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Investigating Coral Bleaching in a Changing Climate: Our State of Understanding and Opportunities to Push the Field Forward

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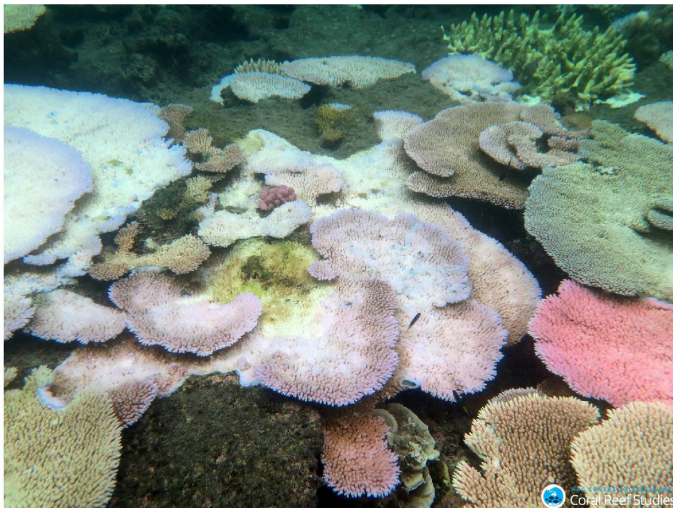

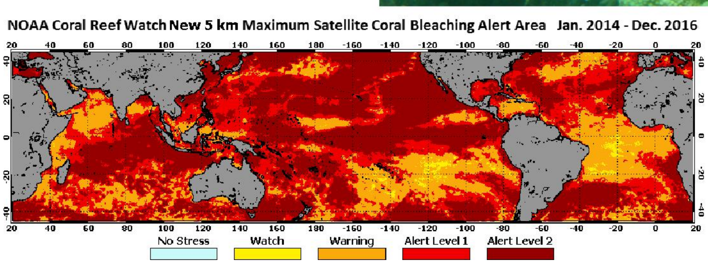

INVESTIGATING CORAL BLEACHING IN A CHANGING CLIMATE: OUR STATE OF UNDERSTANDING AND OPPORTUNITIES TO PUSH THE FIELD FORWARD

Report from the NSF U.S. Investigator Workshop on Coral Bleaching, 17-18 June 2016

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Executive Summary

Coral reefs throughout the world are facing the consequences of large-scale changes in Earth's climate. In particular, ocean warming is leading to frequent coral bleaching, which is threatening the long-term stability of coral reefs. Coral bleaching is a stress response that results in the disassociation of the mutualistic symbioses (i.e., dysbiosis) between corals and their endosymbiotic algae (*Symbiodinium* spp.). In the past two decades, there have been four substantial bleaching events, which have affected large geographic areas across the globe, including the worst recorded bleaching event on the Great Barrier Reef in 2016 (Berkelmans et al. 2004; Eakin et al. 2010; Stella et al. 2016). These large-scale bleaching events, in combination with many local-scale stressors, have contributed substantially to global declines in coral populations. In addition, bleaching may lead to compromised coral immunity, possibly resulting in additional mortality by a range of post-bleaching diseases (Maynard et al. 2015, Randall et al. 2014). Given their link to patterns of global-climate change and projections of increased warming in the coming decades, mass coral bleaching events are a key concern. In addition, current climate projections estimate that global bleaching is expected to occur annually by late this century, with more than 90% of reefs facing long-term degradation (Frieler et al. 2012). Furthermore, in locations such as the Caribbean, frequent thermal anomalies and consecutive annual bleaching events are expected to be common in less than 25 years (van Hooidonk et al. 2015). In fact, large-scale bleaching two years in a row was documented for the first time in 2014-2015 in Hawaii and in the Florida Keys. However, not all corals (and other symbiotic cnidarians) are equally susceptible to thermal stress, and some corals have been shown to recover from bleaching more quickly than others. Likewise, not all reefs are equally susceptible, and depending on local conditions, susceptibility can vary from one event to the next. Such variability in resilience could be a cornerstone to reef persistence over the coming century. However, the research needed to test this hypothesis remains to be performed.

Given the substantial investment that NSF has made by recently funding bleaching-related projects, for example through grants for Rapid Response Research (RAPID), and through regular research proposals in core programs, this workshop provided an opportunity to bring several investigators from the coral-reef community together to discuss current investigations and coordinate future research priorities. To this end, participants of the 2016 Coral Bleaching Workshop were charged with identifying major research gaps in knowledge and central questions concerning coral bleaching that would serve as focal points for formulating key research priorities and questions. These priorities could, in turn, deliver new and important data and provide a framework for future bleaching research**. The group converged on three overarching research themes in need of further exploration:**

1) **Bleaching mechanisms**
2) **Bleaching recovery**
3) **Refugia from bleaching**

These three themes transcended the molecular, physiological, and ecological approaches of the three workshop breakout groups (see below for workshop structure). A critical point, which many participants raised, was that bleaching research would accelerate with questions that integrate across all three approaches.

