Food for Thought

Advanced experimental approaches to marine water-column biogeochemical processes

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This "Food for Thought" article examines the potential uses of several novel scientific and technological developments, which are currently available or being developed, to significantly advance or supplement existing experimental approaches to study water-column biogeochemical processes (WCB-processes). After examining the complementary roles of observation, experiments and numerical models to study WCB-processes, we focus on the main experimental approaches of free-water *in situ* experiments, and at-sea and on-land meso- and macrocosms. We identify some of the incompletely resolved aspects of marine WCB-processes, and explore advanced experimental approaches that could be used to reduce their uncertainties. We examine three such approaches: free-water experiments of lengthened duration using bioArgo floats and gliders, at-sea mesocosms deployed several 100s m below the sea-surface using new biogeochemical sensors, and 50 m-tall on-land macrocosms. These approaches could lead to significant progress in concepts related to marine WCB-processes.

Keywords: at-sea mesocosms, experiments, *in situ* fertilization, water-column biogeochemical (WCB) processes on-land macrocosms.

Focus of this "food for thought"

This "Food for Thought" article develops ideas about novel experimental approaches to investigate marine water-column biogeochemical processes (abbreviated as WCB-processes). This general topic has interested the three authors for many decades, and the present paper examines our ideas about advanced experimental approaches that could be used to revisit selected scientific aspects of WCB-processes that are incompletely resolved.

Biogeochemistry is a subdiscipline of *geochemistry*. The latter considers the cycles of chemical elements and simple substances among the Earth's compartments. *Marine geochemistry* investigates interactions among the atmosphere, the surface ocean including shelf and open-ocean waters, the ocean's interior (subdivided into waters above and below the permanent pycnocline), and the seafloor.

Although the expression "marine biogeochemistry" is sometimes used interchangeably with "biological oceanography", the two approaches are different. Here, marine biogeochemistry refers to the study of cycles of chemical elements that participate in all aspects of marine life, are characteristic of climate-active biogenic molecules and participate in the long-term storage of major bioactive elements. Marine biogeochemistry considers not only the Earth's physical and geological compartments cited in the previous paragraph for marine geochemistry, but also the marine biota and some specific components of organic matter, e.g. long-lived dissolved organic carbon (DOC).

There are numerous processes that drive the cycles of elements among marine biota, the ocean's water column, the atmosphere and the seafloor (Legendre, 2014a, b). This article focuses on processes that occur in the water column. The WCB-processes discussed here mediate cycles of chemical elements across a range of spatial domains both horizontally (e.g. shelf or open-ocean surface waters) and vertically (e.g. above or below the permanent pycnocline).

Finally, the word "experiment" has been used in ocean-related research for both experimental studies and a wide variety of observational and process studies conducted at sea. For example, some process studies that followed time courses of naturally occurring changes in biogeochemical properties within well-constrained and identified patches of water (e.g. eddies) were called experiments. In order to avoid any confusion with such studies, which are included in experiments *sensu lato*, in this article the terms "experiment" and "experimental" we will only be used for procedures designed and carried out to test hypotheses. In other words, we consider that the purpose of an experiment *sensu stricto* is to provide insight into the causal relationship(s) between or among manipulated and response variables. Typically, experiments are characterized by the presence of controls, the manipulation of potentially causal factor(s), and the recording of the state or magnitude of response variable(s). An experimental procedure must be repeatable, and the measurements made during experiments must be amenable to statistical analyses.

This article examines the potential uses of several novel scientific and technological developments, which are currently available or being developed, that could significantly advance or supplement existing experimental approaches. These advanced experimental approaches could produce new knowledge that would be a major step forward for the understanding of the mechanisms that govern WCB-processes.

This article is divided into five sections. We first briefly review the main approaches that are currently in use to investigate WCB-processes, i.e. observation, experiments, and conceptual and numerical modelling. Second, we focus on the existing experimental approaches. Third, we examine some of the incompletely resolved aspects of marine WCB-processes, and fourth, we explore advanced experimental approaches that could be used to reduce the uncertainties of these incompletely resolved scientific topics. Fifth, we conclude on the co-evolution of concepts and technology.

Investigating marine WCB processes

Each of the three main approaches to study WCB processes has a large number of variants, each with its own advantages and drawbacks that can be more or less pronounced depending on the variant considered. For example, many different numerical methods and approaches can be used for comparing large-scale marine ecosystems (Legendre and Niquil, 2013). Table 1 compares key characteristics of observations, experiments and models to study WCB-processes, without considering the wide ranges of their variants.

Field observations

Field observations (Table 1, second column) should, in principle, accurately represent the *in situ* WCB-processes. In practice, however, the choices of the observation site, the frequency of sampling, and the variables sampled may bias the observations relative to the *in situ* conditions. Hence the information from field observations can moderately to highly represent the natural conditions. In addition, rate variables (e.g. fluxes) are much more difficult to determine directly in the field than state variables (e.g. concentrations). Observed relationships determined from correlations or regressions among field variables are often used as bases for causal hypotheses. However causality cannot be tested using only field observations because a correlation can reflect a range of different direct or indirect causal relationships between or among variables and some correlations can be spurious.

Field observations are related to models in many ways. For example, empirical relationships among variables measured in the field are often used as parameters for numerical models, but such empirical relationships do not necessarily reflect causal effects among the variables considered. In addition, the validity of numerical models is often assessed by comparing their outputs with field-observed data. However in cases where field observations are used to both parameterize and validate numerical models, it is essential that parameterization and validation be conducted on different data sets of the observations. Finally, a regression relationship between field-measured variables provides an empirical model that can be used to predict changes in one variable from changes in one or several other variables. However, this can only be done if these changes are within the observed range of variation of the dependent and independent variables used to construct the relationship. Also, when a relationship among field

Table 1. Comparison of characteristics of the three main approaches (field observation, experimentation, and numerical modelling) that are used to investigate WCB-processes.

Characteristic	Field observations	Experiments	Numerical models
Realism	Moderate to high	Low to moderate	Low to moderate
Generation of hypotheses	High	Moderate	Low
Hypothesis testing	None	Moderate to high	Moderate
Generation of parameters	Moderate	High	None
Generation of validation data	High	Moderate	None
Prediction	Low to moderate	Low	High
Replication	None	Moderate to high	High
Possibility of controls	Low	High	High
Regulation of surrounding environmental conditions	None	None to high	High
Largest space scales investigated	Regional to ocean	Local to regional	Regional to ocean
Longest time scales investigated	Months to decades	Weeks to months	Century to millennia

The entry in each cell of the Table corresponds to detailed explanations given in the text.

variables is based on only a few data points or from observations that are distant in space or time, it is not certain if the interpolated values would correspond to the natural conditions.

