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Determining the risk of calcium oxide (CaO) particle exposure to marine organisms

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ABSTRACT

Calcium oxide (CaO) is being considered as a possible treatment for both the control of echinoderm populations and the treatment against sea lice infestation in Norwegian salmon farms. CaO particles produce an exothermal reaction when in contact with water, which can cause epidermal burns and lesions to certain target organisms leading to death. The aim of the present study was to determine the effects of fine (<0.8 mm) and coarse (<2.5 mm) CaO particles to a range of marine species from different taxonomic groups: two echinoderms (*Asterias ruben* and *Strongylocentrotus droebachiensis*); two crustaceans (*Carcinus maenas* and *Tisbe battagliai*); two molluscs (*Mytilus edulis* and *Hinia reticulata*); a polychaete (*Nereis pelagica*); a fish (*Cyclopterus* sp.); and seaweed germlings (*Fucus vesiculosus*). Overall, the fine CaO particles were more toxic to the selected marine species than the coarse particles. Coarse CaO particle effects were only observed in four of the nine species tested (*A. rubens*, *S. droebachiensis*, *N. pelagica*, *T. battagliai*) with similar LC_{50} values between 207 and 268 g/m^2. For the fine CaO particles, the lowest LC_{50} was for the epibenthic copepod (*T. battagliai*) at 3.14 g/m^2, followed by the sea urchin (20.1 g/m^2), starfish (22.2 g/m^2), ragworm (29.6 g/m^2), and netted dog whelk (41.9 g/m^2). Lump sucker fish exhibited significant mortalities only at the highest fine CaO concentration tested (320 g/m^2) and recorded an LC_{50} of 226 g/m^2. The toxicity data were used to generate species sensitivity distributions (SSDs) for both fine and coarse CaO particles. The hazard concentrations for 5% of the species (HC5) calculated from the SSDs, based on NOEC values, for the coarse and fine particles were 35.5 and 1.5 g/m^2 respectively. Using a recommended assessment factor of 5, the Predicted No Effect Concentration (PNEC) was calculated as 7.1 and 0.3 g/m^2 for coarse and fine CaO particles respectively.

1. Introduction

Calcium oxide (CaO), also known as quicklime is being considered as a treatment for the control of sea urchin populations in Norwegian fjords as well as a candidate for the treatment of sea lice in salmon fish farms. Calcium oxide particles have been previously used to successfully control echinoderm populations in California and Nova Scotia for the control of starfish in oyster beds, as well as the control of sea urchins in commercially harvested kelp beds (Bernstein and Welsford, 1982). The practice of controlling echinoderm populations with quicklime is documented back to the start of the 1900 century (Wood, 1908).

Calcium oxide particles when combined with water produce an exothermic reaction, which when in contact with the surface of target organisms, can cause epidermal burns and lesions. In echinoderms, such as starfish and sea urchins, these epidermal burns often result in acute mortalities brought about by osmotic imbalances. Additionally, the burns and lesions can allow bacteria to enter the coelomic fluid resulting in infection and death after a few days (Bernstein and Welsford, 1982). The efficacy of the particles is dependent on dosage, the time after particle reaction with the water, and water temperature, with lower water temperatures likely to limit the rate of bacterial infection. Furthermore, the speed of the exothermic reaction between CaO particles and water is dependent on particle size, with finer particles reacting more quickly and remaining within the water column. Alternatively, larger particles react slower and sink to the seabed much faster. For this reason, an optimal particle size exists for the control of echinoderm populations, to ensure that the particles sink at a speed fast enough so that the reaction and heat produced is not dissipated before reaching the

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target organisms. Application methods have been refined to optimise particle efficacy (Bernstein and Welsford, 1982).

Information on the toxicity of quicklime particles to marine organisms is limited to a few studies where detrimental impacts are only reported in echinoderm species. For example, no effects were found in the blue mussel (*Mytilus edulis*), the blood worm (*Glycera dibranchiate*), the sand worm (*Nereis virens*), the American lobster (*Homarus americanus*), or the periwinkle (*Littorina littorea*) following exposure to CaO particles (Shumway et al., 1988). In contrast, in the same study, 100% mortality was observed in the starfish (*Asterias vulgaris*).

More recent studies have looked at the benefits of dissolved quicklime in reducing the toxicity of certain metals such as copper (Das and Das, 2005; Abdel-Tawwab et al., 2007) and cadmium (Kaviraj and Dutta, 2000). The reduction in toxicity is thought to be partly due to increased competition of calcium and divalent metal ions at the gill surface interface, as well as the increased alkalinity of the water and the reduction in the bioavailable fraction of the metals. However, a clear distinction must be made between dissolved and particle toxicity, and it is the particle impact of quicklime and not the dissolved concentration that is the focus of the current study.

The project was designed to assess the toxicity of CaO particles to a selection of target and non-target marine organisms. A multi-species toxicity assessment was performed to determine those species that may be more vulnerable to CaO particle exposure. The marine organisms tested were carefully selected to represent a wide range of trophic and taxonomic groups typically found in Norwegian coastal waters. Two particle sizes, defined as coarse (<2.5 mm) and fine (<0.8 mm) have been produced for application, and where included in the toxicity assessment. The toxicity data were used to calculate species sensitivity distributions, with the aim to provide a risk assessment for fine and coarse CaO particles in marine coastal waters of temperate regions.

2. Methods

The following protocol describes the procedure for the exposure of marine organisms to CaO (quicklime) particles within controlled laboratory conditions. The design of the laboratory exposure system was intended to represent the application of quicklime when used as a treatment for controlling sea urchin populations. However, although efforts were made to simulate a realistic exposure scenario, practical limitations within the laboratory must be considered. One important consideration includes; a lower dilution of the particles within the recipient water, which may result in an increased pH as well as increased efficacy of the particle. It has been recently demonstrated that the application of CaO particles into coastal waters caused very little change in the pH of the seawater, due to the strong buffering of the seawater (Garmo et al., 2017). The pH was monitored closely during the laboratory exposure and the flow-through rates (when used) were maintained high to reduce the impact of pH on the test species within the aquariums. A total of nine test organisms were assessed. For logistical reasons the tests were divided into three separate exposures.

