



Macrofaunal colonization of mine tailings impacted sediments

Hilde C. Trannum^{a,b,*}, Rita Næss^a, Hege Gundersen^a

^a Norwegian Institute for Water Research, Gaustadalléen 21, NO-0349 Oslo, Norway

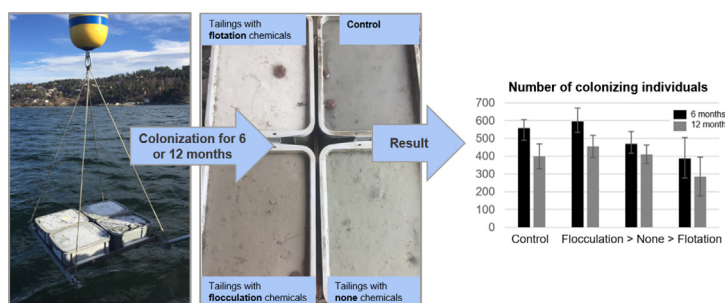
^b University of Agder, Center for Coastal Research, NO-4604 Kristiansand, Norway



HIGHLIGHTS

- Effects of mine tailings on benthic colonization were experimentally studied.
- All sediments capped with mine tailings were successfully colonized within 6 months.
- Abundances of annelids were lower, while mollusks were higher in tailings treatments.
- Fine-grained tailings with remnants of flotation chemicals had most profound effects.
- In the mixing zone outside the STD, the extent of effects will vary between tailings.

GRAPHICAL ABSTRACT



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ABSTRACT

An experiment was conducted to study and compare macrofaunal colonization of thin layers of mine tailings. Experimental boxes filled with marine sediments capped with mine tailings were placed on the seabed and subject to colonization for six and twelve months. Three Norwegian mine tailings, representative of major production processes, were used. In addition, one set of boxes served as control and was not treated with tailings. The layer thickness of the tailings was supposed to represent the thickness in the transition zone between the sea deposit itself and unaffected sediments. The most fine-grained tailings, which also contained flotation chemicals, showed a significantly lower colonization than the control and the other treatments. At the same time, all sediments were successfully colonized and rich in species. In general, the abundance of annelids was lower, while the abundance of mollusks was higher in the tailings-treatments than the controls. There were larger differences in faunal densities between the controls and tailings-treatments after six than twelve months, probably due to coverage by natural sedimentation and mixing of the thin tailings layer with the sediment underneath throughout the experiment. As the tailings initiated varying degree of effects on the benthos, there is expected to be a difference in how far the effects will extend outside the sea deposit. This is the first study where the colonization potential is systematically compared between various tailings, and as colonization is assumed a frequent and important mechanism for faunal restitution after disturbance events, the results are important for the management of tailings placements as well as with regard to other forms of disturbances associated with defaunated areas, like dredging and disposal of contaminated sediments.

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* Corresponding author at: Norwegian Institute for Water Research, Gaustadalléen 21, NO-0349 Oslo, Norway.

E-mail address: hilde.trannum@niva.no (H.C. Trannum).

1. Introduction

Industrial mining is currently in a phase of growth and subject to new environmental laws (Ramirez-Llodra et al., 2015). Many mines are particularly challenged by waste management, as they often produce large quantities of mineral waste (surplus rock from mining and concentrator tailings) during resource extraction. To overcome problems associated with deposition of mineral waste, some mines place the tailings at the seafloor as submarine tailings disposal (STDs). Such under-water disposal has been considered favorable due to the predicted geochemical stability obtained by long-term storage under conditions that promote anoxic conditions (Arnesen et al., 1997; Dold, 2014). Further, in Norway and other coastal countries, sea deposits are considered a favorable option as several mineral ores are in the vicinity of the coast, with local fjord basins that can be filled up (Kvassnes and Iversen, 2013; Ramirez-Llodra et al., 2015).

The soft bottom fauna is the ecosystem component which is expected to be most affected by STDs (Ramirez-Llodra et al., 2015). Most members of the marine invertebrate benthos are infauna, which ingest, burrow in, and construct dwellings from sediment (Kline and Stekoll, 2001). Because of their low mobility and therefore constant contact with the sediment and interstitial water for most of their lives, benthic invertebrates are also sensitive indicators of disturbance effects (Pearson and Rosenberg, 1978; Gray and Elliott, 2009). Potential negative consequences of STDs include hypersedimentation, bioaccumulation of metals, toxicity from process chemicals and dissolved metals, increased turbidity and habitat modification including change in sediment properties like grain size, nutrient content and particle sharpness (Kline and Stekoll, 2001; Ramirez-Llodra et al., 2015; Morello et al., 2016a,b). Negative effects of mine tailings on benthic fauna have been documented in several studies, both on infauna and epifauna, and on biodiversity and functional attributes including functional diversity (e.g. Olsgard and Hasle, 1993; Ellis et al., 1995; Brooks et al., 2015; Trannum et al., 2018; Schaanning et al., 2019; Trannum et al., 2019).

From a management point of view, the environmental effects of STDs should be as small as possible in space and time. An STD can be up to several square kilometers, although totally defaunated sediments are restricted to the seabed which is directly impacted by the tailings plume (Ellis et al., 1995). In the transition zone with thinner layers outside the deposit itself, benthic organisms quickly start colonizing areas that have stabilized (Ellis et al., 1995). Further, after cessation of mining, the entire sea deposit will eventually be subject to recolonization. The rate of such colonization is a function of the natural recruitment potential of the different species and the survival of the recruits in the tailings-impacted sediments (Kline and Stekoll, 2001). The early life stages of benthic invertebrates are generally the most vulnerable (Thorson, 1946; Woodin, 1976; Jablonski and Lutz, 1983; Ramirez Llodra, 2002; Phillips and Shima, 2006; Hori et al., 2009), also regarding disturbance effects (Reish et al., 1976; Elmgren et al., 1983; Bonsdorff et al., 1990; Watzin and Roscigno, 1997; Thompson et al., 2007; Lewis et al., 2008). As pelagic larval recruitment accounts for the largest part of colonization on soft bottom sediments (McCall, 1977; Santos and Simon, 1980; Diaz-Castañeda et al., 1993; Lu and Wu, 2000; Van Colen et al., 2008), colonization experiments are a highly relevant method to study the benthic recovery potential after disturbances, particularly in the cases where defaunated areas frequently occur, like in the case with STDs, as well as for dredging and disposal of contaminated sediments and capping of polluted seabed.

The aim of the present study was to investigate the colonization potential of different tailings currently discharged to sea deposits

in Norway. The focus was on thin layers of tailings, in order to mimic the transition zone from the actual STD to the unimpacted area outside the deposit. In this zone, the effects are assumed to vary according to the particular tailing discharged (Trannum et al., 2018). Effects of thin layers have also been identified as a research-need regarding predictive modelling of the spatial extent of impacted sediments (Skei, 2010). Thus, we have used an experimental approach to:

- 1) Investigate and compare colonization of macrofauna in sediments treated with thin layers of tailings without process chemicals, with flocculation chemicals and with flotation chemicals
- 2) Investigate whether the effects of tailings on the colonization pattern differ between six and twelve months

The results will complement recently conducted studies with mature benthic communities which focused on effects in the actual field situation as well as in a mesocosm setup (Trannum et al., 2018; Trannum et al., 2019), in addition to studies addressing bioavailability and toxic effects of mine tailings (Brooks et al., 2018; Brooks et al., 2019). This is the first study where the colonization potential is systematically compared between various tailings, and as colonization is assumed a frequent and important mechanism for faunal restitution after disturbance events, the results are important for the management of STDs as well as with regard to other forms of disturbances associated with defaunated areas.

