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General considerations to experimental research on ocean alkalinity enhancement

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Abstract. The abundant literature on ocean acidification is documenting the impact of changes in the carbonate chemistry on marine life from genes to ecosystems. A vast majority of the experimental work was performed by manipulating the concentration of carbon dioxide in seawater under constant alkalinity (TA) to simulate near-future ocean acidification. Understanding the impact of changes in alkalinity on marine species and ecosystem is less understood. In the context of ocean alkalinity enhancement (OAE), it is critical to resolve such impacts, alone or in combination with other key trace elements to be co-released during implementation, to ensure that any field manipulation does not translate into damaging biological effects. As for other environmental drivers, this will require a biological understanding across all the levels of biological organizations from species to ecosystems, over relevant time exposure considering the method of deployment and considering factors such as local adaptation. Such complex questions cannot be resolved using a single approach and a combination of monitoring, modeling, laboratory, natural, and field experiments will be required. This chapter summarizes some key general considerations for experimental design as well as compare strengths and weaknesses of the different approaches. We will also consider best-practices relevant to OAE such as the need to properly monitor and consider the addition of trace elements and by-products as well as the potential interactions with other naturally occurring drivers.

1 Comparison of the different research approaches

Every scientific manipulation experiment, either in the field or in the laboratory, is an abstraction of reality. While best practices, in term of experimental design, endpoints measurements or monitoring of environmental conditions, are well-established (see Riebesell et al., 2011 in the context of ocean acidification), the outcome of any scientific study is strongly depending on experimental choices (e.g. tested scenarios, duration, level of biological organization, selected species or population, etc.) These are often resulting from a compromise between the requested design to test a given hypothesis as well as practical constrains and limitations. Understanding the impact of OAE on marine ecosystem is a complex question that can be broke down into multiple hypotheses. For each hypothesis, a strong scientific strategy involving multiple approaches and/or experiments is needed. These different approaches are described in the following chapter (see chapters 4.2 to 4.6). In this section, we will briefly describe and highlight the strengths and limitations of each approach (Figure 1).

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1.1 Laboratory experiments (See chapter 4.3, Iglesias-Rodriguez et al., for more information and references)

Laboratory experiments are classically used as a tool to test hypotheses and attribute biological changes to tested variables beyond the correlative approach often used for field observations and manipulations. A wide variety of approaches exist allowing for small to large size experimental units, single to multiple species or life-history stages, short or long-term exposure, and provide adapted options to work with organisms from bacteria to fish.

Strengths: Experiments in the laboratory offer a wide range of options and have the potential for the highest level of control in the tested parameters (e.g. physico-chemistry, food concentration, species composition, density, etc.) As such, laboratory experiments, in combination with other approaches, is the best alternative to build a mechanistic understanding of the biological impacts of OAE. While not without limitations, some experimental set-ups allow for a high level of replication, allowing to test complex questions highly relevant in the context of OAE (e.g. combined effect of increased alkalinity with trace elements, see below). As for any experimentation on living organisms, there are some ethical and sometime legal aspects associated with biological experimentation. However, those are much easier to resolve than with field approaches.

Limitations: While complex laboratory experiments can have degree of ecological realism, they cannot fully replicate the complexity of a natural ecosystem. For example, it can be highly challenging to include natural variability for all relevant physico-chemical parameters or incorporate the full complexity of an ecosystem. As such, mechanistic models developed from laboratory experiments needs to be validated in more realistic settings (e.g. field experiments).

1.2 Mesocosm experiments (See chapter 4.4, Riebesell et al., for more information and references)

Mesocosms are generally large-scale enclosed body of water, with (benthic) or without (free water column pelagic mesocosms) sediments, including biological communities and related processes that can be experimentally manipulated.

Strengths: Mesocosms experiments can partially compensate for the limitations of laboratory-based experiments. They sit between laboratory and field experiments and allow to evaluate the impact of the tested parameter(s) at the ecological level. Working in a close system minimizes the public concerns and legal requirement when compared to field trials.

