SYMPOSIUM

Physiological Responses to Shifts in Multiple Environmental Stressors: Relevance in a Changing World

Anne E. Todgham[1] and Jonathon H. Stillman

Department of Biology and Romberg Tiburon Center for Environmental Studies, San Francisco State University, San Francisco, CA 94132, USA

From the symposium "Physiological Responses to Simultaneous Shifts in Multiple Environmental Stressors: Relevance in a Changing World" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2013 at San Francisco, California.

[1]E-mail: todgham@sfsu.edu

Synopsis Population response to global change will depend on responses to a multivariate set of changes in abiotic habitat characteristics and biotic interactions. Organismal biologists seeking to make ecological inferences about the impacts of global change by studying physiological performance have traditionally performed carefully controlled experimental studies that examine one variable at a time. Those studies, while of high value, may not lead to accurate predictions of organismal responses in the natural habitat, where organisms experience concomitant changes in multiple environmental factors. The symposium "Physiological Responses to Simultaneous Shifts in Multiple Environmental Stressors: Relevance in a Changing World" focused on physiological studies in which multiple environmental variables were simultaneously examined and brought together an international group of early-career and established speakers with unique perspectives on studies of multistressors. In doing so, the objective of the symposium was to frame the necessary next steps for increasing predictive capacity of organismal responses to environmental shifts in the natural habitat, establish novel collaborations among researchers actively investigating physiological responses to a multivariate environment, and broaden the number of researchers conducting such studies.

Introduction: Why we need to consider multiple stressors in physiological research to improve predictions of organismal responses to global change

Organisms are living in habitats that are increasingly impacted by anthropogenic activities that alter the global climate, shift ecological interactions by invasive species, and increase pollution. Understanding the impacts of global change on the distribution and abundance of organisms in the context of the natural environment is at the forefront of integrative organismal biology. Environmental physiologists and physiological ecologists interested in understanding how the distribution of organisms will be impacted by biotic and abiotic changes in habitats have long used carefully controlled laboratory experiments that manipulate a single environmental variable (e.g., temperature) to determine some aspect of organismal performance, for example, temperature sensitivity (Q_{10}) or tolerance maxima (CT_{max}) (Stillman 2003; Hoffmann 2010; Hofmann and Todgham 2010; Somero 2012). Those parameters of performance are subsequently used to predict where/when shifts will occur (Deutsch et al. 2008). Similarly, ecologists who have documented shifts in the distributions of organisms have used physiological data to attribute shifts in range to changes in the biotic or abiotic environment (Harley et al. 2006; Helmuth 2009). The majority of studies conducted by physiologists examine one environmental variable at a time; yet in nature, organisms are simultaneously exposed to multiple environmental variables, including stressors that may affect different physiological systems. Whether those multiple shifts in environmental variables have an additive, an antagonistic, or a nonlinear synergistic effect on performance (Fig. 1) is critical for properly linking ecological shifts in range to changes in environmental
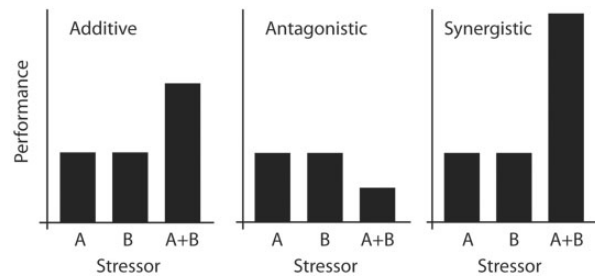
Fig. 1. Conceptual framework for understanding possible interactive effects of two stressors on physiological performance. Multiple stressors can influence performance independently (additive), or interact to either reduce (antagonistic) or enhance (synergistic) performance in a nonlinear, unpredictable fashion.

stressors (Crain et al. 2008; Darling and Côté 2008; Holmstrup et al. 2010). If effects are additive, then small shifts in multiple stressors can still have a small effect on performance, whereas if effects are synergistic, then small shifts in multiple stressors could have a great impact on physiological performance and cause unpredictable responses in terms of species' distributions and abundances. The complex, often nonlinear, interactions observed in studies of multiple stressors suggest that investigations of single stressors have the potential to produce misleading inferences about physiological responses in a multivariate natural environment (see McBryan et al. 2013, this issue; Whitehead 2013, this issue).

