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Free Ocean CO₂ Enrichment (FOCE) systems: present status and future developments

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Abstract. Free Ocean CO₂ Enrichment (FOCE) systems are designed to assess the impact of ocean acidification on biological communities *in situ* for extended periods of time (weeks to months). They overcome some of the drawbacks of laboratory experiments and of field observations by enabling (1) precise control of CO₂ enrichment by monitoring pH as an offset of ambient pH, (2) consideration
5 of indirect effects such as those mediated through inter-specific relationships and food-webs, and (3) relatively long experiments with intact communities. Bringing perturbation experiments from the laboratory to the field is however extremely challenging. The main goal of this paper is to provide guidelines on the general design, engineering, and sensor options required to conduct FOCE experiments. Another goal is to introduce xFOCE, a community-led initiative to promote awareness,
10 provide resources for *in situ* perturbation experiments, and to build a user community. Present and existing FOCE systems are briefly described and examples of data collected presented. Future developments are also addressed as it is anticipated that the next generation of FOCE systems will include, in addition to pH, options for oxygen and/or temperature control. FOCE systems should become an important experimental approach for projecting the future response of marine ecosystems
15 to environmental change.

1 Introduction

The oceans absorb about 25 % of anthropogenic carbon dioxide (CO₂) emissions, moderating the rate and severity of climate change (Le Quéré et al., 2013). While this is a valuable service to human societies, it has important implications for the oceans. The massive input of CO₂ generates critical changes in the chemistry of seawater, especially in the carbonate system. These changes are collectively referred to as “ocean acidification” because increasing CO₂ lowers seawater pH (i.e. increases its acidity). The pH of ocean surface water has decreased by about 0.1 unit since the beginning of the industrial era, corresponding to a 26 % increase in hydrogen ion concentration (Orr, 2011). Earth System Models project a global additional decrease in pH by 2100 ranging from 0.06 to 0.32 units depending on our future emissions, with the latter value, associated with a 163 % increase in hydrogen ion concentration from pre-industrial times, being the best estimate based on our emissions trajectory (Ciais et al., 2013).

Ocean acidification may have profound impacts on marine biota, for example through the direct effects of pH on inter-cellular transport mechanisms that control the physiology and metabolism of marine organisms and the decreased availability of carbonate ions, used by many species to build calcareous shells and skeletons (Kroeker et al., 2013). Other functional groups, such as primary producers that use CO₂ to produce organic matter, may take advantage of its increased availability in the coming decades (Riebesell and Tortell, 2011).

As studies of the consequences of ocean acidification for marine organisms and ecosystems expanded rapidly over the past decade, the methods employed to evaluate the effects of future changes in ocean chemistry have become more sophisticated. Initial studies frequently involved measurements of the survival or physiological response of individuals of marine species to large changes in the partial pressure of CO₂ (pCO₂) or pH, while held in small containers under laboratory conditions with modified seawater, microbes and food conditions (Gattuso and Hansson, 2011). This approach increased the level of understanding of the effects of these environmental changes on individual species but provided little information concerning the response of natural assemblages where indirect effects can be important. Indirect effects occur where species that are not directly affected by changing ocean chemistry nonetheless experience changes in abundance or distribution because their prey, predators or competitors are reduced or increased by ocean acidification. Another example of indirect effects are possible on the substratum, for example dissolution of the calcium carbonate framework.

In order to improve our understanding of the future impacts of ocean acidification on marine life, three major types of *in situ* data can be used: (1) time series of changes in community structure correlated with changes in carbon chemistry (2) observational studies that compare patterns or processes between areas that differ naturally in seawater acidity and (3) perturbation experiments, where environmental conditions are manipulated to compare patterns or processes between artificially acidified and control conditions (Barry et al., 2010). Although time series directly documenting changes in

organismal abundance or distribution in response to ocean acidification are extremely valuable, long-term data sets coupling biological observations and carbon chemistry measurements are generally not available (but see, for example, Wootton et al., 2008). In addition, using time series analysis, it is difficult to disentangle any ocean acidification effects from the effects of other environmental changes, such as climate, pollution or fishing. Comparing contemporary areas with different carbonate chemistry conditions, such as near natural CO₂ vents (Fabricius et al., 2011; Hall-Spencer et al., 2008; Martin et al., 2008) or along other gradients (e.g. Thresher et al., 2011), can provide critical data and an insight on the long-term impacts of ocean acidification on benthic organisms, communities and ecosystems (Hall-Spencer et al., 2008; Martin et al., 2008; Wootton et al., 2008; Fabricius et al., 2011). However, spatial comparisons have several limitations: there is no control over treatment conditions and (in the case of vent studies) organisms are locally exposed to short-term extreme pH levels, and there are confounding factors, such as the release of potentially harmful gases (Boatta et al., 2013) and immigration into the study site of juveniles from outside the study areas, which often make the results difficult to interpret.

The Free Ocean CO₂ Enrichment (FOCE) experimental approach attempts to address some of the limitations of other methods by conducting ocean acidification experiments *in situ*. FOCE experiments are analogous to pelagic mesocosm (Riebesell et al., 2013a) experiments that examined the response of natural plankton communities to controlled pH perturbations, advancing methods of ocean acidification research toward more comprehensive studies of whole communities and embedded processes under mostly natural conditions (e.g. Riebesell et al., 2013b). Free Air CO₂ Enrichment (FACE) experiments in terrestrial systems, which have been providing critical and unexpected data on the impacts of elevated CO₂ on terrestrial ecosystems for more than two decades, and have led to over 120 peer-reviewed publications (Ainsworth and Long, 2005). The first FOCE systems were developed at the Monterey Bay Aquarium Research Institute (MBARI; Walz et al., 2008; Kirkwood et al., 2011), but other FOCE systems have since been deployed or are being planned around the globe (see section 4).

We define FOCE as a technology facilitating studies of the consequences of ocean acidification for marine organisms and communities by enabling the precise control of CO₂ enrichment within *in situ*, partially-open, experimental enclosures. By focusing, for reasons described below, on systems that use partially-open enclosures, this definition does not include all marine *in situ* carbonate parameter manipulation experiments. In pelagic mesocosm experiments (Riebesell et al., 2013a), a bag completely encloses a volume of seawater. The carbonate system is generally altered at the beginning of the experiment through additions of CO₂-enriched seawater, and subsequently drifts in response to biological processes and air-sea gas transfer. A fully enclosed benthic *in situ* CO₂ enrichment system has also been developed. The Submersible Habitat for Analyzing Reef Quality (SHARQ) system has been modified from strictly measuring carbonate parameters (Yates and Halley, 2006) to manipulating carbonate parameters by the addition of CO₂ enriched seawater (Yates,

90 pers. comm.).

CO₂ bubbling in open water without any enclosure has also been used (Arnold et al., 2012). This experiment is more “free ocean” than is encompassed in our FOCE definition, which is restricted to partially enclosed systems. Because the chemical reactions that occur when CO₂ is added to seawater are relatively slow (on the order of 10s of seconds to minutes depending on temperature and pH), the open water approach does not enable precise control of carbonate system parameters by pH monitoring because it does not allow sufficient time to ensure full equilibration of added CO₂ in seawater. Since there are no experimental enclosures to regulate water flow the method generates highly variable pH under variable current speed or direction. This approach is therefore more similar to natural CO₂ vents than to FOCE systems and has the associated advantages and disadvantages.

100 Although straightforward in concept, engineering and logistic aspects of FOCE technology are very challenging to implement. The goal of this paper is to provide information on the general design of FOCE experiments (section 2) as well as recommended sensors, suggested designs depending on scientific needs and details about the pH control system (section 3). It also provides an overview of past and present FOCE systems (section 4), outlines future engineering and research directions (section 5) and describes community building and overarching activities (sections 6 and 7).

2 General design

The key elements of any FOCE experimental units are partially open enclosures that allow control of seawater conditions, but retain through-flow of ambient seawater, a CO₂ mixing system, sensors to monitor ambient and enclosure pH as well as other critical environmental parameters, and a control loop to regulate the addition of gases or liquids to each experimental enclosure (Fig. 1). Ideally, FOCE systems could control the carbonate chemistry and other parameters (e.g. oxygen, temperature) in several relatively large experimental plots (ca. > 10 m²) and would be fully open (i.e. not requiring enclosures). In practice, FOCE systems require far more constrained conditions.

115 Another key element of FOCE systems is the flexibility in the design to enable investigation of different communities and environments (see section 4). The environmental and logistical features of potential experiment sites introduce challenges to configuring a FOCE experiment. The availability of electrical power, sources and delivery of CO₂, experiment enclosure size, and regional seafloor topography and flow patterns are the primary factors that determine the final implementation. Furthermore, the expected duration of the FOCE system and planned experiments as well as the size and the availability of the team available for maintenance and data collection are important considerations for logistics and associated costs.

