Between control and complexity: opportunities and challenges for marine mesocosms

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Between control and complexity: opportunities and challenges for marine mesocosms

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Marine ecologists have a wide array of tools with which to study complex and dynamic systems, but there are cases where neither simple, highly controlled experiments nor largely uncontrolled, more complex field observations provide adequate inferential power. In such cases, mesocosm studies in marine systems may help bridge the gap. Mesocosm studies can facilitate research ranging from basic biology to multifactorial ecosystem studies that involve observation, perturbation, validation, calibration, long-term studies, and testing of new technologies. Although scale, closed boundaries, biodiversity levels, and replication can impose challenges on mesocosm research, these parameters can also help to define research opportunities that are uniquely suited to such controlled environments. Finally, we provide examples of successful marine mesocosm research and discuss opportunities for future work.

Many outstanding questions in marine ecology – including those related to basic life history, carbon cycling, population monitoring, fate and toxicity of contaminants, mechanisms controlling biodiversity, and studies of global change – could benefit from controlled experimentation at relatively large scales. Capacity for studying ecological systems accelerated in the late 20th century with the extensive adoption of manipulative experimental methodologies. One productive response was to perform similar experiments at multiple locations along a biogeographic gradient or in similar ecosystems in different biogeographic regions, to improve understanding of large-scale mechanisms (Menge *et al*. 1994, 2002; Vitousek 2004; Paine 2010). Because not all systems are conducive to this comparative experimental approach, we have seen greater usage and acceptance of observation-based studies, which do not rely on controlled manipulation of variables (Sagarin and Pauchard 2012). Advances in technologies pertinent across scales of biological complexity – from genomics to remote sensing – have amplified the power of observational studies to infer mechanisms underlying ecological phenomena (Pickett *et al*. 2007).

Highly controlled experimental manipulations and open-ended field observations each provide important insights in marine research, but integrating the results from these disparate approaches can be challenging due to the disparity between their spatial and temporal scales. The outcome of a small-scale controlled manipulation may not be consistent with field observations of an unconstrained system, and identifying the reasons why this is so can be beyond the scope of either approach. Research questions arise that cannot be addressed in either the laboratory or the complex and largely uncontrollable field arena (Tyre *et al*. 2003; Biber 2011; Witze 2015). The elucidation of scale- and feedback-dependent processes will ultimately require both fine environmental control and high-resolution observations. This becomes more important as environmental and ecological changes intensify in response to human activity. Understanding, predicting, and managing such change may require knowledge that integrates single-variable manipulative techniques with the complexity of the natural world, where it is nearly impossible to tease apart the many

> **In a nutshell:**
> - Mesocosms are enclosed environments that combine the control of laboratory experiments with some of the complexity of natural ecosystems
> - Although frequently used to investigate terrestrial and freshwater ecosystems, mesocosms are underutilized in marine ecology due to challenges of scale, openness, complexity, and replication
> - By allowing researchers to address critical questions in marine ecology, mesocosms can help bridge the gap between small-scale controlled experiments and field observations
> - As the largest marine mesocosm ever built, the University of Arizona's Biosphere 2 Ocean mesocosm overcomes some of the challenges associated with smaller systems

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variables. This can be particularly difficult today, with the addition of many human-induced stressors such as the introduction of non-native species, overfishing, climate change, and pollution.

A largely under-explored space in marine ecological research that can address some of these gaps is the *mesocosm*, a device that features some of the control of a laboratory (or experimental plot) and some of the complexity and scale of the natural world (eg Wilson *et al.* 2015). In an ideal mesocosm, processes can be controlled and measured across spatial scales, and specific control components can be engaged or disengaged.

Here we discuss some of the opportunities and challenges of marine mesocosm research. Marine ecosystems are large and four-dimensional in scale, and their openness and continuity influence the way in which ecological systems function. As a consequence, processes in marine systems need to be explicitly considered when designing marine mesocosm experiments. For example, nutrients and organisms are retained, diffused, transported, or aggregated by ocean currents that are spatially and temporally variable due to the interaction of multiple physical processes (eg tides, waves, winds, topography, bathymetry) (Denny 1993). In addition, many marine organisms (eg invertebrates and fish) have complex life cycles involving a pelagic larval stage that evolved to match the variable physical environment (Strathmann 1990). Accordingly, while the use of mesocosms in marine ecological research offers unique opportunities, it also comes with challenges. As with other approaches, mesocosm research can be difficult and costly and has limitations in utility and scope. However, the size and control available in such studies can play a critical role in bridging across scales to link laboratory findings with real-world observations.

