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UKOOA Task 3: Colonisation and macrofaunal activity in drill cuttings material – results from small scale laboratory experiments

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Preface

This report presents the results from a laboratory experiment addressing colonisation and bioturbation of drill cuttings material. The work forms a sub-task of Task 3 of the UKOOA Drill Cuttings JIP phase II Task 3 and has been carried out as a joint project between RF and ERTSL.

The laboratory work has been carried out in RF/Akvamiljøs facilities in Stavanger. Several scientists and engineers have contributed to the work:

ERTSL: Annette Woodham (experimental and reporting), Juliana Kerr (data handling), David Runciman (management), Iain Dixon and Brian Roddie.

RF: Øyvind Tvedten (experimental and reporting), Sigfryd Torgrimsen (experimental set up and various analysis), Kjell-Birger Øysæd (experimental planning, THC-analysis), Veslemøy Eriksen (experimental work and analysis), Grete Jonsson (validation THC-analysis), Grethe Kjeilen (project management, reporting and experimental).

The *Capitella* spp specimens used for the experiment were kindly been provided by Dr. Valery Forbes, Roskilde University. She has also given valuable advice on appropriate experimental conditions and how to treat the *Capitella*. Specimens of *Abra alba* were provided by David Murden, University Marine Biological Station, Millport, Isle of Cumbrae.

Stavanger 15. January 20022

Grethe Kjeilen, project manager

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Summary

Macrofaunal colonisation (SSE)

Main goal

Since bioturbation can influence many of the characteristics of sediments, including their stability, microbial populations and contaminant degradation rates, there is a need to assess both the potential for macrofaunal colonisation of cuttings piles and the influences of bioturbation on degradation rates.

Colonisation of cuttings piles is a long-term and complex process, and the approach taken has therefore been to examine a relatively small range of variables in small-scale experiments, in order to simplify interpretation of the results. The aims were to obtain, for different concentrations and types of cuttings, data on:

1. survival of invertebrate species, their growth, burrowing behaviour and disturbance of sediment;
2. effects of invertebrate colonisation on aspects of sediment chemistry relating to degradation processes (THC, redox and pH).

Method used

Two invertebrate species were used separately in the experiments: *Capitella* sp 1 and *Abra alba*. *Capitella* sp 1 is a small, fast-growing opportunistic polychaete, tolerant of sediments polluted with mineral oils and typically among the first colonisers of cuttings piles. It has previously been shown to enhance degradation of organic contaminants in sediments, and its response to differing concentrations and types of cuttings is therefore of relevant interest. *Abra alba*, a bivalve molluscs, was used in the study to represent a larger, slower growing species, with a contrasting feeding and burrowing mode and a greater sensitivity to oily substrates than *Capitella*.

Drill cuttings material from Beryl A (OBM) and Ekofisk 2/4 A (PBM/WBM), was compared. The experiments were carried out using five treatment levels (Figure), chosen to be consistent with the degradation experiments. For each treatment level, separate jars were prepared for *Capitella* and *Abra* and for controls (four replicates of each). The following measurements and observations were made immediately after set-up (T0) and again six weeks (T1) and 14 weeks (T2) after set-up, with some intermediate readings also taken:

- Redox (duplicate) and pH profiles (using microelectrodes)
- Visual observations, including colour of sediment/cuttings and any depth variation, evidence of disturbance of surface, presence and condition of animals on sediment surface, evidence of animal activity and depth of burrows (for *Capitella*);
- *Capitella* abundance and individual lengths, at different depths in the sediment/cuttings;
- *Abra* survival and individual lengths and wet weights;
- THC concentrations

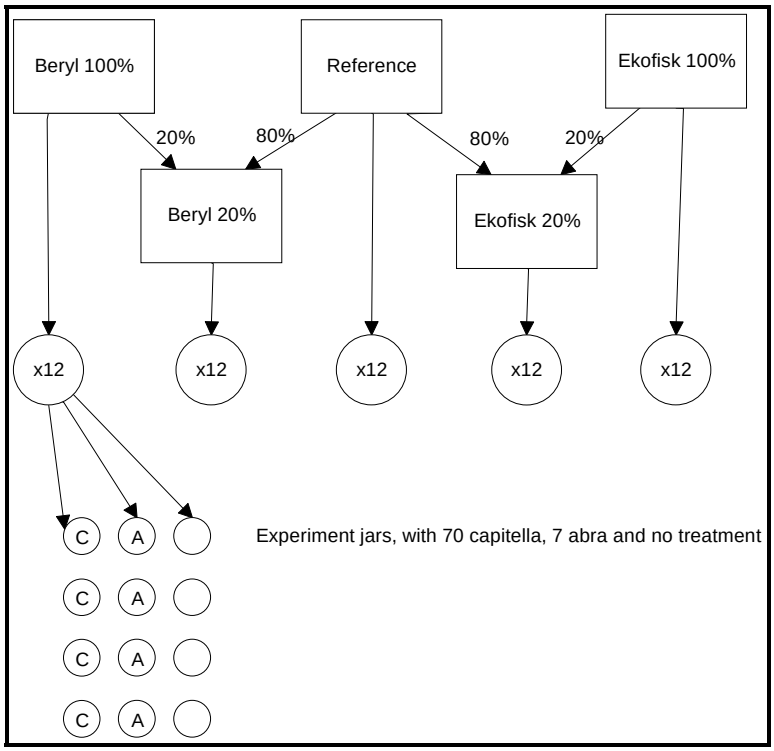

Figure Recolonisation experiment - preparation and set-up. The density of Capitella jars were approximately 8000 ind.m^{-2} (70 per jar), for Abra the density was approximately 1000 ind.m^{-2}. The jars were placed inside aquaria for each treatment level, which were maintained in the dark at 10°C with flow-through seawater. The experiment ran for a total of 14 weeks.

Results and discussion

Survival and growth

Survival of *Capitella* ranged from 37% to 69% for the different treatments after six weeks (T1), and was highest in 20% Ekofisk and lowest in 100% Ekofisk cuttings and in the reference sediment. At the end of the experimental period, survival was only significant in Ekofisk cuttings, especially 100% Ekofisk. Reasons for the death of *Capitella* in the reference sediment and Beryl cuttings could include starvation or toxicity or other unknown factors, but the changes in survival that occurred between T1

and T2 would indicate starvation to be the primary factor. Survival on Beryl cuttings would perhaps be better if an external food source were available. A decision was made not to add food to the experimental chambers, as a build-up of organic matter would affect THC and redox data and confuse the results. The results indicate that the Ekofisk cuttings provided a food source for the *Capitella*, which was probably microbial. Other published studies have demonstrated the tolerance of *Capitella* sp 1 to mineral oils including PAHs. The *Capitella* in the present experiment appeared to thrive and to be reproducing in the 100% Ekofisk cuttings. Average worm length apparently decreased during the experiment. Although this may be an artifact of the measuring process, energy-limited *Capitella* are known to use stored reserves to meet their energy needs.

Abra also showed at least moderately good survival (43% to 71%) in all of the treatments at six weeks and was highest in 20% Ekofisk cuttings and the reference sediment. At 14 weeks, survival was only significant in 20% Ekofisk cuttings (52%) and the reference sediment (62%). Survival in the reference sediment indicates that the lack of provision of food was not a significant factor; *Abra* are known to survive for some time without feeding. The individuals that survived appeared to have lost biomass. Toxicity of the cuttings material is likely to be the main influencing factor in *Abra* survival.

Burrowing behaviour and disturbance of sediment

The most notable feature at T1 was the depth distribution of *Capitella* in the substrate. For the 100% Beryl and 100% Ekofisk cuttings, almost all of the individuals were found in the top 5 mm. For both of the 20% cuttings mixtures, they were distributed mostly within the top 20-30 mm, while in the reference sediment they were distributed throughout the substrate depth, with most found in the bottom 25 mm of the jar. The surviving animals at T2 showed a similar depth distribution as they had at T1.

Abra showed little or no willingness to burrow into 100% Beryl cuttings. Some burial attempts were seen for 100% Ekofisk cuttings, but no significant disturbance of the sediment was observed. The 20% Ekofisk mixture was more favoured than the 20% Beryl mixture, and the majority of the *Abra* became established in the 20% Ekofisk cuttings.

Factors that could influence burrowing behaviour include redox potential and toxicity of the cuttings material. The depth of burrowing of *Capitella* in 100% Ekofisk coincided with the depth at which reducing conditions commenced, and more individuals were found at the bottom of the jar in the Beryl cuttings, which exhibited higher redox values.

Depths of colonisation observed in the small-scale experiments cannot be reliably extrapolated to the field because of the disturbances associated with sampling and the mixing of the cuttings during experimental set-up. Rather, they should be related to cuttings type, THC concentrations and redox potentials. Cuttings piles are not easily categorised, and they also exhibit heterogeneity over the surface of a single pile. Thorough mixing of the cuttings material was conducted prior to experiment set-up, in order to minimise heterogeneity in the experimental chambers.

Evidence of bioturbation in the jars was manifest as the presence of a pelletised layer at the surface, mixing of the surface layers and unevenness of the surface due to *Abra*

burrowing activity. These effects were noted for the Ekofisk and reference sediment jars.

Effects on sediment chemistry/degradation rates

The redox data obtained clearly distinguished the different types and concentrations of cuttings. Even at the start of the experiment, redox values were lower in the Ekofisk cuttings than in the other treatments, and within less than a week the values in the Beryl jars had also fallen to less than those in the reference sediments. After the first week, values stabilised in all of the experimental jars and no further change with time was demonstrated. No clear effects of the presence of *Abra* or *Capitella* on redox potential were demonstrated by the experiment. However, for the Ekofisk cuttings, there was some evidence that the *Capitella* jars had higher redox potential values than the *Abra* jars or the controls, which could be a result of burrowing activity, but further data would be required to demonstrate the significance of the differences.

Although leaching of THC could be expected to occur in the open flow-through system used, only limited leaching was observed in the mesocosm experiments using similar systems (SINTEF-STF66 AO1139), and only from the top few mm of the Beryl cuttings. There were no obvious trends of decreasing THC levels in the current experiment. This was not unexpected, since the degradation tests had already shown a high degree of variability in THC data and consequent difficulty in demonstrating changes in levels over short timeframes. The presence of macrofauna apparently had little impact on THC degradation and/or depletion, as no obvious differences between the *Capitella, Abra* and control jars were seen for any of the cuttings types.

Conclusions

- Survival and burrowing behaviour of both *Capitella* and *Abra* showed clear differences on the two types of cuttings examined. The Beryl cuttings material was shown to be less favourable to the animals than the Ekofisk cuttings, despite containing significantly lower THC levels. These differences were also present in diluted (20%) cuttings.

- *Capitella* appeared to thrive in 100% Ekofisk cuttings at THC levels in the region of 70,000 mg/kg, although the experiment was not long enough to allow the development of generations. Survival in Beryl cuttings and the reference sediment was initially good but had declined by the end of the experiment, apparently due to lack of food.

- Burrowing depth of *Capitella* in cuttings material was clearly limited compared to clean sediments, and showed distinct variation with both cuttings type and concentration. Burrows were mostly limited to the top 5-10 mm in the 100% cuttings and the top 20-30 mm in the 20% cuttings, although for both concentrations penetration was slightly deeper in the Beryl cuttings. Burrowing depth appeared to be related to redox potential, which in turn correlated with THC concentration.

- The more sensitive species, *Abra alba*, only survived in diluted PBM/WBM cuttings, and OBM cuttings were toxic even at THC concentrations in the region of 100 to 200 mg/kg. Information is needed for more species, but the results indicate

that there is a greater potential for the development of more stable communities on PBM/WBM cuttings piles than on OBM piles, and that THC concentrations for the former type may still be too high at present.

- Evidence of sediment disturbance related to both species was apparent in terms of surface layer mixing and alterations in surface texture, and was greater for the PBM/WBM cuttings than for the OBM cuttings.
- Although no significant effects of *Abra* or *Capitella* activity on redox potentials were demonstrated, for the Ekofisk cuttings there was evidence of enhancement related to *Capitella* presence.
- THC data were not expected to be important in demonstrating combined degradation and depletion over the short timeframe of the experiment, due to a high degree of variability in analytical results. No effects of macrofaunal presence on THC degradation were demonstrated, and detectable effects may only occur in the top few mm of cuttings material.

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List of symbols

RF	RF- Rogaland Research
SINTEF	SINTEF Applied chemistry and SINTEF
AEAT	AEA Technology Environment
ERTSL	ERT (Scotland) Ltd
CP	Cuttings pile
DNV	Det Norske Veritas
UKOOA	UK Offshore Operators' Association
OBM	Oil-based drilling mud (also used in this report to describe a cuttings pile containing OBM)
PBM	Pseudo oil-based drilling mud (also used in this report to describe a cuttings pile containing PBM)
WBM	Water-based drilling mud (also used in this report to describe a cuttings pile containing WBM)
THC	Total hydrocarbons
RPD	Redox potential discontinuity layer
PAH	Polycyclic Aromatic Hydrocarbons

1 Introduction

The UKOOA Task 3 work comprises several sub-tasks performed by various members of the project team made up of RF, SINTEF, ERTSL and AEAT. This report deals with the sub-tasks focused on colonisation and consequent bioturbation of drill cuttings material, carried out by RF and ERTSL. Details of the Task 3 project scope are presented in the Phase II Task 3 Joint report (RF-2001/220).

