Mesocosm experiments in ocean alkalinity enhancement research

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Abstract. An essential prerequisite for the implementation of ocean alkalinity enhancement (OAE) applications is their environmental safety. Only if it can be ensured that ecosystem health and ecosystem services are not at risk will the implementation of OAE move forward. Public opinion on OAEs will depend first and foremost on reliable evidence that no harm will be done to marine ecosystems and licensing authorities will demand measurable criteria against which environmental sustainability can be determined. In this context mesocosm experiments represent a highly valuable tool in determining the safe operating space of OAE applications. By combining biological complexity with controllability and replication they provide an ideal OAE test bed and a critical stepping stone towards field applications. Mesocosm approaches can also be helpful in testing the efficacy, efficiency and permanence of OAE applications. This chapter outlines strengths and weaknesses of mesocosm approaches, illustrates mesocosm facilities and suitable experimental designs presently employed in OAE research, describes critical steps in mesocosm operation, and discusses possible approaches for alkalinity manipulation and monitoring. Building on a general treatise on each of these aspects, the chapter describes pelagic and benthic mesocosm approaches separately, given their inherent differences. The chapter concludes with recommendations for best practices in OAE-related mesocosm research.

Kommentiert [UR3]: R2 L 16: 'realism' – This seems unnecessarily contentious; mesocosms are indeed far nearer 'reality' than microcosms and cultures due to their inclusion of a more representative portion of the ecosystem, but it is only a portion. This is clearly reflected in latter parts of the review so a more appropriate term or just leaving it at 'biological complexity' may fit better in the abstract (e.g., 'By combining representative biological complexity with controllability and replication..').

Gelöscht: realism and

Preface

The authors would like to emphasize that this chapter does not intend to cover all aspects of mesocosm experimentation in its full breadth, but rather tries to address aspects specific to research on ocean alkalinity enhancement (OAE) or aspects we consider important to reiterate here. For a more comprehensive presentation of recommendations and guidelines on mesocosm experiments the reader is referred to Chapter 6 of the *Guide for Best Practices on Ocean Acidification Research and Data Reporting* (Riebesell et al. 2010) and references therein as well as Stewart et al. (2013).

Although the general approach to mesocosm experiments is straightforward and basically involves enclosing a body of water with or without sediment in order to monitor responses of the enclosed communities and related processes to the manipulated perturbation over an extended period of time, the specifics of conducting such experiments can vary considerably. These include factors such as the materials, design and location of the enclosures, e.g. fixed structures on land or flexible wall enclosures in situ, as well as the procedures for mesocosm filling, operation, mixing and sampling. While the dimensions of the experimental enclosures can range from less than 1 m^3 to >1000 m^3 depending on the requirements of the experiment, we here adopt the classification set out by the SCOR Working Group 85 in 1991: Microcosms (less than 1 m^3), mesocosms (between 1 and 1000 m^3) and macrocosms (more than 1000 m^3). We note that benthic experimental enclosures can have different size categories.

1 Placing mesocosms in the context of OAE research

Mesocosm experiments provide an essential bridge between the tightly controlled but poorly realistic laboratory culture experiments and the complexity of natural systems. This is particularly important for possible OAE implementations, in order to achieve a sound understanding of the entire process of the proposed OAE strategies, from the dissolution kinetics and effectiveness of the alkalinisation technique, to the potential environmental impacts, risks and co-benefits. This knowledge is crucial prior to any form of OAE application to safeguard the protection of marine ecosystems functioning, biodiversity and related ecosystem services. Moreover, should OAE prove to be a viable approach for marine carbon dioxide removal (mCDR), it will also be crucial to achieve social acceptance for potential OAE implementations. Also in this context mesocosm experiments can serve as a useful tool for proof of concept, the results of which can play an important role in the public discourse about the risks and benefits of mCDR implementation.

Kommentiert [4]: R2 L 42: rather than 'poorly realistic', 'unrealistic'?

Kommentiert [5]: I don't agree with the proposed change. Unrealistic has a very strongly negative connotation

Kommentiert [6]: I agree. Unrealistic is clearly stronger than poorly realistic. I suggest we stick with poorly realistic.

Functional redundancy and species richness in ecosystems allow for some degree of resistance to withstand disturbances and resilience to recover once a disturbance has ended or dissipated. To determine the actual ecological impacts of OAE it is essential, therefore, to test suggested applications at the community/ecosystem level. Doing this in field trials, however, poses serious difficulties, given the hydrographic complexity of most marine systems, with lateral advection (currents, tides), vertical flow (convection, up- and downwelling) and wave-driven mixing. Determining dose-response relationships for environmental impacts is extremely challenging under such conditions. Mesocosm experiments, on the other hand, enable the combination of biological complexity needed for testing resistance and resilience of communities/ecosystems in their natural setting and seasonal succession (in a single experiment where succession occurs on short time scales, e.g. a phytoplankton bloom, or multiple experiments in different seasons using the exact same experimental set-up) with a reasonable degree of control and replication and hence the statistical power to reach reliable conclusions. At the same time, they allow testing the chemical kinetics of mineral dissolution and secondary carbonate precipitation, thereby providing vital information on the efficacy of the suggested OAE applications in a natural setting under a range of environmental conditions (salinity, temperature, carbonate chemistry, inorganic nutrient concentrations, dissolved and particulate organic carbon concentrations etc). Testing them in mesocosm enclosures has the additional benefit of minimizing public concern and regulatory requirements when compared to field trials.

Kommentiert [7]: R1 L 57-58: Therefore, to cover the seasonal succession, it is needed to conduct a mesocosm experiment during several seasons. Could it be possible to maintain such mesocosm experiment during the year(s)? In any case, it could be good to mention something about this aspect as it was suggested above.

Environmental impacts of OAE will be scale- and context-dependent in terms of the physical (e.g. timescales of mixing and CO_2 equilibration, point source vs. diluted release), chemical (e.g. amount/type of alkaline substance, impurities), and biological characteristics (e.g. seasonal succession and related ecosystem vulnerability).

Kommentiert [8]: R1 L66: biological characteristics (e.g. ecosystem vulnerability, time of season). System vulnerability depends on the communities present at the moment of the mesocosm experimentation. So, "communities" could be added as a first example of biological characteristics.

