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A mesocosm experiment on the impacts of water- and olefin-based drill cuttings on benthic communities

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REPORT

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Abstract

A three months simulated seabed experiment on water- and olefin-based drill cuttings has been performed at NIVAs Marine Research Station at Solbergstrand. Twelve box-core samples were transferred from 200 m depth in the outer Oslofjord and treated with 2-4 mm layers of cuttings or sediment particles from the fjord location. In treatments with olefin-based cuttings, microelectrode profiles showed reduced penetration of O_2 into the cuttings layer, and the consumption of O_2 from the overlying water increased after an initial lag phase of 2-3 weeks. The initial (field) structure of the macrobenthic communities was maintained throughout the experimental period, and at community level, no significant difference was observed between treatments at the end of the exposure period. However, three taxa showed reduced abundances in boxes treated with water- and olefin-based cuttings compared with untreated boxes and boxes treated with sediment particles. The effect could not be assigned to olefins or any of the weight materials ilmenite (present in the water-based cuttings) and barite (present in the olefin-based cuttings). Toxic effects of other mud components could not be entirely ruled out, but size or shape of cuttings particles appeared to be a more likely common factor responsible for the observed impact of both types of cuttings.

4 keywords, Norwegian	4 keywords, English
1. Borekaks	1. Drilling mud
2. Marine sedimenter	2. Marine sediments
3. Miljøpåvirkning	3. Environmental effects
4. Bunnfauna	4. Benthic fauna

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A mesocosm experiment on the impacts of water- and olefin-based drill cuttings on benthic communities

Preface

This report has been prepared on request from Akvaplan-niva AS. The report is based on results from a three-month simulated seabed study performed in the soft-bottom mesocosm at Marine Research Station Solbergstrand. The experiment used undisturbed sediment communities transferred to the mesocosm from 200 m depth in the Oslofjord. We thank the crew on RV Trygve Braarud for collecting the sediment samples and Frode Olsgard (NIVA/UiO) and JoLynn Caroll (Akvaplan-niva) for initiation of the work. All NIVA-staff involved at Marine Research Station Solbergstrand and in the laboratory in Oslo is acknowledged for their contributions.

Oslo, 01.02.07

Morten Schaanning

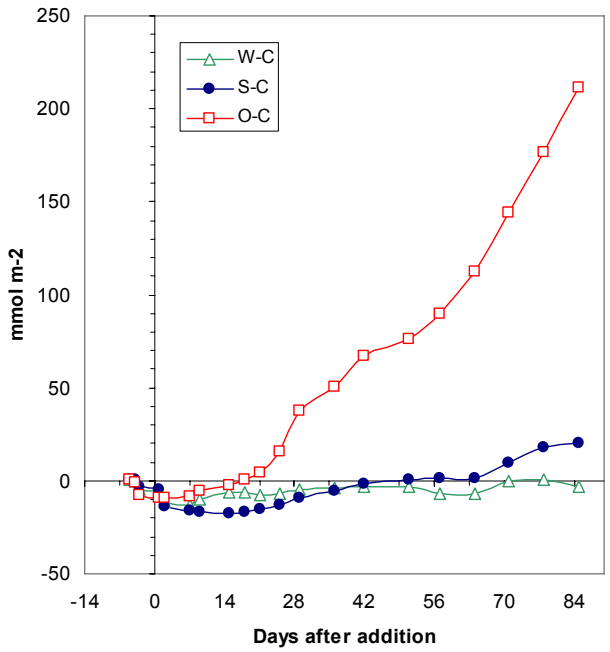
Contents

Summary

Effects of drill cuttings and clean sediment particles on benthic oxygen consumption and macrofauna communities were investigated in a three months mesocosm experiment performed at the Marine Research Station at Solbergstrand, S.E. Norway. 12 sediment samples were collected at 200 m depth in the Oslofjord nearby the research station, using a 0.1 m^2 box corer with internal liners. The liners containing unmixed sections of the top 30 cm of the sediment and a 10-15 cm head-space for overlying water were transferred to the mesocosm and incubated in a flow-through system with fjord water from 60 m depth (~34 PSU, ~7˚C). Three replicate boxes were treated with addition of 2-4 mm layers of clean particles or drill cuttings. The cuttings added were sampled from off-shore drilling operations and contaminated with remnants of either water- or olefin-based muds. The water-based mud was made up from ilmenite weight materials whereas the olefin-based mud was made up from barite. The clean particles were a mixed batch of sediments from the top 0-30 cm layer at the fjord sampling location.

Sediment oxygen consumption was determined both as O_2 penetration into the sediment surface using microelectrodes, and as total consumption measured as the change of the concentration of O_2 in the water flowing through the boxes.

The microelectrode profiles showed that the mean penetration of O_2 into the sediments decreased from 3.6 mm in the boxes treated with water based cuttings, via 3.1 mm in boxes with clean particles and 2.8 mm in control boxes to 1.8 mm in the boxes treated with olefin-based cuttings. Statistical analyses showed that the O_2 penetration was significantly ($p<0.05$) higher in water-based as compared to olefin-based cutting.



The consumption of O_2 from the overlying water (fig. A) was very similar in control boxes and boxes treated with water-based cuttings or clean particles. In the particle treatments the O_2 consumption initially decreased slightly, but towards the end of the experimental period no difference was observed between the control and these two treatments. However, in the olefin-treatments O_2 consumption showed a clear increase after an initial lag phase of 2-3 weeks.

Fig. A. Cumulative oxygen consumption in sediments treated with thin layers of water-based cuttings (W), olefin-based cuttings (O) and clean sediment particles (S) in excess of oxygen consumption in non-treated control sediments (C).

Thus, both methods showed ***increased O_2 consumption in sediments treated with olefin-based cuttings, only.*** It should, however, be noted that in a pilot test performed a few months prior to this test, subsamples of the same batch of water-based cuttings did reveal a short period of increased O_2 consumption immediately after addition of the cuttings. The increase was attributed to the presence of a labile organic phase (probably glycol). The discrepancy between the pilot test and the present study was assumed to result from dissolution and wash-out from the experimental boxes during set-up of the present experiment which involved thin layers only, and exposure of the cuttings particles to a much larger volume of seawater during sedimentation. Whether a similar ***wash-out of the glycol-phase*** will occur during off-shore discharge may depend on the dispersal of cuttings particles in the water column and time of exposure before burial in the sediment.

The macrofauna communities in the box core samples analysed at the end of the experimental period revealed no treatment effects on abundance or biomass. In addition, there was no clear change in community composition according to treatment. Despite of this finding, three individual taxa (the bivalve *Abra nitida*, the sipunculid *Onchnesoma steenstrupi* and the Nemertinea*)* showed decreased abundances when the six boxes with cuttings (water-based and olefins) were compared with the six boxes without cuttings (control and clean sediment) (fig. B). ***The observed effects could not be related to specific mud components such as olefins, ilmenite or barite. Toxicity of other mud components could not be ruled out, but the effects were more likely related to physical properties such as the shape or size of cuttings particles.***

The zero samples from the fjord showed a large similarity with the boxes analysed at the end of the experiment. This confirmed that ***the natural conditions in the sediment had been satisfactorily maintained in the experimental setup.***

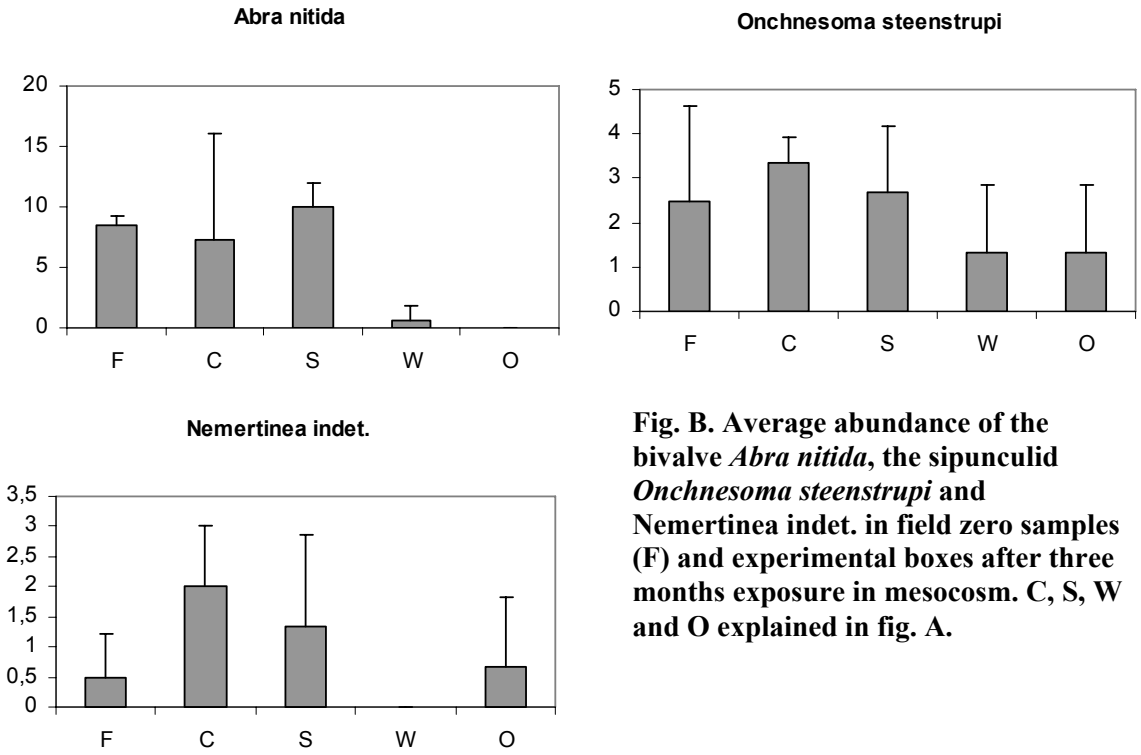


Fig. B. Average abundance of the bivalve *Abra nitida*, the sipunculid *Onchnesoma steenstrupi* and Nemertinea indet. in field zero samples (F) and experimental boxes after three months exposure in mesocosm. C, S, W and O explained in fig. A.

1. Background and objectives

After the ban on discharge of oil based muds in the Norwegian sector of the North Sea in 1992, the Norwegian Institute for Water Research has developed a so-called Simulated Seabed Study for assessment of degradation rates and effects on benthic communities of cuttings deposited in the marine environment (Bakke et al., 1989, Berge, 1995, Schaanning and Bakke, 1997, Schaanning et al., 1996; Schaanning et al., 1997, Schaanning and Rygg, 2002). On request from the oil industry, a number of tests have been performed on OBMs (oil based muds) and muds based on substitute organic phases such as esters, ethers and olefins, often referred to as SBMs (synthetic based muds). Recently, a pilot test on WBM (water based muds) has been performed (Schaanning et al., 2005). In this experiment, a more comprehensive study including benthic abundance data was performed on WBM.

At present, only cuttings drilled with water-based muds are permitted discharged in the Norwegian Sector. Even though monitoring data exists on effects of water based mud on benthic communities, there is a need of more detailed information regarding which factors that are responsible for effects. Synthetic drilling muds are still widely used in other countries, and particularly olefins have been shown to have only moderate effects on the benthos in mesocosm experiments as well as in the field (Schaanning et al., 1996; Schaanning and Bakke, 1997, 2006). It is therefore interesting to make an experimental comparison of effects of olefins and water-based muds.

The present study has been performed on request from Akvaplan-niva AS, and is a follow-up of the previous experiment carried out for the EXPAC-project (Experimental test of petroleum-associated compounds on benthos at community, individual, and cellular levels), see Schaanning et al. (2005). The aim of the present study was to investigate effect of water-based mud and olefins on benthic fauna and benthic respiration, and to compare this with the effect of burial by clean sediment particles. This set-up makes it possible to isolate potential effects of toxic compounds and effects of organic loading in the drilling mud from effects of burial only.

2. Methods

2.1 Collection of test communities

Box core samples were collected 08.08.05 at about 200 m depth in the Oslofjord nearby the Marine Research Station at Solbergstrand. The samples were collected from RV Trygve Braarud, Oslo using a 0.1m^2 KC-Denmark™ box corer modified with internal liners to retrieve undisturbed sediment samples (30 x 33 x 40 cm) in transparent polycarbonate boxes (Figure 1). On deck, most of the overlying water was removed through a siphon to avoid severe erosion of the sediment surface during transportation and handling. The boxes were also covered with black plastic and packed with ice to avoid heating. Fifteen samples were transferred to the mesocosm where they arrived late afternoon 08.08.05.

2.2 Set-up

In the mesocosm laboratory, three samples were set aside with a flow-through of seawater untill next morning when they were sifted for macrobenthic analyses (field samples).

The remaining twelve box core samples were submersed in a tray filled with seawater to the rim of the boxes. The overlying water in each box was continuously exchanged using a Watson-Marlow™ peristaltic pump to maintain a constant flow through the boxes (Figure 2). Throughout the experiment, the water supplied from 60 m depth in the Oslofjord adjacent to the laboratory had a salinity close to 34 PSU and a temperature of 8-10°C. In order to avoid concentration gradients, the overlying water in each box was stirred using timer controlled aquarium pumps which were activated for a period of one minute every two hours.

Treatment with the various particle and contaminant slurries was performed on 18.08.05, after ten days adaptation to the mesocosm environment. The water based cuttings (W) were taken from one of the buckets delivered from Statoil (West Navigator, Well 6507/3-4) prior to the pilot experiment performed in April, 2004 (Schaanning et al., 2005). The cuttings were mixed with water into a slurry (mixing ratio 1:1) using a high-speed stainless steel mixer. The slurry was gently poured into the overlying water in three replicate boxes. Another three boxes were similarly treated with a slurry made up from olefin-based cuttings left over from a previous experiment. Olefin-based cuttings (O) have previously been found to be stable for many years when stored dry, dark and cool. The two types of cuttings also differed with regard to the presence of ilmenite as weight material in the waterbased cuttings and barite in the olefin based cuttings.

Yet another three boxes were treated with a clay/silt sediment (S) from a non-contaminated location in the Oslofjord and the last three boxes were left untreated for control (C) purposes. The treatments are specified in **Table 1**. The estimated layer thickness of approximately 2-4 mm depend on the assumptions performed with regard to water content and wet density of the layers obtained after addition and sedimentation of the particles. After addition, the boxes were left undisturbed until the next day. By then, most of the particles had settled on the sediment surface, but a slight turbidity and some surface foam revealed that some minor fractions were washed out from all treatments when the water exchange was initiated.

Figure 1. Box core sampling. A) Corer on deck with fresh sample. C) Insertion of bottom steel sheet. B) Spade opened and disconnection of steel box with internal liner. D) Internal liner with sediment sample removed from steel box. E) Storage on deck. F) Installed in mesocosm with pump for internal stirring (during operation the perforated pipe is bent down to discharge water jets parallel with sediment surface).

Figure 2. Test set-up showing header tank (down left), 15 channel peristaltic pump, and experimental boxes during addition of slurried particles.

Table 1. Experimental treatments. The table shows the respective amounts of dry sediment and cuttings which were diluted with seawater to slurry volumes of about 600 ml before addition to experimental boxes.

Treatment	Code	Box no.	olefin based cuttings	clean clay/silt sediment	water based cuttings
Control	C	3	0	0	0
Control	C	7	0	0	0
Control	C	10	0	0	0
Clean particles	S	2	0	300g	0
Clean particles	S	5	0	300g	0
Clean particles	S	8	0	300g	0
Water-based	W	4	0	0	300g
Water-based	W	6	0	0	300g
Water-based	W	12	0	0	300g
Olefin based	O	1	150g	150g	0
Olefin based	O	9	150g	150g	0
Olefin based	O	11	150g	150g	0

2.3 Sampling and analyses

2.3.1 Sample collection

Oxygen consumption measurements (see ch. 2.3.2) were performed first time on 22.08.05 (day 4) and repeated 2/week during the next 26 days, and 1/week during the rest of the experimental period.

On day 8, 22 and 89 microprofiles of O_2 were determined in syringe-cores drawn from each box. The cores were mounted on a laboratory stand and measurements were taken at 1 mm intervals from 5 mm above the sediment-water interface down to zero O_2 or maximum 20 mm sediment depth (further described in ch. 2.3.3).