■ What exactly is a mesocosm?

In simplest terms, a mesocosm is an experimental system – with known boundary conditions – that includes both controllability and complexity. A mesocosm typically does not feature the degree of controllability afforded by a laboratory setting, nor is it a perfect replica of a natural system. Rather, it should be viewed as a tool
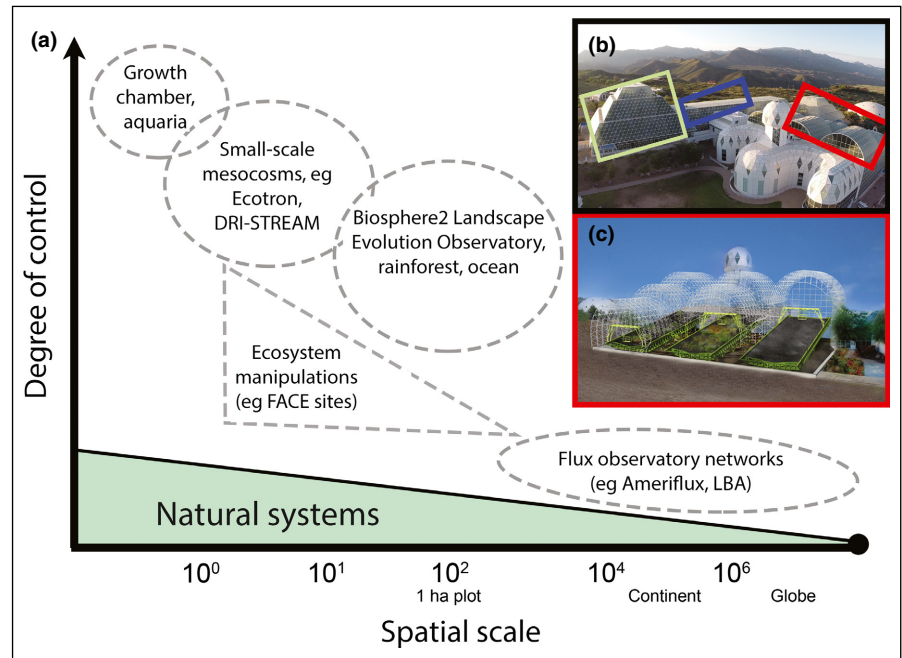
Figure 1. (a) Relative controllability of various sample mesocosms along a compressed size scale. (b) Photograph of Biosphere 2, with approximate locations of the rainforest (light green rectangle), ocean (blue rectangle), and Landscape Evolution Observatory (LEO; red rectangle) mesocosms. (c) Schematic of the LEO. Mesocosm names and websites: Marine Ecosystems Research Laboratory (MERL; www.gso.uri.edu/merl/merl.html), Ecotron (www.ecotron.cnrs.fr), Drought Impacts on Stream Ecosystem Functioning (DRI-STREAM; http://bit.ly/1S5ViYJ). Flux observatory network names and websites: Ameriflux (http://ameriflux.lbl.gov), Fluxnet (www.fluxdata.org). FACE = Free-Air Carbon Dioxide Enrichment; LBA = Large-scale Biosphere–Atmosphere Experiment in Amazonia.

to answer questions that cannot be addressed fully under highly controlled simplistic conditions or complex and largely uncontrolled natural conditions. The areal extent of mesocosms may range from chambers of less than a square meter, to indoor ecosystem experiments close to a hectare, to networked sites spanning continents (Figure 1).