120 It is necessary to measure various parameters in the area inside and outside the controlled volume. In existing FOCE systems, pH data are needed as the perturbed pH is usually determined by an offset to the ambient pH. Also, one of the most important concerns for FOCE experiments is

125 being able to ensure that natural conditions, except for pH, prevail in the experimental enclosures
despite the partial confinement. For that purpose, measurements of salinity, temperature, oxygen,
and a second parameter of the carbonate system outside the controlled volume are desirable. The
deployment of FOCE systems in areas where local physicochemical and biological conditions have
been monitored for several years is recommended in order to place the experimental conditions in a
130 broader timescale.

2.1 Enclosures

The land-based Free-Air CO₂ Enrichment (FACE) experiments involved horizontal or vertical pipes
placed in a circle around areas of forest, grassland or commercial crops using natural and fully
open conditions (Ainsworth and Long, 2005). Although it is desirable for FOCE experiments to
135 mimic FACE and be as open as possible to maintain a natural environment, there are a number
of practical issues that prevent the use of a fully open experiment, the most important being the
slow reaction kinetics of CO₂ chemistry in seawater (see section 2.4). The design of the enclosure
can vary considerably depending on the focus of experiments, but must allow some ventilation of
the enclosed plot with ambient water. Some designs may allow the flow of detritus through the
140 enclosure, while others may be fully closable and function temporarily as respirometric chambers.
Enclosures may be fabricated from various materials. Acrylic may be most appropriate for shallow
water experimental studies that require natural light. Although acrylic is difficult to work with, and
has less intrinsic strength, it has superior optical properties than polycarbonate, and transmits the
full spectrum of natural light. In addition, acrylic does not lose these properties with age, while
145 polycarbonate exposed to sunlight slowly becomes opaque.

The behavior of animals may also influence the design and openness of FOCE experiments. Ex-
perimental enclosures may contain mobile animals which can either swim or burrow out of the
enclosure if or when conditions become adverse. The structure of the FOCE enclosure itself can
attract animals searching for shelter, which may in turn affect the hydrodynamic regime and result
150 in an increase in deposition of nutrient-rich fecal material. The enclosure may also contain prey for
predators able to burrow under the walls into the enclosure. It may, therefore, be desirable to limit
both access and egress to the enclosure (e.g Deep FOCE, thereafter dpFOCE, section 4.1). Alter-
natively, if the FOCE enclosure is large enough, additional cages or baskets may be placed inside it
to safely contain experimental plants or animals. Care must be taken not too anchor the enclosure
155 too deeply into non-cohesive sediment as this may disrupt the pore-water flow and the advective
exchange of material across the sediment-water interface.

2.2 Size and volume

The area and volume of existing and planned FOCE units range from 0.25-2 m² and 0.06-2 m³
(Table 1). Units should be as large as possible as large units enable smoothing out of small-scale

160 changes in the composition of the community and better represent the response of the community.
However, there are critical engineering and financial constraints on the size of FOCE units related
to the amount of CO₂ required, the number of replicate enclosures required, the performance of the
CO₂ control system, and the signal to noise ratio. Small FOCE units can be used to investigate the
response of specific representatives of the ecosystem, for example sedimentary areas within a coral
165 reef or seagrass bed.

Although it would reduce some of the size constraints, manipulating the carbonate system in
lagoons or embayment has never been attempted and the large amount of CO₂ which would need to
be added seems to preclude such experiments. It may be possible, however, to perform FACE-like
experiments in sectors of very shallow areas subject to a unidirectional water flow, such as coral
170 reef flats. Controlling pH upstream would subject the downstream community to future conditions,
although with less ability to control the future pH conditions.

The present and planned FOCE systems have very different designs (see section 4) because they
focus on different biological communities, explore different engineering options, and were subject
to different logistical constraints. There would be considerable benefits to use more standardized
175 systems. It would ensure comparability of results, the development of joint experiments with an
increased number of environmental conditions or increased replication. The size of complete FOCE
systems would benefit from modularity that eases the complexity of shipment and assembly. FOCE
installations in extreme environments may be logistically challenging and will benefit from careful
planning, and preassembly of surface and underwater modules.

180 **2.3 Number of subunits and experimental design**

The design of each FOCE experiment uniquely reflects the goals and the approach taken by the
investigators for the environment and biological community studied. Possible experimental designs
fall into three broad categories (Table 2). The very first FOCE system constructed (dpFOCE) had
only one unit with a manipulated carbonate chemistry. This was primarily an engineering test unit
185 to learn about controlling CO₂ enrichment by monitoring the internal pH offset versus ambient
seawater. Later designs used at least two units, one serving as a control and one or two having their
carbonate system manipulated.

Design should also include randomization, replication, and controls (Hurlbert, 1984; Tilman,
1989). Economic constraints are likely to limit the number of enclosures, but there should be sev-
190 eral, if possible, in order to analyze the data through regression (requiring multiple enclosures with
increasing levels of treatment) rather than through analyses of variance (see Havenhand et al., 2010).

Because of cost and feasibility, replication is challenging for FOCE experiments, particularly for
studies that require (1) large units, (2) deployment in a challenging environment, or (3) lengthy
testing durations. A struggle between the need to replicate and the need to study processes at ap-
195 propriately large natural scales has existed for decades (Carpenter, 1990). Adding to design dif-

200 faculty, replicates collected from within the same mesocosm are considered as pseudo-replicates (Hurlbert, 1984; Gamble, 1990; Heffner et al., 1996). Environmental impact studies have dealt with the “pseudo-replication problem” by sampling through time (Stewart-Oaten et al., 1986; Underwood, 1994; Stewart-Oaten and Bence, 2001). When necessary, FOCE experiments could benefit from a similar, replication in time, approach. Repeating long term experiments can be difficult or impossible, depending on funding. In addition, it is very likely that the FOCE enclosures must be relocated between repeated experiments to increase independence between experimental replicates (in time). Hence, if at all possible, multiple enclosures should be preferred.

205 To date, nested or fully crossed designs to test the impacts of multiple stressors with FOCE have not been performed. However, FOCE systems could be deployed along natural or man-made environmental gradients and used with regression analyses to examine multi-stressor impacts (see section 5 for further discussion). Alternately, researchers could use scenario or orthogonal approaches.

210 Because of the variability in organismal and community processes, differences among treatments need to be large to be detected. Despite replication limitations, Kline et al. (2012) were able to detect consistent differences in the dissolution of calcified structures. The authors also argued that FOCE experiments have inherent value that laboratory experiments do not. Oksanen (2001) made an eloquent argument that an experiment conducted at the ecosystem level with limited replication, if scientifically-grounded, arguably provides a more useful understanding than many well-replicated laboratory investigations. Similarly, Heffner et al. (1996) pointed out that even Hurlbert (1984) in his article on ecological field experiments recognized that the quality of an experiment depends upon more than design. Furthermore, a meta-analysis, which resembles a nested design with replicates, could be conducted with FOCE experiments to identify any unusual experimental outcomes. A “control” enclosure allowed to fluctuate at natural pH levels is necessary in FOCE designs to account for the artificial boundary created by the structure which is absent in nature. Inside the structures, every effort should be taken to mimic natural conditions. Furthermore, the “perturbed” and “treated” environment and organisms should be manipulated in a similar manner. For example, the pumping system used to deliver CO₂ into treated structures should ideally be used in the control enclosure (without CO₂ addition). Also FOCE experiments conducted over several months will need to account for seasonality. In these instances a natural/ambient or reference area, outside the enclosure and open to environment, should be considered in the experimental design.

225
230 Once the experimental design has been defined according to the budget available as well as to technical and logistical constraints, the pH or pCO₂ levels need to be determined. Barry et al. (2010) have provided comprehensive guidance for selecting these levels. Unless the goal of the experiment is to identify physiological and molecular pathways, in which case extreme pH values can be useful, FOCE experiments generally use environmentally relevant carbonate chemistries. The approach usually consists of lowering pH in the perturbed units by a fixed offset value corresponding to a pH value projected according to a CO₂ emission scenario (for example, one of the Representative

Concentration Pathways; van Vuuren et al., 2011). In contrast to many laboratory experiments which used a constant carbonate chemistry, the FOCE technology is one of the approaches that maintains the natural daily and seasonal pH changes. It is worth noting that, to date, no FOCE experiment has attempted a treatment to increase pH to mimic preindustrial (+0.1 unit) or glacial (+0.2 unit) pH, partially due to the chemical difficulty of removing CO₂ from seawater.