2. Materials and methods

2.1. Field work

Test sediments were collected on 9th March 2017 with a 0.1 m² van Veen grab in the outer part of the inner Oslofjord (59°38.574 N/10°37.728 E) with the research vessel “FF Trygve Braarud”. They were homogenized with a cement mixer and filled into 32 0.1 m² propene plastic boxes (height 15 cm), up to 3 cm below the upper edge of the box. The boxes were then frozen at −20 °C. The next day three different kind of mine tailings (described in Section 2.2. below) were added in a nominal layer thickness of 2 cm; 8 boxes of each treatment, in addition to 8 controls with no added material. The boxes were then frozen for additional 7 days. The purpose of the freezing was both to eliminate all living organisms and to reduce loss of material during deployment. Then one replicate of each treatment was mounted in each of eight aluminum frames (Fig. 1). 17th March 2017, the frames were carefully lowered to the seabed at a non-sloping, soft bottom seabed at 26–28 m depth, separated by 10–20 m, in the outer part of the inner Oslofjord (mean position of the frames 59°36'50 N/10°38'50 E). Ropes were attached to each corner of the frame and connected by a buoy approximately 2 m above the frame (Fig. 1). The frames were recollected after six and twelve months (19th September 2017 and 8th March 2018, respectively); 4 frames at each occasion. A Scuba diver put a cover on each box to avoid sediment resuspension and attached a rope to each buoy frame. Then the frames were lifted by the vessel's crane. At the day of the final recollection, four additional ambient sediment samples were collected with a 0.1 m² van Veen grab close to the frames (28.5–30 m depth), for comparison with natural faunal assemblages. For each box as well as the grab samples, one sediment subsample was collected with a hand-corer ($\Phi = 5$ cm) for analysis of grain size and total organic carbon (TOC) and total nitrogen (TN) (0–2 cm). The remaining sediment was sieved through a 1 mm sieve and fixed in 4% buffered formaldehyde stained with Rose Bengal. Munsell Color Chart®

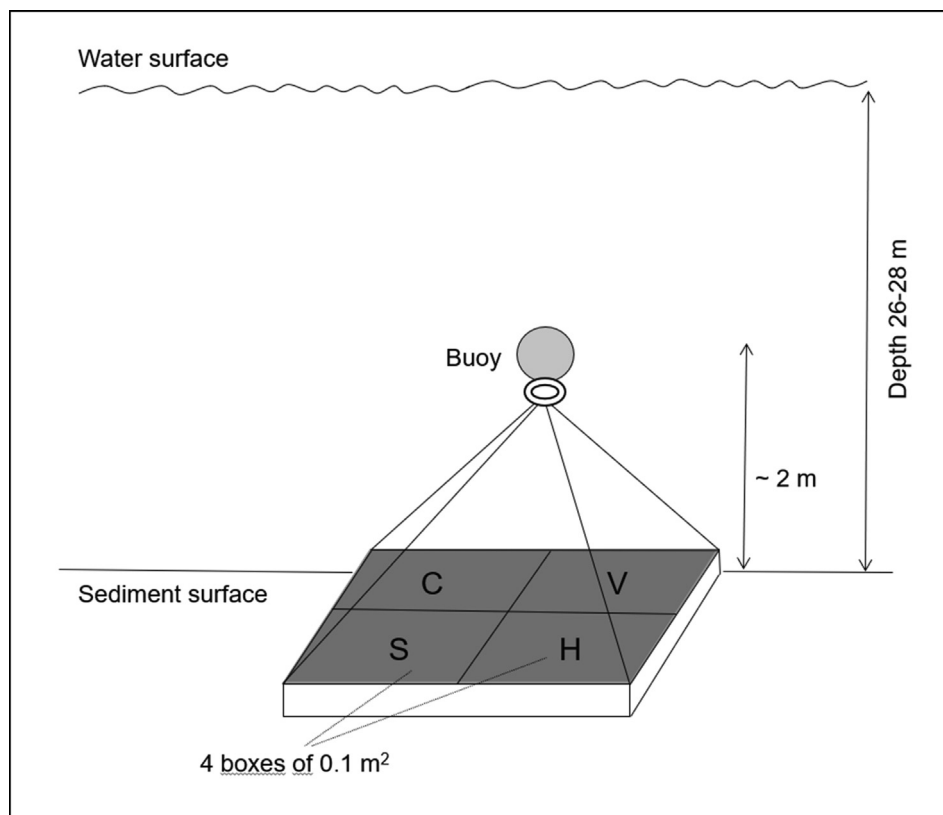


Fig. 1. Illustration of the experimental frames used for the colonization experiment with mine tailings. C = Control, S = Sibelco, V = Sydvaranger, H = Hustadmarmor.

was used to characterize the color of the sediment for the various layer thicknesses.

Mine tailings deposition has taken place or is presently taken place along the Norwegian coast from south to north, and the experiment was conducted with a fjord which is not subject to mine tailings deposition. The selected location in the Oslofjord has also previously been used in colonization experiments (Trannum et al., 2011 and references therein), which was advantageous with regard to existing knowledge on colonization and species patterns. There are no industrial discharges in this part of the fjord. The area is in general considered slightly enriched due to elevated levels of nutrients caused by runoff mainly from agriculture, but also from municipal discharges (Waldy et al., 2017). There is also a small freshwater outlet in the vicinity. In the fjords subject to mine tailings disposal, other sources of disturbances may also be present (available in <https://vann-nett.no/portal/#>). e.g. Frønfjorden, where the Hustadmarmor-tailings are discharged, hosts small municipal discharges and is also subject to discharge of organic material from a dairy. Ranfjorden, another fjord with a STD, but not the tailings tested here, is subject to discharges e.g. from other industrial activities as well as municipal wastewater. Moreover, the recipient for the Sydvaranger-tailings is also influenced by the introduced king crab (*Paralithdodes camtschaticus*). On the other hand, Stjernesundet, where the Sibelco-tailings are disposed, is not supposed to be subject to any other major disturbances. Thus, the degree of background disturbance will vary considerably among the fjords. Therefore, the major question is not the response in one particular fjord, but rather the more general patterns and the comparison between the different tailings. Furthermore, soft bottom communities have been shown to exhibit a high degree of redundancy at higher taxonomic levels and functional attributes

(e.g. Clarke and Warwick, 1998; Olsgard and Somerfield, 2000; Bremner et al., 2006).

2.2. Test materials

Three kinds of mine tailings were used in the experiment, representative of major production processes:

- 1) Without process chemicals (delivered from Sibelco Nordic AS, denoted S);
- 2) With flocculation chemicals (delivered from Sydvaranger Gruve AS, denoted V);
- 3) With flotation chemicals (delivered from Omya Hustadmarmor AS, denoted H).

A layer thickness of 2 cm was used, as this thickness represented a level where the effects on macrofaunal abundance and species number flattened out in a mesocosm-experiment on intact, benthic communities (Trannum et al., 2018). In the Sibelco-mine, the product (nepheline-feldspar concentrate) is separated from the nepheline-syenite rock from a process mainly involving magnetic separation, and no chemicals are added. The ore does not contain heavy metals above natural levels. Sydvaranger Gruve is an iron ore mine, and the tailings contain gangue minerals such as quartz (about 75%), amphiboles and feldspars. Metals are not elevated. The flocculation chemical Magnafloc (composed of poly-DADMAC and polyacrylamide) is added to the tailings. This mine is currently not operative. Omya Hustadmarmor AS receives marble mainly from an open pit mine, and liquid marble is the final product. The discharge consists of 40–50% calcium carbonate, and other minerals are quartz, feldspar, mica and small amounts of iron sul-

fide. Again, the tailings do not contain elevated levels of metals, but both flocculation chemicals (anionic polyacrylamide) and cationic flotation chemicals are used in the process. The flotation chemical is denoted FLOT 2015¹. A more comprehensive description of the tailings and the respective mines can be found in Trannum et al. (2018).

