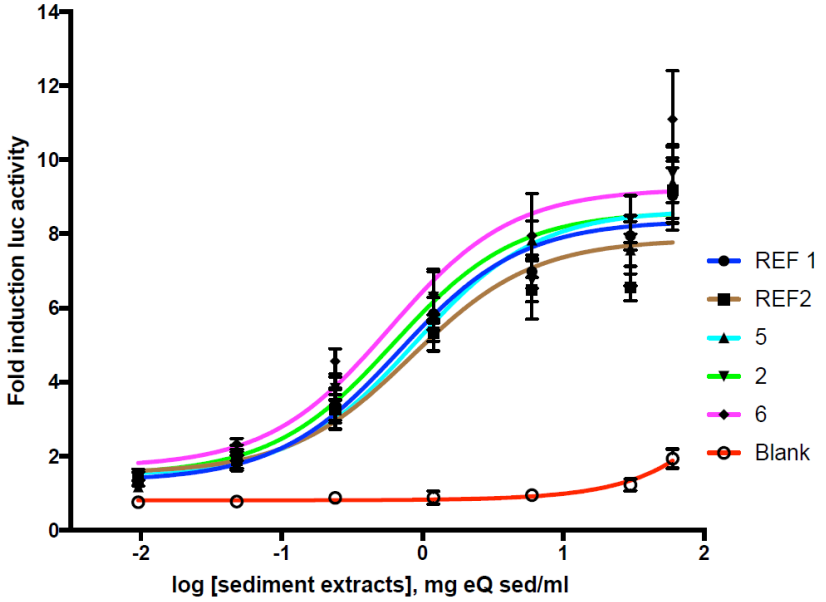


Figure 72. Luciferase activity demonstrating ligand binding activity oh AhR1 receptor in cod following exposure to sediment trap extracts collected from the Ekofisk monitoring stations 2, 5 and 6 and reference stations R1 and R2.

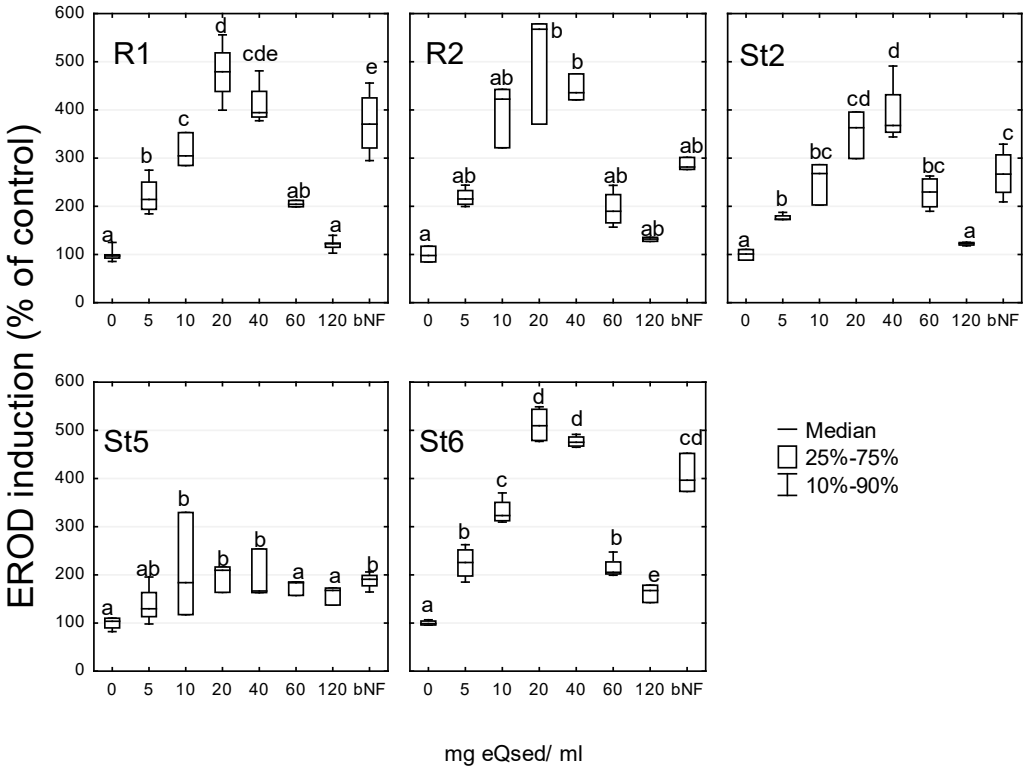


Figure 73. EROD induction presented as % of the control in rainbow trout gill cell line (RT gill-W1) exposed for 24 h to sediment extracts collected from sediment traps at the Ekofisk monitoring stations 2, 5 and 6 and reference stations R1 and R2. Different letters denote significant differences between the different eQsed concentrations from each respective sediment extract (ANOVA, Tukey, p<0.05, Kruskal Wallis for R2).

3.8 Integrated Assessment

3.8.1 Integrated biological response index (IBR/n)

Mussels

The integrated biological response index (IBR/n) was used to combine the individual biomarker results to provide an overall assessment of the mussel health status. Biomarkers included in the IBR/n calculation were CI, SoS, LMS, MN and histological parameters recorded in digestive gland, gill and gonad (Figure 74). The inverse value of CI, SoS and LMS were used in the IBR/n calculation since a lower value is indicative of an adverse response. The star plots show the contribution of the different biomarkers to the overall IBR/n value, with the size of the coloured areas on the star plots dependent on the relative biomarker responses, which are summarised in the bar chart at the bottom of the figure. The IBR/n is a relative score, where the actual value is less important than the comparative differences between the exposure groups.

For the mussels caged at the reference stations, the calculated IBR/n of R2 for both 20 m and 40 m were markedly lower than both R1 depths as well as the T0 mussels. For T0 mussels, CI and gonad and gill histology contributed most to the IBR/n score. Although the similar IBR/n values were calculated for R1 20 and 40 m, the contributions to the IBR/n score were very different. Although all biomarkers contributed to some extent, CI and SoS were more dominant in the R1 40 m mussels whilst gill histology contributed most for the R1 20m mussels.

Highest IBR/n were found in mussels from Station 5 40 m followed by Station 1 20 m and Station 4 40 m. At Station 5 40 m, DG histology and MN had larger contributions, whilst at Station 1 20 m MN and LMS contributed most. For Station 4 40 m all biomarkers contributed with SoS contributing most. Overall, there was no clear pattern in biomarker response and IBR/n was indicative of a low-level biological response throughout all mussel groups. With respect to depth, higher IBR/n values were typically found in the deeper 40 m mussels compared to the 20 m group at each station (exceptions to this were found at Station 1 and 2). In most cases, the whole organism responses of general fitness (i.e. CI and SoS) contributed regularly to the IBR/n of mussels from the 40 m depth.

For the mussels from the three Eldfisk monitoring stations (9-11), higher IBR/n values were calculated in Station 10 mussels. Histological endpoints were mostly responsible for the elevated IBR/n in mussel from Station 10. The closest group to the Eldfisk installation (Station 9) had very low IBR/n and there was no relationship between proximity to the Eldfisk PW discharge and IBR/n value.

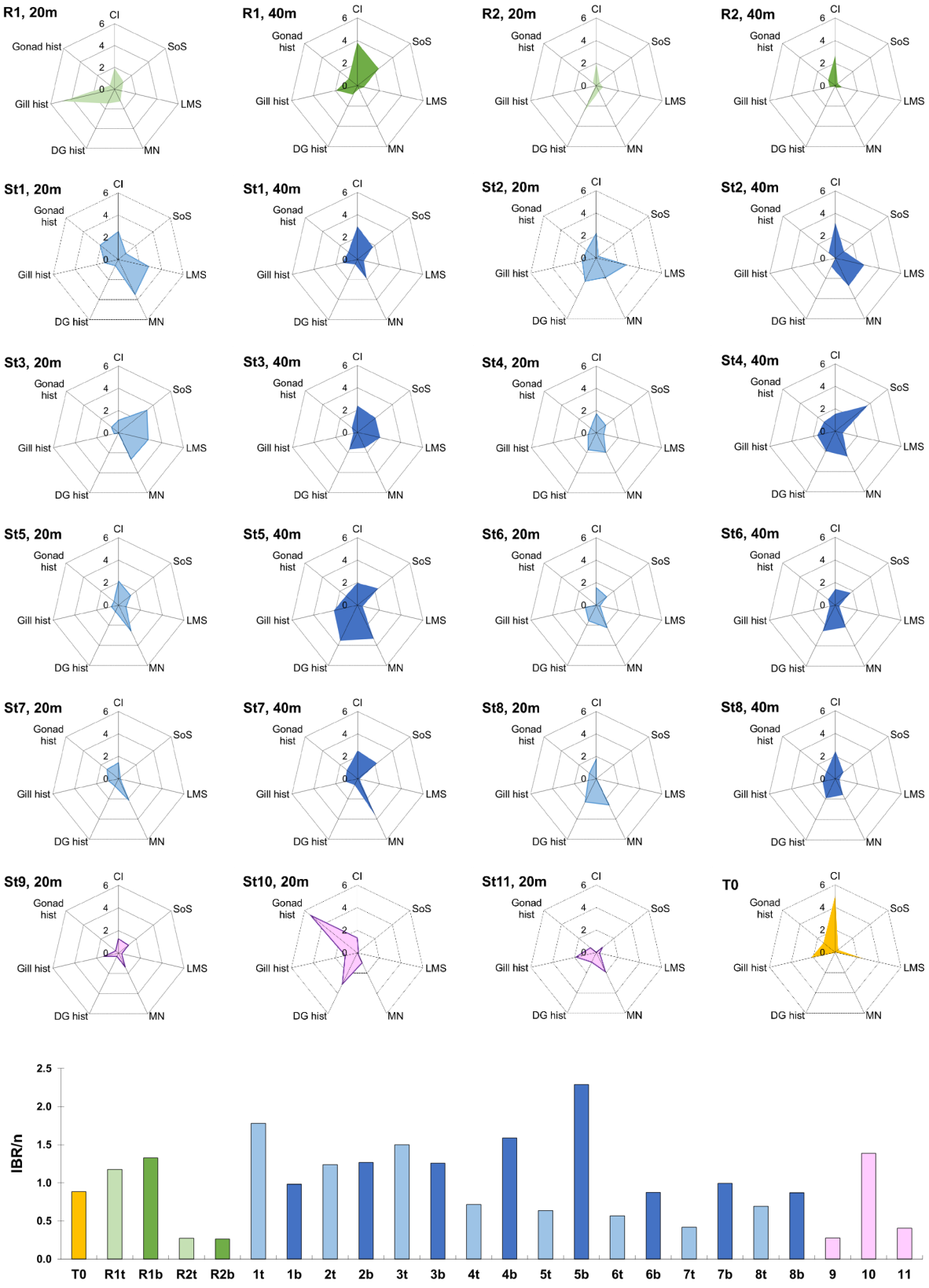


Figure 74. Integrated biological response (IBR/n) calculated from star plots of mean normalised biomarker data in mussels located from the different mussel groups. Mussels held at 20 m (t) and 40 m (b) depths at the reference and Ekofisk stations and at a depth of 20 m for Eldfisk.

Fish

The IBR/n was calculated from biomarker responses in fish collected from the Ekofisk safety zone and three regions of the North Sea, including the wider Ekofisk region as well as Egersundbank and Vikingbank (Figure 75). The IBR/n was calculated from six biomarker responses including AChE, EROD, CYP1A, DNA adducts, liver PAH concentration and liver histology. The selection of the biomarkers was dependent on whether values were available for all fish groups. The comet assay was not included since values were not available for Whiting from Vikingbank. Whilst PAH metabolites were excluded due to the low detection of PAH metabolites among the fish species. The inverse value of AChE was used in the IBR/n calculation since a lower value is indicative of an adverse response. The positioning of the biomarkers on the star-plots influences the IBR/n score, for this reason the biomarkers of similar level and type of response, were placed next to each other as advised (Broeg and Lehtonen, 2006).

Highest IBR/n values were found in Ekofisk dab where the biomarkers EROD, CYP1A, PAH liver and DNA adducts contributed most to the IBR/n score. The IBR/n was also elevated in dab from the Ekofisk region with EROD, CYP1A and DNA adducts contributing most to the IBR/n score. These similar responses from the biomarkers indicates a similar source of impact on the dab from the two localities. In contrast, the IBR/n in dab from Egersundbank was one of the smallest values calculated.

For haddock, very similar IBR/n values were found in Ekofisk region, Egersundbank and Vikingbank. The IBR/n values of haddock were similar in magnitude to the IBR/n of cod from Egersundbank and whiting from Vikingbank scores. The haddock showed similar biomarker responses in all populations shown by the shape of the shaded area in the star plots.

The IBR/n in whiting were overall lower than the other fish species. Lowest IBR/n were calculated in whiting from Ekofisk region and Egersundbank, which were similar in magnitude to the Egersundbank dab. Highest IBR/n values were found in whiting from the Vikingbank region with AChE, liver histology and PAH liver concentrations contributing most to the IBR/n score.

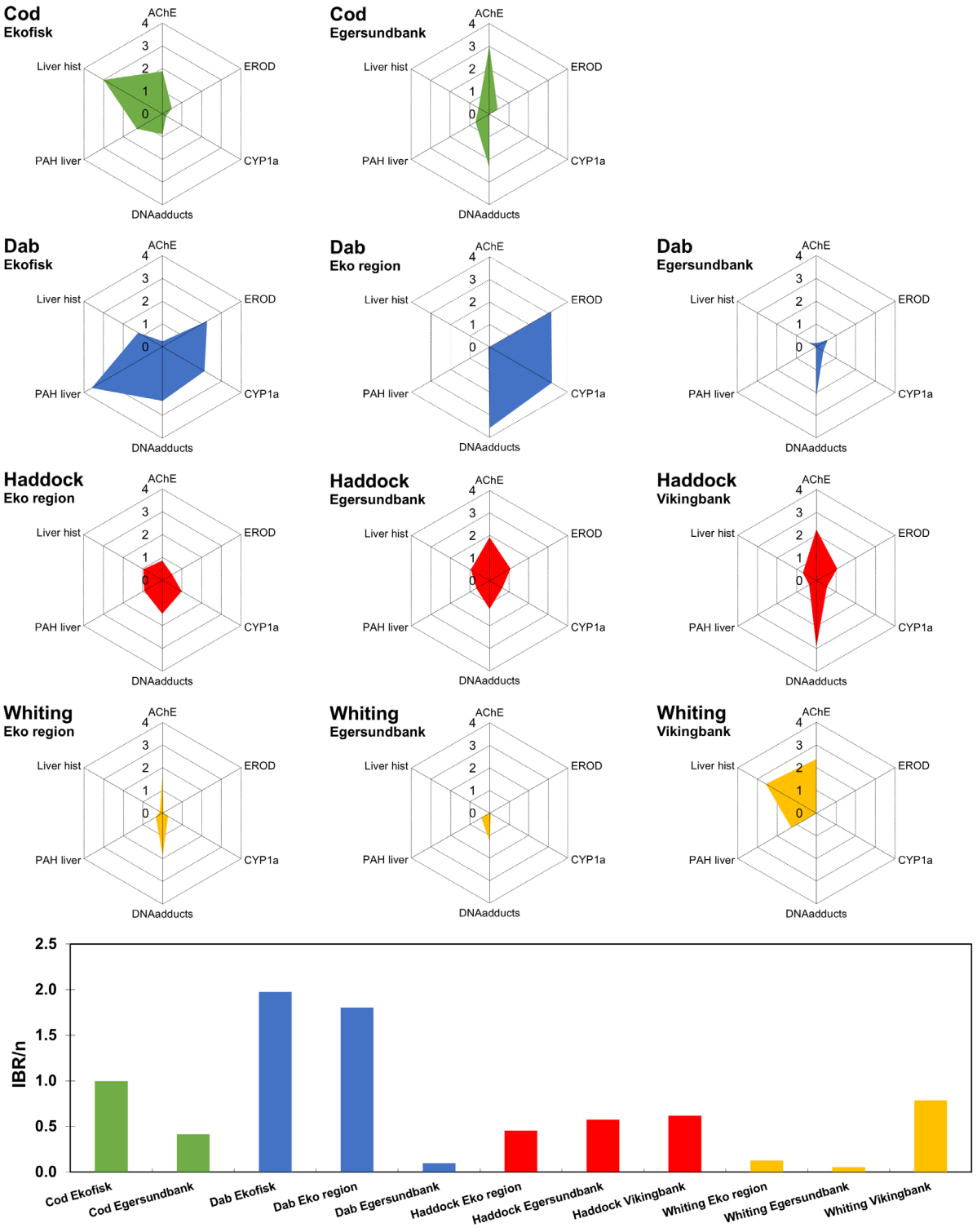
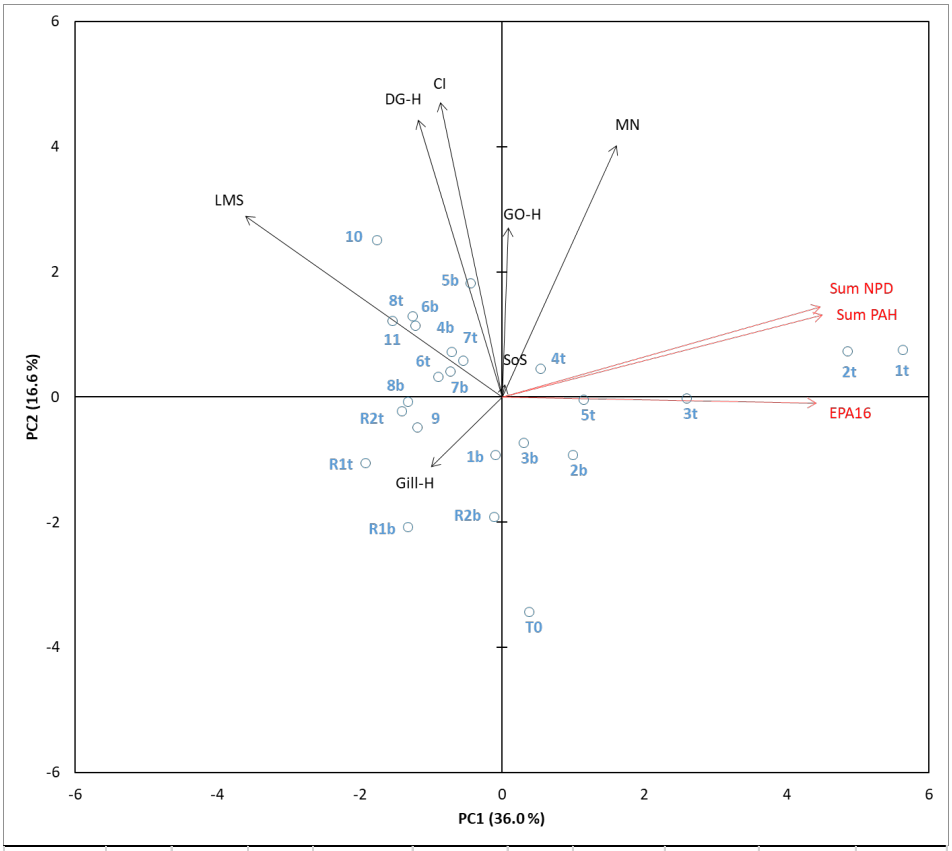