2.1. Test material

Calcium oxide particles (two particles, coarse (<2.5 mm) and fine (<0.8 mm)) were provided as a dry particle (<0.1% water content) by industry (Franzefoss Minerals AS, Fig. 1). The analysis report was provided by the supplier and confirmed a 94% and a 91% CaO content for the fine and coarse particles respectively. In all tests, the exposure concentration was based on the particle weight per surface area, which represented the surface area of the aquarium holding the test species.

2.2. Flow-through dosing system

The flow-through seawater system was set-up at the NIVA marine research station in Solbergstrand, Norway. The system consisted of 11 × 450 L tanks connected to a seawater supply (outer Oslofjord 60 m depth, Na 12620, K 454, Mg 1508, Ca 455, Cl 31236, SO_4 4132 mg/L) with flow rates controlled at approximately 5 L/min to each tank. The depth of water in each tank was standardised at 35 cm. Each tank represented a different test treatment, which included five concentrations of coarse particles and five concentrations of fine particles. The concentration range was 320, 100, 32, 10 and 3.2 g/m^2. A separate tank receiving no particles was used as a control. The same number of test animals were used in each tank.

In the first exposure, five species were used, these included: the common starfish (*Asterias ruben*, 7–15 cm diameter, n = 10 per treatment tank); the green sea urchins (*Strongylocentrotus droebachiensis*, 5 ± 2 cm diameter, n = 10 per treatment tank); the shore crab (*Carcinus maenas*, 6 ± 2 cm carapace width, n = 7 per treatment tank); the blue mussel (*Mytilus edulis*, 4 ± 2 cm length, n = 20 per treatment tank); and the lumpsucker fish (*Cyclopterus* sp., 5 ± 2 g w.w., n = 7 per treatment tank). The lumpsucker fish and the mussels were placed in separate mesh cages to prevent predation from the starfish and crabs. The starfish and sea urchins were collected within the outer Oslo fjord by divers. Mussels and crabs were collected from the rocky shore basins at Solbergstrand, whilst the lumpsucker fish were bought from a known hatchery in Norway (Lerøy Seafood AS). All animals were acclimated to the NIVA 60 m inlet water for approximately two weeks prior to testing.

In the second exposure, following a thorough clean of the exposure system, three marine species were used in the same exposure tanks. These include: the netted dog whelk (*Hinia reticulata*, 2 ± 0.5 cm length, n = 7 per treatment tank); the slender ragworm (*Nereis pelagica*, 11 ± 3 cm length, n = 7 per treatment tank) and germlings of the bladder wrack (*Fucus vesiculosus*, 100 μm, n > 20 per treatment tank). The netted dog whelk and slender ragworm were collected from the rocky shore basins at Solbergstrand. The animals were acclimated to the NIVA 60 m inlet water for approximately one week prior to testing.

Germlings of the bladder wrack (*F. vesiculosus*) were obtained following the procedure described in Brooks et al. (2008). Adult *Fucus*

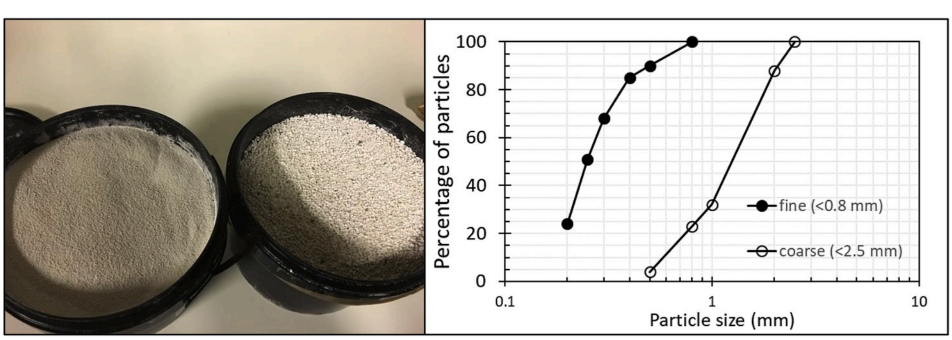

Fig. 1. Calcium oxide particles provided by industry (Franzefoss Minerals AS) including particle size distributions for the fine (<0.8 mm, left) and coarse (<2.5 mm, right) particles.

fronds were collected from the intertidal zone at Ingierstrand, Inner Oslo fjord, Norway (59°49′53.6″N 10°46′13.7″E), and taken to the NIVA laboratory in Oslo where they were subjected to mild desiccation overnight at 15 °C. The receptacles were removed and placed in beakers containing approximately 200 ml of filtered (0.45 μm) seawater. A minimum of 50 receptacles per beaker were used to ensure the presence of both male and female receptacles. Receptacles were left for 2 h to allow the release of reproductive bodies. The resulting zygote suspension was filtered through a 90 μm sieve and collected on a 25 μm sieve. The zygotes were washed from the 25 μm sieve and a zygote seawater suspension of approximately 500 zygotes per ml was used to transplant on to microscope slides. The zygotes, attached to microscope slides, were left to develop into germlings over 7 days before the slides were placed within the flow-through system at Solbergstrand. *Fucus* germlings were measured on day 0 and then at the end of the 10-day exposure. The total length of over 20 germlings were measured for each treatment using a microscope (x200 magnification) and image analysis software (Cell-D, Olympus).

Immediately prior to dosing, all animals were placed on the bottom of the tanks to ensure that all animals were exposed equally. The microscope slides holding the *Fucus* germlings were held in slide racks that were suspended within the water column (top 15 cm) at an angle of approximately 45°. Particle dosing was designed to deliver an even distribution of the measured weight of particles over the entire area of the tank. A perforated grid was used to assist in evenly scattering the particles. A plastic grid tile, held down with a stone weight, was placed in each tank enabling the polychaetes to find shelter during the 10-day exposure. Survival of the test animals in each tank was recorded daily. Dead animals were immediately removed from the tanks at each observation and recorded. The pH, salinity, temperature and dissolved oxygen were measured during the 10-day exposure.

The pH and temperature were measured throughout the 10-day exposure using a Webmaster Industrial Water Controller fitted with calibrated pH and temperature sensors. Salinity and dissolved oxygen were recorded on a WTW handheld meter. Since pH was expected to increase immediately after particle dosing, the pH was measured frequently during this time, particularly in the tanks with the highest CaO particle concentrations.