2.3. Laboratory analyses

Sediment fine fractions (<63 µm) were determined gravimetrically after separation from the coarse fraction by wet sieving. TOC and TN were determined based on chromatographic detection of CO₂ and N₂ gases, respectively, using a CHN (i.e. Carbon, Hydrogen, and Nitrogen) analyzer after removal of inorganic carbons by acidification.

The fauna was sorted in taxonomic groups (Annelida, Bivalvia, Gastropoda, Echinoidea, Asteroidea/Ophiuroidea, Crustacea and "Varia" i.e. other groups), transferred to 80% ethanol and then identified to species or lowest possible taxonomic level. Biomass determination was performed for each taxonomic group (wet weight), and for annelids separate biomass measurement was performed for free-living and tube-building species (where the weight includes the tube). First, the samples were placed on a filter paper, where the organisms were blotted dry for a few seconds, and then placed on a new filter paper which was placed on the weight. The weight was then read immediately; in gram with a resolution of four decimals.

2.4. Data analyses

The univariate statistical analyses of total abundance, total biomass, number of species as well as abundance and biomass of each taxonomic groups were performed using Generalized Linear Mixed-Effects Model (GLMM). Treatment (i.e. the test materials Control (C), Sibelco (S), Sydvaranger (V) and Hustadmarmor (H)) and time (i.e. 6 and 12 months) were included in the modelling as fixed factors. The GLMMs were conducted using the *lme4* library in R (version 3.5.2, R Core Team, 2018), which allows for random effects and family specifications. Interval responses (biomass) were assumed normal distributed (gaussian family, identity link), whereas integer responses (abundance and number of species) were assumed poisson distributed (poisson family, log link), and analysed with the *lmer* and *glmer* functions, respectively. A set of GLMs (library *glm*) were also run on the exact same models, to get standard errors on the predicted estimates in Figs. 2–4, since standard errors are not calculated in the *lmer* and *glmer* methods. All responses of biomass except free-living annelids were log_n-transformed due to lack of residual normality and/or homogeneity. Frame ID was treated as a random factor to account for a potential dependence between samples taken from the same experimental frame. Tukey's tests for multiple comparisons were performed using the library *multcomp*. The test compares the difference between each pair of means with appropriate adjustment for multiple testing.

The species matrix was analyzed with multivariate statistics using the Bray–Curtis similarity index (Bray and Curtis, 1957) calculated from fourth root transformed data. Fixed and random factors were the same as in the univariate analyses. A non-metric MDS-ordination was performed to visualize the faunal patterns. PERMANOVA was used as a permutation test (Anderson et al., 2008) to test effect of treatment and time on community composition, based on the same statistical design as above. Prior to PERMANOVA, the PERMDISP-test was used to check for homogeneity of

variances of the multivariate matrix. Further, pair-wise comparisons between treatments were performed with the PERMANOVA t-statistics. All analyses were done using the PRIMER package version 6.1.13 with the PERMANOVA+ version 1.0.3 add-on.

The ambient samples were not subject to any of the statistical tests, which focused on the treatment effects, but it was included in the nMDS-ordination in order to investigate how similar the colonized communities were to the mature communities. Level for statistical significance was set to $p \leq 0.05$ for all univariate and multivariate analyses.

3. Results

3.1. Visual observations

The surface of the sediment inside the boxes was completely undisturbed after collection, with a thin layer of clear water above the sediment. Also, the tailings layer was intact, and with some newly settled material on the top. More natural sediment was observed after twelve (approximately 5–6 mm) than six months (approximately 2–3 mm). There were some crawling organisms like hermit crabs, other crustaceans and annelids as well as sea urchins (*Strongylocentrotus droebachiensis*) and small brittle stars on the surface. Also, tracks of animals and burrows were visible. The tailings from Sibelco and to some extent Sydvaranger had a very high degree of compaction, while the Hustadmarmor-boxes and controls had a looser surface. During sieving of the Hustadmarmor-boxes, there was some foaming, which was not observed for the other treatments. One of the Hustadmarmor-boxes recaptured in March also had a strong smell, and weaker smell was observed for some of the other boxes from other treatments recaptured both times. Further, two dead sea urchins (*Brisopsis lyrifera*) were recorded in one of the Hustadmarmor-boxes recaptured in September, and one in a Sibelco-box recaptured in March and one in September.

3.2. Sediment characteristics

The control sediment had a fine-fraction of 55%, and the Sibelco and Sydvaranger material was somewhat coarser (Table 1). On the other hand, the fine fraction of Hustadmarmor was as high as 97%. Total organic carbon (TOC) was below the detection limit of 1 µg/mg in the Sibelco and Sydvaranger materials. Hustadmarmor had a higher TOC-content, but this was probably due to incomplete removal of inorganic carbon in the analysis (Trannum et al., 2018). In the experimental treatments, the fine fraction ranged from 46 to 71%, and TOC from 3 to 39 µg/mg. The difference in fine fraction between the treatments was less after twelve than six months. Total nitrogen (TN) is not shown in the table as it was below detection limit in all three tailings, and ranged from 1 to 1.6 µg/mg in the control sediment (test materials and boxes).

3.3. Species composition and univariate patterns

In total 14 244 individuals and 198 species taxa were recorded in the boxes. The boxes recaptured after six months contained in total 8 039 individuals and 130 species, whereas the boxes recaptured after twelve months contained 6 205 individuals and 154 species. Annelids were the numerically dominant phylum, and the small tube-building annelid *Pseudopolydora paucibranchiata* was by far the most abundant species, followed by the tube-building annelid *Galathowenia oculata* (Table 2). The abundance measured for each box ranged from 168 to 676, whereas the number of species ranged from 33 to 57. While the abundance decreased from six to twelve months, the number of species was

¹ FLOT2015 is a fictitious name used for reasons of confidentiality.

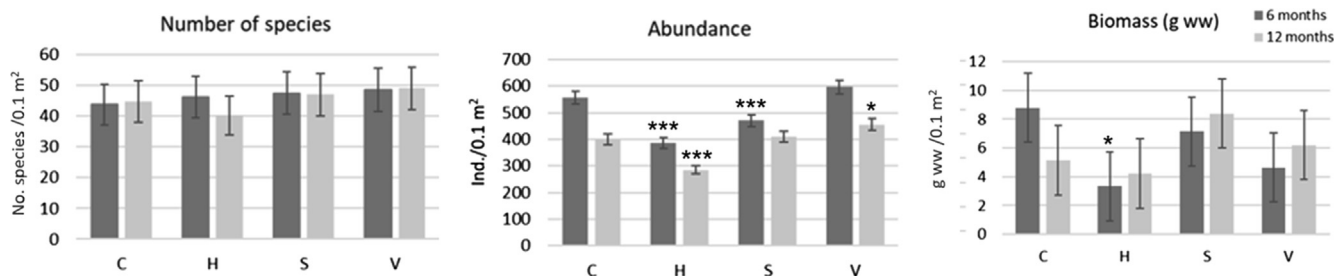


Fig. 2. Average number of species, abundance (number of individuals) and total biomass (Echinoidea excluded) in the experimental treatments. C = Control, H = Hustadmarmor, S = Sibelco, V = Sydvaranger. Estimates are predicted values (± 2 se) based on GLMs including additive effects between treatment and month (random effects are excluded to be able to get standard errors). Values significantly different from control (Tukey's tests, performed for 6 and 12 months separately) are indicated by asterisks; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

