Vol. 543: 273–281, 2016
doi: 10.3354/meps11595

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published February 3

AS WE SEE IT

Rethinking our approach to multiple stressor studies in marine environments

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ABSTRACT: Marine systems experience an unprecedented number of stresses caused by humans. Over the last 25 yr an increasing amount of attention has been given to examining the combined impacts of multiple stressors. Yet, existing studies reveal few patterns that facilitate predicting or understanding when multiple stressors should combine additively, synergistically, or antagonistically. One contributing factor to this lack of clarity may be the lack of a common framework that is based on a mechanistic understanding of stressor impacts. We adapt and advocate a general framework that is employed by the US EPA in terrestrial systems for use in marine systems. This framework involves 3 steps: (1) Mechanistically examine the impacts of multiple stressors on individual organisms. (2) Scale these impacts on individual organisms to population level responses to multiple stressors. (3) Examine context-dependent changes in stressor responses due to changes in community or ecosystem properties. We also argue that 3 specific aspects of previous studies hamper our ability to detect patterns in multiple stressor impact. First, a large number of studies have reported impacts on growth, survival, etc., without elucidating mechanisms. Second, the majority of studies provide insufficient data to determine whether threshold or nonlinear responses to stressors occur. Third, 32% of existing studies transformed data to meet model assumptions, but in so doing, they unknowingly altered the statistical model being tested. We argue that rectifying these 3 conditions will accelerate the detection of patterns in the way that multiple stressors combine to influence marine systems.

KEY WORDS: Anthropogenic stressors · Experimental design · Human impacts · Mechanistic · Phenomenological · Prediction

INTRODUCTION

Ecological systems today face numerous human-induced stressors including, but not limited to, overexploitation of natural resources, habitat destruction, pollution, invasive species, and climate change. The magnitude of many of these stressors in any given area generally increases with the density of human populations, which have grown at an accelerating rate through time (Cohen 2003). However, increases in human populations have not been uniformly distributed geographically; rather, populations have increased disproportionately in coastal regions (Small & Nicholls 2003). Consequently, human-induced stressors are also intense in coastal regions (Lotze et al. 2006), particularly those coastal regions where human population density is highest (Halpern et al. 2008).

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Publisher: Inter-Research · www.int-res.com

These various environmental stressors commonly do not act in isolation, but instead often act together. When they act together, individual stressors may combine additively, or their combined impacts may be greater or less than the sum of their individual impacts (i.e. they may combine synergistically or antagonistically). Crain et al. (2008) reviewed 171 experiments from 92 published studies of multiple stressors in marine systems conducted through 2007. Their review included a range of stressors that are common in coastal areas, including changes in salinity, sedimentation, nutrients, toxins, fishing pressure, sea level rise, temperature, CO_2 (i.e. acidification), UV radiation, invasive species, disease, hypoxia, and disturbance. Studies that were reviewed generally combined 2, and in some cases 3, of these stressors in orthogonal experiments. Crain et al. (2008) concluded that there were no ubiquitous patterns in the way that multiple stressors combined. Rather, published studies provide examples of additive, synergistic, and antagonistic effects of nearly all examined combinations of these stressors. This lack of observable trends limits our ability to extrapolate from existing studies in order to predict the outcome of combined effects of multiple stressors under novel conditions.

As stated by Crain et al. (2008), the reason for this lack of observable trends or ability to predict outcomes is largely because the impacts of multiple stressors may vary with numerous factors. For instance, stressors from the list above may act in any combination, with resulting cumulative impacts depending on the strength of each individual stressor. In addition, individual species may respond differently to stressors, causing potentially novel outcomes for different focal study species. Even within a single species, the response to stressors may be context dependent, changing with different physical environmental conditions. The response of focal species to stressors may also vary depending on the biological context, such as with different assemblages or communities of interacting species. Finally, the response to stressors may also vary with experimental conditions, such as the magnitude or frequency of the stressor presented and the duration of the experiment. Given the dependence of multiple stressors on this list of intrinsic and extrinsic factors, it is not surprising that patterns in the way that organisms respond to the combination of multiple stressors have been difficult to detect.