2.3. Static-renewal dosing system

Due to the difficulty in performing the *Tisbe battagliai* exposure in the flow-through system at Solbergstrand, it was decided to expose the epibenthic copepods within a static-renewal system at the NIVA laboratory. The test was performed according to the ISO guideline (ISO 14669:1999) with slight modifications in that the survival was monitored for an extended period of 10 days instead of 2, and the seawater solutions were replaced every 3 or 4 days.

The marine copepod *T. battagliai* cultures were maintained at the NIVA laboratory. Copepods of 6 ± 2 days old were collected from a *T. battagliai* culture using a 150 μm mesh. Five copepods were transferred into beakers containing 50 ml filtered seawater (0.2 μm filter) collected from NIVA's marine research station at Solbergstrand. Four replicates per treatment were used, resulting in 20 animals per treatment in total). Prior to exposure, the animals were fed with the algae *Rhodomonas baltica* (2×10^5 cells/ml).

The concentration range for both coarse and fine particles was 100, 32, 10, 3.2 and 1 g/m^2 with a control beaker containing no particles. The highest concentration of 320 g/m^2 was excluded due to the expected increase in pH within the semi-static system, as well as the difficulty in observing the animals under the microscope at the high particle density (according to preliminary experiments). Due to the observed increase in pH during exposure, pH controls were included (pH control 9 and 10) by the addition of NaOH to the test natural seawater prior to the addition of the copepods to account for pH effects.

The CaO particles were homogenously distributed over the area of the beakers and the animals were exposed for 1 h. The pH was monitored during the exposure period. At the end of the 1 h exposure the animals were transferred to separate wells of a 6-well plate containing 10 ml of fresh seawater in order to avoid pH effects (based on preliminary experiments), and their survival was monitored for 10 days. The test was performed at a temperature of 20 °C under a photoperiod of 16 h light/8 h dark. The animals that showed no swimming or appendage movement within 10 s of observation were recorded as dead and were removed from the vessels. The seawater containing algal food was renewed on day 4 and 8. Dissolved oxygen, pH and salinity were recorded at the start and end of the test.

3. Results

3.1. Physicochemical properties

The change in pH and temperature over time following the addition of the coarse and fine CaO particles for the highest concentration (320 g/m^2) is shown in Fig. 2. For the coarse particles, a slight rise in pH was observed increasing from 8.0 to 8.4 approximately 30 min after addition. In contrast, the pH rapidly increased following the addition of the fine particles, increasing from 8.0 to 9.1 within the first 10 min. The pH reduced after 10 min and was approaching 8.2 after approximately 2 h. Approximately 2–3 days was necessary to return the pH to starting seawater conditions for both fine and coarse particles. The temperature was less affected by the addition of the CaO particles for both fine and coarse particles. A slight increase in the fine particle tank after 6–7 days was related to the ambient seawater taken from the outer Oslo fjord and was not thought to be due to CaO particle effects.

A summary of the physicochemical measurements of the treatment water from all three exposures are shown in Table 1. Although pH, salinity and dissolved oxygen concentrations were comparable between the exposures, the temperature range for the second exposure was markedly higher. The seawater was taken from the outer Oslo fjord at a depth of 60 m, and since temperature regulation was not actively controlled in the study, this temperature range reflected the natural change in the seawater temperatures, with increased mixing of the bottom waters with the warmer surface waters at the time the study was performed (17th to 27th Oct 2017).

Physicochemical properties of the seawater during the 1 h exposure - 10-day recovery to coarse and fine CaO particles for the semi-static epibenthic copepod (*T. battagliai*) test are shown in Table 2. Dissolved oxygen, temperature and salinity were stable during the 1 h exposure phase and during the observations. For the coarse particles, pH increased to 9 within 1 h at the highest concentration (100 g/m^2), whilst for the fine particles, pH increased to 9.5 at 100 g/m^2, and 8.8 and 8.9 at 10 and 32 g/m^2 respectively.

3.2. Toxicity of the CaO particles

The effects of coarse and fine CaO particles on the survival of the nine marine organisms are shown in Figs. 3 and 4, with calculated toxicity endpoints presented in Table 3. For the coarse particles, no effects on mortality were observed at the highest particle density for the shore crab, the blue mussel, the lumpsucker fish, the netted dog whelk and *Fucus* germlings, with lowest observable effects concentration (LOEC) > 320 g/m^2. Significant effects of the coarse CaO particles were observed in the starfish, the green sea urchin, the slender ragworm and the epibenthic copepod. Of the four affected species, the epibenthic copepod had the lowest LOEC, although the LC_{50} concentrations (concentration causing 50% mortality in the population) were similar between 207 and 264 g/m^2.

The fine CaO particles had more detrimental effect on the survival of the test species. Only the shore crab, the blue mussel and *Fucus* germlings were unaffected by exposure to the fine CaO particles. The most sensitive species tested was the epibenthic copepod, with an LC_{50}