On day 89 the experiment was finalised and the macrofauna retained on a sieve for conservation, sorting and species identification (further described in ch. 2.3.4).

2.3.2 Flux measurements

Fluxes of oxygen (O_2) were determined by successive measurements of concentrations in the inlet water and in the well mixed water above the sediment in each box core. O_2 differences were measured with a precision <0.05 mg O_2 l^{-1} using an oxygen electrode.

The sediment oxygen consumption was calculated from the equation:

$$\mathbf{SOC = (C_i - C_o)\cdot Q\cdot / A}$$

in which

SOC is the flux (µmol $m^{-2}h^{-1}$)
C_i is the concentration in the headertank
C_o is the concentration in the respective box
Q is the flow of water through the respective box
A is the area of the box

The flow of water through each core was measured gravimetrically after collection of outflow water for 5 minutes.

2.3.3 Microelectrodes

Microelectrodes are fast-responding and provide a spatial resolution of two times the tip diameter (Revsbech 1989). In this work, a Unisense microelectrode (OX-50) was used to determine microgradients of oxygen concentration at the sediment-water interface at 1mm depth increments. It was also planned to determine sulphide using a H2S-50 microelectrode. Unfortunately, the two sulphide electrodes which were at hand both failed to work properly and the sulphide measurements were cancelled.

For O_2-measurements a Clark-type OX-50 microelectrode equipped with an internal reference and a guard cathode was used (Revsbech, 1989). This electrode has tip diameter of 50 µm, stirring sensitivities of <1% and a 90% response time of <1 s (Revsbech 1989). Before measurements a two-point calibration was performed in, respectively, oxygen-free water obtained by bubbling with inert gas e.g. N_2 and well aerated water. The OX-50 was connected to a picoammeter and readings were

transferred to an online-pc. After calibration, the electrodes and the core to be measured were mounted on a LS18 laboratory stand (**Figure 3**).

The electrodes were inserted into the overlying water and the measurements were taken at 1 mm intervals from 5 mm above the sediment-water interface down to zero-concentration of O_2 or maximum 20 mm sediment depth, using a manually controlled micromanipulator. Zero depth was assigned at the depth at which the tip of the electrode appeared to touch the first grains of the sediment. However, the tip was frequently difficult to see and hidden behind bumps and hollows on the sediment surface. Probably, the precision with which the sediment water interface was determined in this study, was no better than 3-4 mm.

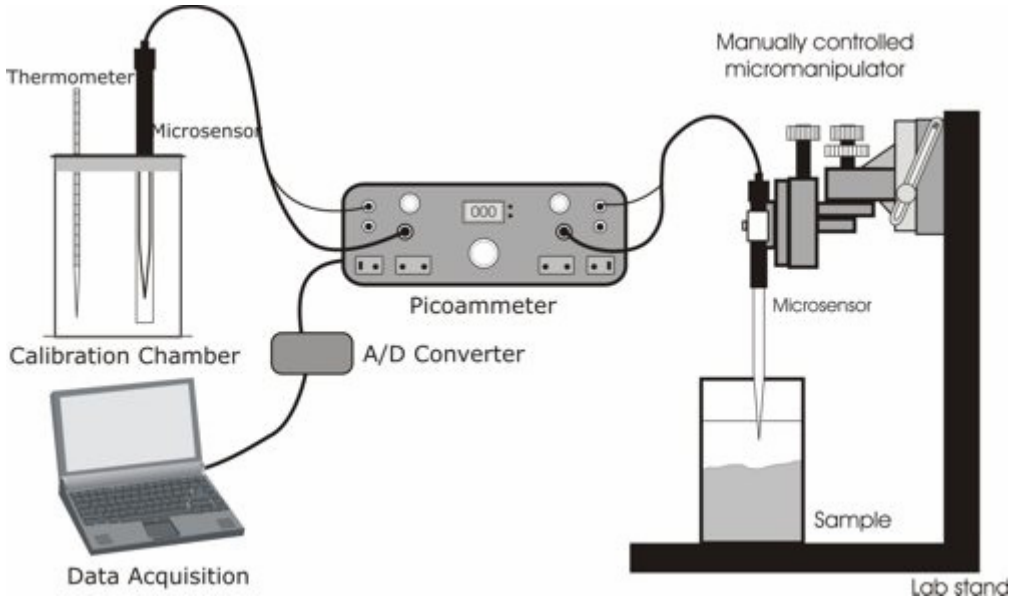


Figure 3. Microelectrode set-up.

2.3.4 Faunal analysis

By the end of the experiment, the sediment in each core was washed through a 1 mm sieve with round holes for macrofaunal analyses. The sieve residues were fixed in 10% buffered formalin, and stored in appropriate containers.

The macrofauna was sorted into main taxonomic groups (mollusca, polychaeta, crustacea, echinodermata and "others") and preserved in 75-80% ethanol. The organisms were identified to species level or, where this proved difficult, to the lowest taxon possible. One of the fjord samples had accidentally not been preserved, and was not included in the analyses. Biomass measurements (g wet weight) were performed for the main taxonomic groups and for selected taxa.

Univariate measures for faunal data for each separate box and the fjord samples included total number of taxa (S), total abundance (N), Shannon-Wiener diversity index calculated with $\log_2$ as the base, Pielou's evenness (J') and ES_{50}, i.e. the number of species expected from 50 randomly selected individuals. To analyse for similarities in the community structure, two multivariate analyses were performed based on the Bray-Curtis similarity measure: a cluster-analysis and MDS (multidimensional scaling). Similarity was calculated based on fourth-root transformed data. To test for significant differences in faunal composition between treatments, an ANOSIM analysis was performed. The calculation of the univariate parameters and the multivariate analyses were performed with the software program PRIMER (version 5.2.9) (PRIMER-E Ltd, 2002).

ANOVA was used to test for significant differences between univariate parameters, incl. selected taxa, and was performed with excel or the software package JMP. Prior to ANOVA, a Levene's test was performed to check for homogeneity of variances. When the ANOVA indicated that there were significant differences within the dataset, Tukey's HSD test was used as a post hoc test between pairs of treatments.

Regression analysis was used to test for correlations between diversity and oxygen consumption or thickness of the oxycline. This was performed with the program R 2.2.1 (R Development Core Team, 2006).

3. Results and discussion

3.1 Oxygen in source water and experimental boxes

Electrode measurements of O_2 concentration in the source water in the header tank (HT) and each box is shown in **Figure 4**. The figure shows some short term variation and a long term decline which occurred concurrently both in the header tank and experimental boxes.

Inaccurate calibration of the O_2 electrode and personal errors may explain some of the short term, random variation in electrode measurements. If, however, the error is the same in both header tank and box, the error will be eliminated in the flux calculated from the difference.

The more long-term trend of decreasing oxygen concentration may result from increased oxygen consumption in the header tank due to accumulated debris and bacteria growth on the bottom of the tank and equipment surfaces. The water flow through the header tank was, however, large compared to realistic rates of O_2 consumption. Therefore, the observed decrease with time is more likely a result of seasonal variations transferred from the fjord water at 60 m depth. This was confirmed by monitoring data from the oceanographic station lm2, which is located not far from the water inlet for Solbergstrand (**Table 2**).

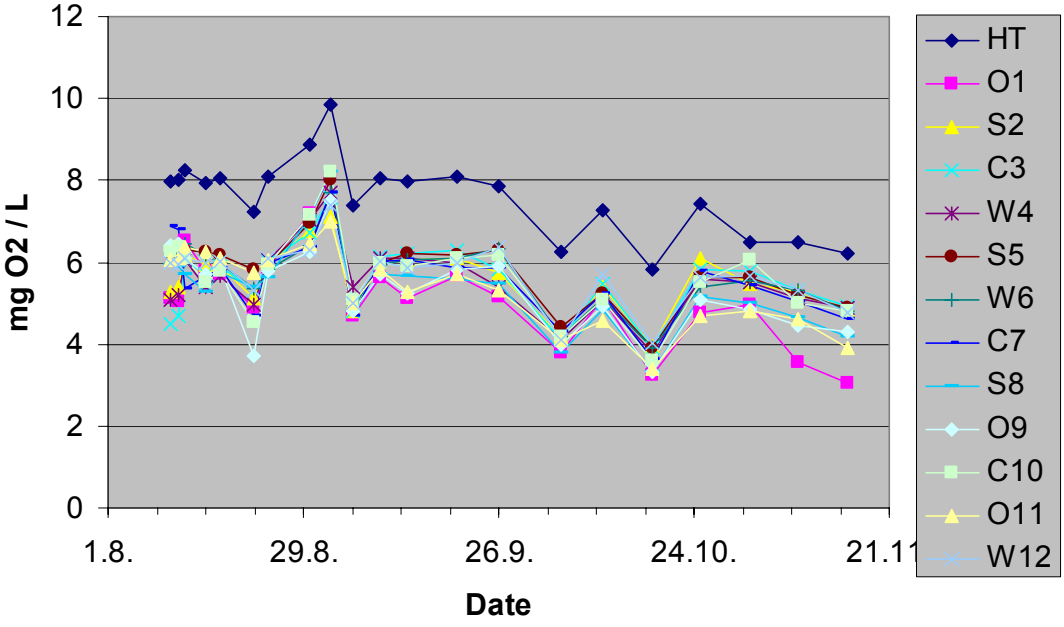


Figure 4. Oxygen concentration determined with electrodes in header tank (HT) and in each box throughout the experimental period.

Table 2. Oxygen determined in fjord water at monitoring station lm2 during the experimental period (Jan Magnusson, pers. com.).

Date \ Depth	50 m	60 m	80 m
15.8.2005	7.8	7.94	7.83
17.10.2005	6.65	6.8	7.22
12.12.2005	6.56	6.64	6.7

3.2 Oxygen concentrations at the sediment water interface

Microelectrode profiles of O_2 saturation are shown in **Figure 5**. The figure shows that in most of the boxes the water column was well mixed with 60-80% O_2 saturation. In the overlying water in most of the boxes the microelectrode showed a decrease of O_2 saturation with time during the study. This was consistent with the changes observed in the fjord water (ch. 3.1, **Figure 4**, **Table 2**).

At a certain depth, O_2 saturation decreased rapidly and reached less than 10% within a depth interval of less than 5 mm. The depth at which the steep decline started varied between +1 and -4 mm relative to the assigned depth zero. As discussed in ch. 2.3.3 s.9, the precision with which the sediment water interface was determined in this study, was no better than 3-4 mm. Thus, the variable position of the oxycline shown in **Figure 5** may have resulted from the unevenness of the sediment-water interface rather than different oxygen penetration into the sediment.

The oxic layer thickness was determined as the distance between the top of the oxycline and the depth at which 10% saturation is reached. As shown in **Figure 6** the oxic layer was thicker during the first survey (mean of all boxes = 3.92 mm, 11 days) than during the second (2.51 mm, 24 days) and third (2.21 mm, 92 days) survey. Statistical analyses (Student's t, Appendix 1) showed that the oxic layer on day 11 was significantly thicker than on day 24 and day 92. This would be expected from the general decrease of O_2 in the source water, but experimental bias such as decreased bioturbation or increased heterotrophic activity in the sediment cannot be ruled out.

During all surveys the thinnest oxic layer occurred in the olefin boxes with 1.66, 1.87 and 2.13 mm observed 11, 24 and 92 days, respectively, after the initial treatment. During the first two surveys, the thickest oxic layer was observed in the boxes treated with water-based cuttings (5.83 on day 11 and 2.77 mm on day 24). At the end of the experiment difference between treatments were smaller: 2.63 mm in control, 2.33 mm in water-based, 2.26 mm in the clean particle treatment and 1.66 mm in the olefin treatment. Statistical analyses (Student's t, Appendix A) showed a significant difference between water-based and olefin cuttings, but neither the water-based nor the olefin cuttings treatments were significantly different from control or clean particle treatments.

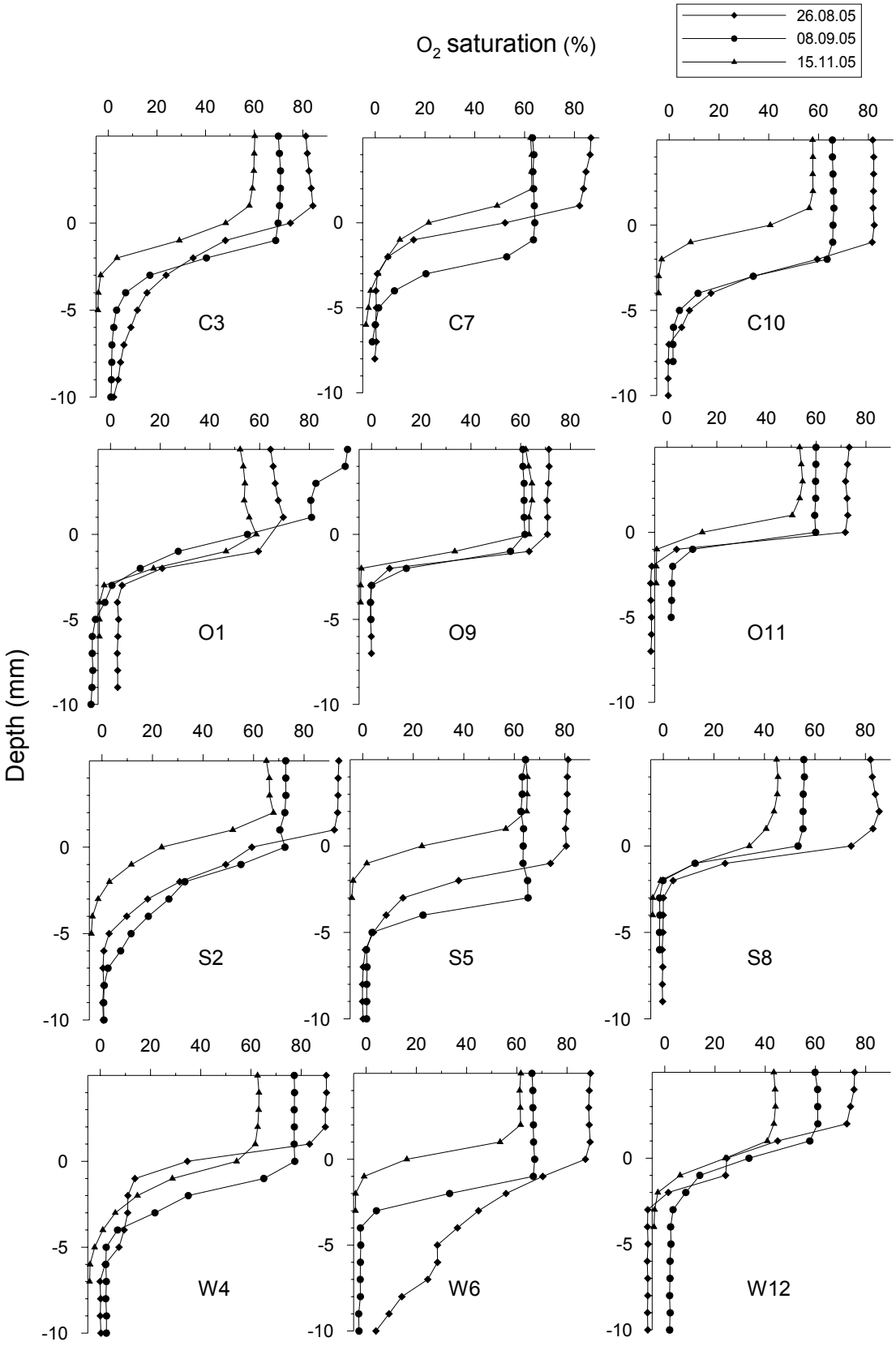


Figure 5. Microelectrode profiles of oxygen saturation measured in each box at three different occasions.

