

Title: A universal theory of biological stress

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What is biological stress?: If you apply stress to the end of a beam of steel, strain will accumulate. If the stress is weak enough, that strain will disappear when the stress is removed. If the stress is more severe, the strain will remain and the beam will be deformed. In extremely stressful situations, the beam will break. Although this stress-strain theory applies to physical systems, stress and strain are also rules of life. The main difference is that life can react to stress and strain to maintain homeostasis up to a point. Here we describe the rules of stress and strain in living systems ranging from microbes to multicellular organisms to ecosystems, with the goal of identifying common features that may underlie a universal biological theory of stress. We then propose to establish a range of experimental, observational, and analytical methods to study stress across scales, including synthetic microbial communities that mimic many of the important characteristics of living systems, thereby enabling a universal theory of biological stress to be experimentally validated without the constraints of timescales, ethics, or cost. A universal theory of biological stress will allow us to predict how living systems respond to stress and strain across different scales of life thus allowing us to improve our ability to address anthropogenic change and human disease.

Organismal stress responses: Hans Selye determined that exposure of rats to vastly different noxious agents (e.g., heat, cold, formalin) led to a trio of repeatable responses: peptic ulcers, increased adrenal gland weight, and reduced size of the thymus gland, an important immune organ. Initially Selye's work indicated that stress (as he termed the response) was elicited by a non-specific stimuli (he termed stressors). Since Selye, we have learned that a wide range of environmental stimuli lead to increased synthesis and release of glucocorticoid hormones (i.e., cortisol in humans, corticosterone in some other animals) from the cortex of the adrenal gland. Additionally we've learned that long-term exposure to glucocorticoids can elevate blood sugar (adrenal diabetes), redistribute fat (Buffalo torso), reduce activity of our inflammatory system, reduce activity of our ability to fight disease and cancer (grieving widow's syndrome, Irwin et al., 1988), reduce life span, reduce the size of certain brain areas involved in short term memory (Finsterwald and Alberini, 2014), lead to growth of some brain areas involved in anxiety and fear (amygdalae) responses, and change feeding patterns (Stengel and Tache, 2014). Release of epinephrine from the medulla of the adrenal gland during stress also causes many changes in physiology and behavior and is probably the leading cause of cardiovascular problems associated with chronic stressor exposure.

Selye folded this idea of stress and stressors into a general theory of adaptation to environmental disturbance called the General Adaptation Theory consisting of three stages: Adaptation, resistance, exhaustion. Complete adrenal gland exhaustion is a rare event in nature, it only has been documented in salmon and marsupial mice males after breeding, both exhibiting semelparous breeding strategies (single breeding event followed by death). During stress the adrenal gland is activated by ACTH released from the pituitary gland, which in turn is controlled by a hormone called CRH secreted from the hypothalamus of the brain. These discoveries have led to a number of antistress drugs being developed and going through clinical trials, largely unsuccessfully. There are no medicines to treat stress in humans or wildlife. There is abundant evidence to support two main ways in which stress is initiated in the vertebrate brain. Psychological or anticipatory stressors (restraint, unpredictability) begin in the limbic system of the brain. Physical or reactive stressors (noxious gasses like ether, chloroform; hemorrhage; increased lactic acid) are detected outside the brain and this information relayed to the brainstem.

Comparative studies (there are no fossil hormones or adrenal glands) indicate that the basic endocrine, behavioral, and physiological response to stressors is hundreds of millions of years old, as the basic response pathways are seen in fishes, amphibians, reptiles, birds, and mammals. Elements of the stress response axis can be found in invertebrate animals. This observation argues against stress being a 'disease' only, i.e. why would the elements have lasted intact so long if only to reduce fitness? Current thinking is that stress in the short term is adaptive, helping animals cope with changes in the environment. Although there are over 131 hypotheses about stress in vertebrate animals (Harris 2020), there is only one type of stress clinically diagnosed in humans- PTSD. Allostasis and allostatic load are terms to describe the cumulative effects of stressors over the lifetimes of humans and wildlife (Seeman et al. 2001), and this concept is most often applied to studies on the effects of low socioeconomic status, pollutant exposures, etc (e.g., Morello-Frosch & Shenassa 2006).

