1

Characterisation of fine-grained tailings from a marble processing plant and their acute effects on the copepod *Calanus finmarchicus*

4

5

6

Julia Farkas[1*], Dag Altin[2], Karen M Hammer[1], Kaja Hellstrøm[1], Andy M. Booth[1] and Bjørn Henrik Hansen[1]

9

[1]SINTEF Materials and Chemistry, Marine Environmental Technology, N-7465 Trondheim, Norway

[2]BioTrix, N-7022 Trondheim, Norway

13

14

15

16

17

18

19

20

21

*Corresponding author

Julia Farkas

SINTEF Materials and Chemistry

Environmental Technology

Brattørkaia 17C

7010 Trondheim

Norway

Email: julia.farkas@sintef.no

Abstract

Submarine tailing disposal (STD) of mining waste is practiced as an alternative to land fill disposal in several countries. Knowledge of the environmental implications of STD on fjord and other marine ecosystems, including the pelagic environment, is scarce. In this study, we characterised the particle shape, size and metal content of the fine-grained fraction of tailings (FGT) from a Norwegian marble processing plant and investigated their acute toxicity and impact on feeding rate in adult *Calanus finmarchicus*. Initial tailing dispersions with a concentration of 1 mg mL^{-1} contained approximately 72 million particles, with 62 % of particles between 0.6 and 1 µm in size. After a sedimentation time of 1 h, 69 % of the particles between 0.6 and 5 µm remained dispersed, decreasing to 22 % after 6 h. When subjected to low energy turbulence in exposure experiments, the formation of fragile agglomerates was observed. The FGT contained Al, Mn, Fe and Ni, with no detectable dissolution occurring during the 48 h exposure period. Acute exposure (up to 4 g L^{-1}) to FGT caused no mortality in *C. finmarchicus*. Similarly, feeding rates determined during a 40 h depuration period, were not significantly impacted. However, surface attachment and uptake of FGT into the digestive tract of the copepods was observed. This indicates that, whilst marble FGT are not acutely toxic to copepods, chronic effects such as impacts on organism's energy budgets could occur, highlighting the need for further research on potential sublethal effects in organisms exposed to fine inorganic particles.

1 Introduction

Demand for mineral resources is driving the rapid increase of mining activities worldwide (Dold, 2014; Ramirez-Llodra et al., 2015). This activity generates large quantities of tailings that require disposal. Land-based disposal in dams is currently the most common practice for industrial-sized mines (Dold, 2015; Ramirez-Llodra et al., 2015). However, finding large areas with suitable conditions (low seismic activity and precipitation) necessary for such disposal represents a major challenge for the mining industry (Kvassnes and Iversen, 2013). As a result, tailing disposal at sea is increasingly being considered as a viable alternative (Dold, 2014, 2015).

The coastal areas of every continent on Earth, with the exception of Antarctica, have been subjected to some form of previous or ongoing tailing disposal in the form of shore, shallow or deep-sea disposal (Koski, 2012). These represent a very broad range of ecosystems from Arctic (e.g. Norway, North America, Greenland) to tropical (Central America, Brazil, Indonesia, Philippines, Benin). Similarly, riverine and lake tailing disposal, together with accidental releases such as the Samarco tailing dam burst in Brazil (Segura et al., 2016) and other international examples (Rico et al., 2008), have the potential to impact both freshwater ecosystems as well as coastal areas through tailing transport. As a result, many coastal locations around the globe are the recipients of tailings, although the chemical composition and physical properties will vary depending on the ore or materials being produced.

In Norway, an increased demand for minerals for green technology solutions (e.g. wind power plants, electric cars) and a greater national focus on alternatives to the oil and gas industry has sparked a revival in the Norwegian mining industry. Several of the major Norwegian mines, quarries and processing plants are located in the vicinity of the coast, and STD is practiced in

several fjords. Currently, there are 6 active and 2 upcoming STD sites (2016) along the Norwegian coast (Figure 1). The tailing release depth varies from emissions in the tidal zone (Stjernøysundet) to 125 m (Rana Gruber, fine particulates) (Norwegian Mining Industry, 2014).

Despite the ongoing practice of STD, there is a lack of scientific literature on the potential for environmental impacts on fjord ecosystems (Skei and Syvitski, 2013; Ramirez-Llodra et al., 2015). One of the most recognised environmental impacts of STD on fjords ecosystems is the destruction of benthic habitats due to hyper-sedimentation (Kvassnes and Iversen, 2013). However, depositing millions of tons of particulate matter will not only have ecological implications for the seabed, but also for the pelagic environment through the spreading of FGT plumes in the water column, potential upwelling processes and slope failure.

Tailing properties are dependent on the characteristics of the ore, with grain size and shape being of significant importance for their environmental impacts (Cheung and Shin, 2005; Dale et al., 2008; Kvassnes and Iversen, 2013). Variations in marine environmental conditions (e.g. salinity, turbidity, concentration of natural organic matter) at different disposal locations will also have a significant impact on the subsequent behaviour and fate of the tailings (aggregation/agglomeration, flocculation, sedimentation, dispersion), especially the FGT fraction. FGTs that do not rapidly settle out of the water column, can increase turbidity, and potentially have impacts on pelagic organisms. The presence of particle-bound metals and potential metal dissolution increases the risk for environmental damage as some metals (e.g. Cd, Cu, Ni, Hg, Ag) are known to elicit toxic effects to marine organisms such as algae, invertebrates and fish (Martin et al., 1981; Fisher et al., 1984; Wood et al., 1999; Hook and Fisher, 2002). Increased Fe tissue concentrations and impaired health was reported in blue mussels (*Mytilus edulis*) caged in the vicinity (0-3 km) of an iron ore STD site (Brooks et al.,

104   2015). Furthermore, floatation chemicals and flocculants, which are used to counteract the

105   spreading of particulates, can potentially have adverse effects on marine organisms (Vigneault

106   et al. 2013).

107

108   The pelagic copepod *Calanus finmarchicus* is a ubiquitously distributed zooplankton species

109   displaying a very high biomass in the North Atlantic, including Norwegian fjords. *C.*

110   *finmarchicus* plays a key role in maintaining the flux of energy from microalgae production to

111   higher trophic levels. In spring, they arise from winter diapause in deeper waters to reproduce

112   in the euphotic zone. The eggs hatch, become nauplii, and develop further into copepodites,

113   which in Norwegian fjords descend to deeper waters for diapause during late summer and

114   autumn when food becomes scarce. Owing to both their geographical and vertical distribution

115   patterns, *C. finmarchicus* can be subjected to FGT exposure during tailing disposal activities.