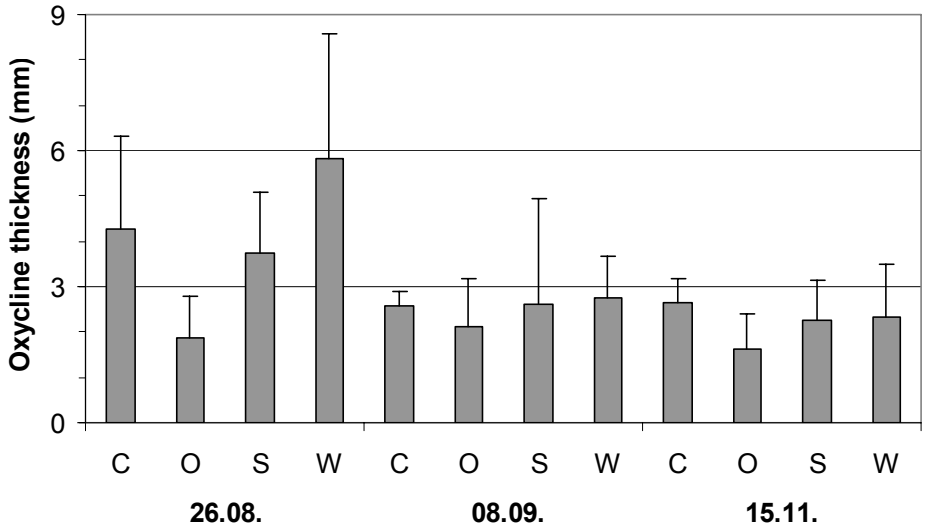


Figure 6. Oxycline thickness determined with microelectrodes at three occasions after treatment with a layer of water- (W) or olefin-(O) based cuttings or clean sediment particles (S). C is control boxes with no addition of particles. The bars show mean value of three replicate boxes and one standard deviation.

3.3 Oxygen consumption from overlying water

The oxygen consumption rates determined in the water flowing through the boxes varied from 444-450 µmol m^{-2} h^{-1} in the three control boxes (mean experimental period, n=20), 406 and 476 µmol m^{-2} h^{-1} in water based, 398-480 µmol m^{-2} h^{-1} in clean particles and 495-532 µmol m^{-2} h^{-1} in the olefin treatments.

The cumulative oxygen consumption in each box is shown in **Figure 7**. For the whole experimental period (10.08.-15.11.), 968±56 mol m^{-2} was consumed in the control boxes as compared to 965±58 mol m^{-2} in water-based, 989 ±100 mol m^{-2} in clean particles and 1179±21 mol m^{-2} in olefin based boxes.

Excess sediment oxygen consumption is the oxygen consumed in excess of the oxygen consumed in the control boxes with no additions. In sediments treated with clean particles and water based cuttings, excess oxygen consumption was close to zero throughout most of the experimental period (Figure 8). Sediments treated with olefin based cuttings did, however, consume more oxygen than control sediments from about day 30 until the end of the experiment.

The increase of the oxygen consumption in olefin treatments was consistent with previous tests which have shown an initial lag phase of about 2-4 weeks before the occurrence of a moderate increase of oxygen consumption in the overlying water. In this study, microelectrodes were used for the first time in such experiments. The electrodes revealed that already on day 11, before any clear effect was observed in the overlying water, the oxic layer in the sediment had already shrunk. An increase in the oxygen consumption in the overlying water was thus not seen until after the microelectrodes showed a reduction in the thickness of the oxic layer. Hence, if the objective is to measure instantaneous changes in sediment heterotrophic activity, the microelectrode may be a favourable tool compared to the measurement of sediment-water fluxes.

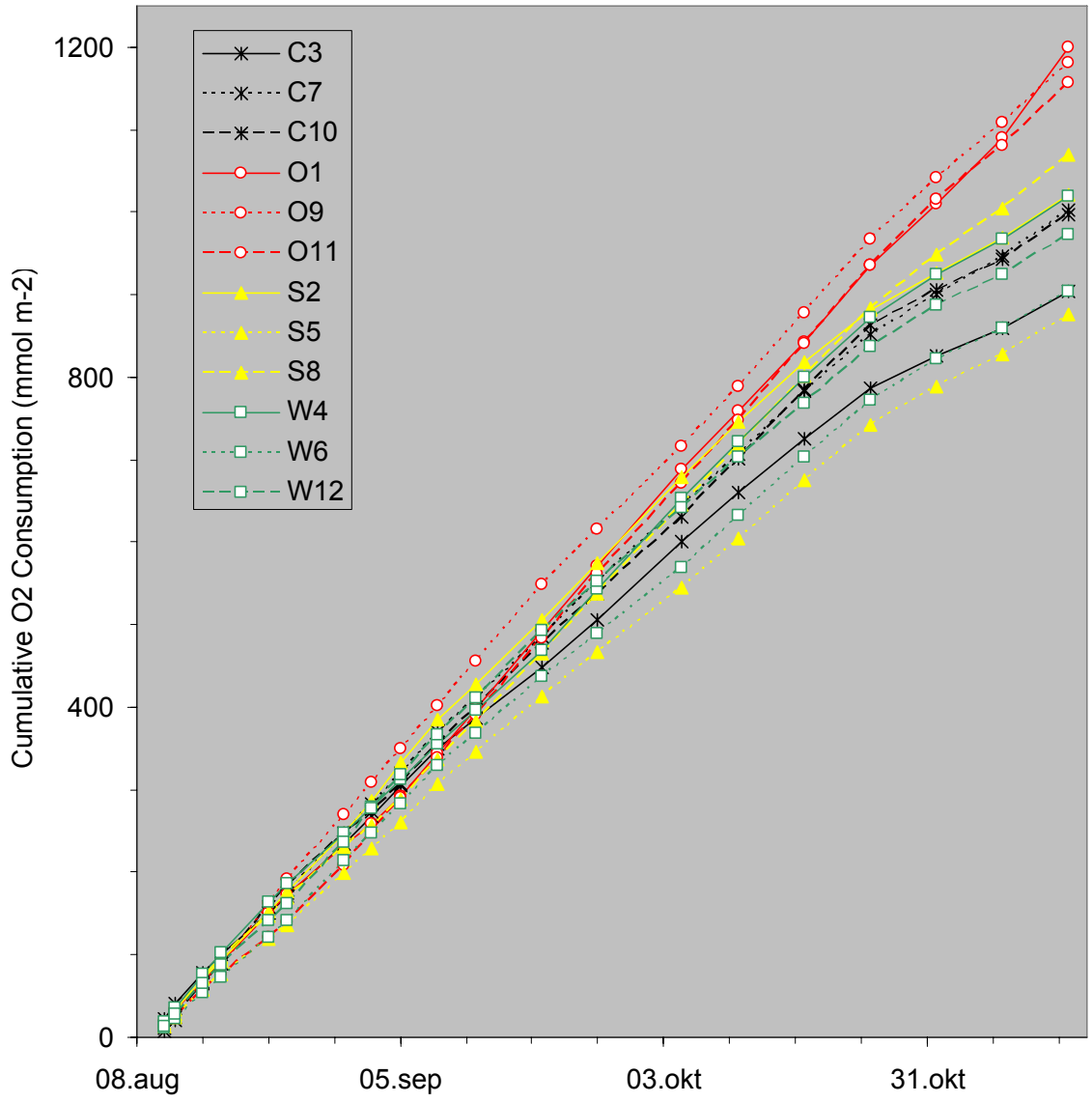


Figure 7. Cumulative oxygen consumption in each box.

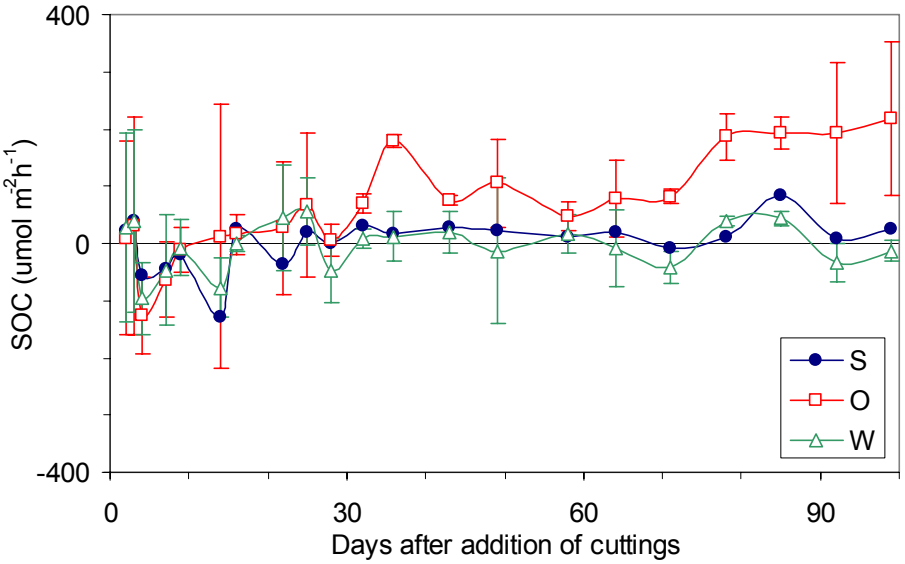


Figure 8. Excess oxygen consumption in sediments treated with 5 mm layers of water- (W) and olefin- (O) based cuttings and clean sediment particles (S). The excess oxygen consumption was calculated as the difference between treated and non-treated control boxes. Each point represent mean of three replicate boxes and vertical bars show ± one standard deviation.

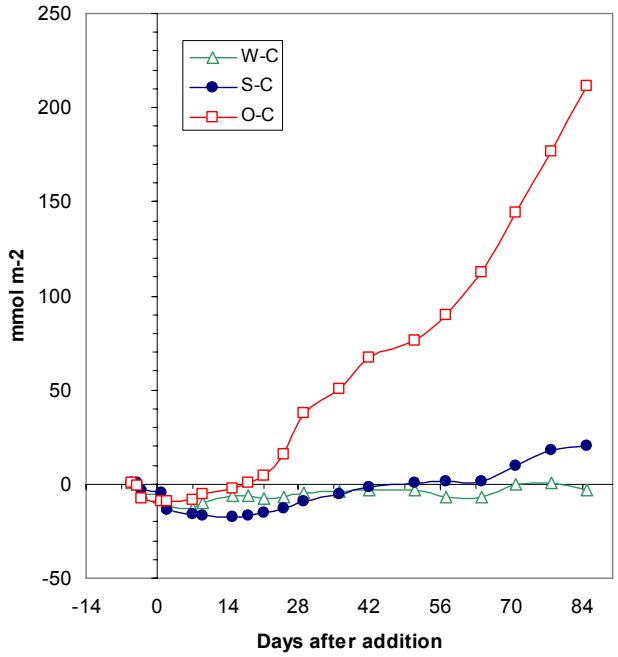


Figure 9. Excess cumulative oxygen consumption in sediments treated with 5 mm layers of water- (W) and olefin- (O) based cuttings and clean sediment particles (S). The excess oxygen consumption were calculated as the difference between treated and non-treated control boxes. Each point represent mean of three replicate boxes.

3.4 Discussion O_2

The presence of an organic, oxygen-consuming phase in water-based cuttings was first indicated by the low redox potentials reported in Bakke et al. (1989).

In a preliminary test on subsamples of the same batch of water based cuttings as in the present experiment, a sharp peak of oxygen consumption was observed about a week after the addition of cuttings (Schaanning et al., 2005). This was attributed to biodegradation of a labile organic phase, probably glycol, present in the cuttings. The fact that similar peaks were not observed in the present study may have been a result of different set-ups. Thus, the pilot study was performed in cores with a much smaller volume of overlying water with which the suspended cuttings are mixed before sedimentation. Glycol is more water-soluble than the organic phases used in synthetic based muds and loss by dissolution and wash-out from the boxes after initiation of the water exchange may have been favoured in the present set-up due to low solid:solution ratios during sedimentation as well as thin cuttings layers after sedimentation. Whether a similar "wash-out" of the glycol-phase will occur during off-shore discharge may depend on the dispersal of cuttings particles in the water column and time of exposure before burial in the sediment. In the ERMS (Environmental Risk Management System) model, easily degradable components in waterbased cuttings are assumed to be completely dissolved in the watercolumn before sedimentation (Henrik Rye, pers. comm.).

Increased oxygen consumption due to biodegradation of olefins was observed both in terms of a thinner oxic layer in the sediments treated with olefin based cuttings and increased consumption of oxygen in the water flowing through the same boxes.

In the water-based cuttings, the oxic layer was larger than in any other treatment and significantly larger than in the olefin treatment. In addition to the obvious increase of oxygen consumption in the olefin treatments, the oxic layer in the water-based treatments may have been increased by reduced oxygen consumption due to inhibited bacterial activity. Inhibitors are frequent additives in drilling muds, but as shown in **Figure 9**, a slight initial inhibition was observed in all treatments during the first 2-6 weeks and the largest inhibition actually occurred in the sediment treated with clean particles.

Disregarding inhibition from mud additives, initial reduction of oxygen consumption may result from a lower initial abundance of labile organic carbon or heterotrophic bacteria. During the course of the experiment such factors will even out due to some natural input of organic carbon from the fjord water and bacterial adaptation to similar environmental conditions. Organic carbon reservoirs may have been less in both the water-based cuttings and clean particles added, than in control sediments. The clean particles were a mixture of the top 30 cm of sediments from the control area and thus dominated by sediment from deeper strata which will be depleted in labile carbon compared to near surface sediments. Initial bacterial populations were most likely smaller in water-based cuttings than in both control and clean particle treatments.

Finally, a higher rate of oxygen diffusion through the water based cuttings due to physical factors such as grain size, grain shape, water content or cohesive forces between cuttings particles may be important to explain why the oxic layer in this treatment was thicker than in the other treatments.

Bioturbation is an important factor mediating O_2 transport through the cuttings layer. However, the macrofauna investigation did not yield any evidence for a different abundance or species composition in the water based treatment, and there is no apparent reason that bioturbation activities of individuals in these boxes should have been positively stimulated by this particular treatment.

Obviously, the major difference between the water and olefin based cuttings is the presence and biodegradability of the mud olefins. However, several of the factors discussed above may have

contributed to explain the more subtle, initial differences in sediment oxygen consumption between the water-based and the other treatments included in this study.

3.5 Benthic fauna

3.5.1 Abundance data

The results of the univariate analysis of the benthic fauna are shown in Table 3 and Figure 10. Complete taxonomic lists are given in Appendix B. In total 63 species were recorded, and 1612 individuals counted. The samples were composed of 13 (W2 and O2) to 31 species (O3) and 55 (O2) to 221 individuals (C1). The Shannon-Wiener diversity ranged from 2.66 (W2) to 3.89 (C1), ES_{50} from 10 (S3 and W2) to 17 (C1 and S1) and the evenness from 0.65 (S3) to 0.80 (F1). From Figure 10 it is evident that there was large variation in the univariate parameters independent of treatments, and no obvious effect of the various additions. This was confirmed by an ANOVA test (lowest p-value = 0.38), see Appendix C. Furthermore, the control samples and the fjord samples were not statistically different from each other regarding the univariate parameters, which indicates that the test communities remained intact throughout the experimental period.

Table 3. Number of species (S), number of individuals (N), Shannon-Wiener diversity (H'), ES_{50} and Pielou's evenness (J') in the fjord samples (F), control (C) and treatments (S, W, O). The highest and lowest values are indicated with bold.