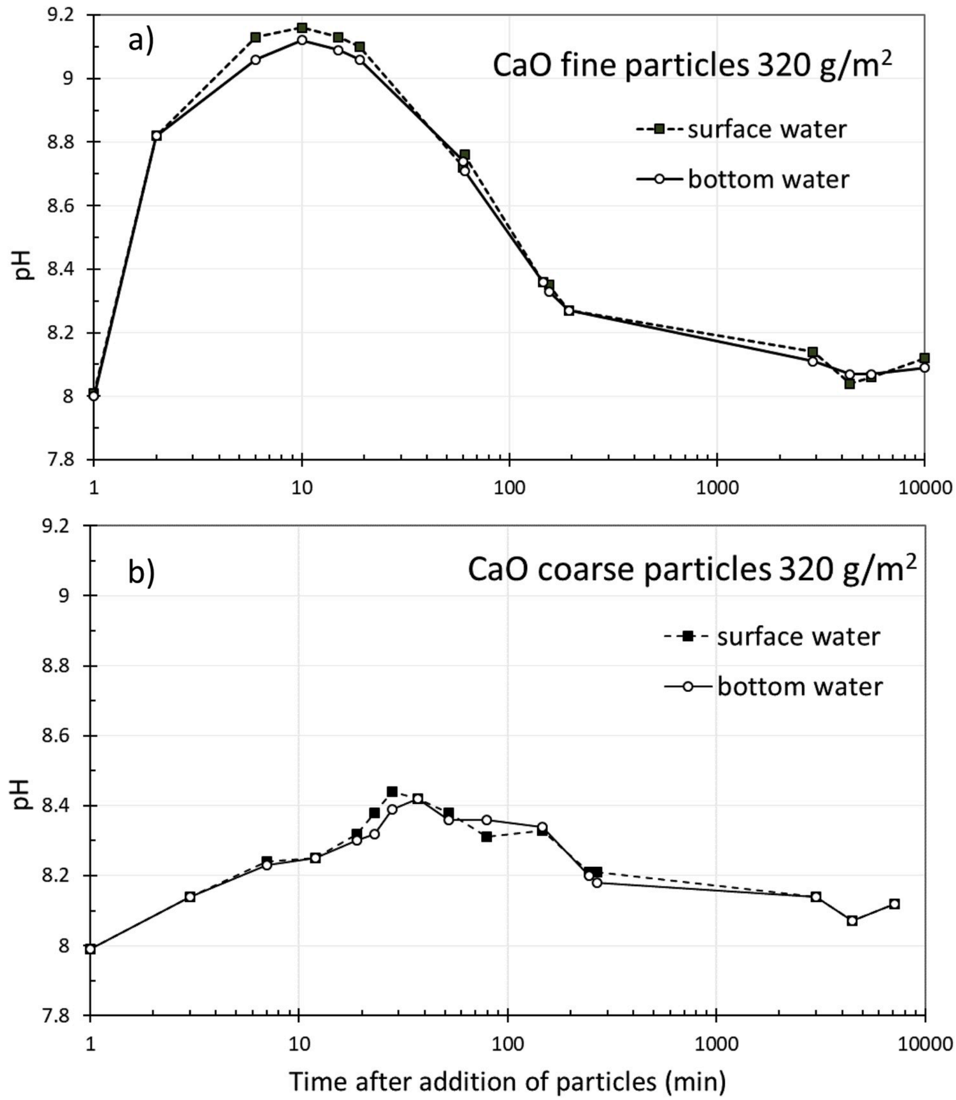

Fig. 2. Changes in seawater pH over time for the highest CaO concentration, following the addition of either a) fine or b) coarse CaO particles.

concentration of 3.14 g/m^2, followed by the green sea urchin and common starfish (LC_{50} 20.1 and 22.2 g/m^2), the slender ragworm (LC_{50} 29.6 g/m^2), the netted dog whelk (LC_{50} 41.9 g/m^2), and the lumpsucker (LC_{50} 226 g/m^2).

Due to the shared exposure tanks, predation was found to have contributed to some of the observed effects. For example, predation of sea urchins by starfish was observed in one of the tanks (10 g/m^2 coarse) and was not attributed to particle exposure. Furthermore, lumpsucker fish escaped their enclosures in the control tank, at 10 g/m^2 for fine CaO particles, and at 100 g/m^2 coarse CaO particles. These were not attributed towards CaO particle toxicity.

3.3. Risk assessment

Species sensitivity distribution (SSD) curves were generated for both fine and coarse particles from the no observable effect concentration (NOEC) values obtained from the 10-day ecotoxicity tests for the nine marine species (Fig. 5). The nine species selected included two echinoderms, two crustaceans (copepod and decapod), two molluscs (gastropod and bivalve), a fish, a polychaete and macro algal germlings. These were considered to represent species typically observed in Norwegian coastal waters and therefore suitable for environmental risk assessment.

A hazard concentration for five percent of the species (HC5) was calculated from the SSD curves and were 35.5 g/m^2 and 1.5 g/m^2 for coarse and fine CaO particles respectively. Based on the European Union Technical guidance document, an assessment factor (AF) between 5 and 1, justified case by case, should be used to calculate the predicted no effect concentration (PNEC) (ECB, 2003). With a recommended AF of 5, the marine PNEC values for the coarse and fine CaO particles were 7.1 g/m^2 and 0.3 g/m^2 respectively (Table 4).

The assessment of contaminant risk in the environment is performed using the equation below.

$$\frac{\textit{Predicted Environmental Concentration (PEC)}}{\textit{Predicted No Effect Concentration (PNEC)}} = \textit{Risk Quotient}$$

If the PEC is greater than the PNEC then the risk quotient (RQ) will be greater than one, which would indicate potential harm and the need for further investigations. Conversely, a RQ less than one would suggest no or low risk of environmental harm.

4. Discussion

This study describes the effects of particle exposure on marine organisms and should be clearly differentiated from a dissolved chemical exposure. For example, CaO dissolved in seawater is not directly harmful

Table 1
Physicochemical properties of the seawater during the 10-day exposure to CaO particles for the flow-through exposure tests.

Particle size	Conc. (g/m^2)	Temp (°C)	pH	Salinity (ppt)	Dissolved oxygen (mg/L)
Flow-through exposure 1 (5 marine species)					
coarse	320	8.6–9.6	7.99–8.44	33.7	7.47–7.66
	100	8.7–8.9	8.02–8.11	33.7	7.56–7.59
	32	8.7–8.8	8.02–8.06	33.6	7.63–7.64
	10	8.9–8.9	8.02–8.02	33.7	7.58–7.64
	3.2	8.8–8.7	8.00–8.02	33.7	7.57–7.64
fine	320	8.6–10.1	8.00–9.16	33.7	7.61–7.63
	100	8.4–9.0	8.03–8.79	33.7	7.59–7.67
	32	8.4–9.0	8.01–8.45	33.7	7.53–7.67
	10	8.8–8.9	8.02–8.19	33.7	7.57–7.65
	3.2	8.7–8.9	8.01–8.07	33.7	7.49–7.60
CONTROL		8.6–10.0	8.00–8.04	33.7	7.59–7.64
Flow-through exposure 2 (3 marine species)					
coarse	320	9.9–14.3	8.10–8.32	33.8	7.21
	100	10.6–14.3	8.10–8.18	33.8	7.26
	32	10.4–14.2	8.05–8.17	33.8	7.40
	10	10.6–14.3	8.04–8.16	33.8	7.28
	3.2	10.4–14.2	8.03–8.18	33.8	7.29
fine	320	10.3–13.9	8.13–9.10	33.8	7.29
	100	10.6–14.4	8.13–8.22	33.8	7.25
	32	10.4–14.1	8.10–8.15	33.8	7.24
	10	10.5–14.3	8.00–8.16	33.8	7.21
	3.2	9.5–14.2	7.99–8.15	33.8	7.27
CONTROL		9.5–14.4	7.99–8.16	33.8	7.60

Table 2
Physicochemical properties of the seawater during the 1 h exposure - 10-day recovery to coarse and fine CaO particles for the semi-static benthic copepod (*T. battagliai*) test.

