

## Effects of mine tailing exposure on the development of early life stages of the marine copepod *Calanus finmarchicus*

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### ABSTRACT

The demand for mineral resources is increasing mining activities worldwide. In Norway, marine tailing disposal (MTD) is practiced, introducing mineral particles into fjord ecosystems. We investigated the effects of two concentrations (high and low) of fine tailings from a CaCO<sub>3</sub> processing plant on early life stages of the marine copepod *Calanus finmarchicus*. Results show that the exposure did not significantly impact hatching success or development in non- and early feeding life stages. However, feeding stage nauplii ingested tailings, which caused a significantly slower development in later nauplii stages in high exposure groups, with most individuals being two stages behind the control group. Further, high mortality occurred in late nauplii and early copepodite stages in low exposure groups, which could be caused by insufficient energy accumulation and depleted energy reserves during development. Individuals exposed to high exposure concentrations seemed to survive by arresting development and potentially by reduced activity, thereby conserving energy reserves. In nature, slower development could affect lipid storage buildup and reproduction.

### KEYWORDS

Mining; marine tailing disposal; zooplankton; developmental stages; *Calanus finmarchicus*

### Introduction

Processing of mineral ores often generate large quantities of non-used material, so-called tailings, that require disposal (Dold 2014; Ramirez-Llodra et al. 2015). Tailing disposal in the sea, marine tailing disposal (MTD) is practiced in some countries including Norway (Dold 2014, 2015). Several of the major Norwegian mines, quarries, and mineral processing plants are located close to the sea and are disposing tailings directly into adjacent fjords, with tailing release depths varying between the tidal zone and 125 m (Norwegian Mineral Industry 2014).

Tailings consist of mineral particles and, depending on the ore and processing, may contain remains of floatation and flocculation polymers, and/or metals originating from the ore. Little scientific data and literature exist on environmental impacts of MTD in fjord ecosystems in Norway (Ramirez-Llodra et al. 2015; Skei and Syvitski 2013). Especially, knowledge on potential impacts from spreading tailing plumes of fine particles on pelagic organisms is scarce.

Pelagic zooplankton species serve as major link in the energy transfer between primary production and higher trophic levels, and thus play a key role in maintaining the energy flux in the marine environment. The filter feeding copepod *Calanus finmarchicus* is one of the most common and abundant copepods in the North Atlantic (Planque and Batten 2000). The geographical and vertical distribution patterns make it a potential target species for tailing exposure.

*Calanus finmarchicus* has a complex life history (Marshall and Orr 1955). After the eggs hatch, they go through six naupliar stages (NI-NVI) and five copepodite stages (CI-CV) before molting into reproducing adults. During the first two naupliar stages (NI-NII), maternally transferred lipids represent their sole energy source and exogenous feeding starts at stage NIII. They develop rapidly through the first stages and start accumulating excess energy as lipids at copepodite stage 3 (CIII) (Marshall and Orr 1955). The buildup lipids are partially utilized during winter diapause, while the main fraction is fueling the gonad maturation and

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transition into reproductive adults in spring. The main egg production is timed to the phytoplankton bloom in early spring (Diel and Tande 1992; Falk-Petersen et al. 2009). Impacts on the individual animals' energy budget can thus have severe implications for survival and reproduction of the population.

In contrast to some other copepod species, *C. finmarchicus* has a nonselective filter-feeding strategy and can ingest non-nutritional particles along with their food (Paffenhöfer 1972; Paffenhöfer and Strickland 1970). Both adults and juveniles were shown to ingest mineral particles deriving from mine tailings and drilling mud (Svendheim et al. 2021; Farkas et al. 2017 and b). While the ingestion of inorganic particles, such as sediment, often does not cause mortality in acute exposures, it can lead to stress and reduced reproduction in exposed organisms (Arendt et al. 2011; Farkas et al. 2017b; Paffenhöfer 1972; Shadrin and Litvinchuk 2005; Sommaruga 2015). Svendheim et al. (2021) reported a developmental delay in *C. finmarchicus* exposed to mine tailings from the NVI life stage. However, knowledge on effects on earlier stages of copepods, including non-feeding stages, remains scarce. In this study, we thus investigated the impacts of mine tailing particles on *C. finmarchicus* from eggs to first feeding nauplii at concentrations that are environmentally relevant for the areas in the vicinity of tailing release points (Davies and Nepstad 2017; Nepstad et al. 2020). Through three experiments covering the (1) non-feeding life stages (NI/NII), (2) the transition between non-feeding and early feeding life stages (NIII/NIV), and (3) the feeding life stages (NIII to CI/CII), we investigated the impact of mine tailings on hatching success, particle uptake as well as survival and development. We further studied developmental stage distribution following a recovery period to determine whether they can compensate for potential impacts when stressor exposure ceases.