1.1 Background

The term colonisation is used here to refer to the establishment of a macro-invertebrate community on cuttings piles. The term re-colonisation is also sometimes used in this context, since the seabed beneath piles has previously supported invertebrates. The original seabed is buried by the cuttings and the animals inhabiting it generally only survive at the peripheries of the piles where the layer of cuttings is very thin. Some species thrive better than others in the altered environment at the edges of piles, resulting in a modified community structure compared to the surrounding unaffected seabed. Colonisation of the piles themselves may occur by a combination of gradual migration of animals from the periphery of the pile and surrounding sediment, and by settlement and survival of planktonic larvae from the water passing over the piles. Colonisation of new sediment typically involves a succession, in which opportunistic early colonisers are gradually replaced with more mature communities, although these may never be identical to those in surrounding areas.

The burrowing and feeding activities of benthic invertebrates cause various types of disturbances to the sediments they inhabit; these disturbances are known collectively as bioturbation. Bioturbation can influence many of the characteristics of sediments, including their structure, stability, chemistry, oxygen levels, nutrient regeneration rates and microbial populations (reviewed in Kjeilen *et al*, 1999). Contaminant degradation rates can be enhanced by the presence of fauna, and erosion processes can theoretically be either enhanced or reduced (Kjeilen *et al*, 1999). In sediments that are not subject to erosion and resuspension, bioturbation can be the most important mechanism for reworking sediments and releasing contaminants (reviewed in Mohanty *et al*, 1998).

The limited available information on the colonisation of cuttings indicates that presently very little bioturbatory activity is occurring in cuttings piles generally. This is because conditions in the piles limit development of the fauna and the activities of the animals that do survive there (Bakke, 1986a, 1989; Kjeilen *et al*, 1999).

In terms of predicting changes in cuttings piles characteristics, there is therefore a need for more information on the potential for macrofaunal colonisation of cuttings piles, the type of bioturbation that could be expected, and the effects of this on cuttings pile biodegradation and erosion.

1.2 Approach

Available information indicates that colonisation of cuttings piles, especially OBM piles, is a slow process, with initial colonisation taking up to a year or more and development of more mature communities taking considerably longer (Dames & Moore, 1999; Cripps *et al*, 1999, Kjeilen *et al*, 1999). The pattern of colonisation is also likely to be highly variable depending on locality and cuttings composition. It was therefore not considered appropriate to examine colonisation rates specifically during the timescale of the experimental work. However, since input is required for Task 4, this is discussed briefly in Section 4 of this report.

The challenge has been to study very long-term processes by means of short-term experiments. The approach taken has been to examine a restricted number of variables in small-scale experiments, in order to simplify interpretation of the results. Further experiments examining the effects of simulated bioturbation of cuttings material are reported in the bioturbation report (RF 2001/219).

The objectives for the present studies were to examine, for different concentrations and types of cuttings:

1. survival of invertebrate species, their growth, burrowing behaviour and disturbance of sediment;

2. effects of invertebrate colonisation on aspects of sediment chemistry relating to degradation processes (redox, pH and THC).

Two invertebrate species were used separately in the experiments: *Capitella* sp 1 and *Abra alba*.

Capitella spp are small polychaetes typically among the first (and sometimes the only) colonisers of cuttings piles, particularly oily piles, and so are highly relevant for the study. They are also often the dominant species in other areas subject to oil pollution and other forms of organic enrichment. Under favourable conditions they exhibit rapid rates of growth and reproduction. *Capitella* sp 1 is the most widespread, tolerant and opportunistic of the several sibling species of this genus (Foss & Forbes, 1997) and it can maintain high densities in sediments contaminated with polycyclic aromatic hydrocarbons (PAHs; Madsen *et al*, 1997). Despite their small size, *Capitella* have been observed to be conveyor-belt bioturbators and have been shown to enhance degradation of organic contaminants in sediments. Méndez *et al* (2001) concluded that processing by *Capitella* spp. may be important in the remediation of PAH-contaminated sediment. For the above reasons, *Capitella* were thought to be highly relevant as test organisms for the experiment. Their response to differing concentrations and types of cuttings, and the effects of their presence on aspects of cuttings chemistry, are of great interest.

Although *Abra* (bivalve molluscs) are not found on cuttings piles, they are readily-available and commonly-used test organisms. They were used in the study to represent a larger, slower growing species, with a contrasting mode of feeding and burrowing (and therefore of bioturbation) to *Capitella*. The species was also expected to exhibit greater sensitivity to oily substrates than would *Capitella*.

2 Material and methods

2.1 Cuttings samples

Drill cuttings material from two different cuttings piles was compared:

- Beryl A (OBM)
- Ekofisk 2/4 A (PBM/WBM)

The piles were sampled as part of the UKOOA Task 1 survey in September 2000. The reference sediment sample used was collected during the same survey, at a set reference location in the North Sea (Westerlund *et al*, 2001). Preparation of the material was the same as that used in the degradation studies (see RF 2001/217) and is summarised below.

The cuttings material used had been stored refrigerated in the dark since it was collected. Sub-samples used in this experiment were taken from two box-corer samples each for Beryl and Ekofisk 2/4 A (see Westerlund *et al*. 2001 for sample identification). The sub-sampled material was homogenised as well as was practically feasible by rotating it in a sealed box in a cement mixer for 30 minutes. The 20% cuttings mixtures were prepared by rotating cuttings (20% w/w) and reference sediment (80% w/w) in the cement mixer for two hours before filling the jars.

2.2 Biota

A culture of *Capitella* sp 1 was obtained from Valery Forbes at the Roskilde University, Denmark, on 19 January 2001. Prior to the start of the experiment, the animals were kept in two holding aquaria for four months to allow the population to establish and increase. The aquaria were maintained at 10°C in the dark with a seawater (34 PSU to 35 PSU) circulation system. The experiment reference sediment (after prior freezing at -80ºC) was used as the substrate, together with some of the sediment from Denmark. Acclimatisation to the experimental conditions was undertaken carefully over a period of several days. The animals were fed about twice a week with aquaculture fish food that had been pulverised and dissolved in water. At the beginning of March, one of the aquariums was moved to a room at 16ºC and the flow of seawater stopped, although aeration was continued. This was done to accelerate the regeneration time for the *Capitella*, since preliminary counting had indicated slow population growth and insufficient numbers of individuals. The *Capitella* appeared to thrive in both aquariums and formed numerous burrows to about 30 mm depth in the sediment, and faecal pellets and some projecting tubes were seen on the sediment surface. The *Capitella* used in the experiments were a mixture from the two aquariums.

Specimens of *Abra alba* were obtained from David J Murden, University Marine Biological Station, Millport, Isle of Cumbrae, Scotland. They arrived in two shipments. The first, of approximately 500 specimens, was received at RF on 8 March 2001 and the animals were maintained in a holding aquarium and gradually acclimatised to experimental conditions, as described above for *Capitella*, over a period of 24 hours.

They were also fed with aquaculture fish food. Initial mortality was high: about 400 had died by the end of March, most of them within a few days of arrival. It is suspected that many were dead before being added to the aquariums, since they had not buried themselves in the sediment, and mortality was possibly caused by the stress of shipment. Subsequent mortality, from the end of March until the experiment started in May, was low. The next shipment, of 230 individuals, arrived on 25 April. Many of these were dead on arrival, possibly due to unfavourable transport conditions, and the remainder died within one week in the aquariums. Sufficient animals survived from the first shipment and were used in the experiment.

Before setting up the experiment, a short and simple pilot experiment was carried out in February to check survival of animals in the cuttings and the effects of handling them. Two 2-litre beakers were set up with 1 to 2 cm of 100% Ekofisk cuttings and aerated seawater. Ten *Capitella* were placed in one beaker and seven *Abra* in the other. After 14 days, eight *Capitella* were living, one was dead and one was not found. The *Abra* fared less well and all were dead after one month. The pilot experiment indicated that *Capitella* could survive in cuttings, while *Abra* may be more difficult to keep.

2.3 Experimental set up

The experiments were carried out using five treatment levels: 100% Beryl cuttings, 20% Beryl cuttings mixed with reference sediment, 100% Ekofisk cuttings, 20% Ekofisk cuttings mixed with reference sediment, and 100% reference sediment. These concentrations of cuttings were chosen to be consistent with the degradation experiments (RF 2001/217). For each treatment level, four glass jars (10-11 cm diameter x 6 cm depth approximately) each were set up for *Capitella* and *Abra* colonisation, and with no animals as a control (Figure 2.1; Table 2.1). The jars were filled to about 1 cm below the rim and the weight of sediment recorded.

Labelled jars were placed inside aquaria for each treatment level, i.e. five aquaria each containing twelve jars. Additional jars (one for each aquarium) were prepared to allow THC sampling at T0 (see below). The jars were positioned randomly within the aquaria, which were maintained in the dark at 10°C with flow-through seawater. The seawater was supplied from 70 m depth in the fjord outside RF research station in western Norway, and had a temperature of around 10°C through the whole experiment and a salinity of 34 PSU to 35 PSU. Each aquarium was filled from the same seawater reservoir (Figures 2.2 and 2.3) and subject to the same flow rate (0.03-0.04 $l.s^{-1}$). The water inlet was designed to minimise water current in the aquarium, to avoid disturbing the jars close to inlet.

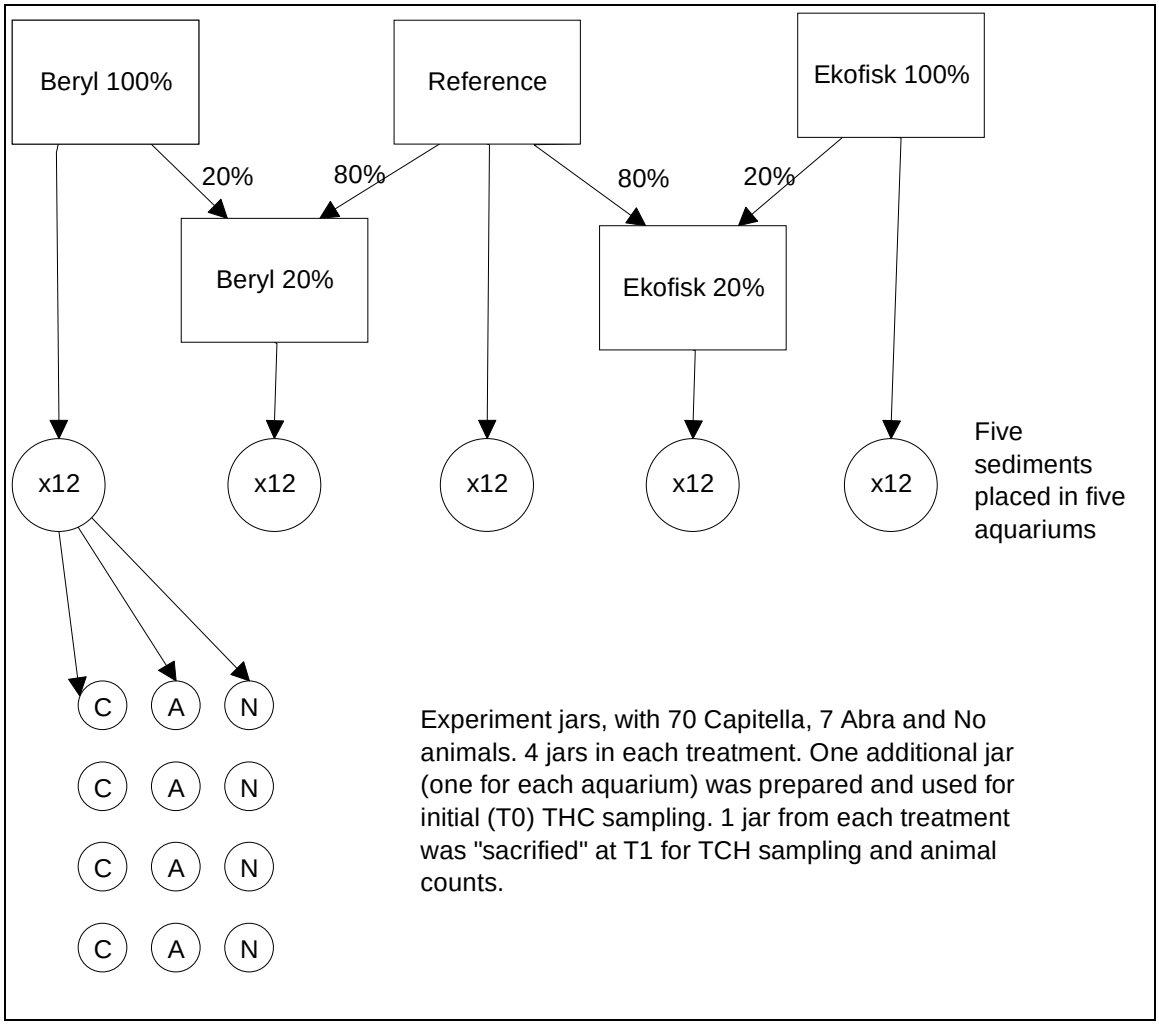


Figure 2.1 Diagram of experiment preparation and set-up.

Table 2.1 Experiment set-up.