Field-observed WCB-processes cannot be experimentally manipulated, i.e. they cannot be replicated or compared to a control as done in experiments (see "Experiments" section, below). A control that is not very robust can be obtained by sampling two or multiple field sites that mostly differ by the presence *vs.* the absence of a natural stimulus, for example areas that are naturally iron fertilized and not fertilized in the Southern Indian Ocean (see "Existing approach 1: Free-water experiments", section below). In addition, researchers cannot regulate the environmental conditions under which measurements are made. The ideas about experimental replication, controls, and regulation of surrounding conditions are further developed in the "Experiments" section just below.

Field-observed WCB-processes may encompass both the past (i.e. paleoceanography) and the contemporary ocean, and their spatial and temporal extent can be quite variable. Concerning space, various remotely sensed observations are used to estimate biogeochemical fluxes at the global scale, and several international sampling programmes have been conducted at the scale of the World Ocean, but most programmes have examined WCB-processes at smaller spatial scales, e.g. regional or multi-basin. Concerning time, some modern-ocean biogeochemical time series span decades, but many are shorter (i.e. months to years).

Experiments

Experiments (Table 1, third column) are carefully designed procedures that are based on a set of objects that are initially equivalent (e.g. several containers filled with water from the same sample). Each of these objects is called *experimental unit*. In some of the units, called *treatments*, one or several variables (e.g. pH, temperature) are modified (or manipulated), whereas the other units, called *controls*, are not modified or treated. Hence, each experimental unit is either subjected to an experimental treatment or used as an untreated control. The same variable(s) (e.g. calcification, respiration) are measured in all units at the beginning and at intervals during the experiment. The effect of natural variability among samples can be estimated by replicating the treatments and controls, but the number of *replicates* is always limited because of logistical complexity and financial costs. The replication allows statistically comparison of the values of the variables measured in the treatments to those in the controls during and at the end of the experiment, taking into account the natural variability among samples. Significant effects in the treatments relative to the controls provide evidence of causal relationships between the *predictor* (manipulated) and the *response* (measured) variables. It is essential that experiments include controls and the replication of treatment conditions.

Given that experiments are designed to test hypotheses, they generally not to represent the full complexity of WCB-processes. Hence, experiments weakly to moderately represent the natural water-column conditions. Well-designed experiments provide the most powerful approach to test hypotheses, but poorly designed experiments or incorrectly analysed experimental results can lead to no conclusion, or even incorrect conclusions. In addition, constraints resulting from the experimental design, especially for experiments carried out in the natural environment from ships or other similar platforms, sometimes allow only a partial test of the

hypotheses. Another important outcome of some experiments is the generation of new hypotheses, but this is not typically the primary purpose of experiments.

Experiments are closely associated to numerical models because experimentally derived relationships are often used to parameterize numerical models. Indeed, some experiments are closely coupled with models, and their results are used to assess the validity of the structure of the model and optimize its parameterization (Mairet *et al.*, 2011; Muñoz-Tamayo *et al.*, 2014).

Experiments usually manipulate single variables at a time, whereas *in situ* environmental conditions are complex and characterized by concurrent changes in multiple interacting variables. As a consequence, the predictive power of most experiments is relatively low for complex WCB-processes, when there are many interactions, or for conditions that are dissimilar to those of the environment where the experiment had been designed.

According to the types of experimental design and approach, it may (or may not) be possible to regulate some of the changes in environmental conditions, thus the degree of environmental regulation of experiments ranges from none to highly regulated. For example, researchers conducting experiments *in situ* cannot prevent (i.e. regulate) natural changes in temperature from occurring in the experimental units, whereas in the case of experiments conducted in containers (on board ships or in the laboratory), surrounding conditions such as temperature can be highly regulated. To prevent confusion, we use the term "control" for the non-manipulated experimental units (see above), and the term "regulation" for the environmental conditions that surround the experiments and are thus not manipulated by the researcher. Regulation will also be used below for the environmental constraints that are imposed on biogeochemical processes within numerical models.

The maximum spatio-temporal extent of experiments on WCB-processes is limited by several technical and experimental constraints. For example, these types of experiments are generally conducted in geographically limited areas, or repeated at only a few locations, hence their spatial scales are local-to-regional. In addition, because of logistical constraints, the duration of such experiments is generally a few weeks only. The constraints on the experimental approaches considered here will be discussed in the "Experimental approaches to marine WCB processes" section.

Numerical models

Numerical models that include equations representing biogeochemical processes (Table 1, fourth column) are necessarily simplifications of the complex biological, chemical and physical characteristics of the natural environment. The simplest biogeochemical-physical models generally did not represent complex water-column processes, but they provided important insights into fundamental biogeochemical interactions (e.g. Fasham *et al.*, 1990). Even advanced models (e.g. ERSEM, Baretta-Bekker *et al.*, 1997; Dynamic Green Ocean; Le Quéré *et al.*, 2016) do not completely represent biogeochemical processes. Comparison of model outputs with validation datasets, which come from either field observations or experiments, provides a way to assess the robustness of the assumptions underlying the models. It is essential however that the parameterization and validation data sets are independent. In some cases, this comparison could lead to the formulation of new or alternative hypotheses. The requirement for processes to be accurately represented in increasingly complex

models (such as biogeochemistry in IPCC-type models, e.g. Le Quéré *et al.*, 2005) stresses the need for fundamental understanding of WCB processes.

Models do not generally generate parameters or validation data, but their numerical formulation and validation requires that modellers assemble and synthesize the data needed to parameterize and validate the models. These steps are essential to ensure that the structure and parameterization of the model are consistent with natural conditions. Inverse models can generate model parameters when the values of both the input and output variables are known from observation. Once a model is parameterized and evaluated, it provides a powerful tool for predicting the responses of WCB-processes, such as vertical carbon fluxes, to natural or anthropogenic changes in forcing conditions including higher temperature, reduced input of nutrients from continents, or changing practices in fisheries.

Models offer the possibility of replication in the form of repeated model runs. Indeed, the same model can be run with variations in starting conditions or parameters, or different variants of the same model can be run and their results assembled in an ensemble. In addition, the results of runs with different forcing conditions (corresponding to different treatments in experiments) can be compared to those of control runs representing "standard" conditions. Finally, the environmental conditions under which a biogeochemical model is executed, e.g. climate conditions, can be precisely prescribed according to the objectives of the study. Hence, this approach leads to high replication, control and regulation.

The spatio-temporal extent of models of WCB-processes can range from regional scales to the World Ocean. Similarly, the models can be used for exploring past conditions, assessing changes to the current ocean conditions, or predicting the consequences of anthropogenic climate change in the future.

Different roles of the three approaches in marine biogeochemistry

Field observations, experiments and numerical models have different and complementary roles in the study of WCB-processes. These roles are summarized as follows:

Field observations provide the closest representation of *in situ* WCB-processes. Information collected during field studies often provides the bases for developing hypotheses that are then tested using either experiments or assessed by numerical models. These are often designed and formulated to explain changes in field observations. The observations also provide parameters and validation data for numerical models, but different sets of field observations must be used for parameter development and model validation. In turn, the results of experiments and the formulation of models can also identify gaps in field data and inspire new observational programmes.

