

LETTER

Interactive and cumulative effects of multiple human stressors in marine systems

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Abstract

Humans impact natural systems in a multitude of ways, yet the cumulative effect of multiple stressors on ecological communities remains largely unknown. Here we synthesized 171 studies that manipulated two or more stressors in marine and coastal systems and found that cumulative effects in individual studies were additive (26%), synergistic (36%), and antagonistic (38%). The overall interaction effect across all studies was synergistic, but interaction type varied by response level (community: antagonistic, population: synergistic), trophic level (autotrophs: antagonistic, heterotrophs: synergistic), and specific stressor pair (seven pairs additive, three pairs each synergistic and antagonistic). Addition of a third stressor changed interaction effects significantly in two-thirds of all cases and doubled the number of synergistic interactions. Given that most studies were performed in laboratories where stressor effects can be carefully isolated, these three-stressor results suggest that synergies may be quite common in nature where more than two stressors almost always coexist. While significant gaps exist in multiple stressor research, our results suggest an immediate need to account for stressor interactions in ecological studies and conservation planning.

Keywords

Antagonisms, cumulative stressors, cumulative threat models, ecosystem-based management, interactions, synergisms, threat-analysis.

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INTRODUCTION

With growing human population comes an increase in the diversity and intensity of anthropogenic stressors, i.e. environmental and biotic factors that exceed their natural ranges of variation due to human activities (Sanderson *et al.* 2002; Halpern *et al.* 2007a). Historically, habitat loss and over-exploitation of key species were the main human impacts on landscapes (Jackson *et al.* 2001). In the last century, this list has grown to include pollution (in particular excess nitrogen), invasive species, and more recently climate change (Wilcove *et al.* 1998; Sala *et al.* 2000; Dulvy *et al.* 2003; Kappel 2005; Dudgeon *et al.* 2006; Venter *et al.* 2006), among many other stressors. While ecological research has begun to document the individual effects of these various stressors on species and ecosystems, research into the cumulative and interactive impacts of multiple stressors is less frequent. Yet natural systems are almost always simultaneously subjected to multiple human-derived

stressors (Breitburg *et al.* 1998; Venter *et al.* 2006; Halpern *et al.* 2007a, 2008b).

The need to better understand the interactive and cumulative effects of multiple stressors was highlighted a decade ago (Breitburg *et al.* 1999) and is still cited as one of the most pressing questions in ecology and conservation (e.g. Sala *et al.* 2000; Zeidberg & Robison 2007). In both theoretical and applied research, the effect of multiple stressors is often assumed to be the additive accumulation of impacts associated with single stressors (Bryant *et al.* 1998; Sanderson *et al.* 2002; Halpern *et al.* 2007a, 2008b; Ban & Alder 2008). However, numerous empirical and correlational studies have documented that multiple stressors can exacerbate negative impacts to systems when acting in concert, such as when increased UV radiation greatly increases the negative effects of a toxin (reviewed by Pelletier *et al.* 2006), raising concern that synergisms may be common in nature. In contrast, other studies show that the effect of multiple stressors in concert can be less than

expected based on their individual effects, potentially even mitigating stressor impacts, such as when nutrient enrichment dampens the negative effect of a second stressor, such as toxins or UV (Breitburg *et al.* 1998; Wulff *et al.* 2000). Given the diversity of stressor pairs that can potentially co-occur across a broad range of ecosystems, it remains difficult to draw general conclusions about the frequency with which various interactions types occur in nature or the cumulative effect of multiple stressors.

Conceptually, there are three broad categories of interaction types describing the outcome of multiple stressors (derived from Folt *et al.* 1999), but the components vary depending on the direction of individual (single stressor) effects (Fig. 1a–c). For the most common case where each stressor has a negative effect when applied individually, e.g. stressor A reduces the response by 'a' and stressor B by 'b', then the cumulative effect under A + B conditions is a reduction of the response from control levels that is additive ($= a + b$), antagonistic ($< a + b$) or synergistic ($> a + b$; Fig. 1a). Folt *et al.* (1999) describe two variations of this interaction model when cumulative effects are the product of individual stress effects (multiplicative model) or when a

single dominant stressor drives the cumulative outcome (comparative model). Here we focus on the additive model described above since it underlies ANOVA models used in factorial experimental studies and is the null hypothesis behind multiple stressor applications (i.e. mapping efforts such as Halpern *et al.* 2008b).

Predicting cumulative stressor effects is challenging due to various potential higher order interactions (HOI sensu Billick & Case 1994). First, the stressors themselves can interact so that impacts change in the presence of additional stressors, for instance if chemical properties of a toxin shift at different temperatures. Second, species' response to a stressor may be context dependent and thus modified by additional stressors, for example, when a species response to invasion varies under different nutrient regimes. Third, species may respond similarly or differently to sets of stressors due to evolutionarily or ecologically derived tolerances, such that the estimated interaction strength in a system depends on which species are present and which are measured (Vinebrooke *et al.* 2004). Finally, community response to stressors can differ due to changing interactions between component species under different stressor sce-

