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PERSPECTIVE

Synergy or antagonism—interactions between stressors on coral reefs

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Abstract Throughout the coral reef scientific literature, there are many examples where the words 'synergy' and 'synergism' are being misused, particularly in the area of study involving interactions between physical stressors. This Perspective discusses the concept of synergy and more generally, interactions; summarises the tools available for detecting and interpreting interactions, including the use of ANOVA, generalized linear models, classification and regression trees and isobolographic analysis; and critically examines specific areas of the scientific literature where synergy has been reported. The aim is to promote further discussion of this topic, avoid future misuse of the term, and assist future experimental design and research into this subject.

Keywords Synergy · Stressors · ANOVA · Isobologram · Coral reefs · Interactions

Defining synergy

From a simple perspective, the dictionary definition of 'synergy' is: "the working together of two things to produce an effect greater than the sum of their individual effects" (Princeton University Wordnet http://wordnet.princeton.edu) or the "combined effect of drugs, organs, etc., that exceeds the sum of their individual effects" (Concise Oxford Dictionary).

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Consider a scenario where there are two physical factors, the effects of which on a biological organism are being examined. Each factor on its own produces a response. When the organism is subjected to both factors simultaneously, the response can either be a simple addition of each individual response, or there may be an interaction. This interaction can result in a synergism or its negative counterpart, an antagonism. The former represents an amplification of the simple additive effect whilst the latter results in a reduction.

Synergy and epidemiology in the 1970s and 1980s

In a scientific context, both 'synergy' and 'interaction' and the relationship between the two terms were extensively discussed by epidemiologists during the late 1970s and early 1980s. These earlier discussions are a useful starting point for examining its use in the context of coral reef science today.

A paper by Rothman (1976) started a lively debate about the definition of these terms, a major conceptual area of epidemiology about which there was still some confusion. Blot and Day (1979) suggested that the word "synergy" should apply only to situations where joint exposure resulted in more than a simple additive effect. At the same time, they noted that an effect could be synergistic even though each factor induced the effect through a different (or independent) biological process. By 1980, Rothman et al. (1980) had proposed four broad contexts within which an interaction could be evaluated. Two of those are relevant here: the statistical context and biological context. The former they defined as "the interdependence between the effects of two or more factors within the confines of a

given model of risk". The latter as "the interdependent operation of two or more causes to produce disease".

Siemiatycki and Thomas (1981) further examined the concept of synergism from a statistical and biological standpoint, pointing out that hitherto a departure from additivity was used to imply that there was biological interaction between the factors. They too distinguished "statistical" from "biological" interactions. In the former case, if an additional parameter was required to describe adequately the risk of joint exposure, then this represented a statistical interaction. This may be illustrated in the simplest form by an algebraic model, such that a purely additive effect is represented by:

$$Y = x\mathrm{A} + y\mathrm{B} + \text{error} \quad (1)$$

where Y is the response and the two factors are represented by A and B.

Whereas an interaction (synergism or antagonism) is shown by the inclusion of an additional parameter term:

$$Y = x\mathrm{A} + y\mathrm{B} + z(\mathrm{A} \times \mathrm{B}) + \text{error}. \quad (2)$$

From the biological standpoint, Siemiatycki and Thomas defined factors as independent if the qualitative nature of the mechanism of action of each is not affected by the presence or absence of the other. Otherwise, they will be said to be biologically interactive.

In Pharmacology, by 1989, the terminology of interactions had been narrowed down to three classes: zero interaction, in which the effect of a combination is that expected from the response of each factor individually; synergy, in which the effect is greater than expected; and antagonism in which it is less (Berenbaum 1989). Here, 'zero interaction' equates with the concept of additivity. In this review, Berenbaum also expressed concern that the analysis of such interactions was in a confused state.

Detecting and interpreting statistical interactions

There are a number of different approaches for examining interactions which are relevant here. I will first deal with classical statistical techniques, of which the analysis of variance (ANOVA) is the foremost example, followed by the use of generalized linear models (GLM) and classification and regression trees (CART). Finally, I will discuss the use of isobolographic analysis.