Biological impacts are determined by exposure time and dose, ranging from acute shock responses on transient and local scales at point sources to chronic effects associated with possible transitions of ecosystem structure and performance at the regional and long-term scale. Key research questions which can be addressed adequately through mesocosm experiments are:

- What is the safe operating space for OAE applications with respect to possible impacts on marine ecosystems functioning, biodiversity, and ecosystem services?
- How could OAE be implemented to reduce the risk of inadvertent negative environmental effects, and maximize co-benefits?
- Which biological indicators can serve as early warning signals or proxies for OAE environmental impacts?
- How do different OAE approaches perform in terms of efficiency (e.g. mineral dissolution, CO_2 uptake) and permanency (e.g. secondary precipitation)?
- Which application sites are most appropriate for which OAE approach?

Kommentiert [9]: R1 L68-69: Key research questions which can be addressed adequately in mesocosm experiments are: ….. The first, second and last mentioned items are not the Key research questions which can be addressed in the mesocosm experiments, but they are requirements which can be established before mesocosm OAE experiment.

Kommentiert [10]: I don't understand this comment, sorry

Kommentiert [11]: I disagree with the reviewer. These ARE key research questions which can be addressed through mesocosm experiments.

Gelöscht: in

2 Strengths and weaknesses of mesocosm experimentation

Mesocosm experiments offer a salient advantage over laboratory-based investigations, as they allow a realistic replication of natural communities. Multiple trophic levels can be confined under natural environmental conditions over a long period of time in a self-sustaining manner. Thereby, the same community can be sampled repeatedly over time. Furthermore, these experiments permit straightforward validation in the context of field research. Mesocosms, in essence, are closer to representing natural ecosystems characterized by carefully defined dimensions and monitored conditions and processes. To ensure realistic ecological boundary conditions, mesocosm experiments should be exposed to meteorological conditions resembling those of the target environment. Notably, the logistical flexibility of mesocosms affords researchers the opportunity to conduct investigations beyond the geographical confines of the environment under investigation. Consequently, mesocosms provide an invaluable avenue for the controlled study of specific environments and the impact of controlled manipulations therein. Given the diverse range of natural processes encountered in mesocosm experiments, external influences may be challenging to control, necessitating a robust monitoring strategy to achieve statistical power by either treatment replication or treatment gradients. Moreover, mesocosm experiments provide extensive multidisciplinary datasets that allow for a high degree of scientific integration and interdisciplinary collaboration. These datasets are valuable for parameterisation and assessment of marine ecosystems and biogeochemical models.

While mesocosm experiments can be considered the preferred tool for the assessment of environmental impacts of OAE applications, they have several weaknesses that need to be considered when interpreting the data and extrapolating the results to the real world. These weaknesses include unnatural mixing and turbulence (in pelagic mesocosm), unnatural flow of bottom water across the sediment (in benthic mesocosms), wall effects and the growth of periphyton and other organisms on the mesocosm walls, spatial heterogeneity in the enclosed sediments and the related difficulties in obtaining representative samples. The larger and more expensive the enclosures become, the more difficult it becomes to have a sufficient number of replicates in a replicated design or treatments in a gradient design. The fact that even the largest mesocosms enclose truncated communities, i.e. exclude higher trophic levels and highly migratory organisms make it difficult to adequately represent the responses of organisms with longer life cycles and the associated impacts on the food web. Another drawback of mesocosm experiments is their limited duration, due to the gradual diversion from their natural counterparts, e.g. due to community shifts, nutrient depletion, and the consequent progressive loss of biological realism. The increasing variability between mesocosms in this process makes it increasingly difficult to identify treatment effects with statistical significance.

3 Experimental design

The primary purpose of a mesocosm experiment is to obtain "near-natural" conditions, that is to say, keeping the abiotic and biotic factors as close to the environment as possible in order to maximize the realism of the tested conditions. In general, time scale is related to mesocosm volume: the shorter the time needed for a controlled experiment, the smaller the enclosure size. Careful consideration should be given to the experimental design to adequately address the specific research questions, account for ecosystem- and site-specific characteristics as well as seasonal variability. The choice of the experimental configuration includes the three key dimensions of time, space and biological complexity, along with the required level of replication. Preference should be given to mimic the natural seasonal succession rather than provoking out-of-season events, e.g. triggering phytoplankton blooms through nutrient addition.

Considering the often limited number of experimental units, a critical consideration concerns the level of replication (Kreyling et al. 2018). The choice is between two basic approaches: (1) replicated ($n \geq 3$) treatments, with limited treatment levels (e.g. Riebesell et al., 2006); (2) a gradient approach with a larger number of non-replicated treatment levels (e.g. Taucher et al., 2017). The statistical power of the two options, using ANOVA statistics for the replicated design and regression statistics for the gradient design, is similar for the small number of experimental units typically available in mesocosm studies (Havenhand et al., 2010). If large within-treatment variation is expected, e.g. due to strong environmental variability or spatial heterogeneity, the replicated approach is recommended. In fact, strong within-treatment variability can easily mask subtle treatment effects. An important advantage of the gradient approach, on the other hand, is that it enables the identification of non-linearities, thresholds and tipping points in biological responses to OAE applications, relevant information for model parameterizations in terms of community functional responses. Knowledge about thresholds and possible tipping points is crucial also in the context of regulatory considerations for OAE implementation.

Kommentiert [12]: R2 L 123: 'relevant information in the context of regulatory considerations' – another important strength (of the gradient approach) is its utility to model parameterization in terms of functional responses of organism physiology and ecosystem function.

Pelagic mesocosms

When aiming to investigate OAE applications in the free water column, pelagic mesocosms are the research tool of choice. Among the various proposed strategies, ocean liming in the wake of ships would consist of sparging high-alkalinity fluids or mineral particles within the surface layer in offshore settings. In this scenario, any chemical perturbation is expected to affect in the first instance the pelagic domain and the planktic component of the marine ecosystem. Also OAE applications at fixed locations with a discharge of alkalinity-enriched water into coastal waters, e.g. desalination plants or sewage treatment plants, are best simulated in pelagic mesocosms. A suitable simulation of OAE approaches in which the alkalising mineral is released in particulate form should ideally have the dissolution rate of the particles known in advance. If the rate is fast enough to ensure complete dissolution in the water column, pelagic mesocosms are well suited. In cases where the dissolution rate is slow compared to the particle sinking rate and particles sink to the seabed before dissolving, the experimental design may require a benthic component.