The University of Arizona's Landscape Evolution Observatory (LEO) at Biosphere 2 (B2) is an example of a terrestrial mesocosm used to answer big-picture ecological questions. The LEO is a set of three replicate 340-m^2 hillslope environments (Figure 1) designed to observe and manipulate the co-evolution of physical, chemical, and biological properties of watersheds and their effects on biogeochemical cycles (Pangle *et al.* 2015). The designers of the LEO created a unique opportunity to develop a large-scale mesocosm experiment from the ground up (Huxman *et al.* 2009). Early experiments at this facility have allowed direct observation of water transit time distributions (impossible in real systems), and the discovery of landscape-scale limiting processes on chemical weathering of basalt substrates. Successful mesocosm projects such as this demonstrate the feasibility of mesocosms and quell skepticism about their value.

■ Marine mesocosm opportunities

Marine mesocosms provide opportunities for diverse research objectives:

(1) *Observation*. Many basic life-history questions regarding marine organisms – such as growth rates, larval longevity and behavior – may be especially suitable for mesocosm experiments. Biochemical cycling may be logistically more feasible to observe in a mesocosm, as studies of carbon cycling in the B2 Ocean revealed (Langdon *et al.* 2003). Moreover, certain applied observations – such as mechanisms that drive the physical and chemical fate of plastic debris (Teuten *et al.* 2009; Law *et al.* 2010) in marine environments – can be made in a mesocosm more effectively than in a small-scale laboratory experiment or the open ocean.

(2) *Perturbation*. The ability to perturb both the ecology and biogeochemistry in a marine mesocosm is a key asset because it is much more difficult to do so in a laboratory or field experiment (Langdon *et al.* 2000, 2003). Because these perturbations can be conducted in parallel, sequentially, or in repeated pulses, marine mesocosms offer the chance to decouple the roles of competing variables of interest, which is usually impossible in the field. The feedbacks among carbon chemistry, individual species, and marine ecosystems, for example, have been identified as a key gap in acidification science (Pfister *et al.* 2014) and could be addressed in a mesocosm.

(3) *Validation*. A marine mesocosm can complement model validations that use historical data by providing instrumental and experimental data under a range of scenarios. Mesocosms may also validate results from small-scale manipulations that are difficult to confirm from large-scale field observations (eg because the system is too complex to tease apart variables). For instance, parallel evolutionary forces (eg immigration, genetic drift) seem to affect species and genetic diversity declines over short timescales following disturbance according to modeling and field studies (Vellend 2005; Cleary *et al.* 2006). However, theory has lagged behind regarding when one can be used to predict the other, in part due to high environmental heterogeneity that may be controlled for in a mesocosm.

(4) *Calibration*. A compelling use of mesocosms is to calibrate response curves between variables to make sense of field observations. One important application of this may lie in quantifying the relationship among genetic and other measures of biodiversity. Although many investigators now use environmental DNA (eDNA) and bioinformatics to measure diversity in the wild (eg Thomsen *et al.* 2012), many questions remain unanswered regarding the sensitivity of the technique when multiple species vary in space and time and how to translate presence/absence data into quantitative estimates (Foote *et al.* 2012; Kelly *et al.* 2014).

(5) *Long-term studies*. Observations are unified and enhanced with long-term observation and monitoring. In particular, non-linear response pathways may emerge only when studies are extended over time (Osmond *et al.* 2004), yielding different predictions and parameterization of system models than short-term studies. In one study, long-term mesocosm experiments measuring the recovery of a system after exposure to toxicants reveal that although these vanish rapidly from the water column, small concentrations of pollutants linger for years in the benthic environment (Oviatt *et al.* 1984).

(6) *Technology test bed*. Development of environmental sensing and exploration technologies faces similar constraints as described above for basic ecological research (Wynn *et al.* 2014). Conditions need to be complex, but controllable. A marine mesocosm with salt water, complex topography, variably controlled water quality and clarity, complex soundscapes, and living organisms – which may interfere with, biofoul, or actively avoid the technology – provides opportunities for developers to study real-world exposures with much lower risk of losing a valuable prototype.