Limitations: While mesocosms allows for a certain level of controls, allowing for other physico-chemical parameters to follow natural variability limits the ability to attribute the observed effects directly to the tested parameter(s). The size and complexity of mesocosms can also limit the number of replicates and then the ability to detect significant effects. Some other limitations include unnatural mixing and turbulence (pelagic mesocosms) or unnatural water flows (benthic mecososms) as well as limitation inherent to a close system.

1.3 Field experiments (See chapter 4.5, Cyronac et al., for more information and references)

Open-system field experiments consist of a direct manipulation (e.g. addition of alkalinity) in a natural system. This approach can be used to simulate an OAE deployment at scale.

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Strengths: This approach allows to evaluate the potential impacts at the ecosystem level in the real world while other environmental parameters naturally fluctuate.

Limitations: Several logistic (e.g. access) and legal challenges (e.g. permit, public acceptance) can be associated with field experiments. Similarly to mesocosm experiments, the cost of the ecological realism is the complexity in attributing the observed effect to the given treatment. It is complicated by the difficulty to truly replicate the experiment and to identify controls. However, this can be partly resolved by substituting space for time and replicating the experiment in time if not strong year-to-year variability is observed.

1.4 Natural analogs (See chapter 4.2, Subhas et al., for more information and references)

As for other physico-chemical parameters, alkalinity is not constant across the ocean. The dynamic alkalinity is linked to cycling of carbon dioxide, calcium carbonate and other minerals. As a consequence, some locations have conditions that can be used as "natural analogs" to OAE deployments. Natural analog sites present environmental conditions that resemble the conditions of an OAE deployment and can then be used as a test bed for both sensor deployments and collection of data on feasibility at scale and potential impacts on key species and ecosystems. These include glacial fjords and runoff into the marine system, seafloor weather of basalts, location of artificial material addition to the marine system, rivers plumes and deltas, and many others.

Strengths: Natural analogs provide the opportunity to work in the field at the ecosystem level and provide a test bed for the interpretation and validation of data collected in laboratory and field experiments as well as models. Different types of analogs can be used to address different space and time processes (Figure 2 in chapter 4.2) from hours at the deployment site to decades at the global level. Observation in natural analogs also have some practical advantages as it can be less costly than experimental approaches (e.g. mesocosms), logistically risky and does not require complex permits to implement (e.g. field manipulation).

Limitations: OAE analogs have the same constrains as any natural analog for other environmental parameters. While working in the field provides opportunities for collection of data at higher level of complexity, it lacks the control over the tested variable making it difficult to attribute any observed effect to one simple parameter. While some statistical options are available to disentangle the individual effects of the different environmental parameters (e.g. multivariate and regression analyses), a full attribution is not possible as many non-linear processes and complex interactions are unavoidable when ecology and multiple stressors are involved. This can be partly solved by incorporating mechanistic understanding and theoretical frameworks coming from more controlled laboratory and field studies. Other limitations include the difficulty of replication and identification of control sites. Natural analogs are also open systems with mobile species and propagule flowing through the ecosystem and introducing confounding factors and noise in the collected data.

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1.5 Modeling considerations (See chapter 4.6, Fennel et al., for more information and references)

The complex scientific questions associated with OAE will require a combination of approach to develop the needed mechanistic understanding and field validation. Models are a critical tool to bridge the different approaches, generate testable hypotheses, upscale from local to global aspects as well as forecast the outcome of different intervention strategies.

2 Identifying a relevant question

A pre-requisite to the selection of a given research approach or strategy is to define a clear question. A safe and efficient implementation of OAE requires to answer several key questions, including:

- What are the optimal implementation methods to optimize efficiency and minimize risks?
- Are the implementation of OAE safe for marine species and ecosystems?