116   Calanoid copepods display very high filtration rates combined with both selective and non-

117   selective filter-feeding behaviour (Meyer et al., 2002). Exposure to tailings results in ingestion

118   of particles (Anderson and Mackas, 1986; Shadrin and Litvinchuk, 2005), yet the toxicological

119   response of these organisms to FGT exposure needs further investigation.

120

121   In this study, we characterised the FGT fraction of tailings from the Omya Hustadmarmor

122   marble processing plant, which is deposited in Frænfjorden, Western Norway (Figure 1).

123   Physicochemical properties including particle number (particles mL^{-1}), volume ($\mu m^3\ mL^{-1}$) and

124   mass (mg L^{-1}), as well as particle (grain) size, settling behaviour, metal content and metal

125   dissolution were studied. The acute toxicity of dispersed FGT and their effects on the feeding

126   behaviour of *C. finmarchicus* were investigated.

127

128
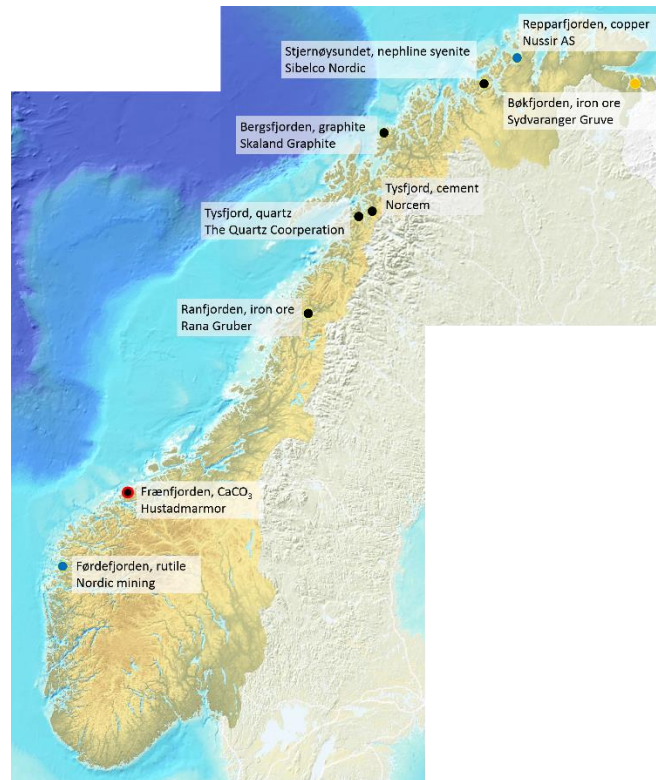
Figure 1. Active mines (black circles), recently active mines (yellow circles) and mines starting operation (blue circles) with submarine tailing disposal (STD) in Norway (as of June 2016). The Omya Hustadmarmor marble processing plant which releases tailings to Frænfjorden is marked with a red outline. Map modified from kartverket.no.

2 Material and Methods

2.1 FGT characterisation

The tailings used in the current study originated from the Omya Hustadmarmor liquid marble production plant (Figure 1). The tailing release at the processing plant occurs at a reported average depth of 20 m (Norwegian Mining Industry 2014). In order to increase aggregation and flocculation, the tailings are pre-mixed with seawater before release. The samples were stored in darkness at 4 °C until use.

143   To determine the dry weight of the material, wet samples of 6.2 ± 2 g were dried at 50 °C and

144   weighed after 24 h, 48 h and 10 days, until a constant dry weight was recorded. Salinity was

145   determined with a refractometer (S/Mill; Atago, Japan) in the supernatant water obtained after

146   centrifugation of 20 g of tailing material at 4000 rpm for 15 min. Phase contrast microscopic

147   images of FGT dispersions were taken (Nikon eclipse 80i; 20x PlanFluor Ph1DLL 0.5NA

148   objective; Nikon, Japan).

149

150   **2.1.1 FGT dispersion preparation and characterisation**

151   Tailing dispersions of 1 g L^{-1} (dry weight) were prepared in filtered seawater (Millipore Sterivex

152   0.2 µm; Merk KGaA, Germany) by stirring and agitation. Larger tailing particles are

153   acknowledged to sediment rapidly following release to the marine environment, and are thus

154   not relevant for widespread exposure to pelagic organisms. In order to prepare the FGT fraction,

155   the larger particles were removed by an initial sedimentation phase of 3 min before the

156   supernatant was decanted. Next, the obtained FGT dispersion was re-suspended and left

157   standing still and in the dark for 24 h at 10 °C. Water column samples of the FGT dispersion

158   were taken at the following time points: immediately (0 min), after 10 min, 30 min, 1 h, 2 h, 3

159   h and 6 h and 24 h.

160

161   To determine the particle load (number, volume, mass) and size distribution (in the range 0.6 -

162   60 µm) of the dispersed FGT fraction, 25 mL samples were taken and analysed with a particle

163   sizer (Coulter counter 4; Beckman Coulter, US) using a 20 µm (size range 0.6 - 18 µm) and a

164   100 µm (size range 2 - 60 µm) aperture. Samples were filtered through 20 µm pore size filters

165   prior to analysis with the 20 µm aperture in order to prevent aperture clogging. Where

166   necessary, samples were diluted with freshly filtered (0.2 µm) seawater. The density of $CaCO_3$

167   was used to calculate the total mass in the samples.

168

2.2 Impacts of FGT on *C. finmarchicus*

2.2.1 Acute toxicity

Seven tailing exposure dispersions (0.2 - 5.0 g L^{-1}) were individually prepared in filtered

seawater (Millipore Sterivex, 0.2 µm, Merck KGaA, Germany) by mixing overnight in 2 L

borosilicate glass bottles with 25% headspace at 0.5 rev. min^{-1}. To remove coarse particles (>

40 µm) the bottles were shaken and left for a 3 min sedimentation period as described above,

before the respective supernatants were decanted into clean 2 L bottles. At onset of the 96 hour

acute toxicity test, the bottles with the supernatant were shaken manually to re-suspend particles

before the contents were divided into three 0.5 L polyethylene terephthalate bottles, which

served as the exposure vessels. The remaining dispersion was used for determining the particle

number with a particle sizer (Coulter counter 3; Beckman Coulter, US), 100 µm aperture, as

described above.