■ Particular challenges of an ocean mesocosm

The most obvious restriction for an ocean mesocosm is scale. Many biological oceanographic features occur across scales that are orders of magnitude larger than any conceivable ocean mesocosm (Mann and Lazier 2006). These features – such as current patterns, larval transport, and nutrient cycling – have real and identifiable consequences at the local scale and cannot be ignored when considering smaller-scale phenomena (eg Selkoe *et al.* 2015). It is critical to deliberately constrain the type of marine environment that is being simulated in the mesocosm and focus on questions directly relevant to that environment. For example, while open-ocean dynamics may be difficult to scale down, nearshore dynamics that occur (in part) on scales of meters to tens of meters, such as nutrient exchange across the land–sea interface, may be possible to replicate in a mesocosm (Álvarez-Romero *et al.* 2011).

A second major challenge for an ocean mesocosm is its closed boundaries relative to the ocean. Wave forces,

Panel 1. The history and future of the Biosphere 2 Ocean

The Biosphere 2 (B2) Ocean is a rare example of a large marine mesocosm with research as its primary rationale. The ocean is a 35-m × 20-m rectangle, with a complex bathymetry featuring a 7-m depth zone that slopes upward to a shallow lagoon and fringing reef with a sandy beach (Figure 2). The ocean volume is approximately 2650 m^3 and its surface area is 711 m^2 (Atkinson *et al.* 1999). Originally, the B2 Ocean biome was created as an integral part of a sealed, self-supporting multi-habitat environment that also included a rainforest, savanna, marsh, desert, and intensive agricultural area, and supported eight human inhabitants for a two-year mission (Marino and Odum 1999; Poynter 2006; Reider 2009).

After the conclusion of the original sealed "manned missions" of B2, Columbia University acquired the facility in 1996 for global change research and teaching. Columbia unsealed and separated the biomes to enable greater control in any given area. A body of marine research on biogeochemistry and ocean–atmosphere gas exchange was developed during this time (eg Atkinson *et al.* 1999; Sweeney 1999; Falter *et al.* 2001), including some of the first long-term experiments on the effects of ocean acidification on coral and ecosystem calcification and on community metabolism (Langdon *et al.* 2000, 2003; Marubini *et al.* 2001).

Columbia's departure from B2 in 2003 resulted in a period of benign neglect that particularly affected the ocean biome.
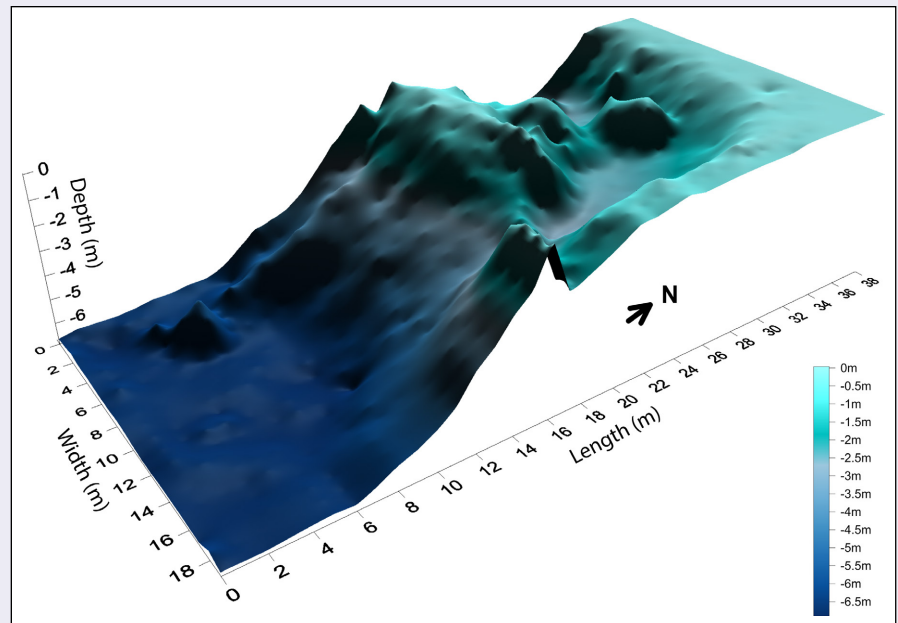

Figure 2. Current bathymetry of the Biosphere 2 Ocean.