Several other reviews of multiple stressors in marine environments have been conducted in recent years, though these have focused more narrowly on specific areas or groups of organisms (coral reefs: Ban et al. 2014; algae on rocky coastlines: Strain et al. 2014) or on specific stressors (warming and acidification: Byrne & Przeslawski 2013, Kroeker et al. 2013). These more focused reviews have detected patterns that provide a clearer picture of the way that organisms and systems respond to multiple stressors than the more general review by Crain et al. (2008), but there is still considerable variation even when focusing on specific systems.

Despite the difficulty of detecting general patterns in the way that multiple stressors combine, the effort to accomplish this goal remains an important objective among scientists working in marine systems. Rudd (2014) surveyed 2179 scientists from 94 countries and asked them to rank the most important outstanding research questions in terms of their potential importance for informing decisions regarding ocean governance and sustainability. The cumulative impact of multiple stressors was identified as the single most important research question in marine systems. However, given the near infinite number of combinations of stressors, focal species, species community combinations, and environmental conditions, combined with the limited resources for marine research available, scientists are unlikely to be able to examine all possible situations. Consequently, approaches other than experimentation, such as expert opinion (Halpern et al. 2007), have been proposed. However, experimentally examining stressor impacts remains a common and robust approach.

We argue that one reasonable means to accelerate progress in understanding the combined impacts of multiple stressors in marine systems is to develop a single, clear framework that guides and structures our collective research efforts. The absence of such a framework results in widely varying approaches that yield different types of information that are difficult to combine and compare. Here we outline a potential framework for future research on multiple stressors in marine systems that, if followed, would facilitate greater comparison across studies, would enhance the use of studies within a predictive framework, and would therefore accelerate progress in this field beyond what it has been to date.

SUGGESTED RESEARCH FRAMEWORK FOR STUDYING MULTIPLE STRESSOR IMPACTS

There is certainly a need for more research on the combined effects of multiple stressors, but this research should be geared towards achieving the ability to predict the impacts of multiple stressors under novel situations and conditions that have not yet been studied experimentally. To achieve this goal, researchers in marine systems could adopt a similar strategy to one that has been established by the United States Environmental Protection Agency for examining the impacts of multiple stressors on terrestrial wildlife populations. This strategy involves 3 major research objectives that begin with a mechanistic understanding at the individual level and then scale up to higher levels of biological organization and spatial and temporal extents (US EPA 2005, Munns 2006). This strategy can be generalized as:

(1) Mechanistically understanding effects of single and multiple stressors at the individual level across different species (i.e. why an individual of one species may respond differently than an individual of another species).

(2) Scale up to population-level responses to stressors based on responses at the individual level.

(3) Evaluate the relative risks for populations within communities and across ecosystems.

This strategy therefore begins with a mechanistic understanding at the individual level and then builds to greater levels of complexity (Fig. 1). This proposed approach is not new. It is the same approach proposed for terrestrial systems, and it is not necessarily different from the approach that has been taken by some researchers in the study of multiple stressors in marine systems (see below, this section). However, it is our hope that by clearly establishing this framework for marine systems, more individual studies and more research programs will take this approach and that this will accelerate progress towards the goal of understanding and predicting responses of marine organisms to multiple stressors. Each component of this 3-tiered approach requires specific types of information to be successful.

