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Effects of mine tailing exposure on early life stages of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*)

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ABSTRACT

Mining and processing of minerals produce large quantities of tailings as waste. Some countries, including Norway, allow disposal of mine tailings in the sea. In this study we investigated the impacts of tailings from a calcium carbonate ($CaCO_3$) processing plant on early live stages of haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*). Fish eggs (3 days post fertilisation; dpf) were exposed for 48 h to three concentrations of tailings, nominally 1 mg L^{-1} (low, L); 10 mg L^{-1} (medium, M) and 100 mg L^{-1} (high, H); with L and M representing concentrations occurring at tailing release points. Results show that tailings rapidly adhered to eggs of both species, causing negative buoyancy (sinking of eggs) in M and H exposures. While tailings remained on egg surfaces in both species also after exposure termination, adhesion seemed more pronounced in cod, leading to larger impacts on buoyancy even after exposure. Tailing exposure further induced early hatching and significantly reduced survival in M and H exposed embryos in both fish species, and in cod from the L exposure group. Moreover, tailing exposure caused reduced survival and malformations in larvae, potentially related to premature hatching. This study shows that mineral particles adhere to haddock and cod eggs, affecting egg buoyancy, survival and development.

1. Introduction

Several anthropogenic activities can increase suspended particle loads in marine environments, for example dredging for marine navigation maintenance, subsea construction including the construction of offshore windfarms, run-off from agriculture, urban development, land-based mining and processing as well as subsea mining activities (Kjelland et al., 2015; Linders et al., 2018).

Mining and ore processing generate large quantities of non-useable finely grinded particulate material, so called tailings, that require disposal (Dold, 2014; Ramirez-Llodra et al., 2015). Land-based tailing placement is common practice for industrial-sized mines, however, finding large suitable areas can in some locations be difficult (Dold, 2015; Kvassnes and Iversen, 2013; Ramirez-Llodra et al., 2015). While banned in most countries, tailing disposal at sea is increasingly considered as viable alternative (Dold, 2014, 2015). Currently, Norway is one out of eight countries worldwide that practises submarine tailing placement (STP) and several Norwegian mines, quarries and processing plants are placing their tailings into adjacent fjords (Ramirez-Llodra et al., 2015).

Tailing plumes from STP can spread in the water column during release and deposition (Davies and Nepstad, 2017). For example, increased turbidity in the pelagic zone was measured several kilometres away from a disposal site in Bøkfjorden, Northern Norway (Berge et al., 2012). Furthermore, upwelling processes and slope failures are sources for fine particulate material into the water column, with the latter being of special concern for seabeds with steep slopes (Ramirez-Llodra et al., 2015). Despite ongoing STP, scientific data and literature on impacts on fjord ecosystems is still scarce, with knowledge on potential impacts on organisms inhabiting the water column being even less (Olsvik et al., 2015; Ramirez-Llodra et al., 2015; Skei and Syvitski, 2013).

The introduction of inorganic particles, potentially in combination

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with metals and processing chemicals, into the water column can potentially affect pelagic organisms, especially their sensitive early life stages. Several fish, including commercially important species such as Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and fish from the flounder family that spawn in Norwegian fjords, have pelagic eggs. Pelagic eggs usually have a thin chorion, which may make them especially vulnerable to mechanical and chemical stress. For example, embryos of cod and haddock were shown to be sensitive to exposure of particulate oil droplets as they adhere to the surface of the chorion, facilitate uptake of oil components and ultimately cause cardiac toxicity and skeletal malformations in developing embryos and larvae (Hansen et al., 2018, 2019; Sørensen et al., 2019; Sørhus et al., 2015). A recent study investigated effects of copper mine tailings on early live stages of Atlantic cod and found that increased amounts of particles attached to the egg's surfaces following exposure (Reinardy et al., 2019). Although embryo mortality did not increase during tailing exposure in that study, larvae mortality was elevated in groups exposed to high tailing concentrations (nominally 20 mg L^{-1}) at an exposure duration of 22 days (Reinardy et al., 2019).

Pelagic fish eggs typically have lower specific gravity than sea water in the upper mixing layer, thus having a slight positive buoyancy in respect to their spawning depth that causes a vertical ascent and prevents sinking into deeper, potentially oxygen depleted layers (Butler et al., 2020; Sundby, 1983; Sundby and Kristiansen, 2015). High-density mineral particles that attach to the egg surface can affect buoyancy and cause the eggs to sink and eventually die, and thus cause a loss for the recruitment of the fish population. Westerberg et al. (1996) showed that cod eggs readily sink after exposure to fine particulate material of glacial clay and limestone sediment, with both particle types causing mortality in cod larvae (Westerberg et al., 1996). In another study, short term exposure (48–96 h) of Pacific herring (*Clupea pallasi*) eggs to suspended sediment at concentrations of 250–500 mg L^{-1} caused self-aggregation, lethal and sublethal impacts (Griffin et al., 2009). In contrast, no impacts were reported for Atlantic herring (*C. harengus*) eggs exposed to 5–300 mg L^{-1} of suspended sediment (Kiørboe et al., 1981).

In the current study we investigated whether a short term (48 h) exposure to calcium carbonate ($CaCO_3$) tailings affects early life stages of haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*). Studied endpoints included the effects of tailing exposure on egg buoyancy, respiration, survival and larvae development.

2. Material and methods

2.1. Tailing suspension preparation and measurements

Tailing samples were obtained from a production plant for $CaCO_3$ products that is placing tailings in the adjacent fjord at approximately 20 m depth. The tailing samples, a slurry consisting of water, residuals of processing chemicals (e.g. flotation and flocculation chemicals), $CaCO_3$ and other mineral particles were taken upstream the release point and were premixed with seawater (personal communication, Ramirez-Llodra et al., 2015). The tailings were previously characterised in detail (Farkas et al., 2017).

The tailing elemental composition was analysed using a modified sequential extraction technique of the Bureau Communautaire de Référence (BCR) and high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) as described in S2.1.

Exposure suspensions were prepared by diluting the obtained tailing sample (slurry, dry mass 34 ± 0.78%) in filtered seawater (Millipore Sterivex 0.2 μm; Merk KGaA, Germany) at a concentration of 1 g L^{-1} (wet weight). As in nature large particles are rapidly removed from the water column due to gravitational sedimentation, a fine particulate fraction was prepared by allowing the tailing suspensions to settle for 20 min. The supernatant was then carefully decanted to avoid resuspension of the settled particles. The obtained suspension represented the high (H) exposure concentration, with an approximated concentration of 100 mg L^{-1} tailing particles (recalculated from particle number and density, see below). Medium (M) and low (L) exposure suspensions were prepared by diluting the H exposure 1:10 and 1:100 with filtered seawater, resulting in nominal exposure concentrations of approximately 10 and 1 mg L^{-1}. The experiments with cod and haddock started at different days (two days apart), and tailing suspensions were freshly prepared for each experiment. Each exposure suspension ($n = 3$) was characterised for particle size distribution, particle number and volume using a particle sizer (Coulter counter 4; Beckman Coulter, US) equipped with a 100 μm (size range 2–60 μm) aperture. The tailing mass in the suspensions was recalculated using the density of $CaCO_3$ (2.71 g cm^{-3}), being the main constituent in the fine tailing fraction (Farkas et al., 2017).

