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Effects of marine mine tailing exposure on the development, growth, and lipid accumulation in *Calanus finmarchicus*

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ABSTRACT

Marine tailing disposal (MTD) is sometimes practiced as an alternative to traditional mine tailing deposition on land. Environmental challenges connected to MTD include spreading of fine particulate matter in the water column and the potential release of metals and processing chemicals. This study investigated if tailing exposure affects the marine copepod *Calanus finmarchicus*, and whether effects are related to exposure to mineral particles or the presence of metals and/or processing chemicals in the tailings. We investigated the impacts of three different tailing compositions: calcium carbonate particles with and without processing chemicals and fine-grained tailings from a copper ore. Early life stages of *C. finmarchicus* were exposed over several developmental stages to low and high suspension concentrations for 15 days, and their development, oxygen consumption and biometry determined. The data was fitted in a dynamic energy budget (DEB) model to determine mechanisms underlying responses and to understand the primary modes of action related to mine tailing exposure. Results show that copepods exposed to tailings generally exhibited slower growth and accumulated less lipids. The presence of metals and processing chemicals did not influence these responses, suggesting that uptake of mineral particles was responsible for the observed effects. This was further supported by the applied DEB model, confirming that ingestion of tailing particles while feeding can result in less energy being available for growth and development.

1. Introduction

Mining activities are rapidly increasing worldwide due to high demands for mineral resources (Ramirez-Llodra et al., 2015; Dold, 2014). There are numerous environmental risks associated with mining (Vogt, 2013). Management of mine tailings is considered the biggest environmental challenge, as only a few percent of the extracted rock contain metals and minerals, resulting in large quantities of tailings to be disposed (Vogt, 2013). Tailings mostly consist of fine crushed rock but may contain heavy metals and chemicals used in the extraction process, as well as sulphide-bearing materials (Ramirez-Llodra et al., 2015; Dold, 2014; Vogt, 2013).

Currently, land-based disposal of tailings is the most common practice for industrial sized mines. Land-based disposal is space demanding, and in regions with rugged terrain and high precipitation, it is often challenging to find suitable deposition areas (Kvassnes and Iversen, 2013). As a result, tailing disposal in the marine environment is sometimes considered as an alternative option (Dold, 2014).

Norway is today one out of only eight countries conducting marine tailing disposal (MTD) (Vogt, 2013). Several of Norway's major mines and production sites are in proximity to the coast, and there are currently five active sites disposing tailings into adjacent fjords, with an additional two sites in the start-up phase (Norwegian Environment Agency, 2020; Setså, 2020).

Depending on the mined ore, MTD will lead to an increased release of inorganic particles, metals, and processing chemicals into the marine

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environment. Even though it has been practiced in Norway for over 30 years, potential environmental impacts on fjord ecosystems are still not sufficiently investigated (Ramirez-Llodra et al., 2015; Skei and Sytski, 2013; Klima-og Forurensningsdirektoratet, 2010). So far, most environmental assessments have been focusing on effects on local benthic habitats, such as smothering of the benthic community by hyper-sedimentation (Trannum et al., 2018; Haugland, 2016; Kvassnes and Iversen, 2013), leaving potential impacts of tailing plumes on pelagic organisms significantly understudied.

Tailing plumes are subjected to tidal currents, upwelling processes, and slope failures, and may spread or resuspend fine particulate matter in the water column (Ramirez-Llodra et al., 2015). For example, remnants of the tailing plumes were detected in the pelagic zone several kilometres from the deposition site in Bøkfjorden, Northern Norway (Berge et al., 2012) and strong wind events caused increased turbidity and spreading of particles in Frænfjorden (Nepstad et al., 2020). Exposure to suspended particles may cause stress and affect pelagic organisms, either through ingestion of particles or attachment of particles to the body surface (Sommaruga, 2015). Also, copepods and other planktonic organisms have limited mobility and rely on ocean currents for horizontal displacement and can thus not actively avoid exposure to tailing plumes. Potentially susceptible organisms are benthic species that have pelagic larvae stages and many zooplankton species, which play a key role in energy transfer from primary producers to higher trophic levels.

The marine filter feeder calanoid copepod *Calanus finmarchicus* has a wide distribution and high biomass in the North Atlantic and Norwegian fjords. *C. finmarchicus* display diel and seasonal vertical migration patterns, where it ascends in spring from winter diapause at depth into the epipelagic zone to reproduce and graze. Due to their wide geographical and vertical distribution patterns they are a potential target species for tailing exposure. *C. finmarchicus* has a complex life cycle, developing though six nauplii stages (NI-NVI) and five copepodite stages (CI-CV) before moulting into adults. The main egg production in spring is timed to and fuelled by the annual phytoplankton bloom (Diel and Tande, 1992; Falk-Petersen et al., 2009). Early development is rapid, and at stage CIII they start accumulating lipids, which is stored for energy consumption during diapause (Kattner and Krause, 1987). The lipids, which are rich in high energy wax esters, are stored in a lipid sac that may take up over 80% of the body cavity in late copepodite stages (Vogedes et al., 2010; Lee et al., 2006). The high abundance and extensive energy storage potential makes *C. finmarchicus* an important food source for fish and sea birds. A previous study showed no acute effects of mine tailing exposure on adult *C. finmarchicus* (Farkas et al., 2017a). However, as early life stages are usually considered to be more sensitive, the effects of tailing exposure on developing *C. finmarchicus* are of interest.

The aim of this study was to determine if mine tailing exposure affects development of early life stages of *C. finmarchicus*, and whether effects vary between different types of tailings, i.e. depend on tailing properties such as particle size, mineral type and presence of chemicals. Early life stages of *C. finmarchicus* were exposed to three different types of mine tailings: tailings from a calcium carbonate processing plant with and without processing chemicals and tailings from a metal ore. Developmental stage distribution, growth, lipid accumulation, and respiration were determined. Based on these data, we applied a dynamic energy budget (DEB) model, which is used to explain how food is used to fuel an individual organism's life history. Changes in life-history traits, like development and growth, imply changes in the uptake or use of available resources, thus underlying mechanisms of responses to stressors can be investigated (Jager, 2020).

2. Material and methods

2.1. Tailing materials and exposure preparation

Tailings were obtained from two different mining/production sites in Norway: a calcium carbonate processing plant (limestone) located in Western Norway, and a copper mine in the start-up phase in Northern Norway. Both production sites have permission to deposit their tailings into the adjacent fjords.

From the calcium carbonate processing site, we obtained tailings (hereafter referred to as Limestone) containing the processing chemicals (flocculation and flotation chemicals) and pure calcium carbonate particles, which did not contain processing chemicals (Particle). Both materials were obtained as a slurry with a dry mass of 42.7 ± 0.14% and 46.4 ± 0.79%, respectively. The drill core tailings from the metal processing mine (Copper) were obtained as dry powder. All tailings were stored refrigerated until use.

Stock (1) suspensions of the three tailing materials were prepared by either adding 2 g L^{-1} of Copper tailing powder or 1500 ml L^{-1} of Limestone or Particle slurry (equalling 2 g L^{-1} dry mass) into filtered (25 μm, then 5 μm, Cuno Aqua-Pure water filter) natural seawater (10 °C).

These stock (1) suspensions were shaken vigorously and then left standing for 1 h to allow settling of large particles. Subsequently stock (1) was carefully decanted to not resuspend settled particles to obtain stock (2) suspensions. Particle concentrations of stock (2) were determined using a particle sizer (Coulter counter 4; Beckman Coulter, US) equipped with a 100 μm (size range 2–60 μm) aperture as described previously by Farkas et al. (2017a). Samples were diluted in filtered (0.2 μm, Millipore Sterivex, Merk KGaA, Germany) seawater to optimise measurements. This resulted in average particle numbers of 2.04 ± 0.13 × 10^6 particles mL^{-1} (2–10 μm, containing >90% of the particles on a number basis). Exposure suspensions were then prepared by diluting stock (2) to approximately 37,500 particles mL^{-1} as low (Low) and to 75,000 particles mL^{-1} as high (High) with filtered seawater (25 μm, then 5 μm, Cuno Aqua-Pure water filter). The exposure suspensions were based on preliminary tests in the lab and results from previous studies (Farkas et al., 2017a) to ensure that they are relevant for what can be expected in nature (Nepstad et al., 2020; Davies and Nepstad, 2017).

