first step towards protection of mesophotic reefs would be to incorporate them into existing MPA networks. In 2009, the Israeli Nature and Parks Authority responded to new evidence of extensive mesophotic reefs in the Red Sea by extending the Coral Beach Nature Reserve to 500 m offshore, providing improved protection for reefs up to 50 m in depth[15].

Adopting a broader ecosystem-scale approach that incorporates deep reefs around the world would have several long-term social and economic benefits. Many mesophotic reefs could be protected from fishing without needing to reclaim fishing grounds in places where deeper reefs are still unrecognized, or infrequently exploited. However, we urge prompt action, because pressure to over-exploit mesophotic reefs will inevitably grow as shallow reefs become depleted. Deep-sea reefs in cold water have already suffered extensive damage worldwide from poorly regulated trawling and mineral exploration, and the limited management actions that have been implemented to protect them have often occurred too late[16]. To avoid a similar fate, proactive management is required to secure the future of tropical mesophotic reefs.

We are not suggesting that better protection of mesophotic reefs is a panacea for everything that ails shallow reefs, and we recognize that deep reefs are by no means immune to human impacts[17]. Moreover, a significant number of species across many taxonomic groups are depth specialists — restricted to either shallow or deep habitats — and seem to be unable to either escape shallow-water disturbance or to recolonize shallow reefs from the deep. Nonetheless, the economic and conservation value of deeper reefs renders them worthy of protection in their own right, and safeguarding mesophotic habitats will also extend continuing efforts in shallow water to protect reef species across their entire depth range. We strongly encourage greater efforts to map the extent of mesophotic reefs and understand patterns of connectivity, and recommend a much greater emphasis on incorporating adjoining habitats into networks of MPAs. ❒

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COMMENTARY:

Framing biological responses to a changing ocean

Philip W. Boyd

To understand how marine biota are likely to respond to climate change-mediated alterations in ocean properties, researchers need to harmonize experimental protocols and environmental manipulations, and make better use of reference organisms.

Great progress has been made since the 1970s in understanding oceanic processes and their wider role in the functioning of the Earth system[1]. Global surveys, such as the Geochemical Ocean Sections Study (GEOSECS) have revealed an ocean comprising many provinces, each composed of differing physical, chemical and biological characteristics[2]. The widespread success of GEOSECS and subsequent mapping of global ocean characteristics has only been possible by implementation of rigorous standardization[3].

Such internationally recognized intercalibration procedures are essential for the direct comparison of observations from different oceanic regions, for example measurements of carbonate chemistry[4]. Similarly, coordinated evaluation, through intercomparisons, of the projections of different climate-change models[5] provides confidence in the trends from a range of model parameterizations that are central to intergovernmental reports on the state of the planet[6].

These climate change model projections have in turn provided compelling evidence that oceanic properties, such as temperature, oxygen, nutrients and irradiance, are being altered simultaneously at fast rates, relative to much of the Earth's geological past[7]. Such dramatic predictions of wholesale environmental change to oceanic conditions have resulted in a proliferation of studies in which conditions, such as temperature, are manipulated to explore the response of biota[8].

So far, individual properties — such as pH — have been manipulated in different studies, which together span a wide range of organisms from microbes to fish[9]. Moreover, investigations of the response of a sole group of organisms, such as phytoplankton, have used a range of protocols to manipulate individual or multiple oceanic properties simultaneously[8]. Taken together, the selection of a variety of experimental organisms, protocols and the manipulation of different clusters[8] of oceanic properties, has resulted in a complex suite of experimental findings. To understand how ecosystems are likely to respond to a changing ocean and to address other similarly fundamental questions, the findings from this disparate range of experimental approaches (protocols, organisms, manipulations and so on) must be readily interpretable.

The broader interpretation of these burgeoning experimental datasets will become increasingly difficult unless there is a harmonization of experimental approaches into the environmental manipulation of ocean biota.

