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Water Column Monitoring 2012
Troll C platform

Final report

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Preface

The Water Column Monitoring (WCM) programme performs investigations into the potential biological effects of offshore oil and gas activity on the biota living within the water column of the Norwegian sector of the North Sea. Oil companies in the Norwegian sector with produced water discharges, who funded the project, are obliged by the Norwegian authorities to perform water column monitoring offshore. The work has been performed at various fields within the Norwegian sector over the last 10 years. The methods used are considered to be the best available technology for the assessment of biological effects monitoring, measuring chemical bioaccumulation of oil related compounds in mussels and passive sampling devices as well as a suite of biomarker responses in mussels. Integration of the chemical and biological effects data enables a comprehensive assessment of the effects of the produced water on organism health.

The WCM programme has been performed through collaboration between the International Research Institute of Stavanger (IRIS) and the Norwegian Institute of Water Research (NIVA). The work participants from these Institutes include:

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Summary

The WCM programme was performed at the Troll field where the potential biological effects of operational discharges were investigated. The 2012 WCM programme focused on caged mussels allowing for an increased number of sampling stations and biological effects endpoints to be measured. Therefore, the programme involved 13 mussel stations including 2 reference stations and 11 exposure stations. The positions of the 11 exposure stations at 500 m, 1000 m and 2000 m from the Troll C platform were positioned with respect to the predicted, and later confirmed, plume direction (Fig 1). Mussels (*Mytilus* spp.) were held at a depth of 15 m at all stations and retrieved after an exposure period of 6 weeks.

The current meter data confirmed the expected dominant direction of the PW plume, and the stations selected for the biomarker analysis were in the PW plume axis direction.

The results of the WCM 2012 survey have shown that mussels caged 500 m from the PW discharge outlet had elevated concentrations of PAH and NPDs compared to mussels at 1000 m and beyond. The maximum PAH16 concentration measured was similar to the maximum concentration measured at Gullfaks C in 2011 but higher than the maximum concentration measured at Ekofisk during the 2006, 2008 and 2009 surveys following similar exposure durations. In addition, PAH levels were above those previously measured in mussel tissue in the vicinity of the Troll and Statfjord B platforms. The mussels positioned at 1000 m and 2000 m contained PAH EPA 16 concentrations typical of offshore background levels of the North Sea.

The NPD concentrations showed the same pattern in concentration with respect to mussel station as that described for PAH EPA16.

With respect to metal bioaccumulation, concentrations of arsenic, barium, copper, cadmium, lead and zinc were found to be higher in the field exposed mussels compared to the pre-exposed group. However, the bioaccumulation of these metals in mussels appeared to be unrelated to the distance from the PW discharge.

Levels of ^{226}Ra in the PW exposed mussels were close to the natural background concentrations measured at reference station and as reported in previous WCM surveys, indicating no accumulation of the radionuclide by the mussels as in previous WCM surveys.

Bioaccumulation data have indicated that only mussels caged at 500 m from the Troll C platform were exposed to PAHs, while the biomarker results (pyrene hydroxylase, lysosomal membrane stability (LMS) in haemolymph and digestive gland, lipofuscin (LF) and neutral lipid (NL) accumulations and condition index (CI)) did not show statistical differences between mussels caged at Troll C compared to the ones at the reference station, except for histopathology.

To summarise the biological marker results, the Integrative Biological Responses (IBR/n) has been applied as in previous WCM projects and other monitoring studies. Different biomarkers (NRRT in blood cells, LMS in digestive gland, LF and NL accumulation and CI, PH) were used to calculate the IBR index. IBR/n discriminated between the different stations. In fact, the IBR/n values were moderately higher in stations 1, 2, 8 and 9 compared to the pre-exposure group. Relative high value was found in mussels caged at the reference station, therefor no difference was found in the IBR/n value of mussels caged around the platform compared to the reference ones.

The biomarker responses were small, a moderate stress response was shown in mussels caged at 500 and 1000 m from the PW discharge, as revealed by the histopathology markers. This indicates a relatively minor effect of the PW discharge in the platform area that decrease with increasing distance from the discharge point.

A clear signal in AP exposure was shown in POCIS extracts collected from stations 1 and 2 (500 m) compared to the reference group.
Sea current measurements and hydrocarbon exposure measures in the caged mussels indicated that the station design of the investigation captured the produced water plume from the Troll C platform.
In conclusion, mussels caged 500 m from the Troll C platform showed significantly higher concentrations of PAH and NPD concentrations then all other mussel groups, indicating a bioaccumulation of these compounds probably due to the PW discharge. All stations at further distance from the platform showed background levels of PAH and NPD. No biological effect responses were recorded in mussels caged around the platform at biochemical and physiological level. Only the histopathological analysis of mussel digestive gland indicated a minor stress condition in mussels caged 500 and 1000 m from the platform.

Oppsummering

WCM programmet ble utført på Troll-feltet hvor potensielle biologiske effekter av operasjonelle utslipp ble undersøkt. I 2012 var fokus i WCM programmet på blåskjell i bur. Dette åpnet for et økt antall prøvetakingsstasjoner og måling av endepunkter av biologiske effekter (13 stasjoner: 2 referansestasjoner og 11 eksponerings stasjoner). Stasjonenes stillinger på 500 m, 1000 m og 2000 m fra Troll C posisjonert i forhold til den forutsagte, og senere bekreftede «plume» retning. Blåskjell (*Mytilus* spp.) ble holdt på 15 meters dyp på alle stasjoner og hentet etter en eksponering periode på seks uker.
Resultatene av WCM 2012 undersøkelsen har vist at blåskjell i bur 500 m fra PW utløp hadde forhøyede konsentrasjoner av PAH og Ods i forhold til blåskjell på 1000 m og utover. Blåskjell plassert på 1000 m og 2000 m inneholdt PAH16 konsentrasjoner som er typiske for offshore bakgrunnsnivåer i Nordsjøen.
Med hensyn bioakkumulering av metall, ble konsentrasjonene av arsenikk, barium, kobber, kadmium, bly og sink funnet å være høyere i felt eksponert skjell sammenlignet med forhåndseksponert gruppe. Bioakkumulering av de nevnte metallene i blåskjell, syntes å være relatert til avstanden fra PW utslipp.
Nivåer av ^{226}Ra i PW eksponerte blåskjell var nær de naturlige bakgrunnskonsentrasjoner målt ved referanse stasjonen og som tidligere rapportert WCM undersøkelsen.
Bioakkumulerings-data har indikert at kun blåskjell i bur 500 m fra Troll C-plattformen ble utsatt for PAH. Samtidig viser biomarkør resultatene ingen statistisk signifikant forskjell mellom blåskjell i bur på Troll C i forhold til de på referanse stasjonen, med et unntak; histopatologi.
For å oppsummere resultatene for biologiske markører, har de Integrative biologiske responser (IBR/n) blitt brukt, i henhold til tidligere WCM prosjekter og andre overvåkingsstudier. Ulike biologiske responser (biomarkører) ble brukt til å beregne IBR indeksen. IBR/n diskriminerte mellom de ulike stasjonene. Faktisk viser IBR/n moderat høyere verdier ved stasjonene 1, 2, 8 og 9 i forhold til pre-eksponering gruppe. Relativ høy verdi ble funnet i blåskjell bur på referansestasjonen, derfor ble det ikke funnet forskjell i IBR/n-verdien av blåskjell bur rundt plattformen i forhold til referanse stasjon.
Biomarkør responsene var små, en moderat stress respons ble vist i blåskjell i bur på 500 og 1000 m fra PW utslipp, bekreftet av histopatologi markører. Dette indikerer en relativt liten effekt av PW utslipp i plattformens område og effekten avtar med økende avstand fra utslippspunktet.
Et klart signal i AP eksponering ble vist i POCIS ekstrakter samlet inn fra stasjonene 1 og 2 (500 m) i forhold til referanse gruppen.

List of Symbols

AP	Alkylphenol
BP	Base pair
Bq	Becquerel
BSA	Bovine Serum Albumin
$C_1 - C_9$	referring to the number of carbons in a side chain (e.g. on a PAH or phenol)
CI	Condition index
DNA	Deoxyribonucleic acid
FTU	Formazin Turbidity Unit
HPLC	High performance liquid chromatography
ICES	International Council for the Exploration of the Sea
IRIS	International Research Institute of Stavanger
LF	Lipofuscin accumulation
LMS	Lysosomal membrane stability
MN	Micronucleus/ micronuclei
NIVA	Norwegian Institute for Water Research
NL	Neutral lipid
NRRT	Neutral red retention time
NPD	Naphthalenes, Phenanthrenes and Dibenzothiophenes
OD	Optical density
OLF	Norwegian Oil Industry Association
PAH	Polycyclic aromatic hydrocarbon
PCR	Polymerase chain reaction
PH	Pyrene hydroxylase
PW	Produced Water
PES	Polyethersulphone
POCIS	Polar organic chemical integrative sampler
RPM	Revolutions per minute
SIM	Selected ion monitoring
SPMD	Semi-permeable membrane device
v:v	Volume:volume
WCM	Water Column Monitoring
w.w.	Wet weight

1. Introduction

1.1 General purpose of the study

The Water Column Monitoring (WCM) programme is designed to determine the potential impact of the offshore oil and gas activities on the local marine environment. Within the Norwegian sector of the North Sea, the offshore operators are obliged to carry out environmental monitoring within the water column in the vicinity of offshore installations. This obligation requires that monitoring of the water column should be carried out in at least one offshore oil and gas field each year. Although approval is required by the Norwegian authorities, the operators can choose the study area and the design of the programme. The operators then select contractors that perform the study based on the proposed program. In 2012, the Troll C platform was chosen as the study site.

1.2 Background for the WCM programme

Organisms living in the water column around offshore oil and gas production facilities are predominantly exposed to chemicals through discharge of production water. Produced water (PW) is the water that is extracted together with oil and gas and usually is a combination of: 1) formation water contained naturally in the reservoir; 2) injected seawater to aid in extraction and 3) treatment chemicals added during the production. However, the specific chemical composition varies between reservoirs and within a reservoir as production proceeds (Røe, 1998).

Some of the organic chemicals found in PW are relatively resistant to biodegradation, have a bioaccumulation potential and may be toxic to organisms in receiving waters (Brendehaug *et al*., 1992; Tollefsen *et al*., 1998; Aas *et al*., 2000).

The general design of the WCM programme is to measure a suite of biomarkers and chemical exposure concentrations in bioindicator species (e.g. caged mussels) in order to determine the potential biological effects of a PW discharge on the local marine environment. Previous survey designs have used at least 6 exposure stations at increasing distances from the discharge point(s) (i.e. 200-300m to 2 km), with two reference stations approximately 50 km away from the studied platform in a 'clean' area. The exposure duration of 6 weeks has been used, which was considered to be sufficient time for significant effects in histological endpoints to occur. Chemical measurements are taken in the whole tissue of mussels and in order to determine exposure to the PW of the exposure animals. Biomarkers measured include specific effect endpoints, genotoxicity and general stress markers.

It is important to ensure that the caged mussels are exposed to the PW plume. Current conditions at Ekofisk used in surveys in 2006, 2008 and 2009 were consistent and reliable and enabled the cages to be positioned within the PW plume. However, this may not be the situation in other areas, e.g. as experienced in Troll (WCM 2005, 2003). The depth at which the bioindicators are held within the water column is dependent on the stratification in the area, with animals positioned above the thermocline at between 10 to 15 m.

Pre-exposure sampling of mussels is performed to determine the chemical concentrations and biomarker levels in animals prior to exposure.

1.3 Objective

The principal objective of the WCM survey 2012 was to assess the extent to which PW discharges from an oil production platform (Troll C) affect organisms living in the water column. To fulfil this objective a combination of chemical concentrations and biological

effects measurements in caged mussels, positioned at strategic locations with respect to the PW discharge, were used.

1.4 Background on the chemical and biological methods used

1.4.1 Mussel PAH bioaccumulation

The organic fraction of PW is dominated by low molecular PAHs, such as NPDs, decalins and their alkyl homologues (Utvik, 1999). High molecular PAHs such as benzopyrene, pyrene and chrysene are also naturally present in effluents of PW from production platforms in the North Sea, although usually at lower concentrations. Many of the low molecular weight PAHs have also been detected in caged organisms deployed downstream from known discharge points (Røe, 1998). This applies in particular to alkyl substituted NPDs, which have been found in higher concentrations than their non-alkylated parent compounds in biological tissues and passive sampling devices (Røe, 1998; Ruus *et al*., 2006). Measurement of contaminant body burdens in caged animals are commonly used to assess the exposure situation in a specified area (Brooks *et al*., 2011a,b; Sundt *et al*., 2011).

1.4.2 ^{226}Radium

Radiation levels in PW related to Technologically Enhanced Naturally Occurring Radioactive Materials (TENORM) is generally relatively low and the typical seawater dilution for offshore discharges is generally high. However, the Norwegian Radiation Protection Authority, Petroleum Directorate and the Pollution Control Authority have emphasized the need for more knowledge regarding radioactive discharges from the oil and gas industry (Anon, 2008) and the environmental authorities have requested monitoring of possible effects on marine organisms. The dominating radionuclides in the PW discharges are ^{226}Ra and ^{228}Ra. At present there is regular monitoring of these radionuclides as well as ^{210}Pb on a monthly or quarterly basis, depending on the volume of the discharged PW. The simplest method for measuring these radionuclides is to use ɣ-spectrometry. This method is used for the monitoring of radioactivity in the PW where the activity of the radium isotopes is in the order of Bq/L. A more sensitive analytical technique based on α-spectroscopy was adapted as part of WCM 2010 (work package 14). This method is suitable for determining the low levels typically present in biota.

1.4.3 *Mytilus* species identification

It has been recently confirmed that a mixed population of *Mytilus* spp. occurs on the Norwegian coastline, with *M. edulis*, *M. trossulus* and *M. galloprovincialis* as well as hybrids between the three species found in a patchy distribution (Väinölä and Strelkov, 2011). The potential differences in the bioaccumulation of contaminants as well as biological responses to stress either environmental or chemical between the different *Mytilus* spp. has the potential to reduce the effectiveness of biomonitoring programmes. For example, with respect to contaminant exposure, higher metal concentrations were detected in *M. trossulus* compared to *M. edulis* when collected from the same habitat and in the same size range (Lobel *et al*., 1990). The differences in the bioaccumulation of metals between the two *Mytilus spp*. was suggested to be due to the slower growth rate of *M. trossulus* compared to *M. edulis* rather than to any direct differences between the element metabolisms of the species. Hence, *M. trossulus* of the same size as *M. edulis* would be in fact older and have a longer exposure history resulting in higher contaminant concentrations. Differences in biomarker responses between the species have not been investigated. However, there are a few cases where differences in general physiology and behaviour of the mussel could affect the overall fitness

and biological response, such as reproductive strategy (Hilbish *et al*., 2002; Dias *et al*., 2011) and susceptibility to parasitism (Coustau *et al*., 1991). Consequently, it is recommended for *Mytilus* species to be determined for biomonitoring programmes and that only one species should be used.

1.4.4 Pyrene hydroxylase

Pyrene hydroxylase (PH) represents an enzymatic phase 1 activity thought to be related to the cytochrome P450 enzymes although not yet confirmed. These enzymes metabolise selected PAHs and consequently alter potentially harmful chemicals to non-toxic and readily excretable end products. Pyrene hydroxylase is considered to be a model PAH with a single phase I metabolite i.e. 1-hydroxypyrene. This conjugates to various phase II metabolites that can be enzymatically deconjugated for quantification of total phase I metabolism (Fillman *et al*., 2004; Jørgensen *et al*., 2005). Pyrene hydroxylase is thought to be induced by certain PAHs and consequently been proposed as a biomarker of exposure to PAH compounds (Fillman *et al*., 2004).

1.4.5 Micronucleus formation

The frequency of MN is regarded as an important tool for in situ monitoring of genotoxicity. This assay has been used for various organic and inorganic pollutants in laboratory studies as well as in field studies (Bolognesi *et al*. 1996; Koukouzika and Dimitriadis, 2005; Baršienė *et al*. 2006) and it has been recommended for offshore biomonitoring (Baršienė *et al*., 2006; Gorbi *et al*., 2008; Sundt *et al*., 2011). This biomarker shows a continuously increasing trend in animals exposed to increasing concentrations of pollutant and exposure times (Viarengo *et al*., 2007a).

Micronuclei (MN) are chromatin-containing structures that are surrounded by a membrane and have no detectable link to the cell nucleus. As an index of chromosomal damage, the MN assay is based on the enumeration of downstream aberrations after DNA damage and reveals a time-integrated response to complex mixtures of pollutants. Therefore, the MN assay provides the evidence of DNA breakage and spindle dysfunction caused by clastogens and aneuploidogenic poisons (Heddle *et al*., 1983, 1991; MacGregor, 1991; Seelbach *et al*., 1993; Kramer, 1998; Zoll-Moreux 1999).

1.4.6 Lysosomal membrane stability

Lysosomes are subcellular organelles surrounded by a semipermeable membrane that contains numerous hydrolytic enzymes involved in a range of cellular processes including digestion, defence, and reproduction (Viarengo *et al*., 2007a and 2007b). Lysosome membrane integrity has been found to be affected by a range of stressors, including metals and organic chemicals. One of the most well-established methods to determine changes in membrane integrity is through measurements of the lysosomal membrane stability (LMS) (Lowe *et al*., 1995).

LMS (assayed as neutral red retention time) is an easy to perform and low cost test to detect impairments of the functional integrity of cells (Livingstone *et al*., 2000; Moore *et al*., 2006; Viarengo *et al*. 2007a). The method uses one of a range of available dyes which will accumulate in the lysosomal compartment of cells. A reduction in membrane integrity will cause the dye to leak back into the cytosol, an effect which can then be quantified. The method is used with circulating cells (e.g. haemocytes), but techniques exist to use a similar method on other tissues (Lowe *et al*., 1995; Viarengo *et al*., 2007a).

The histochemical procedure for LMS determination in frozen samples of mussel digestive gland (Moore *et al*., 2004) is also an alternative for offshore monitoring programs, where the field conditions such as a moving vessel may be a limiting factor (Gomiero *et al*., 2011) .

Digestive gland lysosomes are cell organelles specialised in digestion of both endogenous and exogenous materials. Impairment of lysosomes and, hence, of food assimilation, can result in severe alterations of cells and whole organisms. Lysosomes of the digestive cell of mussels, apart from their main functions in intracellular digestion of ingested material (Robledo *et al*., 2006, Izagirre *et al*., 2008) and autophagic processes (Moore *et al*., 2007), play an important role in responses to toxic compounds through the sequestration and accumulation of toxic metals and organic xenobiotics. Lysosomal responses to pollutants are widely accepted cellular biomarkers of biological effect, especially in mussels and other bivalve molluscs, whose digestive cells possess a very well developed endo-lysosomal system (UNEP/ RAMOGE 1999, ICES 2004).

1.4.7 Lipofuscin accumulation

In mussel, histochemical biomarkers are often analysed in the digestive gland. The digestive gland of molluscs is the main centre for metabolic regulation, participating in the mechanisms of immune defence and homeostatic regulation of the internal medium, as well as in the processes of detoxification and elimination of xenobiotics (Moore and Allen, 2002).
Lipofuscin accumulation represents an oxidative stress biomarker (Viarengo *et al*., 1990; Regoli, 1992). Elevated lipofuscin accumulation reflects degradation of cellular membrane caused by oxidative damage following the action of different pollutants (Moore, 1988). Lipofuscin granules are constituted by oxidatively modified proteins and lipid degradation products, along with carbohydrates and metals. Although lipofuscin composition may be variable, all lipofuscin pigments are not degradable. Therefore, their accumulation in the lysosome vacuolar system represents an indication of the oxidative stress level in the cells and it is related to the level of membrane lipid peroxidation (Viarengo *et al*., 2007a). Increased accumulation of lipofuscin in the lysosome of digestive gland cells has been found to occur following pollutant exposure (Aarab *et al*., 2008; Aarab *et al*., 2011; Shaw *et al*., 2011).

1.4.8 Neutral lipid accumulation

The effects of pollutants are often associated with unbalanced fatty acid metabolisms and the accumulation of neutral lipids in the lysosomal vacuolar system. The lysosomal storage of neutral lipids in mussel digestive glands has been found to be a useful marker of change in the physiology of the cells (Viarengo *et al*., 2007a; Shaw *et al*. 2011). Neutral lipid accumulation appears to be linked to organic chemical pollution (Lowe and Clarke, 1989, Cajaraville *et al*., 1992; Raftopoulou *et al*., 2006). Lipophilic xenobiotics may alter the metabolism of neutral lipids leading to abnormal accumulation of that lipid class inside lysosomes (Moore, 1988).

1.4.9 Histopathology in selected organs

Histological parameters are commonly used as markers of health status in mussels (*Mytilus* spp.). The identification of pathologies and diseases are increasingly being used as indicators of environmental stress since they provide a definite and ecologically-relevant end-point for chronic/ sub chronic contaminant exposure (Mix, 1988). The application of histological markers in mussels can include measures of reproductive and metabolic condition, and allows for the detection of various pathogens that may affect population mortality. The data generated from this type of analysis in various organs (i.e. gills, gonads, digestive gland) is helpful in providing complementary information to support additional cellular and biochemical based biomarkers techniques such as the ones traditionally used in the WCM programme (Bignell *et al*., 2011). Furthermore gonad development is an important aspect of research related to the biological effects of contaminants (COMPRENDO, 2006). Histological

markers assessing the status of gonads may give an indication of contaminant effect on the reproductive performance of mussels (Aarab *et al*., 2006, 2011).

1.4.10 Condition Index

Physiological measurements have been frequently used as a means to integrate the possible effects of complex mixture of contaminants (Pampanin *et al*., 2005). Contaminants can have antagonistic, synergistic, or additive effects on animal health. The identification of easy, practical and low-cost indices capable of recognising physiological stress is required during monitoring activities. Physiological responses are generally nonspecific and are directed at evaluating effects on energy metabolism or influence on growth and reproduction. Their link with effects at higher levels of organisation is very important. In this context, mussel condition index is proposed as an ecophysiological measure of the health status of the organisms, summarizing the physiological activity of mussels (growth, reproduction, secretion, etc.) under given environmental conditions.
Condition indices based on flesh weight relative to whole weight or shell have been used for several years, both in scientific research and in commercial fisheries, and several methods are available (ICES, 2012). The methods may use wet flesh weight, whole weight, and shell size and/or volume. Indices using dry flesh weight are more accurate, particularly when used in relation to internal shell volume. In this case the index were chosen according to previous successful results in environmental monitoring (Pampanin *et al*., 2005), measuring both dry weight of flesh (i.e. ash free dry weight) and shell (an accurate measurement) that gave previously good results.

1.4.11 POCIS

The principle of passive sampling is the placement of a device in the environment for a fixed period of time, where it is left unattended to accumulate contaminants by diffusive and/or sorptive processes. They offer sensitive, time-averaged sampling without confounding factors which may occur when using biomonitoring organisms. In the present study they are used as a support parameter to indicate exposure to groups of chemicals and not to determine effects, which may only be inferred (see Harman *et al*., (2011), for a full discussion). The present passive sampling design has been chosen to focus on the alkyl phenols (AP) most abundant in PW (C1-C5). Biological methods for measuring exposure (not effects) to both these two relevant chemical groups are either lacking or poor. The chosen passive sampling device is the polar organic chemical integrative sampler (POCIS, Alvarez *et al*., 2004), which has previously been shown to be suitable for measuring PW originating AP (Harman *et al*., 2009).

2. Materials and Methods

2.1 Study design

2.1.1 Source of mussels

Mussels (*Mytilus spp.*) were obtained from a clean location in Trondheims fjord, the same population used in the WCM programme in 2006, 2008, 2009 and 2011. Mussels were transported to IRIS Biomljø facility and kept in clean seawater (inlet at 80 m depth) for 3 days prior to pre-exposure sampling and field deployment. Mussels of the same size were used throughout the study (length approx. 50 mm).

2.1.2 Pre exposure sampling

A sub-sample of the mussels collected from Trondheimsfjord were analysed for the same chemical and biomarker endpoints as measured in the mussels used in the field exposure (Table 1). Mussels were sampled after arrival at the IRIS Biomiljø laboratories in Stavanger.

Table 1. Measured chemical and biological endpoints in the mussels.

Method	**Indication of**	**Matrix**
Species confirmation	*Mytilus* spp. (*M. edulis, M. trossulus, M. galloprovincialis*)	Gill
Pyrene Hydroxylase	Exposure to PAHs.	Digestive gland
Lysosomal stability	General stress	Haemocytes and digestive gland
Lipofuscin accumulation	Oxidative stress	Digestive gland
Neutral lipid accumulation	General stress	Digestive gland
Histopathology	General stress	Digestive gland
Gonadal maturation	General stress	Gonad
Condition Index	General health	Whole organism
PAH concentration	PAH exposure	Soft tissue
Lipid content	Used for lipid normalization of PAH	Soft tissue
Micronucleus	Genotoxic exposure	Haemocytes
^{226}Radium	Exposure to ^{226}Ra	Soft tissue

2.1.3 Cage deployment and retrieval

A detailed description of the field work including pre-exposure sampling, deployment cruise and the sampling cruise can be found in the cruise report (Appendix A). A general outline is provided below. The mussel stations were deployed on the 27th and 30th March 2012 and retrieved 7th to 10th May 2012. Mussels were placed at selected locations around the Troll C platform at 500 m, 1000 m and 2000 m as shown in figure 1.

The design of the mussel rigs is reported in details in Appendix A. More than 320 individual mussels were packed in a mussel net bag and held within the water column at a depth of around 15 m. Eleven mussel stations were selected around the platform, with 2 mussel stations located at a reference location approximately 50 km from the platform (Table 2). The higher number of mussel stations used was to increase the likelihood of some mussels lying in the path of the PW discharge plume, with the intention of analysing mussels from 8 stations.

Abiotic parameters, such as temperature and light are always recorded in biomonitoring program, since they can have great influence on biomarkers data. It is important to have a record of these parameters to secure good condition for caged mussels. Moreover, temperature has been always been recorded in previous WCM. Therefore, as an addition to vertical CTD, stationary devices were placed with continuous recording of the temperature. The type of device that was used also logs lighting.

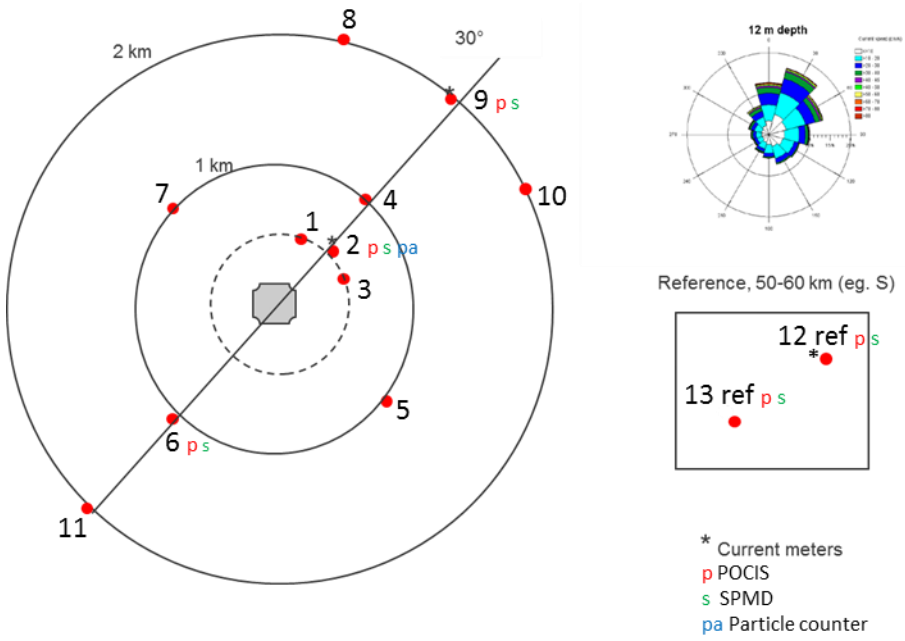

Figure 1 - Overview of the position of the mussel stations around the Troll C platform. The diagram in the upper right corner shows current rose with the dominating current direction based on data from previous measurements. The line between station 11 and 9 represents the predicted residual current direction.

Table 2 -Position of deployed mussel rigs.

Station	Latitude WGS84	Longitude WGS84
Troll C	N 60° 53` 08,98``	E 003° 36` 35,28``
1	N 60° 53` 27,56``	E 003° 36` 44,95``
2	N 60° 53` 25,65``	E 003° 36` 54,42``
3	N 60° 53` 22,48``	E 003° 37` 02,85``
4	N 60° 53` 42,33``	E 003° 37` 14,93``
5	N 60° 52` 49,19``	E 003° 37` 42,92``
6	N 60° 52` 34,63``	E 003° 34` 53,99``
7	N 60° 53` 28,59``	E 003° 35` 25,66``
8	N 60° 53` 11,16``	E 003° 37` 09,83``
9	N 60° 54` 04,53``	E 003° 37` 47,83``
10	N 60° 53` 54,06``	E 003° 38` 08,90``
11	N 60° 52` 13,12``	E 003° 35` 26,93``
12 (ref1)	N 60° 31` 07,69``	E 004° 02` 00,69``
13 (ref2)	N 60° 30` 07,68``	E 004° 02` 00,11``

Current meters were attached to stations 2, 9 and 12 to determine the main current direction during the entire exposure duration.

The collected mussels were sampled on board the vessel and the different matrices collected for specific chemical and biological endpoints as listed in Table 1.

2.1 Temperature depth profiles and current measurements

Mussel rig 2 and 9 and reference rig 12 were fitted with *Aquadopp* current meters by Nortek AS. An *Aquadopp Profiler 400kHz* current meter was used at rig 2. This unit measured current profile between 15 m and 65 m depth. The other two meters were point monitoring instruments and only measured current direction and strength in their fixed position at 15 m depth.

A CTD unit equipped with a particle counter, SD200W was placed at 15 m depth on rig 2 in order to continuously monitor the density of particles like algae during the exposure period of mussels. The unit was programmed to record measurements every 10 min throughout the duration of exposure.

