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Free Ocean CO_2 Enrichment (FOCE) experiments: Scientific and technical recommendations for future in situ ocean acidification projects

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ABSTRACT

Free Ocean CO_2 Enrichment (FOCE) experiments are a relatively recent development in ocean acidification research, designed to address the need for *in situ*, long-term, community level experiments. FOCE studies have been conducted across different marine benthic habitats and regions, from Antarctica to the tropics. Based on this previous research we have formed some core operating principles that will aid those embarking on future FOCE experiments. FOCE studies have potential to provide important insight into the effects of ocean acidification that can add to or refine conclusions drawn from laboratory or single species studies because they are conducted *in situ* on intact assemblages. Scaling up from sub-organismal and individual effects to also include indirect impacts on the ecosystem and ecosystem services, make FOCE experiments essential in filling in current knowledge gaps in our understanding of ocean acidification. While FOCE systems are complex, relatively costly, and somewhat difficult to operate, the challenges they pose are tractable and they have proven to be a useful approach in ocean acidification research. The aim of this paper is to draw from the experiences of past FOCE experiments and provide practical advice for designing, building and operating a FOCE experiment. Some of the most important recommendations include: field testing the system design; having a backup power supply; using replicate treatment enclosures; monitoring and maintaining the chemistry appropriately; allowing sufficient time to achieve near CO_2 equilibrium conditions; and having a scientific focus with a core set of hypotheses. Future FOCE experiments could focus on longer durations, multiple factors, and testing more intact benthic marine communities and ecosystems. We hope this paper will encourage further FOCE deployments and experiments, as well as provide some guidelines to improve future FOCE studies and advance ocean acidification research.

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1. Introduction

Understanding the potential consequences of ocean acidification and other climate-related changes in ocean conditions for marine biological communities has been identified as a priority goal in ocean research (Mason et al., 2017; Rudd, 2014). Ocean acidification, the shift in the carbonate chemistry of the oceans, is a direct result of the rapid rise in atmospheric CO_2 concentrations associated with human activities, particularly fossil fuel use (Caldeira and Wickett, 2003; Canadell et al., 2007; Ciais et al., 2014; Le Quéré et al., 2016; Raupach et al., 2007). It is estimated that the oceans have absorbed 24.6% of the CO_2 emitted to the atmosphere since the beginning of the industrial revolution (Le Quéré et al., 2018), resulting in a 0.1 decline of pH in the upper ocean mixed layer (Bindoff et al., 2007; Rhein et al., 2013; Sabine et al., 2004). Ocean acidification poses multiple challenges for marine organisms from molecular to ecological scales. Changes in ocean conditions can affect populations directly through shifts in the performance (physiology, behavior, survival, growth, reproduction) of individuals, which in turn may drive a cascade of indirect consequences due to changes in the strength of interactions among species (e.g. competition, predation) (Allan et al., 2013; Burnell et al., 2014; Poore et al., 2013).

Laboratory experiments and field observations have documented several ocean acidification impacts. It can cause reduced calcification in marine organisms (Doney et al., 2009; Erez et al., 2011; Kleypas and Yates, 2009; Kroeker et al., 2013), however, impacts are often complex and life-stage dependent (Dupont et al., 2010; Fabry et al., 2008; Hendriks et al., 2010; Iglesias-Rodriguez et al., 2008; Ingels et al., 2012). Furthermore, ocean acidification may have significant impacts when combined with other environmental changes such as hypoxic upwelled waters (Bograd et al., 2008; Nam et al., 2011) and ocean warming (Queirós et al., 2015a; Rühl et al., 2017). For example, the widespread die-offs of oyster larvae at Pacific Northwest commercial hatcheries were due to high pCO_2 associated with upwelling events between 2006 and 2008, severely impacting an industry that generates over US$270 million in annual revenue (Miller et al., 2009). Ocean acidification will very likely cause major ecological, economic, and social impacts on coastal ecosystems. For example, it has been estimated that in the United Kingdom there will be significant regional impacts on the livelihoods of coastal communities reliant on wild fisheries as a result of regional ecological impacts (Fernandes et al., 2017). Technology and methods that enable experiments that can advance our understanding of the consequences of ocean acidification in natural ecosystems over ecological to regional scales are a high priority for ocean research (Andersson et al., 2015).

Riebesell and Gattuso (2015) highlight the need to develop experimental approaches in ocean acidification research that scale up from: (1) single to multiple environmental drivers, (2) single species to communities and ecosystems, and (3) measures of acclimation to species' capacities for adaptation to future conditions. Addressing these challenges requires the development and emphasis of new inferential approaches that can measure the response of species within intact assemblages or communities to multiple environmental changes, over long time scales of weeks to months. Such ecological approaches are vital in addressing more far-reaching goals concerning the ecological and evolutionary responses of marine species and systems to escalating anthropogenic change in ocean conditions, and their implications for society. Alternative experimental approaches that have promoted our understanding of the influence of ocean acidification at the level of species assemblages and communities include experimental or correlative studies along natural gradients in ocean carbonate parameters (e.g. natural CO_2 vents), and pH perturbations in mesocosms enclosing planktonic communities (Riebesell et al., 2008).

Gattuso et al. (2014) defined Free Ocean CO_2 Enrichment (FOCE), "as a technology facilitating studies of the consequences of ocean acidification for marine organisms and communities by enabling the precise control of CO_2 enrichment within *in situ*, partially open, experimental enclosures." FOCE experiments examine the effects of ocean acidification under more natural settings than laboratory conditions, typically consisting of a series of enclosures which are placed on the seabed over a habitat or community of interest. Modified elements (such as biological organisms) can also be added into the enclosure. The enclosure is semi-open, with surrounding ambient seawater flowing through it, which is manipulated to change the pH of the seawater, usually by the addition of CO_2 enriched seawater, which mixes with ambient seawater to produce the desired pH offset. This offset, from pH levels measured in the surrounding environment, is maintained for the duration of the experimental period and reproduces natural variations in environmental pH. Enclosures are sufficiently sealed to permit control of pH and prevent ingress of large mobile organisms. Some examples of manipulated pH conditions in FOCE experiments relative to a control enclosures and ambient conditions are shown in Fig. 1.

In practice, FOCE experiments are complex and difficult to implement, however, there have now been a number of successful experiments completed in a variety of locations, from the tropics to the Antarctic (Table 1, Fig. 2). The aim of this paper is to draw on the knowledge and experience gained from these experiments and provide practical advice for designing, building and operating a FOCE experiment. This paper is a by-product of the xFOCE group, an open and informal group of scientists and engineers created to provide guidelines and best practices information for future users and to encourage open source development of FOCE technology.

1.1. Why do a FOCE experiment?

One of the main gaps in our understanding of ocean acidification are the long term impacts on benthic communities, ecosystems and ecosystem services (Riebesell and Gattuso, 2015). FOCE studies were specifically designed to help address these needs and their rationale has been well summarised by Gattuso et al. (2014). The primary aim of this approach is to move from understanding single organism responses to ocean acidification, to the community and ecosystem level, through the use of *in situ* experimental approaches involving natural marine benthic communities. FOCE experiments address some of the limitations of other approaches for *in situ* ocean acidification experiments, such as time series analyses of changes in communities resulting from changes in carbonate chemistry and in naturally high-CO_2 analogue studies such as CO_2 vents and seeps. Other *in situ* methods have made important contributions to fill gaps in understanding community and ecosystem responses (Molari et al., 2018; Sunday et al., 2017), however, FOCE experiments address an important deficiency in natural analogue studies as they enable the precise control of parameters such as temporal pH variability (Kerrison et al., 2011); control over treatment conditions or reference sites; and movement of organisms into or out of the study area; while maintaining ecological interactions within the enclosures (Gattuso et al., 2014). FOCE experiments complement other methods such as those performed in aquaria, mesocosms, or natural environmental pH gradients. Andersson et al. (2015) and Gattuso et al. (2014) compare the strengths and weaknesses of different experimental approaches, including FOCE studies, and suggest that a combination of approaches are required to understand the impacts of ocean acidification at a range of spatial and temporal scales.

1.2. Capability

The primary capability of FOCE is to provide *in situ* experiments to test the impacts of ocean acidification on benthic communities, with well controlled pH conditions that incorporate natural pH variability. FOCE is thus a holistic experimental approach that can enable the detection of both the direct and indirect effects of ocean acidification on species, communities and processes, potentially providing a more comprehensive assessment of the 'real-world' outcomes of future ocean conditions than other approaches. Environmental conditions such as

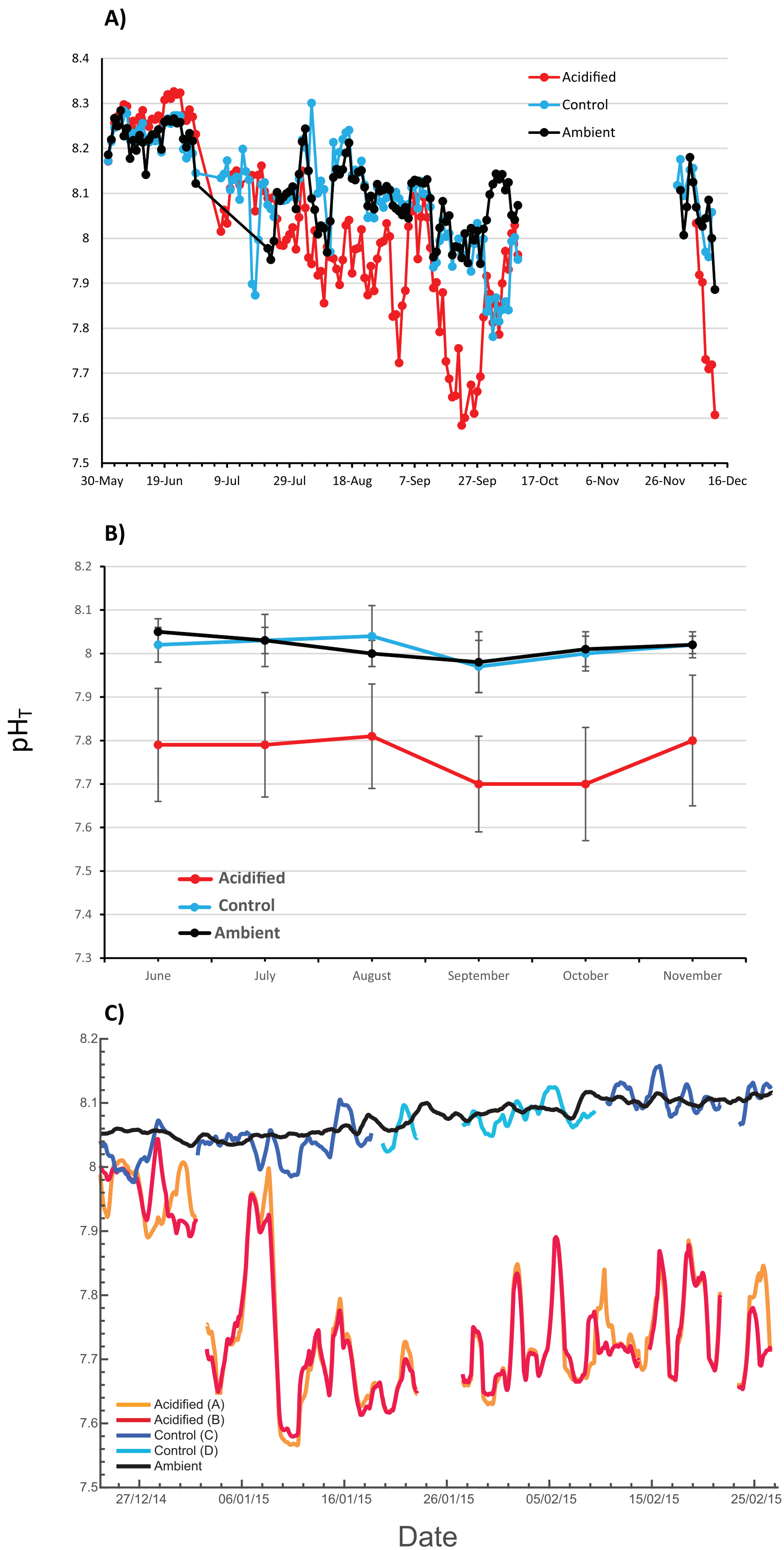

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Fig. 1. Manipulating seawater pH in FOCE experiments. A comparison of pH in control and acidified treatments in relation to ambient pH in three different FOCE experiments: (A) cpFOCE daily average pH, errors not shown, data from (Georgiou et al., 2015); (B) eFOCE monthly averages (± SD), data from (Cox et al., 2016); (C) pH antFOCE experiment (24 h moving average, error not shown), data from (Stark et al., 2018). Gaps in data due to power loss. pH_T = pH total scale. Red/orange line = acidified treatment; blue lines = control treatments; black line = ambient background pH.

temperature, oxygen, light and natural food concentrations are difficult to mimic even in the most controlled laboratory experiment. These advantages make FOCE an ideal approach to help address current gaps in understanding of ocean acidification impacts, including long-term and multi-stressor effects (Riebesell and Gattuso, 2015).

An important goal of FOCE experiments is long term studies, from months to years, with previous experiments ranging from 10 days to 8 months (Table 1). A deep (890 m) offshore version, dpFOCE, operated continuously for 17 months in engineering tests (Kirkwood et al., 2015). The potential exists to run long experiments, particularly now that the technology is proven and system design principles are well established. The main hurdle for running longer experiments is cost, however, the recent emergence of publications from the various FOCE experiments may help to provide justification to obtain the required funding for longer experiments. Compared to the terrestrial Free Air CO_2 Enrichment (FACE) experiments (Norby and Zak, 2011), some aspects of FOCE studies are more expensive to run, largely due to the challenges of logistics at sea, biofouling and system maintenance in the marine environment, although CO_2 costs are typically much lower because FOCE experiments are partially enclosed. The duration of a FOCE experiment also influences the type of response information that can be obtained. Short-term experiments can provide information on stress-responses induced from single species to communities, whereas longer experiments (months to years) can give insights of acclimatization mechanisms of macro-organisms and adaptive responses of micro-organisms, in addition to ecological dynamics (Fig. 3).

One of the most promising capabilities regarding FOCE is the incorporation of multiple environmental stressors into experiments. Some factors would be difficult to manipulate, such as warming a large volume of water flowing through a FOCE system. Manipulating oxygen levels may be more achievable on the spatial and temporal scales that recent FOCE experiments have utilized. It would also be logistically simple to use dosing pumps to dose with different components of pollution or other contaminants. Investigating factors such as contaminants may also be achievable by deploying FOCE experiments in polluted and control, clean areas. The potential to conduct multi-stressor experiments in a FOCE platform provides an exciting and much needed opportunity for future researchers.