	S	N	H'	ES_{50}	J'
F1	19	79	3.40	16	**0.80**
F2	20	101	3.29	15	0.76
C1	31	**221**	**3.89**	**17**	0.79
C2	17	80	3.10	14	0,76
C3	16	73	3.04	14	0.76
S1	24	110	3.60	**17**	0.78
S2	18	124	2.67	11	0.64
S3	17	281	2.65	**10**	**0.65**
W1	17	94	2.97	13	0.73
W2	**13**	109	**2.66**	**10**	0.72
W3	15	84	2.86	12	0.73
O1	15	80	2.77	13	0.71
O2	**13**	**55**	2.81	13	0.76
O3	**28**	140	3.22	14	0.67

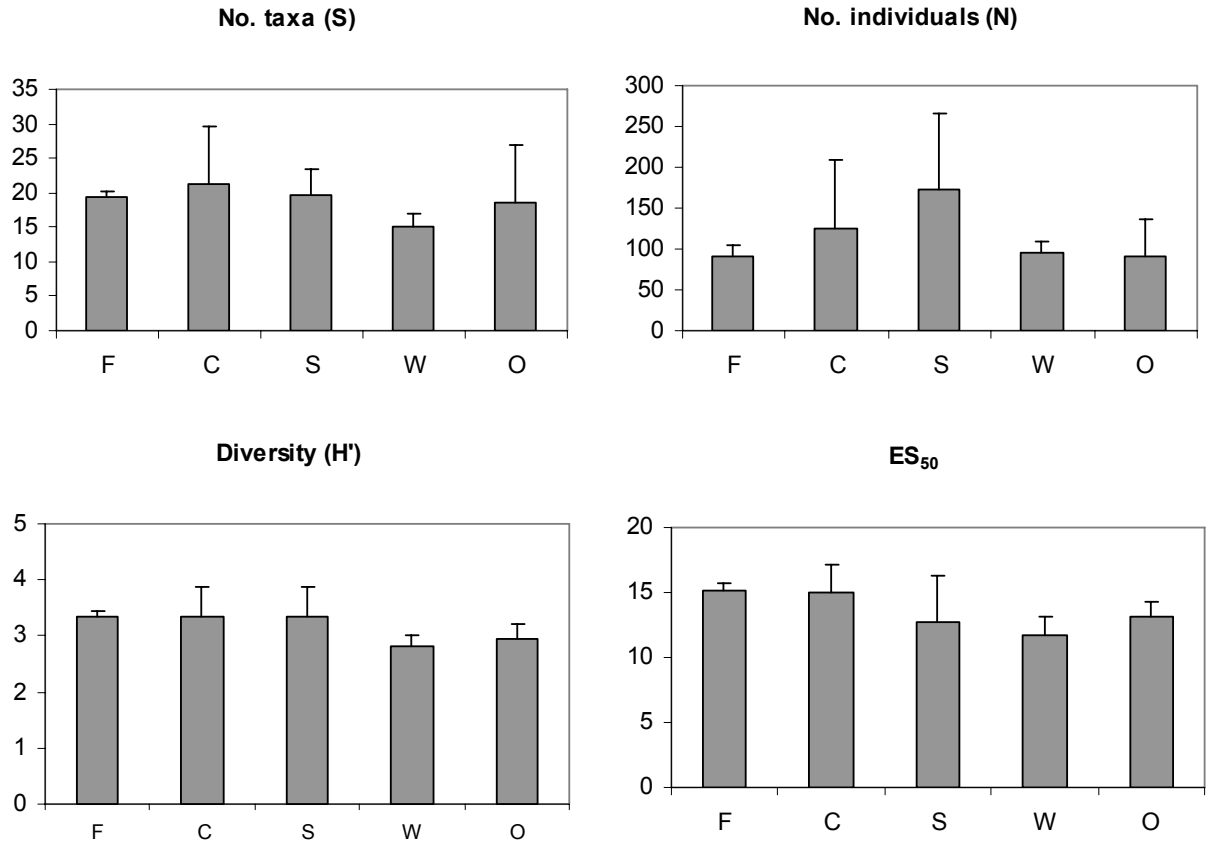


Figure 10. Mean number of taxa, number of individuals, diversity and ES_{50} for the control (C) and treatments (S=sediment, W=water based drill cuttings, O=olefin based drill cuttings) and fjord samples (F), with one standard deviation.

Similarities in faunal structure between the samples are shown as cluster-diagram in **Figure 11** and MDS-plot in Figure 12. As for the univariate analyses, the fjord samples showed a large degree of similarity with the experimental samples, again indicating that there was no effect of the experimental setup on the communities within the time span of this experiment. Furthermore, there was no grouping of samples according to treatment. An ANOSIM analysis on the data confirmed that there were no statistical differences between the various treatments regarding species composition.

The communities were dominated by small bivalves, where *Nucula tumidula* and *Thyasira equalis* were the most dominant, see **Table 4**. These are both subsurface deposit feeders. Also *Abra nitida* was generally quite abundant. This species lives as a suspension/surface deposit feeder. The anthozoa *Paraedwardsia arenaria* was also abundant, particularly in the core samples. This is a sessile burrower, living mainly as a carnivore/omnivore. Of polychaetes, *Melinna cristata* and *Heteromastus filiformis* were the most abundant. *Melinna cristata* is a tube-building surface deposit feeder, while *Heteromastus filiformis* is a subsurface deposit feeder. The large heart urchin *Brissosis lyrifera* was also represented in most boxes, mainly with 1-2 individuals. Although this species only made up a very small part of the total abundance, its size and bulldozing activity as a non-selective subsurface deposit feeder make it an important characteristic of the communities where it is present.

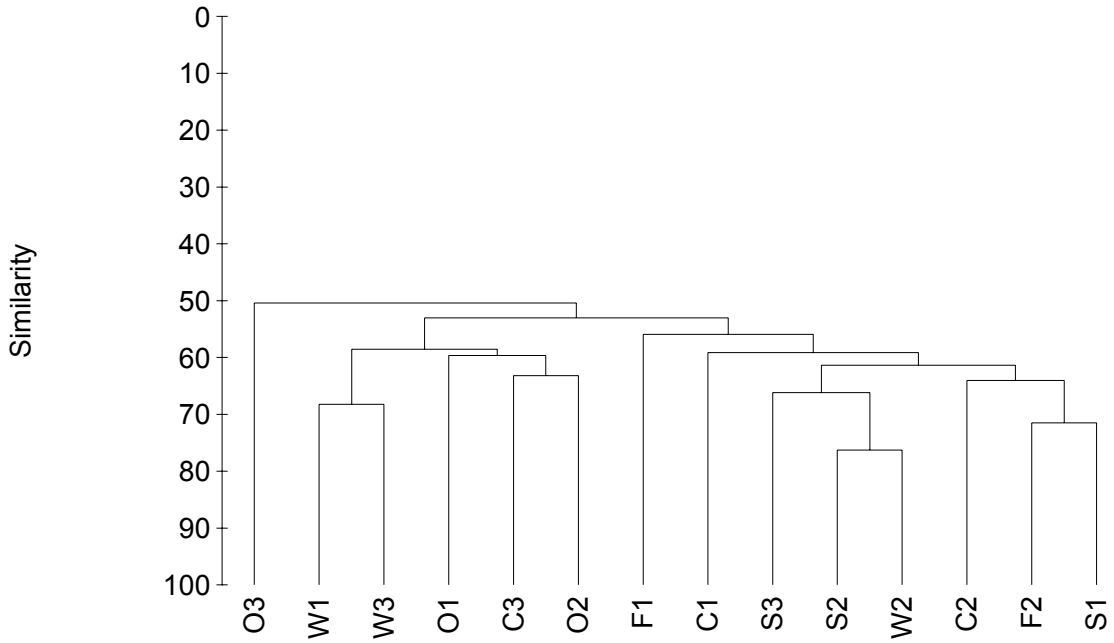


Figure 11. Cluster-analysis of the boxes in the mesocosm experiment.

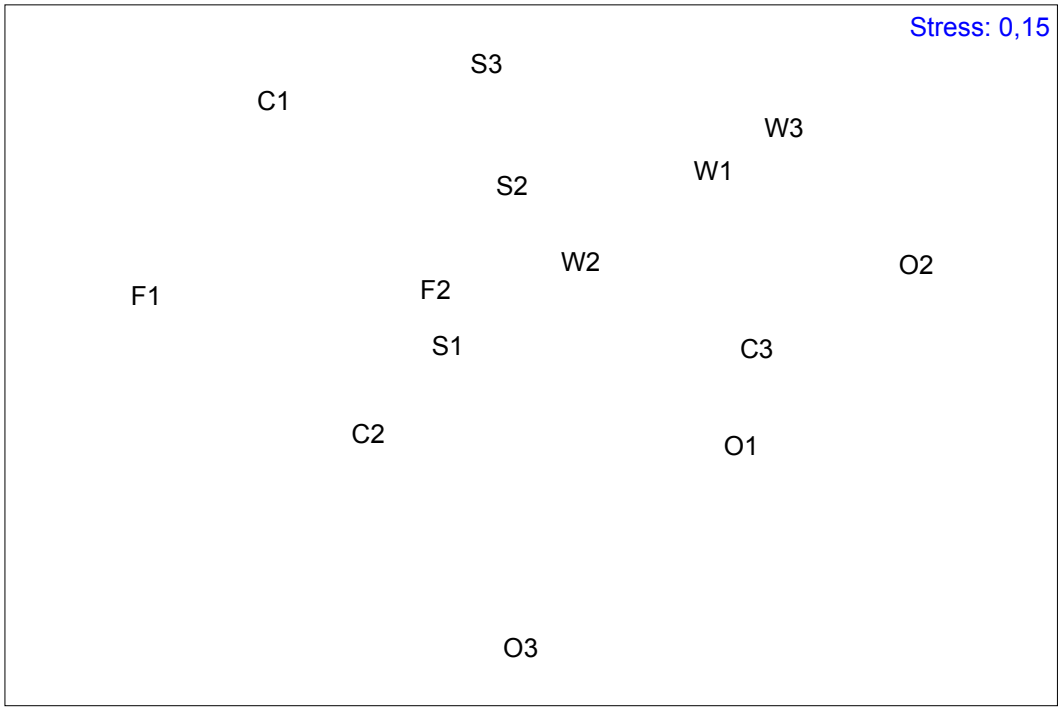


Figure 12. MDS-ordination of the boxes in the mesocosm experiment, shown as a 2-D plot.

Table 4. Overview of the most dominating taxa (total abundance of minimum 2) in the fjord samples (F), control (C) and treatments (S, W, O), normalised for 0.1 m^2. Respective phylum is also presented (A=Annelida, C=Cnidaria, Cr=Crustacea, E=Echinodermata, M=Mollusca, N=Nemertini, S=Sipunculida).

	F		C		S
Nucula tumidula (M)	26,5	Nucula tumidula (M)	31,7	Nucula tumidula (M)	60,0
Thyasira equalis (M)	18,0	Thyasira equalis (M)	26,7	Thyasira equalis (M)	47,7
Abra nitida (M)	8,5	Paraedwardsia arenaria (C)	8,0	Thyasira ferruginea (M)	10,0
Yoldiella lucida (M)	6,0	Abra nitida (M)	7,3	Abra nitida (M)	10,0
Thyasira ferruginea (M)	3,5	Kelliella miliaris (M)	6,7	Paraedwardsia arenaria (C)	8,7
Melinna cristata (A)	3,0	Thyasira pygmaea (M)	4,3	Brissopsis lyrifera (E)	5,3
Heteromastus filiformis (A)	2,5	Thyasira ferruginea (M)	3,3	Kelliella miliaris (M)	4,3
Onchnesoma steenstrupi (S)	2,5	Parvicardium minimum (M)	3,3	Thyasira obsoleta (M)	3,7
Paraedwardsia arenaria (C)	2,0	Onchnesoma steenstrupi (S)	3,3	Heteromastus filiformis (A)	3,0
Thyasira pygmaea (M)	2,0	Heteromastus filiformis (A)	3,3	Montacuta tenella (M)	3,0
Brissopsis lyrifera (E)	2,0	Neoleanira tetragona (A)	3,0	Thyasira pygmaea (M)	2,7
		Yoldiella tomlini (M)	2,7	Onchnesoma steenstrupi (S)	2,7
		Melinna cristata (A)	2,7		
		Nemertinea indet (N)	2,0		
	W		O		
Nucula tumidula (M)	36,0	Thyasira equalis (M)	28,7		
Thyasira equalis (M)	22,3	Nucula tumidula (M)	24,7		
Paraedwardsia arenaria (C)	9,7	Paraedwardsia arenaria (C)	6,3		
Montacuta tenella (M)	3,3	Thyasira pygmaea (M)	6,3		
Eriopisa elongata (Cr)	3,3	Montacuta tenella (M)	4,0		
Melinna cristata (P)	3,0	Kelliella miliaris (M)	2,7		
Thyasira ferruginea (M)	2,3	Nucula sulcata (M)	2,3		
Thyasira pygmaea (M)	2,3	Nereimyra punctata (A)	2,0		

ANOVA was used to investigate whether single species were negatively affected by the cuttings. 59 taxa were counted in the experimental cores in the present experiment (fjord samples not included). Of these, 26 taxa were only found in one box, while only three taxa were found in all twelve boxes. This pattern, which is typical for benthic communities, makes it difficult to perform statistical testing and conclude on the portion of species that are affected. In order to improve the statistical basis tests were also performed with the boxes containing clean sediment or no additions in one group ("clean") and the boxes containing olefin or water-based cuttings in another group ("cuttings"). Three taxa were found to be negatively affected by the cuttings, either between control and treatments and/or when "clean" was tested against "cuttings". These taxa are shown in **Figure 13**, and the ANOVA-results given in Appendix C.

The bivalve *Abra nitida* had clearly lower abundance in the boxes with water-based mud and olefins compared to the fjord, control and clean sediment samples, see **Figure 13**. This species did not have a homogenous variance among the treatments, and the values were transformed with a log-transformation (n+1). After the transformation, the variance was still not homogenous, but it was decided to use ANOVA despite of this as ANOVA is considered a robust test, and as it was convenient to use the same test for the same type of data. The ANOVA analysis based on the log-transformed values confirmed that there was a significant difference in the distribution of this species between the various treatments (p=0.02). However, according to the Tukey's HSD-test, only the difference between the clean sediment and the olefin treatment, was significant. When ANOVA was

performed on the "clean" vs. "cuttings" groups, the difference was highly significant (p=0.003). *Abra nitida* is generally a quite tolerant species (Rygg, 2005). Based on the OLF-database containing all benthic offshore monitoring data, Bjørgesæter (pers. comm.) found that *A. nitida* was tolerant, and in fact showed a positive correlation with several components associated with cuttings including barium. However, in the present experiment it clearly responded negatively to some property of the cuttings added.

Onchnesoma steenstrupi, a surface-deposit feeder sipunculid, was also affected by the cuttings (**Figure 13**). When all treatments were compared separately, no significant effect was found (p=0.19). However, when the treatments were divided into the two groups "clean" or "cuttings" a significant difference was found (p=0.03). *Onchnesoma steenstrupi* is generally considered a sensitive species (e.g. Rygg, 2005). Bjørgesæter (pers. comm.) found this species to be sensitive towards several drill cuttings components (but not barium). In the present experiment it is interesting to note that it appeared to be sensitive to both types of drill cuttings, but not to a similar dose of clean sediment.

The group (phylum) Nemertinea was the third taxon which showed a response towards the cuttings deposition (**Figure 13**). A significant effect was found between "clean" and "cuttings" (p=0.04). Nemertineans live as carnivores/omnivores. They are generally tolerant to disturbances (Rygg, 2005), but despite of this they appeared to be affected by the cuttings. Bjørgesæter (pers. comm.) has not studied this taxon in particular, but based on a smaller data material than for the other taxa, he reports that nemertineans are sensitive towards high concentrations of copper and barium.