Figure 75. Integrated biological response (IBR/n) calculated from star plots of mean normalised biomarker data in fish collected from the Ekofisk 500 m safety zone and three regions of the North Sea, Ekofisk, Egersundbank and Vikingbank for four species of fish (Cod, dab, haddock and whiting). Acetylcholine esterase (AChE) inhibition, ethoxyresorufin O-deethylase (EROD), Cytochrome P4501A (CYP1A), PAH metabolites (PAHmet), liver histology (liver hist).

3.8.2 Principle component analysis (PCA)

Mussels

The PCA was used to separate the main variables responsible for the variance of chemical body burden and biological effects. Overall, the PCA showed a clear spatial differentiation between the 20 m mussel groups closest to the Ekofisk installation (Figure 76). PC1 accounted for 36.0 % of variance and showed a separation between the groups with higher and lower PAH, NPD and PAH EPA16 concentrations. PC2 explained 16.6% of the variance and showed separation of the day zero (T0) mussels from the field exposure groups. The PCA showed that Ekofisk mussels closest to the installation, at the 20 m depth, had highest concentrations of PAHs.

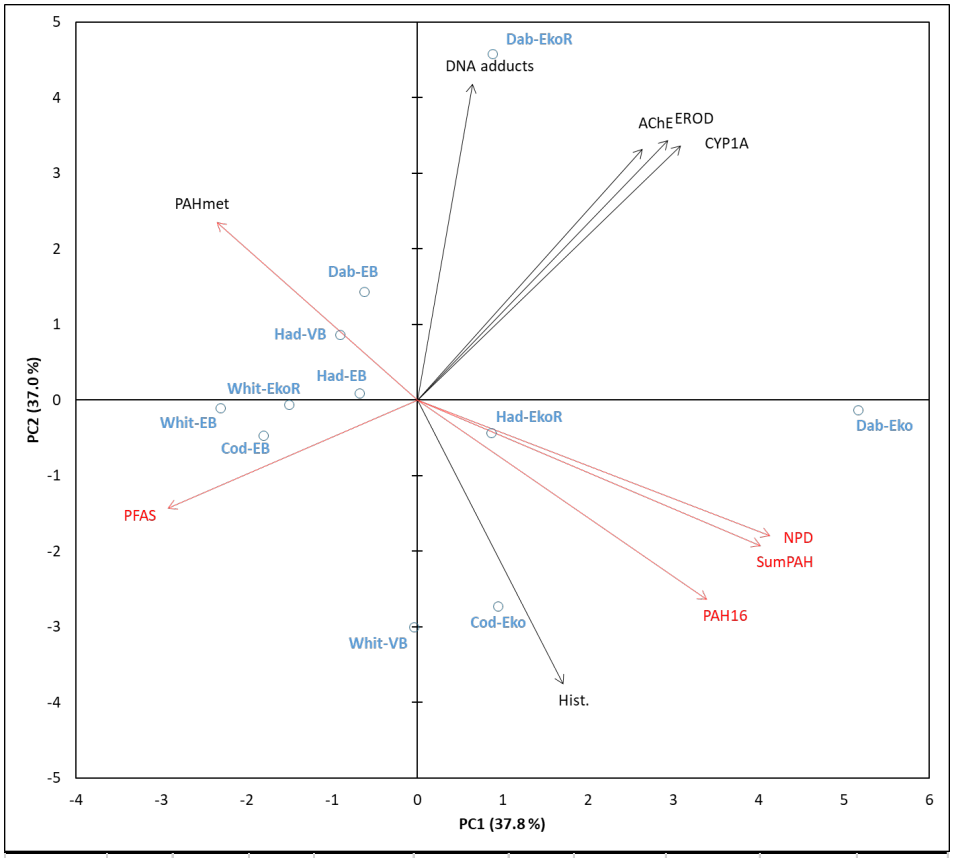


Variables	CI	SoS	LMS	MN	DG-H	Gill-H	GO-H	sumPAH	EPA16	SumNPD
CI	**1**	0.000	0.402	0.257	0.214	-0.030	0.013	-0.045	-0.145	-0.034
SoS	0.000	**1**	0.141	-0.364	0.084	-0.072	0.226	0.055	0.169	0.049
LMS	0.402	0.141	**1**	-0.012	0.322	0.077	0.128	**-0.631**	**-0.674**	**-0.620**
MN	0.257	-0.364	-0.012	**1**	0.099	-0.153	-0.046	0.391	0.175	**0.407**
DG-H	0.214	0.084	0.322	0.099	**1**	0.018	0.297	-0.092	-0.257	-0.075
Gill-H	-0.030	-0.072	0.077	-0.153	0.018	**1**	0.028	-0.139	-0.198	-0.132
GO-H	0.013	0.226	0.128	-0.046	0.297	0.028	**1**	0.081	0.096	0.081
sumPAH	-0.045	0.055	**-0.631**	0.391	-0.092	-0.139	0.081	**1**	**0.914**	**0.999**
EPA16	-0.145	0.169	**-0.674**	0.175	-0.257	-0.198	0.096	**0.914**	**1**	**0.898**
SumNPD	-0.034	0.049	**-0.620**	**0.407**	-0.075	-0.132	0.081	**0.999**	**0.898**	**1**

Figure 76. Principle component analysis of chemical measurements (PAH – red line) and biological responses (black) in mussels held in the water column at approximate depths of 20 m (top, t) and 40 m (bottom, b) at Ekofisk (1-8), Eldfisk (9-11) and reference stations (R1, R2) (blue). Including p-values for the Pearson's correlation between the chemical and biological endpoints. CI – Condition index; SoS – Stress on stress; LMS – Lysosomal membrane stability; MN – micronuclei; Gill -H – Gill histology; GO-H – Gonad histology; DG-H -Digestive gland histology. EPA16 – Sum of EPA PAH16; Sum PAH – Sum of PAH; SumNPD – sum of naphthalene, phenanthrene and dibenzothiophenes alkylated and parent compounds. Significant correlations in bold.

Several statistically significant associations were shown by the correlation analysis between the chemical measurements and the biological responses in mussels from the various stations. Strong positive correlations were found between Sum PAH and Sum NPD and PAH EPA16. A positive correlation was found between Sum NPD and MN formation, whilst negative correlations were detected between LMS and Sum NPD, Sum PAH and PAH EPA16.

Fish



Variables	AChE	EROD	CYP1A	DNA adducts	Hist.	PAHmet	PAH16	NPD	SumPAH	PFAS
AChE	**1**	**0.716**	**0.786**	0.536	-0.330	-0.094	-0.062	0.155	0.114	**-0.653**
EROD	**0.716**	**1**	**0.952**	**0.708**	-0.220	0.179	0.080	0.272	0.242	-0.450
CYP1A	**0.786**	**0.952**	**1**	**0.628**	-0.248	0.105	0.124	0.280	0.246	-0.487
DNA adducts	0.536	**0.708**	**0.628**	**1**	**-0.661**	0.269	-0.364	-0.144	-0.169	-0.215
Hist.	-0.330	-0.220	-0.248	**-0.661**	**1**	-0.598	**0.619**	0.466	0.466	-0.053
PAHmet	-0.094	0.179	0.105	0.269	-0.598	**1**	-0.389	-0.494	-0.471	0.357
PAH16	-0.062	0.080	0.124	-0.364	**0.619**	-0.389	**1**	**0.823**	**0.836**	-0.167
NPD	0.155	0.272	0.280	-0.144	0.466	-0.494	**0.823**	**1**	**0.997**	-0.256
SumPAH	0.114	0.242	0.246	-0.169	0.466	-0.471	**0.836**	**0.997**	**1**	-0.227
PFAS	**-0.653**	-0.450	-0.487	-0.215	-0.053	0.357	-0.167	-0.256	-0.227	**1**

Figure 77. Principle component analysis of chemical measurements (red) and biological responses (black) in fish (dab, cod, haddock (had), whiting (whit)) collected from different locations, the Ekofisk safety zone (Eko), Ekofisk region (EkoR), Egersundbank (EB) and Vikingbank (VB)(blue). Including p-values for the Pearson's correlation between the chemical and biological endpoints. AChE – acetylcholine esterase inhibition; EROD – ethoxyresorufin-O-deethylase; CYP1A – cytochrome P4501a; PAHmet – PAH metabolites; PFAS – perfluorinated substances; PAH16 – Sum of PAH16; SumPAH – Sum of PAH; NPD – Sum of naphthalene, phenanthrene and dibenzothiophenes alkylated and parent compounds; Hist. – liver histology.

PCA was also used to separate the main variables responsible for the variance of chemical body burden and biological effects measured in fish (Figure 77). Overall, the PCA showed a clear spatial differentiation between the fish populations, particularly dab from Ekofisk and Ekofisk region compared to dab from Egersundbank, as well as Ekofisk cod compared to cod from the Egersundbank. Haddock from the Ekofisk region and whiting from Vikingbank also showed separation from their respective populations. In contrast, clustering of the fish species collected from the Egersundbank region was observed. PC1 accounted for 37.8 % of variance and showed a separation between the fish populations with higher concentration of liver PAH, NPD and PAH16 as well as increased EROD and CYP1A. PC2 explained 37.0% of the variance and showed separation with higher concentrations of DNA adducts EROD and CYP1A.

Several statistically significant associations were shown by the correlation analysis between the chemical measurements and the biological responses in the fish groups. Strong positive correlations were found between Sum PAH and Sum NPD and PAH16. A strong positive correlation was found between EROD and CYP1A and DNA adducts, whilst positive correlations were also found between AChE and EROD and CYP1A, and PAH16 and histology. Negative correlations were found between AChE and PFAS and DNA adducts and Histology.

4 Discussion

4.1 Oceanographic conditions and PW exposure

The oceanographic conditions during the period that the monitoring rigs were deployed at sea provided important supporting information on the environment that both mussels and scallops were exposed to. Information collected included temperature, salinity, oxygen saturation, turbidity, chlorophyll a, current speed and direction taken with respect to time, location and depth.

Current measurements recorded during the 6-week deployment period at the Ekofisk and Eldfisk fields confirmed previous investigations that the currents at Ekofisk were influenced strongly by tidal forces. The current direction changed equally between the northeast and the southwest directions with a mean current speed of 0.12-0.15 m/s. The depth of the water column appeared to have no effect on the current direction. This data was used in the DREAM simulations in order to update the prediction on the fate of the PW plume. The simulations showed that the PW plume direction was strongly influenced by the current and changed with the 6 hours tidal cycle between the northeast and southwest directions. This confirmed that the placement of the monitoring stations was suitable for optimal exposure to the PW plumes. However due to the warmer and lighter PW plume entering the colder denser seawater, the plume went to the surface water when released and remained within the top 20 m of the water column. This was despite having deeper discharged depths of 49 m at Ekofisk 2/4 J and between 20 and 40 m at Ekofisk 2/4 M. This information had important implications for the exposure of mussels, scallops and passive samplers within the water column. Items placed on moorings at the deeper depths of 40-45 m would not be expected to be directly exposed to the PW plume. This was confirmed with the bioaccumulations of PAH compounds in mussels from the different depths, which will be discussed in more detail in the following section.

The physicochemical profiles performed during the deployment of the mussel rigs in late March showed that at that time of the year the water column was not stratified, and temperature, salinity and dissolved oxygen remained fairly constant with depth. However, when the profile was taken again when retrieving the monitoring stations in early May, a clear thermocline was measured between 40 and 50 m. This thermocline appeared not to influence the salinity or chlorophyll profile, which remained constant with depth. Chlorophyll sensors were purposefully used in the field to ensure that the mussels placed at the deeper depth of 40 m were not experiencing a shortage of food. In fact, all the chlorophyll measurements showed adequate concentrations of food for the mussels with no obvious differences with depth. Therefore, a difference in food availability was an unlikely confounding factor when observing the health status and biological responses of mussels and scallops at the 40-45 m depth.

Interestingly, the thermocline measured in early May at the Ref2 station was at a depth of around 25 m, much shallower than the 40-50 m thermocline at Ekofisk field. These areas are approximately 50 km apart and are likely to reflect different water bodies, the Ref2 station was also shallower at almost half the depth of the Ekofisk field, which may also influence stratification processes.

The continuous physicochemical data collected for the full duration of the field exposures at selected locations (Ref1 and Station 4 at 20 and 40m) provided important information on the conditions the mussels were experiencing over time. This was used to establish that the conditions experienced by the mussels and scallops at the reference areas were the same as those in the Ekofisk and Eldfisk fields, as well as differences at the two depths (20 m and 40 m). Differences were observed in temperature,

Ref1 rose from 5.5°C to approximately 7°C at 22 m depth, whilst the deeper waters at 48 m remained slightly colder changing from 5.5°C to around 6.5°C. Temperatures experienced by mussels at the Ekofisk field were slightly warmer at the beginning of the exposure than those described at the reference location, starting at approximately 6°C and moving above 7°C towards the end of the exposure. Temperature loggers placed directly on the mussel cages confirmed these temperature differences. Although temperature differences were small, they may have potential impacts on mussel growth and reproductive development, which in turn may influence some of the biological effect parameters that were measured. This will be considered when evaluating the biological effects data.