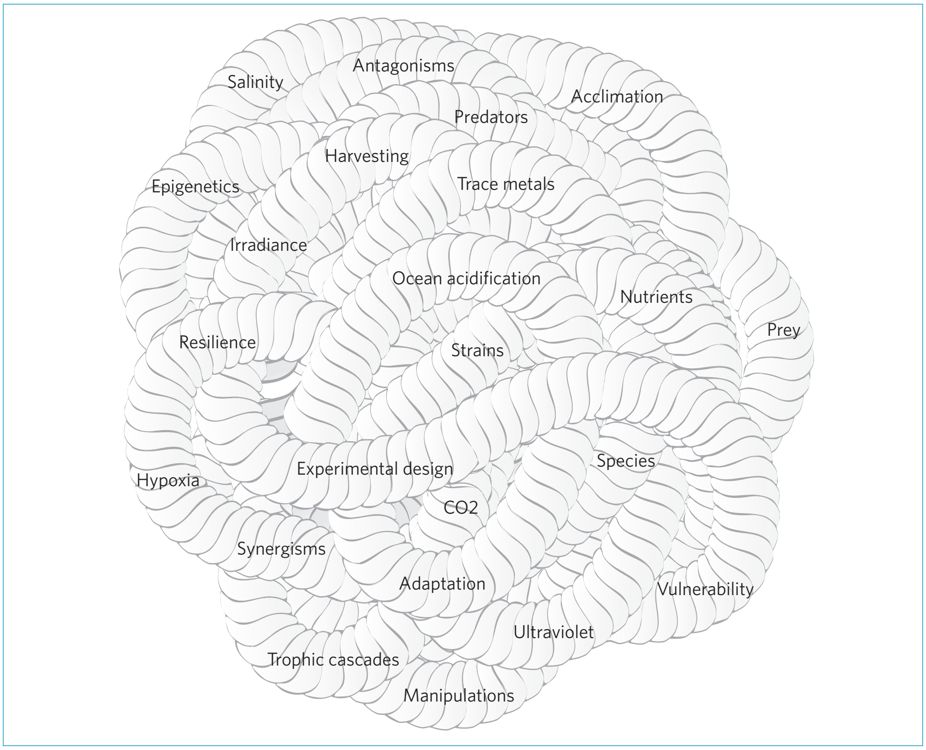

Figure 1 | A Gordian Knot made up of differing thematic information, indicating the wide range of research issues associated with the study of the response of biota to changing oceanic conditions.

A Gordian knot?

If the ocean sciences community continues along its present research trajectory — characterized by a proliferation of experiments using an ever widening range of both study organisms and permutations of environmental manipulation — it will make such intercomparisons less and less tractable. Hence, without advanced planning, our research efforts over the coming decade can only result in a very confused viewpoint of how oceanic ecosystems and biogeochemistry will be altered by climate change.

At present, the increased emphasis and focus on investigating how multiple environmental drivers influence the biota will require more complex experiments[8]. Yet, by conducting more representative experiments there is a risk that — paradoxically —they will, when taken together, result in less clarity with respect to understanding the responses of ocean biota to changing conditions.

Clearly, before research into the cumulative effects of multiple oceanic drivers gains momentum, it is important to develop plans for international coordination, and to agree on ways in which to tackle this issue coherently. A similar debate has taken place in other disciplines — behavioural biology[10], for instance — over the need to be able better to cross-reference the findings from disparate experimental studies and hence maximize the value of such research efforts.

There is a danger of continuing research outputs becoming a Gordian Knot of disparate strands of data (Fig. 1). I now review some potential solutions, and propose the inclusion of biological reference organisms in experiments to cross-link experimental findings better between laboratories.

Potential solutions

A range of approaches to permit more reliable intercomparisons between experiments, with respect to design, manipulation and study organisms, is presented in Box 1. The design of most environmental manipulation experiments is highly complex, relative to the conventional standardization of a chemical measurement protocol[4]. Three methods have been offered so far to harmonize designs better. The production of best practice guides[11] offers a consensus-based recommendation which advocates the best protocols to adopt for experimentation. Such interdisciplinary guides are invaluable to research communities but the need for such detail results in large and cumbersome documents[11] that may be prone to misinterpretation.

Another way to address the need for a common experimental design is that of a scientific community based approach using the breadth of research groups to develop a large coordinated body of observations. This has recently been adopted by ocean scientists[12], who developed agreed-on experimental protocols before conducting manipulation experiments (Box 1). Such an approach is more prescriptive than best practice guides, and will also require that the basic principles of how individual research is funded and the manner in which individual research excellence is recognized by peers to be revisited[13].

Both of these routes would be enhanced by the adoption of standardized apparatus for conducting manipulation experiments

Box 1 | Potential solutions to harmonization of experimental design, environmental manipulation and selection of study organisms.

Experimental design

Best Practice Recommendations. Protocol guides are used routinely to standardize methods for making observations (such as chemical measurements) and rate processes (primary production, for example). As the protocols involved in experimental design are multi-faceted, guides are developed by a panel of field-leading scientists who provide expert advice on different aspects of design — the ocean acidification best practice guide[11] required expertise in carbonate chemistry, bio-statistics, laboratory culture techniques and so on.

Scientific community based studies. These studies use a pre-agreed experimental design to build large internally consistent datasets across several laboratories, either nationally or internationally. This approach enables a wide range of species or strains — for example phytoplankton — to be studied concurrently[12], which is advantageous for climate-change manipulation studies.

Standardized apparatus. Scientists are realising that complex manipulation experiments require interdisciplinary collaborations between chemists, biologists and engineers, for example, to design equipment that can be used widely across research communities. Examples range from the Free-Ocean Carbon Dioxide Enrichment (FOCE)[19] incubator which is deployed *in situ* and is relatively non-intrusive for biota, to customized incubators to conduct ocean acidification experiments under trace-metal clean conditions[15].

