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 (Asteroidea ruben and Strongylocentrotus droebachiensis); two crustaceans (Carcinus maenas and Tisbe battagliai); two molluscs (Mytilus edulis and Hiatia 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. ruben, S. droebachiensis, N. pelagica, T. battagliai) with similar LC50 values between 207 and 268 g/m². For the fine CaO particles, the lowest LC50 was for the epibenthic copepod (T. battagliai) at 3.14 g/m², followed by the sea urchin (20.1 g/m²), starfish (22.2 g/m²), ragworm (29.6 g/m²), and netted dog whelk (41.9 g/m²). Lump sucker fish exhibited significant mortalities only at the highest fine CaO concentration tested (320 g/m²) and recorded an LC50 of 226 g/m². 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 (HCS) calculated from the SSDs, based on NOEC values, for the coarse and fine particles were 35.5 and 1.5 g/m² respectively. Using a recommended assessment factor of 5, the Predicted No Effect Concentration (PNEC) was calculated as 7.1 and 0.3 g/m² 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, SO4 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/m². 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

![Image](image-url)

**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 onto 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 rather than observed. The test was performed according to the ISO guideline (ISO 14669:1999) with slight modifications in that the survival was monitored rather than observed. The pH, salinity, temperature and dissolved oxygen 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/m²) 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/m²), whilst for the fine particles, pH increased to 9.5 at 100 g/m², and 8.8 and 8.9 at 10 and 32 g/m² 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 lump sucker fish, the netted dog whelk and Fucus germlings, with lowest observable effects concentration (LOEC) > 320 g/m². 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 LC50 concentrations (concentration causing 50% mortality in the population) were similar between 207 and 264 g/m².

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 LC50...
concentration of 3.14 g/m², followed by the green sea urchin and common starfish (LC₅₀ 20.1 and 22.2 g/m²), the slender ragworm (LC₅₀ 29.6 g/m²), the netted dog whelk (LC₅₀ 41.9 g/m²), and the lumpsucker (LC₅₀ 226 g/m²).

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/m² coarse) and was not attributed to particle exposure. Furthermore, lumpsucker fish escaped their enclosures in the control tank, at 10 g/m² for fine CaO particles, and at 100 g/m² 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/m² and 1.5 g/m² 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/m² and 0.3 g/m² respectively (Table 4).

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

\[
\text{Predicted Environmental Concentration (PEC)} = \frac{\text{Predicted No Effect Concentration (PNEC)}}{\text{Risk Quotient}}
\]

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.
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 exothermic 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. 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. ruben) 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

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### Table 1

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

| Particle size | Conc. (g/m²) | 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 | 8.02-8.46 | 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 | 8.8-8.8 | 8.02-8.19 | 33.7 | 7.57-7.67 |
| 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 | 8.03-8.16 | 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/m²) | pH | DO (mg/L) | 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 | 7.2 | 7.0 |
| **fine** | | | | | |
| 100 | 8.7 | 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 |
| **CONTROL** | | | | | |
| pH 9 | 9.0 | 8.3 | 8.2 | 7.0 | 7.6 |
| pH 10 | 10.0 | 8.3 | 8.2 | 7.8 | 7.7 |

Salinity (min-max: 35–37), Temperature (min-max: 19.6–20.6 ºC).
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.
similar to the coarse particle in our study. The authors did not provide sure. Due to the nature of particle exposures and the non-standardised created based on the NOEC values from the nine marine toxicity expo could avoid contact with the particles. The effects of fine and coarse CaO particle exposure on germling growth of F. vesiculosus 10-days after exposure to different concentrations of CaO particles (mean ± SD). Since no effects were observed at 32 g/m² coarse CaO, lower concentrations (19 and 3.2 g/m² coarse CaO) were not measured. 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 incomparable. Therefore, the SSDs for coarse and fine CaO particles were made only from the nine marine species. Statistical method, ToxCalc 5.0 (Tidepool scientific software).

| Particle size | Test organism | NOEC (g/m²) | LOEC (g/m²) | LC10 (mg/L) | LC50 (mg/L) | Statistical method |
|---------------|---------------|-------------|-------------|-------------|-------------|-------------------|
| 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 (Tise battagliai) | 32 | 100 | 44.03 | 264 | Logistic curve (GraphPad Prism) |
| Fine          | Common starfish (A. rubens) | 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 (Tise battagliai) | 1 | 3.2 | 0.9 | 3.14 | Logistic curve (GraphPad Prism) |
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 focusing 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/m²) of coarse CaO particles tested had no effect on the survival of the shore crab, the blue mussel, the lump sucker 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 LC₅₀ values between 207 and 268 g/m².