2.2. Cod brood stock, egg fertilisation and transport

Eggs were collected from a spawning brood stock of Atlantic cod and haddock kept at Austevoll Research Station (Institute of Marine Research). Eggs were fertilized immediately and incubated for 24 h (6.2 °C, salinity 34.4‰) before being sent in closed bottles (100 mL eggs in 1 L sea water) in thermo-isolated ice-cooled containers to SINTEF Sealab by air freight. At arrival eggs were transferred to 50 L tanks with flow-through of filtered (1 μm) seawater (8 ± 1 °C) delivering one volume exchange of seawater per day. Natural sea water, collected from a depth of 80 m (below thermocline) in Trondheimsfjord (63°26′ N, 10°23′ E), was supplied by a pipeline system from the source to our laboratories (salinity of 34‰, pH 7.6). Gentle air bubbling kept embryos moving continuously in the tanks. Dead and unfertilized eggs were removed from the tank. Eggs were kept in the tanks until start of exposure (3 dpf).

2.3. Experimental setup

To keep tailings in suspension during exposure, the experiment was conducted in side-flattened 1.2 L glass bottles. An estimated amount of 400–500 fish eggs (3 dpf) was added to each bottle containing the exposure suspensions ($n = 3$) or seawater only in control groups (Ctrl; $n = 5$ for cod, $n = 6$ for haddock). The bottles were filled up to the rim to prevent air bubbles causing additional turbulence and mounted to plankton wheels (carousels) rotating at 13 rpm. The exposure duration was 48 h at a temperature of 10 °C. After 48 h (timepoint 48; TP48), the eggs were transferred into clean 1.2 L bottles containing filtered seawater and placed back on the plankton wheels for another 48 h (timepoint recovery 48; TPR48) to allow for a recovery in motion and to enable the removal of loosely bound tailings from egg surfaces. Oxygen levels and temperature were measured daily during exposure and recovery using a phase fluorometer (NeoFox GT, Ocean Optics, Largo, US) equipped with a R-series FOSPOR electrode (Ocean Optics, Largo, US) and a temperature compensated thermistor probe (NeoFox-TP, Ocean Optics, Largo, US).

2.4. Respiration

To determine if tailings exposure impacts respiration, oxygen consumption was measured in haddock and cod embryos before start of exposure, at 24 h (TP24) and 48 h (TP48) of exposure, and at the end of the recovery period (TPR48) using the Loligo® Microplate Respirometry System (940 μL wells) with the MicroResp™ software version 1.0.4. Ten eggs (6 at TPR48) from each exposure bottle were carefully transferred into the measurement chambers and chambers were sealed using a glass plate. Oxygen consumption was calculated based on the slope of the linear decline in oxygen concentration ($R^2 > 0.9$), corrected for temperature, salinity and air pressure.

2.5. Evaluation of tailing adhesion to eggs

Adhesion of tailings to eggs were determined with two separate

methods: elemental analyses with inductively coupled plasma triple quadrupole mass spectrometry (ICP-MS/MS) and an in-house developed optical method, applying shading (optical density) measurements from digital pictures.

For element analyses, 30 eggs from each replicate were collected after 48 h. Eggs could not be collected after 48 h recovery, as hatching and mortality had reduced the number of available individuals. Collected eggs were quickly rinsed in distilled water to remove excess particles from their surface, dried and frozen at −20 °C until analyses. Samples were digested in ultrapure nitric acid and analysed with ICP-MS/MS as described in more detail in the supplementary information (S2.2.1). Calcium concentrations were used to determine tailing adhesion, as the tailings predominantly consist of $CaCO_3$. The final concentrations were back calculated to ng $CaCO_3 \times egg^{-1}$.

Further, shading measurements were used to determine the degree of particle adhesion to eggs. Measurements were performed on images of 6 eggs (in seawater) from each replicate. Backlight images were taken with a Leica Z6 APO objective using a CMOS camera (MC170HD, Leica Microsystems, Germany) and the differences of opacity of individual eggs was measured and calculated as described in detail in the supporting information (S2.2.2, Fig S1).

2.6. Effects on egg buoyancy

To determine impacts of tailing exposure on the buoyancy of the fish eggs, the vertical velocity and distribution of eggs in the water column were determined after 24 h (TP24) and 48 h of exposure (TP 48), and after recovery for 24 h (TPR24) and 48 h (TPR48). The bottles were removed from the plankton wheels and left standing still for approximately 5 min to avoid turbulence deriving from the rotating motion, and to allow the eggs to position in the water column according to their buoyancy. To determine vertical velocity of eggs in the watercolumn, the bottles were then carefully inverted and positioned in front of a bench-top SilCam imaging system (Davies et al., 2017) imaging the eggs travelling through the water column (i.e. sinking or rising). Obtained images were collected at 15 Hz such that multiple individual eggs could be tracked over several consecutive frames, based on the method for settling velocity measurement described by (Brakstad et al., 2020). One modification was made to this method due to challenges with automatic tracking of high egg concentration and relatively high turbulence caused by egg movement in the confined space, so that manual (human) tracking points were used to identify egg locations over time. Additionally, to determine the relative amount of sinking or rising eggs in the total egg population, images were taken with a handheld digital camera and the number of eggs determined at different positions in the water column after 30, 60, 90 and 120 s following inversion The number of eggs in the areas: top (0 cm, surface), within 5 cm, 10 cm, 15 cm and >20 cm (bottom of the bottle) was determined using image J freeware (https://imagej.nih.gov/ij/) and expressed as relative amount of (%) totally determined eggs.

2.7. Determination of hatching and survival

Following the 48 h recovery in motion, the eggs were transferred into clean 300 mL glass beakers containing filtered seawater to determine hatching success and hatching time. The seawater in the glass beakers was regularly exchanged and the oxygen levels and temperature were measured daily as described above. Hatched larvae were counted daily, carefully collected and transferred into separate glass beakers containing filtered seawater. Dead eggs were removed daily from the Ctrl and L exposure groups. It was not possible to distinguish between alive and dead eggs in the M and H exposure groups as the exposed eggs did not float as alive eggs would normally do, and the egg surface remained covered with tailings. After all larvae in the Ctrl and L group had hatched, unhatched eggs from the M and H exposure group were evaluated under a stereomicroscope to distinguish between live and dead embryos.