1.3. Limitations

In practice, retaining natural variation in most environmental factors must be balanced with the requirements for control of the ocean carbonate system, leading to a flume-like or partially-enclosed mesocosm design for most systems used to date. FOCE enclosures introduce some potentially confounding factors including changes in hydrodynamic conditions (such as restricted flow or reduced sedimentation), alteration of light fields (via transmission properties of enclosure material, and sedimentation and biofouling onto enclosure), exclusion of large mobile organisms, or modification of scale-dependent processes due to enclosure size. This “enclosure effect” is quantifiable when control enclosures (no pH manipulation) and open plots (no enclosure) are included in the experimental design. The enclosure also limits water exchange with ambient seawater, which is of course a requirement of the experimental design to be able to maintain a pH difference. However, an undesirable consequence is that pH diel variability can be magnified in metabolically active communities, as was observed in eFOCE (Cox et al., 2016) and antFOCE (Stark et al., 2018).

One of the main limitations is the difficulty in replicating large enclosures on the seabed (see Section 3). Enclosures are also physically limited in size, due to issues associated with constructing large underwater enclosures and maintaining adequate flow within them, and susceptible to wave and storm energy. The potential size limits of FOCE have not been explored, but the larger the enclosure, the bigger the engineering challenges are in obtaining a uniform pH offset and flow. Nevertheless, it is possible to use enclosures encompassing several square meters of seabed utilizing existing methods.

2. What scientific questions are FOCE systems most capable of addressing?

FOCE systems are amenable to a spectrum of research questions, spanning a range of life history phases of benthic taxa and processes important in shaping natural communities (Fig. 3). FOCE facilities can be used to address questions concerning the influence of ocean acidification on colonization, physiological performance, growth, survival, succession, mediation of predation and competition, community metabolism, nutrient cycling, and other processes in benthic communities. Studies on keystone species (Paine, 1966), foundation species, or other taxa likely to play a key role in regulating community structure or function are likely to be more informative concerning the broader influence of ocean acidification in marine ecosystems.

2.1. What scientific questions have FOCE experiments addressed to date?

FOCE experiments to date have focused on a wide variety of scientific questions. The tropical cpFOCE was the first to demonstrate the ability of FOCE systems to realistically simulate ocean acidification conditions in situ in a shallow water coastal ocean environment (Kline et al., 2012). It was used to investigate calcification of corals (measured as growth and skeletal density) and found that there was no effect on calcification rates or the pH of calcifying fluids (Georgiou et al., 2015). This included an important discovery that some corals are able to maintain pH homeostasis during calcification even under highly fluctuating conditions that reach low pH, and indicating a degree of resilience to ocean acidification (Georgiou et al., 2015). The cpFOCE also revealed an unexpected finding that crustose coralline algae could produce dolomite, a more resistant form of calcium carbonate that would make coralline algae ridges more resistant to ocean acidification than previously thought (Nash et al., 2011; Nash et al., 2012). These results contrasted with theories predicting increased solubility of crustose coralline algae with increasing magnesium content (Andersson et al., 2008), because of this previously unknown calcification mechanism in coralline algae (Kline et al., 2012; Nash et al., 2012). The dpFOCE, deployed on the seafloor at 890 m depth in Monterey Bay, California, investigated effects on urchin behavior and found significant effects on foraging time indicating that ocean acidification could impair chemosensory behavior (Barry et al., 2014). The eFOCE demonstrated the feasibility of manipulating pH at diving depths in a natural intact community. eFOCE investigated the hypothesis that seagrass will benefit from future ocean acidification. It found little evidence to support this hypothesis, casting doubt on theories predicting increased resistance of seagrass to thermal stress and increased buffering capacity under elevated CO_2 (Cox et al., 2016). eFOCE also found evidence of seagrass communities ability to buffer OA effects for many calcifiers; there were very little effects of CO_2 enrichment on epiphytic communities on seagrass with only foraminifera showing any evidence of negative effects among other calcifiers. In comparison, on artificial substrata, there were reductions in recruitment of calcifying species (crustose coralline algae and tube building polychaetes) with sensitive

Table 1
Location and habitat of each of the FOCE experiments attempted to date.

Experiment	Location	Habitat	Depth (m)	Duration of experiment	Principal organisation	Scientific focus and key references
eFOCE – European FOCE	Mediterranean, France	Seagrass	12	4 months ambient, 4 months acidified	Laboratoire d'Océanographie de Villefranche	Seagrass growth and photosynthesis, seagrass epiphytic community, seagrass epibiont recruitment, crustose coralline algal and bulk epiphytic mineralogy (Cox et al., 2017a, 2017b, 2016)
cpFOCE – coral prototype FOCE	Heron Island, Great Barrier Reef, Australia	Coral reef flat	1–4	6 weeks ambient, 6 months acidified	University of Queensland	Coral growth, calcification and photosynthesis; sediment and crustose coralline algae mineralogy (Georgiou et al., 2015; Kline et al., 2015, 2012; Marker et al., 2010; Nash et al., 2012)
dpFOCE – deep FOCE	Monterey Bay Canyon, USA	Sediment	890	17 months test, 32 days acidified	Monterey Bay Aquarium Research Institute	Sea urchin foraging behavior (Barry et al., 2014)
antFOCE – Antarctic FOCE	Casey Station, Antarctica	Under-ice sediment and reef community	14	4 weeks ambient, 8 weeks acidified	Australian Antarctic Division	Sediment community response – microbial, meiofauna, macrofauna, microphytobenthos. Hard substrate community response on settlement panels, in biofilms and artificial substrate units (ASUs). Sediment biogeochemistry and bioturbation (Stark et al., 2018)
swFOCE – shallow water FOCE	Monterey Bay, USA	Sand	13	Test phase only	Monterey Bay Aquarium Research Institute	Focus on nearshore taxa including colonization, succession of kelp bed organisms, calcification, growth, survival, and species interactions.

early development stages (Cox et al., 2017a, 2017b). The utility of the FOCE approach was further demonstrated in the antFOCE experiment in coastal Antarctica, successfully manipulating the carbonate chemistry of subzero temperature waters despite the extended time required for pH equilibration (Stark et al., 2018). Thus, published FOCE studies to date have covered important areas of ocean acidification research, from behavior to effects on primary producers and calcifiers, and have made an important contribution to understanding elevated CO_2 effects in real world environments at community and organism levels.

2.2. Towards a framework of core hypotheses

The FOCE experiments conducted to date have operated completely independently, without common goals or common questions. The development of specific sets of hypotheses, based on well understood ecological theory (Gaylord et al., 2015), that could be pursued in a more coordinated approach, would greatly aid in increasing understanding of future responses to ocean acidification. A set of testable guiding hypotheses proved to be a valuable approach in the well-established terrestrial FACE experiments and extended the understanding of effects of increased CO_2 from the scale of plant responses to ecological responses incorporating large spatial and temporal scales (Norby and Zak, 2011). Such an approach could apply equally well to FOCE and would enable greater comparability among different experiments. In addition, greater efforts to incorporate experiments with ecological theory will increase the capacity to generate insight into the consequences of ocean acidification (Gaylord et al., 2015). We suggest hypotheses based around the following areas as a useful starting point:

- Community structure and biodiversity – Ocean acidification will change marine communities (spanning organisms from microbes to macrofauna and microflora to macroflora)
- Net primary production and plant growth will increase under future elevated CO_2
- Calcification of marine organisms will be negatively affected, leading to dissolution in some species and habitats
- Trophic interactions will change as a result of changes in community structure (including changes to competition and predation)
- Chemosensory responses will be affected leading to changes in animal behaviour and biological interactions
- Important ecosystem functions and ecological processes will be affected, e.g. bioturbation, nutrient cycling (e.g. nitrification in sediments) and carbon cycling

Focus on a core set of hypotheses will accelerate the development of general principles regarding the responses of marine ecosystems to ocean acidification.

2.3. Scaling up and modeling from FOCE studies

Projecting the potential effects of ocean acidification on ecosystems, and how we may shape ocean management efforts to limit its impacts, requires predictive models of marine ecosystem structure and function that operate at this scale (Queirós et al., 2016). Ocean acidification model development has been limited by key aspects such as a mismatch between the scale and diversity of the focus of research, as well as the data type and scaling needs of model development (Koenigstein et al., 2016; Queirós et al., 2015a; Riebesell and Gattuso, 2015). Laboratory studies, where the inherent complexity and variability of natural ecosystems is removed, are important in quantifying and describing mechanisms linked to ocean acidification that can be used to determine mathematical representations to be applied within models. Laboratory studies, however, do not provide information about the interplay between the various processes through which ocean acidification impacts a local community or ecosystem. Ecosystem-level studies of ocean acidification, therefore, remain largely absent. This is where the FOCE

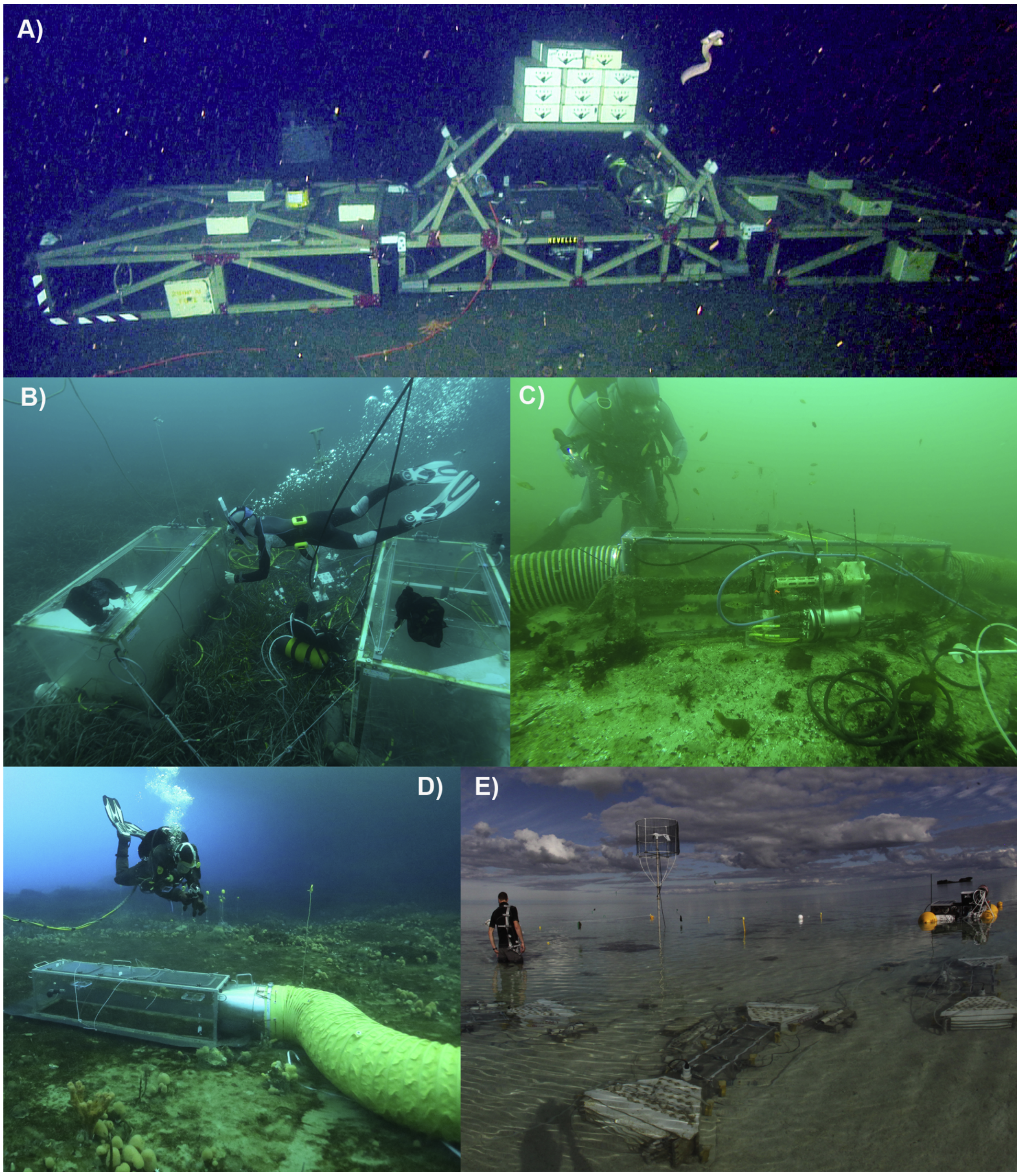

Fig. 2. Examples of FOCE experimental deployments in different habitats and regions. (A) dpFOCE in 890 m depth in Monterey Canyon, California, USA; (B) eFOCE in a shallow (12 m) Mediterranean seagrass bed, Bay of Villefranche, France; (C) swFOCE in 13 m in Monterey Bay, California, USA; (D) antFOCE in 13 m of water underneath sea ice, Casey Station, Antarctica; (E) cpFOCE at low tide on the coral reef flat, Heron Island, Great Barrier Reef, Australia.

approach may provide real value: in the generation of the data required for the development of models that incorporate ecosystem-level understanding of the impacts of ocean acidification. The manipulation of environmental conditions within a whole community/habitat setting offered by FOCE could allow detection of potential changes in the flows of energy and matter between different community components; biogeochemical properties and processes; as well as potential interactions between various community components or processes. Thus, the FOCE approach can provide a more holistic, contrasting, yet complementary approach to other ocean acidification research efforts (Riebesell and Gattuso, 2015).

Furthermore, the use of standardised FOCE methodologies across ecosystems, from tropical to polar regions and shallow to deep environments (Table 1) supports the ability to derive regional responses and general principles about how ocean acidification modifies marine ecosystems. Such coordination may enable differentiation between general responses to ocean acidification and those impacts specific to local ecosystems. These two attributes conferred by a FOCE approach (community level manipulation and standardisation of information across ecosystems) provide clear advantages that will promote progress in developing ecosystem models that may help us forecast ecosystem-level impacts and support adaptive management solutions. As with FACE experiments (Norby and Zak, 2011), models can be used to generate ecosystem level hypotheses that can be tested in a FOCE experiment. Results from FOCE can then feedback into ecosystem model development related to ocean acidification. FOCE experiments can facilitate the testing of hypotheses that contribute to the development of ecosystem models concerning the long-term responses of benthic ecosystems experiencing ocean acidification and associated environmental changes.