## Material and methods

### Tailing suspensions

The tailings were received from a factory for CaCO<sub>3</sub> products placing tailings in the adjacent

fjord. The tailings were received as a slurry with a particle dry mass of 40.5% (70–80% CaCO<sub>3</sub>) containing flocculation and floatation chemicals. Upon arrival, the tailings were aliquoted over in clean containers without headspace to avoid oxidation and stored at 4°C in darkness. A stock suspension of 1 g L<sup>-1</sup> (dry mass) was prepared from the slurry through dilution in natural seawater (35 psu). The seawater was supplied from 70 m depth in the Trondheimsfjorden and filtered through sand and patron filters (1 µm) following intake. To produce the tailing suspension, the seawater was further filtered through 0.2 µm (Millipore Sterivex, Merk KGaA, Germany) prior to use. Following vigorous shaking, the stock suspension was left to settle for 20 min to remove large particles and aggregates from the water column by gravity. The decanted suspension was then used directly as the high exposure and diluted 1:10 with filtered seawater (0.2 µm; Millipore Sterivex, Merk KGaA, Germany) to prepare the low exposure suspension. Particle size distribution and concentration were determined using a particle sizer (Coulter counter 4; Beckman Coulter, US) with a 100 µm (size range 2–60 µm) aperture as described previously by Farkas et al. (2017a). The particle mass was calculated using CaCO<sub>3</sub> density of 2.71 g cm<sup>-3</sup>.

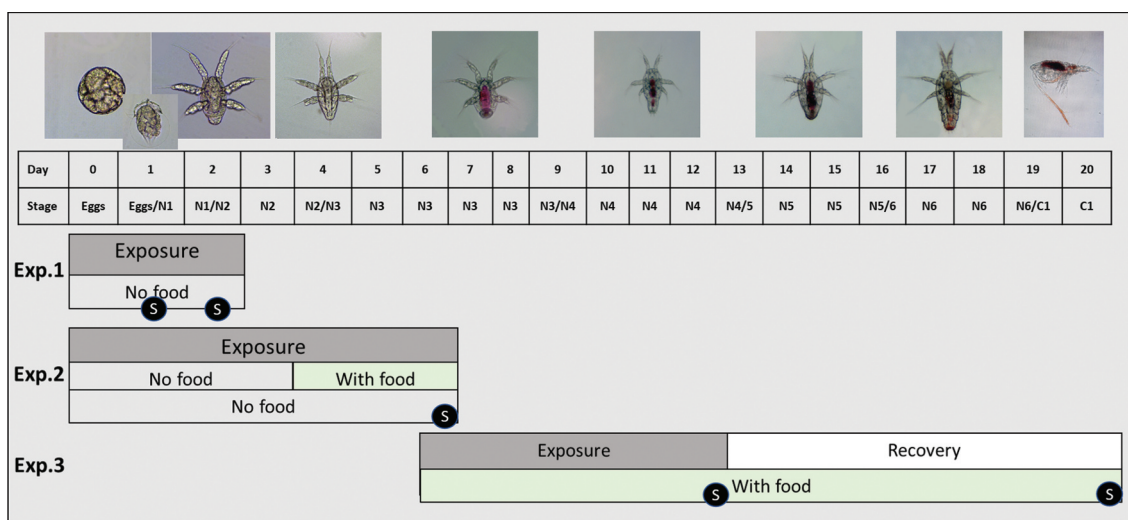
### Effect studies

Three exposure experiments were conducted covering life stages from eggs to early copepodites CI/CII (Figure 1).

#### Experiment 1: impacts of tailings on hatching success

To determine the effects of tailing exposure on hatching success of non-feeding life stages, eggs of *C. finmarchicus* were obtained from females from a continuous in-house culture. The culture was established from stage V copepodites (CV) collected in Trondheimsfjorden, Norway. The culture is kept at 10°C and is continuously reproducing. The animals are maintained with a diet consisting of a mixture of three microalgae species. Culturing conditions are described in detail by Hansen et al. (2007).