Temperature and light sensors (*HOBO pendant*) were put on all rigs. Three sensors were put on the rope of each rig, one close to the mussels, the other two sensors at 2m above and 2 m below the mussels. Light and temperature measurements were recorded every 10 min for the duration of the exposure.

2.2 Passive samplers

Passive samplers in the form of POCIS were deployed at 2 exposure stations (rigs 1 and 2) and 1 reference station (ref 12). Semi-Permeable Membrane Devices (SPMDs) were also used at the same rigs in order to determine chemical uptake rates. POCIS and SPMDs were held in stainless steel cylinders and placed just below the mussels.

2.3 Species determination

Total DNA was extracted from 20-40 mg of gill tissue 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 seconds. Cell debris were then removed by centrifugation at 10,000 g for 10 min (4ºC), before DNA was precipitated from the supernatant by addition of 500 µl 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), and all samples had OD (optical density) 260/280 > 1.8 indicative of pure DNA. For species identification, polymerase chain reaction (PCR) were used to amplify a specific 180 base pair (bp) segment for *M. 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 95ºC followed by 35 cycles of 30 sec at 95ºC, 30 sec at 55ºC, 30 sec at 72ºC, and final extension step of 10 min at 72ºC. 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.

2.4 Analytical methods in mussels

2.4.1 Chemical body burden in mussels

Approximately 15 whole mussels from each station were excised from their shell and transferred to high temperature treated (560^{o}C) glass containers. The mussels were frozen and transported to NIVA on dry-ice. The samples were stored at -20^{o}C until analysis.
A 5g sub-sample of the homogenate was taken and internal standards added (naphthalene d8, biphenyl d10, acenaphthene d8, phenanthrene d10, anthracene d10, pyrene d10, chrysene d12 and perylene d12) before extraction by saponification. Analytes were then 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. Analysis proceeded by GC-MS with the MS detector operating in SIM. 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 'split less' mode. The initial column temperature was 60^{o}C, which after two minutes was raised stepwise to 310^{o}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.

2.4.2 ^{226}Radium in mussel soft tissue

For each individual station, mussel soft tissue was dissected and divided into 3 separate pools each containing between 100-200 g (w.w.). All samples were immediately frozen and the stored at - 20°C until analysis. Due to the ^{226}Ra half-life of 1602 years, degradation by storage for some months prior to analyses is considered insignificant.
Samples were ashed at 600°C, spiked with ^{133}Ba for recovery estimation and subsequently treated with 8 M HNO_3. Radium was then separated by $PbSO_4$ and $BaSO_4$ precipitation before activity of ^{226}Ra was determined by α-spectroscopy. The reported uncertainty is an expanded uncertainty with a coverage factor of 2 (approx. 95 % confidence level).

2.5 Biomarker methods in mussel

Where possible the biological responses were evaluated on the same individuals. This included: pyrene hydroxylase (PH), LMS in both haemocytes and digestive gland, MN, lipofuscin (LF) and neutral lipid (NL) accumulation. Ten mussels were analysed from each station and in the pre exposure group (station 0).

2.5.1 Pyrene hydroxylase

The PH activity method was adapted from a method described in Michel *et al*. (1994). The microsomal fraction (100 µL) was added to sodium phosphate buffer (200 µL, 0.05 M, pH 7.3) containing BSA (2 mg/mL), NADPH (100 µL, 10 mM) and pyrene in acetone (10 µL, 400 µg/mL). The tubes containing the microsomes were incubated on a shaker (room temperature, 30 min.) before the reaction was terminated by adding 500 µL methanol. Internal standard (triphenylamine, 10 µL, 15 µg/ mL) was added to the solution and mixed. The tubes were centrifuged to precipitate protein and the supernatant was injected on a high performance liquid chromatography (HPLC) system for the determination of the metabolite formed.

Determination of metabolites by HPLC

HPLC was performed on a Waters 2695 Separations Module equipped with a 2475 fluorescence detector and fitted with a Waters PAH C18 (4.6 ×250 mm, 5 µm) column. The mobile phase consisted of a gradient from 40:60 acetonitrile: water to 100% acetonitrile at a flow of 1 mL/min, at 35°C. The excitation and emission wavelengths used for detection of pyrene and triphenylamine were 346nm, 384nm and 300nm, 360nm respectively. The injection volume used was 25 µL.

2.5.2 LMS in haemocytes

The determination of the LMS in haemocytes was performed using the neutral red retention time (NRRT) assay. Haemolymph 400 µL was taken from each mussel and mixed with filtered seawater at the ratio 1:1. An aliquot (30 µL) of haemolymph/ seawater mixture was pipetted out on microscope slides, and incubated in a light-proof box for 15 min before 30 µL neutral red (concentration 0.1 µg/µL) was added. The cells were observed after 15, 30, 60, 90, 120, 150 and 180 minutes to determine whether the neutral red dye is retained within the lysosomes of the haemocytes. The time was recorded when more than 50% of all haemocytes had leaked the neutral red dye into the cytoplasm. All analyses were performed blind. For a detailed description of the method see Lowe and Pipe, (1994).

2.5.3 Histochemical preparation

For histochemical examination, small pieces (5 x 5 x 5 mm) of freshly excised digestive gland tissue were placed in cryovials and snap frozen in liquid nitrogen (-196°C). Prior to sectioning the samples were attached to aluminium chucks. Cryostat sections (10 µm) were cut in a cryostat with the cabinet temperature below -25°C and the knife cooled to - 20°C. The sections were transferred to microscope slides. The slides were stored in the freezer at -40 °C before use. Cryostat sections were used for analyses of LMS, lipofuscin and neutral lipid accumulation.

2.5.4 LMS in digestive gland

The determination of LMS was based on the time of acid labilisation treatment required to produce the maximum staining intensity according to UNEP/RAMOGE (1999), after demonstration of hexosaminidase (Hex) activity in digestive cell lysosomes. Slides were incubated at 4 ºC for 30 min and then 10 min at room temperature prior to staining. Serial cryotome sections (10 µm) were subjected to acid labilisation in intervals of 0, 2, 4, 6, 8, 10, 15, 20, 25, 30 40 and 50 min in 0.1 M citrate buffer (pH 4.5 containing 2.5 % NaCl) in a shaking water bath at 37 ºC, in order to find out the range of pre-treatment time needed to completely labilise the lysosomal membrane. Following this treatment, sections were transferred to the substrate incubation medium for the demonstration of Hex activity. The incubation medium consisted of 20 mg naphthol AS-BI-N-acetyl-β-D glucosaminide (Sigma, N 4006) dissolved in 2.5 ml of 2-methoxyethanol (Merck, 859), and made up to 50 ml with 0.1 M citrate buffer (pH 4.5) containing 2.5 % NaCl and 3.5 g of low viscosity polypeptide (Sigma, P5115) to act as a section stabiliser. Sections were incubated in this medium for 20 min at 37 ºC, rinsed in a saline solution (3.0 % NaCl) at 37 ºC for 2 min and then transferred to 0.1 M phosphate buffer (pH 7.4) containing 1mg/ml of diazonium dye Fast Violet B salt (Sigma, F1631), at RT for 10 min. Slides were then rapidly rinsed in running tap water for 5 min, fixed for 10 min in Baker's formol calcium containing 2.5 % NaCl at 4 ºC and rinsed in distilled water. Finally, slides were mounted in Kaiser’s glycerine gelatine and sealed with nail varnish.

The time of acid labilisation treatment required to produce the maximum staining intensity was assessed under the light microscope as the maximal accumulation of reaction product associated with lysosomes (UNEP/RAMOGE 1999). Four determinations were made for each animal by dividing each section in the acid labilisation sequence into 4 approximately equal segments and assessing the labilisation period in each of the corresponding set of segments. The mean value was then derived for each section, corresponding to an individual digestive gland.

2.5.5 Lipofuscin accumulation

The LF content of lysosomes was determined using the Schmorl reaction. Cryostat sections were fixed in Baker`s calcium-formol for 15 min, rinsed in distilled water and immersed in the reaction medium containing an aqueous solution of 1% ferric chloride and 1% potassium ferrocyanide in a ratio 1:1 (v:v). Sections were stained for 2 min, rinsed in acetic acid (1%) for 2 min, washed in running water for 10 min and rinsed in distilled water before mounting using aqueous mounting medium. Slides were subjected to image analysis and results were expressed as pixel density.

2.5.6 Neutral lipid

For the determination of unsaturated NL, cryostat sections were fixed in Baker`s calcium-formol for 15 min, rinsed in distilled water and transferred into 60% triethylphosphate (v/v with distilled water) for 1 min. Sections were stained in 1% solution of Oil Red O in 60% triethylphosphate for 15 min. Then they were rinsed in 60% triethylphosphate for 30 s, washed in distilled water and mounted using aqueous mounting medium. Neutral lipid accumulation was assessed by computer assisted image analysis. Results were expressed as pixel density.

2.5.7 Histopathology in digestive gland

In order to prevent the appearance of post mortem artefacts, specimens were handled with extreme care. Sampled mussels were kept in ice after collections and the dissection were performed immediately on board. Digestive glands were dissected, putted in pre-labelled histocassette and placed into histological fixative (Baker's calcium-solution: 4% formaldehyde, 1% $CaCl_2$, 2.5% NaCl) for wax sections. Tissue samples were no thicker than 1 cm to ensure proper fixation. Samples were then stored at 4˚C until embedding.
When present a piece of gonad was dissected together with the digestive gland and use to determine the gonadal maturation.
Mussel sections were dehydrated in alcohols. The tissues were cleared in methyl benzoate ($C_6H_5CO_2CH_3$), rinsed in benzene (C_6H_6) and embedded in paraffin. Histological sections (5 µm) were cut using a microtome HM 355s (Microm, Bergman), mounted on slides, dried at 37 ºC for 24 hours and stained with haematoxylin and eosin. The tissues were examined for health parameters related to reproductive and physiological conditions, inflammatory and non-specific pathologies and those associated with pathogen and parasites infections. The DG tubules atrophy was recorded using a scoring index ranging from 0 to 3 (Brooks *et al*., 2009). Digestive glands were examined for 11 histopathological alterations related to physiological conditions, inflammatory pathologies and pathogen/parasite infections. Each alteration was scored according to its severity and frequency (0 = absence of alteration, 1 = ≤ 10 % of the histological section showed the alteration, 2 = between 10% and 50% of the histological section showed the alteration, 3 = between 50% and 100% of the histological section showed the alteration) (Benly et al 2008; Sensini et al. 2008).

The reproductive status was determined according to (Seed, 1976). The presence of parasites and non-specific inflammation were scored as absent (0) or present (1). All micrographs were captured using an AxioCam MRc5 (Zeiss) digital camera mounted on a *Zeiss Axioplan 2* light microscope (Göttingen, Germany). The slides were analysed blind.

2.5.8 Condition index

Condition index (CI) was calculated on 20 specimens. Mussel shells were cleaned carefully under fresh water flow and then opened to remove all soft tissue. The shells and soft tissue were dried for 48 h at 90˚C and before being weighed. Afterwards, the remained soft tissue was dried for 4 hours at 470˚C to determine the ash weight. CI was calculated according to the following formula (Lucas and Beninger, 1985): CI= ash free dry weight/dry weight of shell

2.6 Polar chemical integrated sampling devices (POCIS)

2.6.1 Handling, extraction and extract derivitisation

Standard POCIS, with a surface area per mass of sorbent ratio of ca. 180 cm^2 g^{-1} were obtained from ExposMeter (Tavelsjo, Sweden) (Alvarez *et al*., 2004). The 'pharmaceutical' configuration was used, containing Oasis® HLB sorbent between two discs of polyethersulphone (PES) membrane. POCIS were used at specific stations (1, 2, and 12) and deployed in commercially available stainless steel canisters (Environmental Sampling Technologies, Saint Joseph, USA,), which were attached directly to the main rope of each mussel station. After retrieval samplers were frozen until analysis (-20 ^{o}C).

For extraction POCIS were carefully opened and the sorbent washed with water (Option 3, Elga™) into an empty, solvent rinsed solid phase extraction reservoir (International Sorbent Technologies, Hengoed, UK) and dried using nitrogen. The membranes were discarded. Internal standards were added before elution with 20 mL of methanol or acidified methanol. For AP analysis extracts were concentrated to 0.5 mL, derivatised with 10% pentafluorobenzoyl chloride (Sigma-Aldrich, Oslo, Norway) and extracted with 4 mL hexane (Boitsov *et al*., 2004).

2.6.2 Instrumental analysis of AP

Analysis of alkyl phenol pentafluorobenzoate derivatives was carried out using an Agilent Technologies (Santa Clara, USA) 6890 GC linked to a 5973 mass selective detector in selected ion monitoring (SIM) mode. The injection was splitless and a 50m column of 0.25 mm internal diameter and 0.25µm film thickness was used. Temperature at injection was 60°C which was raised stepwise to 290°C. Analytical quantification limits, obtained by the average solvent blank value, (n = 3) + 10 × standard deviation of that average, were below 0.5 ng for all AP, except for phenol (17.0 ng). Further detail concerning the method and the uncertainty etc. are given by Boitsov *et al*., (2004). POCIS blank values are considered separately. Analysis for AP was carried out by IMR, Bergen.

2.7 Statistical methods

Statistical analyses have been carried out using JMP version 5.1 by SAS Institute Inc. First, data from the three different zones has been tested for significant differences by the Wilcoxon/Kruskal Wallis non-parametric test. Then, a comparison between means has been run with the post-hoc all pair Tukey-Kramer test to uncover pair of groups that have significant differences.

Many datasets did not show a normal distribution. This occurs quite often with ecotoxicological data. Thus, most results are presented as quantiles box plots in order to visualise the uneven distribution of data in a best manner. Diagrams show sum of all histopathological lesions that have been found in different tissue: Gonads, DG and Gills as box plots (median value, 0%, 50%, 75% and 100% quantiles) at each station. The horizontal line in the middle of the plot indicates the grand mean. A visualisation of statistical analyses is shown at the right part of each diagram. All red coloured circles mean that there are no significant differences. When a circle appears in a grey tone it indicates a significant different group of data. The size of the circle indicates the power of the data with regard to number of individual samples, e.g. small circle means high power.

3. Results

3.1 Current, temperature, light, turbidity and salinity data

The water currents around Troll C were identified with current meters positioned at station 2, 9 and 12 (Fig 2 and 3, Appendix B). The dominant current direction was towards north and north east and confirmed that the selected stations were in the path of the plume.

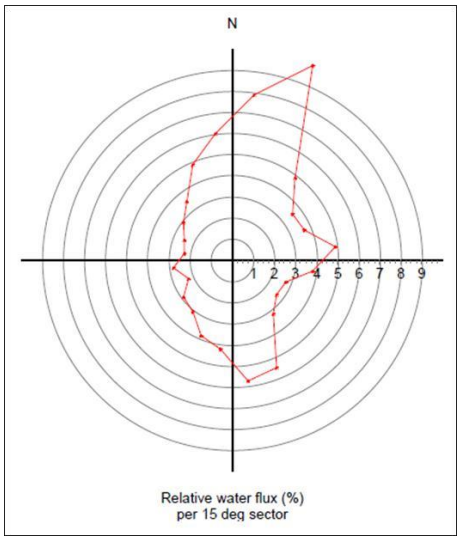

Figure 2 - Sum of relative current at station 9 at 15 m depth (in 15^{o} sectors, cm/s).

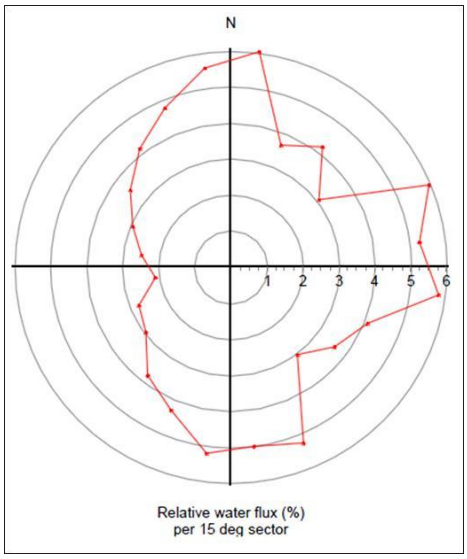

Figure 3 - Sum of relative current at station 2 at 21 m depth (in 15^{o} sectors, cm/s).

With the introduction of temperature and light loggers (Hobo pendants) on each of the mussel stations, water parameters were recorded every 10 minutes throughout the duration of the exposure. Despite the loss of some loggers data are available for all stations (Fig. 4). The temperature profile during the exposure was almost identical at all stations around the platform. At first temperature increased from 7^{o}C to 7.5^{o}C in the first few days before falling to 6^{o}C within the next 10 days and then slowly increasing to 7.5^{o}C at the end of the 6 week exposure. There was not a large difference in temperature between the depths, although in most cases the deeper water tended to be slightly warmer.

The temperature profiles of the two reference stations were also very similar to each other but were markedly different from the stations around the platform. Overall, the temperature at both reference stations slowly increased from below 6.5^{o}C to approaching 7.5^{o}C during the 6 week exposure.

The light data at all stations clearly defined the night and day cycle during the 6 week exposure. As to be expected the loggers located closer to the surface recorded higher light intensities. With exception to station 3, the light profile during the 6 week exposure was very similar at all exposure stations indicating both sunny and cloudy days. The low light readings for both loggers at station 3 may be due to shading of the sensors by the mussels or other objects in the water (e.g. passive sampling equipment). The light profile at the two reference stations showed some resemblance to that of the exposure stations and suggests similar weather conditions between the two locations.

The mean daily temperature, turbidity and depth measurements from station 2 are presented in figure 5. The depth was maintained stable at 27 m throughout the 6 week exposure indicating that the mussel rig remained vertical within the water column. At this depth the temperature fluctuated between 7.5^{o}C and 6^{o}C corresponding with the data from Hobo temperature loggers. The changes in turbidity during the exposure were most pronounced, peaking in the first few days to 15 FTU (Formazin Turbidity Unit) before falling to 2-3 FTU after 10-15 days and then peaking to the highest turbidity (27 FTU) after approximately 25 days. Except for a final peak of 19 FTU on around day 34, the turbidity gradually reduced after day 25 reaching lowest levels in the final few days of exposure.

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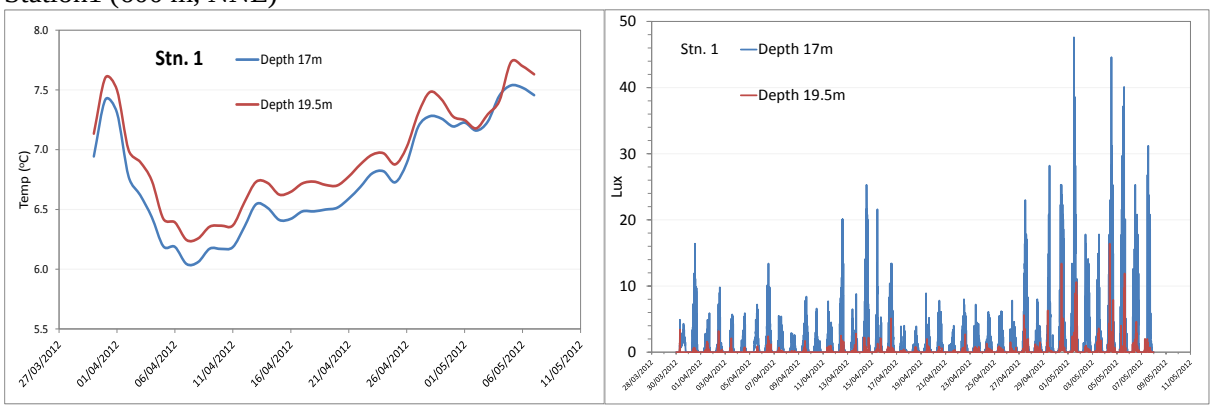
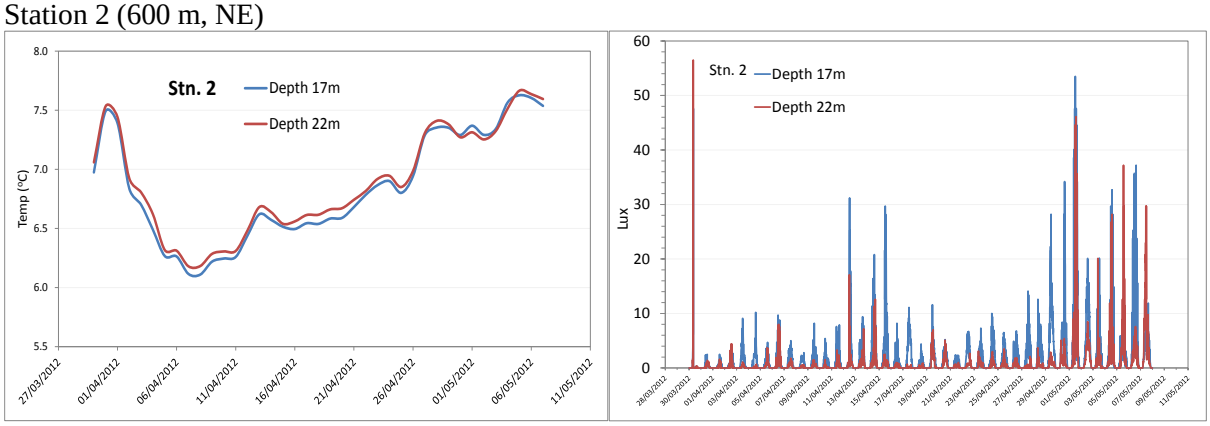
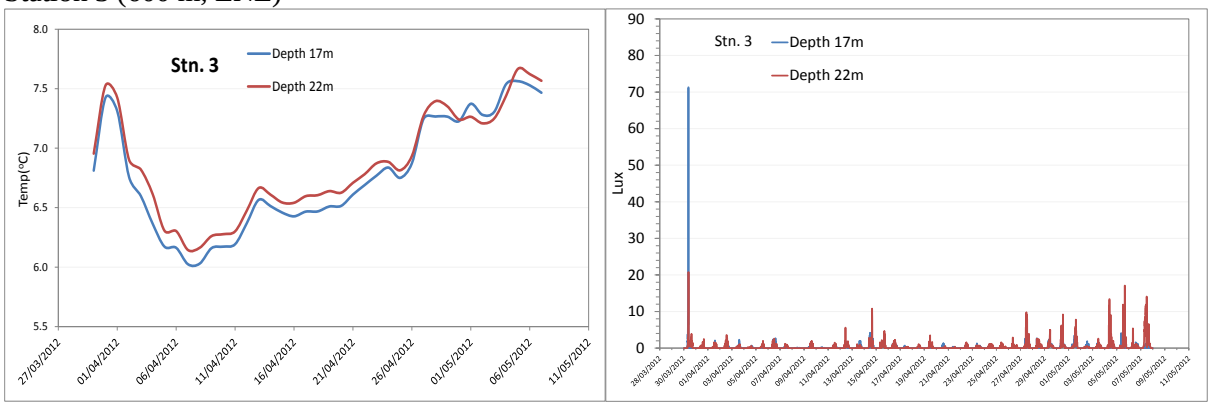


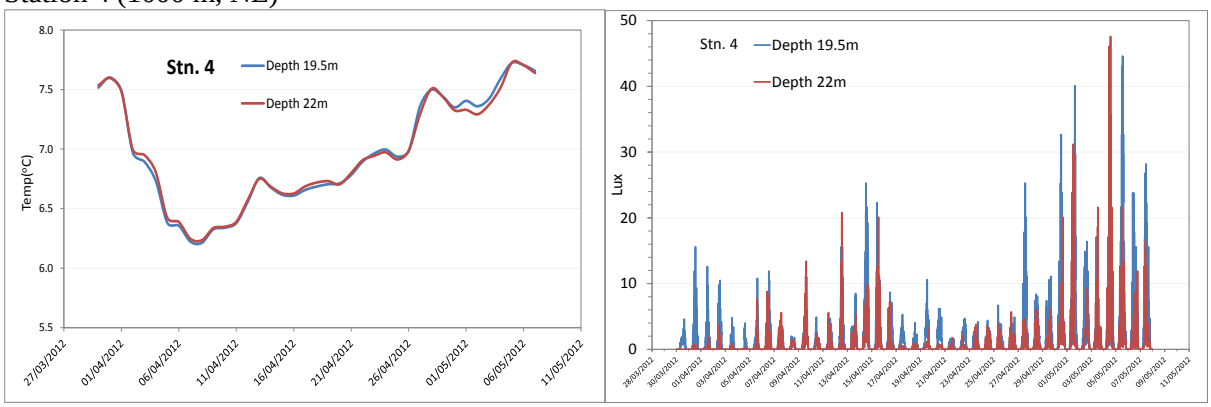
Station 2 (600 m, NE)



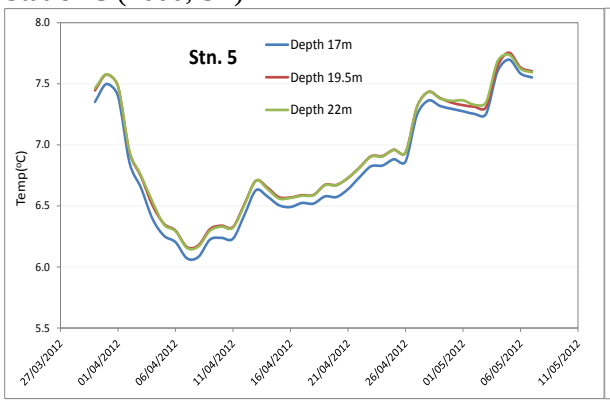
Station 3 (600 m, ENE)

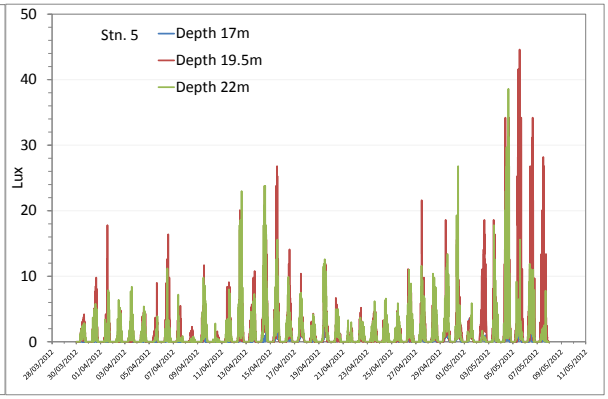


Station 4 (1000 m, NE)

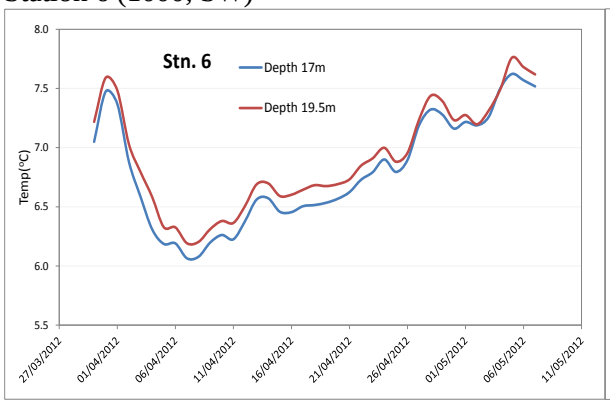


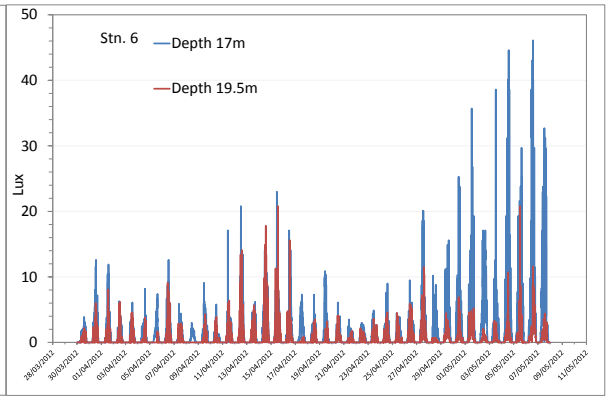
Station 5 (1000, SE)



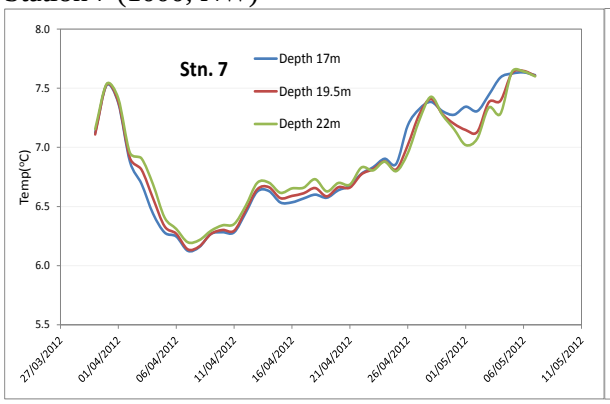


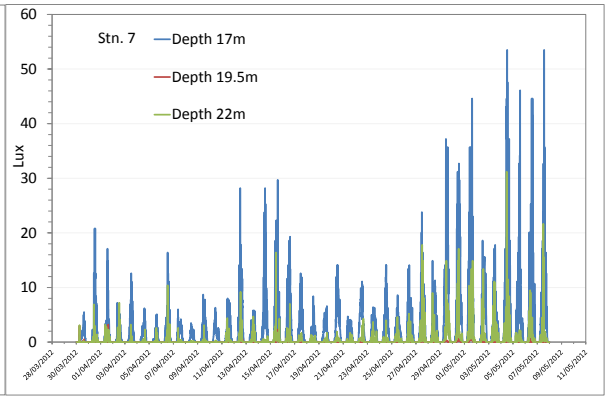
Station 6 (1000, SW)