2.4 pH manipulation

Initial trial experiments using a fully open system have highlighted the difficulties of creating accurate low pH conditions in a freely moving body of seawater (Walz et al., 2008). Subsequently, all chemical equilibrium FOCE experiments have been semi-enclosed and made use of the Zeebe and Wolf-Gladrow (2001) CO₂ kinetic model of e-folding time (τ) to calculate the time delay between CO₂ addition and equilibration for a given pH, CO₂ concentration and temperature. Because the chemical reactions between the various carbonate species are slow, one needs to allow sufficient time for equilibrium to be reached before the introduction of the CO₂ enriched seawater into the experimental chamber. For FOCE experiments, equilibrium is assumed to have been reached at any time past $3 \times \tau$. By using these calculations and continued monitoring of pH, temperature and current velocity, a set pH can be successfully maintained even in areas of low temperature, such as dpFOCE and Antarctic FOCE (antFOCE), and high flow rates and high wave energy, such as Coral Proto FOCE (cpFOCE) and European FOCE (eFOCE).

Early FOCE prototypes developed at MBARI used hydrochloric acid or liquid CO₂ for the early proof of concept experiments. It would also possible to inject CO₂ or CO₂-rich air as this requires special handling and delivering gases is more difficult than delivering water. It also introduces the added difficulty of equilibrating seawater with a gas which is considerably slower than mixing two water bodies. Consequently, all recent FOCE systems have used metered addition of CO₂-enriched, low pH seawater in the enclosures, which is one of the recommended approaches of pH manipulation because it closely mimics the natural process of ocean acidification (Gattuso et al., 2010). The delivery of CO₂-rich seawater, obtained by bubbling pure CO₂ through seawater, is continually adjusted to maintain a constant pH offset relative to ambient pH, or to maintain a constant pH level in experimental enclosures. An example of a pH regulation test in the frame of the dpFOCE project (Fig. 2) demonstrates that precise pH control can be achieved. The negative spikes in ambient seawater trace are due to "blow-back" of CO₂-treated seawater onto the external sensors. This illustrates the need for bi-directional flume operation (so that the outlet is always downstream of the external sensor) or locating the external sensors further away from the test chamber.

The key elements of any pH manipulation system include the CO₂ mixing system, sensors to continuously monitor ambient and enclosure pH, and a control loop to regulate the flow of CO₂-enriched seawater (ESW) to each enclosure. The best option is to set up a supply reservoir that allows for variable delivery on demand. The manufacture of ESW and pH control can be performed either

from a shore station, buoy or a nearby apparatus with a hose delivering the ESW to the enclosures.

270 In general, the rate and range of pH change in experimental enclosures should be aligned with
the pattern of natural pH variation at the study site, which likely reflects the physiological toler-
ances, resilience, and potential for adaptation to pH changes by the local biological assemblage. The
natural range and variability of pH conditions experienced by the organisms may effectively have
275 are exposed to large diurnal fluctuations in pH associated with daytime and nighttime changes in
the intensity of photosynthesis/respiration and/or calcification/dissolution, as well as water advec-
tion owing to tidal cycles and changes in winds (Andersson and Mackenzie, 2012). Note, however,
that alternative treatments such as constant pH or abrupt changes may be required for particular
experimental questions.

280 Hofmann et al. (2011) reported rates of change in pH for various ecosystems. Among them,
upwelling regions, estuaries, coral reefs and kelp forests exhibit large daily fluctuations of about
0.25 units, corresponding to mean rates of pH changes of 0.01-0.04 unit h⁻¹. Diel range similar
to or larger than the changes projected over the next century are often measured in coral reefs (e.g.
Price et al., 2012) and tropical estuaries (Yates et al., 2007). In contrast, changes in pH in the open
285 ocean do not exceed 0.001 unit h⁻¹ (Hofmann et al., 2011). In temperate systems, shallow-water
habitats dominated with macrophytes are also likely to present strong diurnal pH variations with the
highest variability in the shallowest waters and decreasing with increasing water depth (Middelboe
and Hansen, 2007). Diel pH fluctuation in macroalgal-dominated communities may reach 1 unit h⁻¹
(varying from pH 7.8 to 8.6, presumably on the NBS scale; Middelboe and Hansen, 2007). Even
290 larger changes may occur in the tidal zone. For example tidal pools experienced pH oscillations
related to the tide with rapid changes of pH as high as 1 to 3 unit in low-level rock pools in Brittany
(pH_T from 7.7 to 8.6; Egilisdottir et al., 2013) and high-level rock pools in Scotland (pH from 6.4 to
9.4, presumably on the NBS scale; Morris and Taylor, 1983). Organisms exposed to such conditions
may experience rapid pH changes as high as 0.1 to 0.3 pH unit h⁻¹ in the surrounding seawater.
295 However, only the pH of seawater surrounding the organism affects its physiology. The pH at the
surface of the organism (diffusion boundary layer) may differ from the mainstream seawater due
to exchanges related to metabolic activities which may locally modify the pH microenvironment
(Hurd et al., 2011). The seawater flow at the surface of the organism is also important as it sets the
thickness of the layer. For example over a diel cycle coralline algae encounter pH changes of +0.5
300 unit in the light and -0.35 unit in the dark relative to the pH of ambient seawater (Hurd et al., 2011).
Sea urchins may also be subjected to very low pH values (down to 7.5 on the NBS pH scale) at their
surface in slow flows. Conversely, under high flows, pH at the surface of the organisms is close to
ambient pH. The surrounding hydrodynamic environment as well as the organisms' physiology and
morphology should thus be considered to evaluate the differential susceptibility of organisms to pH
305 changes.

The rate of pH decrease to reach the experimental offset used in existing FOCE systems has been highly variable (from hours to month in steps of 0.05 to 0.1 pH unit) and is mainly constrained by the duration of the experiment (Table 3). The ideal situation for the initial rate of perturbation is to avoid exceeding the natural rate of change. An initial rate of pH change of up to 0.05-0.1 unit h⁻¹ should thus be acceptable for most habitats.

2.5 Sampling

Given that FOCE systems represent a large investment in terms of both time and effort, it is not surprising that researchers are keen to maximize the amount of information these experiments generate. Consequently, there is a desire to take a large number and variety of samples from any single FOCE enclosure. This should be encouraged, particularly when complementary samples are taken which support the observation and interpretation of whole system effects or which identify key trade-offs between biological responses. However, heavy sampling from such spatially restricted systems (current FOCE systems containing typically less than 2 m² of sea floor) can have a number of negative impacts on the representativity and effectiveness of these experiments. This is most obvious for samples that are destructive in nature, such as the direct removal of material. In such cases it can be argued that, once destructive sampling has been conducted within the enclosure, all subsequent responses to the CO₂ perturbation could be compromised. Therefore, it is recommended that destructive sampling should only be conducted at the end of the experimental exposure period or be of sufficiently small scale so as to not significantly impact upon the structure and the function of the community within the enclosure. Biological samples required to describe the initial or starting conditions, prior to the CO₂ exposure, could be collected from areas close to the enclosures, rather than inside them, if impact is a concern.

For non-destructive samples of sufficiently small scale, such as direct visual observations, settlement panels or chemical and physical sensors, it is possible to make more frequent observations throughout the exposure period. However, even if no material is actually removed from the enclosure, it is still possible that non-destructive sampling can have a negative impact upon the health of organisms contained within the enclosure. For example, the continued physical disturbance caused by divers entering the enclosure during preliminary testing of the eFOCE system (see section 4.3), resulted in a noticeable degradation in the seagrass habitat in the enclosure relative to habitat outside the enclosure. A possible solution to this problem is to automate sampling as much as possible through the deployment of cameras and auto-sampling devices, thereby reducing the frequency at which observers need to directly interact with the enclosure. Disturbance is also an issue to be considered when planning regular cleaning and maintenance, where greater automation would also be a benefit. Additionally, the design of the enclosure should allow easy access for sampling and maintenance in order to minimize disturbance.

Finally, consideration should be given to the timing of sampling or observation activities. A

strength of FOCE systems is their exposure to environmental conditions which are generally more realistic than laboratory-based studies. They, thus, are subjected to natural short-term (e.g. tidal, diurnal) and long-term (e.g. seasonal) cycles experienced in the field. Whilst this adds to the realism of FOCE experiments it also presents a few challenges with respect to sampling. Care should be taken to ensure that data are collected and interpreted in the context of these cycles.

2.6 Data access and density

FOCE sensors collect a large amount of data that need to be monitored, processed, stored, and accessible from the storage system. The data serve two purposes: (1) monitor the electronics and system functionality and (2) collect meaningful data to understand the effects of ocean acidification. The frequency for sensing should be based upon the ability and accuracy of the sensors and upon knowledge of past environmental fluctuations at the study site. If the sensors, such as pH electrodes, are used for feedback control, higher sampling rates are required to provide rapid enough data for feedback decisions. Researchers need to have rapid access to the sensor data, ideally in real-time, to ensure that the system is functioning properly and to identify extreme values.