1. Bleaching mechanisms

Mechanisms of bleaching refer to the entire set of processes that lead to individual and large-scale coral bleaching events. Historically, there has been considerable emphasis on bleaching mechanisms, at least compared with the other two research themes of this document. This appreciable attention to bleaching mechanisms in the past is reflected in the summary, and in the body of this document. Such a historical emphasis is not, however, meant to diminish the importance of the other research themes. Still, many knowledge gaps remain on the mechanisms of bleaching from the cellular to organismal scales. In particular, few studies have examined molecular-level pathways of stress in conjunction with organismal-level responses. Better integration of knowledge between symbiosis establishment and breakdown (i.e. dysbiosis) across scales is needed. There is mounting evidence for multiple pathways of thermal stress affecting both the host and its endosymbionts. While much is known concerning some of the initial triggers of thermal stress in reef corals and their endosymbiotic algae (*Symbiodinium* spp.), less is known about the role(s) of other components of the holobiont (e.g., the microbiome comprising viruses, bacteria, archaea and microeukaryotes other than *Symbiodinium*). In many cases, the variation of experimental designs, and inconsistency in parameters measured during natural or experimentally-induced bleaching, rarely allow for comparisons across studies, thereby slowing progress. Thus, there is an urgent need for standardized experimental best-practices and metrics to enable broader comparative analyses. Paradoxically, there is a pressing need to focus and develop several experimental 'model' systems amenable to laboratory and field manipulation, and simultaneously expand the range of scleractinian species under study. A better characterization of bleaching mechanisms and stress tolerance to elevated temperature will also lead to an improved understanding of thermal acclimatization, phenotypic plasticity, and adaptation. While there is considerable interest in investigating mechanisms of thermal tolerance at the molecular and cellular scale (e.g., phenotypic plasticity vs. standing genetic variation vs. epigenetic and trans-generational modification), it will be critical also to address possible larger-scale physiological trade-offs (e.g., decreased calcification) in organisms exhibiting higher thermal/stress tolerance. At the environmental and macro-ecological scale, there is a need to characterize and resolve the physical processes that lead corals to bleach, and determine what alleviates coral bleaching. Current methods that forecast and assess bleaching (e.g., satellite observations) do not have the temporal or spatial resolution to capture small-scale variability on coral reefs. Higher resolution environmental data and model development at smaller spatial scales ($< 1\ km^2$) would allow for better bleaching projections. Formulation of hydrodynamic models that account for temperature variability, and the deployment of instrumentation to test thermal models on selected reefs, would better inform our design of bleaching projections and define regions more susceptible to bleaching. The three research priorities highlighted under the bleaching mechanism theme were:

(i) Define the mechanisms of bleaching at the molecular and corresponding organismal scale.

(ii) Formalize a standard set of best practices (e.g., experimental exposures and/or response metrics) for bleaching studies that allow cross comparisons among focal organisms, geographic locations, and laboratories.

(iii) Identify the physical drivers of bleaching, their inherent variability within reef systems, and the selective pressures that may slow or override thermal stress.

2. Bleaching recovery

While coral bleaching is predicted to increase in frequency and severity; we know very little about the processes governing recovery dynamics following bleaching. The majority of recovery studies to date have focused on larger-scale trends such as mortality and regrowth at the population and community level. This work has provided valuable information to the field, yet we lack critical detail about molecular to physiological processes that affect recovery at the organismal scale. Phenotypic plasticity, acclimatization, and adaptation, may influence recovery success. Furthermore, our understanding of how, or if, *Symbiodinium* populations change after bleaching has been characterized for only a small number of corals and even less is known with concern to how such changes in *Symbiodinium* alter coral physiology or recovery. In most cases, we lack baseline knowledge about the diversity and stability of *Symbiodinium* spp. in coral communities around the world. Moreover, little is known about the spatial and temporal dynamics of viral, bacterial, archaeal, fungal, and other microeukaryote communities, and how they may change during and after episodic stress or influence the outcome of recovery. The interval following a bleaching event is a critical period when the coral's immune response and energetic demands are especially taxed, thereby making corals more susceptible to disease and other threats in their physiologically compromised state. Recovery thresholds at the ecosystem scale will also be important to characterize, as we do not yet know whether specific thresholds, or tipping points, occur, beyond which reef ecosystems may display phase shifts, and will be unlikely to recover to their previous state. Thus, given the relevant timelines/durations of these processes, many thorough studies for bleaching recovery will require more time than conventional funding periods typically allow (3–4 years). Research priorities for investigating bleaching recovery included the following:

(i) Determine the systems level processes, spanning molecular to organismal, involved in bleaching recovery.
(ii) Determine the links between bleaching recovery and patterns of disease susceptibility.
(iii) Characterize mechanisms and dominant attributes of coral-reef ecosystems that facilitate or hinder recovery.

3. Bleaching refugia

While many models suggest that the majority of coral reefs will not survive current IPCC projections for global warming (Frieler et al. 2012), other work has documented areas of bleaching resistance or refugia (van Woesik et al. 2012; Schmidt et al. 2016). Further, while some species may be driven to extinction, a subset of contemporary species may persist (Pandolfi et al. 2011; Edmunds et al. 2014; Grottoli et al 2014). Regional or habitat refugia may be determined by specific physical or biological attributes that protect species that are vulnerable to thermal stress. Other coral species may successfully grow under adverse conditions and have already adapted to withstand further thermal stress and subsequent bleaching (Hume et al. 2013; Palumbi et al. 2014; Richards et al. 2015).

Locating and investigating such bleaching refugia will entail a range of approaches, and has direct links to our continued understanding of what causes and prevents bleaching. Furthermore, harnessing our current and future knowledge of bleaching resistance into possible conservation strategies, such as the assisted migration or transplantation of thermally tolerant corals, as a means of restoration, are also key areas of interest by many in the scientific community, which also could benefit from future efforts in characterizing climate-change refugia. Three research priorities were identified under the refugia theme:

(i) Determine patterns of bleaching resistance across multiple coral taxa within multiple populations.
(ii) Locate and define refugia at the physical and biological scale.
(iii) Characterize the potential for survival and persistence of coral phenotypes in a range of habitats.