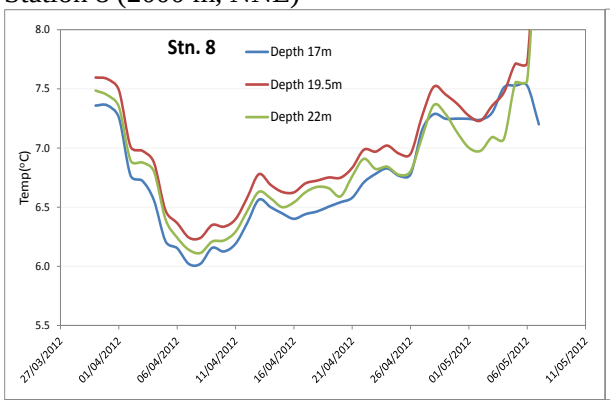


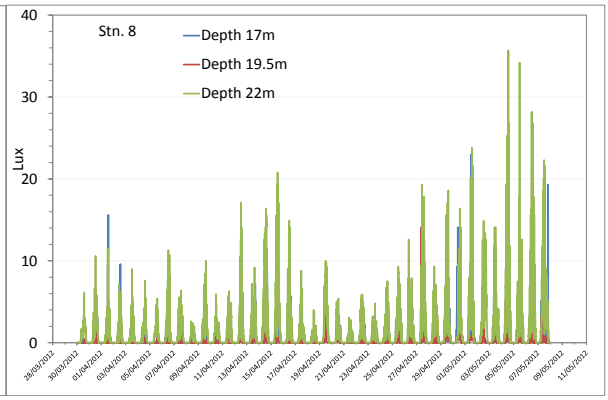
Station 7 (1000, NW)



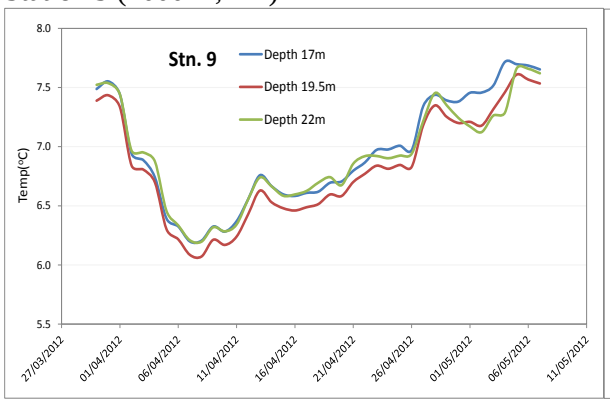


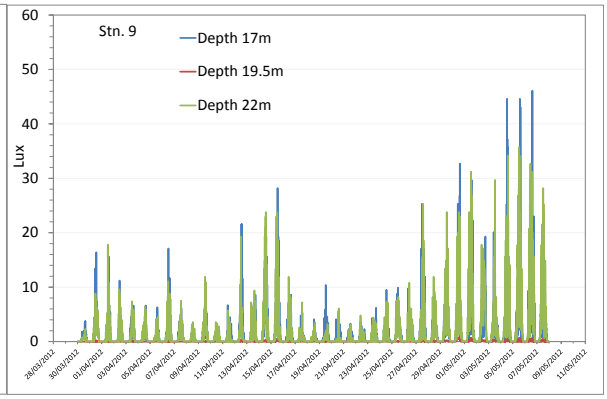
Station 8 (2000 m, NNE)



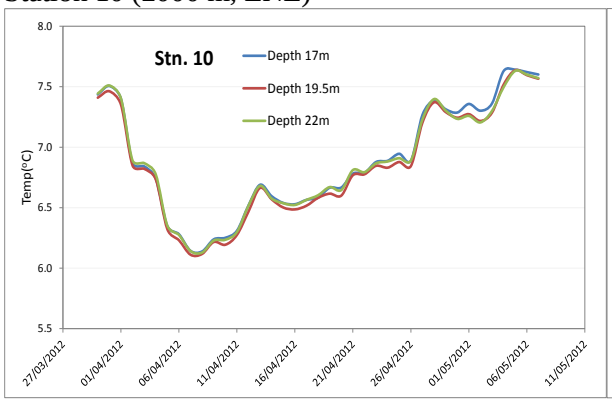


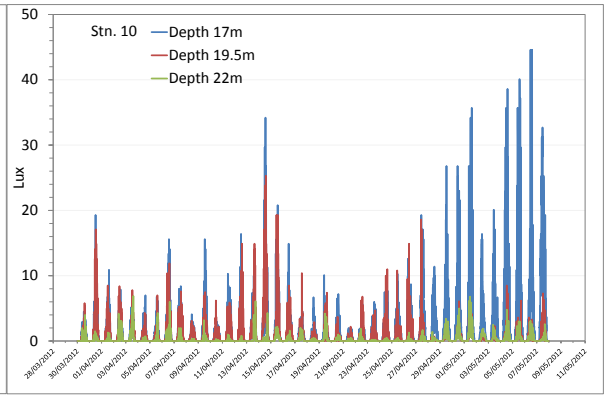
Station 9 (2000 m, NE)



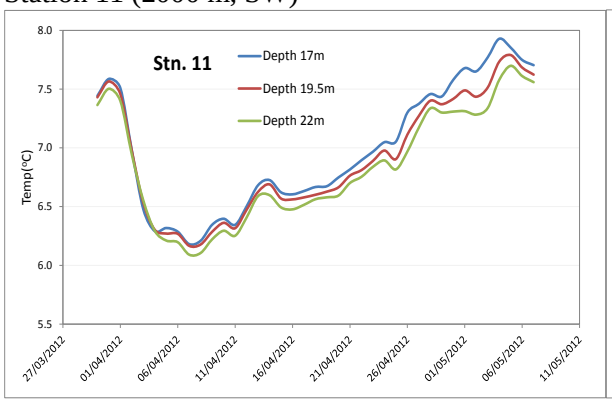


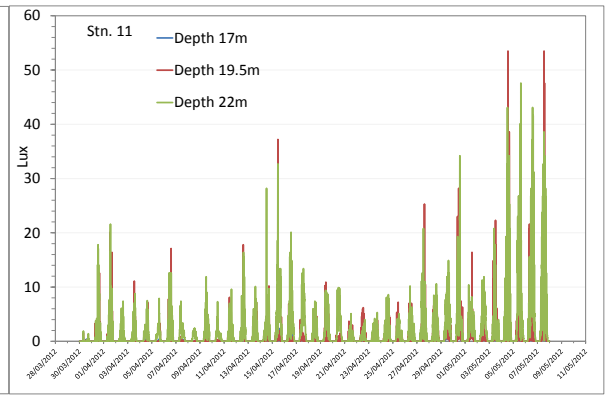
Station 10 (2000 m, ENE)



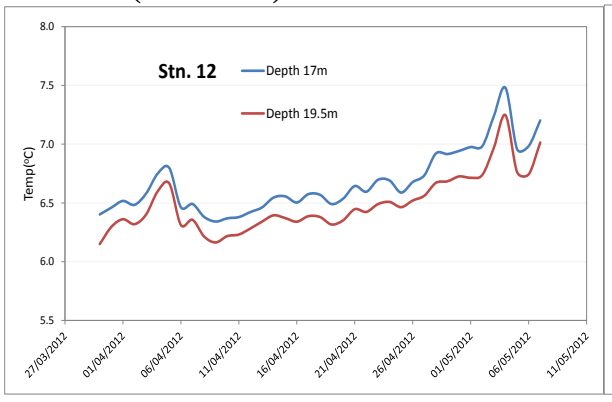


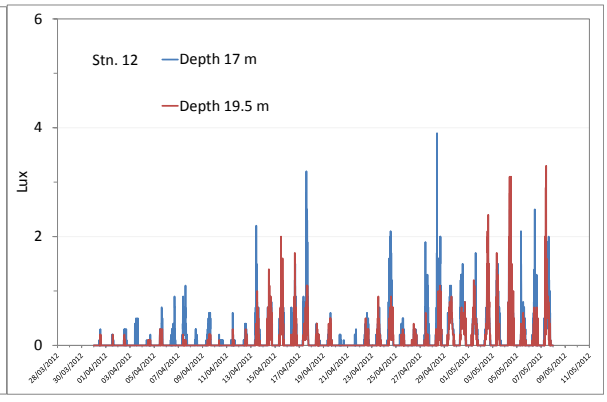
Station 11 (2000 m, SW)





Station 12 (reference 1)





Station 13 (reference 2)

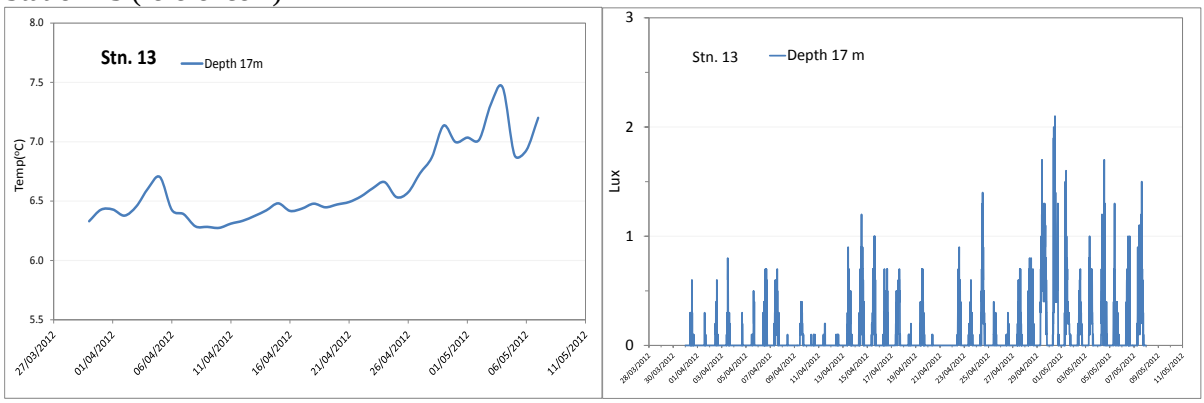


Figure 4 – Temperature profile and light data (for easier visualisation of the results, the y-axis does not reach the origin).

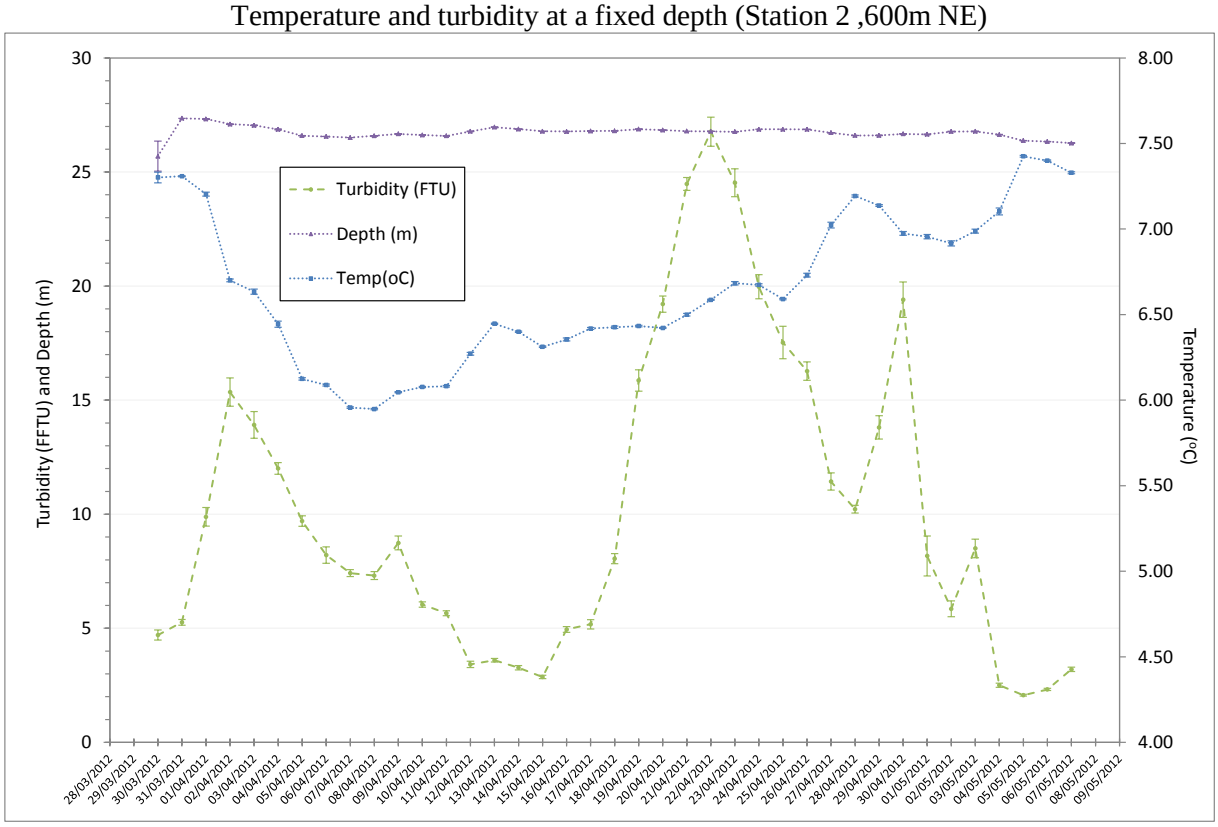


Figure 5 – Temperature and turbidity measurements recorded at station 2 (600 m NE) at a recorded fixed depth during the 6 week exposure.

The data from the salinity and temperature depth profiles taken during the deployment cruise at 600 m, 1000 m and 2000 m from the platform are shown in figure 6. At all three distances from the platform a weak thermocline was present. At 600 m, the thermocline was present between 20 and 30 m whilst at both 1000 m and 2000 m the thermocline was more defined at 20-25m. The salinity appeared to correspond with the temperature data, with a weak thermocline between 20 and 30 m, although this was more defined at 1000 m and 2000 m from the platform.

There has been a technical issue. The rig design was made so that all equipment including mussels, temperature loggers and current meters were placed on a 5 m long rope that was connected just below the buoys (see Fig 2 appendix A). The temperature loggers were placed at the top middle and bottom of each 5m rope with the mussels placed towards the top of the rope and the current meters placed about 1 to 2 m below the mussels. Therefore, either the depth of the current meters has been wrongly reported or the depth of the mussels is incorrect. Due to the design the current meter cannot be more than 3 m away from the mussels and typically 1-2 m below the mussels.

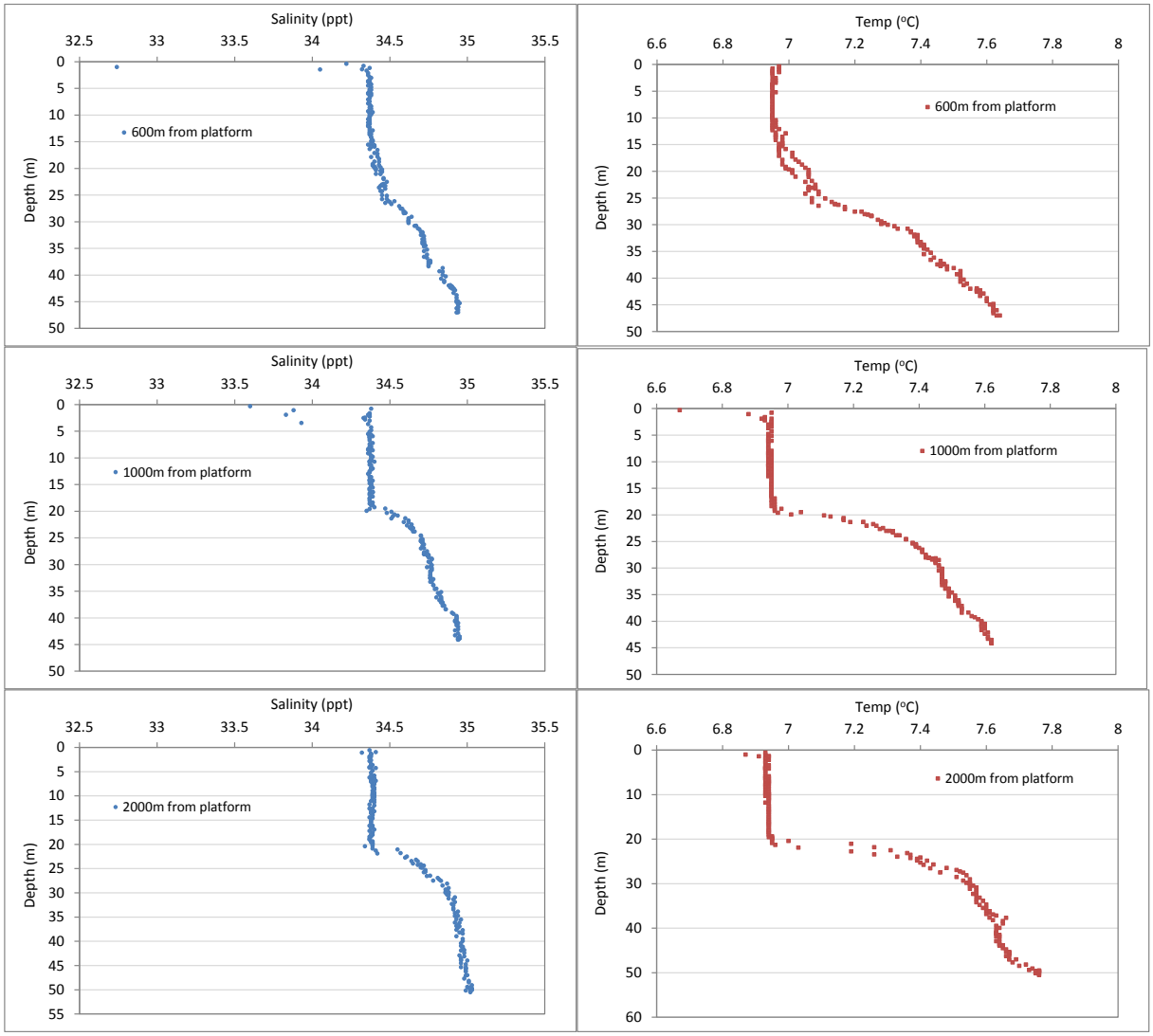


Figure 6 – Temperature and salinity depth profiles taken from the vessel at known distances from the Troll platform during the deployment cruise (27th-30th April).

3.2 Identification of *Mytilus* spp.

The *Mytilus* species were identified from a sub sample of 66 randomly selected mussels from the same mussel population that was used in the monitoring programme (Fig. 7). Of the 66 individuals sampled 87.9% were identified as *Mytilus edulis*, 1.5% *M. trossulus* and 1.5% *M. galloprovincialis*. The remaining 9.1% were hybrids of *M. edulis*, with *M. edulis*/ *M. trossulus* and *M. edulis*/ *M. galloprovincialis* making up 7.6% and 1.5% respectively.

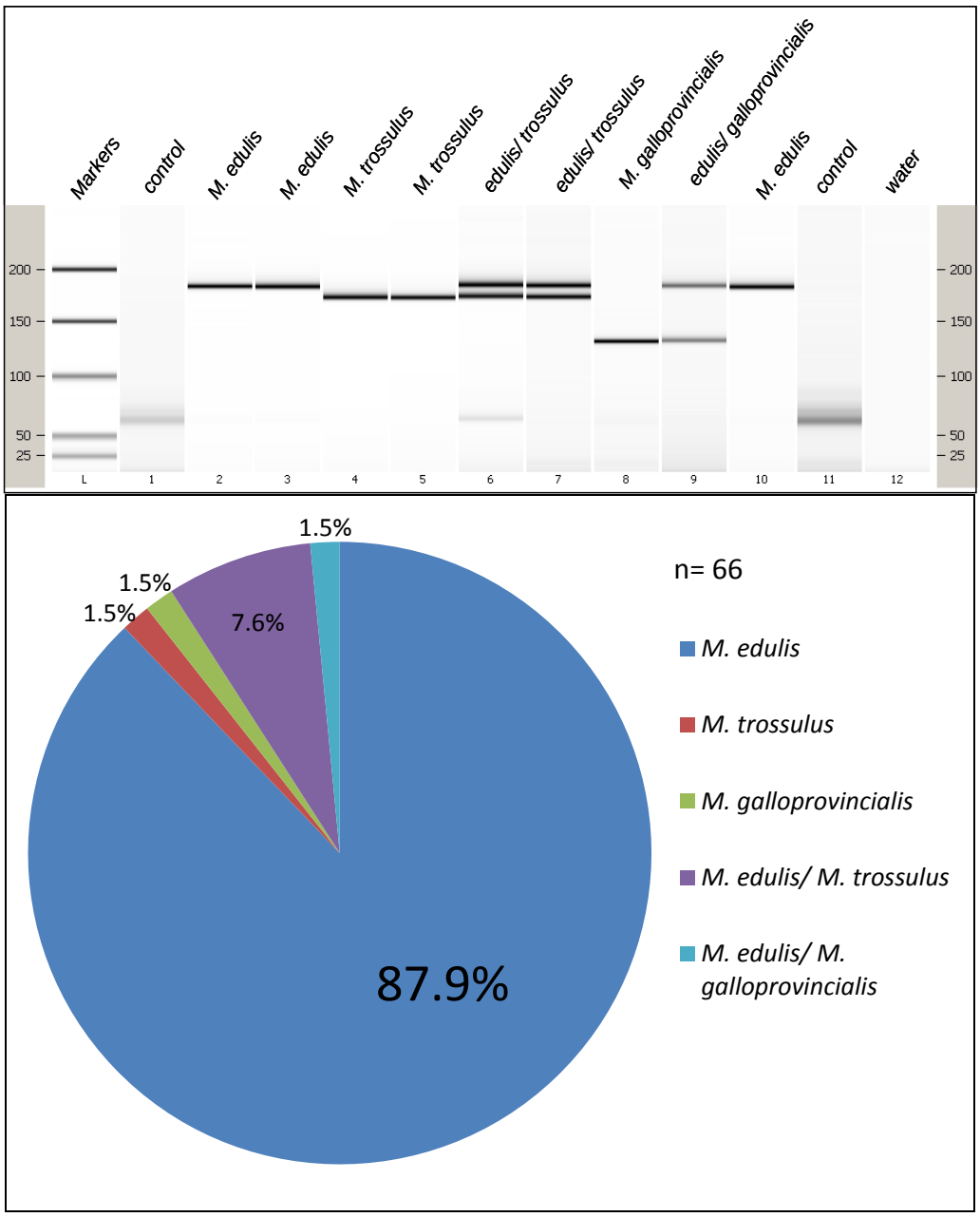


Figure 7 - The percentage distribution of *Mytilus* spp. and their hybrids from a sub-sample of the exposure mussels (n = 66).

3.3 Chemical body burden data in mussels

Triplicate pooled mussel samples from fourteen groups were analysed for PAHs including NPDs, and metals. These included eleven exposure stations in the vicinity of the platform (Stations 1 to 11), two reference stations (Stations 12-13) and the pre-exposure mussels (Station 0). The chemical analysis was carried out by NIVA and the raw data is provided in the appendices.

With exception of the pre-exposure mussels (Station 0), which had higher lipid content than the other groups (1.1-1.2%), median lipid content for all mussel groups was between 0.7 and 0.9 % (Fig. 8). The data for PAHs and NPDs are presented on a wet-weight basis (µg/ kg) and where not normalised with respect to lipid content. Due to the small sample size (n=3 in each

group), statistical evaluation was not performed. Groups with no overlapping values where regarded as significantly different.

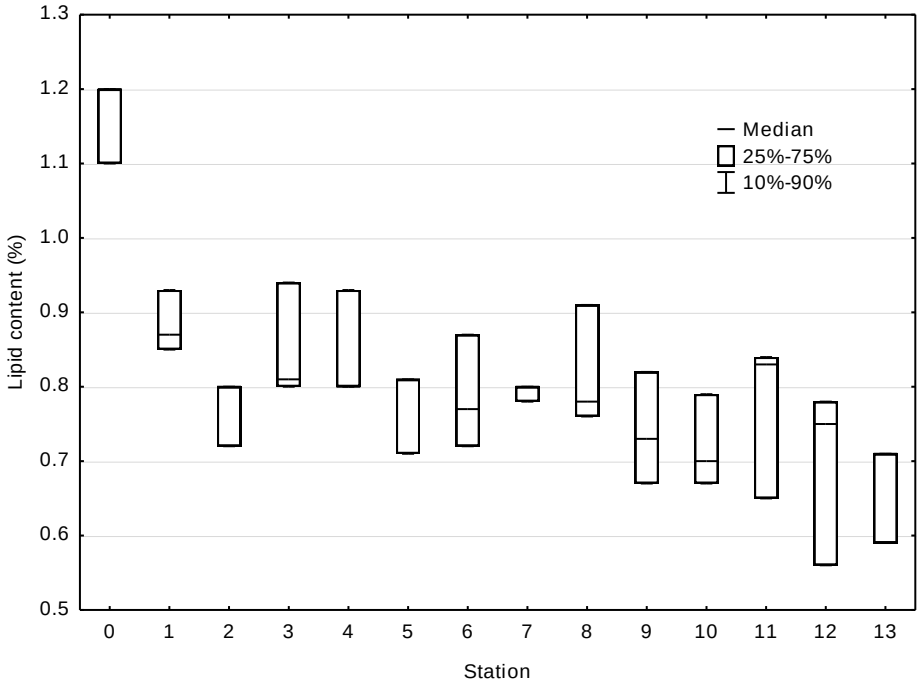


Figure 8 - Lipid content of mussels from the groups indicated. The figure displays median and minimum/ maximum values (n=3). Station 0 refers to the pre-exposed mussels (for easier visualisation of the results, the y-axis does not reach the origin).

3.3.1 PAH and NPD concentrations in mussels

The sum of PAH16 concentration showed a good relationship with respect to distance from the platform. Highest PAH16 values were found in mussels from the closest stations (1-3, 600 m) with station 1 having significantly higher concentrations (31-32 µg/ kg w.w.) than stations 2 and 3 (Fig. 9). The stations positioned 1000 m from the platform (stations 4-7) had median PAH16 concentrations ranging between 9 and 17 µg/ kg w.w., whilst those positioned approximately 2000 m from the platform (stations 8-11) ranged between 5-10 µg/ kg w.w. The mussel from the reference stations (station 12-13) had the lowest concentrations that were marginally above or lower than the detection limit. The mussels from the pre-exposure group (station 0) had PAH16 concentrations in the range of that found in the mussels approximately 1000 m from the platform.

The pattern of PAH16 concentration in relation to distance from the platform was consistent for all other PAHs measured including Sum of PAH (Fig. 10), sum of NPD (Fig. 11) and total naphthalenes (Fig. 12), total phenanthrenes & anthracenes (Fig. 13), and total dibenzothiophenes (Fig. 14). The PAH concentration was dominated by the NPD compounds at all stations. The sum of PAH compounds was highest at station 1 (1550µg/ kg w.w.), of which approximately 99% was made up of NPD compounds (48% Naphthalenes, 41% Phenanthrenes and 11% Dibenzothiophenes). A similar relationship was seen at all exposure stations.

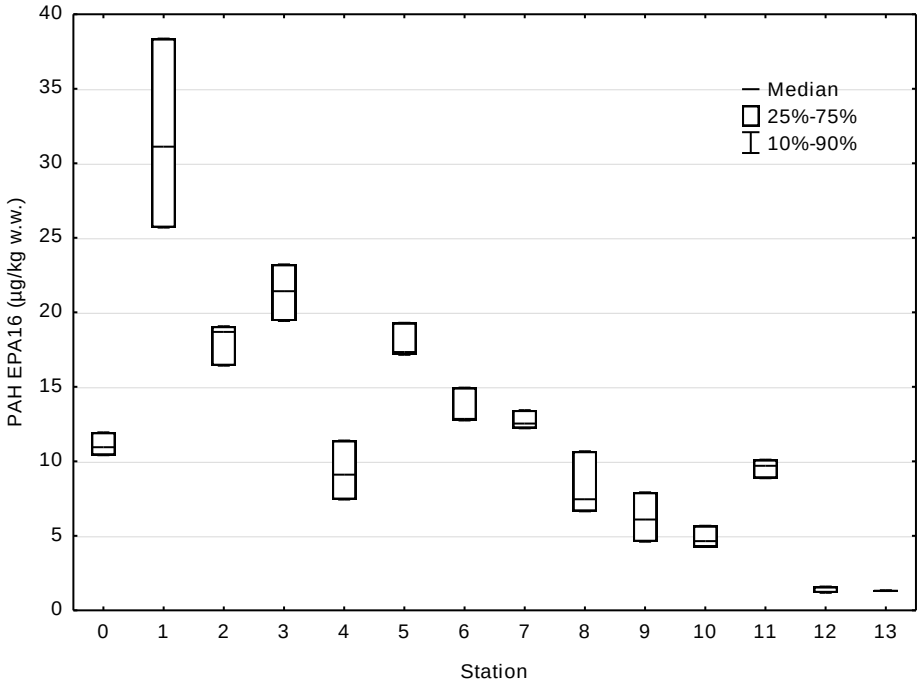


Figure 9 - Sum of PAH16 concentrations in mussels from the groups indicated. The boxes display median and minimum/maximum values (n=3). Station 0 refers to the pre-exposed mussels.

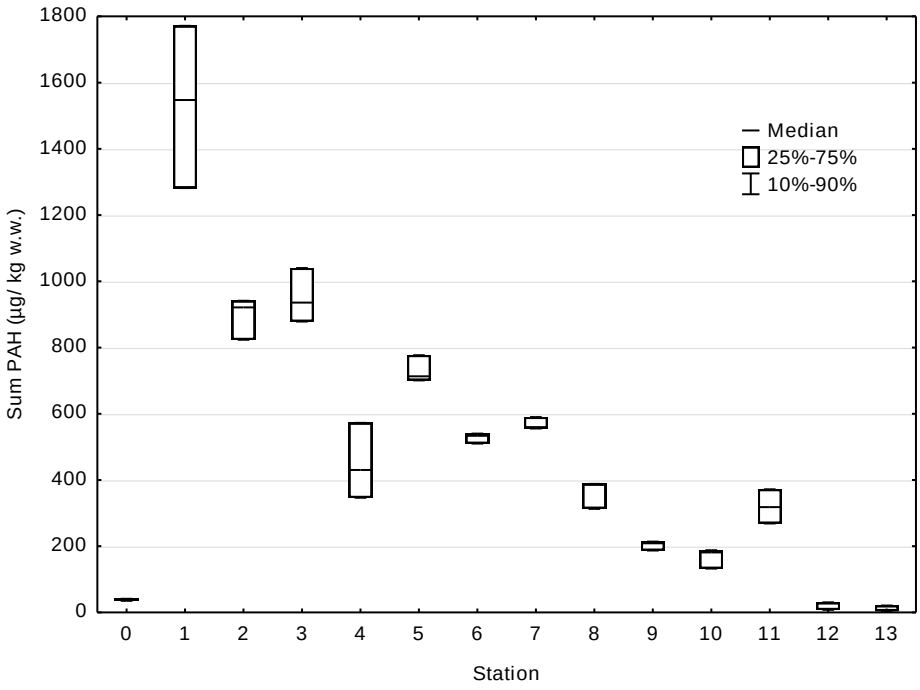


Figure 10 - Sum of total PAH concentrations in mussels measured in the groups indicated. The boxes display median and minimum/maximum values (n=3). Station 0 refers to the pre-exposed mussels.