For this study, ovulating females were transferred to flat bottomed 50 L HD polyethylene



**FIGURE 1.** Timeline of experiments (Exp.) 1, 2, and 3 and sampling points (S) during early developmental stages of *Calanus finmarchicus* including eggs, nauplii, and CI stages.

tanks and eggs were collected from the tanks after 18 h. Twenty eggs were distributed into glass vials containing 40 mL high or low tailing suspensions, or clean natural seawater as negative control (control) ( $n = 6$  replicate vials per treatment). The vials were kept in slow rotation (15 rpm) on plankton wheels to keep the tailings in suspension during exposure (10°C, darkness). At 24 h and 48 h, three replicate vials from each exposure condition ( $n = 3$ ) were sampled (Figure 1; Exp. 1) to determine the hatching success.

#### **Experiment 2: impact of tailings on early feeding nauplii stages (NIII/NIV)**

To study the effects on early feeding nauplii stages, *C. finmarchicus* eggs were collected as described above and transferred into glass vials (40 mL,  $n = 40$  eggs per vial) containing either clean seawater as negative control (control) and low or high exposure suspensions ( $n = 6$  replicate vials per treatment). The vials were kept in rotation on plankton wheels for 144 h (6 days; 10°C, darkness). After 72 h, half of the exposure groups ( $n = 3$  vials from each group, Figure 1; Exp. 2) were fed with 7500 cells  $\text{mL}^{-1}$  of the microalgae *Rhodomonas baltica* to determine whether there were differences in tailing uptake and development in the presence and absence of food. This selected algae concentration equals approximately 320  $\mu\text{g C L}^{-1}$ , which ensures normal growth at the relevant live stages (Campbell et al. 2001). Feeding was repeated at 96

h. The other half of the replicates were exposed without feeding throughout the experimental period. At the end of the exposure period, the animals were collected and imaged with a microscope (Eclipse 80i, Nikon Inc., Japan equipped with a MC170 HD camera, Leica Microsystems, Germany). Images were used for assessment of developmental stages and to determine presence of tailings into the gut.

#### **Experiment 3: exposure and recovery of feeding stages NIII to CI/CII**

In this experiment, we wanted to study whether tailing particles are taken up and can impact the survival and development of feeding nauplii stages, and whether a recovery phase diminishes or compensates potential effects (Figure 1; Exp. 3).

Eggs were collected and incubated in 2 L glass bottles in filtered seawater at 10°C for 6 days. Subsequently, actively moving individuals were collected, and approximately 50 individuals were transferred into 1 L glass bottles containing tailing exposure suspensions (low and high,  $n = 6$  bottles per treatment) prepared as described above, or filtered seawater as negative control (control) ( $n = 6$  bottles). *R. baltica* were added to the bottles to reach 7500 cells  $\text{mL}^{-1}$  as food. The bottles were filled without headspace to avoid the presence of air bubbles and were kept in rotation at 15 rpm, 10°C on plankton wheels in darkness. Every 48 h, the number of algal cells was determined in the

controls using a Coulter Multisizer 4 particle counter, and fresh algae were added to the exposure bottles to reach a final number of 7500 cells mL<sup>-1</sup>. Due to measurement interference of the tailing particles, the number of algae cells could not be measured in the tailing exposure bottles. Therefore, average algal cell concentrations in controls were used to calculate the number of algal cells to be added. This approach could lead to differences of algae cell numbers between control and exposure bottles, if mine tailings treatment alter feeding activity. In a previous study, we determined feeding rates following tailing exposure in adult *C. finmarchicus* and found no differences between exposed and unexposed groups (Farkas et al. 2017a). Furthermore, while *C. finmarchicus* feed on algae in a certain size range (our feed algae are around 7–10 µm), they appear not selective regarding “material” (e.g. organic vs inorganic particles) (Paffenhöfer and Strickland 1970; Paffenhöfer 1972; Farkas et al. 2017a; personal observation).

After 6 days of exposure, three replicates of each treatment were terminated. The animals were collected and imaged as described above to determine the developmental stage and the uptake of tailings in the guts. Three classes of tailing uptake were distinguished as shown in figure 3: no uptake, uptake 1 (low to moderate uptake: some tailings visible in the gut), and uptake level 2 (high uptake: substantial amounts of tailings in the gut). As animals in the high exposure groups were observed to be much less active, short videos were taken using a stereo microscope (Leica Z16APO with 2.0× PlanApo objective, Leica Microsystems, Germany) mounted on a transmitted light base (Leica TL/DF, Leica Microsystems, Germany) and equipped with a CMOS camera (MC170 HD, Leica Microsystems, Germany).