The three focal themes of the workshop presented above, and the accompanying ideas and questions covered below, provide a road map for initiating potentially transformative bleaching research into the next decade and beyond. A key conclusion of this workshop was that many of these ideas and questions are well beyond the scope of any one laboratory. This point is especially germane when considering how to approach many of the research themes across scales of study. Such efforts will take a large consortium of investigators to leverage the necessary expertise that is needed to tackle these questions fully.

Workshop Organization and Structure:

This white paper summarizes the efforts of a workshop entitled, "Investigating coral bleaching in a changing climate: Our state of understanding and mapping opportunities to push the field forward," which was held June 17–18, 2016 at the Hawaii Prince Hotel in Waikiki. The motivation behind the workshop was to assess the culmination of several recent years of focused research on coral bleaching by many U.S. investigators, which was made even more significant by the widespread bleaching events throughout the world that corresponded with a strong El Nino in 2014–2016. The workshop was strategically timed to precede the largest international scientific conference on coral reefs, the 13th International Coral Reef Symposium, which was also held in Honolulu Hawaii.

In October of 2015, Warner made initial contact with possible attendees to gauge initial interest in the possible meeting and timing. In December of that year, he invited several scientists from the U.S. coral-reef community, representing a range of age and academic rank, to join the steering committee to help organize the format of the workshop. The steering committee was composed of the following scientists:

Dan Barshis, Old Dominion University
Sarah Davies, Boston University
Andréa Grottoli, Ohio State University
Todd C. LaJeunesse, Pennsylvania State University
Mark Warner, University of Delaware
Robert van Woesik, Florida Institute of Technology

The steering committee formed a final list of participants (see Appendix 2 for complete list of participants) intended to capture a broad array of expertise in reef coral biology, and in academic experience. In addition, each participant was asked to recommend at least one advanced Ph.D. student, or postdoc, from their group, who they thought would benefit from participating in the meeting. The total number of participants was kept intentionally low to facilitate as much discussion as possible.

In April, all participants were asked to provide a one-page statement that included a brief summary of each person's current research focus related to coral bleaching, as well as a short list of no more than five key questions, or knowledge gaps, that they perceive in studying bleaching. Participants were encouraged to consider questions and gaps that may be outside their immediate expertise but were still connected to their larger research interests. Based on these summaries, and the lead participant's research history, the steering committee placed each participant into one of the three following breakout groups: (1) Cellular & Molecular level processes, (2) Organismal-level processes and (3) Ecological & Large-scale processes. The steering committee then split into these three groups to compile all of the participant summaries and assemble a short 20-minute presentation that would serve as a 'jumping off' point at the start of the workshop with the entire group. After further deliberation, the steering committee determined that it would be beneficial for one additional presentation at the start of the workshop which would be designed to summarize the status of the links between coral bleaching, microbial interactions, and disease. Two participants, Rebecca Vega-Thurber (Oregon State University) and Erinn Muller (Mote Marine Lab), kindly agreed to provide this presentation (see Appendix 3 for the workshop schedule). Virginia Weis (Oregon State University) kindly joined the molecular group leaders to help provide a broader cellular context for the meeting introduction. The steering committee agreed that our central focus should be on the discussion of coral bleaching in the context of specific climate events related to elevated or prolonged heating of seawater and high light exposure. While other large-scale factors, such as ocean acidification, and local stressors, such as coastal development, pollution, and turbidity may play important roles in influencing the outcome of bleaching, it was determined that we could not adequately address all of these issues given the time constraints of the meeting.

After the introductory presentations and discussions of the participant summary statements, all participants were charged with coming up with what they perceived as key research priorities for their respective breakout group. In particular, they were also encouraged to consider the direct applications and links that their questions had with the other two working groups. At the end of Day one, the entire group met to summarize the results. On Day two, each breakout group was charged with distilling their central ideas into no more than five research topics, and one steering committee/working group discussion leader also moved to a new breakout group to facilitate integration across groups. These distilled central ideas were then presented and discussed among the entire group. The entire group then worked together to condense these ideas further into the three themes listed below. The workshop concluded with a general discussion of the final product, and any additional modifications were made. Despite the varied perspectives of the participants, all three breakout groups converged on three central research themes that should be given equal weight for future investigations on coral bleaching. Summaries of group discussions for each theme and their **research priorities** and research questions are presented in the following sections.

1. Bleaching mechanisms

Our current knowledge of cellular and organismal responses to thermal stress in general, and coral bleaching in particular, is extensive, but only for specific aspects of thermal stress and only for some coral species. Substantial progress has been made in understanding the establishment of coral symbiosis, and in understanding the disestablishment of coral symbiosis, or dysbiosis. However, major gaps remain in our understanding of the mechanisms of these processes at the molecular, cellular, and organismal levels. At the cellular scale, several independent sets of studies have examined the mechanisms of symbiosis recognition, regulation, and re-establishment, as well as studies that have examined mechanisms of dysbiosis. A comprehensive evaluation of the overlap in these mechanisms is lacking and would provide substantial insight into the common cellular and molecular machinery that is responsible for the coral-bleaching response. This point is especially important when considering the potential targets for shifts in thermal resilience, either by adaptation or acclimatization. At the macro-scale, there was considerable discussion for better hydrodynamic and temperature model development for predicting and characterizing bleaching. Within the bleaching mechanism foci, three research priorities were identified as follows:

(Research Priority 1.1) Define the mechanisms of bleaching at the molecular and corresponding organismal scale.

