Title: Disentangling effects of multiple stressors on biological robustness and resilience

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STATEMENT OF FUTURE WORK

The four authors listed above produced this concept paper during the limited time at the Atlanta jumpstart. We appreciate all of the input from folks in Atlanta and we plan to move forward with developing these ideas further in spring 2020. Clearly, there is much work to be done developing this idea, including spending more time exploring the literature with respect to existing models and gaining perspectives from a diverse array of colleagues. Thus, we leave some comments here for all to see and expand on. These occur in the section that urges for a standard set of definitions and show there is already constructive conversations regarding how these terms are and should be applied across disciplines. We welcome further input and interdisciplinary collaboration from jumpstart participants across locations or others that may be appropriate as this process moves forward.

INTRODUCTION

A long-term goal of biology is forecasting how biological systems will function under future conditions given that environments are often in a state of change rather than at equilibrium. In particular, how do we disentangle the impacts of multiple stressors on biological robustness and resilience, across biological systems and hierarchical scales? To properly predict the robustness and/or resilience of biological systems in the face of perturbation by environmental stressors, we must consider that systems often/always experience pressure from multiple stressors simultaneously and/or sequentially across hierarchical scales. Creating a common framework for this research is the next frontier in increasing our fundamental understanding of biological systems, and will aid our ability to make informed predictions about the future performance of systems. This work will inform our understanding of how cells to communities survive and rebound from stressors (abiotic and biotic, acute and chronic) from the responses of humans and domesticated animals when exposed to environmental toxicants and ecosystem-level functions such as pollination services for crops or the emergence and prevalence of disease.

For a few systems, there is substantial information about how multiple stressors interact to affect system performance. For example, many studies have manipulated water availability and temperature and/or other parameters factorially in plant systems in relation to photosynthesis, growth, and fruit yields, among other performance traits. Some studies have even scaled up in estimating the effects of multiple abiotic and biotic stressors on the structure and functions of plant communities and their associated herbivores and microbial symbionts through factorial experiments in microcosms. Arguably, general rules may be emerging in these well characterized plant systems. Additionally, substantial progress has been made in the field of toxicology towards understanding the multiple-stressor challenge, where models can predict how multiple toxicants affect organismal performance. Finally, substantial progress has been made in bacterial systems, where responses to individual and combined stressors have been studied in depth.

In contrast to our understanding of plant communities and toxicology, in many other systems and at other scales, we lack a basic understanding of the interactive effects of stressors and the responses to them. For example, the effects of multiple stressors on animal systems are dramatically less well-explored at all scales, with a fundamental gap between studying single stressors in isolation versus the multifarious nature of stressors experienced by biological systems, which has been recognized for decades. We know organisms experience multiple types of stress all together and/or in sequence, but predicting the direction, magnitude, and consequences of multiple types of interacting stress across levels in biological systems has been challenging. Additionally, the ability to make accurate predictions in toxicological models is rooted in a fundamental understanding of the modes of action of each toxicant (target sites) as well as how shared mechanisms for detoxification may affect organismal responses to toxins in combination. Yet, for many environmental stressors we do not understand the modes of action well enough to build predictive models about the directionality or magnitude of effects of experiencing multiple stressors on the performance of biological systems, particularly across scales.

Given our deep knowledge of some systems and extremely limited knowledge of others, to what extent will generalized rules about the interactions between stressors allow us to make predictions about biological systems where multi-dimensional stresses have not been well characterized? For example, what can we take away from existing knowledge about the robustness of plant communities when exposed to both drought and thermal stress when trying to predict how coral reef systems will respond to the combination of ocean acidification and thermal stress? How do these response mechanisms scale hierarchically, from cells to ecosystems? What are the commonalities and differences across those scales in terms of response to multi-stressors? Given the rapidly increasing speed of climate change, the far-reaching anthropogenic impacts (e.g., trash in the depths of the ocean, impacts of humans where they rarely or have not gone before), and their combined potential to exacerbate biotic stresses (e.g., emergent diseases, expanded disease ranges, biological invasions), now is the time to understand the impact of multi-stressors across scales to better understand how the world is changing.