2.2. Exposure organisms

C. finmarchicus were obtained from a continuously reproducing in-house culture (for more information see Hansen et al., 2007). Eggs were collected from ovulating females and kept in incubation tanks (50 L) provided with a flow through of natural filtered seawater (25 μm followed by 5 μm Cuno Aqua-Pure water filter) at 10 °C. The organisms were kept for 14 days and were provided algae as food (*Rhodomonas baltica* and *Dunaliella tertiolecta* in an approximately 1:1 mixture based on algal carbon and more than 150 μg C L^{-1} to support normal growth and development) until the start of the experiment. When most of the animals reached nauplii stage 6 (NVI), the tank was drained and the nauplii gently collected using a sieve (64 μm) submerged in water to avoid injury of the nauplii. Nauplii were transferred to Petri dishes to verify the stage before stage NVI were distributed to exposure bottles.

2.3. Exposure set-up

Exposure suspensions for each of the three tailing types of Particle, Limestone and Copper in low (Low) and high (High) suspensions were prepared as described above and transferred to exposure bottles (1 L), with six bottles per exposure treatment (n = 6). Natural filtered seawater (as described above) was used as control (n = 6). Calanus NVI (n = 60) and algae (at a similar concentration as described above) were added to the exposure bottles before the bottles were carefully closed to avoid air bubbles and placed on rotating wheels (1 rpm) to keep particles and algae in suspension. The wheels were kept in a temperature-controlled room (10 °C). The bottles were closed with caps that allowed water exchange (through a sieve protected outlet) during the exposure period to ensure sufficient oxygen levels and organisms feeding. Six times during the exposure, at day one, 4, 6, 8, 11 and 15, sampling was conducted by terminating one bottle per treatment, with all copepods being

sampled and randomly distributed for analysis of developmental stage, biometry, and oxygen consumption. At each sampling point, half the exposure suspensions of remaining bottles were replaced with fresh suspensions containing both tailings and algae.

While we are aware that the use of one replicate bottle per exposure and timepoint is not optimal, the focus of this study was to obtain a time resolution for data integration into the applied DEB-model. The lack of replicate bottles will have no impact on the modelling of the data. Also, all animals derived from the same tank, and the bottles for each treatment were filled and refilled from a larger container, ensuring the same particle suspension in the bottles. We thus would not expect a significant variation between replicate exposure bottles.

2.4. Oxygen and temperature

Each time, before animals were collected, oxygen and temperature were measured. Oxygen in the samples was measured with a phase fluorometer (NeoFox GT, Ocean Optics, Largo, US) equipped with a R-series FOSPOR electrode (Ocean Optics, Largo, US) and temperature compensated by a thermistor probe (NeoFox-TP, Ocean Optics, Largo, US). The fluorometer was controlled by NeoFox Viewer (version 2.66, Ocean Optics, Largo, US) software. Before use, the fluorometer was calibrated in seawater either de-oxygenated by bubbling with nitrogen gas or fully saturated with ambient air until stable readings for the tau-value. The salinity correction in the software was set to 33 ppt, and ambient temperature for the measurements was used in calibration.

2.5. Copper concentrations in exposures

To determine Cu release from Copper tailings, water samples were collected from 3 bottles per treatment at the beginning, during the exposure exchange and at the end of the experiment. Samples were collected and filtered through 0.45 μm filters (polyethersulfone membrane, VWR). Subsequently, samples were acidified with 3 droplets of 50% v/v nitric acid (HNO_3, ultrapure grade, purified from HNO_3, AnalaR NORMAPUR®, VWR, by distillation with Milestone SubPur, Sorisole, BG, Italy) to achieve final concentration 0.1 M HNO_3, and stored in the dark at 4 °C prior to elemental analysis. To determine concentrations of trace elements, analyses of HR-ICP-MS were performed by a Thermo Finnigan model Element 2 instrument (Bremen, Germany) (See SI for a detailed description).

2.6. Respiration measurements

Respiration was measured at every sampling point. For the first four sampling points (at day one, 4, 6 and 8), the PyroScience FireStingO_2 3.07 with Pyro Oxygen logger software version 3.315 (PyroScience, Germany) calibrated with oxygen saturated sea water at 10 °C and 34 ppm in 220 μL vials were used. Respiration measurements were performed in duplicates in closed 220 μL vials at 10 °C, with natural filtered seawater (as described above) for 30–45 min. The vials were placed in a water bath with circulating 10 °C water to keep a constant temperature during the measurements. Due to the small size of the copepods, individuals were pooled to get good respiration readings in the available timeframe (30–45 min). The first sampling point, at day one, was the only sampling consisting of nauplii and 10 nauplii were pooled for each measurement. At the following sampling points, at day four, six and eight, the measurements were performed with eight copepods per chamber. When the copepods reached copepodite stage 4 (CIV), the PyroScience system respiration chambers were too small. To avoid inducing stress, measurements were thereafter performed with the Loligo® Microplate Respirometry System with the MicroPlate™ software version 1.0.4 with 940 μL wells (Loligo Systems, Denmark). The system was calibrated with oxygen saturated sea water at 10 °C and 34 ppm and de-oxygenized distilled water (by adding sodium sulphite). During the 30–45 min respiration measurement the plates were placed in a water bath with circulating 10 °C water to ensure constant temperature. Due to the larger number of individual wells in the Loligo system, four parallel chambers were used per treatment, each with three copepods at day 11 and two copepods at day 15.

Respiration was calculated based on the oxygen concentrations at the start and end of each respiration measurement based on a linear decline in oxygen concentration ($r^2 > 0.9$), and corrected for temperature, salinity, and air pressure. The oxygen saturation in the chambers never went below 80%.

2.7. Biometry analysis and developmental stage determination

Copepods used for respiration measurements were subsequently used for biometry measurements with an additional 10 animals imaged per treatment. Imaging was conducted using a Leica Z6APO macroscope with mounted Leica MC170HD camera. Additionally, images of faecal pellets and particle aggregations were taken to determine whether tailings/particles were ingested.

From each image life stage, prosome length and lipid area were determined. Lipid sac area is previously shown to be a good indicator of total lipid content in *Calanus* sp. (Vogedes et al., 2010). Prosome length, body and lipid sac area was automatically measured as shown in Fig. 1, using the MASK-R CNN neural net architecture (He et al., 2017) trained on 169 manually annotated images of *C. finmarchicus*. This neural net architecture outputs outlines of the body (shown in red/outer line, Fig. 1) and lipid sac (shown in green/inner line, Fig. 1), where the area and prosome length were calculated using automated image processing techniques such as topological structural analysis (TSA) (Suzuki and Abe, 1985) and ellipse fitting (Fitzgibbon and Fisher, 1995).

For analysis and modelling purposes (described below), lipid area was converted into lipid volume (Vogedes et al., 2010). Prosome length and projected area were converted into total body volume, assuming a cylindrical shape. Structural body volume was subsequently calculated from the difference of total and lipid volume and converted into a length measure (volumetric length) by taking the cubic root. Details are provided in the supporting information. In addition, the applied DEB model (see section 2.9) uses time passed as a reference on the x-axis (the independent variable), and not developmental stage, as is often common with copepod studies. This most likely increased variation in the measurements, as different developmental stages were present in the measurements for each sampling day. However, as the copepods progress in developmental stage with time, body length, lipid accumulation and respiration also increase with time, hence, increased variation caused by different developmental stages in the measurements will most likely even out. Also, at each sampling day, except for sampling at day six, one

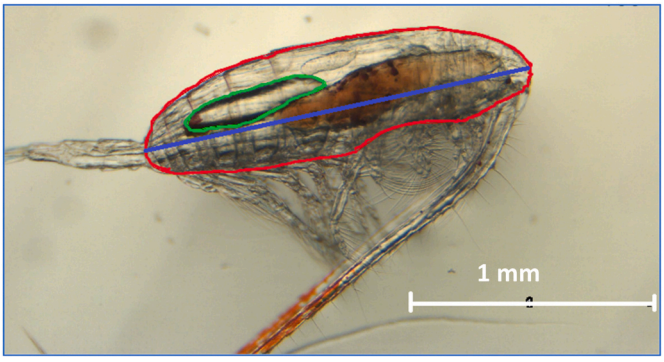

Fig. 1. Automated biometry measurements. Image of *C. finmarchicus* (stage CIV), showing the computer automated measurements of the copepod. In outer red line is the outline of the copepod's body, while the inner green line outlines the lipid sac. In blue is the computer calculated prosome length, used for DEB model calculations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

developmental stage was more abundant than others in all treatments, which resulted in a close to stage like distribution in the measurements regardless.

2.8. Mortality

The total mortality was calculated for each bottle at the time of bottle termination. Due to the experimental setup and bottle cap connection to the water exchange system, it was not possible to count dead animals in each bottle every day. Also, Dead copepods decompose rapidly. Therefore, mortality was estimated based on the number of copepods present in the bottles at the beginning and the copepods removed from the bottles during sampling. Copepods that were not used for analyses described in previous chapter were counted, developmental stage was determined, and number of dead animals recorded.