Table 1
Particle number, volume, mass and size (volume-based measurements) of the exposure suspensions in the size range 2–60 μm at the start of the exposure experiments for haddock (HAD) and cod (COD). Mean ± SD.

Exposure	Particle concentrations			Particle size
	Particle number (mL^{-1}) x 10^3	Particle volume ($\mu m^3\ mL^{-1}$) x 10^6	Estimated mass ($mg\ L^{-1}$)	Diameter (μm)
HAD Low	18.7 ± 0.52	0.42 ± 0.05	1.20 ± 0.1	5.35 ± 0.47
COD Low	20.0 ± 1.40	0.41 ± 0.11	1.20 ± 0.3	4.81 ± 0.56
HAD Med	181 ± 3.78	4.92 ± 0.65	12.9 ± 1.2	5.91 ± 0.58
COD Med	186 ± 11.6	5.27 ± 1.11	12.6 ± 0.7	5.95 ± 0.55
HAD High	1770 ± 740	43.0 ± 13.9	116 ± 3.8	5.33 ± 0.24
COD High	1810 ± 108	48.4 ± 5.13	128 ± 18.5	5.66 ± 0.29

2.8. Larvae mortality, malformations

The fish larvae collected at different days were held separately, while larvae from exposure replicates that hatched the same day were pooled. Larvae were followed up until 17 dpf and mortality was determined daily with dead larvae being removed. Oxygen and temperature were measured daily as described above, and water was regularly exchanged. Larvae were immobilised using a methylcellulose gel and imaged through a Leica Z6 APO objective using a CMOS camera (MC170HD, Leica Microsystems, Germany) to determine malformations.

2.9. Statistics and data treatment

Data analyses were performed with GraphPad Prism 7 (Graph-Pad Software Inc., USA). To determine statistical differences between groups, data sets were analysed for normality (Shapiro-Wilk normality test) and analysed with either one-way ANOVA or Kruskal-Wallis tests and associated post-hoc tests to determine differences between groups. For hatching and mortality, data was fitted using non-linear regression curves. Detailed statistical information and results are provided in the supplementary information.

To determine mortality, we followed the approach of Kotani (Kotani et al., 2011) to correct for errors deriving from the removal of eggs and larvae due to successive sampling during the experiment. This approach assumes a constant mortality over the experimental duration and assigns a mortality to sampled individuals as well.

3. Results and discussion

3.1. Tailing characteristic and exposure

The elemental composition of the tailings was described previously (Farkas et al., 2017) and results from the sequential extraction are given in Tab S1. After the initial 20 min settling time, >99% of the particles (number) and 88 ± 5% of the particle volume were <20 μm in size. Particle number, volume, size distribution and mass of the particles (<20 μm) in the tailing exposure suspensions are presented in Table 1. The exposure suspensions used in the experiments with haddock and cod were prepared at different days and thus presented separately. The amount of particles was slightly, but not significantly ($p > 0.05$) higher in cod exposure suspensions compared to haddock exposure suspensions (Table 1). Particle sizes less than 2 μm were not recorded, which can cause a slight underestimation of the total mass. The volumetric size

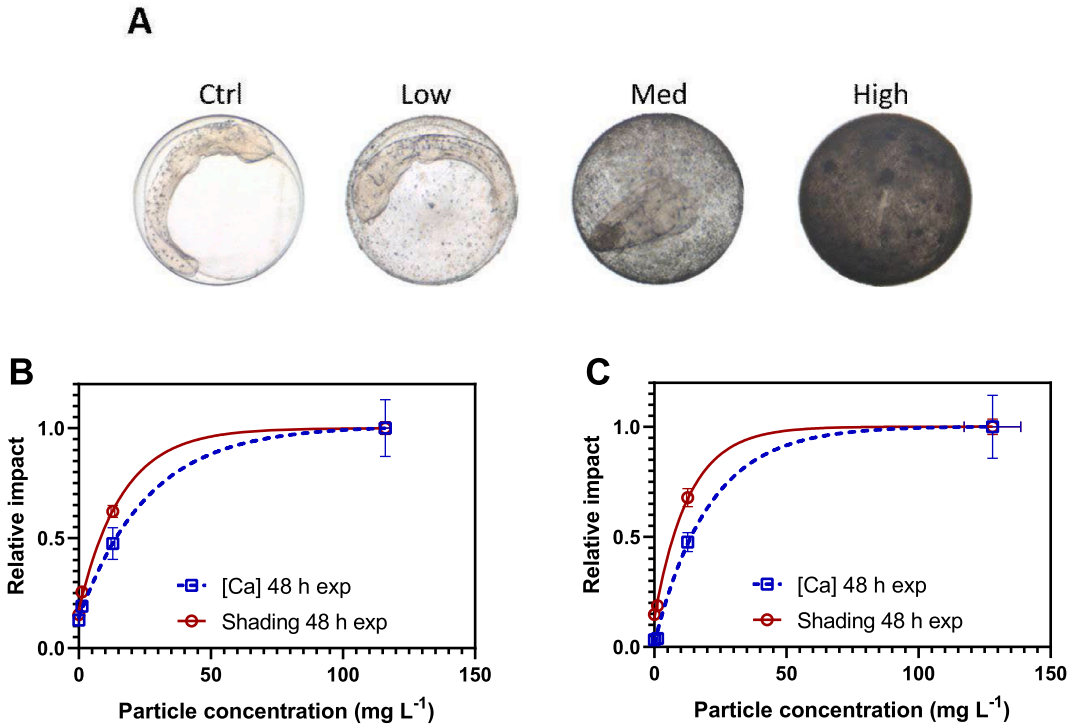


Fig. 1. A: Examples of haddock eggs after 48 h in seawater only (Ctrl) and exposure to low, medium and high concentrations of tailings. Comparison of the relation between exposure concentration and adhesion of particles for haddock (B) and cod (C) measured by associated Ca concentration (element analyses) and shading in backlight images relative to the highest exposure. Non-linear curve fit; one-phase association.

distributions of the particles were approximately in the range 2–10 μm, and extrapolating this distribution to below 2 μm indicate that the undetected mass was in the range 10–20% of the total mass.