At the molecular scale, a community effort to conduct a meta-analysis of the thermal-stress response (e.g., from transcriptomic datasets) in a range of corals and their symbionts could provide some convergence on common mechanisms of both bleaching susceptibility and resistance, and help to define the bleaching phenotype. Considerable discussion focused on the need for many of the mechanistic-cellular questions to be first characterized in a model organism, to arm the community with the necessary understanding to interpret these processes across other corals, *Symbiodinium* species, and other coral-associated microbial diversity. For example, a 3-part model system could prove useful that uses organisms that are: (i) nonsymbiotic and non-calcifying (e.g., the sea anemone *Nematostella vectensis*), (ii) symbiotic and non-calcifying (e.g., the sea anemone *Aiptasia pallida*[1] or the jellyfish *Cassiopeia*), and (iii) an aposymbiotic or symbiotic organism that calcifies (e.g., the coral *Astrangia poculata*). An additional coral, preferably one that is tropical, symbiotic and can be reared in the lab for multiple generations, was also discussed (e.g., the Caribbean species *Favia fragum*) as a promising system in need of further development. Also, there was discussion about the need to converge on a single or set of *Symbiodinium* model species that could be grown in culture. Such model systems could prove important in interrogating candidate stress pathways to demonstrate their role in bleaching. While not discussed as extensively across the break-out groups, there remains a pressing need to better define symbiont species and the microbial communities across corals, as taxonomic differences within and across these constituents can play a significant role

[1] This genus has recently undergone taxonomic revision and Grajales and Rodriguez (2016) have recommended the assignment of the new genus *Exaiptasia*.

in interpreting the bleaching response. Collaborations with a large group of molecular and cellular biologists could be fruitful in helping to address mechanisms and pathways of bleaching. In addition to the aforementioned model systems, participants also raised the point that the number of coral species under current intense physiological study is limited and should be expanded, encompassing a broad geographic range, to more broadly represent the diversity of natural reef systems. All of these efforts would better define the breadth of bleaching phenotypes, which may be similar or dissimilar among various host-symbiont combinations, and could provide a holistic understanding of the bleaching response.

The metabolic responses and dysfunction of photosynthesis to thermal stress are well characterized for some species of *Symbiodinium*. Nevertheless, more data are needed to fully characterize the range of physiological responses which are likely to occur across different coral species and their symbionts. We also need a deeper understanding of how host-generalist vs. host-specialist *Symbiodinium* interact within a range of coral taxa when subjected to thermal-stress conditions. Similarly, the direct links between cellular stress responses (e.g., production and release of reactive oxygen species (ROS) from *Symbiodinium* and/or host mitochondria) and the breakdown of the coral-*Symbiodinium* symbiosis are not well understood. In addition, several studies have hypothesized that the animal (e.g., impairment of coral immune response during symbiosis breakdown) and/or the microbiome responses could be as important as the resident *Symbiodinium* in inducing bleaching in some coral species (Bourne et al. 2008; Diaz et al. 2016), and in the commencement of post-bleaching disease (Vega Thurber et al. 2009; Maynard et al. 2015). Still, the mechanistic pathways involved in the onset of post-bleaching disease have yet to be characterized or even fully defined.

Research questions and topics to address bleaching mechanisms included:

- How conserved are the cellular mechanisms underlying mutualistic symbioses and dysbiosis across host taxa?
- Are there common mechanisms of adaptation, resilience, acclimation, and acclimatization to thermal stress?
- To what extent do coral-associated microbes (e.g., viral, bacterial, and fungal) function in the process of bleaching?
- To what extent are certain partner combinations (coral, *Symbiodinium*, intracellular and extracellular microbiome) resistant to stress, and what are the physiological tradeoffs inherent among these combinations?

(Research Priority 1.2) Formalize a set of best practices for bleaching studies that allow cross-comparison among focal organisms, geographic locations, and laboratories.

There is a pressing need for greater standardization of some elements of coral-bleaching experiments. Studies employ a range of experimental designs that tend to utilize either rapid, acute heating, or slow, more chronic, heating protocols. Likewise, while the interaction between light and temperature exposure in bleaching is well established, variability in light quantity and quality (e.g., photosynthetic active radiation, ultraviolet light) also contributes to differences across experimental bleaching studies. Many of these studies may have different goals (i.e., quantify the transcriptomic vs. the organismal response, or determining what is the cause of slow vs. rapid thermal priming). This makes comparisons across studies especially challenging. While one set protocol for testing bleaching susceptibility may not be applicable in all situations, there was considerable discussion on how we must reconcile possible differences between such experimental designs. Equally important is the need for the community to converge on common "currencies" or units for expressing response metrics. Hence a community effort to standardize heat-stress experiments, while still considering the length of time in the field, necessary equipment, and feasibility across field sites and lab groups was seen as a valuable research priority. Such an effort would allow for better mobilization of resources across laboratories when attempting to measure corals before, during, and after future bleaching events.

Ideally, biochemical and organismal-level responses (e.g., photosynthesis, respiration, calcification, feeding, carbon translocation, *Symbiodinium* density, microbiome composition, pigment concentration) should be compared against molecular and cellular level (e.g., transcriptome, proteome, and genome) responses for all focal organisms. However, participants acknowledged that this would not always be feasible, and hence the need for a refined list of standard metrics and a comparative analysis of the pros and cons of each is required. 'Best practices' development must consider the ease of use, broad applicability, feasibility, and access to specific equipment. Despite these challenges, participants noted that in many cases several laboratories have the capacity to measure a common set of variables that would allow for better cross-study comparisons.