Chlorophyll and turbidity measurements were also recorded continually at the Ekofisk area (Station 4) and Ref1 during the entire mussel development. Chlorophyll measurements confirmed the presence of food at the two depths where mussels and scallops were placed so that lack of food was not a confounding factor in the assessment of mussel health. Turbidity values appeared to indicate episodes of high turbidity, which were thought to be related to storm events or rough weather conditions during the deployment period.

4.2 Chemical accumulation

4.2.1 Chemical accumulation in mussels

PAH accumulation in mussels positioned downstream from the Ekofisk installation provided some very interesting evidence of exposure to the PW plume and appeared to corroborate the information provided by the DREAM simulations. Highest concentrations of PAH were measured in the whole soft tissue homogenates of mussels closest to the Ekofisk PW discharge outlet and reduced gradually with distance away reaching close to offshore background concentrations approximately 4000 m from the installation. Highest concentrations of Sum PAH in mussels from the two closest stations were approximately 140 ng/g (w.w.). Previous investigations at Ekofisk in 2006, 2008 and 2009, where PAH concentrations were also measured in mussels positioned within the Ekofisk safety zone for 6 weeks (Brooks et al., 2011a) can be used for comparison. In 2006 and 2008, highest Sum PAH concentrations in mussels were approximately 700 ng/g (w.w.), reducing to approximately 300 ng/g (w.w.) in 2009. These values were much higher than the Sum PAH concentration in the current investigation. The PW volumes between these years based on the annual discharge reports indicates that PW volumes have increased from 8 million m^3 in 2006 to 13.5 million m^3 in 2021. Improved treatment systems at Ekofisk have reduced the oil in water concentrations from an annual average of 20-10 mg/L between 2006-2009 to 5 mg/L in 2021, which has resulted in a reduction in the amount of oil discharged to sea.

In previous WCM surveys using mussels, the closest stations 500 m downstream of the installation have recorded much higher Sum PAH concentrations than the maximum concentration of Sum PAH in the current Ekofisk study. These include Statfjord A and B in 2017 (Pampanin et al., 2019), Troll in 2012 (Pampanin et al., 2013) and Gullfaks in 2011 (Brooks et al., 2011b), where Sum PAH concentrations of 450, 1500 and 1600 ng/g (w.w.) were recorded respectively.

The concentrations of PAH EPA16 were approximately 10% of the Sum PAH concentration, with around 90% of the PAH made up of the alkylated NPD compounds measured. This as expected, clearly points to petrogenic sources of oil accumulation from the PW discharge.

In contrast to the Ekofisk mussels, there was no PAH concentration gradient in mussels away from the Eldfisk installation. Mussels caged at 500 m, 1000 m and 2000 m southwest of the Eldfisk platform had almost identical sum PAH concentrations and were similar to those sampled 4000 m from the Ekofisk

installation, as well as both reference stations. The PW discharge at Eldfisk, based on the quantity of oil released to sea was approximately 10% of the Ekofisk discharge. Although it was believed, based on the ocean current data, that the monitoring rigs were suitably placed to ensure exposure of the PW plume to the mussels, PAH accumulations were not elevated above offshore background concentrations.

Metal concentrations were also measured and despite the apparent exposure to the PW plume, as shown with the PAH accumulation, there were no relationships found between metal concentration and proximity to the Ekofisk (or Eldfisk) installations. It appears that the PW exposure did not contribute to metal accumulation in mussels.

The Norwegian classification scheme for selected metal concentrations in mussel tissues have been developed (Molvær et al., 1997; Table 9). Based on the classification scheme, As, Cd, Cr, Pb, Hg, Ni and Zn concentrations were below the lowest classification scheme of I, indicating insignificant levels of contamination. Only Cu concentrations showed 4 values above the insignificant (I) level, with Station 5 40 m, Station 7 20 m and Station 8 20 and 40 m indicating moderate (II) levels of contamination. Since there was no obvious relationship between proximity to the Ekofisk installation and Cu concentrations in the mussels, it is difficult to link the Ekofisk installation as a source of the elevated Cu concentration.

Table 9. The Norwegian classification scheme for the relative risk of metal concentrations present in the soft tissue of marine mussels (mg/kg w.w.) (Molvær et al., 1997).

Metal (mg/ kg w.w.)	Classification (upper limit for class I to IV)				
	Insignificant (I)	Moderate (II)	Marked (III)	Severe (IV)	Extreme (V)
As	10	25	70	140	>140
Cd	0.4	1.8	4	8	>8
Cu	2	6	20	40	>40
Cr	0.2	1	3	10	>10
Pb	0.6	3	8	20	>20
Hg	0.04	0.1	0.3	0.8	>0.8
Ni	1	5	10	20	>20
Zn	40	80	200	500	>500

4.2.2 Chemical accumulation in passive sampler

The range of silicone rubber sampling rates observed during this sampling event (3 - 13 L/d for 500 cm^2 of sampling surface) is generally in agreement with sampling rates obtained for coastal deployments of silicon rubber samplers (Monteyne et al., 2013; Pintado Herrera et al., 2020). Harman et al. (2009a) found sampling rates in the range of 4 - 15 L/d for SPMDs (similar sampling surface area) deployed at this location in 2008. In a specific channel set-up, Glanzmann et al. (2022) found that a mass transfer coefficient for the boundary layer, k_w of 3 - 4 µm/s for a water velocity of 5 cm/s. Considering the water velocity measured at selected stations as part of this study was 12 - 15 cm/s, the use of metal canisters and spider holders used for the deployment of silicone rubber samplers that likely reduce the water

velocity in the vicinity of the samplers, our estimated average k_w of 2.3 µm/s is relatively consistent with water velocities measured in this study. However, a pattern in sampling rates was observed with the lowest Rs found at the reference stations. The highest R_s values were found for exposure stations closest to Ekofisk (Stations 1 - 4) at both depths. It is difficult at this stage to explain these differences conclusively. Water turbulences and velocity may have been lower at reference stations. Direct exposure of silicon rubber samplers to oil in water could potentially enhance PRC dissipation and result in the apparent increase in R_s observed for stations 1 - 4. These are suppositions and more information or data would be needed to confirm either possibility or whether these differences are the result of other unknown artefacts. Sampling rates for POCIS-like devices are lower and expected to be homogenous across all sampling stations. This means that signals from naphthenic acids from non-target screening of extracts from the different samplers from the different stations can be compared.

In general, no major differences in total hydrocarbon (THC) were observed between different stations on the Ekofisk and Eldfisk transects. Slightly higher levels were found for samplers deployed at 20 m depth. These results are perhaps a little surprising considering how close the exposure stations were and that silicone rubber is well suited to the sampling of hydrocarbons. THC amounts absorbed during exposure were relatively similar for all stations and higher concentrations calculated for the reference stations result from the low sampling rates found for these stations. Levels of THC in the range of 0.13-2.3 µg/L estimated here are in line with levels quantified in UK marine waters (Law, 1981). In case the sampling rates found for the reference stations are real, these waters may present levels of contamination above those found in the vicinity of Ekofisk and Eldfisk.

The freely dissolved concentration data for PAH16 showed a similar pattern as for THC with only minor differences in concentrations at the different stations along the transects. Concentrations of PAH16 were clearly higher for the two reference stations. Freely dissolved concentrations of individual PAHs do not vary appreciably between stations. For example, concentrations of fluoranthene in the vicinity of Ekofisk varied in the narrow range of 0.17 to 0.38 ng/L. This range is slightly lower than that reported for previous measurements with SPMDs (Harman et al. 2009a). In that study, significant increases in concentrations of individual PAHs were seen for acenaphthene, dibenzothiophene, phenanthrene, but increases between the sites exhibiting highest and lowest remained well under an order of magnitude. The slightly stronger PAH signal seen at the reference sites may be due to sediment resuspension (i.e. during storm events) and subsequent release of sorbed chemicals. These could also reflect general contaminant levels for this area of the North Sea.

Diagnostic PAH ratios can sometimes be used in a forensic approach to identify possible differences in contamination sources observed at different locations in the environment. The use of PAH ratio based on fluoranthene and pyrene has advantages. These compounds have similar hydrophobicities and their exchange kinetics into passive samplers are similar. Ratios of these compounds are not really influenced by dissolved concentration calculation procedures. They are also not influenced by possible analytical inter-batch variability. Here, it is possible to observe a difference in ratios between the reference and Eldfisk stations and stations 1 - 4, closest to Ekofisk. Diagnostic ratios in the range of 0.93 - 0.96 were found by Harman et al. (2009a) for reference stations and ratios in the range of 0.76 - 0.90 for stations in the vicinity of Ekofisk. This is in line with our finding a decade or more later. These differences in diagnostic ratios can be explained by higher pyrene concentrations for stations 1 - 4, the closest to Ekofisk.

Concentration profiles for pyrene were shown through masses absorbed in silicone rubber and with benchmarking with HCB (Allan et al., 2021). Hexachlorobenzene is ubiquitous in the marine environment and levels at regional level are very uniform. This means we can use masses absorbed or freely dissolved concentrations for benchmarking masses absorbed or concentrations for other

chemicals. Possible effects of the uncertainty in sampling rate and freely dissolved concentration estimation are therefore minimised.

Alkylated PAHs (except for C1-naphthalenes consistently below LOQ) tend to show elevated freely dissolved concentrations in water at Ekofisk locations when compared with reference sites. Estimated concentrations are generally higher than for PAH16. Concentrations of C3-naphthalenes in the range of 10-12 ng/l closest to Ekofisk are in line with previously reported concentrations of 10-21 ng/l (Harman et al., 2009a; Harman et al., 2010). A similar concentration gradient and at similar levels to previously measured away from the Ekofisk platform were found for C2-phenanthrene at both 20 m and 40 m. C2 and C3 dibenzothiophenes were in a concentration range of 0.4 to 1.2 ng/l and were similar to those found with SPMDs and reported in 2009 (Harman et al 2009a). These results are in agreement with previous studies showing that alkylated PAHs tend to form a significant fraction of the dissolved phase contamination seen in the vicinity of PW emission points.

The picture of alkylphenol contamination along the transects at Ekofisk or Eldfisk is unclear. Some alkylphenols such as 4-n-hexylphenol, 4-n-octylphenol or 4-n-nonylphenol are consistently detected at stations 5 - 8. Alkylphenols are generally below limits of quantification with sometimes some relatively high values due to blank levels in the laboratory and the low polymer-water partition coefficients for the least hydrophobic alkylphenols. In general, silicone rubber samplers and sampling rates in the range of 4 - 12 L/d allow to obtain suitable limits of quantification for the more hydrophobic of the alkylphenols. These were not as effectively sampled by POCIS principally because of sorption to the PES membranes (Harman et al., 2009b). The generally low polymer-water partition coefficients for the least hydrophobic alkylphenols result in relatively high limits of quantification and rapid time to equilibrium. In other terms, the concentrations measured for these alkylphenols reflect water concentrations for the last days of exposure, not the entire sampler exposure duration. This is valid for PAHs with log K_{sw} < 4. It may be that the use of the POCIS with a stainless steel-mesh membrane and HLB disc is more suitable for the integrative sampling of these more polar compounds, as was shown for the naphthenic acids.

The sampler that was deployed for sampling naphthenic acids was based on the original POCIS configuration but adapted to: (i) minimise the impact of contaminant sorption to the membrane; (ii) attempt to sample as wide array of naphthenic acids as possible; and (iii) minimise the effect of water turbulences on sampling rates. Contaminant diffusion across the stainless-steel mesh and into the HLB disc is likely to control the rate of naphthenic acid uptake into this sampler, with an expected amount of water sampled in the range of 8 to 12 L. This deployment of samplers showed that it was possible to identify and distinguish naphthenic acid isomers with a number of carbons ranging from 10 to 35. Most of the naphthenic acid isomers however are in the range of 15 to 25 in agreement with past studies of produced waters from Norwegian oil fields (Samanipour et al., 2020; Samanipour et al., 2018). The original version of POCIS was able to demonstrate the presence of naphthenic acid with z values between 2 and 8 and carbon numbers from C7 to C14 (Harman et al., 2014). It is likely that some naphthenic acids sorb to the membrane in this version of the device. Our new version enhanced the distribution of naphthenic acids that could be sampled. Although no clear pattern of distribution of naphthenic acid with distance from discharge points could be seen, station 3 near Ekofisk showed the highest detection frequency together with station 11 near Eldfisk. The source of the signal of naphthenic acids at the reference sites remains unclear.

4.3 Biological effects responses in mussels

To limit the impact of potential confounding factors on the biological responses in mussels exposed to the PW effluent, general parameters, including length of the mussel as an indication of mussel age and mussel speciation, were recorded. Since mussels were selected randomly from a single year class to represent the different groups, differences in biometry between the groups were not expected. In fact, only two mussel groups, Ref 2 20 m and Station 3 40 m, were found to be significantly different from each other. The median length from each group ranged from 51 mm to 56 mm, which were representative of approximately 4 - 6-year-old mussels (Sukhotin et al., 2007).

Since it has been previously shown that differences in chemical bioaccumulation and biological responses to contaminant stress can vary between *M*. *edulis*, *M. trossulus* and *M. galloprovincialis* (Brooks et al., 2015a), it was important to determine the proportion of the three species and hybrids in the mussels used in this study. As expected, the dominant species within the sub-sampled group was the native species *M*. *edulis* accounting for 90 % of individuals. Similar, dominance of *M*. *edulis* in the WCM2017 and also the WCM2012 study, where mussels were first analysed for species identification under the WCM programme, suggest that these studies can be used for comparison and overall mussel speciation was unlikely to be a confounding factor for either chemical accumulation or biological responses in mussels.

The CI of mussels measured in the day zero (T0) and field deployed groups showed some differences, that were not likely to do with exposure to the PW discharge from Ekofisk, but more reflective of natural pressures and seasonal differences. For instance, T0 mussels were found to have the lowest CI of all mussel groups and significantly different to some groups. The most likely explanation for the lower CI in the day 0, was natural season variation, with the T0 mussels measured at the beginning of the exposure in March, whilst the field deployed mussels were sampled in May. Hence, field mussels had 6 weeks of growth and reproductive development with increased seawater temperatures in May. Since CI is measured as the dry weight of the soft tissue divided by the dry weight of the shell multiplied by 100, having an increased meat weight would result in a higher CI in mussels when sampled later in May. Seasonal variations in CI have been previous reported for *M*. *edulis* from the Puget Sound (United States) with a marked increase in CI between March and the peak spawning period of late May to early June (Kagley et al., 2003). Furthermore, season changes in CI for *M*. *edulis* from Scottish coastal waters have been reported (Okumuş and Sterling, 1998). In both these cases, temperature and food availability were thought to be responsible for the change in CI.

Although it was not found to be significantly different, there was a tendency for all the deeper (40 m) mussels to have a lower CI than the 20 m mussels for each station. The apparent lowering of the CI in 40 m mussels was unlikely a response to PW exposure but may reflect the additional pressures on the mussels at deeper depths. Food availability could play a part although chlorophyll measurements during the deployment period confirmed that food was equally available at this depth. Although mussels can survive at deeper depths, it is less certain whether they can thrive. Mussels are not typically found in deeper waters and may be slightly less adapted and/or experience higher energy demands to live there. An additional factor that may influence the CI is the seawater temperature. Measurements taken continuously during the mussel deployment revealed a small difference in temperature (< 0.5°C), with mussels at 40 m exposed to slightly colder water. It is known that mussel growth and reproductive development are strongly influenced by temperature (Okumuş and Sterling, 1998) and may have contributed to the lower CI in the mussels at the deeper depth.

An indication of the overall fitness of the mussels based on the available energy to maintain shell closure and withstand desiccation can be determined with the simple SoS assessment. Overall, the

mussel LT_{50} values were indicative of healthy mussels above the ICES BAC of 10 days in all 20 m mussels, except for those at Station 3. These Station 3 mussels had a LT_{50} just below 8 days between the BAC and EAC values to indicate some stress but compensating. The lack of reduction in the 20 m mussels at the closest stations (Station 1 and 2) to the Ekofisk platform, and mussels with the highest PAH burden, would suggest that the reduction in SoS in mussels from Station 3 was not likely due to exposure to the PW plume.

Incidentally, all Eldfisk mussels had LT_{50} values above the BAC and were indicative of healthy mussels, which also fits with the low PAH accumulation and low exposure to the PW chemicals in these mussels.