	Number of replicates		
	Capitella (70 individuals)	*Abra* (7 individuals)	Control (no animals)
100% Beryl	4	4	4
20% Beryl	4	4	4
100% Ekofisk	4	4	4
20% Ekofisk	4	4	4
Reference sediment	4	4	4

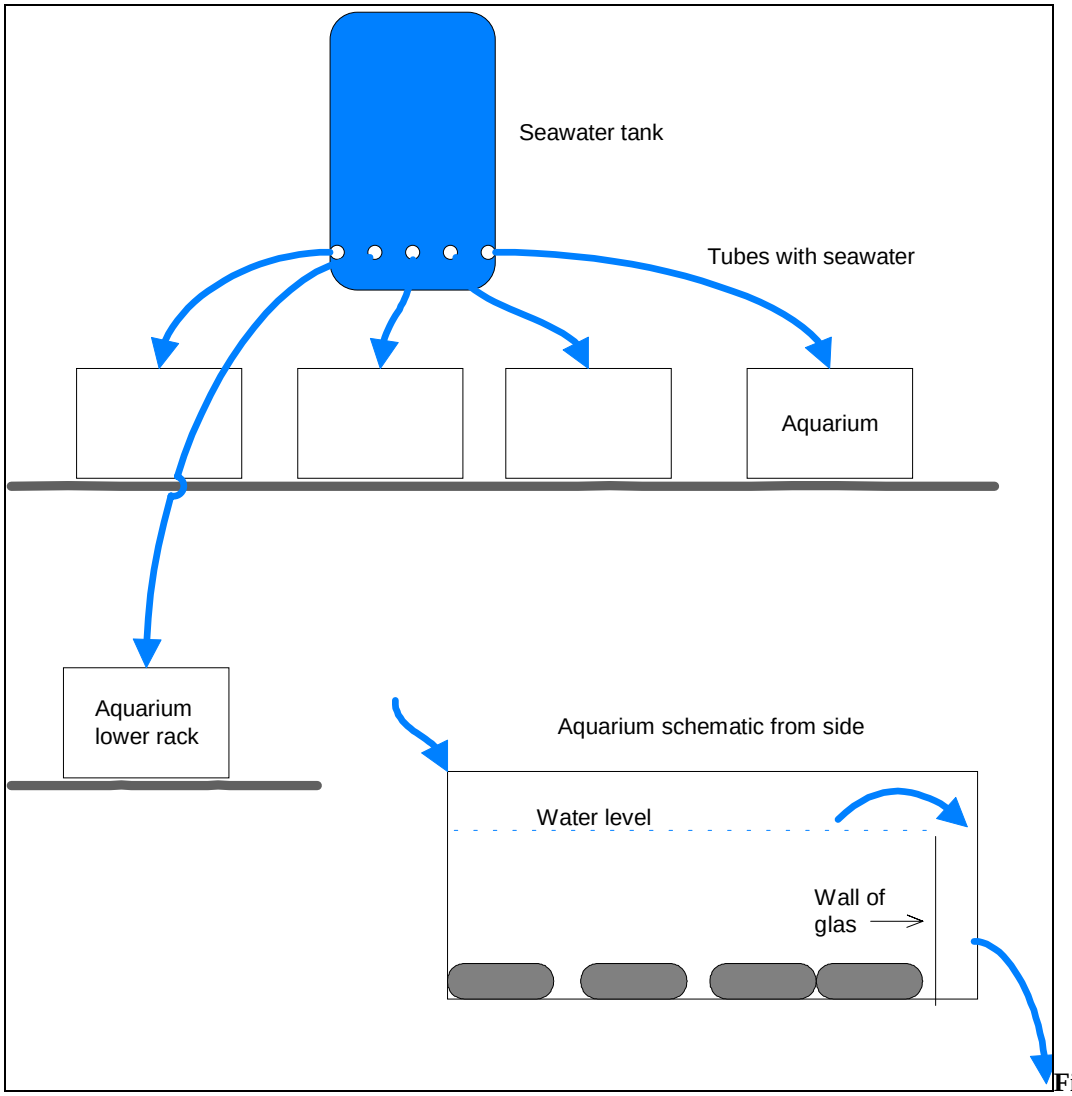


Figure 2.2 Aquarium arrangement and water flow system

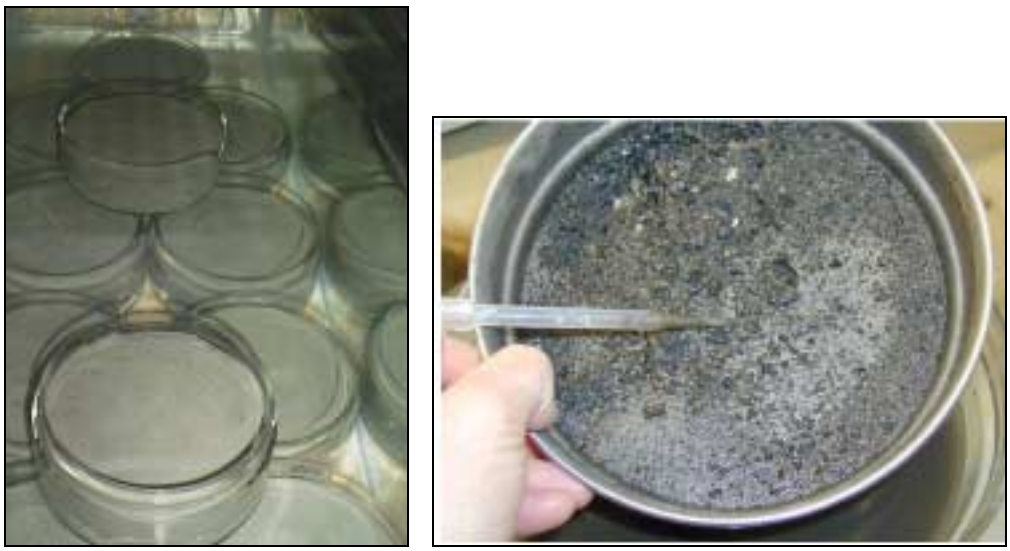


Figure 2.3 Jars with 100% Beryl cuttings placed in aquarium before animals were added (Photo left) Sieved sediment with *Capitella* (right).

To seed the jars, the sediment in the holding aquaria was sieved and the animals removed from the residual sediment by pipette or forceps while it was kept in seawater (Figures 2.3 and 2.4).

The size distribution of the *Capitella* population was determined by measuring a representative subsample of 154 individuals (see Section 2.4.3.1 for method). The *Capitella* jars were then seeded with 70 individuals, which were placed on the surface of the sediment/cuttings material, corresponding to a density of approximately 0.8 ind.cm^{-2} or 8000 ind.m^{-2}. This number was based on available animals and on the densities observed in the field. In Task 1, the numbers of *Capitella capitata* were found to be about 2000 and 5000 ind.m^{-2} in the Beryl and Ekofisk cuttings piles respectively (Westerlund *et al*., 2001). Previously, they have been recorded at densities in excess of 4000 ind.m^{-2} (equivalent to 0.4 ind.cm^{-2}) on cuttings piles and peripheral areas, although 1000 ind.m^{-2} is more typical (Kjeilen *et al*, 1999). A higher density than this was used in the experiment because the initial survival rate was unknown, the animals were relatively small (see Section 3.3) and a greater density was more likely to show an effect on degradation rates over the period of the experiment. Densities are much higher than this in heavily organically enriched areas such as underneath fish farm cages, and experimental work in these areas has been based on *Capitella* densities of 30,000 ind.m^{-2}.

The *Abra* jars were seeded with seven individuals selected to represent the size range of the specimens available. They were placed on the surface of the sediment/cuttings material, corresponding to a density of approximately 0.1 ind.cm^{-2} or 1000 ind.m^{-2}. The length of each specimen was recorded, together with the total wet weight of the *Abra* in each jar. More individuals would have been preferred, but with the high mortality on transportation, that was not possible. For comparison, the density of *Abra* used is higher than the density of bivalves (all species) found at the Beryl and Ekofisk piles and their reference locations (Westelund *et al*, 2001). The highest number of bivalves was found at the Beryl reference station where the density was about 250 ind.m^{-2}.

Figure 2.4 *Abra* specimens before being placed in the jars.

The aquaria with the filled jars were flushed with seawater at the experimental conditions described for about 5 days before the animals were added at T0. No additional food was added to any jars during the course of the experiment. The experiment ran for a total of 14 weeks. A set of samples, measurements and observations were taken immediately after set-up (T0) and again six weeks (T1) and 14 weeks (T2) after set-up. Intermediate observations and measurements of redox and pH were also taken, usually at weekly intervals, as detailed below.

2.4 Analysis parameters

2.4.1 Redox and pH

Redox (duplicate) and pH measurements were made using microelectrodes: ORP-M1-80-401 (platina), REF-M1-402 (Ag|AgCl) and pH-M1-407 (Microelectrodes Inc. USA). The two parameters were measured at the following depths in the sediment: 0 mm, 2 mm, 5 mm, 10 mm, 15 mm, 20 mm and (where required) 30 mm. To obtain the depth profile, the jar was raised by elevating the base while measuring the distance with a ruler (Figure 2.5). The pH electrode was calibrated before each day of measurement. The redox electrode was checked against a standard solution. The half-cell potential on the electrode is 205 mV (Microelectrodes Inc, pers. comm.), thus the Eh values presented in this report were obtained by adding 205 mV to the instrument readings.

Figure 2.5 Set-up for redox and pH measurement. Detail to the right.

Readings were taken immediately after experiment set-up (T0; 9 May 2001). Subsequent readings were taken at variable intervals in single representative *Capitella*, *Abra* and control jars for each treatment level. At T1 (18/19 June 2001), readings were taken for two representative jars in each case, one of which was then ‘sacrificed’ for THC sampling and animal counts and measurements. At T2 (13-15 August 2001), readings were taken from all of the remaining replicates (three for each treatment level).

2.4.2 Visual observations

The following visual observations were made throughout the course of the experiment, particularly at T0, T1 and T2:

(1) Sediment/cuttings material (all jars)

- Colour of sediment and any depth variation;
- Evidence of disturbance of surface;

(2) *Capitella* jars

- Presence of animals on sediment surface;
- Evidence of animal activity on sediment surface (tubes, mucous trails, faecal pellets);
- Depth and orientation of burrows (seen through sides of jars);

(3) *Abra* jars

- Presence and condition of animals on sediment surface;
- Evidence of animal activity on sediment surface (furrows from burrowing activities, emergent siphons).

2.4.3 Animal counts and measurements

2.4.3.1 Capitella

Before seeding the jars, the size distribution of the *Capitella* population was determined by measuring a subsample of individuals. At T1, one representative jar from each treatment level was 'sacrificed' and the animals removed for counting and measuring. The remaining three replicate jars were analysed at T2.

The cuttings/sediment was removed from the jars and sieved over a 0.180 or 0.125 mm mesh. In order to determine the depth distribution within the jars, this process was carried out for three layers: top approximately 0.5 mm, next approximately 20 mm and bottom section (approximately 25 mm). These depths were chosen to reflect largely the changes seen in redox potential. The contents of the sieve were washed into white trays and *Capitella* were picked out from the remaining sediment under a binocular microscope, counted, and each individual measured. Length measurements were obtained by carefully removing remains of tubes from the worms and waiting until they reached their maximum length while actively moving along the base of a petri dish, which was positioned over graph paper.

2.4.3.2 Abra

Before placing them in the jars, each individual *Abra* was measured (length) with a ruler and the combined blotted wet weight obtained on a balance.

At T1, one of the replicate jars for each treatment level was 'sacrificed' and the animals removed to see how many were alive and to measure and weigh surviving individuals. This was repeated for the remaining three replicates at T2.

2.4.4 THC

Degradation and/or depletion of hydrocarbon and other organic compounds of the cuttings material was assessed by analysing for THC in the samples. Results from the Task 3 degradation tests had already shown a high degree of variability in THC data and consequently a difficulty in demonstrating changes in levels over time. THC data were therefore recognised as being of less importance than first anticipated in terms of demonstrating combined degradation and depletion over the short timeframe of the experiment. THC analyses were still carried out, however, in order to provide rough comparisons with other results.

Samples of sediment/cuttings material for THC analysis were removed from the additional jars at the start of the experiment (T0). At T1, a small sub-sample of approximately 8 cm^2 of the sediment/cuttings material was removed from each of the jars being sacrificed. The sub-samples were obtained using a modified plastic syringe. Two sub-samples of approx. 4 cm depth were taken from each jar, and the samples were placed in glass vials. Similar samples were taken from the remaining three replicate samples for each treatment level at T2. These were and mixed together prior to analysis to provide an average value, since the degree of variability in the THC had already been established.

Extraction and analysis methods are described in detail in the degradation report (RF 2001/217).

3 Results

3.1 Redox and pH

Redox and pH profiles for each treatment level at T0, T1 and T2 (based on averages of replicate data) are illustrated in Figures 3.1 and 3.2. The full data are included in Appendix 1.

T0 redox readings, taken the day after experimental set-up, were similar for the different treatment levels and generally fell only slightly with depth down to 20 mm in the sediments. The exceptions were the Ekofisk cuttings, particularly Ekofisk 100%, which exhibited lower redox potentials below the surface than the other treatments, with a discontinuity at around 5 mm depth.

The jars with cuttings showed a general fall in redox values after T0. The Ekofisk cuttings continued to exhibit the lowest values, particularly Ekofisk 100% in which a marked shift in redox was seen between depths of 0 to 10 mm at both T1 and T2. This shift was seen with all jar types. While there were no apparent differences between the *Abra* and control jars, the shift was less distinct with the *Capitella* jars, especially at T1

but also to some extent at T2. Whether the differences were significant is difficult to say.