Research questions and topics to develop best practices for bleaching studies included:

- What common methods (e.g., active Chl *a* fluorescence via PAM fluorometry, calcification via buoyant weight and/or linear extension) could be combined across field studies to draw a more holistic picture of the short and long-term bleaching response, and result in common metrics to enable comparisons across studies?
- How consistent are coral phenotypic responses to short- and long-term experimental heating exposures and how do they relate to bleaching responses in the field?
- Design and implementation of archival protocols (e.g., preservation of samples for later molecular 'omics' analyses) are needed for better downstream analyses of field-based studies that may not be technically, financially, or temporally feasible at the time of the study and would allow for better capture of "samples of opportunity" among different investigators.

(Research Priority 1.3) Identify the physical drivers of bleaching, their inherent variability within reef systems, and the selective pressures that may slow or override thermal stress.

The physical oceanography of reef systems can modulate water chemistry within and across the reef and influence the response of the benthic community. The current assessment of coral-bleaching events is based largely on satellite observations, often with follow-up field assessments. By contrast, coral bleaching is predicted using global climate models (GCMs), which project open-ocean temperatures. Yet, the temporal and spatial resolutions of both observations are too coarse to characterize variability on coral reefs. Bleaching predictions may be improved by identifying the most important physical drivers of bleaching and projecting these parameters to the scale of reefs. In this context, many agreed that a central goal should be to identify the methods needed to forecast bleaching at high-resolution. Such improvements would allow for better estimates of whether bleaching events are increasing in frequency and severity, and whether and where the spatial distribution of bleaching is changing over time.

Research questions to identify physical drivers of bleaching and their variability include:

- What are the physical and biological context dependencies of coral bleaching?
- At what spatial and temporal scale is temperature variability important in determining bleaching?

Possible steps forward include the construction of hydrodynamic models to quantify temperature variability and the deployment of instrumentation to test these models on selected reefs. The results would determine which proxies are the most accurate to assess bleaching events, and develop methods to determine how the intensity and frequency of other processes may slow or override the thermal tolerance of some reef corals

2. Bleaching recovery

Although coral bleaching is predicted to increase in frequency and severity, we know very little about the processes governing recovery from individual corals up to whole reefs, and whether that recovery is locally or regionally variable. While individual studies have documented bleaching recovery at the ecological scale, to date, few studies have closely followed the progression of field or experimental bleaching and subsequent recovery, at the organismal and molecular scales. By their very nature, large-scale field-based bleaching studies tend to include only a few metrics such as visual inspection, mortality, and regrowth. While such investigations are important and necessary, more detail is required at the organismal scale (e.g., reproductive output, calcification, energy reserves, and carbon budgets) to form accurate projections of how coral populations will recover in the future. In turn, it is valuable to determine the extent to which biogeographic variability in *Symbiodinium* specificity and host coral genetic diversity influence physiology (e.g., host energetic reserves and biomass) and tolerance across coral species. Lastly, more information is needed on the influence of diminished physiological state on disease outbreaks and susceptibility to annual bleaching. Similar to the focus on bleaching mechanisms described above, participants noted the need to define physical, chemical, and biological attributes that facilitate recovery at the macro-scale. Three research priorities were identified for bleaching recovery, as follows:

(Research Priority 2.1) Determine the systems level processes, spanning molecular to organismal, involved in bleaching recovery

Research questions to identify processes involved in recovery included:

- Which genotypes/populations/species are more likely to recover and why?
- What drives stability vs. flexibility between host-*Symbiodinium* interactions?
- Are there markers (i.e., phenotypes, genotypes, gene loci, etc.) that could be used to identify individuals or populations that exhibit high rates of recovery?

- What is the potential for organismal acclimatization (at both the individual and trans-generational scale), and how does this compare with standing genetic variation in explaining organismal responses and recovery potential?
- What is the impact of bleaching on fecundity, and thus what are the subsequent effects of bleaching on recruitment and coral community recovery?
- How do the responses to all of these questions change under annual/repeat bleaching stress?

(Research Priority 2.2) Determine the links between bleaching recovery and patterns of disease susceptibility

Research questions to identify links between recovery and disease included:

- Can the microbiome facilitate colony recovery?
- Does bleaching facilitate the growth of disease-causing opportunistic microbes during recovery?
- Which microbes are benign for corals under normal temperatures, but become problematic at elevated temperatures?

(Research Priority 2.3) Characterize the mechanisms and dominant attributes of reef ecosystems that facilitate or hinder recovery

Research questions regarding characterizing attributes of reefs that facilitate or hinder recovery included:

- What metrics are useful in defining baselines and recovery (i.e., population abundance, demographic structure, species diversity, composition, and ecosystem function)?
- What biotic (e.g., herbivory, predation), abiotic (e.g., nutrients, pH), and historical factors influence recovery rates and trajectories after bleaching?
- Where do thresholds (i.e., tipping points) occur beyond which reef ecosystems display hysteresis and do not recover to their previous state?
- What role do remnants (i.e., surviving colonies) play as a source of new recruits versus recruitment from other source populations?
- What role do deep and mesophotic reefs play in providing sources of new recruits?