It was noted overall that mussels from the 40 m depth had lower LT_{50} values, with 7 out of the 10 mussel groups recording LT_{50} values below the BAC, including one of the reference stations. The reasons for the slight reduction in the general fitness of mussels placed for 6 weeks at 40 m compared to 20 m, maybe similar to the pressures effecting the CI, as discussed above. The fact that mussels are not often naturally found at 40 m depths, may suggest that they are not fully adapted to deeper waters and although have the ability to survive, assuming sufficient food is available, they may face additional stressors that could interfere with energy budgets and general condition. What is clear is that the impacts on CI and SoS in 40 m mussels were not caused by exposure to the PW, since the PW plume was directed towards the surface immediately upon release and had limited impact on the 40 m mussel.

When comparing LT_{50} values in the current study with previous WCM campaigns, similar LT_{50} values (between 8 and 14 days) were found in mussels positioned for 6 weeks at the Statfjord A and B oil and gas field (Pampanin et al., 2019). In addition, mussels transplanted into the Bøkfjord, Norway for 6 weeks to measure the potential impact of a tailing discharge from an iron ore mine showed LT_{50} values between 8 and 12 days (Brooks et al., 2015b). In other coastal studies, mussel SoS values between 6.5 and 8.2 days (Brooks et al., 2021) and 5.6 and 9.8 days (Brooks et al., 2022a) were recorded after mussels were transplanted for 6-weeks into the seawater recipient of two separate aluminium smelters. Overall, the LT_{50} values of the mussels placed in the recipient of the Ekofisk and Eldfisk installations were indicative of healthy individuals and appeared not to be affected by exposure to the PW plume, although SoS is a relative crude effect parameter.

LMS was one of the few biological responses measured in mussels that appeared to show an adverse effect in relation to proximity to the Ekofisk installation. Lowest labilisation times, indicating a stress response, were observed in mussels from the three closest stations to the Ekofisk installation for the 20 m group and 2 of the 3 closest stations from the 40 m group. However, they were only marginally below the ICES BAC value of 20 min, indicating a weak stress response. Mussels approximately 1000 m and more away from the Ekofisk installation recorded LMS labilisation times similar to those from the reference stations and those from Eldfisk, and were all above 30 min. In a similar study, where mussels were placed around the Johan Sverdrup installation for 6 weeks, LMS values between 24 and 35 min were recorded (Brooks et al., 2022b). These values are comparable to the field values at Ekofisk and Eldfisk. In contrast, mussels placed 500 m downstream of the Statfjord A PW discharge showed LMS labilisation times as low as 6 min (Pampanin et al., 2019). This value was below the ICES EAC of 10 min and indicative of severe stress, which would likely lead to impacts on mussel health. PAH bioaccumulations 3 times higher than that measured in the current study were considered to be responsible for the impacts on LMS in mussels from the Statfjord A study.

MN frequencies were above ICES BAC values (2.5 MN per 1000 cells) in all field stations, with only T0, R1 40 m and R2 20 and 40 m recording below background levels. Although the field exposed mussels at both 20 m and 40 m were not significantly different from each other, all groups demonstrated a

reasonable level of genotoxicity with Ekofisk mussels ranging from 8.7 to 4.2 MN/ 1000 cells. In comparison, the frequency of MN was lower in the Eldfisk mussels ranging between 3 and 5 MN per 1000 cells. Despite the ICES BAC of 2.5, typical baseline levels for mussels between 0.5 and 5 MN per 1000 cells have been suggested (Bolognesi and Fenech, 2012), which would categorise the Eldfisk mussels within background levels.

For comparison, mussels placed 500 to 2000 m downstream of Statfjord A and B installations showed MN frequencies between 2.6 and 10.2 MN per 1000 cells (Pampanin et al., 2019). A range of values that overlap the MN frequencies in mussels from the Ekofisk field in the current study. Overall, genotoxicity was experienced in all field exposed mussels from the Ekofisk field and to a slightly lower extent in mussels from the Eldfisk field compared to the reference groups. Advancements in the automated scoring of MN in mussel haemocytes has progressed with recent WCM R & D activities (Gomes et al., in prep.). The labour-intensive manual scoring system, currently used, will be superseded by an almost completely automated image analysis system. This will enable more cells to be counted from individual mussels (and other species including fish) and will reduce the operator bias in manual scoring of MN frequencies. This will increase the overall quality of the data and improve the ability to differentiate between mussels experiencing different levels of genotoxic exposure.

The mussels were analysed for the presence of lesions in their selected tissues, as a potential indication of damage at a higher level of biological organisation. Histological lesions in mussels are widely used as biological effects markers and recommended for environmental monitoring as they are endpoints for the evaluation of chemical contaminants that relate to the health and fitness status of individuals (Howard, 2004; Yancheva et al., 2016; Beyer et al., 2017; ICES, 2021). Histopathological evaluations of tissues can also be used to detect early signs of disease and predict harmful effects. For example, histological lesions found in gonads of mussels may indicate potential impairment of their reproductive capacity, while gill and digestive gland lesions indicate harmful effects, which may compromise survival and fitness of affected individuals (Balk et al., 2011).

Digestive epithelium is composed of two types of cells, digestive cells and basophilic cells. Digestive cells take up material through pinocytosis and digest it within vacuoles, while basophilic cells synthesise and secrete enzymes into the lumen for extracellular digestion (Cajaraville et al., 1990). Digestive glands also play an important role in the storage of metabolic reserves, which are used as a source of energy during gametogenesis or periods of physiological stress (Purchon, 1976). Epithelial degeneration, a disintegration and subsequent absence of digestive and basophilic cells within digestive epithelia, was observed in field exposed mussels although no clear relationship to the discharge outlet at Ekofisk or Eldfisk was observed and no significant differences between the mussel stations were found. When exposed to contaminants, digestive tubules can present some degree of tissue breakdown, provoked by the release of hydrolytic enzymes. It has also been demonstrated that regression of digestive tubules can be an indicator of a “reconstituting phase” as an attempt to restore the detoxification potential of digestive glands (Stara et al., 2020).

Lesions were measured using a traditional approach with a specialist reader recording either presence or absence or using severity scales between 0 - 2. However, a more sensitive evaluation of histological parameters can be achieved by using whole slide readings in an automated way. This approach to histological scoring is under development and is proposed for future WCM programmes to secure more accurate evaluations.

Parasites of the digestive gland were equally present amongst all mussel groups and no differences were found with proximity to the installations or between 20 m and 40 m mussels. The identification

of the specific parasite might help differentiate between stations and drawing conclusion about implication for the organism health, although this was not performed in the current study.

Changes in gonadal tissue were also evaluated, as contaminants in PW may impair or even prevent gametogenesis, impacting the reproductive potential of a whole population (Auffret, 1988). ADP tissue was significantly lower in mussels before the deployment (T0) in comparison to Station 1 and 2 at 20 m and Ref1 at 40 m, showing an impact of PW exposure. Significant differences were also observed in gonadal stage, where significantly higher stages were found in T0 and Station 11 mussels compared to Ref1 and Station 2, and between Station 6 and Station 2. It is important to be aware that mussel developmental stage can also be influenced by season, food availability and depth (Beyer et al., 2017) and all of these factors must be taken into account when assessing the developmental stage of the mussels.

As for most of the gonad histology markers, there was no significant differences found between the groups and it was therefore assumed that the level of PW exposure did not impair the reproductive ability of mussels in the study. In fact, the dominant gonadal stage in all stations were 3 and 4, indicating a well-developed gonad ready for a typical mid-May spawning.

Mussel gills are associated with a variety of essential functions including respiration, filter-feeding, nutrient uptake, blood haematopoiesis, and bacterial symbiosis (Gosling, 2003). As one of the first lines of defence against contaminants and xenobiotic compounds, gills often reflect adverse effects through histological changes and abnormalities (Au, 2004; Beyer et al., 2017). Morphological changes are considered to be early adaptations to xenobiotic exposures in gills that can alter uptake pathways or compensate for the impaired capacity of feeding and gas exchange processes (Pagano et al., 2016). Despite this, there were no significant differences observed for any of the gill histological markers between mussels from the various stations, and the level of PW exposure appeared not to cause any measurable response. As described for the digestive gland, a more sensitive evaluation of the gill lesions may be obtained using an automated scoring system. Such a system is currently under development and will be proposed for future WCM programmes.

Overall, it could be argued that a six-week exposure to moderate/low levels of PW exposure was not long enough time to develop high order histological alterations. Additionally, mussels placed offshore were provided with optimal growth conditions, which can lead to developing better overall health then pre-transplanted mussels, demonstrated with improved CI in field mussels compared to the day zero (T0) group.

4.4 Chemical accumulation and biological responses in scallops

Scallops were added to the sampling program at four stations as an additional trial species. They were selected as they are depth appropriate for the 40 m depth deployments, are self-sustaining in terms of feeding, and they provide ample tissue for multiple biological effects measurements from each individual animal. Mussels were also deployed at each of these four stations at 40 m depth and so provide a direct comparison to the results generated by scallops for chemical accumulation and biological responses.

Measurement of PAHs in scallops, both PAH EPA16 and total PAH, showed low levels at all stations, with no individual station showing a higher value than that recorded in samples taken from scallops prior to the deployment (T0). Similar results were recorded for total NPD analyses. Measurements in mussels at 40 m depth showed a lower level of PAH EPA16 than the scallops, but as with scallops these

did not show any clear differences between the T0 samples, the reference sites, and the stations close to the outfall. Total PAH concentrations measured in mussels at 40 m are generally similar to those measured in scallops and indicates low exposure to the PW plume.

Four of the 15 metals measured in scallop tissues did show a significant difference among sites when compared against T0 concentrations. These were relatively small changes and of these, three of the metals recorded lower than T0 values with only molybdenum showing an increase. As with the mussel analyses, there was no clear association found between metal concentrations and the distance from the discharge outlets. When scallops and mussels from the same deployment depth at Ref1 were compared there were marked differences in some analyses with increased concentrations of Ag, As, Cd, Fe, Mn and Mo found in the scallop tissues. This may reflect differences in the size of tissue mass and age of the two species.

Biological responses measured in scallops included CI where all deployment stations recorded very similar values indicating no relationship with distance from the platform discharge point. The day zero (T0) scallop CI value was significantly lower than that of the deployed animals and likely reflected the seasonal availability of food. CI values for mussels deployed at the same stations were similar to the scallops. LMS analyses showed similar values throughout the stations, with station 3 recording the lowest value. All stations showed reduced values when compared with the T0 value. The reason for this is unclear. LMS values from mussels sampled at the same stations were broadly similar, with station 3 also showing the lowest value of deployed animals, but here the T0 mussels recorded the lowest value of all. MN analysis showed some variation in deployed scallops among the stations, though there was no indication of a gradient in these responses to suggest it was related with proximity to the discharge source. In general, the MN count was higher in scallops than it was for mussels. For mussels deployed at the same stations there was an indication that proximity to the discharge could have driven the increases in micronuclei observed.

The successful use of scallops as an additional species in this monitoring program proved that this bivalve could be effectively collected, transported, and deployed for six weeks. There was no mortality during deployment and the improvement observed in CI confirmed they were able to feed and increase in weight when caged at sea. All required tissues were collected and analysed. If future monitoring programs require caging of a depth appropriate bivalve, then scallops can perform this role. The size of tissue mass available from each individual serves to reduce the number of animals required to complete the planned assays which is in line with the recommendations of the authorities overseeing the scientific use of animals.

4.5 Chemical exposure and biological effects responses in fish

PAH compounds are rapidly metabolised in the liver of fish during phase I and phase II metabolism where the PAHs are made more water soluble (Meador et al., 1995). The resulting PAH metabolites are stored temporarily in the bile prior to excretion. PAH liver concentrations in fish therefore represent relatively recent exposure to PAH compounds (Varanasi et al., 1989). Elevated sums of PAH liver concentrations were found in dab from the Ekofisk safety zone, compared to the PAH liver concentrations in dab from the Ekofisk region and Egersundbank region. PAH concentrations in dab liver were higher than those observed in the other species, even in cod specimens also collected from

within the Ekofisk safety zone. It was noted that approximately 90 % of the sum of PAH was made up of alkylated NPD compounds strongly indicating exposure to petroleum sources.

The higher concentrations of PAH in dab may reflect its habitat. Since dab are flatfish and the only true benthic fish species used in the study, they live on the sea floor and are exposed to environmental chemicals through diet, feeding on benthic invertebrates and small fish, and direct contact with contaminated sediment (Hinz et al., 2005). Offshore installations such as Ekofisk, which with over fifty years of oil and gas production and well development, has amassed significant quantities of drill cuttings on the seafloor, as well as leakages from well deposits and the sedimentation of particles from the PW discharges. It seems reasonable to expect therefore why the sediment dwelling dab were more likely to experience exposure to PAH contaminated sediment in and around the Ekofisk safety zone. Furthermore, dab do not move around like pelagic fish (Rijnsdorp et al., 1992) and therefore contaminant concentrations in their tissues are more representative of exposures in the area from which they were collected.

This was the first time PAH has been measured in fish liver within the WCM programme and therefore comparisons between earlier investigations were not possible. However, PAH concentrations were measured in species of grouper collected throughout the Gulf of Mexico between 2011 and 2017 in order to provide information in the aftermath of the Deepwater Horizon oil spill (Pulster et al., 2020). Mean concentrations of PAH in the liver of groupers ranged between 400 and 5010 ng/g (w.w.) from 584 sampled fish. Additionally, PAH liver concentrations in the deep-water benthopelagic hake (*Urophycis* spp.) measured in the Gulf of Mexico between 2012 and 2015 were between 50 and 150 ng/g (w.w.) (Struch et al., 2019). These studies indicate large species differences in PAH liver accumulation, although both studies reported higher PAH liver concentrations than our study, where median concentrations ranged between 10 and 70 ng/g (w.w.).

Due to the rapid metabolism of PAH in fish tissues, including the liver, the measurement of PAH metabolites in the bile of the gall bladder can serve as an additional approach for assessing environmental PAH exposure in fish (Beyer et al., 2010). The measurement of PAH metabolites of naphthalene, phenanthrene and pyrene were highly variable for all species. Detection of the PAH metabolites in the fish bile showed exposure of all fish to PAH compounds. However, it was not possible to specify the level of exposure based on the metabolite data.

The large variation in PAH metabolites can be dependent on the natural feeding behaviour of the fish species and the feeding state of the individual fish when sampled (Brumley et al., 1998). For example, it is known that during periods of starvation the amount of PAH metabolites in bile increases. When the fish starts to feed again bile fluid is released into the intestine and the gall bladder becomes empty. Shortly after feeding the gall bladder is refilled with water resulting in diluted bile fluid. Therefore, the concentration of PAH metabolites can vary markedly depending on food availability and whether the sampled fish have recently consumed food. Measurements of the bile pigment biliverdin has been suggested as a means to normalise for this filling and emptying of the gall bladder during feeding (Richardson et al., 2004). However, biliverdin was not measured in the current programme and could not be used to normalise the PAH metabolite data.

In addition to PAH exposure in fish, the presence of PFAS in blood samples of collected fish were measured to determine whether the Ekofisk installation as a whole was acting as a source of PFAS into the North Sea. Of the 36 PFAS targeted, only 14 were measured above detection limits, and since 2 or these 14 PFAS were only detected in one sample, essentially 12 PFAS were detected (PFOA, PFNA, PFDA, PFUdA, PFDoDA, PFTrDA, PFBS, PFOS, br-PFOS, PFOSA, PFBSA and PFHxSA).

PFOSA was the most dominant PFAS compound detected, although PFOSA was below or just above the LOD for dab and haddock, median concentrations of PFOSA in cod and whiting ranged between 5 and 15 ng/ml. PFOSA was also the most common PFAS compound measured in fish blood from previous WCM campaigns in 2013, 2014 and 2017. PFOSA concentrations were highest in ling (*Molva molva*) from reference locations up to 53 ng/ml and 128 ng/ml for WCM2013 and WCM2014 respectively. In WCM2017, PFAS was only measured in cod and saithe where PFOSA concentrations ranged between 6 and 15 ng/ml, a very similar concentration range as measured in cod and whiting in the present study.

Overall, the current study at Ekofisk was in good agreement with the earlier WCM investigations that measured PFAS in fish blood, in that offshore oil and gas installation did not appear to significantly contribute to PFAS load in local fish populations. Long range transport of these highly persistent compounds has made them ubiquitous in the marine environment and they are therefore detected equally in offshore reference fish as those caught in the vicinity to offshore installations.

