

## RESEARCH REVIEW

# A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae

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## Abstract

Marine organisms are simultaneously exposed to anthropogenic stressors with likely interactive effects, including synergisms in which the combined effects of multiple stressors are greater than the sum of individual effects. Early life stages of marine organisms are potentially vulnerable to the stressors associated with global change, but identifying general patterns across studies, species and response variables is challenging. This review represents the first meta-analysis of multistressor studies to target early marine life stages (embryo to larvae), particularly between temperature, salinity and pH as these are the best studied. Knowledge gaps in research on multiple abiotic stressors and early life stages are also identified. The meta-analysis yielded several key results: (1) Synergistic interactions (65% of individual tests) are more common than additive (17%) or antagonistic (17%) interactions. (2) Larvae are generally more vulnerable than embryos to thermal and pH stress. (3) Survival is more likely than sublethal responses to be affected by thermal, salinity and pH stress. (4) Interaction types vary among stressors, ontogenetic stages and biological responses, but they are more consistent among phyla. (5) Ocean acidification is a greater stressor for calcifying than noncalcifying larvae. Despite being more ecologically realistic than single-factor studies, multifactorial studies may still oversimplify complex systems, and so meta-analyses of the data from them must be cautiously interpreted with regard to extrapolation to field conditions. Nonetheless, our results identify taxa with early life stages that may be particularly vulnerable (e.g. molluscs, echinoderms) or robust (e.g. arthropods, cnidarians) to abiotic stress. We provide a list of recommendations for future multiple stressor studies, particularly those focussed on early marine life stages.

**Keywords:** arthropod, cnidarian, combined effect, echinoderm, meta-analysis, mollusc, multifactor

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## Introduction

Increasing atmospheric CO<sub>2</sub> has concurrently caused ocean acidification and a global increase in temperatures, as well as likely contributing to an increased frequency of extreme climate events (IPCC, 2013). By 2100, ocean temperatures in the top hundred metres are projected to increase by 0.6–2.0 °C, and the pH of the ocean's surface will have decreased by 0.1–0.4 units (IPCC, 2013). The increasing frequency of heatwaves, cyclones and other intense storms may cause localized high temperatures and pulses of rainfall and terrestrial run-off (IPCC, 2014). In addition, stressors associated with urban, industrial and coastal development will also continue to alter environmental conditions [e.g. dredging (Cooper *et al.*, 2011), nutrient enrichment

(Koop *et al.*, 2001), metal contaminants (Lewis *et al.*, 2013) and sedimentation (Risk 2014)]. Thus, marine organisms are exposed not only to natural environmental stressors, but also the compounding effects of anthropogenic stressors, notably increasing global temperatures, reduced pH and pulses of decreased salinity (Hughes & Connell, 1999; Przeslawski *et al.*, 2008; Shi *et al.*, 2008). The magnitude of the changes will vary among regions due to influence by ocean circulation, local temperature and water chemistry (e.g. Range *et al.*, 2012; Hobday & Pecl, 2013).

Early life stages of marine organisms have been recognized as potentially vulnerable to the stressors associated with climate change and ocean acidification (Pechenik, 1987; Przeslawski *et al.*, 2008; Byrne, 2011), particularly regarding their calcification and survival (Kroeker *et al.*, 2013). Meta-analyses have revealed increased sensitivity of early life stages to stressors compared to adults (Darling & Cote, 2008; Harvey

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*et al.*, 2013; Kroeker *et al.*, 2013), although this pattern is not ubiquitous among all taxa or response variables. Moreover, these analyses did not target early life stages and may have been limited by low sample sizes. Indeed, recent research suggests that juveniles and young adults of three species of Antarctic echinoderms and one species of mollusc are no more vulnerable to thermal stress than adults (Peck *et al.*, 2013), and it remains unknown if early life stages are indeed more vulnerable than adults for most taxa. Nevertheless, consideration of early life stages and multiple stressors is crucial in global change impact studies; lest conclusions show no effects when these stages actually do represent a major bottleneck for population persistence under stressful conditions (Pechenik, 1987; Byrne & Przeslawski, 2013).