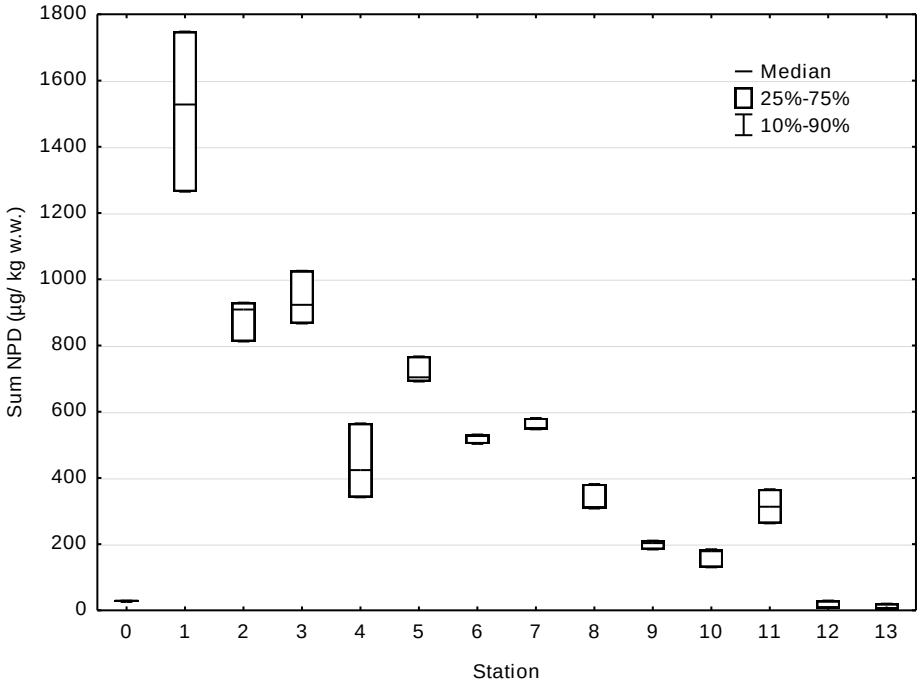


Figure 11 - Sum of NPD concentrations shown in mussels from the groups indicated. Boxes display median and minimum/ maximum values (n=3). Data includes the parent compound and alkylated C1 to C3. Station 0 refers to the pre-exposed mussels.

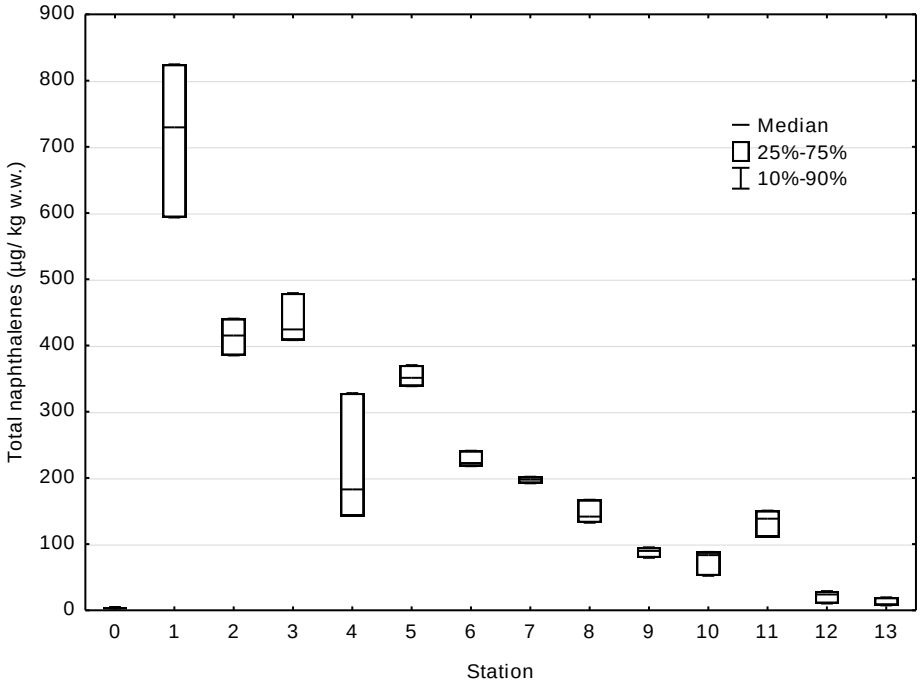


Figure 12 - Total naphthalene concentrations shown in mussels from the groups indicated. Boxes display median and minimum/ maximum values (individual observations since n=3). Data includes the parent compound and alkylated C1 to C3. Station 0 refers to the pre-exposed mussels.

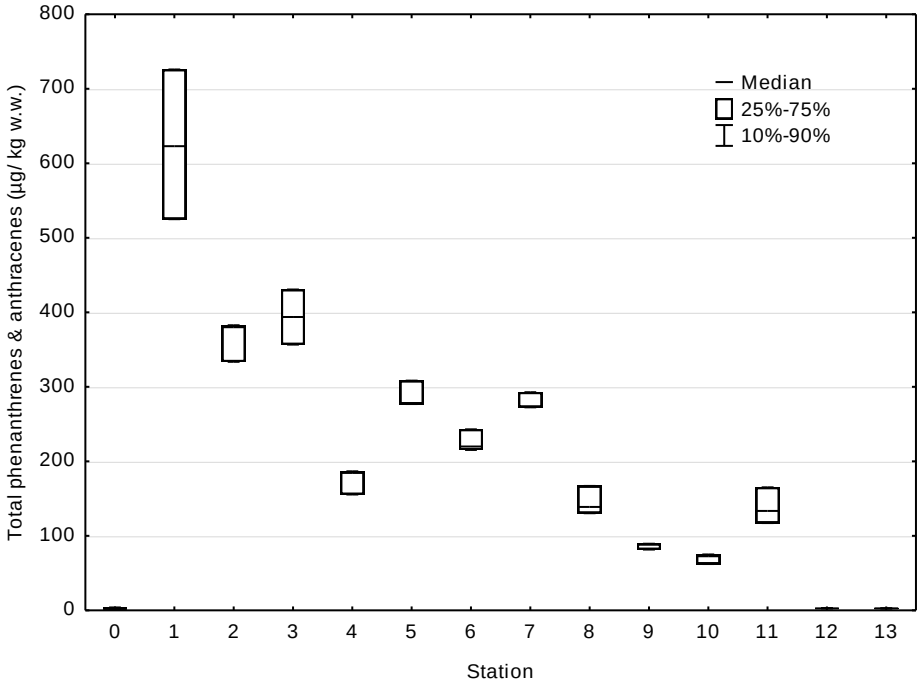


Figure 13 - Total phenanthrene/ anthracene concentrations shown in mussels from the groups indicated. Boxes display median and minimum/ maximum values (n=3). Data includes the parent compound and alkylated C1 to C3. Station 0 refers to the pre-exposed mussels.

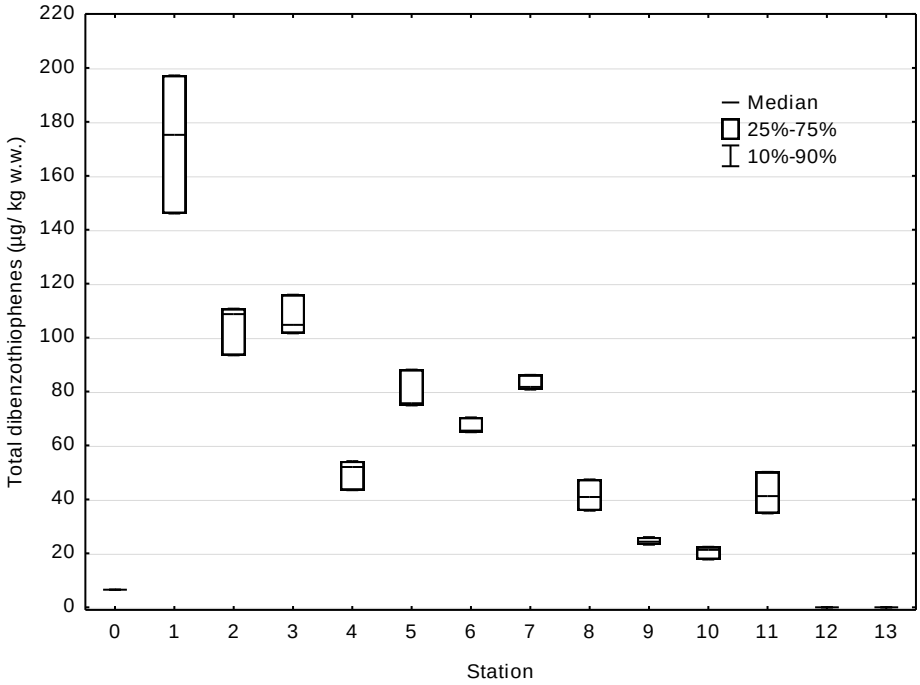


Figure 14 - Total dibenzothiophene concentrations shown in mussels from the groups indicated. Boxes display median and minimum/ maximum values (n=3). Data includes the parent compound and alkylated C1 to C3. Station 0 refers to the pre-exposed mussels.

Ratio of alkylated to non-alkylated NPDs

PAHs found in coal and petroleum will typically contain one or more methyl (C1), ethyl (C2), propyl (C3), butyl (C4), or occasionally higher alkyl substituents on one or more of their aromatic carbons. These alkylated PAHs are generally more abundant than the parent PAH in petroleum, but are less abundant than the parent PAH in pyrogenic PAH mixtures. The ratio of alkylated compounds: parent-compounds for the mussels with respect to the NPDs are shown (Figure 15 to Figure 17). As would be expected, higher ratios were observed in mussels caged within the vicinity of the platform, with very low ratios in both the pre-exposure and reference mussels.

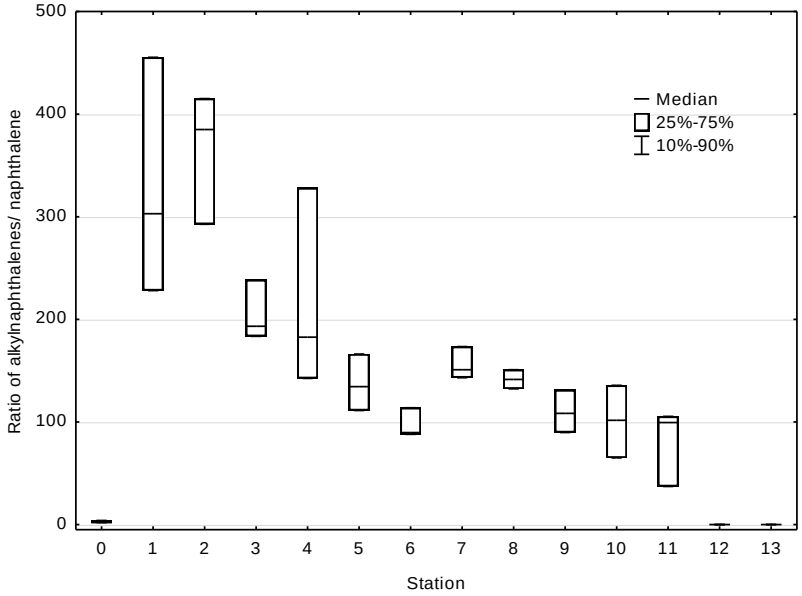


Figure 15 - Ratio of alkylated naphthalenes/ naphthalene. Boxes display median and minimum/ maximum (n=3). Alkylated groups include C1 to C3. Station 0 refers to the pre-exposed mussels.

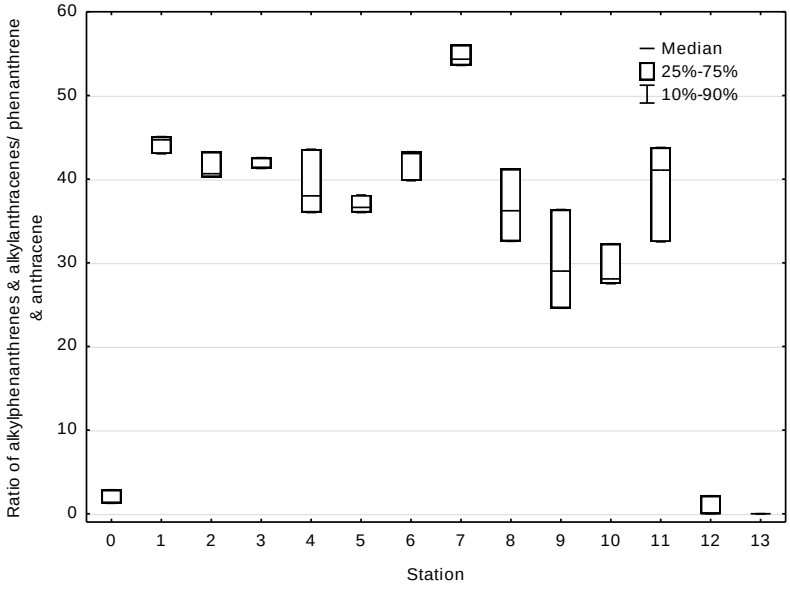


Figure 16 - Ratio of alkylated phenanthrenes & anthracenes/ phenanthrene and anthracene. Boxes display median and minimum/ maximum (n=3). Alkylated groups include C1 to C3. Station 0 refers to the pre-exposed mussels.

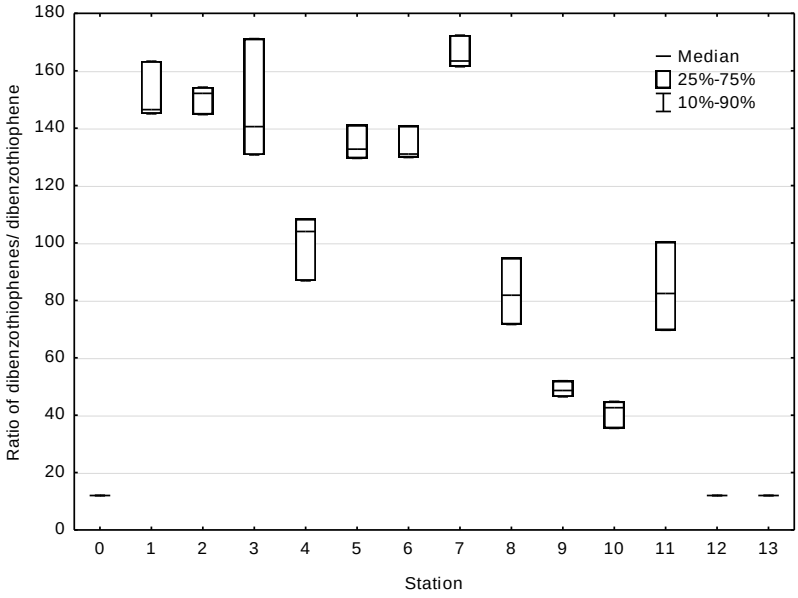


Figure 17 - Ratio of alkylated dibenzothiophenes/ dibenzothiophene. Boxes display median and minimum/ maximum (n=3). Alkylated groups include C1 to C3. Station 0 refers to the pre-exposed mussels.

3.3.2 Metal concentrations

A total of 11 metals were measured in the pooled whole mussel homogenates of pre-exposed and caged mussels (Fig. 18a and 18b). There were no obvious relationships observed between metal concentration and the distance from the platform for any of the metals measured. The pre-exposed mussels had lower concentrations of arsenic, barium, copper, cadmium, lead and zinc compared to the field exposed mussels, whilst the opposite was true for iron. Cobalt and nickel were not measured above their detection limits of 0.1 mg/ kg w.w. at any station.

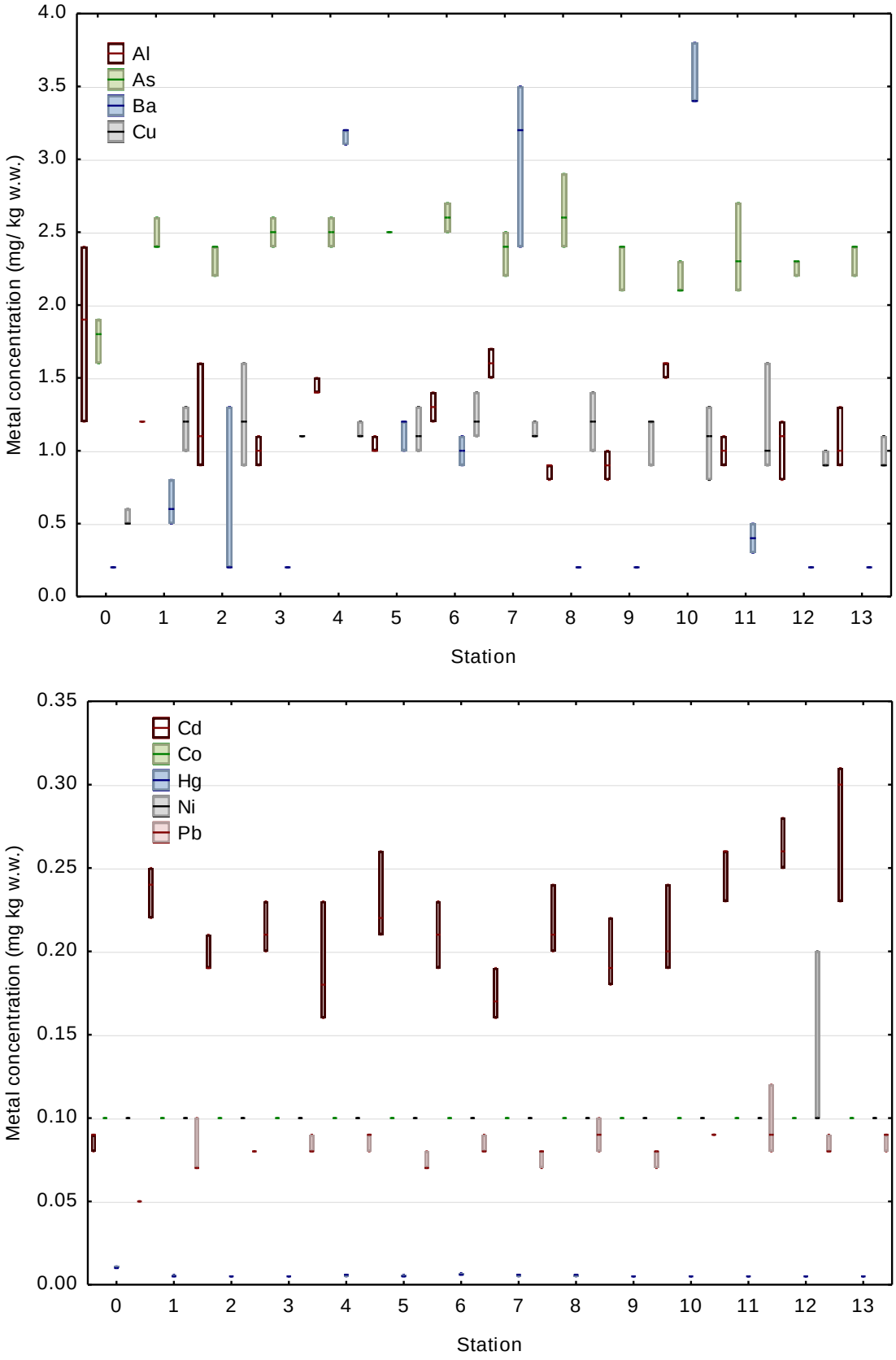


Figure 18a - Metal concentrations in pooled samples whole tissue homogenates of mussels from the stations indicated. Data expressed as mg/ kg w.w. (n=3).

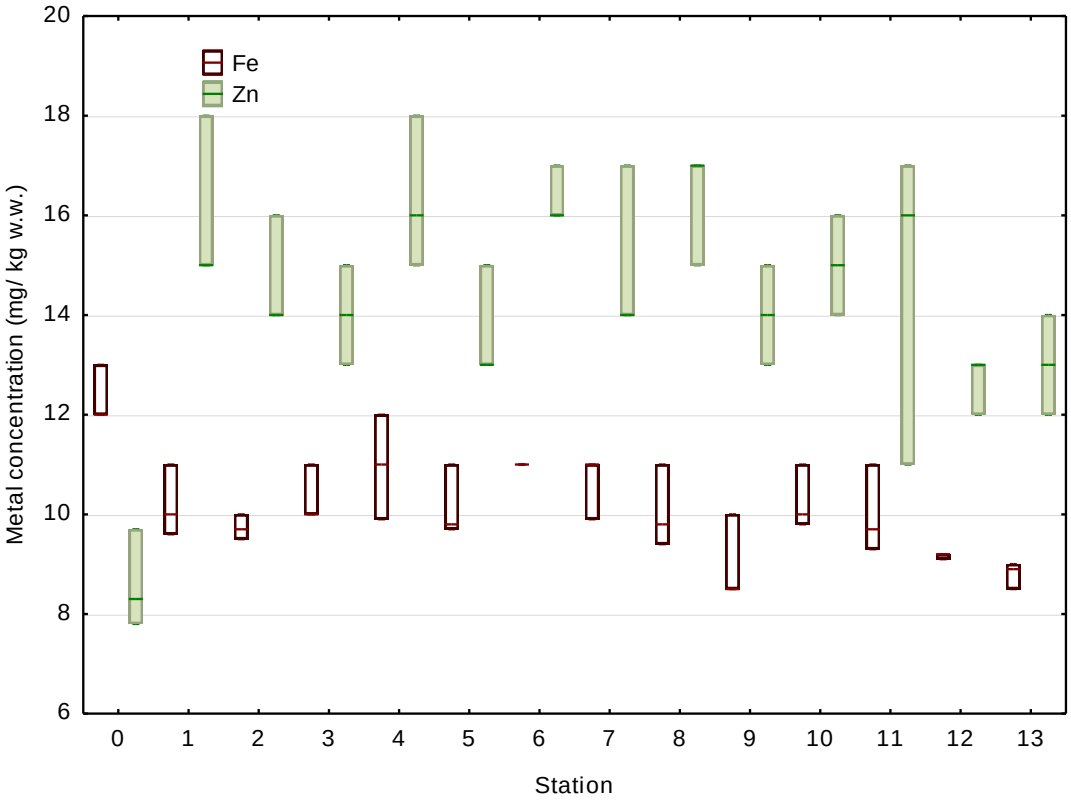


Figure 18b - Metal concentrations in pooled samples whole tissue homogenates of mussels from the stations indicated. Data expressed as mg/ kg w.w. (n=3) (for easier visualisation of the results, the y-axis does not reach the origin).

3.3.3 ^{226}Radium concentrations

Results indicate that levels of ^{226}Ra in the PW exposed mussels were close to the natural background concentrations measured at reference station and as reported in previous WCM surveys, indicating no accumulation of the radionuclide by the mussels (Table 3). This confirms the findings from mussels from previous WCM surveys.

Table 3 - Concentrations of ^{226}Ra in pooled mussel soft tissue analysed by α-spectrometry (>20 mussels per pool). Data are reported as mean ± s.d..

Sample	^{226}**Ra** (mBq/kg w.w)
Station 1 A	47 ± 9
Station 1 B	46 ± 10
Station 1 C	54 ± 12
Station 2 A	21 ± 7
Station 2 B	33 ± 6
Station 2 C	34 ± 6
Station 3 A	41 ± 10
Station 3 B	19 ± 6
Station 3 C	30 ± 7
Station 9 A	17 ± 5
Station 9 B	18 ± 6
Station 9 C	18 ± 6
Station 12 A	32 ± 8
Station 12 B	24 ± 7
Station 12 A	35 ± 8
Station 13 B	30 ± 8
Station 13 C	34 ± 7
Station 13 C	28 ± 7

3.4 Biomarkers in mussels

3.4.1 Pyrene hydroxylase

There were large variations observed in PH activity within each station with no significant differences in PH between the stations (Fig. 19). Mussels from station 1 and 2, which were found to contain the highest tissue concentrations of PAH showed the lowest median PH activity.

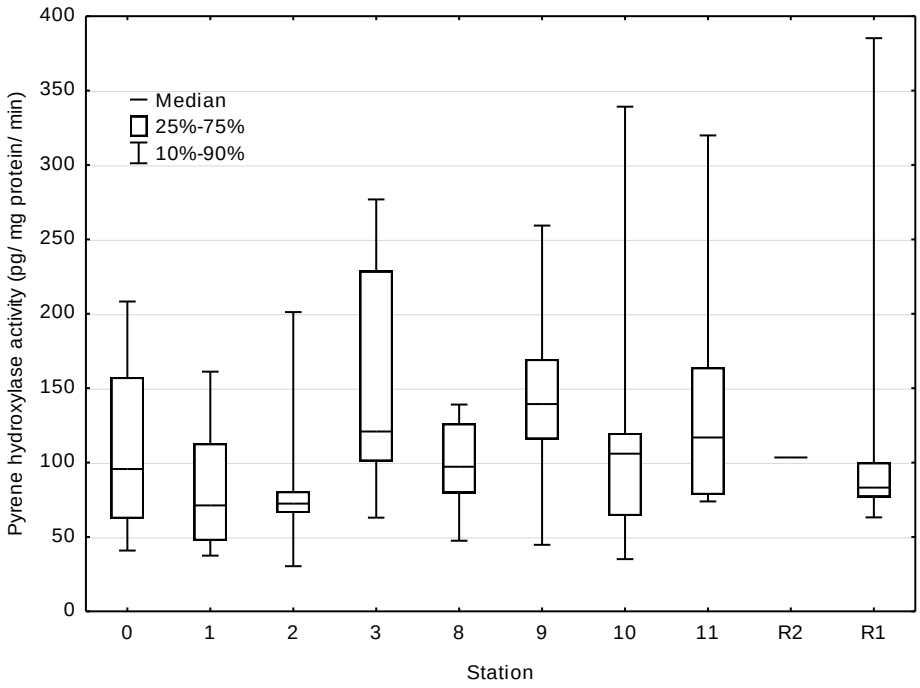


Figure 19 - Pyrene hydroxylase activity in the digestive gland of mussels from the stations indicated. Data expressed as median, quartiles and 10/90-percentiles. (R1=station 12, R2= station 13)

3.4.2 Micronucleus assay

Microscope slides for the assessment MN frequency in the mussel haemocytes were prepared on board the research vessels as in previous WCM campaigns. However, during the laboratory assessment it was discovered that in almost all cases the haemocytes were heavily clustered and cells appeared crushed with damaged cell membranes. This unfortunately resulted in insufficient numbers of viable haemocyte cells for the assessment of micronuclei formation. Therefore, the MN data for the different mussel groups are not presented.
This one time accident won't be repeated abd strategy to avoid unfortunate sampling has been established.

3.4.3 Lysosomal membrane stability in haemocytes

LMS was measured in mussel haemocytes (15 individuals at 10 different stations) as NRRT (min) and ranged from 90 to 180 min (end of the test time) (Fig. 20). Most of the individual showed a NRRT of 150 min. For healthy mussels NRRT of 120 min is considered as the threshold level. There was no statistical difference found between mussels caged around the discharge point compared to each of the reference stations.

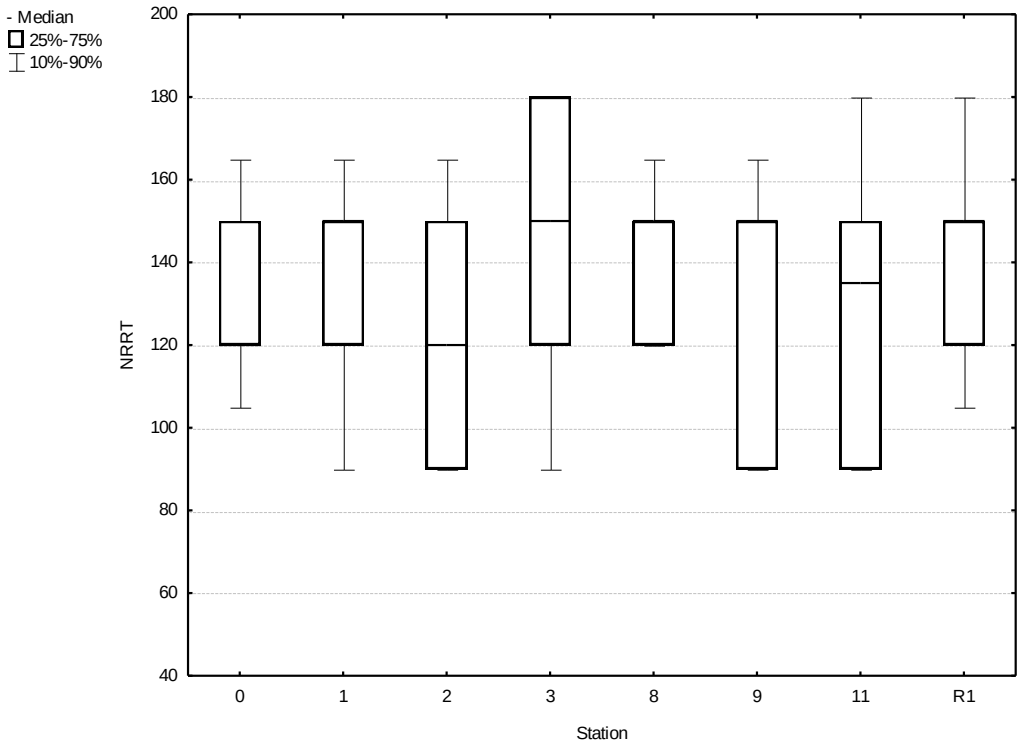


Figure 20 - Lysosomal membrane stability measured as NRRT [min] in haemocytes of mussels from the stations indicated. No significant difference was found. Data are expressed as the group mean. Each box has lines (whiskers) that extend from the ends of the box to the outermost data point that falls within the distances computed (here 90/120-180 min). (n=13 in station 8, n=14 in station 3 and 12 (R1), n=15 in remaining stations) (for easier visualisation of the results, the y-axis does not reach the origin).