3 Key sensors and scientific needs

FOCE experiments depend on careful monitoring and control of the carbonate system. In addition, other parameters are typically monitored to examine various processes (e.g. metabolic rates). Because the sensor system is so critical, we discuss in this section issues related to measuring “essential parameters” considered necessary for FOCE systems and “optional parameters” that may be required for specific applications.

3.1 Essential parameters

3.1.1 pH

Considering that a primary goal of FOCE systems is to control of seawater pH within experimental enclosures, pH sensors and their calibration protocols must be selected carefully. Ocean acidification experiments involve pH perturbations of up to 0.4 pH unit below ambient. Sensors with a precision better than 0.003 pH unit are required in order to have a good pH control. To date, FOCE systems have used potentiometric pH electrodes, which often exhibit significant drift with time due to diffusion of seawater into the KCl reference solution. Therefore, such electrodes require frequent calibration and comparison with pH measurements from discrete water samples. The pH of discrete water samples can be accurately measured spectrophotometrically (accuracy ± 0.005 pH units; Table 4) or computed based on total alkalinity (A_T) and total inorganic carbon (C_T) measurements (accuracy ± 0.007 pH units; Table 5). The electrode used for controlling pH must be calibrated when the measured pH differs from the pH value estimated from discrete sample by more than a threshold

375 value (e.g. 0.05 pH unit), Calibration is performed in the laboratory using buffers at a temperature close to *in situ* temperature as described by Dickson et al. (2007).

There are few commercially-available potentiometric pH electrodes designed for underwater deployment with the required precision and accuracy. One example is the Seabird SBE 18 pH sensor which uses a pressure-compensated glass electrode Ag/AgCl-reference probe to provide *in situ* measurements at depths up to 1200 m. The earlier version of this electrode was modified by MBARI 380 to reach a precision of 0.0003 pH units. The most recent commercial version reaches a similar precision. Both versions drift due to seawater contamination of KCl reference buffer; therefore the accuracy varies depending upon the frequency of calibration and duration of deployment (drift decreases with time). As alternative to the potentiometric electrode, an ion sensitive field effect transistor (ISFET) sensor can be used, such as the Honeywell Durafet™. The advantages of the ISFET 385 include robustness, stability and precision that make it suitable for ocean pH measurements. An ISFET sensor has been incorporated into the deployable SeaFET™ package developed at MBARI and the Scripps Institution of Oceanography (Martz et al., 2010) and commercialized by Satlantic. SeaFET™ sensors have been successfully deployed in shallow water applications monitoring (Hofmann et al., 2011) and will be used in antFOCE (see section 4.5). Current ISFET sensors are only 390 deployable to a depth of ca. 70 m, but versions that can operate at greater depth are in development. A recent XPRIZE contest (<http://oceanhealth.xprize.org>) is helping spur the development of new ocean pH sensors and it is anticipated that more options may become available soon.

3.1.2 Salinity, temperature and dissolved oxygen

395 Salinity and temperature are required to assess the carbonate system parameters and control pH (see section 2.4). Dissolved oxygen is a key parameter characterizing the environmental setting and can be used to assess net community production and respiration based on closed chamber incubations. For example, community metabolism can be measured by observing changes in oxygen levels in a temporarily closed FOCE enclosure. Photosynthesis-irradiance curves of *Posidonia* seagrass 400 communities in the bay of Villefranche have been derived from changes in oxygen concentration measured during 24 h incubation with the eFOCE system (Fig. 3).

FOCE units should therefore be equipped with "conductivity temperature depth" (CTD) instruments. Most commercial CTDs have specifications suitable for FOCE systems, with accuracies for temperature and salinity better than 0.002 °C and 0.001, while typical drifts are about 0.0002 °C and 405 0.0001 month⁻¹, respectively. Various optical oxygen sensors have successfully been tested in the field (see <http://www.act-us.info>) and are commercially available. They typically exhibit a precision and accuracy of about 2 and 3 μmol kg⁻¹ respectively.

3.1.3 Additional parameter of the carbonate system

The carbonate system can be characterized when at least two of its parameters are known (Zeebe
410 and Wolf-Gladrow, 2001)). Table 4 presents the precision and accuracy of the different methods
used to measure these parameters and Table 5 shows the errors associated with the computation
of each parameter as a function of the pair of parameters measured. Measuring $p\text{CO}_2$ and pH
continuously using *in situ* sensors is attractive as it could provide a continuous characterization of
the carbonate chemistry (with real-time temperature and salinity data, see section 3.1.2). However,
415 this pair of parameters leads to large uncertainties in the computation of the other parameters of
the carbonate system (Table 5). It is therefore recommended to sample water in the experimental
enclosures as well as in the external environment on a regular basis (e.g. once a week or more
if an automated sampling technique is available). Sampled seawater can be used to independently
measure parameters of the carbonate system such as A_T , C_T and/or pH. It should also be noted
420 that A_T can be overestimated due to alkalinity associated with dissolved organic matter, leading to
significant errors in the derived parameters (Hoppe et al., 2012). Koeve et al. (2012) recommended
to over-determinate the CO_2 -system in experimental ocean acidification studies to safeguard against
possibly large errors in estimated $p\text{CO}_2$. It is out of the scope of this paper to detail all analytical
procedures and we refer to Dickson et al. (2007) for a comprehensive description of the protocols.
425 Instruments for continuous measurement of C_T *in situ* have recently been developed (Liu et al.,
2013). A_T can be measured continuously in the laboratory (Li et al., 2013), but it is not clear when
a system that can be deployed on the sea floor will be available.

3.2 Optional sensors and parameters

3.2.1 Net calcification

430 Ocean acidification is detrimental to the net precipitation of calcium carbonate (CaCO_3) shells and
skeletons by many marine organisms (Gazeau et al., 2013; Kroeker et al., 2013). Net calcification is
therefore an important process in FOCE experiments involving calcifying organisms. It can be as-
sessed by collecting organisms, measuring their buoyant weight in the laboratory and bringing them
back in the enclosures. This approach is very time-consuming and involves a lot of manipulation of
435 the organisms. Calcification of the entire community can be measured using the alkalinity anomaly
technique (Smith and Key, 1975) when the units are closed. This technique makes the assumption
that the production of 1 mole of CaCO_3 decreases total alkalinity (A_T) by 2 moles.

Since there is currently no total alkalinity sensor that can be deployed *in situ*, measuring the
change in A_T must rely on discrete water samples collected during incubations. Calculating total
440 alkalinity from pH and $p\text{CO}_2$ (the only two parameters that can be conveniently and continuously
monitored) leads to unacceptably large errors ($\pm 88 \mu\text{mol kg}^{-1}$; Table 5). In most cases, the increase
or decrease of A_T generated by the precipitation and dissolution of CaCO_3 is much lower than the

accuracy that can be achieved using measurements of only pH and pCO₂.

Another non-destructive technique is to estimate net calcification rates from changes in C_T during incubations, assuming that C_T is only controlled by net community production (NCP) and net calcification. NCP can be estimated using changes in the concentration of dissolved oxygen (see section 3.1.2) converted to carbon units (e.g. Gattuso et al., 1999). Since this method is based on the measurements of two parameters and on the use of a theoretical $C_T:O_2$ ratio, it provides estimates with a relatively high uncertainty compared to the alkalinity anomaly technique.

450 3.2.2 Nutrients

Modifications in the functioning of benthic communities as a response to ocean acidification has the potential to significantly alter nutrient cycling and the role of these communities as source or sinks of various elements such as nitrogen and phosphate (Barry et al., 2011). In addition to the direct effects on rates of primary production and associated nutrient requirements, several laboratory experiments have demonstrated modifications of shallow sediment-water nutrient fluxes due to the impacts of ocean acidification on the macro-organisms responsible for bioturbation and bioirrigation activities in the sediment (Barry et al., 2011). Therefore, the measurement of macronutrients (nitrate, ammonium and phosphate) changes during closed incubations appear of great interest. Sensors allowing the continuous measurement of nitrate *in situ* have been developed since the 1980's (Johnson et al., 1989). Available commercial sensors currently reach a precision and accuracy of respectively 0.5 and 3 μmolL^{-1} (see <http://www.act-us.info/evaluations.php>). Although, this could be sufficient for highly active communities, in most cases, laboratory measurements of discrete samples must be performed.

3.2.3 Light

465 FOCE systems deployed in the euphotic zone benefit from having photosynthetically active radiation (PAR) sensors inside the enclosures in order to relate the community metabolism to irradiance. A surface PAR sensor is also useful to model the optical characteristics of the water column. Ultraviolet sensors are also optional sensors if UV is a parameter of interest at the study site and if partial confinement is achieved by UV-transparent material.