Experiments on WCB-processes are very powerful approaches to test hypotheses. Experiments offer the advantages of replication and controls, and some also provide the possibility of regulating environmental conditions. Experiments can generate high-quality parameters as well as validation data for numerical models. Hypotheses concerning WCB-processes are can be based on field observations as well as on results of experiments or models.

Numerical models are powerful tools for predicting WCB-processes under a wide range of spatio-temporal conditions. The validation of models with field or experimental data provides a test of their underlying assumptions. Model runs can be replicated and controlled, and the environmental conditions under which models are running can be regulated. In turn, the assumptions on which the structure of a model is based and its parameterizations are based on field observations and experimental results.

The above description shows that experimental approaches are critical for the quantitative understanding of WCB-processes. Marine biogeochemistry has now reached a stage in its evolution when some key experimental approaches could undergo major developments leading to significant advances. The remainder of this paper is focused on experimental approaches.

Experimental approaches to marine WCB processes

Three main types of water-column experimental approaches

There are three broad categories of experimental systems to investigate WCB-processes. These systems are either "free water" or "enclosed", with the latter type being subdivided into systems that can be deployed at sea or are erected on land. In free-water experiments, a large body of water is modified and the time courses of changes in chemical, biological, and/or biogeochemical characteristics of the modified water column are monitored. There have been many such experiments in freshwaters since the 1970s, including the manipulation of whole lakes (e.g. Van Donk *et al.*, 1990; Koschel, 1995; Stokstad, 2008), whereas marine free-water experiments started in the 1990s with the *in situ* enrichment of limiting inorganic nutrients i.e. iron and phosphorus. In enclosure experiments, a volume of water is incubated in a container and response variables are measured after varying one or more conditions (independent variables). Experimental enclosures can be classified, based on size, as microcosms (from 10^{-3} to 1 m^3), mesocosms (between 1 and 10^3 m^3), and macrocosms ($>10^3$ m^3), terms recommended by Unesco (1991), and based on their aspect ratio (i.e. ratio of width to height), as 1:3 and taller, between 1:3 and 1:1, and 1:1 and wider. Experimental enclosures used *in situ* (at sea) and built on land have different sizes and shapes.

This article is focused on WCB-processes, and the relevant experimental approaches here are the free-water experiments, and the meso- and macrocosms with aspect ratios of 1:3 and taller, which adequately represent a water column. For meso- and macrocosms, we examine separately the structures deployed at sea and erected on land. Some of the key characteristics of the three experimental approaches are summarized in Table 2, and discussed in the "Existing approach 1" to "Existing approach 3" sections.

Existing approach 1: Free-water experiments

Ecosystem-scale experiments in freshwater systems have assessed responses of both communities and biogeochemical processes to a range of environmental manipulations including nutrients, temperature, acidification, radionuclides, geomorphology and ultraviolet exposure (Likens, 1985; Carpenter *et al.*, 1995). Results from whole-lake free-water experiments showed that high nutrients rather than carbon were responsible for eutrophication, and N_2 could be drawn from the atmosphere to sustain cyanobacterial blooms (Schindler, 1977). These experiments also showed that

Table 2. Characteristics of the at-sea experiments, and at-sea and on-land meso- and macrocosms that have been, or are presently being used to investigate WCB-processes.

Characteristics	Free-water *in situ* experiments	At-sea meso- and macrocosms	On-land meso- and macrocosms
Size	$\sim$300 to $>$ 2000 km^2	$\sim$30 to $\sim$1300 m^3	$\sim$100 to $>$ 1000 m^3
Medium	Natural	Natural	Natural or artificial
Replication	None	High	None to moderate
Control(s)	Moderate	High	None to moderate
Regulation of surrounding environmental conditions	None	None to low	None to high

The entry in each cell of this Table corresponds to explanations in the text. Suggestions for improvements are described in the "Looking beyond current experimental approaches" section.

eutrophication could be controlled by managing the sole inputs of P (Schindler, 2001, Schindler *et al.*, 2016). Whole-lake acidification experiments demonstrated that effects were transmitted through food webs to affect populations of fishes and aquatic birds and cause indirect effects that cascaded through food webs to alter productivity and nutrient cycling (Schindler, 1990, Carpenter *et al.*, 1995). The marine equivalent of whole-lake experiments is not feasible because both the spatial and depth scales over which processes occur in oceans are much larger than in lakes and the ocean regions are interconnected.

New approaches were developed in the early 1990s to address experimentally the idea that the availability of iron limited primary productivity in large high-nutrient and low-chlorophyll (HNLC) areas of the ocean (Martin and Fitzwater, 1988). These regions account for $>$35% of the area of the global ocean. According to this "iron hypothesis", iron availability controls the biological carbon pump (i.e. photosynthetic production, food-web transfers and downward export of biogenic carbon), and had a significant effect on atmospheric carbon dioxide concentrations over glacial–interglacial cycles. The effect of iron on phytoplankton production was first tested during experiments using bottle incubations on board ships. This was followed by "free-water" mesoscale fertilization experiments in diverse areas of the World Ocean between 1993 and 2009 (Boyd *et al.*, 2007).

The mesoscale experiments varied in their fertilized areas from $\sim$25 to $>$300 km^2 and lasted $\sim$nine to 70 days, and the maximum extent of the fertilized area exceeded 2000 km^2 with a volume of $\sim$3 $\times$ 10^{20} m^3. Iron was added to 12 sites in polar, sub-polar and tropical HNLC regions, phosphorus was added to one site in the ultra-oligotrophic eastern Mediterranean Sea, and iron plus phosphorus were added to one site in the Northeast Atlantic. Ocean fertilization purposefully alters the chemistry of the water column, and the biological and biogeochemical processes. The design of mesoscale nutrient enrichment experiments required that the addition of the dissolved nutrient (i.e. iron or phosphorus) be simultaneously released with a conservative tracer (i.e. sulphur hexafluoride, SF6) to track the distribution and dilution the nutrient-enriched patch (de Baar *et al.*, 2005). A number of parameters were monitored in these experiments to assess the biological (i.e. plankton abundance, growth and species composition) and the chemical (nutrients, carbon dioxide and organic and inorganic nutrients) responses and the downward carbon export using sediment traps and radionuclides. Measurements were conducted outside and inside the fertilized patch, thus providing one control and one treatment.

Due to the size, logistical complexity and cost of establishing and monitoring a mesoscale patch of nutrient enriched water,

ocean iron (or phosphorus) fertilization experiments have not been replicated (i.e. two or more patches at the same location and time). Since the fertilized patch was not replicated, it was difficult to statistically assess the magnitude of the planktonic response to the nutrient fertilization (and subsequent carbon export to the ocean interior in the case of Fe-fertilization). For example, Hale *et al.* (2006) assessed the effects of iron fertilization on heterotrophic bacteria in one experiment, using the Analysis of Covariance where time (i.e. "sampling day") was included as covariate. Although these authors found significant changes in many of the variables inside the Fe-enriched patch relative to outside (e.g. bacterial biomass and production, and community respiration), they could not distinguish between the direct (i.e. enhanced growth and production of heterotrophic bacteria) or indirect (i.e. changes in extracellular release of nutrients by phytoplankton and trophic cascades) effects of Fe addition on heterotrophic bacteria. In addition, the short durations of these experiments precluded extrapolation of the results to longer time scales. An alternative to these short-term experiments was to study the phytoplankton and WCB-processes in response to naturally occurring iron fertilization. Examples were the studies on natural iron fertilization in the Southern Ocean, in the Crozet Island region (Pollard *et al.*, 2009) and on the Kerguelen Plateau (Blain *et al.*, 2007; Van der Merwe *et al.*, 2015) where long-term effects of these processes were compared in naturally fertilized and non-fertilized areas.