A missing component in all closed-system mesocosm experiments is the dilution through mixing with non-perturbed waters. Switching to an open system, where the enclosed water is partially replaced by non-alkalised water, places much greater demands on monitoring and complicates the interpretation of the observed responses, to the extent that it may be impossible to establish a reliable dose-response relationship. This experimental artifact is exacerbated when repeated additions of alkalinity are applied. Incorporating naturally occurring dilution in the experimental design can be done by applying the OAE treatment to only part of the enclosed water column and allowing for gradual mixing with the untreated water. The time until mixing can be controlled by stratifying the water column through a salinity gradient (adding fresh water into the upper layer or brine into the bottom layer, whereby the salinity change should be at a low enough level not to cause a biological response, e.g. a few tens of a salinity unit) or via a temperature stratification. Break-off of the stratification can be gradual or abrupt through active mixing. Parallel sampling of the OAE treated and untreated water bodies can provide insights about the compensating effect of dilution.

There is a wide range of enclosure volumes and structures used in pelagic mesocosm experimentation. Among the various available solutions, the most obvious difference is the placement of the mesocosm: 1) stable, permanent structures on land, or 2) floating bags in the water. All materials that come into contact with the enclosed water/sediment must be chemically inert, i.e. they must not leach or actively absorb any substances. Some technical details of the mesocosm design can markedly affect some abiotic factors, such as thermal characteristics, light conditions or mixing intensity of the enclosed water column. Most pelagic mesocosm enclosures are made of transparent material supported by a mini-mal rigid framework, with the intent to keep light conditions as in nature. Most materials, however, change the spectrum of the transmitted light, for example are not transparent for UV-light. As enclosure depth is often lower than the mixed layer depth of the natural environment, natural light conditions are not well represented in mesocosms, with light intensities averaged over the mesocosm depth often higher than those averaged over the mixed layer depth.

Benthic mesocosms

Benthic mesocosm experiments offer the unique chance to study OAE-mineral addition to the seafloor in a controlled set-up. In comparison to experiments in laboratory settings, often small in scale with respect to mineral weathering, benthic mesocosms are more likely to mimic natural seafloor conditions and allow the coupling of biogeochemical processes at larger spatial and temporal scales. Key research questions on seabed alkalinisation to be addressed in benthic mesocosm experiments include: 1) What are alkaline mineral dissolution rates under mesocosm conditions? 2) Do secondary minerals form that may compromise the net CO_2 sequestration efficiency of this method? 3) How are microbial communities and macrofauna affected by mineral dissolution? 4) Is there a release and accumulation of heavy metals related to addition of silicate-based minerals and how does their toxicity affect the community/ecosystem?

Continuous water flow system: In this set-up, a continuous flow of ambient seawater, preferably bottom water, over the sediment (Fig. 2), likely best resembles natural seafloor conditions. It is recommended to remove larger

Kommentiert [13]: R1 L141-143: addition of freshwater into the upper layer or brine into the bottom layer of a mesocosm, could be considered as a new treatment engendering marine organism responses to less or more saline water. This could provide additional complication for interpretation of the results regarding the effect of OAE.

Gelöscht: ,

Gelöscht: ,

Gelöscht: To the best of our knowledge, benthic mesocosm experiments in OAE research have been conducted to date at GEOMAR (Germany) and at the University of Antwerp (Belgium) and preliminary data has been presented at EGU and ASLO and in 2023 international conferences (Hylén et al. 2023). Therefore, the following sections are based on personal experiences and the challenges encountered.

Gelöscht: screen the incoming bottom water to debris that could obstruct the water supply using a sediment trap (Fig. 2), whilst allowing small particulate matter to enter the mesocosms. The supply of particulate matter is essential to sustain natural microbial metabolism in the sediments and to provide food for filter-feeding macrofauna that colonize the sediment surface within a short period of weeks to months (Fig. 2). A relatively high flow rate is required (between 5000 to 10000 L d^{-1}) to keep the seawater well oxygenated and guarantee the survival of fauna and for maintaining the natural microbial communities as closely as possible to in situ conditions. With this set-up, the bottom water should be monitored to trace seasonal changes in physical and chemical properties of the incoming seawater.

Water circulation approach: The benthic mesocosm set-up with a seawater circulation approach consists of two tanks stacked on top of each other, with the upper tank housing the benthic ecosystem with sediments and organisms and the lower tank is functioning as a seawater reservoir from which water is pumped into the upper tank (Fig. 3). Thus, a constant flow of water is generated through the water in- and outflow and the height of the water column in the upper tank can be controlled by the vertical positioning of the outflow. The tanks for the benthic mesocosms have a volume of approximately 1 m^2 and are situated outdoors and exposed to natural temperature fluctuations.

Based on the water circulation approach, the closed system allows for the detection and accumulation of weathering products and to focus on a specific process or reaction, such as the dissolution kinetics of silicate minerals in the case of the University of Antwerp study (Fig. 3). After a defined timespan (flux session) the total amount of water is replaced and accumulation of weathering products starts again from initial values. In terms of this experiment design, ≥3 replicates of benthic mesocosms are crucial to ensure that results are statistically significant and can be generalized to the broader ecosystem being studied (e.g. Wadden Sea).
The total experiment duration as well as the sampling strategy is defined by the research questions and longer experiments may be necessary to capture seasonal or long-term trends in the system. The use of natural sediment and the inclusion of a dominant bioturbating organism (e.g. *Arenicola marina*) in benthic mesocosm experiments is a crucial step toward making the experimental setup more representative of real-world conditions. However, it's important to emphasize that the choice of sediment type and benthic organisms should be aligned with the specific research objectives and questions being addressed.

In OAE studies involving benthic mesocosms, various types of sediments can be considered, ranging from fine-grained sediments to rocky substrates. The selection of sediment type should be guided by factors such as the local environmental conditions, the availability of sediment types that reflect the targeted ecosystem, and the specific geochemical interactions being investigated. For studies related to carbonate dissolution and alkalinity enhancement as given above, fine-grained or sandy sediments are most suitable, given their potential to facilitate mineral dissolution and subsequent alkalinity release.