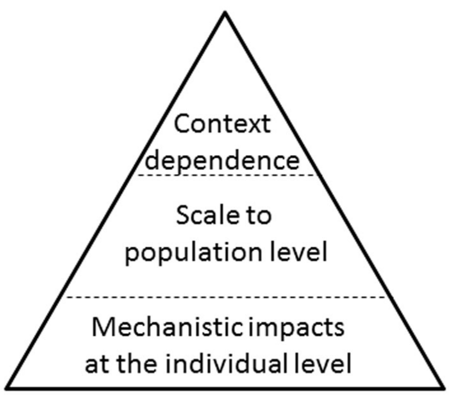

Fig. 1. Suggested 3-tiered approach for examining combined impacts of multiple stressors in marine systems. Effective research builds up from the bottom of the triangle. Modified from an approach developed by the US EPA for terrestrial systems (Munns 2006)

The first component of this proposed approach requires focusing on the mechanisms of individual stressor impacts. Several excellent mechanistic approaches are possible. For instance, previous studies have examined the genetic basis of stressor impacts via stressor-induced changes in gene expression (e.g. Dangre et al. 2010, Fleming & Di Giulio 2011). These studies both demonstrated that hypoxia modified the expression of genes normally expressed in the metabolism of toxic compounds. Other successful approaches have mechanistically examined physiological (e.g. Enzor & Place 2014), or behavioral responses to stressors (e.g. Queirós et al. 2015). The key to these approaches is to provide sufficient mechanistic detail at the individual level to understand why the response has occurred and how it could potentially differ if examined in a different species (Segner et al. 2014). The benefit of this approach can be seen by examining a study by Cabrerizo et al. (2014) that compared the combined impacts of UV radiation, nutrient concentration, and temperature on 4 species of microalgae. They showed that as levels of the 3 stressors simultaneously increased, 2 of the species (a dinoflagellate and a chlorophyte) decreased photosynthesis and repair rates, while the other 2 species (a diatom and a haptophyte) increased photosynthesis and repair rates and decreased respiration rates. Contrasting the different responses across species to increased stressor levels allowed these researchers to predict that diatoms and haptophytes are likely to become competitively dominant under continued environmental change due to their favorable responses. This example highlights both the benefit of mechanistically examining the response to multiple stressors and the benefit of contrasting this response across different species.

The second component is scaling from small-scale impacts measured under controlled conditions on single individuals, to impacts on entire populations of the focal species. In practice, this scaling-up can most effectively be done using ecological models. Models based on a mechanistic understanding, known as process-based models (or mechanistic models), may be most suitable, as these are better than other modeling approaches, such as statistical extrapolation, for predicting ecological responses and processes under changing environmental conditions (Cuddington et al. 2013).

Successfully accomplishing this component requires 3 pieces of information in addition to the mechanistic understanding of individual stressor impacts identified under component one above. First, it requires understanding how the mechanistic impacts of stressors specifically influence vital rates that are important to population dynamics. For instance, this requires linking mechanistic changes in behavior, physiology, or gene expression to eventual consequences for growth, reproduction, and survival. Fortunately, these end results are often examined more simply than stressor mechanisms and are in fact the response variables that have commonly been measured to date. Second, scaling to the population level requires an understanding of the variation in stressor exposure across individuals within a population, for instance, along an estuarine gradient, with distance from shore, or with distance or time from some point source of pollution. And third, it requires the derivation of mathematical functional relationships between stressor intensity and mechanistic response that can be used to parameterize the process-based models. If individual responses change nonlinearly with stressor intensity, then simply using average values of effects size for scaling up will provide spurious results due to Jensen's inequality (Ruel & Ayres 1999). This means that, when responses scale nonlinearly with stressor intensity, it is not possible to extrapolate across a range of stressor intensities from effect sizes that have been calculated, for example, from an ANOVA that has compared some organismal response in the presence and absence of a stressor. Determining the presence of nonlinear or threshold responses requires experiments that are conducted using several stressor intensities over the range of values that may be experienced by individuals of a population. These thresholds and functional relationships can then be used to parameterize process-based models that can then be used to scale from the individual to the population level.

While most of the multiple stressor studies to date have examined only 2 (present/absent) or at best 3 (high, low, absent) stressor levels, studies that examine a greater number of stressor levels indicate that nonlinear responses may be common. For instance, Peachey (2005) examined the impacts of 4 levels of crude oil extracts on the survival of larval crabs from several species and found nonlinear impacts that were species-dependent. Mortality of some species increased asymptotically with oil concentration, while others appeared to have a threshold response. In each case, the shape of the response to oil concentration only became apparent in the presence of UV radiation stress.