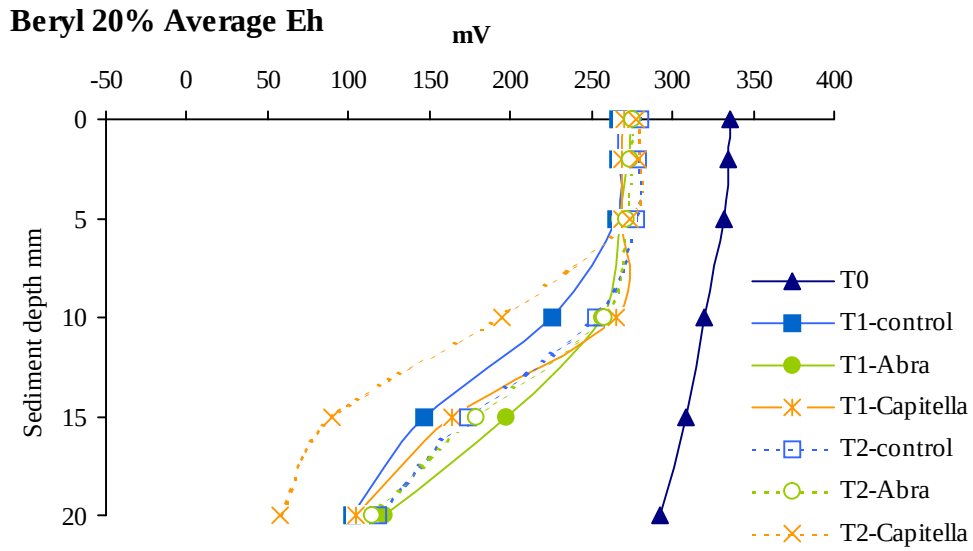
Similar observations were made for Ekofisk 20%, but here the shifts in redox potential were less distinct and were greatest at between 5 and 15 mm depth. There were apparently fewer differences between the *Abra* and *Capitella* jars than observed for Ekofisk 100%, but the shift in the *Capitella* jars at T2 occurred more at 10 to 15 mm. Both of the Ekofisk treatments showed generally higher redox values in the *Capitella* jars than in the controls and the *Abra* jars. This was most marked in the case of T1 *Capitella* for Ekofisk 100%, and for T2 *Capitella* for Ekofisk 20%.

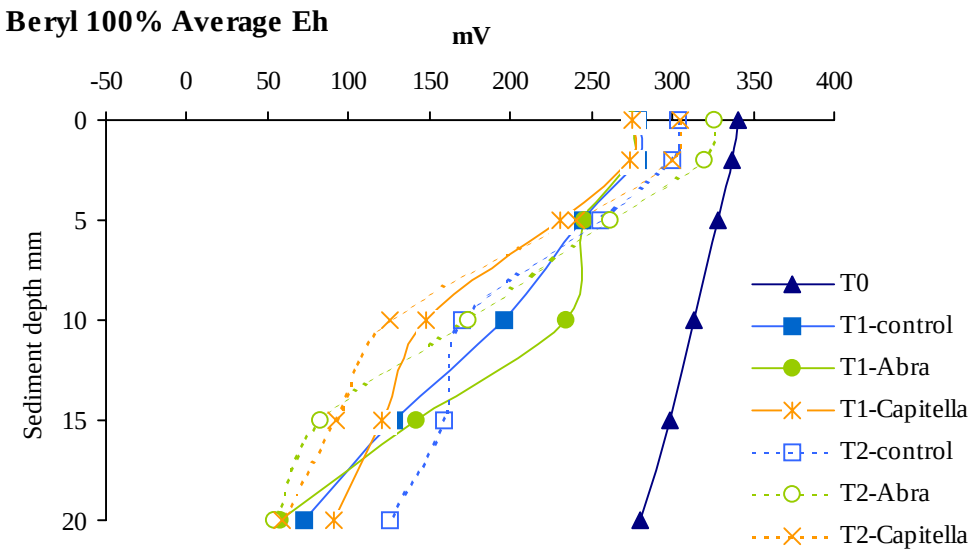
The redox patterns seen in the Beryl cuttings at T1 and T2 were similar to those for Ekofisk, although the redox potentials did not reach such low values. Also, the shifts with depth were less marked and deeper in the sediment, compared to the Ekofisk jars. In the Beryl cuttings, there was no significant difference between the jars seeded with animals and the controls.

The reference sediment jars showed little change between T0 and T1. There was very little or no obvious shift in redox values within the measured layer down to 20mm, although the T1 control jars showed a marked fall, on average, between 15 and 20 mm. At T2, a slight fall had occurred for all treatments in the deeper parts of the depth range examined.

The reference sediment samples exhibited a consistent slight fall in pH with depth in the sediment at T0 and T1, while all of the cuttings samples showed a slight rise with depth (Figure 3.2). There were no consistent trends associated with the presence of either *Abra* or *Capitella* for any of the treatments.

Several factors may have influenced the pH of the samples. Microbial activity would most likely produce acidic degradation products, and hence will lower the pH. The seawater will act as a buffer counteracting the implications of this. In addition, an array of seawater, sediment and degradation-product interactions can be expected that can affect the pH in different directions. The complex pH chemistry will not be dwelt upon; of more importance in this context are the differences between treatments.





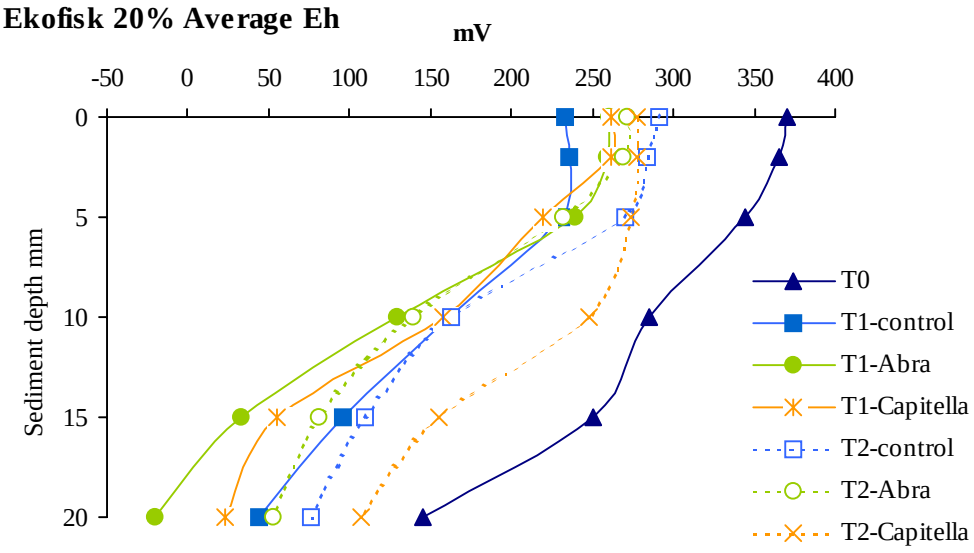


Figure 3.1 Average redox profiles at T0, T1 and T2 for each treatment levels. Cont. next page.

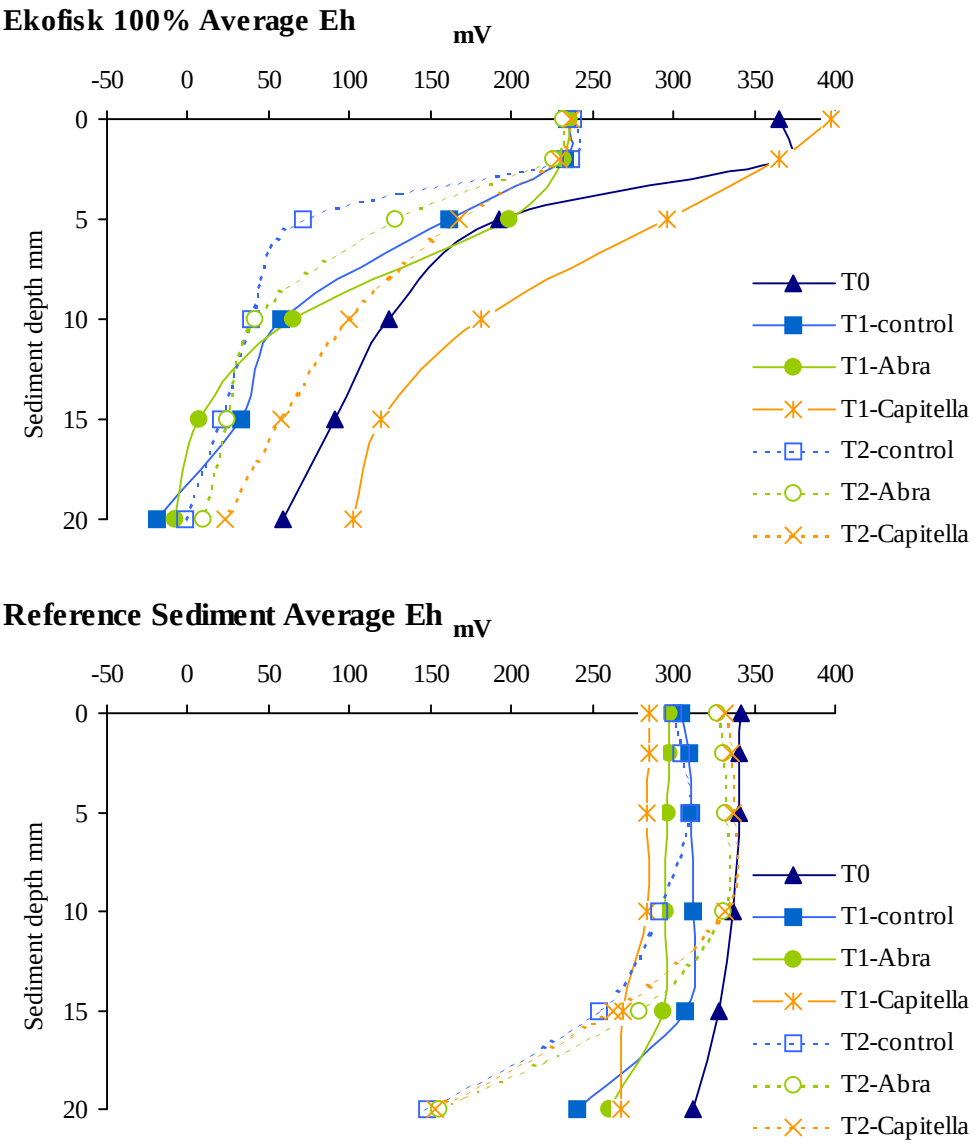


Figure 3.1 Average redox profiles at T0, T1 and T2 for each treatment levels.

3.2 Visual observations

Copies of all of the recording sheets completed during the course of the experiment are contained in Appendix 2. The observations made are summarised below. At the start of the experiment, the appearances of the sediments/cuttings were described as follows:

Reference sediment: colour greenish, consistent throughout the depth of the jar; surface uneven, with some loose, easily suspended material at the top.

100% Beryl cuttings: colour grey, consistent throughout the depth; surface uneven, cohesive, clay-like, with some very fine loose material on the top.

100% Ekofisk cuttings: colour brown, with the top 7 mm lighter than the rest; surface uneven, cohesive, clay-like, with a little loose fine material at the surface.

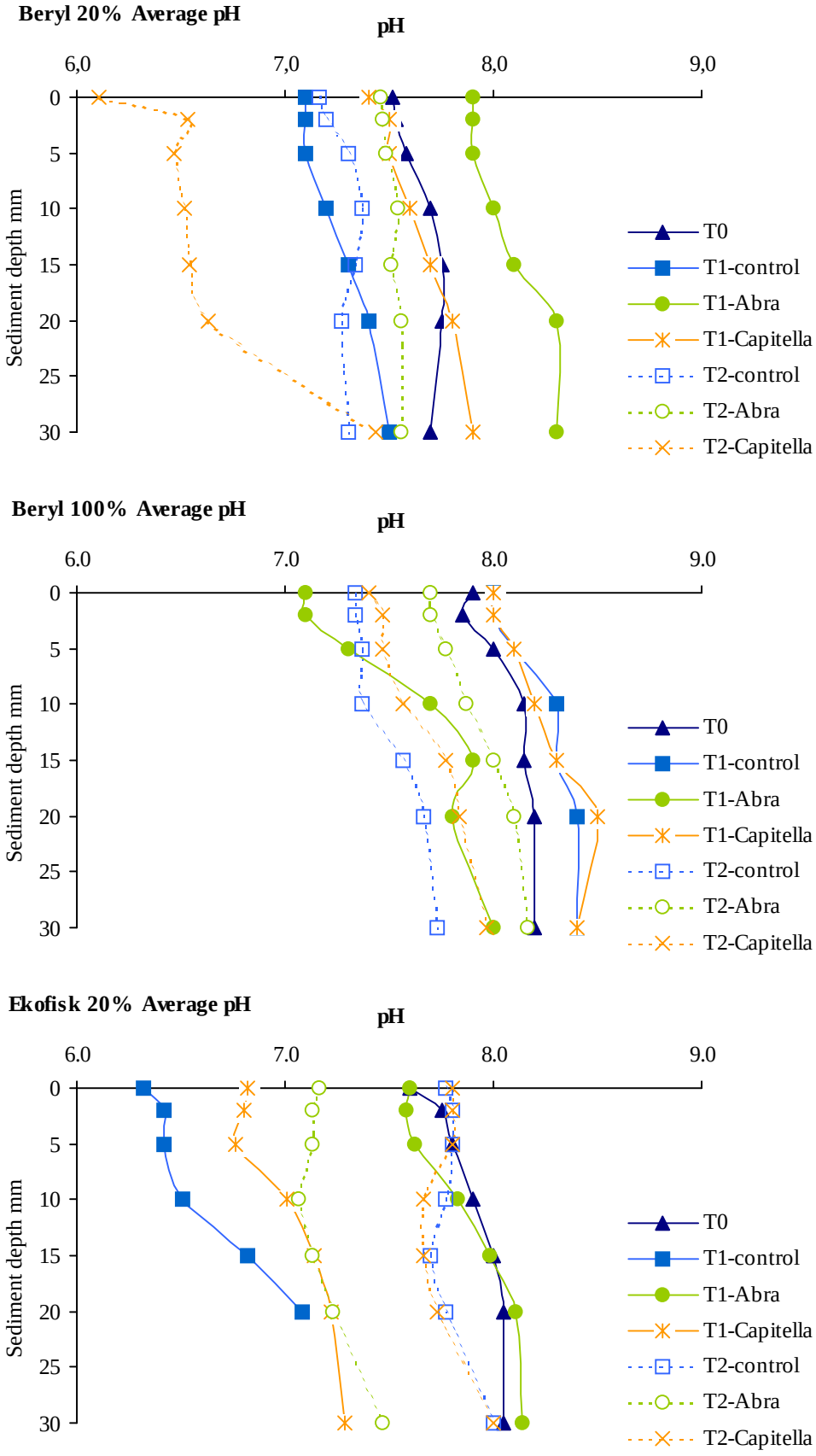


Figure 3.2 pH profiles at T0, T1 and T2 for each treatment level. Cont. next page.