In the acute test, filtered natural seawater was used as negative control and a 0.85 mg L^{-1}

solution of 3,5-dichlorophenol (3,5-dcp) was used as positive control to assess the sensitivity

of the test animals. To avoid loss of 3,5-dcp during the exposure, the study was performed in

0.5 L borosilicate glass bottles capped with Teflon lined screw caps. The test was performed in

triplicate for the positive controls and the particle dispersions, and in sextuple for the negative

controls. After adding seven *C. finmarchicus* at the copepodite V or early female (non-

ovulating) stage, the exposure vessels were topped to remove any headspace and finally capped.

To avoid settling of particles during the exposure, the exposure vessels were secured axially on

a plankton wheel set at 0.5 rev. min^{-1} and placed in a temperature-controlled room at 10±0.5 °C

under dim light conditions at a 16:8 light:dark cycle. The test animals were not fed during

192   exposure, and the exposure solutions were not renewed. Animal survival was assessed daily

193   over the 96 h exposure period.

194

195   **2.2.2 Impacts on feeding rates**

196   For the 48 h exposures with subsequent feeding tests, three exposure concentrations based on

197   the sedimentation experiment described above were prepared by sedimentation of the FGT

198   stock dispersion (for preparation see 2.1.1) for 0 min (high concentration; H), 1 h (medium

199   concentration; M) and 6 h (low concentration; L). The respective supernatants were transferred

200   to prewashed (acid and MilliQ) 2 L glass flasks and equilibrated on turning plankton wheels

201   for 12 h. Filtered seawater was used as the control in the experiments.

202

203   2.2.2.1 Tailing characterisation in exposure solutions

204   At the start (0 h) and the end (48 h) of the exposure period, the exposure dispersions were

205   characterised for particle number, particle mass and particle size distribution. Twenty five mL

206   samples were taken from the water column and analysed as described above (2.1.1). In order to

207   determine the concentration of selected elements in the particulate and dissolved fractions, 10

208   mL samples were taken at the start and end of the exposure. Five mL of each sample was then

209   preserved unfiltered, while the remaining 5 mL were passed through a 0.1 µm Omnipore PTFE

210   filter (MerkMillipore Ltd, Ireland) to remove the particles. All samples were then preserved

211   with ultraclean HNO_3 (2 % final concentration) and analysed with inductively coupled plasma

212   triple quadrupole mass spectrometry (ICP-QQQ, Agilent 8800; Agilent Technologies, USA).

213   Samples were analysed for Al, Mn, Fe, Ni, Ca, Pb, Hg, Cd, Cu, Co, Ca and As. ^{115}In and ^{89}Y

214   were used as internal standards and quantified against standards from Inorganic Ventures (US).

215

216   2.2.2.2 *Calanus finmarchicus* exposure

Female *C. finmarchicus* were exposed to FGT dispersions L, M, H and control for 48 h. Exposures were performed in two groups, with one group being exposed to FGT only (tailing group; T) and the other group being fed with approximately 7500 cells L^{-1} of the unicellular algae *Rhodomonas baltica* during exposure (feeding group; F). Copepod density in the exposures was 10 individuals L^{-1}. The ambient exposure temperature was 10 °C. Exposures flasks were kept on a plankton wheel in slow rotation (0.5 rotations min^{-1}) in order to prevent settling of both the algae and FGT. Feeding groups (F) received fresh algae after 24 h to restock to 7500 cells L^{-1}. All conditions were conducted in triplicates (*n*=3) or in quadruplicates (*n*=4) for microscopy.

2.2.2.3 Uptake of particulate material and impacts on feeding rate

After termination of the 48 h exposure, animals from both exposure groups (P, F) were transferred to 2 L flasks with clean, filtered seawater. Algae were added to reach an initial feeding concentration of approximately 7500 cells L^{-1} (7550±165 cells L^{-1}). After 20 h a 25 mL sample was taken and the number of algae analysed with a particle sizer (Coulter counter 4; Beckman Coulter, US). Subsequently, 25 mL of filtered seawater spiked with an individually specified amount of algae stock were refilled in each exposure flask in order to raise the algae cell number to initial concentrations. A second 25 mL water sample was taken after 40 h and the algae number measured once again.

In order to investigate the occurrence of surface attachment and determine uptake of FGT particles, individual *C. finmarchicus* were sampled (i) after 48 h exposure, and (ii) after feeding depuration period (48 h exposure + 40 h feeding). Individuals were anesthetised with tricaine methanesulfonate (Finquel, Argent Laboratories, USA; 1.5g/L stock solution in seawater) and observed with a dissecting microscope (Leica MZAPO, Leica Microsystems, Germany).

243 **2.3 Statistics**

244 Data analyses were performed with GraphPad Prism 7 (GraphPad Software Inc., USA). Data

245 sets were analysed for normality (Shapiro-Wilk normality test) and analysed with one way

246 ANOVA. In order to compare elemental concentrations statistically, random values (0-LOD)

247 were calculated and assigned to samples that were below the detection limit (control groups).

248

249 # 3 Results and Discussion

250 **3.1 Mine tailing characteristics**

251 The obtained tailing material had a water content of 19.4±0.1% and a salinity of 25‰.

252 Microscope images showed the presence of a large number of FGT particles, which exhibited

253 slightly edged triangular and rectangular, as well as spherical particle shapes (supporting

254 information, Figure S1).

255

256 **3.1.1 Sedimentation behaviour in undisturbed seawater**

257 Despite the initial sedimentation phase of 3 min, the remaining FGT dispersions contained a

258 large amount of particulate material. Measurements of particle number, volume and mass in

259 overlapping size ranges were generally in good agreement between the 30 µm and 100 µm

260 apertures. The number of particles above 18 µm in the FGT dispersions accounted only for

261 approximately 0.3 % of the total particle number (100 µm aperture, size rage >2 - 60 µm). Thus,

262 most reported data derives from 30 µm aperture measurements (0.6 - 18 µm particle size) if not

263 stated otherwise.

