1 **META-ANALYSIS USING NEW METHODS FOR THREE-STRESSOR COMBINATIONS REVEAL**
2 **SUBSTANTIAL HIGHER-ORDER INTERACTIONS AND EMERGENT PROPERTIES**
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5 Eleanor S. Diamant[1*], Sada Boyd[1*], Natalie Ann Lozano-Huntelman[1*], Vivien Enriquez[1], Alexis
6 R. Kim[1], Van M. Savage[1,2,3], and Pamela J. Yeh[1,3**]
7
8 *Authors contributed equally*
9
10 [1]Ecology and Evolutionary Biology, University of California, Los Angeles, USA
11
12 [2]Computational Medicine, David Geffen School of Medicine, University of California, Los Angeles,
13 USA
14
15 [3]Santa Fe Institute, Santa Fe, New Mexico, USA
16
17 **Corresponding author: Phone (310) 825-7441. Fax (310) 206-0484. Email:
18 pamelayeh@ucla.edu
19
20 Mailing address: 621 Charles E. Young Drive South, Los Angeles, California 90095, U.S.A.
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Abstract

Although natural populations are typically subjected to multiple stressors, most past research has focused on single stressors and two-stressor interactions, with little attention paid to higher-order interactions among three or more stressors. However, higher-order interactions increasingly appear to be widespread. Consequently, we used a recently introduced and improved framework to re-analyze higher-order ecological interactions. We conducted a literature review of the last 100 years (1920-2020) and reanalyzed 151 ecological three-stressor interactions from 45 published papers. We found that 89% ($n=134$) of the three-stressor combinations resulted in new or different interactions than previously reported. We also found substantial levels of emergent properties—interactions that are only revealed when all three stressors are present. Antagonism was the most prevalent net interaction whereas synergy was the most prevalent emergent interaction. Understanding multiple stressor interactions is crucial for fundamental questions in ecology and also has implications for conservation biology and population management.

Introduction

Individuals in natural populations almost always face multiple stressors that affect their ability to survive and to find food, shelter, mates, and safety (Blaustein & Kiesecker 2002; Côté *et al.* 2016). These stressors include changes to biological or environmental factors that can result in unfavorable responses within a population (Vinebrooke *et al.* 2004), leading to unfavorable responses across ecological systems (Jackson *et al.* 2016, 2021). Over the past century, ecological stressors such as climate change, pollution, and habitat destruction have adversely affected natural systems, contributing to biodiversity loss and a continuing threat to populations and ecosystems (Didham *et al.* 2007; Butchart *et al.* 2010; Halpern *et al.* 2015). These stressors rarely occur in isolation. Instead, they often interact—potentially changing the overall impact on populations (Crain *et al.* 2008; Côté *et al.* 2016). Therefore, there is a great need to properly assess and predict stressor interactions to mitigate their cumulative effects.

When the combined impact of two stressors is equal to the amount of the individual effects in isolation, the interaction type is defined as an additive interaction, or no interaction (Bliss 1939; Loewe 1953; Folt *et al.* 1999; Yeh *et al.* 2006; Piggott *et al.* 2015; Jackson *et al.* 2016). Alternatively, two stressors could interact synergistically—increasing the overall effects—or antagonistically—decreasing the overall effects (Bliss 1939; Loewe 1953; Folt *et al.* 1999; Yeh *et al.* 2006; Piggott *et al.* 2015). For example, synergistic interactions were observed when the combined effects of high temperatures and low pH decreased calcification (the production of shells and plates) in certain marine animals when compared to the individual effects of each stressor (Rodolfo-Metalpa *et al.* 2011). On the other hand, antagonistic interactions were found in coral (*Pocillopora meandrina*) microbiome response to multiple stressor interactions (Maher *et al.* 2019). Specifically, increased temperature and coral scarring both decreased the abundance of the

83 dominant taxon (*Endozoicimonacae*) in coral microbiomes. However, the combined stressor effect

84 led to a lower magnitude response than predicted if there were no interactions between the stressors

85 (Maher *et al.* 2019). An extreme form of antagonism is termed suppression—one stressor reverses

86 another stressor's effects (Yeh *et al.* 2006; Chait *et al.* 2007; Singh & Yeh 2017). For example,

87 the combined effect of carbaryl and nitrate decreased green frog (*Rana clamitans*) tadpole growth

88 despite their individual positive effects on tadpole growth (Boone *et al.* 2005).

89 Pairwise interactions–the effects of two stressors in combination compared to individual effects–

90 have been well studied in the ecological literature. Empirical work on pairwise stressor interactions

91 (e.g., Hesse *et al.* 2012; Cramp *et al.* 2014; Van Praet *et al.* 2014; Sniegula *et al.* 2017; Delnat *et*

92 *al.* 2019), literature reviews, and meta-analyses (Crain *et al.*, 2008; Darling & Cote, 2008; Ban *et*

93 *al.*, 2014; Piggott *et al.*, 2015; Jackson *et al.*, 2016; Matthaei & Lange, 2016; Côté *et al.*, 2016;

94 Villar-Argaiz *et al.*, 2018; Tekin *et al.*, 2020) have revealed their substantial influence across

95 biological systems and scales. Yet, there are likely more than two stressors acting on all, or almost

96 all, wild populations. In fact, multiple stressor interactions are more frequent than previously

97 thought, despite having received less attention (Beppler *et al.* 2016; Tekin *et al.* 2018a).

98 When studying interactions, higher-order combinations—defined here as a combination of three

99 or more stressors—have long been ignored (Pomerantz 1981) despite their importance in

100 ecological communities (Billick & Case 1994; Levine *et al.* 2017). The assumptions that have been

101 used to justify this include (1) paired interactions or single-stressor effects provide the main effects,

102 so one only needs to worry about paired interactions or single effects; higher-order interactions,

103 therefore, provide negligible effects (Pomerantz 1981; Wootton 1994; Ban & Alder 2008; Wood

104 *et al.* 2012; Wood 2016); (2) higher-order interactions are complex and depend on accurate,

105　specific parameters and underlying null models that are often not available for effective and

106　reliable testing (Billick & Case 1994; Thompson *et al.* 2018); and, (3) from an experimental

107　standpoint, the collection of higher-order interactions, whether in the lab or the field, can be

108　onerous, time-consuming, and logistically difficult as increasing stressor combinations could

109　theoretically lead to an exponentially large number of experiments, which has given rise to

110　research on approximating multi-stressor responses from single pairs (Billick & Case 1994; Côté

111　*et al.* 2016; Wood 2016; Zimmer *et al.* 2016; though see: Levine *et al.* 2017).

112　A recently introduced framework to examine interactions was used specifically to analyze both

113　pairwise and higher-order interactions (Tekin *et al.* 2018b, 2020). This framework, the Rescaled

114　Bliss Independence (RBI), was originally drawn from the pharmacology and microbiology fields

115　(Beppler *et al.* 2016; Tekin *et al.* 2016). The RBI has several key advantages compared to the

116　most-used method to examine interactions in ecology, ANOVA. First, ANOVA incorporates

117　several assumptions that are often violated or not tested (Text S1). Second, RBI enables direct

118　comparisons of interaction effects from absolute to relative fitness. Third, the framework allows

119　for straightforward generalization from pairwise to higher-order interactions while keeping the

120　ability to rescale interaction terms—that is, to normalize interaction values relative to a natural

121　baseline, much like the way we typically measure fitness as relative fitness rather than absolute

122　fitness (see Text S2 for details). Finally, and crucially, the framework enables the identification of

123　emergent properties—that is, what interactions arise that are the result of all three stressors

124　together, rather than just being the dominant effect due to a pairwise interaction dominating the

125　landscape of multiple stressors.

126 Emergent properties only arise from three or more stressors; there are no emergent properties in

127 two-stressor interactions because the interaction between the two stressors is what emerges from

128 the combination of the stressors. However, in three or more stressor combinations, the interaction

129 from three stressors could be coming primarily from a strong two-stressor interaction—a non-

130 emergent interaction—or the interaction could be the result of all three stressors together—an

131 emergent interaction (Beppler *et al.* 2016). Together, pairwise and emergent interactions constitute

132 the observed net three-way interaction (Figure 1A). For example, imagine ecological stressors A,

133 B, and C all are impacting the growth of a population X. Let's say that the interaction among all

134 three stressors together is synergistic. But this three-stressor interaction could arise primarily from

135 a strong synergistic pairwise interaction between two of the three stressors, say A and B, which

136 would obscure all other interactions (described in Figure 1B). This would be a case of a non-

137 emergent interaction. Or this three-stressor interaction could need all three stressors present to

138 show a synergistic interaction, which would be an emergent interaction (Beppler *et al.* 2016; Tekin

139 *et al.* 2016). Thus, it is imperative to quantify both pairwise and three-stressor combinations to

140 determine the nature of population X's response to A, B, and C.