3.4.4 Lysosomal membrane stability in digestive gland

The observed lysosomal responses in mussel digestive gland cryo-sections from both T0 and the reference station are within the normal range of destabilization times usually observed for blue mussels in unexposed areas (Fig.21). No significant difference was found among all investigated sites (ANOVA, Tukey HSD, $p<0.05$).

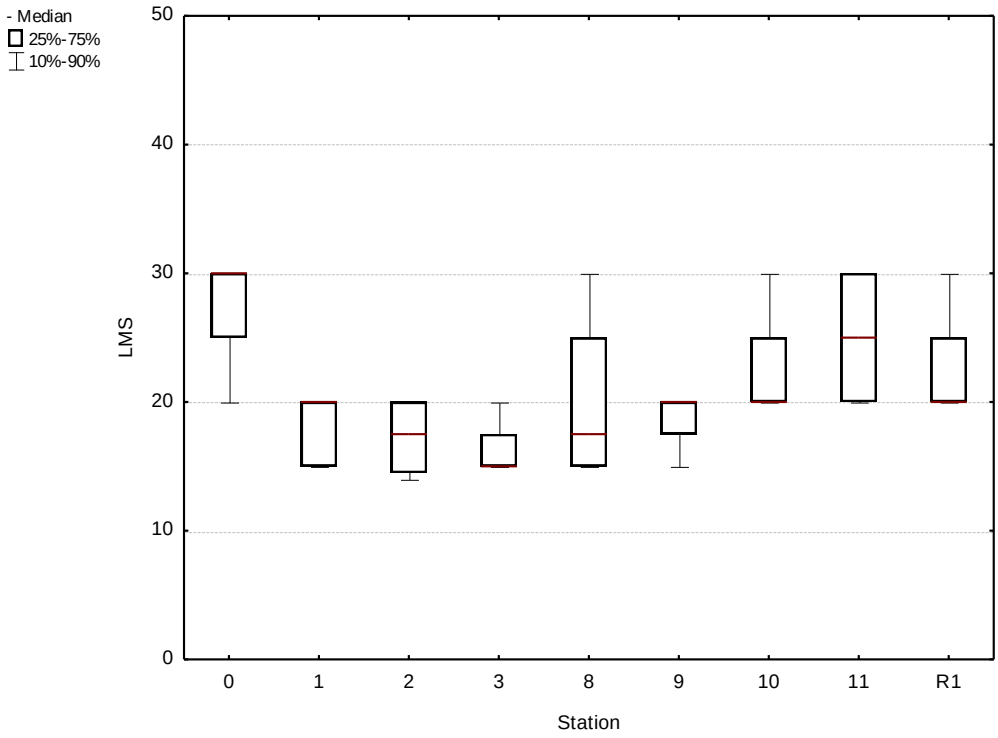


Figure 21 – Results of lysosomal membrane stability (LMS) measured as the time of acid labilisation period in frozen sections of mussel's digestive glands sampled from the stations within the biomonitoring program. No significant difference was found. Data are expressed as the group mean. (R1=station 12)

3.4.5 Lipofuscin accumulation

LF accumulation in lysosomes of mussel digestive gland was lower in the stations around the platform, a statistical difference was found only for station 3 compared to the reference station (R1=station12) (Kruskal-Wallis, multiple comparisons, P<0.05) (Fig. 22). LF accumulation is the result of peroxidation of authophagocytosed proteins associated with protein aggregates and oxidatively damaged organelles.

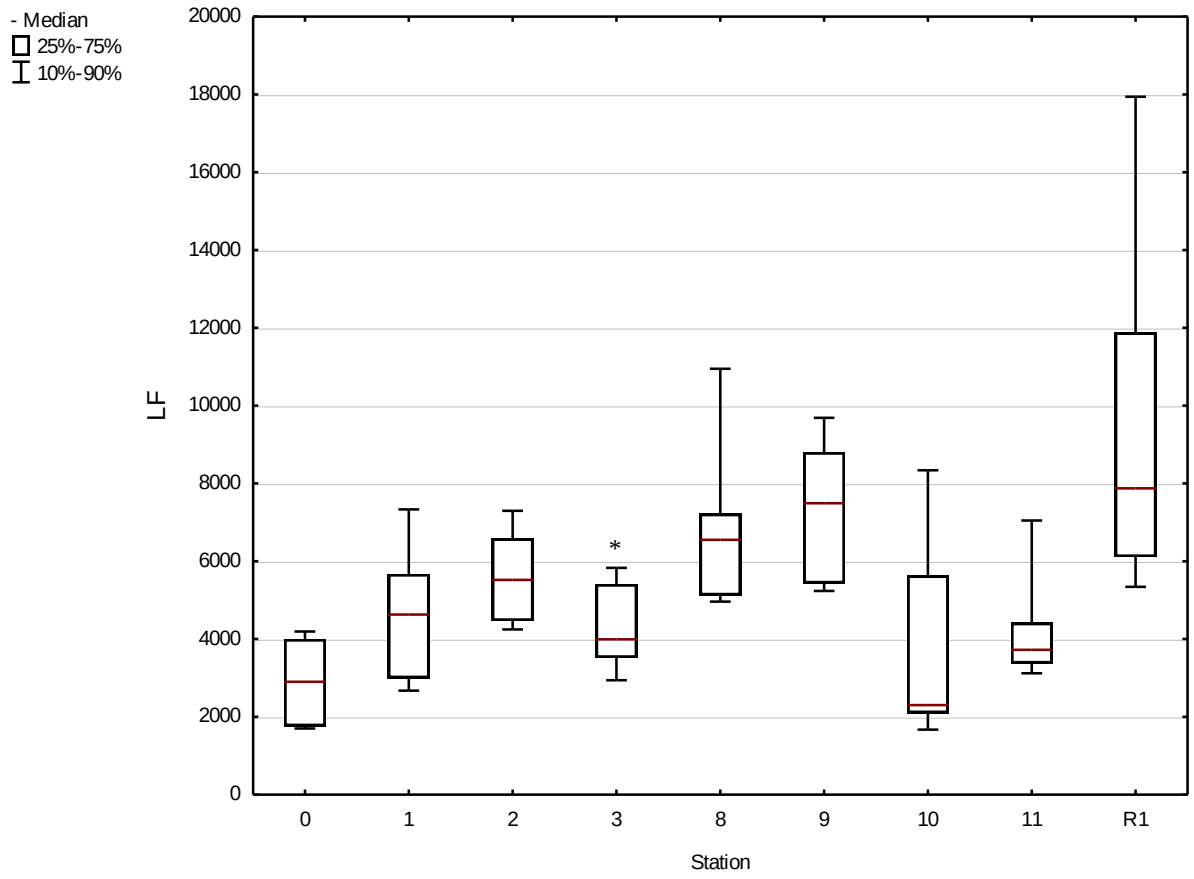


Figure 22 - Lipofuscin (LF) accumulation in mussel lysosomes given as optical density from the groups indicated. The figure shows median, quartiles and 10/90-percentiles. *=significantly different, p<0.05.

3.4.6 Neutral lipid accumulation

NL accumulation was significantly lower in station 3 compared to the reference station (R1=station 12) (Kruskal-Wallis, multiple comparisons, P<0.05) showing the inhibition of this class of lipid accumulation close to the discharge.

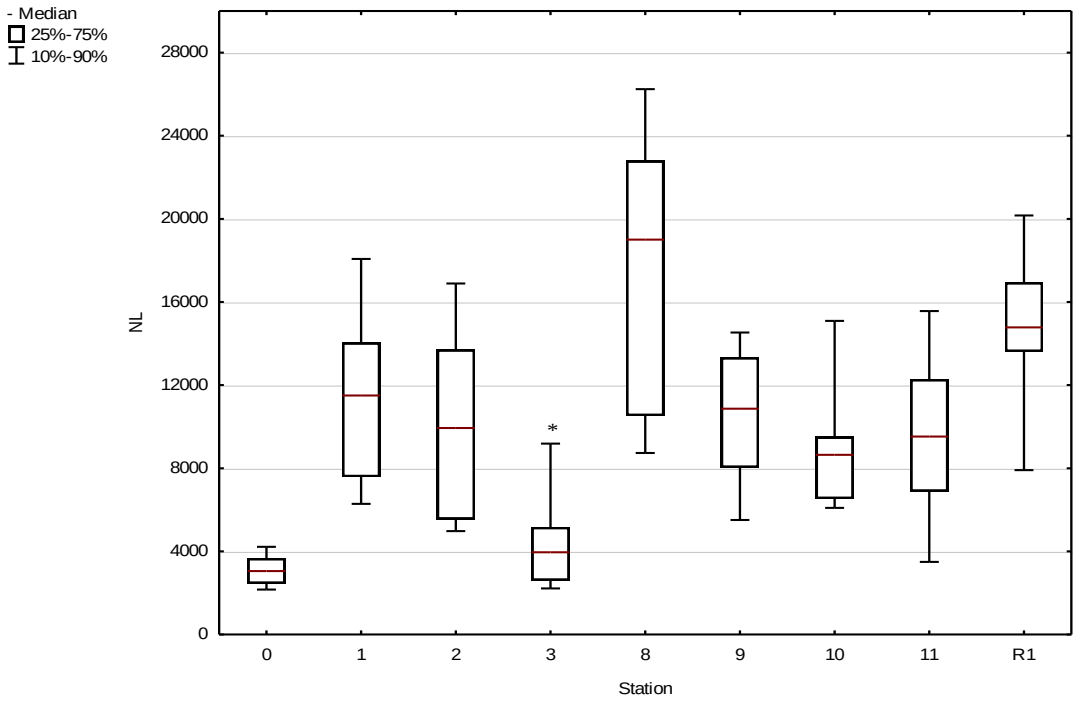


Figure 23 - Neutral lipid accumulation given as optical density in mussels from the groups indicated. The figure shows median, quartiles and 10/90-percentiles. (R1=station12), *=significantly different, p<0.05.

3.4.7 Histopathology in selected tissues

Several of the examined mussels showed histopathological alterations in digestive gland and gonads. These alterations have been scored for each individual mussel. By looking at the colours in the score diagram it is possible to see that there are differences in mean score values in some groups compared to the mussels sampled at station 12 (Fig. 24).

By using the statistical analysis software, a more objective analysis of the score values is presented. The overall histopathology results indicated that mussels caged at station 1, 10, 2, 3 and 9 were statistically different from the one caged at reference station. Results are presented as quantiles box plots in order to visualize the uneven distribution of data in an appropriate manner. Diagrams show sum of all lesions that have been found in the digestive gland as box plot at each station. A visualisation of statistical analyses is shown at the right part of the diagram. All red coloured circles mean that they are not significant differences. When a circle appears in a grey tone it indicates a significant group to the reference one. The analysis shows also mussels caged at station 11 are having low abnormalities compare to the reference one.

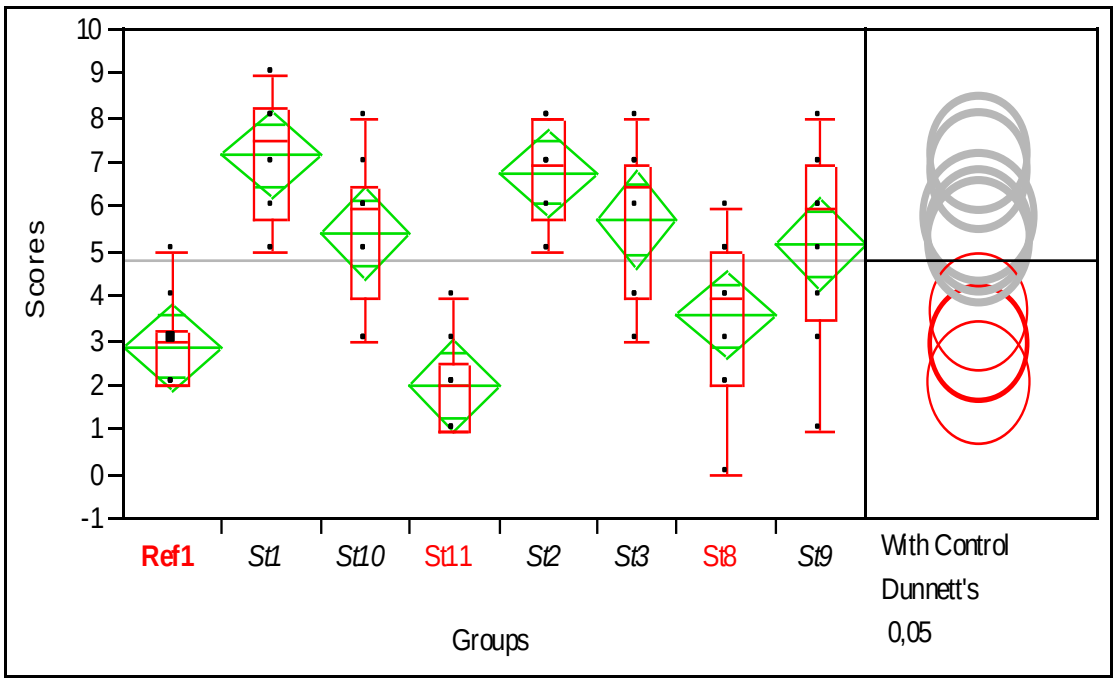


Figure 24 - Statistical comparison using one way ANOVA of the different mussel stations according to their abnormalities in digestive gland. (Ref1=station 12) (for easier visualisation of the results, the y-axis does not reach the origin).

3.4.8 Condition Index

The condition index (CI) is commonly applied in biomonitoring surveys as it is a physiological measurement that summarises the overall condition of the animals. It has been used as a physiological response that describes the impact of pollutants on the normal functioning of the organisms (growth and reproduction) (Pampanin et al., 2005). In general, CI values of sampled organisms were comparable to those calculated for the reference station 12 with the exception of station 8 (fig. 25).

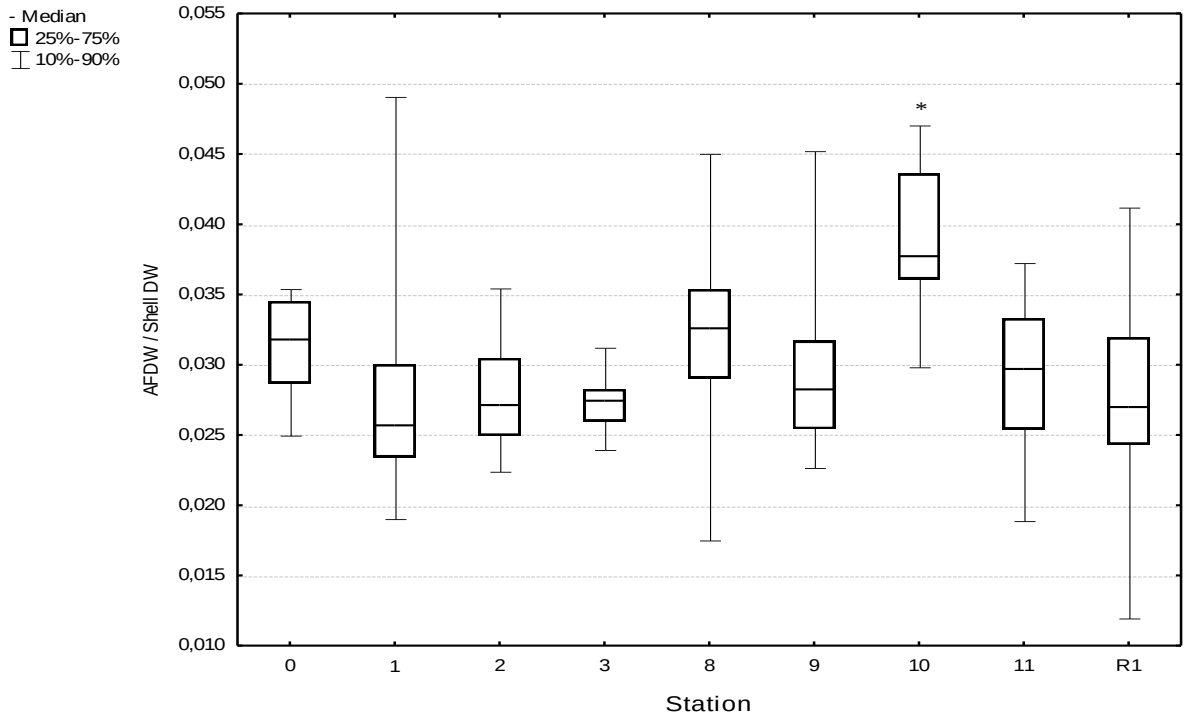


Figure 25. Condition index of mussels. Data expressed mean, standard error. AFDW=mussel ash free dry weight, DW=dry weight,*= significantly different, $p<0.05$ (for easier visualisation of the results, the y-axis does not reach the origin).

3.4.9 Integrated Biological Response (IBR)

The IBR/n index was calculated from star plots of normalised biomarker data from, NL, LF, PH, LMS (histochemical method) and NRRT (Fig. 26). The IBR/n was zero in mussels from the pre-exposure group.

The IBR/n value was low in the pre-exposure group (T0). The highest IBR/n value was found in mussels caged at station 2. Relative high and spread IBR/n value was recorded in mussels caged at the reference station (R1=station 12). All analysed biomarkers (NRRT, LMS, NL, PH, LF and CI) contributed to the IBR/n score of mussels, with different weight at different stations.

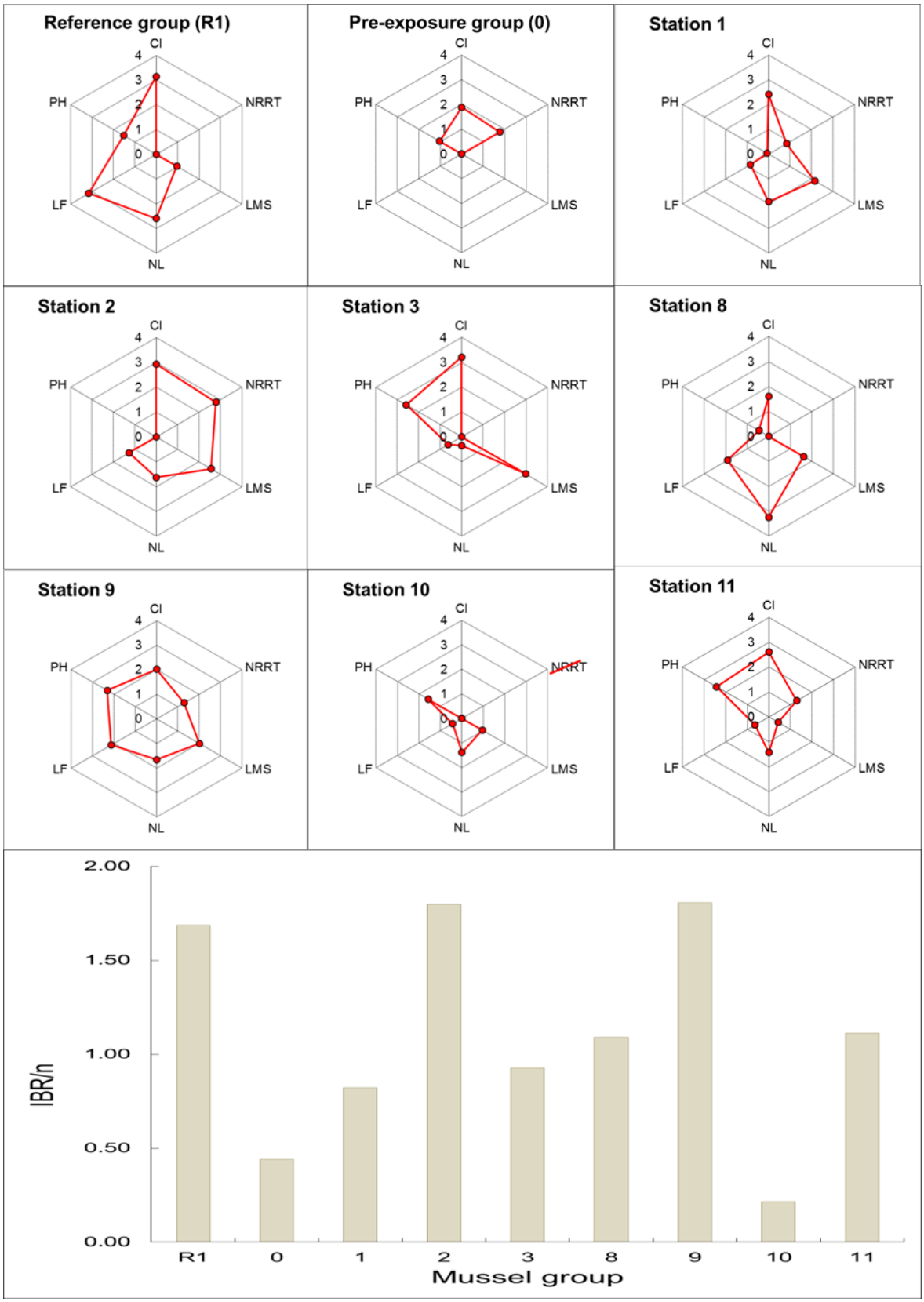


Figure 26 - Statistical comparison using one way ANOVA of the different mussel stations according to their abnormalities

3.5 POCIS extracts

3.5.1 Alkyl phenols concentrations

The POCIS extracts were analysed for over 50 different alkylphenol compounds from three stations, including the reference station (REF1), and the two closest stations (stations 1 and 2) and are presented in table 5 and Fig. 27. The data displayed in the table shows both alkylphenol data that was measured above their respective limit of quantification (>LOQ) as well as all values including those below the LOQ (<LOQ). In order to provide a clearer visual assessment, the >LOQ data was also displayed in the figure. Based on the >LOQ data, a clear signal in AP exposure was shown in stations 1 and 2 compared to the reference group. The most abundant APs were the C2 , cresol and C3 phenols.

Table 5 - Alkylphenol concentrations measured in POCIS extracts placed at the stations indicated for 6 weeks. Values are calculated concentrations in water based on the uptake rate coefficients determined from SPMDs (the best available at present to estimate water concentrations) (mean values, n=3). The data is presented both as compounds detected above the level of quantification (>LOQ) as well as all values including those below the LOQ.

	ng/L >LOQ (mean ± SD)				ng/L <LOQ (mean)		
	REF1	STN1	STN2		REF1	STN1	STN2
∑Cresol	0.00	9.29 ± 3.069	1.63 ± 2.827		<18.99	<21.09	<19.07
Σ C2 phenol	0.00	7.84 ± 0.565	5.73 ± 0.571		<24.26	<29.38	<27.41
Σ C3 phenol	0.00	3.00 ± 0.429	2.05 ± 0.572		<2.00	<3.78	<3.16
Σ C4 phenol	0.00	0.13 ± 0.041	0.09 ± 0.035		<6.15	<6.21	<6.20
∑ C5 phenol	0.00	0.03 ± 0.007	0.02 ± 0.009		<0.43	<0.45	<0.44
Σ C6 phenol	0.00	0.00	0.00		<1.13	<1.13	<1.13
Σ C7-C9 phenol	0.03 ± 0.001	0.04 ± 0.006	0.03 ± 0.002		<6.11	<6.12	<6.11
Σ 4-tert nonylphenols	0.03 ± 0.002	0.05 ± 0.003	0.05 ± 0.010		<151.93	<151.95	<151.95
TOTAL	0.06	20.37	9.60		<211.00	<220.10	<215.46

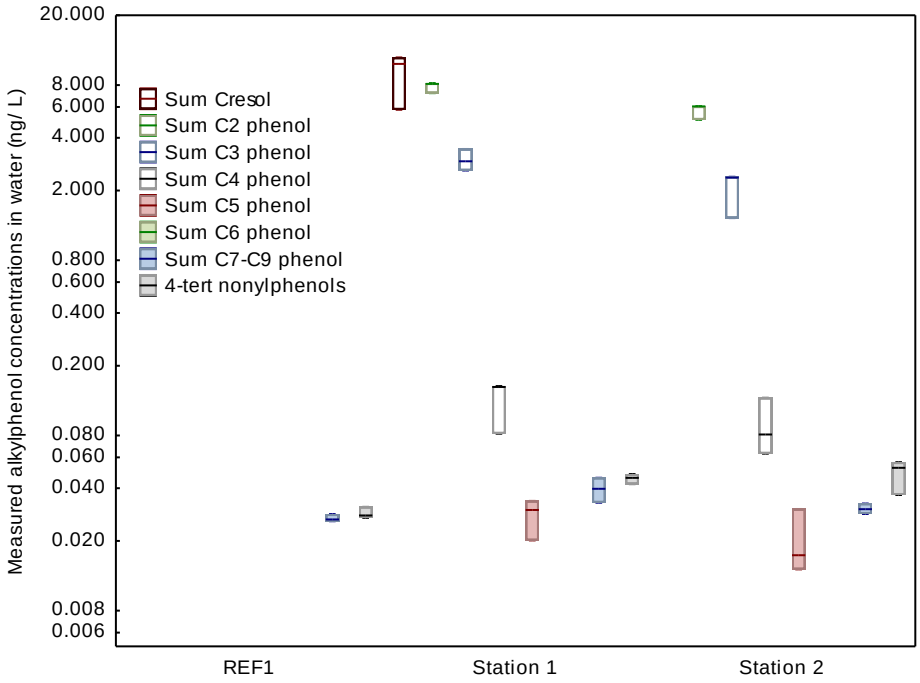


Figure 27 - Alkylphenol concentrations measured in POCIS extracts placed at the stations indicated for 6 weeks. Values are calculated concentrations in water, based on the uptake rate coefficients determined from SPMDs (median, min and max values, n=3). The data is presented as compounds detected above the level of quantification (>LOQ) only (for easier visualisation of the results, the y-axis does not reach the origin).

4. Discussion

The physicochemical data revealed a weak density stratification of the water column with respect to salinity and temperature that was considered unlikely to have any major effect on the distribution and direction of the PW discharge. However, such stratification may partially reduce mixing of the surface plume with the deeper water, thereby reducing dilution of the PW plume and ensuring that the mussels held in the upper water column were exposed to the discharge plume. Consequently, the depth at which the mussels were held during the exposure was deemed suitable for exposure to the PW plume.

The current meter data confirmed the expected dominant direction of the PW plume, and the stations selected for the biomarker analysis were in the PW plume axis direction.

4.1 PAH concentrations

The results of the WCM 2012 survey have shown that mussels caged 500 m from the PW discharge outlet had elevated concentrations of PAH and NPDs compared to mussels at 1000 m and beyond. The PAH16 concentration was particularly high only at station 1 (about 30 µg/kg (w.w.)). This is probably an indication of the fact that these mussels had the highest exposure of all the stations during the 6 week of caging.
Station 2 and 3 had similar levels of PAHs, this can suggest that the PW discharge plume from Troll C was rather narrow. This supports the importance of using multiple stations even when the average current direction and speed is previously known.
The maximum PAH16 concentration measured was similar to the maximum concentration measured at Gullfaks C in 2011 but higher than the maximum concentration measured at Ekofisk during the 2006, 2008 and 2009 surveys following similar exposure durations (Brooks *et al*., 2011a). In addition, PAH16 concentrations were above those previously measured in mussel tissue in the vicinity of the Troll and Statfjord B platforms (Utvik, 1999 and Hylland *et al*., 2008 respectively). However, the maximum recorded value was still within the lower end of the range of the concentrations found in mussels from coastal waters of the Nordic countries, where background concentrations have been found to range from 10 to 111 µg/ kg w.w. (Granby & Spliid, 1995).
The results clearly show that mussels at all stations were exposed to the plume from Troll C or possibly adjacent platforms, which means that stations in the opposite direction and at right angles to the predicted direction of the residual current were also exposed.
The mussels positioned at 1000 m and 2000 m contained PAH EPA 16 concentrations typical of offshore background levels of the North Sea.
The NPD concentrations showed the same pattern in concentration with respect to mussel station as that described for PAH EPA16. Exposure decreased with distance from the platform, although NPD concentrations at stations furthest away from the platform (2000 m) were still above the background levels measured in the reference mussels.
Overall total naphthalene and total phenanthrenes/ anthracenes were measured at similar concentrations at all stations and were approximately 3 fold higher than total dibenzothiophenes. NPD concentrations were particularly elevated at one of the stations closest to the platform (station 1) with total naphthalene, total phenanthrenes/ anthracenes and total dibenzothiophenes measured at 730 µg/ L, 620 µg/ L and 175 µg/ L respectively. These values were approximately 2-fold higher than the maximum concentrations measured in mussels from Ekofisk in 2006, 2008 and 2009 (Brooks *et al*., 2011a) and similar, if not

slightly higher, than concentrations measured in mussels from Gullfaks C in 2011 (WCM, 2011).

4.2 Metal concentrations

Special selection of metal analysis is not performed, all monitoring programs employ established parameters that are primarily based on their relevance to the environment but also technical analysis possibilities.
With respect to metal bioaccumulation, concentrations of arsenic, barium, copper, cadmium, lead and zinc were found to be higher in the field exposed mussels compared to the pre-exposed group. However, the bioaccumulation of these metals in mussels appeared to be unrelated to the distance from the PW discharge. Therefore single metal concentration results are not discussed.

4.3 ^{226}Radium concentrations

Levels of ^{226}Ra in the PW exposed mussels were close to the natural background concentrations measured at reference station and as reported in previous WCM surveys, indicating no accumulation of the radionuclide by the mussels as in previous WCM surveys.

4.4 Biological responses

Bioaccumulation data have indicated that only mussels caged at 500 m from the Troll C platform were exposed to PAHs, while the biomarker results did not show statistical differences between mussels caged at Troll C compared to the ones at the reference station (station 12), except for histopathology.

4.4.1 Pyrene hydroxilase

Pyrene hydroxylase (PH) is an MFO enzyme involved in PAH metabolism within the mussel digestive gland. Its activity has therefore been previously proposed as a biomarker of exposure to PAHs (Fillman *et al*., 2004, Bebianno and Barreira 2009). This enzymatic activity has been extensively studied as an exposure marker of organic xenobiotics in mussels and clams (Michel *et al*., 1994, Peters *et al*., 1999, Nasci *et al*., 2000, Porte *et al*., 2001). In this study, PH activity was similar in all the stations. In general the levels were one order of magnitude lower than the one recorded at Gulfaks C (WCM 2011), but at the same level measured at Ekofisk in 2008 and 2009 (Brooks *et al*., 2011a).