The highest concentration of fine CaO particles had no effect on blue mussel survival or Fucus germling growth. For the fine particles, the

| Table 4 |

| CaO particle | HC5 | AF | PNEC (g/m²) |
|--------------|-----|----|------------|
| Coarse       | 35.5| 5  | 7.1        |
| Fine         | 1.5 | 5  | 0.3        |

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.
lowest LC₅₀ (most sensitive) was the epibenthic copepod at 3.14 g/m², followed by the sea urchin (20.1 g/m²), starfish (22.2 g/m²), ragworm (29.6 g/m²), and netted dog whelk (41.9 g/m²). Lumpsucker fish exhibited significant mortalities only at the highest fine CaO concentration tested (320 g/m²) and recorded an LC₅₀ of 226 g/m².

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/m² 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 Geor-gantzopoulou: Methodology, Software, Validation, Formal analysis, Writing - review & editing. Joachim Tarum Johansen: Methodology, Investigation. Martin Mengede: Methodology, Writing - review & editing.

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Appendix A. Supplementary data

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References

Abdel-Tawwab, M., Mousa, M.A.A., Ahmed, M.H., Sakr, S.F.M., 2007. The use of calcium pre-exposure as a protective agent against environmental copper toxicity for juvenile Nile tilapia, Oreochromis niloticus (L.). Aquaculture 246, 236–246.
Bernstein, B.B., Wehford, R.W., 1982. An assessment of the feasibility of using high-calcium quicklime as an experimental tool for research into kelp bed/sea urchin ecosystems in Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 968.
Brooks, S.J., Bolam, T., Tolfurst, L.E., Basset, J., La Roche, J., Wallock, M., Barry, J., Thomas, K.V., 2008. Dissolved organic carbon reduces the toxicity of copper to the germings of the macroalga, Fucus vesiculosus. Ecotoxicol. Environ. Saf. 70, 88–98.
Das, B.K., Das, N., 2005. Impacts of quicklime (CaO) on the toxicity of copper (CuSO₄, 5H₂O) to fish and fish food organisms. Chemosphere 61, 186–191.
European Chemicals Bureau (ECB), 2003. Technical Guidance Document on Risk Assessment. European Commission joint research centre EUR20418-EN/2, p. 337pp.
Filbee-Dexter, K., Wernberg, T., 2018. Rise of turf: a New Battlefront for globally declining kelp forests. Bioscience 68, 64–76.
Garmo, Ø., With Fagerli, C., Segtman, O.A., 2017. Overvåking av pH under dosering av brent kalk i Slettnesfjorden. In: NIVA notat. Journal no. 1587/17. (in Norwegian). IMR report, 2017. TARE - Restaurering Av Tareskog Som Økosystem I Hammerfest, Finnmark. Prosjekt Rapport 2015, p. 269272. RFFNord, prosj. nr.
International Organisation for Standardization (ISO), 1999. Water Quality- Part 5: Biological Methods- Section 5.24: Determination of Acute Lethal Toxicity to Marine Copepods (Copepoda, Crustacea) (ISO 14669).
Kaviraj, A., Dutta, T.K., 2000. Use of quicklime (CaO) as a means to reduce cadmium toxicity in common carp, Cyprinus carpio. J. Appl. Aquacult. 10, 87–95.
Norderhaug, K.M., Christie, H., 2009. Sea urchin grazing and kelp re-vegetation in the NE Atlantic. Mar. Biol. Res. 5, 515–528.
Rinde, E., Christie, H., Fagerli, C.W., Bekkby, T., Gundersen, H., Norderhaug, K.M., Hermann, D., 2014. The influence of physical factors on kelp and sea urchin distribution in previously and still grazed areas in the NE Atlantic. PloS One. https://doi.org/10.1371/journal.pone.0100222.
Shumway, S.E., Card, D., Getchell, R., Newell, C., 1988. Effects of calcium oxide (quicklime) on non-target organisms in mussel beds. Bull. Environ. Contam. Toxicol. 40, 503–509.
Strand, H.K., Christie, H., With Fagerli, C., Mengede, M., Moy, F., 2019. Optimizing the Use of Quicklime (CaO) for Sea Urchin Decimation – A Lab and Field Study (in press).
Wood, F.B., 1908. Enemies and Perils of the Oyster. Report State of Connecticut Shellfish Commissioners 1907-1908. Dec No 30, Appendix to Rept, pp. 94–98. In Shumway SE, Card D, Getchell R, Newell C. 1988. Effects of calcium oxide (quicklime) on non-target organisms in mussel beds. Bull. Environ. Contam. Toxicol. 40:503-509.