The following day, the remaining groups ( $n = 3$  of each treatment) were collected, carefully imaged to determine the number of live and dead animals, and the alive individuals were transferred to clean seawater containing algae (7500 cells mL<sup>-1</sup>). The animals were kept in clean natural seawater for recovery for 7 days and were provided fresh algae every 48 h based on average concentrations of algae cell numbers as described above. If the algae clearance was measurable, but low, algae were added to reach an additional 1000 cells mL<sup>-1</sup>. At the end of

the recovery period, the animals were collected and counted using the stereo microscope as described above. Images for stage determination were taken with a microscope as described above.

To visualize the movements of the animals from videos taken after exposure (MC170 HD, Leica Microsystems, Germany; from at least two replicates), composites of three 1-second image averages from the video frames taken at 0–1 s, 5–6 s, and 10–11 s into each video were created. As the images were backlit, the nauplii are dark and movement trails were also dark (i.e. low pixel values), and thus the composite was created by finding the minimum intensity for each pixel over all the three 1-second periods in order to retain movement trails. Such a composite allows the trails of the nauplii to be visible over a longer period of time than averaging, and thus represents a total of 3-seconds of movement sampled at three separate times within the video.

### Statistics and data treatment

Data are presented as means and standard deviations (mean ± SD). Data analyses were performed with GraphPad Prism 7 (GraphPad Software Inc., USA). Data sets were analyzed for normality (Shapiro-Wilk normality test) and analyzed with one-way ANOVA followed by Tukey’s multiple comparisons test. Sidak’s multiple comparisons test was applied to compare uptake rates in low and high exposed groups.

As stage distribution was spread out over six live stages in the last experiments, ranks (1–6) were assigned to each live stage and compared using Kruskal-Wallis test. Statistical significance was set to  $p < 0.05$ .

## Results and discussion

### Exposure suspensions

The high exposure suspensions contained  $2.24 \pm 0.07 \times 10^6$  particles mL<sup>-1</sup> in the size range 2–10 µm. Based on particle number, >90% of the particles were smaller than 5 µm. Related to particle volume,  $38 \pm 6\%$  of the particles were <5 µm and  $63 \pm 1\%$  <10 µm in size. From previous studies we know that there is also



a fraction of finer particles ( $<2\ \mu\text{m}$ ). However, here we focus on particles above  $2\ \mu\text{m}$ . A more in-depth characterization of the particle size distribution, including the presence of particles  $<2\ \mu\text{m}$  and contents of other elements, is provided in a previous study (Farkas et al. 2017b).

The calculated particle mass ( $<10\ \mu\text{m}$ ) in the high exposure concentration was  $120 \pm 7\ \text{mg L}^{-1}$ . The low exposure suspensions were prepared by diluting the high suspension 1:10 and particle numbers were not determined separately.

### Effects on early life stages

#### Experiment 1: impacts of tailings on hatching success

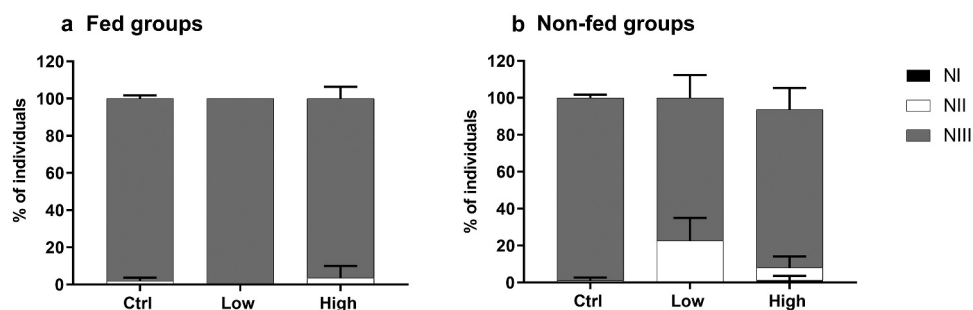
Even at high concentrations, the tested tailings did not reduce hatching success after 24 h and 48 h ( $p > 0.05$ ). After 24 h,  $69 \pm 12\%$  of the eggs in the control series had hatched, with similar hatching success in the low ( $65 \pm 25\%$ ) and high ( $62 \pm 18\%$ ) groups. After 48 h, hatching success was  $93 \pm 7\%$ ,  $86 \pm 13\%$ , and  $94 \pm 1\%$  in the control series, low and high exposures, respectively. Similar 48 h hatching success rates have previously been reported for *C. finmarchicus* (at  $10^\circ\text{C}$ ; Hansen et al. 2017, 93%). These results showed that neither the mineral particles nor the processing chemicals were acutely toxic to *C. finmarchicus* eggs.