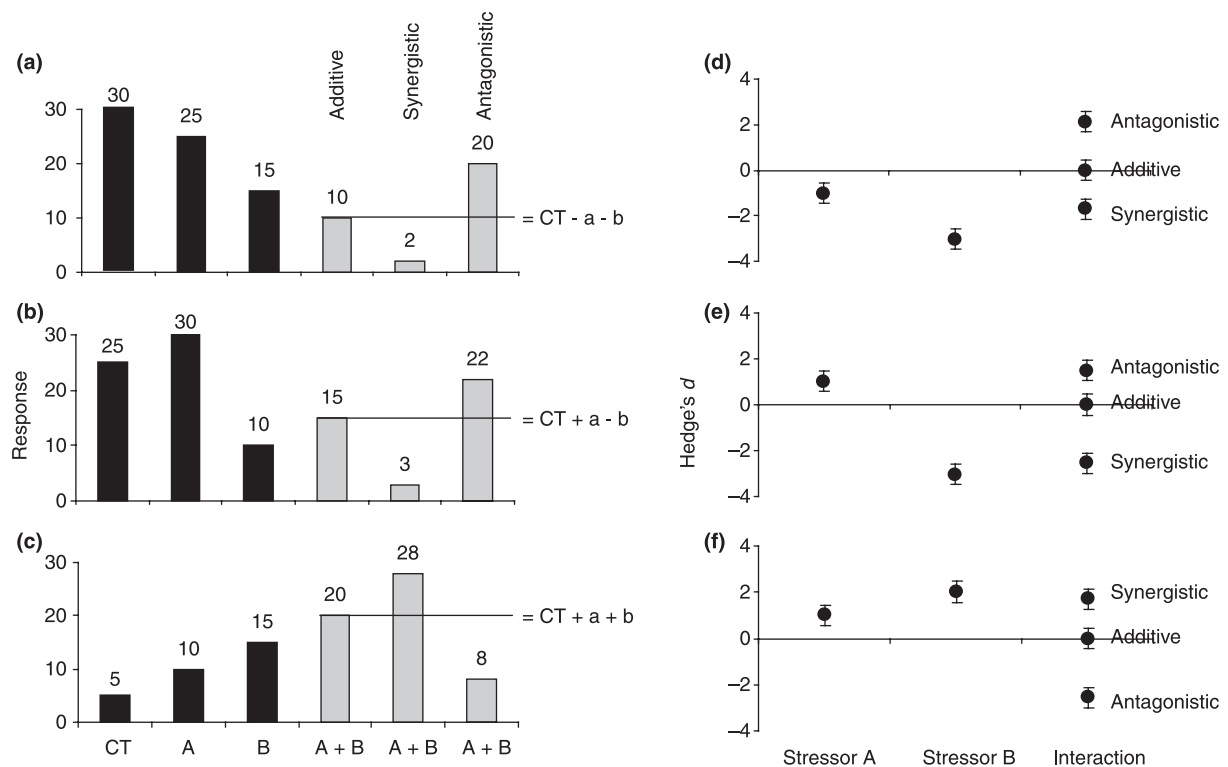


Figure 1 Conceptual approach to interpreting interaction types from population or community response data presented in factorial studies (a–c) and corresponding individual and interactive effect sizes measured with Hedge's *d* (d–f) before combining across studies using meta-analysis. Treatments in factorial studies include control (CT), with stressor A (A), with stressor B (B), and with both stressors (A + B). Interaction types (additive, synergistic and antagonistic) vary depending on A + B response and are illustrated here for stressors that have double negative (a and d), opposing (b and e), and double positive (c and f) main effects on the response variable of interest.

narios, for instance when species switch from competitive to facilitative interactions under different salinity regimes (e.g. Crain 2008). Predicting community or ecosystem response to multiple stressors is additionally complicated by HOI among the component species themselves and factors such as species diversity (and associated issues of redundancy and resilience), openness of a system that can influence dynamics of disturbance recovery, and environmental stochasticity (Breitburg *et al.* 1998). Temporal patterns of stressor occurrence (simultaneous vs. consecutive, frequency of stressor occurrence, etc.), the intensity of the stressor (especially for nonlinear responses), and which response variable is measured (e.g. Relyea & Hoverman 2006) also influence our estimates of cumulative stressor effects. Understanding the dynamics of multiple stressor interactions can thus be dauntingly complex, but enough studies of interactions between and among multiple stressors now exist to allow for some predictive understanding of when and where various interaction types are expected to occur.

Here we synthesize findings from multiple stressor studies to expose general patterns in cumulative stressor effects. We address three core questions: (i) what is the average interaction strength across studies and how does it vary by key ecological and methodological variables, (ii) does the frequency of interaction types shift due to context-dependency, and (iii) which pairs of stressors have been evaluated empirically and, in contrast, where do key research gaps exist for multiple stressors. We focus on the body of research that addresses multiple stressors in marine ecosystems because marine systems are affected by multiple human threats, particularly coastal ecosystems that sit at the interface of land and sea and so are subject to the consequences of human activities in both realms (Stoms

et al. 2005; Tallis *et al.* 2008). In addition, the growing interest and application of ecosystem-based approaches to marine resource management has made cumulative impacts from multiple stressors a focal topic in marine conservation and management (Rosenberg & McLeod 2005; Leslie & McLeod 2007). Thus the opportunity exists for our synthetic findings to feed directly into management efforts.

METHODS

Database development

We searched the literature using Web of Science for factorial experimental studies in marine systems (any intertidal, nearshore or offshore marine or estuarine habitats) that manipulated two or more of thirteen pre-identified stressors and measured the direct (rather than indirect) impact of the stressors (see Fig. 2 and Table S1). As in Breitburg *et al.* (1999), we defined stressor in a broad sense as any environmental or biotic factor that exceeds natural levels of variation. The stressor list was derived from a previous list of 20 human threats to the marine environment identified by Halpern *et al.* (2007a), but modified to reflect the stressors associated with human activities that are amenable to experimentation (e.g. freshwater increase/decrease was replaced with 'salinity'). Stressors generally caused a negative effect on the response of interest, however, several stressors (nutrients, UV, CO₂) can have a positive effect on given populations; all response types were included in our analyses. Search terms included the stressor itself or other bi-products of the stressor that could be specifically manipulated in experiments and clearly related to the stressor (e.g. sea level rise and flooding depth). Further