The tools and methods for extending individual impacts of stressors to the population level are still developing. One common tool that has developed rapidly is the implementation of the process-based modeling approach within individual-based simulation models that are ideal for incorporating both spatial dynamics and individual variation (Grimm & Martin 2013). Such models can easily incorporate intraspecific interactions and density-dependent processes. A recent special issue of *Ecological Modelling* highlights recent progress in extending individual impacts to populations, primarily using individual-based modeling from an ecotoxicology perspective (Grimm & Thorbeck 2014). While these studies focus on single stressors, they highlight the advances that have been made in using mechanisms of stressor impacts at the individual level to scale up to population responses.

Other approaches besides process-based modeling have also been developed for scaling up impacts of multiple stressors from the individual to the population level. For instance, King et al. (2015) present methods for assessing population impacts of noises and other stressors on marine mammals. Their approach was based on using expert opinion to parameterize models when empirical data is sparse. While this may be the best available option in many cases, scaling up using process-based models is generally a more robust approach and so is preferable when the necessary data are available for model development (Cuddington et al. 2013).

The third component of the 3-tiered approach that we advocate is to place the population response to multiple stressors within the broader ecological context by examining the response embedded within a community and across ecosystems. This component amounts to examining the context-dependency of multiple stressor impacts. Kroeker et al. (2013) in their review of studies examining the combined impacts of warming and acidification, highlight the context-dependency of organismal responses, which varied with nutritional status, source population of study organisms, etc. Further, this review found that variability in species' responses to multiple stressors was enhanced when species were in multispecies assemblages rather than examined independently, suggesting that community interactions are important in determining individual species responses to stress. The specific contexts that should be examined will therefore vary across stressors and systems and may be better decided based on what is pertinent rather than on any set of prescribed criteria.

Multiple examples of feasible approaches exist for examining multiple stressors in a community or ecosystem context. For instance, Breitburg et al. (1999) examined the combined effects of nutrient addition and a mixture of trace elements (arsenic, copper, cadmium, zinc, and nickel) across 5 different experimental levels of increasing community complexity by sequentially including (across treatments) phytoplankton, zooplankton, fish, sediment, and benthic invertebrates. They measured biomass at each level of the community and also mechanistically examined changes in bottom up community dynamics by measuring phytoplankton and bacterial production at regular intervals throughout the experiment. Similarly, Martínez-Crego et al. (2014) studied the combined impacts of nutrient and CO_2 addition at multiple levels of organization within a mesocosm, including at the individual (biochemical impacts), population (changes in production, reproduction and/or abundance), community (species interactions and global metabolism), and ecosystem (detritus release and carbon sink) levels. Finally, Jordan et al. (2008) provide an alternative theoretical approach. They examined the impacts of multiple stressors (loss of submerged aquatic vegetation and shoreline hardening) on blue crab populations via its impact on recruitment. They then extended this to the ecosystem level using data on the distribution of habitat types throughout Mobile Bay, AL, USA, allowing them to determine the total blue crab recruitment from this system. This recruitment projection was then fed into a Gulf of Mexico fishery model to project how the Gulf of Mexico blue crab fishery was impacted by altered recruitment due to changes in submerged aquatic vegetation and shoreline hardening in the Mobile Bay system.

Thus, there are numerous successful approaches that have and can be applied to address each level of this 3-tiered framework. However, the breadth of this recommended framework may be more amenable to development of a research program examining the impacts of multiple stressors, as opposed to inclusion of all of these aspects within individual studies. Yet, a review of the existing literature suggests that, regardless of which level of this general framework a study focuses on, there are specific things that we can do to improve the usefulness of individual studies in advancing the field. Below we provide 3 specific recommendations that should be followed to accelerate progress of this field.