Static-renewal exposure (*T. battagliai*)								
Particle size	Conc. (g/m^2)	pH				DO (mg/L)		
		0.5h	1h	4d	8d	1h	4h	8d
coarse	100	8.7	9.0	8.3	8.3	7.0	7.2	7.0
	32	8.2	8.5	8.3	8.2	7.0	7.2	6.9
	10	8.0	8.1	8.3	8.2	7.0	7.2	7.0
	3.2	8.0	8.1	8.3	8.2	7.0	7.1	6.9
	1.0	8.0	8.0	8.3	8.2	7.0	7.2	6.9
	Ctrl	8.2		8.1	8.2	7.1	7.1	6.9
fine	100	9.5	9.5	8.3	8.3	7.0	7.2	7.0
	32	8.9	8.9	8.3	8.3	7.0	7.2	6.9
	10	8.7	8.8	8.3	8.3	7.0	7.2	7.0
	3.2	8.3	8.3	8.3	8.3	7.0	7.2	7.0
	1.0	7.9	8.0	8.3	8.3	7.0	7.2	6.9
	Ctrl	7.8		8.4	8.1	7.1	7.1	6.9
pH 9 ctrl		9.0		8.3	8.2	–	7.6	7.6
pH 10 ctrl		10.0		8.3	8.2	–	7.8	7.7

Salinity (min-max: 35–37), Temperature (min-max: 19.6–20.6 °C).

to marine organisms, as long as the pH isn't elevated above tolerance limits, and can be potentially beneficial to marine organisms against divalent metal toxicity (Das and Das, 2005). However, particle toxicity through the exothermal reaction of CaO particles when in contact with seawater is harmful to a range of marine organisms as shown in this study.

The results showed that of the nine species tested only three were unaffected by CaO particle exposure at the concentrations tested for both fine and coarse particles. These included the shore crab (*C. maenas*), the blue mussel (*M. edulis*) and germling growth in the bladder wrack (*F. vesiculosus*). As expected, both echinoderm species, the starfish (*A. ruben*) and the green sea urchin (*S. droebachiensis*), were sensitive to CaO particle exposure, with both the coarse and fine particles impacting survival. However, clear differences between particle size were observed with the fine particles exhibiting approximately 10 times greater efficacy than the coarse particles. The larger surface area to volume ratio of the smaller particles would likely lead to a greater reactivity, increasing the exothermic impact. This could be seen when measuring the change in pH over time, with fine CaO particles causing a rapid increase in pH reaching a maximum level of 9.2 after 10 min and reducing to near 8.2 after 2 h. In contrast, the coarse CaO particles had a weaker and slower influence on the pH of the seawater, reaching a maximum pH of only 8.4 after 30 min. The increased effects of the fine CaO particles on the echinoderms as well as the other marine species tested was therefore likely due to the greater exothermic reaction or burning on the external surface of the organisms. However, the large increase in seawater alkalinity provided an additional stress, which may have contributed partly to the observed effects.

The most sensitive response was observed in the epibenthic marine copepod (*T. battagliai*) when exposed to the fine CaO particles, producing a LOEC and LC_{50} concentration of 3.2 and 3.14 g/m^2 respectively. This was almost ten times the sensitivity of the two echinoderms with LOEC and LC_{50} values of 32 and 20.1, and 32 and 22.2 g/m^2 for *S. droebachiensis* and *A. ruben* respectively. The flow-through system was used in order to increase dilution and enhance the pH buffering capacity of the seawater. However, the flow-through design was not suitable for the epibenthic marine copepod and a static renewal system was employed. Since it was acknowledged that the elevation in pH could impact the copepods, the exposure was shortened to a 1 h period after which time the copepods were transferred to fresh seawater and survival observed over 10 days. In order to account for the effects of pH only on the copepods, two pH controls were used. Impacts on survival were observed in these pH controls, with 75% survival (25% mortality) at pH 9 and 60% survival (40% mortality) at pH 10. From the pH measurements of the different treatments, it appears that pH may have contributed to copepod mortality at the higher exposure concentrations.

For the coarse particles, only the highest concentration (100 g/m^2) raised pH to 9 within the 1 h of exposure. Reduction in copepod survival to 70% was shown following exposure to 100 g/m^2 CaO coarse particles. This was only marginally below the effects of pH 9 alone on copepod survival and suggests that pH and particles are having a combined effect at 100 g/m^2 coarse CaO concentration. However, for the fine particles, significant reduction in copepod survival (50%) occurred at 3.2 g/m^2, when pH was 8.3. Therefore, at this concentration, the particles were solely responsible for the impact on copepod survival. At 10 g/m^2 CaO fine particles, pH increased to 8.8, which may have partially contributed to the effects of the particles and the observed 20% survival of the copepod. At 32 g/m^2 (pH 8.9) and particularly 100 g/m^2 (pH 9.5) of fine CaO particles, pH stress would have contributed more to the copepod mortalities, although the impact of the particle burning effect on the surface of the organism would still be by far the biggest reason for the observed effects.

A further factor that may have influenced the high sensitivity of the copepod to the CaO particles was the temperature at which the test was performed. The *Tisbe* acute toxicity test is a standard regulatory test (ISO 14669), which is typically performed at 20 ± 2 °C, this was higher than the temperature of the flow-through exposures (10–15 °C). Previous research has found that the epidermal burns and lesions caused by contact with the reactive CaO particles, enables bacteria to enter the body resulting in infection and death after a few days (Bernstein and Welsford, 1982). Higher temperatures are thought to proliferate bacterial growth and reduce survival in infected organisms.