141 The importance of identifying emergent properties lies in our ability to understand the impacts of

142 specific stressors, even when most populations experience multiple stressors in combination. In

143 fields such as conservation ecology or climate change biology, there is often an emphasis on

144 conserving and bolstering endangered and threatened populations by mitigating at least one of the

145 stressors affecting population survival and growth (Brown *et al.* 2013). However, if we do not

146 clearly understand how stressors interact, we could be mitigating the wrong stressors, or at least,

147 not the optimal stressors. A striking example of the importance of emergent interactions can be

148 seen in the field of pharmacology, where the combination of three antibiotic stressors

149   trimethoprim, streptomycin, and erythromycin have a synergistic effect, efficiently reducing

150   bacterial population size. However, if one of the drug stressors (for example, erythromycin) is

151   removed from this combination the overall killing efficiency actually *increases* (Beppler *et al.*

152   2017). This results in the population of concern, the bacteria, decreasing more when there are only

153   two stressors, rather than three (Beppler *et al.* 2017). This is an example of a critical emergent

154   interaction. In much the same way, understanding multiple stressors and their emergent, higher-

155   order, effects could be crucial for understanding how best to conserve species and populations.

156   Unlike ANOVA and similar approaches, the Rescaled Bliss Independence framework is conducive

157   to rescaling the interaction measure (e.g., normalization), which allows for more easily measuring

158   and identifying the strength of an interaction. Similar to absolute versus relative fitness, a rescaled

159   interaction value is normalized by a natural baseline (e.g., lethality). Rescaling results in a

160   multimodal distribution with clearer cut-offs around values. Therefore, interaction types are more

161   easily distinguishable (Segrè *et al.* 2005; Tekin *et al.* 2016, 2020). Without the rescaling step, raw

162   interaction values can be exactly the same even though they may represent different interaction

163   types, leading to incorrect interpretations (Tekin *et al.* 2016). Therefore, rescaling is critical to

164   comparing interaction measures and identifying interaction types.

165   Here we conduct a literature search of the last 100 years (January 1920-November 2020) and re-

166   analyze stressor interactions using the new Rescaled Bliss Independence (RBI) framework (Tekin

167   *et al.* 2016), recently applied to ecological studies to identify two-stressor interactions (Tekin *et*

168   *al.* 2020). For simplicity, we define "stressors" as factors that affect population growth or fitness.

169   While most of these "stressors" decrease population growth or fitness, a few stressors in the studies

170   examined here actually increase population growth and/or fitness. We aim to obtain a more

171   detailed, accurate, and complete understanding of higher-order ecological stressor interactions.

172 Specifically, we use this framework to reanalyze the data from previously published papers (that

173 used traditional methods i.e., ANOVA, General Linear Model, or log-logistic) that measure three-

174 stressor interactions and all the lower-order interactions: all three pairwise combinations of

175 stressors and all three single-stressors. We ask three questions: (1) How well does this new

176 framework match previously published interaction results? (2) How often do emergent properties

177 appear in higher-order ecological interactions? (3) Can we find patterns of emergent properties—

178 that is, for example, do they primarily occur in synergistic interactions or antagonistic

179 interactions?

180

Materials and Methods

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182 *Study Selection and Criteria*

183 We conducted a literature search using the *Web of Science* database to select the studies included

184 in our analysis. We searched one hundred years of published literature, from January 1920-

185 November 2020, using the following key terms included in the papers' keywords, title, and/or

186 abstract: "multiple stressors," "multiple antagonism," "multiple synergy," "multiple disturbance,"

187 "multifactor," "additions," "indirect interactions," and "stressors" (Supplemental Figure S1).

188 Then, we further filtered the search results by selecting the following specific topic categories to

189 reflect our interest in ecological studies: agriculture dairy, animal science, biodiversity

190 conservation, biology, biotechnology applied microbiology, ecology, environmental sciences,

191 evolutionary biology, genetics heredity, marine freshwater biology, microbiology, and zoology.

192 We removed duplicate studies and only selected papers that measured growth, mortality, and/or

193 survival at the population level for a specific species. Next, we examined the remaining papers to

determine the presence of the following criteria: the study had (1) three individual stressors and a full multi-factorial design was implemented, (2) quantitative response variables, and (3) explicit control treatments. From the remaining papers, we extracted growth, mortality, and survival data from figures and tables from each of the qualifying studies. We included the following: stressor type, stressor units, responses for individual stressors, responses for combinations of stressors, responses for control variables, sample size, species of the organism tested, species natural habitat, and the interaction type between stressors determined by the original authors (i.e., additive, synergistic, or antagonistic). If a study did not specify if there was an interaction among the three stressors, we determined whether the authors specifically sought to investigate an interaction. Additionally, if a study reported that there was no interaction among the stressors—but the authors explicitly sought to investigate an interaction—we classified the interaction as additive because additivity is the null hypothesis when testing for interactions. Most of the quantitative responses from each study reflected mean values generated from raw values by the authors, often summarized from tables or figures provided in the studies. Other quantitative responses were directly obtained from raw data. We recorded the latest time point as the response value if mean or raw data were presented as a time series. Importantly, these factors could enhance or inhibit growth.

Finally, we filtered out combinations that would not work with the RBI framework. Specifically, RBI only works with uniform factors (e.g., all inhibitors or all enhancers of growth). Therefore, combinations that affected populations in opposite directions (e.g., a stressor decreasing population size and one increasing population size) were removed. Combinations that had a positive control value of zero could not be analyzed and were removed from the dataset. Combinations that had a lethal single stressor or a single stressor with no effect were assessed

217   separately. In instances where one or more of the single stressors was lethal, we could only

218   accurately identify the presence of a net suppressive interaction, and not an emergent effect. In this

219   case, distinguishing additive, synergistic, or antagonistic interactions is not possible since a

220   population cannot exhibit growth or survival less than zero. However, if the population

221   demonstrates growth or survival in the presence of the three stressors combined, we could

222   determine a suppressive interaction when at least one of the individual stressors is lethal.

223   Additionally, there were cases where a single stressor had no effect. This can be problematic

224   because it is unclear if the single stressor in combination with additional stressors has any effect

225   or if we only see the effects of the additional stressors. Since we can only identify synergistic

226   combinations under these circumstances, those cases were not included in our analysis. Many of

227   the papers compared multiple combinations of three stressors. In total, 151 stressor combinations

228   from 45 papers met the requirements needed for our study (Table S1).

229

230   *Data analysis*

231   The RBI framework has previously been used to examine drug interactions and pairwise stressor

232   interactions by relying on Bliss Independence as the additive model to determine if there is an

233   interaction between stressors on a population (Beppler *et al.* 2016; Tekin *et al.* 2016, 2018a,

234   2020). We applied this framework to ecological studies exploring the impact of three stressors in

235   each environment. Within this framework, there are a total of seven possible measurements one

236   can take among the three stressors (stressor A, stressor B, and stressor C) acting simultaneously.

237   These are: (1) the effects of A alone, (2) the effects of B alone, (3) the effects of C alone, (4) the

238   pairwise effects of A and B by themselves, (5) the pairwise effects of A and C by themselves, (6)

239   the pairwise effects of B and C by themselves, and (7) the effects of all three stressor A, B, and C

240   together. The net interaction—termed deviation from additivity (*DA*) (Equation 1)—occurs when

241   we remove the effects of the individual stressors from consideration. Removing the result of the

242   pairwise interactions produces the emergent effect (*E3*) (Equation 2). Further, we can rewrite

243   Equation 2 to only reflect relative fitness effects (Equation 3).

244    $$\textbf{Equation 1. } DA = w_{ABC} - w_A w_B w_C$$

245    $$\textbf{Equation 2. } E3 = w_{ABC} - DA_{AB}w_C - DA_{AC}w_B - DA_{BC}w_A - w_A w_B w_C$$

246    $$\textbf{Equation 3. } E3 = w_{ABC} - w_{AB}w_C - w_{AC}w_B - w_{BC}w_A + 2w_A w_B w_C$$

247

248   Upon calculating these initial interactions, rescaling methods and cutoff values used by Tekin *et*

249   *al.* (2018b) were used to further investigate and identify interactions. After rescaling (see Text S2),

250   both net and emergent interaction values below -0.5 were synergistic, values between -0.5 and 0.5

251   were additive, and values above 0.5 were considered antagonistic (where values above 1.3 were

252   considered suppressive). For more information about rescaling and details on how to determine

253   interactions for combinations where all stressors increase growth, see Text S2 and S3.

254   The framework described above requires that all single stressors have a non-lethal effect on relative

255   fitness ($0 < w \neq 1$). This is because if a single stressor is lethal ($w = 0$) or if a single stressor has no

256   effect ($w = 1$) we would not be able to identify all interaction types. For example, if the use of one

257   stressor results in complete lethality one cannot determine if a combination with that stressor

258   interacts synergistically or additively if the combination also results in complete lethality.

259   Similarly, if a single stressor appears to have no effect ($w = 1$) there is no way to distinguish if that

260   stressor interacts at all with the system and is relevant or if the other stressor in the combination

261 acts additively. This framework also requires relative fitness to be calculated with reference to a

262 positive control (the growth of the population under no stressor present).