(1) Focus on mechanisms

Ideally, multiple stressor studies should uncover the mechanism underlying stressor impacts on focal organisms. A mechanistic understanding is necessary because it provides sufficient understanding of cause and effect at the individual level to increase predictive power (Helmuth et al. 2005, Kearney & Porter 2009). As such it offers the ability to extend results beyond the specific studied conditions to different focal species, different community contexts, or different environmental conditions. Stressor impacts may act via a broad range of mechanisms, and it has been argued that a common metric is needed to integrate the impacts of multiple stressors (Segner et al. 2014). Some mechanisms may be particularly useful in terms of their ability to integrate the impacts of disparate types of stressors. For instance, individual energetic state and energy-limited tolerance has been proposed as a unifying mechanism that likely responds to a wide range of stressors and that could therefore be used to integrate impacts of multiple stressors using a common metric (Sokolova 2013). The explanatory power of this mechanism is likely high because of the strong association between energetics and physiological condition from the cellular to the organismal level (Sokolova et al. 2012) and the importance of this association for growth, reproduction, and survival rates that form the link between individual performance and population dynamics.

A large portion of empirical multiple stressor studies to date have not been based on a mechanistic understanding of stressor impacts. We revisited the 92 studies identified by Crain et al. (2008) and also identified 51 additional studies published since that review. For each of these 143 studies, we assessed whether the study was phenomenological or mechanistic (Table S1 in the Supplement at www.int-res.com/larticles/suppl/m543p273_supp.pdf). Crain et al. (2008) assessed each individual experiment separately, yielding 171 experiments in the 92 published studies. In contrast, we assessed each published study holistically, rather than separately assessing multiple experiments that were published together in the same paper. We examined studies holistically because we were interested in determining whether studies as a whole were mechanistic, rather than whether each individual experiment examined mechanisms. We defined a phenomenological study as one that measured the effect(s) of multiple stressors in terms of their impacts on the abundance, survival, growth rate, biomass, or other characteristic of a focal organism, without explicitly examining the underlying cause or pathway of that effect. We defined a mechanistic study as one that not only measured the effect(s) of multiple stressors on a focal organism but that also attempted to identify, via the collection of additional data, the physiological, behavioral, or ecological pathway by which that effect was incurred. A simple example may help to clarify and distinguish these 2 categories. If a study examined the combined influence of increased temperature and decreased pH on coral growth by simply measuring growth under different experimental conditions, this study was classified as phenomenological. If the same study had additionally examined changes in the rate of photosynthesis by zooxanthellae under the different experimental conditions as a means of understanding changes in coral growth, this study was classified as mechanistic. Additionally, studies that did not explicitly measure mechanisms, but that tested *a priori* hypotheses that were based on known mechanistic understanding of the system, were also classified as mechanistic.

This simple exercise clearly showed that the rate of publication over the last 25 yr has increased for both phenomenological and mechanistic studies of multiple stressors. However, the publication of mechanistic studies has increased at only approximately 65% the rate that phenomenological studies have increased over the long term (Fig. 2), and the increase in mechanistic studies appears to have been even slower than this in the time since the review published by Crain et al. (2008). These studies that document the impacts of multiple stressors without examining the mechanisms are a necessary precursor to the framework presented here. These phenomenological studies have laid the foundation by raising awareness of the combined impacts of multiple stressors. Future studies should, where possible, focus more directly on the mechanisms of stressor impacts.

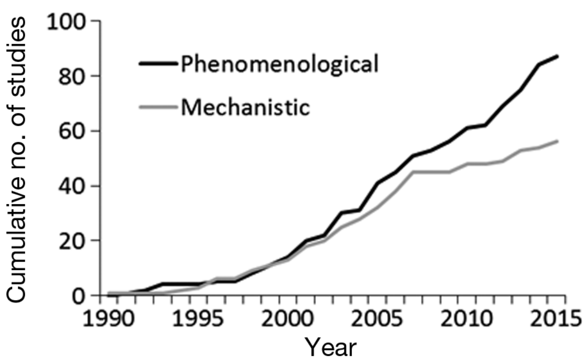

Fig. 2. Cumulative number of studies through time that experimentally examined multiple stressors using either a phenomenological or mechanistic approach