Particle concentrations used in this study reached from approximately 1.2 mg L^{-1} (L), over 13 mg L^{-1} (M) to 130 mg L^{-1} (H) (Table 1; measurement range 2–60 μm, mass recalculated from particle number using a $CaCO_3$ density of 2.71 g cm^{-3}). Due to the complex behaviour of tailing particles, it is difficult to determine environmental their concentrations in the watercolumn after release into the environment. However, Davies and Nepstad (2017) used *in situ* optical particle measurement techniques to determine particle concentrations and transport in the vicinity of the release point of the STP site from which the tailings used in the present study were obtained. Measurements showed that most large tailing particles deposit close to the release point, while finer particles (measured down to 10 μm) were detectable within 3 km from the release point (Davies and Nepstad, 2017). The reported (volume) concentration of fine particles was up to 5 μl L^{-1}, i.e. 5 (μm^3 mL^{-1}) x 10^6 (Davies and Nepstad, 2017), which corresponds approximately the particle volume of the M exposure concentration in our study (Table 1). Moreover, Nepstad and co-authors recently applied high-resolution numerical modelling approaches to estimate particle concentrations and distribution around the STP discharge point (Nepstad et al., 2020). Predictions show that, especially during strong wind events, particle concentrations can exceed 1 mg L^{-1} several km from the release point and can reach more than 100 mg L^{-1} close to the release point (Nepstad et al., 2020). This indicates that the concentrations used in this study (Table 1) can be regarded as environmentally relevant, at least in the vicinity of the release point.

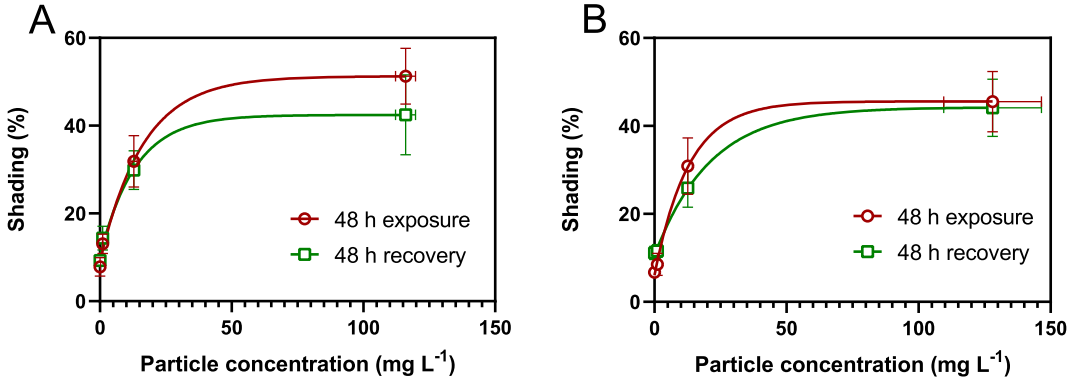


Fig. 2. Shading by eggs exposed to different concentrations of mine tailings for 48 h (5 dpf) before transferred to clan seawater for a subsequent 48 h (7 dpf). A; haddock, B; cod. Vertical and horizontal bars in A and B represent standard deviation, and the number of individuals measured were 18 individuals (from 3 replicates) except for tree groups (haddock recovery low and cod exposure low and recovery medium group) which contains 12 individuals (only 2 replicates). Non-linear curve fit; one-phase association.

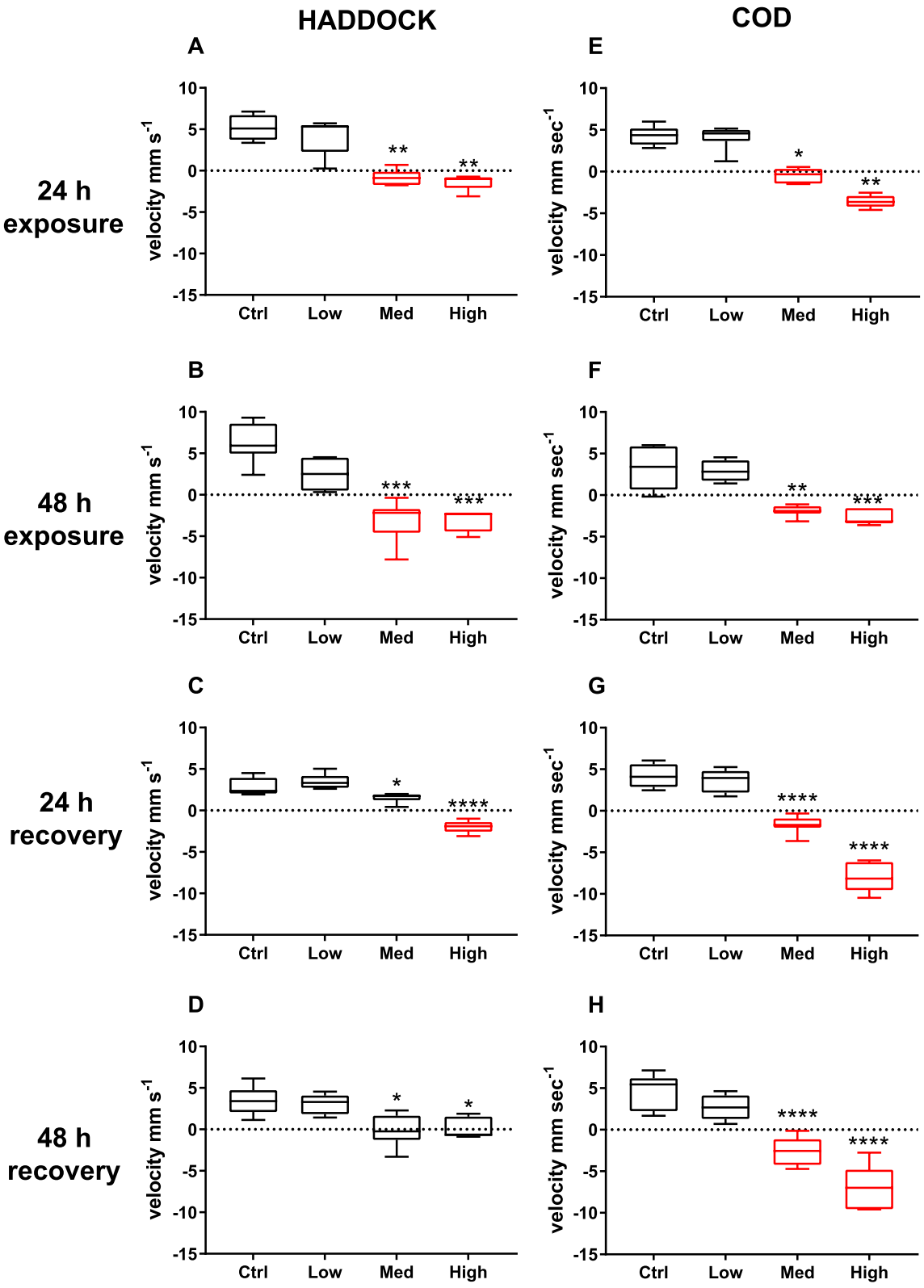


Fig. 3. Vertical velocity (mm s^{-1}) of haddock (A, B, C, D) and cod (E, F, G, H) eggs after exposure to no (Ctrl), low, medium (Med) and high concentrations of tailings for 24 h and 48 h, and a subsequent 24 h and 48 h recovery period. Groups that experienced a loss of positive buoyancy are plotted in red. Data presented as median with 25th and 75th percentile ± min/max, $n = 6–9$. Significant differences between exposure and Ctrl are indicated with *($p < 0.05$) **($p < 0.01$) and ***($p < 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