470 3.2.4 Camera

Imagery is not necessary for operating a FOCE system but it is can be useful for monitoring the status of the system and make scientific observations. Cameras allow the non-destructive collection of data, such as changes in the density and composition of epifaunal and epifloral communities, as well as behavioral responses of conspicuous species. Ideally camera observations should be via continuous video but time-lapse photography can also be appropriate.

Very simple techniques can be employed to make inexpensive cameras. For example, the Linkspit™

is a single board camera with a very small foot print and several lens options. It comes with open source software and can easily be interfaced with Arduino™ computer boards. Another approach for additional performance is to use off-the-shelf security cameras which have additional features, such as pan and tilt, but are more costly. Both types of cameras require a simple housing for submerged operations. Underwater cameras are available but the cost of a full-featured system can be prohibitive for FOCE experiments.

3.2.5 Flow measurements

Water flow influences virtually all aspects of the biology of marine benthic organisms. Hydrodynamics has wide-ranging roles. For example, it controls the availability of nutrients and food particles and also affects the dispersion of gametes (Vogel, 1994). Flow measurements can therefore be extremely useful in a FOCE system and it is crucial to maintain a flow regime in the enclosures similar to ambient. Even if the system is only partly open, the structure will limit natural flow patterns, affecting processes from molecular exchange rates to the transport of waste (i.e. mussel beds) or export of dead leaves (seagrass and macroalgae). This could influence the results obtained in the enclosures and limit upscaling of the results to natural ecosystems.

Flow can be monitored using instruments such as acoustic doppler current profiler (ADCP), acoustic doppler velocimeter (ADV) or cheaper instruments. Fans or water pumps integrated into the FOCE design can achieve the simulation of environmental flow rates. Replication of average flow rates is reasonably easy in areas with moderate flow environments. However maintaining very large flow rates is challenging. It is also an issue to maintain very slow flow rates due to the equilibration of the carbonate system in the enclosure (low pH spikes).

3.2.6 Sensor biofouling protection

For longer term experiments, biofouling protection is necessary to limit the growth of organisms that can alter sensor function. If possible, mechanical biofouling protection such as rotating brush or plate is preferable but often more expensive. Chemical biofouling protection such as copper or tributyl tin is commonly used in instruments such as CTDs, O₂ sensors and pH sensors but care must be taken not to place them within the enclosures where the chemicals could affect the organisms inside. When instruments with chemical biofouling protection are used, it is recommended to include them in a pumping system that pulls the water from the enclosures through the instruments and then disposes the water well away from the experimental area.

4 Past, current and planned FOCE implementations

The main characteristics of the past, current and planned FOCE systems are summarized in Table 1.

4.1 Deep FOCE (dpFOCE)

510 A FOCE system for studies of deep-sea benthic communities (designated dpFOCE; Fig. 4A) was developed by MBARI. The dpFOCE project, deployed at a depth of 880 m, was attached to the Monterey Accelerated Research System (MARS) cabled seafloor observatory in Monterey Bay, central California, approximately 52 km from Moss Landing where the cable makes landfall. The system used a flume concept where the pH offset vs ambient seawater was monitored to maintain control of

515 CO₂ enrichment within the experimental enclosure, while allowing access to natural seafloor sediments and suspended particulate material. Time-delay wings attached to either end of the dpFOCE enclosure allowed for injection of CO₂-rich seawater into either end depending on the direction of near-bottom currents, and provide sufficient time for full hydration of the injected CO₂-enriched seawater before entering into the experiment enclosure. Fans were integrated into the dpFOCE design to control water flow through the experimental enclosure and to simulate typical local-scale flow

520 conditions. Multiple sensors (pH, CTD, ADV, and ADCP) used in conjunction with the fans and the enriched seawater injection system allowed the control loop software to achieve the desired pH offset. dpFOCE was connected to shore via the MARS cabled observatory, which provided power and data bandwidth. Enriched CO₂ seawater was produced from ambient seawater flowing under a pool

525 of liquid CO₂ held in a small container near the dpFOCE enclosure; dissolution of liquid CO₂ into the seawater stream produced a CO₂-rich mixture used for injection into the dpFOCE enclosure. The dpFOCE system operated over 17 months and verified the effectiveness of the design hardware and software (Kirkwood et al., 2011). dpFOCE has performed a number of long term (4 weeks) pH control tests and has also proven to be an ideal platform to explore sub-lethal effects on animals that

530 may be observed as changes in typical movement, posture and feeding rates (Barry et al., 2013).

4.2 Coral Proto FOCE (cpFOCE)

A shallow water FOCE system that could be deployed in coral reef habitats was developed at the University of Queensland. The cpFOCE system used replicate experimental flumes to enclose sections of a coral reef and dose them with CO₂-enriched seawater using peristaltic pumps with computer

535 controlled feedback loops to maintain a specified pH offset from ambient conditions (Fig. 4B). The system was powered by solar and wind energy and the data was transmitted to a nearby laboratory for real time monitoring and data management (Marker et al., 2010). A cpFOCE enclosure had forward and rear flow conditioners on either end to accommodate bidirectional ocean currents. The openings were placed parallel to the dominant axis of tidal currents over the reef flat, and each enclosure was

540 anchored with sand stakes. The flow conditioners were attached to maximize turbulence and to provide passive mixing of the CO₂ enriched seawater. Four of the tubes in the flow conditioners furthest from the enclosure had small holes along their length through which low pH water was pumped to dispense it evenly along the entire width and height of the conditioner. The cpFOCE system was

545 deployed at Heron Island (Great Barrier Reef) to investigate the response of coral communities to ocean acidification (Kline et al., 2012) and an 8 month duration experiment was performed.

4.3 European FOCE (eFOCE)

The European FOCE (eFOCE; <http://efoce.eu>) is currently in the initial deployment stage and comprises two enclosures (control and experimental) as well as a surface buoy housing the electronics and pumps to produce CO₂-enriched water (Fig. 4C). The system is powered by solar and wind
550 energy. Data are sent to the nearby laboratory and can be monitored on the internet. The eFOCE system is currently deployed in the bay of Villefranche-sur-mer (France) at about 12 m depth and 300 m offshore. The eFOCE project has been developed to investigate the long-term effects of acidification on benthic marine communities of the North West Mediterranean Sea, especially *Posidonia* seagrass beds. Over a 3-year period, the aim of the project is to perform a relatively long (> 6
555 months) experiment.

4.4 Shallow water FOCE (swFOCE)

In collaboration with Hopkins Marine Station and the Center for Ocean Solutions, MBARI is developing a swFOCE system to examine the effects of ocean acidification on shallow subtidal communities in central California. swFOCE will use a shore station for the seawater control system
560 and production of CO₂-enriched seawater, and will also use an existing cabled observational and research platform to connect the swFOCE node. Initially, two swFOCE enclosures will be installed at a depth of 15 m, approximately 250 m offshore from Hopkins Marine Station. The nearby node of the cabled observatory (Kelp Forest Array) has instruments to monitor local currents, temperature, pH, and O₂ in real-time, as a cabled observatory platform for scientific research.

565 4.5 Antarctic FOCE (antFOCE)

The first polar FOCE, antFOCE, experiment was proposed and awarded funding in November of 2012, followed by design and concept work in 2013 (Fig. 5) and engineering tests before deployment in 2014. The antFOCE units will be placed on the seabed near Casey Station, Eastern Antarctica (2 control experimental enclosures and 2 high CO₂/low pH experimental enclosures). One of the
570 difficulties with deploying FOCE infrastructure in a polar region will be both sea- and fast-ice. To circumvent issues with ice, scientific divers will deploy each unit through the sea-ice early in the Antarctic summer field season and the units will be retrieved in open water conditions late in the summer. Connecting the sea-floor units to topside power (through sea-ice or open water) will be achieved with a purpose designed heated sea-ice buoy to avoid land, fast-ice and freezing of the
575 seawater in the in/out flow.

5 Future developments

As FOCE technology matures, one will be increasingly faced with the decision of which communities to target and where to locate new FOCE experiments, both in terms of complementing existing FOCE research and expanding into areas of particular concern including regions of high biodiversity, 580 providing key economic, cultural and/or ecosystem services, or particularly sensitive. Key locations could also be identified in order to benefit from established infrastructure and ocean acidification monitoring programs. While ocean acidification is of global concern, upwelling regions, coral reefs and polar areas are especially sensitive to ocean acidification (Gattuso et al., 2011) and are therefore good candidates for FOCE experiments.

585 Areas of particular difficulty for FOCE experiments include regions of heavy wave action, large tidal currents, rapid flow and heavily polluted, disturbed or unstable sediments. To increase the likelihood of success for future FOCE experiments we recommend partnering with marine science institutes having the multi-disciplinary expertise and infrastructure necessary to undertake such activities.