Biological responses in fish

A range of supporting parameters in fish were measured in order to assist in the interpretation of the biological effects data and to ensure that any confounding factors (i.e., size, sex, age, reproductive status) that could influence the biological effects responses were identified. Although biometric differences were expected between species it is helpful for comparative reasons for within species difference to be kept to a minimum. However, with the differences in fishing methods and the time restraints in working offshore, it is often necessary to sample what is available rather than being able to select a specific size category. Observations of fish length and weight detected within species differences for cod, dab and whiting, which were mostly due to the age of the different cohorts. Older cod were sampled from the Egersundbank (2 years) compared to Ekofisk (1 year), whilst older dab individuals were sampled from Ekofisk (6 years) compared to both Ekofisk region and Egersundbank (5 years). Whiting from Ekofisk region (2 years) were younger than those from Egersundbank (3 years) and Vikingbank (4 years). These differences may have implications for at least some of the biological effects responses and will be considered in later discussions.

As a simple comparison, cod, haddock and whiting were also the monitoring species used in WCM2017 at the Statfjord A installation and two regions of the North Sea including Tampen and Egersundbank (Pampanin et al., 2019). In that study much older cod (2.7 – 6.7 years), haddock (2.5 – 4.9 years) and whiting (4.0 - 6.1 years) were sampled.

Despite the differences in age there were no significant differences in the CI for each individual fish species. The CI provides a simple measurement of individual fitness, which can be influenced by natural factors such as food availability and temperature. Interestingly, when comparing the CI between species, whiting from all populations sampled remained slightly lower than cod and haddock. This finding was also observed in WCM2017 and was likely to reflect the general difference in body shape between the species, with whiting exhibiting a slightly lighter and longer body shape compared to haddock and cod rather than any overall impact on fitness.

The LSI can provide an indication of the nutritional status of the fish. Within species differences were found in dab, where a significantly higher LSI was found in dab from Egersundbank compared to Ekofisk, and a significantly higher LSI in haddock from the Ekofisk region than both Egersundbank and Vikingbank. A higher LSI would indicate a heavier liver relative to body weight. In controlled feeding studies, differences in macronutrient contributions to the diet were found to have significant effects on the LSI, with high protein high carbohydrate diets increasing LSI and high protein low carbohydrates reducing LSI in gilthead sea bream (Metón et al., 1999). In addition, periods of starvation were found

also to decrease LSI values. The present study showed differences in nutritional status in haddock and dab populations, which may indicate differences in food availability and food sources in the different regions.

The GSI provides an indication of the reproductive status of the fish with a large GSI indicating a more developed gonad relatively to body size. GSI values for males and females were markedly different for some of the fish species such as dab and whiting, indicating that the female dab and whiting were sexually mature. However, there were no within species difference with respect to the different populations for either male or female fish, and reproductive status was unlikely to be a confounding factor in the interpretation of the biological effects data.

Significant responses in EROD activity were found in dab from both the Ekofisk and the Ekofisk region compared to the Egersundbank. EROD activity is a measure of the CYP1A enzyme activity in fish and is well documented to be induced by exposure to PAH compounds and associated with phase I biotransformation processes (Stagg et al., 1995; Aas and Klungsøyr, 1998). This shows a good relationship between increased EROD activity and elevated concentrations of liver PAH and PAH metabolites in dab from Ekofisk and Ekofisk region. A similar relationship between PAH liver concentration and EROD induction has also been demonstrated in dab measured around offshore oil and gas installations in the British sector of the North Sea (Stagg et al., 1995). Interestingly, much higher EROD activities between 1000 and 4000 pmol/min/mg protein were reported, which were 5 to 10-fold higher than that measure in dab in the present study. These differences may be due to differences in methodology, since the current study used the adapted microplate version of the method, which is a more high-throughput approach and although able to differentiate between the groups, may have resulted in lower activity levels than the traditional approach of Stagg et al. (1995). Furthermore, sex difference in EROD activity in dab were reported in this study with approximately 5 times higher activity in males than females (Stagg et al., 1995). Similar differences in EROD activity in dab were found in our study with higher activities found in male dab, although only a 2-to-3-fold increase was observed. Interestingly, both male and female dab showed a similar profile in response with respect to the different locations, with EROD activity highest at the Ekofisk region, lower at Ekofisk and lowest from the Egersundbank for both males and females. Consequently, differences in EROD activity between males and females although acknowledged, were considered not to influence the main outcome of the investigation. However, this does highlight the importance of controlling for sex differences in biological response in field surveys, particularly when EROD and also CYP1A are measured. In the current WCM programme, gender was recorded in the individual fish so that confounding effects could be accounted for. The study assumed a normal distribution of male and female in the population and in most cases a reasonable division of males to females was achieved. However, in dab this was not optimal at all locations with a ratio of 5:25 and 23:7 males to females sampled from Egersundbank and Ekofisk, respectively. Although in many fish, sex is only confirmed following internal examination, in dab sex can be identified from the size and shape of the gonad externally. Therefore, it is possible to provide a 50:50 sex ratio for all locations and this is strongly advised when dab are used in future investigations.

Impacts of other confounding factors on EROD activity have been well documented, including temperature, reproductive status, and season (Sleiderink et al., 1995a; Lange et al., 1999; Kammann et al., 2005). Although the present study was designed to reduce these factors, differences in reproductive status and temperature may have had some influence on EROD. However, temperature differences between the locations based on the temperatures measured on the mussel monitoring rigs would only suggest a less than 1°C difference and unlikely to have a major impact. In addition, no significant differences in GSI were found between the different locations for male or female dab, which may indicate similarities in reproductive status. Confirmation of reproductive status through gonad

histology would have been useful in this context. With regards to seasonal variations in EROD activity, the fish sampling at Ekofisk, Ekofisk region and Egersundbank (as well as Vikingbank) took place within 2 weeks of each other to limit any influence of seasonal effects.

CYP1A protein concentrations measured with ELISA showed elevated concentrations in CYP1A in dab collected from the Ekofisk installation and Ekofisk region compared to the Egersundbank region and supported the findings of CYP1A activity levels measured with EROD in these fish species. It is well documented that CYP1A is induced following exposure to planar compounds including PAHs (Whyte et al., 2000). The close relationship between increased CYP1A protein concentration, enzyme activity (EROD) and PAH liver concentration in dab supports this relationship and shows an exposure and response to PAH in dab populations living in areas of oil and gas activity compared to the reference population. The other fish species (cod, haddock, and whiting) overall showed low PAH concentrations in the liver and low CYP1A enzyme activity (EROD) and low CYP1A protein concentrations as a result. As reported for EROD, sex differences in CYP1A protein concentration were found in dab with highest concentrations in males than females. As described for EROD, the relative CYP1A concentrations were 2-to-3-fold higher in males than females from the respective locations. As also found for EROD both male and female dab showed a similar profile in response with respect to the different locations, with CYP1A concentrations highest at the Ekofisk region, lower at Ekofisk and lowest from the Egersundbank for both males and females. Similar differences in CYP1A protein concentrations have been found between male and female dab (Sleiderink et al., 1995b), which highlights the importance of considering gender and optimising sex ratios in sampling protocols. Some other confounding factors (i.e., temperature, reproductive status, and season) described above for EROD are also relevant for CYP1A protein measurements. The impact of these confounding factors where minimised in the study design as described.

Unfortunately, CYP1A gene expression was not performed in dab, so the results of EROD activity and CYP1A protein concentrations in dab could not be compared to the gene expression. CYP1A gene expression was measured in cod and haddock. Although no differences were observed between the cod populations, significant differences in haddock populations were observed with significantly higher CYP1A expression in haddock from the Ekofisk region compared to the Egersundbank and Vikingbank.

The level of DNA damage and genotoxicity experienced by the fish in the study was assessed by both DNA strand breaks in blood cells, using the comet assay, and the frequency of DNA adducts in fish liver and intestine samples. Significant levels of DNA strand breaks, quantified as % comet tails, were higher in both dab and cod collected from the Ekofisk safety zone compared to their reference populations. Median comet tails at Ekofisk were 24% in cod and 16% in dab. Compared to previous WCM surveys where comet was measured in the same fish species, cod collected from the safety zone of the Statfjord A installation showed significantly higher % comet tails (median 15%) compared to Egersundbank and Tampen regions (Pampanin et al., 2021). The % comet tails previously measured in dab from several location in the North Sea and the coast of Iceland showed median values of 30% from the Firth of Forth, Scotland, 10% from the German Bight and from Ekofisk, whilst baseline levels of 2% were found in dab from Iceland and Dogger Bank (Hylland et al., 2017). The median comet tail of 10% at Ekofisk was comparable to that measured around the Ekofisk platform in the current study (16%).

In earlier WCM programmes where comet was used (WCM2013 and 2014), high background levels of % comet tails were found in all groups, which was thought to be due to the difficulties in applying this method offshore, particularly since this was the first time the comet assay was adapted to the offshore scenario. Improvements in the comet assessment in how samples were preserved and stored prior to electrophoresis in the laboratory have reduced the background levels of DNA damage. Incidentally the background levels of % comet tails recorded in the current study were considered low. However, since

all fish groups had median % comet tails above the ICES BAC of 5% indicating genotoxicity, it does raise the question whether methodological challenges are partly responsible for some of the DNA damage and whether the ICES BAC is suitable for offshore field observations when time between sample preparation and electrophoresis is unavoidable.

The measurement of DNA adducts in fish liver samples did not support the results of DNA strand breaks (comet) and there were no differences found with overall low concentrations of DNA adducts in cod and dab from all locations including the Ekofisk safety zone. Interestingly, haddock were found to have slightly elevated concentrations in Egersundbank and Vikingbank regions, although median DNA adduct levels were still on or just above the ICES BAC of 3 per 10^9 nucleotides. The DNA adducts in the intestine samples of the four fish species were also low and did not differentiate between the populations. There is a tendency for DNA adducts concentrations to increase with age partly due to the potential increase in exposure to DNA damaging contaminants and the reduced ability to repair damaged DNA with age (Akcha et al., 2004). Despite differences in age between populations of dab, cod and whiting, there was no obvious impact of age on DNA adducts. The significant differences observed in haddock were not related to differences in age.

DNA adduct analysis in environmental monitoring using the method of ^{32}P post-labelling has become difficult to perform due to the scarcity of European laboratories that perform this technique. In previous WCM campaigns of 2013, 2014 and 2017, a French laboratory ADnTox carried out the work. However, this laboratory was no longer operational for the current investigation and help was provided by the Institute of Experimental Medicine AS located in Prague, Czech Republic. As a quality control step and to provide information on the comparability of the method adopted by the new laboratory to that of the French laboratory, samples from the WCM2017 campaign that were stored at -80°C were also analysed by the lab from the Czech Republic (Table 10). Despite the higher levels of DNA adducts measured in the nine samples in 2017, only four of the nine samples detected measurable concentrations of DNA adducts. In those four samples that had measurable concentrations of DNA adducts, the levels were between 2 to 20-fold lower than those previously measured. This makes comparison to previous studies difficult.

Table 10. Comparison of the relative adduct levels measure in the same fish liver samples by AdnTox in 2017 and the Institute of Experimental Medicine in 2021 by ^{32}P post labelling. (n.d. not detected).

Fish liver sample	Relative adduct level (RAL) x 10^{-9}	
	WCM2021	WCM2017
Cod	0.69	10.3
Cod	n.d.	16.3
Saithe	n.d.	13.8
Saithe	n.d.	24.2
Whiting	n.d.	12.5
Whiting	n.d.	13.1
Haddock	1.45	18.3
Haddock	26.92	47.1
Haddock	3.17	72.6

When considering the WCM2017 results where DNA adducts were measured in cod, haddock and whiting, haddock and whiting recorded mean adduct levels of 4.9 and 3.6 per 10^9 nucleotides at the Statfjord A installation respectively, whilst cod had only 1.2 per 10^9 nucleotides (Pampanin et al., 2019).

These levels were comparable to those measured in the current study despite the apparent differences in sensitivity showed by the control samples.

Intestine DNA adduct concentrations were also measured in WCM2017 where much high levels of DNA adducts were found including median concentrations of 27 and 18 per 10^9 nucleotides for cod and ling at Statfjord A respectively. These values were much higher than the values reported in the current study and may be related to methodological differences between the laboratories performing the analysis in the different studies as discussed earlier.

Neurotoxicity measured through the inhibition of AChE in fish fillet showed no impact on fish from the Ekofisk installation. Conversely, a relatively higher neurotoxic response was observed in cod from Egersundbank compared to the Ekofisk platform and also in haddock from Egersundbank and Vikingbank compared to the Ekofisk region. Inhibition of AChE has been reported following exposure to environmental contaminants including pesticides, organophosphate and carbamate (Galgani and Bocquene, 1990) as well as heavy metals and PAHs (Kang and Fang, 1997). With respect to PAH exposure, 3-ring PAHs and above were thought to be responsible for inhibiting AChE activity in controlled studies with the electric eel (Kang and Fang, 1997). The lack of AChE inhibition from the Ekofisk safety zone populations of cod and dab compared to their comparative regional populations, would suggest that these fish were not exposed to high enough concentrations of 2-3 ring PAHs that would cause an inhibition of AChE activity.

Effect directed analysis (EDA) reported two compounds in PW (butylated hydroxytoluene and 4-phenyl-1,2-dihydronaphthalene) that were responsible in causing inhibition of AChE activity (Froment et al., 2016). Interestingly, *in vitro* experiments revealed both AChE inhibiting and AChE stimulating compounds in PW (Holth and Tollefsen, 2012). Unidentified aromatic compounds in the oil/particulate fraction were found to be responsible for inhibition of AChE, whilst polar compounds in both the oil/particulate and water-soluble fractions of the PW caused stimulation in the electric organ of the Japanese eel (*Electrophorus electricus*). The composition of the PW appears to be therefore important in the resulting effect on AChE, with a potentially cancelling out effect. Such a cancelling out effect cannot be ruled out in the present study.

Histological endpoints were measured in the liver of fish from the Ekofisk safety zone and three regions of the North Sea. Only hepatocyte vacuolisation measurements in haddock and whiting showed statistically significant differences between groups. This measurement has been previously associated with exposure to contaminants, including microplastics (Jacob et al., 2022). Haddock from the Ekofisk region had significantly higher frequencies of hepatocyte vacuolisation compared to the those caught at Egersundbank and Vikingbank. In contrast, whiting from Vikingbank had significantly higher frequencies of hepatocyte vacuolisation compared to whiting from Ekofisk region and Egersundbank. This may be due to the different exposure routes, as haddock is a demersal fish species, which lives in contact with the sediment and can be exposed to contaminants present in this matrix, as for example cutting piles. While whiting, a benthopelagic fish, is more mobile and becomes exposed through the diet or from the water column.

In general, when comparing the present results with the WCM2017 findings, histological lesions were not significantly present in liver of fish caught in Ekofisk region, Egersundbank and Vikingbank. Although no significant differences were found, it is important to continue to assess histological lesions when monitoring the ecosystem, as these measurements carry a higher biological meaning than molecular and biochemical markers.

4.6 Integrated assessment

The methods selected for the integration of the responses in mussels and fish included the integrated biological response (IBR/n) and the principle component analysis (PCA). The IBR/n was able to combine the biomarkers results to provide an overall score of impact. As stated earlier, the position of the biomarkers can influence the biomarker score, which has led to authors devising an alternative calculation to include all possible combinations and provide a mean IBR/n score for each group (Devin et al., 2014). Alternatively, it has been indicated that placing biomarkers that are measuring similar responses, such as oxidative stress, genotoxicity, cellular toxicity, whole organism responses and histology should be placed together to provide a more representative response (Broeg and Lehtonen, 2006). This approach of arranging similar biomarkers together was selected in the current study. Although the IBR/n is accepted as a useful tool in biological effects assessments, it is important to understand that it is an oversimplification of a very complex environmental exposure. It is therefore important not to over-interpret the results of the IBR/n but use it to provide guidance on the general impacts following environmental exposure that may require further investigation.