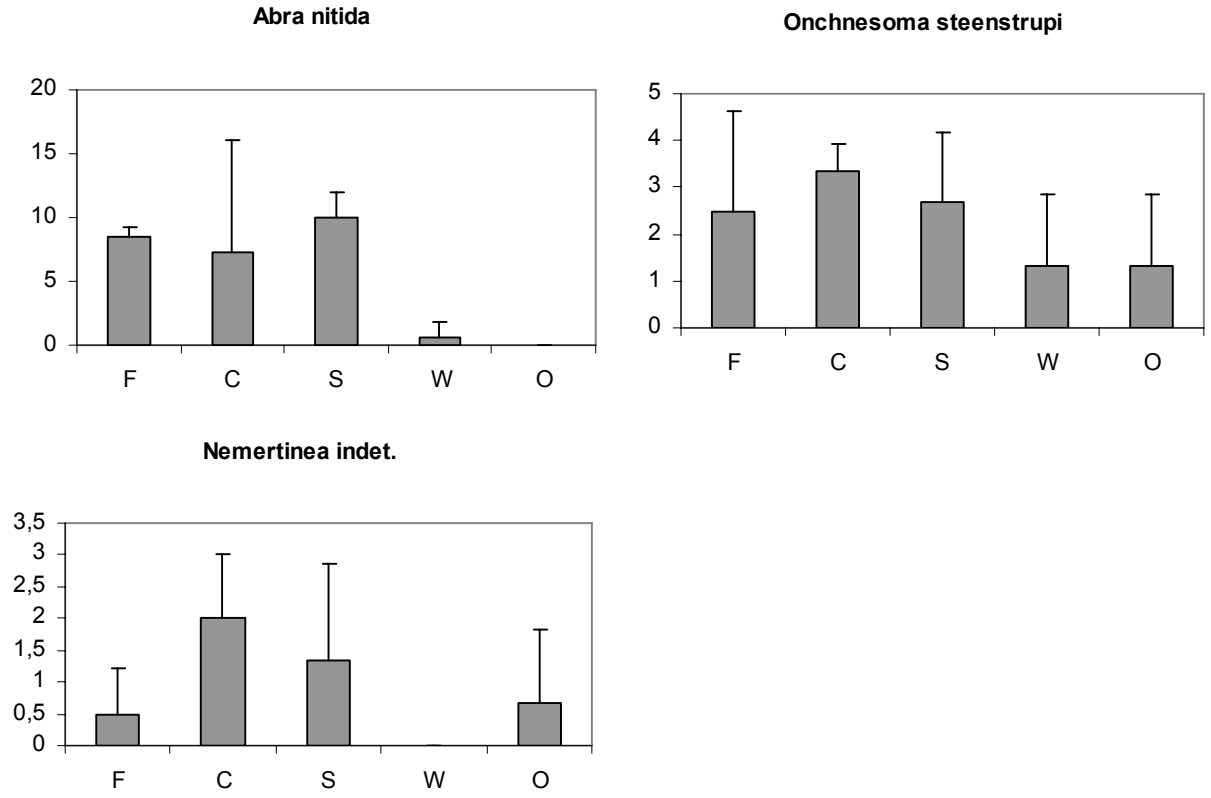


Figure 13. Average abundance of the bivalve *Abra nitida*, the sipunculid *Onchnesoma steenstrupi* and Nemertinea indet. in control (C) and treatments (S, W, O), with one standard deviation.

Although the various treatments did not have any overall effect on the faunal composition, with exception of a few taxa, it was investigated to which extent the communities were influenced by the oxygen conditions in the sediment, independent of treatments. The diversity (H') was used as an indicator of the state of the faunal communities. First, a regression analysis was performed to investigate whether there was a correlation between cumulative sediment oxygen consumption and the diversity. This relationship was not significant (see Appendix C). Next, the relationship between the oxycline thickness, which was measured three times throughout the experiment (chapter 3.2) and the diversity measured at the end was investigated, again by a regression analysis. This analysis showed that there was no correlation between the thickness of the oxycline and the diversity at neither point in time (see Appendix C). Thus although the thickness of the oxycline gradually became more narrow throughout the experiment, this did not seem to have any effect on the fauna.

3.5.2 Biomass

The biomass of each box is given in **Figure 14**. The total biomass ranged from 5 g (C2) to 53 g (O2), and there was large variation between boxes independently of treatments. It is evident that the sea urchin *Brissopsis lyrifera* made up a large part of the biomass where it was present. In order to get a more detailed picture of the biomass of the other groups, a separate figure is presented, where *B. lyrifera* is excluded, as well as another large animal *Myxine glutinosa,* which was only found in one box. However, the variation between boxes was still large, and again no effect of treatment was evident. Furthermore, there is no indication of differences between the fjord and box core samples, which indicates that the experimental conditions maintain the natural conditions very well.

It was also investigated whether the mean individual weight of selected taxa were influenced by the treatments. The sea urchin *Brissopsis lyrifera* and the anthozoa *Paraedwardsia arenaria* were weighted separately, in addition to bivalves, see **Figure 15**. Again there were large variations in weight independent of treatment, and whether taken from the fjord or cores.

3.6 Discussion, fauna

The fjord samples and the control samples contained approximately the same number of species and individuals. Furthermore, in the multivariate analyses the fjord samples did not form a separate group, but were quite well mixed with the other samples. This result means that the core communities appear to have tolerated the experimental conditions very well. In a similar experiment carried out by Schaanning et al (2003), field reference samples contained more species and more individuals than the experimental control samples at the end of the experiment. In that experiment, however, the fauna was exposed to the experimental conditions for seven months, i.e. four months longer that in the present experiment.

No overall faunal effects were observed as a result of the treatments, which is evident from both the univariate and multivariate analyses on the abundance data, as well as from the biomass measurements. Thus for the duration of the present experiment, the doses of the various additions were too low to induce any effects on the composition of macrofauna. As discussed above, the olefin-cores had larger oxygen consumption than the other cores, but this was not reflected in the abundance or biomass data. However, the abundances of the bivalve *Abra nitida*, the sipunculid *Onchnesoma steenstrupi* and the nemertineas were significantly lower in the treatments with cuttings (water-based and olefins) compared to clean sediment (control and sediment), and these three taxa appear to be

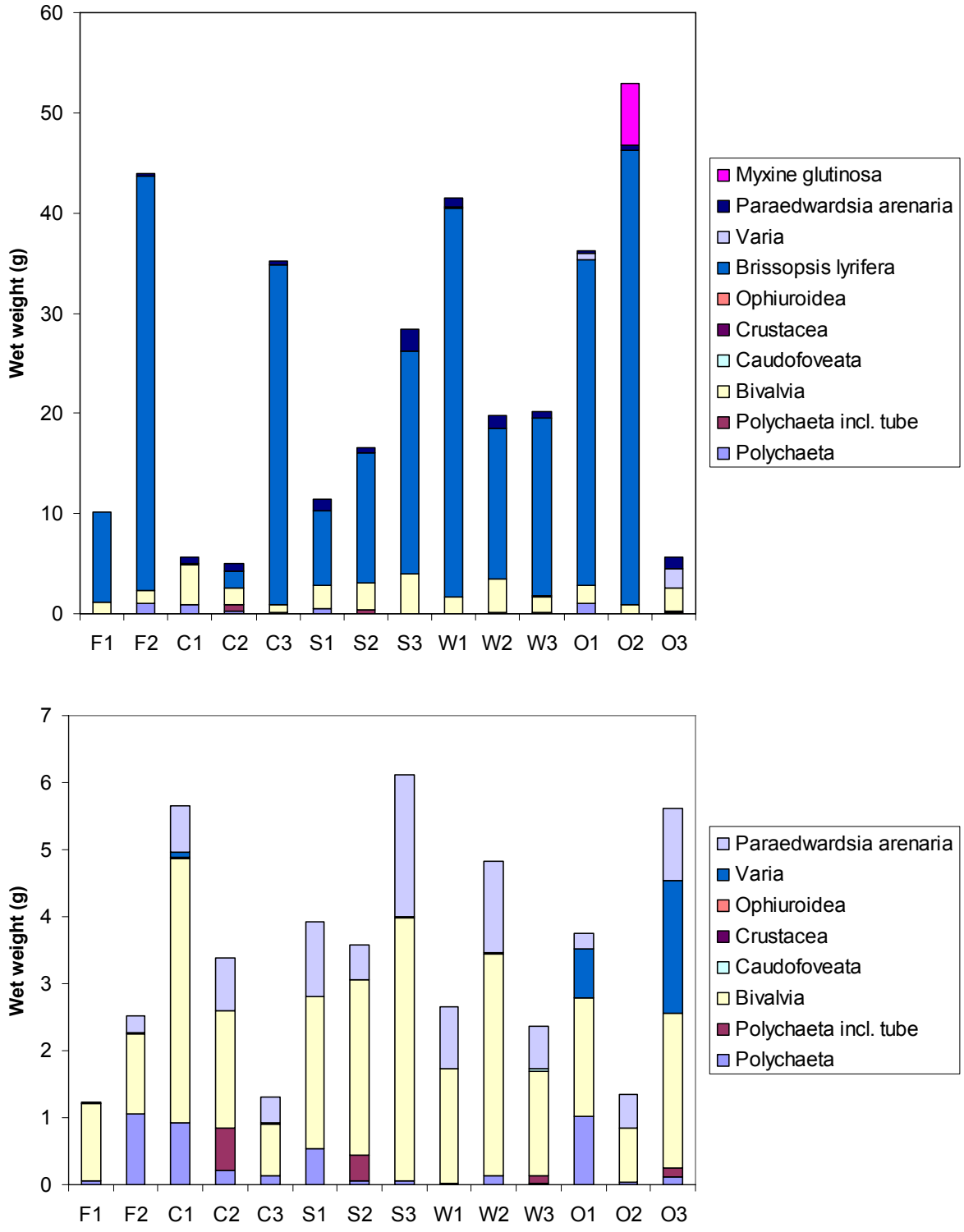


Figure 14. Biomass of selected phyla (g wet weight) for each box, including *Brissopsis lyrifera* and *Myxine glutinosa* in the upper figure and excluding these taxa in the lower figure.

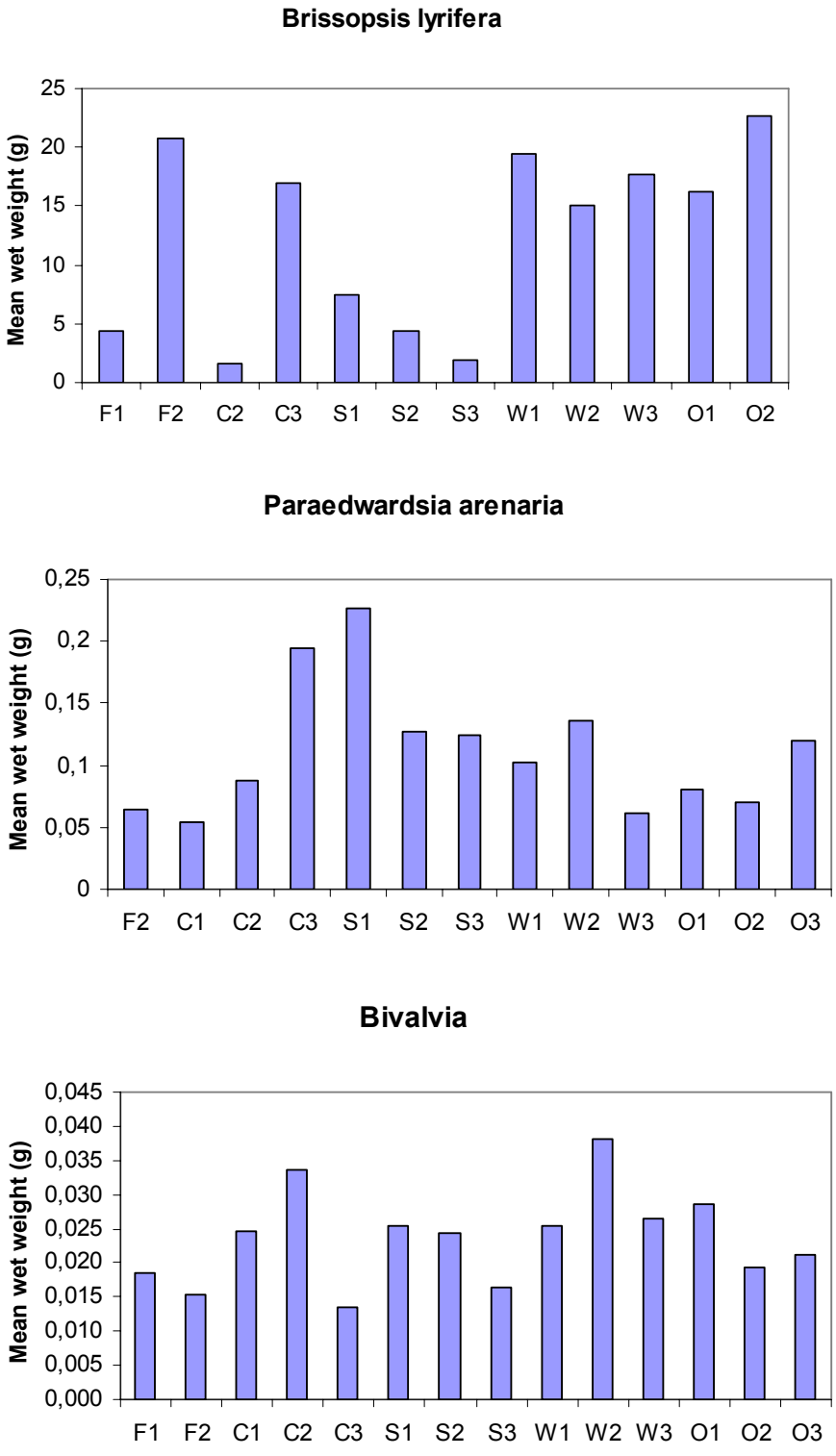


Figure 15. Mean weight of the sea urchin *Brissopsis lyrifera*, the anthozoa *Paraedwardsia arenaria* (below) for boxes where they were present.

particularly sensitive towards cuttings deposition. It is interesting to note that these taxa respond to the deposition of cuttings and not to the deposition of clean sediment. This revealed a harmful effect of some property present in the cuttings.

Toxic effects of water based muds have not previously been reported, but there are indications that physical properties such as the shape and size of particles may affect proper functioning of certain organs, through physical interactions with gill, the gastrointestinal tract and integument (Neff, 2005). Both weight materials are characterised by small grain size and high specific gravity. The properties of bore hole cuttings themselves will depend on the local mineralogy, but sharp edges of machined stone are likely to represent at least one common factor of all cuttings wether they contain ilmenite, barite or water- or olefin-based muds.

The communities were dominated by subsurface deposit feeders. Compared to other groups, this group is generally tolerant towards disturbances (e.g. Pearson and Rosenberg, 1978) such as burial (e.g. Holte and Gulliksen, 1998) and contamination (e.g. Gaston et al., 1998). Suspension feeders, on the other hand, which were not very abundant in the present study, have been shown to be more sensitive towards increased sedimentation (e.g. Hyland et al.,1994; Holte and Gulliksen, 1998). This corresponds well with the finding that the bivalve *Abra nitida* appeared to be negatively affected by cuttings deposition in the present experiment. Suspension feeders are generally more frequent at exposed habitats, where more particles may be captured from the overlying water. Future studies should directed towards communities representative of such habitats, i.e. coarser sediments, in order to investigate whether they have similar tolerance towards drill cuttings. This is particularly relevant since coarser sediments seem to be common in the vicinity of several new potential exploration sites, e.g. in the Barents Sea.

In the DREAM-model for drill cuttings the PNEC-value for burial is 6.5 mm (Smit et al., 2006). The PNEC for burial is derived on a probabilistic basis, and should *a priori* safeguard at least 95% of the benthic species present. The layer thickness in the present experiment was approximately 2-4 mm, which means that one should expect less than 5% affected species. 59 taxa were counted in the experimental cores in the present experiment (fjord samples not included). Of these, 26 taxa were only found in one box, while only three taxa were found in all twelve boxes. This makes it difficult to conclude on the proportion of species that is affected. However, even if one uses 33 species as the basis (the number of species which were recorded in two or more boxes), one ends up with more than 5% affected taxa. It is important to have in mind that the data basis is sparse, and further documentation appears to be required before conclusions are drawn on the actual risk. The experiments that will be performed within the PEIOFF project are specially designed to investigate effects of various layer thicknesses of cuttings and clean sediment, and will provide important information on the PNEC for burial. Also data from another ongoing experiment at Solbergstrand on metal mobility from various water-based cuttings and weight minerals may contribute further to a better understanding of the impacts of water based cuttings on benthic communities.

4. Conclusions

A seasonal decrease of oxygen at 50-60 m depth in the fjord water was transferred to the mesocosm, causing gradually decreased concentration of O_2 in the overlying water and decreased thickness of the oxic layer in the sediment in all treatments.

Biodegradation of olefins resulted in a further reduction of the thickness of the oxic layer and increased consumption of oxygen from the overlying water in the boxes treated with olefin-based cuttings.

Unlike a previously reported pilot experiment, the water-based cuttings provided no increase of O_2 consumption. This indicated that an easily degradable organic phase, probably glycol, had been washed out during or shortly after addition of cuttings. Finely dispersed cuttings, low solid/solution ratios and thin layers on the sediment surface may have contributed to rapid dissolution of the glycol phase in the present experiment.