3. Experimental design

3.1. Factors to consider for FOCE experimental design

The strength of FOCE experiments as community level, realistic, large scale manipulations also leads to their main weakness: the

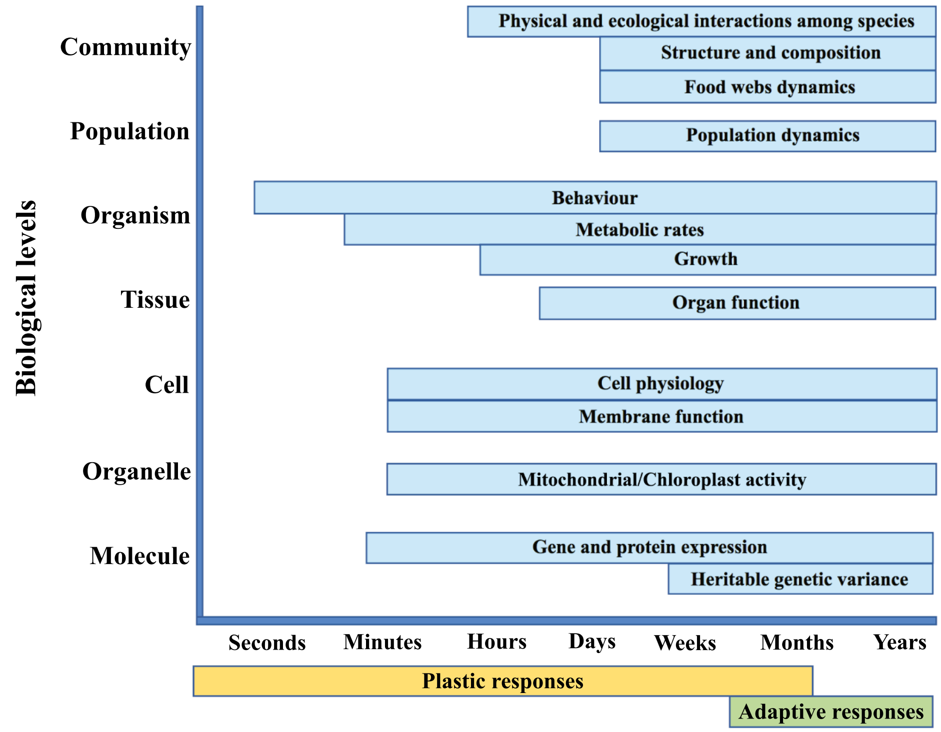

Fig. 3. Conceptual relationships between scale of biological organisation level and time required to obtain information. Experiment duration has an important effect on the types of questions and information obtained.

difficulty in replicating experimental units and the implications for obtaining sufficient statistical power. However, as recent reviews on the subject of limited replication have stated, this does not detract from their potential value and contribution to ecological theory and there are many ways to deal with this issue (Davies and Gray, 2015; Kreyling et al., 2018; Oksanen, 2001). Experimental design as it relates to ocean acidification research has been well examined (Cornwall and Hurd, 2016; Gattuso et al., 2014; Havenhand et al., 2010), but here we explore some of the issues specifically related to design and analysis of FOCE experiments.

The basic design for a FOCE system consists of perturbed and control enclosures, and in some previous experiments, open control enclosures, or plots in the ambient environment. In the perturbed enclosure, carbonate parameters are manipulated to mimic a designated future scenario of high CO_2 conditions. The control enclosure experiences all of the experimental artifacts in the perturbed enclosure (e.g. altered local hydrodynamics) but maintains the natural carbonate chemistry environment that exists at the experimental site. Open control plots (i.e. areas of un-enclosed habitat) represent the natural baseline of the habitat studied, and are used to account for possible artifacts created by the addition of a physical structure in the environment (e.g. changes to local water movement, predation). For example, through use of open plot controls, the eFOCE experiment found patterns in mineral changes and organism abundances were most closely related to the effect of the enclosure structure and not related to lowered pH (Cox et al., 2017a).

3.2. Replication

The challenges of replicating multiple enclosures at each treatment level (due to the expense and manageability of deployment and maintenance) has led to minimal or unreplicated FOCE experiments. This can be dealt with using the following approaches:

i. Understanding the limitations on interpretation of results if replication is minimal or if the experiment is un-replicated, and exercising caution when drawing conclusions. FOCE findings should be placed into the context of other study outcomes drawn from the literature.
ii. Quantitative assessment of spatial variation in target habitat/communities prior to implementation to inform robust design. There is strong potential to confound treatment effects with spatial variation, as benthic systems, their species and processes, are spatially heterogeneous (Barry and Dayton, 1991; Pischedda et al., 2008). This applies in particular to examination of community responses, whereby communities may differ naturally at the spatial scale of the experimental layout (Kendall and Widdicombe, 1999; Morrisey et al., 1992). For FOCE, one approach to account for spatial variability but limit costs is to increase the number of replicate open plots to determine if the acidification (or enclosure) effect is outside the bounds of natural variability. Although technically challenging, another approach would be to increase the size of the enclosures to encompass as much of the fine scale spatial variability as possible. The size of the enclosures determines the spatial scale of the variability captured, however, the spatial scale at which natural variability operates is relative to the size-class of the organisms (e.g. variability of microbial and meiofauna communities will operate on a smaller scale than macrofaunal communities). Thus, replication and sub-sampling strategies and the related decision of capturing an appropriate degree of variability for different organisms requires careful planning.
iii. Increased temporal replication within the same enclosures in a repeated measures design (Green, 1993; Stewart-Oaten et al., 1986). Such designs are prevalent where the cost of establishing additional experimental treatments is prohibitively time consuming or expensive compared with taking repeated samples in established units (Underwood, 1997). For example, (Cox et al., 2016) used repeated monthly sampling both before and during the pH manipulation in eFOCE to study the effects of lowered pH/increased pCO2 on seagrass leaf traits. Any large change or deviation in pattern through time within the pH manipulated enclosure from the control enclosure and open plot, associated with timing of pH manipulation,

would indicate an ocean acidification effect. This type of design, however, introduces temporal non-independence among sampling times leading to increased or decreased probability of Type I errors (Underwood, 1997). In cases where there are replicate enclosures, the temporal trend in each enclosure should be used to test the hypothesis that "temporal trends will differ under acidification treatments", whereby each temporal trend is considered a replicate (Underwood, 1997).

iv. Repeat FOCE experiments and effectively replicate through time, however, this approach has not been attempted and has limitations. Responses of organisms and processes to ocean acidification can be heavily influenced by the time or season in which the experiment is run (Godbold and Solan, 2013; Queirós et al., 2015b; Zhang et al., 2015).

v. The use of a regression or gradient design rather than a replicated ANOVA design (see Section 3.3) is another promising approach (Kreyling et al., 2018) and is useful for revealing underlying processes. For example 6 FOCE chambers could be used to test 6 different pH levels and compare the community response to this pH gradient rather than having 3 replicate controls and 3 replicate low pH chambers.

It is highly desirable for future FOCE designs to replicate enclosures beyond two per treatment, the maximum number used to date. Increasing replication will be particularly challenging for FOCE experiments that require large units; deployment in a challenging environment; multiple exposure levels; or lengthy experimental periods. Ideally, experimental designs for *in-situ* long-term studies would include both spatial and temporal replication.

The nature of mesocosm or enclosure experiments means that many replicate sub-samples are usually taken from within each structure, raising the issue of non-independence of observations within and between treatment groups. While multiple measures within one enclosure may be non-independent, they are vital for understanding intra-enclosure variation and obtaining reliable and representative estimates of average responses of each enclosure to the experimental conditions (Havenhand et al., 2010). To estimate within treatment variation, however, replicate enclosures are necessary, as this is the level at which the response to the experimental manipulation is measured, for which multiple, independent measures are needed. Replication at levels other than that at which the treatment has been applied was termed pseudoreplication by Hurlbert (1984). However this term is often used to imply a design is fatally flawed, and is perhaps overused, leading to unwarranted stigmatization of reasonable ways to test large scale ecological questions (Davies and Gray, 2015; Oksanen, 2001).

3.3. Experimental design: discrete versus continuous gradient treatments

FOCE experiments to date have addressed the effects of specific future scenarios, and have used discrete or categorical levels of treatment conditions. For example, antFOCE used CO_2 levels projected by the Representative Concentration Pathways as recommended by Barry et al. (2010) with a treatment offset pH of −0.4. Discrete treatment levels favors traditional ANOVA analysis and are suited to answer questions such as "Does the response variable (Y) (e.g. species composition, calcification) differ across different levels of the independent variable X (e.g. pH)?", and test the null hypothesis of no difference among treatments. This requires only two treatment levels, a control and a modified environmental variable (e.g. pH). Thus, where replication of experimental enclosures is limited by cost and difficulty, a discrete concentration or scenario design is suitable, as it allows estimation of within and among treatment variation.

Regression style experiments with a gradient of treatment levels are designed to determine how the response variable (Y) changes with the independent variable (X) by building a quantitative model to describe the relationship. This involves using several treatment levels, often unreplicated, and tests the null hypothesis that Y is not predicted by a simple linear function of X. An issue with simple regression style approaches is that they assume the relationship is linear, however segmented regression, threshold detection and nonlinear responses may also be important and should be considered (Kreyling et al., 2018). Regression style approaches have been shown to be more powerful when basic assumptions are met (Cottingham et al., 2005) and are also more conducive to the types of mathematical representations required to build ecosystem models. Given their limitations, however, FOCE experiments are not currently suitable for defining the precise relationship between pH and a particular biological process.

3.4. Multifactor designs

To date no FOCE design has used multi-factor approach, but there is a need to examine the effects multiple drivers together with acidification including warming, reduced oxygen, and eutrophication (Riebesell and Gattuso, 2015). The additional complexity of full factorial designs with multiple factors will be financially and logistically challenging in FOCE experiments. For such studies, scenario or collapsed factorial designs (e.g. Boyd et al., 2016, 2018) are likely to be more appropriate. This approach requires identifying the dominant physiological control of interest (such as acidification) and collapsing or grouping remaining factors into one combined factor. The relative importance of the dominant factor can be assessed, as well as together with the combined factors and their interaction (Boyd et al., 2016).

4. Required components for a FOCE system

Ocean acidification researchers using FOCE systems need to consider costs and risks when determining whether the system can effectively fulfill their research objectives. In this section, we present basic FOCE concepts, core components, highlight key design elements and implementation decisions. We provide suggestions for balancing requirements against budget, schedule and programmatic risk. A schematic example of a fully developed FOCE system (antFOCE) showing all components can be seen in Stark et al. (2018, Supplementary Fig. S3).

4.1. FOCE fundamental elements

There are several key components that all FOCE experiments have in common and these may be implemented in many different ways; these implementation choices are driven by experiment design, and drive the cost and technical risk. An example of some of the major components from the antFOCE system can be seen in Fig. 4.

Energy: Power requirements are dominated by the flow thrusters and pumps for the enriched seawater delivery. Power is also needed for sensors and the control system. The power budget is critical and is largely dependent on the number and size of replicate experimental enclosures, the size of the pumps required to deliver the enriched seawater, and the number of sensors and other instruments connected to the system. The energy supply in previous FOCE systems proved to be a source of problems and the use of a backup power supply will prevent system outages. antFOCE used an independent power source consisting of a large diesel generator housed in a container (Fig. 4A), which was subject to failures (due to a faulty oil pressure sensor) causing stoppages of the system. The eFOCE used solar panels and a wind generator on a floating platform and these caused a consistent problem as the amount of power generated on cloudy days was insufficient for full system operation and batteries had to be physically replaced to maintain functionality. The cpFOCE also used solar panels and a wind generator on a floating platform (Fig. 2E) as well as cabled power from the research station as back up. In contrast swFOCE used shore based mains power which may be the most reliable option, but in this case frequently experienced ground faults related to failure of connectors or other subsurface components. Power distribution will require properly

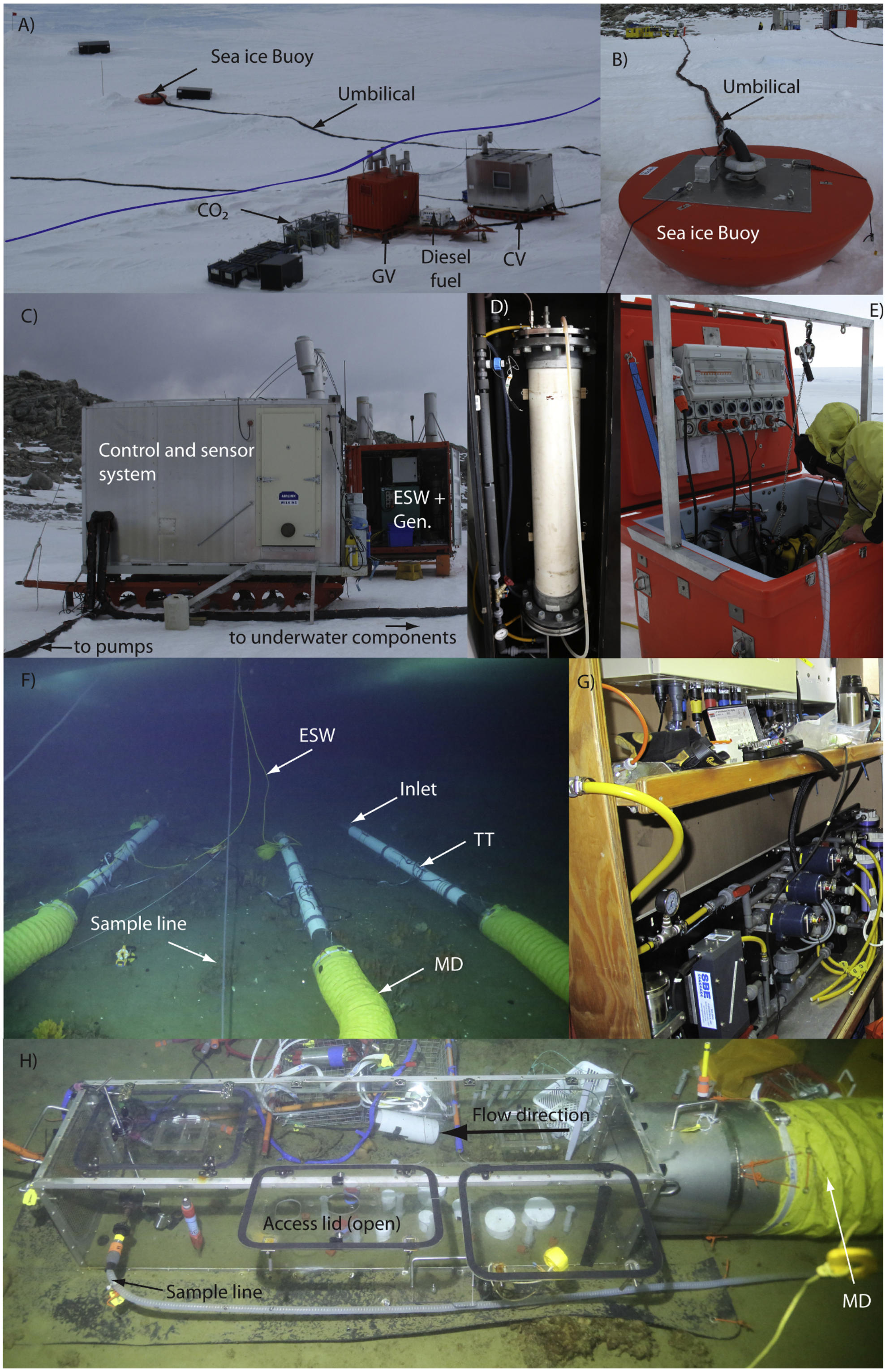

Fig. 4. Examples of major components of an in-situ FOCE system from antFOCE. (A) Overview of the antFOCE site showing the surface infrastructure including the generator van (GV) and the control van (CV) mounted on sleds; the umbilical; the CO_2 supply and the fuel supply; and the sea ice buoy, with the edge of the sea ice shown as a blue line; (B) Insulated, heated umbilical housing the cables (including water for CO_2 enriched seawater (ESW) generation, sample line from enclosures, ESW for dosing) and wires (power/sensor cables) to the subsurface components. The umbilical penetrates the sea ice buoy through a central steel pipe allowing protected access through the sea ice; (C) The sled mounted vans containing the control, sensor and sampling systems and the van containing the electrical generator and ESW production unit (ESW + Gen.). Also visible are umbilicals connected to pump system and to underwater components; (D) the CO2 enriched seawater (ESW) unit which doses seawater with CO_2, and also contained small inert plastic balls to increase total surface area available for dissolution of CO_2 into seawater; (E) Insulated box housing the pumps used in the antFOCE system including to collect seawater for ESW dosing, to pump ESW into enclosures on seabed and to pump sample water to sensor system on surface; (F) Three of the thruster tubes (TT) on seabed, which contain a small propeller which draws water into the mixing ducts (MD) through the inlet end where ESW is also introduced. Thruster tubes also contained a flow meter to measure water volume flowing through each enclosure. Also visible is a sampling line from an enclosure; (G) The sensor panel (in lower part of photo) and control system (upper part of photo) used to measure conditions inside enclosures and control ESW dosing. Water samples could also be taken from the sensor panel from each enclosure; (H) One of the seabed enclosures in the antFOCE experiment during the sampling phase, showing open access lids. Also visible are the sample line and water intake which pumped water to the sensor system on the surface, and the end of the 40 m long mixing duct where the ESW treated or control water flows into the enclosure.