The only previous studies of CaO particle exposures in controlled laboratory experiments were performed over 30 years ago (Shumway et al., 1988). In these experiments only the starfish (*A. vulgaris*) was significantly impacted by the addition of CaO particles with 100% mortality, whilst polychaetes, gastropods and mussels were unharmed. However, major differences between the design of the exposure system between this study and the present study would likely explain many of the differences observed. Although flow-through seawater systems were used, Shumway et al. (1998) provided sand and gravel within the exposure tanks, enabling the organisms to acclimatise for one week. Organisms such as the blood worm (*G. dibranchiata*), the sand worm

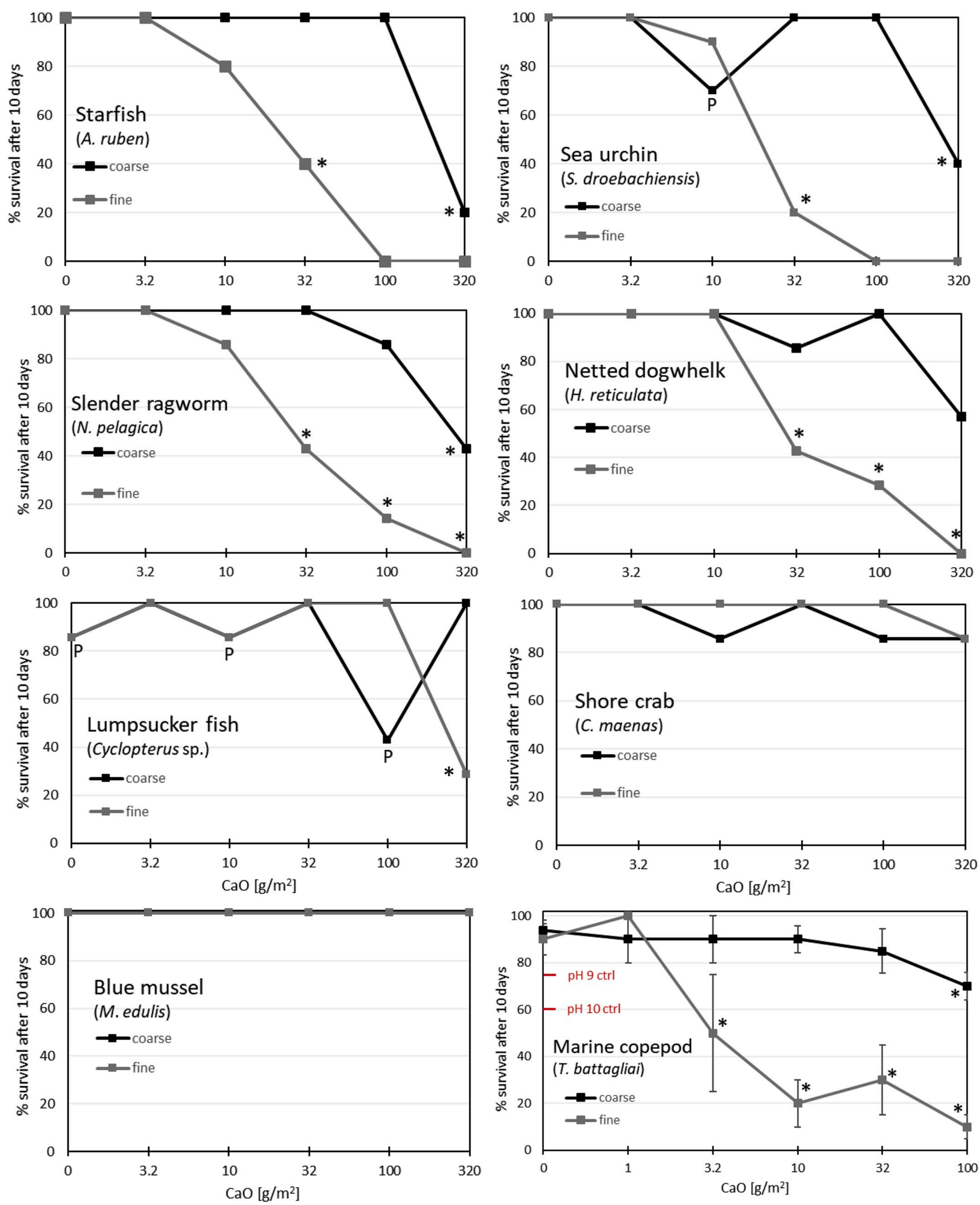

Fig. 3. The effects of fine and coarse CaO particles on the survival of the selected marine organisms. Survival 10-days after exposure to different concentrations of CaO particles. P, predation. * Significant difference from control value ($p < 0.05$). pH 9 and 10 controls *for T. battagliai* indicate 75% and 60% survival respectively.

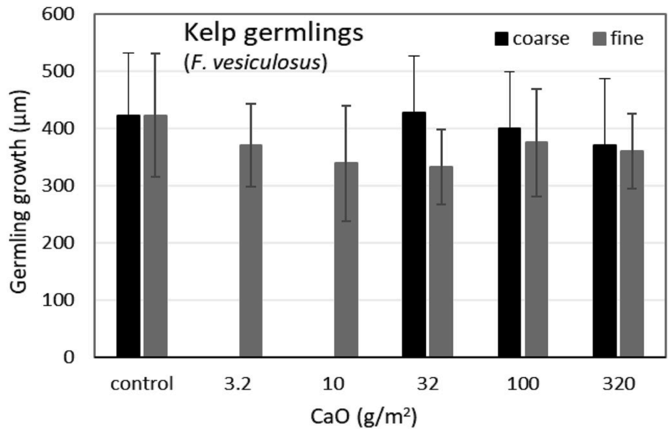

Fig. 4. The effects of fine and coarse CaO particle exposure on germling growth of *F. vesiculosus* growth 10-days after exposure to different concentrations of CaO particles (mean ± SD). Since no effects were observed at 32 g/m^2 coarse CaO, lower concentrations (19 and 3.2 g/m^2 coarse CaO) were not measured.