Even prior to the widespread acknowledgement of anthropogenic climate change and ocean acidification, there was recognition of the need for ecological realism in experiments through multifactorial designs to facilitate a greater understanding of subtle ecological relationships, interactions and sensitivities (Kinne & Kinne, 1962). For example, if natural stressors are not considered in combination with stressors related to climate change or ocean acidification, the effects of these processes may be underestimated (Daufresne & Boet, 2007). Consideration of multiple stressors is also crucial in fishery and aquaculture studies to identify optimal conditions for production (e.g. Munari *et al.*, 2011). Interactions between stressors can be broadly classified into three types: (1) additive effects in which stressors independently affect an organism such that their combined effects are simply the sum of the individual effects (note that this includes instances when one or more stressors do not have a significant effect), (2) antagonistic effects in which one stressor offsets the effect of the other and (3) synergistic effects in which stressors interact such that their combined effects are greater than the sum of their individual effects (Folt *et al.*, 1999). The importance of multifactorial analysis has become increasingly recognized among ecologists, with 35% of climate change marine experiments conducted during 2000–2010 involving two or more stressors (Wernberg *et al.*, 2012).

Multifactorial experiments have been considered in several qualitative (Wahl *et al.*, 2011; Wernberg *et al.*, 2012; Byrne & Przeslawski, 2013) and quantitative (i.e. meta-analysis) (Crain *et al.*, 2008; Darling & Cote, 2008; Harvey *et al.*, 2013; Kroeker *et al.*, 2013; Ban *et al.*, 2014; Strain *et al.*, 2014) reviews of marine biota, although many are dominated by single-stressor studies (Wahl *et al.*, 2011; Wernberg *et al.*, 2012; Harvey *et al.*, 2013; Kroeker *et al.*, 2013). None of these, however, have focussed on the impacts of

simultaneous exposure to multiple stressors (using only multifactorial studies) on premetamorphic life stages, as we do here. See Table 1 for a list of previous meta-analyses on multiple stressors in marine environments and their key findings.

Summarizing results and identifying patterns across studies, species and diverse response variables are challenging and seem to be particularly difficult among embryos and larvae, likely due to large intra-specific variation due to maternal investment, phenotypic plasticity or genetic differences (Przeslawski & Webb, 2009); disparate methods (Byrne 2012) or the variety of metrics used to assess larval fitness (Vagner *et al.*, 2007). There is tremendous variation in the magnitude and even direction of individual and combined effects of environmental stressors on marine early life stages (Byrne & Przeslawski, 2013). The ecological realism of stressor studies has been questioned, with calls for more complex (e.g. mechanistic) and long-term experiments over multiple life stages and generations (Dupont & Pörtner, 2013). While these types of experiments are indeed very important, they are difficult to achieve with multiple stressors to identify vulnerabilities for the vast majority of marine invertebrate species in a changing ocean. When performed in an appropriate prescribed manner (see Rosenberg *et al.*, 2013), meta-analyses of data from multifactorial studies remain an important tool in modelling potential future impacts to species and marine ecosystems at broad spatial (e.g. global), temporal (e.g. decadal), phylogenetic (e.g. all marine species) or response (e.g. all sub-lethal effects) levels (Nakagawa & Poulin, 2012).

In this study, we review available literature on the effects of simultaneous exposure to multiple stressors on early life stages and perform the first meta-analysis of the results of multiple stressor studies that have targeted early marine life stages to (1) identify knowledge gaps in research on multiple stressors and early life stages of marine organisms, (2) quantify the effects of multiple stressors on early marine life stages (see hypotheses below), (3) assess whether broad patterns are detectable under the current paradigm of multifactorial design and (4) provide recommendations for future research on the impacts of environmental stress on early life stages. While quantifying the effects of multiple stressors, we tested the following simple hypotheses that have been suggested, assumed or supported by previous studies:

- 1 Synergistic interactions are more common than additive or antagonistic interactions (Harvey *et al.*, 2013).
- 2 Early embryonic life stages are less vulnerable to stress treatments than later larval stages (Byrne *et al.*, 2009; Ericson *et al.*, 2012) (e.g. due to loading of protective stress proteins in Hamdoun & Epel, 2007).