ANOVA

Almost without exception, the analysis of interactions in coral reef publications to date has involved the use of factorial ANOVA, whereby a linear model describes the relationship between the predictor variables (factors) and the response variable. A comprehensive treatment of factorial ANOVA can be found in Underwood (1997) so only a brief discussion of the specific use of ANOVA for interpreting interactions will follow.

In the simplest situation where there are only two factors, A and B, there will be three null hypotheses; the *first* that there is no difference between levels of factor A independent of factor B; *secondly* that there is no difference between levels of factor B independent of factor A; and *thirdly* that differences among levels of factor A (if they exist) are independent of differences among levels of factor B (if they exist). This third null hypothesis is the potential 'interaction' between the two factors and must be considered first. Rejecting this third null hypothesis demonstrates an interaction between the two factors. The other two hypotheses are logically analysable only if there is no interaction.

If the interaction term is significant, this can involve an amplified effect (synergism) or a reduced effect (antagonism). The test statistic alone will not reveal this, and simple plots of the means against each factor should be used. Figure 1 shows three possible outcomes. In Fig. 1a, the two lines are parallel and there is no interaction, furthermore, each factor causes a significant increase in the dependent variable (the ANOVA main effect terms). In Fig. 1b, there is an interaction which is synergistic and in Fig. 1c, there is again an interaction, which is antagonistic.

Once again, it is necessary to reiterate that if the interaction term is significant interpretation of the main effects from the ANOVA (hypothesis 1 and 2) is impossible or at best unreliable (the reason that the main effects are not reported in Fig. 1). To examine these main effects, *a posteriori* analysis using a multiple comparison test such as Student–Newman–Keuls (SNK) is run for each factor comparing the means of one factor separately at each level of the other factor and vice versa. It is important to note, however, that the multiple test only adjusts for alpha within each analysis and an additional adjustment to control for Type I errors must be made to reflect the total number of separate tests. In the simplest case with two factors and two levels, there are four separate multiple comparisons requiring the alpha value to be further adjusted by $\alpha/4$. Thus, at the normal $\alpha = 0.05$, significance would be judged where $P < 0.0125$ in any of the SNK results.

This use of ANOVA to detect and interpret a synergism or antagonism relies upon several assumptions being met. One of these is that the sampled means are approximately normally distributed, although ANOVA is very robust to most violations of this requirement in large experiments or where variances are similar and sample sizes are the same. Another is that the response is linear over the range being examined; however, biological responses to stressors are frequently non-linear. Care must therefore be exercised in