3. Bleaching refugia

Locating and protecting coral communities found in refugia from bleaching is of critical importance to their persistence and conservation in a warming ocean. Bleaching and mortality are not always uniform across a region, and we need to better identify geo-spatial scales where certain locations or habitats may act as refugia. Likewise, certain populations of corals appear to be less susceptible and more tolerant of bleaching than others. Thus, we need to define the physical and biological characteristics that will most likely support coral communities into the future. The physiological plasticity of some corals during thermal disturbance in such locations is just beginning to be characterized at the molecular, cellular, and organismal scales. A central challenge will be how we define (i.e., both physically and biologically) and locate refugia. Pockets of bleaching resistance have already been located (Cacciapaglia and Woesik 2015) and many more potentially exist (e.g., shallow areas of mesophotic reefs (30-60 m)). Several participants also noted that such refugia could be used as important locations for investigating methods in coral restoration, possibly by means of transplanting or breeding corals with high thermal tolerance. However, research concerning reef restoration by transplantation or selective breeding may not be under the purview of many core programs at NSF, but could be of interest within other NSF programs or other agencies such as NOAA. Additionally, such efforts will need to carefully incorporate detailed analyses to decipher possible drivers of local adaptation (D'Angelo et al. 2015). Importantly, we have yet to predict where reef refugia will be located in the future. A first step toward understanding these locations will be to establish sentinel sites to track the performance of these systems and to identify the oceanographic and biological factors that confer resistance and resilience, and that modulate the severity of bleaching. For example, developing methods to identify local parameters that correspond to bleaching resistance similar to the accessible community proxy for thermal perturbation – Degree Heating Weeks (DHW) (e.g., internal waves, bleaching history, variation in temperature, light, flow, nutrients, etc.) – could prove useful in this effort. Within the refugia component, three research priorities were identified as follows:

(Research Priority 3.1) Determine patterns of bleaching resistance across multiple coral taxa within multiple populations.

Research questions regarding bleaching resistance included:

- Are there specific genetic and/or physiological markers for bleaching resiliency and/or propensity for recovery, and can these be used to identify refugia populations?
- How ubiquitous is bleaching resistance and/or propensity for recovery across multiple coral taxa at refuge sites?

(Research Priority 3.2) Locate and define refugia at the physical and biological scale.

Research questions and topics to help characterize bleaching refugia included:

- At what spatial and temporal scales do locations act as refugia, and are they stable or unstable at these scales?
- What sea-surface temperature parameters are the best indicators of local, regional and global refugia?
- What other physical and biological parameters (e.g., flow, upwelling, turbidity, nutrients, dissolved organic matter, particulate organic matter) best characterize refugia at the local, regional and global scale?
- What characteristics of the biotic community (e.g., species richness, functional redundancy, genetic variation) are the best indicators of local and regional refugia?
- Integrate times-series data on both biotic and abiotic parameters, and build and validate models to predict refugia locations.

(Research Priority 3.3) Characterize the potential for survival and persistence of coral phenotypes in a range of habitats.

Research questions and topics regarding bleaching-resistant corals that may characterize a refuge included:

- Identify resilient coral populations or genotypes of corals, irrespective of their current geographic location, to provide possible source populations for "reseeding" decimated reefs with larval recruits.
- Are traits that improve coral persistence representative of phenotypic plasticity and/or genotypic diversity, and are they heritable?
- What trade-offs exist between thermal adaptation and growth, fecundity, and other climate change stressors such as acclimatization to ocean acidification?
- Can we optimize Marine Protected Area (MPA) management to increase resilience and recovery from bleaching events?

Implementing Future Research

This workshop converged on several research themes across the breakout groups. Importantly, there was considerable agreement among participants that the sheer scope and scale of many of the resulting questions within each research priority will require a coordinated effort across numerous research disciplines and groups. While attendees represented a subset of U.S. scientists, a larger bleaching research consortia should include other U.S. investigators and international scientists. In this regard, organization of future bleaching research collaborations, through such funding platforms as the National Science Foundation – Research Coordination Networks (RCN), could serve as a catalyst to increase the multidisciplinary exchanges. Such a research network (or groups of networks) could facilitate the coordination of planned experiments, establish consistency in experimental protocols and field monitoring efforts, and develop an integrated modeling approach.

While research coordination is one path toward more collaboration, many participants agreed that several of the research priorities compiled from this workshop are timely and certainly approachable within the current framework of funding opportunities, such as those across several programs within the National Science Foundation. Likewise, some of these research topics may be addressed in part by better use of existing research frameworks. For example, at the field work scale, some new efforts could take advantage of current initiatives like the NSF-LTER site, while meta analyses and theoretical approaches will benefit from strong data archiving from established repositories (e.g., BCO-DMO) and new initiatives now underway (e.g., Coral Traits Database, the Coral Reef Science & Cyberinfrastructure Network). Participants agreed that national and international collaboration would be an important component to fully optimize expertise across disciplines, and to minimize duplication of efforts. A few of the initial topics that were discussed for forming such research collaborations included: integration of molecular to organismal-scale mechanisms of bleaching and recovery across several model systems (RP 1.1, RP 2.1), resolving fine-scale physical (e.g., temperature and irradiance) bleaching drivers and their variability for better targeted model development (RP 1.3), and determining the physical and biological characteristics of bleaching refugia (RP 3.2). Addressing the priorities provided in this summary will deliver critical information that many in the community see as necessary to significantly advance our understanding of this globally important phenomena.

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Appendix 1 - Community Comments

Comments from all workshop participants were solicited as well as many U.S. coral reef investigators who were unable to attend the workshop. In several cases, comments were directly incorporated into the final document, and additional discussion points provided by the community are included below and are organized by topic or section of this document. Brief responses to these comments are provided in italics.

General comments / Executive summary:

"In my view, it will be tough to predict where and when bleaching occurs under 21st century climate change without a better handle on the complexities of ocean warming on the scale of a reef. It will also be tough to identify whether or not acclimation/adaptation is occurring if we don't have a good handle on the reef *in situ* conditions during an open ocean warm anomaly. My second suggestion is that we badly need an historical perspective. Are we convinced that we have the data to conclude that the frequency and severity of bleaching events is increasing? I'm not convinced we have the data because I don't believe we have a sufficiently lengthy frame of reference. ...how and where did coral reefs respond, bleach, die, not die, and how fast did they recover? Thinking bigger and longer scales: have coral reefs periodically died back, and recovered? In my view, documenting and understanding the history of coral reef responses to warm events is as important for predicting the future, and we need to develop the tools and approaches to investigate these questions with confidence."

