

Optimizing the use of quicklime (CaO) for sea urchin management — A lab and field study



Hans Kristian Strand^{a,*}, Hartvig Christie^b, Camilla With Fagerli^b, Martin Mengede^c, Frithjof Moy^a

^a Institute of Marine Research, P.O box 1870 Nordnes, NO-5817 Bergen, Norway

^b NIVA, Gaustadalleen 21, NO-0349 Oslo, Norway

^c Franzefoss Minerals AS, Olav Ingstadsvei 5, NO-1309 Rud, Norway

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ABSTRACT

Mass blooms of sea urchins sometimes cause kelp forest collapses that can last for decades. Quicklime has historically been used to reverse those conditions, but the efficacy of liming has varied along latitudinal and temperature gradients for reasons that are not fully understood.

To evaluate the feasibility and ecological impacts of liming in a high latitude area in Northern Norway (70°N), we conducted a field pilot study in 2008–2011, a follow-up lab study in 2017, and a further field study in 2018–2019, with the latter evaluating and implementing the previous results in a site high in refuges. It was found that liming can reduce sea urchin densities sufficiently for macroalgal revegetation to occur, and that the mobile fauna species richness and abundance increased in the re-vegetated in comparison to the barren control fields. Also, the remaining sea urchins in the treated fields increased their roe content to commercial levels after 2 years.

The lab experiments in 2017 indicated that the liming method is season/temperature-independent, as mortality remained at the same level irrespective of whether treatment started in the spring, when the sea temperatures were 2 °C, or in autumn when the temperatures were closer to 10 °C. The most important factor in treatment efficacy in the lab was particle size. With similar doses, the particles in the smallest size range (0–0.5 mm) caused 100% mortality, while the 0.5–2 mm and 2–4 mm fractions caused only 13% and 2% mortality respectively.

In 2018–2019 we tested the fine CaO fraction (0.1–0.6 mm) and the medium fraction (0.5–2 mm) in a field experiment in areas characterized by high levels of refuges. Within 11 days, the sea urchin densities in the three fields treated with the fine lime were reduced to levels that theoretically should allow revegetation, but only in one of those fields was that potential partly realized after 1 year. The lack of effect in the two other fields was probably due to urchins protected by the substrate during treatment reappearing in sufficient numbers to prevent macroalgal regrowth, demonstrating that CaO treatment can be less effective on substrates where part of the sea urchin population hides among stones.

Of the three variables held up as potential explanations for the different effects of CaO treatment in previous studies, we conclude based on our experiments that the presence of refuges and particle size were probably more important than temperature. Further improvements for larger scale treatments are discussed.

1. Introduction

Grazing fronts of green sea urchins (*Strongylocentrotus droebachiensis*) have caused large-scale mass destruction of kelp forests all along the coast of Central and Northern Norway (Sivertsen, 1997), resulting in denuded sea urchin barrens that have persisted for more than 45 years (Norderhaug and Christie, 2009). These kinds of destructive grazing events of varying durations have been described in many parts

of the world (Elnor and Vadas, 1990; Estes et al., 2004; Steneck et al., 2004; Norderhaug and Christie, 2009). Macroalgal beds and kelp forests are highly productive (Pedersen et al., 2012) and provide shelter, habitat and feeding areas for fish and a high diversity of invertebrates (Norderhaug et al., 2003; Norderhaug et al., 2005; Christie et al., 2009). Hence, methods to control sea urchin populations to reestablish rich and diverse kelp ecosystems have been requested, among others by the Norwegian authorities (Sakshaug et al., 2002).

* Corresponding author.

E-mail address: hansks@hi.no (H.K. Strand).

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Fig. 1. Substrate types treated with CaO in the 2008–2011 pilot study (a) and 2018–2019 field experiment (b and c).

High mortality rates within sea urchin populations have resulted in regrowth of kelp forest in temperate areas (Elner and Vadas, 1990; Estes et al., 2004; Steneck et al., 2004). In Norway, kelp growth has also responded to reduced sea urchin densities caused both naturally (Christie et al., 1995; Norderhaug and Christie, 2009) and by artificially removing sea urchins (Leinaas and Christie, 1996). The latter study involved the time-consuming job of divers using a hammer to kill sea urchins, and more efficient methods must be developed if sea urchins are to be removed from larger areas.

Quicklime has mainly been used to remove starfish from mussel farms, and it is known to be harmful to echinoderms but much less harmful to other organism groups (Shumway et al., 1988). As sea urchin grazing is mainly a major problem in cold water areas in Northern Norway (65–71° N) with water temperatures below 10 °C for large parts of the year, the potential for successful liming at these high latitudes has been questioned. The reservation with respect to temperature stems from Bernstein and Welsford (1982), who reported that CaO was less effective in Canadian than in Californian waters. In Nova Scotia, approximately 1000 g CaO m⁻² sea floor was needed to achieve a sea urchin mortality rate greater than 70%, while only a quarter to half this dose was needed to achieve 95% mortality rates in Californian waters.

In addition to the lower temperatures, the fact that the Nova Scotian location had a more complex bottom structure higher in refuges on the sea floor than the Californian ones and the large maximum particle size in the Canadian experiments compared to the grades used in California were highlighted as potential causes for the discrepancy in efficacy (Bernstein and Welsford, 1982).

Rich mobile fauna components have been described in the Norwegian kelp forests (Christie et al., 2003), but the fauna dispersal have only been studied meters out from the kelp forests (Jørgensen and Christie, 2003; Waage-Nielsen et al., 2003). There is a lack of studies on fauna on urchin barrens and on mobile fauna re-establishment in kelp forests at previous barrens with long distances to potential sources.

The aim of this study was threefold. The first aim was to monitor the initial response of marine life to application of CaO on urchin-dominated barren grounds (pilot study 2008–2011). The second aim was to evaluate the season-dependence and the potential to optimize CaO treatment criteria in the lab (lab study 2017). Finally, we aimed to test the efficacy of promising lab results and a moderate dose (300 gm⁻²) on urchin-dominated habitats high in refuges (field study 2018–2019).

The results presented and discussed span several years, and field as well as lab studies. For better readability we merged the specifics of the material section with the corresponding result section for each experiment but kept the introduction and discussion sections general.

2. Methods and results

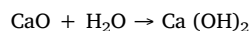
2.1. General

Calcium oxide is usually made by the thermal decomposition of materials, such as limestone, that contain calcium carbonate (CaCO₃;

mineral calcite) in a lime kiln. This is accomplished by heating the material to above 1050 °C in a process called calcination or lime burning, which liberates a molecule of carbon dioxide (CO₂), leaving CaO:



In an exothermic reaction, calcium oxide particles and water produce a strong base, which can cause epidermal lesions when it comes into contact with the surface of target organisms.



Protective clothes, glasses, and face masks were used during all the liming operations.