(*N. virens*), and the periwinkle (*L. littorea*) were allowed to burrow within the sediment and avoid or reduce direct contact with the CaO particles. In our study, the netted dog whelk and the slender ragworm are comparable species to those used by Shumway et al. (1998). However, the design of our experiments ensured direct contact with the CaO particles resulting in adverse impact on organism survival.

Particle size may also explain some of the observed differences between the studies. The fine CaO particles used in our investigation are considered too small for surface application to control echinoderm populations, since they would sink too slowly to the target area of the sea floor and provide sufficient time for the exothermic reaction to dissipate before contact with the target organisms. Although smaller particles can be used to make a CaO slurry for application on the seafloor, it was thought that Shumway et al. (1988) used a larger particle, similar to the coarse particle in our study. The authors did not provide information on the size of the particles used, although since they were supplied by the Great Eastern Mussel, Tenant's harbour, Maine and used to control starfish predation of mussel hatcheries, they were considered to be a relatively coarse particle.

The lack of hiding places to avoid particle contact, was an intentional design of the present study in order to ensure contact of the particles with the test organisms. However, it is noted that in their natural habitats mobile species would have the potential to avoid CaO particle exposure by hiding under rocks or in crevices or buried within the sediment. The toxicity data observed in this study should therefore be treated as a worst-case scenario and a conservative estimate for risk assessment.

Mussels (*M. edulis*) and the shore crab (*C. maenas*) were resistant to CaO particle exposure, which was in agreement with previous laboratory investigations for mussels and decapods, *H. americanus* (Shumway et al., 1988). Observations during the exposure noted the closure of the mussel shell and absence of filtering during particle scattering, whilst the high mobility of crabs enabled them to avoid areas of high particle load and remove CaO particles that had landed on their carapace and appendages. Well used tracks around the outside of the circular tanks were created by the crabs producing particle clear zones so that the crabs could avoid contact with the particles.

To provide a simple risk assessment of the coarse and fine CaO particles in marine waters, species sensitivity distributions (SSDs) were created based on the NOEC values from the nine marine toxicity exposures. Due to the nature of particle exposures and the non-standardised methods that are used, it was not possible to use existing data for input into the SSDs. Published data to date on CaO particle toxicity, was only available from Shumway et al. (1988) where significant differences in the exposure systems, as previously discussed, make the data

Table 3
Summary of the toxicity data for coarse and fine CaO particles calculated for all nine marine species. Statistical method, ToxCalc 5.0 (Tidepool scientific software).

Particle size	Test organism	Toxicity (g/m^2)				Statistical method (ToxCalc 5.0)
		NOEC	LOEC	LC10	LC50	
Coarse	Common starfish (*A. rubens*)	100	320	–	207	Spearman-Karber
	Green Sea Urchin (*S. droebachiensis*)	100	320	–	264	Spearman-Karber
	Shore crab (*C. maenas*)	320	>320	No significant effect observed		
	Blue mussel (*M. edulis*)	320	>320	No significant effect observed		
	Lump sucker fish (*Cyclopterus* sp.)	320	>320	No significant effect observed		
	Netted dog whelk (*H. reticulata*)	320	>320	No significant effect observed		
	Slender Ragworm (*N. pelagica*)	100	320	89.9	268	Spearman-Karber
	Fucus germlings (*F. vesiculosus*)	320	>320	No significant effect observed		
	Epibenthic copepod (*Tisbe battagliai*)	32	100	44.03	264	Logistic curve (GraphPad Prism)
Fine	Common starfish (*A. ruben*)	10	32	8.3	22.2	Max. likelihood probit
	Green Sea Urchin (*S. droebachiensis*)	10	32	10.1	20.1	Max. likelihood probit
	Shore crab (*C. maenas*)	320	>320	No significant effect observed		
	Blue mussel (*M. edulis*)	320	>320	No significant effect observed		
	Lump sucker fish (*Cyclopterus* sp.)	100	320	–	226	Spearman-Karber
	Netted dog whelk (*H. reticulata*)	10	32	13.1	41.9	Max. likelihood probit
	Slender Ragworm (*N. pelagica*)	10	32	8.56	29.6	Max. likelihood probit
	Fucus germlings (*F. vesiculosus*)	320	>320	No significant effect observed		
	Epibenthic copepod (*Tisbe battagliai*)	1	3.2	0.9	3.14	Logistic curve (GraphPad Prism)

incomparable. Therefore, the SSDs for coarse and fine CaO particles were made only from the nine marine organisms in the present study. Following the European Union technical guidance document (ECB, 2003) on risk assessment, the PNEC for coarse and fine CaO particles were calculated. Unsurprisingly the PNEC of the fine CaO particles were approximately 20 times lower than the PNEC of the coarse CaO particles. The lower PNEC of the fine particles is mostly driven by the sensitivity of the marine copepod. Although pH effects were described in this study, the NOEC and LOEC values used to develop the SSD were not influenced by pH and only reflect the exothermal particle effect on the copepod.

The rapid growth of sea urchin populations in coastal waters can result in the collapse of kelp forest ecosystems, with their impacts lasting for decades (Filbee-Dexter and Wernberg, 2018; Rinde et al., 2014; Norderhaug and Christie, 2009). Quicklime has historically been used to reverse such conditions, but efficacy of CaO application along latitudes