2.9. Dynamic energy budget (DEB) model

To elucidate mechanisms underlying the responses to mine tailing exposure, a dynamic energy budget (DEB) model *(DEB model)* was applied. Here, the simplified DEBtox model from Jager et al. (2020) was chosen as it better suited the data collected. Nevertheless, several parameters established from the detailed DEB model for *C. finmarchicus* (Jager et al., 2017) were used, thereby decreasing the number of parameters that needed to be fitted in this study. A complete overview of the model equations, including the model parameters and data translations is provided in the SI. Below a conceptual summary is provided.

The simplified DEBtox model only considers juveniles and sub-adults (as defined in Jager et al., 2017) as the animals developed from stage NVI to CIV during the experimental period. The switch between these two stages is determined by a fixed body length, which in DEB terminology, could be called "puberty" as it marks the start of investment in reproduction (lipid storage was treated as a reproduction buffer), and coincides with a step-up in growth rate, attributed to an increase in maximum feeding rate (Jager et al., 2017). To explain growth and lipid storage in the Control, the only parameters needed to be fitted were the initial volumetric length and the maximum lipid storage rate. The other model parameters were directly taken or recalculated from the parameter values in Jager et al. (2017).

Respiration is difficult to interpret in a DEB context since it is influenced by several processes. Here, we opted for a simplified description of respiration rate, as a weighted sum of an area-specific and a volume specific process (see Kooijman, 2009). The two proportionality constants, representing the surface related and volume related processes, were fitted together on the data from the Control treatment.

Stress affects mass/energy fluxes in the model through a physiological mode or mechanism of action (pMoA). The simplified DEBtox model (Jager, 2020) offers a range of possible pMoA's, of which the most likely for mine tailing would be a decrease of feeding and/or assimilation, or an increase in the maintenance costs, based on the lack of acute toxicity and ingestion of particles seen in a previous study (Farkas et al., 2017a). The DEBtox model provides the equations to apply the stress factor on feeding/assimilation (s_A) or maintenance costs (s_M) and calculates the consequences for growth and reproduction (here lipid storage). The stress factor was assumed to remain constant over time and was fitted for each treatment, for both pMoAs, on the data for body size and lipid storage over time. The respiration data was excluded from the fit, because of the simplified modelling approach for this trait. Instead, a prediction was made based on the selected pMoA's. The prediction for respiration relies on the simplifying assumption that assimilation stress (s_A) affects the surface-area related component of respiration, and maintenance stress (s_M) the volume-related one. This gives an indication of the plausibility of different pMoAs.

Juveniles (i.e., animals before reaching the size at puberty) seemed to be less affected by the treatments. To explore this observation, we included an additional parameter representing a factor by which stress (sA or sM) is reduced in the juveniles. Including an additional model parameter allows testing whether this apparent reduction is significant. If the CI for the juvenile stress reduction factor excludes one, we can conclude that juveniles are indeed less sensitive than sub-adults.

The model was implemented in the general modelling platform BYOM in Matlab (https://www.debtox.info/byom.html). The model in the package DEBtox2019 was adapted to deal with the step-up in feeding and the non-chemical stressor and to include the respiration equation. Model optimization was based on the likelihood function following from the assumption of normally distributed residuals. Observations on body size and lipid storage contribute to one overall likelihood function and are treated as independent observations. Confidence intervals (CI) were generated by profiling of the likelihood function, which is essentially a series of likelihood-ratio tests. If the CI for the stress factor excludes zero, we can conclude that the observed treatment effects on body size and lipid storage over time are significant.

2.10. Statistical analysis

Data analyses and visualization were completed using GraphPad Prism 8 (GraphPad Software Inc., USA) and R studio (R Core Team, 2019). All measurements are given as mean ± SE. The data were analysed for normality (Shapiro-Wilk) and homogeneity of variance (Bartlett) before applying a main effect Ancova (adjusted for heteroscedasticity where necessary) and/or One-way Anova. Tukey's post hoc tests were used to identify differences between groups. For non-normal data, Kruskal-Wallis tests were performed, followed by Dunn's multiple comparisons tests. The significance level was set to $p \leq 0.05$.

3. Results

3.1. Exposure characterization and conditions

During the experimental period, the temperature varied between 9.32 and 10.09 °C in the bottles, with an average of 9.70 ± 0.22 °C. Oxygen concentrations were between 7.09 and 8.87 mg L^{-1}, with an average of 8.35 ± 0.51 mg L^{-1}. Oxygen concentrations in the bottles were above 8 mg L^{-1} up till the last sampling day, when oxygen most likely dropped due to the larger size of the copepods and thus increased oxygen demand (see SI for all measurements).

Table 1 gives an overview over particle numbers, volumes, and weights for each treatment at the beginning of the experiment. In the High exposure concentrations, 99 ± 0.0046% (Particle), 99.2 ± 0.15%

Table 1
Particle data. Overview over particle numbers, volume, and weight for each treatment at the beginning of the experiment. Values are given as mean ± SD.

Treatment	Particle number (per mL)	Particle volume (μm^3 mL^{-1})	Particle weight (mg L^{-1}) 2–10 μm	Particle weight (mg L^{-1}) 2–60 μm
Particle Low	37,361 ± 73	$0.45 \times 10^6 \pm 0.01 \times 10^6$	2.38 ± 0.09	5.83 ± 3.02
Particle High	76,757 ± 1046	$1.51 \times 10^6 \pm 0.05 \times 10^6$	4.09 ± 0.15	8.52 ± 0.84
Limestone Low	52,017 ± 436	$0.43 \times 10^6 \pm 0.02 \times 10^6$	2.49 ± 0.04	6.13 ± 1.26
Limestone High	85,727 ± 899	$1.51 \times 10^6 \pm 0.21 \times 10^6$	4.08 ± 0.06	8.25 ± 0.70
Copper Low	48,499 ± 373	$0.42 \times 10^6 \pm 0.01 \times 10^6$		
Copper High	75,373 ± 290	$1.75 \times 10^6 \pm 0.02 \times 10^6$		

(Limestone) and 100% (Copper) of the measured (2–60 μm fraction) particles were in the size range 2–10 μm, based on particle numbers. Due to flocculation of the particles during the experiment, quantification of particle numbers was not possible at the end of the experiment. The mass (mg L^{-1}) of Particle and Limestone tailings was calculated using density of calcium carbonate (2.71 g cm^{-3}) (Farkas et al., 2017a). As the composition of Copper tailings is not known in detail, mass exposure concentrations were not calculated.

There was no evidence of substantial copper leaching into the water from the tailings. In Control the copper concentration ranged from below detection limit (LOD) to 7.13 μg L^{-1}. The copper concentration ranged between 0.55 and 9.59 μg L^{-1} for the low treatments and from 1.73 to 12.54 μg L^{-1} for the high treatments (see SI). The highest concentration of copper was found in the Limestone treatment, both for the low and high treatments.

3.2. Copepod development

3.2.1. Mortality

Dead animals in Control ranged from 10 to 40% over the experimental period. For the treatments, mortality ranged from 0 to 27.7%, 11.7–33.3%, 10–43.3%, 18.3–50%, 8.3–36.7% and 11.7–38.3% for Particle Low, Particle High, Limestone Low, Limestone High, Copper Low and Copper High, respectively (See SI). There was no significant difference in mortality between treatment groups over time, nor any noticeable increase with time.

3.2.2. Stage progression

This experiment followed the development of *C. finmarchicus* from NVI to CIV, i.e., the last nauplii stage to the second to last copepodite stage before adulthood. Fig. 2 shows the developmental stage distribution at each sampling point for each treatment group (n = 30–62 copepods per bottle). At the first sampling (day one), the proportion of stage CI copepodites was lowest in the Control group. The following sampling (day 4), developmental stage CII were present in all treatment groups except for Control. Similarly, the proportion of stage CII copepodites was again lowest in the Control group after 6 days. This trend changed from day 8, where the first presence of developmental stage CIII was recorded in all treatment groups except for Particle High and Limestone High. The proportion of stage CIII copepodites at day 11 was overall higher in the Control group and low treatments, with CIII's accounting for 82.4, 93.5, 85.2 and 73.9% of the individuals in Control, Particle Low, Limestone Low and Copper Low, respectively, while Particle High, Limestone High, and Copper High had a proportion of 74.5, 50.0 and 51.4% stage CIII copepodites, respectively. In addition, developmental stage CIV were recorded only in the Control group and the Particle High treatment. Stage CIV copepodites were present in all groups at day 15, however, the proportion of stage CIV copepodites were lower in all high treatments, and in Particle Low. In the Control group, Limestone Low and Copper Low, stage CIV copepodites accounted for 58.3, 69.2 and 68.4% of the individuals, while for Particle Low, Particle High, Limestone High and Copper High, the proportion of stage CIV were 14.0, 42.5, 36.7, and 25.6%, respectively. When the experiment ended, developmental stage CV was not recorded in any of the treatments.