Currently, the ocean is very low in diversity and population density of animals – with no live corals and an estimated 90–95% algal cover on hard surfaces – as compared to the Biospherian and Columbia eras. This situation provides an opportunity to re-envision the B2 Ocean and move away from the energy- and maintenance-intensive coral reef model.

Our vision for the B2 Ocean, moving forward, emphasizes the importance of understanding "Desert Seas". Worldwide, a substantial fraction of the coastal ocean abuts arid or semi-arid lands
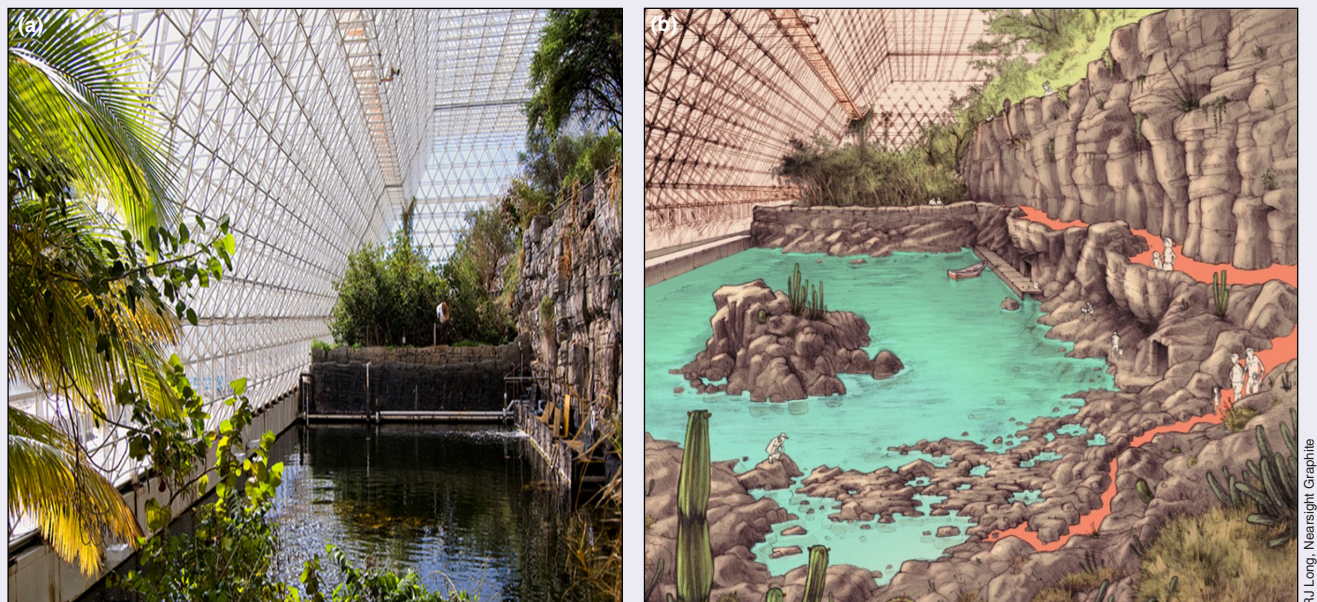
Figure 3. (a) Current Biosphere 2 Ocean mesocosm. Visitor viewing is from the top of the rock wall on the right. (b) First draft of possible transformation to a Gulf of California model mesocosm; orange pathways indicate new access/viewing points for visitors.

in the subtropics. These oceans support diverse marine ecosystems along with local economies, cultures, and food systems. The scientific goals of the project will address biological diversity in an era of anthropogenic change. This is a globally relevant vision that ties into local expertise in semi-arid ecosystems and the B2's nearest marine environment, the Gulf of California. Research on environmental change and biodiversity will provide a pedagogical link between global processes and the changes occurring in the Gulf (Sagarin *et al.* 2008; Brusca 2010).

Nonetheless, the original B2 Ocean was not built specifically for manipulative research projects. The sandy beach, for example, erodes quickly when wave action is increased in the ocean and thus has limited research into biophysical processes. Accordingly, design plans could include physical modifications of the mesocosm, such as transforming the beach into a rocky shoreline with tidal pools, that would provide the public with

a sense of the high-diversity Gulf of California environments and scientists with more robust platforms to conduct research (Figure 3).