Ecosystem Disturbance as Biological Stress: Disturbance is a relatively discrete event that removes organisms directly (mortality at organismal scale) or otherwise disrupts the community indirectly by influencing the availability of space, food, or other resources or by changing the physical environment (conditions). Disturbances can either increase (intermediate disturbance hypothesis: with infrequent and mild disturbance only a few species come to dominate the system, while frequent and intense disturbance allows only pioneer species or no life to exist [Connell 1978]) or decrease complexity of the ecosystem. Strength of disturbance can refer to frequency, intensity, extent, and duration. Disturbance leads to ecological succession, a non-seasonal, directional, and continuous pattern of colonization and extinction at a site by species leading to sequential change in community composition subsequent to disturbance. Pioneer species often facilitate the ability of other species to colonize the system through their altering of conditions and resources to match the niche requirements of additional species. Monitoring of community responses to disturbance in ecosystems occurs through measurement of species richness and abundance and calculation of diversity indices, as well as depictions of interactions through food webs.

Disturbance can lead to alternative stable states when the system passes a tipping point causing a regime shift. It is difficult if not impossible to return to the original state once a tipping point has been passed (i.e., hysteresis). Examples include desertification, algal blooms, shifts from coral to algal reefs, grass-dominated to tree-dominated savannas, and microbial gut communities after antibiotic treatment. Restoration ecology is the practical utilization of these concepts to avoid tipping points and counteract disturbance. How a community/ecosystem responds to disturbance, or its stability, depends on its resilience, the speed with which a community returns to its former state after being disturbed, and its robustness, the ability of a community to avoid displacement from its present state by a disturbance. Resilience can be thought of as the system's flexibility, such as how far a rubber band can be stretched without breaking, while robustness can be thought of as the strength of disturbance needed to initiate a regime shift, such as the thickness of a rubber band. There are four main hypotheses regarding community stability, all of which assume some form of an increase in species richness leads to increased stability of ecological function: MacArthur's (1955) complementarity (each species contributes equal but different functions), Lawton's (1994) idiosyncratic (effects depend on species interactions, with an overall trend of an increase in stability with more species, but with each additional species, there can be a loss of stability depending on how that particular species affects the system through its interactions with other species), Ehrlich and Ehrlich's (1981) redundancy (species share roles in a community and their functions overlap thus enhancing resilience if only some of each functional group are lost after disturbance), and Walker's (1992) drivers and passengers (some species play a more critical role to stability, such as keystone species, than others).

Experimentally Integrating Microbes & Ecosystems into a Network Framework: Although stress physiology and disturbance ecology are well developed disciplines with similar terminology and described processes, little integration has occurred between the two. Building off of these two fields, we will begin to develop our

universal theory of biological stress, which will then be extended to other scales. Utilization of network science and systems biology will become critical as we model effects of stress on parts and interactions of parts of a system and the consequence for functioning of that system across scales. Bacteria are an optimal tool for mesocosm stress studies as they can be easily manipulated. As a result, there are many studies of stress effects on bacteria that can inform study designs (Manzoni et al. 2012, Barkay, 1987, Evans and Wallenstein 2012, Wittebolle et al. 2009). Both small aquatic systems (Solé et al. 2008, Moore and Folt 1993, Feld and Hering 2007) and grasslands (Milchunas and Lauenroth 1995, Van den Brink and Braak 1999, Liancourt et al. 2005) can also be manipulated fairly easily and have previous stress studies. By approaching the scales of life each as a system of nodes and interactions leading to the emergent property of the above scale (e.g., cells interact to form tissues, individuals interact to form populations, species interact to form communities, etc.), we will be able to examine how stress first leads to a decrease in functionality and eventually can lead to a collapse of the system. Only through integrating the relationship between systems functioning and stress across scales will we be able to fully grasp how biological systems operate to maintain optimal functionality in the face of external changes.

Potential Impact: Development of a universal theory of biological stress will enable us to infer how living systems have responded to disturbances in the past, to explain how they will respond in the present, and predict how they may respond in the future. Bacterial communities can be used experimentally to then scale up to multicellular organisms since they utilize cellular signaling to transfer information in stressful environments. Studying how microbial communities, including synthetic microbes, could mimic more complex systems has the potential for providing insightful information as it relates to complex diseases, including cardiovascular disease. By synthesizing terminology, concepts, and theory from across scales of life, including disturbance ecology, stress physiology, and microbiology, we will be able to evaluate similarities and differences in how parts and systems of each level respond to stress and how these dynamics scale across life. Doing so will be a first step to predicting and engineering robust systems in the face of global change, especially if common tipping points can be determined. Results from microbial communities can scale to macro organism communities such as tropical forest ecosystems.