263

264 **Results**

265 For our analysis, we collected data from multi-stressor ecological studies published over the last

266 100 years. Most of these studies were published in the past 10 years (Figure S2). We subsequently

267 applied the RBI framework to reanalyze three-stressor interaction data derived from those studies.

268 Our findings were then compared to those from the original studies. We found new net interactions

269 that were previously unidentified by the original authors and net interactions that we reclassified

270 based on our methods (Figure 2). Of the 151 interactions, nearly half, 42% (*n=64*), were

271 interactions that were previously untested (e.g., experiments were conducted but no statistical

272 analysis on the interactions themselves were reported) but classified as an interaction using RBI.

273 We classified 17% (*n=25*) of previously tested but unspecified interactions (e.g., an interaction

274 was found using statistical analyses by previous authors though the type of interaction was not

275 explicitly stated). Only 11% (*n=17*) of the total interactions reanalyzed by RBI were re-classified

276 with the same interaction type previously reported in the original studies while 30% (*n=45*) were

277 different from what was previously reported. Collectively, 89% of interactions were newly

278 classified or reclassified by RBI.

279 Of the combinations that resulted in the same interaction type when applying both the original

280 method described in the published studies and the RBI, 41% (*n=7*) were additive, 53% (*n=9*) were

281 synergistic, and 6% (*n=1*) were antagonistic (Table 1). Among the interactions reclassified by the

282 RBI, 71% (*n=32*) were previously published additive interactions, all of which were reassigned as

283 antagonistic (Figure 3). The remaining interactions reclassified by RBI were synergistic and

284 reclassified as antagonism (22%, $n=10$) or additive (7%, $n=3$). We found that 82% ($n=32$) of

285 additive and 45% ($n=10$) of synergistic net interactions were reclassified as antagonistic net

286 interactions using RBI (Table 1). No previously identified interaction type ($n=62$) was newly re-

287 classified as synergy, but 41% ($n=9$) of interactions reported as synergy were confirmed using RBI

288 (Table 1, Figure 3).

289 We also examined the frequency of interaction types among net and emergent three-stressor

290 combinations (Figure 4). Of the net interactions identified, we found that antagonism was the most

291 prevalent interaction type at 69% ($n=104$). Of the antagonistic interactions, 34% ($n=35$) were

292 suppressive. The remaining net properties were composed of 18% ($n=27$) synergistic and 13%

293 ($n=20$) additive interactions (Figure 4A). Of the emergent interactions, we found that synergy and

294 additivity were the leading interaction types across emergent properties–accounting for 47%

295 ($n=71$) and 40% ($n=61$), respectively (Figure 4B). Antagonism accounted for 13% ($n=19$) of

296 emergent interactions (Figure 4B).

297 When comparing the frequency of interaction types among the net and emergent properties, we

298 found that there were over three times as many instances of additivity in emergent interactions

299 (emergent: $n=61$, net: $n=20$) (Figure 4C). There were also substantially fewer instances of

300 emergent antagonistic interactions ($n=19$), including fewer suppressive interactions ($n=7$) among

301 emergent when compared to net properties ($n=104$ total antagonism, including $n=35$ suppressive

302 interactions). We then examined if there was a correlation between the net and emergent

303 interactions. We did not find a significant correlation after performing a Spearman's correlation (p

304 = 0.12) (Figure 4D). We also observed more synergistic emergent interactions (emergent: $n=71$)

305 than synergistic net interactions (net: $n=27$). Across all combinations, 65% ($n=98$) were found to

306 have instances of "hidden suppression" where a pairwise combination is suppressed by the

307   presence of a third stressor (e.g., better fitness with three stressors than with two stressors for

308   negative stressor combinations). These interactions are only present in 3+ stressor interaction

309   combinations. The comparison of the interactions' distributions can be seen in Figure 5.

310

311   **Discussion**

312   In this study, we surveyed ecological literature published between 1920-2020 that examined the

313   effect of three stressors simultaneously on population mortality, survival, or growth. After re-

314   analyzing data from previously published results using a newly introduced framework, the RBI,

315   we identified 151 interactions. We found that only 11% of the results generated by RBI matched

316   those in the original studies, meaning that nearly 90% of interactions were classified as new (either

317   unspecified or not investigated by the previous authors) or different interaction types (Figure 2).

318   Our results show that methods used in the original studies may have difficulty identifying

319   antagonistic interactions (Figure 3). When comparing our findings using the RBI framework to

320   those of the original findings, we found that interactions were more often reclassified as

321   antagonisms than synergy, and antagonisms made up most interaction types (nearly 69%).

322   Furthermore, there was only one instance of both RBI and original methods classifying

323   combinations as antagonistic—although crucially, the one case that met the criteria of this study

324   where a three-stressor interaction was previously classified as "antagonism." In one example, an

325   interaction was classified as synergistic using restricted maximum likelihood ANOVA methods

326   when assessing the combined effects of UV-radiation, water temperature, and salinity stress on

327   mollusk embryos (Przeslawski *et al.* 2005). However, during our reanalysis, we reclassified the

328   interaction as antagonistic. Similar results were observed when using RBI to reanalyze work on

329   insecticide combinations in anuran species (Boone 2008), pesticide combinations and food

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330   limitation in *Daphnia magna* (Shahid *et al.* 2019), and a combination of abiotic and biotic stressors

331   in a seagrass (*Zostera noltei*) (Vieira *et al.* 2020). Traditional ANOVA and log-logistic methods

332   initially classified these combinations as synergy, but, using RBI, they were reclassified as

333   antagonism.

334   Higher-order interactions involving three or more stressors and emergent properties that arise from

335   higher-order combinations are still poorly understood (Beppler *et al.*, 2016; Tekin *et al.*, 2018).

336   We asked how often emergent properties appear in higher-order ecological stressor interactions.

337   We find that emergent effects occur nearly 60% of the time, suggesting that emergent properties

338   are common among higher-order ecological interactions. Moreover, we demonstrate that the RBI

339   framework can identify higher-order emergent interactions that are overlooked or not explicitly

340   explored when using traditional methods. For example, when using a general linearized model

341   (GLM) to investigate the effect of pH, temperature, and oxygen availability on moon jellyfish,

342   additive interactions were reported (Algueró-Muñiz *et al.* 2016). However, using RBI, we did not

343   confirm the original authors' conclusion and instead found antagonistic net interactions, and we

344   also identified synergistic emergent interactions.

345   Our data also demonstrate that emergent properties persist across all interaction types—synergy,

346   additivity, and antagonism. We demonstrated that synergy and additivity are the leading effects

347   across emergent interactions—accounting for 47% and 40% of the total interaction types identified

348   in our study, respectively (Figure 4B). Among the 13% of emergent antagonistic interactions we

349   identified by RBI, 37% of them were characterized as suppressive (where one stressor reverses

350   another stressor's effects) (Figure 4).

351   In contrast to our findings, some prior studies observed more synergistic net interactions in

352   comparison to antagonistic interactions when examining three-stressor interactions, even if

353   antagonism was more common in two-stressor combinations (Crain *et al.* 2008; Maher *et al.* 2019).

354   Our study examined over three times as many interactions as Crain *et al.* (2008) ($n=151$ and $n=48$

355   respectively). Maher and colleagues (Maher *et al.* 2019) found that synergies dominate three-

356   stressor interactions in the coral microbiome using GLM and LMM models rather than RBI, which

357   could explain the finding of synergistic interactions. This study also focused on a different

358   biological scale—the microbiome *community* rather than a unique population's fitness.

359   On the other hand, one major three-stressor interaction study, which observed the impact of low

360   food, thermal stress, and elevated toxin levels on *Daphnia* populations, demonstrated that most

361   interactions were antagonistic (Folt *et al.* 1999), similar to what we found here. Although emergent

362   properties were not explicitly stated, the severity of the combined antagonistic effects differed

363   from what would be predicted based on the sum of individual effects (Folt *et al.* 1999), suggesting

364   that emergent properties played a role in population response. From a very different field, that of

365   microbiology, antibiotic-combination studies reveal that higher-order emergent interactions were

366   most often antagonistic than synergistic (Beppler *et al.* 2016; Tekin *et al.* 2018a). By identifying

367   emergent interaction types, we can determine the combined effects of specific factors on

368   populations in complex habitats which are subjected to multiple stressors at any given time.

369   Although our study addresses three-stressor interactions, our results are comparable to the

370   previously mentioned two-stressor interaction studies in that additivity and antagonism were also

371   found to be the most prevalent interaction types in a reanalysis of two-way interactions using RBI

372   from ecological studies within the past 25 years (Tekin *et al.* 2020). In the two-stressor studies,

373   41% ($n=286$ of 840) of interactions were identified as additivity and 40% ($n=278$ of 840) as

374   antagonism (Tekin *et al.* 2020). Interestingly, those results correspond well with previous reports

375   that were not using RBI (Darling & Cote 2008; Côté *et al.* 2016; Jackson *et al.* 2016). This provides

376   support for the idea that synergy may be overemphasized in the literature and that antagonism may

377   occur more often than previously thought (Darling & Cote 2008), at least for net interactions.