	Salinity	Sediment	Nutrients	Toxins	Fishing	SLR	Temp	CO ₂	UV	Invasives	Disease	Hypoxia	Disturbance	Total by stressor
Salinity	0	9	9	0	16	13	5	5	0	1	0	0	0	58
Sedimentation		5	0	0	0	2	1	0	1	0	0	0	0	9
Nutrients			17	3	7	8	2	5	0	2	0	0	0	56
Toxins				0	0	7	0	40	0	0	1	0	0	54
Fishing					0	1	0	0	0	0	0	0	0	4
SLR						0	1	0	0	0	0	0	3	27
Temperature							5	18	0	5	2	1	0	62
CO ₂								3	0	1	0	0	0	18
UV									0	0	0	0	0	71
Invasives										0	0	0	0	1
Disease											0	0	0	12
Hypoxia												0	0	3
Disturbance													0	7
Total studies = 202														

Figure 2 Stressor matrix listing the number of independent factorial experimental studies found meeting the criteria listed in the text for each stressor pair. This list includes studies that did not report error and were therefore not included in further analyses calculating Hedge's *d*. **Fishing × nutrients pair has been reviewed and examined elsewhere (see Borer *et al.* 2006) and was therefore not included in our analysis.

searches in cited literature or by polling experts were conducted to identify missing literature. Thirteen total stressors were investigated; giving 78 potential stressor pairs (see Fig. 2).

Some multiple stressor research has focused on a combination of direct and indirect impacts (e.g. algal response to nutrients and fishing, via trophic cascades). These studies were excluded from our database for two reasons: (i) there is a need to first understand interactions among direct effects before addressing indirect effects, and (ii) the individual impact (positive or negative) of indirect interactions will vary depending on subsequent biotic interactions within the community, so that the interaction type is not easily classified (Fig. 1a–c). For example, the indirect impact of fishing stress on primary producers can occur via an increase or decrease in grazing pressure depending on whether the fished species is itself a predator or grazer. Experimental manipulations that remove all grazing pressure do not clearly mimic fishing stress as removing top predators likely increases and decreases populations of various herbivores. This criterion of measuring direct effects eliminated from our analyses most (but not all) studies that included fishing pressure as a stressor. In a few cases (e.g. Miller & Hay 1996) large-mesh exclusion cages were used that mimic direct fishing pressure by excluding only large herbivorous fish (but also urchins), in places where these herbivores are subject to a fishery and so these studies were included in our analyses. The full set of trophic cascade and grazer-nutrient studies have been synthesized elsewhere (Micheli 1999; Borer *et al.* 2006; Burkepile & Hay 2006; Gruner *et al.* 2008) and provide important comparative results for our current analyses.

Studies were only included when multiple stressors were manipulated in a factorial manner with an appropriate control within a single experimental design. Data were mined from the published studies on each response type in four treatments: control, with stressor A, with stressor B, and with stressor A + B. This criterion excluded a number of studies that examined the effect of a stressor in the presence and absence of a second stressor, but did not have a control treatment where neither stressor was applied (e.g. disease severity at different temperatures without a non-diseased control). In addition, numerous studies looked at the effect of one stressor on a second stressor (nutrients on invasive species) but did not measure a common response in the absence of both stressors and were therefore excluded. To be included, studies had to test stressors of a magnitude deemed by the authors to be in a range that could induce 'stress' in the response variables. Therefore, studies examining responses across a normal range of temperature and salinity were not included since it was unclear which level was the stressor treatment. Studies that did not report error were counted in the total number of studies (202), but could

not be included in calculations of Hedge's d and associated analyses (171 total studies).

We included studies that measured community or population response variables. Community-level metrics included species richness, total biomass, and whole-system productivity [production, respiration, P : R ratio, or quantum yield (F_v/F_m)], and population-level metrics included biomass, abundance, survivorship, growth rate, disease severity (inverse) and species-level productivity (photosynthetic rate, respiration rate, P : R ratio, quantum yield or metabolic rate). When multiple response types were provided for a single study we used the most inclusive response variable (community over population and biomass or abundance over rates). In cases where more than two stressors were manipulated, responses to each stressor pair of interest were extracted at ambient levels of the third stressor. In cases where multiple levels of a factor were tested, we examined responses at the ambient and greatest level of the stressor (before complete mortality, across treatments) as the core analysis of interest. Similarly, if responses were recorded over time, we used the final time, unless complete mortality for numerous treatment groups obscured relative effects, in which case we used data from the time period where at least three treatment groups still persisted.

Effect size calculations

We calculated individual, main, and interaction effect sizes for each study using Hedge's d according to the methods for factorial meta-analysis (Gurevitch *et al.* 2000). Individual and main effects differ in that individual effects represent the response in the presence of a stressor alone vs. the control, while main effects compare the net effect of a stressor in the presence and absence of a second stressor, similar to main effect tests in ANOVA. Individual effects were used to classify studies into one of the three potential categories of studies to allow for proper identification of interaction type (Fig. 1a vs. b vs. c). For each study with the four treatment groups – control (CT), plus stressor A (A), plus stressor B (B), and with both stressors (A + B) – the main effect of stressor A (d_A), stressor B (d_B) and their interaction (d_I) were calculated as:

$$d_A = [(Y_A + Y_{AB}) - (Y_B + Y_{CT})/2s] J(m)$$

$$d_B = [(Y_B + Y_{AB}) - (Y_A + Y_{CT})/2s] J(m)$$

$$d_I = [(Y_{AB} - Y_B) - (Y_A - Y_{CT})/2s] J(m)$$

where Y is the main performance of the group indicated by the subscript, s the pooled standard deviation, and $J(m)$ is a correction term for small sample bias (Gurevitch *et al.* 2000). Individual effects are depicted with a lowercase subscript and were calculated as: $d_a = [(Y_A - Y_{CT})/s] J(m)$ and $d_b = [(Y_B - Y_{CT})/s] J(m)$.