rated cables and suitable submersible connectors to minimise local electrocution hazards and eliminate earth leakage. Using low voltages in FOCE experiments can reduce this risk, for example many of the FOCE systems such as the cpFOCE operated at 12–24 V, to minimize any electrocution hazards.

CO_2 and enriched seawater system: A supply of gas or liquid CO_2 is required to generate low-pH seawater to treat experimental enclosures. CO_2 enriched seawater (ESW) can be generated in several ways (see section 5) and can have CO_2 concentrations close to saturation, with a resulting very low pH. ESW is then pumped into the system through hoses (Fi. 4a – f) and introduced to the mixing duct along with water drawn from the surrounding environment (Fig. 4F) to create the desired pH treatment.

Experiment control system and data acquisition: This controls pH and water flow through the system, monitors the system and collects and archives data (Fig. 4C). A large variety of software and computing solutions are available to do this and are dependent on the users needs and experience. The control system includes pumps to supply ESW and sensors (Fig. 4E, G). Sensors can be either placed in-situ in the enclosures, or on the surface (e.g. Fig. 4G), where water from the enclosures is pumped to them (see section 5.5).

FOCE enclosures and equilibration system: Enclosures on the seabed containing natural benthic communities are the key element of the FOCE system (Figs. 2 and 4H). An essential feature of the system are the equilibration ducts or flumes, which allow ESW to mix and come to equilibrium with surrounding seawater drawn through the enclosures. The mixing ducts require a thruster to draw in seawater and an intake point. The types of connections between the components also need to be considered.

Data and telemetry: Data must be available in real time to operators and other users, including physico-chemical parameters in enclosures (e.g. pH, salinity, temp.) and system status information such as flow rate, thruster and pump status. It is critical that the data is robustly stored and backed up to minimize data loss.

There are three basic configurations for FOCE systems: shore; surface; and subsurface (Fig. 5), which vary in the placement of core elements. Each has a slightly different path for analysis of costs and risks. Shore-based FOCE systems, with much of key infrastructure (CO_2 enrichment, control system) located on land adjacent to the FOCE site, have the advantage of easy access and maintenance of some system elements (Fig. 5A). The antFOCE used a mobile shore design with surface components installed on portable sleds for deployment at a remote site, including a sampling and sensing manifold (Fig. 4G), ESW (Fig. 4D) and power systems (Fig. 4A, C and 5A). Shore based control systems that pump water above sea level (e.g. antFOCE) may require larger pumps and more power than surface systems, depending on the distance from the FOCE enclosures to the sensors. Another advantage of shore based systems can be the ability to take water samples without diving where the design enables pumping of water to surface sensors as part of the pH manipulation.

Surface designs are characterized by locating the majority of the infrastructure on an offshore surface platform (e.g., boats, buoys) and are suited to locations that are too distant to cost-effectively operate from shore, for example, eFOCE used a surface design (Fig. 5B). cpFOCE combined elements of both shore and surface designs, with the CO2 enrichment system ashore and the ESW delivery system positioned on a floating platform (Figs. 2E and 5C). There are several options for implementing a surface platform but all require adequate anchoring and protection for components from severe environmental conditions. Although floating platforms require shorter cables and hoses connecting with FOCE enclosures, they may be very exposed to the full range of weather conditions and can be difficult to access quickly should problems arise. Durable surface platforms are an established commercially available technology. The ocean acidification elements added to the surface platform (CO_2 and energy sources) must be sufficient to maximize maintenance intervals and reduce operational costs. In practice, this implementation generally has a smaller enriched seawater capacity, but this results in relatively less power being required so it supports smaller-scale experiments relatively well. However, a surface architecture can be scaled up to support larger experiments.

A completely subsurface FOCE configuration has not yet been developed, but there is interest in designing a subsurface system for use in remote (e.g. deep-sea) locations. Several aspects of fully subsurface systems are challenging, including power, CO2 enrichment, and maintenance. Deep-sea environments however, provide greater stability for long duration experiments where power and CO_2 can be co-located in situ. MBARI's dpFOCE experiment (Fig. 5D) closely resembled the subsurface configuration, operating continuously for 17 months at a depth of 900 m, generating enriched seawater in situ using liquid CO_2.

4.2. Design process and technology selection criteria

Implementing FOCE experiments requires expertise in electrical, mechanical and software engineering. Applying an engineering design process is critical to success and will help to ensure a cost-effective implementation of a specific FOCE system. Critical steps in the design process include (1) gathering of science and engineering requirements; (2) trade studies to evaluate technical approaches and selection of key components; (3) reviews of requirements and designs before implementation to ensure common understanding and engineering oversight; and (4) review of the project budget to determine what will be feasible considering the scientific, technical and budget considerations. Science priorities determine the operating requirements for the FOCE system. FOCE design development can then proceed by considering the cost and benefit of FOCE components and design elements (e.g. shore or surface based) in relation to the operational priorities. Cost, performance and risk are the fundamental criteria for selecting system architecture and key components of all FOCE systems. Some of the factors affecting performance, cost and risk trade-offs are shown in Table 2 and provide a framework for making technical decisions.

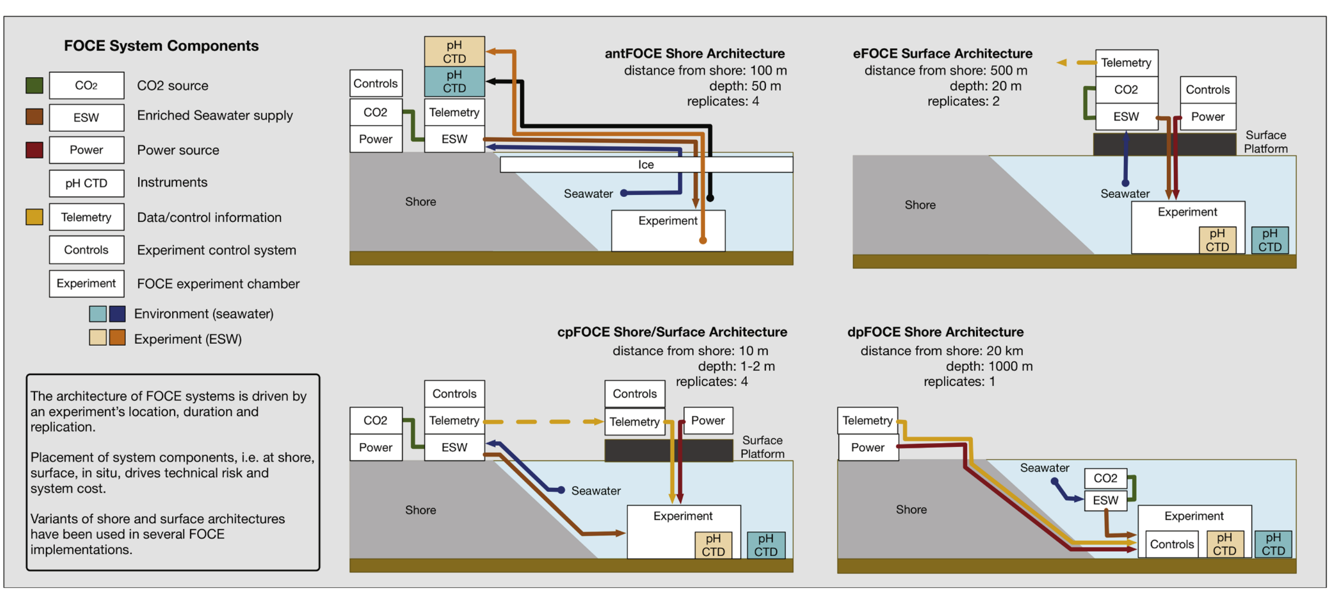

Fig. 5. Schematic diagrams of FOCE components and different architectures. The essential FOCE system elements are shown with relation to different system setups for (A) antFOCE; (B) eFOCE; (C) cpFOCE; and (D) dpFOCE.

Table 2
Technology selection criteria and tradeoff considerations that effect performance, risk and cost.

Trade criteria	Comparative specifications	Tradeoff considerations
Performance	Energy	Alternative sources: generator, battery, mains
	CO_2 use	Alternative sources: gas, liquid, exhaust (generator)
	Function	Ease of use: simple vs. complex operation
	Reliability, fault tolerance	Redundancies, spare components
Risk	Environment	Depth, distance from shore, habitat
	Schedule	Duration of experiment, build and deployment
	Technical	Low tech versus high tech operation
	Budget	Risk decrease with increasing budget
	Skill	Difficulty of operation, training
	DIY Potential – build in-house	Capacity, engineering and technical skills
Cost	Construction	Replication, materials, components
	Installation	Replication, ease of deployment, habitat
	Operation and maintenance	Maintenance schedule of different components
	Decommission	Reusable system, effects on habitat

Once the architecture is selected, trade studies are used to determine the most effective way to implement the key components. For a specific FOCE application, quantitative trade studies for each element should be undertaken to make the best choices for meeting science requirements and minimizing risks. Trade off decisions also hinge on other factors, such as schedule and budget, the technical skills available and additional resources. There are often commercial off-the-shelf options available, which are almost always cheaper when development time is considered, but sometimes it is necessary to build a solution. Fig. 6 gives a very general comparison of solutions to the elements outlined in Table 2, and broadly reflects a collective sense of what has worked best, based on actual FOCE implementations and the trade studies leading up to them. Taking an iterative and incremental approach to implementation using these guidelines has proven to be the most successful path to developing in situ ocean acidification experiments using FOCE technology.

4.3. Enclosure design

To maximize the value of FOCE experiments, effort should be taken to allow the natural conditions, processes, cycles and interactions to persist in both the perturbed and control enclosures. This requires that the enclosures be as open as possible to the surrounding environment whilst still being able to reliably modify and maintain the carbonate chemistry. This represents a delicate balance and the precise engineering of the enclosures to achieve this will vary among habitats. FOCE enclosures used to date have generally been long and narrow as this facilitates the even flow of water through the enclosure and the minimization of low-flow areas or dead spots. Flow has also generally been laterally directed along the long axis (in both uni- and bi-directional setups). In contrast, eFOCE used chambers ($2 \times 1 \times 0.85$ m) in which seawater was injected in multiple holes at the bottom and flow directed through tubing to different heights within the chamber. Initially chambers were completely open at the top, however, tests revealed that controlling pH using such a design was not feasible and it was modified to allow water to exit from two holes located at the top. Design considerations should also consider wave energy and storm surges as tall designs are less stable than lower configurations.

Experimental artifacts from enclosure material should be considered. Many plastic materials attenuate certain wavelengths of light, such as UV, and consideration needs to be given to the effect that might have on photosynthetic responses. Both acrylic and polycarbonate plastics have been used in previous FOCE experiments. Polycarbonate transmits less PAR (McMahon et al., 1990) but is stronger and less brittle than acrylic. In antFOCE for example, polycarbonate was chosen due to its strength, to reduce risks of damage during transport and deployment. In contrast, acrylic was used in the shallow cpFOCE experiments as it transmits UV which is important in shallow coral reef ecosystems.

4.4. Costing a FOCE experiment

The costs of a FOCE experiment can be broadly divided into (1) system equipment and components; (2) labor and expertise required to design, build and test a system; (3) operating and maintaining the field experiment; and (4) post experiment analysis. These can vary considerably depending on size and replication of enclosures and habitat and location of the experiment. Each deployment is uniquely adapted to a particular habitat and scientific focus, which drives the modifications to the xFOCE system concept, leading to a range of different approaches used to enrich and control CO_2 in enclosures and a wide range of costs (Table 3). An example of costs across different FOCE experiments is shown in Table 3, however these should be viewed with caution as costs (parts and operation) will differ significantly between systems, environments, experimental aims and countries or funding agencies.

An important factor to consider is the tradeoff between reliability and cost of components. Cheaper components, although less reliable, may be a better option if they are modular and can be easily and quickly replaced when they become problematic in a FOCE system. A good example of this are the thrusters used in the antFOCE experiment to draw in ambient water to mix with the enriched seawater. Low flow thrusters are difficult to find and are either very inexpensive and prone to failure, or prohibitively expensive. In the antFOCE experiment the thrusters were designed to be able to be changed over in a single dive, so that when they failed the system was offline for less than 24 h.