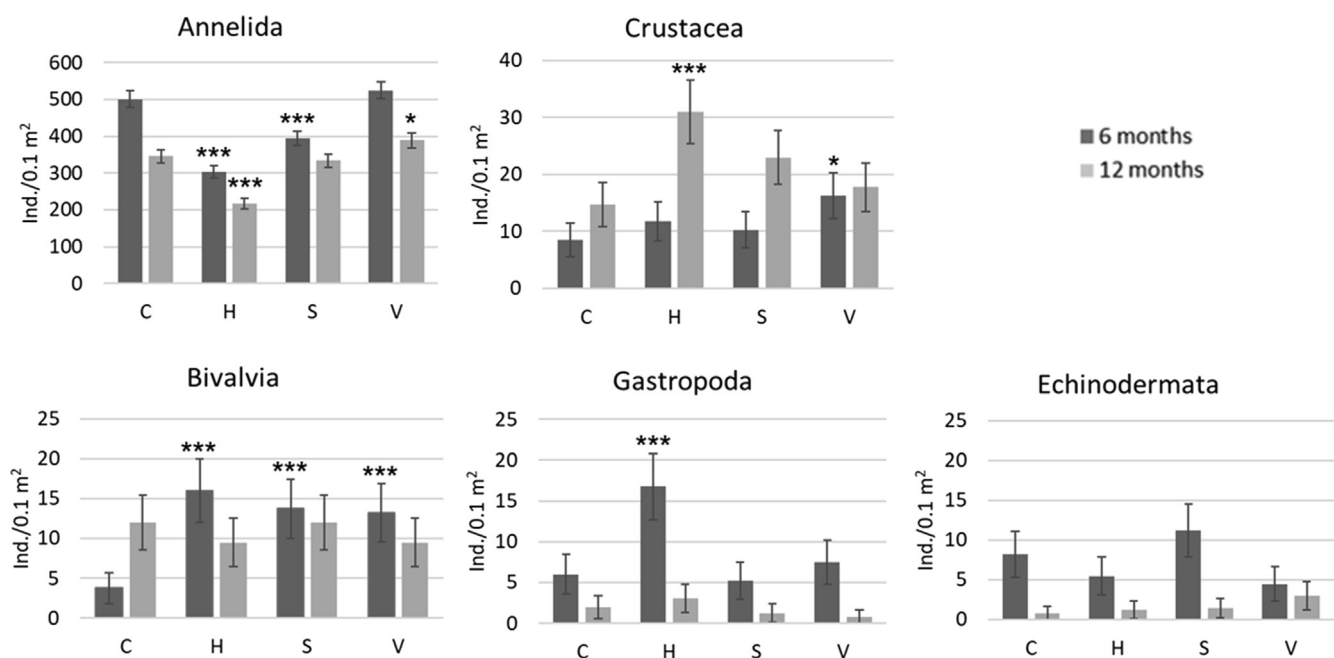


Fig. 3. Average abundance of main taxonomic groups in the experimental treatments. C = Control, H = Hustadmarmor, S = Sibelco, V = Sydvaranger. Estimates are predicted values (± 2 se) based on GLMs including additive effects between treatment and month (random effects are excluded to be able to get standard errors). Note different scale on the y-axis. Values significantly different from control (Tukey's tests, performed for 6 and 12 months separately) are indicated by asterisks; *** $p < 0.001$, * $p < 0.05$.

relatively stable. The ambient communities ranged from 260 to 445 individuals and from 29 to 41 species per grab. The complete species list is given in [Supplementary Material 1](#).

The GLMMs showed that there was a significant effect of treatment for total abundance, total biomass, biomass of free-living annelids, abundance of annelids, and abundance of gastropods (Table 3). Also, there was a significant effect of time for all measures of abundance, except bivalves. Only in two cases (total biomass and abundance of gastropods), there was a significant interaction, i.e. a time-specific effect of treatment (Table 3). The Tukey test revealed which of the pairs of mean that were significantly different, while adjusting for multiple testing. The complete list of Tukey comparisons is given in [Supplementary Material 2](#). For simplicity, only differences between control at tailings are shown in Figs. 2–4, where the main faunal patterns are presented. For total abundance, significantly fewer individuals were observed for Hustadmarmor and Sibelco compared to control after six months, and also after twelve months in Hustadmarmor (Fig. 2). Hustadmarmor also had significantly fewer individuals than the other treatments both after six and twelve months. On the other hand, Sydvaranger had significantly higher abundance than control

after twelve months, as well as higher than Sibelco after six and twelve months. For number of species and biomass, no significant differences between the control and treatments were observed. For biomass, there was a large variation also within the treatments, but it can be noted that all tailings-treatments, and Hustadmarmor and Sydvaranger, in particular, had lower biomass than the control after six months. Also, the biomass was significantly lower in Hustadmarmor than Sibelco after twelve months. Furthermore, the biomass decreased in the control from six to twelve months, was stable in Hustadmarmor, but increased in the last two tailings-treatments. Another finding to note, but not significant, was that number of species declined slightly in the Hustadmarmor-boxes from six to twelve months, but was stable in the other treatments.

For the taxonomic groups, both the Hustadmarmor- and Sibelco-boxes contained significantly fewer annelids than controls after six months, and for Hustadmarmor also after twelve months (Fig. 3). One of the species underlying this response was the small tube-building annelid *Pseudopolydora paucibranchiata*, which also was the numerically dominant species (Table 2). In the Hustadmarmor-boxes, it still had a lower abundance than the control after twelve months, but not markedly lower in the Sibelco-

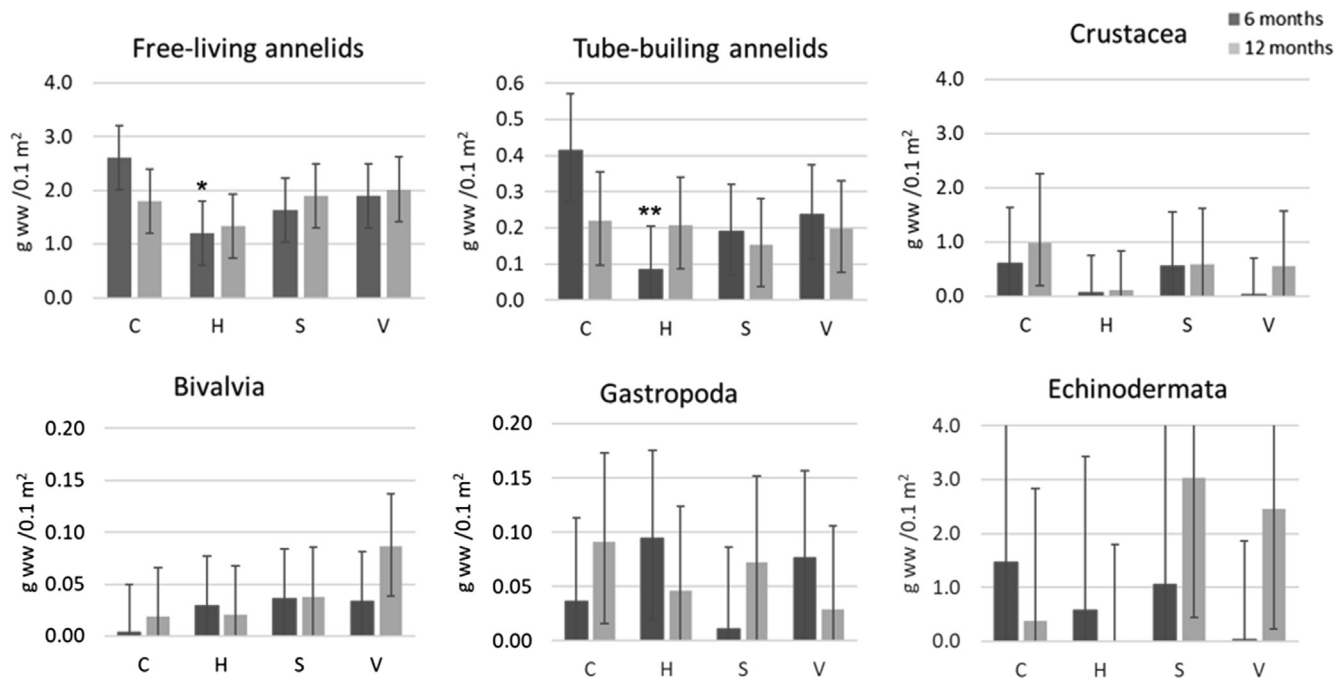


Fig. 4. Average biomass of main taxonomic groups in the experimental treatments. C = Control, H = Hustadmarmor, S = Sibelco, V = Sydvaranger. Estimates are predicted values (± 2 se) based on GLMs including additive effects between treatment and month (random effects are excluded to be able to get standard errors). Note different scale on the y-axis. Values significantly different from control (Tukey's tests, performed for 6 and 12 months separately) are indicated by asterisks; ** $p < 0.01$, * $p < 0.05$.