Habitats

The substrate in the fields treated in the 2008–2011 pilot study was dominated by the sloping underwater parts of skerries with few refuges (Fig. 1 a), while those treated in the 2018 field experiment were rich in refuges either due to several layers of cobbles or due to piled and tilted boulders (Fig. 1 b and c, see also video in supplementary data).

In order to monitor the benthic macro algal and faunal response to CaO treatment, field experiments lasted for at least one year. The sea urchin removal took place in autumn, before the spore release period of seaweeds (winter), then with recordings of macroalgal growth the following spring and summer. Lab experiments, on the other hand, lasted maximally for 75 days, as their main objective was to evaluate effects of potential CaO treatment regimens on sea urchin mortality (Fig. 2).

2.2. Pilot study 2008–2011

The study area is situated in Porsangerfjorden (70.5°N 25.3°E) in the far north of Norway. Hard substrates, suitable for kelp and sea urchins, are confined to a narrow belt of the sublittoral zone; in the upper meters there are smooth bedrock surfaces and lower down there are cobblestone bottoms, while a transition to sand and soft bottoms occurs at a depth of approximately 10 m.

A pilot liming experiment aimed at sea urchin removal was conducted at two small, isolated islets, both dominated by sea urchins and completely barren for years (own observations). The other parts of the surveyed area (Hamnholmen and Veidneset) were kept as control areas. Skarveskjær was treated in autumn 2008 and Storskjær in 2009 with one and two tons of CaO respectively. Nonetheless, since the area surrounding Storskjær is almost twice as large as the area around Skarveskjær, the concentrations of CaO added per unit area at the two treatment sites were approximately the same. The treatment in 2008 was conducted in October when sea temperatures were 5–6 °C and the one in 2009 in September when temperatures were 8–9 °C.

CaO (0–2 mm particle size) was applied at a depth of 0–5 m by sprinkling the particles at the water surface manually from a small boat. This application technique did not allow quantification of the exact amount of CaO added per unit area, but an identical application

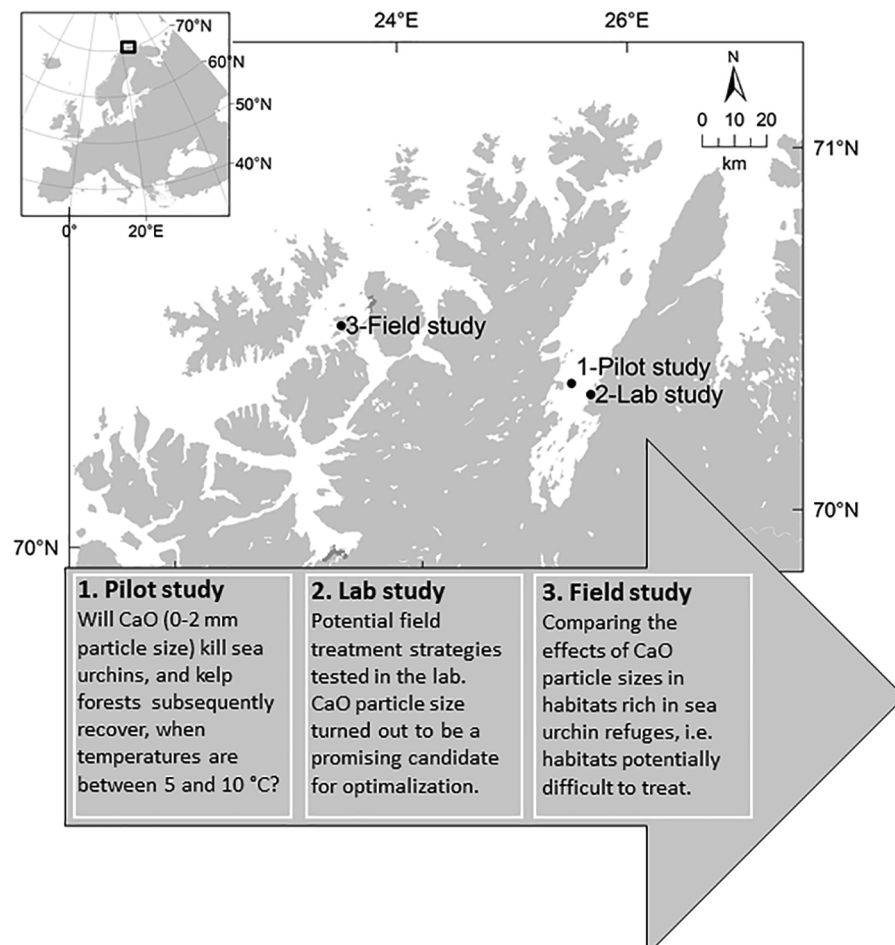


Fig. 2. Locations and methodology for the Pilot study (2008–2011, Locality 1), 2017 Lab study (Locality 2) and Field study (2018–2019, Locality 3).

technique was used throughout the study and ensured the use of a standardized method. It was observed that the particles settled and stuck to the urchins, but also that a proportion drifted out of the area.

Pre-lime (August 2005) and post-lime (August 2010 and 2011) surveys were performed to map the distribution of sea urchins and benthic macroalgal cover before and after liming. Sea urchin density and algal cover was estimated by scuba divers who placed frames ($n = \geq 10$, see Fig. 3) randomly on the sea floor, each frame measuring 0.25 m^2 . The number of sea urchins was counted within each frame and the overall, relative cover of benthic macroalgae was estimated. The procedure was repeated at depths of 2 and 5 m.

In 2010 a representative population sample of sea urchins was collected for gonad index (GI) analysis (gonad wet weight*100/total ww of the urchin). At least 20 sea urchins were collected from the survey sites.

Standardized bundles of rope (3 m of sisal rope in a bundle of $5 \times 10 \text{ cm}$) were used as traps for collecting mobile fauna (see Christie et al. (2009)).

Statistics

To examine the effects of CaO treatment on spatial patterns in sea urchin abundance and benthic macroalgal cover, a generalized linear mixed models (GLMM) were applied. GLMM allows for random factor model parameters. The explanatory factors “depth” (two levels: 2 and 5 m), “treatment” (two levels: CaO and no treatment) and “stage” (four levels: before and one, two and three years after CaO treatment) were included as fixed factors while “site” (two levels: Skarveskjær, Storskjær) and “year” was included as random factors. Interactions

between “depth” and “treatment” were included in the full model to account for treatment and depth specific density variations. The Akaike Information Criterion (AIC) was used for model selection. Interannual variation at the control sites was tested in separate models with “depth” and “year” as the fixed factors and “site” (Hamnholmen and Veidneset) as the random factor. Due to overdispersion of the sea urchin abundance data, the analysis was performed with package lme4 (Bates et al., 2012) in R version 3.5.3 (R-Core-Team, 2016), which allowed negative binomial distributed response variables. Arcsine transformation was applied for the percentage cover of macroalgae (cf. Crawley (2007)). A linear model was used to test for differences between sites and CaO treatment effects with respect to GI levels of sea urchins. Faunal community effects (abundance of taxa and individuals) of CaO treatment were analysed with a permutational multivariate analysis of variance in PRIMER 6 (Anderson et al., 2008). The analyses were undertaken on a Bray-Curtis matrix derived from square-root transformed differences.