Hedge's d is constructed similar to ANOVA where a significant interaction effect signifies deviation from the null model of additivity (Gurevitch *et al.* 2000). The alternative metric, log response ratio (LnRR), uses a multiplicative model for calculating interactions based on proportional changes (Hawkes & Sullivan 2001; Morris *et al.* 2007). However, because most multiple threat models and individual studies based on ANOVA assume additivity, our hypothesis was to specifically test how often multistressor effects deviate from additive so we used Hedge's d for our analyses. Additionally, the same data may be classified differently depending on the underlying model, with an additive model assigning more antagonisms and multiplicative model assigning more synergisms (Sih *et al.* 1998; Folt *et al.* 1999). Thus the additive model is more conservative in terms of quantifying the negative effect of multiple stressors.

Frequency of interaction types

Considering the direction of individual effects, we used the interaction effect size and 95% confidence interval (as in Fig. 1d–f) to classify each study as synergistic, antagonistic or additive (see Table S1). Additive interactions were ones whose 95% confidence intervals overlapped zero. For stressor pairs whose individual effects were either both negative or one negative and one positive, interaction effect sizes less than zero were synergistic and greater than zero antagonistic. Following from the equations above, a synergism occurs when the cumulative effect of both stressors reduces a response more than the sum of the individual stressor effects so that the term $(Y_{AB} - Y_B)$ is greater than $(Y_A - Y_{CT})$ leading to a negative interaction effect. In cases where individual effects were both positive, interactions are interpreted in the opposite manner (> 0 is synergistic and < 0 antagonistic). Using these criteria, interaction effects were characterized for each study in the database. The frequency distribution of interaction types was calculated for the entire database and for various subsets including response level (community vs. population level studies), trophic level (autotrophs vs. heterotrophs), and each stressor pair. We tested if distributions differed due to response and trophic levels using chi-square.

Meta-analysis

Meta-analyses were performed across all studies in the database and for stressor pairs that had five or more studies with error estimates reported. Responses were combined across studies using the methods of Gurevitch *et al.* (2000) for fixed-effects factorial meta-analysis using Microsoft excel. Main (stressor A pooled across levels of stressor B, and vice versa) and interaction effects described above were combined across studies using weighted Hedge's d to

calculate overall effects. Because studies with two positive individual effects had interaction terms opposite from the majority of studies with negative individual effects, the interaction effect sizes for these studies were inverted for inclusion in the analyses. While low sample sizes within stressor pairs limited the power of our pair-wise meta-analyses, we did not find that pairs with more replicate studies always had significant interactions while those with few studies were additive (null hypothesis), so we picked a minimum of five replicate studies as a reasonable cutoff for testing hypotheses despite limited data.

In cases where sufficient studies were available, we conducted meta-analysis on subsets of the total data sets that could potentially inform what drives variation in multiple stressor effects. For the entire database, we conducted meta-analysis on subdivisions based on trophic level (autotroph vs. heterotroph) and response level (population vs. community). Within stressor pairs, we tested the effect of trophic level on interaction effects for three stressor pairs (temperature \times UV, toxin \times UV and nutrients \times toxin) with sufficient data. Given the large number of studies that evaluated the combination of UV and toxins, we reran the overall meta-analysis with these studies removed to test for potential biases driven by this particular stressor pair. We also conducted the meta-analysis separately for studies within each of the three types of experiments (lab, mesocosm, and field) to test for potential methodological biases.

Context dependency

To test if stressor interactions vary depending on context, we compared stressor pair interactions that were manipulated at various levels of a third factor, generally a third stressor. Within our database we found 48 studies testing three-way interactions among stressors and we recorded pair-wise data (control, stressor A, stressor B, stressors A + B) at ambient and elevated levels of a third stressor. For each study, we calculated Hedge's d_i and 95% CI at both levels of the third factor and asked whether interaction effect sizes were significantly different (non-overlapping CI) or were classified as different interaction types at different levels of the third stressor.

RESULTS

Database development

We found a total of 202 studies that met our criteria for factorial experimental studies of multiple stressors, but only 171 had error estimates and were included in the analysis of interaction effects. Of the total studies, the majority of experiments were conducted in the lab (148) or mesocosms

(35), with very few in the field (19). The studies were heavily weighted towards single species (157) vs. community-level (45) experiments.

Of the 78 pairs of stressors in our database, we found no factorial experimental studies meeting our criteria for 46 pairs of stressors (Fig. 2). A further 19 pairs had fewer than five studies (with errors reported), leaving only 13 pairs for which meta-analysis could be conducted and only five pairs with $N > 10$.

Frequency of interaction types

In the complete database, cumulative effects of any two stressors were distributed among all interaction types with 26% additive, 36% synergistic and 38% antagonistic. Within stressor pairs, interactions were also distributed among types, particularly as the number of replicate studies increased (Fig. 3). All pairs with over five replicate studies had every interaction type represented. Other subgroups of the total database had equally diverse and similar distributions of interaction types. Frequencies of additive, synergistic and antagonistic interactions were, respectively, 26, 29

and 45% for autotrophs, 25, 42 and 33% for heterotrophs, 27, 37 and 36% for studies that measured population-level responses, and 23, 30 and 47% for studies that measured community-level responses, with neither comparison significantly different (trophic level chi-square = 1.47, $P = 0.48$; response level chi-square = 0.71, $P = 0.73$).