For the mussels, seven biological responses were used in the calculation of IBR/n, which were arranged in terms of whole organism responses (CI and SoS) cellular responses (LMS, MN) and tissue level responses (DG, gill and gonad histology). Using a high number of endpoints provides the IBR/n with an increased robustness with not one biological response having too much influence over the IBR/n score. As discussed earlier for the mussels, bioaccumulation of PAH, which is considered to be the most toxic component of the PW effluent, did show a profile away from the Ekofisk complex but the PAH concentrations were much lower compared to previous WCM campaigns. Due to this low PAH exposure the biological responses were also generally low. This was reflected in the IBR/n values that did not appear to show a clear pattern of effect. However, when plotting the mussel IBR/n scores with distance from the Ekofisk complex there was a weak trend observed (Figure 78). Overall, similar IBR/n scores were found in mussel within 1000 m of the Ekofisk installation and reduced in mussels 2000 m and 4000 m away.

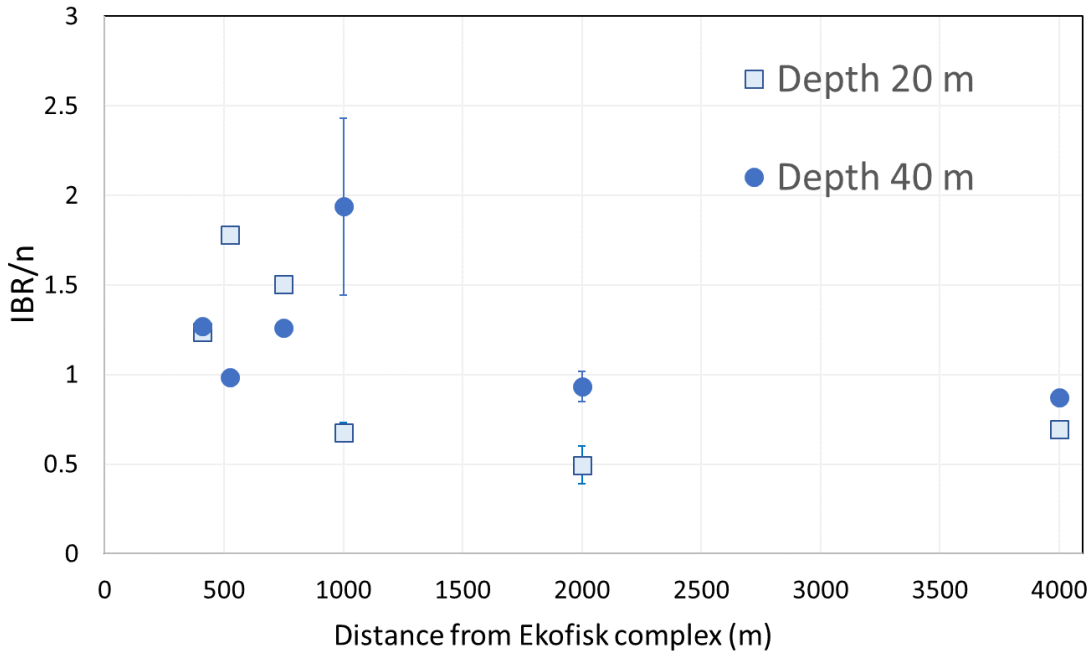


Figure 78. IBR/n scores in mussel groups with estimated distance from the Ekofisk complex.

The PCA was employed to separate the main variables responsible for the differences in the chemical accumulation and biological effects in the mussels from the different groups. The PCA revealed a strong relationship between PAH accumulation in mussel tissue and distance from the Ekofisk complex for the 20 m mussels. This was not shown in the 40 m mussels and reflects the low exposure of the 40 m mussels to the PW plume. A weak but significant positive correlation was found between MN and Sum NPD concentrations, whilst negative correlations were found between PAH (EPA16, Sum PAH, Sum NPD) and LMS. This indicates that increasing PAH concentrations in mussel tissue causes a decrease in LMS and to a lesser extent increase in MN and this occurs more in the mussel groups closer to the Ekofisk installation at the 20 m depth.

Integration of the biological responses in fish using the IBR/n showed a clear differentiation in response between the fish from the different locations. In addition to the biomarker data that were available for all groups (i.e., AChE, EROD, CYP1A, DNA adducts, Liver histology), PAH concentrations of the liver were also included due to the important relationship between PAH exposure and CYP1A induction. Cod and dab from the Ekofisk safety zone showed highest IBR/n scores than all groups including their reference Egersundbank populations, indicating that the Ekofisk safety zone is having some impact on fish health. The biomarkers responsible for the higher IBR/n in cod where different to those in dab. Ekofisk dab had the highest IBR/n score with CYP1A, EROD and PAH liver contributing most. The elevated IBR/n in dab from Ekofisk region was mostly due to CYP1A and EROD but with no contribution with PAH liver. This was because PAH concentration in dab liver from Ekofisk region were low, although higher levels of PAH metabolites were found in this species group. Due to the patchy distribution of PAH metabolite measurements, they were not included in the IBR/n calculation. DNA adducts also contributed to the IBR/n of dab from Ekofisk and Ekofisk region, despite DNA adducts showing low concentrations in the individual biomarker data and highlights the caution that should be taken when interpreting IBR/n output.

The PCA was able to separate the main variables responsible for the variance of chemical body burden and biological effects measured in the fish populations. Clear spatial separation between fish populations were observed, particularly dab from Ekofisk and Ekofisk region compared to the Egersundbank population as well as Ekofisk cod compared to Egersundbank cod. The main factors responsible for the separation in the dab populations include PAH concentrations of the liver, particularly for Ekofisk dab and CYP1A, EROD and AChE for dab from the Ekofisk and Ekofisk region. The PCA supported the findings of the IBR/n, with strong positive correlations found between PAH liver concentrations and EROD activity and CYP1A protein concentration. Positive correlations were also found between DNA adducts and EROD/ CYP1A activity.

Overall, the integrated data clearly point to dab collected from the Ekofisk safety zone and dab from the Ekofisk region to be distinct from the other populations. Chemical concentrations in PAH liver and PAH metabolites showed that these dab populations were recently exposed to PAH compounds prior to sampling and were accompanied with elevated EROD and CYP1A activity levels, which would be expected with such an exposure. However, despite the measurable PAH exposure and clear cellular responses described, higher level orders of biological response such as histological changes in the tissue of the dab were absent.

4.7 Sediment trap material

The formation of ROS was monitored after exposing RTgill-W1 cells to the sediment extracts (5 – 120 mg eQsed/ml) for 60 min. The results showed that Station 5 and 6 produced the highest ROS levels in RTgill-W1 cells. As previously shown, heavy metals, as well as other environmental compounds, are

known to promote ROS production (Andersen et al., 1996; Kljaković-Gašpić et al., 2010). As described in Goksøyr et al. (2021), the presence of high levels of ROS-forming agents in sediment extracts can be linked to the presence of contaminants including PAHs. The high concentrations of PAH detected in the sediment trap material were therefore considered to be the contaminants most responsible for ROS production and were capable of causing significant increases in ROS production even at the lowest concentrations (10 and 20 mg eQsed/ml) for Station 5 and 6.

EROD activity, a catalytic measure of CYP1A induction, is one of the most widely used biomarkers in fish biomonitoring studies. It is a marker of exposure to AhR agonists (such as TCDD, dioxin-like PCBs, PAHs, and others), but also a biochemical response that could precede adverse effects at various levels of biological organization (Whyte et al., 2000). CYP1A induction has often been correlated with the concentration of PAHs in marine sediments (Traven et al., 2008). Bell-shaped dose-response curves were found for EROD activity following exposure to the sediment extracts from all sediment traps. A similar shaped dose-response curve has been reported in several studies for typical EROD activity and has been attributed to overlapping effects of inducing and inhibitory compounds (Bols et al., 1999; Bosveld et al., 2002; Brack and Schirmer, 2003). In addition, the obtained results are comparable to those obtained by Blanco et al. (2018), showing that a significant response in the activity of EROD is due to the presence of a significant amount of AhR agonists in sediment collected from all sediment trap extracts.

Based on these two *in vitro* assays and on the results of PAH concentrations, station Ref2 was the one with the highest concentration of PAH, as also evidenced by EROD activity, while at Station 6, despite a lower concentration of PAHs was found to have comparable EROD activity to Ref2 and was the most affected site. Combined effects of PAH and other contaminants, such as heavy metals may be responsible since heavy metals can significantly induce both ROS production and EROD activity.

The AhR2 ligand activation assay supports results from the EROD activity assay and demonstrates that PAH extracted from sediments are able to activate AhR2 receptor and induce levels of CYP1A. However, we could not see clear differences in ligand binding activity between the reference stations and the three sediment traps from the gradient from the Ekofisk discharge point, nor clear differences in PAH levels. Sum NPD constituted approx. 36 % of Sum PAH from the sediment traps and indicates that both produced water discharges and long transported PAH compounds contribute to the PAH levels in the sediment traps.

5 Conclusion

Exposure and Environmental conditions

- DREAM simulations and current data showed that the PW plume direction was strongly influenced by the current and changed with the 6-hour tidal cycle between the northeast and southwest directions. This confirmed that the monitoring stations were suitably positioned for optimal exposure to the PW plumes.
- Despite the deeper discharged outlets of 49 m at Ekofisk 2/4 J and 20 - 40 m at Ekofisk 2/4 M, the warmer and lighter PW plume went towards the surface waters when released and remained within the top 20 m of the water column. For this reason, PW exposures at the deeper depths of 40 - 45 m were considered to be minimal.

- At Ekofisk and Eldfisk a thermocline at around 50 m was only present towards the end of the exposure in May and was thought not to influence the direction of the PW plume and subsequent exposure with regards to mussels/ scallops and PSDs. Incidentally, a shallower thermocline at 25 m deep was found in May at the reference station (Ref 2).
- Overall, the reference stations experienced slightly lower temperatures (a difference of <1°C) compared to Ekofisk and Eldfisk stations. This was not thought to have any significant impact on the chemical and biological effects results. Chlorophyll measurements indicated no lack of food for mussels from any station.

Chemical accumulation in passive sampling devices

- A gradient of dissolved concentration of C3-naphthalenes can be observed with increasing distance from Ekofisk at 20 m depth. This is as expected considering these PAH compounds are present in PW in relatively high concentrations in PW.
- Highest freely dissolved concentrations measured with silicone rubber passive samplers were seen for alkylated PAHs with the strongest signal of C3-naphthalenes, C1-dibenzothiphenes, C2-and C3-phenanthrenes closest to Ekofisk. The sum of concentrations of alkylated PAHs reached a few tens of ng/L. A clear signal of alkylated PAHs was also observed for Eldfisk when compared with reference stations.
- No clear profiles for PAH16, THC or alkylphenols could be observed.
- The modified version of POCIS was able to sample effectively a relatively large range of naphthenic acids with 0- to 6 rings and carbon atoms number from 9 to 35, but no clear profiles for these contaminants could be observed.

Chemical and biological responses in mussels

- Highest concentrations of PAH were measured in the whole soft tissue homogenates of mussels closest to the Ekofisk PW discharge outlet and reduced gradually with distance away reaching close to offshore background concentrations approximately 4000 m from the installation.
- Highest concentrations of sum PAH in mussels from the two closest stations were approximately 140 ng/g (w.w.). These were markedly lower than sum PAH measured in almost identical exposures in mussels at Ekofisk from WCM2006 and 2008 (700 ng/g w.w.) and 2009 (300 ng/g w.w.).
- Mussels caged at 500 m, 1000 m and 2000 m southwest of the Eldfisk platform had almost identical sum PAH concentrations and were similar to those sampled 4000 m from the Ekofisk installation, as well as both reference stations.
- *Mytilus edulis* accounting for 90 % of individuals and overall mussel speciation was unlikely to be a confounding factor for either chemical accumulation or biological responses in mussels.
- Although not significantly different, consistently lower CI and SoS values were measured in mussels from 40 m compared to their comparative 20 m group. The differences were not related to PW exposure but discussed in relation to mussels being less adapted to the deeper waters, such as impacts on energy budgets.
- A weak stress response was observed for LMS in mussels closest to the Ekofisk station and was one of the few biological responses measured in mussels that appeared to show an adverse effect in relation to proximity to the Ekofisk installation.
- Overall, based on MN frequencies in mussel haemocytes, genotoxicity was experienced in all field exposed mussels from the Ekofisk field and to a slightly lower extent in mussels from the Eldfisk field compared to the reference groups.
- The higher-level biological effects of digestive gland, gill and gonad histology showed no adverse impact with proximity to the Ekofisk or Eldfisk installations.

Chemical and biological responses in scallops at 40 - 45 m

- Scallops (*P. maximus*) were added to the sampling program at four stations as a trial species in deeper waters (40 - 45 m).
- Measurement of PAHs in scallops, both PAH EPA16 and total PAH, showed low levels at all stations, with no individual station showing a higher value than that recorded in samples taken from scallops prior to the deployment (T0).
- Sum PAH concentrations measured in scallops at 40 m were generally similar to those measured in mussels at the same depth and reflect the low exposure to the PW plume at this depth.
- The biological responses (CI, LMS) measured in scallops showed no relationship with proximity to the Ekofisk installation, a weak gradient away from the installation was reported for MN.
- Scallops were successfully used as an additional species in this monitoring program and were found robust enough for field transplantation.

Chemical and biological responses in fish

- Higher sum of PAH liver concentrations were found in dab from the Ekofisk safety zone, compared to the PAH liver concentrations in dab from the Ekofisk region and Egersundbank region.
- Detection of the PAH metabolites in the fish bile showed exposure of all fish to PAH compounds. However, it was not possible to specify the level of exposure based on the metabolite data.
- Possible relationship between PAH liver concentrations and PAH metabolites in dab have indicated influence of time after exposure, influenced by food availability. The presence of either indicates exposure to PAH as shown in dab populations from Ekofisk and Ekofisk region.
- PFOSA was the most dominant PFAS compound detected, although PFOSA was below or just above the LOD for dab and haddock, median concentrations of PFOSA in cod and whiting ranged between 5 and 15 ng/ml.
- Overall, the current study at Ekofisk was in good agreement with the earlier WCM investigations that measured PFAS in fish blood, in that offshore oil and gas installation did not appear to significantly contribute to PFAS load in local fish populations.
- Older cod were sampled from the Egersundbank (2 years) compared to Ekofisk (1 year), whilst older dab individuals were sampled from Ekofisk (6 years) compared to both Ekofisk region and Egersundbank (5 years). Whiting from Ekofisk region (2 years) were younger than those from Egersundbank (3 years) and Vikingbank (4 years). These differences may have implications for at least some of the biological effect responses.
- Significant responses in EROD activity were found in dab from both the Ekofisk and the Ekofisk region compared to the Egersundbank. A good relationship between increased EROD activity and elevated concentrations of liver PAH and PAH metabolites in dab.
- Elevated concentrations of CYP1A in dab collected from the Ekofisk installation and Ekofisk region compared to the Egersundbank region and supported the findings of CYP1A activity levels measured with EROD in these fish species.
- In dab, significant differences in EROD activity and CYP1A concentration were found between male and female dab with higher values found in males.
- The close relationship between increased CYP1A protein concentration, enzyme activity (EROD) and PAH liver concentration in dab shows an exposure and response to PAH in dab populations living in areas of oil and gas activity compared to the reference population.

- The other fish species (cod, haddock and whiting) overall showed low PAH concentrations in the liver and low CYP1A enzyme activity (EROD) and low CYP1A protein concentrations as a result.
- Significant levels of DNA strand breaks, quantified as % comet tails, were higher in both dab and cod collected from the Ekofisk safety zone compared to their comparative populations.
- The measurement of DNA adducts in fish liver samples did not support the results of DNA strand breaks (comet) and there were no differences found with overall low concentrations of DNA adducts in cod and dab from all locations including the Ekofisk safety zone.
- Haddock were found to have slightly elevated DNA adduct concentrations in Egersundbank and Vikingbank regions, although median DNA adduct levels were still on or just above the ICES BAC of 3 per 10^9 nucleotides.
- Neurotoxicity measured through the inhibition of AChE in fish fillet showed no impact on fish from the Ekofisk installation.
- Histological lesions were not significantly present in liver of fish caught in Ekofisk region, Egersundbank and Vikingbank.