#### Experiment 2: impact of tailings on early feeding nauplii stages (NIII/NIV)

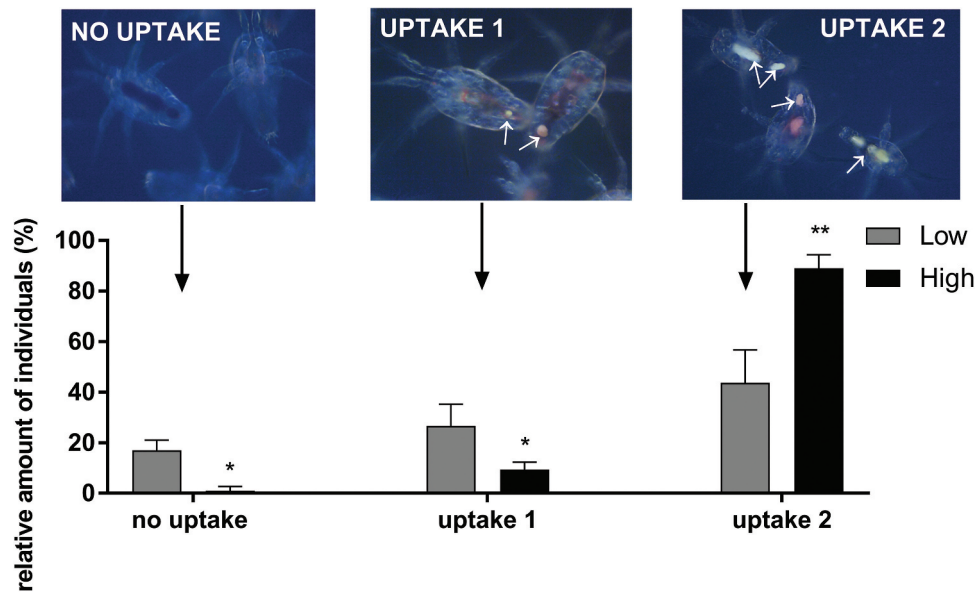
In the first-feeding exposure experiment (eggs to NIII), all exposed animals, both fed and non-fed groups, were found to have taken up tailings in their guts (qualitative observation). There was no statistically significant developmental delay

( $p > 0.05$ ) in any of the tailing exposed groups compared to the controls, and most individuals had reached developmental stage NIII (Figure 2 a,b). However, in the groups that were not fed, there was a non-significant, but slightly higher number of nauplii still at developmental stage NII in low ( $23 \pm 12\%$ ) and high ( $7 \pm 6\%$ ) exposures compared to control groups, where  $99 \pm 2\%$  of the nauplii had already reached stage NIII (Figure 2b). One NI individual was found in the high non-fed group. We visually observed that most of the animals were inactive or immobilized in both non-fed exposure groups (low and high), which was not observed in the non-fed controls, suggesting an impact of the combined exposure to mine tailing particles and absence of food.

As the nauplii are dependent on internal energy reserves during the first two non-feeding stages, the presence of particles may have little effect on development until they start to feed at stage NIII. This further suggests that the effects are mostly related to non-food particles and not to the presence of processing chemicals that would also cause effects on non-feeding live stages. This is in agreement with a previous study that showed no significant differences in the effects of tailings containing processing chemicals and tailing particles without chemicals (Svendheim et al. 2021). One explanation for the lack of motility may be the ingestion of tailing particles in the non-fed exposure groups, leading to the depletion of energy reserves in nauplii using energy to filter the water for food without being able to extract any energy from ingested material.



**FIGURE 2.** Developmental stages (% of total animals) of *Calanus finmarchicus* after a 6-day exposure to low and high concentrations of tailing particles in the presence (a) and absence (b) of food (Exp 2). Data are shown as mean  $\pm$  SD. No significant ( $p < 0.5$ ) differences between treatments were found.