5. CO_2 enrichment in the FOCE enclosures

A FOCE experiment requires that CO_2 enrichment of seawater occurs prior to entering the experimental enclosures. There are various ways in which to adjust seawater to different pCO_2 levels to mimic future CO_2 levels in the ocean (Gattuso et al., 2010). Preferred methods for FOCE include gas bubbling and addition of CO_2 enriched seawater as these increase the pCO_2 while not affecting the total alkalinity, as this is the situation predicted to occur in future oceans. While introducing the CO_2 directly may seem the easiest to control, experience has shown that the mixing of gaseous or liquid CO_2 (e.g. below 400 m depth where pure CO_2 is in the liquid phase – see Supplementary Fig. S1) presents several difficulties, as neither phase mixes quickly with seawater. At depths of 350 – 550 m and low temperatures (0–16 °C) CO_2 hydrates form around the CO_2 gas bubbles that then physically impede the mixing process. Given that the extent of the enrichment in enclosures is small, on the order of 100 µmoles of CO_2 per litre of seawater, there is a problem of metering a small amount of CO_2 into a much larger volume of seawater flowing through the enclosures. As FOCE systems are semi-open with water flowing constantly through them, the retention period of gas bubbles is reduced. To avoid these problems, FOCE systems to date have all used CO_2 enriched seawater in a two-step approach where first a working solution is prepared, which is then delivered into the FOCE

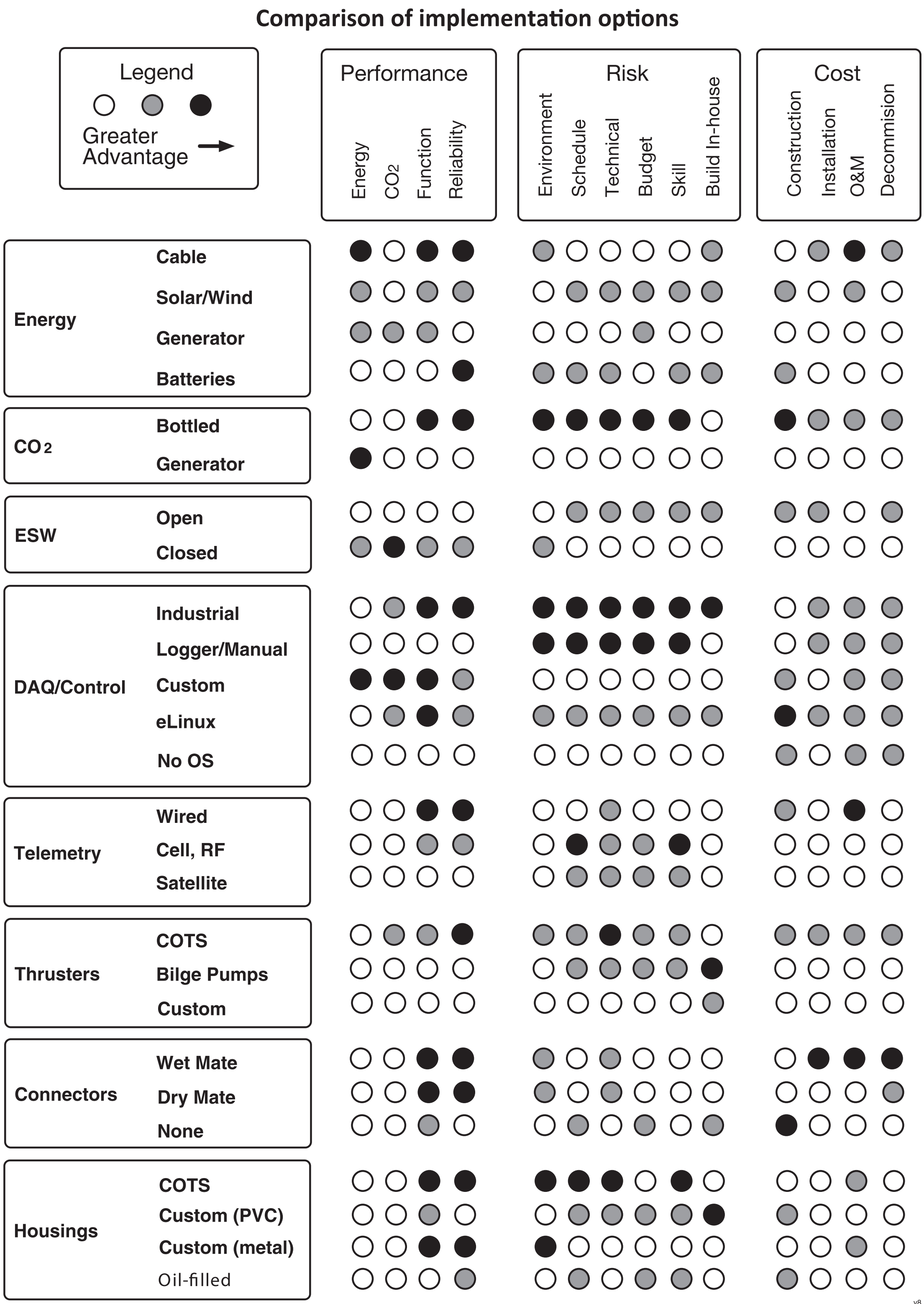

Fig. 6. Framework for comparison of performance, risk and costs for differing technology implementations. Trade off options showing advantages of potential system elements against cost, performance and risk. COTS = Commercial off the shelf; DAQ = Data acquisition, ESW = CO_2 enriched sea water.

enclosure under well controlled conditions, usually via a mixing duct or flume, where it mixes with surrounding seawater and results in the desired pH offset (see below). The problem of mixing phases in the experimental enclosure is avoided, and the mixing of the CO_2-enriched seawater with background seawater is easily facilitated. The R package seacarb (Gattuso et al., 2018) with the function *pmix* can be used to estimate carbonate chemistry parameters after mixing of two water samples.

5.1. Alternatives for producing CO_2 enriched seawater

For shallow water FOCE systems, two approaches have been used to produce CO_2 enriched seawater: 1) bubbling CO_2 through an open container of seawater at atmospheric pressure (e.g. eFOCE); and 2) spraying seawater into a closed vessel where the gas phase is a mix of air and CO_2 (e.g. cpFOCE and swFOCE used a 50/50 mix – see

Table 3
Comparison of approximate costs to run previous FOCE experiments (not including cost of core scientific personnel).

Experiment	antFOCE	eFOCE	cpFOCE
	No. of enclosures		
	4	2	4
System components	€220,000	€160,000	€200,000
System-build labor costs	€225,000	€100,000	€130,000
Field costs	€645,000	€250,000	€97,000
Post-field analysis costs	€64,000	€50,000	€32,000
Experiment duration	12 weeks	8 months	8 months
Location	Antarctica	Mediterranean	Great Barrier Reef

Supplementary Fig. S2). In both cases, a 40–50 mMolar solution of CO_2 in seawater with a pH of approximately 5.0 is obtained where the concentration of CO_2 varies in response to the temperature of the seawater, as colder seawater absorbs more CO_2.

5.1.1. Open container CO_2 addition

Some fraction of CO_2 added is lost to the air as not all of the CO_2 will dissolve in the seawater as the bubbles rise to the surface. Some fraction of the air dissolved in the seawater is also removed by the rising CO_2 bubbles resulting in a reduced oxygen and nitrogen content. This loss is most likely small compared to the large volume of seawater to which it will be added, but should be considered.

5.1.2. Closed container CO_2 addition

Spraying seawater through a mixed atmosphere of air and CO_2 is more complicated, but avoids the loss of air from the seawater. Saturation of the seawater with CO_2 and air occurs quickly but results in a solution that has a total partial pressure of dissolved gases of 2 atm. The mixture must be contained at this pressure (or greater) otherwise out-gassing of the dissolved gases will occur. Hence, it cannot be used in experiments deployed at depths less than 10 m, but works well at depths below this point. Maintaining the 50/50 mixture of air and CO_2 in the mixing chamber is quite simple. By using surface seawater fully saturated with air as the seawater supply to the mixing chamber, a P (air) = 1 atm is easily maintained. Pressurizing the system to P (abs) = 2 atm with a CO_2 gas supply results in the desired 50/50 air/CO_2 mix as $P(CO_2) = P(Total) - P(air)$. A 50/50 mix of CO_2 and air is not essential, as simply 1 atm of air (absolute) is needed and enough CO_2 to achieve the required level of concentration in the enriched seawater. In the antFOCE experiment, the pressure in the enriched seawater system was run at around 1.6 atm to ease the load on the pump that had to push water into the enclosure. This resulted in sea water with enough CO_2 in it that the dosing system was running at 80–90% (i.e. with enough headroom for some feedback control) and achieving the 0.4 pH offset. The ratio can be adjusted either way: lower CO_2 to help the water injector pump; or higher to achieve a greater CO_2 enrichment.

Deep FOCE experiments present more difficult challenges. The dpFOCE experiment in Monterey Bay (Barry et al., 2014), at 890 m depth, used an in situ source of CO_2-enriched seawater (Kirkwood et al., 2015). This was comprised of CO_2 in liquid phase in a rectangular box (240 L), with seawater. The liquid CO_2 was less dense than seawater, so it formed the upper phase, with ambient seawater in the lower portion, which as it became saturated with CO_2 at the hydrate-skin interface, sank to the bottom of the box where it was delivered by pumps to dpFOCE (Kirkwood et al., 2015).

5.2. Control of CO_2 enrichment in enclosures/control of pH

There are two strategies for CO_2 control in enclosures: (1) quantitative control of the mixing of seawater with the CO_2 enriched seawater; or (2) feedback control of the addition of the CO_2 enriched seawater based upon fully mixed conditions in enclosures. Either way requires monitoring the extent of CO_2 enrichment to enable system control and to document the environmental conditions of the experiment. A direct measure of the CO_2 level is difficult as pCO_2 sensors for seawater are currently prohibitively expensive. Discrete sampling and analysis is too slow for providing an effective feedback signal in real-time. However if the alkalinity of the background seawater is relatively constant or if its variation is essentially linear with salinity, then pH can be used to calculate total CO_2 concentration and provide the necessary information in real-time. There are systems other than pH meters for measurement of pCO_2 based on equilibrators that may be useful in monitoring the extent of enrichment but detailing these is beyond the scope of this paper. For example the antFOCE experiment used a mass flow meter on the CO_2 supply into the enriched seawater, so was able to calculate how much CO_2 was added to achieve a particular offset (assuming no leaks), as an additional check in combination with feedback control of pH in enclosures.

5.2.1. Quantitative control of enriched seawater addition

If the concentration of CO_2 in the CO_2 enriched seawater is known, the ratio of CO_2 enriched seawater to seawater mixing is easily calculated, and the experiment can proceed using a fixed CO_2-enriched seawater addition rate at a constant seawater flow through rate. If the background seawater CO_2 concentration and pH varies during the experiment, the constant mixing ratio will maintain the constant CO_2 enrichment while the experimental pH drifts in response to the changes in the ambient conditions. However, the CO_2 calculations to set a constant pH shift rather than a constant CO_2 enrichment are more complex. The amount of CO_2 required must be calculated as function of the ambient background seawater CO_2 system parameters. Maintaining a constant mixing ratio is no longer sufficient to maintain a constant pH offset as the changing background pH will require more or less dissolved inorganic carbon (DIC) per liter to achieve the desired pH offset.

5.2.2. Feedback control based upon pH offset

Feedback control can be achieved by using an enriched seawater source with a nearly constant CO_2 concentration and a control loop that monitors the pH in the enclosures and controls the enriched seawater dosing pump. This avoids having to make calculations regarding how much enriched seawater or CO_2 is required. In the antFOCE experiment the pH was measured in ambient seawater and in experimental enclosures and the experiment was controlled by feeding a very small amount of enriched seawater into the treatment enclosures. The resulting pH was then lowered by incrementally increasing the amount of enriched seawater pumped into the system until the desired offset of approximately 0.4 was achieved. Variations in background pH were then incorporated into the treatment. In experiments where a constant pH offset is desired, pH feedback control is required. Alternatively a pH-stat system can be set up to control the pH within the enclosures as an offset from the pH measured in the environment.

5.3. Environmental effects on CO_2 enrichment

The main factors that influence CO_2 enrichment level in a FOCE experiment include the ambient background seawater pH, temperature and system parameters, most notably the flow rate through the enclosures. For preparing the CO_2-enriched seawater, temperature and pressure are the primary controls on CO_2 saturation as CO_2 is more soluble in cold seawater, and solubility follows Henry's Law. At cold temperatures (< 10 °C) and high pressures (> 10–30 atm depending upon the temperature) CO_2 can form a clathrate hydrate with seawater (Supplementary Fig. S1), which limits the solubility of CO_2 in seawater.

The stability of the CO_2 enrichment and resulting lower pH is an important concern in FOCE experiments. CO_2 speciation must be as close to equilibrium in the experimental enclosures as possible, to avoid fluctuations that are not present in background conditions. For open systems such as FOCE, where there is constant mixing of seawater of different compositions and pH, the kinetics of the CO_2 system equilibration are crucial and the time constant for equilibrium becomes important. This is especially important for flow through systems where flow rates may be high and the residence time of the seawater in the enclosure is small. The 1/e-folding time constant (τ) for obtaining equilibrium when mixing is a function of the end-point pH (Zeebe and Wolf-Gladrow, 2001) (Fig. 7). In warm waters, the reaction is faster at all pH conditions than in colder waters, but given the higher pH of surface waters, the end-point pH can sometimes be at the slowest reaction rates (Fig. 7). The approach used in FOCE experiments to ensure > 95% equilibrium in the carbonate chemistry is a requirement of 3 tau (3τ) (Gattuso et al., 2014). While tau is dominated by temperature, the concentration of the various carbonate species also play an important role, so for a precise estimate of the equilibration time tau should be calculated for the actual carbonate concentrations of the specific site or experiment. The equations and instructions for this calculation can be found in Appendix C.7 of Zeebe and Wolf-Gladrow (2001). Care should be taken to ensure that water used for enriched seawater production comes from the same place as water being pumped through control enclosures, to prevent confounding factors such as different properties, e.g. biological propagules.

5.4. How much CO_2 gas is used?

The most important factors that affect the amount of CO_2 used in FOCE experiments include the size of the enclosure (cross section of volume), the number of enclosures, the rate of seawater turnover within the enclosure, and the enrichment (pH offset) required. For example in the case of a 50 µmol·L^{-1} CO_2 enrichment, with a cross-sectional area of the enclosure of 1 m^2, and a seawater flow rate of 2 cm·s^{-1}, then the amount of CO_2 required per minute per enclosure is:

CO_2 = 1 m^2 × 0.02 m·s^{-1} × 60 s·min^{-1} × 1000 L·m^{-3} × 50 µmol·L^{-1} = 60 mmol·min^{-1}.

Once the amount of CO_2 required per unit time is known, it is simple to determine how much CO_2 will be needed for the duration of an experiment. Several examples are provided in the supplementary information: Supplementary Table S1 shows example calculations of the change in TCO_2 required for a −0.5 pH-shift in several different water masses; Supplementary Table S2 shows calculations of the change in TCO_2 required for pH offsets ranging from −0.1 to −1.0 for the dpFOCE site; Supplementary Table S3 shows calculations of the change in TCO_2 required for pH offsets ranging from −0.2 to −1 for the antFOCE experiment in Antarctica; and Supplementary Table S4 shows example calculations for the swFOCE in Monterey Bay (Supplementary Table S3 and S4 are available as spreadsheets for download).