The macrobenthic communities in the box core samples showed a large similarity with the zero samples taken from the fjord. This showed that transplantation from field to mesocosm and three months maintenance in the mesocosm had been performed without significant change in the macrobenthic community structure.

Neither cuttings nor clean sediment addition had significant effects on the overall composition or biomass of macrofauna in the box cores. Despite of this 3 of the taxa (*Abra nitida*, *Onchnesoma steenstrupi* and Nemertinea indet.) showed significantly reduced abundances ($p<0.05$) in sediments treated with cuttings compared with untreated mesocosm control and sediments treated with clean sediment particles. The effect on these taxa occurred independently on whether the mud was water- or olefin-based and whether it was made up from ilmenite or barite.

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Anders Bjørgesæter, PhD-student, University of Oslo.

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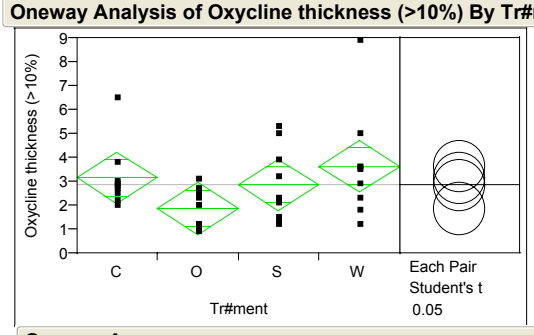
Appendix A.

Statistical analyses (JMP statistical software) on the variation of the oxic layer with treatment (left-hand side) and time (right-hand side).

Fit Y by X Group

Oneway Analysis of Oxycline thickness (>10%) By Tr#ment



Oneway Anova

Summary of Fit

Rsquare	0.156134
Adj Rsquare	0.077021
Root Mean Square Error	1.590794
Mean of Response	2.886111
Observations (or Sum Wgts)	36

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Tr#ment	3	14.983056	4.99435	1.9736	0.1377
Error	32	80.980000	2.53063		
C. Total	35	95.963056			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C	9	3.15556	0.53026	2.0754	4.2357
O	9	1.87778	0.53026	0.7977	2.9579
S	9	2.86667	0.53026	1.7866	3.9468
W	9	3.64444	0.53026	2.5643	4.7246

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]

	W	C	S	O
W	0.0000	0.4889	0.7778	1.7667
C	-0.4889	0.0000	0.2889	1.2778
S	-0.7778	-0.2889	0.0000	0.9889
O	-1.7667	-1.2778	-0.9889	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

t	Alpha
2.03693	0.05

Abs(Dif)-LSD

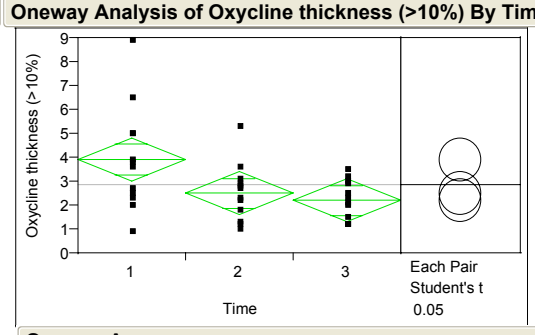
	W	C	S	O
W	-1.5275	-1.0386	-0.7497	0.2392
C	-1.0386	-1.5275	-1.2386	-0.2497
S	-0.7497	-1.2386	-1.5275	-0.5386
O	0.2392	-0.2497	-0.5386	-1.5275

Positive values show pairs of means that are significantly different.

Level		Mean
W	A	3.6444444
C	A B	3.1555556
S	A B	2.8666667
O	B	1.8777778

Levels not connected by same letter are significantly different

Oneway Analysis of Oxycline thickness (>10%) By Time



Oneway Anova

Summary of Fit

Rsquare	0.208072
Adj Rsquare	0.160076
Root Mean Square Error	1.517532
Mean of Response	2.886111
Observations (or Sum Wgts)	36

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time	2	19.967222	9.98361	4.3352	0.0213
Error	33	75.995833	2.30290		
C. Total	35	95.963056			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1	12	3.92500	0.43807	3.0337	4.8163
2	12	2.51667	0.43807	1.6254	3.4079
3	12	2.21667	0.43807	1.3254	3.1079

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]

	1	2	3
1	0.0000	1.4083	1.7083
2	-1.4083	0.0000	0.3000
3	-1.7083	-0.3000	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

t	Alpha
2.03452	0.05

Abs(Dif)-LSD

	1	2	3
1	-1.2604	0.1479	0.4479
2	0.1479	-1.2604	-0.9604
3	0.4479	-0.9604	-1.2604

Positive values show pairs of means that are significantly different.

Level		Mean
1	A	3.9250000
2	B	2.5166667
3	B	2.2166667

Levels not connected by same letter are significantly different

Appendix B.

Species composition of boxes and fjord samples:

	C1	C2	C3	F1	F2	O1	O2	O3	S1	S2	S3	W1	W2	W3
Anthozoa indet	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Cerianthus lloydi	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Paraedwardsia arenaria	13	9	2	0	4	3	7	9	5	4	17	9	10	10
Nemertinea indet	3	1	2	0	1	0	0	2	3	0	1	0	0	0
Nematoda indet	1	0	0	0	0	0	0	1	0	0	0	0	0	0
Paramphinome jeffreysii	4	0	0	0	0	0	1	1	0	1	0	0	2	2
Neoleanira tetragona	6	3	0	0	3	3	0	0	2	0	0	0	0	0
Pholoe assimilis	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Nereimyra punctata	0	0	4	0	0	3	1	2	0	0	0	1	0	0
Ceratocephale loveni	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Onuphis fiordica	0	2	0	0	0	0	0	1	1	0	0	1	0	0
Onuphis quadricuspis	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Lumbrineris sp	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Levinsenia gracilis	1	0	0	2	1	0	0	2	0	0	0	0	0	0
Paradoneis eliasoni	2	0	0	0	0	1	0	1	2	0	1	0	0	1
Prionospio cirrifera	1	0	0	0	1	0	0	0	1	1	0	0	0	0
Raricirrus beryli	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Caulleriella serrata	0	1	0	1	0	0	0	1	0	0	0	0	0	0
Cirratulidae indet	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Ophelina norvegica	0	0	0	1	2	0	0	0	0	0	0	0	0	0
Heteromastus filiformis	7	3	0	4	1	0	0	1	3	1	5	2	0	2
Rhodine loveni	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Myriochele oculata	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Melinna cristata	6	1	1	0	6	0	1	1	1	1	1	3	5	1
Mugga wahrbergi	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Sosanopsis wireni	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Terebellides stroemi	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Euchone papillosa	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Sabellidae indet	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Oligochaeta indet	4	0	0	0	0	0	0	0	0	0	0	1	0	2
Philine scabra	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Caudofoveata indet	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Bivalvia indet	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Nucula sulcata	0	0	1	0	1	0	2	5	1	1	1	0	0	3
Nucula tumidula	52	29	14	26	27	34	4	36	26	57	97	31	45	32
Nuculoma tenuis	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Nuculana minuta	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Yoldiella lucida	5	0	0	5	7	0	0	0	2	0	0	3	0	0
Yoldiella tomlini	6	0	2	3	0	0	0	0	0	1	0	0	0	1
Delectopecten vitreus	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Thyasira equalis	39	13	28	10	26	18	23	45	25	26	92	25	24	18
Thyasira ferruginea	10	0	0	5	2	1	2	0	4	7	19	1	6	0
Thyasira obsoleta	1	0	1	0	2	0	1	1	0	3	8	2	0	0
Thyasira pygmaea	6	5	2	1	3	4	2	13	4	1	3	0	7	0

Montacuta tenella	1	0	2	0	0	4	8	0	3	0	6	5	1	4
Astarte montagui	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Parvicardium minimum	9	1	0	1	0	0	0	0	2	0	0	0	0	0
Abra nitida	17	5	0	8	9	0	0	0	8	10	12	0	2	0
Arctica islandica	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Kelliella miliaris	14	0	6	1	1	1	0	7	11	2	0	0	2	1
Cuspidaria obesa	1	0	0	2	0	0	0	0	0	0	0	0	0	0
Tropidomya abbreviata	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Philomedes lilljeborgi	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Ianira maculosa	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Eriopisa elongata	1	0	1	1	0	0	0	0	0	1	1	5	1	4
Bathymedon saussurei	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Onchnesoma steenstrupi	4	3	3	4	1	2	0	1	1	3	4	1	3	0
Sipunculida indet	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Ophiuroidea indet	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ophiura sp	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Brissopsis lyrifera	0	1	2	2	2	2	2	0	1	3	12	2	1	1
Echinocardium cordatum	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Myxine glutinosa	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Appendix C.

ANOVA, univariate parameters (excel)

No. species
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	64,66667	3	21,55556	0,556272	0,658409	4,066181
Within Groups	310	8	38,75			
Total	374,6667	11				

No. ind.
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12248,25	3	4082,75	0,904597	0,480355	4,066181
Within Groups	36106,67	8	4513,333			
Total	48354,92	11				

H’
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,451	3	0,150333	0,991645	0,44439	4,066181
Within Groups	1,2128	8	0,1516			
Total	1,6638	11				

ES50
ANOVA

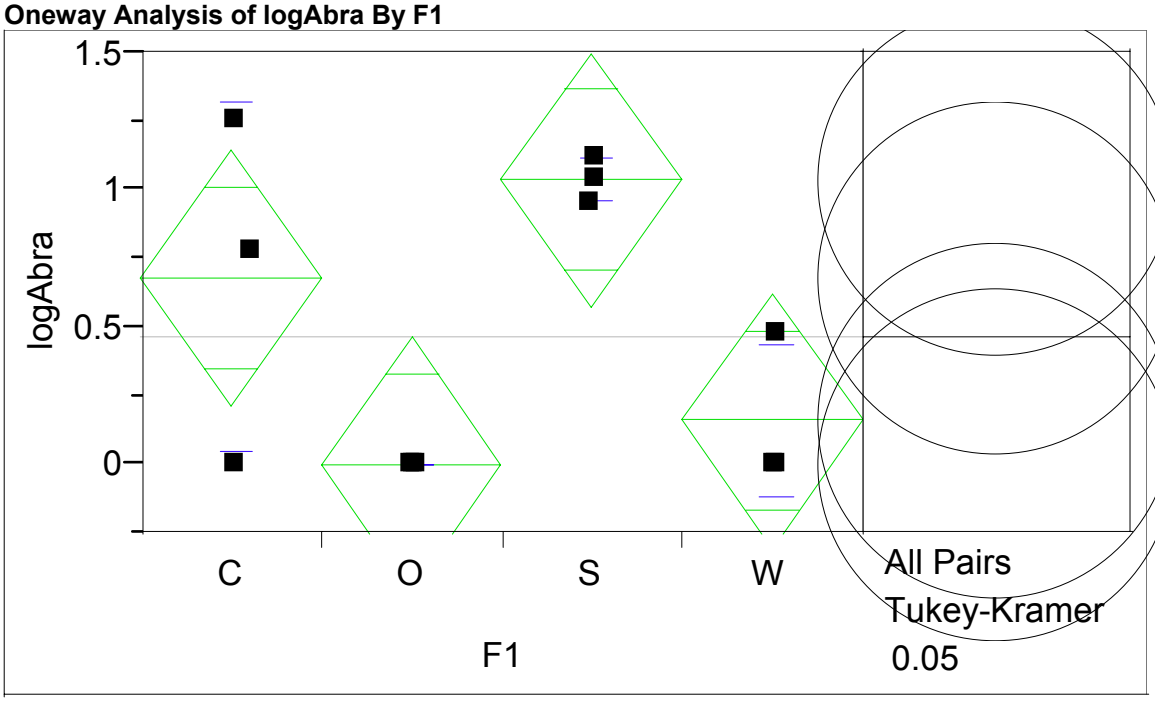
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17,66667	3	5,888889	1,177778	0,377235	4,066181
Within Groups	40	8	5			
Total	57,66667	11				

J’
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,451	3	0,150333	0,991645	0,44439	4,066181
Within Groups	1,2128	8	0,1516			
Total	1,6638	11				

Statistical analyses on the number of individuals of selected species in each treatment (JMP statistical software).
F1 = C vs O vs S vs W.
Column 2 = C&S vs O&W.

Oneway Analysis of logAbra By F1



Oneway Anova
Summary of Fit

Rsquare	0.678854
Adj Rsquare	0.558424
Root Mean Square Error	0.347762
Mean of Response	0.468344
Observations (or Sum Wgts)	12

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
F1	3	2.0451628	0.681721	5.6369	0.0226
Error	8	0.9675085	0.120939		
C. Total	11	3.0126713			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C	3	0.67781	0.20078	0.2148	1.1408
O	3	0.00000	0.20078	-0.4630	0.4630
S	3	1.03653	0.20078	0.5735	1.4995
W	3	0.15904	0.20078	-0.3040	0.6220

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	S	C	W	O
S	0.0000	0.3587	0.8775	1.0365
C	-0.3587	0.0000	0.5188	0.6778
W	-0.8775	-0.5188	0.0000	0.1590
O	-1.0365	-0.6778	-0.1590	0.0000

Alpha=
0.05

Comparisons for all pairs using Tukey-Kramer HSD

q* Alpha

q*	Alpha
3.20238	0.05

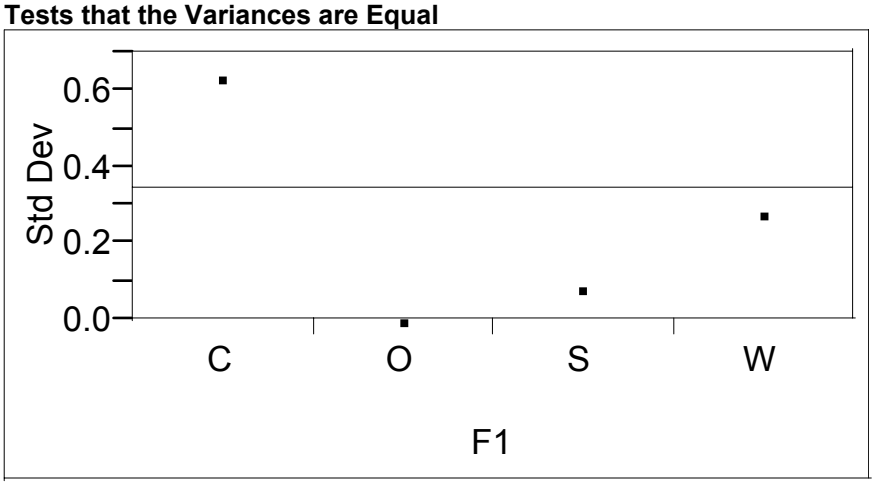
Abs(Dif)-LSD	S	C	W	O
S	-0.90930	-0.55059	-0.03182	0.12722
C	-0.55059	-0.90930	-0.39054	-0.23150
W	-0.03182	-0.39054	-0.90930	-0.75026
O	0.12722	-0.23150	-0.75026	-0.90930

Positive values show pairs of means that are significantly different.