**Table 1** Compilation of quantitative meta-analyses of the effects of multiple stressors on marine organisms. Key findings include results related to multiple stressors and early life stages

Reference	Ecosystem/ taxa	Stressors	Life stages	Responses	No studies/ observations	No species	Effect size	Key findings
Crain <i>et al.</i> (2008)	Marine	Variety of abiotic and biotic, only studies with $\geq 2$ stressors	Not differenti- ated	Not differentiated	171/201	88*	Hedges <i>d</i>	Cumulative additive effects occurred in 26% of studies, synergistic in 36%, antagonistic in 38%. Interaction type varied by response level, trophic level, and stressor pair. Addition of a 3rd stressor doubled number of synergies. 1/3 of experiments showed synergies.
Darling & Cote (2008)	Marine, Freshwater, Terrestrial	Variety of abiotic and biotic, only studies with $\geq 2$ stressors	Embryo, juvenile, adult	Survival	23/112	30	In- transformed response ratio	
Harvey <i>et al.</i> (2013)	Marine	Temperature, acidification, includes single- factor studies	Embryos, larvae, juveniles, adults	Calcification, growth, photosynthesis, reproduction, survival	107/623	128†	In- transformed response ratio	Biological responses varied among taxa, ontogenic stage, trophic level. Synergistic interactions detected in all responses except growth. Combined effects were higher for juveniles than adults (calcification) and larvae than juveniles (survival).
Kroeker <i>et al.</i> (2013)	Marine	Temperature, acidification, includes single- factor studies	Larvae, juvenile, adults	Calcification, growth, photosynthesis, survival, development, abundance, metabolism	228/532	224‡	In- transformed response ratio	Biological responses varied among taxa and ontogenic stage. Negative effects of acidification are enhanced with concurrent exposure to elevated temperature. Mollusc larvae are more sensitive to acidification, but this does not apply to other taxa.
Ban <i>et al.</i> (2014)	Marine/ scleractinian corals	Temperature, irradiance, only studies with $\geq 2$ stressors	Not differenti- ated	Symbiont photosynthesis	26	Not specified	Hedges <i>d</i> §	Insufficient evidence exists to suggest synergisms are prevailing interaction type
Strain <i>et al.</i> (2014)	Marine/ algae	Variety of abiotic and biotic, only studies with $\geq 2$ stressors	Not differenti- ated	Survival, growth	118	Not specified	Hedges <i>d</i> §	Additive effects were the most common. Synergies were most common between nutrient enrichment and other stressors
Current study	Marine	Variety of abiotic ¶, Temperature/ salinity/pH **, only studies with $\geq 2$ stressors	Embryos, larvae	Survival, sublethal (growth, developmental rate, calcification, abnormality rate)	60/168¶ 54/177**	73¶ 55 **	Hedges <i>d</i>	Synergistic interactions were the most common. Responses varied among response and ontogenic stage. Early life stages of echinoderms and molluscs are most vulnerable to stress, while

Table 1 (continued)

Reference	Ecosystem/ taxa	Stressors	Life stages	Responses	No studies/ observations	No species	Effect size	Key findings
								arthropods are most resilient. Calcifying larvae are more vulnerable than noncalcifying.
*This value includes four instances of multiple species groups (listed as bacteria, phytoplankton) and excludes 42 observations from studies from which no taxonomic information is supplied.								
†This value includes eight instances of multiple species groups (listed as community, cyanobacteria, diatoms, dinoflagellates, phytoplankton or turf algae).								
‡This value includes 37 instances of multiple species groups (listed in Supplementary Material Table 1 of Kroeker <i>et al.</i> , 2013) and excludes 18 studies from which no taxonomic information is supplied.								
§Referred to as Hedges <i>g</i> (note that this is the same as Hedges <i>d</i> ).								
¶Associated with studies and individual tests included in the analysis of interaction types (does not include effects of individual stressors for individual tests).								
**Associated with studies and individual tests included in the GLM Model (includes effects of both individual and combined stressors).								