4.4.2 Lysosomal membrane stability

Lysosomal membrane stability (LMS) is one of the most used biomarker in monitoring activities and laboratory studies and has been proposed as a screening test for field surveys, in particular for PW monitoring (Viarengo *et al*., 2007b, Sundt *et al*., 2011). Biomarker based risk assessment is often limited by a lack of connection between marker levels and effect data. However, for LMS (as NRRT assay) threshold levels have been defined (ICES/OSPAR, 2009). Mussels are considered to be healthy if the NRRT value is above 120 min, stressed but capable of compensating it if the value is between 120 min and 50 min and severely stressed and probably exhibiting pathology if the value is below 50 min. LMS, evaluated through the usual NRRT assay, did not show any statistical difference between the groups and all mean values were equal or above 120 min indicating healthy mussels at all stations.
LMS in the digestive gland of mussels indicated relatively good conditions in mussel caged around the platform area and no statistical difference was observed for any station compared

to the reference one. These results agreed well with the NRRT analysis method. Assessment criteria have not yet been clearly established for LMS in caged mussels. However, in native shore mussels LMS values over 20 min indicate a healthy condition; <20 and >10 min indicate a minor stress, whereas LMS values lower than 10 min indicate a severe stress situation (Viarengo *et al*., 2000). Based on these assessment criteria, pre-exposed mussels and those from stations 11 were indicative of healthy mussels, whilst all other mussels exhibited minor stress.

Enhanced LF accumulation has been consistently observed in digestive gland of mussels exposed to PAHs in both laboratory and field studies (Au, 2004). In this case, LF accumulation was not statistically different in any station around the platform compared to the reference site (with only one exception, station 3).

Neutral lipid accumulation in digestive cells may be considered indicative of exposure to organic chemicals of different physicochemical properties (Brooks *et al*., 2011a). No statistical difference was observed between stations, with only one exception (station 3). Values were in general higher in mussels caged around the platform compared to the reference station, underlining a possible minor stress condition. High NL accumulation is indicative of general stress effects in the mussel digestive glands. Assessment criteria are currently not available for NL accumulation in field mussels.

4.4.3 Histopathology

Histopathological examination of the mussel tissues provides information at tissue/organ level. Identification of bivalve organ alteration and pathologies has been more and more used as indicators of environmental stress, since they provide a definite and ecologically-relevant biological end-point of chronic contaminant exposure (Au, 2004; Bignell *et al*., 2011). Histological lesions have been used as sensitive and reliable indicators to determine the health status of aquatic species in a wide range of studies (Au, 2004; Aarab *et al*, 2006, 2008 and 2011; Stentiford *et al*., 2009; Bignell *et al*., 2011).

The digestive gland of mussels is the main centre for metabolic regulation, participating in the mechanisms of immune defence and homeostatic regulation of the internal medium, as well as in the processes of detoxification and elimination of xenobiotics (Moore and Allen, 2002). Mussel digestive gland alterations are a reflection of distrubances at the tissue level and identification of these distrubances help in understanding of whole animal impact due to pollutants and other stress factors. Exposure to pollutants causes severe changes in the relative occurrence of cell types in the digestive gland epithelium. Moreover the increase number of basophilic cells is recognised as an indication of stress (Marigomez *et al*., 1998).

Mussel digestive glands were analysed for histopathological alterations related to physiological conditions, inflammatory pathologies and pathogen/parasite infections. The applied scoring system is the commonly used for mussels (Benly *et al*., 2008; Sensini *et al*., 2008).

Mussels caged at Troll C showed a loss of histological integrity in digestive gland tissue and other alterations, which was typical of a minor stress conditions (with two exceptions, stations 8 and 11) (Lowe *et al*., 1981; Cajaraville *et al*., 1990, 1992; Marigómez *et al*., 2006; Wedderburn *et al*., 2000; Usheva *et al*., 2006; Kim *et al*., 2008).

ICES guidelines for conducting histopathology on mussels have been initiated through the Working Group on the Biological Effects of Contaminants (i.e. both NIVA and IRIS are participating). The development of grading criteria for histological health parameters, together with adequate SOP and QA controls, will strengthen their use and inclusion in future monitoring programmes.

4.4.4 Condition index

Physiological markers are aiming to summarise the general status of the analysed organisms, providing an overview of the animal conditions and an indication of the level of damage caused by pollutant exposure (Pampanin *et al*., 2005). Even though, there could be physiological changes no directly connected to mussel body and shell weight. No differences were found in the condition index values of mussels caged near the platform and the reference group, indicating general good health conditions of the animals.

4.5 Integrative Biological Response (IBR/n)

Single biomarker information is not indicating a specific stress condition in mussels caged around the Troll C platform (except for some indication in the histopathology results). Integrative Biological Responses (IBR/n) has been applied as in previous WCM projects and other monitoring studies (Baussant *et al*., 2009; Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006; Brooks *et al*., 2011b; Damiens *et al*., 2007; Pytharopoulou *et al*., 2008; Raftopoulou and Dimitriadis, 2010.). Four different biomarkers (NRRT, NL, LF accumulation, LMS in digestive gland) were used to calculate the IBR index developed by Belaieff and Burgeot (2002).

Integrative Biological Responses (IBR/n) has been previously applied to fish and mussels including different suites of biomarkers (Baussant *et al*., 2009; Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006; Brooks *et al*., 2011b; Damiens *et al*., 2007; Pytharopoulou *et al*., 2008; Raftopoulou and Dimitriadis, 2010.). Five different biomarkers (NRRT in blood cells, LMS in digestive gland, LF and NL accumulations and CI) were used to calculate the IBR index developed by Belaieff and Burgeot (2002), and they were orderly represented in the five axes of star plots according to their biological complexity level. IBR/n discriminated between the different stations. In fact, the IBR/n values were moderately higher in stations 1, 2, 8 and 9 compared to the pre-exposure group. Relative high value was found in mussels caged at the reference station, therefor no difference was found in the IBR/n value of mussels caged around the platform compared to the reference ones.

Overall, the results are in agreement with the location of the stations in relation to both the PW discharge distance and the PW plume direction (as revealed by the current data). The biomarker responses were small, a moderate stress response was shown in mussels caged at 500 and 1000 m from the PW discharge, as revealed by the histopathology markers. This indicates a relatively minor effect of the PW discharge in the platform area that decrease with increasing distance from the discharge point.

4.6 Alkyl phenols and naphthenics in POCIS

A clear signal in AP exposure was shown in POCIS extracts collected from stations 1 and 2 compared to the reference group. The most abundant APs measured were cresol, C2 and C3 phenols, although only in the low ng/ L range and 5 to 10 times lower than that detected around the Gullfaks C platform as part of last year's WCM (WCM 2011). Previous measurements of APs from POCIS extracts have found far higher concentrations around Ekofisk in 2008 (< 55 ng/ L) and in 2009 (< 200 ng/ L) and Statfjord B in 2004 (< 70 ng/ L) (Harman *et al*., 2009; 2010; 2011).

Although the chemical uptake by POCIS cannot be corrected for differences in exposure conditions between laboratory and field, hydrophobic samplers (e.g. SPMDs) have been used for this purpose (Harman *et al*., 2008; 2009). Consequently, water concentrations of APs obtained from POCIS should be considered as semi-quantitative and the environmental effects of such concentrations must only be inferred. However, the concentrations of APs measured

in POCIS positioned closest to the platform (600 m) were several orders of magnitude below the levels considered to illicit biological effects in marine organisms.
Although many AP have been reported to have some degree of estrogenic potency, the estrogenicity is markedly higher in the C8 and C9 AP (Tollefsen *et al*., 2008). In the present study, accumulation of C8 and C9 APs were below the detection limit and unlikely to pose any estrogenic risk to marine life

5. Conclusions

Sea current measurements and hydrocarbon exposure measures in the caged mussels indicated that the station design of the investigation captured the produced water plume from the Troll C platform.

Mussels from stations located 500 m from the Troll C platform showed significantly higher concentrations of PAH and NPD concentrations than all other mussel groups, indicating a bioaccumulation of these compounds probably due to the PW discharge. All stations at further distance from the platform showed background levels of PAH and NPD.

No biological effect responses were recorded in mussels caged around the platform at biochemical and physiological level.

Histopathological analysis of mussel digestive gland indicated a minor stress condition in mussels caged 500 and 1000 m from the platform.

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7. Appendices

Appendix	Description
A	Cruise final report-WCM 2012
B	Current meter measurements
C	Chemical analyses
D	Quality assurance

Appendix A

Jan Fredrik Børseth (IRIS), Daniela Pampanin (IRIS) and Steven Brooks (NIVA)

Water Column Monitoring 2012
Cruise Report

Preface

This report details the activities of two research cruises that were part of the Water Column Monitoring programme of 2012. The two cruises consist of a deployment cruise and a retrieval cruise. Caged mussels were deployed to the produced water discharge at Troll C platform for a period of six weeks to determine the potential biological effects of the discharge on animals living within the water column. Additional work activities included; the trial of particle counters to monitor algae densities, and the use of polar passive samplers for the detection of alkylphenols and naphthenic acids.

Stavanger, October 2012

Daniela M. Pampanin, Project Manager

Contents

1. Pre-exposure sampling

Mussels (*Mytilus spp.)* were collected from Trondheim Fjord, the same location as used previously for the WCM surveys. The mussels were collected by Dag Altin (Altins Biotrix) two weeks before the deployment cruise. The mussels were sent immediately on ice via overnight courier to the IRIS Biomiljø facility for the pre-exposure sampling. The mussels were held in clean filtered sea water taken from a depth of 80 meters for 6 days prior to tissue sampling. Sampling was conducted by IRIS personnel and distributed to the relevant laboratories for analysis. Body fluids and tissues, including haemolymph, digestive gland, gills and whole soft tissue were sampled and used for biological and chemical endpoints as described in Table 3.

2. First cruise: deployment of the mussel cages

2.1 Research vessel and scientific personnel

The M/S Northern Commander (

Figure 1) departed from Ågotnes offshore base outside Bergen on Tuesday 27th April in the evening and arrived back on Friday 30th in the evening. The scientific personnel onboard the vessel consisted of two from IRIS; Jan Fredrik Børseth and Stig Westerlund, and two from NIVA; Steven Brooks and Christopher Harman. In addition, Rolf Sundt (Statoil) was onboard and represented the client (OLF).

Figure 1. The MS Northern Commander used for the deployment of mussel cages and field equipment.

2.2 Stations and rig equipment

The design of the mussel rigs is shown in

Figure 2. More than 320 individual mussels were packed in a mussel net bag. The bag was then tied to the upper part of a mussel/equipment rope witch was approximately 5m long. This rope was then attached to a shackle at the top of the main rig rope (Spectra quality, 6mm thick) in order to prevent knots made on the main rope which could weaken its strength.

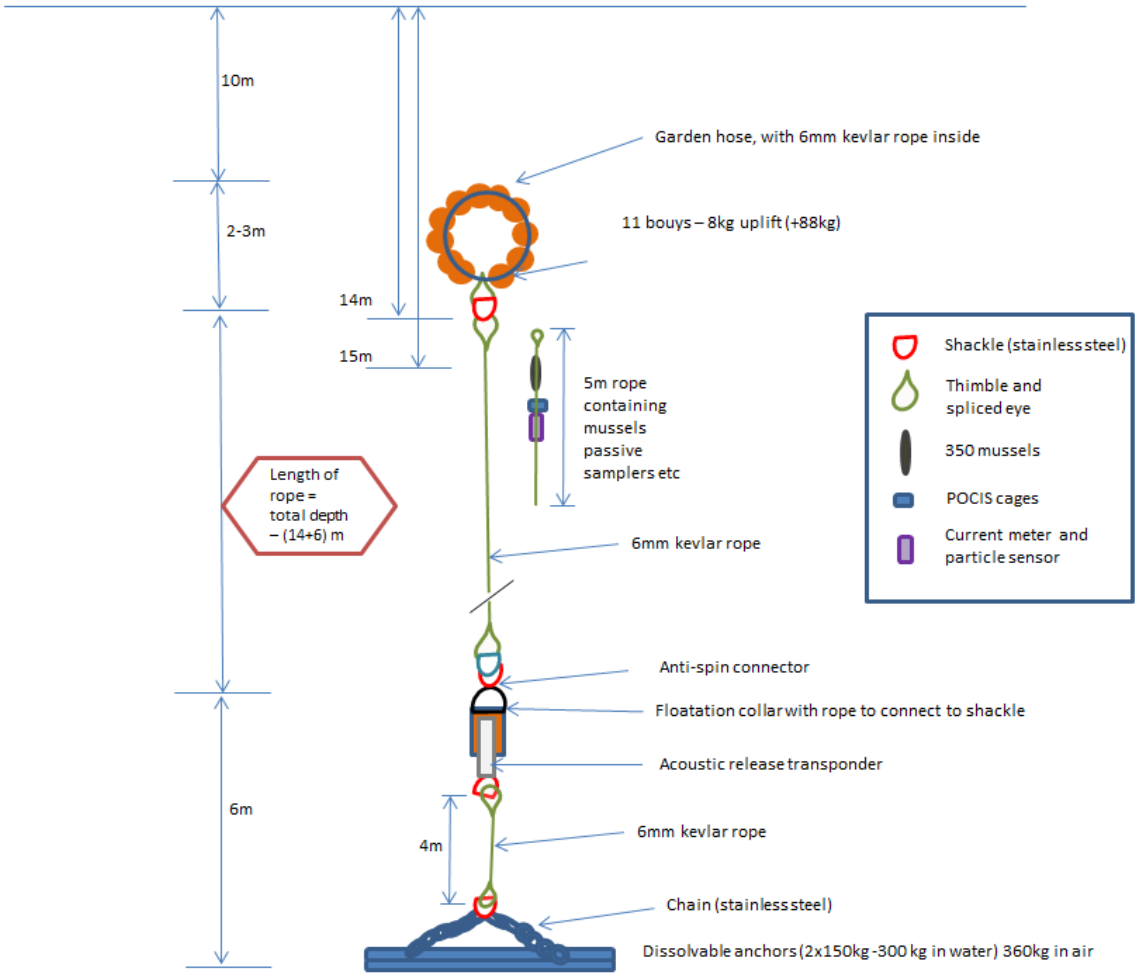


Figure 2. The design of the mussel rigs.

An acoustic release transponder (cNODE) and a positioning system (HPR418P) were hired from Kongsberg Maritime AS, and was planned for the retrieval of the rigs after the exposure duration. A special anchor for the rig was delivered by EMGS AS. The anchor was manufactured with a self-dissolving concrete material that dissolves after typically 6-8 months on the sea floor
The acoustic release transponders were used at all 13 stations to avoid the use of surface buoys in the study area.

The planned grid design of the mussel stations was based on the main current direction within the area and is shown in

Figure, with the planned co-ordinates shown in Table 1. The majority of the stations were positioned North by North-East of the platform along the dominant current direction. The three closest stations were positioned 600 m from the platform, four stations were at 1200 m and four stations at 2000 m. All mussels were held within the water column at approximately 15 m. The exposure duration was approximately 6 weeks. The vessel was equipped with dynamic positioning (DP), thus the specific co-ordinates for each station were regarded as quite accurate.
The crew of the MS Northern Commander had no previous experience with the water column survey. However, despite this, they were able to learn quickly and used the ship's resources (crane on starboard side and winch) to deploy the mussel rigs safely and reasonably quickly. One station was deployed at day 1 (station 11), five stations on day 2 (station 4, 5, 8, 9, 10) and seven stations on day 3 (station 1, 2, 3, 6, 7, 12-ref1, 13-ref2).
The accuracy of the given data on water depth for each station was unclear. Thus, each rig was deployed with an extra rope to the surface in order to be able to lift the rig up if necessary and adjust the length of the rope between mussel cage and bottom anchor. According to the plan, the mussel cage must be immersed to 15 m below surface. The extra rope to the ship had marks for each meter and a hook with release mechanism at the rope end. Fortunately, all data on water depth was correct and all mussel cages ended up at 15 ±1 meter depth.

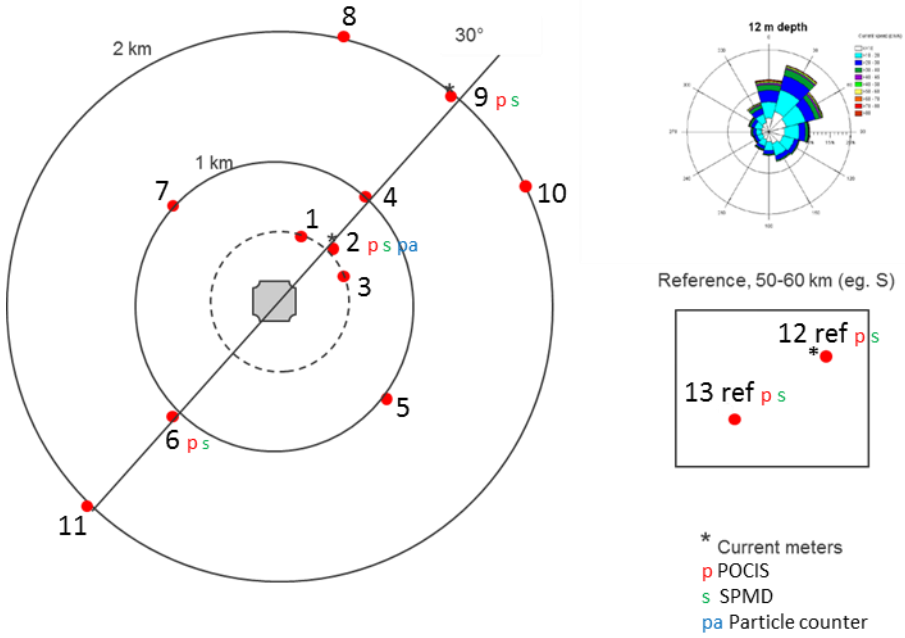


Figure 3. An overview of the planned position of the mussel stations around the Troll C platform. The diagram in the upper right corner shows current rose with the dominating current direction based on data from previous measurements.
The accurate position of deployed mussel rigs as well as sequence and time of deployment is given in Table 2 -. Additional information during the deployment work was registered in a log book and a résumé of these notes is presented in Table 3.
Table 1. Planned coordinates of the mussel stations. Distance and angle with regard to platform, as well as water depth, are given. Reference stations were planned in a suitable area in South-Easterly direction.

Station	Degree	Distance (m)	E UTM-ED50-S31N	N UTM-ED50-S31N	Depth (m)
Troll C	0	0	533190	6750440	-
1	15	600	533345	6751020	-338,2
2	30	600	533490	6750960	-338,1
3	45	600	533614	6750864	-338,2
4	30	1200	533790	6751479	-339,9
5	120	1200	534229	6749840	-338,2
6	210	1200	532590	6749401	-336,8
7	300	1200	532151	6751040	-336,7
8	15	2000	533708	6752372	-341,6
9	30	2000	534190	6752172	-341,2
10	45	2000	534604	6751854	-341,4
11	210	2000	532190	6748708	-336,0
12 (ref1)	-	~60000	556832	6709852	~-340
13 (ref2)	-	~60000	556852	6707996	~-340

Table 2. Position of deployed mussel rigs as well as sequence and time of deployment.

Station	Latitude WGS84	Longitude WGS84	Deployment sequence, time and date
Troll C	N 60° 53` 08,98``	E 003° 36` 35,28``	---
1	N 60° 53` 27,56``	E 003° 36` 44,95``	9th 08:45 29.03
2	N 60° 53` 25,65``	E 003° 36` 54,42``	10th 09:45 29.03
3	N 60° 53` 22,48``	E 003° 37` 02,85``	11th 10:30 29.03
4	N 60° 53` 42,33``	E 003° 37` 14,93``	5th 22:25 28.03
5	N 60° 52` 49,19``	E 003° 37` 42,92``	6th 23:15 28.03
6	N 60° 52` 34,63``	E 003° 34` 53,99``	7th 06:40 29.03
7	N 60° 53` 28,59``	E 003° 35` 25,66``	8th 07:30 29.03
8	N 60° 53` 11,16``	E 003° 37` 09,83``	2nd 18:35 28.03
9	N 60° 54` 04,53``	E 003° 37` 47,83``	3rd 19:35 28.03
10	N 60° 53` 54,06``	E 003° 38` 08,90``	4th 21:00 28.03
11	N 60° 52` 13,12``	E 003° 35` 26,93``	1st 19:50 27.03
12 (ref1)	N 60° 31` 07,69``	E 004° 02` 00,69``	12th 14:15 29.03
13 (ref2)	N 60° 30` 07,68``	E 004° 02` 00,11``	13th 15:10 29:03

Table 3. Log of activities during the deployment cruise on board MS Northern Commander.

Date	Events
Tuesday 27th March	Packaging of mussels, tank, pump, current meters, clothing, etc. 8-9:30. Departure with Hertz van from Mekjarvik (Stavanger) to Lindenes wharf at Ågotnes (Bergen). Arrival 14:00. Loading equipment and then boat security equipment training. Delayed delivery of acoustic transponders at 17:00. Dinner at 17:30. Review with 19 crew with regard to launching rigs and followed with safe job analysis. Main message: stay away from the suspended load! Pinch Hazard concrete anchor. Preparing a rig on deck ready for start 8:00 the next day. Start Station 11, 336 m deep. Roll, snatch block, out on the starboard side. Double helping rope into the roll first. Anchor laid out on deck before departure 24:00. Codes for triggering of acoustic transponders noted. Tested a transponder under the boat with release from the bridge.
Wednesday 28th March	Start 8:00. Powered seawater pump for mussels and runs until 12:00. Struggling throughout the morning to connect with transponders. Testing three transponders. Different transport projects for Statoil Marine between two rigs operations. Duration of transport task was 4 hours. Various telephone contact with Kongsberg office onshore to find fault. Seems to have to do with clarification from Huston. Appointment to access to land and get service personnel from Kongsberg onboard tonight. Seem for a while to have a breakthrough: something with 'disabled' and channel shift (later turned out to be wrong). Testing one more time at sea and return to station 11. Station 11: At 20:00 rig no.11 is on the seafloor. Now going to Ågotnes approximately 50 nautical miles, plan to arrive at about 1:00 tonight. Service man from Kongsberg comes at 8:00 to check transponders that does not work. Equipment on Rig 11: two Temp / Light loggers, 7 kg mussels, POM.
Thursday 29th March	Arrives CCB at about 2:00 in the morning. Service man (Thomas) on board around 8:30. Changing frequencies and completed work at about 10:30. Agreed on next order of stations and prepared the next pitch on a roll. Finished work at 12:00. A transponder completely defective. Departure at 13:00. Arriving at station 8 at 18:00. At 18:45 on the bottom and precise reading of 15 m below surface from shackle! Was 7 kg shells, 3 temp loggers, and POM No. 8. Moves to Station 9. Sea wave height 4-5 m. Station 9: rig deployed 8:15. Does not manage to get contact with transponder, either from the bridge or portable device. Put on a small flashy trawl ball into thin polyester rope to surface, about 22 m long. Shackle hung in the middle of the rope to lower the rope from the surface. Rope is not powerful enough to lift rig! Unfortunately, a small knot was on the lower end of the rope at the launching. Should now more easily find the position and lower the vehicle to buoyancy ball ring. Rope is attached at the top of the ball circle. Set out Akvadupp meter (Troll 1), POCIS, POM no. 9, two temp loggers and 8 kg shells. Station 10 at 21:20 on the bottom; 8 kg shells, temp (1, 3 and 5 m from top of rope), POM. Do not achieve contact with transponder. Station 4 at 10:30 on the bottom. 8 kg shells, temp, and POM. Station 5 at 11:15 on the bottom. 7 kg shells, temp, POM.

Friday 30th March	Station 6 at 6:45 down. 7 kg shells, temp, POCIS, POM no.6. Station 7 at 7:30 down. 7 kg shells, temp, POM no.7. Station 1 at 8:45 down. 8 kg shells, temp, POM no.19 (NB number is 19 and not 1, not because no. unit is lacking). Taking simultaneously CTD profile down to 50 m depth. Station 2 at 9:30 down. Full "package"; 8 kg shells, temp, POCIS, POM no.2, particle counter, profiling current meter (set to measure 70 meters downwards, no point to measure from 15 m and up, according to Stig). Station 3 at 10:40 down. 7 kg shells, temp, POCIS, POM no.3. Stops at 1000m and 2000 m circle on the way to the reference area to take CTDs. The reference station 12 at 14:00 down. 7 kg shells, temp, POCIS, POM no.12, Aquadopp current meter, particle counter. The reference station 13 at 15:00 down. 6 kg shells, temp, POCIS, POM no.13. Packaging of equipment. Return travel onshore 4:30 hours to CCB Ågotnes.

2.3 CTD profiles

In order to provide temperature, conductivity, and salinity profiles from the water column around the platform, an *SBE Seabird 901* probe was deployed at 600 m (from rig 1), 1200 m (near rig 4) and 2000 m (nr rig 9) during the deployment cruise. The CTD recorded measurements every 1 sec during the controlled lowering and raising of the unit down to a depth of approximately 50 m. A marked rope was used to estimate water depth.

2.4 Monitoring of seawater current, particle density and light condition

Mussel rig 2 and 9 and reference rig 12 were fitted with *Aquadopp* current meters by Nortek AS. An *Aquadopp Profiler 400kHz* current meter was used at rig 2. This unit measured current profile between 15 m and 65 m depth. The other two meters were point monitoring instruments and only measured current direction and strength in their fixed position at 15 m depth.
A CTD unit equipped with a particle counter, SD200W was placed at 15 m depth on rig 2 in order to continuously monitor the density of particles like algae during the exposure period of mussels. The unit was programmed to record measurements every 10 min throughout the duration of exposure
.
Temperature and light sensors (HOBO pendant) were put on all rigs. Three sensors were put on the rope of each rig, one close to the mussels, the other two sensors at 2m above and 2 m below the mussels. Light and temperature measurements were recorded every 10 min for the duration of the exposure.

2.5 Passive samplers

Passive samplers in the form of Polar Organic Integrated Chemical Samplers (POCIS) were deployed at 3 exposure stations (rigs 2, 6 and 9) and both reference stations (ref 1 & 2). Semi-Permeable Membrane Devices (SPMDs) were also used at the rigs 2 and 9 and both reference stations in order to determine chemical uptake rates. POCIS and SPMDs were held in stainless steel cylinders and placed just below the mussels.

3. Second cruise: Retrieval and sampling

3.1 Research vessel and scientific personnel

The M/S Scandi Stord (

Figure 4) was ready on Monday 7th May in the morning at the Coast Centre Base (CCB) and mobilisation started at 7:00. The vessel departed from Ågotnes at noon and arrived back on Thursday 10th at 20:00.
The scientific personnel onboard the vessel consisted of two from IRIS; Jan Fredrik Børseth and Claudia Lucas, and four from NIVA; Steven Brooks, Christopher Harman, Bjørnar Andre Beylich and Eivind Farmen. In addition, Rolf Sundt (Statoil) was onboard and represented the client (OLF).

Figure 4. The MS Scandi Stord used for the retrieval of mussel cages and field equipment.

3.2 Locating and retrieval of mussel rigs

The strategy of using acoustic release units for retrieval of rigs was new this year. In theory, this method is well proven and is known to save a lot of time and effort during retrieval. There is also no need for surface buoys, which is well appreciated when operating close to a platform with lots of ship traffic around. However, the experiences we had this year with acoustic release units was really a big disappointment; it caused a lot of frustration and created a lot of extra work. Even with a Kongsberg technician onboard as support, we did not manage to solve the problem. The latest feedback from Kongsberg was that they checked the equipment after the field work and realise that technically the transponders were not in a proper condition for use when they were delivered us. The company apologize for this and will not invoice us for hire of equipment and technician. They hope we still will consider hiring their transponders in future projects despite the extraordinary experience we had this time.

A log of activities during the retrieval cruise can be found in Table 4.

Table 4. Log of activities during the retrieval cruise on board MS Scandi Stord.