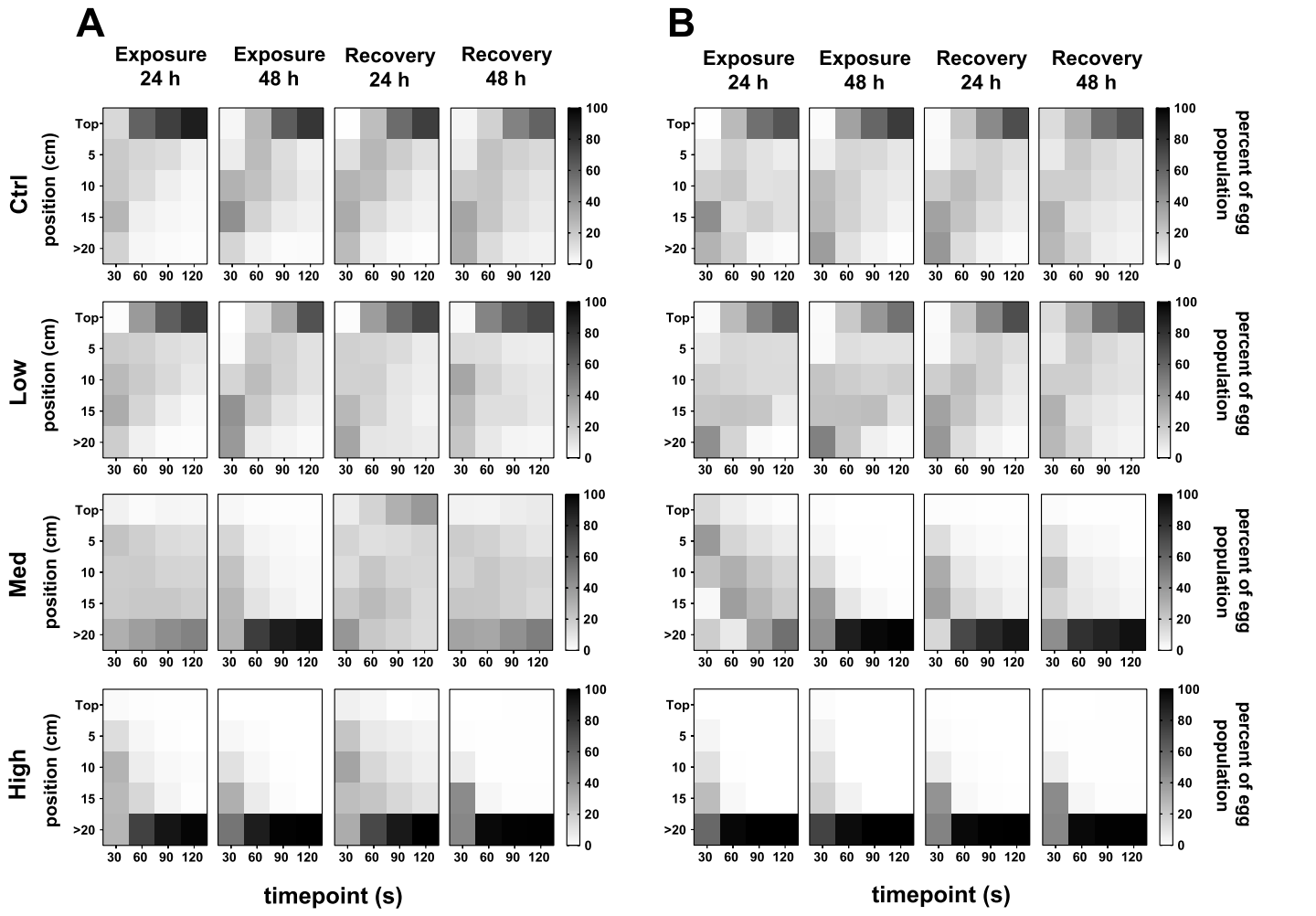


Fig. 4. Position of relative amount of eggs (% of total population; data presented as mean, $n = 3$ to 6) in different sections of the exposure bottle (top, 5, 10, 15 and > 20 cm) after 30, 60, 90 and 120 s following exposure bottle inversion. Results are shown for haddock in A and cod in B measured after 24 h and 48 h exposure, and 24 h and 48 h recovery.

3.2. Adhesion of tailing particles to eggs

Tailings were observed to rapidly attach (within minutes in H exposure concentrations; visual observation) to both haddock and cod eggs and remained attached to the chorion throughout the exposure and recovery period until hatch (Fig. 1A, S2, S3). Images, elemental analyses and optical measurements of shading show an exposure dependent increase of tailings associated with exposed eggs (Fig. 1A and B,C).

Elemental analysis show that Ca concentrations were higher in L exposed eggs (443 ± 50 ng egg^{-1}) in comparison to Ctrl eggs (298 ± 17 ng Ca egg^{-1}), and significantly higher in M and H eggs than in Ctrl, with 1107 ± 528 ng Ca egg^{-1} ($p = 0.0028$) and 2331 ± 523 ng Ca egg^{-1} ($p < 0.001$), in M and H, respectively.