3.2.3. Growth and lipid accumulation

Tailing exposure affected both body length and lipid accumulation. While an increase in volumetric body length was seen in all groups during the experiment, individuals in the Particle Low treatment and all high tailing treatment groups had slightly lower body lengths than the Control group and the Limestone Low and Copper Low treatment groups (Fig. 3a). After 15 days, copepods in Particle Low and High, Limestone High, and Copper High treatment groups had reached a mean volumetric body length of 0.43 ± 0.01, 0.52 ± 0.01, 0.47 ± 0.02 and 0.48 ± 0.02 mm, respectively, compared to 0.55 ± 0.01, 0.57 ± 0.01 and 0.54 ± 0.02 mm for copepods in the Control group, and Limestone Low and Copper Low treatment groups. Lipid accumulation (Fig. 3b) followed the same pattern with copepods in Particle Low and High, Limestone High, and Copper High groups reaching a mean lipid volume of 0.0003 ± 0, 0.002 ± 0, 0.002 ± 0,0002, and 0.001 ± 0,0002 mm^3, respectively, compared to 0.0037 ± 0.001, 0.0029 ± 0.001 and 0.0026 ± 0,0003 mm^3 for copepods in the Control group and Limestone Low and Copper Low groups.

The difference in both volumetric body length ($H(7) = 50.72$, $p < 0.0001$) and lipid volume ($H(7) = 55.29$, $p < 0.0001$) were significant.

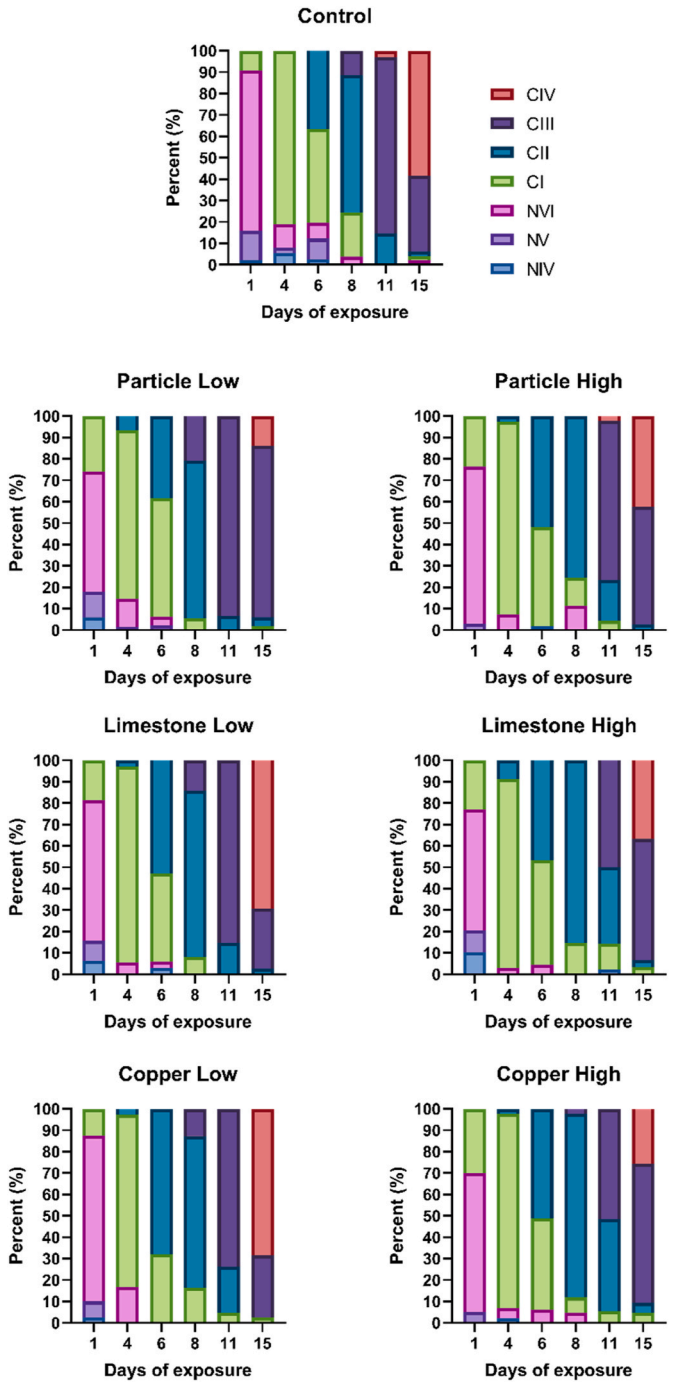

Fig. 2. Stage distribution of *C. finmarchicus*. The proportion of each stage present is shown in percent (%) calculated from the total number of animals present in each sampled bottle (n = 30–62) at each sampling time and presented for each treatment.

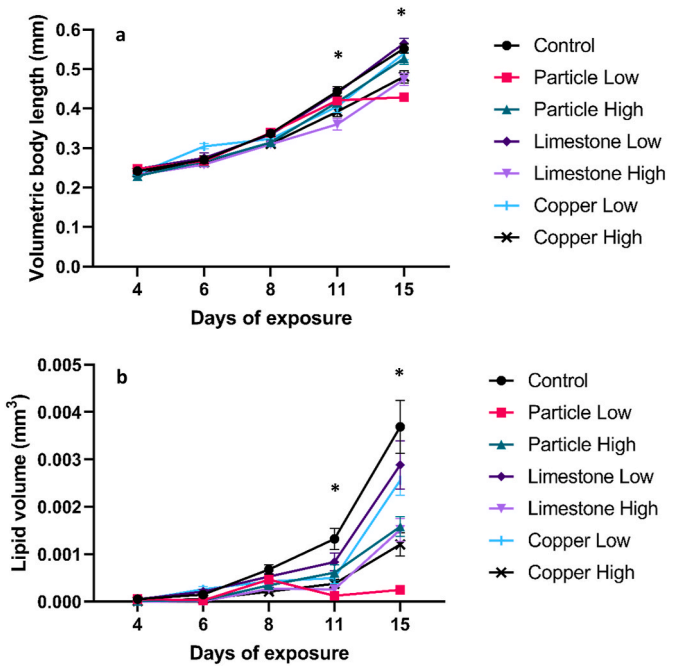

Fig. 3. Biometric measurements. The increase in volumetric body length (a) and lipid sac volume (b) in *C. finmarchicus* is shown for each of the five sampling days and for each treatment during the 15-day exposure period. Volumetric body length is given in mm and lipid sac volume in mm^3 (values are presented as mean ± SE, n = 11–32). Days marked with * indicate days with significant differences in body length or lipid volume based on Kruskal-Wallis tests.

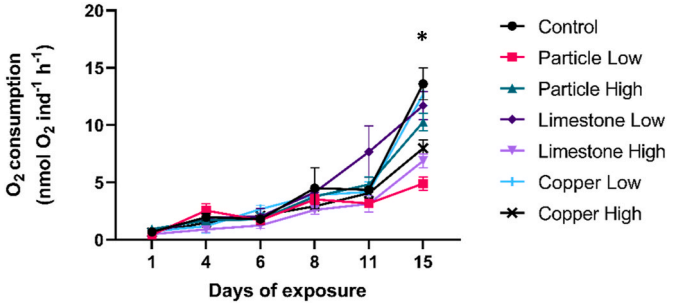

Fig. 4. Oxygen consumption. Mean ± SE oxygen consumption (nmol per individual per hour) in *C. finmarchicus*. Copepods were pooled for measurements, n = 2 for day 1 through 8, and n = 4 for day 11 and 15 (see section 2.6 for explanation). The day marked with * indicate the day with significant difference in oxygen consumption based on one-way Anova.

Dunn's post hoc comparisons showed a significant lower volumetric body length in copepods from the Particle Low ($p < 0.0001$), Limestone High ($p = 0.013$) and Copper High ($p = 0.039$) treatment groups compared to the copepods from the Control group. In addition, copepods from the Particle Low ($p < 0.0001$), Limestone High ($p = 0.002$) and Copper High ($p = 0.007$) treatments also had significantly lower volumetric body length than copepods from the Limestone Low treatment, while Particle Low ($p < 0.0001$) and Limestone High ($p = 0.04$) copepods were significantly shorter than Copper Low copepods.