378   Synergy has been overemphasized in other biological disciplines, including research on antibiotic

379   resistance (Singh & Yeh 2017).

380   Historically, whether three-stressor interactions exist and, if they do, to what extent they affect

381   natural populations and ecosystems has been a subject of debate since the 1960s (e.g., Vandermeer

382   1969; Pomerantz 1981; Abrams 1983; Billick & Case 1994). At the population level, one major

383   limitation in understanding these interactions is determining an applicable and generalizable

384   model. By applying RBI, we were able to properly assess three-stressor interactions and determine

385   that not only do emergent properties exist across biological systems but that they are also relatively

386   common. Thus, a population's response cannot necessarily be predicted by assuming additivity

387   across stressors.

388   Further work still needs to be done to scale from population dynamics to community and

389   ecosystem functioning across time (Côté *et al.* 2016; Brooks & Crowe 2019; Jackson *et al.* 2021).

390   Beyond the scale of single-species populations, interactions between species and resources (Coyte

391   *et al.* 2015; Butler & O'Dwyer 2020) and higher-order interactions between species (Kelsic *et al.*

392   2015; Grilli *et al.* 2017) have been shown to be important in modeling stability in ecological

393   communities. Additionally, evolution in response to multiple stressor interactions and the fitness

394   landscapes they form could influence adaptive dynamics (Ogbunugafor *et al.* 2016), population

395   outcomes (Venturelli *et al.* 2015), and therefore broader eco-evolutionary dynamics. Indeed,

396   understanding selection can help determine trajectories populations may take in adapting to given

397   stressors (e.g., Toprak *et al.* 2012).

398   The implications of finding higher-order interactions extend beyond basic science. There is

399   growing awareness that stressor interactions are crucial for population management and response

400   predictions across systems. Pairwise interactions have received plenty of attention and have been

401   the subject of many studies. In contrast, higher-order interactions and emergent interactions in 3+

402   stressor systems—which almost certainly present a more accurate representation of what natural

403   populations face and will continue to face—remain less understood. Properly identifying these

404   interactions is critical for managing ecological stressors (Brown *et al.* 2013; Piggott *et al.* 2015).

405   The finding that the majority of emergent interactions are synergistic or additive rather than

406   antagonistic, while the majority of combinations also exhibit hidden suppression, suggests that we

407   need to identify *which* stressors are involved in a given system and what the impact may be if a

408   given stressor is removed or decreased.

409   Crucially, identifying emergent properties can reveal hidden suppressive interactions (i.e.,

410   suppressive interactions that only occur among higher-order interactions). These hidden

411   suppressive interactions could be particularly important for the ecological management of at-risk

412   populations. In a three-stressor combination, the addition of a third stressor may suppress a two-

413   stressor interaction. For example, when examining the combined effects of acidification, drought,

414   and warming, the interaction between drought and acidification is suppressed by the effect of

415   higher temperature on plankton producer biomass resulting in more biomass with all three stressors

416   than with two (Christensen *et al.* 2006). Such an observation may be important for incorporating

417   necessary mitigation strategies. In this example, alleviating acidification would result in lower

418   biomass because it would undo the suppression of the interaction between warming and drought.

419   Instead, mitigating warming and drought would be a better strategy if the goal were to increase

420   biomass. If the stressor interactions are not clearly understood or identified, the wrong stressor

421   could be mitigated. These hidden suppressive interactions are common: the majority (65%) of our

422   re-analyzed stressor combinations revealed hidden suppression between a pairwise interaction and

423   a third stressor.

424   Throughout the ecological literature, investigations of three-stressor interactions involving biotic

425   and environmental stressors remain scarce. We completed a thorough literature search of

426   thousands of relevant results over the last 100 years and found that only 0.3% of research articles

427   examining three ecological stressors qualified for our meta-analysis. Comparatively, when

428   exploring pairwise interactions across the ecological literature, Tekin *et al.* (2020) found that

429   nearly 8% of search results were applicable. Nevertheless, over the last decade, studies

430   investigating higher-order interactions across disciplines have increased dramatically (Figure S2).

431   Most of the studies that qualified for re-analysis here occurred within the past five years. The

432   recent increase in higher-order interaction studies highlights how crucial it is that we extend our

433   research beyond pairwise interactions to more accurately examine the effect of stressors in

434   combination.

435   In conclusion, we show that this new RBI framework can be generalized from pairwise interactions

436   to three or more stressors to examine how multiple stressors interact. RBI can distinguish between

437   net and emergent interactions, providing greater insight into complex biological systems. From a

438   basic science perspective, predicting higher-order interactions is essential to understanding how

439   the combined effects of multiple stressors interact and impact diverse biological systems. From a

440   conservation perspective, multiple stressor interactions can influence the population size of species

441 of concern. Thus, correctly characterizing multiple stressor interactions can be crucial for

442 developing management strategies to mitigate biodiversity loss.

443

451

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599   drug dose responses based on measurements of drug pairs. *Proc. Natl. Acad. Sci.*, 113,

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601

602 **Tables and Figures**

603 Table 1. Comparison counts for each net interaction type originally reported in previously

604 published results and how they were re-classified using Rescaled Bliss Independence (RBI).

605 "Inconclusive" interactions under "Previously Published Results" correspond to cases for which

606 no explicit interaction type is reported or investigated.

607

Net Interactions		Rescaled Bliss Independence (RBI)		
		Synergy	Additive	Antagonism
Previously Published Results	Synergy	9	3	10
	Additive	0	7	32
	Antagonism	0	0	1
	Inconclusive	18	10	61

608

609
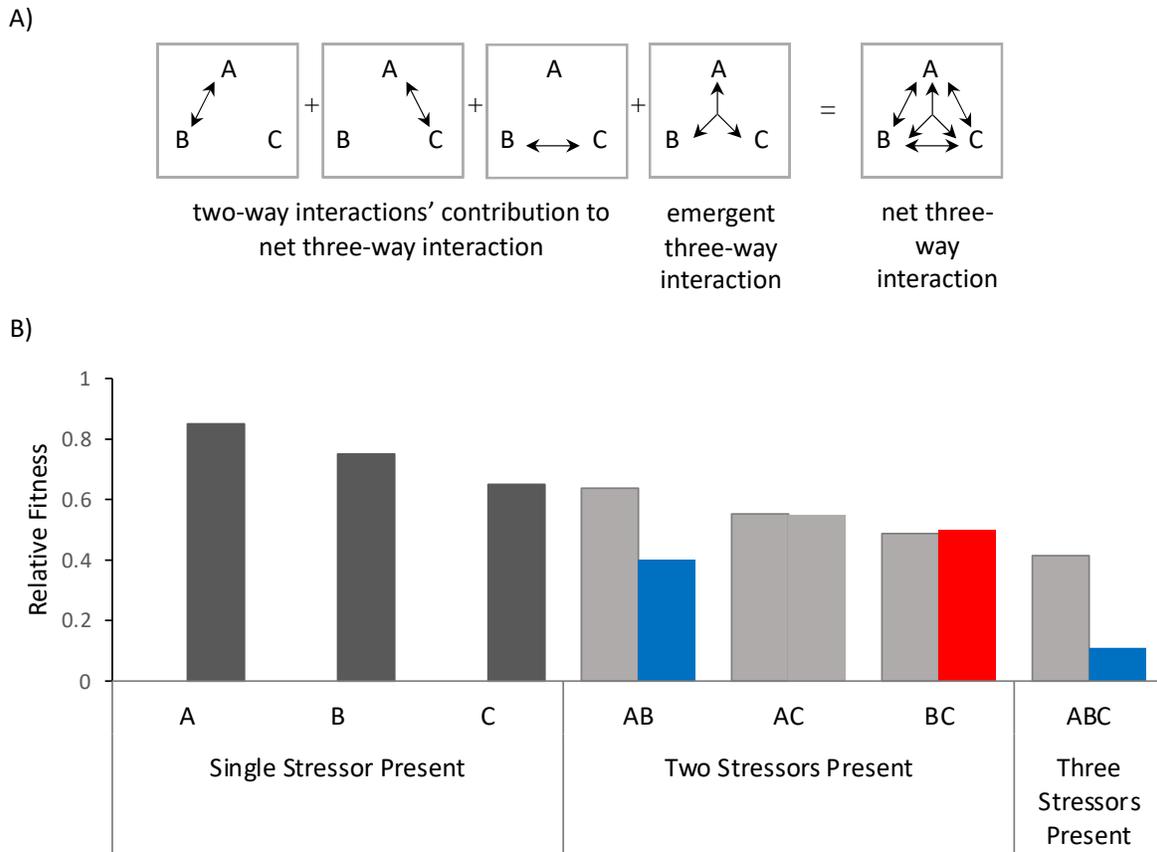
Figure 1. The components needed to assess three-stressor interactions. A) Net three-way interactions are composed of lower-order two-way interactions between given stressors (A, B, and C) and the higher-order emergent three-way interaction that is only quantifiable with all three stressors present and when all two-way interactions are known. Together, these compose the net three-way interaction. Figure 1 is partially adapted from (Tekin *et al.* 2018b). B) An example of the contribution of two-way interactions on net three-way interactions. Here, single stressors all decrease relative fitness. In two-stressor and three-stressor interactions, light gray represents the additive expectation based on single-stressor effects on fitness. Blue represents synergistic interactions and red represents antagonistic interactions. In this example, the strong synergistic two-stressor interaction between A and B, rather than an emergent interaction,

621   overshadows the additive interaction between A and C and the slightly antagonistic interaction

622   between B and C. This leads to a synergistic net three-way interaction between A, B, and C.