264

265 The FGT dispersions initially contained 72.3±4.2 million particles mL^{-1} in the size range 0.6 -

266 18 µm, which decreased to 50±3.9 million after 1 h, 15.7±0.13 million after 6 h, and 0.59±0.06

267 million particles mL^{-1} after 24 h of settling. This represents a decrease of the total particle

268 number to 69 %, 21 % and 0.8 % after 1 h, 6 h and 24 h settling time, respectively. The

269 calculated total particle mass (0.6 - 18 µm) was 312±20, 165±23, 46±3 and 0.9±0.1 mg L^{-1} at

270 time points 0, 1, 6 and 24 h, respectively. The particle load (number and volume) in the size

271 fractions 0.6 - 1 µm, 1 - 2 µm, 2 - 5 µm and 5 - 18 µm is presented in Figure 2. The particle

272 number was highest in the two small size fractions 0.6 - 2 µm (Figure 2 A,C), accounting for

273 94 % of the total particle number. Being the most stable in the undisturbed water column, these

274 fractions represented 96% and 99% of the total particle number after 6 and 24 h, respectively.

275 In contrast, particle volume dominated in the two larger fractions 2 - 5 and 5 - 18 µm, accounting

276 for 63% of the total particle volume in the initial settling phase (0 and 30 min), but gradually

277 decreased thereafter to 16% at 24 h (Figure 2 B,C). Our results show that the FGT dispersion

278 contained a large number of small particles which remain dispersed for several hours in the

279 water column when undisturbed.
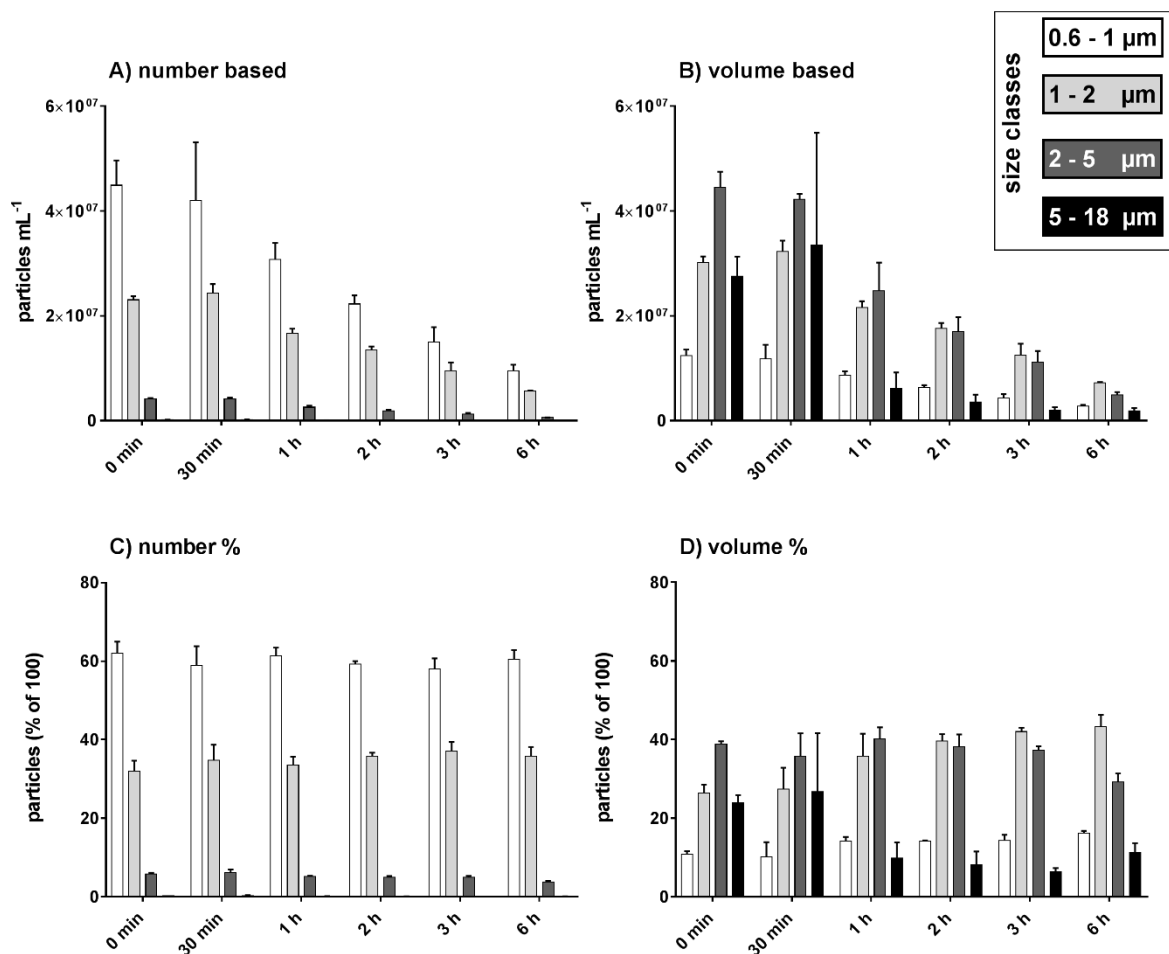
Figure 2. Concentration of particles in different size classes during 6 h of settling presented as number based (A) and volume based (B). The relative amount (%) of particles in the different size classes is shown for particle number (C) and volume (D). Mean ± SD, $n=3$.

3.2 Impacts of FGT on *C. finmarchicus*

3.2.1 Acute toxicity

The total particle number (2 - 60 µm) and particle mass in the exposures is presented in the supporting information (Table S1). The measured total particle number (2 - 60 µm) was 15 million particles in the highest exposure concentration (4 g L^{-1}) and 0.59 million particles in the lowest exposure concentration (0.23 g L^{-1}). At the nominal exposure concentration of 1.07 g L^{-1}, 2.9 million particles were measured, which corresponds well to results of the settling

292   experiment of 3.1 million particles (2 – 60 µm, data not shown). No mortality occurred at any

293   of the tested concentrations, showing that the FGT are not acutely toxic (Table S1). The results

294   are consistent with previous studies that have reported the ability of copepods to handle very

295   high particle (suspended sediment) loads for short periods of time (Arendt et al., 2011).