Results

A high local and temporal variation in the urchin density was found, as well as a generally higher sea urchin abundance at a depth of 2 m than at 5 m (Fig. 3). The largest temporal change in sea urchin density was observed at a depth of 2 m at Skarveskjær between 2005 (before CaO treatment) and 2010, the second year after CaO treatment, where the mean ($\pm \text{SE}$) density of sea urchins was reduced from $102 (\pm 12.1) \text{ m}^{-2}$ to $4 (\pm 1.2) \text{ m}^{-2}$. In general, the initial sea urchin density and the relative decrease in sea urchin density were greater at depths of two meters than at five meters (Fig. 3). Although sea urchin density varied between surveys, the variation was not well explained by the stage of

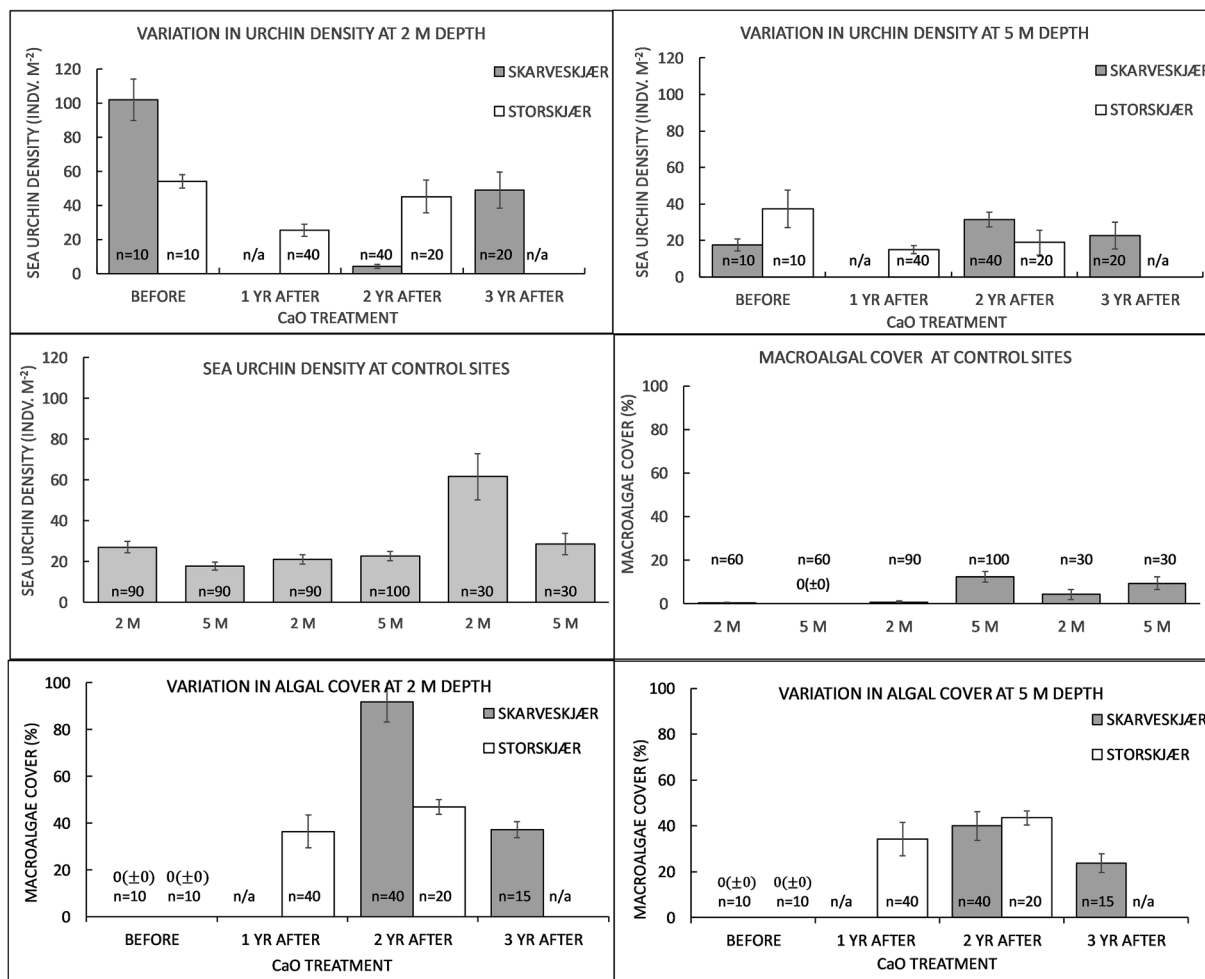


Fig. 3. Mean (\pm SE) density of sea urchins (upper panels) and benthic cover of macroalgae (lower panels) at depths of 2 m (left panels) and 5 m (right panels) at treatment sites along the pilot study timeline (before treatment (Skarveskjær/Storskjær), one/two years after (Storskjær), two/three years after (Skarveskjær)). Middle panels show mean density of sea urchins (left panel) and macroalgal cover (right panel) estimated from control sites in 2005, 2010 and 2011, at depths of 2 m and 5 m.

the study timeline. Nonetheless, the density of sea urchins was significantly lower after the “CaO treatment” and at five meters than at two meters (GLMM $p < 0.05$).

Analyzing interannual variability of the sea urchin density at control sites only showed significant effects for the interaction between “depth” and “year”, implying that interannual fluctuations in the sea urchin abundance are inconsistent at depths of 2 and 5 m.

Benthic macroalgae colonized the treatment sites (Storskjær and Skarveskjær) after liming, and algal cover was higher at treatment sites than at control sites in 2010 and 2011, subsequent to the CaO treatment (Fig. 3). A variety of the species of kelp and macroalgae were recorded among the recovered macroalgal community at the treatment sites. Of the kelps, *Alaria esculentus*, *Saccharina latissima* and *Sacchoriza dermatodea* were the most common, while *Laminaria digitata* and *Laminaria hyperborea* were rare. Other common macroalgae included *Chorda filum* and *Desmarestia* spp. Grazing from remaining and/or invading sea urchins led to a reduction in algal cover at the treatment sites in 2011.

Statistical analysis found that the macroalgal cover at the treatment sites (Skarveskjær and Storskjær) most strongly related to the “stage” of the study timeline and to “depth” (GLMM $p < 0.05$). Fig. 3 show a high macroalgal cover (>40% increase) the second year after CaO treatment at both treatment sites, and at 2 and 5 m depth. Depth were found significant explaining the macroalgal cover at the control sites. Although the macroalgal cover increased at the control sites during the survey period, the presence of macroalgae was low (<15% cover)

compared to macroalgal cover at the treatment sites (Storskjær and Skarveskjær) after CaO treatment.