(2) Conduct studies across a range of stressor levels

A key requirement for extrapolating experimental results to novel conditions that lie beyond the specific study conditions that have been empirically examined is determining the functional form of stressor impacts across a range of levels. Of particular importance are any nonlinear or threshold responses to stressor intensity. This is necessary because stressor intensity may change over space or time for the population of interest. As part of the review described in the preceding section, we also assessed the number of stressor levels experimentally presented in each multiple stressor study published to date to gauge whether studies collected sufficient data to determine whether the response to stressors scaled nonlinearly with stressor intensity. The majority of studies examined stressors across just 2 or 3 levels of each stressor (Table 1), yielding insufficient data to determine whether responses to stressors scaled nonlinearly with stressor intensity. Examining numerous levels of each stressor becomes increasingly more difficult in orthogonal experimental designs, particularly when the number of stressors examined exceeds 2. A feasible approach may be to examine stressors individually across a broad range of stressor intensities to determine the form of the functional relationship between stressor and response, followed by combinations of multiple stressors across a more restricted range of stressor levels to explore interactions between stressors. This approach enables researchers to use the functional relationships detected for individual stressors to strategically choose

Table 1. Number of studies conducted with a given number of experimental levels for the 2 stressors combined. Controls without stressors were considered as treatments, so the presence/absence of a stressor was considered as 2 levels, while experiments with ‘high’, ‘low’, and ‘none’ were considered to have 3 levels. Several studies also examined a third factor. Three of these included 3 levels of the third factor, while each of the others included 2 levels of the third factor

		Number of levels of Stressor 2						
		2	3	4	5	6	7	8
Number of levels of Stressor 1	2	67						
	3	31	15			1		
	4	5	5			1		
	5	4	1	4	1			
	6	1				1		
	7	1	1					
	8	1	1					

stressor levels employed in multiple stressor trials (e.g. to encompass a known threshold). This suggested approach certainly increases the workload relative to the standard 2 × 2 factorial experimental design that is commonly employed (presence/absence of each stressor), but this may be necessary in order to make substantial progress in understanding multiple stressor impacts.

(3) Understand what statistical model is being tested

Ultimately, ecologists would like to make comparisons across different types of stressors and how these influence different taxonomic or functional groups of organisms. Making these comparisons based on published studies has been hampered thus far by statistical practices that have unknowingly used different null models. A common experimental approach to studying multiple stressor impacts is to orthogonally present multiple stressors using 4 treatments: Stressor 1 only, Stressor 2 only, Stressors 1 and 2 combined, and a no-stressor control. This type of experiment is then commonly analyzed using a 2-way ANOVA, where a significant interaction term indicates departure from additivity in the impacts of the 2 stressors. In order to use this approach, experimental data must conform to the assumptions of ANOVA, and data that do not conform to these assumptions (particularly the assumption of homogeneous variances) are often log-transformed to help meet this statistical assumption.

However, log-transformation of the data alters the underlying model being tested. A 2-way ANOVA performed on nontransformed data tests the assumption that 2 stressors combine additively. However, because of the properties of logarithms ($\log ab = \log a + \log b$), the same ANOVA performed on log-transformed data tests the assumption that 2 stressors combine multiplicatively (Sih et al. 1998). This distinction has been recognized for decades in a branch of ecology that examines the combined impacts of multiple predator species on shared prey (Soluk & Collins 1988) and was thoroughly described by McArdle & Anderson (2004) but was only recently recognized in the plant enemy literature (Stephens et al. 2013) and has gone largely unnoticed in the multiple stressor literature. A single multiple stressor study by Folt et al. (1999) acknowledges that stressors may combine additively or multiplicatively and accounts for this in its analysis, and this distinction was noted in the review by Crain et al. (2008). However, neither of these papers acknowledges that the transition from one model to the other is produced through data transformation. In fact, Crain et al. (2008, p. 1305) explicitly state that they focus their review on the additive model 'since it underlies ANOVA models used in factorial experimental studies'.