These questions are too big and complex to be resolved by a single experiment or approach. Addressing fully these would require a large-scale involvement of the scientific community and strong international and multi-disciplinary collaboration. Exposure to elevated alkalinity at different rates and intensity, potentially combined with the other elements such as silicate, calcium, magnesium and trace metals (e.g. iron, nickel, cobalt, chromium) would expose natural ecosystems to conditions that strongly deviate from the present range of natural variability and has then the potential to drive stress responses. At present, these impacts are poorly understood. Understanding the impact of multiple environmental changes (alkalinity and the consequence for the carbonate chemistry, as well as other elements) on key marine ecosystems requires research at the crossroad between physiology, ecology, and evolution. As a comparison, after more than 2 decades of research on ocean acidification and the publication of more than 10000 scientific articles, we are still lacking the full mechanistic understanding that would allow to bridge chemical and biological changes and the forecasting ability required for science-based management (Cooley et al., 2022).

Regarding the urgency of the climate crisis and the limited resources, it is critical to quickly identify the key sub-questions that need to be urgently answered to provide informed guidance to if, how and when OAE should be implemented. These priorities should be identified in the spirit of the United Nation Decade of Ocean Science for Sustainable Development (The science we need for the ocean we want) and focus on the trade-off between the desirable level of understanding, the time needed to collect such data and how they can translate into concrete actions. Each question can organically translate into a research strategy and the selection of the appropriate approach(es), species/ecosystem, or experimental design (see next section).

Examples of key sub-questions to resolve the potential impacts of OAE on marine ecosystems include:

- What is the best material for a safe implementation of OAE?
- What is the safest deployment method?
- What makes a species or an ecosystem sensitive to OAE?

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Resolving these questions would allow to identify the best sites and methods for safe implementation but would require a complex experimental strategy combining laboratory studies (e.g. identify thresholds, resolve the combined effect of multiple drivers, develop a mechanistic understanding of how species and ecosystem resilience to OAE links to factors such as present natural variability, taxonomy, physiology, life-history strategies, trophic levels, etc.) as well as field experimentations, including in mesocosms, to validate mechanistic models.

3 Best practices: from a scientific question to an experimental strategy

A full consideration of best practices for experimental design is beyond the scope of this chapter. We will summarize some key general and OAE specific consideration while designing an experimental strategy or experiment. Adapting the famous quote by George Box, we can say that essentially, all experiments are wrong, but some are useful. Each research approach is associated with its own set of strengths and limitations (Figure 1) that combined with practical and technical constrains such as time, space, manpower, money, or expertise, lead to decisions that limit the relevance of the collected data. The full picture can only come from a combination of different approaches and different experimental decisions.

There are, however, some general best practices that should be followed including the importance of defining proper controls, properly monitor the physico-chemical parameters following established procedures, including proper calibration and use of reference materials, use the appropriate level of true replication, and follow best-practices for the measured endpoints.

Following best practices optimize the chance to identify the impact of a given environmental change. Variability is the rule in any biological data and can have different sources: technical (e.g. quality of the method used for the manipulation of a parameter or the measurement of an endpoint), experimental noise (e.g. confounding factors), and biologically relevant (e.g. genetic diversity or driven by the manipulated parameter). Each experiment should be designed to minimize unwanted variability. This includes randomization of the experimental units, proper training of the person(s) taking care of the experiments, or measuring the endpoints, etc.

For each question and associated experimental design, one must take the following decisions:

- What is my model organism or ecosystem?

One approach is to follow the Krogh's principle: For such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied. A given species can be selected for its life-history trait, longevity, physiology, phylogenetic position, sensitivity to the tested parameter, or role in the ecosystem. For example, to study the potential for genetic adaptation to OAE, a species with short generation time would be most appropriate. Model species may be considered when specific techniques are needed (e.g. functional genetics). Additional factors also need to be considered including size, life-history stage, age, weight, sex, etc. Different ecosystems can also be considered, number of trophic levels, level of complexity, etc.

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- Where to sample or perform the experiment?