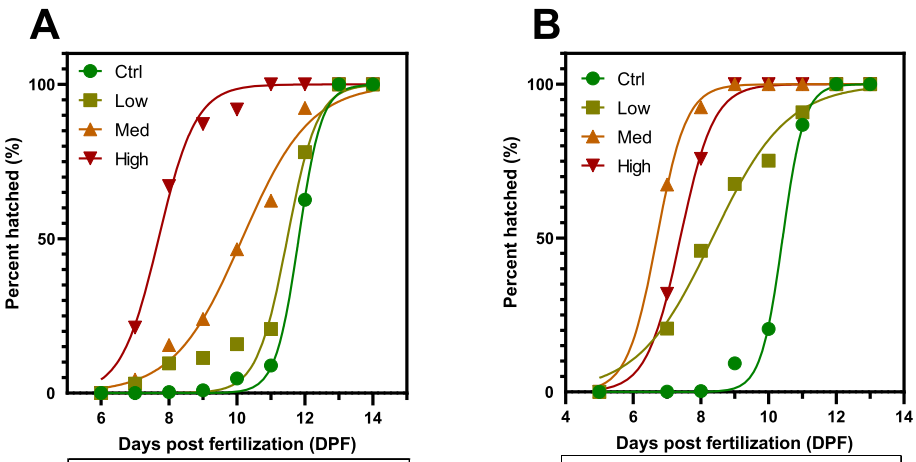
In cod, Ca concentrations were 198 ± 37 ng Ca egg^{-1} in Ctrl, 236 ± 18 ng Ca egg^{-1} in L, and significantly higher compared to Ctrl in M and H eggs with 2925 ± 376 ($p = 0.0043$) and 6136 ± 1527 ($p < 0.0001$) ng Ca egg^{-1}. Further, Ca concentrations were higher in cod eggs compared to haddock eggs in the M ($p = 0.0047$) and H ($p < 0.0001$) exposure groups.

Optical measurements reveal a similar exposure-dependent increase in tailing attachment. After exposure, shading was significantly different in all haddock exposure groups from the Ctrl ($p = 0.042$ for L, $p < 0.0001$ for M and H). Whereas in cod, the L group was similar to the Ctrl ($p = 0.92$) with the M and H groups being significantly different ($p < 0.0001$) from the Ctrl.

The deviation from linearity in both Ca concentration and optical measurements (Fig. 1B and C) suggests that the egg surfaces are becoming increasingly "saturated" with particles at exposure concentrations above approximately 50 mg L^{-1}. The shading curves are, however, steeper initially than the fitted Ca concentration curves (Fig. 1B and C), which is potentially due to particle self-shading at high exposure concentrations. In contrast to the ICP-MS/MS results, shading measurements do not indicate higher adhesion of tailings in cod than in haddock (Fig. 2A and B). This can either be due to the beforementioned self-shading, or differences in egg handling/preparation during sampling, such as the inclusion of washing steps for ICP-MS/MS sampling that could remove loosely bound tailings.

While both applied methods have limitations, they proved useful in determining particles adhesion to fish eggs. Elemental analysis using ICP-MS/MS are quantitative; however, a larger sample (high number of eggs), higher costs and processing times are limiting factors. In contrast, optical measurements are relatively rapid and inexpensive and do not require many individuals, however measurements are not quantitative and issues such as self-shading at higher concentrations need to be taken into account.

Shading measurements were further used to determine particle adhesion after recovery. In both species, eggs in M and H groups still had significantly more tailings adhered compared to Ctrl groups ($p < 0.0001$) after recovery. Tailing adhesion was further similar between exposure and recovery (Fig. 2A and B), except for the H exposure in



HAD		
Exposure	50 % hatch	95 % conf. int.
Ctrl	11.8	11.8 - 11.9
Low	11.5	11.2 - 11.7
Med	10.2	9.8 - 10.5
High	7.7	7.4 - 8.0

COD		
Exposure	50 % hatch	95 % conf. int.
Ctrl	10.4	10.3 - 10.5
Low	8.3	7.8 - 8.9
Med	6.7	6.6 - 6.8
High	7.4	7.2 - 7.6

Fig. 5. Cumulative hatching in haddock (A) and cod (B) exposed to high, medium (Med), low and no (Ctrl) tailings as embryos. The time (days) of 50% hatched with confidence intervals are shown in the table below each graph.

haddock, which had less tailings attached to the chorion after the 48 h recovery than after exposure (Fig. 2A; $p < 0.0001$). This shows that in the current exposure settings, tailings effectively adhered to the chorion of both haddock and cod eggs and stayed attached also during recovery in motion. Results further indicate that tailing adhesion was probably more effective in cod than in haddock, at least in H exposure concentrations.

This contrasts with a previous study, reporting that adhesion of oil droplets to eggs was more efficient in haddock than in cod (Hansen et al., 2018), probably reflecting differences in structure and potentially charge distribution on the egg chorion of the two species as described by Hansen et al. (2018).

3.3. Effects on egg buoyancy

Egg buoyancy was affected by tailing exposure. Control eggs of both species were positively buoyant at all times during the documentation period (96 h), with average rising velocities of 4.6 ± 2 mm s^{-1} (median: 4.6 mm s^{-1}; 2.6–6.1 25th and 75th percentiles) for haddock, and 3.9 ± 1.6 mm s^{-1} (median: 4.4 mm s^{-1}; 2.9–5.1 25th and 75th percentile) for cod. This is higher than the previously reported modelled/calculated rising velocities for haddock and Atlantic cod of approximately 1–2 mm s^{-1} (Butler et al., 2020; Sundby, 1983). The higher rising velocity in the present study compared to those previously modelled, may arise from altered seawater density used in our facility compared to the where the eggs were produced, or from measurement artefacts such as background turbulence created by drag in the exposure bottles used in this study.

Eggs of both species remained positively buoyant in L exposures throughout the exposure and the recovery time (Fig. 3). However, after 48 h of exposure, haddock eggs in the L exposures were ascending significantly slower compared to Ctrl eggs ($p = 0.0049$), with an average rising velocity of 2.5 ± 1.8 mm s^{-1} (median: 2.5; 0.5–4.5 25th and 75th percentile) in L groups compared to 6.3 ± 2.3 mm s^{-1} (median 5.9 mm s^{-1}; 4.9–8.6 25th and 75th percentile) in the Ctrl. Similarly, the overall (all timepoint average) rising velocity was significantly ($p = 0.0239$) lower in L exposed haddock (average 3.3 ± 1.6 mm s^{-1}) compared to Ctrl (4.6±2mmsec^{-1}). In contrast, no significant effects were found for cod eggs in L exposure groups as compared to the Ctrl groups. For both fish species, eggs in M and H exposure groups became negatively buoyant during exposure (Fig. 3). While haddock eggs, at least partially, regained buoyancy during the recovery phase in motion, cod eggs in M and H groups remained negatively buoyant throughout the exposure and recovery period (Fig. 3). This is in agreement with tailing adhesion measurements (Fig. 2A and B) that indicated a more efficient tailings adhesion in cod than in haddock eggs.

The water column distribution of eggs (proportion of the total population) during exposure and recovery are presented in Fig. 4. The results show that, while the majority of the egg population in Ctrl and L exposed eggs of both species retain a positive buoyancy (rise to top), eggs exposed to M and H tailing concentrations sink after 24 and 48 h exposure (Fig. 4 A, B).