Shifting the mindset of physiologists to consider whether experiments that focus on single environmental variables are appropriate for understanding responses to global change in the natural, multivariate, environment is an important challenge. Comparative physiologists went through a similar shift in their thoughts about inferring adaptive significance following the paper by Garland and Adolph (1994) on how two-species comparisons must be replaced by phylogenetic methods in comparative physiology. Future environmental physiological studies performed in the context of a multivariate set of environmental changes will likely produce outcomes that increase precision of the predicted ecological consequences of global change.

Major challenges in integrative organismal physiology that can be addressed in multistressor studies

Improving predictive capabilities through a better understanding of the mechanistic underpinnings of organismal responses to multiple stressors

An important challenge for developing a predictive understanding of how organisms respond to multiple

stressors is to describe the mechanisms by which a first stressor modulates physiological responses to a second stressor. At the organismal level, a first stressor may either elevate tolerance to a second stressor (cross-tolerance) or cause the organisms to be more susceptible to the second stressor (cross-susceptibility). For example, in tidepool sculpins, *Oligocottus maculosus*, a preliminary $+12^\circ$C heat shock increased a fish's tolerance to hypoxic and hyperosmotic challenges (cross-tolerance), whereas a slightly higher preliminary $+15^\circ$C heat shock significantly decreased tolerance to a subsequent severe stressor (cross-susceptibility) (Todgham et al. 2005). Cross-tolerance to heat and salinity has also been demonstrated in long-term studies of *Daphnia pulex*, in which populations exposed to low thermal variability have low tolerance to heat and hyperosmotic conditions, but when thermal variability increases, tolerance both to heat and salinity increases through cross-tolerance (Chen and Stillman 2012). Similarly, populations exposed to elevated salinity increased their tolerances to salinity and heat, but only when long-term acclimation was at lower temperatures. At elevated temperatures, increased variation in salinity decreased tolerances to heat and salinity, an example of cross-susceptibility (Chen and Stillman 2012). Predicting when multiple stressors may result in cross-tolerance or cross-susceptibility, and understanding the adaptive capacity of organisms to respond to future multifactorial environmental change, could be aided by a better understanding of the mechanistic bases driving the responses to multistressors (Sinclair et al. 2013, this issue; Verberk et al. 2013, this issue). At the cellular level, multiple pathways, each stimulated by a separate stressor, may converge on one physiological function, or each stressor may modulate the same pathway, producing distinct physiological outcomes. Those alternatives, termed "cross-talk" and "cross-tolerance," are discussed by Sinclair et al. (2013, this issue) in the context of insects' responses to cold and desiccation and their regulation of immune function, and by Verberk et al. (2013, this issue) in the context of insects' responses to hypoxia and heat stress. Both cross-talk and cross-tolerance may be adaptive, but with differing consequences for responding to future global change, due to their mechanisms of action.

Another important challenge for developing predictive models of how multiple stressors in a changing climate will impact the distribution and abundance of organisms is an understanding of the bioenergetic costs of environmental stress and downstream consequences for fitness. Dependent on the magnitude and duration of stress, organisms must

divert variable amounts of available energy away from growth and reproduction to cellular defense and maintenance of homeostasis. Sokolova (2013, this issue) reviews the concept of energy-limited tolerance to stress and outlines the development of bioenergetic markers of aerobic scope that can be used to integrate the effects of multiple stressors and distinguish between combinations that are sustainable from the perspective of energy allocation and those that are unsustainable and would ultimately lead to long-term population decline.