Here we discuss the challenges, barriers, and gaps in our understanding of interactions between multiple stressors and subsequent mechanisms of robustness and resilience. We offer insight and potential ways forward to address these complex problems, emphasizing the necessity for collaboration across sub-fields and scales to create a robust multi-faceted understanding of these complex interactions. Addressing these gaps and working to improve understanding of the complexities of multi-stressor robustness and resilience will encourage reintegration across biological fields and contribute to: **1)** improved prediction of system outcomes under predicted future multiple stress scenarios (e.g., chronic and acute, biotic and abiotic); improved understanding of the rate and capacity for adaptation to environmental changes, **2)** increase our ability to preserve and restore ecosystems; improve our ability to understand the effects of species introduction (purposeful or invasive) on ecosystems**, 3)** improve understanding of complex issues related to human health (e.g., cancer, microbiome function), **4)** improve crop and livestock production in the face of rapidly changing climates, **5)** increase our understanding of capacity and rate of species adaptation to environmental changes, and **6)** consider natural selection as a stressor driving evolutionary change across species.

MAJOR BARRIERS

1. *A set of standard definitions for associated terms (e.g., robustness, resilience, resistance, tolerance)*

To quantify the effects of multiple stressors on robustness and resilience in biological systems, a standard set of definitions is needed. Within scientific literature, these and other associated terms are often used interchangeably, often with vastly different meanings. Thus, it is necessary to develop consensus definitions of relevant terminology. This will improve comparability across studies, synthesis, and integration across sub-fields and hierarchical scales. Here we propose several definitions which we will use for the duration of this paper. We define stressor as a pressure, tension, or impact on an entity that may be abiotic (e.g., temperature, toxin, salinity) or biotic (e.g., predator, disease, parasite). In contrast perturbation is the response of an entity, defined as a deviation from regular or normal functional state caused by the stressor in question. Entities may be robust or resilient to these stressors. Herein we define robustness as the ability for a system to avoid perturbation by a stressor(s), whether through phenotypic plasticity or other means. This is synonymous with the concept of resistance in infection literature. In contrast, resilience is the ability to maintain or return to adequate performance (defined in context) despite the stressor(s). This takes two forms: **1)** systems can be perturbed, stay perturbed, and maintain performance, or **2)** systems can quickly regain performance after a depression due to stressors. This is similar to the concept of disease tolerance. Notably, some forms of robustness and resilience are difficult to disentangle experimentally, in which case we suggest the use of the term hardiness, which reflects general lack of sensitivity to stress. Finally, we use the term system to define a group of interacting entities, characterized in networks, that are applicable across hierarchical scales. It is important to note that the components of a system are best defined by the users in question: the necessary scales are highly context and question dependent.

Kommentar [1]: I think I would disagree that a perturbation is a response. Rather stressors are an example of a perturbation. Entities respond to perturbations (and stressors).

Kommentar [2]: I tend to agree

Kommentar [3]: We are using the term as defined in a dictionary. Prior to this, we specifically state that we need to determine specific definitions for these terms, for the purposes of this paper. We recognize these terms could change in future iterations.

Kommentar [4]: At least in bacterial systems, resistance is defined as the ability to fend off the stressor (e.g. pumping out salt, for haloresistant species) while tolerance is the ability to "live" with the stressor (e.g. adapting internal machinery to operate at higher salt concentrations: halotolerant species).

Kommentar [5]: Thanks for this comment Ivan. Under our definitions, both of these examples are two sub-types of tolerance 1) living with the perturbation and still maintaining performance, or 2) snapping back from the perturbation.