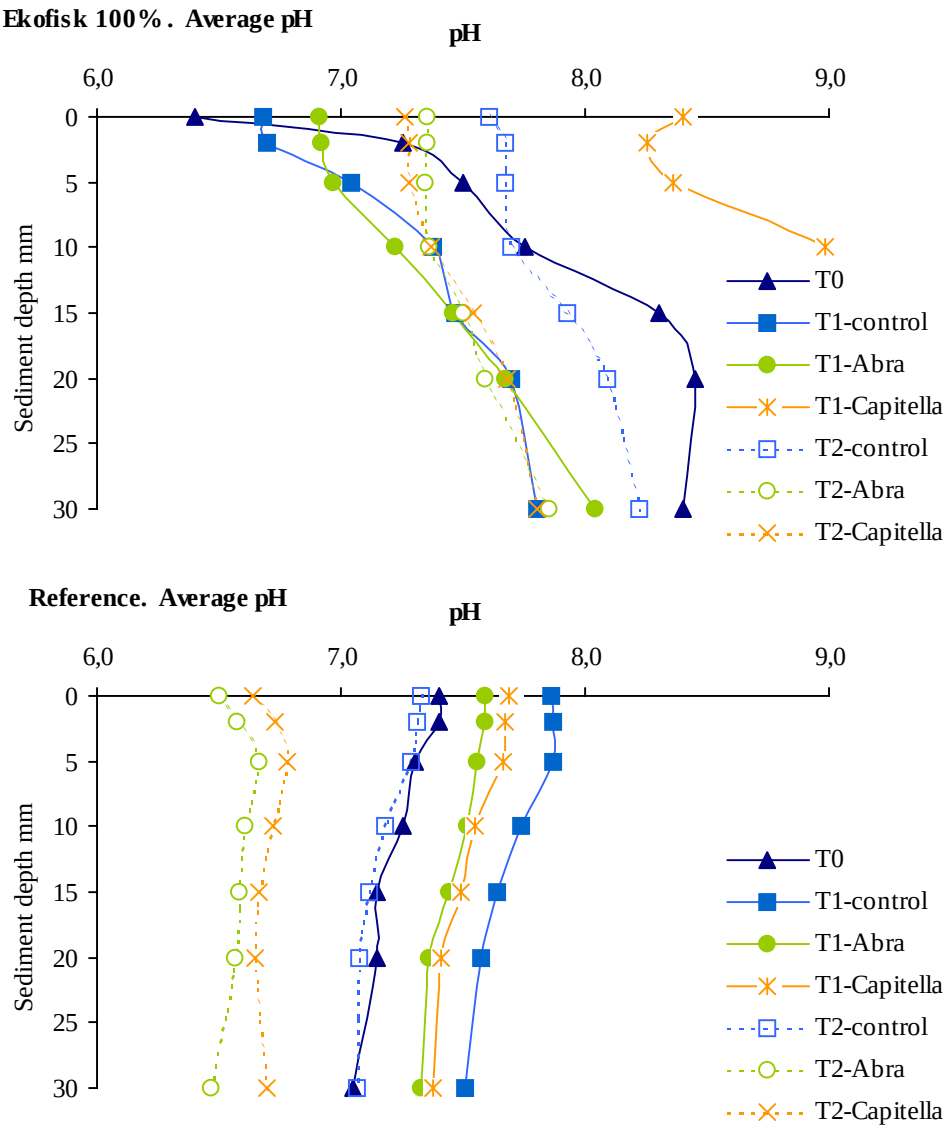


Figure 3.2 pH profiles at T0, T1 and T2 for each treatment level

20% Beryl cuttings: colour greenish like the reference sediment; surface fairly flat, with some loose fine material at the top.

20% Ekofisk cuttings: colour greenish, slightly lighter at the very surface; surface uneven, cohesive, with some loose, easily suspended material on the top.

3.2.1 *Capitella*

3.2.1.1 20% Beryl cuttings mixture

T0 Limited burrowing had occurred. Most of the animals remained on the surface, but were well covered in sediment particles. Burrowing was to 0-6 mm in the four jars, and mostly orientated horizontally, with a few deeper ones at 45º. Some tubes projected above the surface, and there were some mucus trails.

T1 No individuals remained on the surface. Most burrows were shallow, but a few reached the bottoms of the jars. *Capitella* themselves were visible in shallow burrows at the edge of the jars. Some tubes projected above the surface.

The sediment remained olive green throughout its depth with a little flocculent material at the surface, and showed little evidence of disturbance.

T2 Few *Capitella* themselves could be seen. Burrows were mostly down to 5 mm, but some reached 20 mm.

The sediment appeared unchanged.

Summary Burrowing had commenced after one day, but at six weeks the *Capitella* were well established and forming mostly shallow burrows, although some penetrated deeper.

3.2.1.2 100% Beryl cuttings

T0 Limited burrowing had occurred. Most of the animals remained on the surface, some of them in clumps, and were not covered in sediment particles. There were many mucus trails. Several individuals were lying around the edges of the jars. Burrowing was to 0-8 mm in the four jars.

T1 No individuals remained on the surface. Burrows were limited to the top few mm. Some tubes extended above the surface and showed a patchy distribution.

The cuttings were pale grey at the surface and slightly darker grey below 2 mm. This was also the case for the controls.

T2 Few *Capitella* themselves were seen. Burrows extended to the bottoms of the jars, but most were within the top 10 mm and were probably old.

Below the surface, some dark spots (possibly sulfide) had appeared. This was also the case for the controls.

Summary The *Capitella* were slow to commence burrowing. After six weeks they were well established but in the surface layers only. There were no apparent effects on the sediment.

3.2.1.3 20% Ekofisk cuttings

T0 The vast majority of the animals had already burrowed into the sediment. The burrows extended to 2-4 mm below the surface and were long, mostly horizontal in orientation. Many tubes projected above the surface. There were a few mucus trails on the surface.

T1 Evidence of burrowing was seen all over the sediment and down to about 20 mm. The burrows were mostly oriented horizontally to 45º.

The sediment remained olive green throughout its depth, with a little flocculent material at the surface, and showed little evidence of disturbance.

T2 Most burrows were in the top 5 mm, but a few extended to 20-30 mm and the bottom of the jar.

The sediment was lighter in the top 0-5 mm. This was also the case in the control jars, which, additionally, had dark spots (possibly sulfide) beneath the surface. The surface in the *Capitella* jars was highly pelletised and with discarded tubes.

Summary Burrowing was fairly quick and extended to 20-30 mm although was concentrated in the surface layer. The surface had become pelletised after 14 weeks. Possible sulphide-rich areas had developed in the controls after 14 weeks. This was not apparent in the *Capitella* jars.

3.2.1.4 100% Ekofisk cuttings

T0 The *Capitella* had not burrowed into the cuttings, and remained exposed on the surface, not covered in sediment, either in one large clump or several smaller clumps. Where there were separate clumps, these were joined by long mucus trails. In one jar, several individuals were lying in straight lines around the edge of the jar.

T1 Very few individuals remained on the surface. Burrows extended to 5 mm and were mostly horizontal.

The surface of the cuttings was light brown, darker and grey-green below 2-3 mm; same as for the control jars. The surface was mostly undisturbed, but there was some granular material that could be faecal pellets, and a few mucus trails.

T2 No individuals remained on the surface. Extensive burrows were seen to 10 mm depth.

The lighter surface layer was slightly less distinct than in the control jars and the surface was granulated. One of the jars had black spots (possibly sulfide) beneath the surface.

Summary The *Capitella* were slow to commence burrowing, but after six weeks only a few remained on the surface. Burrows were horizontally oriented in the top layer (5 mm), but some had reached 10 mm after 14 weeks. The surface became granulated as a result of worm activities.

3.2.1.5 Reference sediment

T0 Most of the *Capitella* had burrowed into the sediment. Burrows could be seen reaching down to 12-25 mm in the four jars, and were generally vertical in orientation. Some tubes projected above the surface. Some of the individuals remained on the surface, but these were all well covered with sediment, and there were only a few mucus trails.

T1 No individuals remained on the surface. Burrowing was extensive down to the bottom of the jar, about 40 mm. Some tubes extended above the surface.

The sediment was a greeny grey/brown colour throughout. The surface was smooth with some granular material that could be *Capitella* faecal pellets.

T2 Burrows extended to the bottoms of the jars, but most were in the top 20 mm.

The sediment was olive green and did not change significantly with depth.

Summary Most of the *Capitella* burrowed into the sediment very quickly (<l day) and burrows, many vertically oriented, were established to 25 mm, increasing to 40 mm at six weeks. The surface became granulated as a result of worm activities.

3.2.2 Abra

3.2.2.1 20% Beryl cuttings mixture

T0 More than half of the *Abra* remained on the surface or were only partially buried, but they all seemed to be alive.

T1 6 or 7 individuals in each jar remained on the surface and some were dead.

Some depressions at the surface may have been *Abra* movements/burial attempts.

T2 All but one of the *Abra* were on the sediment surface and were dead.

The mixture was olive green colour throughout its depth, with some flocculent material on the surface.

Summary The *Abra* attempted to burrow in the sediment, causing some disturbance to it, but after six weeks the majority had not survived.

3.2.2.2 100% Beryl cuttings

T0 All of the *Abra* remained on the cuttings surface, and some were dead while others appeared to be nearly dead.

T1 All but one individual remained on the cuttings surface. Some in each jar were dead.

The cuttings were pale grey in the top 2 mm, and darker below.

T2 Nearly all of the *Abra* were on the surface of the cuttings and the majority were dead.

The surface 2-3 mm of the cuttings remained a paler colour than the rest, with a little flocculent material on the top. There were also darker spots (possibly sulfide) beneath the surface, which was also seen in the controls.

Summary The *Abra* showed no attempt to bury themselves in the cuttings, and many were dead at six weeks, the majority at 14 weeks.

3.2.2.3 20% Ekofisk cuttings mixture

T0 All of the *Abra* were either completely or partially buried in the sediment, and several pairs of active siphons were seen.

T1 Most of the *Abra* remained below the surface and active siphons were seen. 0-3 individuals per jar were dead.

The surface 3-4 mm was lighter in colour than the remainder, but it was partly mixed down to 10 mm.

T2 Some more animals had returned to the surface, and 3-4 individuals per jar were dead.

The lighter surface layer had extended to 15-20 mm, clearly distinguishing the *Abra* jars from the controls (2-4 mm).

Summary The *Abra* readily buried into the sediment and survival was fairly good. Their presence caused mixing and oxygenation of the top 15-20 mm of the sediment.

3.2.2.4 100% Ekofisk cuttings

T0 The *Abra* were on the surface, half buried or completely buried, but all seemed to be alive.

T1 Between 4 and 6 individuals in each jar remained on the surface. In three of the jars, at least half of these were dead, but in the fourth jar 6 were clearly alive with active siphons.

The surface 2-3 mm of the cuttings was brownish, with a darker grey-green colour below; same as the control jars. There was some evidence of disturbance of the surface.

T2 6-7 individuals were on the surface in each jar and were dead.

The lighter surface layer was very thin, 1-3 mm, with some loose flocculent material at the top.

Summary The *Abra* showed some variability in their response, but some did burrow into the cuttings, although most individuals did not survive to 14 weeks. No significant disturbance of the sediment was observed.

3.2.2.5 Reference sediment

T0 All of the *Abra* had successfully buried themselves beneath the surface, and several sets of active siphons could be seen.

T1 Most of the *Abra* remained below the surface. 0-2 individuals per jar were on the surface and/or dead.

The sediment was an even greeny grey/brown colour. The surface was slightly uneven with some possible *Abra* faecal pellets in patches.

T2 In one jar all of the *Abra* were below the surface, in the other two there were 3 or 4 on the surface and dead.

The sediment remained an even colour with depth, but there were some darkerspots (possibly sulfide) below the surface.

Summary The *Abra* readily burrowed into the sediment and survival was generally good. The surface was disturbed by the presence of the bivalves.