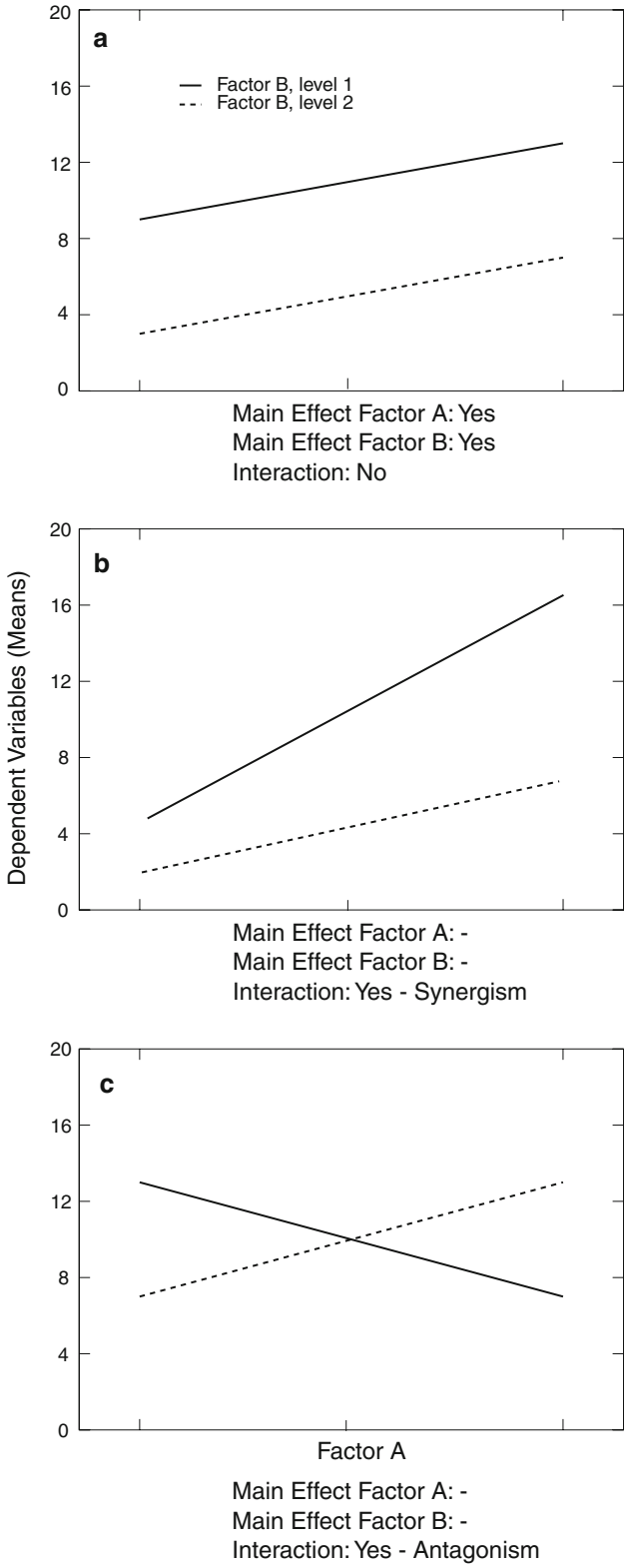

Fig. 1 Examples of interaction plots and their interpretation. In (**a**) there is no interaction, in (**b**) there is a synergy, and in (**c**) an antagonism. Main effects in (**b**) and (**c**) are not reported because of the significant interaction (see text)

using and interpreting ANOVA, and prior knowledge of the dose/concentration–response relationship is invariably advisable. Caution is also required where data transformations are required in order to satisfy the assumptions of normality or homoscedasticity, since such transformations can alter the underlying model; an illustration of this problem is given in Billick and Case (1994).

Generalized linear model

An alternative method of analysis where the assumptions of ANOVA cannot be met is the generalized linear model (GLM), allowing the evaluation of cases where the dependent variable is non-normal (e.g. a binomial distribution, or a Poisson distribution) or where the relationship is non-linear. The GLM is simply a more generalised version of the general linear model of which the ANOVA is a special case. Thus, instead of a least squares fitting of a linear relationship, the GLM normally uses a maximum likelihood estimation method to fit the response variable via a specified link function (e.g. Log, Inverse) that encompasses the non-linearity. Interpretation and investigation of interaction terms follows a similar protocol to ANOVA.

Classification and regression trees (CART)

Classification and regression trees (Breiman et al. 1984) are a more recent development for modelling non-linear interactions involving several different variable types (categorical or numeric). The CART methodology uses a tree building algorithm to determine a set of 'if–then' logical conditions which repeatedly partition the data according to the predictor variables (e.g. physical stressors) into a nested series of mutually exclusive groups, each as homogeneous as possible with respect to the response (e.g. coral bleaching). The branching structure of the resulting decision tree reveals non-additive (synergistic) effects, and the primary splits represent the most important stressor as well as indicating the best competitive stressors that show similar classification power (Olden et al. 2008). CART methodology is particularly well suited for data mining tasks where there is little a prior*i* knowledge nor any coherent set of theories or predictions regarding which variables are related and how. There is also no implicit assumption that the relationship between the predictor variable and the response is linear (c.f. ANOVA) or follows some specific non-linear link function (c.f. GLM), and is unaffected by data transformations.