296

297   **3.2.2 Impacts of FGT on *C. finmarchicus* feeding**

298   3.2.2.1 FGT characteristics in exposure solutions

299   The particle loads in the three exposure groups (L, M, H) were characterised at time point 0 h

300   (start) and 48 h (end) of the exposure experiment (Figure S2). The calculated total particle mass

301   at the start of the exposure experiment was 314±35, 176±10 and 62±9 mg L^{-1} for the H, M and

302   L exposure groups, respectively. Total particle numbers in all exposure groups at time point 0

303   h were similar (p>0.05) compared to those in the sedimentation experiments (H = 0 min, M =
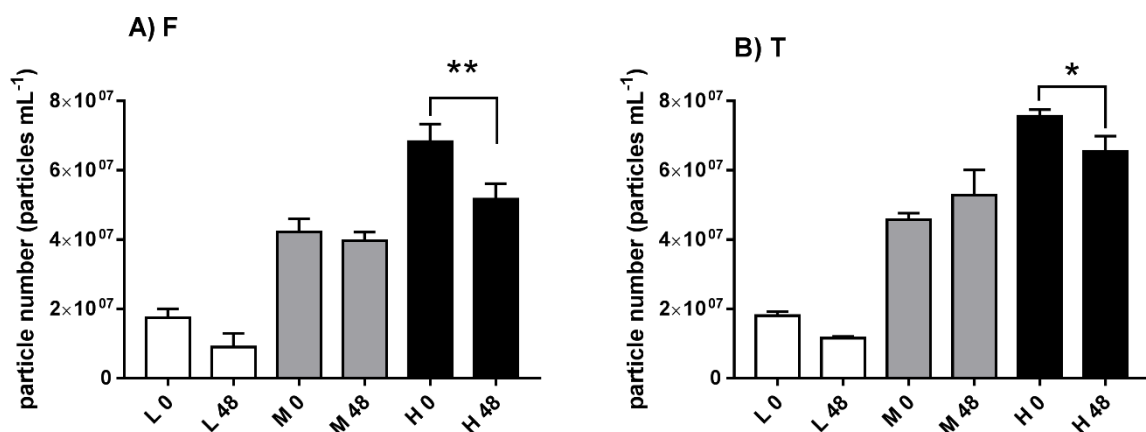
304   1 h, L = 6 h).

305

306   In addition to the continued presence of dispersed particles, the formation of agglomerates

307   (flocs) was observed (visual observation) over the exposure period for all exposure groups. The

308   formation of large flocs in low energy environments has been previously described (Skei and

309   Syvitski, 2013), and thus the floc formation in the current study is likely derived from the use

310   of gentle rotation during the exposure. However, the flocs were very fragile and readily

311   dissociated during sampling and analysis meaning that floc size could not be determined.

312

313   Despite reduced particle numbers in most exposure groups at the end of the experimental period

314   (Figure 3), a significant reduction in total particle number was only determined in the H

315   exposures in both, feeding (F; p<0.0006) and tailing only (T; p<0.0156) groups (Figure 3A and

316   B). Comparison of the particle number across the different size classes in the H exposure groups

revealed a significant decrease of 0.6-1 µm (p<0.01) and 1-2 µm (p <0.01) sized particles. Furthermore, a small increase in particle number was observed in the two larger size classes (2-5 µm and 5-18 µm). This indicates that agglomeration processes are the primary mechanisms driving the reduction in particle number. This agglomeration was more efficient in exposures with high particle concentrations due to the increased frequency of particle-particle interactions (Skei and Syvitski, 2013). The decrease in particle number between the start and end of the exposure was slightly more pronounced in feeding exposures (Figure 3A) compared to particle only (Figure 3B) exposures. Results suggest that homoagglomeration occurs between FGT particles as well as heteroagglomeration between FGT particles and algal cells in these samples. The formation of FGT-derived agglomerates and flocs will also occur to various extents after tailing release in fjords. Floc size and stability will depend on factors such as FGT particle concentration, turbulence conditions (Skei and Syvitski, 2013) and the presence of other, naturally occurring inorganic and organic (e.g. algal cells) particulate matter. Furthermore, water parameters such as salinity and concentration of dissolved natural organic matter are known to influence the fate of small particles in the environment (Wang et al., 2014; Booth et al., 2015).

Figure 3. Total number of particles in *C. finmarchicus* exposures at time point 0 and after 48 h. A) in the feeding exposure group (F). B) in the tailing only exposure group (T). Data shown as Mean ± SD. *n*=3. Significant differences are indicated (p<0.05*; p<0.01**).

Total elemental analysis (combined dissolved and particulate contribution) of the exposure samples revealed increased concentrations of Fe, Al, Ni, Mn and Ca in the particulate samples compared to the seawater controls (Table 1). Ca was analysed as indicator element for the marble tailings ($CaCO_3$). The Ca concentrations determined in the control samples were 380±26 mg L^{-1}, and thus corresponded well to a typical seawater Ca concentration of 400 mg L^{-1}. Compared to the seawater controls, concentrations of Al and Fe were increased by approximately 10, 50 and 100 times in the L, M and H exposure groups, respectively (Table 1). Concentrations of Al, Mn, Fe (>99%) and Ni (>84%) were positively correlated to Ca concentrations in the exposure samples (Figure S3 and Figure S4), confirming their origin from the FGT in the exposures. Based on the Ca content determined in $CaCO_3$, the FGT contained 0.39±0.04% Al, 0.013±0.001% Mn, 0.42±0.01% Fe, and 0.0016±0.001% Ni. The relative concentrations were similar in L, M and H exposures (settling time 0, 1 and 6 h), indicating that the elements are associated only with the particulate fraction. Similarly, elemental concentrations were reduced to seawater control levels after filtration, indicating no significant dissolution into the seawater under the exposure conditions employed (60 h, pH 7.8, salinity 33.5‰, temperature 10°C).

The concentrations of the individual metals presented in Table 1 can be compared to the Criterion Maximum Concentrations (CMC) provided by the US EPA (US EPA, 2016), which represent recommended exposure limits for acute toxicity in seawater. The total Ni concentrations (particulate and dissolved) in the current study range from 2.9 (L) - 4.8 (H) µg

360 L^{-1}, and are thus significantly below the 74 µg L^{-1} CMC (dissolved concentration). Seawater

361 CMC values for Al, and Fe are not provided by the US EPA, despite being listed as pollutants

362 in the Water Quality Criteria Table and where freshwater data is available for Al (freshwater

363 Al CMC value is 750 µg L^{-1}). None of the metals identified as components of the FGT used in

364 the current study are considered priority environmental pollutants except Ni. However, tailings

365 and FGT from other mining operations in Norway and globally will contain their own unique

366 metal profile, possibly containing high priority metals, and should be considered on a case by

367 case basis.