The lipid volume of the copepods in the Particle Low ($p < 0.0001$) and Copper High ($p = 0.003$) treatments were significantly lower than copepods from the Control group. Moreover, copepods from the Particle Low treatment had significantly lower lipid volume in comparison to copepods from the Particle High ($p = 0.005$), Limestone Low ($p < 0.0001$), Copper Low ($p < 0.0001$) and Limestone High ($p = 0.02$) treatment groups.

3.2.4. Respiration

Respiration measurements showed an overall increase in oxygen consumption, reflecting the progression in development/growth during the experimental period (Fig. 4). Copepods in the Control group had an increase in oxygen consumption from 0.69 ± 0.07 nmol O_2 ind^{-1} h^{-1} at day one to 13.6 ± 2.8 nmol O_2 ind^{-1} h^{-1} after 15 days, with the main increase taking place after day 11. The same pattern can be seen for the treatment groups. At day 6, all groups were respiring at similar levels, while subsequent measurements show an increase in variation between the different exposure groups. After 15 days, respiration rates in descending order were 13.6 ± 2.8, 12.7 ± 2.0, 11.7 ± 2.4, 10.3 ± 1.5, 8.0 ± 1.5, 6.9 ± 1.2 and 4.9 ± 1.2 nmol O_2 ind^{-1} h^{-1} for copepods in the Control, Copper Low, Limestone Low, Particle High, Copper High, Limestone High, and Particle Low groups, respectively.

Differences in oxygen consumption were significant between the treatment groups ($F(6, 21) = 11.96$, $p < 0.0001$) after 15 days of exposure. Tukey's multiple comparisons tests revealed a significantly lower oxygen consumption at day 15 for copepods in the Particle Low ($p < 0.001$), Limestone High ($p = 0$ 0.001) and Copper High ($p = 0.0032$) treatment groups compared to copepods from the Control group. In addition, oxygen consumption of copepods from the Particle Low treatment was significantly lower than copepods from the Particle High ($p = 0.0109$), Limestone Low ($p = 0.0007$) and Copper Low ($p = 0.0002$) groups. Also, copepods from Limestone Low and Limestone High ($p = 0.0214$) and Copper Low and Copper High ($p = 0.0156$) differed significantly from each other.

3.3. Documentation of particle ingestion

Faecal pellets from *C. finmarchicus* exposed to particles (Low and High groups) contained a high proportion of particles (selected images are shown in Fig. 5, see SI for all pictures). The faecal pellets of copepods

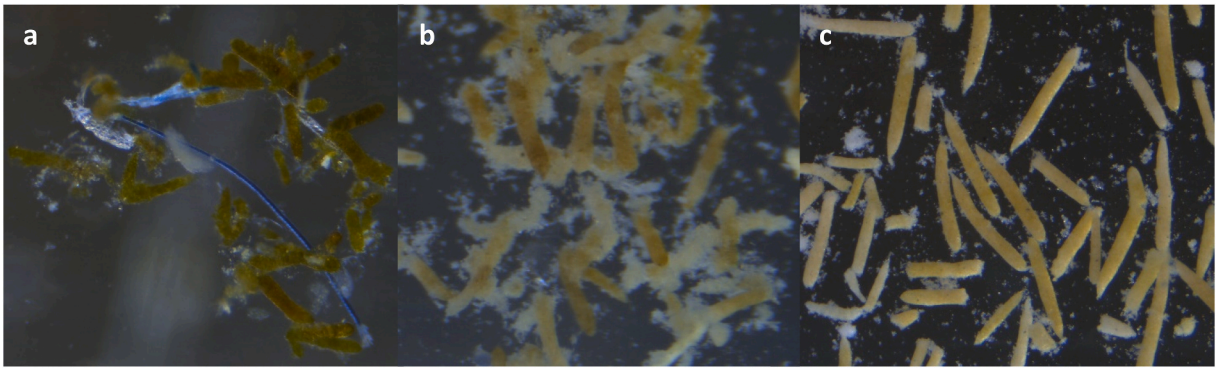

Fig. 5. Faecal matter. *C. finmarchicus* faecal pellets from Control (a) Copper Low (b) and Copper High (c) at sampling at day 11 show a whiter colour indicating presence of particles in the low and high exposure (b and c) compared to the green colour of Control (a), consisting mostly of algae. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in the Control groups contained mostly algae (green pellets, Fig. 5a), while the whiter coloration of faecal pellets in copepods from the treatment groups indicates presence of particles (Fig. 5b and c). Quantification of faecal pellets were not possible due to particle aggregation, broken pellets and some faecal matter being lost during sampling.

3.4. DEB model approach

To assess the physiological mode of action of particle exposure, i.e., the underlying responses to particle exposure, we used a simplified DEB model to test two different pMoA's, assimilation and maintenance. An overview of stress levels and fit for each pMoA is shown in Table 2. Fig. 6 shows the model fit for volumetric body length and lipid volume, in addition to the model prediction for respiration, with assimilation as pMoA.

The model fits for volumetric body length were good, with $r^2 > 0.96$ for both pMoA's (Table 2). This trait by itself is thus insufficient to distinguish between the pMoA's. In addition, the step-up in growth at puberty (as established by Jager et al., 2017) was captured nicely by the model (Fig. 6, days after start of exposure) for Control, Limestone Low, Copper Low and Particle High. The fit for lipid accumulation also visually corresponded well with the observed data (Fig. 6), even though the r^2-value was somewhat lower (0.68–0.94) than the fit for volumetric body length. The r^2 values for the assimilation pMoA were better than for maintenance. Since the two pMoA's do not represent nested models, there is no formal test for significance to decide which one is the better explanation. However, the AIC clearly points at assimilation to be the better model for all particle types. This conclusion is strongly supported by the predictions for respiration, which gave a r^2-value < 0 for the prediction using maintenance as pMoA, while it rendered a much better fit for assimilation, with $r^2 > 0.90$ (Table 2). Effects on maintenance imply an increase in respiration while the opposite effect was observed.

For all fitted stress factors, the CI excludes zero. Since the CI resulted from likelihood profiling, this implies that the effects on volumetric length and lipid storage are significant in a likelihood-ratio test ($p < 0.05$). Preliminary data analysis showed that juveniles were less affected by the stressors than sub-adults. For the overall model, a stress reduction factor on the juveniles was included, where a factor of 0 indicated no stress on juveniles at all. For assimilation, the CI for the stress-reduction factor excludes one in all treatments, which implies that juveniles experience significantly less stress from the treatments than sub-adults. The CIs include zero, which indicate that juveniles might even experience no stress at all. However, the confidence intervals were rather wide, for Limestone Low in particular. When considering assimilation as pMoA, the lowest overall stress factor was 0.051 for Limestone Low followed by Copper Low (0.17), Particle High (0.25), Limestone High (0.32) Copper High (0.37) and Particle Low (0.48). For Limestone and Copper, the high treatments showed higher stress factors than the low treatments (CIs do not overlap). However, for the Particle treatment, the opposite was observed.

Table 2
Fit of treatment data. Low and high treatment fitted simultaneously. Fitted parameter are two stress levels (one for each treatment) and a stress-reduction factor for juveniles (zero means no stress on juveniles). The r^2 (calculated on the mean response) is shown for the three endpoints length, lipids, and respiration, but note that the model is fitted to the data for volumetric body length and lipid volume only, with a prediction for respiration rate. For the maintenance fits, the difference in Akaike Information Criterion (model evaluation criterion) with the corresponding assimilation fit is shown.