A comprehensive explanation and illustration of the use of CART in the context of coral reef research is contained

in an analysis of survey data of soft coral taxa and physical and environmental information by De'ath and Fabricius (2000). In this case, they demonstrated its use on a complex and large ecological dataset. Further extensions of this technique are the multivariate regression tree (MRT) (De'ath 2002) and the aggregated boosted tree (ABT) (De'ath 2007) both of which have been demonstrated in a biodiversity study of octocorals (Fabricius and De'ath 2008).

Isobolographic analysis

In the fields of pharmacology, toxicology and pathology, a well established and frequently used qualitative method for assessing the nature of interactions is the isobologram (Tallarida 2001). This is a refinement of the dose–response graph, which now includes two or three factors rather than just one. The advantage of this technique is that it makes no assumptions about the shape of the single factor dose–response curve (Nelson and Kursar 1999). Furthermore, it is a generally valid procedure for analysing interactions irrespective of their biological mechanisms of action (Berenbaum 1989). In this respect, it thus avoids any dispute over the choice of a valid null model for a parametric statistical test as occurred in Pennings (1996) and Hay (1996). Because it is mechanism free, the isobologram focuses first on detecting interactions and then allowing the mechanisms to be deduced. Examples of isobolographic analyses have occasionally appeared in plant studies (Nelson and Kursar 1999), and for marine sponges (Jones et al. 2005). Nelson and Kursar (1999) have given a wider discussion of the advantages of this type of analysis over ANOVA and illustrations of its use.

In this technique, a traditional dose–response (or concentration–response) relationship is constructed by experimentally measuring the response at a variety of doses or concentrations of the factors both individually and in combination. Thus, to achieve a detailed relationship requires a number of measurements.

In a two-factor isobologram, the X and Y axes of a two-dimensional graph are the dose axes of each individual factor. The points on the graph represent the combination of the two factors that are isoeffective for a given response (for example 50% bleaching). If the factors do not interact, the points will form a straight line as shown in Fig. 2. When factors are more effective than expected from their individual response curves, smaller amounts of each are required to produce the same effect and the line of points will fall below and to the left of the zero interaction line. Conversely, where there is antagonism the line of points will be above and to the right. The degree of deviation from the straight line can be used as a measure of the degree of

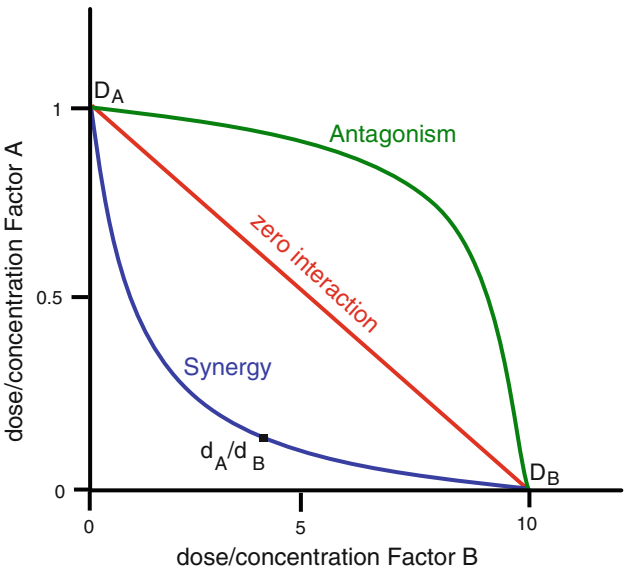

Fig. 2 An isobologram showing the line of zero interaction, and an example of a synergy and antagonism. The interaction index (see Eq. 3) for the synergy line shown is 0.5

synergism or antagonism, the 'interaction index' (Tallarida 2002):

$$\text{Index} = d_A/D_A + d_B/D_B \quad (3)$$

where d_A, d_B is a point on the graph, and D_A and D_B are the respective doses of each factor (A and B) in the absence of the other such that the index for a zero interaction = 1; synergism < 1; antagonism > 1. For further techniques of statistical analysis of the isobologram, see Nelson and Kursar (1999) and Tallarida (2000, 2002) and the use of the software PharmTools Pro (2009).