623

624
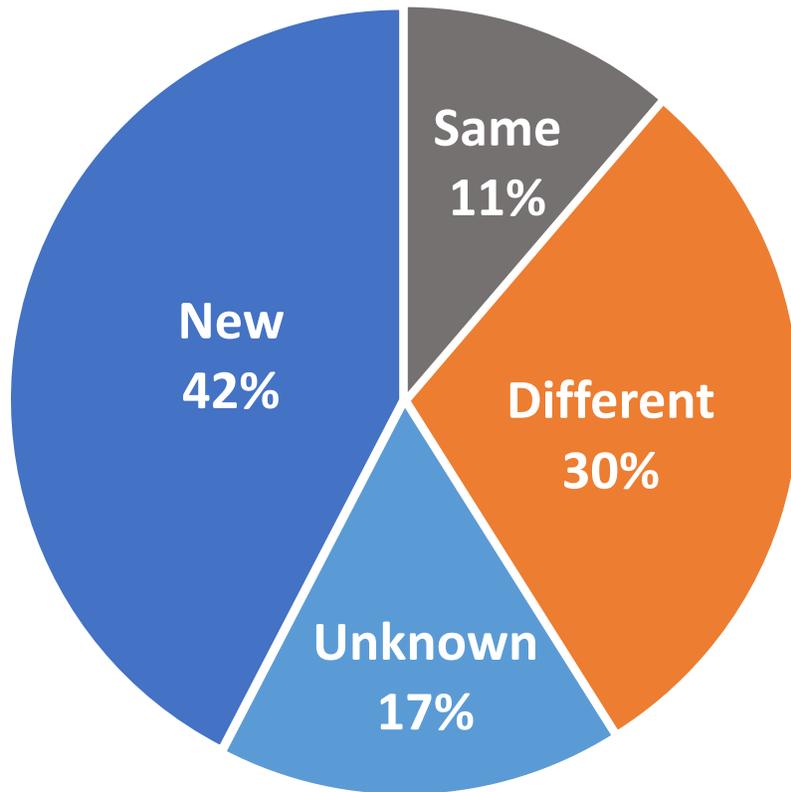
Figure 2. Total interactions identified by Rescaled Bliss Independence (RBI) contrasted to previously published results. Over half (59%) of the total interactions examined were untested and are therefore "new" interactions (42%) or were tested in the original study, but the interaction type was not specified or classified by the original study, and are therefore "previously unknown" interactions (17%) now classified using RBI. Only 11% of interactions analyzed with RBI remained the same as previously published results; 30% resulted in a different interaction than previously reported.

Different Results

Category	Value
Antagonism to Additive	0
Antagonism to Synergy	0
Additive to Antagonism	32
Additive to Synergy	0
Synergy to Antagonism	10
Synergy to Additive	3

632
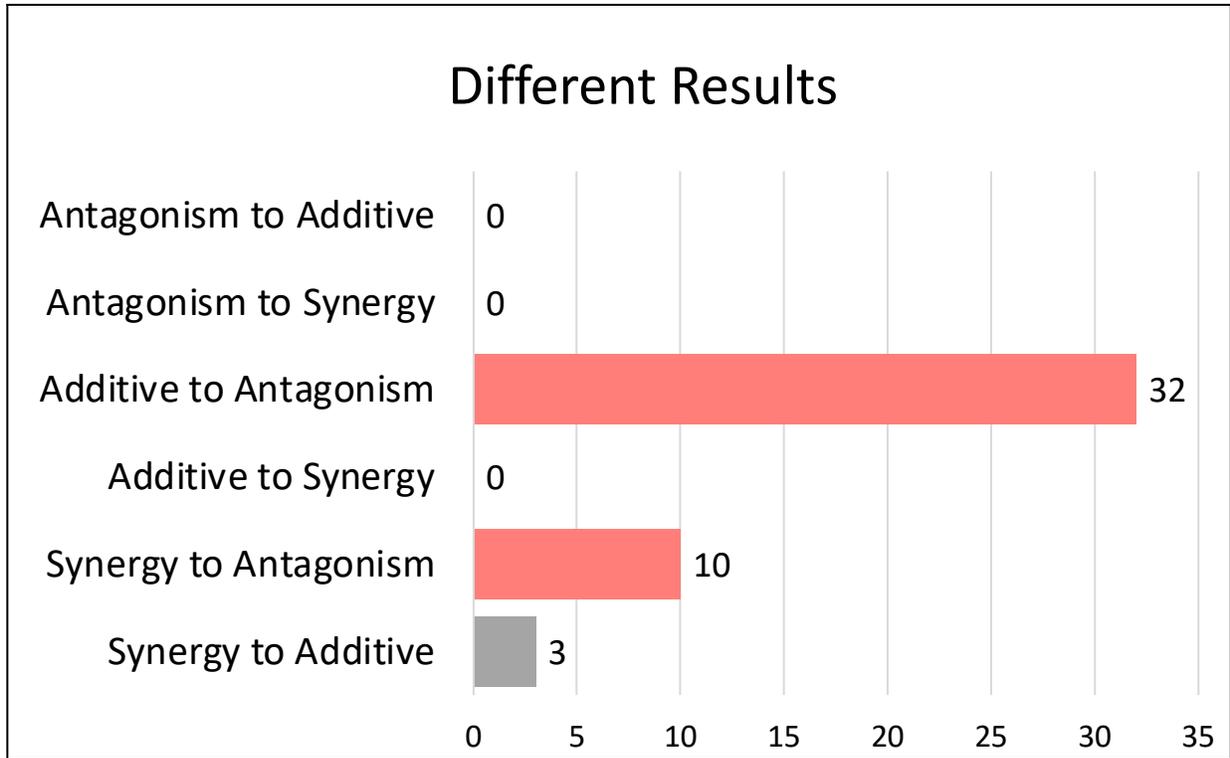
Figure 3. Traditional methods have difficulty identifying antagonistic interactions. Each bar shows the number of combinations that demonstrated a change from the previously published interaction type to the type of interaction with RBI.
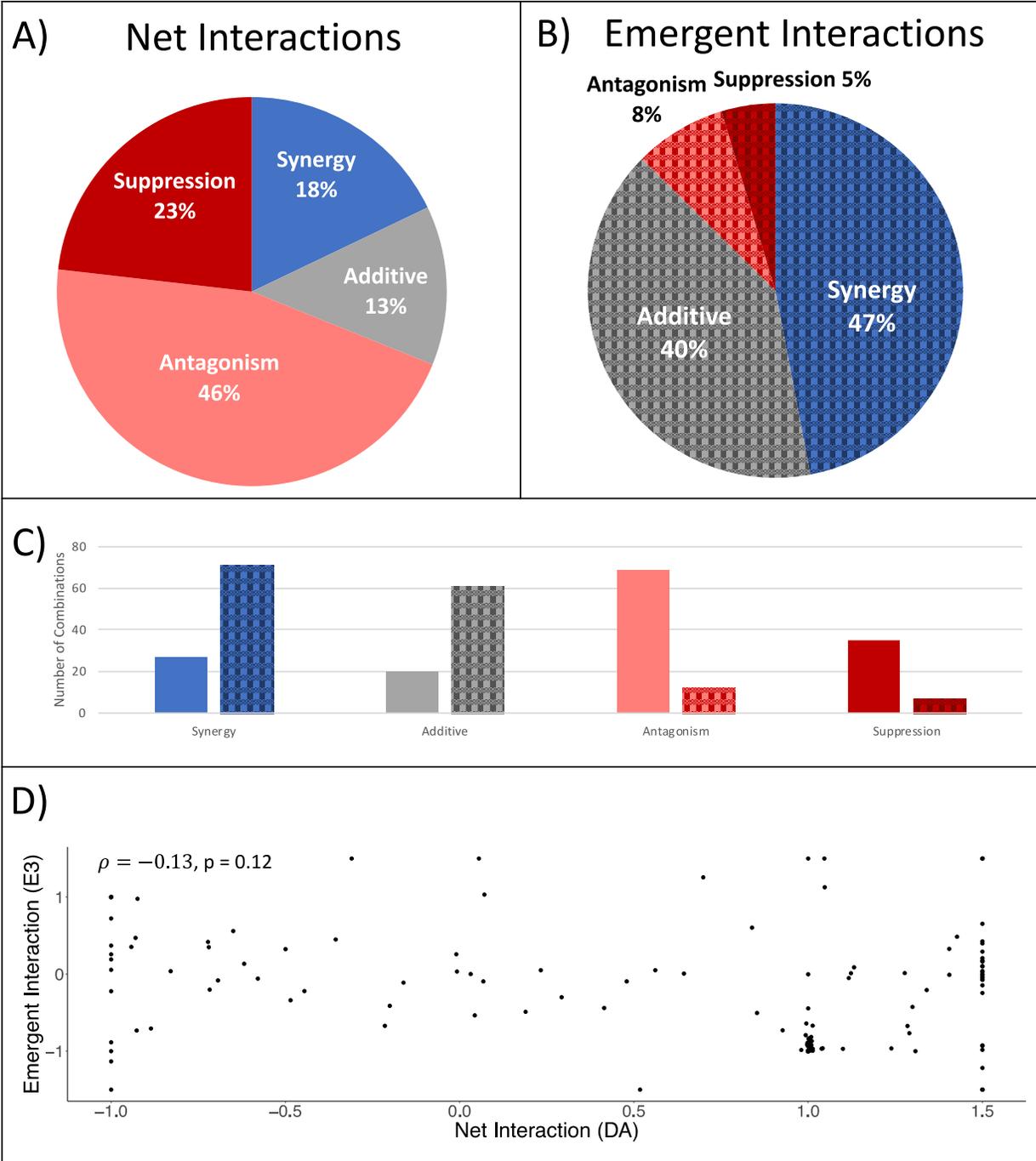
Figure 4. The composition of the net and emergent interactions using the RBI method. In each panel, gray represents additivity, blue represents synergism, and red represents antagonism. A darkening red illustrates an intensifying antagonism (e.g., antagonism → suppression). The plaid pattern represents emergent interactions, while solid colors represent net interactions. Panels A)