590 The ocean acidification community recently emphasized the need to investigate the combined effects of ocean acidification and other environmental drivers (Hoegh-Guldberg and Bruno, 2010; Boyd, 2011). Two other major global drivers are ocean warming and the spreading of hypoxic zones where the concentration of dissolved oxygen is lower than $60 \mu\text{mol kg}^{-1}$ (Hofmann et al., 2011). The combination of global warming, ocean acidification and hypoxia can have additive, synergistic 595 or antagonistic effects on organisms performances (Boyd and Hutchins, 2012), advantaging or disadvantaging some species or group of species (e.g. calcifiers, fleshy autotrophs) to the detriment of others, affecting the distribution of species and ultimately leading to ecosystems shifts. For example, in tropical regions, the coincidence of warming and acidification is expected to cause shifts from coral reefs to seagrass- and seaweed-dominated ecosystems (e.g. Diaz-Pulido et al., 2011; Fabricius 600 et al., 2011). In temperate areas, the same drivers are expected to cause shifts from forests and meadows of perennial algae to mats of ephemeral and opportunistic algae such as turfs (Connell and Russell, 2010).

Very few experiments have considered warming, acidification and hypoxia together and all were performed in the laboratory. To date, no FOCE experiment has controlled a parameter other than 605 the carbonate system. While FOCE systems are being developed, deployed and data analyzed, it is important to develop the next generation of FOCE systems that should be able to manipulate multiple variables. For example, to simulate warming and hypoxia as offsets from ambient values.

Manipulating the temperature of underwater open-top enclosures seems unrealistic because it would require large amounts of energy due to the high thermal conductivity of seawater, the “in- 610 finite” thermal capacity of the ocean (at the scale of a FOCE system) and the enhanced outflow of seawater at the top of the enclosure due to the increased buoyancy of warmer water. Temperature control would therefore require enclosing seawater much more than the current FOCE systems do.

Early calculations suggest that simultaneous control of pH and temperature can be accomplished, with closed enclosures of 0.25 m³ flushed once every hour, and higher operational electrical costs
615 (Mahacek, unpublished data). Cross flow heat exchange techniques can be used to significantly reduce the heating energy requirement.

Hypoxia can be simulated with the injection of O₂ deprived seawater (e.g. 60 μmol kg⁻¹). O₂ can be decreased in an intermediary reactor by physical outgassing, through the injection of N₂ (g), or by chemical scavenging through the injection of sulfite ions. Although toxic, sulfites rapidly react with
620 O₂ to produce sulfate ions (Wong and Zhang, 1992). Surface seawater O₂ concentration is about 230 μmol kg⁻¹ (at salinity = 35 and temperature = 20 °C) and the depletion to 60 μmol kg⁻¹ will generate approximately 170 μmol kg⁻¹ sulfate ions, which is negligible compared to the natural seawater concentration (ca. 28 mmol kg⁻¹ at salinity 35). Unlike for pCO₂ and temperature, the concentration of dissolved oxygen can be easily increased to simulate preindustrial concentrations by
625 direct injection of O₂ (g) in the reactor. Designs for manipulating oxygen are underway at MBARI and a future upgrade of the swFOCE system is planned in order to control the concentration of dissolved oxygen, in line with the expected expansion of a dead zone in Monterey Bay.

6 Building a community of FOCE users: the xFOCE approach

xFOCE is an open source package of plans and software developed at MBARI to make the design and fabrication of FOCE systems more accessible to a wide range of ocean acidification researchers
630 (<http://www.xfoce.org>; Kacey et al., 2013).

Developing new systems for *in situ* experiments can be expensive, and requires time and expertise to develop. xFOCE provides reference designs at no cost to enable users to develop a simple application relatively quickly, and to refine those designs as research questions evolve. The key elements
635 of xFOCE reference designs include modularity, robustness, ease of use, and adaptability. While it is not possible to build a single solution suited to every possible FOCE experiment, the developers goal has been to provide fundamental building blocks that are easily used and modified to suit many configurations. Designs will include a system for delivering CO₂ enriched seawater, a modular experimental enclosure and anchoring system, a power distribution system for a cabled observatory, as
640 well as electronics and software for collecting data and controlling the FOCE instrument.

7 Overarching activities

A globally coordinated network of FOCE experiments in regions known to be particularly at risk of increasing CO₂ absorption, from the tropics to the poles, in partnership with leading FOCE technology specialists, will contribute the kind of community-scale information required to influence
645 ocean-research, -policy and -technology agenda's to better manage at-risk marine communities and regions in the high-CO₂ future. In order to successfully build an efficient and sustainable long-term

FOCE network it will be useful to put into place a number of joint overarching activities alongside the purely scientific work associated with each individual FOCE system. Examples of such activities are capacity building, communication and effective management of the data that will emerge from the network. These activities will need a strong collaborative effort among the various xFOCE partners and should be organized taking into account existing efforts and opportunities for coordination with other projects and initiatives. Experience from past multi-national projects such as the European Project on Ocean Acidification (EPOCA) has shown the added value of such activities, e.g. in terms of creating a collaborative work atmosphere and adding to the integrity and legacy of the project.

655 **7.1 Capacity building and collaboration**

Training and capacity building are of particular importance for the xFOCE network, especially during its initial build-up phase, due to the complex technology involved. Proper training and knowledge transfer is important for data quality assurance and comparability. It would be beneficial to plan for scientists and engineers within the network to visit one or several of the other FOCE set-ups in order to increase knowledge transfer, promote problem-solving and encourage opportunities of collaboration. This has been done on an ad-hoc basis so far, especially for engineers, but no specific funding source has been identified so far. If possible and practicable, it would also be valuable for scientists and students outside the network to visit FOCE facilities to get trained and initiate collaborations so as to increase the potential of widening the network and maximizing the use of the technique. Two workshops bringing together FOCE network partners have been organized in 2012 and 2013. The meetings brought together about 20 scientists and engineers and provided an opportunity to present recent progress and the next generation of systems, discuss difficulties encountered, common pitfalls and best practices, as well as overarching network activities such as data management and outreach. It is hoped that these annual meetings can continue to facilitate the development of the network.

670 **7.2 Inter-comparison exercises**

Inter-comparison exercises are an important means to achieve quality assurance and control at a network level. It would be advantageous for the FOCE community to organize inter-comparison exercises between the different FOCE units. Such exercises would allow comparisons of results emerging from the different systems and ensure data quality.

675 **7.3 Data management**

The real-time data collection in FOCE experiments generates a relatively large amount of information. Large data sets are not unique to FOCE experiments and are effectively dealt with, for example by the ocean observing community. It is important to ensure robust data quality assurance through a common data policy and data reporting guidelines for all FOCE systems participating in the network. Care will be taken to ensure appropriate FOCE representation and integration with

existing and emerging efforts on the compilation and archiving of ocean acidification data, for example the data compilation of the Ocean Acidification International Coordination Centre (OA-ICC; Nisumaa et al., 2010, <http://www.iaea.org/ocean-acidification/page.php?page=2195>;), and through participation in expert workshops. For example, FOCE users were represented at the first international workshop on the management of ocean acidification biological response data organized by the
685 OA-ICC in 2014 (<http://tinyurl.com/oaicc-data-mtg>).

7.4 Dissemination and outreach

Communicating the goals and results of FOCE studies to interested stakeholders (e.g. scientists, policy makers, schools, news media, and the public) is an important activity for those involved
690 in FOCE activities. FOCE experiments are charismatic and visually attractive. They can be used to increase public attention and contribute to ocean and climate literacy. Several FOCE products are already available, including videos and animations (<http://efoce.eu/media-efoce/videos>; http://www.dailymotion.com/video/xvfv42_ocean-acidification_news?search_algo=2), fact sheets and a leaflet (<http://www.efoce.eu/media-efoce/documents>). Web sites for eFOCE and xFOCE are already
695 available (<http://efoce.eu>; <http://www.xfoce.org>). A 52' documentary on FOCE systems in general and eFOCE in particular (Mediterranean 2100 – towards a record acidity) was produced in 2013 by Com On Planet (<http://vimeo.com/56970910>).

Organizations and projects such as the Center for Ocean Solutions (<http://www.centerforoceansolutions.org>), the OA-ICC (<http://www.iaea.org/ocean-acidification>) and the Ocean Acidification International Reference User Group (<http://www.iaea.org/ocean-acidification/page.php?page=2198>) could
700 assist and facilitate several of the activities mentioned above.

8 Conclusions

All approaches available to investigate the effects of ocean acidification have benefits and limitations (Table 6) and there is not a single ideal approach. It is the combination of information collected in
705 laboratory experiments, field observations (monitoring), CO₂ vents, laboratory mesocosms, FOCE, and modeling that will allow to provide the needed mechanistic understanding and predictive power that is needed (Dupont and Pörtner, 2013).