Level			Mean
S	A		1.0365262
C	A	B	0.6778079
W	A	B	0.1590404
O		B	0.0000000

Levels not connected by same letter are significantly different

Tests that the Variances are Equal



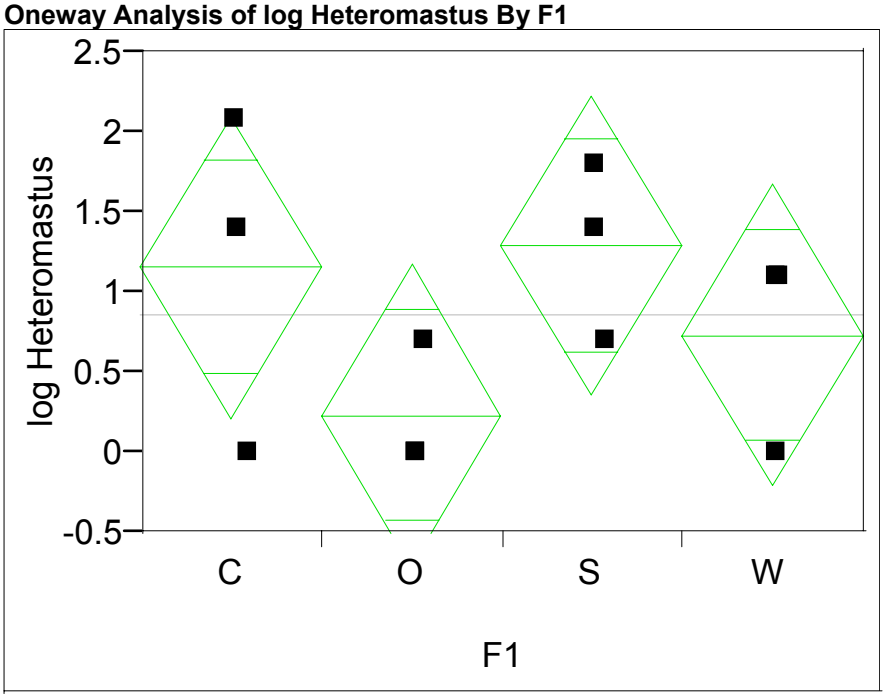
Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
C	3	0.6336236	0.4518719	0.5774646
O	3	0.0000000	0.0000000	0.0000000
S	3	0.0799616	0.0548558	0.0774172
W	3	0.2754661	0.2120539	0.1590404

Test	F Ratio	DFNum	DFDen	Prob > F
O'Brien[.5]	1.5399	3	8	0.2776
Brown-Forsythe	7.5033	3	8	0.0103
Levene	4.6633	3	8	0.0363
Bartlett	.	3	.	0.0000

Warning: Small sample sizes. Use Caution.
Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
.	3	.	.

Oneway Analysis of log Heteromastus By F1



Oneway Anova
Summary of Fit

Rsquare	0.339954
Adj Rsquare	0.092436
Root Mean Square Error	0.705719
Mean of Response	0.852276
Observations (or Sum Wgts)	12

Analysis of Variance

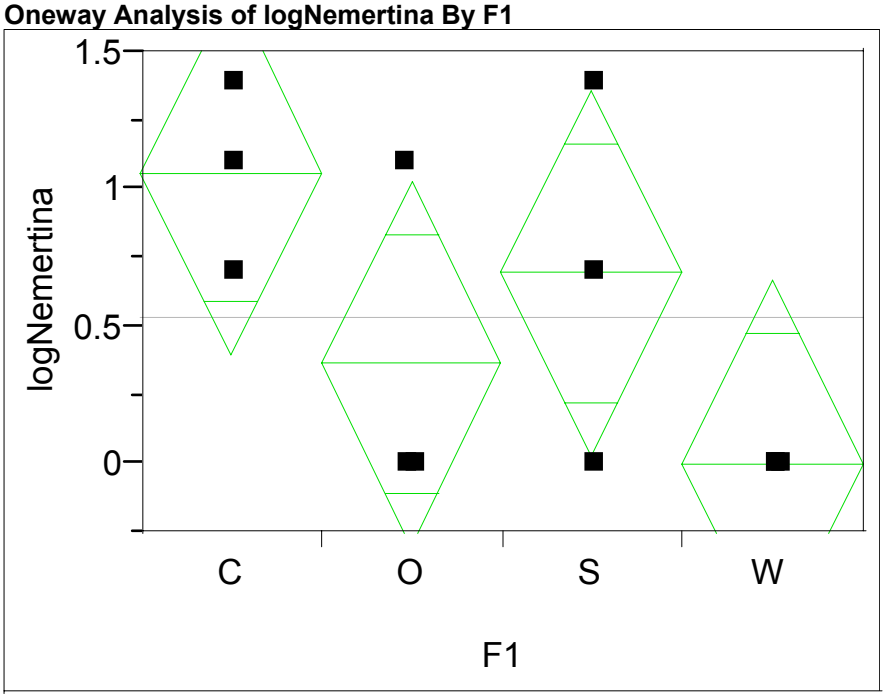
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
F1	3	2.0521036	0.684035	1.3735	0.3190
Error	8	3.9843167	0.498040		
C. Total	11	6.0364203			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C	3	1.15525	0.40745	0.2157	2.0948
O	3	0.23105	0.40745	-0.7085	1.1706
S	3	1.29040	0.40745	0.3508	2.2300
W	3	0.73241	0.40745	-0.2072	1.6720

Std Error uses a pooled estimate of error variance

Oneway Analysis of logNemertina By F1



Oneway Anova
Summary of Fit

Rsquare	0.478659
Adj Rsquare	0.283156
Root Mean Square Error	0.501009
Mean of Response	0.529676
Observations (or Sum Wgts)	12

Analysis of Variance

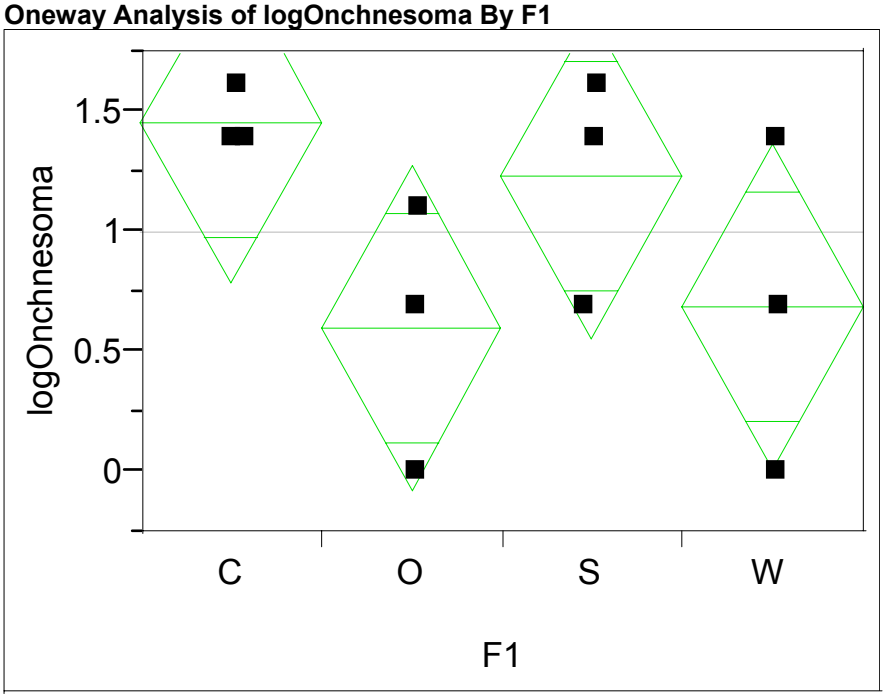
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
F1	3	1.8436754	0.614558	2.4483	0.1385
Error	8	2.0080773	0.251010		
C. Total	11	3.8517527			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C	3	1.05935	0.28926	0.3923	1.7264
O	3	0.36620	0.28926	-0.3008	1.0332
S	3	0.69315	0.28926	0.0261	1.3602
W	3	0.00000	0.28926	-0.6670	0.6670

Std Error uses a pooled estimate of error variance

Oneway Analysis of logOnchnesoma By F1



Oneway Anova
Summary of Fit

Rsquare	0.430566
Adj Rsquare	0.217028
Root Mean Square Error	0.508427
Mean of Response	0.995176
Observations (or Sum Wgts)	12

Analysis of Variance

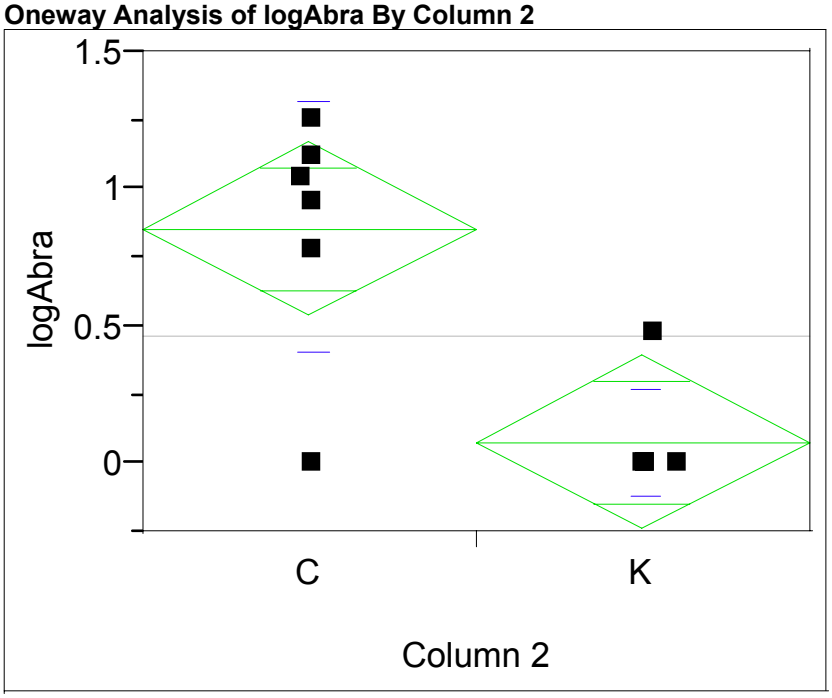
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
F1	3	1.5636626	0.521221	2.0163	0.1903
Error	8	2.0679810	0.258498		
C. Total	11	3.6316435			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C	3	1.46068	0.29354	0.7838	2.1376
O	3	0.59725	0.29354	-0.0797	1.2742
S	3	1.22963	0.29354	0.5527	1.9065
W	3	0.69315	0.29354	0.0162	1.3701

Std Error uses a pooled estimate of error variance

Oneway Analysis of logAbra By Column 2



Oneway Anova
Summary of Fit

Rsquare	0.602191
Adj Rsquare	0.56241
Root Mean Square Error	0.346189
Mean of Response	0.468344
Observations (or Sum Wgts)	12

t Test

Assuming equal variances

	Difference	t Test	DF	Prob > |t|
Estimate	0.777647	3.891	10	0.0030
Std Error	0.199872			
Lower 95%	0.332304			
Upper 95%	1.222990			

UnEqual Variances

	Difference	t Test	DF	Prob > |t|
Estimate	0.77765	3.891	6.81632	0.0063
Std Error	0.19987			
Lower 95%	0.30243			
Upper 95%	1.25286			

Analysis of Variance

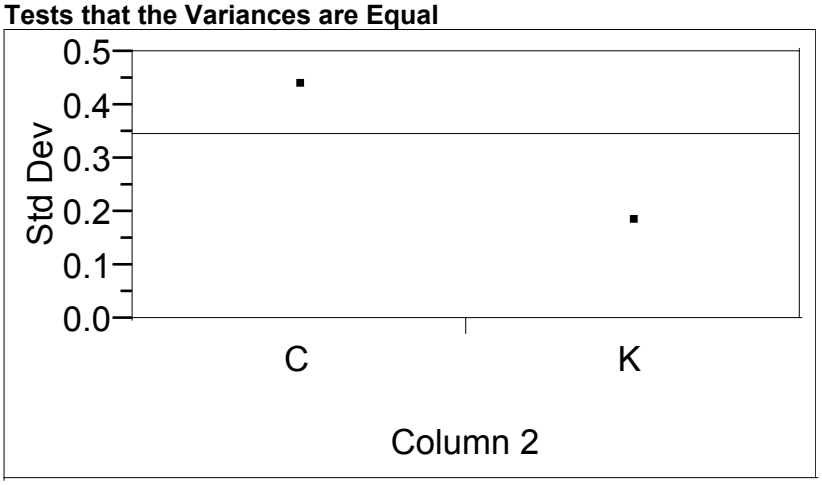
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 2	1	1.8142038	1.81420	15.1377	0.0030
Error	10	1.1984675	0.11985		
C. Total	11	3.0126713			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C	6	0.857167	0.14133	0.5423	1.1721
K	6	0.079520	0.14133	-0.2354	0.3944

Std Error uses a pooled estimate of error variance

Tests that the Variances are Equal



Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
C	6	0.4491689	0.3120610	0.2797025
K	6	0.1947839	0.1325337	0.0795202

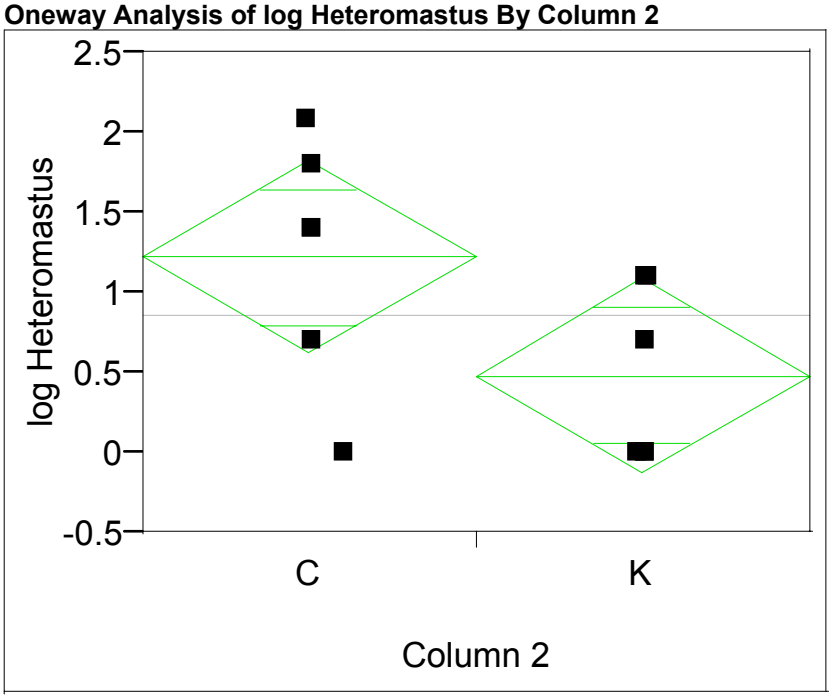
Test	F Ratio	DFNum	DFDen	Prob > F
O'Brien[.5]	1.0513	1	10	0.3294
Brown-Forsythe	1.4181	1	10	0.2612
Levene	1.9004	1	10	0.1981
Bartlett	2.8607	1	.	0.0908

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
15.1377	1	6.8163	0.0063

t Test
3.8907

Oneway Analysis of log Heteromastus By Column 2



Oneway Anova
Summary of Fit

Rsquare	0.272953
Adj Rsquare	0.200249
Root Mean Square Error	0.662477
Mean of Response	0.852276
Observations (or Sum Wgts)	12

t Test

Assuming equal variances

	Difference	t Test	DF	Prob > |t|
Estimate	0.741094	1.938	10	0.0814
Std Error	0.382481			
Lower 95%	-0.11113			
Upper 95%	1.593316			

UnEqual Variances

	Difference	t Test	DF	Prob > |t|
Estimate	0.7411	1.938	9.09453	0.0843
Std Error	0.3825			
Lower 95%	-0.1228			
Upper 95%	1.6050			

Analysis of Variance

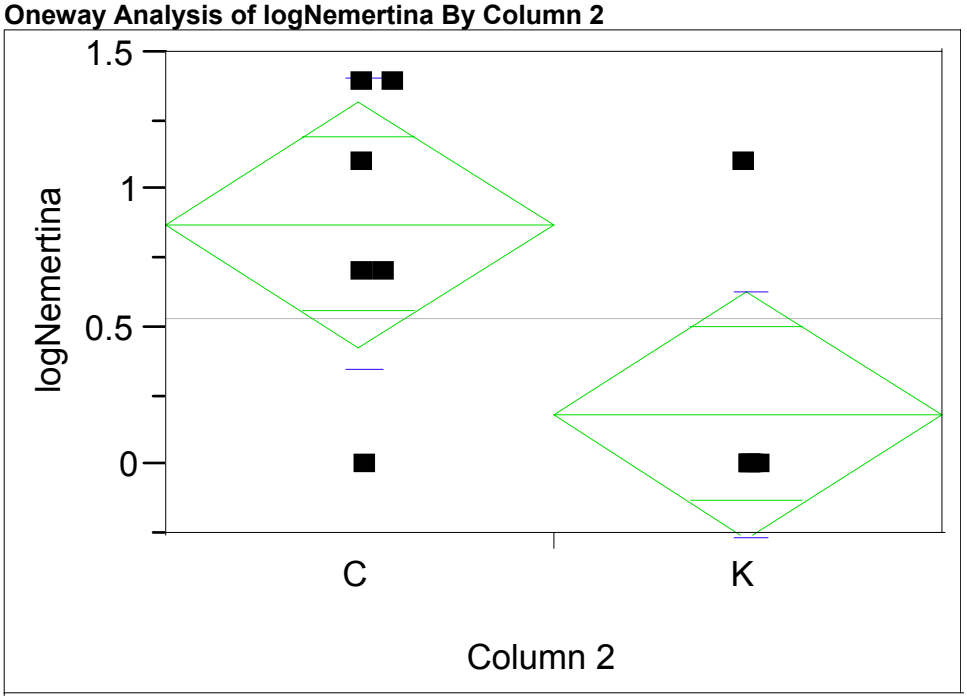
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 2	1	1.6476618	1.64766	3.7543	0.0814
Error	10	4.3887585	0.43888		
C. Total	11	6.0364203			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C	6	1.22282	0.27046	0.6202	1.8254
K	6	0.48173	0.27046	-0.1209	1.0843