641   and B) demonstrate the composition of the net and emergent interactions, respectively. Panel C)

642   shows a direct comparison between the net and emergent interactions for each interaction type

643   (synergy, additive, antagonism, buffering, and suppression). Panel D) shows no significant

644   correlation between the net and emergent interaction values (Spearman correlation, $\rho =$

645    $-0.13, \mathrm{p} = 0.12$).

646
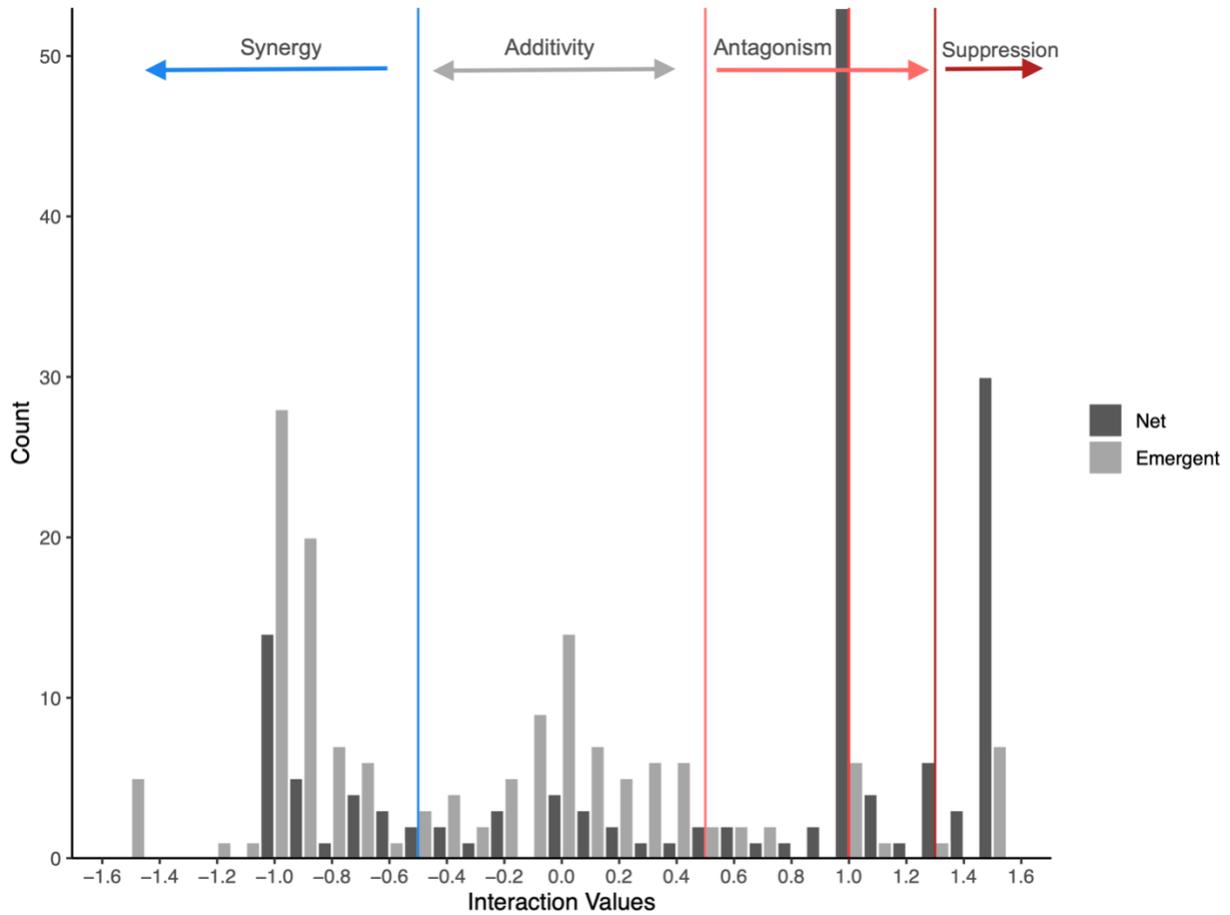
Figure 5. The distribution of interaction values of both net and emergent interactions. The

distribution of net (DA) and emergent (E3) values. Cut-off values for each interaction type are as

followed: synergy is less than -0.5; additivity is between -0.5 and 0.5; antagonism is above 0.5,

suppression is above 1.3. Rescaled values are distributed across multimodal peaks. Thus,

rescaling aids in the identification of interaction types through the use of these peaks.

Supplementary Material

Text S1. A summary of the issues with using ANOVA (ANalysis Of VAriance) for

classifying interaction types as outlined in Tekin *et al.* (2020).

The limitations and potential false inferences of applying ANOVA to test for and classify

interactions are fourfold. **First**, variance in response measures within and across all treatments is

determined by the number of experimental replicates, which are often limited in stressor-

combination studies. This then leads to poor estimates of higher-order moments (e.g., kurtosis

and skewness), potentially leading to inaccurate results. **Second**, though hidden replication (e.g.,

assuming variance is constant across treatments) is often used to justify applying ANOVA in

these scenarios, hidden replication rests on the assumption that there is *no interaction* between

variables (Welham *et al.* 2014). Thus, employing hidden replication when testing for interaction

often invalidates the findings themselves. Further, non-linear pairwise interactions require

carefully chosen data transformations when the assumptions of ANOVA are otherwise not

violated (Pomerantz 1981; Billick & Case 1994; Gotelli *et al.* 1999). It is particularly important

to transform the underlying additive model to a multiplicative model when stressors have large

effects on populations (Segrè *et al.* 2005; Tekin *et al.* 2018a). **Third**, ANOVA assumes Gaussian

or parametric distributions when comparing variances between treatments. Therefore, a large

number of replicates per treatment would be necessary to accurately assess the variance of non-

normal distributions and subsequently reconstruct ANOVA based on the non-parametric null

model. **Fourth**, ANOVA methods do not allow for rescaling. Rescaling or normalization,

relative to control baselines (e.g., population fitness with no stressors present), is often necessary

to classify interaction types because different interaction types may result in similar unscaled

674 values. Additionally, rescaling results in multimodal distribution of interaction values, aiding

675 interaction classifications (Figure 5) (Segrè *et al.* 2005; Tekin *et al.* 2018b, 2020).

676

677 **Text S2. Mathematics of Rescaling Bliss Independence with Multiple Stressors that Inhibit**

678 **Growth.**

679 Once interaction values are calculated as described in the methods it may be hard to distinguish a

680 cut-off value for each type of interaction. To do this, rescaling is needed to transform a unimodal

681 distribution of the raw interaction scores to become trimodal, allowing for clearer distinctions of

682 truly antagonistic and synergistic interactions. For this study, we followed protocols developed

683 by Tekin *et al.* (2016) to rescale. For both net and emergent synergistic interactions, we

684 normalized to a lethal case because when measuring growth rates, relative fitness cannot be

685 below zero.

686
$$DA_{rescaled} = \frac{DA}{|0 - w_A w_B|} \qquad DA_{rescaled} = \frac{DA}{|0 - w_A w_B w_C|}$$

687 When rescaling occurs for non-synergistic net interactions the interaction value is normalized to

688 the minimum of the single stressor effects.