3.3 Animal counts and measurements

3.3.1 *Capitella*

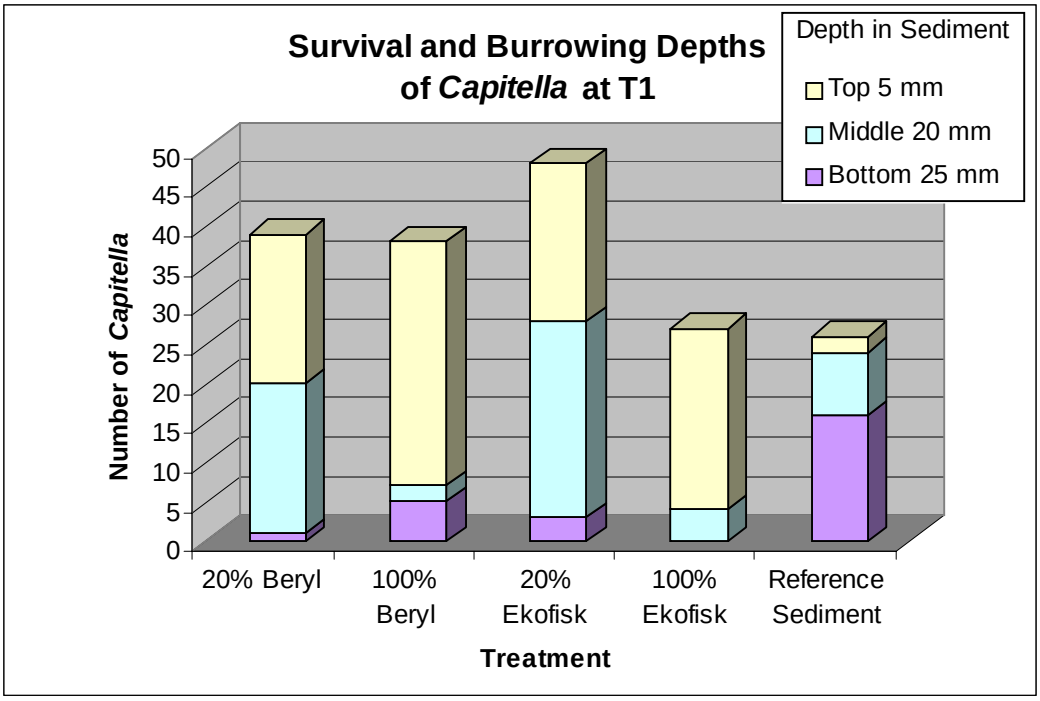
3.3.1.1 Survival and burrowing depths

Six weeks after the start of the experiment (T1), the number of *Capitella* remaining in the jars ranged from 26 to 48, representing survival of approximately 37% to 69%. Survival was highest in the 20% Ekofisk cuttings mixture, and lowest in the 100% Ekofisk cuttings and in the reference sediment.

The most notable feature at T1 was the depth distribution of *Capitella* in the substrate. For both the 100% Beryl and the 100% Ekofisk cuttings, the vast majority of the individuals were found in the top 5 mm (Figure 3.1). For both of the 20% cuttings mixtures, they were distributed mostly within the top 25 mm, while in the reference sediment they were distributed throughout the substrate depth, with most found in the bottom 25 mm of the jar. These data correlated well with the visual observations made of the locations of borrows (Section 3.2.1; Appendix 2).

By T2, very few *Capitella* remained in the 20% and 100% Beryl cuttings and in the reference sediment (Figure 3.1). The few individuals that were found showed a similar depth distribution as they had at T1. Survival after three months was much better in the Ekofisk cuttings, particularly in the 100% cuttings. The majority of the *Capitella* were found in the top 5 mm of the substrate, although in the 20% cuttings a few were in the bottom 25 mm of the jar, as at T1.

a) T1



b) T2

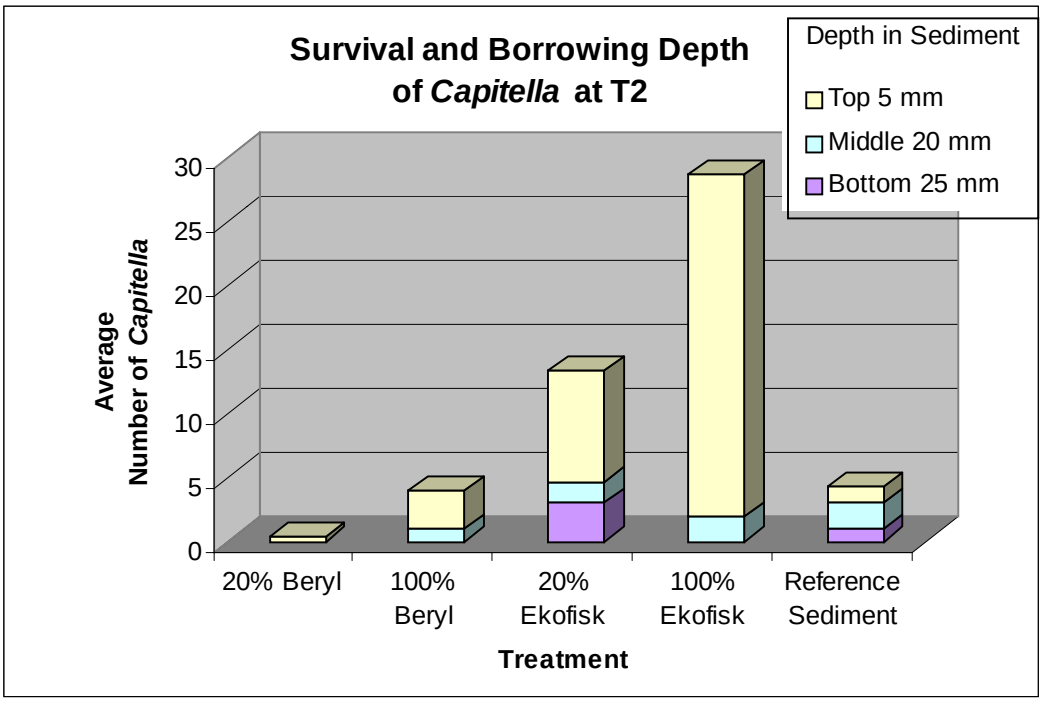


Figure 3.1 Survival and burrowing depths of *Capitella* at T1 and T2

3.3.1.2 Size frequency histograms

The subsample of *Capitella* measured from the holding aquaria immediately prior to the start of the experiment showed a unimodal size distribution with a modal size of 9 to 12 mm length (Figure 3.2). Individuals ranged in size from 4 to 20 mm.

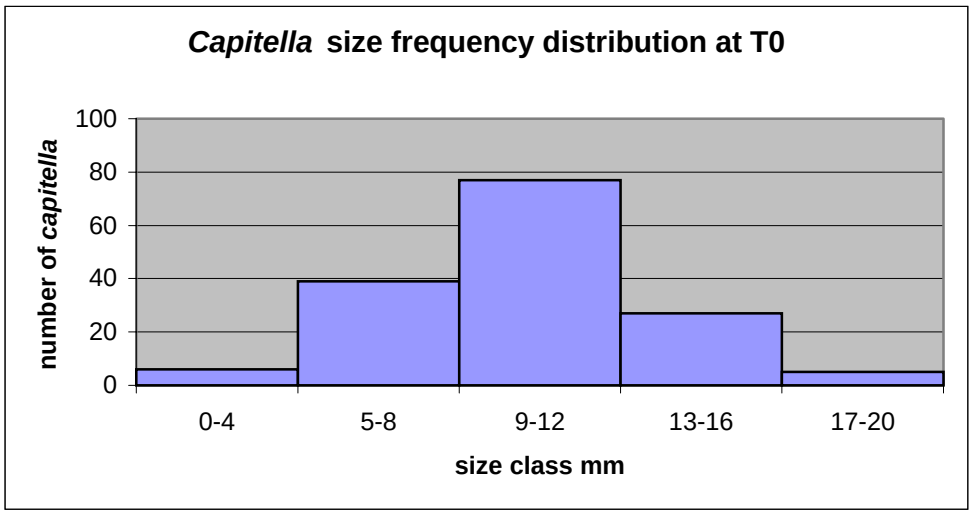


Figure 3.2 *Capitella* size frequency distribution at start of experiment (T0)

At T1, the size range remained the same overall but the modal size had decreased for all treatments (Figure 3.3).

For most of the treatments, few individuals survived until T2. In the 100% Ekofisk cuttings, however, there was an apparently thriving population with most individuals between 5 and 12 mm long (Figure 3.4). Several of the larger individuals contained mature eggs, and there were also some very small individuals, about 2 mm long, that were not present at the start of the experiment.

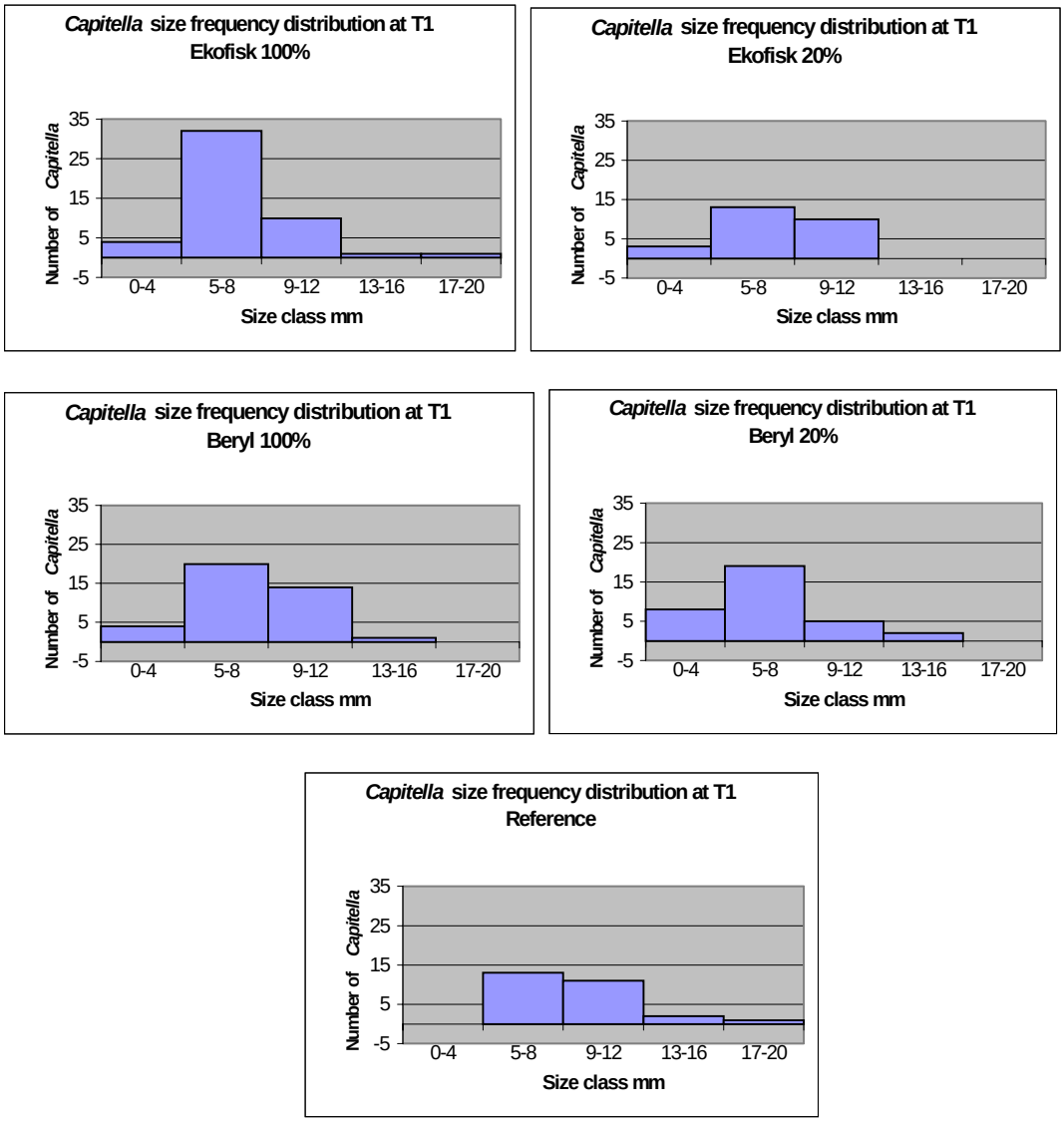


Figure 3.3. *Capitella* size frequency distributions at T1 for different treatments.

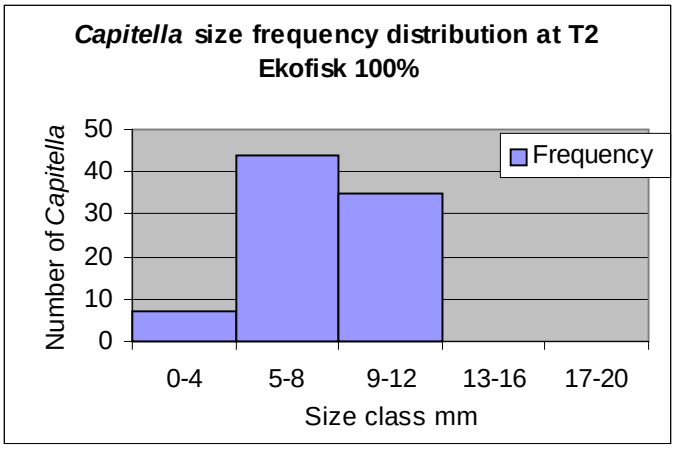


Figure 3.4 *Capitella* size frequency distributions at T2 for 100% Ekofisk cuttings.

3.3.2 Abra

3.3.2.1 Survival

Six weeks after the start of the experiment (T1), the number of *Abra* surviving in the single replicate that was sacrificed ranged from 3 to 5, representing survival of approximately 43% to 71%. Survival was highest in the 20% Ekofisk cuttings mixture and the reference sediment, and lowest in the 20% Beryl cuttings mixture and the 100% Ekofisk cuttings (Figure 3.5).

By T2, very few *Abra* had survived in the 20% and 100% Beryl cuttings and in the 100% Ekofisk cuttings (Figure 3.5). Survival in 20% Ekofisk cuttings and in the reference sediment was over 50% at 14 weeks.