As a consequence of local adaptation, species and ecosystems evolved different strategies to cope with different locations and environment. For example, different populations of the same species can have contrasting sensitivity to the same changes in the carbonate chemistry (Vargas et al., 2022). In the context of OAE, physical environment can also influence dilution rates, the distribution of the particles or the turbidity, the chemistry can also impact the dissolution, and the modulate other drivers or combined effects. The biological characteristics can also influence the potential sensitivity to changes (e.g. natural variability, redundancy, endangered species, other drivers).

- How to design my experimental unit?

To avoid introducing confounding factors, it is critical that the design of the experimental unit fits the tested species, community, or ecosystem. This includes using the right volume, density of biological unit, open vs. flow through, density of food, water used, aeration, currents, other physico-chemical parameters, etc.

- How long shall I conduct my experiment or observations?

Based on the question, different durations should be considered to ensure that the observed effect can truly be representative of the treatment. For example, this can be short-term, chronic, or dynamic depending on the tested OAE scenario.

- What is the general experimental design?

Two general experimental approaches can be used: the replicated scenario “ANOVA” approach and the gradient “regression” approach (Figure 2). There are pros and cons to both approaches. The regression approach allows to identify non-linear processes, resolve performance curves, and identify potential thresholds. However, there is the risk of not being able to properly analyze the collected data if no obvious trend is present. It is also possible to combine both approaches using a collapsed design (Boyd et al., 2018).

- Do I have the proper control(s) and treatment(s) to test my hypothesis?

All research approaches should consider the proper controls taking into account the present natural variability at the relevant spatio-temporal scale as well as conditions in the context of the implementation of OAE. The treatments can mimic a deployment of OAE and cover a wide range of alkalinity (e.g. 1500 to 4000 µmol Kg-1) and other parameters for a more mechanistic approach. Intensity or concentrations are not the only parameters to consider as the duration and dynamic of exposure can strongly vary depending on the implementation method.

- What endpoint(s) to measure?

A wide variety of endpoints and methods are available to evaluate biological impacts, including indicators of biodiversity, ecosystem health, and individual fitness. A rule of thumb is to use an endpoint that is as close as possible to the process under

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evaluation. For example, transcriptomic is often used to infer on organism physiology while there is very poor correlation between these two endpoints (Feder and Walser, 2005).

4 Best practices: Specificities to OAE

4.1 Manipulation of alkalinity

The desire to increase the alkalinity of aquatic environments is not new and predates the concept of OAE. For example, aquaculture farmers are traditionally using liming agents or sodium bicarbonate to restore pond alkalinity to increase photosynthesis and fish production, and to better buffer production water against possible pH changes over time. The so-called "liming" has been used through various materials or chemicals applied in ponds such as agricultural limestone, alkaline slag, agricultural gypsum (calcium sulfate), calcium chloride, slaked lime, quicklime, and lime liquor. While all these compounds mainly neutralize soil acidity before the filling-in with water the pond, some are more convenient or more effective than others (Boyd and Tucker, 1998). On a smaller scale, aquarists who farm ornamental marine life such as fish, crustaceans, and corals also carefully monitor seawater alkalinity. They use different methods to activate calcium and alkalinity such as additional water changes, kalkwasser (lime water), “balling” and devices such as calcium reactors containing alkaline material that can produce high-alkalinity liquid upstream of the aquarium (Goemans, 2012).

In the context of OAE, different methods of manipulating alkalinity are proposed. Two main options are generally considered:

- Addition of ground alkaline material or in situ enhanced weathering (best size, etc.)
- Pre-dissolution of alkaline materials or agents prior pouring the resulting liquid in studied waters.

These can be directly used in experiments while a more controlled manipulation of the chemistry (alkalinity and other substances) can be used to resolve the mechanisms and modes of action.

When alkaline materials are used, other compounds or impurities can also be released, such as silicate, calcium, magnesium and various trace metals (e.g. iron, nickel, cobalt, chromium). The main elements released through the use of lime, olivine or magnesite are Mg and Ca ions, along with minor elements like iron and trace elements, that occur at relatively low concentrations in seawater. While the concentration is low, their levels could be sufficient to affect marine organisms (e.g. Hauck et al., 2016; Moore et al., 2013). Therefore, the seawater contamination by the compounds and impurities inherent to alkaline materials has to be properly monitored and included in impact studies.