368

369 Bioaccumulation of metals from the dissolved phase, as well as from ingested food particles,

370 has been reported in marine copepods (Fisher et al., 2000). Although the bioaccumulation

371 potential of the detected FGT-associated metals was not assessed in the current study, the

372 bioavailability of the FGT-associated metals is considered as low as metal analysis after

373 removal of the particulate material (0.1 µm filtration) showed there was no significant

374 dissolution of metal ions. A recent study comparing the bioavailability of metals from different

375 origins suggests that those present in metal sulphide minerals were considerably less

376 bioavailable compared to dissolved metals associated with sediments (Simpson and Spadaro,

377 2016). However, the acidic and suboxic–anoxic environment of the copepod gut may support

378 metal dissolution that otherwise are not favoured in the ambient seawater (Tang et al., 2011).

379

380 Table 1. Total (dissolved and particulate) element concentrations in the Ctrl, L, M and H

381 exposures, shown for both feeding + tailing, and tailing only exposures. Values given as Mean

382 ± SD. Significant differences from controls (p<0.05 *; p<0.01**) are given. Exposure groups

383 featuring more than 50 % of the samples with concentrations below the detection limit are

384 presented as <LOD with the LODs given in parentheses (italic).

385

386

Exposure	Al (mg L^{-1})	Mn (mg L^{-1})	Fe (mg L^{-1})	Ni (mg L^{-1})	Ca (mg L^{-1})
Feeding - Ctrl	0.015 (± 0.0076)	<LOD (*0.005*)	<LOD (*0.01*)	0.00167 (±0.00095)	386 (±37.9)
Feeding - Low FGT group	0.168 (±0.0711)	0.0077 (±0.00095)	0.176 (±0.014)	0.00292 (±0.0005)	396 (±2.89)
Feeding - Medium FGT group	0.749* (±0.105)	0.0282** (±0.006)	0.728** (±0.072)	0.00313 (±0.0004)	448* (±7.79)
Feeding - High FGT group	1.620** (±0.437)	0.062.3** (±0.0005)	1.765** (±0.195)	0.00448** (±0.0008)	549** (±33.4)
Tailing - Ctrl	<LOD (*0.01*)	<LOD (*0.005*)	<LOD (*0.01*)	0.00157 (±0.00073)	372 (16.7)
Tailing - Low FGT group	0.161 (±0.0442)	0.0074 (±0.00085)	0.185 (±0.0067)	0.00292 (±0.00102)	397 (±7.1)
Tailing - Medium FGT group	0.698** (±0.229)	0.0228** (±0.00104)	0.689** (±0.0256)	0.00364* (±0.00044)	442** (±11.5)
Tailing - High FGT group	1.47** (±0.178)	0.0645** (±0.00624)	1.72** (±0.16)	0.0048** (±0.00113)	539** (±21.6)

387

388

3.3.2.2 FGT uptake and impacts on feeding

Uptake and surface attachment on *C. finmarchicus* was investigated both directly after exposure

and after a 40 h depuration phase with feeding (algae). FGT were clearly observed throughout

the whole digestive tract of animals during exposure (Figure 4B), indicating rapid and efficient

uptake. The limited ability of *C. finmarchicus* to distinguish between food and non-food

particles, and the ingestion of clay/silt particles has been previously described (Arendt et al.,

2011). In addition to uptake of FGT, their attachment to *C. finmarchicus* surfaces, especially to

the filtering apparatus and furcal setae, was observed (Figure 4C and 4D). Whilst the contents

of the digestive tract were cleared of FGT during the 40 h depuration and algal feeding period,

398 some surface attachment was still observed after this time on fine structures such as the furcal

399 setae feathers (Figure 4E).

400

401 *C. finmarchicus* typically filters particles up to 50 µm (Hebert and Poulet, 1980), but can in fact

402 filter larger particles, i.e. cannibalistic ingestion of nauplii (Basedow and Tande, 2006).

403 Although the lower limit of particle size that copepods are able to filter is not known,

404 phytoplankton as small as 3 µm in diameter are commonly used in laboratory experiments

405 (Nejstgaard et al., 1995; Båmstedt et al., 1999). The primary particle size in the settling and

406 exposure experiments showed that most of the particles (number based) are between 0.6 and 2

407 µm. However, the formation of loose agglomerates, as observed under gentle motion in the

408 exposure conditions, could also cause a shift to a more preferred feeding size range. The extent

409 to which dispersed FGT and FGT flocs *in situ* correspond to copepod feeding size requires

410 assessment in future studies.

411
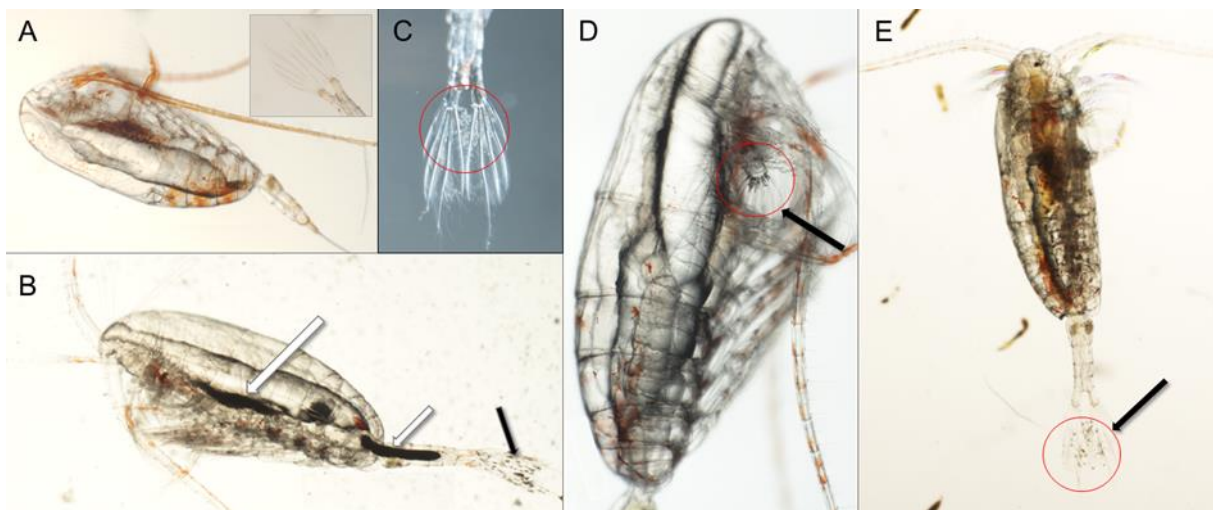
412

413 Figure 4. Images of *C. finmarchicus*. A) seawater control; B) ingested FGT in digestive tract

414 (white arrows) and attached to furcal setae (black arrow); C and D) FGT attached to the furcal

415 setae and filtering apparatus after 48 h exposure; E) FGT remaining attached to the furcal setae

416 after 40 h depuration phase.