2. *What is the mode of action of stressors?*

An assumption of our approach to generating a consensus on general rules for understanding interactions among stressors is that disentangling the effects of stressors on biological systems and the potential mechanisms that confer resilience or robustness, it would be useful to understand the mode of action of both abiotic and biotic stressors. When the mode of action of a specific stressor is well understood and characterized, groups can develop models to predict the effect of a specific stressor or multiple stressors. Toxicological work on herbivorous insects that feed on hosts containing distasteful/toxic compounds highlighted the importance of alterations to the chemical's target binding site, metabolism of the compound, rapid excretion of the chemical to minimize exposure, or some combination of these responses in generating resilience within the insect. Much of the toxicological research has focused on the mode of action of a single toxin (stressor) or a suite of chemically/structurally similar compounds, and the results of these studies demonstrate that a system or entity's response to toxins with similar modes of action can be conserved. However, our understanding of the mode of action of many non-chemical stressors, and the responses that contribute to robustness/resilience within systems/entities to some chemical stressors with known modes of action remains poorly understood. Consuming a toxic substance is expected to stress a system in a different manner than an increase in environmental temperature, which may be related to the location that the stressor impacts on an individual (e.g., more localized impact by particular toxin vs. stressor that impacts multiple cellular components like temperature). But to what extent can we use knowledge of mechanisms or robustness/resilience to predict where effects of stressors will overlap and where they will not?

Further complicating our understanding of robustness and resilience in biological systems is the fact that a system can and will often experience multiple stressors at the same time or sequentially across time. This complicates our ability to quantify the effects of the stressors for several reasons: **1)** it has been demonstrated that when multiple stressors co-occur the mode of action of the individual stressors (observed in isolation) can be altered. In some systems, the mode of action of the different stressors can become additive or non-linear (see figure 1). **2)** When systems experience multiple stresses that occur sequentially, the initial stress can prime the system to become more resilient to later stress (hormesis) or more susceptible to later stress. **3)** Entities within a system experience the stressors as chronic or acute depending on their scale. However, different entities in a single system can experience the stressors at different scales depending on their life history (e.g., a stressor might be acute for a longer lived entity but chronic for those with a significantly shorter lifespan, priming and memory with respect to stressors and how that can modify the response). Thus, a clearly articulated understanding of the mode of action of stressors individually and when occurring in the presence of other stressors is needed for the development of networks that can be used to characterize and predict biological robustness and resilience in a system. For example, drought and an unusually warm spell of two weeks may have a greater impact on the population dynamics of an insect pollinator with a 2-week lifespan than populations of a perennial plant the insect may pollinate.

3. ***How might we disentangle roles of plasticity, epigenetic, genetic variation, and adaptation in robustness/resilience?***

Additionally, we must consider the nonlinear mechanisms are largely goal dependent, and based on the natural frequency of stressors in question, and time span being considered. Thus, unified rules would include modeling both plasticity and heritable changes in organisms due to stress exposure and the extent to which the multivariate nature of experiencing multiple stressors may affect system performance.

4. ***Experimental barriers: creating systems that maintain naturalistic characteristics but are manipulatable in realistic ways***

A large challenge to understanding robustness and resilience to multiple stressors across systems lies in experimental design. Current approaches to understanding these topics are plagued by a lack of reproducibility in methodology and standard environmental conditions, producing context-specific effects. This necessitates an approach that allows for creation of manipulatable systems that maintain naturalistic characteristics (both abiotic and biotic). Arguably this is most feasible on the organismal level, though to this point repeatable experimental methods have yet to be developed for most organismal systems. However, we envision the need to develop reproducible designs for manipulating sub-organismal systems (e.g., sets of cells in organoids to model tissues in organisms coupled with *in vitro* tissue culture) and higher-level ecological systems up to complex communities that could be manipulated in microcosms (e.g., rapidly deployable and inexpensive field manipulations akin to FACE sites for multiple stressors). Beyond the necessary advances in infrastructure and technology, improved communications between groups studying similar species and systems is necessary to tackle this challenge. For example, a key component of approaching this barrier is producing common benchmarks for stressors across scales and organisms/ecosystems (e.g., set changes in temperature, acidification), that will then increase the replicability of studies. In sum, it is essential to reduce the amount of non-relevant noise between and within studies to truly understand mechanisms of robustness and resilience.