Post-treatment observations revealed aggregation behaviour by the remaining or invading urchins attracted by the kelps, leading to high-density grazing fronts and resulting in neighboring counting frames varying between high kelp density and high urchin density. Observations also found that the control areas contained ephemeral filamentous algae during the summer season in contrast to the perennial kelps at the treatment sites.

At the treatment sites the remaining sea urchin aggregations grazed the algal cover in patches during 2011 and achieved a high Gonad Index (GI). There were significant differences in GI levels between the sites and between the sea urchins sampled at the limed sites and at the control sites ($r^2 = 0.65$; $p < 0.01$, Fig. 4).

The recovery of macroalgae led to an increase in associated fauna at the treatment sites, and there were significantly higher numbers of taxa and individuals identified from the fauna traps at the treatment sites than at the control sites (PERMANOVA, $p < 0.05$). The most common species identified from the traps was the small amphipod *Ischyroceros angipes*, which increased in density from 2010 to 2011, while other amphipod species and gastropods (particularly *Margarites* sp. and *Lacuna* sp.) also became more common. Juveniles of the common mussel (*Mytilus edulis*) were the only abundant species in the control traps on barren ground. Mean (\pm SE) number of taxa and individuals identified from the replicate fauna traps across treatment the sites was

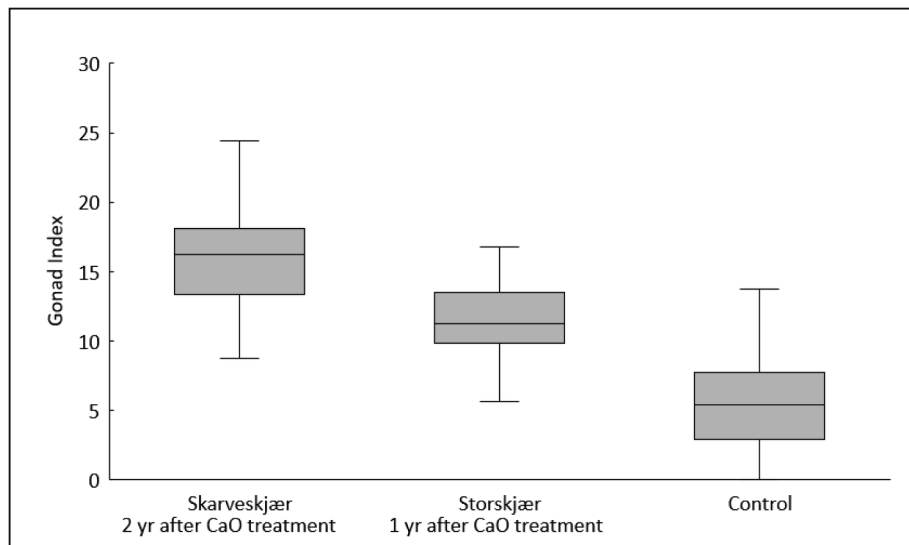


Fig. 4. Between-site comparison of reproduction potential (Gonad Index) estimated from *S. droebachiensis* sampled from frames at two CaO treatment sites and two control sites (2011). Boxes: lower and upper quartiles; lines: median value; whiskers: 5th and 95th percentiles.

10.4 (± 1.6) species and 676 (± 69) individuals, while the corresponding numbers identified at the control sites was estimated at 4.1 (± 1.0) taxa and 81 (± 27) individuals.

2.3. 2017 Lab study

Potential CaO field-treatment strategies were tested in the lab from May to October 2017. Experiment 1: spring simulation, where treatment efficacy was tested at low initial temperatures (2 °C) starting in early May. Experiment 2: autumn simulation, where initial temperatures were close to maximum (9 °C), starting in late August. Experiment 3: test of whether a CaO dose was more effective when applied in two half doses spaced at a 2-week interval rather than in one operation. Experiment 4: test of whether different particle fractions (0–0.5, 0.5–2, 2–4 mm) produced different mortality rates. Sea urchins were generally not fed during the experimental period, but in Experiments 1 and 2 two tanks were allocated to testing whether feeding ad libitum with sugar kelp (*Saccharina latissima*) would facilitate healing of wounds in sublethally exposed urchins. Experiments lasted for 75 (Experiment 1), 42 (Experiment 4) and 36 days (Experiments 2 and 3), based on temperatures during the experimental period and mortality leveling off.

In the lab experiments 1, 2, 3, we used particles retained by the 0.5 mm tray of a sieve that had passed through a 2 mm tray. This fraction between 0.5 and 2 mm was designated M (for medium). In experiment 4, the M fraction was tested against the 0–0.5 mm fraction (designated F for Fine) and the 2–4 mm fraction (designated C for coarse). The doses used were 0.03, 0.06, 0.12, 0.24 and 0.48 g/individual. Thus, results from sea urchins exposed to e.g. 0.12 g CaO from the fine fraction were designated 0.12F.

Tests were conducted in round conical tanks with a perforated plate covering the bottom such that feces and other materials could fall through and drain through the bottom. The effective holding volume for the sea urchins was 170 l (60 cm diameter \times 60 cm depth). Each tank was supplied with ambient sea water from a depth of 5 m at a rate of approximately 4 l/min.

The sea urchins were collected by scuba diving within a 50-m radius from the test facilities and water intake a few hours prior to the start of the experiments.

A total of 1200 sea urchins were used in the 4 lab experiments. Their average diameter (\pm SD) was 47 \pm 6 mm. The doses applied were 0.03, 0.06, 0.12, 0.24 and 0.48 g/individual. Sea urchins were exposed for 3–5 min in their individual application chambers. During the

application period, they typically climbed up the side walls of the chambers, and often also turned themselves almost upside down at the water surface. Some particles usually fell off during this behavior, particularly those in the M and C fractions. Sea urchins exposed to the F particles typically retracted their tube feet and did not climb up the reaction chamber wall.

After exposure, the urchins were transferred to the bottom of tanks holding a total of 15 specimens. The first sea urchins typically climbed up the tank wall within a few hours after transfer and usually close to 100% were situated on the wall within less than a week.

Test doses were weighed in single doses into 2.5 \times 2.5 cm plastic beakers and applied individually to 15 sea urchins (one tank) each placed at the bottom of 1 l beakers with approximately 7 cm of water above their highest point. The application procedure for 15 urchins took approximately 2 min. After application, the sea urchins were left for 3 min before they were carefully placed in their respective tanks. Control urchins were subjected to the same treatment, except for the application of CaO. Each dose was tested in triplicate, but in experiment 3 and 4 we used only duplicate control groups. The effect of feeding on wound healing was also only tested in duplicate (experiment 1 and 2).

Dead urchins were removed daily, and their diameter was measured to the nearest mm with a ruler. At the end of the experiments, each remaining urchin was also measured with a ruler, and it was also recorded whether the urchins had any damage to their exoskeletons. The criterion for counting as damage was that some white part of the exoskeleton should be visible. Typically, the lesions developed within one to two weeks.