Similarly, the choice of benthic organisms should be tailored to the research objectives. While many benthic organisms can be tested in mesocosms, it's important to consider the life history, behavior, and ecological role of the selected species (Bach et al. 2019; Flipkens et al. 2023). For instance, if the experiment spans a year and aims to study the recruitment and life cycle of benthic organisms that have a pelagic phase, careful planning is required.

Kommentiert [14]: R1 L175 : Please give examples for the monitoring frequency: high frequency? every some hours? daily, weekly? etc.

Kommentiert [15]: R1 L179: … a constant flow of water. In the area with the tide water movement, the flow of water could/should be adjusted regarding the tide water movement? It is necessary or not?

Kommentiert [16]: Respond: Tidal cycles might influence the general alkalinity cycles due to current induced advective fluxes. However, this experiment is designed to investigate the interactions of OAE source material compared to non-OAE source material present and fluxes are induced by bioirrigation.

Gelöscht: account inner dimensions

Kommentiert [17]: R1: L177-182: Water circulation approach. It could be useful to refer in the text, the number of the figures presented in the manuscript (Fig. 3). It was mentioned in L185, but it could be also indicated before.

Kommentiert [18]: Respond: We considered your suggestion and the referring Figure (Fig. 3) is mentioned now in the first sentence of the paragraph.

Kommentiert [19]: R1 L186: Please provide examples about replicates (at least 2?, 3?, > 3?).

Kommentiert [21]: Respond: Thank you for this comment. The paragraph has been rewritten and extended to illustrate the variety of possible applications for mesocosms related OAE research.

Furthermore, an extended experimental design could connect pelagic mesocosms and benthic chambers to serve as an approach to investigate benthic-pelagic coupling in the future but until now OAE research is still in the phase of processed-based studies.

Gelöscht: The use of natural sediment and the inclusion of a dominant bioturbating organism (e.g. *Arenicola marina*) can help make the mesocosm more representative of natural conditions. However, it is important to note that the specific organism and sediment type may vary depending on the research question being addressed.

Monitoring larval settlement, growth, and interactions with the sediment during their benthic phase becomes integral to such investigations.

As an illustrative example, consider an OAE study targeting the enhancement of carbonate precipitation through the addition of alkalinity. In a coastal setting, sandy sediments rich in carbonate minerals might be chosen, given their potential for mineral dissolution and subsequent bicarbonate formation. Benthic organisms like filter-feeding mollusks and burrowing polychaetes could be tested to assess their responses to altered alkalinity levels.

Finally, the water circulation approach should be carefully designed to ensure consistency in water flow rates and initial seawater chemistry. Sedimentation in the water reservoir tank has to be prevented to avoid secondary sediment surfaces and a continuous monitoring system (salinity, temperature) is recommended to estimate evaporation rates. In addition, regular sampling of environmental conditions (humidity, pCO_2) as well as carbonate system parameters and nutrients, can ensure that the experiment proceeds as planned and that the results are reliable.

4 Mesocosm operation: filling, sampling, wall cleaning

Filling of the mesocosms is a delicate process that, if not done with care, can jeopardize the entire experiment. A key aspect is to ensure identical starting conditions, both for the abiotic and biotic conditions in all mesocosms. Between mesocosm differences in baseline conditions can cause divergence of the enclosed communities and severely hamper the detection of treatment effects. As the filling often represents a major perturbation itself, some time of equilibration may be needed before applying the treatment manipulation and starting the actual experiment. The time for equilibration may differ for pelagic and benthic habitats as well between different ecosystems and seasons. Adequate monitoring during this pre-manipulation phase can determine when a new steady state is reached and confirm whether all mesocosms have similar starting conditions. Key parameters for which equal starting conditions among mesocosms need to be ensured include temperature, salinity, inorganic nutrient concentrations, the carbonate chemistry (pH, pCO2, DIC TA), dissolved and particulate organic matter concentrations, community composition and diversity, and standing stocks of the dominant taxonomic groups across trophic levels.

Another critical aspect of mesocosm operation is taking representative samples. The enclosed water bodies and sediments typically show spatial heterogeneity (vertical gradients in the water column and sediments, patchiness in the distribution of larger organisms). The spatial variability of the target variables of the enclosed system should be determined prior to deciding on the best sampling strategy. Sampling bias related to vertical gradients, e.g. water column nutrient concentration and phytoplankton biomass, can be overcome by taking depth-integrated water samples (Fig. 4). Some species may even perform diurnal vertical migration, which also should be accounted for in the sampling strategy.

Mesocosm enclosures are always associated with additional surfaces, the mesocosm walls, that are not present in the natural environment. The smaller the mesocosms, the larger the additional surface area relative to the enclosed volume. Free surfaces are generally subject to rapid biofilm formation, followed by colonization of larger

Kommentiert [22]: L200-206: The time for equilibration may differ for pelagic and benthic habitats. Adequate monitoring during this pre-manipulation phase can determine when a new steady state is reached and confirm whether all mesocosms have similar starting conditions.

Kommentiert [23]: Reviewer: "This means that the T0 samples of all mesocosms should be taken and analyzed and if the results are similar for all mesocosms, thereafter the real manipulation can be started and monitored. Please provide the type of samples (physical, chemical and/or biological), with some examples, which can be taken and analyzed during this pre-manipulation phase. This information will also help better understanding of the L255-257."

Kommentiert [24]: R2 Ln 204-205: 'the time for equilibrium may differ for pelagic and benthic habitats' – is variability in the equilibrium time for different ecosystems and seasons another consideration that needs to be made?

Kommentiert [25]: R1 L212-213: Some species may even perform diurnal vertical migration, which also should be accounted for in the sampling strategy. Which sampling strategy should be considered related to diurnal vertical migration of organisms? Sampling during the night? At which depths? Because "taking depth-integrated water samples » cannot help to study diurnal vertical migration.