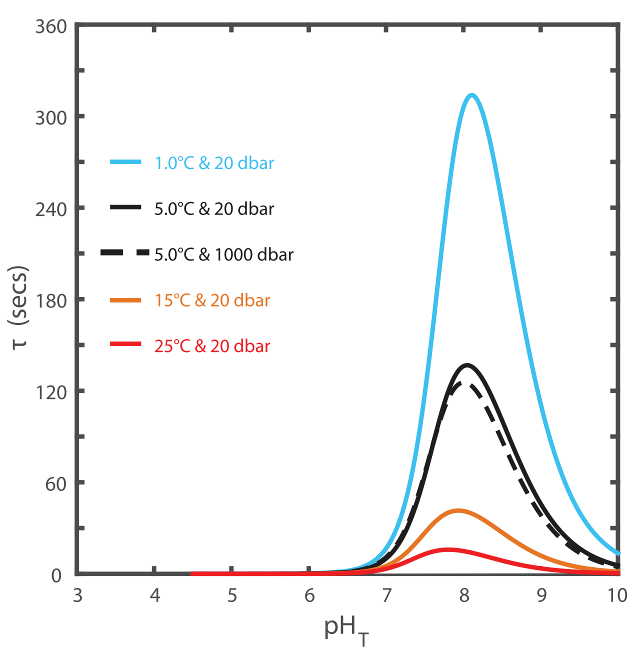

Fig. 7. The effect of temperature and pressure on mixing time for pH equilibrium in sea water. The relationship between the 1/e-folding time constant (tau τ) for the kinetics of the CO_2 system equilibrium as a function of the end-point pH at selected temperatures and pressures. For this plot seawater properties were: S = 34.5, phosphate = 1.5 µM, silicate = 50 µM and total alkalinity held constant at 2300 µmol/kg. pH was varied by adjusting the total CO_2 concentration. pH_T = pH total scale.

5.5. In situ underwater sensors (in enclosures) vs. surface sensors

In situ environmental sensors offer several advantages in terms of measuring the experimental conditions in real-time and without concern for alteration of water properties (such as pH or temperature) during transport or analysis. Underwater sensors are required for each enclosure, thus more power and additional cabling is required to operate them. Underwater sensors are subject to bio-fouling which can affect their performance. Sensors for salinity, temperature and dissolved oxygen are well known to the oceanographic community and have a reputation for reliability and robustness. Unfortunately, pH sensors are not as robust and many are problematic. Sensors employing combination electrodes with KCl reference electrodes are particularly sensitive to seawater fouling during deployment, and electrical interference from pumps and valves if the electrical systems are not properly isolated and protected. Recent developments in ion-sensitive field-effect transistor (ISFET) sensors have improved the quality and stability of field pH measurements (e.g. the seaFET™ or the Durafet™ sensors), particularly for shallow water, longer term deployments, and may be the most suitable choice for FOCE systems.

An alternative to *in situ* sensors is the use of remote sensing where seawater from enclosures is pumped to sensors on the surface (e.g. antFOCE, Fig. 4G, H). This situation allows for the use of laboratory grade sensors that do not need protection from pressure or submersible electronics. These sensors are cheaper and can easily be replaced. There is potential, however, for seawater contamination or alteration in the transfer lines that brings the seawater into the laboratory. Depending upon the depth and distance to the experiment, there can be considerable time delays between events inside the experimental enclosure and the sensing of these changes by the surface sensors, introducing a lag between adjustments made to the system and resulting enrichment/pH offset. As with many other elements of system design, it is a trade-off between cost and performance. As all replicate experimental enclosures and a minimum of one control should be monitored (to give a baseline from which to offset pH), there is an increased cost of equipment (hoses, pumps, sensors) required to bring several streams of water to the surface, although this is more than offset by not having sensors in every enclosure. The antFOCE experiment reduced costs by using a switching system to alternate between the different water sources flowing through the sensor panel, which included sensors for salinity, temperature, pH and dissolved oxygen. Used in combination with a reduced set of underwater sensors (temperature, salinity) the changes in water properties during transport were able to be measured and adjustments made to CO_2 enrichment. Bringing the water to the surface also introduces the ability to easily take water samples for further characterization of carbonate chemistry, such as measurement of DIC, total alkalinity (TA) or nutrients, without the need for diving.

6. What makes a good FOCE site/location?

6.1. Site characterization

Site selection is crucial to successfully running a FOCE experiment. The key considerations with respect to both onshore and underwater

Table 4
Factors to consider when selecting a FOCE site.

	Influences
Shore based factors	
Location on shore	Connection to power supply, access for operation and maintenance, sufficient space for all components, housing to protect equipment
Water access	For boats or divers to access experiment. For cables to run from shore to underwater equipment.
Safety	Barriers to keep out public from shore site, power etc. Signage and buoys to warn of sensitive underwater equipment
Sample collection and transport	Sample storage on site, transport, laboratory for on-site processing
Site access	24 h/7 day access required
Regulatory framework	Permits and compliance with local laws (including marine components)
Underwater factors	
Depth	CO_2 kinetics, dive times
Tides, waves and currents	CO_2 kinetics, sedimentation, damage to equipment, orientation of enclosures
Oceanographic conditions	pH, temperature, salinity influence CO_2 kinetics and determine mixing duct length
Seabed habitat	Scientific focus, placement of enclosures, uniformity between enclosure sites and open plots
Bottom topography	Placement of enclosures, seal of enclosures and carbonate chemistry

site selection, based on past experience, are outlined in Table 4. Habitat is one of the most important considerations in selecting a FOCE site and will drive the scientific focus, such as effects on primary production (e.g. seagrass in eFOCE) as compared to calcification (e.g. corals in cpFOCE). Suitable areas of habitat on which to locate experimental enclosures need to be identified, as the bottom topography will have an effect on the sealing of the enclosures. Gaps in the seal with the seabed may affect flow rates through the enclosures as well as carbonate chemistry conditions, affecting control of the experiment. Areas of flat bottom are much easier to seal and thus soft sediment habitats are particularly suitable but FOCE systems can be deployed on hard bottom habitats such as coral reefs and rocky reefs by developing suitable sealing systems. The use of rubber skirting around the base of enclosures has proven to be an effective way to provide a seal in some habitats and could be customized for specific enclosure placements.

6.2. Oceanographic conditions

Long-term datasets of local environmental conditions are ideal if they exist, particularly when they provide measures of variation over several time scales (e.g. diel, seasonal and annual variation).

6.2.1. Hydrodynamics

The hydrodynamic environment will critically affect the experimental system in several ways. Local current regimes will determine the residence time of the seawater flowing through the enclosure. A high flow site with low residence time can be extremely challenging to manipulate the carbonate chemistry and large dosing pumps or flow restriction in chambers could be required. Previous experience suggests that sites with flow rates above 5–10 $cm{\cdot}s^{-1}$ will be very challenging for a FOCE experiment (Kline et al., 2012; Marker et al., 2010). Strong currents or wave induced turbulence will also require the enclosures to be fixed to the seabed to prevent movement or undercutting. For the swFOCE experiment, a system of rigid stainless steel frames were fixed to the seabed, into which the enclosures were then attached with a large rubber mat around the base to control erosion of the bottom around the enclosure. The local currents should be recreated inside the enclosures to mimic local conditions as closely as possible. This can be done by aligning enclosures with prevailing currents (as was done for cpFOCE) or by the use of thrusters or impellers to draw water through the ducting at the required velocity. The swFOCE used a spring loaded paddle system which moved with the shoreline wave surge to produce some turbulence within chambers.

6.2.2. Temperature

The main effect of temperature in a FOCE experiment is on the rate of CO_2 equilibration into the enriched CO_2 treatments. Depending on the desired flow rate, temperature affects the time required for enriched seawater to equilibrate with ambient seawater (see Section 4). For example in antFOCE, where water temperatures ranged from −1.5 to −0.5 °C, the maximum flow rate of 5 $cm{\cdot}s^{-1}$ required 40 m long ducts on each enclosure to enable the enriched seawater to mix with ambient seawater and come to a stable pH (see Section 4). In contrast cpFOCE, conducted at 20–29 °C, used a flow conditioner only 1 m long, while in Monterey Bay the swFOCE used ducts that were 5 m long at ambient temperatures of 12–14 °C.

6.2.3. pH

Natural pH levels can vary considerably in different habitats, for example in upwelling areas, kelp forests, estuarine ecosystems and shallow benthic habitats such as reef flats there can be annual fluctuations that range by over 0.4 pH units or higher (Hofmann et al., 2011; Kline et al., 2015; Saderne et al., 2013). The implications for large natural variability of pCO_2 in benthic marine ecosystems are clearly important when examining the effects of elevated pCO_2. It is important prior to deployment to have an understanding of pH variation at the experiment site to select meaningful ocean acidification offset treatment levels.

6.3. Biological factors

Understanding the spatial variation of communities being studied is important to avoid or limit spatial confounding factors (Morrisey et al., 1992) potentially introduced by the size and spatial arrangement of enclosures. Together with consideration of distribution patterns of the different-sized organisms operating at different scales, these factors should inform the design of an appropriate sampling scheme to avoid confounding treatment effects with natural variability. Very diverse benthic habitat such as a coral reef may be more challenging to experimentally enclose and replicate similar within-enclosure diversity.

FOCE enclosures and sensors should be inspected and cleaned regularly to limit biofouling, which is dependent on the region and site. Areas such as Monterey Bay and Heron Island had extremely high biofouling rates, requiring regular cleaning of the system. In the cpFOCE the enclosure and sensors had to be cleaned every other day to prevent significant sensor drift and to prevent the growth of calcifying biofouling organisms that were hard to remove. In contrast the biofouling rates in Antarctica are extremely slow and the antFOCE system did not require cleaning over a period of 12 weeks. Inline pre-filters (500 µm) were used before the surface sensor panel in antFOCE and were changed every two weeks. When using a sensor array on the surface, sections of clear hose will provide a quick visual indication of internal biofouling within the system. It is also recommended that surface sensor arrays are modular, easy to access and clean. Heavy fouling could potentially reduce sensor accuracy, light levels, current velocity, dissolved oxygen and food availability. Sedimentation may be an issue in some habitats, either through resuspension of surrounding sediments onto the enclosure, or via precipitation of sediment and organic matter from the water column. There is a tradeoff between the regular work required to clean and maintain a FOCE system versus the cost to automate biofouling protection with systems such as sensor wipers, biofouling inhibitors such as copper or enclosures that can be readily removed and replaced with pre cleaned enclosures. One of the largest costs associated with a long-term (multiple month) FOCE experiment is the amount of labor required to clean, calibrate and maintain the FOCE sensors and enclosures. Maintenance becomes more challenging with depth as deeper than 3 m generally requires diving and depths greater than 10 m have increasingly limited safe diving times.

7. Field deployment, operation and maintenance of FOCE experiments

Prior to deployment of FOCE underwater components consideration should be given to issues such as minimizing habitat disturbance during deployment; testing the workability of connections between system components and sampling procedures, given the restrictive confines presented by an enclosure. For example the antFOCE experiment was deployed onto soft sediments and buoyancy devices were used to manipulate the heavy components (such as enclosures) onto the seafloor to prevent disturbance of fine sediments. In this case there was a thin layer of diatoms growing on the surface that was easily disturbed by turbulence and every effort was made to prevent its removal. Similarly, the connections between the enclosures and equilibration ducts had not been properly tested by divers and proved very difficult to join without disturbing the seabed.

7.1. Team profile

A range of engineering expertise is required to design, build, operate and maintain a FOCE experiment, including mechanical and electrical engineers, and software engineers to manage feedback control of pH and data telemetry. Ideally this expertise should be available to deploy and operate the experiment. With respect to scientific expertise, a marine or carbonate chemist is vital to ensure accurate and reliable characterization of CO_2 enrichment, measurement and calculation of carbonate system parameters. Deploying and operating the experiment will require a mix of technicians, divers and scientists. The underwater components of a FOCE system are difficult to deploy and highly experienced divers are required with a high degree of buoyancy control to avoid disturbing the habitat being studied. Physical disturbances created by divers working near the bottom can result in resuspension and redistribution of sediments and result in burial, loss or movement of mobile species, or damage to fragile or sensitive species.

7.2. System maintenance

After the intense activity of deployment and commissioning phases, regular visits will be necessary to monitor and maintain both surface and underwater systems. As FOCE systems are complex and parts of them operate underwater, inevitably there will be problems and the best way to deal with these is to have the designers available to assist with maintenance and repairs. Designing underwater components in a modular, easy to remove/replace configuration can also reduce downtime. For example, the antFOCE used modular underwater thrusters units that were relatively quickly replaced when issues arose. To reduce system downtime it is important that the capacity exists to visit the site at relatively short notice. The remoteness of a site and bad weather may influence response plans. Remote monitoring of the system is highly useful to alert the occurrence of failures. Pumps, solenoids, water quality monitoring, and environmental parameters such as temperatures can all be monitored and telemetered to the internet to identify faults or problems, potentially preventing system failures.

Pumps are vital to FOCE systems and ideally should be running below maximum capacity, constraining pump selection. The electrical current draw of a pump can be monitored and an increase can indicate imminent failure, requiring replacement. Spare pumps are recommended, particularly if cheap pumps are being used.

Regular calibration and maintenance of any sensors should be performed at least at intervals recommended by the manufacturer. Sufficient spare sensors should be at hand to change out any failed sensors with minimum downtime. Certified reference materials and standards and TRIS and AMP buffers should be used to callibrate pH sensors (Dickson et al., 2007).

FOCE studies of natural communities over long-term durations (months) need to consider the impact of sampling. Monitoring and sampling water quality, biota and the sediment within the enclosures needs to be done so not to disturb long-term experiments and environmental conditions within the enclosures. A clear protocol for sampling can be prepared prior to initiating the system, so all divers/operators are aware of what must be left undisturbed. Non-destructive sampling techniques are preferable and destructive techniques should be withheld or limited until the end of study. For example, cores are commonly used to sample benthic infauna but they are highly destructive. Therefore, in the eFOCE and antFOCE studies, cores were collected only at two times; at the beginning in the ambient area outside the enclosures and then at the end of the experiment from within and outside enclosures.