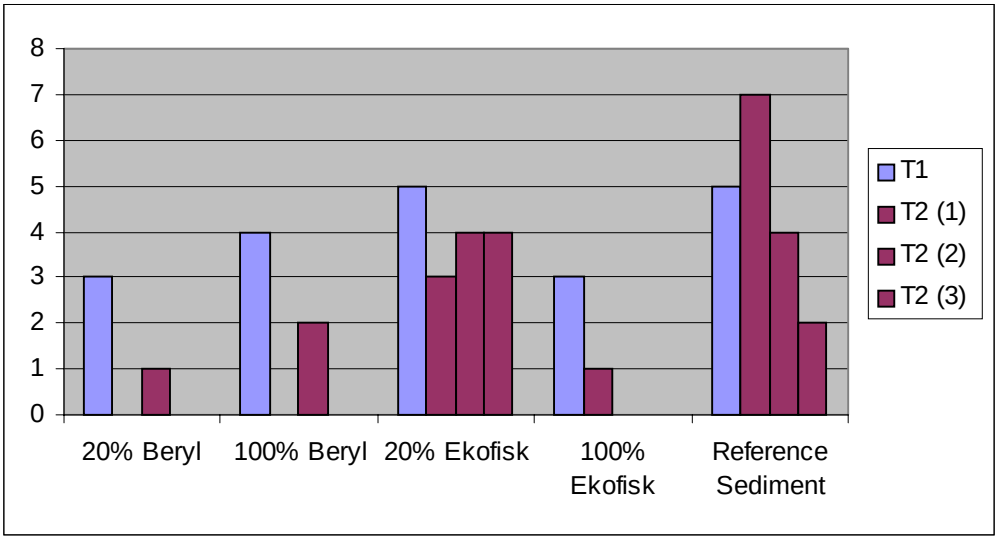


Figure 3.5. Survival of *Abra* at T1 (six weeks) and T2 (14 weeks) with replicates.

3.3.2.2 Length and biomass

At the start of the experiment, the *Abra* specimens ranged in length from 11 to 23 mm, with a modal size of 19 to 20 mm (Figure 3.6). The mean biomass (blotted wet weight) in each jar was 5.30 g.

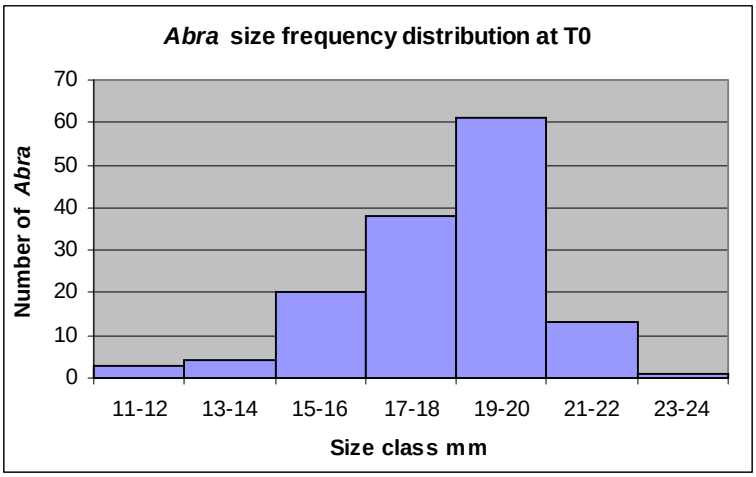


Figure 3.6 *Abra* size (length) frequency distribution at T0.

For reference sediment jar #5, in which all of the *Abra* survived to T2, the lengths measured were exactly the same (to the nearest mm) as at the start of the experiment. This is borne out by the length frequency distribution of individuals in all of the reference sediment jars at T2 (Figure 3.7), which closely reflects the distribution at T0. However, total biomass for the seven *Abra* had decreased from 4.94 g to 4.33 g, a loss of over 12%. Unfortunately, biomass could not be compared for other jars/treatments because only total biomass for each jar was recorded at T0, rather than for each individual *Abra*.

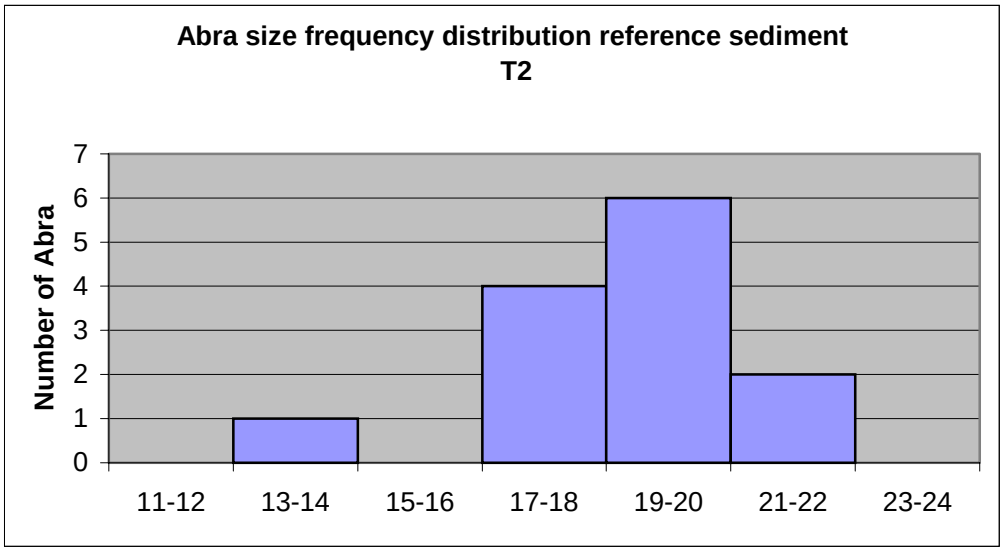


Figure 3.7 *Abra* size (length) frequency distribution in reference sediment at T2 (all three jars).

3.4 THC

There were marked differences in the THC concentration in the four cuttings treatments, with the two Ekofisk treatments exhibiting the highest values of more than 70,000 mg/kg dry weight in Ekofisk 100% and more than 10,000 mg/kg in Ekofisk 20% (Table 3.1).

Table 3.1 THC concentrations for each treatment level at T0, T1 and T2

	THC, mg/kg dry weight		
		weeks	
Treatment	0 (T0)	6 (T1)	14 (T2)
Beryl 100 % - control	675	937	1491
Beryl 100 % - *Abra*	675	1656	1001
Beryl 100 % - *Capitella*	675	2878	1211
Beryl 20 % - control	81	218	134
Beryl 20 % - *Abra*	81	260	176
Beryl 20 % - *Capitella*	81	269	111
Ekofisk 100 % - control	73248	66877	62916
Ekofisk 100 % - *Abra*	73248	68373	68872
Ekofisk 100 % - *Capitella*	73248	65042	76008
Ekofisk 20 % control	11410	12689	10103
Ekofisk 20 % - *Abra*	11410	8428	8536
Ekofisk 20 % - *Capitella*	11410		10066

Changes in THC concentrations over the course of the experiment are illustrated in Figures 3.8 and 3.9.

It appears from these data that the THC concentrations in 20% Beryl and 100% Beryl all increased after T0, which is very unlikely to have occurred. The T0 100% and 20% Beryl concentrations in this experiment were notably lower than those for all other Beryl starting concentrations for the other Task 3 experiments (RF-2001/217). THC measurements for Beryl cuttings for several day 0 and day 1 samples in the degradation experiments were in the range 2,500 to 4,000 mg/kg, suggesting the present data are not representative. However, no specific methodological reason has been found to explain the aberrant results. If the T0 measurements are taken out or set at an average level of the other measurements, Beryl THC concentrations do not show an increase.

No estimations of the accuracy of the THC data have been carried out within this sub-task, but some effort has been used to examine this in the degradation sub-task (RF-2001/217). The results of the degradation work indicate a 25-30% accuracy, which suggests that no significant increases or decreases in THC have been demonstrated in this experiment.

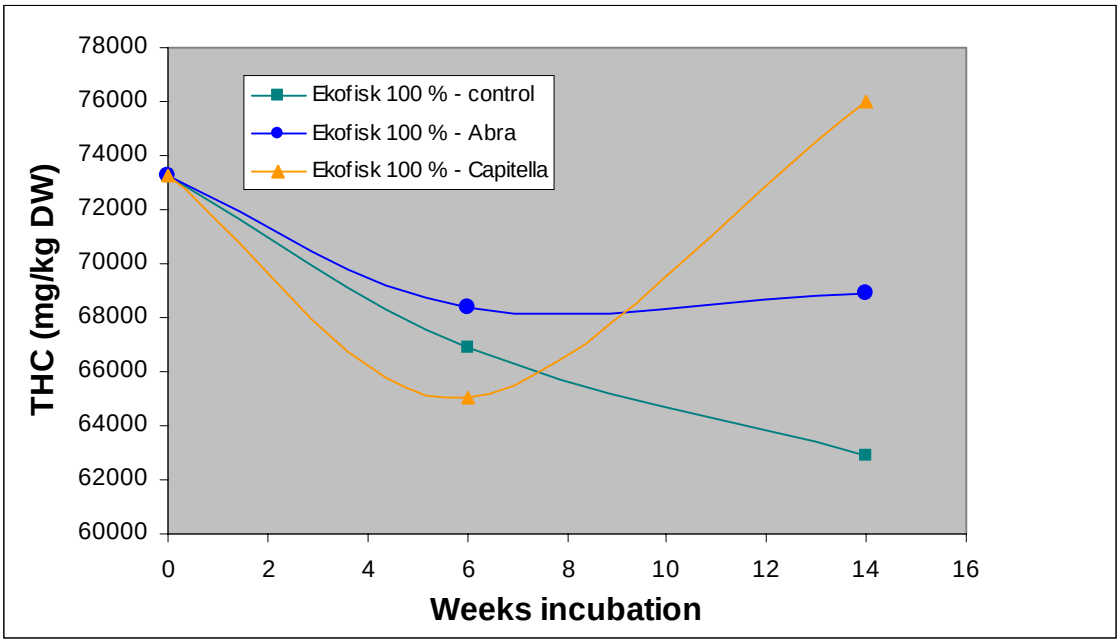


Figure 3.8 Changes in THC in 100% Ekofisk cuttings over the course of the experiment.

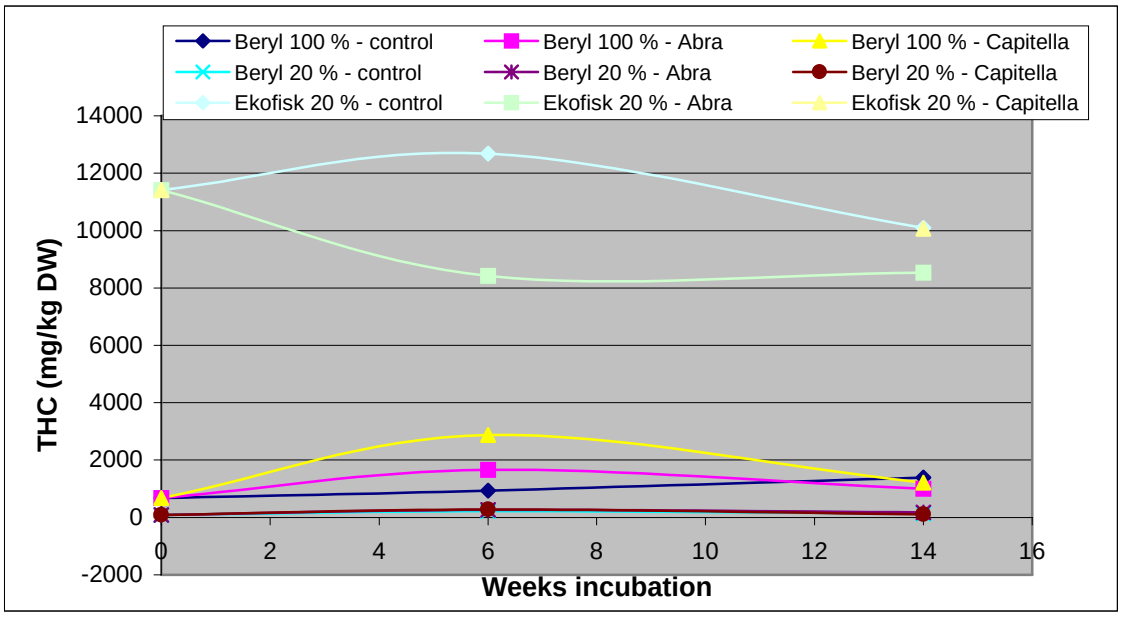


Figure 3.9 Changes in THC in 20% Ekofisk, 100% Beryl and 20% Beryl cuttings over the course of the experiment.

4 Discussion

4.1 Introduction

Available information on the macrofauna inhabiting cuttings piles was reviewed during UKOOA Drill Cuttings JIP Phase I (Projects 2.1 and 2.3 - Dames & Moore, 1999; Kjeilen *et al*, 1999). Only limited data were available from ROV and grab-sampling surveys, and by their nature provided little information on spatial or temporal colonisation patterns or the mechanism of the colonisation process. The fauna of three Ekofisk cuttings piles, after four to six years of dormancy, was dominated by small surface deposit feeding polychaetes (Cripps *et al*, 1999). Where oil additives had been discharged with the cuttings, and PAH contamination was recorded in the pile (though not necessarily at the pile surface), these included *Capitella capitata* and *Cirratulus cirrat*us. At West Ekofisk, where no oil additives were thought to have been discharged, the fauna was dominated by *Myriochele oculata* and another fast growing opportunistic polychaete, *Scoloplos armiger*. The diversity and evenness of the communities were depressed compared to uncontaminated sediments, and this was a reflection of the dominance of the fauna by one or two species (Cripps *et al*, 1999; summarised in Kjeilen *et al*, 1999). Similar results were obtained at Ekofisk in 2000, after six to eight years of dormancy (Westerlund *et al*, 2001).