Ocean iron fertilization experiments have provided unambiguous support for the first component of the iron hypothesis, i.e. the enhancement of phytoplankton production by iron addition in HNLC regions. However, these experiments have not unequivocally demonstrated the second component of the hypothesis, i.e. the transfer of the resulting biogenic carbon to depth. The studies carried out in the Southern Ocean on the Kerguelen Plateau indicate that fertilization duration and microbial dynamics may be important factors in controlling the Fe-enhanced export of carbon.

Existing approach 2: at-sea mesocosms and macrocosms

At-sea (or *in situ*) mesocosms and macrocosms have their origin in the freshwater enclosures (limnocorrals) first used in 1957 in a Czechoslovakian reservoir to study the effects of algicides on phytoplankton (O'Brien *et al.* 1992). In marine systems, such large incubation systems were typically deployed *in situ* to ′capture′ a column of water, or the cylinders were filled otherwise with natural seawater (Grice and Reeve, 1982; Lalli, 1990). The first marine mesocosm was the Kiel Plankton Tower (Kiel Bight), which consisted of four 16-m high and $\sim$30-m^3 cylindrical PVC enclosures

that extended from the surface to sediments (11-m depth), and were used to study the annual cycle of phytoplankton and nutrient dynamics and sediment-water interactions (Bodungen *et al.*, 1976; Smetacek *et al.*, 1976).

There have been several other at-sea cylindrical mesocoms used in Norway (Hamburg Enclosures), Holland (Den Helder Enclosure), Scotland (Loch Ewe Enclosure), and Canada (Controlled Ecosystem Pollution Experiments) (Grice and Reeve 1982), and China (Wu *et al.*, 1992). Enclosure volumes ranged from ∼5 to 40 m high and ∼30 to ∼1300 m^3. In addition, large spherical mesocosms were deployed in Canadian coastal waters (McAllister *et al.*, 1961). These ∼6-m diameter mesocosms were generally moored and did not include sediments, and they were used to investigate a wide range of processes including plankton production, nutrient cycling, and potential toxic effects of petroleum hydrocarbons and trace metals on the planktonic food web. There are presently many mesocosm facilities in Europe, North America, Asia, and elsewhere (e.g. https://www.mesocosm.eu/).

In the last decade, technological advances in highly flexible, non-toxic plastic films have permitted the use of large-volume (>50 m^3) experimental enclosures to study the effects of micronutrients, atmospheric nutrient delivery (via dust particles) and ocean acidification, with replicated systems (e.g. Riebesell *et al.*, 2010). Some of these studies were carried out in high latitude and coastal areas, whereas others were conducted in oligotrophic waters such as the Mediterranean Sea. The latter experiments required ultra-clean mesocosms and operations because the very low nutrient concentrations made the experiments very sensitive to potential chemical contamination (e.g. Guieu *et al.*, 2014).

At-sea mesocosms are highly versatile experimental systems. In addition, because several mesocosms can be deployed in a relatively small area, the experimental design can incorporate replication for both control and treatment conditions. However the temperature and light fields of these experimental systems cannot be regulated and the conditions thus vary as those *in situ*. Because turbulence and vertical mixing inside the enclosures differ from those in the surrounding environment, mesocosms offer a limited amount of regulation of this environmental condition. The use of at-sea mesocosms has advanced our understanding of aquatic and ecological processes, and these have been described and reviewed in the literature (Petersen *et al.*, 2009; Stewart *et al.*, 2013).

Existing approach 3: on-land mesocosms and macrocosms

On-land mesocosm experiments have an important role in increasing our understanding of the functioning of marine ecosystems, and there is a long and rich history of such facilities. Initially, these systems were not used to study WCB-processes, but other marine biological phenomena. The first reported experimental on-land enclosure (∼1936) was an indoor tank (12 m tall × 2 m diameter; 38 m^3) at the Oceanographic Institute in Göteborg, Sweden. It was used to study plankton dynamics under controlled abiotic conditions with natural gradients in light and temperature. There was no replication of experimental conditions, and temperature was not regulated. Three decades later (∼1965), the Deep Tank Facility, an outdoor tank (10 m tall × 3 m diameter; 73m^3) at the Scripps Institute of Oceanography, CA, USA, was constructed and used mainly for research in biological oceanography, phytoplankton and zooplankton dynamics, and

pollution. There was no replication of experimental conditions and the temperature was not regulated.

There are at least two large on-land facilities that are currently in use for experimental studies (https://www.mesocosm.eu/). The Aquatron Tower Tank Facility (since 1975) is an indoor water-column enclosure (10 m tall × 3.6 m diameter; 113 m^3) at Dalhousie University, Nova Scotia, Canada, which has been used for a variety of biological oceanographic and plankton- and life-history related studies. Temperature is regulated, but there is no replication (i.e. single tank). The Marine Ecosystem Research Laboratory (since 1976) consists of outdoor tanks (5.5 m tall × 1.8 m diameter; 31 m^3) at the Environmental Protection Agency laboratories in RI, USA. The 14 enclosures have been used to study long- and short-term influence of pollution, nutrients, salinity, acidification, and stratification. There is replication of experimental conditions, but the temperature is not regulated.

Most on-land mesocosms have been small (typically <100 m^3), and lacked the regulation of environmental conditions such as temperature, light and mixing. In addition, mesocosms >100 m^3 lacked the replication of treatments or controls, both of which are essential to obtain experimental results that can be statistically analysed to test hypotheses. Mesocosms and marcocosms could substantially contribute to bridging the gap between small-scale laboratory and large-scale *in situ* environmental studies provided that these mesocosm and macrocosm facilities allow replications and their environmental conditions are regulated, thus allowing for robust statistical analyses. The use of on-land mesocosms has advanced our understanding of aquatic and ecological processes, and these have been described and reviewed in the literature (Petersen *et al.*, 2009; Stewart *et al.*, 2013).

In the "Three incompletely resolved research topics" section, we describe three important, yet incompletely resolved aspects of marine WCB-processes, and in "Looking beyond current experimental approaches" section, we suggest improved or alternative experimental approaches to address these research topics. These proposed advanced approaches extend the currently existing free-water experiments, at-sea mesocosms and on-land macrocosms whose characteristics were summarized in this section.