Examining important co-variates that can influence how responses to multiple stressors are interpreted

Mechanistically, the manner in which multiple phenotypes respond to multiple stressors could vary dramatically within a population depending on the plasticity of the pathways involved. For example, if there are epigenetic and/or posttranslational modifications that alter the relative proportions and/or nature of one or more components of pathways, that could alter whether multiple stressors are interactive, as well as the nature of any interaction. Such modifications may be apparent through correlations among distinct performance traits. Calosi et al. (2013, this issue) demonstrate correlations between metabolic rate and either Na^+/K^+ ATPase activity or heat tolerance in individual amphipods that had been exposed to elevated CO_2, to elevated temperature, or to elevations both of CO_2 and temperature, suggesting that responses to multiple stressors do vary across individuals. Responses to multiple stressors may also be modulated by important co-variates, although Klok and Harrison (2013, this issue) found that none of the canonical environmental characteristics (e.g., temperature, elevation) helped explain the temperature-size rule (TSR) in a wide range of insects. However, insects' body size was an important co-variate, as the TSR was more strongly pronounced in small-bodied taxa. Understanding the biological and/or environmental parameters that may alter responses to defined sets of multiple stressors is an important component of predicting capacity.

Identifying unifying themes in physiological responses to multistressors

Much of our understanding of the molecular, biochemical, and physiological mechanisms that organisms have available to respond to changes in environmental conditions is grounded in the fundamental principle that animals use a diversity of responses or solutions to a common set of problems, that is, unity in diversity (Somero 2000).

Comparative physiology has been extremely successful in identifying the processes that are conserved among species in response to a single stressor (e.g., conservation of membrane fluidity across temperatures), and this has lead to an appreciation of the key aspects of an organism's environment that limit physiological processes. Much of the same approach is needed as we begin to identify unifying themes in physiological responses to multistressors (Kroeker et al. 2013). Meta-analyses of the individual effects of temperature (Sunday et al. 2012) and ocean acidification (Kroeker et al. 2010) have been extremely useful in identifying species and ecosystems that are vulnerable to global change. Byrne and Przeslawski (2013, this issue) present a review of the combined impacts of elevated pCO_2 and temperature on early life-history stages of invertebrates. Their analysis demonstrates that in 16 of the 23 species studied so far, warming and acidification of oceans had interactive effects on organisms' responses. Additive negative effects were the most common with some antagonistic interactions where warming reduced the negative impact of ocean acidification. Interestingly, no synergistic interactions were found between these two stressors in invertebrates. Furthermore, by comparing across life-history stages, Bryne and Przeslawski (2013, this issue) provide evidence that fertilization and early embryonic development of echinoderms are more robust in their capacity to tolerate elevated pCO_2 and temperature when compared to echinoderm larvae and juveniles.

Can we infer interactive effects based on the study of single stressors?

Multiple stressors are inherently difficult (or, potentially, impossible) to study, as a well-replicated study has an exponentially increasing number of units with each added stressor examined. Whether interactive effects of multiple stressors can be inferred from logistically feasible studies of single stressors is addressed by Whitehead (2013, this issue) and Schulte and coworkers (McBryan et al. 2013, this issue) through examination of changes in response to a second environmental factor following acclimation to a first environmental factor. For example, in killifish acclimated to a particular temperature, but then exposed to acute changes in temperature and oxygen, there was a strong negative relationship between hypoxia tolerance and increasing temperature such that even within the optimal temperature range for the fish, additional hypoxia in the environment would likely impact the fish profoundly (McBryan et al. 2013, this issue). Importantly, the effects of

temperature on tolerance to hypoxia were greater than would have been predicted from Q_{10} alone, suggesting that these stressors act synergistically and hence we cannot make inferences about them from studies of single stressors (McBryan et al. 2013, this issue). Whitehead (2013, this issue) similarly demonstrates that a suite of naturally encountered stressors that are typical of oil spills (e.g., salinity, hypoxia, and pathogens) exacerbate the direct toxic effects of the oil's chemical components. In these examples, synergistic effects of multiple stressors highlight the importance of multifactorial studies, and the potential for inaccurate inferences to be drawn from results of studies of single stressors placed in a multiple-stressor context.

Importance of characterizing the multivariate environment.