There is also the possibility of a more complex interaction, such as a synergy at one dose combination and an antagonism at another. In this case, the isobole will take on a more complex shape, such as a wave-like form, crossing the zero interaction line at one or more points. A three-factor interaction may also be represented in a similar manner to the two factor except that here the isobole surface will be represented by a flat plane in a three dimension plot.

Experimental design for constructing an isobologram

In practice, it may be convenient to run preliminary experiments to determine the dose/concentrations for each factor which produce the desired effect (e.g. 50% bleaching) thus avoiding out of range dose/concentrations. When the main experiment has been designed and run for sufficient combinations of the factors, the isoeffective points can be interpolated from the results and used to plot the isobologram. Inevitably, this will mean that a number of

experimental combinations will be required to generate each point on the isobole in order to achieve an accurate interpolation (see Tammes (1964) for a brief illustration of the technique).

Examples of synergies reported in the coral reef literature: errors and confusion

In this section, reference will be made only to the primary sources in the coral reef literature where experimental evidence of synergism was presented. Publications which hypothesise or simply re-iterate these primary sources are not included. This is also not intended to be a comprehensive review although the more important instances are covered.

In probably the first investigation into the interactions of temperature, salinity and light on coral survival, pigmentation, ^{14}C fixation and growth (Coles and Jokiel 1978), the authors titled their paper "Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*". This paper is often cited as evidence of a synergism. Clearly though, from an examination of the experimental results and the text, these authors were misusing the term synergy to mean 'combined effect', for no evidence or analysis of an interaction or synergy was presented.

As interest in coral bleaching grew during the late 1980s/early 1990s, the prospect of a synergy between light and temperature as causative factors began to be investigated. For a minor mass bleaching of corals in Tahiti, Drollet et al. (1994) analysed a variety of physio/chemical factors, stating in their Abstract that "The results are consistent with a synergistic interaction between temperature and UV-B radiation, possibly associated with total solar irradiance". However, once again no evidence or analysis of an interaction was presented.

In 1988, Lesser et al. (1990) conducted an experiment on a coral reef anthozoan (*Palythoa caribaerum*) under a regime of differing temperature, irradiance and ultraviolet (UV) radiation (three factors, two levels of each) and analysed changes in zooxanthellae numbers, chlorophyll, UV absorbing compounds, protein, and enzyme activity using a three-way ANOVA. For the enzyme catalase in zooxanthellae the authors reported a significant interaction between irradiance and temperature ($P = 0.048$); for UV absorbing compounds based on dry weight, an interaction between irradiance and temperature ($P = 0.047$), and between irradiance and UV ($P = 0.045$); and for animal superoxide dismutase (SOD) between irradiance and UV ($P = 0.02$). A posteriori analyses conducted here (with the adjustment for six separate tests: $\alpha = 0.05/6 = 0.0083$) show that the increased temperature (26–31°C) caused a synergistic increase in zooxanthella catalase activity when both UV was present and irradiance was high (1,700 μmol m^{-2} s^{-1}). Similar analyses, also made here, of the UV absorbing compounds and animal SOD interactions reveal no significant differences for any factor. The authors, however, reported an additional 'synergy' for a non-significant interaction (temperature and UV) on UV absorbing compound concentrations, other 'significant' effects when their own analyses showed the contrary, and also incorrectly used main effect terms where there had been significant interactions. Their discussion is not surprisingly confused and erroneous.