4.2 Monitoring compounds and impurities

There are many analytical methods available for measuring trace metals or other elements. The full process of collecting samples, analyzing dissolved trace elements is time consuming and complex. The existence of multiple chemical forms (speciation), specialized procedures for different elements due to speciation effects and contamination; such analytical works has to be coordinated with specialized laboratories/chemists. One of the major challenges in determining trace metals is indeed preventing contamination of environmental water samples during sampling and analysis (Benoit et al., 1997). Nevertheless,

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there are some good procedures available online validated by experts to collect and handle of sample for dissolved trace elements analysis (e.g. GEOTRACES, 2017; Noble et al., 2020). Among the different research methods discussed in this section, the survey of dissolved trace metals or other elements inherent in alkaline substances in seawater is easier to plan and to realize in laboratory experiments than in the field as the collection and handling of the samples is more straight forward, and the risk of contaminating samples are much lower.

An exhaustive list of analytical equipment available to analyze all possible compounds and pollutants released into the ocean from each candidate alkaline material is outside the scope of this chapter. The most suitable approach may be to combine a seawater preconcentration system (automated, such as seaFAST or non-automated; Hirata et al., 2000; Wuttig et al., 2019) with inductively coupled plasma-mass spectrometry (ICP-MS). There are exceptions for some elements, but this approach works for most elements expected to be released. Furthermore, the use of passive samplers has the advantage of better temporal and spatial resolution of marine pollution risks compared to discrete samples (Schintu et al., 2014; samples have then subsequently been analyzed in laboratories).

3. Combined effects of increased alkalinity and compounds and impurities inherent to alkaline materials

While many questions remained to be answered to fully address the potential ecological impacts of OAE, understanding the combined effects of increased alkalinity with other compounds and impurities is a tremendous challenge. Such questions require specific best practices and strategies (Boyd et al., 2018, IOC UNESCO, 2022). Changes in the carbonate chemistry and other dissolved elements are very likely to have different modes of actions that can lead to complex interactions. For examples, changes in the seawater chemistry can affect the chemical form and bioavailability of a given element (Millero et al., 2009). Resolving these interactions requires a combination of mechanistic studies resolving the mode of action, modeling and complex multi-stressor experiments.

Nickel may be one of the most important trace metal pollutants in olivine-based ocean alkalization, but there are other potential bioavailable trace metals (such as Cr, Cu or Cd; Bach et al., 2019) which all can be to a certain extent be bioaccumulated (Metian et al., 2007; Hédouin et al., 2010; Eisler, 2009). There is a large body of literature detailing the toxicity, subtoxic concentrations or bioaccumulation potential of many of the compounds release by OAE in marine organisms (e.g. compendium edited by Eisler is one of the most comprehensive sources of information; most elements have an extremely wide range of species - protozoa to vertebrates; Eisler 2009, 2010). However, the effects of some elements found in rocks have not been studied or are poorly reported (e.g., zirconium).