Table 1
Sediment fine fraction (% < 63 μ m) and content of total organic carbon (TOC, μ g/mg) of the test materials and in the boxes after 6 and 12 months (0–2 cm, average per treatment). C = Control, H = Hustadmarmor, S = Sibelco, V = Sydvaranger, A = Ambient. Values for the test materials are taken from Trannum et al. (2018).

| | Test materials | | 6 months | | 12 months | |
|---|----------------|------|---------------|------|---------------|------|
| | Fine fraction | TOC | Fine fraction | TOC | Fine fraction | TOC |
| C | 55 | 14.6 | 53 | 12.5 | 66 | 16.0 |
| H | 97 | 6.4 | 71 | 9.7 | 65 | 39.0 |
| S | 41 | <1 | 46 | 3.0 | 52 | 8.9 |
| V | 40 | <1 | 53 | 3.5 | 54 | 3.4 |
| A | | | | | 41 | 4.7 |

treatment. The annelid *Ophelina acuminata*, which had a low overall abundance, was not recorded in the Hustadmarmor-boxes after six months (Table 2). On the other hand, the annelids *Amphitrite cirrata* and *Therochaeta flabellata* seemed to increase in the Hustadmarmor-boxes. Another finding was that number of annelids was higher in the Sydvaranger-boxes than the control after twelve months (Fig. 3). Between the tailings-treatments, annelids had significantly higher abundance in Sydvaranger than the other two treatments, and in Sibelco than Hustadmarmor ($p < 0.001$ for all differences, see Supplementary material 2). Number of bivalves, and in Hustadmarmor also gastropods, was significantly higher in all tailings-treatments than in the control after six months (Fig. 3). After six months, gastropods were also significantly higher in Hustadmarmor than the other two treatments. Furthermore, number of crustaceans was significantly higher in Sydvaranger than the control after six months. Hustadmarmor contained more crustaceans than the control and Syd-Varanger after twelve months. No significant differences were observed for echinoderms between the controls and the treatments, but Sibelco had significantly more echinoderms than Syd-Varanger after six months.

With regard to biomass, there was generally high variance between the boxes also within the treatments (Figs. 2, 4), and less significant differences were observed than for the abundances. Nevertheless, there were significantly lower biomass of free-

living and tube-building annelids in the Hustadmarmor-boxes than control after six months (Fig. 4).

3.4. Multivariate pattern

In the nMDS-ordination of the faunal communities (Fig. 5), the four ambient samples were isolated in the right side of the plot. Then, there was a grouping of the experimental boxes according to time; where the boxes recaptured after twelve months were more similar to the ambient communities than the boxes recaptured after six months. Furthermore, after 6 months, the controls were aligned outside the other boxes, and also the Hustadmarmor-boxes were to some extent placed in the periphery of the other boxes. After twelve months, the Hustadmarmor-boxes were again placed outside the main group. Also, one of the Sibelco-boxes and one of the controls were isolated. Lastly, the boxes were more similar to each other after six than twelve months.

The differences in faunal composition were tested in PERMANOVA (Table 4). Here a significant effect of both experimental treatment and time was evident. On the other hand, the interaction between treatment and time was not significant. The pairwise test showed that there was a significant difference between the control and Hustadmarmor-boxes (Table 5). Moreover, the difference between Sydvaranger- and Hustadmarmor-boxes was significant.

Table 2

Average abundance (number of individuals per 0.1 m²) of the most dominant species for the recolonized and ambient communities in Oslofjorden in 2017/2018. Species selection is based on the eight most dominant species per treatment. C = Control, H = Hustadmarmor, S = Sibelco, V = Sydvaranger, A = Ambient. The listed species constitute at least 34% of the total abundance. See [Supplementary Material 1](#) for a complete list of species.

| 6 Months | | | | | | |
|---------------------------------------|------------|------|------|------|------|------|
| Species | Group | C-6 | H-6 | S-6 | V-6 | A-6 |
| <i>Pseudopolydora paucibranchiata</i> | Annelida | 262 | 69 | 123 | | 235 |
| <i>Galathowenia oculata</i> | Annelida | 88 | 70 | 110 | | 134 |
| <i>Scalibregma inflatum</i> | Annelida | 57 | 37 | 22 | | 37 |
| <i>Amphitrite cirrata</i> | Annelida | 8 | 41 | 49 | | 19 |
| <i>Edwardsia</i> sp. | Anthozoa | 23 | 23 | 26 | | 23 |
| <i>Prionospio fallax</i> | Annelida | 17 | 15 | 18 | | 20 |
| <i>Therochaeta flabellata</i> | Annelida | 3 | 15 | 18 | | 14 |
| <i>Hermania Scabra</i> | Gastropoda | 5 | 16 | 5 | | 7 |
| <i>Jasmineira caudata</i> | Annelida | 13 | 10 | 12 | | 14 |
| <i>Ophelina acuminata</i> | Annelida | 10 | 0 | 1 | | 6 |
| 12 months | | | | | | |
| Species | Group | C-12 | H-12 | S-12 | V-12 | A-12 |
| <i>Pseudopolydora paucibranchiata</i> | Annelida | 172 | 85 | 155 | 159 | 127 |
| <i>Galathowenia oculata</i> | Annelida | 59 | 51 | 68 | 103 | 96 |
| <i>Jasmineira caudata</i> | Annelida | 36 | 20 | 23 | 32 | 7 |
| <i>Anobothrus gracilis</i> | Annelida | 0 | 1 | 0 | 1 | 31 |
| <i>Edwardsia</i> sp. | Anthozoa | 14 | 16 | 26 | 20 | 3 |
| <i>Ampelisca tenuicornis</i> | Annelida | 8 | 23 | 12 | 9 | 9 |
| <i>Scalibregma inflatum</i> | Annelida | 10 | 7 | 14 | 17 | 0 |
| <i>Prionospio fallax</i> | Annelida | 8 | 8 | 13 | 14 | 11 |
| <i>Chaetozone setosa</i> | Annelida | 9 | 6 | 9 | 11 | 14 |
| <i>Nemertea</i> indet. | Nemertini | 5 | 4 | 6 | 6 | 10 |
| <i>Amphitrite cirrata</i> | Annelida | 7 | 3 | 9 | 4 | 0 |
| <i>Therochaeta flabellata</i> | Annelida | 5 | 9 | 2 | 5 | 0 |
| <i>Thvasira sarsii</i> | Bivalvia | 0 | 0 | 0 | 0 | 8 |

Table 3

Results based on p-values for all variables included in the GLMMs. *** p < 0.001, ** p < 0.01, * p < 0.05, · p < 0.1, ns = not significant.