8. Ancillary short-term process studies

FOCE systems, while principally designed to allow long term examination of the effects of enriched pCO_2 (weeks to months), also provide an opportunity to examine short term responses (hours to days). These may correspond to different physiological processes and ecological interactions that are important in interpreting future ocean acidification effects. Short term studies can be practically achieved in two ways: (1) by designing elements that can be easily deployed and retrieved from FOCE enclosures such as sensors and instruments (e.g. PAM fluorometers to investigate photosynthesis), or elements such as settlement panels; or (2) by running parallel experiments in small independent enclosures, which draw water from the main FOCE supply of modified and equilibrated enriched CO_2 seawater. Short-term (e.g. physiological) and longer term ecological FOCE experiments provide complimentary investigations of ocean acidification, but have different limitations. Open, flow-through FOCE experiments are not suited to examine physiological responses, but they can be temporarily closed to make these measurements. Short term processes like metabolic response, net production and photosynthetic rate are most commonly looked at in isolation using a static water renewal approach. For example the cpFOCE use guillotine style doors and thrusters to provide water movement when respiration and photosynthesis measurements were made with the doors installed for 1–2 h. Considerations for ancillary studies should include: (1) physiological processes that can be linked or have relevance to longer FOCE experiments/ecosystem responses (e.g. photo-physiology links to biomass changes in FOCE chambers); (2) organism lifecycle processes in context of the study time (e.g. keystone species or primary producers with short lifecycles); (3) experimental and statistical design; and (4) design of ancillary equipment.

Replication of additional small enclosures is likely to be restrictive, due to the additional effort required to deploy and run them and the need to limit the amount of water taken from the FOCE system. However at least one control and one acidified chamber would be required and replication could be obtained by repeating experiments, or alternatively via one ancillary chamber per FOCE enclosure.

The ratio of chamber volume to biomass of study organisms to residence time of water in the incubation chambers should be considered as this will affect the carbonate chemistry and the detectability of the metabolic signal or “effect size”. It is strongly recommended that test experiments are conducted to optimize this ratio for the ecosystem and organisms being examined. Parallel short-term incubation experiments can be made partly independent of FOCE systems by utilising their own power supply and data loggers. In the antFOCE experiment, parallel short-term experiments, which focused on benthic microalgae, were conducted in an independent add-on system of two 27 L chambers, which drew on enriched CO_2 and control seawater from the antFOCE system to conduct incubations of multiple days.

9. Evaluating FOCE and future directions

FOCE experiments are a recent development and it is premature to evaluate their contribution to our understanding of the potential effects of ocean acidification on marine ecosystems. The early results emerging from FOCE experiments, however, are beginning to justify the efforts involved, with important discoveries that included evidence of resilience to ocean acidification in corals (Georgiou et al., 2015), in coralline algae (Nash et al., 2011; Nash et al., 2012), changes in chemosensory perception and behaviour in urchins (Barry et al., 2014), and limited effects on seagrass production and seagrass buffering of ocean acidification for sensitive epiphytic calcifiers (Cox et al., 2017a; Cox et al., 2017b; Cox et al., 2016). Ultimately, their contribution to climate change science will be evidenced by a greater understanding of community and ecosystem level effects and incorporating these discoveries into the larger body of ocean acidification research. There are distinct parallels with the terrestrial FACE experiments, which provide encouragement and some important lessons for the ocean acidification research community in attempting further FOCE experiments. The early FACE experiments provided a deeper understanding of forest response to projected increases in CO_2 concentration in the atmosphere (Norby et al., 2016) including tests of assumptions that are being used to project future interactions between forest productivity and the atmosphere in terrestrial biosphere models (Norby et al., 2016). The next generation of FACE experiments are being done across a global range of ecosystems and integrate model-data interaction as part of the experimental design to investigate cross- experiment questions on a range of key issues (Norby et al., 2016).

The development of a set of testable hypotheses should be at the forefront of future FOCE planning. Such hypotheses were valuable in FACE experiments in extending the understanding of responses to increased CO_2 from species up to ecosystem levels, despite the mismatch between the resolution of these hypothesis and the larger spatial and temporal scales required for evaluating feedback between the biosphere, atmosphere and climate (Norby and Zak, 2011). As stated by Norby and Zak (2011) "*The value of FACE experiments has been in defining ecological processes and mechanisms of responses that can inform conceptual and quantitative models of ecosystem responses to* eCO_2.". Whether FOCE experiments can expect to attain a similar contribution is dependent on their continued use and further uptake and development by the ocean acidification community. Thus this paper is an attempt to encourage their careful implementation alongside consideration of the most appropriate scientific hypotheses to test in situ in a FOCE system, in order to better understand how marine ecosystems will respond to future ocean CO_2 concentrations.

Much ocean acidification research to date has considered species as the experimental unit with regard to the conclusions and models derived, however, different populations can exhibit different responses to ocean acidification (Gaitán-Espitia et al., 2017; Padilla-Gamiño et al., 2016). Spatial replication of future FOCE experiments, that examine responses of different populations of the same species but in different areas with different environmental variability, are needed in order to gain a more realistic idea of the species/community responses.

FOCE technology is well developed with a growing body of scientific literature. Capitalizing on the main advantages of FOCE will help address many of the shortcomings identified in our understanding of ocean acidification. The next generation of FOCE experiments ideally would include increased replication, longer experiments to encompass multi-generational times for benthic species and whole and multiple seasonal cycles; multiple environmental-factor approaches, and the use of ancillary incubation chambers for examination of short-term physiological and behavioral responses. Furthermore the field would benefit from coordinated FOCE experiments and research questions across study systems. In addition, there are potentially substantial benefits in re-using FOCE equipment and sharing expertise across institutes and countries to facilitate and increase FOCE accessibility and deployments. The knowledge gained from further FOCE experiments would make an important contribution to improving our ability to forecast the impacts of ocean acidification on natural ecosystems and to better support the management of its impacts.

Declaration of interest

Conflicts of interest: none.

Author contributions

All authors were either participants in the workshop or were invited to contribute to the manuscript (DK, JB, EP, WK, TEC). All authors contributed to the planning and discussion of the manuscript, its preparation and editing. All authors approved the final submission.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pocean.2019.01.006.

References

Allan, E.L., Froneman, P.W., Durgadoo, J.V., McQuaid, C.D., Ansorge, I.J., Richoux, N.B., 2013. Critical indirect effects of climate change on sub-Antarctic ecosystem functioning.

Andersson, A.J., Kline, D.I., Edmunds, P.J., Archer, S.D., Bednaršek, N., Carpenter, R.C., Chadsey, M., Goldstein, P., Grottoli, A.G., Hurst, T.P., 2015. Understanding ocean acidification impacts on organismal to ecological scales. Oceanography 28.

Andersson, A.J., Mackenzie, F.T., Bates, N.R., 2008. Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. Mar. Ecol. Prog. Ser. 373, 265–273.

Barry, J.P., Dayton, P.K., 1991. Physical heterogeneity and the organization of marine communities. In: Kolasa, J., Pickett, S.T.A. (Eds.), Ecological heterogeneity. Springer-Verlag, New York, pp. 270–320.

Barry, J.P., Lovera, C., Buck, K.R., Peltzer, E.T., Taylor, J.R., Walz, P., Whaling, P.J., Brewer, P.G., 2014. Use of a Free Ocean CO2 Enrichment (FOCE) system to evaluate the effects of ocean acidification on the foraging behavior of a deep-sea urchin. Environ. Sci. Technol. 48, 9890–9897.

Barry, J.P., Tyrell, T., Hansson, L., Plattner, G.-K., Gattuso, J.-P., 2010. Atmospheric CO_2 targets for ocean acidification perturbation experiments. In: Riebesell, U., Fabry, V.J., Hansson, L., Gattuso, J.-P. (Eds.), Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union, Luxembourg, pp. 53–66.

Bindoff, N., Willebrand, J., Artale, V., Cazenave, A., Gregory, J., Gulev, S., Hanawa, K., Le Quéré, C., Levitus, S., Nojiri, Y., 2007. Observations: oceanic climate and sea level. In:/Climate change 2007: The physical Science Basis. Contribution of Working Group I to the Fourth Assessment report of the Intergouvernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, and New York, USA. pp. 385–432.