Integrated Assessment

- For the mussels, seven biological responses were used in the calculation of IBR/n, which were arranged in terms of whole organism responses (CI and SoS) cellular responses (LMS, MN) and tissue level responses (DG, gill and gonad histology).
- The IBR/n values did not appear to show a clear pattern of effect, although when plotting the mussel IBR/n scores with distance from the Ekofisk complex there was a weak trend observed. Overall, similar IBR/n scores were found in mussel within 1000 m of the Ekofisk installation and reduced in mussels 2000 m and 4000 m away.
- The PCA revealed a strong relationship between PAH accumulation in mussel tissue and distance from the Ekofisk complex for the 20 m mussels. This was not shown in the 40 m mussels and reflects the low exposure of the 40 m mussels to the PW plume.
- Correlation analysis revealed a relationship between increasing PAH concentration in mussel tissue and a decrease in LMS and to a lesser extent increase in MN, which occurs in the mussel groups closer to the Ekofisk installation at the 20 m depth.
- Cod and dab from the Ekofisk safety zone showed highest IBR/n scores than all groups indicating that the Ekofisk safety zone is having some impact on fish health.
- The PCA was able to separate the main variables responsible for the variance of chemical body burden and biological effects measured in the fish populations. Clear spatial separation between fish populations were observed, particularly dab from Ekofisk and Ekofisk region compared to the Egersundbank population as well as Ekofisk cod compared to Egersundbank cod.
- The main factors responsible for the separation in the dab populations include PAH concentrations of the liver, particularly for Ekofisk dab and CYP1A, EROD and AChE for dab from the Ekofisk and Ekofisk region.
- The PCA supported the findings of the IBR/n, with strong positive correlations found between PAH liver concentrations and EROD activity and CYP1A protein concentration.
- Chemical concentrations in PAH liver and PAH metabolites showed that dab populations (Ekofisk and Ekofisk region) were recently exposed to PAH compounds prior to sampling and were accompanied with elevated CYP1A enzyme activity and protein concentrations, which would be expected with such an exposure. However, despite the measurable PAH exposure and clear cellular responses described, higher level orders of biological response such as histological changes in the tissue of the dab were absent.

6 Recommendations and future developments

To develop and improve the WCM approach for future campaigns, it is important to reflect on some of the positive aspects of the monitoring programme as well as learn from those parts that may require further development or refinement. Overall, the WCM2021 was successful in applying an integrated biological effects monitoring study, in multiple species, to determine the potential impacts of Ekofisk and Eldfisk installations on the marine environment. The design of the programme considered the main sources of contamination from oil and gas installations, principally the PW discharges as well as contamination from the sea floor.

The program benefitted from the PW plume modelling work that was firstly used to inform on the positioning of the monitoring stations for optimal exposure and was then later used to confirm the actual dynamics of the plume during the field exposure. This helped greatly in the interpretation of the PAH bioaccumulation in the mussels and scallops, confirming that the plume mostly occupied the upper 20 m of the water column and the deeper waters were not exposed to the plume to any great extent. Modelling the PW plume in order to provide valuable information on exposure is very important and should be used in future campaigns.

The accumulation of PAH in mussels was an effective tool showing a strong gradient away from the platform. This has been replicated continuously in previous WCM campaigns and demonstrates one of the many important features of mussels in biological effects monitoring. However, it was shown that the PAH bioaccumulations in mussels were lower than previous studies performed at Ekofisk, and this low exposure resulted in a low level of biological response in the mussels. It could also be interpreted that the biological effects methods used in the mussels were not sensitive enough to determine effects and differentiate between the monitoring stations at low exposure concentrations to PAH and other PW components. Developments in some of the methods such as MN and histology as well as comet to fully automate the assessment has potentially major advantages in increasing replication and improving the sensitivity of the methods. Automated scoring of MN and comet should be adopted in mussels, fish and potentially other monitoring species, to improve the diagnostic tool for the assessment of genotoxicity. Genotoxic responses have been reported in other WCM surveys and with the recent analytical limitations in measuring DNA adducts, due to lab closures, providing sensitive methods to differentiate organisms that have been exposed to low concentrations of genotoxic compounds would prove highly valuable.

The study indicated that the mussels were less adapted to deeper water. The successful trial of the scallop, natural adapted to deeper waters may therefore be a possible replacement in surveys where deeper investigations are required. Biomarker responses in scallops are not very well established and development of assessment criteria in this species is recommended. This would increase the quality of the biomarker results and aid in its interpretation.

PAH concentrations measured in the liver of fish, proved valuable in assessing PAH exposure and together with PAH metabolites provided information of recent exposure to PAHs and should continue in future programmes. A strategy that proved to be valuable in the WCM2021 programme was the inclusion of measured CYP1A activity by both enzyme (EROD) and protein measurements. The strong agreement between the responses in EROD and CYP1A protein together with the associated increase in PAH liver and PAH metabolite in dab provided a measurable exposure and effects response. It was unfortunate that methods to measure CYP1A gene expression in dab were not available and this should be developed for future programmes.

Passive sampler exposure showed it was possible to detect signals of alkylated PAHs and naphthenic acids from the platforms, but some methodological improvements are needed to ensure efficient sampling of alkylphenols and increase the time integrative nature of the sampling of low molecular weight PAHs. This can be done by modulating for example the volume to surface ratio of silicone samplers. It may be beneficial to design a passive sampler exposure system that allows to transpose easily the information regarding contaminant exchange/uptake kinetics gathered by the silicone membranes to POCIS devices.

The inclusion of sediment traps provided a useful link between the water column and the sea floor. The large volumes of sediment in the traps and the high concentrations of PAH in the sedimented material highlight the important of particulates in the water column and the potential impacts these can have on marine life. It also highlights the challenges in finding suitable references locations in the North Sea due to the chronic exposure to PAH.

7 References

Aas E, Klungsøyr J. 1998. PAH metabolites in bile and EROD activity in North Sea fish. Marine Environmental Research. 46:229-232.

Akcha, F., Leday, G., Pfohl-Leszkowicz, A. 2004. Measurement of DNA adducts and strand breaks in dab (*Limanda limanda*) collected in the field: effects of biotic (age, sex) and abiotic (sampling site and period) factors on the extent of DNA damage. Mutat. Res. Fundam. Mol. Mech. Mutagen. 552:197-207.

Allan IJ, Vrana B, de Weert J, Kringstad A, Ruus A, Christensen G, Green NW. 2021. Passive sampling and benchmarking to rank HOC levels in the aquatic environment. Scientific reports, 11(1), 1-12.

Andersen V, Maage A, Johannessen PJ. 1996. Heavy metals in blue mussels (*Mytilus edulis*) in the Bergen Harbour area, western Norway. Bulletin of Environmental contamination and Toxicology, 57(4), 589-596.

Au DWT. 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Marine Pollution Bulletin. 48:817-834.

Auffret M. 1988. Histopathological Changes Related to Chemical Contamination in *Mytilus edulis* from Field and Experimental Conditions. Marine Ecology Progress Series 46: 101–7.

Bakke T, Klungsøyr J, Sanni S. 2013. Environmental impacts of produced water and drilling waste discharges from the Norwegian offshore petroleum industry. Mar. Environ. Res. 92:154–169.

Balk L, Hylland K, Hansson T, Berntssen MHG, Beyer J, Jonsson G, et al. 2011. Biomarkers in Natural Fish Populations Indicate Adverse Biological Effects of Offshore Oil Production. PLoS ONE 6(5): e19735.

Beliaeff B, Burgeot T. 2002. Integrated biomarker response: a useful tool for ecological risk assessment. Environmental Toxicology and Chemistry. 21:1316-1322.

Beyer J, Green NW, Brooks SJ, Allan IJ, Ruus A, Gomes T, Bråte ILN, Schøyen M. 2017. Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A review. Marine Environmental Research.130: 338-365.

Beyer J, Jonsson G, Porte C, Krahn MM, Ariese F. 2010. Analytical methods for determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile: A review. Environmental Toxicology and Pharmacology.30:224-244.

Blanco M, Pérez-Albaladejo E, Piña B, Kušpilić G, Milun V, Lille-Langøy R, Karlsen OA, Goksøyr A, Porte C. 2018. Assessing the environmental quality of sediments from Split coastal area (Croatia) with a battery of cell-based bioassays. Sci. Total Environ. 624: 1640–1648.

Bocquené G, Galgani F. 1998. Biological effects of contaminants: Cholinesterase inhibition by organophosphate and carbamate compounds. International Council for the Exploration of the Sea: 1998.

Boitsov S, Klungsøyr J, Jensen HKB. 2020. Background concentrations of polycyclic aromatic hydrocarbons (PAHs) in deep core sediments from the Norwegian Sea and the Barents Sea: A proposed update of the OSPAR Commission background values for these sea areas. Chemosphere. 251: 126344.

Bolognesi C, Fenech M. 2012. Mussel micronucleus cytome assay. Nature Protocols 7(6): 1125-1137.

Bols NC, Schirmer K, Joyce EM, Dixon DG, Greenberg BM, Whyte JJ. 1999. Ability of polycyclic aromatic hydrocarbons to induce 7-ethoxyresorufin-o-deethylase activity in a trout liver cell line. *Ecotoxicology and environmental safety*, 44:118-128.

Booij K, Hofmans HE, Fischer CV, Van Weerlee EM. 2003. Temperature-dependent uptake rates of nonpolar organic compounds by semipermeable membrane devices and low-density polyethylene membranes. *Environmental Science and Technology*, *37*(2), 361-366.

Booij K, Smedes F. 2010. An Improved Method for Estimating in Situ Sampling Rates of Nonpolar Passive Samplers. Environmental Science and Technology, 44: 6789-6794.

Bosveld AT, De Bie PA, Van den Brink NW, Jongepier H, Klomp AV. 2002. *In vitro* EROD induction equivalency factors for the 10 PAHs generally monitored in risk assessment studies in The Netherlands. Chemosphere, 49:75-83.

Brack W, Schirmer K. 2003. Effect-directed identification of oxygen and sulphur heterocycles as major polycyclic aromatic cytochrome P4501A-inducers in a contaminated sediment. *Environmental science & technology*, 37(14):3062-3070.

Bradford 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72:248-254.

Broeg K, Lehtonen KK. 2006. Indices for the assessment of environmental pollution of the Baltic Sea coasts: integrated assessment of the multi-biomarker approach. Marine Pollution Bulletin 53:508-522.

Brooks SJ, Gomes T, Grung M, Macken A. 2022a. Determining the effects of the discharge effluent from the Karmøy aluminium smelter using an integrated biological effects approach. NIVA report. 65pp.

Brooks SJ, Bamber S, Gomiero A. 2022b. Biological effects monitoring of a thermomechanical cleaned cuttings discharge from the Johan Sverdrup Installation. NIVA report 7768-2022.

Brooks SJ, Gomes T, Grung M, Macken A. 2021. Integrated biological effects assessment of the discharge water into the Sunndalsfjord from an aluminium smelter. NIVA report.

Brooks SJ, Farmen E, Sørlie Heier L, Blanco-Rayón E, Izagirre U. 2015a. Differences in copper bioaccumulation and biological responses in three Mytilus species. Aquatic Toxicology. 160:1-12.

Brooks SJ, Harman C, Hultman M, Berge JA. 2015b. Integrated biomarker assessment of the effects of tailing discharges from an iron ore mine using blue mussels (*Mytilus* spp.) Science of the total environment. 524-525:104-114.

Brooks SJ, Pampanin DM, Harman C, Grung M. 2014. Water column monitoring 2014. Determining the biological effects of an offshore platform on local fish populations. NIVA report 6735-2014, pp. 75.

Brooks SJ, Pampanin DM, Harman C, Dunaevskava E. 2013. The Water Column Monitoring Programme 2013: Determining the biological effects of two offshore platforms on local fish populations. NIVA report 6595-2013, pp. 62.

Brooks SJ, Harman C, Grung M, Farmen E, Ruus A, Vingen S, Godal BF, Baršienė J, Andreikėnaitė L, Skarphéðinsdóttir H, Liewenborg B, Sundt RC. 2011a. Water column monitoring of the biological effects of produced water from the Ekofisk offshore oil installation from 2006 to 2009. Journal of Toxicology and Environmental Health. 74: 582-604.

Brooks SJ, Pampanin DM, Harman C, Aarab N, Sundt RC. 2011b. Water column monitoring 2011. NIVA report 6237-2012, pp. 61.

Brumley CM, Haritos VS, Ahokas JT, Holdway DA. 1998. The effects of exposure duration and feeding status on fish bile metabolites: Implications for biomonitoring. Ecotoxicology and Environmental Safety, 39(2), 147-153.

Brunborg G, Jackson P, Shaposhnikov S, Dahl H, Azqueta A, Collins AR, Gutzkow KB. 2014. High throughput sample processing and automated scoring. Front. Genet. 5:373.

Cajaraville MP, Diez G, Marigomez IA, Angulo E. 1990 Responses of basophilic cells of the digestive gland of mussels to petroleum hydrocarbon exposure. Diseases of aquatic organisms,.9:221-228.

Cheong HSJ, Seth I, Joiner MC, Tucker JD. 2013. Relationships among micronuclei, nucleoplasmic bridges and nuclear buds within individual cells in the cytokinesis-block micronucleus assay. Mutagenesis 28(4): 433–440.

Davenport J, Chen X. 1987. A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.) J. Moll. Stud, 53:293-297

Davies IM, Vethaak AD. 2012. Integrated marine environmental monitoring of chemicals and their effects. ICES Cooperative Research Report No. 315. 277 pp.

de la Ballina NR, Maresca F, Cao A, Villalba A. 2022. Bivalve Haemocyte Subpopulations: A Review. Frontiers in Immunology, 13: 826255.

Devin S, Burgeot T, Giambérini L, Minguez L, Pain-Devin S. 2014. The integrated biomarker response revisited: optimization to avoid misuse. Environmental Science and Pollution Research. 21:2448-2454.

Dignam JD. 1990. Preparation of Extracts from Higher Eukaryotes. I: Methods in Enzymology. Vol 182. M.P. Deutscher (Ed) Academic Press Inc. San Diego, New York, Berkley, Boston, London, Sydney, Tokyo, Toronto.

Eggens ML, Galgani F. 1992. Ethoxyresorufin-O-deethylase (EROD) activity in flatfish: Fast determination with a fluorescence plate-reader. Marine Environmental Research. 33:213-221.

Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7, 88-90.

Estrada N, Velázquez E, Rodríguez-Jaramillo C, Ascencio F. 2013. Morphofunctional study of hemocytes from lions-paw scallop *Nodipecten subnodosus*. Immunobiology 218: 1093– 1103.

Fernandes D, Pujol S, Perez-Albaladejo E, Tauler R, Bebianno MJ, Porte C. 2014. Characterization of the environmental quality of sediments from two estuarine systems based on different in-vitro bioassays. Mar. Environ. Res. 96: 127–135.

Froment J, Langford K, Tollefsen KE, Nerland IL, Brooks SJ, Thomas KV. 2016. Identification of petrogenic produced water components as acetylcholine esterase inhibitors. Environmental Pollution. 215: 18-26.

Galgani F, Bocquene G. 1990. In vitro inhibition of acetylcholinesterase from four marine species by organophosphates and carbamates. Bulletin of Environmental Contamination and Toxicology. 45:243–249.

Gingstad GA, Aasberg M, Durie R, Norland AK. 2022. Utslippsrapport 2021 Ekofisk Feltet. ConocoPhillips report. 76 p.

Glanzmann V, Booij K, Reymond N, Weyermann C, Estoppey N. 2022. Determining the Mass Transfer Coefficient of the Water Boundary Layer at the Surface of Aquatic Integrative Passive Samplers. Environmental Science and Technology, 56: 6391-6398.

Goksøyr SØ, Sørensen H, Grøsvik BE, Pampanin DM, Goksøyr A, Karlsen OA. 2021. Toxicity assessment of urban marine sediments from Western Norway using a battery of stress-activated receptors and cell-based bioassays from fish. Environmental Toxicology and Pharmacology. 87:103704.

Gomes T. in prep. Water column Monitoring Research and development programme: Automated analysis of micronuclei in fish and mussel haemocytes. NIVA report.

Gosling E. 2003. Bivalve Molluscs. In Bivalve Molluscs, Biology Ecology and culture i–x. John Wiley & Sons, Ltd.

Harman C, Langford K, Sundt RC, Brooks S. 2014. Measurement of naphthenic acids in the receiving waters around an offshore oil platform by passive sampling. Environmental toxicology and chemistry, 33(9), 1946-1949.

Harman C, Allan IJ, Vermeirssen ELM. 2012. Calibration and use of the polar organic chemical integrative sampler—a critical review. Environmental Toxicology and Chemistry, 31: 2724-2738.