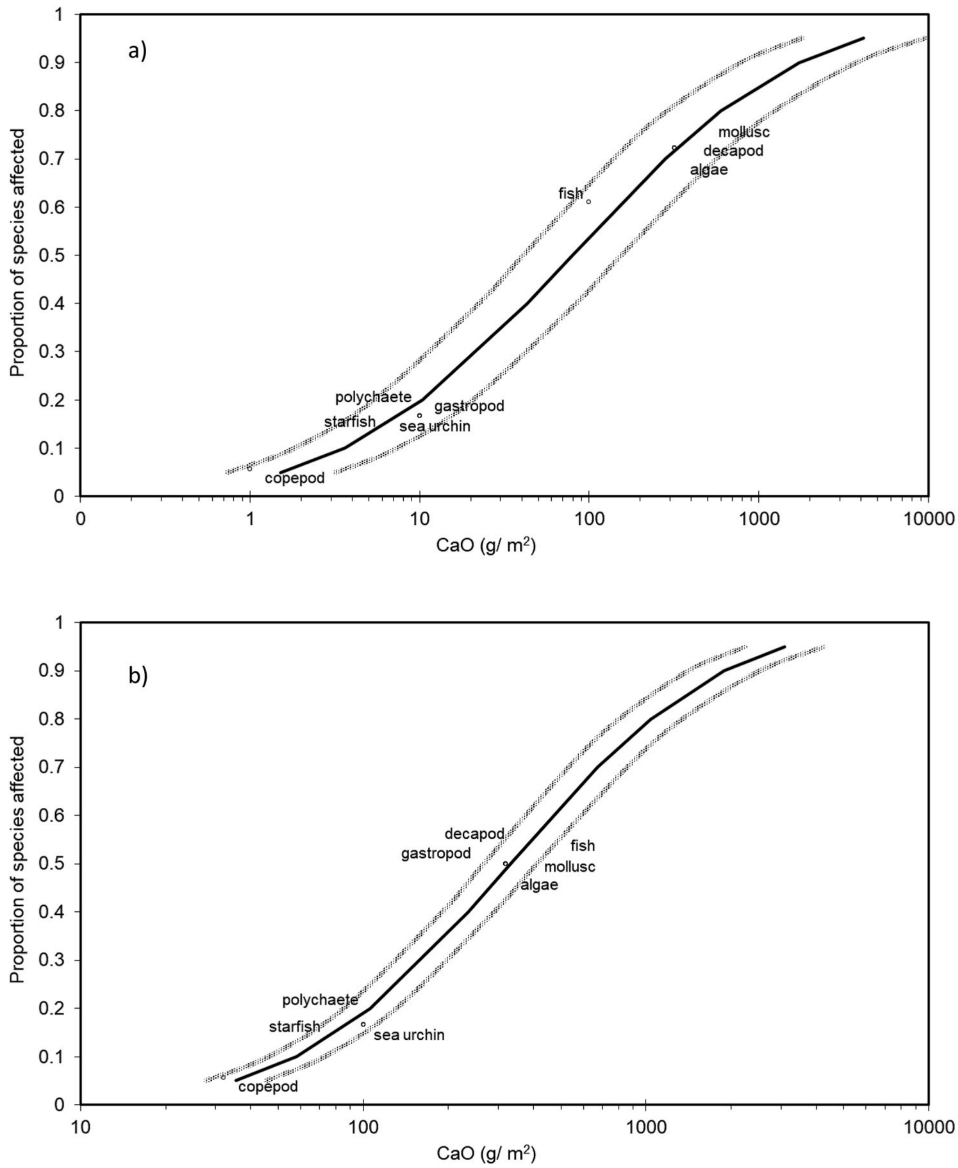

Fig. 5. Species sensitivity distributions (SSDs) for a) fine and b) coarse CaO particles. Lines calculated from the no observable effect concentration (NOEC) values from the 10-day toxicity tests for all nine marine species from the different trophic groups.

Table 4
Calculation of the marine Predicted No Effect Concentration (PNEC) for coarse and fine CaO particles from the SSD curves generated with NOEC values of selected marine species. Hazard concentration (HC), Assessment factor (AF).

CaO particle	HC5	AF	PNEC (g/m^2)
Coarse	35.5	5	7.1
Fine	1.5	5	0.3

and temperature gradients has been variable for reasons not fully understood. In Norway, laboratory and field investigations have taken place to optimise CaO application for the control of sea urchin populations. In the Porsanger fjord, attempts to restore the kelp forests by removing grazing sea urchins through CaO application have been performed (Strand et al., 2019, in press). Initial experiments have shown that the treatment methods are effective, the sea urchin population is immediately reduced, and the vegetation grows at the treated sites. Similar investigations focussing on the restoration of kelp forest ecosystems have taken place in other Norwegian fjords, including Slettnes fjord, Store Fagervika and Hammerfest in Finmark (IMR report, 2017). It appears that ecosystem enhancement can be achieved through the removal of grazing pressures with CaO application. The environmental benefits of CaO application will need to be evaluated alongside the impacts of the particles on non-target species such as those reported in the present study.

5. Conclusions

CaO particles were found to have significant effects on marine species from different trophic groups and the effects were not limited to echinoderm species as previously indicated. The fine CaO particles were overall more toxic to the selected marine species than the coarse particles. The highest concentration (320 g/m^2) of coarse CaO particles tested had no effect on the survival of the shore crab, the blue mussel, the lumpsucker fish or germlings of the bladder wrack. The coarse CaO particles were equally toxic to the two echinoderms (starfish and sea urchin), slender ragworm and the epibenthic copepod with LC_{50} values between 207 and 268 g/m^2.

The highest concentration of fine CaO particles had no effect on blue mussel survival or *Fucus* germling growth. For the fine particles, the lowest LC_{50} (most sensitive) was the epibenthic copepod at 3.14 g/m^2, followed by the sea urchin (20.1 g/m^2), starfish (22.2 g/m^2), ragworm (29.6 g/m^2), and netted dog whelk (41.9 g/m^2). Lumpsucker fish exhibited significant mortalities only at the highest fine CaO concentration tested (320 g/m^2) and recorded an LC_{50} of 226 g/m^2.

The risk assessment was based on SSDs developed from NOEC values from the nine marine species. With an assessment factor of 5, the PNEC was calculated as 7.1 and 0.3 g/m^2 for coarse and fine CaO particles respectively.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The work was funded by Industry (Franzefoss Mineral AS, Norway) and Martin Mengede an employee of Franzefoss Mineral AS is a co-author on the manuscript. However, the work was performed independently by researchers at the Norwegian Institute for Water Research and the results have not been manipulated or influenced by industrial involvement.

CRediT authorship contribution statement

Steven J. Brooks: Conceptualization, Methodology, Software, Validation, Formal analysis, Writing - original draft. **Anastasia Georgantzopoulou:** Methodology, Software, Validation, Formal analysis, Writing - review & editing. **Joachim Tørum Johansen:** Methodology, Investigation. **Martin Mengede:** Methodology, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2020.104917.

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