Three incompletely resolved research topics
Criteria for selecting the research topics

There are many aspects of marine WCB-processes that are incompletely resolved. Here we use two criteria to select three general topics to be further explored. Our first criterion is the relevance of these topics to the biologically driven sequestration of carbon in the ocean, which has been of interest to the three co-authors for decades. Our second criterion was the possibility of using novel experimental approaches to improve the fundamental understanding of the selected topics.

The three research topics are: the downward export of organic matter from the euphotic zone; the transformation of organic matter below the euphotic zone; and the effects of temperature on the initiation of phytoplankton blooms and the metabolic balance of the ocean, and subsequent effects on WCB-processes. The first two topics are related to key steps of the ocean's biological carbon pump, which is an important determinant of carbon sequestration below the top of the permanent pycnocline (whose depth varies between <500 and 2000 m), i.e. in deep waters and sediments (Guidi *et al.*, 2015). The third topic relates to the ongoing anthropogenic warming of the global ocean.

Production of organic matter in the euphotic zone and its downward export

There is a general relationship between phytoplankton production in the euphotic zone and the downward export of organic matter at large spatial (ocean basin) and temporal (years) scales, and temperature is one of the key factors that govern this relationship (e.g. Laws et al., 2000). However, the processes of primary production and downward export are often not tightly coupled at small spatio-temporal scales (e.g. Henson et al., 2012). Indeed, organic carbon synthesized by phytoplankton is largely transformed by the pelagic food web within the euphotic zone, where food-web processes remineralize a large part of the organic carbon back to CO_2 (ca. 80–85%), and generate a wide variety of particulate and dissolved forms of organic matter (POM and DOM, respectively) and inorganic nutrients. The organic matter that is not remineralized in situ by the food web can be exported downwards, or transformed to long-lived, refractory DOM.

Several mechanisms have been proposed to explain the different observed relationships between primary production and export from the euphotic zone. These mechanisms include differences in phytoplankton community structure (Guidi et al., 2009), the ballasting of particles by minerals such as calcite or opal (Moigne et al., 2014), the selective remineralization of POM and DOM by microbes (Letscher and Moore, 2015), and the possibility that aerobic anoxygenic phototrophic bacteria (AAPB) which require less organic carbon (due to their unique physiology) than non-AAPB heterotrophic bacteria, could leave more carbon for export (Jiao et al., 2007). Another proposed explanation is the accumulation in surface waters of a large pool of aggregates made of organic particles combined with non-sinking transparent exopolymer particles (TEP) (Mari et al., 2017). The quantitative and relative importance of the various proposed mechanisms in determining downward export remains to be established by experiments conducted at scales that are relevant to processes that occur in the ocean's water column.

Organic matter transformation below the euphotic zone

The POM and DOM that is exported downwards ("Production of organic matter in the euphotic zone and its downward export" section) can be further modified and remineralized during their vertical transit through the mesopelagic layer and into the deep ocean. The fraction of the exported organic carbon that escapes remineralization during its downward transit and reaches depths below the top of the permanent pycnocline is considered to be sequestered. The carbon transformation processes below the euphotic zone include the consumption, repackaging and remineralization of POM, and both the assimilation, increase of lifetime and remineralization of DOM (Robinson et al., 2010). Because the transformation and remineralization of POM and DOM during their downward transit largely determines the fraction of the exported biogenic carbon that is sequestered, the biological mechanisms involved are of critical importance for the quantitative study of WCB-processes.

Regional differences in carbon sequestration below the top of the permanent pycnocline can be caused by differences in the rate of carbon export from the euphotic zone and remineralization of carbon during its downward transit (Guidi et al., 2015). Key mechanisms for the transformation of POM below the euphotic zone include the degradation or ingestion of sinking organic particles by the pelagic food web, which can remineralize up to 90% of the exported POM back to CO_2 on average, and the repackaging of small particles into larger ones by e.g. appendicularians (Gorsky et al., 1991; Robison et al., 2005). Repackaging of small into large particles generally increases the sequestration of carbon because larger particles sink more rapidly, and thus have a higher probability of escaping remineralization above the depth of sequestration.

Most of the DOC is consumed and remineralized to CO_2 by bacteria during its downward transit. As a consequence, the contribution of DOC to carbon sequestration via vertical transport processes (e.g. vertical mixing, thermocline ventilation) is thought to be small. However, not all DOC is consumed, and part of it is transformed into long-lived DOC (i.e. average lifetime >100 years) that biochemically sequesters carbon at any depth in the water column. The suite of microbial processes that transfer carbon to long-lived DOC is called the Microbial Carbon Pump (Jiao et al. 2010). Legendre et al. (2015) and Walker et al. (2016) estimated the magnitude of the microbial carbon pump in the World Ocean to be 0.2 and 0.11–0.14 Pg C y^{-1}, respectively. The corresponding values reported by Guidi et al. (2015) for the biological carbon pump were 0.7 and 0.3 Pg C y^{-1} at the top of the permanent pycnocline and at 2000 m (i.e. the depth used as reference in several sediment-trap studies), respectively. Quantifying the biogeochemical processes of organic matter transformations that take place in the mesopelagic layer requires novel experimental approaches to be conducted in situ, deep in the water column.

WCB processes affected by global ocean temperature

The overall fate of biogenic carbon in oceans, i.e. its remineralization vs. sequestration, is largely determined by key WCB-processes. Some of these processes have been incorporated into hypotheses and conceptual models, but empirical testing of their predictions is often lacking. We selected here two hypotheses whose predictions may be affected by the change in global ocean temperature, which would thus influence the biologically mediated carbon transformation and sequestration.

The first process considered here is the set of conditions that lead to the initiation of the spring phytoplankton bloom. The generally accepted explanation of this phenomenon is provided by the critical depth hypothesis. Although proposed more than six decades ago by Sverdrup (1953) and successfully used in many papers (compiled in Sathyendranath et al., 2005), this hypothesis has not been rigorously tested by lack of some key in situ measurements (Franks, 2015; see also related papers in the July/August 2015 issue of the ICES Journal of Marine Science). This hypothesis has also been used to explain phytoplankton blooms that occur at other times of year (Legendre, 1990). Many factors have been invoked to explain the timing and magnitude of the spring bloom (Sathyendranath et al., 2015), and most of these relate directly or indirectly to temperature, irradiance and stratification. Hence global warming could affect the timing and magnitude of phytoplankton blooms, and thus the biological carbon pump.

The second process examined here is how the temperature-dependence of both primary production and heterotrophic respiration affects the metabolic balance of the ocean. Based on the metabolic theory of ecology, López-Urrutia et al. (2006) predicted that in the surface ocean, the rate of respiration by heterotrophs would increase more rapidly than photosynthesis, thus leading to a decrease in the net flux of CO_2 into the ocean due to climate warming and increased ocean temperature. Global warming

related changes in the metabolic balance of the ocean could change the magnitude of the biological vs. the microbial carbon pumps.

The hypotheses concerning the effect of temperature on the above two key WCB-processes are generally supported by modelling and field and laboratory data. However, these hypotheses presently lack rigorous experimental testing.