418 Despite significant uptake and surface attachment of FGT in the 48 h exposures, *C.*

419 *finmarchicus* feeding rates assessed during a depuration phase were not significantly impacted

420 relative to controls (at 20 or 40 h) (Figure S5). In medium concentration exposures (M) after a

421 20 h depuration, the FGT-only exposure group had a significantly higher feeding rate compared

422 to the FGT+algae exposure group (p=0.0041). No further significant differences in depuration

423 phase feeding rates between those organisms exposed only to FGT and those exposed to

424 FGT+algae were found. This indicates that the digestive tract was cleared successfully after

425 exposure termination and the filtering apparatus was not damaged during short-term exposure

426 and FGT attachment. However, the chronic exposure to high loads of inorganic particles can

427 have negative implications in copepods (Sommaruga, 2015). Arendt et al. (2011) report reduced

428 ingestion of Chlorophyll *a* in *C. finmarchicus* during exposure to fine suspended sediments

429 (most abundant particle size 2 - 3 µm) at concentrations above 20 mg L^{-1} and suggested this is

430 due to unselective feeding. Furthermore, a reduction in *C. finmarchicus* egg production was

431 observed consequently to the reduced food uptake (Arendt et al., 2011). This indicates that

432 chronic exposure of *C. finmarchicus* and other copepods to FGT may lead to a reduced energy

433 intake (Paffenhöfer, 1972; Shadrin and Litvinchuk, 2005). Finally, FGT uptake and surface

434 attachment was shown to result in reduced buoyancy for copepods (Shadrin and Litvinchuk,

435 2005).

436

437 **4 Conclusions and implications for other marine environments**

438 FGT from the Hustadmarmor marble processing plant contain a large number of small (0.6 - 1

439 µm) particles. The FGT remained dispersed in undisturbed seawater for several hours, but

440 formed lose agglomerates after being subjected to gentle motion in exposure experiments.

441 Importantly, FGT were not acutely toxic to *C. finmarchicus* adults, and nor did they have a

significant impact on feeding rates during a depuration phase following exposure. However, FGT were found to be taken up and ingested, as well as attaching to the copepod surfaces. This ingestion and attachment has the potential to cause long-term effects on the animals' energy budget, especially for sensitive life stages such as nauplii. Copepods such as *C. finmarchicus* are a key component of the food chain in coastal areas in Norway, serving as energy transfer link between trophic levels. In order to investigate the effects of MT release on fjord ecosystems more thoroughly, further research into the impacts of chronic tailing exposure and potential effects on the sensitive juvenile life stages of pelagic filter feeders such as *C. finmarchicus* are needed.

The data reported in this study is specifically generated for improving our understanding of the acute effects of suspended FGT from STD in Norwegian fjord ecosystems. However, knowledge on FGT behaviour, fate and potential impacts has a broader significance for other marine environments which receive fine particulate material deriving from anthropogenic activities including mining, disposal of drill cuttings and future deep sea mining activities. The current study shows that FGT can remain suspended in the water column for significant periods of time and are thus relevant for exposure to organisms such as zooplankton and fish. However, the proportion of FGT from tailings which remains suspended in a specific marine environment will depend on both the physicochemical properties of the particles and the environmental conditions at the release or deposition location. Furthermore, the FGT studied here come from a marble mine and contain mostly $CaCO_3$ with limited amounts Al, Fe, Ni and Mn. None of these metals exhibit rapid dissolution under the exposure conditions used and are not considered priority metal toxins. Mine tailings from other sources will exhibit their own unique physicochemical profile, possibly containing elements and chemicals which have a higher toxicity and/or which may undergo a more rapid dissolution. It is therefore necessary to gain a

467 better understanding of the environmental fate and behaviour of different types of FGT in

468 different marine environments. Similar studies using organisms from other geographical marine

469 environments receiving FGT would significantly improve our general understanding of their

470 fate and effects.

471

Acknowledgements

References

Anderson, E.P., Mackas, D.L., 1986. Lethal and sublethal effects of a molybdenum mine tailing on marine zooplankton: Mortality, respiration, feeding and swimming behavior in Calanus marshallae, Metridia pacifica and Euphausia pacifica. Marine Environmental Research 19, 131-155.

Arendt, K.E., Dutz, J., Jonasdottir, S.H., Jung-Madsen, S., Mortensen, J., Moller, E.F., Nielsen, T.G., 2011. Effects of suspended sediments on copepods feeding in a glacial influenced sub-Arctic fjord. J Plankton Res 33, 1526-1537.

Basedow, S.L., Tande, K.S., 2006. Cannibalism by female Calanus finmarchicus on naupliar stages. Mar Ecol Prog Ser 327, 247-255.

Booth, A.M., Størseth, T., Altin, D., Fornara, A., Ahniyaz, A., Jungnickel, H., Laux, P., Luch, A., 2015. Freshwater dispersion stability of PAA-stabilised cerium oxide nanoparticles and toxicity towards Pseudokirchneriella subcapitata. Science of the Total Environment 505, 596-605.

Brooks, S.J., Harman, C., Hultman, M.T., Berge, J.A., 2015. Integrated biomarker assessment of the effects of tailing discharges from an iron ore mine using blue mussels (Mytilus spp.). Sci Total Environ 524, 104-114.

Båmstedt, U., Nejstgaard, J.C., Solberg, P.T., 1999. Utilisation of small-sized food algae by *Calanus finmarchicus* (Copepoda, Calanoida) and the significance of feeding history. Sarsia 84, 19-38.