Meta-analysis

Meta-analysis across all studies revealed a significant synergistic overall interaction effect (Fig. 4). Results were nearly identical with the UV \times toxin studies removed (all studies: $d = -0.18 \pm 0.12$, $n = 169$; UV \times toxin removed: $d = -0.16 \pm 0.13$, $n = 136$), but varied by experiment type, with lab studies producing synergies ($d = -0.33 \pm 0.14$, $n = 122$), field studies being additive, but with a trend towards synergies ($d = -0.11 \pm 0.46$, $n = 15$), and mesocosm studies producing antagonistic interactions ($d = 0.28 \pm 0.27$, $n = 32$). When the database was subdivided by groups, the overall interaction effect was antagonistic for autotrophs and community level studies and synergistic for heterotrophs and population level studies (Fig. 4).

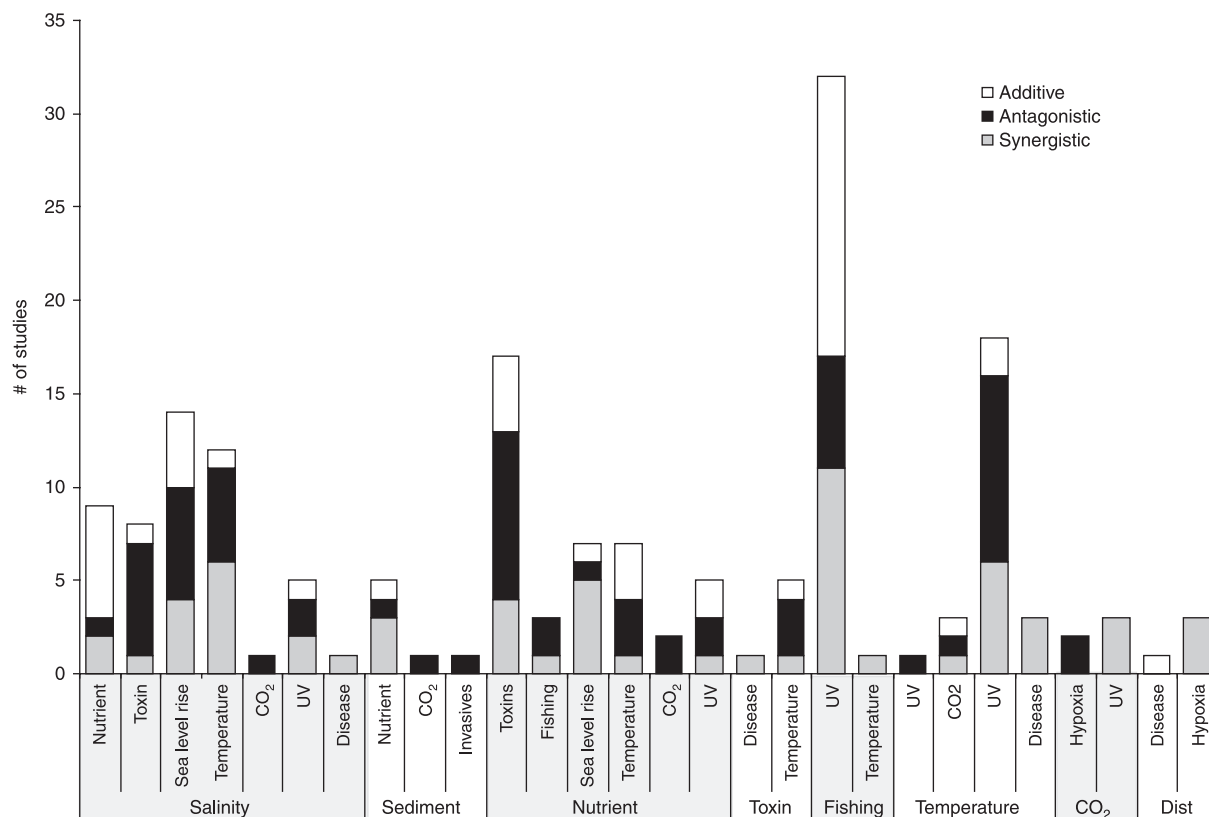


Figure 3 Frequency distribution of interaction types (synergistic, antagonistic and additive) across stressor pairs. Stressor pairs are indicated within blocks on the x-axis that list one stressor horizontally (e.g. salinity) with all stressor combinations listed vertically (e.g. nutrients).

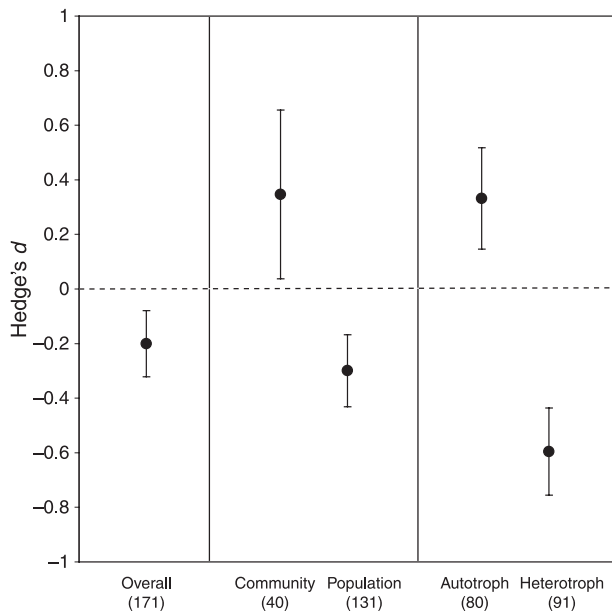


Figure 4 Interaction effect sizes (Hedge's d) and 95% CI for the complete database (overall) and subsets based on response level (community vs. population) and trophic level (autotroph vs. heterotroph). The number of studies included in each analysis is noted in parentheses.