Based on information provided by authors in each of the 143 marine multiple stressor studies published to date, we determined that fully 1/3 of these studies log-transformed their data to meet assumptions of ANOVA without acknowledging the impact this had on the model tested (Table S1). Rather, each of the studies that made this transformation interpreted results of their analyses as if it were the additive model being tested. The proportion of published studies making this error (32%) has remained constant before and after the review by Crain et al. (2008). Thus, while Crain et al. (2008) intended to focus exclusively on comparing additive effects of multiple stressors in their review, 1/3 of the studies they examined unknowingly tested multiplicative effects, and this failure to recognize the statistical model being tested may have contributed to the inability of that review to detect any consistent trends in the combined impacts of multiple stressors.

The additive model produces predicted impacts of the stressors that are systematically biased relative to the multiplicative model, as the additive model always predicts higher combined stressor impacts, and the discrepancy between the 2 models increases with the magnitude of the impacts of the 2 stressors. Interpreting statistical results therefore requires understanding which model is being tested. If data are analyzed using a multiplicative model due to the log transformation, and then this analysis is interpreted as if it had been a test of the additive model, this can lead to spurious conclusions. In fact, if the impacts of both stressors are large when presented alone, it is entirely possible to conclude that stressors combine antagonistically based on expectations of the additive model, but that they combine synergistically based on expectations of the multiplicative model. The analysis of transformed and non-transformed data, often within the same study, without recognizing the implications of the transformation for the model being tested, may reduce our chances of detecting general patterns in the way that multiple stressors combine.

There are instances where a multiplicative model is entirely appropriate from an ecological or biological perspective. Imagine a study examining the impacts of 2 stressors on the biomass of some focal organism. Assume that Stressors 1 and 2 when presented alone reduce organismal biomass relative to the control treatment by 60 and 70%, respectively. Then the interaction term in an ANOVA based on the additive model tests for whether Stressors 1 and 2 together reduce biomass by 130%, a nonsensical prediction. Using the multiplicative model in this case accounts for the fact that the exact same impact cannot be created twice in the same individual. Thus, using the multiplicative model we would predict that Stressors 1 and 2 together reduce biomass by 88%.

Understanding which model is being tested is crucial for interpreting results appropriately and for making comparisons across results of different studies. Ultimately, the choice of whether to test the additive or the multiplicative model should be based not on statistical convenience or convention but on which type of model makes biological and ecological sense for the system under study.

CONCLUSIONS

Coastal marine systems today are experiencing a wide range of human-induced stressors that often occur in conjunction. Determining the combined impacts of multiple environmental stressors on marine systems is one of the most important challenges facing marine scientists today. Research to date has clearly demonstrated that multiple stressors often act in concert and that they can have additive, synergistic, or antagonistic impacts. However, given the rapid rate of environmental change, the nearly limitless number of novel situations involving multiple stressors, and the limited time and resources available to pursue research in this field, the next challenge is to progress beyond simply documenting multiple stressor impacts under a limited set of conditions, and to focus instead on developing the capacity to predict the combined impacts of multiple stressors under novel conditions that have not yet been explicitly measured. Towards this goal, we have advocated a general research framework that, if followed, has the potential to accelerate and streamline progress in this field. Individual studies can best contribute to this framework by conducting studies that (1) elucidate the mechanism(s) underlying stressor impacts and how those mechanisms differ across species, (2) examine a range of stressor levels enabling detection of nonlinear or threshold responses across the range of stressor intensities encountered by study organisms under field conditions, and (3) recognizing the underlying statistical model being tested and how data manipulation influences these models.

Acknowledgements. The authors have no conflicts of interest. This work was supported by NSF grant no. OCE-1129166.

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Editorial responsibility: Tim McClanahan, Mombasa, Kenya

Submitted: May 27, 2015; Accepted: December 24, 2015
Proofs received from author(s): January 27, 2016