Statistics

Differences in mortality rates between replicates and between groups in the lab experiments were tested for significance using a Chi-squared test with Yates' continuity correction, in R (R-Core-Team, 2016). The Yates correction is a correction made to account for the fact that chi-square test is biased upwards, which if uncorrected tend to make results larger than they should be. No significant differences between the replicates were demonstrated and these were therefore pooled for further comparisons. The student's *t*-test was used to compare the average size of surviving and dead CaO-challenged sea urchins, as there was no average size difference between them. Its non-parametric version, The Wilcoxon Rank-Sum Test, was used to compare the size of cannibalized sea urchins to those surviving the experimental

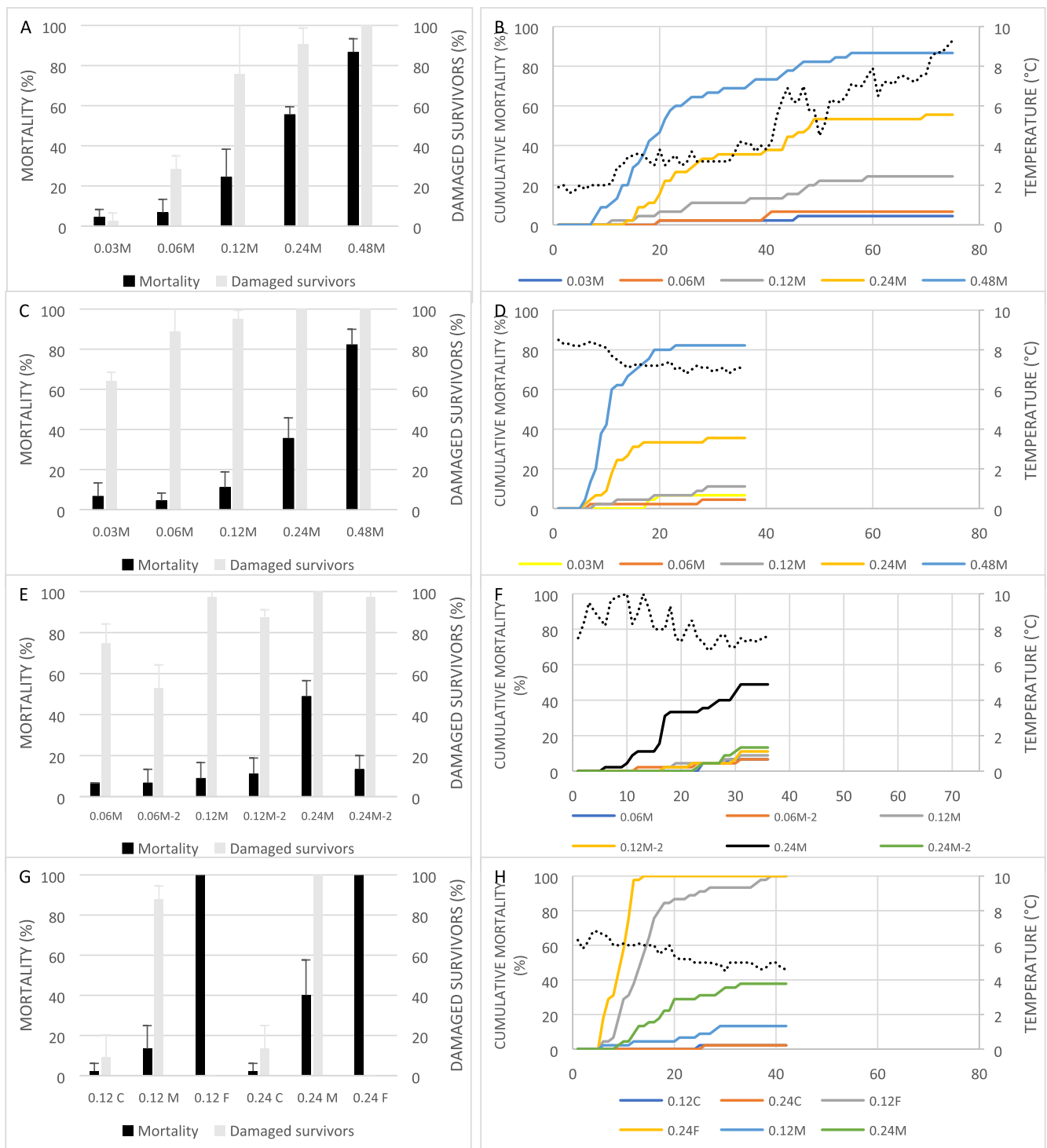


Fig. 5. Total mortality and survivors with visible damage in the exoskeleton in the left column, and cumulative mortality and temperature on a day-to-day basis in the right column. Spring simulation (A and B), Autumn simulation (C and D), One whole vs two half doses (E and F) and different particle sizes (G and H). F = Fine (0–0.5 mm), M = medium (0.5–2 mm) and C = coarse (2–4 mm) particle diameters. SD are shown as vertical bars.

period, as the smaller average size of those cannibalized indicated a non-random sample from the general population in the experimental tank. SDs are shown in brackets.

Results

The sea urchins started dying approximately one week after CaO application, and the steepness of the mortality curve from then varied with both temperature and particle size. No size-specific vulnerability

was found, as those surviving the experiments measured on average 46.8 (6.1) mm, while those that were killed by CaO measured 46.4 (6.3) mm. Mortalities were slightly higher in the spring than in the autumn treatment group, but not significantly so ($\chi^2 = 1.90$, $p > 0.05$). The temperature fluctuated more in the spring than in the autumn experimental period (Fig. 5 B and D).

In the split-dose experiment, the 0.24 g dose caused a significantly higher mortality when administrated in one vs two rounds of 0.12 g spaced at two-week intervals ($\chi^2 = 11.67$, $p < 0.01$, Fig. 5 E and F).



Fig. 6. Sea urchins alive at the end of the experimental period, but with different levels of damage to their exoskeletons. Apparently healthy specimen on the left and from minor to major damage moving right.

The largest difference in survival between the treatment groups was related to the CaO particle size, where 0.12 g/individual caused 100% mortality in the 0–0.5 mm particle groups, while in the corresponding coarse particle groups (2–4 mm), only 2% of the sea urchins died. For the Medium particle size distribution (0.5–2 mm) mortality was 13 and 40% respectively (Fig. 5G and H).

For most cases the cause of mortality (infection, osmotic stress, etc.) was not determined, but some cases of cannibalism were observed; 1.4% and 2.0% of the mortality in the spring and autumn treatment groups were attributed to cannibalism, respectively. The act of cannibalism was observed as one sea urchin clinging on to another, with the result that parts of the prey's exoskeleton were damaged. While the general diameter in the population was 46.6 mm, those preyed upon was smaller and on average measured 41.2 mm (Wilcoxon Rank-Sum Test, $p < 0.05$).

There were no mortality, cannibalism or damaged individuals among sea urchins in the control groups.