Kommentiert [26]: What the reviewer is asking for ("Which sampling strategy should be considered related to diurnal vertical migration of organisms? Sampling during the night? At which depths?") depends on the behavior of the migrating organisms. The sampling strategy needs to be adapted accordingly. I think it is beyond the scope of this paper to specify sampling strategies for the various vertical migrators.

organisms. The associated microbial community can significantly influence water column processes, which is of particular concern in pelagic mesocosms. To minimize such wall effects, cleaning of the mesocosm walls can be useful. Specific to OAE mesocosm experimentation is that under conditions where the water column is highly oversaturated with respect to calcium carbonate, mesocosm walls can provide free surfaces for secondary precipitation of carbonates. Under these circumstances, wall cleaning can scrape off these carbonates, creating additional precipitation nuclei in the water column. If wall cleaning is continued under these circumstances, possible effects caused by this, e.g. enhancement of secondary precipitation in the water column and increased ballasting of particulate matter, should be seen as artifacts and interpreted as such. If wall cleaning is discontinued and the biofilm on the walls grows to a significant biomass compared to the suspended biomass, this may limit the duration of the experiment. The decision for or against wall cleaning must be made on a case-by-case basis and depends, among other things, on the severity of wall growth, the duration of the experiment and the specific research questions to be investigated.

Kommentiert [27]: R1 L214-221: What are the conclusions of this paragraph? In the OAE mesocosm experiment, cleaning of the mesocosm walls can/should be done or not? If yes, this additional precipitation nuclei in the water column could not provide the artifacts in the experiment and result interpretation?

Pelagic mesocosms

Different techniques have been employed for filling pelagic mesocosms, including (1) direct pumping from the sea in cases where mesocosms are placed *in situ* or close to natural waters, (2) collection in tanks when source waters need to be transported over some distance and subsequent pumping from the tanks into the mesocosm, (3) lowering a flexible bag like a curtain over an undisturbed water column. In all cases care should be taken to fill the mesocosms with identical source waters. Considering that water masses may change over the filling procedure, this can best be achieved by filling the mesocosms in parallel through a distributor system (Fig. 4). Likewise, if several tanks are needed to obtain the required source water volume, the water of each tank should be distributed evenly into all mesocosm units. The source water should be representative for the targeted ecosystem. This concerns the depth at which the source water is collected and, when diurnally vertically migrating organisms are present, the time of day. When pumping is applied some damage to fragile organisms, e.g. gelatinous zooplankton, is unavoidable. It is therefore recommended to use pumps that ensure a smooth flow of pumped water, e.g. peristaltic pumps (Fig. 4). To prevent large and rare organisms from entering and being unevenly distributed in the mesocosms, some screening can be applied at the intake of the pumping hose.

As mentioned above a typical artifact of mesocosm enclosures is the reduced level or absence of turbulence. In mesocosms with solid wall structures it may be useful to apply some form of mixing of the water column, considering that turbulence (including its absence) is known to strongly affect the plankton community composition and succession. In floating enclosures with flexible walls some turbulence is induced by surface wave action, below surface water movement and variability in water currents, but the vorticity of the enclosed water is still always much reduced compared to that of the natural environment. Somewhat related to the mixing regime is another potential artifact in mesocosms where settling particulate matter is continuously resuspended from the bottom. Resuspension of degrading organic matter, which under natural conditions would sink out of the upper mixed layer, exaggerates the heterotrophic processes in the system. Collecting and removing the sedimented matter in cone-shaped sediment traps which form the bottom of the mesocosms can avoid this problem (Fig. 4).

Benthic mesocosms

A particular challenge in benthic mesocosm experiments concerns the filling with sediment from the seafloor. Depending on the size of the tanks and the sediment height, it may be necessary to transfer several hundreds of kilograms of sediment from the seafloor to the tanks. Near intact sediments (undisturbed vertical stratification) may be collected relatively easily in sub-tidal areas. At sea, undisturbed sediments may be retrieved using a box corer or similar device, although this may be a tedious exercise involving multiple deployments of the coring equipment. Large amounts of sediment can be gathered relatively easily and quickly using a sediment grab, but disturbance of the sediment matrix is inevitable, and longer equilibration times for the sediment geochemistry to stabilize will be required before experiments can be started. In any case, benthic communities within mesocosms may be altered from those in natural ecosystems and a sound understanding of the equilibration period is crucial to allow for changes in benthic communities and the establishment of a new steady state within the benthic mesocosm. This equilibration period should be determined based on the specific conditions of the mesocosm experiment, including the number of replicates, environmental parameters, and the selected organisms. Adequate monitoring and sampling during the equilibration period are essential to ensure that the experimental conditions have stabilized and the ecosystem has reached a new steady state which in turn increases material and labour requirements. Robust control units are crucial in benthic mesocosm experiments and should ideally consist of the same number of replicates as the treatment group to ensure that any observed changes are due to the experimental treatments rather than natural variability. Sampling and monitoring should be in the same manner as the treatment group.

5 Alkalinity manipulation and monitoring

Different minerals, waste materials and electrochemical products have been suggested as feedstock for ocean alkalinity enhancement (for a comprehensive introduction to potential source materials see Eisaman et al. 2023). Most source materials do not come as pure alkalinity, but contain other substances, such as silicate, calcium, magnesium and various trace metals (e.g. iron, nickel, cobalt, chromium). OAE can be achieved by addition in dissolved form, which requires dissolution of the feedstock before its release into the sea, or in particulate form, after grinding of the feedstock, with the grain size being one important factor determining the dissolution rate. OAE can further be conducted in a CO_2-equilibrated mode, which involves some form of active injection of CO_2 into the alkalinity-enriched source water prior to its release, or in a non-equilibrated mode, which relies on air-sea gas exchange to provide the additional CO_2 that the alkalinized seawater can absorb. In case of the latter it is important to keep in mind that the time scales for CO_2 equilibration are on the order of months and can only occur as long as the alkalinized seawater is in contact with the atmosphere. (see Schulz et al., 2023 for further details)

Taken together, this results in a wide range of possible application scenarios, not all of which can be tested with the same scrutiny in mesocosm experiments due to the high financial and personnel costs involved. Hence, it is important to focus on those OAE application scenarios which are most likely to be implemented. As the field of OAE R&D is developing rapidly and dynamically, there will likely be changes in what is considered the most suitable OAE application approaches, in terms of cost, efficiency, environmental safety, friendliness in terms of monitoring, verification and reporting (MRV), technological readiness, as well as the regulatory requirements for their implementation. Mesocosm research in this field should maintain sufficient flexibility to respond to those changes and aim for testing 'real-world' scenarios of OAE applications. On the other hand, because the results

Kommentiert [28]: R1 L262: Pleased named here these different minerals, waste materials and electrochemical products and provide the references.