Bograd, S.J., Castro, C.G., Di Lorenzo, E., Palacios, D.M., Bailey, H., Gilly, W., Chavez, F.P., 2008. Oxygen declines and the shoaling of the hypoxic boundary in the California Current. Geophys. Res. Lett. 35.
Boyd, P., Dillingham, P., McGraw, C., Armstrong, E., Cornwall, C., Feng, Y., Hurd, C., Gault-Ringold, M., Roleda, M., Timmins-Schiffman, E., Nunn, B., 2016. Physiological responses of a Southern Ocean diatom to complex future ocean conditions. Nat. Clim. Change 6, 207–213.
Boyd, P.W., Collins, S., Dupont, S., Fabricius, K., Gattuso, J.-P., Havenhand, J., Hutchins, D.A., Riebesell, U., Rintoul, M.S., Vichi, M., 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change—a review. Glob. Change Biol. 24, 2239–2261.
Burnell, O.W., Russell, B.D., Irving, A.D., Connell, S.D., 2014. Seagrass response to CO_2 contingent on epiphytic algae: indirect effects can overwhelm direct effects. Oecologia 176, 871–882.
Caldeira, K., Wickett, M.E., 2003. Oceanography: anthropogenic carbon and ocean pH. Nature 425, 365.
Canadell, J.G., Le Quéré, C., Raupach, M.R., Field, C.B., Buitenhuis, E.T., Ciais, P., Conway, T.J., Gillett, N.P., Houghton, R., Marland, G., 2007. Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. Proc. Natl. Acad. Sci. 104, 18866–18870.
Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., 2014. Carbon and other biogeochemical cycles. Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 465–570). Cambridge University Press.
Cornwall, C.E., Hurd, C.L., 2016. Experimental design in ocean acidification research: problems and solutions. ICES J. Marine Sci.: Journal du Conseil 73, 572–581.
Cottingham, K.L., Lennon, J.T., Brown, B.L., 2005. Knowing when to draw the line: designing more informative ecological experiments. Front. Ecol. Environ. 3, 145–152.
Cox, T., Nash, M., Gazeau, F., Déniel, M., Legrand, E., Alliouane, S., Mahacek, P., Le Fur, A., Gattuso, J.-P., Martin, S., 2017a. Effects of in situ CO_2 enrichment on *Posidonia oceanica* epiphytic community composition and mineralogy. Mar. Biol. 164, 103.
Cox, T.E., Díaz-Castañeda, V., Martin, S., Alliouane, S., Mahacek, P., Le Fur, A., Gattuso, J.-P., Gazeau, F., 2017b. Effects of in situ CO_2 enrichment on epibiont settlement on artificial substrata within a *Posidonia oceanica* meadow. J. Exp. Mar. Biol. Ecol. 497, 197–211.
Cox, T.E., Gazeau, F., Alliouane, S., Hendriks, I.E., Mahacek, P., Le Fur, A., Gattuso, J.-P., 2016. Effects of in situ CO_2 enrichment on structural characteristics, photosynthesis, and growth of the Mediterranean seagrass *Posidonia oceanica*. Biogeosciences 13, 2179–2194.
Davies, G.M., Gray, A., 2015. Don't let spurious accusations of pseudoreplication limit our ability to learn from natural experiments (and other messy kinds of ecological monitoring). Ecol. Evol. 5, 5295–5304.
Dickson, A.G., Sabine, C.L., Christian, J.R., (Eds.), 2007. Guide to best practices for ocean CO_2 measurements.
Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: the other CO_2 problem. Annual Rev. Marine Sci. 1, 169–192.
Dupont, S., Dorey, N., Thorndyke, M., 2010. What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? Estuar. Coast. Shelf Sci. 89, 182–185.
Erez, J., Reynaud, S., Silverman, J., Schneider, K., Allemand, D., 2011. Coral calcification under ocean acidification and global change. In: Dubinsky, Z., Stambler, N. (Eds.), Coral Reefs: An Ecosystem In Transition. Springer, pp. 151–176.
Fabry, V.J., Seibel, B.A., Feely, R.A., Orr, J.C., 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J. Marine Sci.: Journal du Conseil 65, 414–432.
Fernandes, J.A., Papathanasopoulou, E., Hattam, C., Queirós, A.M., Cheung, W.W., Yool, A., Artioli, Y., Pope, E.C., Flynn, K.J., Merino, G., 2017. Estimating the ecological, economic and social impacts of ocean acidification and warming on UK fisheries. Fish Fish. 18, 389–411.
Gaitán-Espitia, J.D., Villanueva, P.A., Lopez, J., Torres, R., Navarro, J.M., Bacigalupe, L.D., 2017. Spatio-temporal environmental variation mediates geographical differences in phenotypic responses to ocean acidification. Biol. Lett. 13, 20160865.
Gattuso, J., Epitalon, J., Lavigne, H., 2018. seacarb: seawater carbonate chemistry. R package, Version 3.2.6. http://CRAN.R-project.org/package=seacarb.
Gattuso, J., Lee, K., Rost, B., Schulz, K., 2010. Approaches and tools to manipulate the carbonate chemistry. In: Riebesell, U., Fabry, V.J., Hansson, L., Gattuso, J.-P. (Eds.), Guide for Best Practices in Ocean Acidification Research and Data Reporting. pp. 41–52.
Gattuso, J.P., Kirkwood, W., Barry, J.P., Cox, E., Gazeau, F., Hansson, L., Hendriks, I., Kline, D.I., Mahacek, P., Martin, S., McElhany, P., Peltzer, E.T., Reeve, J., Roberts, D., Saderne, V., Tait, K., Widdicombe, S., Brewer, P.G., 2014. Free Ocean CO_2 Enrichment (FOCE) systems: present status and future developments. Biogeosciences 11, 4057–4075.
Gaylord, B., Kroeker, K.J., Sunday, J.M., Anderson, K.M., Barry, J.P., Brown, N.E., Connell, S.D., Dupont, S., Fabricius, K.E., Hall-Spencer, J.M., 2015. Ocean acidification through the lens of ecological theory. Ecology 96, 3–15.
Georgiou, L., Falter, J., Trotter, J., Kline, D.I., Holcomb, M., Dove, S.G., Hoegh-Guldberg, O., McCulloch, M., 2015. pH homeostasis during coral calcification in a free ocean CO_2 enrichment (FOCE) experiment, Heron Island reef flat, Great Barrier Reef. Proc. Natl. Acad. Sci. 112, 13219–13224.
Godbold, J.A., Solan, M., 2013. Long-term effects of warming and ocean acidification are modified by seasonal variation in species responses and environmental conditions. Phil. Trans. R. Soc. B 368, 20130186.
Green, R.H., 1993. Application of repeated measures designs in environmental impact and monitoring studies. Aust. J. Ecol. 18, 81–98.
Havenhand, J., Dupont, S., Quinn, G.P., 2010. Designing ocean acidification experiments to maximise inference. In: Riebesell, U., Fabry, V.J., Hansson, L., Gattuso, J.-P., (Eds.). Guide to Best Practices for Ocean Acidification Research and Data Reporting. pp. 67–79.
Hendriks, I.E., Duarte, C.M., Álvarez, M., 2010. Vulnerability of marine biodiversity to ocean acidification: a meta-analysis. Estuar. Coast. Shelf Sci. 86, 157–164.
Hofmann, G.E., Smith, J.E., Johnson, K.S., Send, U., Levin, L.A., Micheli, F., Paytan, A., Price, N.N., Peterson, B., Takeshita, Y., Matson, P.G., Crook, E.D., Kroeker, K.J., Gambi, M.C., Rivest, E.B., Frieder, C.A., Yu, P.C., Martz, T.R., 2011. High-frequency dynamics of ocean pH: a multi-ecosystem comparison. PLoS ONE 6 e28983.
Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54 (2), 187–211.
Iglesias-Rodriguez, M.D., Halloran, P.R., Rickaby, R.E., Hall, I.R., Colmenero-Hidalgo, E., Gittins, J.R., Green, D.R., Tyrrell, T., Gibbs, S.J., von Dassow, P., 2008. Phytoplankton calcification in a high-CO_2 world. Science 320, 336–340.
Ingels, J., Vanreusel, A., Brandt, A., Catarino, A.I., David, B., De Ridder, C., Dubois, P., Gooday, A.J., Martin, P., Pasotti, F., Robert, H., 2012. Possible effects of global environmental changes on Antarctic benthos: a synthesis across five major taxa. Ecol. Evol. 2, 453–485.
Kendall, M.A., Widdicombe, S., 1999. Small scale patterns in the structure of macrofaunal assemblages of shallow soft sediments. J. Exp. Mar. Biol. Ecol. 237, 127–140.
Kerrison, P., Hall-Spencer, J.M., Suggett, D.J., Hepburn, L.J., Steinke, M., 2011. Assessment of pH variability at a coastal CO_2 vent for ocean acidification studies. Estuar. Coast. Shelf Sci. 94, 129–137.
Kirkwood, W.J., Walz, P.M., Peltzer, E.T., Barry, J.P., Herlien, R.A., Headley, K.L., Kecy, C., Matsumoto, G.I., Maughan, T., O'Reilly, T.C., 2015. Design, construction, and operation of an actively controlled deep-sea CO_2 enrichment experiment using a cabled observatory system. Deep Sea Res. Part I: Oceanograp. Res. Papers 97, 1–9.
Kleypas, J.A., Yates, K.K., 2009. Coral reefs and ocean acidification. Oceanography 22 (4), 108–117.
Kline, D.I., Teneva, L., Hauri, C., Schneider, K., Miard, T., Chai, A., Marker, M., Dunbar, R., Caldeira, K., Lazar, B., 2015. Six month in situ high-resolution carbonate chemistry and temperature study on a coral reef flat reveals asynchronous pH and temperature anomalies. PLoS ONE 10 e0127648.
Kline, D.I., Teneva, L., Schneider, K., Miard, T., Chai, A., Marker, M., Headley, K., Opdyke, B., Nash, M., Valetich, M., Caves, J.K., Russell, B.D., Connell, S.D., Kirkwood, B.J., Brewer, P., Peltzer, E., Silverman, J., Caldeira, K., Dunbar, R.B., Koseff, J.R., Monismith, S.G., Mitchell, B.G., Dove, S., Hoegh-Guldberg, O., 2012. A short-term in situ CO_2 enrichment experiment on Heron Island (GBR). Sci. Rep. 2, 413–1411.
Koenigstein, S., Mark, F.C., Gößling-Reisemann, S., Reuter, H., Poertner, H.-O., 2016. Modelling climate change impacts on marine fish populations: process-based integration of ocean warming, acidification and other environmental drivers. Fish Fish. 17, 972–1004.
Kreyling, J., Schweiger, A.H., Bahn, M., Ineson, P., Migliavacca, M., Morel-Journel, T., Christiansen, J.R., Schtickzelle, N., Larsen, K.S., 2018. To replicate, or not to replicate–that is the question: how to tackle nonlinear responses in ecological experiments. Ecol. Lett. 21 (11), 1629–1638.
Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.-P., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob. Change Biol. 19, 1884–1896.
Le Quéré, C., Andrew, R.M., Canadell, J.G., Sitch, S., Korsbakken, J.I., Peters, G.P., Manning, A.C., Boden, T.A., Tans, P.P., Houghton, R.A., Keeling, R.F., Alin, S., Andrews, O.D., Anthoni, P., Barbero, L., Bopp, L., Chevallier, F., Chini, L.P., Ciais, P., Currie, K., Delire, C., Doney, S.C., Friedlingstein, P., Gkritzalis, T., Harris, I., Hauck, J., Haverd, V., Hoppema, M., Klein Goldewijk, K., Jain, A.K., Kato, E., Körtzinger, A., Landschützer, P., Lefèvre, N., Lenton, A., Lienert, S., Lombardozzi, D., Melton, J.R., Metzl, N., Millero, F., Monteiro, P.M.S., Munro, D.R., Nabel, J.E.M.S., Nakaoka, S.I., O'Brien, K., Olsen, A., Omar, A.M., Ono, T., Pierrot, D., Poulter, B., Rödenbeck, C., Salisbury, J., Schuster, U., Schwinger, J., Séférian, R., Skjelvan, I., Stocker, B.D., Sutton, A.J., Takahashi, T., Tian, H., Tilbrook, B., van der Laan-Luijkx, I.T., van der Werf, G.R., Viovy, N., Walker, A.P., Wiltshire, A.J., Zaehle, S., 2016. Global Carbon Budget 2016. Earth Syst. Sci. Data 8, 605–649.
Le Quéré, C., Andrew, R.M., Friedlingstein, P., Sitch, S., Pongratz, J., Manning, A.C., Korsbakken, J.I., Peters, G.P., Canadell, J.G., Jackson, R.B., Boden, T.A., Tans, P.P., Andrews, O.D., Arora, V.K., Bakker, D.C.E., Barbero, L., Becker, M., Betts, R.A., Bopp, L., Chevallier, F., Chini, L.P., Ciais, P., Cosca, C.E., Cross, J., Currie, K., Gasser, T., Harris, I., Hauck, J., Haverd, V., Houghton, R.A., Hunt, C.W., Hurtt, G., Ilyina, T., Jain, A.K., Kato, E., Kautz, M., Keeling, R.F., Klein Goldewijk, K., Körtzinger, A., Landschützer, P., Lefèvre, N., Lenton, A., Lienert, S., Lima, I., Lombardozzi, D., Metzl, N., Millero, F., Monteiro, P.M.S., Munro, D.R., Nabel, J.E.M.S., Nakaoka, S.I., Nojiri, Y., Padin, X.A., Peregon, A., Pfeil, B., Pierrot, D., Poulter, B., Rehder, G., Reimer, J., Rödenbeck, C., Schwinger, J., Séférian, R., Skjelvan, I., Stocker, B.D., Tian, H., Tilbrook, B., Tubiello, F.N., van der Laan-Luijkx, I.T., van der Werf, G.R., van Heuven, S., Viovy, N., Vuichard, N., Walker, A.P., Watson, A.J., Wiltshire, A.J., Zaehle, S., Zhu, D., 2018. Global Carbon Budget 2017. Earth Syst. Sci. Data 10, 405–448.
Marker, M., Kline, D., Kirkwood, W., Headley, K., Brewer, P., Peltzer, E., Miard, T., Chai, A., James, M., Schneider, K., 2010. The coral proto-free ocean carbon enrichment system (CP-FOCE): Engineering and development. OCEANS 2010 IEEE-Sydney. IEEE. pp. 1–10.
Mason, J.G., Rudd, M.A., Crowder, L.B., 2017. Ocean research priorities: similarities and differences among scientists, policymakers, and fishermen in the United States. Bioscience 67, 418–428.
McMahon, M., Kelly, J., Decoteau, D., 1990. Spectral transmittance of selected greenhouse construction and nursery shading material. J. Environ. Horticult. 8, 118–121.
Miller, A.W., Reynolds, A.C., Sobrino, C., Riedel, G.F., 2009. Shellfish face uncertain

future in high CO_2 world: influence of acidification on oyster larvae calcification and growth in estuaries. PLoS ONE 4 e5661.
Molari, M., Guilini, K., Lott, C., Weber, M., de Beer, D., Meyer, S., Ramette, A., Wegener, G., Wenzhöfer, F., Martin, D., 2018. CO_2 leakage alters biogeochemical and ecological functions of submarine sands. Sci. Adv. 4 eaao2040.
Morrisey, D.J., Howitt, L., Underwood, A.J., Stark, J.S., 1992. Spatial variation in soft sediment benthos. Mar. Ecol. Prog. Ser. 81, 197–204.
Nam, S., Kim, H.J., Send, U., 2011. Amplification of hypoxic and acidic events by La Niña conditions on the continental shelf off California. Geophys. Res. Lett. 38.
Nash, M., Troitzsch, U., Opdyke, B., Trafford, J., Russell, B., Kline, D., 2011. First discovery of dolomite and magnesite in living coralline algae and its geobiological implications. Biogeosciences 8, 3331–3340.
Nash, M.C., Opdyke, B.N., Troitzsch, U., Russell, B.D., Adey, W.H., Kato, A., Diaz-Pulido, G., Brent, C., Gardner, M., Prichard, J., Kline, D.I., 2012. Dolomite rich coral reef coralline algae resist dissolution in acidified conditions. Nat. Clim. Change 3, 268–272.
Norby, R.J., De Kauwe, M.G., Domingues, T.F., Duursma, R.A., Ellsworth, D.S., Goll, D.S., Lapola, D.M., Luus, K.A., MacKenzie, A.R., Medlyn, B.E., 2016. Model–data synthesis for the next generation of forest free-air CO_2 enrichment (FACE) experiments. New Phytol. 209, 17–28.
Norby, R.J., Zak, D.R., 2011. Ecological lessons from free-air CO_2 enrichment (FACE) experiments. Annu. Rev. Ecol. Evol. Syst. 42, 181.
Oksanen, L., 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue? Oikos 94, 27–38.
Paine, R.T., 1966. Food web complexity and species diversity. Amer. Nat. 100, 65–75.
Padilla-Gamiño, J.L., Gaitán-Espitia, J.D., Kelly, M.W., Hofmann, G.E., 2016. Physiological plasticity and local adaptation to elevated pCO_2 in calcareous algae: an ontogenetic and geographic approach. Evol. Appl. 9, 1043–1053.
Pischedda, L., Poggiale, J.-C., Cuny, P., Gilbert, F., 2008. Imaging oxygen distribution in marine sediments. The importance of bioturbation and sediment heterogeneity. Acta Biotheor. 56, 123–135.
Poore, A.G., Graba-Landry, A., Favret, M., Brennand, H.S., Byrne, M., Dworjanyn, S.A., 2013. Direct and indirect effects of ocean acidification and warming on a marine plant–herbivore interaction. Oecologia 173, 1113–1124.
Queirós, A.M., Fernandes, J.A., Faulwetter, S., Nunes, J., Rastrick, S.P., Mieszkowska, N., Artioli, Y., Yool, A., Calosi, P., Arvanitidis, C., 2015a. Scaling up experimental ocean acidification and warming research: from individuals to the ecosystem. Glob. Change Biol. 21, 130–143.
Queirós, A.M., Huebert, K.B., Keyl, F., Fernandes, J.A., Stolte, W., Maar, M., Kay, S., Jones, M.C., Hamon, K.G., Hendriksen, G., 2016. Solutions for ecosystem-level protection of ocean systems under climate change. Glob. Change Biol. 22, 3927–3936.
Queirós, A.M., Stephens, N., Cook, R., Ravaglioli, C., Nunes, J., Dashfield, S., Harris, C., Tilstone, G.H., Fishwick, J., Braeckman, U., 2015b. Can benthic community structure be used to predict the process of bioturbation in real ecosystems? Prog. Oceanogr. 137, 559–569.
Raupach, M.R., Marland, G., Ciais, P., Le Quéré, C., Canadell, J.G., Klepper, G., Field, C.B., 2007. Global and regional drivers of accelerating CO_2 emissions. Proc. Natl. Acad. Sci. 104, 10288–10293.
Rhein, M., Rintoul, S.R., Aoki, S., Campos, E., Chambers, D., Feely, R.A., Gulev, S., Johnson, G.C., Josey, S.A., Kostianoy, A., Mauritzen, C., Roemmich, D., Talley, L., Wang, F., 2013. Observations: ocean. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge. pp. 255–315.
Riebesell, U., Bellerby, R., Grossart, H.-P., Thingstad, F., 2008. Mesocosm CO_2 perturbation studies: from organism to community level. Biogeosciences 5, 1157–1164.
Riebesell, U., Gattuso, J.-P., 2015. Lessons learned from ocean acidification research. Nat. Clim. Change 5, 12–14.
Rudd, M.A., 2014. Scientists' perspectives on global ocean research priorities. Front. Mar. Sci. 1, 36.
Rühl, S., Calosi, P., Faulwetter, S., Keklikoglou, K., Widdicombe, S., Queirós, A.M., 2017. Long-term exposure to elevated pCO_2 more than warming modifies early-life shell growth in a temperate gastropod. ICES J. Mar. Sci. 74, 1113–1124.
Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., Wanninkhof, R., Wong, C.S., Wallace, D.W.R., Tilbrook, B., Millero, F.J., Peng, T.-H., Kozyr, A., Ono, T., Rios, A.F., 2004. The Oceanic Sink for Anthropogenic CO_2. Science 305, 367–371.
Saderne, V., Fietzek, P., Herman, P.M.J., 2013. Extreme variations of pCO_2 and pH in a macrophyte meadow of the Baltic Sea in summer: evidence of the effect of photosynthesis and local upwelling. PLoS ONE 8 e62689.
Stark, J.S., Roden, N.P., Johnstone, G.J., Milnes, M., Black, J.G., Whiteside, S., Kirkwood, W., Newbery, K., Stark, S., van Ooijen, E., Tilbrook, B., Peltzer, E.T., Berry, K., Roberts, D., 2018. Carbonate chemistry of an in-situ free-ocean CO_2 enrichment experiment (antFOCE) in comparison to short term variation in Antarctic coastal waters. Sci. Rep. 8, 2816.
Stewart-Oaten, A., Murdoch, W.M., Parker, K.R., 1986. Environmental impact assessment: 'pseudoreplication' in time? Ecology 67, 929–940.
Sunday, J.M., Fabricius, K.E., Kroeker, K.J., Anderson, K.M., Brown, N.E., Barry, J.P., Connell, S.D., Dupont, S., Gaylord, B., Hall-Spencer, J.M., 2017. Ocean acidification can mediate biodiversity shifts by changing biogenic habitat. Nat. Clim. Change 7, 81.
Underwood, A.J., 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge p. 504.
Zeebe, R., Wolf-Gladrow, D., 2001. CO2 in Seawater: Equilibrium, Kinetics, Isotopes. Elsevier Oceanogr. Book Series 65. Amsterdam Elsevier. p. 346.
Zhang, Q., Warwick, R.M., McNeill, C.L., Widdicombe, C.E., Sheehan, A., Widdicombe, S., 2015. An unusually large phytoplankton spring bloom drives rapid changes in benthic diversity and ecosystem function. Prog. Oceanogr. 137, 533–545.