Damaged survivors

Individuals of *S. droebachiensis* were defined as damaged if at the end of the experiment any parts of their exoskeleton were exposed as white patches. Mortality varied between groups, but the percentage of damaged survivors was generally high (Figs. 5 and 6). As shown in Fig. 6, the degree of damage did, however, vary greatly between individuals. Generally, the lower doses and the coarse particle sizes produced the smallest exposed areas.

All the urchins that were sublethally exposed to CaO (0.06 g/ind) were observed to feed, but the percentages of damaged individuals were almost identical in the fed (23% and 90%) and unfed groups (28% and 89%) during the spring and autumn simulations respectively. In the spring simulation, by the end of the experiment most damaged individuals had developed a purple layer covering the white exposed spots on their exoskeletons. They were removed from the “damaged” category, but it is not known whether they were in the process of healing.

2.4. Field study 2018–2019

Three experimental fields were established in sea urchin barrens in autumn 2018 in a fjord system somewhat west of the one chosen for the 2008–2011 pilot study (Fagervika at 70.36°N 23.25°E). Each field measured 100 × 15 m and extended from the shoreline down to a depth of approximately 5 m. While the fields 1 and 3 consisted largely of cobble stones, the field 2 consisted of larger boulders (Fig. 1 b and c). All fields were completely barren before treatment. Control fields were established next to each of the experimental field.

The experimental fields not only differed in substrate morphology, the fields 1 and 3 consisted of cobble stones while field 2 was dominated by large boulders (Fig. 1), but also sea urchin size and initial densities. Before the treatment, there was a significant difference in the sea urchin diameters between the fields, measuring 22 (±7) mm and 18

(±9) mm in the fields 1 and 3 respectively and 35 (±6) mm in the field 2 (ANOVA $p < 0.05$). Pairwise comparisons revealed that the sea urchins in field 2 were significantly larger than in fields 1 and 3 (Tukey HSD, $p < 0.05$), while the latter two were not significantly different from one another (Tukey HSD, p greater than 0.05). At day 11, average sea urchin sizes had not changed significantly from the pre-treatment values within each field (Tukey HSD, p greater than 0.05).

Each experimental field was split in two sections: one half was treated with the medium CaO fraction (0.5–2 mm) and the other half treated with the fine CaO fraction (0.1–0.6 mm), in which the lower dust fraction had been removed and the upper cut-off point moved up from 0.5 to 0.6 mm, compared to the fine fraction used in the lab experiments in 2017. The two sections in each field were marked with small floats. Control fields were established next to each experimental field.

CaO was applied at a dosage of 300 gm⁻² and was distributed manually on the surface from a boat at low tide and in quiet weather using beakers. The water temperatures during the application and the inspection on day 11 varied between 10.1 and 10.7 °C.

Sea urchin densities (#m⁻²) and size (diameter) were measured the day before and 11 days after treatment, and densities were measured again one year after treatment. 60 sea urchins were collected for size measurements and an assessment of damage before treatment, and at day 11 another 60 sea urchins were collected from each of the areas treated with fine or medium CaO, i.e. 120 from each field. As in the pilot study (2008–2011), the number of sea urchins was counted and relative benthic macroalgal cover was estimated within each frame ($n = 15$) between a depth of 0 and 2 m in the fields 1 and 3, and additionally at a depth of 5 m in the field 2, since the large size of the urchins and substrate with boulders made such estimates feasible. The sea urchins were counted before treatment, on day 11 and 1 year after treatment.

Observations were made by snorkeling at low tide, and a measured line was connected to the frame for depth estimation the day before

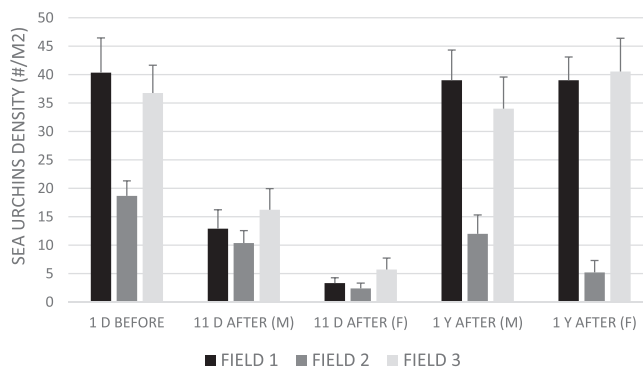


Fig. 7. Average density of sea urchins in experimental fields 1 day (D) before, 11 days after, and one year (Y) after treatment with medium (M) and fine (F) fractions of CaO respectively. Error bars = SD.

treatment, as well as 11 days after treatment. Densities before and after liming are presented in Fig. 7.

Statistics

Shapiro-Wilk's method was used for normality test of data. It is based on the correlation between the data and the corresponding normal scores. Average sea urchin diameters between the three fields before CaO application were compared using a one-factor ANOVA, and a Tukey HSD test was applied for pairwise comparison of fields. A student's *t*-test was used for pairwise comparisons of average sea urchin sizes before and after treatment within each field. Within each field the student's *t*-tests were also used for pairwise comparisons of sea urchin densities 11 days and one year after CaO application with pre-application levels. Statistics were computed with the software R (R-Core-Team, 2016).

Results

There was a dramatic reduction in sea urchin densities following the CaO treatment. Before treatment, the mean density was estimated at 40, 19 and 37 sea urchins per m² in the fields 1, 2 and 3 respectively. 11 days after treatment, the sea urchin densities were reduced to 3, 2 and 6 sea urchins per m² in the fields treated with the fine lime fraction, while the corresponding numbers for fields treated with the medium fraction were 13, 10 and 16 sea urchins per m². The average percentage reductions in the sea urchin densities were 56% ($\pm 12\%$) and 88% ($\pm 4\%$) in the fields treated with medium and fine fraction CaO respectively. The effect of the treatment on the sea urchin densities was significant compared to pre-treatment levels in all the three fields and for both CaO fractions (*t*-test, $p < 0.01$). Furthermore, the density of sea urchins in the fields treated with the fine CaO fraction was only 1/3 of the density in those treated with the medium CaO fraction. In the control fields, the densities 11 days after the treatment varied, compared to the pre-treatment count, by +14%, +22% and -21% in fields 1, 2 and 3, but the differences were not significant (*t*-test, $p > 0.05$).

On average $37 \pm 16\%$ of the surviving sea urchins at day 11 had damaged exoskeletons due to the CaO treatment. The rate of damaged individuals in the control field at day 11 was $4.0 \pm 1.7\%$, but the damaged area typically consisted of a small patch where the spines had been broken and it was clearly distinguishable from the damaged areas in urchins from the treated fields. These kinds of damage were not included in the measurements from the treated fields.

After one year there were still no macroalgae present in the fields 1 and 3 or in the control areas. In the field 2, however, filamentous macroalgae covered 28% of the area treated with the fine lime fraction the previous year (see also [video in supplementary data](#)). No perennial macroalgal species were observed.