In 1992, but not reported until 2000, D'Croz and Mate (2000) conducted an experiment on *Pocillopora damicornis* in which colonies were first bleached at high temperature (30.76°C) and then monitored in recovery under two temperature regimes (28.7 and 30.3°C) and two UV regimes (natural UV present or absent). Of the several responses, they found one significant interaction between UV and temperature (two-way ANOVA interaction term $P = 0.038$) whereby the recovery of zooxanthellae numbers in the bleached corals was suppressed. However, it is difficult to evaluate the robustness of this finding in the light of several factors: (1) the data seems to have been log transformed prior to analysis (presumably to satisfy the assumptions of ANOVA); (2) no a posteriori tests were conducted to identify the respective effects of temperature and UV; (3) and finally the authors reported that "corals under heated treatments remained bleached during these 48 days of additional exposure to elevated water temperature, and polyp condition steadily deteriorated during this time". In biological terms the conditions at the elevated recovery temperature seems to have been pathological regardless of the presence or absence of UV. Possibly with more appropriate analysis a clearer picture could have been obtained.

In 2004, the first indication of a true synergism between light and temperature for photoinhibition in corals arose by chance and was unnoticed at the time of an experiment by Bhagooli and Hidaka (2004). The corals *Stylophora pistillata* and *Playtgyra ryukyuensis* were subjected to different temperature (26, 32, 34°C) and light stresses (0, 110, 520, 1,015 μmol m^{-2} s^{-1}) and changes in the maximum quantum yield (F_v/F_m) were recorded. A three-way ANOVA (species, light, temperature) gave significant interactions for the factors temp × light ($P = 0.0302$), and several species-related interactions (species × temp $P = 0.0019$; species × light $P = 0.0001$; species × temp × light $P = 0.0028$). The authors then used a posteriori tests (Tukey HSD) on each species separately but only within each temperature treatment for different light levels. In each case, alpha did not seem to have been adjusted for the number of separate tests (three tests; corrected

$\alpha = 0.0167$). The authors reported that at irradiances below 110 μmol m^{-2} s^{-1} neither light nor temperature had any effect on F_v/F_m for *P. ryukyuensis*, but above 520 μmol m^{-2} s^{-1} at all temperatures (26, 32, 34°C) there was a significant decline, suggesting that the observed decline was most likely due to light in excess of this threshold without an interaction. *Stylophora pistillata* was more sensitive such that at 32°C and 110 μmol m^{-2} s^{-1} a significant decrease in F_v/F_m was detected, and at 34°C this also occurred in the dark treatment. The differences in this case suggest initially a temperature and light synergism and then a temperature effect alone at 34°C, the former interaction having been detected by the ANOVA. Although this is one of the few instances where synergy may have occurred for one coral species, the authors themselves did not recognise it as such, and as a result the opportunity to examine and discuss the data in this context was missed.

In an experiment specifically designed to examine "the synergistic role of solar radiation on the severity of the thermal stress response in the Caribbean coral, *Montastraea faveolata*", Lesser and Farrell (2004) subjected *M. faveolata* to different solar radiation regimes during a period when sea temperature in the treatments rose from 28 to 31°C. They commenced by introducing the reader to an earlier paper (Lesser et al. 1990) as prior evidence of a synergistic effect of irradiance (and/or UV) with temperature in causing coral bleaching, and yet that earlier study (already discussed earlier) had shown that there was no interaction between irradiance, temperature or UV on chlorophyll concentrations or zooxanthellae numbers (both of which are the proxy parameters of bleaching). In the present experiments, they noted that concurrent with the rising sea water temperature, corals in the high light treatment (up to 2,100 μmol m^{-2} s^{-1}) visibly bleached but not under low light (550 μmol m^{-2} s^{-1}) and described this in terms of "synergistic effects of irradiance and elevated sea temperatures" notwithstanding the absence of any data or analysis to support this. Their subsequent discussion claimed that "The results from the laboratory bleaching studies described here and elsewhere (Lesser 1996; Bhagooli and Hidaka 2004) clearly show that the underwater light field, both PAR (photosynthetically active radiation) and UVR, play an important synergistic role in the photoinhibition of the photosynthetic apparatus and subsequent bleaching of corals"; a statement unsupported by any evidence from this experiment or from Lesser (1996), and only loosely supported in the context of PAR from Bhagooli and Hidaka (2004). Later in their discussion, they again assert synergism deduced from the literature, in the context of reactive oxygen species (ROS): "Symbiotic cnidarians routinely experience elevated pO_2 within the host tissues (Dykens and Shick 1982), and UVR acts synergistically with sublethal temperature perturbations and physiological hyperoxia to produce ROS in host tissues (Dykens et al. 1992)". Once again examination of Dykens et al. (1992) reveals no mention or consideration of interactions or synergism.