| Response | Treatment | Time | Treatment:Time |
|------------------------|-----------|------|----------------|
| No. species | · | ns | ns |
| Abundance | | | |
| Total abundance | *** | * | ns |
| Annelida | *** | ** | ns |
| Crustacea | ns | * | ns |
| Echinodermata | ns | ** | · |
| Bivalvia | ns | ns | · |
| Gastropoda | *** | *** | ** |
| Biomass | | | |
| Total biomass | * | ns | * |
| Free-living annelids | * | ns | ns |
| Tube-building annelids | · | ns | ns |
| Bivalvia | ns | ns | ns |
| Crustacea | ns | ns | ns |
| Echinodermata | ns | ns | ns |
| Gastropoda | ns | ns | ns |

4. Discussion

4.1. General colonization pattern

In general, the boxes were species-rich and had a high abundance. The first six months from spring to autumn were sufficient time to establish species-rich communities. This result accords with a previous colonization experiment performed from March to September at the same location (Trannum et al., 2011). The time and frequency of larval recruitment is an important factor in determining benthic community structure, especially during the early stages of succession (Rosenberg, 1976). Larval settlement is generally highest during spring/summer, and also in previous colonization experiments abundance and number of species colonizing defaunated sediments were highest in this period (Arntz and Rumohr, 1982; Zajac and Whitlatch, 1982; Bonsdorff and

Österman, 1985; Lu and Wu, 2007). Also in compliance with earlier recruitment studies, annelids were the most abundant group (McCall, 1977; Berge, 1990; Diaz-Castañeda et al., 1993; Olsgaard, 1999; Trannum et al., 2004; Trannum et al., 2011).

The abundance decreased from six to twelve months, indicating stronger biological interactions through time. Number of species was more or less constant. In the nMDS-plot a grouping according to time was evident (Fig. 5), and the boxes recaptured after twelve than six months were most similar to the ambient communities which indicates a succession through time towards the climax community. However, also an effect of seasonality may have been involved for this pattern, as the ambient communities only were sampled after twelve months, and possibly may have changed slightly through time. Notably, a larger variance in the colonized communities was observed after twelve than six months, also for the controls. This may be caused by an initial colonization with the same set of typically opportunistic species. Then, when biological interactions become stronger and also more specialized species are established, more patchiness seems reasonable. The composition of early colonists often alters the community structure at later succession stages (Van Colen et al., 2008; Como and Magni, 2009), which supports such explanation.

After twelve months, the colonized communities contained approximately the same number of individuals, but slightly more species than the ambient communities. This somewhat higher species number may again be due to the fact that biological interactions were still less strong in the boxes than in the mature community. The most abundant colonizers in the controls after both six and twelve months were the tube-building annelids *Pseudopolydora paucibranchiata* and *Galathowenia oculata* (Table 2). *P. paucibranchiata* is generally tolerant towards high organic load, and is typically among first- or second-order colonizing species (Pearson and Rosenberg, 1978; Borja et al., 2000). *G. oculata* is somewhat less tolerant, but may still be facilitated by slight disturbance, according to its classification in AMBI (AZTI Marine Biological Index) (Borja et al., 2000). It can also be recorded when there

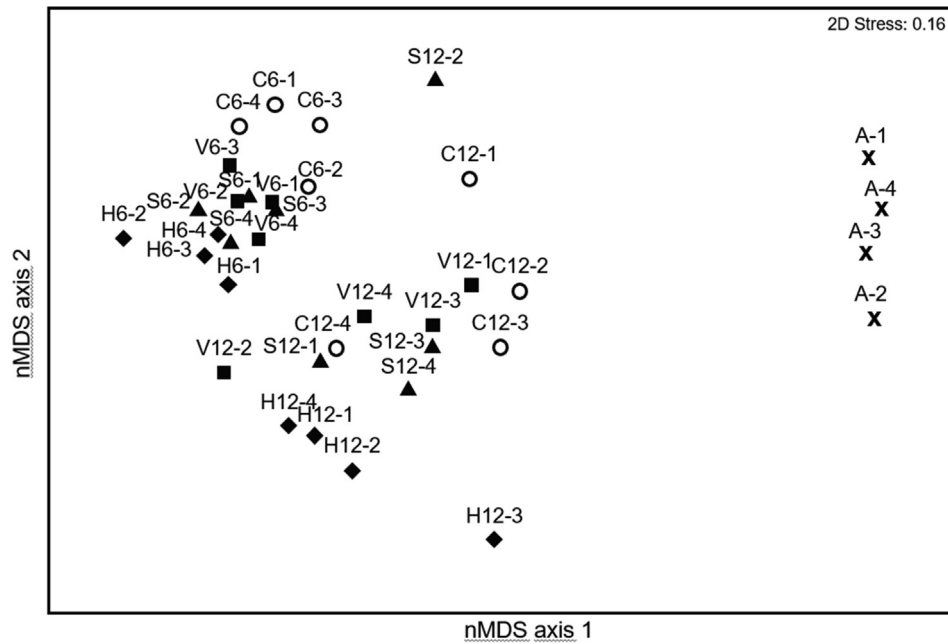


Fig. 5. nMDS-ordination of recolonized faunal communities (fourth root transformed data, Bray Curtis similarity); o = C (Control), ◆ = H (Hustadmarmor), ▲ = S (Sibelco), ■ = V (Sydvaranger); first number = month (6, 12), second = frame (1, 2, 3, 4), x = A (Ambient).

Table 4

Summary of three-way PERMANOVA for the recolonized communities, using Bray–Curtis distances. Values in bold indicate significant differences; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, $p < 0.1$ (p-values calculated by permutations of residuals under a reduced model).

| | df | SS | MS | Pseudo-F | P (perm) |
|-------------------|----|--------|-------|----------|------------------|
| Treatment | 3 | 3897 | 1299 | 1.926 | 0.0005*** |
| Time | 1 | 6270 | 6270 | 6.178 | 0.0001*** |
| Frame | 6 | 6089 | 1015 | – | – |
| Treatment × Time | 3 | 2460 | 820.0 | 1.216 | 0.1251 |
| Treatment × Frame | 18 | 12,140 | 674.5 | – | – |
| Total | 31 | 30,857 | | | |

Table 5

Pairwise PERMANOVA-tests for the recolonized communities, using Bray–Curtis distance. Values in bold indicate significant differences; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, $p < 0.1$ (p-values calculated by permutations of residuals under a reduced model). C = Control, H = Hustadmarmor, S = Sibelco, V = Sydvaranger.

| | t | P (perm) |
|------|-------|----------------|
| C, H | 1.783 | 0.0136* |
| C, S | 1.246 | 0.1567 |
| C, V | 1.309 | 0.1191 |
| S, V | 1.093 | 0.3275 |
| S, H | 1.214 | 0.1884 |
| V, H | 1.522 | 0.0335* |

are only a few species present (Rygg, 2002). The same species dominated in the ambient community, where also other tolerant species were present, like the annelid *Chaetozone setosa* and the bivalve *Thyasira sarsi*. Thus, the ambient Oslofjord benthic community is not completely pristine, and a shorter time for establishment of communities resembling the ambient community may be expected compared to a site with a completely non-disturbed native community. As mentioned above (Section 2.1), the area is considered slightly enriched in nutrients, which may have influenced on this pattern.