Reply: We agree that a longer historical context for coral responses to previous warming events will help inform future bleaching projections. The historical perspective did provide the basis for many of our conversations. However, a longer review of this point is outside the scope of this workshop summary and more appropriate for a review / opinion paper on the topic of historical bleaching.

"I worry that it lacks a little bit of context from the history bleaching. The document reads a little bit like this is a 'new' problem and we are starting out from near zero. I think this may be problematic... it encourages earlier career scientists (and grads) not [to] familiarize themselves with the literature, what we know already, and from what platform the new push is launching."

Reply: The workshop participants were charged with discussing current and future approaches to investigating coral bleaching. We agree that a historical perspective on what we have learned to date is important and this did provide the basis for many of our conversations. However, such a review is outside the scope of this workshop summary and more appropriate for a review / opinion paper on the topic of bleaching.

Regarding Research Priority 1.1 (Define the mechanisms of bleaching at the molecular and corresponding organismal scale):

"My one main comment/criticism is that the paper is nearly 100% scleractinian coral-centric... in that section you talk about model systems, including sea anemones. Yet, sea anemones are seldom studied in nature in terms of global climate change... anemone and anemonefish numbers plummeted by 86 % and 74 %, respectively [in the Gulf of Eilat]. We concluded that the anemonefish demise was probably driven by anemone decimation. This drop is in the Gulf of Eilat (Aqaba) where the scleractinian corals, for the most part, are doing fine. So, even in places where corals do not seem to be affected (yet), [for] regions that are considered refugia... other cnidarians may be the prelude for things to come. Furthermore, in the Caribbean, gorgonians actually dominate many reefs and they have been doing so as far back as reports by Cary in 1914. Hence, assessment of Caribbean reefs is incomplete without assessing other cnidarians."

Reply: We agree with the main point here and also included the phrase in the executive summary for "other symbiotic cnidarians." While the loss of scleractinian corals is a central concern given the topographic complexity and underlying biodiversity that they support, the comment is correct that other symbiotic organisms, including some sponges, foraminiferans, as well as gorgonians and giant clams can contribute substantially to tropical ecosystems and should also be studied in the context of thermal bleaching in the field and in laboratory-based studies. The purpose of the workshop was to focus on coral bleaching. Therefore, the focus of this document is also on corals with an acknowledgment that other organisms are affected by thermal anomalies that lead to bleaching and could be considered in the context of future bleaching investigations as well.

Regarding Research Priority 1.2 (Formalize a set of best practices for bleaching studies that allow cross-comparison among focal organisms, geographic locations, and laboratories):

"Another part of this that doesn't get much exposure is integrative organismic modeling that could provide a vehicle to analyze groups of data within a meta-analysis framework. DEB (dynamic energy budgets) are one way ahead, and while this has remained a complex body of theory with few experts, progress is being made in applying these tools."

Reply: DEBs are one approach as are some of the multi-variate techniques beginning to be used in this area. The exact tools used to address best practices should be explored in an open forum, and other research opportunities, such as a Research Coordinated Network (RCN), could provide a good opportunity to address these points.

"I would add here that there is also a need for interdisciplinary scientists to work together on bleaching experiments, to more comprehensively address the role(s) of the different components of the holobiont during bleaching (e.g, we may need larger project budgets to comprehensively conduct these experiments!)"

Reply: We agree and have addressed this specifically in several areas of this white paper.

"I am always nervous of institutionalized 'standard' methodologies as I worry that this stifles creativity. I agree that there is a strong need to be able to compare results, but it is valuable to note this can be achieved through multiple mechanisms such as explicitly supporting means for inter-calibration."

Reply: Several participants raised these points as well with concern to best practices restricting creativity. However, many considered that the field has converged on several experimental design 'norms' but the range in their use (e.g., the rate of heating with regard to acute vs. chronic exposure as well as light levels used and their ecological context) has created difficulty in comparing data across studies and hence adds to the difficulty in understanding the variance in bleaching responses. We agree that better community intercalibration would be one way forward, and this priority was also seen as a way to encourage sample archiving within the community for future work that may reach beyond the original scope of any one project.

With regard to the use of model laboratory and field-based organisms:

"Perhaps this could be broadened to an appeal for a phylogenetic perspective? One suspects that at least some of variation in species represents aspects of phylogeny that we have not yet grasped."

Reply: We agree that placing this work into a larger phylogenetic context will be important and should be considered as one of many logical approaches when working with or comparing data across multiple organisms.

With regard to our limited knowledge of the spatial and temporal dynamics of viral, bacterial, archaeal, fungal and other microeukaryote communities and how they may change during and after episodic stress:

"I think this is a really important point for NSF to hear. They don't like to fund methods projects, but without the methods we really can't answer these questions."

Reply: We agree and hope that this workshop and summary here will provide a platform for the community to communicate with NSF about needs within the discipline. We also note that potential to support work related to technique and methodology development through NSF is still a possibility through avenues such RCN's and research-based workshops

Regarding Bleaching Recovery

"It might be helpful to draw a clearer distinction among at least three functional levels - (1) organismic, (2) populations, and (3) communities. I think there is a tendency to aggregate these all together, and I think this promotes a lack of clarity. Community recovery brings the thorny issue to the forefront of whether ecological theory predicts the identical state to re-assemble, in particular as broader ecosystem and environmental issues change in the new millennium."