Type	Low (CI)	High (CI)	δ_s Juv.stress red.	r^2 (length, lipid, resp.)
		pMoA assimilation		
Particle	0.48 (0.44–0.55)	0.25 (0.21–0.30)	0 (0–0.044)	0.96, 0.84, 0.90
Limestone	0.051 (0.013–0.10)	0.32 (0.20–0.42)	0.26 (0–0.78)	0.99, 0.94, 0.92
Copper	0.17 (0.11–0.22)	0.37 (0.29–0.43)	0.037 (0–0.28)	0.99, 0.94, 0.91
		pMoA maintenance		
Particle	5.6 (4.8–6.3)	2.6 (2.0–3.2)	0 (0–0.088)	0.96, 0.68, <0 (ΔAIC = 50)
Limestone	0.33 (0.036–0.75)	2.7 (1.2–4.0)	0.49 (0.15–1.5)	0.99, 0.92, <0 (ΔAIC = 7.5)
Copper	1.3 (0.80–2.0)	3.2 (2.1–4.4)	0.27 (0.047–0.65)	0.98, 0.89, <0 (ΔAIC = 22)

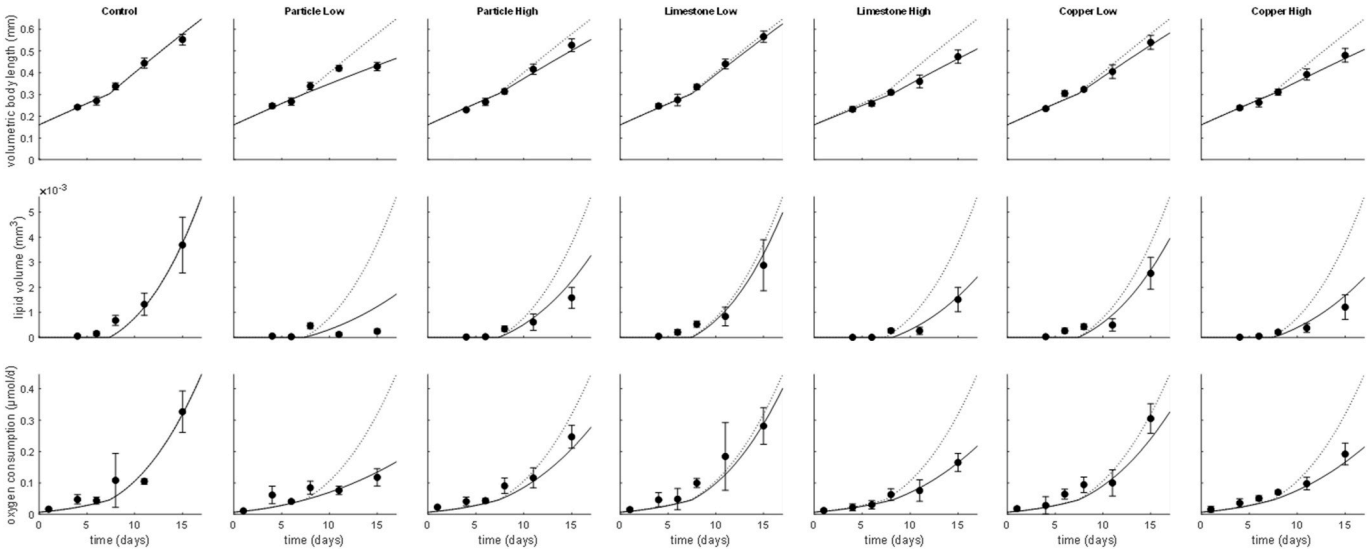

Fig. 6. Model fit for volumetric body length and lipid sac volume for all treatments, assuming a stress on assimilation. The model curve for respiration is a prediction; these data were not included in the fit. Error bars are approximate confidence intervals on the means (2 X SE). Dotted lines in the treatment plots show the control response.

4. Discussion

This study shows that mine tailing exposure results in slower development and growth, lower lipid accumulation and reduced respiration in developing *C. finmarchicus*. However, no clear difference in mortality was observed between treatments, suggesting that the tailing particles themselves are not acutely toxic to the animals. Other studies have shown that copepods are able to tolerate high sediment loads, as least for shorter time periods (Farkas et al., 2017a; Arendt et al., 2011; Shadrin and Litvinchuk, 2005), and our results support these findings. Mortality did not increase throughout the experiment, indicating that the main mortality occurred in nauplii and younger stages, which naturally have a higher mortality than older stages (Cook et al., 2007).

The limestone particle suspensions used in this study, were comparable to what can be expected in nature around the deposition site. Two studies have investigated the particle concentrations and particle transport in the vicinity of the deposition site for the limestone tailings used in this study. Optical particle measurements showed that larger particles settle close to the release point, while the finer fraction, down to 10 μm, could be detected up to 3 km from the deposition point (Davies and Nepstad, 2017). The particle concentrations reached a particle volume of up to 5 μl L^{-1} (i.e., 5×10^6 μm^3 mL^{-1}) (Davies and Nepstad, 2017), which is higher than the concentrations used in this study. High resolution modelling also shows that, especially during strong wind events, particle concentrations can reach levels higher than 100 mg L^{-1} close to the tailing pipe and exceed 1 mg L^{-1} several km away (Nepstad et al., 2020). In fjords with mine tailing discharge, copepods might therefore encounter higher particle suspensions than the ones used in this study some distance away from the deposition site.

We did not detect significant differences in the measured parameters between the tailing types containing processing chemicals (Limestone) or metals (Copper). Our results thus suggest that the particles themselves are the main effect components. There were no significantly larger effects of Copper tailings compared to other exposures, indicating that at least under the applied experimental conditions, heavy metals present in the tailings do not play a significant role for the observed effects. In addition, we did not discover substantial copper leaching from the copper-ore tailings to the water. Even though we did not find any specific metal related effects in our exposures, we cannot exclude any additional metal related effects on a molecular level in the copepods. Overall, higher particle concentrations resulted in stronger responses, highlighting the particles as drivers of observed effects (the strongest response, which was found for Particle Low, will be discussed in detail further down).

This is in good agreement with the DEB model results, as stress through reduced energy assimilation was identified as pMoA, and not increased maintenance costs (e.g., increased regulation costs for detoxification). At this moment, we cannot entirely exclude other pMoAs, or combinations of pMoAs. However, reduced assimilation is most promising since the effect of mine tailings is likely mechanical rather than toxicological (as supported by the lack of acute toxicity (Farkas et al., 2017a)). Assimilation stress due to particle exposure is most likely linked to particle ingestion.

Replacing food with ingested particles might leave less energy for development and reproduction (Shadrin and Litvinchuk, 2005). Images revealed a high load of particles in the faecal pellets, confirming that the copepods had ingested particles from the exposure suspensions. Similar findings have been reported in earlier studies showing that copepods readily ingest particles from suspended sediments, marine snow, detritus, and even crude oil droplets (Farkas et al., 2017a, 2017b; Hansen et al., 2012, 2017; Arendt et al., 2011; Kattner et al., 2003; Dilling et al., 1998; Paffenhofer and Knowles, 1979). It has been suggested that some copepods are able to differentiate between inorganic particles and algal cells (Cowles et al., 1988), however, according to Meyer et al. (2002), this selectivity is probably linked to particle size. Moreover, with a high concentration of inorganic particles, it will be harder for the animals to selectively choose food, potentially resulting in a higher intake of particles. Sew et al. (2018) found that particle size and particle to algae ratio was an importing factor determining particle ingestion rates, with small particles in the presence of low algae concentrations having the strongest impact. Therefore, even if the copepods can differentiate between food and particles, this selectivity could become impaired at higher particle concentrations (Sew et al., 2018). This was reflected in the higher stress factor on assimilation for copepods in the high tailing exposure groups, indicating a higher particle ingestion in these groups, further supporting a stronger response on development.

Interestingly, Particle Low caused the strongest effect of all exposures, with copepods from this treatment having the shortest body length and lowest lipid volume at the end of the study. This was also reflected in the highest stress factor (Table 2) for this treatment. As Koski et al. (2017) discovered that calanoid copepods had a higher feeding rate when feeding on dispersed food compared to aggregated food, this could be due to an increased suspended particle load in the Particle low exposures. The Limestone tailings contain a flocculation chemical enhancing particle aggregation and sedimentation, which is not present in the Particle exposure groups. Further, the higher particle number in the Particle high exposure can have caused increased collision frequency/likelihood and thus aggregation and flocculation. Thus, Particle low could contain more smaller and unaggregated particles that are available for ingestion by the copepods. On the other hand, similar strong effects were not observed for the Copper tailing exposures, which also do not contain flocculation chemicals. This can be explained by Copper tailings having different composition and shape, which in turn may lead do different flocculation properties and thus bioavailability.

Effects of low food assimilation on zooplankton development have been investigated in several studies and corresponds well with our results. Paffenhofer (1972) showed that ingestion of red mud particles delayed growth and development in *Calanus helgolandicus*. Copepods and nauplii subjected to low food quality, or low food concentrations, were shown to generally have slower growth and lower lipid accumulation (Chen et al., 2018; Daase et al., 2011; Cook et al., 2007; Ceballos and Alvarez-Marques, 2006; Mayor et al., 2006). The lower respiration rates for the treatment groups further supports this, as starving animals were reported to have lower respiration rates than fed ones (Kiørboe et al., 1985; Ikeda, 1977:1971; Mayzaud, 1976).

Interestingly, we found a significantly lower stress effect on juveniles, i.e., before the animals reached development stage CIII or "puberty" in DEB terminology (around eight days in this experiment). Growth, lipid accumulation and respiration in exposed copepods differed most profoundly from the Control after eight days. This could be linked to the observed increase in growth rate, most likely related to an increase in feeding rate after this point (Jager et al., 2017). Increased respiration rates have been documented after the CIII stage (Marshall and Orr, 1958), which has been related to the start of lipid accumulation (Kattner and Kruse, 1987), and gonad maturation, which starts at stage CIV (Tande and Hopkins, 1981).