4.2. Effect of tailings

4.2.1. Overall faunal effects

The univariate (Figs. 2 and 3) and multivariate analyses (Fig. 5, Tables 4 and 5) indicated that thin layers of tailings affected the colonization pattern. Significantly fewer individuals had colonized the Hustadmarmor- and Sibelco-boxes than the control during the first six months, and for Hustadmarmor there were significantly fewer individuals also after twelve months. Moreover, Hustadmarmor had a lower abundance than the other two tailings treatments both after six and twelve months, while Sibelco had a lower abundance than Sydvaranger. For number of species and biomass, no significant differences between the control and treatments were observed, but it can be noted that the Hustadmarmor-boxes showed the lowest species number and biomass of the various treatments after twelve months. In the multivariate test, only a significant difference was found between Hustadmarmor and the control, as well as between Hustadmarmor and Sydvaranger. Thus, the overall response was in general most pronounced for the Hustadmarmor-treatment, and weaker for Sibelco. For Sydvaranger, no significantly lower abundances compared to the control were observed; in fact the abundance was significantly higher than the control after twelve months. A similar response order of these three tailings was found in a multi-species mesocosm-experiment with the same tailings (Trannum et al., 2018) as well as in a sediment contact assay where survival of the amphipod *Corophium* sp. was measured (Brooks et al., 2019).

The difference in faunal abundances between the control and tailings-treatments was less pronounced after twelve than six months. The tailings were probably mixed with the sediment above and below the tailings layer by bioturbation throughout the experiment, and further, more natural sedimentation was observed after twelve than six months (see Section 3.1). Thus a dilution effect seems probable. This is supported by the grain size data which show more even values after twelve than six months (Table 1). On the other hand, the nMDS-analysis pointed to more distinct differences in the faunal composition after twelve than six months, particularly for the Hustadmarmor-treatment vs. the other treatments. As for the general colonization pattern discussed above, this finding is probably due to an initial colonization by typically tolerant, r-selected species, but later in the succession more k-selective species with more specific habitat requirements are established. E.g. the opportunistic capitellid annelid *Heteromastus filiformis* was only recorded after six months, while the terebellid annelid *Terebellides stroemi* was only recorded after twelve months.

In the mesocosm-experiment with the same three tailings (Trannum et al., 2018), the effects were in general more pronounced than in the present experiment. In that setup the layers were in general thicker and the doses were added four times to mimic a more continuous discharge situation. Repeated deposition may initiate more severe responses than single instances (Lohrer et al., 2004; Bolam et al., 2006). Furthermore, the mesocosm experiment was conducted with intact, mature communities, and only mortality was studied as colonization was not possible. While that experiment was considered to represent a worst-case scenario, the present experiment is on the other end of the scale with communities in a relatively early succession stage which were subject to colonization throughout the experiment, and with thin layers added only once and prior to faunal establishment. Nevertheless, as mentioned above, the impact order was the same in both experiments, i.e. most effects of Hustadmarmor, followed by Sibelco, and least for Sydvaranger.

The present study thus points to a rapid colonization of thin layers of mine tailings impacted defaunated patches. Also in field studies of STDs, a rapid initial colonization has been observed, but at the same time it can take up to several years or even decades before the fauna has returned to its original state (Olsgard and Hasle, 1993; Burd, 2002; Josefson et al., 2008; Schaanning et al., 2019). It is also important to be aware that the present study was designed to represent the transitional zones outside the STD rather than the deposit itself. Moreover, the tailings were present prior to faunal colonization, and the organisms were therefore not affected by smothering. Lastly, the defaunated patches were only 0.1 m², and the frames were surrounded by undisturbed sediment and fauna, thus providing high connectivity. Indeed both scale and frequency are highly important for recovery patterns after a disturbance (Miller, 1982; Thrush et al., 1996; Bolam et al., 2004; Svensson et al., 2009; Norkko et al., 2010), and recovery depends on the ability of undisturbed, surrounding sediments to supply recruiting larvae and migrating adults (Zajac and Whitlatch, 1982; Bolam and Fernandes, 2002). Thus, the results from the present study cannot be transferred to a situation where the deposition of tailings ceases and the entire STD of several tens of meters, typically a fjord basin, needs to be recolonized.

In a colonization study in Repparfjorden, a more pronounced effect of mine tailings was observed, with reduced colonization at layer-thicknesses lower than in the present experiment (Trannum, unpublished data). The different response compared to the present experiment may either be due to more harmful tailings and/or that this Arctic fjord hosts a more vulnerable fauna. The tailings had a high level of copper, but also a highly different grain size than the original substrate. However, independently of the underlying mechanisms for the observed difference in effects

between these experiments, it is clear that the response of tailings on benthic colonization may be site-specific.

The finding in the present as well as previous studies that the tailings differed in their degree of harmfulness on marine biota (Brooks et al., 2018; Trannum et al., 2018; Brooks et al., 2019), indicates that there will be a difference in how far the effects reach in the transition zone outside the deposit itself. This information should preferably be used in the STD management with the aim of minimizing the environmental impacts, through pre- and post-processing of tailings.

4.2.2. Differences between taxonomic groups

Annelids as a group showed a reduced colonization in the Hustadmarmor-boxes compared to control after both time-intervals, and also in the Sibelco-boxes after six months (Fig. 3). The species underlying this response was in particular the small, tube-building annelid *Pseudopolydora paucibranchiata*. After six months, the density in the Hustadmarmor-boxes was only one fifth compared to the controls. After twelve months, the difference was less, but still half of the controls. In a previous experiment, this species also showed reduced colonization in sediment capped with drill cuttings and in a coarse vs. a fine sediment (Trannum et al., 2011). Such response can be both due to habitat selection during settlement or due to different post-settlement migration or mortality. In general, organisms preferentially settle or accumulate in sediment treatments that characterize their natural adult habitat (Butman, 1987). Larvae of annelids with sedentary adult stages have been observed to be better adapted to discriminate among sediments compared to errant annelids (Gray, 1971), and tube-building species may exhibit more sediment-specific preferences during settlement (Pinedo et al., 2000; Duchêne, 2010). In Frænfjorden where the Hustadmarmor-tailings are deposited, a reduction of tube-building species in particular has been observed in tailings-impacted stations compared to less impacted (Brooks et al., 2015; Trannum et al., 2019), in accordance with the present experiment.

Of free-living annelids, *Ophelina acuminata* was reduced in all tailings-treatment, and again particularly reduced in the Hustadmarmor-tailings with only one individual recorded in total (Table 2, Supplementary Material 1). It lives as a deposit-feeding burrower. This species responded to drill cuttings in a previous colonization-experiment (Trannum et al., 2011), but it has been assumed to be relatively tolerant to disturbances (Josefson et al., 2009). Nevertheless, it was negatively affected by mine tailings.

A couple of annelid species increased in the tailings treatments; *Amphitrite cirrata* and *Therochaeta flabellata* (Table 2, Supplementary Material 1). Both these species mainly live as surface deposit feeders, as does *Pseudopolydora paucibranchiata*, and may have benefited from less interspecific competition.

Bivalves, and to a lesser extent gastropods and crustaceans, increased in the tailings-treatments. A similar response has been recorded in colonization-experiments with heavy metals and drill cuttings (Trannum et al., 2004; Trannum et al., 2011). This finding is interpreted as exploitation of available niches due to less biological interactions rather than a positive settlement cue by the tailings. A "trophic group amensalism" (Rhoads and Young, 1970) has been described between annelids and mollusks, and tube-building annelids may decrease growth and survival of bivalves through competition, physical harm and increased sedimentation (reviewed by Noji and Noji, 1991). *Pseudopolydora paucibranchiata* in particular exhibits interspecific interference with other species (Levin, 1982), and the decline in this species may thus have facilitated other species. Anyway, bivalves and crustaceans must have tolerated the tailings better than the annelids. The bivalve's shell may protect them for direct exposure to the environment, as well as the fact that they often feed on the sediment surface by a siphon.

Crustaceans usually live on the sediment surface, which may protect them from hostile conditions within the sediment.

For echinoderms, no significant responses were detected. A patchy distribution of large sea urchins resulted in large variances particularly in biomass (Fig. 4), but notably, dead *Brissopsis lyrifera* were recorded in some boxes with tailings from Hustadmarmor and Sibelco.