Kommentiert [29]: The reader is referred to the overview on feedstock materials by Eisaman et al.

Gelöscht: OAE

Gelöscht: MRV

Kommentiert [30]: L277: Please use entire words for MRV at the first use in the text (Monitoring, Reporting and Verification?).

obtained from mesocosm studies will likely be context-specific (depending on e.g. ecosystem type, time of year, latitudinal location, hydrographic setting and depend on the mesocosm set-up and operation itself, it takes multiple such studies for a given OAE approach to reach robust conclusions about its environmental safety. To facilitate inter-comparison between results it would be favorable to use standardized mesocosms and follow common protocols for mesocosm experimentation.

Gelöscht: , …)

From an experimental perspective, there is a trade-off between testing pure alkalinity enhancement and feedstocks which involve the release of other biologically active components. While the latter is more in line with real-world applications, it complicates the interpretation of the observed responses due to confounding factors and limits the extrapolation of the findings, considering that the stoichiometric composition differs between feedstocks. As the field is currently still at an early stage and considering that the number of mesocosm studies will likely be small due to their high costs, it seems beneficial to first establish a basic understanding of alkalinity effects in isolation, before turning to more feedstock-specific testing. This being said, we note that the above-mentioned confounding effects may actually be the intended research question or that the focus may be on a specific feedstock likely to be utilized widely. In general, we recommend designing mesocosm experiments with a more generic approach first and address feedstock-specific in smaller scale laboratory-based experiments.

Kommentiert [31]: R1 L285-293: This paragraph is very important and recommendations are very reasonable and logic.

Pelagic mesocosms

Alkalinity manipulations in pelagic mesocosms are fairly straightforward when done in dissolved form. Dissolving the alkaline feedstock in freshwater or deionized water prevents secondary carbonate precipitation during preparation of the concentrated solution (we note that the use of freshwater for feedstock dissolution may not be practical for large-scale implementation of OAE). To avoid confounding effects of the freshwater addition on the mesocosm community, the volume should be kept to a minimum. Using source materials with a high solubility in water, such as $NaHCO_3$, Na_2CO_3, $Ca(OH)_2$ or NaOH enables highly concentrated alkaline source water (Hartmann et al., 2023). To simulate CO_2-equilibrated alkalinisation $NaHCO_3$ and Na_2CO_3 can be combined in appropriate proportions (Subhas et al., 2022), for non-equilibrated alkalinisation carbonate-free source materials such as NaOH and $Ca(OH)_2$ can be used (Moras et al., 2021). To avoid prolonged pH peaks and secondary precipitation during the injection procedure it needs to be assured that the concentrated solution is mixed in rapidly. One way to achieve a uniform alkalinity enhancement across the water column is to move a distribution device with multiple outlets up and down the mesocosms at a constant speed (Fig. 5). Flocculent precipitates that form directly at the injection site are usually not stable and disappear quickly when further diluted through mixing. Care should be taken to ensure that the added alkalinity is evenly distributed throughout the enclosed water column.

Kommentiert [32]: R1 L296-307: Some references are welcome for this section.

Alkalinity enhancement in particulate form is far less practical. If the particles sink faster than they dissolve, they accumulate on the mesocosm floor or sink directly into the trap in mesocosms with a sediment trap at the bottom. Accumulation and subsequent dissolution at the bottom might lead to highly concentrated alkalinity enrichment, enhancing the risk of secondary precipitation and of strong negative impacts in bottom waters. Alkaline particles sinking into the sediment trap would be lost from the mesocosm enclosure during the next trap sampling. In both cases it would be considered an experimental artifact. It is therefore recommended to use minerals with high dissolution rates (e.g. NaOH, CaO, $Ca(OH)_2$, $Mg(OH)_2$) and small grain sizes to ensure dissolution before the mineral particles reach the bottom of the mesocosms (see Eisaman et al. 2023 for a detailed description of technical aspects of OAE).

Kommentiert [33]: R1 L314-315: It is therefore recommended to use minerals with high dissolution rates (e.g. CaO, Ca(OH)2) and small grain sizes to ensure dissolution before the mineral particles reach the bottom of the mesocosms. As the mesocosms are not very deep, and regarding to estimation of settling rate of these grains, what size these grains should have to dissolute before reaching the bottom of the mesocosm? It could be useful to provide in the text a notion about the grain sizes (less than ??) which are recommended to be use regarding the mesocosm deep.

Monitoring of seawater carbonate chemistry in the water column should adhere to the guidelines provided in Schulz et al., 2023. High levels of non-equilibrated alkalinisation can lead to secondary precipitation, triggering a process termed "runaway precipitation" (Moras et al., 2022; Hartmann et al., 2023), whereby carbonate formation can consume more alkalinity than initially added. It seems that the initiation of this process can occur both in the water column and on the mesocosm walls. As the carbonate crystals grow in size, their sinking velocity increases. When incorporated in organic matter aggregates they serve as ballast, thereby increasing the vertical flux of organic matter. In addition, carbonate crystals could affect mobility and feeding of plankton organisms, with possible adverse effects on food web interactions and trophic transfer. Secondary precipitation also increases seawater turbidity, affecting light attenuation and possibly primary production. Collecting this sinking particulate matter in sediment traps at the bottom of the mesocosms enables the quantification and identification of the precipitates and provides information about the chemical reactions leading to their formation. In mesocosms without integrated sediment traps, simple traps can easily be set up on the bottom and sampled through a tube that reaches the surface.

Kommentiert [34]: R1 L318: What could be the interaction of "secondary precipitation" with organisms in the water column of the mesocosm or in the mesocosm wall? It could be useful to provide some insights about this potential interaction(s), or if the effect of secondary precipitation on organisms is not clear, it can be mentioned in the text.