Looking beyond current experimental approaches
Recent scientific and technological advances

We summarize here three types of recent technological and scientific advances in marine water-column studies that, when combined with existing technologies, could result in major advances in experimental approaches. We foresee that such new experimental approaches could lead to significant progress in the quantitative understanding of WCB-processes.

The first technological and scientific advance is the development of bioArgo floats, which are biogeochemical versions of the standard Argo floats. About 4000 standard Argo free-drifting profiling floats are currently deployed in the World Ocean, and each of them robotically measures temperature (T) and salinity (S) in the upper 2000 m of the World Ocean every 10 days. The new bioArgo floats will measure T and S as well as biogeochemically important variables including downwelling irradiance at three wavelengths (380, 412, and 490 nm), photosynthetically available radiation, fluorescence of coloured dissolved organic matter and chlorophyll *a* (chl *a*), backscattering coefficient at 700 nm (a proxy of suspended particles and DOM), dissolved O_2, attenuation coefficient (660 nm), pH, and NO_3. A recent study shows that combining T, S and dissolved O_2 values using a neural network method can generate reliable estimates of macronutrient (NO_3, PO_4, and $Si(OH)_4$) concentrations and parameters of the carbonate system (pH, total alkalinity, total dissolved inorganic carbon, and partial pressure of CO_2) in the upper 1500 m of the water column (Sauzède *et al.*, 2017). Hence the standard Argo floats could provide, if progressively equipped with O_2 sensors, information not only on T, S, and O_2, but also on the macronutrients NO_3, PO_4 and $Si(OH)_4$ and the parameters of the carbonate system above 2000 m. Increasing numbers of gliders are now being equipped with the same sensors as the bioArgo floats. In addition, autonomous systems have been developed to estimate the particle flux *in situ*, e.g. bio-optical profiling floats (Estapa *et al.*, 2013) and the Carbon Flux Explorer (Bishop *et al.*, 2016).

The second advance is the present and developing technology for *in situ* and miniaturized sensors to measure relevant biogeochemical variables (Estapa, 2017; Johnson, 2017). Below are selected examples for different categories of sensors. Increasingly diverse *in situ* miniature sensors are used at sea, e.g. on bioArgo floats and gliders (previous paragraph), and recently developed sensors, e.g. a multichannel fluorometer able to identify *in situ* phytoplankton functional types, should be deployed in the near future. Approaches are being investigated for the *in situ* measurement of low-concentration nutrients, e.g. microfluidic chips and electrochemical sensors, and it may be possible to adapt other miniature sensors for use at sea, e.g. the small mass spectrometer *Ptolemy* that equipped the lander *Philae* of space probe *Rosetta*. In addition, some bulky *in situ* instruments could be or are being miniaturized, e.g. the *in situ* flow cytometer Imaging FlowCytobot, for particles 10–150 μm, the Underwater Vertical Profiler, for particles >100 μm and automatic identification of >80 zooplankton

types, the Laser In Situ Scattering Transmissometer StreamLined profiler, for particle-size distribution and concentration, and *in situ* mass spectrometers. Finally, bench-top instruments for marine biogeochemical variables (e.g. the Contros-Kongsberg analysers for seawater pCO_2 and total alkalinity) may have *in situ* versions in the future. With the extensive deployment of additional sensors on various platforms, moored and autonomous (e.g. profiling floats, gliders), combined with the satellite remote sensing of surface marine biogeochemical properties, there is the potential to extend the understanding of WCB-processes beyond the presently conceivable horizon.

The third advance is the possible development of currently existing on-land mesocosms into larger systems, i.e. macrocosms. Indeed, a persistent challenge has been how to scale up the experimental results from smaller to larger systems, including from the scale of mesocosms to that of the water column *in situ* (Petersen *et al.*, 2009). This difficulty could perhaps be resolved by scaling up the experimental systems themselves, i.e. using macrocosms, instead of trying to scale up the results of experiments conducted on small-scale mesocosms.

Advanced approach 1: free-water experiments of lengthened duration

In response to the addition of a limiting nutrient, a portion of the organic carbon produced by phytoplankton would be remineralized by the food web in or below the euphotic zone, with the remainder being exported below the euphotic zone, representing the first step leading to carbon sequestration at depth. The temporal and spatial scales for the export and sequestration are generally weeks to months and 10s–100s of km, respectively. Scale appears to be important variable that influences the ecosystem response to iron fertilization (Boyd *et al.*, 2002, Watson *et al.*, 2008). The spatial scale of the iron fertilization experiments conducted in different areas of the World Oceans between 1993–2009 that tested the iron hypothesis may have been too small ($<2000 \, km^2$) and too short (<70 days) to assess unambiguously the biogeochemical fate of the newly synthesized organic carbon ("Existing approach 1: Free-water experiments" section). Moreover the impossibility of regulating the environmental conditions inside and outside the fertilized patch ("Experiments" section) made it even more difficult to draw clear conclusions concerning the fate of the new organic carbon inside the fertilized patch. Furthermore, there have been no (published) free water mesoscale nutrient fertilization experiments where replicate nutrient enriched patches were created and monitored. Because of the lack of replication, robust statistical analyses comparing treatments (i.e. Fe fertilization) with unfertilized control site were not possible.

There are large uncertainties with constraining, quantifying and understanding the functional relationships between the production of organic matter in the euphotic zone and its downward export ("Production of organic matter in the euphotic zone and its downward export" section) Significant downward export of organic particles was not observed following the addition of Fe in most of the iron fertilization experiments, and the reasons invoked for this include: consumption and respiration back to CO_2 in surface waters of the organic carbon produced in response to the iron enrichment; occurrence of downward particulate export after the observation period; or intervention of other processes that retarded or prevented the production and sedimentation of

organic particles, such as the production of TEP. To further assess and perhaps distinguish among these options and to specifically address whether the observation period was insufficient to detect particle export, the period of observation of the enriched waters, which progressively occupy an increasing large surface because of horizontal diffusion, should be lengthened significantly, e.g. to two to three months. Is it possible to achieve this needed lengthening of free-water experiments given the high cost of maintaining a fleet of oceanographic ships on site during several months?