What essential information could this provide us with today?: Biologists in various sub-disciplines have developed 'local' understanding of how stress impacts their study 'living system'. Microbiologists study microbial stress responses in order to bioremediate, mitigate adverse effects of pollutants, or understand the impact of microbiomes on their hosts. Physiologists and endocrinologists study the health related effects of acute and chronic stress in wildlife and humans. Ecologists study how disturbance relates to the resilience and robustness of ecosystems. Paleobiologists study how disturbance may lead to global ecosystem turnover or passage through major evolutionary transitions like the development of complex multicellularity. However, tipping points have often escaped many of these studies and little communication occurs across scales. Developing a universal theory of stress will help us identify these tipping points and therefore those living systems most likely to cease functioning from extrinsic stressors, such as ecosystems affected by anthropogenic change, individuals suffering from cardiovascular disease, or microbes experimentally stressed in a laboratory setting. Evolutionary biologists and ecologists have recognized the power of using synthetic microbial communities and grasslands as 'natural' simulations to test rules of life, and here these communities will assist with identification of tipping points related to stress.

Barriers and Challenges: Different biological sub-disciplines have their own vocabulary to describe how a living system responds to a stressor or disturbance. Placing these different descriptions into a common conceptual framework will be a necessary first step toward achieving a universal theory of biological stress. Direct experimental manipulation would be the most straightforward way to test whether there are universal strains that stress puts on living systems. Time and cost constraints, however, make it impossible to observe how most organisms or ecosystems respond to experimental disturbances on timescales ranging

from the life span to geologic. Likewise experimental manipulation of macrobiota (for example, humans) is often impossible due to ethical concerns. It is conceivable that we can eventually engineer microbes to proxy the initial steps in the signal path of various stress responses. Many reactive 'stressors' initiate local inflammatory responses (noxious gasses, for example, Emmert and Herman, 1999) and we know a good deal about how inflammatory mediators trigger chemosensory neurons that play the initial role in the signal path (Manou-Stathopoulou et al., 2019). Similarly conceptual models explain how local changes in pH caused by increased lactic acid levels causes panic attacks and strain on animals (Esquivel et al., 1999). In order to make the synthetic microbial communities reflective of other 'living systems' (for example, organs, higher organisms, and ecosystems) it will be important to identify critical characteristics of those systems that can be mimicked accurately by creative assembly of the microbial communities as well as those characteristics that cannot. Defining these characteristics will also allow us to distinguish this approach from previous work on microbial mats/biofilms.

What are the state-of-the-art technologies and applications?: We will leverage developments in microbial biology and engineering to develop complex, multiscale microbial communities that mimic higher-level 'living systems'. For example, synthetic microbial communities can be constructed to mimic human organs or high complexity ecosystems and can be manipulated through stress or disturbance.

Broader Impacts: Through a universal theory of biological stress, we will be able to (1) build more resilient and robust coupled natural-human systems; (2) develop a predictive understanding of human health and disease; and (3) test how stress affected major transitions in the history of life (e.g., mass extinctions). Insight will inform our understanding of aging, mortality, and collapse of life, at both the level of parts and the integrated whole at each scale (e.g., cells and tissues, organs and individuals, species and ecosystems).

How does it reintegrate biology?: This vision reintegrates biology by utilizing and expanding theory, methodology, and data from multiple scales of biological sciences that study the effects of stressors or disturbances on living systems to evaluate if similar processes occur leading up to cessation of functioning of living systems regardless of the scale (e.g., cell death, organismal mortality, ecosystem collapse). Disturbance to a system causes biological changes that allow the system to express both resilient and robust properties. Some systems adapt to the acquired stress and thus genetic changes could be introduced to allow those adaptive properties to be displayed. We are proposing the use of microbial communities that could be used in multiple organismal scales including ecological systems as well as human health and disease models. Microbial communities may help us define global changes caused by stressors or disturbances and assist in cross comparing the stressor effect across different biological systems. By perturbing different systems, we see this approach as a means to help us understand the occurrence of stress and how it both negatively and positively impacts different biological systems. Stress at other levels will be examined as a team of experts is built, including the role of quorum sensing, oxidative stress, behavioral response and social interactions, and coupled natural-human systems. Scientists across multiple disciplines, including biology, psychology, anthropology, and environmental science, as well as professionals in human and veterinary medicine and environmental and wildlife management will be targeted audience for this paper given the central role of stress across these fields.

References Available Upon Request