Ultimately, the goal of the ocean transformation project is to provide a platform for marine research that can be used by scientists or technology developers on a subscription or user-fee basis, similar to how many telescope facilities and marine biology laboratories operate today. To make this platform more useful, a series of design charrettes – beginning in 2013 – have brought marine biologists, conservation biologists, ecologists, aquarists, and exhibit designers to B2 to conceptualize the research, design, and outreach priorities for the project. A challenge in this design process, and for all marine mesocosms, is finding ways to combine a strong and adaptable platform for marine research with a compelling space for public science outreach.

currents, ocean–atmosphere interactions, and direct or indirect biological transport provide numerous ways in which nutrients, pollutants, and biological organisms can enter, exit, and organize a marine system (Siegel *et al.* 2003; Cowen and Sponaugle 2009). The physical walls around the mesocosm and the manner in which seawater and organisms are introduced affect this kind of transport. This produces both a challenge and opportunity. The challenge is to design a system and experimental design in which inputs and outputs to the system (as well as the intervening dynamics) are carefully tracked. Extensive (spatially dense), real-time environmental sensors as well as basic population monitoring are therefore both necessary and feasible for marine mesocosms. Introduction and removal of species will usually be deliberate, although unintentional introductions are still inevitable, particularly in the microbiome. The ability to track these flows within mesocosms – enhanced relative to field studies by the mesocosm structure and by new sensor and genomic technologies – presents a clear opportunity to address questions that are logistically difficult or impossible to answer in the field.

A third challenge is maintaining an appropriate level of biodiversity, which is related to both issues of scale and physical boundaries in marine mesocosms. By not capturing the large-scale dynamics of currents and larval pools that drift and swim within, mesocosms can only approximate the major source of diversity input (Watson *et al.* 2011; Treml *et al.* 2015). Additionally, organisms that might normally escape predation may literally have nowhere to hide. Design considerations – such as creating complex topography and habitat, large open-water zones, and tidal systems (that might limit the mobility of certain predators or herbivores) – can create refugia for species, and may also be used to test theories about the relationship between habitat complexity and diversity (Kostylev *et al.* 2005). Species composition can be manipulated to avoid incompatibilities and to include symbiotic partners and desirable habitat "engineers". Thus, the ideal suite of species for a mesocosm to function may be different than

any current or past assemblage of species in a real-world ecosystem.

A fourth challenge is replication. Unlike treatments conducted in 20-gallon aquarium tanks, it is logistically difficult to replicate large-scale ocean mesocosms. As we move to larger scales of space and increased complexity, true replication becomes exceedingly difficult. At B2, to address replication concerns, the LEO project created three identical hillslope platforms arranged side by side within an enclosed 5000-m^2 section. Yet even in this highly controlled structure, there will be slight differences due to ambient light and temperature between the westernmost, easternmost, and central bays. The difficulty of balancing replication with achieving reasonable inference in complex systems is one that both field and laboratory ecologists have recognized and debated for some time (eg Schindler 1998; Oksanen 2001).

Many concerns about mesocosm research reflect long-standing debates in ecology (Hurlbert 1984; Naeem 2001). Marine ecologists have long accepted that even field studies capture only a small part of natural oceanic variability. In making necessary spatial, temporal, and taxonomic simplifications to field or lab studies, we draw boundaries around our systems just as surely as we must with mesocosms.

◼ Examples of successful marine mesocosm research

Although mesocosms have been under-used in marine ecology, marine researchers have demonstrated their utility. Below are examples of how marine mesocosms have addressed questions related to ecological processes, the fate and toxicity of environmental contaminants and eDNA – questions that can be intractable using laboratory experiments or field observations alone.

Marine mesocosm experiments perform as ideal platforms for conducting ecological research with small

larvae or propagules of marine organisms, including observing larval growth and survival in real-time (Davis *et al.* 1996) and the evolution of aquatic organisms. In a recent study, Scheinin *et al.* (2015) measured evolutionary trends against CO_2 enrichment that mimics future environmental change conditions. Marine mesocosms have also been used to validate the effectiveness of different seeding techniques on genetic diversity before conducting restoration efforts in the field (Ort *et al.* 2014).