Images taken of the copepods after monitoring of respiration showed a low presence, if any, of particles in the gut. This indicates that the animals in addition to readily ingesting particles, can efficiently evacuate them from the gut. This was also shown by Farkas et al. (2017a), who documented that stage CV *C. finmarchicus* exposed to limestone tailings for 48 h, were able to excrete the particles after a depuration period of 40 h. Effective excretion may however have a negative impact on the energy uptake, as the few food particles present in the gut may not be fully utilized by the copepods. Shadrin and Litvinchuk (2005) discovered in *Acartia clausi* that copepods that ingested limestone particles used less time to produce a faecal pellet, and that the pellets were

not fully digested, than the faecal pellets from copepods in the control group. This might result in lower assimilation of available food particles at high particle concentrations.

Since the clearance rate of particles can be high, it can be assumed that the period of food limitation when encountering tailing plumes in the pelagic zone will be short in duration. However, Malzahn and Boersma (2012) showed that even a two-day exposure to low quality food reduced development in *Acartia tonsa*, with approximately one developmental stage. This effect was non reversible even when *A. tonsa* was fed high quality food after exposure. Longer exposure to low food quality delayed development even more. In addition, Hirche et al. (1997) found that pre-starved *C. finmarchicus* females continued to produce less eggs also after being transferred to high food conditions. However, there are indications that even if development is highly dependent on food availability, the copepods tolerance to starvation increases with age (Calbet and Alcaraz, 1997). Hence, copepods that are exposed to tailing plumes at a later live stage may be less affected.

For *C. finmarchicus* delayed development and low lipid accumulation may have large implications as lipid stores are utilized during diapause to support metabolism, moult into adults, for gonad maturation and to start reproduction start (Tarrant et al., 2014; Falk-Petersen et al., 2009; Niehoff, 2004; Rey-Rassat et al., 2002). Therefore, the capacity to successfully overwinter depends on sufficient lipid stores (Sargent and Falk-Petersen, 1988; Rey-Rassat et al., 2002).

In conclusion, marine tailing disposal has the potential to cause adverse effects for *C. finmarchicus*. Despite high mortality in the nauplii stages and the absence of a dose-response relationship, the results from this study indicate that a delay in development and lower lipid accumulation was a result of exposure to suspended particles with subsequent particle ingestion, resulting in reduced energy uptake in *C. finmarchicus*. While this study included different tailing types and compositions, observed effects seem to be mostly related to particle exposure itself. Further, more knowledge is needed on other potential toxicological effects (i.e., responses to heavy metals and processing chemicals attached to ingested particles) to advance our understanding of the effects of marine tailing disposal on marine copepods.

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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.chemosphere.2021.131051.

References

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 (10), 1526–1537. https://doi.org/10.1093/plankt/fbr054.

Berge, J.A., Bjørnar, B., Brooks, S., Jaccard, P.-F., Tobiesen, A., Øxnevad, S., 2012. Overvåkning av Bøkfjorden 2011 og giftighetstesting av gruvekjemikaliene Magnafloc LT38 og Magnafloc 10. NIVA Report L.N.R 6310–2012, 123.

Calbet, A., Alcaraz, M., 1997. Growth and survival rates of early developmental stages of *Acartia grani* (Copepoda:Calanoida) in relation to food concentration and fluctuations in food supply. Mar. Ecol.-Progr. Ser. 147, 181–186. https://doi.org/10.3354/meps147181.

Ceballos, S., Alvarez-Marques, F., 2006. Reproductive activity and physiological status of the calanoid copepods *Calanus helgolandicus* and *Calanoides carinatus* under food-limiting conditions. J. Exp. Mar. Biol. Ecol. 339 (2), 189–203. https://doi.org/10.1016/j.jembe.2006.07.021.

Chen, L., Li, C.L., Zhou, K.L., Shi, Y.Q., Liu, M.T., 2018. Effects of nutrient limitations on three species of zooplankton. Acta Oceanol. Sin. 37 (4), 58–68. https://doi.org/10.1007/s13131-017-1122-z.

Cook, K.B., Bunker, A., Hay, S., Hirst, A.G., Speirs, D.C., 2007. Naupliar development times and survival of the copepods *Calanus helgolandicus* and *Calanus finmarchicus* in relation to food and temperature. J. Plankton Res. 29 (9), 757–767. https://doi.org/10.1093/plankt/fbm056.

Cowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100 (1), 41–49. https://doi.org/10.1007/BF00392953.

Daase, M., Soreide, J.E., Martynova, D., 2011. Effects of food quality on naupliar development in *Calanus glacialis* at subzero temperatures. Mar. Ecol. Prog. Ser. 429, 111–124. https://doi.org/10.3354/meps09075.

Davies, E.J., Nepstad, R., 2017. In situ characterisation of complex suspended particulates surrounding an active submarine tailings placement site in a Norwegian fjord. Reg. Stud. Mar. Sci. 16, 198–207. https://doi.org/10.1016/j.rsma.2017.09.00.

Diel, S., Tande, K., 1992. Does the spawning of *Calanus finmarchicus* in high latitudes follow a reproducible pattern? Mar. Biol. 113 (1), 21–31. https://doi.org/10.1007/bf00367634.

Dilling, L., Wilson, J., Steinberg, D., Alldredge, A., 1998. Feeding by the euphausiid *Euphausia pacifica* and the copepod *Calanus pacificus* on marine snow. Mar. Ecol. Prog. Ser. 170, 189–201. https://doi.org/10.3354/meps170189.

Dold, B., 2014. Submarine tailings disposal (STD)—a review. Minerals 4 (3), 642–666. https://doi.org/10.3390/min4030642.

Falk-Petersen, S., Mayzaud, P., Kattner, G., Sargent, J., 2009. Lipids and life strategy of Arctic Calanus. Mar. Biol. Res. 5 (1), 18–39. https://doi.org/10.1080/17451000802512267.

Farkas, J., Altin, D., Hammer, K.M., Hellstrøm, K.C., Booth, A.M., Hansen, B.H., 2017a. Characterisation of fine-grained tailings from a marble processing plant and their acute effects on the copepod *Calanus finmarchicus*. Chemosphere 169, 700–708. https://doi.org/10.1016/j.chemosphere.2016.11.118.

Farkas, J., Badsvik, C.Y., Altin, D., Nordtug, T., Olsen, A.J., Hansen, B.H., 2017b. Acute and physical effects of water-based drilling mud in the marine copepod Calanus finmarchicus. J. Toxicol. Environ. Health 80 (16–18), 907–915. https://doi.org/10.1080/15287394.2017.1352197.

Fitzgibbon, A., Fisher, R.B., 1995. A buyer's guide to conic fitting. In: British Machine Vision Conference (BMVC), Birmingham, vol. 1995, pp. 513–522.

Forurensningsdirektoratet, Klima- og, 2010. *Bergverk Og Avgangsdeponering. Status, Miljøutfordringer Og Kunnskapsbehov* (TA 2750-2010). Klima- og Forurensningsdirektoratet, Norway.

GraphPad Prism Version 8.4.3 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com.

Hansen, B.H., Altin, D., Nordtug, T., Olsen, A.J., 2007. Suppression subtractive hybridization library prepared from the copepos *Calanus finmarchicus* exposed to a sublethal mixture of environmental stressors. Comp. Biochem. Physiol. Genom. Proteonomics 2 (3), 250–256. https://doi.org/10.1016/j.cbd.2007.04.006.

Hansen, B.H., Altin, D., Olsen, A.J., Nordtug, T., 2012. Acute toxicity of naturally and chemically dispersed oil on the filter-feeding copepod *Calanus finmarchicus*. Ecotoxicol. Environ. Saf. 86, 38–46. https://doi.org/10.1016/j.ecoenv.2012.09.009.

Hansen, B.H., Altin, D., Nordtug, T., Overjordet, I.B., Olsen, A.J., Krause, D., Storseth, T. R., 2017. Exposure to crude oil micro-droplets causes reduced food uptake in copepods associated with alteration in their metabolic profiles. Aquat. Toxicol. 184, 94–102. https://doi.org/10.1016/j.aquatox.2017.01.007.

Haugland, J.K., 2016. *Miljøovervåkning Frænfjorden 2015 -Bløtbunnsfauna, Sediment- Og Hydrografiundersøkelse.* (2016-0146, Rev.01). Norway: DNV GL AS Oil & Gas.

He, K., Gkioxari, G., Dollár, P., Girshick, R., 2017. Mask R-CNN. *2017 IEEE International Conference On Computer Vision (ICCV)*, pp. 2980–2988. https://doi.org/10.1109/ICCV.2017.322. Venice, 2017.