Harman C, Farmen E, Tollefsen KE. 2010. Monitoring North Sea oil production discharges using passive sampling devices coupled with in vitro bioassay techniques. Journal of Environmental Monitoring, 12:1699-1708.

Harman C, Thomas KV, Tollefsen KE, Meier S, Bøyum O, Grung M. 2009a. Monitoring the freely dissolved concentrations of polycyclic aromatic hydrocarbons (PAH) and alkylphenols (AP)

around a Norwegian oil platform by holistic passive sampling. Marine Pollution Bulletin, 58:1671-1679.

Harman C, Bøyum O, Thomas KV, Grung M. 2009b. Small but different effect of fouling on the uptake rates of semipermeable membrane devices and polar organic chemical integrative samplers. Environmental Toxicology and Chemistry: An International Journal, 28(11):2324-2332.

Hinz H, Kröncke I, Ehrich S. 2005. The feeding strategy of dab *Limanda limanda* in the southern North Sea: linking stomach contents to prey availability in the environment. Journal of Fish Biology, 67: 125-145.

Holth TF, Tollefsen KE. 2012. Acetylcholine esterase inhibitors in effluents from oil production platforms in the North Sea. Aquatic Toxicology. 112:92-98.

Hylland K, Skei BB, Brunborg G, Lang T, Gubbins MJ, le Goff J, Burgeot T. 2017. DNA damage in dab (*Limanda limanda*) and haddock (*Melanogrammus aeglefinus*) from European seas. Mar Environ Res. 124:54-60.

Hylland K, Tollefsen KE, Ruus A, Jonsson G, Sundt RC, Sanni S, Røe Utvik TI, Johnsen S, Nilssen I, Pinturier L, Balk L, Barsiene J, Marigòmez I, Feist SW, Børseth JF. 2008. Water column monitoring near oil installations in the North Sea 2001-2004. Mar Pollut Bull. 56(3):414-29.

Hylland K, Lang T, Vethaak AD. (Ed.) 2006. Biological effects of contaminants in marine pelagic ecosystems. Society of Environmental Toxicology and Chemistry (SETAC): Pensacola. ISBN 1-880611-84-8. XXIX, 474 pp.

Hylland K, Ruus A, Sundt RC, Feist S, Marigomez I, Balk L, Abrehamson A, Barsiené J. 2005. Water Column Monitoring 2004. Summary Report. Report by NIVA and RF for Statiol ASA on behalf of the OLF WCM coordination group. NIVA-report no. 499-2005, 59 pp. + appendix.

Howard DW. 2004. Histological techniques for marine bivalve mollusks and crustaceans. Vol. 5. NOAA, National Ocean Service, National Centers for Coastal Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, Cooperative Oxford Laboratory.

ICES. 2021. Working Group on Biological Effects of Contaminants (WGBEC). ICES Scientific Reports. 3:65. 90 pp.

Inoue K, Waite H, Matsuoka M, Odo S, Harayama S. 1995. Interspecific variations in adhesive protein sequences of *Mytilus edulis*, *M*. *galloprovincialis*, and *M*. *trossulus*. Biol. Bull. 189:370-375

Jacob H, Besson M, Oberhaensli F, Taylor A, Gillet B, Hughes S, Melvin SD, Bustamante P, Swarzenski PW, Lecchini D, Metian M. 2022. A multifaceted assessment of the effects of polyethylene microplastics on juvenile gilthead seabreams (*Sparus aurata*). Aquatic Toxicology 241, 106004

Jonsson G, Beyer J, Wells D, Ariese F. 2003. The application of HPLC-F and GC-MS to the analysis of selected hydroxy polycyclic hydrocarbons in two certified fish bile reference materials. Journal of Environmental Monitoring. 5:513-520.

Jonsson G, Taban IC, Jørgensen KB, Sundt RC. 2004. Quantitative determination of de-conjugated chrysene metabolites in fish bile by HPLC-fluorescence and GC-MS. Chemosphere. 54:1085-1097.

Julshamn K, Maage A, Norli HS, Grobecker KH, Jorhem L, Fecher P. 2007. Determination of arsenic, cadmium, mercury, and lead by inductively coupled plasma/mass spectrometry in foods after pressure digestion: NMKL1 interlaboratory study. Journal of Aoac International 90:844-856.

Kagley AN, Snider RG, Krishnakumar PK, Casillas E. 2003. Assessment of Seasonal Variability of Cytochemical Responses to Contaminant Exposure in the Blue Mussel *Mytilus edulis* (Complex). Arch. Environ. Contam. Toxicol. 44: 43-52.

Kammann U, Lang T, Vobach M, Wosniok W. 2005. Ethoxyresorufin-O-deethylase (EROD) Activity in Dab (*Limanda limanda*) as Biomarker for Marine Monitoring. Environmental Science and Pollution Research. 12:140-145.

Kang J-J, Fang H-W. 1997. Polycyclic aromatic hydrocarbons inhibit the activity of acetylcholinesterase purified from electric eel. Biochemical and Biophysical Research Communications. 238:367-369.

Kljaković-Gašpić Z, Herceg-Romanić S, Kozul D, Veza J. 2010. Biomonitoring of organochlorine compounds and trace metals along the Eastern Adriatic coast (Croatia) using *Mytilus galloprovincialis*. Marine pollution bulletin, 60(10), 1879-1889.

Lacroix C, Coquillé V, Guyomarch J, Auffret M, Moraga D. 2014. A selection of reference genes and early-warning mRNA biomarkers for environmental monitoring using *Mytilus* spp. as sentinel species. Marine Pollution Bulletin. 86:304-313.

Lange U, Goksoyr A, Siebers D, Karbe L. 1999. Cytochrome P450 1A-dependent enzyme activities in the liver of dab (*Limanda limanda*): kinetics, seasonal changes and detection limits. Comparative Biochemistry and Physiology B—Biochemistry and Molecular Biology, 123:361–371.

Law RJ. 1981. Hydrocarbon concentrations in water and sediments from UK marine waters, determined by fluorescence spectroscopy. Marine Pollution Bulletin, 12(5):153-157.

Lille-Langøy R, Jørgensen KB, Goksøyr A, Pampanin DM, Sydnes MO, Karlsen OA. 2021. Substituted Two- to Five-Ring Polycyclic Aromatic Compounds Are Potent Agonists of Atlantic Cod (*Gadus morhua*) Aryl Hydrocarbon Receptors Ahr1a and Ahr2a. Environ Sci Technol. Nov 16;55(22):15123-15135.

Lindberg HK, Wang X, Järventaus H, Falck, GCM, Norppa H, Fenech M. 2007. Origin of nuclear buds and micronuclei in normal and folate-deprived human lymphocytes. Mutation Research 617: 33–45.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin Phenol Reagent, Journal of Biological Chemistry. 192:265-275.

Luzhna L, Kathiria P, Kovalchuk O. 2013. Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. Frontiers in Genetics 4, 131.

M-408|2020. Guidelines for environmental monitoring of petroleum activities on the Norwegian continental shelf (Revised in 2020).

Meador J, Stein JE, Reichert WL, Varanasi U. 1995. Bioaccumulation of Polycyclic Aromatic Hydrocarbons by Marine Organisms. Reviews of environmental contamination and toxicology. 143. 79-165.

Metón I, Mediavilla D, Caseras A, Cantó E, Fernández F, Baanante IV. 1999. Effect of diet composition and ration size on key enzyme activities of glycolysis–gluconeogenesis, the pentose phosphate pathway and amino acid metabolism in liver of gilthead sea bream (*Sparus aurata*). British Journal of Nutrition 82:223-232.

Mjanger H, Svendsen BV, Senneset H, Fotland Å, Mehl S, Salthaug A. 2017. Håndbok for prøvetaking av fisk og krepsdyr. Institute of Marine Research, Bergen, Norway. 1-194 pp. Northeast Fisheries Science Center. 2018. Ecosystems Surveys Branch.

Molvær J, Knutzen J, Magnusson J, Rygg B, Skei J, Sørensen J. 1997. Klassifisering av miljøkvalitet i fjor der og kystfarvann. Veiledning. Statens forurensingstilsyn (SFT): TA-1667/1997. 36 s.

Monteyne E, Roose P, Janssen CR. 2013. Application of a silicone rubber passive sampling technique for monitoring PAHs and PCBs at three Belgian coastal harbours. Chemosphere, 91(3): 390-398.

Moore MN. 1976. Cytochemical demonstration of latency of lysosomal hydrolases in digestive cells of the common mussel *Mytilus edulis*, and changes induced by thermal stress. Cell and Tissue Research, 175:279-287.

Nilsen BM, Berg K, Goksøyr A. 1998. Induction of Cytochrome P450 1A (CYP1A) in fish. A biomarker for environmental pollution, Methods Mol Biol. 107:423-38.

Okumuş I, Stirling HP. 1998. Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. Aquaculture, 159: 249-261.

Pagano M, Capillo G, Sanfilippo M, Palato S, Trischitta F, Manganaro A, Faggio C. 2016. 'Evaluation of Functionality and Biological Responses of *Mytilus galloprovincialis* after Exposure to Quaternium-15 (Methenamine 3-Chloroallylochloride)'. Molecules 21 (2): 144.

Pampanin DM, Brooks S, Grøsvik BE, Sanni S 2019. Water Column Monitoring 2017. Environmental monitoring of petroleum activities on the Norwegian continental shelf 2017. NORCE-Environment REPORT 007 – 2019, pp 92.

Pampanin DM, Brooks S, Børseth JF, Harman C, Gomiero A, Farmen E, Aarab N, Nerland IL, Westerlund S, Grung M, Lucas C, Strålberget E. 2013. Water Column Monitoring 2012 Troll C platform. IRIS Final report 2013-252, pp. 95.

Pampanin DM, Sydnes MO. 2013. Polycyclic aromatic hydrocarbons a constituent of petroleum: Presence and influence in the aquatic environment. In Kutcherov V, Kolesnikov A, eds, Hydrocarbon. InTech, London, UK.

Pampanin DM, Volpato E, Marangon I, Nasci C. 2005 Physiological measurements of native and transplanted mussel (M*ytilus galloprovincialis*) in the canals of Venice. Survival in air and condition index. Comparative Biochemistry and Physiology A, 140:41-52.

Pintado-Herrera MG, Allan IJ, González-Mazo E, Lara-Martín PA. 2020. Passive samplers vs sentinel organisms: one-year monitoring of priority and emerging contaminants in coastal waters. Environmental Science & Technology, 54(11): 6693-6702.

Pulster EL, Gracia A, Armenteros M, Carr BE, Mrowicki J, Murawski SA. 2020. Chronic PAH exposures and associated declines in fish health indices observed for ten grouper species in the Gulf of Mexico. STOTEN, 703:135551

Purchon R. 1976. Marine mussels. Nature 262, 728.

Rahmanpour S, Ghorghani NF, Ashtiyani SML. 2014. Polycyclic aromatic hydrocarbon (PAH) in four fish species from different trophic levels in the Persian Gulf Environmental Monitoring and Assessment. 186:7047–7053.

Richardson DM, Gubbins MJ, Davies IM, Moffat CF, Pollard PM. 2004. Effects of feeding status on biliary PAH metabolite and biliverdin concentrations in plaice (*Pleuronectes platessa*). Environmental Toxicology and Pharmacology, 17(2):79-85.

Rijnsdorp AD, Vethaak AD, van Leeuwen PI. 1992. Population biology of dab *Limanda limanda* in the southeastern North Sea. Marin Ecology Progress Series 91:19-35.

Rusina TP, Smedes F, Koblizkova M, Klanova J. 2010. Calibration of silicone rubber passive samplers: Experimental and modelled relations between sampling rate and compound properties. Environ. Sci. Technol. 44:362–367.

Samanipour S, Baz-Lomba JA, Reid MJ, Ciceri E, Rowland S, Nilsson P, Thomas KV. 2018. Assessing sample extraction efficiencies for the analysis of complex unresolved mixtures of organic pollutants: A comprehensive non-target approach. Analytica Chimica Acta, 1025:92-98.

Samanipour S, Reid MJ, Rundberget JT, Frost TK, Thomas KV. 2020. Concentration and Distribution of Naphthenic Acids in the Produced Water from Offshore Norwegian North Sea Oilfields. Environ. Sci. Technol. 54:2707–2714.

Sleiderink HM, Beyer J, Scholtens E, Goksøyr A, Nieuwenhuize J, Van Liere JM, Everaarts JM, Boon JP. 1995a. Influence of temperature and polyaromatic contaminants on CYP1A levels in North Sea dab (*Limanda limanda*), Aquatic Toxicology. 32:189-209.

Sleiderink HM, Oostingh I, Goksøyr A, Boon JP. 1995b. Sensitivity of cytochrome P450 1A induction in dab (*Limanda limanda*) of different age and sex as a biomarker for environmental contaminants in the southern North Sea. Archives of Environmental Contaminants and Toxicology. 28:423-430.

Smedes F. 2019. SSP silicone–, lipid–and SPMD–water partition coefficients of seventy hydrophobic organic contaminants and evaluation of the water concentration calculator for SPMD. Chemosphere, 223, 748-757.

Stagg RM, McIntosh A, Mackie P. 1995. Elevation of hepatic monooxygenase activity in the dab (*Limanda limanda* L.) in relation to environmental contamination with petroleum hydrocarbons in the northern North Sea. Aquatic Toxicology, 33:245-264.

Stara, A, Pagano M, Capillo G, Fabrello J, Sandova M, Vazzana I, Zuskova E, Velisek J, Matozzo V, Faggio C. 2020. Assessing the effects of neonicotinoid insecticide on the mussel mollusc *Mytilus galloprovincialis*. Science of the total environment 700: 134914.

Struch R, Pulster EL, Schreier AD, Murawski SA. 2019. Hepatobiliary Analyses Suggest Chronic PAH Exposure in Hakes (*Urophycis* spp.) Following the Deepwater Horizon Oil Spill. Environ Toxicol Chem. 38(12):2740–2749.

Sukhotin AA, Strelkov P, Maximovich NV, Hummel H. 2007. Growth and longevity of *Mytilus edulis* (L.) from northeast Europe. Marine Biology Research 3(3):155-167.

Traven L, Zaja R, Lončar J, Smital T, Mićović V. 2008. CYP1A induction potential and the concentration of priority pollutants in marine sediment sample – *In vitro* evaluation using the PLHC-1 fish hepatoma cell line. *Toxicology in vitro*, 22(6), 1648-1656

UNEP/RAMOGE, 1999. Manual on the biomarkers recommended for the MED POL biomonitoring programme. UNEP, Athens.

Varanasi U, Stein JE, Nishimoto M. 1989. Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAH) in fish. In: Varanasi U (ed) Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press, Boca Raton, FL, pp 94-149.

Verreault J, Muir DC, Norstrom RJ, Stirling I, Fisk AT, Gabrielsen GW, Derocher AE, Evans TJ, Dietz R, Sonne C, Sandala GM, Gebbink W, Riget FF, Born EW, Taylor MK, Nagy J, Letcher RJ. 2005. Chlorinated hydrocarbon contaminants and metabolites in polar bears (*Ursus maritimus*) from Alaska, Canada, East Greenland, and Svalbard: 1996-2002. Science of the total Environment. 351-352(1):369-90.

Yancheva V, Velcheva I, Stoyanova S, Georgieva E. 2016. Histological biomarkers in fish as a tool in ecological risk assessment and monitoring programs: a review. Applied ecology and environmental research 14:47-75.

Whyte JJ, Jung RE, Schmitt CJ, Tillitt DE. 2000. Ethoxyresorufin-O-deethylase (EROD) Activity in Fish as a Biomarker of Chemical Exposure. Critical Reviews in Toxicology, 30:347-570.

Appendix A.

Appendix List

Number	Description
A	WCM 2021 Cruise report
B	Data report
C	DREAM simulations
D	Zooplankton report
E	DNA adduct report

NIVA: Norway´s leading centre of competence in aquatic environmentes

The Norwegian Institute for Water Research (NIVA) is Norway's leading institute for fundamental and applied research on marine and freshwaters. Our research comprises a wide array of environmental, climatic and resource-related fields. NIVA's world-class expertise is multidisciplinary with a broad scientific scope. We combine research, monitoring, evaluation, problem-solving and advisory services at international, national and local levels.

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