Experimental barriers become much more significant at the community level. While knowledge gained from organismal studies could be summed into mathematical models that predict community function, robustness, and resilience in response to multiple stressors, this approach is inherently limited. Responses of organisms in isolation often differ substantially from *in situ* responses, where interactions between organisms may have complex effects on individual organism robustness. These interactive effects quickly become difficult to capture in a controlled laboratory setting: large mesocosm studies can only capture so many players in a community or ecosystem and fail to represent the true natural complexity of system. Additionally, the frequency of disturbance/stress events must also be considered to accurately predict response of individuals to communities. Furthermore, studying such responses *in situ* presents challenges for controlling conditions that are not of interest, and scale quickly becomes an issue (e.g., how to accommodate for large migratory species). Thus, there is a need to harmonize best practices in studying mechanisms of robustness/resilience at the community level in general across systems, and in response to multiple stressors.

The use of macrosystem scale infrastructure could allow for the determination of acute or chronic stressors across spatial and temporal scales, such as NEON (for terrestrial systems), MarineGEO (in coastal environments), Marine Reserves (for subtidal environments), National Data Buoy Center (for open ocean), Long-term Ecological Research Centers (for terrestrial and coastal systems) and ForestGEO (for forest systems). Considering these across countries and continents, particularly for macrosystem scale studies leads to the need for this infrastructure in other countries (for example, Brazil and Colombia, which have NEON-like infrastructure). There is also the potential to use latitudinal and elevational gradients to understand the role of stressors across organisms/populations/communities by comparing populations or similar communities across these broad-scale gradients as a proxy for understanding chronic stressors (e.g., temperature change across latitude, salinity change across estuary).

5. *Analytical barriers: the need for complex large data approaches and models*

Understanding robustness and resilience across multiple scales in response to multiple stressors requires increasingly complex network and multi-directional correlative approaches. While analytical and computational approaches are rapidly advancing, there is a need to provide greater access to model increasingly complex, multi-scale systems. The development of new computational tools that can incorporate components identified above (e.g., modes of action, interaction effects) will be essential for analyzing and identifying mechanisms of robustness and resilience and using this information to develop rules for how organisms may be robust/resilient to multiple stressors that can be applied to systems with limited information. This also requires an ontological framework to drive forward the discussion to enable inter-discipline approaches. It will be essential for scientists to leverage aspects of the big data revolution and advances in supercomputing computational power to address these complex questions and systems.

ON THE NECESSITY OF INTEGRATIVE APPROACHES

Concepts of robustness and resilience in systems are applicable to biologists studying life at all hierarchical scales (molecules to ecosystems) and across disciplinary boundaries. Understanding how systems respond to multiple stressors is a key component of many different subfields of biology. To truly understand these ideas, and create a universal framework for approaching these complex problems, we must integrate a variety of perspectives. Approaches from fields such as immunology, physiology, ecology, human health, biochemistry, cell biology, evolutionary biology, toxicology, systematics, and computational biology must all be incorporated in order to truly approach questions of robustness and resilience. Thus, this question necessitates re-integration of biology across almost all major sub-disciplines and hierarchical scales. Most importantly, accurate understanding of robustness and resilience to multiple stressors requires uniting these fields around a clear set of terms, and standardized methods, concepts, and frameworks. Only by using this integrative approach will we truly understand diversity and constraint in mechanisms of robustness and resilience to multiple stressors.