Such systems can also be ideal for validating the fate and impact of contaminants over longer timescales or that were observed in a laboratory and/or suggested by a model. For example, to measure the rate of ecosystem recovery from polluted sediments, Oviatt *et al.* (1984) used fourteen cylindrical mesocosms, 1.8 m in diameter and 5.5 m in depth, that were scaled to a natural system in terms of sunlight, temperature, tidal mixing and flushing, and mimicked the behavior of the natural system with regard to phytoplankton, zooplankton, benthos, metabolism, and nutrient concentration. Vethaak *et al.* (1996) performed a long-term (3-year) study using large mesocosms (40 m × 40 m × 3 m) to determine whether diseases in European flounder (*Platichthys flesus*) were directly related to pollution. The mesocosm made it possible to separate pollution from other potential causes of disease, such as perturbation due to climate change or non-native species. Their results demonstrated liver neoplasia (tumor formation) in fish under simulated conditions with environmentally relevant levels of pollutants.

Marine mesocosm research at California's Monterey Bay Aquarium developed and calibrated eDNA techniques to detect mitochondrial DNA of bony fish and identify individuals to the family- or genus-level (Kelly *et al.* 2014). This approach could improve the cost, efficiency, and sensitivity of current marine monitoring programs. Mesocosm-based research also allowed scientists to test different techniques to quantify the number of target DNA copies, showing droplet digital polymerase chain reaction (PCR) had higher accuracy than quantitative PCR, especially at low eDNA concentrations (Doi *et al.* 2015). By moving beyond taxonomic identification, such techniques may allow estimation of the abundance and biomass of aquatic species with eDNA methods in the field. A recent study applied this approach to characterize patterns of eukaryotic diversity on oyster reefs in Virginia and Florida (Leray and Knowlton 2015).

The future of mesocosms

We contend that continued and increased use of mesocosm infrastructure can help answer complex questions in a changing world. The B2 Ocean is an example of a unique, large-scale, multi-purpose, marine mesocosm that supported fundamental advances in our understanding of ocean acidification by measuring coral reef community responses to changes in pH (Langdon *et al.* 2000, 2003). Although currently being redeveloped as a community platform for marine research (Panel 1), the B2 Ocean will become available for use for a variety of experiments and by a diversity of researchers.

In addition to scientific limitations, the cost and complexity of building large mesocosms are another reason that so few of these systems – either terrestrial or marine – are active. We cannot take full advantage of mesocosm research opportunities when such infrastructure is under-supported. However, by incorporating substantial public outreach efforts into new projects, both the public and scientific mission may benefit. By opening facilities to members of the public and educational groups, fees from visitors can help defray costs associated with research and maintenance. Incorporating projects in which the public can participate (eg citizen science) or learn provides additional data and supports "broader impacts" programs that are part of the mission of public and private foundations, many of which can and do serve as critical sources of funding.

We argue that marine mesocosms have a central role to play in advancing marine science and (potentially) public understanding. By providing a central stage around which diverse researchers can organize, mesocosms offer a platform for developing collaborative approaches and resolving complex ecological questions. The opportunities offered by mesocosms suggest that there is abundant room for exploring their diverse promise in marine research.

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In Memoriam: Raphael (Rafe) Sagarin

Rafe Sagarin – eminent marine biologist, friend, father, husband, brother, and son – lost his life to a drunken driver while riding his bicycle near Biosphere 2 in Oracle, AZ, on 28 May 2015. Rafe, an Associate Research Scientist at Biosphere 2, was an amazing individual whose intellect and caring attitude inspired others. One of his greatest gifts was translating science to the general public – something few of us do very well. With infectious enthusiasm for all that he did, Rafe was deeply passionate about the oceans, particularly the Sea of Cortez (Gulf of California). The real world *was* his laboratory. He made connections where no one else would and, by applying an open mind and a creativity that had little patience for boundaries separating academic disciplines, he formed a rare bridge between academic and non-academic sectors. He strongly believed that holistic approaches could transcend barriers (between science and art, society and nature, observation and experimentalism) and that such approaches were key to resolving the scientific and societal challenges that we face.