Our ability to predict how organisms will respond to changes in environmental conditions is rooted in an understanding of which aspects of environmental variation matter to the animal and therefore those attributes to which the organism is responding either successfully (distribution should remain unchanged) or unsuccessfully (distribution will ultimately be impacted). An essential question for future studies of multistressors is: How well does the natural habitat need to be characterized, over temporal and spatial scales, in order to properly predict responses to a complex environment? As a research community, we need to define the "environment" in terms of variation in environmental complexity at the scale of the local population over which single or multi-stressor studies should be conducted to make the best predictive inferences. This type of approach has been extremely informative in understanding the thermal heterogeneity and stochasticity in the intertidal zone and in assessing those aspects of temperature are likely important in setting the limits to species' distributions and abundances in a highly variable environment (Helmuth et al. 2002; Denny et al. 2009; Denny and Dowd 2012). Buckley et al. (2013, this issue) investigated multiple stressors across elevations and their research highlights the problems with predicting thermal stress based solely on air temperatures along elevational and latitudinal gradients. Much in the same way that biophysical models have identified which aspects of heat transfer predominantly affect body temperature in intertidal organisms (e.g., rock temperature in limpets) (Denny and Harley 2006), Buckley et al. (2013, this issue) demonstrate the importance of considering humidity and solar radiation, in addition to air temperature,

to more accurately predict body temperatures of grasshoppers.

How is the choice of organisms relevant or important for understanding complex issues in biology?

Studies of multiple stressors are extremely important for making accurately informed policy decisions as organisms live in complex, dynamic, and multivariate environments. Equally important is the choice of experimental organism(s) that either can target organisms of conservation concern (e.g., endangered delta smelt) (Hasenbein et al. 2013, this issue), provide insight into the immediate impacts of a severe and acute environmental perturbation (e.g., oil spill) (Whitehead 2013, this issue), or provide a more comprehensive picture of impacts to biodiversity through meta-analyses (e.g., Byrne and Przeslawski 2013, this issue) or macrophysiological approaches (Gaston et al. 2009). Applying physiological tools to elucidate root causes of conservation problems is not new; however, there is a renewed appreciation of the importance of integrating physiological knowledge into ecological models to more accurately predict the impacts of global environmental change on contemporary organisms (Gaston et al. 2009; Cooke et al. 2013). Fangue and coworkers (Hasenbein et al. 2013, this issue) illustrate a conservation-physiology approach to understanding the multiple abiotic factors that influence organismal performance of the endangered delta smelt in hopes of providing accurate decision managers with information on optimal habitat conditions that must be preserved for this species' persistence.

Concluding remarks

The papers presented in this symposium highlight the importance of considering multiple environmental factors to improve predictions of how organisms are likely to respond to global environmental change. Improvements in our mechanistic understanding of how multiple environmental factors operate to influence physiological performance could lead to a better understanding of the cellular pathways involved, and yield insight into the basic, but complex, manner in which physiological systems interact. Studies of multistressors are challenging to conduct, and sometimes are difficult to interpret, but because of nonlinear synergistic or antagonistic interactions between or among stressors, they are necessary in order to best understand how organismal physiology is likely to respond to future environments.

Acknowledgments

Funding

References

Buckley LB, Miller EF, Kingsolver JG. 2013. Thermal stress and specialization across altitude and latitude. Integr Comp Biol published online (doi:10.1093/icb/ict026).

Byrne M, Przeslawski R. 2013. Impacts of ocean warming and acidification on benthic marine invertebrates, a multistressor perspective. Integr Comp Biol published online (doi:10.1093/icb/ict049).

Calosi P, Turner LM, Hawkins M, Bertolini C, Nightingale G, Truebano-Garcia M, Spicer JI. 2013. Multiple physiological responses to multiple environmental challenges: an individual approach. Integr Comp Biol published online (doi:10.1093/icb/ict041).

Chen X, Stillman JH. 2012. Multigenerational analysis of temperature and salinity variability effects on metabolic rate, generation time, and acute thermal and salinity tolerance in *Daphnia pulex*. J Therm Biol 37:185–94.

Cooke SJ, Sack L, Franklin CE, Farrell AP, Beardall J, Wikelski M, Chown SL. 2013. What is conservation physiology? Perspectives of an increasingly integrated and essential science. Conserv Physiol 1:1–23.

Crain CM, Kroeker K, Halpern BS. 2008. Interactive and cumulative effects of multiple human stressors on marine systems. Ecol Lett 11:1304–15.

Darling ES, Côté IM. 2008. Quantifying the evidence for ecological synergies. Ecol Lett 11:1278–86.