Also in tailings-impacted fjords, a gradient with lower dominance by annelids and high abundance of other taxonomic groups towards the vicinity of the discharge has been observed. For instance, in the vicinity of the Hustadmarmor-outlet in Frænfjorden, there is a high abundance of bivalves and partly crustaceans (DNV, 2014; Trannum et al., 2019), which accords very well with the present experiment. Moreover, in the old deposition-site for mine-tailings in Jøssinfjorden, there is a high abundance of bivalves and echinoderms compared to undisturbed fjords (Schaanning et al., 2019).

4.2.3. How do tailings affect the fauna?

Several underlying factors for the differences in the colonization pattern were probably involved, and it is not possible to distinguish between these. Firstly, as mentioned above, active substrate selection may explain some of the differences. If the sediment surface is physically or chemically altered or constantly unstable, the settlement cues might be missing and thereby prevent recolonization by the larvae (Menzie, 1984; Hyland et al., 1994; Shin et al., 2008; Lam et al., 2010). In addition, post-settlement mortality due to intolerable conditions or biological interactions like predation and competition can underly the responses, which explains the observed mortality of sea urchins. Lastly, some species actually increased in the tailings-treatments, which points to a benefit due to reduced settlement of other species and thereby less biological interactions as discussed above. For Sydvaranger there appeared to be an overall increased abundance after 12 months, i.e. a possible stimulation effect. Although there were no indications of a higher nutrient level in these boxes (Table 1), the presence of the flocculation-chemical may have stimulated bacterial degradation, and subsequently the macrofauna.

The finding of a most profound effect of the Hustadmarmor-tailings mainly points to an effect of the flotation chemical and/or the very fine-grained sediment (Table 1). The flotation chemical contains a cationic tensioactive-type substance (foamer), which can be hydrolyzed in the environment. Even after twelve months, foaming was observed during sieving, which shows the presence of this substance. In addition to a toxic effect which was documented in the above mentioned sediment contact assay with *Corophium* sp. (Brooks et al., 2019), the chemical can be degraded and reduce the oxygen-level, in accordance with the strong smell of H₂S in one of the Hustadmarmor-boxes (see Section 3.1). Also in the Hustadmarmor-STD, such smell has been recorded (Trannum et al., 2019), and sediment profile images show blackening of the sediment indicating oxygen depletion (Schaanning et al., 2009). In the ecotoxicological assessment conducted by Brooks et al (2019), interference of the fine particles of Hustadmarmor with the gill epithelia was assumed to have contributed to the toxicity. This conclusion was based on a study where fine silt and clay sized mineral particles were responsible for cytotoxicity in gill epithelial cells of fish (Michel et al., 2014). Moreover, previous investigations on the filter feeding mussel *Mytilus edulis* positioned within 2 km of the discharge outlet showed that process chemicals were present in the tissue of mussels (Brooks et al., 2018). In addition, a suite of biomarkers measured in the mussels indicated a clear stress response including reduced fitness, which was correlated with chemical bioaccumulation and proximity to the discharge outlet (Brooks et al., 2018). Thus, the biological responses observed were interpreted as exposure to the suspended particles from the

discharge. A similar explanation seems reasonable also with regard to the effects observed in the present study, and is also supported by a strong decline in benthic suspension-feeders close to the Hustadmarmor-outlet (Trannum et al., 2019).

The fine Hustadmarmor-tailings are known to cause high turbidity in the receiving waters (Brooks et al., 2015), and may thus potentially harm also pelagic species. Based on these findings, Brooks et al (2019) particularly recommended to determine the interactions of Hustadmarmor fine tailings on gill epithelial cells of marine organisms. This should be conducted both for benthic and pelagic species.

The difference in grain size between the control and Hustadmarmor-boxes in particular, independently of associated chemicals, is considered to be a contributing factor for the difference in the colonization pattern observed here. Previous recolonization experiments have demonstrated that different grain size lead to different recolonization patterns (Trannum et al., 2011; Kanaya, 2014). A more homogenous sediment is also a typical characteristics for tailings deposition (Morello et al., 2016). A decrease in sediment heterogeneity was for instance documented in a field-gradient outside the Hustadmarmor-deposit in Frænfjorden (Trannum et al., 2019). Less heterogeneous sediment may reduce available niches and thus benthic biodiversity (Gray, 1974; Etter and Grassle, 1992), and a more homogenous sediment may also reduce sediment oxygen penetration (Näslund et al., 2012).

Another particle property that can be altered due to tailings deposition, is shape and angularity. In the SEM-EDX analysis carried out by Trannum et al. (2018), edged triangular and rectangular shaped particles were evident for all three tailings. Ingestion of sharp-edged mine tailings by the marine copepod *Calanus finmarchicus* was considered to have contributed to adverse effects in a test with Hustadmarmor-tailings (Farkas et al., 2017), and may likewise have been involved for the present responses as well.

Lastly, nutrient depletion can potentially explain the lowered faunal abundance and biomass in some of the tailings-treatments. Tailings are in general assumed to have a low organic matter content (Shimmield et al., 2010), here shown by less total nitrogen and total organic carbon in the tailings (except Hustadmarmor where the TOC probably also reflects some inorganic carbon) than the control-sediment. In the present study where the added layer was relatively thin, and the boxes were subject to natural sedimentation, nutrient depletion has probably been reduced throughout the experiment, in line with more pronounced effects after six than twelve months.

4.3. Conclusion and recommendations

The present study points to a rapid initial colonization and thus a rapid recovery potential of sediments capped with thin layers of mine tailings. The response of the tailings varied, with the strongest effect of sediments treated with fine-grained particles with remnants of flotation chemicals, which showed a significantly lower colonization than the control and the other tailings treatments throughout the one-year experiment. At the same time, all colonized communities were species rich, and there were no significant differences in species numbers. Among the taxonomic groups, annelids, and tube-building annelids in particular, were most sensitive to the tailings. The lower colonization of annelids in the tailings-treatments compared to controls was to some extent compensated for by increased colonization of other taxonomic groups and mollusks in particular.

It is important to be aware that the recolonized communities still were in a relatively early successional phase, and that effects not directly can be transferred to mature communities. It is also highly important to be aware that the scale was small both with

regard to the layer thickness and the sediment area subject to colonization; indeed to represent defaunated, small patches rather than an entire sea deposit.

The finding in this study as well as in previous studies that the faunal response to thin layers of mine tailings vary between different tailings, shows that the extent of effects in space and time will depend on the type of tailings disposed. This information should be used by the industry and the management, and is valid not only with regard to STDs, but also for DSTPs (deep-sea tailings placement). For instance, selection of the least harmful chemicals and optimization by preventing over-dosage of process chemicals and flotation chemicals in particular is necessary. Furthermore, as it was the most fine-grained material which was most harmful, it is important to minimize the spreading of this fraction into the receiving water bodies, e.g. in Frænfjorden, where the Hustadmarmor-tailings are discharged, fine particles have been recorded out to 3 km away from the discharge (Davies and Nepstad, 2017). Lastly, at the seabed, it is important that the deposit area is carefully selected, and that the tailings plume deposited on the seafloor is contained within the permitted impact area.

In addition to the particular issue with tailings placement, the results are relevant also with regard to deposition of other kinds of contaminated sediments as well as dredging for maintenance of channels and harbors and disposal of contaminated sediments. Although a rapid colonization seems to be universal, the colonization pattern may vary between different sediments, even with only thin layers of the deposited material. Lastly, the methodology used in the present experiment could be used to predict the responses of different materials with regard to their colonization potential and long-term succession, including materials used in capping of contaminated sediment in remediation purposes where it is an aim to reestablish faunal communities resembling the original community.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.134866>.

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