After 48 h of exposure (TP48), 29 ± 26% of the M and 54 ± 7% of the H exposed haddock eggs had reached the bottom 30 s after inverting the exposure bottles. After 120 s, 93 ± 4% of the M and 100 ± 0% of the H exposed haddock eggs had reached the bottom of the exposure bottle. In contrast, only 2 ± 1 and 2 ± 2% had sunken to the bottom in L and Ctrl groups, while 79 ± 9 and 67 ± 1% of the eggs had risen to the surface (top section of the bottle) after 120 s.

In cod, 43 ± 7% of the M exposed group, and 73 ± 14% of the H exposed group had sunken to the bottom within 30 s, while 99 ± 1% of the M, and 100 ± 0% of the H exposed eggs reached the bottom after 120 s. In contrast, 77 ± 19% of Ctrl, and 55 ± 9% in the L exposed group reached the surface, and only 1–2% respectively sank to the bottom. After 48 h recovery in clean seawater, 36 ± 8 and 30 ± 7% of M and H exposed haddock eggs were at the bottom after 30 s following bottle inversion, which increased to 50 ± 7 and 81 ± 6% for M and H, respectively, after 120 s. Exposed cod eggs were sinking to a larger extend after 48 h recovery, with a total of 44 ± 15% of the M, and 47 ± 20% of the H exposed eggs at the bottom after 30 s, which reached 93 ± 3 and 99 ± 0.6% after 120 s. In L and Ctrl groups, most eggs were positively buoyant also in the recovery phase, with 61 ± 18 and 71 ± 22% of Ctrl and L exposed haddock eggs risen to the surface after 120 s. For Ctrl cod eggs the relative amount that reached the surface in 120 s was, with 67 ± 25% similar to those of haddock eggs, which as slightly, but not significantly lower in L exposed cod (41 ± 15%, $p = 0.2$ compared to cod Ctrl).

These results corroborate the findings on tailing attachment and sinking velocity, indicating that both haddock and cod eggs lose their positive buoyancy following exposure to relatively high tailing concentrations. While measurements of the sinking velocity of selected eggs indicate that haddock eggs at least partly regain neutral buoyancy (Fig. 3 C, D), the analyses of whole egg populations show that most

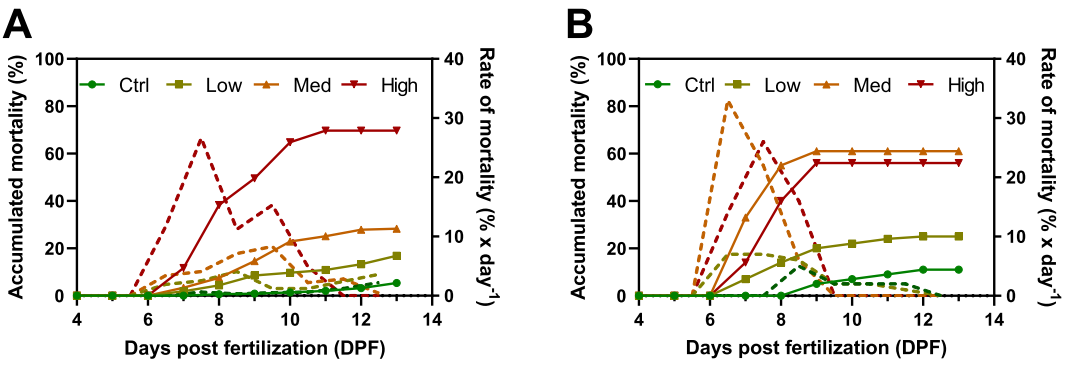


Fig. 6. Accumulated mortality (solid line with symbols) and daily mortality (broken lines) show that there was a dose related increase in mortality, and that the mortality in the exposed groups mainly occurred during and shortly after the hatching period. Haddock (A) and cod (B), respectively.

haddock eggs (50% in M and >80% in H exposures) still sink after recovery (Fig. 4). This discrepancy is probably related to the small sample size for velocity measurements, and the limited measuring time. However, results support the finding that buoyancy of cod eggs are more affected compared to haddock and that tailings seem to attach more efficiently (for a longer time) to cod than to haddock.

To our knowledge, previous studies on the impact of suspended (mineral) particles on the buoyancy of pelagic fish eggs are limited. In a study investigating the effects of clay and limestone particles deriving from dredging operations, Westerberg showed that cod eggs readily sink after exposure to fine particulate material in concentrations relevant for surroundings of dredging areas (Westerberg et al., 1996). Reinardy et al. (2019) found that tailing particles attached to the chorion of cod eggs, however, impacts on buoyancy were not reported. A positive or neutral buoyancy is crucial for pelagic fish eggs for their vertical positioning in the water column as eggs sinking to deeper layers or the sea bottom may be lost to the recruitment of the year due to oxygen depletion, benthic predation or after hatch, limited availability of food (Sundby and Kristiansen, 2015; Vikebø et al., 2007). The loss of positive buoyancy due to tailing attachment could thus impact the survival of fish early live stages and overall recruitment success.

3.4. Effects of tailings on embryonic respiration and hatching

Measurements of embryonic respiration rates showed a significant response to tailing exposure only in haddock. Respiration in haddock embryos was significantly reduced in M ($p = 0.01$) and H ($p = 0.048$) groups after a 48 h exposure, from 2.69 ± 0.05 nmol O_2 ind^{-1} h^{-1} in Ctrl groups to 2.32 ± 0.11 nmol O_2 ind^{-1} h^{-1} in M and 2.42 ± 0.11 nmol O_2 ind^{-1} h^{-1} in H, respectively. No effects were observed in the L exposure group or in any of the exposure groups after an exposure duration of 24 h. Similarly, no effects were seen in exposed cod embryos, neither at 24 nor 48 h. The relatively limited or lacking reduction in embryonic respiration despite the high degree of eggs surface coverage by tailing particles is rather unexpected. It is however possible that bacterial respiration can contribute to the measured oxygen decrease, as the tailings contain polymers processing chemicals that are prone to biodegradation (Trannum et al., 2020).

Exposure to tailings caused a significant reduction in overall hatching success in H exposed groups in both haddock ($p = 0.0009$) and cod ($p < 0.0001$), with no significant impacts in L and M exposed groups (Fig. S4 A). Exposure further initiated concentration-dependent premature hatching in both fish species (Fig. 5A and B). Hatching of haddock larvae in the H exposure group started about 3–4 days earlier than in the Ctrl group, with 50% of the H larvae hatched at 7.7 dpf, compared to Ctrl larvae, where 50% had hatched at 11.8 dpf ($p < 0.0001$; Fig. 5A). Similarly, exposed cod larvae hatched earlier than Ctrl with 50% hatched at 7.4 dpf in comparison to 10.4 dpf in Ctrl larvae ($p < 0.0001$; Fig. 5B). Early onset of hatching has previously been described for pacific herring embryos exposed to suspended sediments after fertilisation (Griffin et al., 2009). Previous studies show that early hatching is an escape strategy in fish under unfavourable conditions such as hypoxia or osmoregulatory stress (Oppen-Berntsen et al., 1990; Ord, 2019). The trigger for premature hatching, or combination of triggers such as hypoxia, limitations of osmoregulation capacity or reduced light conditions, that induce early hatching following particle exposure remain to be investigated.