Kommentiert [35]: Add here something like this: Secondary precipitation may interact mechanically with plankton mobility and reduce the water transparency, hence hampering primary producers

Benthic mesocosms

Alkalinity enhancement in the benthic mesocosm approach is achieved by mineral addition, which dissolves in the surface sediment over time. In general, the addition of sedimentary OAE source materials (e.g. siliciclastic minerals, carbonates; Eisaman et al., 2023) modifies the grain size distribution of the sediment and thus affects the porosity, permeability, and water flow through the sediment. The changing sediment structure can impact living conditions for organisms, as well as the distribution and abundance of organisms living in the sediment and their behavior and ecology. With respect to mineral addition, the grain size selection is important, as a trade-off between grain size and production costs is required (e.g. Hartmann et al., 2013). Previous studies have investigated the relationship between CO_2-sequestration efficiency and grain sizes and there is a general assumption that small grain sizes reveal higher dissolution rates and CO_2 sequestration rates due to larger reactive surface areas, whereas more grinding energy is required generating a higher CO_2 footprint and lower CO_2-sequestration efficiencies (Köhler et al., 2010; Renforth and Henderson, 2017; Foteinis et al., 2023). Clearly, the CO_2 emissions during production and transport must be significantly lower than the potential CO_2 sequestration of benthic mineral dissolution (see Eisaman et al., 2023). The selection of appropriate grain sizes for the addition of alkaline minerals is a critical consideration for experimental studies, particularly in the context of the target environment's geological setting. From an environmental perspective, it is recommended to choose comparable grain sizes that are stable under in-situ hydrodynamic conditions. For highly dynamic ecosystems such as the Wadden Sea, estuaries and wave-dominated coastal areas, a range of grain sizes from fine to coarse sand (0.075 to 2 mm) may be appropriate for experimental approaches. However, in low-dynamic systems such as lagoons, enclosed bays, or shelf regions, grain sizes from silt to very fine sand (<0.075 mm) can be considered for investigation. This approach would also help to ensure that the sedimentary structure and settings for organisms in the mesocosms are representative of the natural conditions of the target environment.

Kommentiert [37]: L328: Please identify the mineral which was added.

Kommentiert [38]: Here we could also refer to Eisaman et al's chapter

Gelöscht: see also

It may be practical to interrupt the water circulation system during mineral deployment in order to allow sedimentation of the suspended matter. To achieve a uniform alkalinity enhancement in the benthic mesocosms, minerals should be evenly distributed. To induce a measurable effect on alkalinity changes in the envisioned experimental time, grain sizes smaller than 1 mm are desirable (Strefler et al., 2018). The addition to the marine environment could best be achieved through a mixture of natural seawater, marine sediments, and OAE source materials. This may ensure a more uniform distribution and reduce the purity of industrially produced OAE source materials, which are poor in nutrients and microbial organisms. Thus, this approach is also recommended for the addition of silicates to benthic mesocosms. By using a mixture, the potential effects of silicate addition can be more accurately evaluated because the experimental conditions are more similar to those in the natural environment.

For calcium carbonate, it may be reasonable to use the annual flux of POC to the seafloor as an upper estimate of the required mineral to be added. The underlying assumption here is that the added mineral can completely neutralize the natural CO_2 produced from organic matter degradation. However, this assumes that mineral dissolution efficiency is close to 100 %, which may not be the case if it is mixed below the undersaturated layers. Adding minerals in large excess risks clogging the surface layer and creating a physical barrier against effective benthic-pelagic coupling of solute fluxes. Finding the optimal mineral dosage to achieve a balance between dissolution efficiency and dissolution rate would likely be specific to the local environmental characteristics and require testing at each potential mineral addition site. For silicate minerals (e.g. olivine), the upper limit of mineral addition per square meter will also depend on the trace metal concentrations (Flipkens et al., 2021). Based on the variation in Ni content of marine sediments (prior to the addition of olivine), this implies that the allowable range for the addition of olivine is between 0.059 and 1.4 kg per square meter of seafloor without posing a risk to benthic biota. This threshold is based on Environmental Quality Standards (EQS), which are derived from metal toxicity data using methods such as species sensitivity distributions (SSDs). They provide threshold metal concentrations in seawater or sediment that are considered protective for the aquatic environment and are used by industries, governments, and environmental agencies to guide regulations. So far, these guidelines are only appropriate to specific regions and environments and may need to be re-evaluated for a broader use in OAE applications.

Gelöscht: rain rate

Kommentiert [39]: A. Poulton "It is not clear whether the authors mean rain ratio (PIC:POC) or sedimentation rate in this line. Based on the rest of the paragraph it is likely the former but this line is unclear. Please clarify and rephrase."

Kommentiert [40]: Now clarified.

Monitoring of mineral dissolution will be determined by the experimental design. A major drawback of a high through-flow is that rapid dilution and flushing of geochemical tracers emitted from the sediment compromises the analytical detection of dissolving alkaline minerals in the overlying water and the reliable assessment of the effectiveness of the method (see also section 4.4.3). In this case, alternative ways of mineral dissolution detection may be required. For instance, alkalinity enhancement may be detectable in pore fluids, which can be extracted using filters (e.g., rhizones) inserted horizontally through holes pre-drilled vertically in the tank (Fig. 6). However, the vertical sampling resolution may be too coarse to detect mineral dissolution close to the sediment surface. Microelectrodes for O_2, pH and H_2S are arguably a better alternative to detect changes in surface geochemistry in the uppermost centimeters after mineral addition. An advantage of the high dilution factors is the potential suppression of secondary mineral formation such as phyllosilicates and/or carbonates, that could reduce the net CO_2-sequestration efficiency of OAE (Fuhr et al., 2022, Moras et al., 2022, Hartmann et al., 2023). Secondary mineral formation is a common process in marine seafloor sediments, potentially impacting global carbon and element cycles on a global scale, the controlling factors are not unambiguously identified to date (e.g. Rahman et al., 2017; Torres et al., 2020; Geilert et al., 2023).

The deployment of benthic incubation chambers within the mesocosms themselves is a non-invasive method for detecting alkalinity release following mineral addition (Fig. 6). These benthic chambers enclose a certain area of the surface sediment and allow the accumulation of alkalinity and other components of interest over time, from which benthic fluxes can be determined. Mineral dissolution rates can be estimated by comparison with control mesocosms where no minerals were artificially added. Fluid sampling can be achieved by hand via suction using connected tubing and syringes. Care is needed to prevent hypoxia or anoxia inside the chambers due to respiration by benthic biota, which may be observable by a blackening of the sediment surface due to precipitation of iron sulfide minerals. Low oxygen levels will result in an interruption to the normal respiration rates of animals causing them to resurface. This may alter natural sediment mixing rates as well as mineral saturation states via changes in biogeochemical turnover rates and pathways in the sediment. Together, these undesired artifacts may be reflected in unrealistic fluxes of alkalinity and other solutes from the sediment. Completely interrupting the water flow to the whole benthic mesocosm in order to detect changes in bottom water alkalinity will only serve to magnify these side effects.