689
$$DA_{rescaled} = \frac{DA}{|\min(w_A, w_B) - w_A w_B|} \qquad DA_{rescaled} = \frac{DA}{|\min(w_A, w_B, w_C) - w_A w_B w_C|}$$

690

691 When rescaling occurs for non-synergistic emergent interactions we chose to normalize the

692 interaction value to the minimum of the pairwise interactions. Tekin *et al.* (2016) recommend

693 this normalization option because it may be more biologically relevant than other options.

694
$$E3_{rescaled} = \frac{E3}{|\min(w_A DA_{BC}, w_C DA_{AC}, w_C DA_{AB}) - w_A DA_{BC} - w_B, DA_{AC} - w_C DA_{AB}|}$$

695

696

Text S3. Mathematics of Rescaling Bliss Independence with Stressors That Increase

Growth

For combinations that only had stressors that increased growth, we adapted the protocols

developed by Beppler *et al.* (2016) and Tekin *et al.* (2016) to rescale. For the initial net (DA) and

emergent (E3) interactions, the signs were reversed to keep synergistic interactions as negative

values and antagonistic interactions as positive values following the equations below.

$$DA = w_A w_B w_C - w_{ABC}$$

$$E3 = w_{AB} w_C + w_{AC} w_B + w_{BC} w_A - 2 w_A w_B w_C - w_{ABC}$$

When rescaling synergistic interactions, the interaction value is normalized to the maximum

positive value because there is no upper limit to what a synergistic combination of promoters can

be. Ideally this maximal value would be infinity, however this is not practical. In an attempt to

estimate this maximal value, we used twice the amount of the relative fitness of the highest-order

combination.

$$DA_{rescaled} = \frac{DA}{|2w_{AB} - w_A w_B|} \qquad DA_{rescaled} = \frac{DA}{|2w_{ABC} - w_A w_B w_C|}$$

When rescaling occurs for non-synergistic net interactions, the interaction value is normalized to

the maximum of the single stressor effects. This was done to keep with the same definition of

rescaling to buffering, as described in Tekin *et al.* (2016): buffering normalizes to the most

extreme of the single stressors.

$$DA_{rescaled} = \frac{DA}{|\max(w_A, w_B) - w_A w_B|} \qquad DA_{rescaled} = \frac{DA}{|\max(w_A, w_B, w_C) - w_A w_B w_C|}$$

716

717

718 When rescaling occurs for non-synergistic emergent interactions we chose to normalize the

719 interaction value to the maximum of the pairwise interactions. Tekin *et al.* (2016) recommend

720 this normalization option because it may be more biologically relevant than other options.

721 Again, we chose to use the maximum of the single and pairwise interactions following the

722 definition of buffering that normalizes to the most extreme effect of the single stressors and

723 lower-order combinations that interact additively with the third stressor (Tekin *et al.,* 2016).

724 When all stressors inhibit growth, the most extreme effect would result in the minimum amount

725 of growth. Whereas, in combinations of stressors that each increased growth, the most extreme

726 effect would result in the maximum amount of growth.

727 $$E3_{rescaled} = \frac{E3}{|\max(w_A DA_{BC}, w_C DA_{AC}, w_C DA_{AB}) - w_A DA_{BC} - w_B, DA_{AC} - w_C DA_{AB}|}$$

Table S1. Research articles included in our study. The author(s), publication year, habitat type, species, unique stressors, response

variable, net interactions reported, and interactions identified by RBI are provided, including the emergent and net

interactions. Growth responses vary in "units reported" as some are growth rates determined by change in population size or mass

divided by experimental days ("growth rate"). Other growth responses are raw population size or mass at the conclusion of the

experiment ("growth"). When reporting interactions, we list the type of interaction followed by the number of instances for that

interaction type in that study (interaction type: number of instances).

habitat	source	species	stressors	responses (units reported)	interaction reported (net)	interaction by RBI (emergent)	interaction by RBI (net)
Estuary	(Gobler *et al.* 2018)	*Menidia berylina*	pCO$_2$, temperature, food limitation	growth (mm), survival (%)	Synergy: 2	Additive: 2	Antagonism: 2
Freshwater	(Boone 2008)	*Rana clamitans*	carbaryl, malathion, permethrin	growth (g)	Synergy: 1	Synergy: 1	Additive: 1
	(Buck *et al.* 2012)	*Rana cascadae*	carbaryl, *Batrachochytrium dendrobatidis, Pseudacris regilla*	growth rate (mg/d)	Additive: 1	Additive: 1	Antagonism: 1
	(Chen *et al.* 2008)	*Simocephalus vetulus*	triclopyr, pH, food availability	survival (%)	Additive: 1	Additive: 1	Antagonism: 1
	(Chen *et al.* 2004)	*Simocephalus vetulus*	herbicide, pH, food availability	survival (%)	None: 2	Synergy: 2	Antagonism: 2
	(Davis *et al.* 2018)	*Agapetus fuscipes, Silo pallipes*	sediment level, phosphorus, nitrogen	growth (individuals/mesocosm)	None: 6	Additive: 1, Antagonism: 3, Synergy: 2	Antagonism: 6
	(De Coninck *et al.* 2013)	*Daphnia magna*	parasites, carbaryl, carbaryl pre-sensitivity	survival (%)	Synergy: 1	Additive: 1	Additive: 1
	(Dinh *et al.* 2016)	*Coenagrion puella*	temperature, food limitation, chlorpyrifos	growth rate (ln(final mass)-ln(initial mass))/days)	Synergy: 1	Additive: 1	Synergy: 1

|						
(Hasenbein *et al.* 2018)	*Hyalella azteca*	salinity, temperature, bifenthrin	survival (%)	None: 6	Additive: 5, Antagonism: 1	Antagonism: 3, Synergy: 3
(Hatch & Blaustein 2000)	*Rana cascadae*	pH, nitrate, UVB	survival (%)	Additive: 4	Additive: 4	Additive: 2, Antagonism: 2
(Hintz *et al.* 2019)	*Physella acuta*, *Helisoma trivolvis*	nutrients, predator presence, non-invasive snails	growth (g)	Additive: 1, None: 1	Additive: 1, Synergy: 1	Antagonism: 2
(Houde *et al.* 2019)	*Oncorhynchus tshawytscha*	salinity, temperature, hypoxia	survival (count)	None: 2	Additive: 2	Antagonism: 2
(Jansen *et al.* 2011)	*Daphnia magna*	carbaryl, *Pasteuria ramosa*, predation threat	survival (%)	Additive: 1	Synergy: 1	Antagonism: 1
(Janssens & Stoks 2013)	*Ischnura elegans*	food limitation, chlorpyrifos, temperature	growth rate ((ln(final mass)-ln(initial mass))/days)	Additive: 2	Additive: 2	Additive: 2
(Manzi *et al.* 2020)	*Daphnia longispina* x *galeata* hybrids	temperature, low food quality, parasite infection	growth rate (per capita rate of increase per day)	None: 2	Additive: 2	Additive: 1, Antagonism: 1
(Op de Beeck *et al.* 2018)	*Ischnura elegans*	temperature, CPF, density	survival (proportion intact dead larvae per mesocosm)	None: 2	Synergy: 2	Antagonism: 2
(Piggott *et al.* 2015)	*Gomphonema minutum*, *Encyonema minutum*, *Fragilaria vaucheriae*, *Gomphoneis minuta* var. *cassiae*	temperature, sediment, nutrients	growth (cells per cm^2 x1000)	None: 13	Additive: 5, Antagonism: 1, Synergy: 7	Antagonism: 13
(Relyea 2006)	*Rana clamitans*	predator presence, high pH, high carbaryl	growth rate (mg/d)	Additive: 1	Additive: 1	Antagonism: 1
(Shahid *et al.* 2019)	*Daphnia magna*	food limitation, Esfenvalerate, Prochloraz	survival (%)	Synergy: 5, None: 5	Additive: 4, Antagonism: 4, Synergy: 2	Antagonism: 1, Synergy: 9