3.5. Implications of short-term embryonic tailing exposure on survival and larvae malformation

The exposure to tailings reduced the overall survival (embryos and larvae) in a concentration dependent manner in both cod and haddock (Fig. S 4 B). In Ctrl groups the overall survival until the end of the experiment (17 dpf) was similar for the two fish species, with an average survival of $80.6 \pm 2.6\%$ and $80 \pm 4\%$ for haddock and cod, respectively (Fig. S 4 B). In the H exposure groups, the overall survival was reduced significantly for both species compared to Ctrl ($p < 0.0001$), with only $15.7 \pm 1\%$ of haddock and $12.3 \pm 3\%$ of cod surviving until 17 dpf. Survival was also significantly lower in L ($64.6 \pm 4.4\%$; $p = 0.0413$) and M ($28.5 \pm 0.7\%$; $p < 0.0001$) exposure groups in cod, and M ($58.5 \pm 5\%$; $p < 0.0001$) exposure groups in haddock (Fig. S4 B). Regarding survival, cod was more sensitive to tailing exposure as the overall survival was significantly less ($p < 0.0009$) in cod compared to haddock, potentially due to the prolonged adhesion of tailings to cod compared to haddock.

Larvae survival after hatch was impacted by embryonic tailing exposure. The accumulated mortality of hatched larvae for haddock and cod per day (symbols and solid lines) and the mortality rate of larvae hatched at each day (symbols with dashed lines) are presented in Fig. 6. Larvae that were exposed to M and H tailing concentrations as embryos hatched early and died shortly after hatch. The hatching timepoint also influenced larvae survival in Ctrl groups, where mortality decreased with later hatching. Approximately 30% (haddock) to 35% (cod) of the few Ctrl larvae that hatched at 9 dpf died shortly after hatch (data not shown). In contrast, only 6% of haddock, and 7% of cod Ctrl larvae that hatched later died during the experiment. This indicates that early hatching is a factor for increased larvae mortality in M and H exposed groups.

Studies investigating effects of mine tailings or other suspended particles on early life stages of pelagic fish are so far scarce. Reinardy et al. (2019) did not find significantly reduced hatching rates (embryo mortality) in cod embryos exposed to tailings from a copper processing plant (Reinardy et al., 2019). However, the maximum determined exposure concentration to tailings in their study was 3.2 mg L^{-1}, which is between the L and M concentration in our study, and which, as in the present study, did not induce significant embryo mortality. In agreement with our study, Reinardy et al. (2019) report early hatching in their high exposure groups. The authors further report increased mortality in cod

Fig. 7. Example images of haddock (A–D) and cod (E–F) larvae (3 dph). Controls for cod and haddock are given in A and E, respectively. Minor (B and F), moderate (C and G) and severe (D and H) deformations are displayed as spine, craniofacial and jaw deformations with increasing severity.

larvae continuously exposed for 22-days. However, the larvae themselves were exposed, which was not the case in our study, making the results in that study and the present study not directly comparable. Increased mortality of cod embryos and larvae, with larvae being more sensitive, following suspended sediment exposure are further reported by Westerberg et al., 1996 . Griffin and co-workers also reported early hatching and increased larvae mortality for pacific herring exposed to suspended sediment as embryos (Griffin et al., 2009).

While effect mechanisms remain to be determined, the possibility for leaching of potentially toxic metals/metalloids to the seawater seems to be limited. Concentrations of As, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn bound in the labile, exchangeable, acid soluble and reducible fractions were relatively low for the tailing type used in this study (Farkas et al., 2017; Tab. S1). An element driven toxicity can thus, at least under the tested experimental conditions, be excluded as the main driver of effects. Example images of the Ctrl larvae and some of most frequently observed malformations are shown in Fig. 7, with haddock in the top row and cod larvae presented in the bottom. Marginal finfold, spine and jaw malformation were most observed abnormalities. In haddock, the frequencies of several types of malformations were exposure concentration dependent (Fig. S5). Heart deformations were observed, but were not common (data not shown). The overall percent of malformations (relative number of fish with at least one deformation) in haddock were 23.5% in Ctrl, 45.85% in L, 55.76% in M and 91.40% in H exposure groups.

Hatching timepoint and malformations were correlated in Ctrl, L and M exposure groups. Early hatched larvae showed higher deformation frequencies, with significant relationships between hatching timepoint and malformations in Ctrl ($r^2 = 0.75$, $p = 0.025$), L ($r^2 = 0.68$, $p = 0.022$) and M ($r^2 = 0.81$; $p = 0.005$) exposures. This was not observed in H exposure groups, where malformations were consistently high at all hatching days and varied between 70 and 100%. It has previously been described that early hatched larvae, also unexposed individuals, are often deformed (Blaxter, 1977). Our results thus indicate that the induction of earlier hatching could lead to, or at least contribute to increased deformations in L and M groups. While malformations were observed in cod (Fig. 7), we were unable to quantify malformation in cod due to challenges with sampling.

4. Conclusion

The results of our study show that tailings rapidly attached to haddock and cod eggs and that a 48 h exposure had significant effects on buoyancy, hatching time, survival and larval development in both species. Survival of cod was significantly reduced at concentrations that are assumed to be environmentally realistic within some km away from the release point. However, contact times of fish eggs and mineral particles at tailing release sites are not known to our knowledge. To predict risks related to positive buoyancy loss in real environmental scenarios, further research is needed to determine the likelihood and duration of particle-fish egg encounters at tailing placement sites. Further, egg-tailing contact time in relation to exposure concentration causing impacts on buoyancy need to be investigated.

While this study focused on mineral particles deriving from STP, findings are further relevant for other anthropogenic activities that increase the amount of fine particles in the water column like dredging and submarine construction. Furthermore, changing climatic conditions can increase terrigenous runoff and thus suspended particle loads in receiving environments such as fjords. With survival of pelagic embryos and larvae being related to their vertical and horizontal transport in the water masses it is critical to study impacts of different types of particles on fish egg buoyancy, especially in species such as haddock and cod, which are of great ecological and economical importance.

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.envres.2021.111447.

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