Std Error uses a pooled estimate of error variance

Oneway Analysis of logNemertina By Column 2



Oneway Anova
Summary of Fit

Rsquare	0.374209
Adj Rsquare	0.311629
Root Mean Square Error	0.490958
Mean of Response	0.529676
Observations (or Sum Wgts)	12

t Test

Assuming equal variances

	Difference	t Test	DF	Prob > |t|
Estimate	0.693147	2.445	10	0.0345
Std Error	0.283455			
Lower 95%	0.061571			
Upper 95%	1.324723			

UnEqual Variances

	Difference	t Test	DF	Prob > |t|
Estimate	0.69315	2.445	9.73354	0.0351

	Difference	t Test	DF	Prob > |t|
Std Error	0.28345			
Lower 95%	0.05922			
Upper 95%	1.32707			

Analysis of Variance

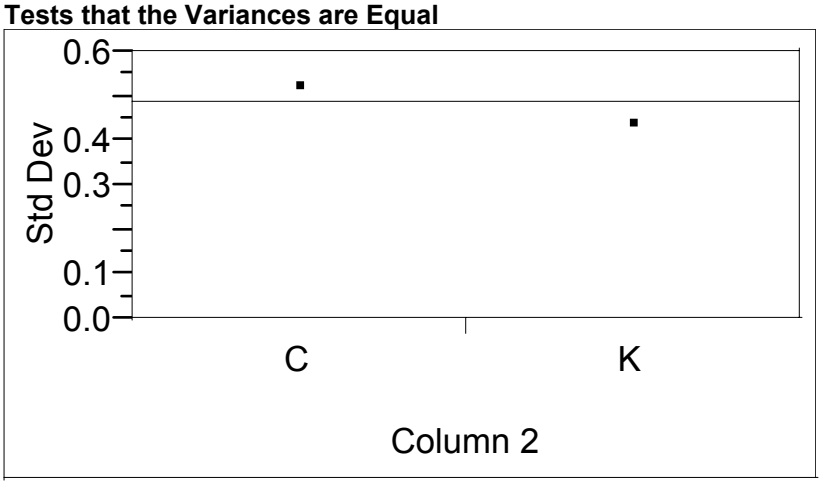
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 2	1	1.4413590	1.44136	5.9798	0.0345
Error	10	2.4103936	0.24104		
C. Total	11	3.8517527			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C	6	0.876249	0.20043	0.4297	1.3228
K	6	0.183102	0.20043	-0.2635	0.6297

Std Error uses a pooled estimate of error variance

Tests that the Variances are Equal



Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
C	6	0.5300194	0.4141511	0.4141511
K	6	0.4485066	0.3051701	0.1831020

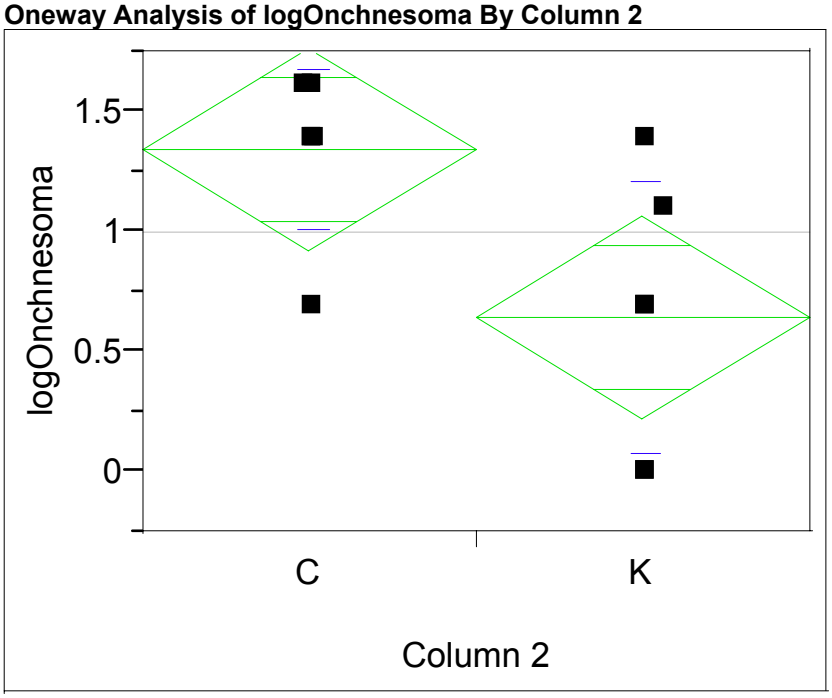
Test	F Ratio	DFNum	DFDen	Prob > F
O'Brien[.5]	0.1114	1	10	0.7455
Brown-Forsythe	1.1575	1	10	0.3073
Levene	0.4332	1	10	0.5253
Bartlett	0.1262	1	.	0.7224

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
5.9798	1	9.7335	0.0351

t Test
2.4454

Oneway Analysis of logOnchnesoma By Column 2



Oneway Anova
Summary of Fit

Rsquare	0.404719
Adj Rsquare	0.34519
Root Mean Square Error	0.464957
Mean of Response	0.995176
Observations (or Sum Wgts)	12

t Test

Assuming equal variances

	Difference	t Test	DF	Prob > |t|
Estimate	0.699951	2.607	10	0.0262
Std Error	0.268443			
Lower 95%	0.101822			
Upper 95%	1.298079			

UnEqual Variances

	Difference	t Test	DF	Prob > |t|
Estimate	0.69995	2.607	8.17312	0.0307
Std Error	0.26844			
Lower 95%	0.08319			
Upper 95%	1.31671			

Analysis of Variance

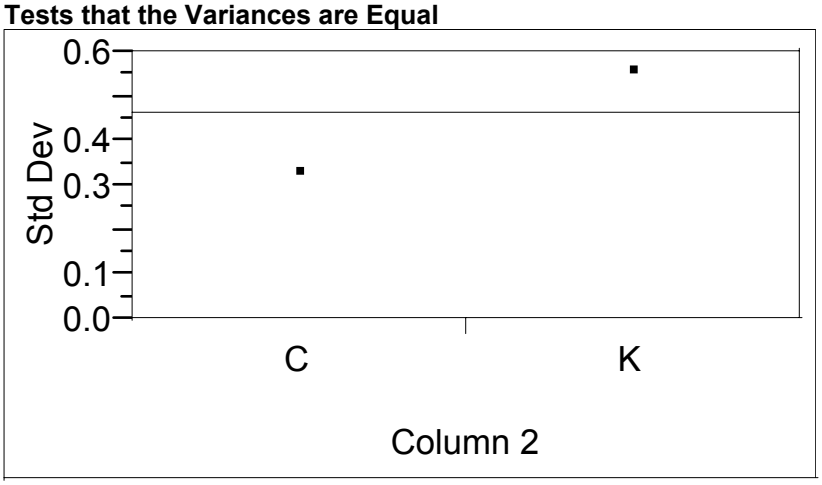
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 2	1	1.4697936	1.46979	6.7988	0.0262
Error	10	2.1618500	0.21618		
C. Total	11	3.6316435			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
C	6	1.34515	0.18982	0.92221	1.7681
K	6	0.64520	0.18982	0.22226	1.0681

Std Error uses a pooled estimate of error variance

Tests that the Variances are Equal



Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
C	6	0.3376040	0.2173346	0.1899057
K	6	0.5642637	0.4301334	0.4141511

Test	F Ratio	DFNum	DFDen	Prob > F
O'Brien[.5]	1.7006	1	10	0.2214
Brown-Forsythe	1.6044	1	10	0.2340
Levene	1.7681	1	10	0.2132
Bartlett	1.1499	1	.	0.2836

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
6.7988	1	8.1731	0.0307

t Test
2.6074

Results from multivariate statistics (software package PRIMER)

Similarity

Create triangular similarity/distance matrix

Worksheet

```
File: K:\Prosjekter\Sjøvann\O-25213-WABASCU\statistikk\splist.xls
Sample selection: All
Variable selection: All
```

Parameters

```
Analyse between: Samples
Similarity measure: Bray Curtis
Standardise: No
Transform: Square root
```

Outputs

```
Worksheet: Sheet2
```

CLUSTER

Hierarchical Cluster analysis

Similarity Matrix

```
File: Sheet2
Data type: Similarities
Sample selection: All
```

Parameters

```
Cluster mode: Group average
Use data ranks: No
```

Samples

```
 1  C1
 2  C2
 3  C3
 4  F1
 5  F2
 6  O1
 7  O2
 8  O3
 9  S1
10  S2
11  S3
12  W1
13  W2
14  W3
```

Combining

```
10+13 -> 15 at 76,31
5+9 -> 16 at 71,5
12+14 -> 17 at 68,25
11+15 -> 18 at 66,15
2+16 -> 19 at 64,04
3+7 -> 20 at 63,23
18+19 -> 21 at 61,4
```

6+20 -> 22 at 59,64
1+21 -> 23 at 59,14
17+22 -> 24 at 58,57
4+23 -> 25 at 55,94
24+25 -> 26 at 53,02
8+26 -> 27 at 50,42

Outputs

Plot: Plot1

MDS

Non-metric Multi-Dimensional Scaling

Similarity Matrix

File: Sheet2
Data type: Similarities
Sample selection: All

Best 3-d configuration (Stress: 0,08)

Sample	1	2	3
C1	-1,08	0,47	-0,58
C2	-0,62	0,12	0,72
C3	0,73	-0,49	0,29
F1	-1,26	-0,79	0,14
F2	-0,45	-0,37	0,15
O1	0,63	-0,10	0,84
O2	1,14	-0,66	-0,29
O3	-0,10	1,27	0,25
S1	-0,49	0,15	0,21
S2	-0,12	-0,33	-0,32
S3	-0,14	-0,15	-0,89
W1	0,63	0,43	-0,36
W2	0,14	-0,05	-0,02
W3	0,98	0,48	-0,13

Best 2-d configuration (Stress: 0,15)

Sample	1	2
C1	-1,07	0,72
C2	-0,71	-0,58
C3	0,81	-0,25
F1	-1,57	-0,04
F2	-0,44	-0,02
O1	0,75	-0,63
O2	1,44	0,08
O3	-0,11	-1,42
S1	-0,39	-0,24
S2	-0,14	0,39
S3	-0,24	0,86
W1	0,64	0,44
W2	0,12	0,09
W3	0,92	0,61

STRESS VALUES

Repeat	3D	2D
1	0,08	0,15
2	0,09	0,15
3	0,09	0,16
4	0,08	0,16
5	0,09	0,15
6	0,12	0,18
7	0,08	0,16

```
     8  0,09       0,17
     9  0,09       0,16
    10  0,11       0,17
    11  0,12       0,19
    12  0,09       0,16
    13  0,11       0,15
    14  0,09 **    0,15
    15  0,08       0,16
    16  0,11       0,16
    17  0,09       0,16
    18  0,09       0,16
    19  0,11       0,18
    20  0,11       0,16

** = Maximum number of iterations used

3-d : Minimum stress: 0,08 occurred 4 times
2-d : Minimum stress: 0,15 occurred 5 times

Outputs

Plot: Plot2
```

ANOSIM

Analysis of Similarities

One-way Analysis

Global Test

```
Sample statistic (Global R): 0,185
Significance level of sample statistic: 8,6%
Number of permutations: 999 (Random sample from 1401400)
Number of permuted statistics greater than or equal to Global R: 85
```

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
C, F	-0,333	100,	10	10	10
C, O	-0,111	70,	10	10	7
C, S	-0,222	90,	10	10	9
C, W	0,185	20,	10	10	2
F, O	0,417	10,	10	10	1
F, S	0,25	40,	10	10	4
F, W	0,833	10,	10	10	1
O, S	0,407	10,	10	10	1
O, W	0,074	30,	10	10	3
S, W	0,667	10,	10	10	1

Regression analyses (computed in software package R)

Regression analysis sediment oxygen consumption and diversity:

```
> cSOC=c(904,1003,998,1021,877,1069,1018,903,1069,1200,1181,1157)
> plot(h~cSOC)
> abline(lm(h~cSOC))
> summary(lm(h~cSOC))

Call:
```

```
lm(formula = h ~ cSOC)

Residuals:
    Min      1Q  Median      3Q     Max
-0.45645 -0.18881 -0.08494  0.11556  0.78193

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  3.7236437  1.1620617   3.204  0.00942 **
cSOC        -0.0006809  0.0011190  -0.609  0.55640
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4005 on 10 degrees of freedom
Multiple R-Squared: 0.03571,    Adjusted R-squared: -0.06072
F-statistic: 0.3703 on 1 and 10 DF,  p-value: 0.5564
```

Regression analysis thickness of oxycline and diversity:

```
> oxy1=(6.5,2.5,3.8,5,3.9,2.3,5,8.9,3.6,2.7,2,0.9)
Error: syntax error in "oxy1=(6.5,"
> oxy1=c(6.5,2.5,3.8,5,3.9,2.3,5,8.9,3.6,2.7,2,0.9)
> plot(h~oxy1)
> abline(lm(h~oxy1))
> summary(lm(h~oxy1))

Call:
lm(formula = h ~ oxy1)

Residuals:
   Min     1Q  Median     3Q     Max
-0.5172 -0.2381 -0.1166  0.1677  0.7886

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  2.89596    0.24575  11.784 3.46e-07 ***
oxy1         0.03160    0.05522   0.572     0.58
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4014 on 10 degrees of freedom
Multiple R-Squared: 0.03172,    Adjusted R-squared: -0.06511
F-statistic: 0.3276 on 1 and 10 DF,  p-value: 0.5797

> oxy2=c(2.7,2.8,2.2,5.3,1.3,1.2,3.6,1.8,2.9,3.1,2.3,1)
> plot(h~oxy2)
> abline(lm(h~oxy2))
> summary(lm(h~oxy2))

Call:
lm(formula = h ~ oxy2)

Residuals:
   Min     1Q  Median     3Q     Max
-0.3408 -0.2188 -0.1629  0.0887  0.8415

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
```

```
(Intercept)  2.62847    0.25001   10.51 1.00e-06 ***
oxy2         0.15557    0.09044    1.72    0.116
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3583 on 10 degrees of freedom
Multiple R-Squared: 0.2283,     Adjusted R-squared: 0.1512
F-statistic: 2.959 on 1 and 10 DF,  p-value: 0.1161

> oxy3=c(2.9,3,2,3.2,1.5,2.1,3.5,2.3,1.2,2.5,1.2,1.2)
> plot(h~oxy3)
> abline(lm(h~oxy3))
> summary(lm(h~oxy3))

Call:
lm(formula = h ~ oxy3)

Residuals:
    Min       1Q   Median       3Q      Max
-0.37704 -0.30907 -0.04114  0.14296  0.73025

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   2.5667     0.3168   8.102 1.05e-05 ***
oxy3          0.2045     0.1346   1.519    0.160
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3677 on 10 degrees of freedom
Multiple R-Squared: 0.1875,     Adjusted R-squared: 0.1062
F-statistic: 2.307 on 1 and 10 DF,  p-value: 0.1598
```