Hirche, H.J., Meyer, U., Niehoff, B., 1997. Egg production of *Calanus finmarchicus* : effect of temperature, food and season. Mar. Biol. 127 (4), 609–620. https://doi.org/10.1007/s002270050051.

Ikeda, T., 1971. Changes in respiration rate and in composition of organic matter in *Calanus cristatus* (crustacea copepoda) under starvation. Bull. Fac. Fish. Hokkaido Univ. 21, 280–298.

Ikeda, T., 1977. The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton. IV. Changes in respiration and excretion rates of boreal zooplankton species maintained under fed and starved conditions. Mar. Biol. 41 (3), 241–252. https://doi.org/10.1007/BF00394910.

Jager, T., 2020. Revisiting simplified DEBtox models for analysing ecotoxicity data. Ecol. Model. 416, 108904. https://doi.org/10.1016/j.ecolmodel.2019.108904.
Jager, T., Salaberria, I., Altin, D., Nordtug, T., Hansen, B., 2017. Modelling the dynamics of growth, development and lipid storage in the marine copepod *Calanus finmarchicus*. Mar. Biol. 164 https://doi.org/10.1007/s00227-016-3030-8.
Kattner, G., Krause, M., 1987. Changes in lipids during the development of *Calanus finmarchicus* s.l. from Copepodid I to adult. Mar. Biol. 96 (4), 511–518. https://doi.org/10.1007/BF00397968.
Kattner, G., Albers, C., Graeve, M., Schnack-Schiel, S., 2003. Fatty acid and alcohol composition of the small polar copepods, Oithona and Oncaea: indication on feeding modes. Polar Biol. 26, 666–671. https://doi.org/10.1007/s00300-003-0540-x.
Kiørboe, T., Møhlenberg, F., Hamburger, K., 1985. Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. Mar. Ecol.-Progr. Ser. 26, 85–97. https://doi.org/10.3354/meps026085.
Kooijman, S.A.L.M., 2009. Dynamic Energy Budget Theory for Metabolic Organisation. Third Edition, third ed. Dynamic Energy Budget Theory for Metabolic Organisation, pp. 1–514. https://doi.org/10.1017/CBO9780511805400.
Koski, M., Boutorh, J., Rocha, C., 2017. Feeding on dispersed vs. aggregated particles: the effect of zooplankton feeding behavior on vertical flux. PloS One 12. https://doi.org/10.1371/journal.pone.0177958.
Kvassnes, A.J.S., Iversen, E., 2013. Waste sites from mines in Norwegian fjords. Miner. Prod. 3, A27–A38.
Lee, R., Hagen, W., Kattner, G., 2006. Lipid storage in marine zooplankton. Mar. Ecol.-Progr. Ser. 307, 273–306. https://doi.org/10.3354/meps307273.
Malzahn, A.M., Boersma, M., 2012. Effects of poor food quality on copepod growth are dose dependent and non-reversible. Oikos 121 (9), 1408–1416. https://doi.org/10.1111/j.1600-0706.2011.20186.x.
Marshall, S.M., Orr, A.P., 1958. On the biology of *Calanus finmarchicus*. X. Seasonal changes in oxygen consumption. J. Mar. Biol. Assoc. U. K. 37 (2), 459–472. https://doi.org/10.1017/S002531540002381X.
Mayor, D.J., Anderson, T.R., Irigoien, X., Harris, R., 2006. Feeding and reproduction *of Calanus finmarchicus* during non-bloom conditions in the Irminger Sea. J. Plankton Res. 28 (12), 1167–1179. https://doi.org/10.1093/plankt/fbl047.
Mayzaud, P., 1976. Respiration and nitrogen excretion of zooplankton. IV. The influence of starvation on the metabolism and the biochemical composition of some species. Mar. Biol. 37 (1), 47–58. https://doi.org/10.1007/BF00386778.
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*. Helgol. Mar. Res. 56 (3), 169–176. https://doi.org/10.1007/s10152-002-0105-3.
Nepstad, R., Liste, M., Alver, M.O., Nordam, T., Davies, E., Glette, T., 2020. High-resolution numerical modelling of a marine mine tailings discharge in Western Norway. Reg. Stud. Mar. Sci. 39, 101404. https://doi.org/10.1016/j.rsma.2020.101404.
Niehoff, B., 2004. The effect of food limitation on gonad development and egg production of the planktonic copepod *Calanus finmarchicus*. J. Exp. Mar. Biol. Ecol. 307 (2), 237–259. https://doi.org/10.1016/j.jembe.2004.02.006.
Norwegian Environment Agency, 2020. Norske utslipp, 22/9/2020 from. https://www.norskeutslipp.no/.
Paffenhoefer, G.A., Knowles, S.C., 1979. Ecological implications of fecal pellet size, production and consumption by copepods. J. Mar. Res. 37 (1), 35–49.
Paffenhofer, G.A., 1972. The effects of suspended 'red mud' on mortality, body weight, and growth of the marine planktonic copepod. Calanus helgolandicus. Water, Air, and Soil Pollut. 1 (3), 314–321. https://doi.org/10.1007/bf00294007.
R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Ramirez-Llodra, E., Trannum, H.C., Evenset, A., Levin, L.A., Andersson, M., Finne, T.E., 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 (1–2), 13–35. https://doi.org/10.1016/j.marpolbul.2015.05.062.
Rey-Rassat, C., Irigoien, X., Harris, R., Carlotti, F., 2002. Energetic cost of gonad development in *Calanus finmarchicus* and *C. helgolandicus*. Mar. Ecol. Prog. Ser. 238, 301–306. https://doi.org/10.3354/meps238301.
Sargent, J.R., Falk-Petersen, S., 1988. The lipid biochemistry of calanoid copepods. Hydrobiologia 167 (1), 101–114. https://doi.org/10.1007/BF00026297.
Setså, R., 2020. Et kvantesprang for sjødeponi, 23/09/2020 from. https://www.geoforskning.no/nyheter/bergverk/2309-et-kvantesprang-for-sjodeponi-3.
Sew, G., Calbet, A., Drillet, G., Todd, P.A., 2018. Effects of concentration and size of suspended particles on the ingestion, reproduction and mortality rates of the copepod, *Acartia tonsa*. Mar. Environ. Res. 140, 251–264. https://doi.org/10.1016/j.marenvres.2018.06.016.
Shadrin, N., Litvinchuk, L., 2005. Impact of increased mineral particle concentration on the behavior, suspension-feeding and reproduction of *Acartia clausi* (Copepoda). Comparat. Roles of Suspension Feeder. Ecosyst. 137–146.
Skei, J.M., Syvitski, J.P.M., 2013. Natural flocculation of mineral particles in seawater - influence on mine tailings sea disposal and particle dispersal. Miner. Prod. 3, A1–A10.
Sommaruga, R., 2015. When glaciers and ice sheets melt: consequences for planktonic organisms. J. Plankton Res. 37 (3), 509–518. https://doi.org/10.1093/plankt/fbv027.
Suzuki, S., Abe, K., 1985. Topological structural analysis of digitized binary images by border following. Comput. Vis. Graph Image Process 30 (1), 32–46. https://doi.org/10.1016/0734-189X(85)90016-7.
Tande, K.S., Hopkins, C.C.E., 1981. Ecological investigations of the zooplankton community of Balsfjorden, Northern Norway - the genital system in *Calanus finmarchicus* and the role of gonad development in overwintering strategy. Mar. Biol. 63 (2), 159–164. https://doi.org/10.1007/bf00406824.
Tarrant, A.M., Baumgartner, M.F., Hansen, B.H., Altin, D., Nordtug, T., Olsen, A.J., 2014. Transcriptional profiling of reproductive development, lipid storage and molting throughout the last juvenile stage of the marine copepod *Calanus finmarchicus*. Front. Zool. 11 (1), 91. https://doi.org/10.1186/s12983-014-0091-8.
Trannum, H.C., Næss, R., Borgersen, G., 2018. *Overvåking Av Marin Bløtbunnsfauna for Titania A/S I 2018* (7291-2018). NIVA, Norway.
Vogedes, D., Varpe, O., Søreide, J.E., Graeve, M., Berge, J., Falk-Petersen, S., 2010. Lipid sac area as a proxy for individual lipid content of arctic calanoid copepods. J. Plankton Res. 32 (10), 1471–1477. https://doi.org/10.1093/plankt/fbq068.
Vogt, C., 2013. International Assessment of Marine and Riverine Disposal of Mine Tailings. Final Report Adopted by the International Maritime Organization, London Convention/Protocol, IMO, p. 138.