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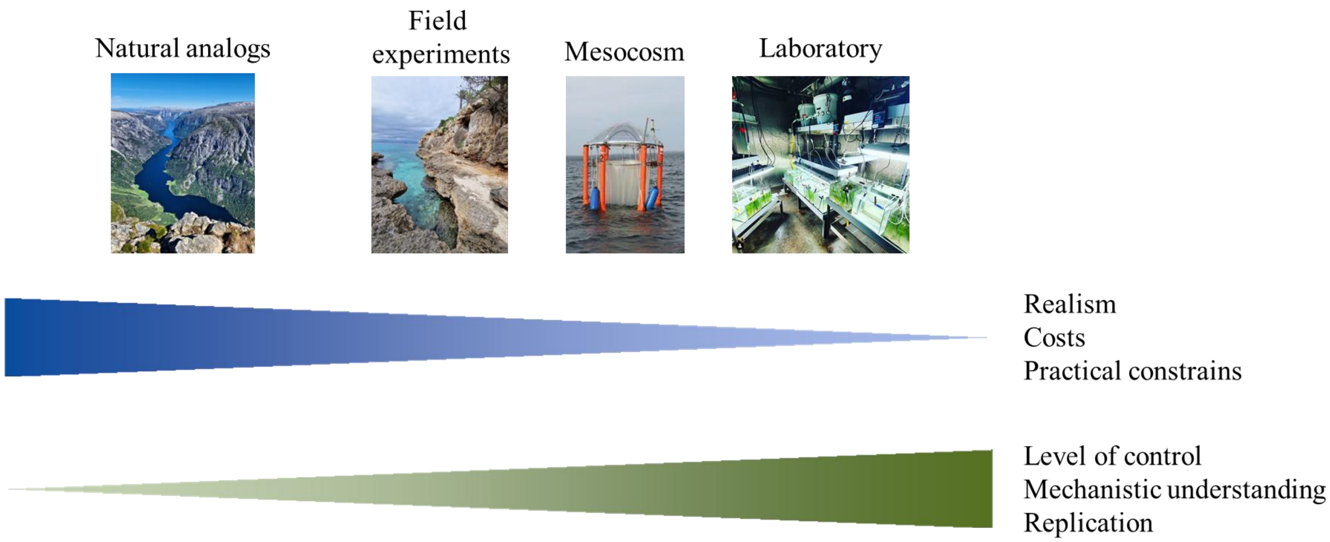

Figure 1: Simplified version of the strengths and limitations of different complementary research approaches. While the level of environmental and ecological realism decreases from natural analogs to laboratory experiments, field-based approaches are facing other complexities: high costs, legal and practical constrains, lower control and attribution to the tested parameters, lower level of replication. The selection of approach should be based on the question and most questions requires a strategy combining multiple approaches.

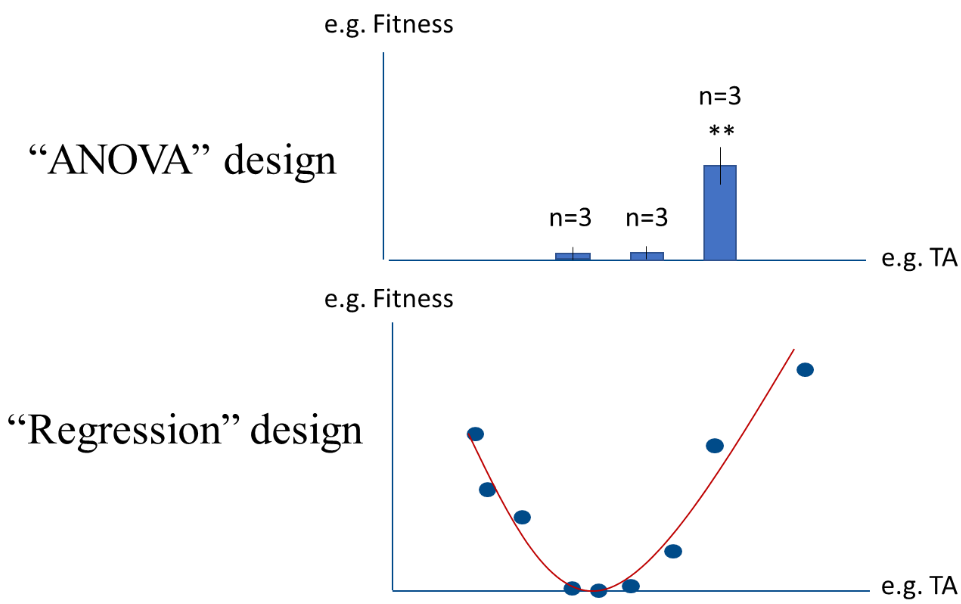

Figure 2: Illustration of two complementary experimental approaches using the same level of replication.