Experimental manipulations

Standardization of perturbations. Conventionally, ocean acidification manipulation experiments use three CO_2 concentrations: 280 ppmv (Last Glacial Maximum); 400 ppmv (present day, control treatment); and 750 ppmv (future ocean scenario based on Intergovernmental Panel on Climate Change (IPCC) emissions scenarios for 2100[5,6]). However, as more studies investigate the effects of multiple drivers on biota, consensus is needed to select a set of coordinated perturbations for nutrients, trace metals, oxygen, irradiance and so on. As for ocean acidification, these could be based on IPCC climate change scenarios; however, in some cases, such as for trace metals, the magnitude/sign of the change is relatively uncertain[8].

Acclimation. Early manipulation experiments used quasi-instantaneous perturbations (such as from 400 ppmv to 750 ppmv CO_2). In many recent studies, including laboratory cultures, an acclimation period is used before experimentation. In addition, manipulations are run for a range of timeframes, from days to years. Wide-ranging discussions are urgently needed about criteria to set the duration of both acclimation and the subsequent experiment. A suite of standard durations could be used to take into account the range of issues from short term physiological/'omics' to long-term micro-evolutionary adaptive responses that researchers wish to address.

Experimental organisms

Biological Reference Organisms. It is evident, mainly from ocean acidification research, that the selection of study organism(s) is equally influential to the experimental outcome[16,17] as the choice of experimental design or environmental manipulation. Reference organisms would help to demarcate the relative influence of design, organisms and manipulation on experimental outcomes, and assist with the intercomparison of experimental findings. For example, recent physiological studies on polar diatoms used comparisons with physiologically well characterized temperate diatom species as cross-referencing checks. These cross-checks revealed large differences between polar and temperate diatoms in their response to manipulation of iron supply and light levels[20], indicating that the choice of study organism drove the experimental outcome. Such an approach could be internationally coordinated for key groups, including nitrogen fixers or coccolithophores.

Natural communities. The large-scale manipulation of resident oceanic biota — using *in situ* mesoscale perturbations or medium scale mesocosms[18] — can also circumvent the confounding range of responses from single species laboratory studies. However, natural community studies provide fewer insights into the physiological mechanisms that may underlie changes in community structure due to altered environmental conditions.

(Box 1). At present such equipment is largely designed for exclusive use in a particular laboratory. For example, although the number of ocean acidification manipulation experiments increased exponentially in the last decade, most studies used different methods to manipulate pH. This resulted in a tiny subset of studies in which the consequences of using different approaches could be rigorously compared[14]. There is a growing need for custom-built experimental manipulation chambers to conduct increasingly sophisticated experiments. However, with enhanced international cooperation it should be possible to adapt existing systems, or to design systems that can be commercially produced to pre-agreed specifications, to minimize the inadvertent introduction of confounding experimental artefacts. These systems could be used to focus on particular clusters of properties, including pH and trace metals (Box 1).

The term experimental manipulation refers to the magnitude, complexity (sole versus multiple environmental properties) timescales and duration of manipulations (Box 1). This facet of experiments also requires coordination because as manipulations become more complex, the number of permutations (degree of acclimation, length of incubations and interplay of individual properties) will increase markedly. For example, the confounding effects of synergisms (amplification of the effects of individual properties) and antagonisms (diminution) can influence the selected organisms in subtly different ways, and also alter the intended environmental manipulation—for example, pH effects on trace metal availability[15]. A range of measures from careful reportage of the manipulation, to a nested suite of recommended experimental manipulations, is advocated.

Biological reference organisms

The selection of study organisms is based on their ecological or biogeochemical role, the nature of the planned environmental perturbation and/or their regional or global importance. For example, calcifying phytoplankton (coccolithophores), and in particular the species *Emiliania huxleyi*, have been popular model organisms in ocean acidification studies. This is because they are ubiquitous, important in the ocean's carbon cycle and ocean acidification may have a detrimental effect on their ability to calcify. However, ocean acidification experiments comparing *E. huxleyi* strains[16] or different coccolithophore species[17] reveal a wide range of responses, providing a confusing array of results. This lack of consensus hinders the understanding of the environmental controls on this group — which is essential information for modellers[8]. I advocate the use of reference organisms to cross-link experiments across different laboratories, and to help tease apart the relative roles of design, study organism and/or environmental manipulation in influencing experimental outcomes. Selection criteria for a reference organism should capitalize on the increasing power of 'omics' in characterizing functional differences between organisms. Such an approach, if combined with a common experimental design, would boost our confidence in the power of metanalyses in pinpointing trends across large datasets[9].

The use of reference organisms is not possible in large-volume enclosure experiments where natural communities are manipulated environmentally. However, large-volume experiments offer valuable and complementary insights into the ecological connections across food webs, and how they are altered at the community level by changing ocean conditions[18].

The discussion and eventual implementation of this harmonization is essential before the community embarks on the major challenge of investigating how a diverse ocean biota will respond to a dramatically changing ocean. The time invested in moving towards uniformity will provide great benefits to making progress in this demanding and difficult research area. ❐

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