Date	Events
Monday 7th May	Equipment from Stavanger carried by Kuhn & Nagel on last Friday. Delivered on Ågotnes and Scandi Stord at 7:30 on Monday. Stavanger personnel takes 6:40 flight from Sola and arrive the boat at 8:10. Problems with finding equipment from the deployment cruise that were stored in Statoil's onshore stock. Floating rope to NIVA arrived by error in Haugesund. A follow-up of this task was cancelled. Clothing etc. from NIVA not arrived on time (Schenker). Clothing finally arrived at 12 o'clock. Departing right after this. On the way out, we have a briefing meeting with the crew with regard to the mission and run through a secure job analysis. Arrive at field approximately 18 o'clock. Attempting to connect to transponder St.1 without success. Continue to St.8 (did have contact here during the deployment) and manage to release rig acoustically. Use MOB-boat to pick up rig no.3. People aboard MOB-boat included Chris from NIVA. Taking full test suite including Neutral Red test. Finished at 11:00 and 0:30 with Neutral Red. Attempting to connect at St.11 and succeed 100 m aside the given position. Going to sleep after this.
Tuesday 8th May	Starting at 6 o'clock. Check first if there is contact with transponder at 350 m distance from position, to get an impression of how close from the rig we have to stay in order to communicate. The attempt is successful. Going to position, ok signal, and then to 100 m away. Ok signal and trying to release, but does not work, even if the battery test shows ok. Thomas, the technician from Kongsberg call home office to discuss solutions. Negative. Some theories are that transponder might be damaged in the release mechanism due to physical contact with bottom, or ropes going into the release mechanism. Conducted a briefing meeting on the "lasso method" and continue by making lasso with different splice rope to approximately 750 m and with a short pieces of iron chain as sinkers. Moves to position 9 to make a trial. It should be a small surface buoy here, but are not able to relocate this. No contact with transponder here. Lay out the long spectra line in a loop on the surface and get contact with the rig at about 11:00. The upper part of the rig comes up about 12:00. The samples are intact and we run a full suite of mussel samples. We have to be extra careful with storage of transponder on deck when because there is risk of water penetration and contact with lithium batteries that could lead to an explosion in air. At 16 o'clock, retrieve the next station no.2. All intact and taking new full test suite. Proceeding to st.3. Earlier, Statoil offered Kongsberg to have a vessel with HiPAP system, but they did not need it. After all the problems experienced now, and after Thomas has consulted with the home office, it is suddenly interesting to have HiPAP anyway! Get an offer from MS Møkster Stril, another standby vessel, to try with their HiPAP system. They try to connect with St.3 and 1 without success (19 o'clock). We then proceed to St.3 and continues with a final fishing effort with the lasso method before it gets dark. We are successful with this and finish the retrieval at 21:30.
Wednesday	Starting at 7:30 to lay out the loop with the rescue boat. Still a lot of waves and wind,

9th May	at the limits to continue working due to security, but the captain finally approves the operation. St.1: succeeded in getting the line under the transponder. Rig on deck at 8:00. The driver of the rescue boat thrown into the water when they are entering the vessel again with the rescue boat. The captain wants to wait 2 hours for the weather to calm down before continuing. Use the time to go down to the reference stations and to ensure that we get at least one of these. At 12:00 lay out loop on the southern reference (hereafter referred to as Position 2). This is equivalent to deployment no. 13. Changes rope as there are endless wear. In addition, we get away all the shackles on the rope that make it difficult to get past the shackles of the winch. Splices two long pitches so that the rope is now 900 m long. Shell package etc. up 13:30 and transponder up at 14:00. Taking full package of shells, POCIS and POMS. Orders the rope to the next "lasso catch". Temperature loggers stripped off. Go to the next reference is position (Reference 1, deployment no. 12). Went well on the first try and we retrieve current meter, particle counter, POCIS, POMS, etc. Taking the CTD measurement for 0-50m deep. At 16:00, going back to the Troll C and St. 4 for further retrieval work. Thomas from Kongsberg checked both references transponders on deck with negative results (no physical damages). By keeping the submersible transponder head up to trigger transponder both work when they came on deck. Observing some corrosion of hook area, but not critical. So far, all the valves have been found tightened according to procedure before deployment. Thomas still do not know what caused the error. He says everyone has been used before, because they were reprogrammed to another channel frequency than what is standard delivered from the factory. Choosing to measure Neutral red retention in mussels from both references, although not made this previous years. Planning to try to retrieve at a final station today, but this was cancelled after MOB-boat personnel reports sharp waves that break and threatens to overturn the boat. Stop further attempt that night, because time is 21:00 and working legislation on working hours for boating personnel do not permit a longer working day 6:00-20:00 several days in a row.
Thursday 10th May	Start extra early. Precise 6:00 MOB-boat goes in the water. Finished with Station 10 and 4 at 9:15. Proceed with Station 7 and 11. Finished at 12:30 and finished with Station 5 at 14:00. Moves to the last Station 6, where there is uncertainty with regard to coordinates. Use coordinates provided by Statoil as a starting point, since the reference from the vessel log book is incorrect. Succeed in the first trial! Travels back to CCB Ågotnes and arrives here at 20:30. Demobilise equipment and personnel.

3.3 Mussel sampling

The different samples taken from the mussels were performed according to the plan presented in Figure 5.

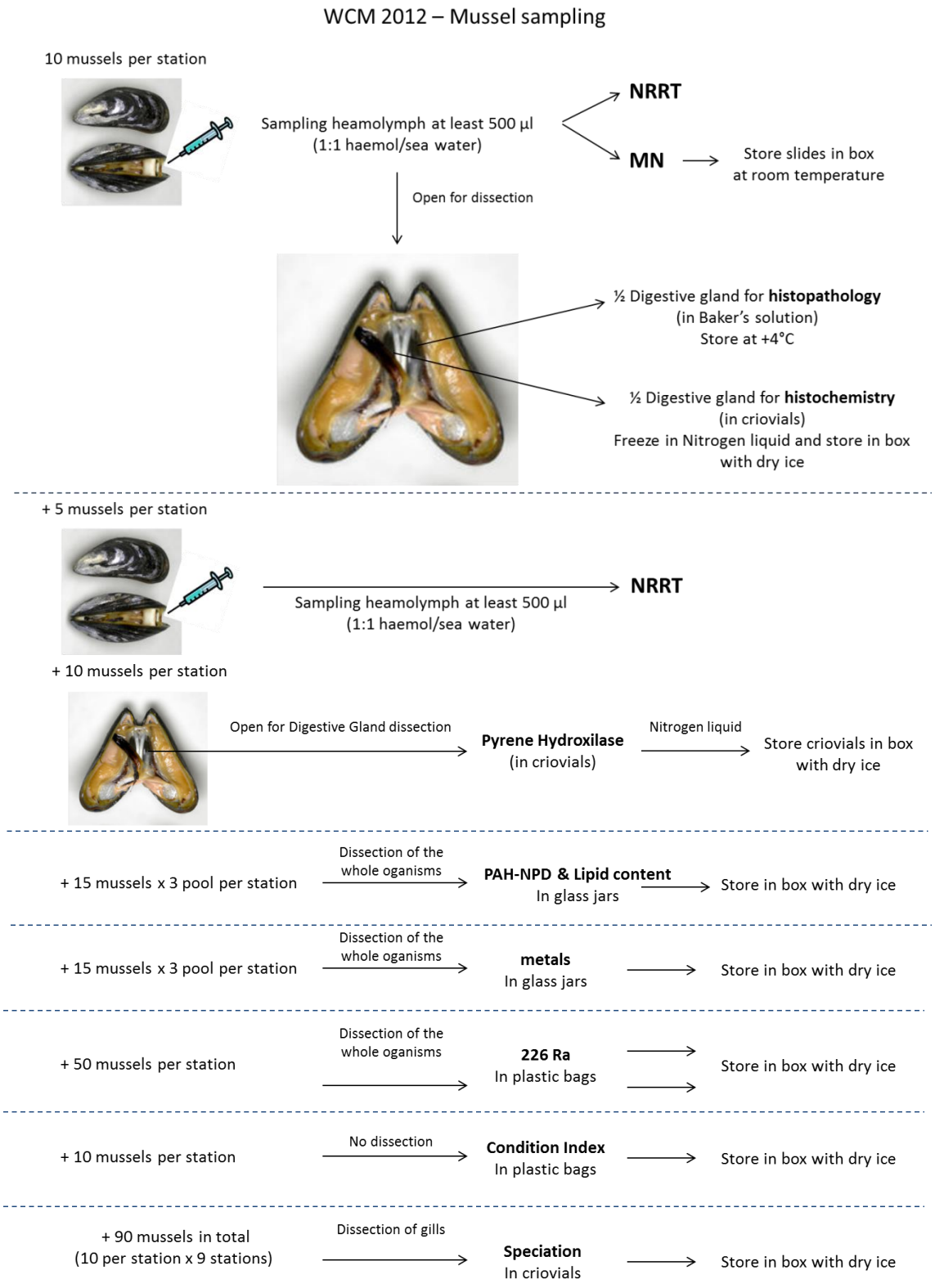


Figure 5. Schematic overview of the mussel sampling plan.

3.4 Instrumentation and passive samplers

The POCIS were retrieved from stations 2, 6, 9 and both reference stations. This POCIS were in good condition without significant fouling. The POCIS were removed from the outer cage and disconnected from the spider assemblage before wrapped carefully in foil containers and stored at -20^{o}C within the ships freezer facilities. The SPMDs, used to obtain chemical uptake rates, were retrieved from stations 2, 9 and both reference stations. The SPMDs were placed back in their original tins and stored at -20^{o}C until analysis.

The CTD containing the particle density meter (SD200W) was safely retrieved from rig 2. The units was returned to NIVA and the stored data was collected.

4. Conclusion and recommendations

The problems we experienced with the acoustic transponders clearly show that there is a risk for malfunction that always must be considered carefully. We were well aware of this. However, there was a case with the Kongsberg company that seems to be the main factor of our difficulties experienced. This time, we made an order with Kongsberg office in Horten, but due to lack of sufficient number of units on storage in Norway, transponders were sent directly from a department in Houston. It seems afterwards that the quality assurance work done by Kongsberg personnel in Houston was not good at all and was the main reason for our trouble.
Despite problems with the acoustic transponders we managed to retrieve all rigs this year, which is a very good result. The use of the alternative "lasso method" to catch rigs showed to be very successful. However, this method is not recommended as a regular strategy for retrieval, because there is a considerable risk having personnel to operate for longer periods in a rescue boat in the open ocean. Also the launch and return of the small boat from the deck of mother vessel and seaside is dangerous under heavy weather conditions.
The deployment of each rig with an anchor made of 300 kg dissolvable concrete was a challenge this time. The deck railing on the side of the boat was several meters high. The lifting operation over the railing was secured and controlled by two personnel, each with a rope connected to the anchor in a way that swinging could be controlled.
If acoustic release is to be used in future surveys, we recommend to either scientific personnel get coursed in the use of communication command systems like the HiPAP. Kongsberg regularly offer such courses. Alternatively, a technician from the transponder company should be hired for the job. It is not obligatory to have a boat with HiPAP system installed, but it is a great advantage.
More spawning activity was observed among mussels than previous years. After dialog with the company that supplied us with mussels, we were informed that sea temperature measured in the shellfish farm the last couple of years showed increased temperature and mussels were observed to start spawning earlier than normal. This might influence our monitoring data and should be considered when more results are available from laboratory analyses. The first impression during retrieval of rigs at Troll C was that mussels looked healthy, but with some few empty and dead shells observed within nets.
The use of hi-tech Spectra ropes was very successful. Ropes were light, soft and easy to handle on deck and splices could be made easily when the right procedure for splicing Spectra ropes was followed. The rig design also functioned well. We managed to place mussel nets at the regular 15 m depth, even without any permanent rope to surface. The design where a shorter rope, separate to the main rope, was used for mussel nets and monitoring equipment, made it easier to lift off mussels and equipment from the rig while it was hanging with a heavy anchor at the end and alongside the boat.
Due to the technical problem with acoustic release system, the time schedule ended up to be very busy. At normal circumstances we would have normal work conditions. Instead of having to wait with retrieval of rigs because dissection of mussels from last rig was not finished, this time the question was if we managed to retrieve all rigs within the working time window of the crew and the boat.

5. Appendix: Sampling data

Code	Station	length(mm)	NRRT	MN	Pyr Hyd	Histopath	Histochem	PAH-NPD, metals, lipids	226Ra	speciation
1	8	54	x	x		x	x			x
2	8	51,1	x	x		x	x			x
3	8	46,6	x	x		x	x			x
4	8	47,2	x	x		x	x			x
5	8	46,1	x	x		x	x			x
6	8	49,6	x	x		x	x			x
7	8	42,8	x	x		x	x			x
8	8	51,4	x	x		x	x			x
9	8	42,2	x	x		x	x			x
10	8	47,1	x	x		x	x			x
11	9	58,4	x	x		x	x			x
12	9	53,6	x	x		x	x			x
13	9	49,4	x	x		x	x			x
14	9	44,3	x	x		x	x			x
15	9	53,7	x	x		x	x			x
16	9	55,8	x	x		x	x			x
17	9	49,1	x	x		x	x			x
18	9	52,4	x	x		x	x			x
19	9	56,3	x	x		x	x			x
20	9	54,3	x	x		x	x			x
21	2	48,3	x	x		x	x			x
22	2	51	x	x		x	x			x
23	2	51,6	x	x		x	x			x
24	2	56,5	x	x		x	x			x
25	2	59,7	x	x		x	x			x
26	2	56,1	x	x		x	x			x
27	2	55	x	x		x	x			x
28	2	51,2	x	x		x	x			x
29	2	50,5	x	x		x	x			x
30	2	52,3	x	x		x	x			x
31	3	50	x	x		x	x			x
32	3	52,5	x	x		x	x			x
33	3	63,3	x	x		x	x			x
34	3	49,3	x	x		x	x			x
35	3	49,2	x	x		x	x			x
36	3	56,2	x	x		x	x			x
37	3	48,4	x	x		x	x			x
38	3	53,2	x	x		x	x			x
39	3	54,2	x	x		x	x			x
40	3	52,2	x	x		x	x			x

Code	Station	length(mm)	NRRT	MN	Pyr Hyd	Histopath	Histochem	PAH-NPD, metals, lipids	226Ra	speciation
41	1	51,2	x	x		x	x			x
42	1	60,1	x	x		x	x			x
43	1	51,7	x	x		x	x			x
44	1	52,8	x	x		x	x			x
45	1	49,5	x	x		x	x			x
46	1	50,4	x	x		x	x			x
47	1	46,7	x	x		x	x			x
48	1	47,1	x	x		x	x			x
49	1	54,3	x	x		x	x			
50	1	46,4	x	x		x	x			x
51	ref2	45	x	x		x	x			x
52	ref2	44,1	x	x		x	x			x
53	ref2	52,4	x	x		x	x			x
54	ref2	47,1	x	x		x	x			x
55	ref2	47,6	x	x		x	x			x
56	ref2	53,6	x	x		x	x			x
57	ref2	44,3	x	x		x	x			x
58	ref2	47,5	x	x		x	x			x
59	ref2	50,7	x	x		x	x			x
60	ref2	52,9	x	x		x	x			x
61	ref1	48,1	x	x		x	x			x
62	ref1	55,8	x	x		x	x			x
63	ref1	56,2	x	x		x	x			x
64	ref1	50,5	x	x		x	x			x
65	ref1	49,7	x	x		x	x			x
66	ref1	47,4	x	x		x	x			x
67	ref1	44	x	x		x	x			x
68	ref1	49,8	x	x		x	x			x
69	ref1	51,5	x	x		x	x			x
70	ref1	48,6	x	x		x	x			x
71	10	52,1		x		x	x			x
72	10	54,6		x		x	x			x
73	10	58,1		x		x	x			x
74	10	57,5		x		x	x			x
75	10	52,1		x		x	x			x
76	10	51,6		x		x	x			x
77	10	56,6		x		x	x			x
78	10	51,3		x		x	x			x
79	10	47,6		x		x	x			x
80	10	42,8		x		x	x			x

Code	Station	length(mm)	NRRT	MN	Pyr Hyd	Histopath	Histochem	PAH-NPD, metals, lipids	226Ra	speciation
81	4	47,4	x	x		x	x			x
82	4	54,6	x	x		x	x			x
83	4	45,4	x	x		x	x			x
84	4	44,8	x	x		x	x			x
85	4	45,9	x	x		x	x			x
86	4	49,6	x	x		x	x			x
87	4	50,7	x	x		x	x			x
88	4	47,8	x	x		x	x			x
89	4	49,8	x	x		x	x			x
90	4	48,8	x	x		x	x			x
91	7	46,6		x		x	x			x
92	7	47,9		x		x	x			x
93	7	52,5		x		x	x			x
94	7	49,8		x		x	x			x
95	7	53,1		x		x	x			x
96	7	48,2		x		x	x			x
97	7	53,6		x		x	x			x
98	7	54,6		x		x	x			x
99	7	51,6		x		x	x			x
100	7	55,9		x		x	x			x
101	11	51	x	x		x	x			x
102	11	54,3	x	x		x	x			x
103	11	49,6	x	x		x	x			x
104	11	54	x	x		x	x			x
105	11	51,7	x	x		x	x			x
106	11	53	x	x		x	x			x
107	11	48,5	x	x		x	x			x
108	11	47,5	x	x		x	x			x
109	11	50,7	x	x		x	x			x
110	11	45,9	x	x		x	x			x
111	5	47,2	x	x		x	x			x
112	5	50,2	x	x		x	x			x
113	5	51,6	x	x		x	x			x
114	5	51,2	x	x		x	x			x
115	5	53,1	x	x		x	x			x
116	5	55,1	x	x		x	x			x
117	5	51,2	x	x		x	x			x
118	5	51,4	x	x		x	x			x
119	5	54,7	x	x		x	x			x
120	5	50,9	x	x		x	x			x

Code	Station	length(mm)	NRRT	MN	Pyr Hyd	Histopath	Histochem	PAH-NPD, metals, lipids	226Ra	speciation
121	6	49,7		x		x	x			x
122	6	49,3		x		x	x			x
123	6	52,1		x		x	x			x
124	6	56		x		x	x			x
125	6	49,5		x		x	x			x
126	6	55,6		x		x	x			x
127	6	49,2		x		x	x			x
128	6	47,4		x		x	x			x
129	6	59,7		x		x	x			x
130	6	48,9		x		x	x			x
Code	Station	length(mm)	NRRT	MN	Pyr Hyd	Histopath	Histochem	PAH-NPD, metals, lipids	226Ra	speciation
1	8	49,8			x					
2	8	48			x					
3	8	59,9			x					
4	8	53			x					
5	8	52,9			x					
6	8	49,2			x					
7	8	56,2			x					
8	8	44,2			x					
9	8	45,9			x					
10	8	51,2			x					
11	9	50			x					
12	9	48,3			x					
13	9	58			x					
14	9	50,1			x					
15	9	51,1			x					
16	9	46,1			x					
17	9	54,2			x					
18	9	51			x					
19	9	56,2			x					
20	9	47,3			x					
21	2	52,3			x					
22	2	51			x					
23	2	44,5			x					
24	2	55,4			x					
25	2	57			x					
26	2	51,2			x					
27	2	47,1			x					
28	2	49,6			x					
29	2	54			x					
30	2	43,8			x					
31	3	50,8			x					
32	3	49,2			x					
33	3	50,1			x					
34	3	43,9			x					
35	3	59,1			x					
36	3	48,8			x					
37	3	46,5			x					
38	3	49			x					
39	3	51,2			x					
40	3	48,3			x					

Code	Station	length(mm)	NRRT	MN	Pyr Hyd	Histopath	Histochem	PAH-NPD, metals, lipids	226Ra	speciation
41	1	47,5			x					
42	1	45,5			x					
43	1	48,2			x					
44	1	55,1			x					
45	1	47			x					
46	1	54,6			x					
47	1	45,4			x					
48	1	51			x					
49	1	47,1			x					
50	1	47			x					
51	ref2	49,4			x					
52	ref2	58			x					
53	ref2	41,1			x					
54	ref2	54,8			x					
55	ref2	50,4			x					
56	ref2	48,1			x					
57	ref2	50			x					
58	ref2	51,9			x					
59	ref2	46,8			x					
60	ref2	43,5			x					
61	ref1	47,3			x					
62	ref1	44			x					
63	ref1	50,1			x					
64	ref1	49,6			x					
65	ref1	44,2			x					
66	ref1	44,8			x					
67	ref1	52			x					
68	ref1	49,1			x					
69	ref1	49,9			x					
70	ref1	48,6			x					
71	10	48,4			x					
72	10	53,2			x					
73	10	48			x					
74	10	48,4			x					
75	10	50,1			x					
76	10	55,6			x					
77	10	60			x					
78	10	48,9			x					
79	10	49,8			x					
80	10	52			x					

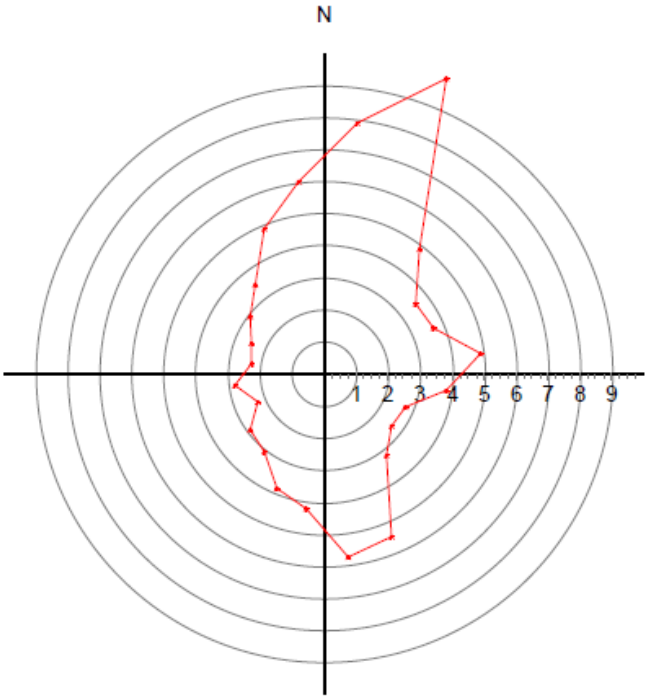
Code	Station	length(mm)	NRRT	MN	Pyr Hyd	Histopath	Histochem	PAH-NPD, metals, lipids	226Ra	speciation
81	4	50,3			x					
82	4	54,6			x					
83	4	56,4			x					
84	4	51,9			x					
85	4	52,1			x					
86	4	51,7			x					
87	4	60			x					
88	4	51,7			x					
89	4	48,9			x					
90	4	50,4			x					
91	7	54,2			x					
92	7	57,4			x					
93	7	54,8			x					
94	7	46,2			x					
95	7	48,4			x					
96	7	49			x					
97	7	46,2			x					
98	7	46,8			x					
99	7	56,1			x					
100	7	60,5			x					
101	11	47,8			x					
102	11	50,1			x					
103	11	52			x					
104	11	51,7			x					
105	11	60,2			x					
106	11	48,2			x					
107	11	48,2			x					
108	11	45,3			x					
109	11	47			x					
110	11	52,9			x					
111	5	49,8			x					
112	5	50,1			x					
113	5	52,4			x					
114	5	61,8			x					
115	5	58,6			x					
116	5	61,6			x					
117	5	50,6			x					
118	5	50,2			x					
119	5	52,4			x					
120	5	53			x					

Code	Station	length(mm)	NRRT	MN	Pyr Hyd	Histopath	Histochem	PAH-NPD, metals, lipids	226Ra	speciation
121	6	60,4			x					
122	6	59,8			x					
123	6	55,6			x					
124	6	53			x					
125	6	58,5			x					
126	6	55,4			x					
127	6	49,6			x					
128	6	51,5			x					
129	6	50,1			x					
130	6	55,4			x					