Denny MW, Harley CDG. 2006. Hot limpets: predicting body temperature in a conductance-mediated thermal system. J Exp Biol 209:2409–19.

Denny MW, Hunt LJH, Miller LP, Harley CDG. 2009. On the prediction of extreme ecological events. Ecol Monogr 79:397–421.

Denny MW, Dowd WW. 2012. Biophysics, environmental stochasticity, and evolution of thermal safety margins in intertidal limpets. J Exp Biol 215:934–47.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc Natl Acad Sci USA 105:6668–72.

Garland T Jr, Adolph SC. 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. Physiol Zool 67:797–828.

Gaston KJ, Chown SL, Calosi P, Bernardo J, Bilton DT, Clarke A, Clusella-Trullas S, Ghalambor CK, Konarzewski M, Peck LS, et al. 2009. Macrophysiology: a conceptual reunification. Am Nat 174:595–612.

Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL. 2006. The impacts of climate change in coastal marine systems. Ecol Lett 9:228–41.

Hasenbein M, Komoroske LM, Connon RE, Geist J, Fangue NA. 2013. Turbidity and salinity affect feeding performance and physiological stress in the endangered delta smelt. Integr Comp Biol published online (doi: 10.1093/icb/ict082).

Helmuth B. 2009. From cells to coastlines: how can we use physiology to forecast the impacts of climate change? J Exp Biol 212:753–60.

Helmuth B, Harley CDG, Halpin PM, O'Donnell M, Hofmann GE, Blanchette CA. 2002. Climate change and latitudinal patterns of intertidal thermal stress. Science 298:1015–7.

Hoffmann AA. 2010. Physiological climatic limits in *Drosophila*: patterns and implications. J Exp Biol 213:870–80.

Hofmann GE, Todgham AE. 2010. Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Annu Rev Physiol 72:127–45.

Holmstrup M, Bindesbøl A-M, Oostingh GJ, Duschl A, Scheil V, Köhler H-R, Loureiro S, Soares AMVM, Ferreira ALG, Kienle C, et al. 2010. Interactions between effects of environmental chemicals and natural stressors: a review. Sci Total Environ 408:3746–62.

Klok CJ, Harrison JF. 2013. The temperature size rule in arthropods: independent of macro-environmental variables but size-dependent. Integr Comp Biol published online (doi:10.1093/icb/ict075).

Kroeker KJ, Kordas RL, Crim R, Singh GS. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol Lett 13:1419–34.

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duartes CM, Gattuso J-P. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biol 19:1884–96.

McBryan TL, Anttila K, Healy TM, Schulte PM. 2013. Evolution of tolerance to multiple interacting stressors in fish. Integr Comp Biol published online (doi: 10.1093/icb/ict066).

Sinclair BJ, Ferguson LV, Salehipoushirazi G, MacMillan HA. 2013. Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. Integr Comp Biol published online (doi:10.1093/icb/ict004).

Sokolova IM. 2013. Energy-limited stress tolerance as a conceptual framework to integrate the effects of multiple

stressors. Integr Comp Biol published online (doi:10.1093/icb/ict028).

Stillman JH. 2003. Acclimation capacity underlies susceptibility to climate change. Science 301:65.

Somero GN. 2000. Unity in diversity: a perspective on the methods, contributions, and future of comparative physiology. Annu Rev Physiol 62:927–37.

Somero GN. 2012. The physiology of global change: linking patterns to mechanisms. Annu Rev Mar Sci 4:39–61.

Sunday JM, Bates AE, Dulvy NK. 2012. Thermal tolerance and the global redistribution of animals. Nat Clim Change 2:686–90.

Todgham AE, Schulte PM, Iwama GK. 2005. Cross-tolerance in the tidepool sculpin: the role of heat shock proteins. Physiol Biochem Zool 78:133–44.

Verberk WCEP, Sommer U, Davidson RL, Viant MR. 2013. Anaerobic metabolism at thermal extremes: a metabolomic test of the oxygen limitation hypothesis in an aquatic insect. Integr Comp Biol published online (doi:10.1093/icb/ict015).

Whitehead A. 2013. Interactions between oil-spill pollutants and natural stressors can compound ecotoxicological effects. Integr Comp Biol published online (doi: 10.1093/icb/ict080).