FOCE systems are designed to assess the impact of ocean acidification, in isolation or in combination with other drivers, on biological communities. They overcome some of the drawbacks of
710 laboratory experiments and of observations in locations naturally enriched in CO₂. It is expected that the next generation of FOCE systems will include, in addition to pH, options for oxygen or temperature control. Bringing perturbation experiments from the laboratory to the field is extremely challenging. All current FOCE users have experienced setbacks due to engineering issues or failures

¹. It is hoped that the present paper and the products of the xFOCE initiative will assist in the use
715 and development of FOCE systems for studies of the response of marine communities to changing
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¹For example, IEB6 and IEB8 Impulse underwater connectors, first units of Seabird SBE 37 SMP-ODO, KC Denmark
Multiple Water Sampler. More information is available from the authors.

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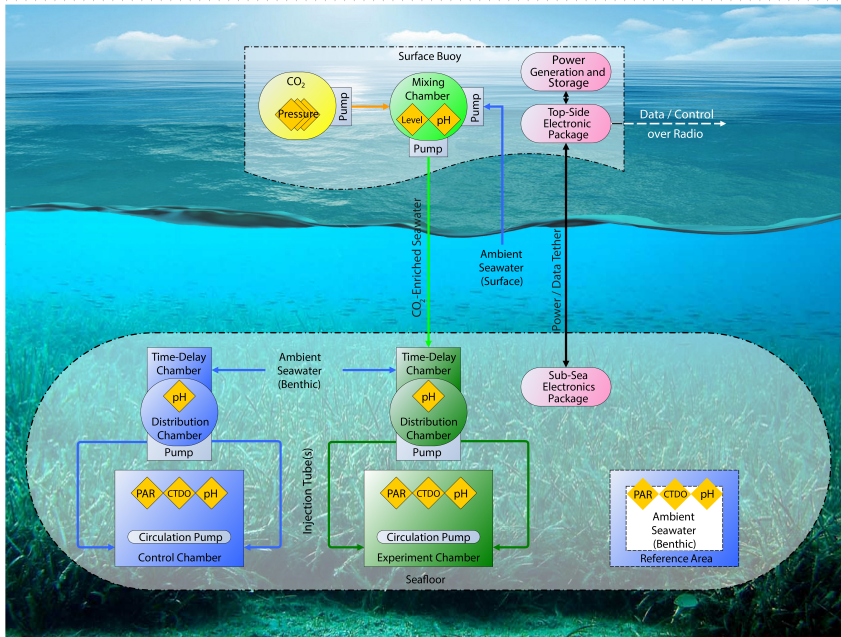


Fig. 1. Conceptual diagram of a FOCE system. The number of control, experiment and reference areas vary depending on the experimental design and budget. Additional sensors are also optional. PAR: photosynthetically active radiation; CTDO: conductivity, temperature, salinity, oxygen sensors. See the main text for definitions of the sub-components.

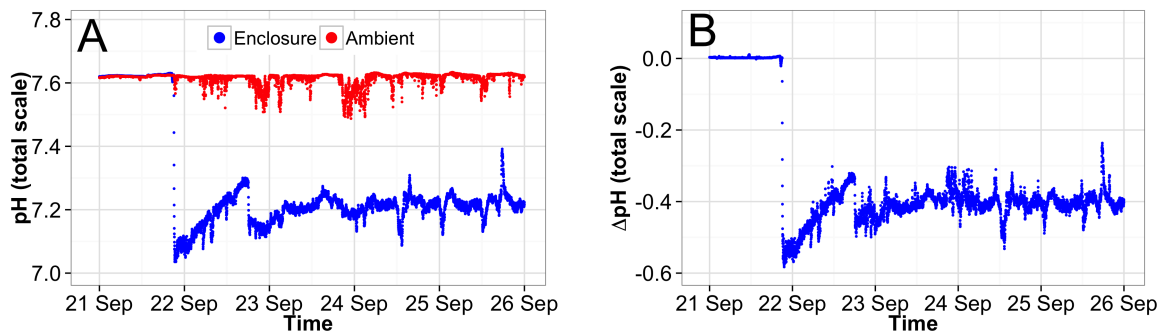


Fig. 2. pH regulation test performed in the frame of the dpFOCE project, targeting a pH offset of -0.4 unit.

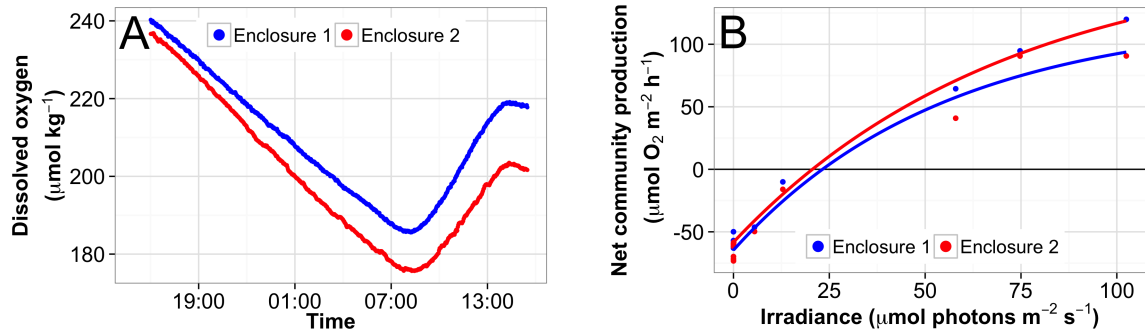


Fig. 3. Measurements of the community metabolism of a *Posidonia* seagrass bed in the northwest Mediterranean Sea performed in the frame of the eFOCE project. Oxygen variations (A) and photosynthesis-irradiance curves (B) during incubations performed in closed-chambers conditions.

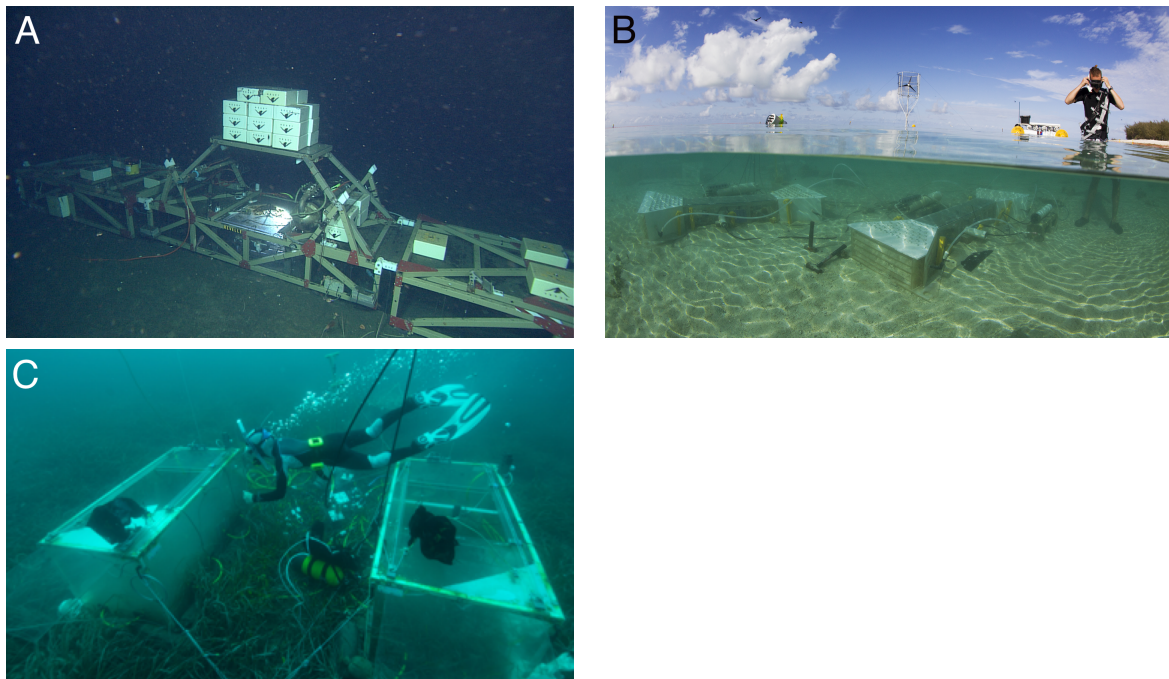


Fig. 4. A, deep-water FOCE (dpFOCE); B, Coral Proto FOCE (cpFOCE); C, European FOCE (eFOCE).