Harrington et al. (2005) investigated the effects of the herbicide diuron and sediment on the photosynthesis of the crustose coralline algae (CCA) *Hydrolithon reinboldii*. They demonstrated that the presence of diuron in sediment treatments depressed both the photosynthetic efficiency and subsequent recovery compared to the effect of sediment or diuron alone. The authors implied that this represented a synergy and also included 'synergistic' in the title. No interaction term from the ANOVA was reported and inspection of the graphical plots reveals what might have been a synergistic effect at lower diuron concentrations at certain time points but an antagonism at the highest concentration. Also, at many time points, there was no effect for diuron alone. Overall, the results present a rather uncertain and confused outcome which the authors did not investigate.

A recent study on early life stages of soft corals (Zeevi-Ben-Yosef and Benayahu 2008) robustly claimed to demonstrate a synergistic effect of temperature and artificial UV on survival, but in fact only demonstrated the differential effects of temperature in the presence of UV without interaction. This is a good example of a fundamental misunderstanding of the meaning of synergy.

As the focus of attention has turned to the effects of ocean acidification in recent years, experiments have appeared to test relationships between elevated pH and temperature and irradiance on the bleaching of corals. Anthony et al. (2008) looked at bleaching, productivity and calcification responses of crustose coralline algae (CCA), and branching (*Acropora*) and massive (*Porites*) corals. They used a combination of two temperature ranges (25–26°C and 28–29°C) and three CO_2 dosing regimes (a control at pH 8.0–pH 8.4; and treatments at 7.85–7.95 and 7.6–7.7), all treatments and control were under an irradiance of ~1,000 μmol m^{-2} s^{-1}. The results were analysed using a two-way ANOVA. For bleaching, none of the interaction terms were significant for CCA, *Acropora*, or *Porites* (respectively $P = 0.153$, 0.237, 0.433). Notwithstanding this, the authors concluded that for bleaching in *Porites* "there was a strong synergy between CO_2 and temperature…", and also reported that "high temperature thus amplified the bleaching responses by 10–20% in CCA and *Acropora*, and up to 50% in *Porites*", thereby also suggesting a synergy between these two factors for CCA and *Acropora* as well. These are erroneous conclusions given the statistical results. They also concluded that "interestingly, for the CCA and *Acropora*, the effect of CO_2 dosing on bleaching was stronger than the effect of temperature", notwithstanding that the ANOVA main

effect for temperature on *Acropora* was not significant ($P = 0.195$). This overall misinterpretation is compounded by the fact that the Abstract specifically reports this synergism, declaring "Results indicated that high CO_2 is a bleaching agent for corals and CCA under high irradiance, acting synergistically with warming to lower thermal bleaching thresholds". Although their results gave significant interaction terms for productivity in CCA, *Acropora*, and *Porites*, and for calcification in CCA alone, the authors did not examine whether this represented a synergy or antagonism. Once again, the term 'synergy' has been misused while interaction terms in the ANOVA have been ignored or misconstrued.

Amongst these documented errors in the use of ANOVA, one paper describing the interactions between salinity and temperature stresses on *Montastraea annularis* deserves mention as an example of both the correct use and interpretation of a 2×2 factorial ANOVA. Porter et al. (1999) reported that their data met the assumptions of ANOVA before showing that a significant interaction term represented a "mitigative interactive model rather than the exacerbative interactive model"; this is a clear demonstration of antagonism.