After one year, the sea urchin densities were back to the baseline levels, except for the section of field 2 treated with the fine lime. In this section, the density was 5 per m², which was significantly higher than at day 11 but significantly lower than before liming (*t*-test, $p < 0.05$) (Fig. 7).

In the experimental fields, particularly in the fields 1 and 3, most of the sea urchins were partly hidden between and below the cobble stones and were not affected by the CaO.

3. General discussion

The main finding of this multiyear and multi-discipline approach to CaO treatment is that the method can be used successfully to reduce the number of sea urchins and thereby allow for a rapid macroalgal recovery in urchin-dominated areas, even at high latitudes. Nonetheless, our studies show that the success level will depend on various factors, such as the initial sea urchin density, the substrate type, the water depth and the particle fraction of the CaO. The pilot field experiments

indicated a higher success rate for urchin removal in the shallow zone where the highest sea urchin density was found (depth of 2 m vs 5 m). These differences can be attributed to lower doses and larger, less efficient particles hitting the sea urchins at greater depth due to drift and dilution of the CaO particles while sinking through the water column.

Discontinuous monitoring and the large time intervals between the pilot surveys of sea urchin density and macroalgal recovery contribute to uncertainty about the results. Nonetheless, at both control sites the interannual fluctuations in sea urchin density were low and macroalgae were scarce during the entire timeline of the pilot experiment. In contrast, the development of high macroalgal cover at the treatment sites implies that the sea urchin density had been sufficiently reduced beyond the threshold that allowed macroalgal recovery (Leinaas and Christie, 1996; Ling et al., 2015). The pilot study also demonstrated that mobile fauna abundance and species richness quickly increases in newly vegetated areas.

CaO is considerably more harmful to echinoderms than to most other marine species typically found on barren grounds (Bernstein and Welsford, 1982; Shumway et al., 1988), and no toxic residues will form during breakdown. These facts led the U.S. Environmental Protection Agency to remove CaO from its list of hazardous substances (Shumway et al., 1988). The chemical is also listed on the OSPAR Commission's PLONOR list (List of Substances Used and Discharged Offshore which Are Considered to Pose Little or No Risk to the Environment) (OSPAR, 2019). Sea urchin barrens have been considered a long-lasting and stable state (Norderhaug and Christie, 2009; Ling et al., 2015) with low productivity (Chapman, 1981) and diversity (Ling, 2008), and CaO treatment stands out as a potential method for controlling sea urchins and revegetation of the barrens. For biological and financial reasons, it is important to use as little lime as possible. Adequate doses have, however, varied between southern Californian and northern Canadian waters for reasons that are not fully understood. Based on the present results, we discuss some possible reasons for the discrepancy as well as prospects for further methodological improvements.

Effect of particle size

Particles in the smallest size range (0–0.5 mm) in the 2017 lab experiments caused 100% mortality, while the 0.5–2 mm fraction caused only 13% mortality and the 2–4 mm fraction only 2% mortality at the same dose. The same pattern appeared in the 2018–2019 field experiments, with the number of surviving sea urchins at day 11 being on average 3–4 times higher in the fields treated with the medium fraction than in those treated with the fine fraction.

Both in California and Canada the sea urchins were treated with a mix of particles and slurry containing CaO particles ranging in size from dust to 15 mm in diameter, while in our field experiments CaO was sprinkled dry on the water surface. Bernstein and Welsford (1982) concluded that low water temperature (10 °C) was probably an important reason that 250–500 g CaO m⁻² was enough in Californian waters while close to 1000 gm⁻² was needed in Canadian waters to obtain 70% sea urchin mortality. Our lab experiments demonstrated that the particles larger than 2 mm had almost zero effect on mortality at the same doses that caused 100% mortality with particles in the size range 0–0.5 mm, and in our 2018–2019 field experiment, the reduction in sea urchin density was still approximately 70% after 1 year in field 2, which had been treated with 300 gm⁻² and the finest lime fraction. Thus, small differences in the particle size ranges or pre-application procedures that altered the quantity of the smallest particles could probably have contributed to the differences between the Californian and the Canadian field trials.

The greater efficacy of small particles in our lab and field experiments is probably related to at least two mechanisms. First, from the lab experiments we observed that when sea urchins were hit by the 0.5–2 mm and 2–4 mm fractions in the reaction chamber, they typically climbed up the wall and turned themselves partly around, apparently in

a somewhat successful attempt to rid themselves of particles (see also [video in supplementary data](#)), while those exposed to the 0–0.5 mm fraction retracted their appendages and typically did not climb up the walls, with the result that they did not get rid of any particles. Second, it was observed that some of the largest particles were caught between the sea urchin spines and only partly made direct contact with the exoskeleton, as was also noted by [North and Shaefer \(1963\)](#). As part of the reaction of these particles will happen in the water and not at the urchin-particle interface, we believe they are less efficient.

Effect of temperature and season

The 2017 lab experiments indicated that temperature is probably of minor importance to sea urchin mortality, at least in the range 2–10 °C and when CaO is mainly applied as particles that stick to the sea urchin surface. The assumption made by [Bernstein and Welsford \(1982\)](#) that low water temperature was probably an important reason a dose 2–4 times as high was necessary in Canadian waters to obtain the same mortality rate as in California, was, however, based on [North and Shaefer \(1963\)](#). The latter authors demonstrated that lowering the water temperature from 20 to 15 °C prolonged the time to mortality from 4 to 13 days in *Strongylocentrotus purpuratus* and that one individual exposed at the lowest temperature (10 °C) recovered. However, the sea urchins were exposed to CaO at the same temperature (16 °C for 7 min.), rinsed and then allocated to different temperature regimes, so the results are not directly comparable to ours. Furthermore, the number of specimens was only four per test temperature, and pooling the results from the high temperatures (15 and 20 °C, 8 individuals, 100% mortality) and comparing them to the lowest temperature (10 °C, 4 individuals, 75% mortality) does not disprove a null hypothesis that there is no effect of temperature on mortality (Chi-squared test, $p = 0.7$). Nonetheless, in the Canadian and Californian experiments, a mix of particles and slurry were used, and we do not exclude the possibility that sea urchins spending a fixed amount of time in slurry will suffer higher mortality rates at higher temperatures.

The 2017 lab experiments nevertheless indicated that the method is season-independent at a high latitude when CaO is applied as particles, which, if true, increases the potential treatment window. A wider treatment window would make it easier to perform repeated treatments, as well as treatments optimized regarding times of the year when e.g. weather conditions, spore formation in seaweeds, and aggregations of sea urchins are considered optimal.