The observation period for some of the iron fertilization experiments was extended beyond the ship-based observations by using remotely sensed colour images of ocean chl *a* (Moore and Doney, 2006; Boyd *et al.*, 2007). These have shown a continued expansion of the area of the phytoplankton bloom. However, in order to assess the biogeochemical consequences of a mesoscale nutrient fertilization (e.g. downward flux of organic particles), it would be necessary to observe over the long term a wide area (i.e. the diffusing-advecting patch, and a non-nutrient-enriched reference site as statistical control) of both surface and subsurface indices of biomass changes and particle flux. This could be achieved (Figure 1) by using a large number of bioArgo profiling floats or gliders with advanced instrumentation, standard Argo floats or gliders equipped with O_2 sensors, and autonomous particle flux systems (see "Recent scientific and technological advances" section). These could be deployed within and outside the nutrient fertilized area before the fertilization, and programmed to vertically profile the water column for a predetermined period (e.g. 2–3 months). Inside and outside the nutrient-enriched patch, high-density spatio-temporal information on vertical changes in biogeochemical characteristics will be provided by the profiling floats, and on particle flux by the relevant autonomous systems. Operational forecast of 3-D ocean circulation (e.g. Mercator Ocean, Lellouche *et al.*, 2013) could be used to predict the horizontal diffusion/advection of the patch, and these predictions could be refined by assimilating in higher resolution
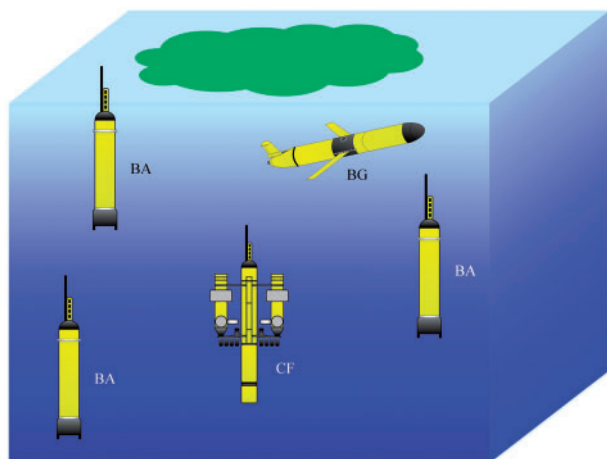
regional numerical models remotely sensed satellite data of sea-surface temperature, salinity and sea surface height (altimetry) in the region of interest. Combining information from the profiling floats and remote sensing of the surface ocean could allow the observation period of free-water experiments to be increased from at most a few weeks presently to several months in the future.

This advanced experimental approach could launch a new generation of free-water experiments, which would take advantage of both the information from the previous generation of nutrient enrichment experiments and the novel, rapidly developing bioArgo technology and associated scientific advances. This coupled approach could directly address some of the incompletely resolved scientific challenges concerning the biologically driven sequestration of carbon in the deep ocean.

Advanced approach 2: Water-column at-sea mesocosms
The deployment of mesocosms at sea has provided novel experimental information on biogeochemical processes in the surface ocean ("Existing approach 2: At-sea mesocosms and macrocosms" section). Similarly, the deployment of various types of enclosures on the sea bottom has provided unique experimental information on biogeochemical processes at the water-sediment interface (Jahnke, 1996; Glud, 2008). However, similar experimental approaches using enclosures have not been used to investigate biogeochemical processes in the water-column located between the upper 100 m of the ocean and the seafloor. This reflects the lack of appropriate experimental systems.

The mechanisms regulating the transformation of organic matter below the euphotic zone are often inferred from indirect measurements. Adapting aspects of mesocosm methodology developed for surface waters and applying them to directly measure the biogeochemical consequences of processes occurring *in situ* in the mesopelagic layer ("Organic matter transformation below the euphotic zone" section) may significantly improve the quantification and parameterization of these processes. Indeed, it may be possible to deploy mesocosm-type enclosures (e.g. cylindrical bags ≥15 m long and ≥2 m in diameter) suspended several 100s m below the surface (Figure 2). These enclosures could be closed at the bottom to permit particle collection (similar to the approach currently used in most surface-water mesocosms), or they could be open at the bottom to allow free vertical circulation of the water through the enclosure, with a particle collector affixed below to collect the POM sinking from the bag. The biogeochemical mechanisms that influence the downward POM flux could be studied experimentally by manipulating (i.e. modifying) the magnitude of the POM flux in the enclosures. This could be done by installing, at the top of the enclosure, a horizontal louver whose mobile slats could be fully open (i.e. control), or partly or completely closed, to change the flux of sinking particles (i.e. treatments). A large number of biogeochemical variables would be measured automatically at various levels inside the treatment and control enclosures, and also outside the enclosures in the adjacent water column (this is called 'negative control' in an experimental design). These variables could include T, S, dissolved O_2, macronutrients, parameters of the carbonate system, DOM, POM, chl *a*, particles and zooplankton, and other relevant biogeochemical data such as those provided by new *in situ* and/or miniaturized sensors become available ("Recent scientific and technological advances" section).

Figure 1. Conceptual representation of advanced experimental approach 1 (i.e. free-water experiments of lengthened duration): autonomous robots could be deployed in the region of an experimental nutrient fertilization to sample WCB properties under the fertilized water patch and outside. Examples of autonomous platforms are BioArgo floats (BA), gliders with biogeochemical sensors (BG), and systems to estimate the particulate carbon flux (CF). Not drawn to scale.
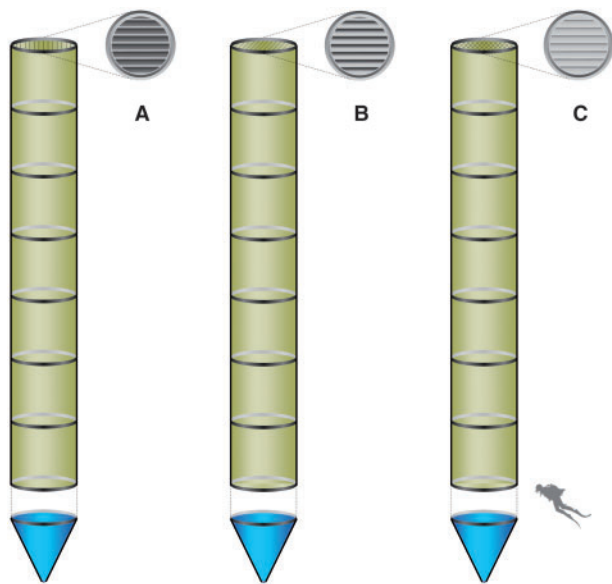
Figure 2. Schematic representation of advanced experimental approach 2 (i.e. water-column at-sea mesocosms): mesocosm-type enclosures (e.g. cylindrical bags ≥15 m long and ≥2 m in diameter) could be suspended several 100's m below the water surface, with a particle collector attached to their bottom. The flux of sinking particles could be modified by (A) fully opening, (B) partly closing, or (C) completely closing the mobile slats of a horizontal louver system at the top of each enclosure. Biogeochemical properties within each mesocosm would be monitored by *in situ* sensors. The diver provides the scale of the mesocosms (although there would be no divers at such depths).

Moored and free-drifting surface tethered sediment traps are known to influence the flow of fluid (hydrodynamic focusing), which in turn alters the collection of particles (Buesseler *et al.*, 2000, 2007). Similarly, the walls of water-column enclosures alter the water column mixing and water flow regimes (Riebesell *et al.*, 2010, Striebel *et al.*, 2013), and thus affect the distribution of particles. Hence the water-column enclosures proposed here would likely cause similar perturbations. However, given that the purpose of the enclosures is to conduct experiments comparing biogeochemical variables under a range of vertical flux regimes through the enclosures (i.e. the treatments), the perturbations caused by the enclosures should be similar among the different experimental units (i.e. the enclosures) and thus not prevent a meaningful comparison of the treatments.