For the 13 stressor pairs with enough replicate studies to allow for pair-specific meta-analysis, seven had additive, three antagonistic and three synergistic overall cumulative effects (Fig. 5). Three stressor pairs had sufficient sample size to allow for analysis of differential responses between trophic levels. Interaction effect switched significantly from antagonistic for autotrophs to synergistic for heterotrophs in the temperature \times UV ($d \pm 95\%CI = A: 1.47 \pm 0.81$, H: -1.21 ± 0.29) and toxin \times UV (A: 0.838 ± 0.800 , H: -1.32 ± 0.523) pairs and was not significantly different in the nutrients \times toxin pair.

Context dependency

When interaction effect sizes from individual studies ($N = 48$) were compared at different levels of a third factor, interactions changed significantly in a majority of cases. Pair-wise interactions at two levels of a third factor had significantly different interaction effect sizes in 67% of cases and changed classification type (e.g. from additive to synergistic) in 64% of pairs, the majority of which (75%) shifted to a more negative (antagonistic \rightarrow additive \rightarrow synergistic) interaction type. Synergisms occurred in 33% of two stressor studies at ambient levels and 66% of studies at increased levels of a third stressor.

DISCUSSION

The meta-analysis across all studies revealed a significant overall synergistic interaction effect (Fig. 4), indicating that cumulative effects of multiple stressors will often be worse than expected based on single stressor impacts. Previous meta-analyses of a single stressor pair (nutrient enrichment and reduced grazing pressure) in marine systems also found synergistic interactions (Burkepile & Hay 2006; Gruner *et al.* 2008). In contrast, Borer *et al.* (2006) did not find a significant interaction between predator removal (e.g. fishing) and nutrient addition, although this analysis averaged across ecosystem types, which have been shown to respond differently to multiple stressors (Shurin *et al.* 2002, Gruner *et al.* 2008), and differs from our analysis here in that they measured interaction effects between direct and indirect stressors. Together, these studies provide robust evidence that multiple stressors generally interact synergistically in marine systems. Past studies documenting synergisms (Hughes & Connell 1999; Jackson 2001; Przeslawski *et al.* 2005) have raised concern over these examples of 'ecological surprises' (*sensu* Paine *et al.* 1998), which can be devastating where they occur. Nevertheless, all three interaction types were commonly found among individual studies (see Fig. 3), suggesting that while synergisms may dominate overall effects, outcomes will vary in specific scenarios. Covariates and context dependency play a major role in driving multiple stressor effects, and exploring these drivers can help better predict how specific stressors interact.

Not surprisingly, stressor identity influences interaction effects. For the stressor pairs with enough replicate studies to allow for pair-specific meta-analysis, the majority had additive cumulative effects, but almost as many had antagonistic or synergistic cumulative effects (Fig. 5). These results differ substantially from the overall effect and shows that on average many specific stressor pairs are additive, lending support to cumulative models that assume additivity of stressor effects (Bryant *et al.* 1998; Sanderson *et al.* 2002; Ban & Alder 2008; Halpern *et al.* 2008a), but also indicating that certain stressor pairs have overall interactive effects worth incorporating in future modelling efforts.

Exploring which stressor pairs deviated from additive may provide insight into when multiple stressors have interactive effects. For instance, nutrients and toxins had opposing main effects and interacted antagonistically, indicating that the positive effect of nutrients can overcompensate for the negative effect of toxins. In contrast, a synergistic interaction was seen between nutrients and sea level rise (SLR), also with opposing main effects. In this case, all replicate studies came from intertidal marshes where nutrient enrichment seems to exacerbate the negative effects of sea-level rise on marsh plants. Why the positive effect of