Credits: A, MBARI; B, D. I. Kline (Scripps Institution of Oceanography); C, D. Luquet (CNRS-UPMC)

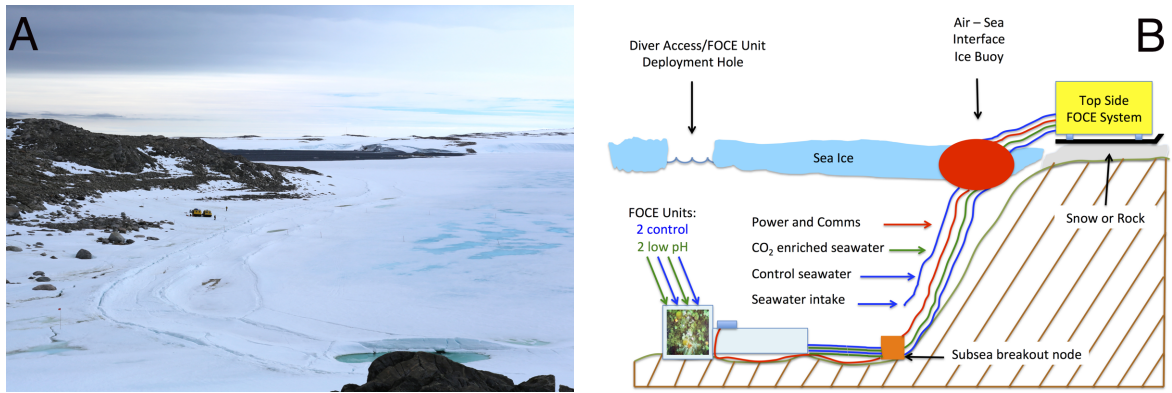


Fig. 5. A, antFOCE Site (O'Brien Bay, Antarctica); B, simplified sketch of the antFOCE system. Credits: A, G. Johnstone (AAD) and B, W. Kirkwood (MBARI) & J. Reeve (AAD).

Table 1. Design of past, current and planned FOCE systems.

	dpFOCE	cpFOCE	eFOCE	swFOCE	antFOCE
Location	North Eastern Pacific	Heron Island, Great Barrier Reef	NW Mediterranean Sea	North Eastern Pacific	Antarctica
Habitat	Deep-sea sediment	Coral reef flat	Seagrass meadow	Nearshore sand flat	Polar sediment
Time of operation	2009-2012	2009-2011	2013-present	2014	2014-2015
Depth (m)	890	3	12	15	20
Number of units	1	4	2	2	4
Duration	4 weeks	(1) 10 days; (2) 6 months	tbd	tbd	tbd
Dimension of experimental units (L × w × h in m)	1 x 1 x 0.5	1 x 0.25 x 0.25	2 x 1 x 1	2 x 0.5 x 0.5	2 x 0.5 x 0.5
Experimental design	Ambient and -0.45 pH offset	(1) ambient, -0.15 and -0.25 pH offsets, with a reference plot (2) 2 ambient and 2 with -0.25 pH offset, with a reference plot	Ambient, -0.3 pH offset, with a reference plot	Ambient and pH offset to be determined, with a reference plot	2 x ambient + 2 x -0.4 pH offset, with 2 reference plots
pH control	MBARI modified Seabird SBE18 pH sensor (6 each), metered addition of high-CO ₂ seawater	Seabird MBARI modified SBE18 pH sensors, metered addition of high-CO ₂ seawater	Seabird SBE18S pH sensors, metered addition of high-CO ₂ seawater	Seabird SBE18S sensors, metered addition of CO ₂ enriched seawater	Seabird SBE18S pH sensors and Durafet™ pH sensors, metered addition of high-CO ₂ seawater

Table 1. Continued.

	dpFOCE	cpFOCE	eFOCE	swFOCE	antFOCE
Other sensors and equipment	Seabird SBE 52 CTD with SBE 43 O ₂ sensor, Nortek Vector flow sensor (2 ea), RDI Workhorse ADCP, camera and lights	4 Seabird SBE 16+version CTDs, RINKO Li-Cor LI-192 PAR sensors, Nortek Vector flow sensors	3 Seabird SBE 37 SMP-ODO CTDs with Seabird SBE 63 O ₂ optodes, 4 Li-COR LI-192 PAR sensors	Seabird SBE 52 CTD w/ SBE 43 O ₂ , flow sensor (FM6550), RDI Sentinel ADCP, Satlantic PAR-SER ICSW irradiance sensor, camera and lights	ADCP (NORTEK Aquadopp), TSG Seabird SBE 45), Anderaa 3835 O ₂ sensor, Li-Cor LI-190SA PAR sensor, Tidbit temperature sensors, GoPro camera
Temperature (°C)	3.7-5.1	20.3-27.5	13.5-24.4	9-14	-1.0 to +1.6
pH _T	7.609-7.634	7.9-8.3	8.024-8.140	7.6-8.2	tbd
pCO ₂ (µatm)	1014-1075	148-1800	339-462	250-1200	tbd
Total alkalinity (µmol kg ⁻¹)	2358-2374	2357-2560	2240-2420	2200	tbd

Table 2. Possible experimental designs of FOCE experiments.

	Single factor	Two factors
Orthogonal	-	Two levels of two factors require at least 4 units. Complete or partial replication possible.
Gradient	At least 2 levels (ambient and elevated CO ₂). Additional levels can be added. Complete or partial replication possible.	See orthogonal design above if each factor has two levels. The levels of parameters can be unequal in nested designs. For example 3 pH levels and 2 temperature levels (6 units required without replication). Complete replication possible but technically difficult to implement.
Scenarios	Same as above with CO ₂ levels corresponding to specific scenarios for the location.	The two factors are not manipulated in isolation. For example: present, year 2050 (medium change in pH and temperature), and year 2100 (large changes in pH and temperature).

Table 3. Initial change in pH performed (or planned) during the different FOCE experiments.

FOCE system	Timeframe	Rate of change	Time to reach targeted Δ pH
dpFOCE	18 month deployment with 1 month experiments	0.3 to 0.4 unit min ⁻¹	1 min
cpFOCE	8 month experiment	-0.1 unit every 20 days	2 months
eFOCE	Gradual + natural cycle	0.05 pH unit min ⁻¹	1 week
swFOCE	1 to 6 month	0.3-0.4 unit min ⁻¹	1 min
antFOCE	4 month experiment	0.3-0.4 unit min ⁻¹	1 min

Table 4. Precision and accuracy of the measurements of carbonate chemistry parameters based on various *in situ* or laboratory techniques. $p\text{CO}_2$ is in μatm , A_T and C_T are in $\mu\text{mol kg}^{-1}$. IRGA: infrared gas analyzer.

Parameter (method)	Precision	Accuracy	Reference
pH (<i>in situ</i> , potentiometric)	± 0.003	± 0.01	Peltzer (pers. obs.)
pH (laboratory, spectrophotometric)	± 0.001	± 0.005	Dickson et al. (2007)
$p\text{CO}_2$ (<i>in situ</i> , IRGA or colorometry)	$\pm 5\text{-}10$	$\pm 7\text{-}8$	Tamburri et al. (2011)
$p\text{CO}_2$ (laboratory, IRGA)	± 1	± 2	Dickson et al. (2007)
A_T (laboratory, potentiometric titration)	± 1	± 2	Dickson et al. (2007)
C_T (laboratory, coulometer)	± 1.5	± 4	Dickson et al. (2007)

Table 5. Relative uncertainties in pH, total alkalinity (A_T in $\mu\text{mol kg}^{-1}$), dissolved inorganic carbon (C_T in $\mu\text{mol kg}^{-1}$) and partial pressure of CO_2 ($p\text{CO}_2$) resulting from the uncertainties (accuracies) shown in Table 4 (the *in situ* uncertainties are considered for pH and $p\text{CO}_2$). These relative uncertainties have been computed as described in Dickson (2010), assuming uncertainties in temperature and salinity *in situ* of 0.002°C and 0.001 , respectively.

Pair of parameters	$u\text{pH}$	uA_T	uC_T	$up\text{CO}_2$
pH - $p\text{CO}_2$	-	± 88	± 73	-
pH - A_T	-	-	± 7	± 11
pH - C_T	-	± 9	-	± 10
$p\text{CO}_2$ - A_T	± 0.007	-	± 5	-
$p\text{CO}_2$ - C_T	± 0.008	± 8	-	-
A_T - C_T	± 0.007	-	-	± 10

Table 6. Benefits and limitations of the five main approaches used to investigate the effects of ocean acidification. A 5 stage ranking is used: very high, high, medium, low and very low. Modeling approaches are not considered in this table.

Approach	Cost	Replicability	Ecological relevance
Laboratory experiments	Very low	Very high	Very low
Field observations (monitoring)	Medium	Very low	Very high
CO_2 vents	Low	Very low	Very high
Laboratory mesocosms	Medium	Medium	Medium
FOCE	High	Medium	High