What then are the main reasons for this lack of understanding of interactions and synergisms in coral reef research? From a reading of the literature earlier, there appear to be a number of contributory reasons: (1) sometimes due to a fundamental misunderstanding of the term 'synergy'; (2) occasionally due to straightforward misreporting; (3) frequently due to errors in the use of ANOVA, and in particular the interpretation of interaction terms and the conduct of appropriate *a posteriori* tests; (4) partly due to ignorance of the alternative techniques available to investigate synergy; and finally (5) the complexity of the experimental designs that are required to demonstrate synergisms and antagonisms.

The last point was mentioned by Reynaud et al. (2003) in their study on the "Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral", where they commented that "Numerous biotic and abiotic factors control the structure and function of marine ecosystems. Their synergistic or antagonistic interactions are poorly known because physiological responses are primarily investigated by manipulating one parameter at a time". Their own experiments at "normal" and "elevated" temperatures, and "normal" and "elevated" pCO_2, showed that calcification in the coral *S. pistillata* increased when temperature was increased from "normal" (25.3°C) to "elevated" (28.2°C) when pCO_2 was "normal", but that at "elevated" pCO_2 the increase in temperature caused a drop in calcification. A two-way ANOVA detected this interaction ($P < 0.001$), however, in their discussion they implied that the results demonstrated a synergistic interaction, and yet closer examination using interaction plots show that this was, in fact, an antagonism. This experiment also illustrates the need for prior examination of the dose–response relationship, because at "normal" CO_2 calcification was increasing up to 28°C, but this was the top of their experimental range. It has since been suggested (Silverman et al. 2009) that 28°C represented the optimum temperature for calcification in this coral, the antagonism that they observed may therefore be a special feature of this range, with calcification peaking at about 28°C and either levelling out thereafter or decreasing. Had they looked at higher temperatures they may well have detected an entirely different effect. Furthermore, there are potential issues of non-linearity which may affect the use of ANOVA even for the range examined, since calcification has been demonstrated to be 'modal' in its temperature dependency with a non-linear function either side of a maximum (Cooper et al. 2008).

Finally, although this Perspective is primarily about stressors, one study related to reefs deserves mention. Amongst the errors of analysis and interpretation highlighted earlier, it is an example where appropriate techniques were used so that robust conclusions could be reached. Jones et al. (2005) studied eight Caribbean sponges to examine whether potential chemical and physical defences operated in isolation or interacted. Using binomial data, they fitted a generalized linear model to their data and used this to construct two and three dimensional isobolograms from which they were able to demonstrate synergy in a clear and unambiguous manner. This paper, published in the mainstream marine and ecological literature shines through as an example where appropriate experimental design and analysis led to a rigorously tested scientific conclusion.

Concluding remarks

This brief foray into interactions and synergy has highlighted that there are many potential pitfalls, not least from careless use of what is a precise terminology. The reliance on ANOVA as the preferred statistical tool for investigating interactions has also led to a blinkered approach, where at the worst the underlying assumptions may have been violated; interaction terms misinterpreted; or at best there has been a failure to appreciate the necessary *a posteriori* testing required to correctly identify an antagonism or a synergy. With greater knowledge of techniques which are emerging in the field of ecology or borrowed from other fields of science, it is possible to detect and analyse data in a more robust and meaningful way. Finally, it should be remembered that statistical or mathematical evidence of an interaction is not the end-point of an investigation. To

understand the importance or indeed whether an interaction is biologically meaningful, the interaction needs to be interpreted within the context of the system being studied. Indeed, Pennings (1996) and Hay (1996) suggested that ecologists should seek first to understand the physiological mechanisms in order to predict or better comprehend the more complex interactions before embarking on experimentation and analysis. This approach has much to commend it, for it has the potential to produce a well-designed experiment and ensure the choice of the correct analytical tools for data analysis.

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