**FIGURE 3.** Uptake of tailings in Exp 3. Example images (dark field) of uptake categories in *Calanus finmarchicus* nauplii: no uptake, uptake 1 (low-medium uptake), and uptake 2 (high uptake). Tailings show in white, algae in red. B: relative number of animals (% of total) showing: no uptake, uptake 1, and uptake 2 in the low (gray bars) and high (black bars) exposure groups. Data are presented as mean  $\pm$  SD ( $n = 3$ ). Statistical differences (\* $p < 0.05$ ; \*\* $p < 0.01$ ) in uptake between low and high exposure groups are indicated by asterisks.

### Experiment 3: exposure and recovery of feeding stages NIII to CI/CI

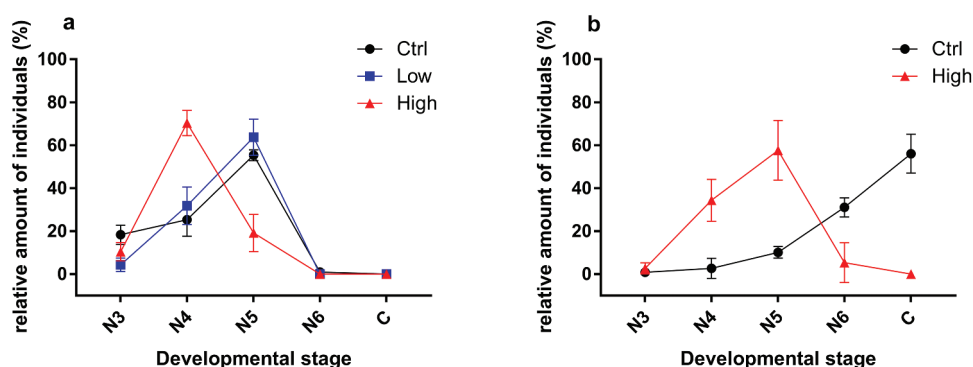
Following 6-day exposure, individuals in the low and high exposure groups had accumulated tailings in their gut (Figure 3a). In the high exposures, almost all individuals (98%) had taken up tailing particles, and a higher percentage ( $89 \pm 5\%$ ;  $p < 0.0001$ ) had a high amount (uptake 2) of tailings in their guts compared to low exposure groups, in which  $44 \pm 13\%$  were classified as high uptake (Figure 3b).

No significant differences in numbers of alive animals compared to stocking density were found at the sampling day at the end of the exposure, indicating no significant mortality (data not shown). However, the following day during transfer of individuals from exposure to recovery, around 80% of the individuals in the low exposure groups were found dead in all three replicates. Such a degree of mortality was not found in either the control group or the high exposure groups. This contrasts with Svendheim et al. (2021), who did not report significant mortality caused by mine tailing exposure for 15 days between developmental stage NVI and CIV. However, exposure concentrations in that study were slightly lower

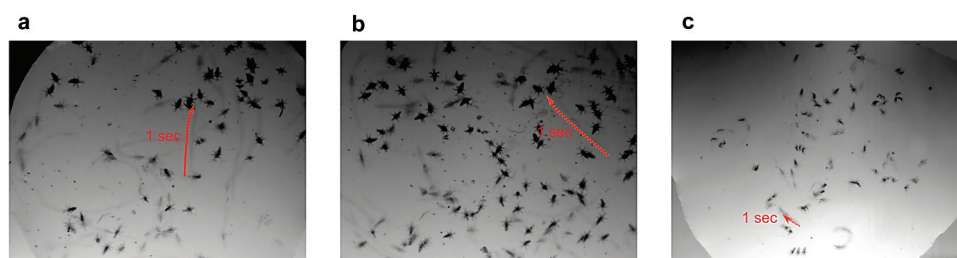
(particle number  $2-60 \mu\text{m} < 100\ 000$ ), with a later exposure onset (NVI).

At the end of the exposure period (6 days), the developmental stage distribution differed between the exposure groups, with a significant delay in development observed in the high exposure groups compared to the low and control groups ( $p = 0.0010$ ,  $p < 0.0001$ ; Figure 4 a). Most of the nauplii were at stage NIV ( $70 \pm 10\%$ ) in the high exposure groups, whereas the control and low exposure groups were dominated by NV ( $>55\%$ ). The stage distribution was similar in the control groups and the low exposure groups (Figure 4 a), indicating no effect of low exposure on nauplii development in the presence of food for the tested exposure duration (6 days). The fraction of nauplii at stage NV was marginally higher in the low exposure groups ( $64 \pm 8\%$ ) compared to the control groups ( $55 \pm 3\%$ ), but the difference was not statistically significant.