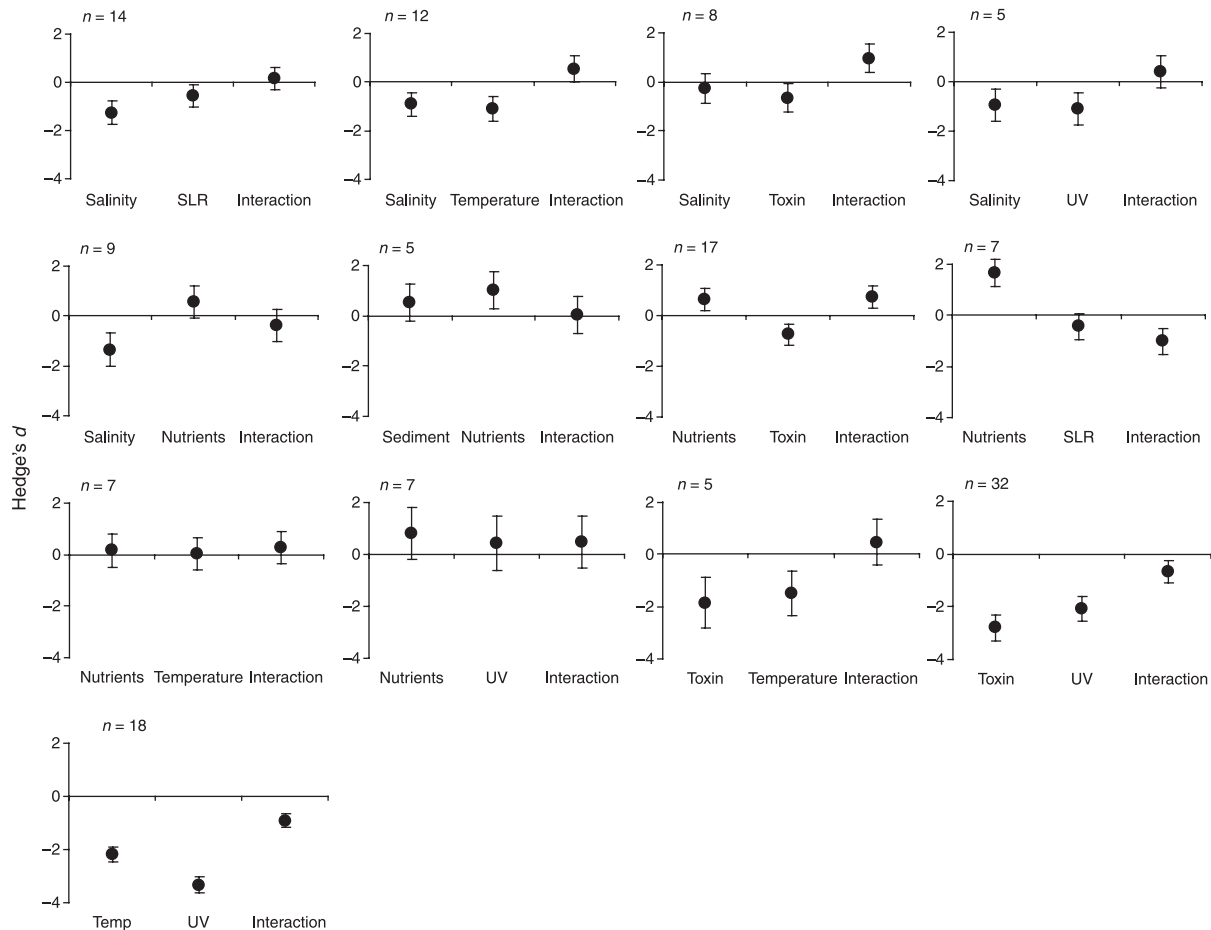


Figure 5 Results of meta-analyses using weighted Hedge's d for main and interactive effects of given stressor pairs.

nutrients leads to synergisms or antagonisms when paired with various negative stressors requires further attention, particularly since the only pairs with opposing main effects in our study (e.g. Fig. 1b) had significant interactive cumulative effects.

Antagonistic effects were also seen for salinity paired with both temperature and toxins and may have arisen because these stressors potentially mitigate each other or because one stress reduced the response to such a degree that the second stress has no room for an additional effect (comparative model sensu Folt *et al.* 1999). Alternatively, in cases where stressors are applied consecutively rather than simultaneously, a negative effect of the first stressor may pre-condition the species or community to be less sensitive to the second stressor. Synergistic interactions were found for UV paired with both temperature and toxins; both pairs had double negative main effects for which synergisms are particularly devastating (e.g. Fig. 1a). These synergisms may occur because chemical reactions change when these stressors are combined, such as has been found

with phototoxicity, the increasingly negative effect of toxins in the presence of UV (reviewed by Pelletier *et al.* 2006). Alternatively, with consecutive exposure, populations or communities that survive UV exposure could be more sensitive to a second stressor.

Understanding the mechanisms by which each stressor individually drives population or community response may help interpret or predict when and where cumulative stressors interact. For instance, in Mora *et al.* (2007), two stressors that affect population size directly (overexploitation and immigration due to habitat patchiness) had additive effects. However, when these stressors were each paired with temperature increases due to global warming, the pairwise interactions were both synergistic. Because response to warming depends on resiliency and therefore to some extent on genetic diversity, decreasing population size (i.e. due to overexploitation) may disproportionately affect the response to warming. Thus, stressors acting through similar mechanisms may be additive, while those acting through alternative but dependent pathways may be synergistic. Similarly,

Christensen *et al.* (2006) suggest that non-additive interactions are driven by the degree of similarity of individual stressor effects. They suggest that stress-induced species tolerances lead to antagonistic interactions since tolerance to one stressor can improve tolerance to a second stressor that acts through similar mechanisms (Blanck 2002), while ecological trade-offs lead to synergistic interactions since exposure to one stressor will select for species or individuals robust to that stressor but susceptible to a second stressor (Kneitel & Chase 2004). Thus understanding the mechanisms and effects of single stressors may be key to predicting the nature of stressor interactions, but these hypotheses require validation through continued empirical tests.

The high variance in interaction type both within (Fig. 3) and among (Fig. 5) stressor pairs suggests that other variables, such as trophic level, response type, ecosystem type, starting abundance, reproductive rate, magnitude of the stressor, or length of experiment may be responsible for driving interaction types. For instance, trophic level may be an important driver of interaction type because organisms with fundamentally different methods of energy acquisition may respond differently to stressors (i.e. increased CO₂ can benefit plants but harm invertebrates due to increased ocean acidification). Three stressor pairs had sufficient sample size to allow for analysis of differential responses between trophic levels, and two of these pairs switched from overall synergistic interaction effects for heterotrophs to antagonistic interaction effects for autotrophs. In studies of trophic cascades (indirect effects of fishing \times nutrients; Borer *et al.* 2006), interaction effects were slightly antagonistic for plants and synergistic for herbivores, but these effects were not significantly different from additive. This pattern among trophic levels within stressor pairs is consistent with our results from trophic level analysis across the complete database where autotroph studies had significantly antagonistic and heterotroph studies had significantly synergistic cumulative effects (Fig. 4). Taken together, our results indicate that interactive effects of multiple stressors could be more negative for organisms at higher trophic levels. A similar trend for single stressors to harm consumers but benefit producers has been attributed to loss of biological insurance as taxonomic, physiological and genetic diversity decrease with increasing trophic level (Christensen *et al.* 2006 and references therein).

Cumulative stressor effects may also depend on the response level (species vs. community) examined since species-level impacts can be dampened or exacerbated through species interactions. When analyzed across all studies, interaction effects were significantly synergistic in population level studies and antagonistic in community level studies (Fig. 4). These results suggest that species interactions within communities dampen and diffuse the impacts

of multiple stressors that can have strong negative effects on a given species. Consequently, species-level data may have limited utility in predicting community or ecosystem response to multiple stressors. Our synthesis is heavily weighted toward single-species studies (78% of studies). The impact of multiple stressors on marine systems will depend not only on species-level responses, but additionally on species interactions, species diversity and redundancy, trophic complexity, ecological history, and ecosystem type (Vinebrooke *et al.* 2004), suggesting a clear need to increase research on community-level or whole-ecosystem responses to multiple stressors (Breitburg *et al.* 1998).