Recommendations

General

- Use inert materials for mesocosm hardware (e.g. plastics, stainless steel)
- Select the mesocosm size and experimental duration according to the enclosed community and processes studied
- Choose the experimental design to maximize the statistical power and report it
- Maximize similarity in starting conditions between mesocosms during enclosure filling
- Monitor starting conditions before applying experimental treatment
- Allow for the natural (e.g. seasonal) succession and avoid out-of-season events
- Avoid confounding factors and perturbations other than the intended treatments
- Adapt the sampling frequency to the dynamics of the processes studied
- Determine spatial heterogeneity and take account of it in the sampling strategy
- Apply depth-integrated sampling in case of vertical gradients (pelagic mesocosms)
- Minimize wall growth, e.g. by regularly cleaning the walls

OAE-specific

- Test real-world OAE scenarios, focusing on those most likely to be implemented
- Keep some flexibility to respond to changes in the OAE R&D field
- Monitor carbonate chemistry with at least two carbonate system parameters and watch out for secondary precipitation
- Maximize transferability of results by testing generic OAE approaches
- Take note of the context-specificity of the observed ecosystem responses
- Provide detailed information of the feedstock composition utilized for experimental manipulations

Kommentiert [41]: L386-387: Care is needed to prevent hypoxia inside the chambers. How does know the hypoxia occurred inside the chambers. By measuring continuously at high frequency oxygen concentrations inside the chambers? Otherwise?

Kommentiert [42]: Ln 396: Can the authors give some examples of the inert materials that should be considered?

Kommentiert [43]: R2 Lns 407-414: These recommendations are really important to the fledgling field of OAE research; should they be incorporated into the abstract to ensure they are taken up by the research community? The fourth (transferability) and sixth (feedstock) are essential for the community to ensure consistent and value-for-money OAE research that advances the field rather than causing confusion.

Competing interests

The contact author has declared that none of the authors has any competing interests.

Acknowledgements

This is a contribution to the "Guide for Best Practices on Ocean Alkalinity Enhancement Research". We thank our funders the ClimateWorks Foundation and the Prince Albert II of Monaco Foundation. Thanks are also due to the Villefranche Oceanographic Laboratory for supporting the lead authors' meeting in January 2023. UR acknowledges funding from the European Union's Horizon 2020 Research and Innovation Program under grant 869357 (project OceanNETs: Ocean-based Negative Emission Technologies analysing the feasibility, risks, and co-benefits of ocean-based negative emission technologies for stabilizing the climate). DB acknowledges funding from the Prince Albert II of Monaco Foundation for the OACIS project "Ocean alkalinity enhancement: a mesocosm-scale approach". SG and UR acknowledge funding from the German Federal Ministry of Education and Research (Grant No 03F0895) Project RETAKE, DAM Mission "Marine carbon sinks in decarbonization pathways" (CDRmare).

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Figure 1. Pelagic mesocosm facilities currently used in OAE research. *top left:* Land-based mesocosms (1 m^3) at the University of Vigo, Spain. *top right:* In situ on-shore mesocosms (10 m3) operated by GEOMAR, here employed on Gran Canaria, Spain. *bottom left:* Kiel Off-Shore Mesocosms for Ocean Simulations (KOSMOS), here employed in the Raunefjord, Norway. *bottom right:* Sketch of a KOSMOS mesocosm unit (55 m3). Photo/graphic sources: *ul:* Daniela Basso, University of Milano-Bicocca, *ur:* Ulf Riebesell, GEOMAR, *bl:* Uli Kunz, *br:* Rita Erven, GEOMAR.

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Figure 2. *top:* Benthic mesocosm units currently (2022-2023) installed at the Kiel Fjord, Germany. *bottom:* Sketch of the experimental set-up for the benthic mesocosms shown in top picture. Photo/graphic source: top: Sonja Geilert; bottom: Rita Erven, GEOMAR.

Figure 3: In the benthic mesocosms at the University of Antwerp the dissolution kinetics of silicate minerals and the impacts on the benthic fauna in coastal environments are monitored since 2019. The system comprises 20 units with two stacked tanks, the upper tank is housing the benthic ecosystem, and the lower tank is functioning as a water reservoir. Natural sediment of 40 sediment height with a mean grain size of 123 µm (3.0 phi) was collected from an intertidal sand flat in the Oosterschelde (Netherlands) and mixed with olivine sand of similar grain size. Water from the Easter Scheldt Estuary (salinity 32-35) is used to conduct flux-sessions of 5 weeks (weekly sampling). At the end of each session, the total volume of water in each unit (~500 L) is renewed (Drawing: A. Hylén, Photo: M. Kreuzburg, https://www.coastal-carbon.eu/, GeoBiology Research Group, Experimental Design and coordinating principal investigator: Prof. Filip Meysman, University of Antwerp).

Figure 4: *Upper left:* Distributor control system enabling parallel filling of all mesocosms. *Upper right:* Peristaltic pump ensuring smooth flow of source water during filling of the mesocosms, keeping damage to fragile organisms at a minimum. *Lower left:* Sediment traps forming the bottom of in situ mesocosm enclosures. *Lower right:* Programmable water sampler, enabling dept-integrated water samples over the entire mesocosm depth (or parts thereof). (Photo sources: *ul, ur:* Ulf Riebesell, *ll:* Michael Sswat, *lr:* Solvin Zankl)

Figure 5: *Left:* Distribution device used for alkalinity addition; by moving it up and down in the water column during alkalinity injection at constant speed a uniform alkalinity enhancement can be achieved. *Right:* Milky water at the outlet of the injection tubes indicates temporary precipitation which, however, quickly disappears as the highly concentrated alkalinity solution dilutes. Photo sources: Ulf Riebesell

Figure 6. *left:* Pore fluid sampling using rhizons. *right:* benthic incubation chamber to assess alkalinity enhancement with respect to mineral dissolution in benthic mesocosm experiments. Photo sources: left Sonja Geilert, right Michael Fuhr, GEOMAR.