We observed a significant difference in development between control and high exposure groups also after a 7-day recovery ( $p = 0.001$ ; Figure 4b), even though all visible tailing particles had left the gut during recovery. While more than 50% ( $55 \pm 8\%$ ) of the individuals in control groups had developed into early copepodite stages, only a few



**FIGURE 4.** Developmental stages (% of total animals) of *Calanus finmarchicus* after a 7-day exposure to a low and high concentration of  $\text{CaCO}_3$  tailings (a), and after a 7-day recovery following the exposure (b) in Exp 3. Due to the high mortality, data for the low exposure group are not shown in b. Data are shown as mean  $\pm$  SD.



**FIGURE 5.** Composite of three 1-second image averages from videos taken of control (a), low (b), and high (c) exposure groups after exposure in Exp 3. Nauplii and movement trails appear dark. Each movement trail represents the movements of a nauplii during 1 second. In each image one movement trail is highlighted in red for better illustration.

individuals from the high exposed group had reached NVI (Figure 4 b). Most individuals in the high groups were two developmental stages behind the control groups. Despite the high mortality, some individuals were recovered in the low exposed group. The 20% animals recovered alive in the low exposed group were also less developed than control animals ( $p < 0.001$ ; data not shown in graph). Our results are in agreement with Jensen et al. (2016), who found that exposure to barite particles caused developmental delays in development of *C. finmarchicus* nauplii. Similarly, Svendheim et al. (2021) reported developmental delays, reduced  $\text{O}_2$  consumption, and a reduced accumulation of lipids in *Calanus* following mine tailing exposure for 15 days. The applied energy budget model indicated that reduced energy assimilation in the presence of particles was the likely cause of observed effects (Svendheim et al. 2021). Developmental delays were also reported for nauplii of *Calanus* species reared under no food conditions that arrested development at NIII, with nauplii reared in low-quality food conditions

developing slower, and showing higher mortality compared to high-quality food conditions (Cook et al. 2007; Daase, Soreide, and Martynova 2011; Jung-Madsen and Nielsen 2015). It was further shown that copepods feeding on a low-quality diet were not able to compensate even after receiving high-quality food (Malzahn and Boersma 2012), which is consistent with findings in our study.

In the current study, individuals in the high treatment groups were observed to move less and shorter distances. An example visualization of differences in swimming activity is shown in Figure 5, which shows that the individuals in the control group (Figure 5 a) and low exposure group (Figure 5 b) move more and over longer distances than individuals from high exposure groups (Figure 5 c). Moving distances are visible as light gray lines, with one movement trail (distance traveled in 1 s) highlighted in red for each exposure group for better visualization (Figure 5 a-c). It has to be acknowledged that our observation cannot be used to quantify activity levels with the given image

quality, different number of animals present, and different size of animals; however, a better suited measurement setup and data integration tools are currently being evaluated.

## Conclusions and outlook

The data of this first assessment of tailing impacts on *C. finmarchicus* eggs and early nauplii stages show that already early stage nauplii can take up mine tailing particles at both high and low exposure scenarios. Results further show that such exposure causes developmental delays that were not compensated during a 7-day depuration period. In nature, such impacts could have implications for copepod populations. Findings of this study are also relevant beyond MTD activities. For example, rivers can transport high suspended sediments loads, especially during snowmelt, ice-breakup, and high discharge events reaching concentrations of >100 mg/L (for example: Beltaos and Burrell, 2021; Syvitski 2002), which is comparable with concentrations used in this study. Climate change is expected to further increase the frequency and extent of extreme weather events that can cause high terrigenous runoff and thus inorganic particle loads in receiving environments such as fjords.

## Highlights

- Exposure to tailings did not impact hatching success
- Feeding early life stages were ingesting mine tailing particles
- Mine tailing uptake affected the development of early life stages of copepods
- Developmental delays were likely caused by impacts on the energy budget

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability statement

Raw data were generated at SINTEF Ocean. Derived data supporting the findings of this study are available from the corresponding author J.F. on request.

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