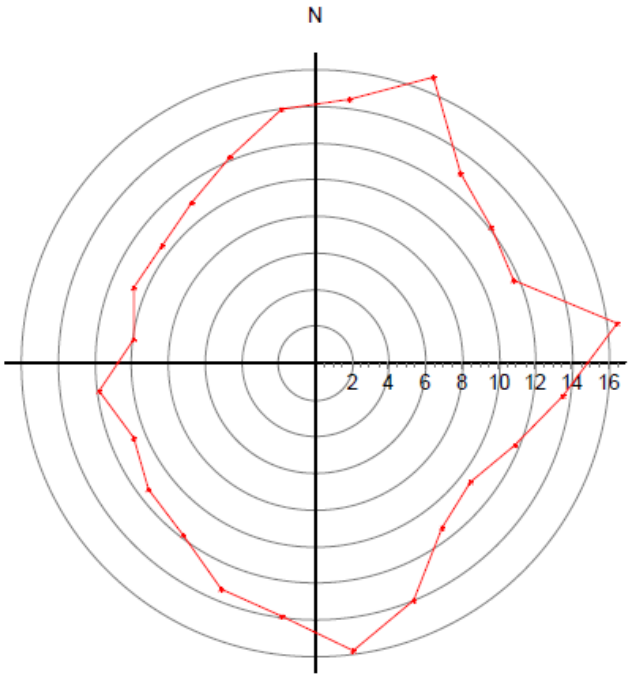
Appendix B

Current meter measurements at Troll 29 –Mars- 7 May 2012

Station 9, 15 m depth

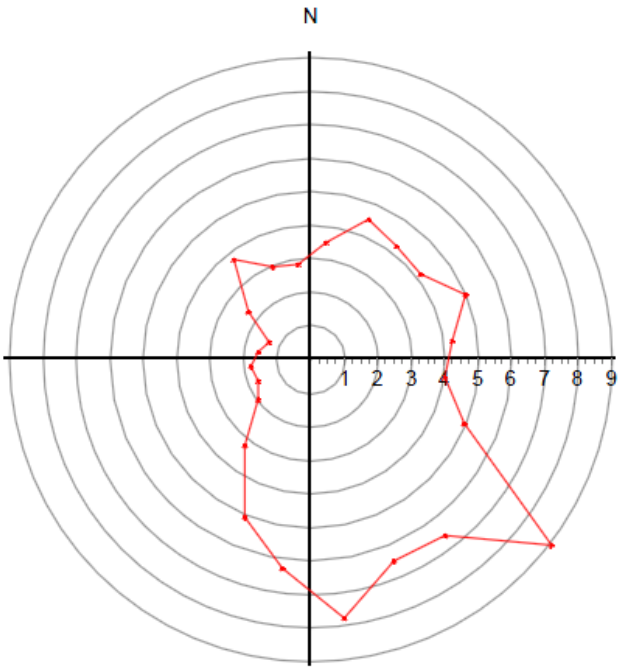


Relative water flux (%)
per 15 deg sector

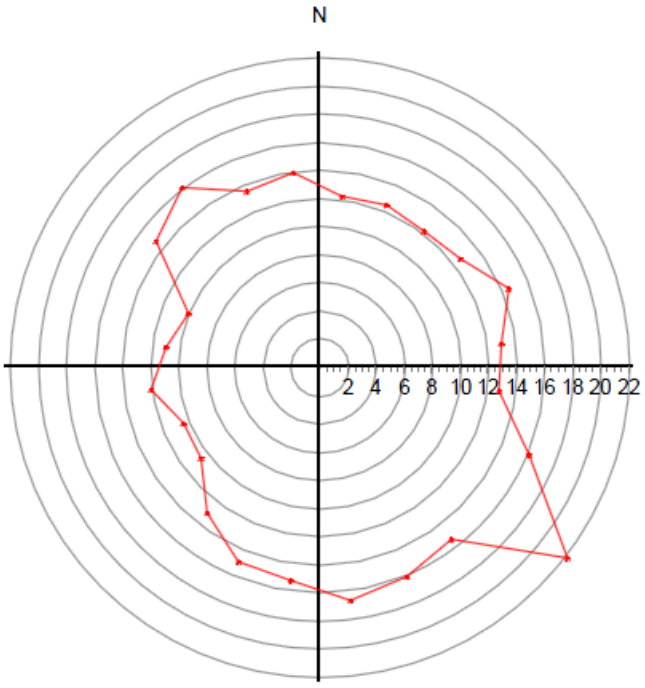


Mean velocity (cm/s)
per 15 deg sector

Station 12, 15 m

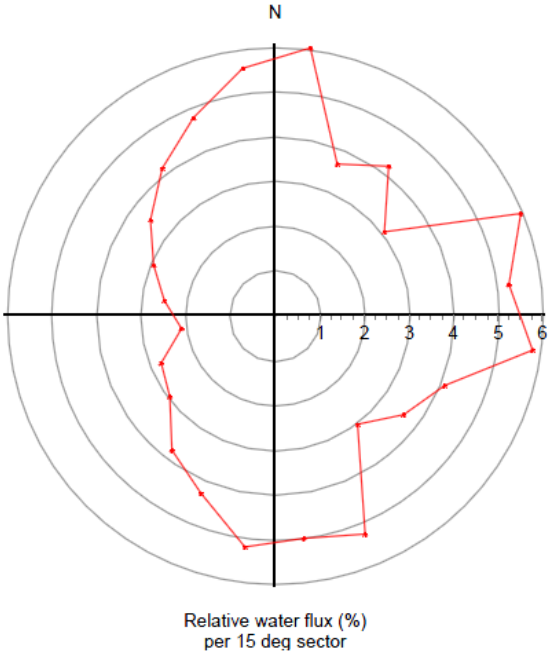


Relative water flux (%)
per 15 deg sector

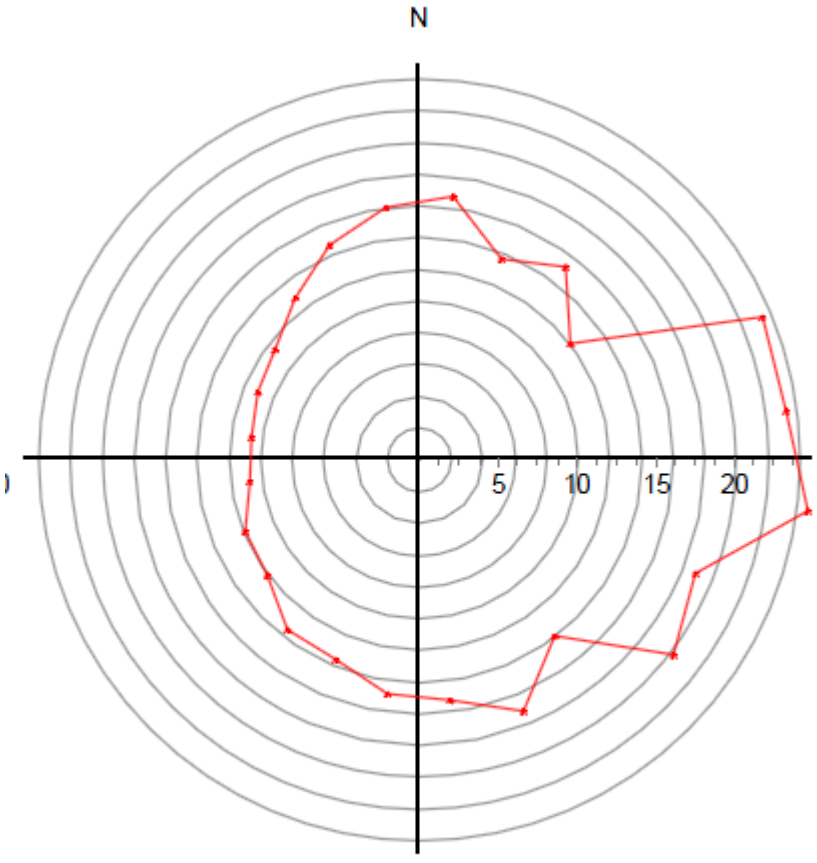


Mean velocity (cm/s)
per 15 deg sector

Station 2, 21 m

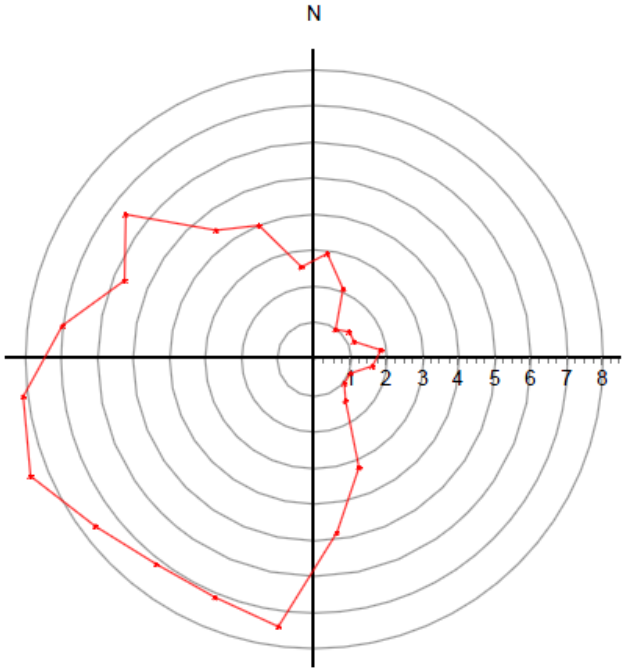


Relative water flux (%)
per 15 deg sector

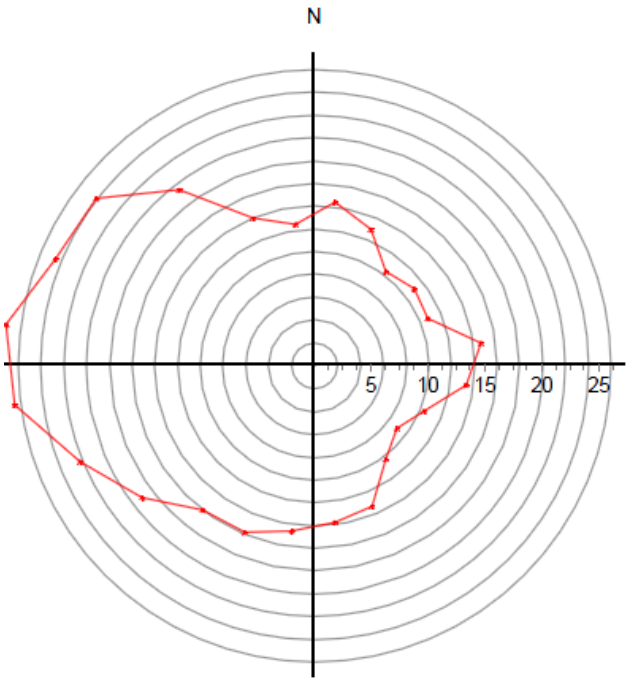


Mean velocity (cm/s)
per 15 deg sector

Station 2, 31m

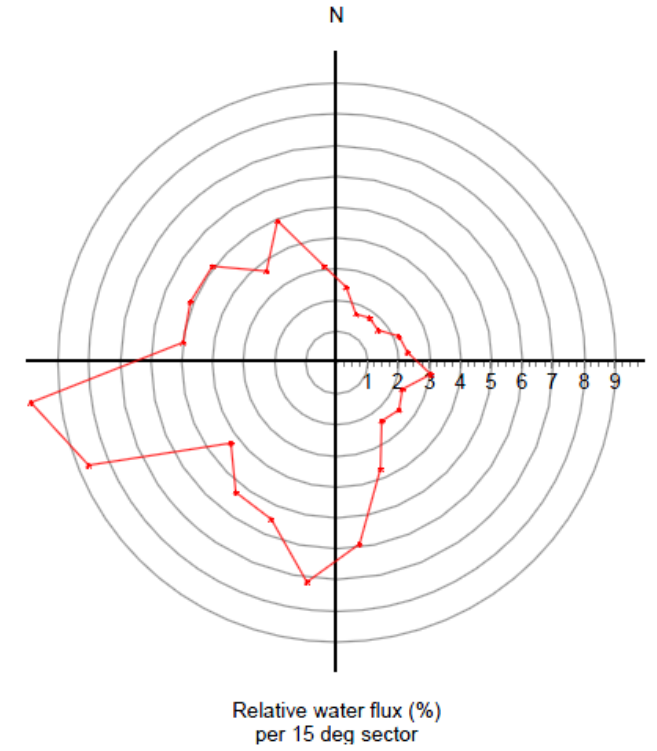


Relative water flux (%)
per 15 deg sector

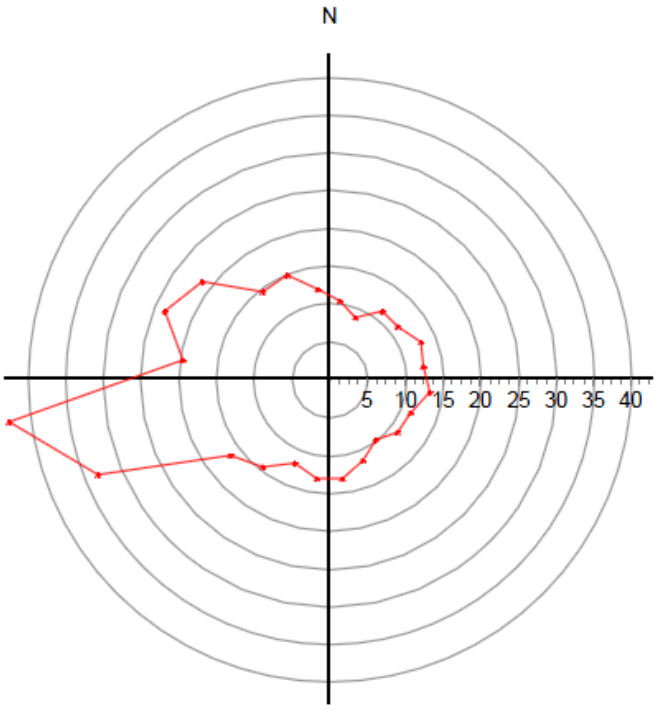


Mean velocity (cm/s)
per 15 deg sector

Station 2, 61 m



Relative water flux (%)
per 15 deg sector



Mean velocity (cm/s)
per 15 deg sector

Appendix C

	TTS/%	Fett-%	Al/MS-B	As/MS-B	Ba/MS-B	Cd/MS-B	Co/MS-B	Cu/MS-B
	%	% pr.v.v.	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Station 1.1	16	0,85	1,2	2,4	0,8	0,24	<0,1 *	1
Station 1.2	17	0,93	1,2	2,6	0,5	0,22	<0,1 *	1,3
Station 1.3	16	0,87	1,2	2,4	0,6	0,25	<0,1 *	1,2
Station 2.1	15	0,72	1,1	2,2	<0,2 *	0,21	<0,1 *	0,9
Station 2.2	16	0,8	1,6	2,4	13	0,19	<0,1 *	1,2
Station 2.3	15	0,8	0,9	2,4	0,2	0,19	<0,1 *	1,6
Station 3.1	16	0,81	1	2,4	0,2	0,21	<0,1 *	1,1
Station 3.2	17	0,8	1,1	2,6	0,2	0,23	<0,1 *	1,1
Station 3.3	16	0,94	0,9	2,5	0,2	0,2	<0,1 *	1,1
Station 4.1	16	0,8	1,5	2,6	3,2	0,18	<0,1 *	1,1
Station 4.2	16	0,8	1,4	2,4	3,2	0,16	<0,1 *	1,1
Station 4.3	17	0,93	1,4	2,5	3,1	0,23	<0,1 *	1,2
Station 5.1	17	0,81	1	2,5	1,2	0,21	<0,1 *	1,3
Station 5.2	17	0,81	1	2,5	1,2	0,22	<0,1 *	1,1
Station 5.3	17	0,71	1,1	2,5	1	0,26	<0,1 *	1
Station 6.1	17	0,77	1,3	2,7	1	0,21	<0,1 *	1,4
Station 6.2	17	0,72	1,4	2,5	1,1	0,23	<0,1 *	1,2
Station 6.3	16	0,87	1,2	2,6	0,9	0,19	<0,1 *	1,1
Station7.1	16	0,8	1,5	2,4	2,4	0,16	<0,1 *	1,1
Station7.2	17	0,8	1,7	2,2	3,2	0,19	<0,1 *	1,1
Station7.3	17	0,78	1,6	2,5	3,5	0,17	<0,1 *	1,2
Station 8.1	17	0,78	0,9	2,9	<0,2 *	0,2	<0,1 *	1,2
Station 8.2	16	0,91	0,8	2,6	<0,2 *	0,24	<0,1 *	1,4
Station 8.3	16	0,76	0,9	2,4	<0,2 *	0,21	<0,1 *	1
Station 9.1	15	0,82	1	2,4	<0,2 *	0,18	<0,1 *	1,2
Station 9.2	15	0,67	0,8	2,1	<0,2 *	0,19	<0,1 *	0,9
Station 9.3	16	0,73	0,9	2,4	<0,2 *	0,22	<0,1 *	1,2
Station 10.1	16	0,79	1,6	2,3	3,8	0,24	<0,1 *	1,3
Station 10.2	16	0,7	1,6	2,1	3,4	0,2	<0,1 *	1,1
Station 10.3	16	0,67	1,5	2,1	3,4	0,19	<0,1 *	0,8
Station 11.1	16	0,83	0,9	2,3	0,4	0,26	<0,1 *	0,9
Station 11.2	16	0,65	1	2,1	0,3	0,23	<0,1 *	1
Station 11.3	17	0,84	1,1	2,7	0,5	0,26	<0,1 *	1,6
Station 12.1	15	0,56	1,1	2,2	<0,2 *	0,25	<0,1 *	1
Station 12.2	15	0,78	1,2	2,3	<0,2 *	0,26	<0,1 *	0,9
Station 12.3	16	0,75	0,8	2,3	<0,2 *	0,28	<0,1 *	0,9
Station 13.1	17	0,71	1	2,4	<0,2 *	0,31	<0,1 *	1,1
Station 13.2	15	0,59	1,3	2,4	<0,2 *	0,23	<0,1 *	0,9
Station 13.3	15	0,71	0,9	2,2	<0,2 *	0,3	<0,1 *	0,9

	Fe/MS-B	Ni/MS-B	Pb/MS-B	Zn/MS-B	NAP-B	NAPC1-B	NAPC2-B	ACNLE-B
	mg/kg	mg/kg	mg/kg	mg/kg	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.
Station 1.1	9,6	0,1	0,07	15	1,3	12	80	<0,5
Station 1.2	10	0,1	0,07	15	3,6	21	110	<0,5
Station 1.3	11	0,1	0,1	18	2,4	18	99	<0,5
Station 2.1	9,5	0,1	0,08	14	<1	12	53	<0,5
Station 2.2	9,7	0,1	0,08	14	<1	9,1	56	<0,5
Station 2.3	10	<0,1 *	0,08	16	1,5	13	76	<0,5
Station 3.1	11	0,1	0,08	13	2,3	11	61	<0,5
Station 3.2	10	0,1	0,09	14	2,1	12	64	<0,5
Station 3.3	10	<0,1 *	0,08	15	2	12	65	<0,5
Station 4.1	11	<0,1 *	0,09	16	<1	7,6	35	<0,5
Station 4.2	9,9	0,1	0,08	15	<1	4,5	18	<0,5
Station 4.3	12	0,1	0,09	18	<1	160	38	<0,5
Station 5.1	9,7	0,1	0,07	13	3,3	16	61	<0,5
Station 5.2	9,8	<0,1 *	0,07	13	2,5	13	53	<0,5
Station 5.3	11	0,1	0,08	15	2,1	14	65	<0,5
Station 6.1	11	0,1	0,09	17	2,1	10	39	<0,5
Station 6.2	11	0,1	0,08	16	2,5	11	39	<0,5
Station 6.3	11	0,1	0,08	16	2,4	8,3	27	<0,5
Station7.1	9,9	<0,1 *	0,07	14	1,3	5,2	21	<0,5
Station7.2	11	0,1	0,08	14	1,4	6,4	24	<0,5
Station7.3	11	<0,1 *	0,08	17	1,1	7,8	33	<0,5
Station 8.1	11	0,1	0,1	17	<1	8,5	23	<0,5
Station 8.2	9,8	<0,1 *	0,09	17	1,1	9	27	<0,5
Station 8.3	9,4	0,1	0,08	15	<1	4,3	18	<0,5
Station 9.1	10	0,1	0,08	15	<1	3,8	13	<0,5
Station 9.2	8,5	<0,1 *	0,07	13	0,6	2,7	17	<0,5
Station 9.3	8,5	<0,1 *	0,08	14	0,87	4,3	19	<0,5
Station 10.1	11	<0,1 *	0,09	16	0,86	5,4	22	<0,5
Station 10.2	10	0,1	0,09	15	0,79	3	7,4	<0,5
Station 10.3	9,8	<0,1 *	0,09	14	0,61	3,8	23	<0,5
Station 11.1	9,7	<0,1 *	0,09	16	1,5	6,1	23	<0,5
Station 11.2	9,3	<0,1 *	0,08	11	2,9	6,8	22	<0,5
Station 11.3	11	0,1	0,12	17	1,3	6,1	21	<0,5
Station 12.1	9,2	0,1	0,08	12	<0,5	<2	11	<0,5
Station 12.2	9,2	0,2	0,08	13	<0,5	<2	<4	<0,5
Station 12.3	9,1	0,1	0,09	13	<0,5	2,1	13	<0,5
Station 13.1	8,9	0,1	0,09	14	<0,5	<2	<4	<0,5
Station 13.2	8,5	<0,1 *	0,08	12	<0,5	<2	8,7	<0,5
Station 13.3	9	<0,1 *	0,09	13	<0,5	<2	<4	<0,5

	NAPC3-B	ACNE-B	FLE-B	DBTHI-B	PA-B	ANT-B	PAC1-B	DBTC1-B
	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.
Station 1.1	500	1	2,9	0,99	11	<0,5	84	15
Station 1.2	690	1,2	4,3	1,2	16	<0,5	120	20
Station 1.3	610	1,1	3,4	1,2	13	0,53	100	18
Station 2.1	320	0,62	1,9	0,61	7,6	<0,5	56	9,8
Station 2.2	350	0,59	2,2	0,76	8,7	<0,5	64	12
Station 2.3	350	0,68	2,1	0,7	8,1	<0,5	62	11
Station 3.1	350	0,62	2,2	0,74	8,8	<0,5	65	10
Station 3.2	330	0,6	2,2	0,59	7,7	<0,5	59	10
Station 3.3	400	0,82	2,6	0,88	9,7	<0,5	71	12
Station 4.1	140	<0,5	1	<0,5	3,7	<0,5	29	5,2
Station 4.2	120	<0,5	0,83	<0,5	3,5	<0,5	27	4,4
Station 4.3	130	<0,5	1,2	<0,5	4,5	<0,5	30	5
Station 5.1	290	0,65	2,1	0,62	7,4	<0,5	51	8,5
Station 5.2	270	0,54	2	0,56	6,9	<0,5	49	8,3
Station 5.3	270	0,55	2	0,58	7	<0,5	47	8,1
Station 6.1	190	<0,5	1,4	<0,5	4,9	<0,5	35	6,5
Station 6.2	170	<0,5	1,3	<0,5	4,4	<0,5	34	5,9
Station 6.3	180	<0,5	1,3	<0,5	5	<0,5	38	6,4
Station7.1	170	<0,5	1	<0,5	4,5	<0,5	39	6,7
Station7.2	170	<0,5	1,2	<0,5	4,8	<0,5	40	7,2
Station7.3	150	<0,5	1,1	<0,5	4,3	<0,5	37	6,7
Station 8.1	110	<0,5	0,94	<0,5	3,4	<0,5	22	3,8
Station 8.2	130	<0,5	1,1	<0,5	4	<0,5	27	4,4
Station 8.3	110	<0,5	0,94	<0,5	2,8	<0,5	21	3,9
Station 9.1	73	<0,5	0,66	<0,5	3	<0,5	14	2,4
Station 9.2	59	<0,5	0,57	<0,5	1,7	<0,5	12	2,3
Station 9.3	71	<0,5	0,63	<0,5	2,5	<0,5	14	2,4
Station 10.1	60	<0,5	0,61	<0,5	2,1	<0,5	12	2,2
Station 10.2	41	<0,5	<0,5	<0,5	1,7	<0,5	9,5	<2
Station 10.3	56	<0,5	0,55	<0,5	1,7	<0,5	11	2,2
Station 11.1	120	<0,5	0,94	<0,5	3,2	<0,5	24	4,2
Station 11.2	79	<0,5	0,68	<0,5	2,3	<0,5	18	2,8
Station 11.3	110	<0,5	0,93	<0,5	3,5	<0,5	22	4,2
Station 12.1	13	<0,5	<0,5	<0,5	0,62	<0,5	<2	<2
Station 12.2	6,3	<0,5	<0,5	<0,5	0,81	<0,5	<2	<2
Station 12.3	14	<0,5	<0,5	<0,5	0,73	<0,5	<2	<2
Station 13.1	3,4	<0,5	<0,5	<0,5	0,74	<0,5	<2	<2
Station 13.2	11	<0,5	<0,5	<0,5	0,64	<0,5	<2	<2
Station 13.3	4,9	<0,5	<0,5	<0,5	0,69	<0,5	<2	<2

	PAC2-B	PAC3-B	DBTC2-B	DBTC3-B	FLU-B	PYR-B	BAA-B	CHR-B
	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.
Station 1.1	270	160	55	75	2,5	2,1	0,69	2,8
Station 1.2	370	220	76	100	3,7	3,4	0,87	3,4
Station 1.3	320	190	66	90	3,2	2,6	0,69	2,6
Station 2.1	170	100	35	48	2	1,5	<0,5	1,8
Station 2.2	190	120	42	56	2,4	1,8	<0,5	1,9
Station 2.3	190	120	42	55	2,5	1,7	<0,5	1,6
Station 3.1	200	120	40	54	2,6	1,8	<0,5	2
Station 3.2	180	110	39	52	2,3	1,9	<0,5	1,7
Station 3.3	220	130	45	58	2,9	2	<0,5	2
Station 4.1	94	60	21	28	1,9	0,89	<0,5	0,98
Station 4.2	78	47	17	22	1,6	0,69	<0,5	0,82
Station 4.3	91	59	20	27	2	0,85	<0,5	1
Station 5.1	160	90	33	46	2,2	1,3	<0,5	1,5
Station 5.2	140	82	29	37	2	1,2	<0,5	1,4
Station 5.3	140	83	29	38	2,1	1,3	<0,5	1,3
Station 6.1	110	70	26	33	2,1	0,91	<0,5	0,78
Station 6.2	110	67	25	34	2	1	<0,5	1,1
Station 6.3	120	80	27	37	2,4	1,1	<0,5	1,3
Station7.1	140	89	31	43	2,3	1,2	<0,5	1,4
Station7.2	150	98	32	47	2,1	1,4	<0,5	1,6
Station7.3	140	92	30	45	2,1	1,2	<0,5	1,5
Station 8.1	66	39	14	18	1,8	0,66	<0,5	0,66
Station 8.2	84	52	18	25	2,1	0,79	<0,5	0,95
Station 8.3	71	44	15	22	1,5	0,65	<0,5	0,76
Station 9.1	44	28	8,9	13	2	0,8	<0,5	0,87
Station 9.2	42	26	8,9	12	1,1	<0,5	<0,5	0,64
Station 9.3	45	28	9,6	14	1,3	<0,5	<0,5	0,8
Station 10.1	38	23	8,1	11	1,5	<0,5	<0,5	0,61
Station 10.2	31	20	6,8	8,9	1,2	<0,5	<0,5	0,56
Station 10.3	37	23	8,2	12	1,2	<0,5	<0,5	0,59
Station 11.1	84	54	18	28	1,8	0,72	<0,5	1
Station 11.2	60	37	13	19	1,3	1,7	<0,5	0,68
Station 11.3	68	40	15	22	1,8	0,62	<0,5	0,72
Station 12.1	2,4	<2	<2	<2	0,56	<0,5	<0,5	<0,5
Station 12.2	<2	<2	<2	<2	0,79	<0,5	<0,5	<0,5
Station 12.3	<2	<2	<2	<2	0,84	<0,5	<0,5	<0,5
Station 13.1	<2	<2	<2	<2	0,61	<0,5	<0,5	<0,5
Station 13.2	<2	<2	<2	<2	0,68	<0,5	<0,5	<0,5
Station 13.3	<2	<2	<2	<2	0,61	<0,5	<0,5	<0,5

	BBJF-B	BKF-B	BEP-B	BAP-B	PER-B	ICDP-B	DBA3A-B	BGHIP-B
	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.
Station 1.1	1,4	<0,5	2,7	<0,5	1,2	<0,5	<0,5	<0,5
Station 1.2	1,9	<0,5	3,3	<0,5	1,7	<0,5	<0,5	<0,5
Station 1.3	1,6	<0,5	3,1	<0,5	1	<0,5	<0,5	<0,5
Station 2.1	1	<0,5	2	<0,5	0,63	<0,5	<0,5	<0,5
Station 2.2	1,1	<0,5	2,2	<0,5	0,65	<0,5	<0,5	<0,5
Station 2.3	0,89	<0,5	1,9	<0,5	0,59	<0,5	<0,5	<0,5
Station 3.1	1,1	<0,5	2	<0,5	0,55	<0,5	<0,5	<0,5
Station 3.2	0,92	<0,5	1,8	<0,5	0,5	<0,5	<0,5	<0,5
Station 3.3	1,2	<0,5	2,3	<0,5	0,63	<0,5	<0,5	<0,5
Station 4.1	0,64	<0,5	1,2	<0,5	<0,5	<0,5	<0,5	<0,5
Station 4.2	<0,5	<0,5	1	<0,5	<0,5	<0,5	<0,5	<0,5
Station 4.3	0,65	<0,5	1,2	1,2	<0,5	<0,5	<0,5	<0,5
Station 5.1	0,85	<0,5	1,5	<0,5	<0,5	<0,5	<0,5	<0,5
Station 5.2	0,8	<0,5	1,5	<0,5	<0,5	<0,5	<0,5	<0,5
Station 5.3	0,8	<0,5	1,5	<0,5	<0,5	<0,5	<0,5	<0,5
Station 6.1	0,55	<0,5	1,4	<0,5	<0,5	<0,5	<0,5	<0,5
Station 6.2	0,55	<0,5	1,3	<0,5	<0,5	<0,5	<0,5	<0,5
Station 6.3	0,73	0,72	1,5	<0,5	<0,5	<0,5	<0,5	<0,5
Station7.1	0,84	<0,5	1,9	<0,5	<0,5	<0,5	<0,5	<0,5
Station7.2	0,94	<0,5	2	<0,5	<0,5	<0,5	<0,5	<0,5
Station7.3	0,92	<0,5	1,9	<0,5	<0,5	<0,5	<0,5	<0,5
Station 8.1	<0,5	<0,5	0,93	<0,5	<0,5	<0,5	<0,5	<0,5
Station 8.2	0,64	<0,5	1,1	<0,5	<0,5	<0,5	<0,5	<0,5
Station 8.3	<0,5	<0,5	0,88	<0,5	<0,5	<0,5	<0,5	<0,5
Station 9.1	0,59	<0,5	0,82	<0,5	<0,5	<0,5	<0,5	<0,5
Station 9.2	<0,5	<0,5	0,65	<0,5	<0,5	<0,5	<0,5	<0,5
Station 9.3	<0,5	<0,5	0,7	<0,5	<0,5	<0,5	<0,5	<0,5
Station 10.1	<0,5	<0,5	0,64	<0,5	<0,5	<0,5	<0,5	<0,5
Station 10.2	<0,5	<0,5	0,59	<0,5	<0,5	<0,5	<0,5	<0,5
Station 10.3	<0,5	<0,5	0,61	<0,5	<0,5	<0,5	<0,5	<0,5
Station 11.1	0,54	<0,5	1	<0,5	<0,5	<0,5	<0,5	<0,5
Station 11.2	<0,5	<0,5	0,91	<0,5	<0,5	<0,5	<0,5	0,56
Station 11.3	<0,5	<0,5	0,92	<0,5	<0,5	<0,5	<0,5	<0,5
Station 12.1	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Station 12.2	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Station 12.3	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Station 13.1	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Station 13.2	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Station 13.3	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5

	Sum PAH	Sum PAH16	Sum KPAH	Sum NPD
	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.	µg/kg v.v.
Station 1.1	1281,58	25,69	6,19	1264,29
Station 1.2	1771,57	38,37	9,77	1747,8
Station 1.3	1547,42	31,12	7,29	1527,6
Station 2.1	823,46	16,42	2,8	812,01
Station 2.2	921,4	18,69	3	908,56
Station 2.3	941,26	19,07	3,99	929,3
Station 3.1	935,71	21,42	5,4	922,84
Station 3.2	878,31	19,42	4,72	866,39
Station 3.3	1040,03	23,22	5,2	1025,58
Station 4.1	430,11	9,11	1,62	423,5
Station 4.2	346,34	7,44	0,82	341,4
Station 4.3	572,6	11,4	2,85	564,5
Station 5.1	776,92	19,3	5,65	766,82
Station 5.2	700,7	17,34	4,7	691,26
Station 5.3	713,33	17,15	4,2	703,78
Station 6.1	533,64	12,74	3,43	526,5
Station 6.2	510,05	12,85	4,15	502,8
Station 6.3	540,15	14,95	5,15	531,1
Station7.1	559,34	12,54	3,54	550,7
Station7.2	590,04	13,44	3,94	580,8
Station7.3	555,62	12,22	3,52	546,9
Station 8.1	312,69	7,46	0,66	307,7
Station 8.2	388,18	10,68	2,69	381,5
Station 8.3	316,73	6,65	0,76	312
Station 9.1	208,84	7,92	1,46	203,1
Station 9.2	187,16	4,61	1,24	184,2
Station 9.3	214,1	6,1	1,67	210,67
Station 10.1	188,02	5,68	1,47	184,66
Station 10.2	132,44	4,25	1,35	130,09
Station 10.3	181,46	4,65	1,2	178,51
Station 11.1	372	9,7	3,04	366
Station 11.2	268,63	10,12	3,58	262,8
Station 11.3	318,09	8,87	2,02	313,1
Station 12.1	27,58	1,18	0	27,02
Station 12.2	7,9	1,6	0	7,11
Station 12.3	30,67	1,57	0	29,83
Station 13.1	4,75	1,35	0	4,14
Station 13.2	21,02	1,32	0	20,34
Station 13.3	6,2	1,3	0	5,59

Appendix D - Quality assurance

The following is a description of the quality assurance measures that were taken for each procedure. For all laboratory analysis, standard operating procedures of the analysing laboratory were adhered to. For the chemical analysis, accredited procedures were used.

Quality assurance measures taken

Procedure	**Quality measure**
Sampling protocols	All samples were collected by trained scientific personnel. All samples were clearly marked in pre-labelled vials with individual labels and stored in the appropriate conditions prior to analysis. All biological data was recorded and stored in a project folder.
PAH body burden	Accredited method complying with the requirements of NS-EN ISO/IEC 17025.
Species Identification	Established methods based on Inoue et al., (1985). Mussels selected at random.
Pyrene hydroxylase	The samples were randomised before analysis took place and analysed blind. The samples were analysed within 24 hour of work-up. When determining hydroxy pyrene by HPLC in the samples, two procedural blanks were run for each series of samples. These include 1) a sample without pyrene, and 2) a sample containing pyrene but without microsomes. The median of the blank 2 was subtracted from each sample. A series contained not more than 30 samples. When analysing a series, a standard was run every 10-20 samples, to check that the levels of hydroxy pyrene were correct.
LMS (NRRT)	IRIS SOP NRRT (Lysosomal Membrane Stability) Haemolymph cells . The method is described in Lowe et al., (1995). One batch of neutral red stock solution was used for all individuals. The light level was kept to a minimal tolerable level and maintained throughout the subsequent analysis. Examination time for each slide was kept less than a minute.
Neutral lipid	IRIS 2.2-421 SOP - Neutral lipid accumulation Rev 02 A piece of digestive gland of mussels were immediately put in a cryovial and frozen in liquid nitrogen after dissection. Neutral lipids were detected in cryostat sections (10 µm) by the Oil Red O technique according to Bayliss, (1984). All slides were stained using one batch of the Oil Red O stock solution. Two pictures of each individual were taken using an objective lens of 400 x magnifications and the image analysis program AxioVision (Zeiss). For every picture taken similar settings were used for both microscope and image analysis program. A scale bar was added in each picture. The measurements of neutral lipids were carried out in each picture using an automatic measurement program (AxioVision). Only the secondary tubules in the digestive gland were measured.
Lipofuscin accumulation	IRIS 2.2-423 SOP - Lipofuscin accumulation Rev 02 A piece of digestive gland of mussels were immediately put in a cryovial and frozen in liquid nitrogen after dissection. Demonstration of lipofuscin was performed histochemically in cryostat sections (10 µm) using Schmorl's method (Pearse, 1985). All slides were stained using one batch of the stock solution. Two pictures of each individual were taken using an objective lens of 400 x magnifications and the image analysis program AxioVision (Zeiss). For every picture taken similar settings were used for both microscope and image analysis program. A scale bar was added in each picture. The measurements of lipofuscin were carried out in each picture using an automatic

	measurement program (AxioVision). Only the secondary tubules in the digestive gland were measured.
Lysosomal membrane stability in digestive gland	IRIS 2.2-420 SOP – Lysosomal membrane stability test Cryostat sections (10 µm) were cut in a cryostat with the cabinet temperature below -25°C, object temperature – 18 °C and knife temperature - 20°C. The sections were transferred to "warm" slides (20 °C). The slides were stored for more than 24 hours in the freezer at -40°C (or -20°C) before use motorized cutting. Serial cryostat sections are exposed to artificial acid and temperature stress in intervals for 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45 and 50 minutes in Citrate buffer in a shaking water bath at 37°C. By the difference of intensity of staining in each picture the two peaks of maximum staining of lysosomes were identified. After implementation of the new macro, automatic measurement of number and percentage of dark stained compartments in the tissue were identified by image analysis. Areas with artifacts like cracks and folding were not used for the assessment. Chose areas had with clearly identified digestive secondary tubuli.
Alkyl phenols in POCIS	Analytical limits of detection/quantification set to 3 and 10 times the standard deviation of four analysed solvent blanks plus the average. POCIS blanks (n=3) are reported alongside exposure POCIS for comparison as it is incorrect to subtract these (depends on stage of equilibrium). Water concentrations provided are semi-quantitative, due to the lack of an exposure correction method. Samples analysed blind.
Data archive	All raw data were stored electronically in the appropriate project folder on the secured NIVA and/or IRIS hard drives. Data will be stored for a minimum of five years.