Marine	(Alguero-Muniz *et al.* 2016)	*Aurelia aurita*	pH, temperature, oxygen availability	survival (%)	Additive: 10	Synergy: 10	Antagonism: 10
	(Andrew *et al.* 2019)	*Phaeocystis antarctica*	temperature, light, iron	growth rate (μ)	None: 1	Additive: 1	Additive: 1
	(Blake & Duffy 2016)	*Zostera marina*	shade, temperature, grazers	growth (g)	Additive: 2	Additive: 2	Additive: 1, Antagonism: 1
	(Büscher *et al.* 2017)	*Lophelia pertusa*	elevated CO_2, temperature, low food availability	survival (% per day)	Antagonism: 1	Synergy: 1	Antagonism: 1
	(Castro-Sanguino *et al.* 2017)	*Halimeda heteromorpha*	light exposure, herbivory, nutrient enrichment	growth (new segment count)	None: 4	Synergy: 4	Antagonism: 4
	(Dineshram *et al.* 2016)	*Crassostrea gigas*	temperature, reduced salinity, pH	survival (%)	Additive: 1	Additive: 1	Additive: 1
	(Gamain *et al.* 2018)	*Zostera noltei*	temperature, pesticide mixture, copper	growth rate ((ln(final biomass)-ln(initial biomass))/days)	None: 1	Additive: 1	Synergy: 1
	(Gil *et al.* 2016)	*Porites rus*	nutrient enrichment, sedimentation, overfishing	survival (% live tissue cover per colony)	Additive: 2	Synergy: 2	Antagonism: 2
	(Gobler *et al.* 2018)	*Menidia beryllina*	diet, pCO_2, temperature	survival (%)	Synergy: 1	Additive: 1	Synergy: 1
	(Hoadley *et al.* 2016)	*Symbiodinium trenchii*	temperature, nutrients, pCO_s	growth (cells cm^{-2})	Additive: 1	Additive: 1	Antagonism: 1
	(Maulvault *et al.* 2019)	*Diplodus sargus*	temperature, triclosan exposure, acidification	growth (cm)	None: 1	Additive: 1	Additive: 1
	(Oliver *et al.* 2019)	*Crassostrea gigas*	Imidacloprid, handling, air exposure	survival (%)	Additive: 1	Synergy: 1	Antagonism: 1
	(Przeslawski *et al.* 2005)	*Dolabrifera brazieri, Bembicium nanum, Siphonaria denticulata*	temperature, salinity, light	survival (proportion)	None: 16, Synergy: 8	Synergy: 24	Antagonism: 24
	(Kriegisch *et al.* 2019)	*Ecklonia radiata*	nutrient, sediment, density	growth (% cover)	Additive: 1	Synergy: 1	Antagonism: 1

	(Vasquez *et al.* 2015b)	*Limulus polyphemus*	temperature, salinity, oxygen	survival (%)	None: 8	Additive: 6, Antagonism: 2	Additive: 1, Antagonism: 4, Synergy: 3
	(Vasquez *et al.* 2015a)	*Limulus polyphemus*	temperature, oxygen, H_2S	survival (%)	None: 1	Additive: 1	Additive: 1
	(Vasquez *et al.* 2017)	*Limulus polyphemus*	temperature, salinity, oxygen	survival (%)	None: 12	Additive: 9, Antagonism: 3	Additive: 3, Antagonism: 5, Synergy: 4
	(Vieira *et al.* 2020)	*Zostera noltei*	nutrients, sediment, density	growth (shoot density per corer)	Synergy: 1	Synergy: 1	Additive: 1
Terrestrial	(Bednarska & Laskowski 2009)	*Pterostichus oblongopunctatus*	temperature, Chlorpyrifos, nickel	survival (%)	Synergy: 2	Antagonism: 1, Synergy: 1	Synergy: 2
	(Dyer *et al.* 2003)	*Spodoptera frugiperda*	Piplartine, 4`-desmethylpiplartine, cenocladamide	growth (mg)	None: 1	Synergy: 1	Synergy: 1
	(Janssens *et al.* 2017)	*Lestes viridis*	egg temperature, larval temperature, previous esfenvalerate concentration	survival (%)	None: 1	Additive: 1	Antagonism: 1
	(Morgado *et al.* 2016)	*Porcellionides pruinosus*	Chlopyrifos, Mancozeb, soil moisture	survival (%)	None: 2	Additive: 1, Synergy: 1	Additive: 1, Synergy: 1
	(Relyea 2006)	*Rana catesbeiana, Rana clamitans*	predator presence, high pH, carbaryl	survival (%)	Additive: 2	Additive: 1, Synergy: 1	Antagonism: 2
	(Stevens & Gowing 2014)	*Anthoxanthum odoratum*	clipping, *Plantago lanceolata, Prunella vulgaris*	growth (g)	Additive: 1	Antagonism: 1	Additive: 1
	(Ward *et al.* 1995)	*Meleagris gallopavo* f. *domestica*	dietary copper, water copper, Coccidiosis infection	survival (%)	Additive: 4	Antagonism: 2, Synergy: 2	Antagonism: 4
	(Wilsey 1996)	*Stipa occidentalis*	CO_2, clipping, urea treatment	growth (g/plot)	Additive: 3	Antagonism: 1, Synergy: 2	Additive: 1, Antagonism: 2
	(Wong *et al.* 2015)	*Spartina maritima*	nutrient availability, inundation, soil type	survival (%)	None: 1	Synergy: 1	Synergy: 1

734

735   **Supplementary Figures**

736

737   **PRISMA 2009 Flow Diagram**

738

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┌──────────────┐
│              │   ┌─────────────────────────┐   ┌─────────────────────────┐
│ Identifica-  │   │ Records identified through│  │ Additional records       │
│   tion       │   │ database searching        │  │ identified through other │
739             │   │ (n = 72,340)              │  │ sources (n =0)           │
│              │   └─────────────────────────┘   └─────────────────────────┘
└──────────────┘
740
```

741   Records after duplicates removed
                 (n = 69,062)

742

```
┌──────────┐
│          │
│Screening │
743         │
│          │          Records screened        Records excluded
744         │          (n = 69,062)            (n = 48,150)
│          │
└──────────┘
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745

746

```
┌───────────┐
│           │       Full-text articles        Full-text articles
│Eligibility│       assessed for eligibility  excluded by criteria
747         │       (n = 20,912)              given in methods
│           │                                 (n = 20,833)
748         │
│           │
└───────────┘
```

749   Studies included   Studies excluded
                    before data filtering   through data filtering
                    (n = 79)   (n = 34)

750

```
┌──────────┐
│          │
751         │
│ Included │       Studies included in
│          │       quantitative synthesis
752         │       (meta-analysis)
│          │       (n = 45)
753         │
└──────────┘
```

754

Figure S1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-

Analyses) (Moher *et al.* 2009) Flow Diagram. Using the *Web of Science* database, 45 out of

20,912 studies (records) were identified and included in our meta-analysis, resulting in 151

unique interactions.

759

760
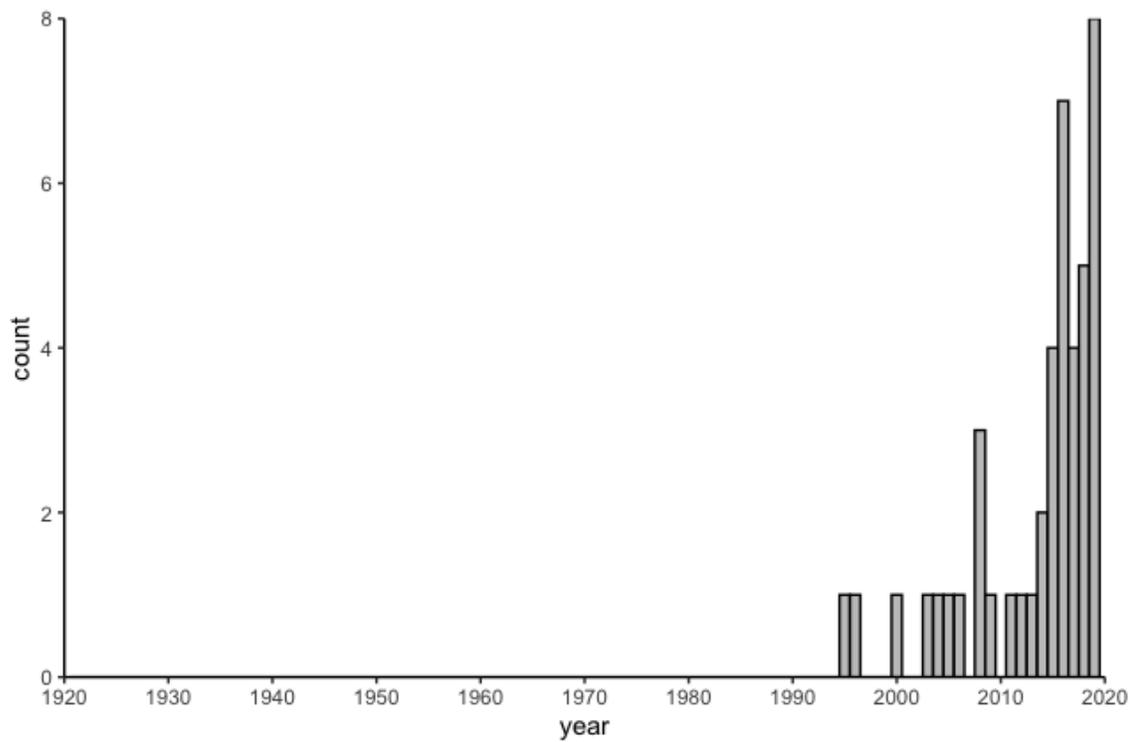
761

762 **Figure S2. The number of qualifying three-stressor studies that examined growth,**

763 **mortality, or survival at the population level between January 1920-November 2020, by**

764 **year.** Across a 100-year timespan, we identified 45 unique papers that were conducted in a

765 factorial design that fit our data quality requirements needed for RBI. Most qualifying studies

766 were conducted from 2016 to 2020 ($n=24$).

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