Cheung, S.G., Shin, P.K.S., 2005. Size effects of suspended particles on gill damage in green-lipped mussel Perna viridis. Marine Pollution Bulletin 51, 801-810.

Dale, T., Kvassnes, A.J.S., Iversen, E.R., 2008. Risikoen for skader på fisk og båsjell ved gruveaktivitet på Engebøneset. En litteraturstudie om effekter av metaller og suspenderte partikler.

Dold, B., 2014. Submarine Tailings Disposal (STD)-A Review. Minerals-Basel 4, 642-666.

Dold, B., 2015. On/land or submarine tailings disposal? - Pros and cons. 10th International Conference on Acid Rock Drainage & IMWA Annual Conference, Chile.

Fisher, N.S., Bohe, M., Teyssie, J., 1984. Accumulation and toxicity of Cd, Zn, Ag, and Hg in four marine phytoplankters. Marine Ecology - Progress Series 18, 14.

Fisher, N.S., Stupakoff, I., Sanudo-Wilhelmy, S., Wang, W.X., Teyssie, J.L., Fowler, S.W., Crusius, J., 2000. Trace metals in marine copepods: a field test of a bioaccumulation model coupled to laboratory uptake kinetics data. Mar Ecol Prog Ser 194, 211-218.

Hebert, R., Poulet, S.A., 1980. Effect of modification of particle size of emulsions of venezuelan crude oil on feeding, survival and growth of marine zooplankton. Marine Environmental Research 4, 121-134.

Hook, S.E., Fisher, N.S., 2002. Relating the reproductive toxicity of five ingested metals in calanoid copepods with sulfur affinity. Mar Environ Res 53, 161-174.

Industry, N.M., 2014. Norwegian experiences with the sea disposal of mine tailings. p. 34.

Koski, R.A., 2012. Metal dispersion resulting from mining activities in coastal environments: A pathways approach. Oceanography 25, 170–183.

Kvassnes, A.J.S., Iversen, E., 2013. Waste sites from mines in Norwegain Fjords. Mineralproduksjon 3, 12.

Martin, M., Osborn, K.E., Billig, P., Glickstein, N., 1981. Toxicities of ten metals to Crassostrea gigas and Mytilus edulis embryos and Cancer magister larvae. Marine Pollution Bulletin 12, 5.

Meyer, B., Irigoien, X., Graeve, M., Head, R.N., Harris, R.P., 2002. Feeding rates and selectivity among nauplii, copepodites and adult females of Calanus finmarchicus and Calanus helgolandicus. Helgoland Marine Research 55, 169-176.

Nejstgaard, J.C., Bamstedt, U., Bagoien, E., Solberg, P.T., 1995. Algal constraints on copepod grazing - Growth-state, toxicity, cell-size, and season as regulating factors. ICES Journal of Marine Science 52, 347-357.

Paffenhöfer, G., 1972. The effects of suspended "red mud" on mortality, body weight, and growth of the marine planktonic copepod, *Calanus helgolandicus*. . Water, Air and Soil Pollution 1, 8.

Ramirez-Llodra, E., Trannum, H.C., Evenset, A., Levin, L.A., Andersson, M., Finne, T.E., Hilario, A., Flem, B., Christensen, G., Schaanning, M., Vanreusel, A., 2015. Submarine and deep-sea mine tailing placements: A review of current practices, environmental issues, natural analogs and knowledge gaps in Norway and internationally. Mar Pollut Bull 97, 13-35.

Rico, M., G. Benito, G., Salgueiro, A.R., D´ıez-Herrero, A., Pereira, H.G., 2008. Reported tailings dam failures: A review of the European incidents in the worldwide context. Journal of Hazardous Materials 152, 846–852.

Segura, F.R., Nunes, E.A., Paniz F.P., Paulelli, A.C.C., Rodrigues, G.B., Braga, G.U.L., dos Reis Pedreira Filho, W., Barbosa Jr, F., Cerchiaro, G., Silva, F.F., Batista, B.L., 2016. Potential risks of the residue from Samarco's mine dam burst (Bento Rodrigues, Brazil). Environmental Pollution, 218, 813-825.

Shadrin, N., Litvinchuk, L., 2005. Impact of Increased Mineral Particle Concentration on the Behavior, Suspension-Feeding and Reproduction of Acartia clausi (Copepoda). in: Dame, R.F., Olenin, S. (Eds.). The Comparative Roles of Suspension-Feeders in Ecosystems: Proceedings of the NATO Advanced Research Workshop on The Comparative Roles of Suspension-Feeders in Ecosystems Nida, Lithuania 4–9 October 2003. Springer Netherlands, Dordrecht, pp. 137-146.

Simpson, S.L., Spadaro, D.A., 2016. Bioavailability and Chronic Toxicity of Metal Sulfide Minerals to Benthic Marine Invertebrates: Implications for Deep Sea Exploration, Mining and Tailings Disposal. Environmental Science & Technology 50, 4061-4070.

Skei, J.M., Syvitski, J.P.M., 2013. Natural flocculation of mineral particles in seawater-influence on mine tailings sea disposal and particle dispersal Mineralproduksjon 3, 10.

Sommaruga, R., 2015. When glaciers and ice sheets melt: consequences for planktonic organisms. J Plankton Res 37, 509-518.

Tang, K.W., Glud, R.N., Glud, A., Rysgaard, S., Nielsen, T.G., 2011. Copepod guts as biogeochemical hotspots in the sea: Evidence from microelectrode profiling of Calanus spp. Limnology and Oceanography 56(2), 666–672.

US EPA, 2016. National Recommended Water Quality Criteria - Aquatic Life Criteria Table. https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table. Accessed November 2016.

Wang, H., Burgess, R.M., Cantwell, M.G., Portis, L.M., Perron, M.M., Wu, F., Ho, K.T., 2014. Stability and aggregation of silver and titanium dioxide nanoparticles in seawater: role of salinity and dissolved organic carbon. Environmental Toxicology and Chemistry 33 (5), 1023-1029.

Wood, C.M., Playle, R.C., Hogstrand, C., 1999. Physiology and modeling of mechanisms of silver uptake and toxicity in fish. Environmental Toxicology and Chemistry 18, 71-83.