An indication of the rates of colonisation of cuttings piles is provided by field studies in the Dutch Sector and by experimental work carried out in Norway on thin layers of cuttings (reviewed by Dames & Moore, 1999; Kjeilen *et al*, 1999). Dames & Moore (1999) concluded that the accumulated evidence, though limited in nature, suggests that colonisation of cuttings piles begins during the first one or two years following the cessation of cuttings discharge. However, the degree of oil contamination has a major effect on the rate of colonisation and any succession that may occur (Kjeilen *et al*, 1999). Field and experimental evidence suggests that, in common with other deposited sediments such as dredge spoil, recolonisation of cuttings piles is initially by opportunistic species that are well adapted, by virtue of their rapid rate of reproduction and growth, to colonisation of deposits that are subject to frequent disturbance (Kjeilen *et al*, 1999). Early colonising species are generally small sedentary, often tube-dwelling, surface deposit feeders, while species characteristic of more stable communities tend to be larger, more mobile animals that are deposit and/or suspension feeders (Newell *et al*, 1998). The development of these more stable communities on cuttings piles appears to depend on the degree of oil contamination, but the low diversity and predominance of surface deposit feeders at Ekofisk West suggests that establishment of a stable community may take a considerable time. In experiments with 10 mm layers of OBM cuttings on top of natural sediment, even after more than five years, the major bioturbators of the surrounding seabed had been prevented from colonising the substrates; recolonisation was only superficial and did not penetrate the cuttings (Bakke *et al*, 1986a, 1986b, 1989).

As discussed in Section 1.2, an experimental study of colonisation patterns is not appropriate within a short-term experimental programme. The present study aimed to compare survival, growth, activity and effects of two contrasting invertebrate species in different types and concentrations of cuttings. Cuttings piles are not easily categorised, not least due to the combinations of drilling muds used at different times during their formation. They also exhibit heterogeneity over the surface of a single pile. Due to disturbances associated with sampling and mixing, the cuttings materials used in the experiment were not truly representative of conditions at any single point on the surface of the two piles involved, but were selected to provide representative and contrasting conditions. Thorough mixing of the cuttings material was conducted prior to experiment set-up, in order to minimise heterogeneity in the experimental chambers.

4.2 Survival and growth

Survival of *Capitella* was fairly good in all of the treatments after six weeks (T1), and was highest in 20% Ekofisk and lowest in 100% Ekofisk cuttings and in the reference sediment. At the end of the experimental period, however, survival was highest in Ekofisk cuttings, especially 100% Ekofisk. Reasons for the death of *Capitella* in the reference sediment and Beryl cuttings could include starvation or toxicity or other unknown factors, but the changes in survival that occurred between T1 and T2 would indicate starvation to be the primary factor. Survival on Beryl cuttings would perhaps be better if an external food source were available. Westerlund *et al* (2000) found densities of 2000 ind/m^2 on the cuttings pile, but these abundances were less than half those recorded at Ekofisk. A decision was made not to add food to the experimental chambers, as a build-up of organic matter would affect THC and redox data and confuse the results. The results indicate that the Ekofisk cuttings jars provided, directly or indirectly, a source food for the *Capitella*, which was probably bacterial. Linke-Gamerick *et al* (2000) found that survival and reproduction in *Capitella* sp 1 were affected little, if at all, by high PAH (fluoranthene) exposures, and concluded that the species is particularly well adapted to persist in stressed (eg hypoxic or sulfide-rich) environments.The *Capitella* in the present experiment appeared to thrive and to be reproducing in the 100% Ekofisk cuttings. Average worm length appeared to decrease during the experiment. Although this may be an artifact of the measuring process, energy-limited *Capitella* are known to use stored reserves to meet their energy needs (Forbes *et al* in Méndez *et al*, 2001)

Although there was a loss of *Abra* immediately after arrival at the laboratory, those that survived were kept for a considerable time without further losses prior to the start of the experiment, and so were not considered to be moribund. *Abra* also showed at least moderately good survival in all of the treatments at six weeks, but at 14 weeks survival was only significant in the reference sediment and the 20% Ekofisk cuttings. Survival in the reference sediment indicates that the lack of provision of food was not a significant factor; *Abra* can survive for some time without feeding (David Murden, pers comm). The individuals that survived appeared to have lost biomass. Toxicity of the cuttings material is likely to be the main influencing factor in *Abra* survival

Even though the THC level of the Ekofisk cuttings is higher than the Beryl, both of the test organisms seemed to thrive better in the Ekofisk cuttings. Apparently, the toxicity of the Beryl material is higher despite the lower measured THC. This is also evident from both small and meso-scale degradation studies (RF-2201/217, SINTEF STF66A01139).

4.3 Burrowing behaviour and disturbance of sediment

The different cuttings/sediment treatment levels were clearly distinguished by the responses of the invertebrate species, in terms of the degree and speed of burrowing, the depth of burrowing (*Capitella*), and the degree of disturbance of the sediment.

Both species readily burrowed into the reference sediment, the *Capitella* rapidly forming vertically oriented burrows and reaching the bottom of the jar (50 mm). With both species, the surface became more granular with the production of faecal pellets, and the *Abra* jars showed general disturbance of the surface layers.

For both of the 100% cuttings treatments, *Capitella* burrowing was slow to start, and then limited to the top 10 mm, with most burrows aligned horizontally and in the top 5 mm. The degree of *Capitella* burrowing had developed between T1 and T2. The population in the 100% Beryl jars had declined over the same period, due to lack of a suitable food source or to toxicity. The surface of the 100% Ekofisk cuttings was altered by the presence of granular faecal pellets, but this was not observed for the 100% Beryl cuttings.

Abra showed little or no willingness to burrow into 100% Beryl cuttings. Some burial attempts were seen for 100% Ekofisk cuttings, but no significant disturbance of the sediment was observed. These observations again indicate that Beryl cuttings were more toxic than the Ekofisk cuttings.

As may be expected, the 20% cuttings mixtures elicited intermediate responses from the invertebrates. Burrowing by *Capitella* was quicker in 20% Ekofisk than in 20% Beryl cuttings, and the surface of the former mixture also displayed a change in surface texture at 14 weeks due to the actions of the worms. The 20% Ekofisk mixture was also more favoured than the 20% Beryl mixture by the *Abra*, the majority of which became established in the 20% Beryl cuttings and caused mixing and oxygenation of the top 15-20 mm of the sediment.

Factors that could influence burrowing behaviour and depth include redox potential and toxicity of the cuttings material. The depth of burrowing of *Capitella* in 100% Ekofisk coincided with the depth at which reducing conditions commenced, and more individuals were found at the bottom of the jar in the Beryl cuttings, which exhibited higher redox values. Burrowing behaviour also indicates Beryl cuttings are more toxic than Ekofisk.

Depths of colonisation observed in the small-scale experiments cannot be reliably extrapolated to the field because of the disturbances associated with sampling and the

mixing of the cuttings during experimental set-up. Rather, they should be related to cuttings type, THC concentrations and redox potentials.

The observed depth and orientation of the *Capitella* burrows in the different treatments is consistent with other studies. Madsen *et al* (1997) and Holmer *et al* (1997) indicated that reworking influence on the sediment by *Capitella* sp 1 may be restricted to the top 10 to 20 mm of the sediment. However, Madsen *et al* (1997) also showed that at very high densities (30,000 ind/m^{-2}) in a microcosm it had a profound effect on sediment appearance and reworked the sediment in a conveyor-belt fashion. A light brown oxidised later of coarser texture, consisting mainly of faecal pellets, occupied the top 15 to 20 mm. The separation between anoxic and oxic sediment was not as apparent as in uninhabited microcosms, and sediment near worm burrows was often an intermediate grey colour. Burrows occurred most frequently in the vicinity of the redox profile discontinuity (RPD), which was most distinct at the base of the pellet layer. The burrows tended to be more vertically oriented within the upper pelletised area and more horizontally oriented near the RPD.

Evidence of bioturbation in the experimental jars was present in the form of the presence of a pelletised layer at the surface, mixing of the surface layers, and unevenness of the surface from *Abra* burrowing activity. These effects were noted for the Ekofisk and reference sediment jars. The experiment did not include study of rates of bioturbation. Small polychaetes like *Capitella* are not expected to have a great bioturbatory potential. Also, in conditions in OBM-contaminated cuttings, bioturbation will be limited because of the restrictions on burrowing depth. Mendez *et al* (2001) recently investigated sediment processing rates of five species of *Capitella*. The experiments were carried out in the presence of the PAH fluoranthene. Processing rates were higher for larger species of *Capitella*, but the small *Capitella* sp 1 had a relatively high size-specific processing rate of 12 x body wt/day. Each worm processed an average of 5.3 mg of sediment per day. The fluoranthene concentrations used, representing moderately to highly contaminated conditions, had only marginal effects on sediment processing and growth rates of the *Capitella* spp.

4.4 Effects on sediment chemistry/degradation rates

The redox data obtained clearly distinguished the different types and concentrations of cuttings. Even at the start of the experiment, redox values were lower in the Ekofisk cuttings than in the other treatments, and within less than a week the values in the Beryl jars had also fallen to less than those in the reference sediments. After the first week, values stabilised in all the experimental jars and no further change with time was demonstrated. Throughout the experiment, values were consistently lower in the Ekofisk cuttings than in the Beryl cuttings, and for both types of cuttings the 20% cuttings mixture displayed higher values than the 100% cuttings. Redox values show a correlation with THC levels. In addition to differences in hydrocarbon content, differences in particle size may have been an influencing factor in differentiating the cuttings from the reference sediment, which was noticeably coarser in texture.

No clear effects of the presence of *Abra* or *Capitella* on redox potential were demonstrated by the experiment. However, for the Ekofisk cuttings, there was some evidence that the *Capitella* jars had higher redox potential values than the *Abra* jars or the controls, which could be a result of burrowing activity, but further data would be required to demonstrate the significance of the differences.

In the microcosm degradation sub-task (RF-2001/217), closed systems were used, and hence depletion of THC due to factors other than degradation was expected to be at a minimum. In open flow-through systems as used in the present experiment, leaching could be expected to occur. Only limited leaching was observed, however, in the mesocosm experiments (SINTEF-STF66A01139), and only from the top few mm of the Beryl cuttings. There were no obvious trends of decreasing THC levels in the current experiment, although with the limited accuracy of the THC data such processes may have been masked. The presence of macrofauna apparently had little impact on THC degradation and/or depletion as no obvious differences between the *Capitella, Abra* and control jars were seen for any of the cuttings types. However, degradation could have been masked because the THC samples were taken from the top 40 mm of the sediment, ie beyond the depth at which burrowing occurred in the cuttings. Although feeding and burrowing activities can increase fluxes of contaminants into the water column, they can also lead to their burial deeper in the sediment (Kjeilen *et al*, 1999).

Capitella spp have been shown to promote the degradation of organic matter in sediments. For example, Chareonpanich *et al* (1994) demonstrated the effectiveness of *Capitella* sp 1 in treating organically polluted fish-farm sediments. The organic matter was very efficiently decomposed and oxidation of the reduced sulphides in the sediment was also promoted. *Capitella* sp 1 has also been demonstrated to stimulate microbial activity and degradation of PAH/crude oil in sediments (Holmer *et al*, 1997; Madsen *et al*, 1997; Bauer *et al*, 1988; reviewed in Kjeilen *et al*, 1999).

5 Conclusions

Survival and burrowing behaviour of both *Capitella* and *Abra* showed clear differences on the two types of cuttings examined: PBM/WBM (Ekofisk) and OBM (Beryl). The Beryl cuttings material was shown to be less favourable to the animals than the Ekofisk cuttings, despite containing significantly lower THC levels (approximately 2500 ppm or less compared to approximately 70,000 ppm). These differences were also present in diluted (20%) cuttings.

Capitella sp 1survived well in the Ekofisk cuttings to the end of the experiment (14 weeks), and this was probably because it could utilise the hydrocarbons in the cuttings, directly or indirectly, as a food source. *Capitella* appeared to thrive in the 100% Ekofisk cuttings, at THC levels in the region of 70,000 mg/kg, although the experiment was not long enough to allow the development of generations. Survival in Beryl cuttings and the reference sediment was initially good, but had declined by the end of the experiment, apparently due to lack of food (none was added during the experiment), although the toxicity of the cuttings may have been a factor in the Beryl treatments. The potential for colonisation of OBM piles may therefore be dependent on the availability of external food sources.

Burrowing depth of *Capitella* in cuttings material was clearly limited compared to clean sediments, and showed distinct variation with both cuttings type and concentration. Burrows were mostly limited to the top 5-10 mm in the 100% cuttings and the top 20-30 mm in the 20% cuttings, although for both concentrations penetration was slightly deeper in the Beryl cuttings. Burrowing depth appeared to be related to redox potential, which in turn correlated with THC concentration.

The more sensitive species, *Abra alba*, only survived in diluted PBM/WBM cuttings, and OBM cuttings were toxic even at THC concentrations in the region of 100 to 200 mg/kg. Information is needed for more species, but the results indicate that there is a greater potential for the development of more stable communities on PBM/WBM cuttings piles than on OBM piles, and that THC concentrations for the former type may still be too high at present.

Evidence of sediment disturbance related to both species was apparent in terms of surface layer mixing and alterations in surface texture, and was greater for the PBM/WBM cuttings than for the OBM cuttings.

Although no significant effects of *Abra* or *Capitella* activity on redox potentials were demonstrated, for the Ekofisk cuttings there was evidence of enhancement related to *Capitella* presence.

THC data were not expected to be important in demonstrating combined degradation and depletion over the short timeframe of the experiment, due to a high degree of variability in analytical results. No effects of macrofaunal presence on THC degradation were demonstrated, and detectable effects may only occur in the top few mm of the cuttings material.

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Appendix 1

Full redox and pH data

Appendix 2

Copies of all of the recording sheets completed during the course of the experiment.

Appendix 3

Animal counts, size

Appendix 4

Photos of *Abra*, Time series

Appendix 5

Capitella photos