This advanced experimental at-sea approach would extend the existing mesocosm science and technology from its present surface-ocean focus to the ocean's interior, by making extensive use of presently existing and developing biogeochemical sensors. This would directly address the mechanisms and magnitude of organic matter transformation below the euphotic zone, which determine the attenuation of the POM flux in the water column and thus largely govern the biologically-driven sequestration of carbon in the deep ocean.

Advanced approach 3: Scaled-up on-land macrocosms

On-land mesocosms are the only large experimental systems that offer the possibility of fully regulating the environmental conditions of experiments, can be replicated, and can thus be used to

investigate complex biogeochemical processes. Indeed, some studies using on-land mesocosms have led to advances concerning WCB-processes ("Existing approach 3: On-land mesocosms and macrocosms" section). However, it may be difficult to impossible to scale up to the ocean water column the experimental results about WCB-processes that are obtained with relatively small mesocosms (i.e. ≤100 m^3) to the ocean water column (Petersen *et al.*, 2009). However if scaling problems could be resolved, on-land mesocosms would provide unique experimental quantitative and mechanistic information to address the effects of changes in global ocean temperature on the timing, development and fate of phytoplankton blooms and on the metabolic balance of the ocean ("Global ocean temperature and WCB processes" section). Until now, the use of on-land mesocosms as robust experimental systems for investigating WCB-processes has been limited by small size, lack of regulation of critical environmental variables such as temperature, light or turbulence, and the lack of replications of treatment and control conditions.

An approach to resolve the difficulty of scaling up experimental results from the scale of the mesocosm to that of the water column would be to make the two scales equal, i.e. to build on-land mesocosms at the scale of the *in situ* water column, including the presence of a seasonal pycnocline. This would mean developing on-land mesocosms about 50-m tall, that could include the possibility of a pycnocline at a depth that is representative of *in situ* conditions, i.e. 10–30 m (Figure 3). These very large experimental systems, i.e. macrocosms, would have volumes >1000 m^3 ("Three main types of water-column experimental approaches" section). In order to carry out true experiments, with replicated controls and treatments, the facility should include at least six macrocosms, i.e. three replicates each for the control and treatment. In the case of two treatments, nine macrocosms would be required, i.e. three replicates each for the control and the two treatments. There is presently a project for building such a macrocosm facility in China, called "Marine Ecosystem Experimental Chamber System" Jiao *et al.*, 2015).

Since the mesocosm/macrocosm will be used to conduct carefully controlled experiments, a major challenge will be to ensure that the conditions in the replicate controls and treatments are the same at the start of each experiment and remain the same during its time course. In other words, the controls and treatments must be replicated, which may be a challenge for macrososms.

This advanced experimental on-land approach could bring the long-existing on-land mesocosms methodology into a new realm of pertinence for ocean studies. This would be achieved by eliminating the step of scaling up the results from mesocosms to make them relevant to the *in situ* water column. The use of scaled-up on-land macrocosms would allow researchers to conduct highly regulated, full-scale experiments on WCB-processes that have, until now, eluded robust and statistically relevant quantification.

Conclusion

The analysis of interactions between technological advances and scientific discoveries has shown that there is a general co-evolution of concepts and technology (Legendre, 2004, 2008). In the last decades, our understanding of marine WCB-processes has greatly progressed in field observation (e.g. satellite remote sensing, and miniaturized sensors embarked on profiling floats and gliders) and modelling (e.g. inclusion of an increasingly wider array of biogeochemical processes in ocean general
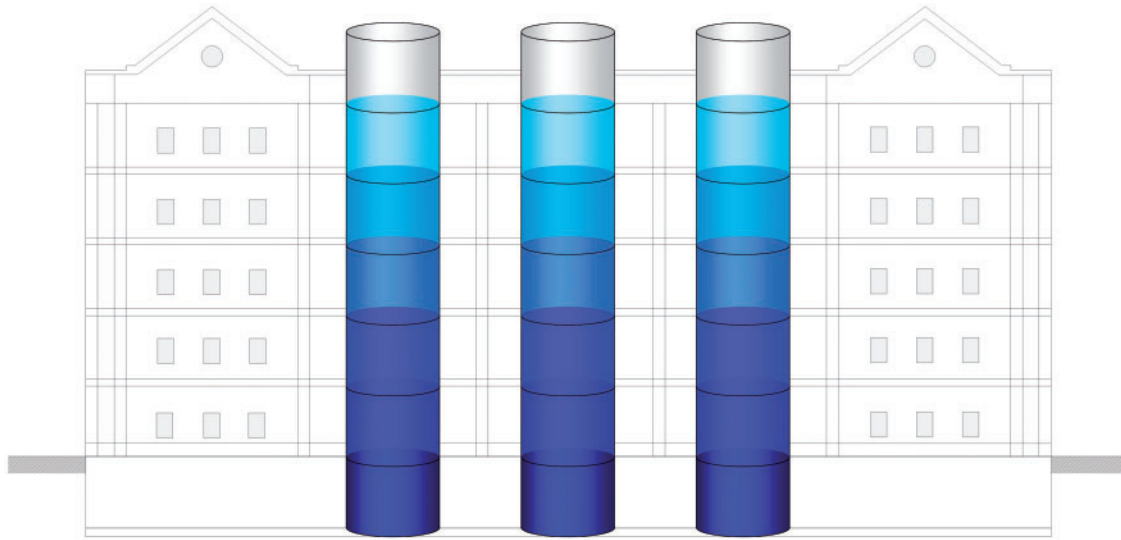
Figure 3. Schematic representation of advanced experimental approach 3 (i.e. scaled-up on-land macrocosms): enclosures up to 50-m tall could be erected on land. Such systems could be used to conduct experiments with treatments, controls and replicates, under fully regulated environmental conditions. The background building provides the scale of the enclosures, whose bottom parts are located partly underground.

circulation models), leading to the improvement of concepts (e.g. effects of mesoscale dynamics on large-scale ocean properties). These gave rise, in turn, to further methodological developments (e.g. bioArgo floats).

During the same period, experimental approaches to study WCB-processes did not seem to progress as rapidly as field observations and models. Major advances in experimentation cited above include the free-water fertilization experiments involving iron and phosphorus, and the at-sea deployments of large mesocosms to assess the effects of both ocean acidification and fertilization of oligotrophic waters by nutrients and trace elements from atmospheric inputs. However, the basic approach to free-water fertilization experimentation remained relatively unchanged between 1993 and 2009, the at-sea deployment of mesocosms has been limited to surface waters, and on-land mesocosms have continued to be confronted to the recurrent upscaling difficulty. The present paper indicates that the time may be ripe for major advances in the three existing types of experimental methods, i.e. free-water, at-sea and on-land. These could include robotically assisted free-water fertilization experiments, the deployment of mesocosms in the water column hundreds on metres below the surface, and the construction of arrays of on-land macrocosms that would allow true experiments on real-size water columns. These could lead to significant progress in concepts related to marine WCB-processes.

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