There are a number of state-of-the-art technologies, many of which require expertise in disparate methods and technologies, that can address these issues on a fundamental/mechanism level. First, advances in sequencing technology, including the omics "revolution" (e.g., gen-, transcript-, metageno-, metabol-) allows us to assess the mechanistic responses of organisms, populations and communities to stressors. Additionally, gene editing tools, such as CRISPR/Cas9, allow for direct manipulation of gene function to determine redundancy in genomes/transcriptomes and the impact of stressors across genes. We also have the ability to synthesize compounds/proteins for experimental use, allowing the use of large inputs of compounds/proteins as stressors (e.g., nutrient addition, toxins) in experiments. Advances in sensors for monitoring stressors allows us to determine what stressors are present, for how long, and observe if those stressors had an impact. While, connecting data generated from these advanced technologies across hierarchical scales remains a challenge, we now have access to supercomputers that contain the increased computational power to answer or address large complex problems, using tools like multi-directional correlative analysis. The improved application of these computing resources will serve to improve integration of data and understanding of complex robustness/resilience.

BROAD IMPACTS OF ADDRESSING THESE CHALLENGES

Climate Change/Ecosystem Restoration- In an increasingly and rapidly changing world, the ability to understand how multiple stressors affect biological entities across scales is paramount. Improving understanding of how systems respond to and endure interactive stressors will directly increase understanding of the effects of changing environments (i.e. climate change). Furthermore, this knowledge will improve our ability to predict future trajectories at levels from individual species to communities in the face of predicted environmental change. Beyond this, improved understanding of complex robustness/resilience will improve ecosystem preservation and restoration efforts, allowing for proper re-assembly of degraded or destroyed ecosystems with communities that can withstand natural multi-stressors in that environment and help to identify the properties that make systems more or less impacted by stressors. Understanding mechanisms of robustness/resilience to multiple stressors will aid in the identification of characteristics of robust/resilient ecosystems. By understanding the factors contributing to complex ecosystem robustness we can better preserve and restore these factors, while understanding population sustainability.

Human Health- Understanding of the effects of multiple stressors also applies to numerous topics in human health and disease. Understanding interactive effects of stressors on robustness/resilience of tumors, neoplasia, and tissues will improve cancer therapies. Additionally, applying similar concepts to microbial communities will address current uncertainties regarding the robustness of the microbiome and links between robust structure of these communities and overall organism health, including the utility/effectiveness of pro-biotics. These concepts also apply to the responses of zoonotic pathogens and how they impact wildlife and humans. Finally, these concepts extend down to smaller, pathway and molecular scales. Understanding robustness/resilience of critical cellular pathways in the context of multiple stressors can aid in understanding diseases and conditions resulting from the breakdown of these pathways, such as through an Adverse Outcome Pathway conceptual framework.

Agriculture- Understanding responses of entities to multiple stressors, and mechanisms of robustness/resilience to combinatorial stressors will greatly improve future agricultural efforts (e.g., aquaculture, plant crops). Understanding how key crops and livestock species respond to multiple stressors will allow for identification of robust/resilient species and genotypes under current and future scenarios. These efforts have the potential to improve crop yields and agricultural output, addressing a critical challenge presented by climate change and other human impacts on ecosystems.

Restoration and Wildlife Re-integration/De-extinction- With a broad understanding of robustness and resilience across organisms/populations/communities, we could understand the impacts of restoration and wildlife re-integration. This would allow for the understanding of what stressors re-introduced wildlife would experience and how they would respond to those stressors. Additionally, we would understand what stressors the re-introduced wildlife would cause in the communities/ecosystems that they are introduced to. Finally, it is possible that understanding the impact of stressors would result in more robust restoration techniques with better long-term consequences.

CONCLUSIONS

In conclusion, we contend that understanding of multi-stressor robustness and resilience is currently limited, and fragmented across hierarchical scales. While significant challenges exist in developing a universal framework to study and describe how stressors interact and effect entity robustness and resilience, the way forward clearly necessitates an integrative approach. Working together across sub-disciplines to improve understanding of concepts of multi-stressor robustness and resilience across scales and systems has impacts for a variety of pertinent challenges in biology ranging from ecosystem preservation, to climate change and human health.