Reply: We agree that studying recovery at these different scales requires different approaches, but all are ultimately related. The response and recovery of communities is dependent on the response and recovery of different species populations, and populations are dependent on the response and recovery of individuals. We chose to take a general and broad view of this topic and did not expound too deeply on aspects of what this would entail at each scale of organization. The comment raises a good point in that defining resilience or recovery at the *cellular or organismal scale may be equivalent to returning to a pre-defined state or set of measures, but at higher macroscales this may not be possible.*

"Recovery is not simply when a coral regains normal pigmentation. It will be important to decide how do we actually define homeostasis and at what scales should we measure this (biochemical, organismal, population etc.). Also, can organismal attributes be informative for ecosystem-wide processes?

Reply: We agree. Similar to the comment preceding it, it will be necessary to differentiate how we define bleaching recovery at a particular biological scale.

Regarding research to study the effect of introducing transplanted coral species within established populations in refugia:

"I would like to see more results of the practicalities (and outcome) of this work before it is advocated as a priority. Plus, it assumed that we have sort of given up on other solutions, and it would be sad to be at that point."

"I would favor caution in this area as I think more needs to be known before this should be advocated as a way ahead."

Reply: Several participants also voiced similar concerns about this particular topic. We stress that it was viewed as one aspect of study under the larger priority of characterizing and establishing reef refugia and significant quantitative evidence that will be needed prior to justification of any transplant studies.

Appendix 2 - Workshop Participants:

Last name	First name	Affiliation
Adam	Tom	University of California, Santa Barbara
Baker	Andrew	University of Miami
Barshis	Dan	Old Dominion University
Baum	Julia	University of Victoria
Baums	Iliana	Pennsylvania State University
Burkepile	Deron	University of California, Santa Barbara
Castillo	Karl	University of North Carolina, Chapel Hill
Chan	Andrea	Pennsylvania State University
Coffroth	Mary Alice	State University of New York at Buffalo
Cohen	Anne	Woods Hole Oceanographic Institution
Conley	Daniel	Scripps Institution of Oceanography
Davies	Sarah	Boston University
deCarlo	Thomas	Woods Hole Oceanographic Institution
Dobson	Kerri	Ohio State University
Eakin	Mark	NOAA
Fitt	Bill	University of Georgia
Grottoli	Andrea	Ohio State University
Hancock	Harmony	Old Dominion University
Hawkins	Thomas	University of Delaware
Hoadley	Kenneth	University of Delaware
Iglesias-Prieto	Roberto	Pennsylvania State University
Jury	Chris	Hawaii Institute of Marine Biology
Kellner	Julie	NSF
Kemp	Dustin	University of Georgia
Klepac	Courtney	Old Dominion University
Kline	David	Scripps Institution of Oceanography
Kuffner	Ilsa	U.S. Geological Survey
LaJeunesse	Todd	Pennsylvania State University
Lewis	Cindy	Florida International University
Manzello	Derek	NOAA, AOML
McLachlan	Rowan	Ohio State University
McMinds	Ryan	Oregon State University
Merselis	Daniel	Florida International University
Morikawa	Megan	Stanford University
Muller	Erinn	Mote Marine Lab
Palumbi	Steve	Stanford University
Putnam	Hollie	Hawaii Institute of Marine Biology

Randall	Carly	Florida Institute of Technology
Riegl	Bernhard	NOVA Southeastern University
Ritson-Williams	Raphael	Hawaii Institute of Marine Biology
Rodriguez-Lanetty	Mauricio	Florida International University
Santos	Scott	NSF
Thornhill	Dan	NSF
van Woesik	Rob	Florida Institute of Technology
Vega Thurber	Rebecca	Oregon State University
Warner	Mark	University of Delaware
Weis	Virginia	Oregon State University

Appendix 3 – Workshop Schedule:

2016 U.S. Investigator Coral Bleaching Workshop
Hawaii Prince Hotel Waikiki, June 17th & 18th 2016

Friday, June 17th:

8:00–8:45	**Hale-Kila Room**, Check in and continental breakfast
	Short summary presentations
8:45–9:00:	Greeting and Introduction: Mark Warner and NSF Program Officers "High Altitude" Goals of our meeting
9:00–9:15:	The molecular scale (Dan Barshis, Sarah Davies, Virginia Weis)
9:15–9:30:	Microbial links, disease (Becky Vega Thurber, Erinn Muller)
9:30–9:45	The organismal scale (Andrea Grottoli, Todd LaJeunesse, Mark Warner)
9:45:–10:00	Higher level / ecological, geological, and global scales (Rob van Woesik)
10:00–10:10	Break
10:10–12:00	Break-Out Discussion Groups (Hale-Kila Room, Board Room & Captain's Room)
12:00–1:00	Lunch (Hale-Kila Room)
1:00–2:45	Break-Out Discussion Groups (Hale-Kila Room, Board Room & Captain's Room)
2:45–3:00	Coffee break
3:00–4:00	Day 1 discussion Summary (Hale-Kila Room)

Saturday, June 18th:

8:00–8:45	Hale-Kila Room, Continental breakfast
9:00–9:10	Goals for the day
9:10–11:50	Break-Out Discussion Groups (Hale-Kila Room, Board Room & Captain's Room)
12:00–1:00	Lunch (Hale-Kila Room)

1:00–2:00	Break-Out Discussion Groups (Hale-Kila Room, Board Room & Captain's Room)
2:00–4:00	White Paper Development (with caffeine infusion) and Adjournment.