Effect of refuges

The 2008–2011 pilot experiments showed that the barrens were partially revegetated with kelp 1–3 years after the treatment. Nonetheless, despite high initial mortalities in the 2018–2019 field experiment, the sea urchins quickly regained high abundance, preventing the development of macroalgae. Our lab experiments showed that approximately 280 gm^{-2} of CaO particles in the medium size range (0.5–2 mm) caused 80% mortality in both the spring and autumn simulation experiments. However, when we applied 300 gm^{-2} in the 2018–2019 field experiment, only 56% mortality was estimated after 11 days, and after 1-year the sea urchin densities were back to the pre-treatment levels. The only exception was the test field consisting of large boulders that were treated with the smallest CaO particle fraction. The discrepancy might be due to less exact application of dosages in the field experiment, but perhaps a more likely explanation is that many sea urchins in these habitats were either partly or fully shielded from the CaO particles by the cobblestones and boulders. In the 2017 lab experiments, 100% of the sea urchins that survived the 280 gm^{-2} dose had damage to their exoskeletons, as did close to 90% of those that received half that dose. “Damage” was defined as a visual observation of a white spot on the sea urchin surface, where spines had been lost and the white calcified skeleton was exposed. The damaged areas

appeared approximately one week after CaO application. Generally, the higher doses produced the largest damaged areas. In the 2018–2019 field experiment, only 37% of surviving sea urchins sampled at day 11 had damage to their exoskeletons. This suggests that a large proportion of the sea urchins in the 2018–2019 field experiment had not been exposed to CaO due to refuges in their habitat. Furthermore, [North and Shaefer \(1963\)](#) found that small specimens were able to escape treatment by hiding in cavities in the rock. The greater efficacy of fine particles than medium-sized particles was probably caused both by the particles being more harmful and by them being more able to enter between cobblestones, into the large crevices and underneath tilted surfaces of large boulders. After an initial CaO treatment, the settlement of organisms on the top of the surfaces will attract the urchins hiding between the stones, and repeated treatments will probably decrease the urchin density sufficiently for revegetation to occur.

Damaged individuals

Even though the lab results didn't show signs of recovery from damage in the sea urchins fed ad libitum with sugar kelp, further studies must be conducted under field conditions to establish whether sea urchins have the potential to recover from damage caused by CaO under natural conditions. A much smaller damaged area was sufficient to cause mortality when it was caused by CaO than by mechanical removal of spines ([North and Shaefer, 1963](#)). Damaged urchins will probably be more susceptible to cannibalism and also experience increased mortality relative to predator abundance in the treated habitat. [North and Shaefer \(1963\)](#) reported that fish were frequently found to attack the spineless portions of damaged individuals and that the stomach contents in each of four sheepshead (*Pimelemetopon pulchrum*) specimens caught in treated areas consisted almost 100% of sea urchin remains. Such “secondary” causes of mortality might cause treatment efficacy to differ between apparently similar physical habitats. In the spring 2017 lab simulation experiment, there were lower percentages of damaged individuals at the two lowest doses than in the autumn simulation experiment. This could, however, be a difference caused by the criteria used to define damage, as we cannot say whether the purple layer covering damaged areas meant they were in the process of healing or not.

Strategies to prevent new grazing events

The 2008–2011 and 2018–2019 field experiments showed that application of CaO can reduce sea urchin abundance sufficiently for rapid kelp and other macroalgae recovery on barren grounds. However, if the treatment areas are small, as in the 2008–2009 field experiments, and/or rich in refuges, as in the 2018 field experiment, sea urchins may reappear after treatment in sufficient quantities to keep the area barren. Alternatively, the recovered macroalgae might soon be lost to new grazing events.

As a lack of sea urchin predators may cause kelp forest collapse ([Steneck et al., 2002](#)), it follows that establishing new sea urchin predators could prevent new grazing events. In the 2008–2011 field experiments, it was observed that the remaining sea urchins aggregated at grazing fronts, a behavior previously described for this species ([Vadas et al., 1986](#); [Elnor and Vadas, 1990](#)), and could then more easily be taken out by repeated treatments with CaO ([North and Shaefer, 1963](#)). There is also a financial incentive to harvest them due to the remaining urchins' large size and commercially viable high gonad index. This could indicate a possible management procedure: first create patches of rich kelp beds by using CaO, then harvest sea urchins in the transition areas as a profitable resource when roe contents have increased. When commercial harvesting became viable off Maine, sea urchin densities were reduced to levels which resulted in the re-establishment of kelp forest ([Steneck et al., 2004](#)). Then, a dynamic could develop of new kelp beds being gradually gained while new transition areas for urchin harvesting also emerge.

4. Conclusion and suggestions for methodological improvements

Bernstein and Welsford (1982) inspected their treated fields on average 105 days after CaO application, and thus in many instances too much time had passed to adequately evaluate the immediate effect of treatment. Our results demonstrate the potential for sea urchin densities to recover between 11 days and 1 year after a successful treatment if the substrate is rich in refuges, as was the Canadian habitat compared to the Californian ones. Also, our results show that particle size is decisive with regard to effect, and this parameter also differed between the Canadian and Californian trials. Thus, of the three variables held up as potential explanations for the different effects of CaO treatment in Canadian and Californian waters, we conclude that the presence of refuges and suboptimal particle size were probably more important than temperature.

The most conspicuous result in the current experiments was the superior efficacy of the small CaO particles compared to the large ones, and the fact that the “large” particles in our experiments were still substantially smaller than those used by others in previous experiments. The most effective particles tested in our experiments ranged in diameter from 0 to 0.6 mm (0–0.5 mm in the 2017 lab study and 0.1–0.6 mm in the Field study (2018–2019)). Nevertheless, if the smallest and the most effective particle sizes were to be applied during management operations in the field, an unknown proportion would dissolve before reaching the sea floor or form a “dust bowl” and drift out of the treatment areas, as was also noted by North and Shaefer (1963).

As strong currents and depths greater than 3–5 m will often be encountered in sea urchin-infested areas, sprinkling the particles on the surface will probably produce mixed results. We therefore suggest that an application method should be developed where the particles are released under water at depths greater than 3 m, maybe 1–2 m above the sea floor. The interaction with the sea urchins should happen as soon as possible after the CaO particles mix with water. Our 2017 lab results showed that it was more effective to treat the sea urchins with one full dose compared to two half doses spaced at a one-week interval. However, this result was obtained when the same sea urchin was subjected to one or two treatments. In the field the main issue will be whether the sea urchin is hit at all during the first treatment, due to refuges in the habitat. In line with the procedures developed by North and Shaefer (1963), we agree that one should inspect treated areas after application of CaO and repeat the procedure if the densities are not below a certain threshold, e.g. less than 5 sea urchins per m² (Leinaas and Christie, 1996). The exact maximum sea urchin density that will still allow kelp reforestation to occur will probably vary locally depending on the species, temperature, depth, season and local particularities.

CaO treatment may be applied successfully along high latitude barren coasts to alter the benthic production and biodiversity, and to improve the commercial value of the remaining sea urchins. However, as the stability of the newly vegetated state will be at least partly dependent on a balance between sea urchins and its predators, additional measures should be considered to prevent new grazing events.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.eccoena.2020.100018>.

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