Variation in multiple stressor effects suggests that context matters and our comparison of stressor interactions at various levels of a third factor confirmed this pattern. In two-thirds of the studies, differences in context changed stressor interaction effect sizes significantly and changed the interaction classification to a more 'negative' type. The number of synergistic interactions doubled (from 33% to 66%) in ambient vs. increased levels of the third stressor. As the number of stressors in a system increases (most natural systems are subject to more than two stressors), stressor pair interactions become increasingly complex and more commonly synergistic.

Despite these clear results, significant and striking gaps exist in research on multiple stressors. A majority of human-induced marine stressor pairs have not been experimentally studied in controlled factorial experiments (46 of 78; Fig. 2), leaving large gaps in our understanding of the interactive effects of multiple stressors in marine ecosystems. Even for pairs that have been researched, few replicate studies in most cases limits the strength of our conclusions. The stressors examined in this study have been shown to commonly co-occur in nature (Venter *et al.* 2006; Ban & Alder 2008; Halpern *et al.* 2008b), and our results show that interactions are common, so filling the gaps in cumulative stressor research is of high ecological and conservation significance. Perhaps most notably, the paucity of studies that have carefully controlled for and tested the direct effect of fishing and how it interacts with other stressors is a critical research need given the demonstrated importance of fishing in changing marine ecosystems (Worm *et al.* 2006).

The majority of experiments included in our analysis were conducted in the lab (73%), with fewer studies in mesocosms (17%) or in the field (10%). While factorial experiments in marine systems are much easier to conduct in the lab, relying on lab experiments to predict community and ecosystem responses in the field may lead to spurious conclusions because lab studies remove the important contextual factors of real ecosystems. Indeed, we found significant differences in overall interaction type depending on experimental methods. Lab studies were strongly synergistic, possibly because researchers target potentially

negative interactions to study in the lab. Mesocosm studies were significantly antagonistic, possibly because they were mostly community-level studies that we have shown are more commonly antagonistic. The few field studies were additive, perhaps balancing the complex interactions found in a field setting. Alternatively, differences in interaction effects could be a byproduct of the stressor pairs studied using different methodologies. For instance, many studies of nutrient \times toxin, an antagonistic pair, were conducted in mesocosms. However, a large diversity of stressor pairs were represented in all experimental methods, so stressor pair bias is unlikely. Future efforts to evaluate field studies that use correlation and historical baselines to infer multiple stressor effects could complement findings here and address stressors that are difficult to manipulate in factorial studies (e.g. environmental stressors and disease: Lenihan *et al.* 1999; global change and invasives: Dukes and Mooney 1999, Stachowicz *et al.* 2002; temperature, hypoxia and CO₂: Portner *et al.* 2005; temperature and disease: Harvell *et al.* 1999; historical multiple stressors: Hughes & Connell 1999).

Other examples of research bias exist in our database of studies and may skew overall results. Certain stressors, such as UV and toxins, lend themselves to factorial experimentation while others, such as species invasions, hypoxia, or over-fishing, are extremely difficult to address in this way (Fig. 2). Important work on these stressors has been conducted using other methods that could not be appropriately combined with studies in the current analysis (e.g. Lenihan *et al.* 1999 and others). Similar meta-analyses on factors related to fishing (trophic cascades (Borer *et al.* 2006) and grazer manipulations (Burkepile & Hay 2006; Gruner *et al.* 2008)) indicate that synergies between factors related to fishing and other stressors are common and clearly merit greater research attention. In addition, the concern over synergistic interactions has likely resulted in a focus on potentially synergistic pairs, such as for UV \times toxin (Fig. 2). Researchers often conduct factorial studies when interactions are expected and the interest in discovering synergistic effects in particular may skew overall results and interaction frequencies. Finally, some stressor pairs are studied in certain habitats and not others, potentially restricting the generality of findings. Sea-level rise, for example, is of major concern to intertidal biologists, and all studies in our database testing its effects come from intertidal marshes.

Understanding variation in cumulative stressor effects helps to inform management strategies such as ocean zoning (Crowder *et al.* 2006) and changes expectations for outcomes of various conservation and management efforts. For additive effects, reducing the magnitude of any stressor should lead to a corresponding increase in the response of interest, which would be predictable from single-stressor studies. Such situations allow for straight-forward expectations of response to management and conservation actions.

In contrast, mitigation of stressors involved in synergistic or antagonistic interactions with other stressors will lead to greater than or less than (respectively) predicted results based on additive models. Antagonistic stressors, in particular, create management challenges, as all or most stressors would need to be eliminated to see substantial recovery, except in cases where the antagonism is driven by a dominant stressor (e.g. Folt *et al.* 1999), such that mitigation of that stressor alone would substantially improve the state of species or communities. Synergisms, in contrast, may respond quite favourably to removal of a single stressor as long as the system has not passed a threshold into an alternative state. Ocean management can no longer focus on single-sector issues that address individual stressors (Halpern *et al.* 2007b), but must incorporate cumulative stressor effects. Our findings suggest that in general cumulative stressor outcomes are additive for stressor pairs, but are non-additive in specific comparisons 75% of the time and heavily weighted toward synergies when more than two stressors interact. This is a sobering reality for how humans are affecting our planet, but also suggests a real opportunity for greater-than-expected return on our conservation and management efforts as the number and intensity of synergisms are removed.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Table S1 Comprehensive listing of studies used for analysis of cumulative effect of stressor pairs.

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