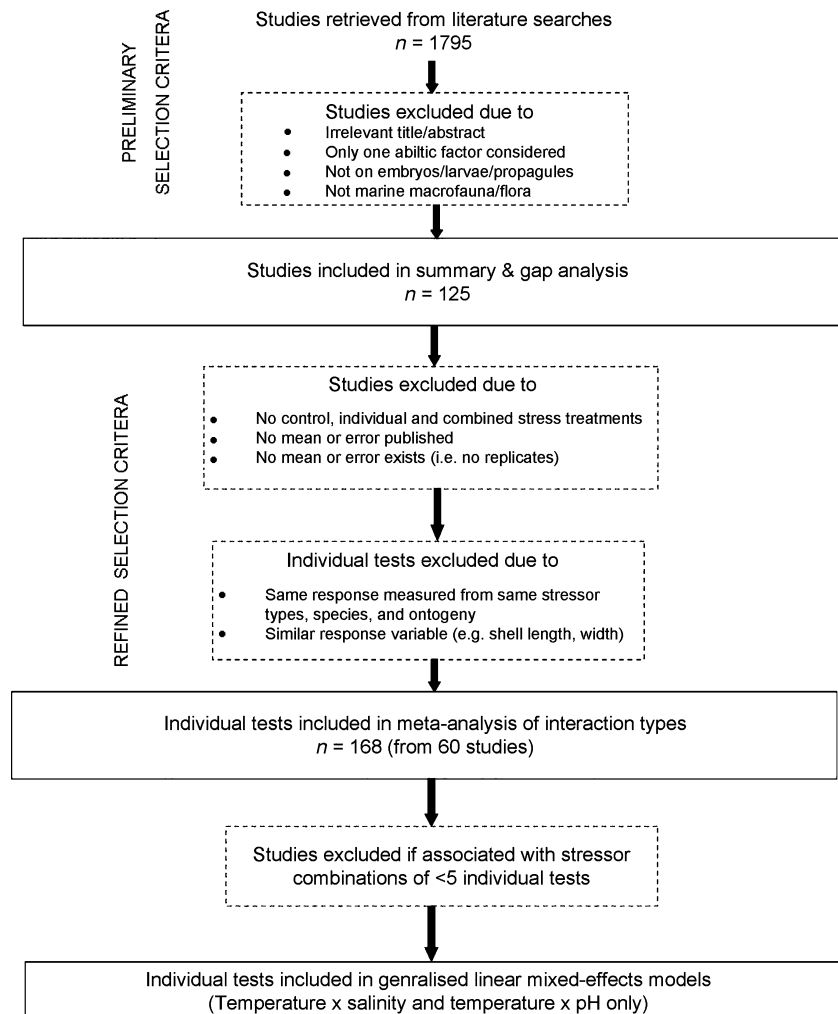
- 3 Increased  $p\text{CO}_2$ / decreased pH levels at those expected over the next 100 years are more likely to result in sublethal effects during experimental duration (Todgham & Hofmann, 2009; Beniash *et al.*, 2010; Stumpp *et al.*, 2012; Thomsen *et al.*, 2013) than temperature and salinity treatments which are more likely to result in lethal effects (Przeslawski, 2004).
- 4 Interaction types (synergistic, additive, antagonistic) between stressors differ among phyla, ontogenies and biological responses (Harvey *et al.*, 2013, Kroeker *et al.*, 2013).
- 5 Ocean acidification is a greater stressor to calcifying than noncalcifying larvae (Byrne & Przeslawski, 2013).

This review focuses on life stages of marine macrofauna and macroflora between postfertilization and settlement stages. We confined our review to premetamorphic stages due to the challenge of investigating such stages in the field and the need to understand their reactions to multiple stressors as potential bottlenecks for population success (Pechenik, 1987; Byrne, 2011). Although we consider all abiotic stressors in our gap analysis and overall meta-analysis of interaction types, our models incorporate only temperature, salinity and pH as these had the minimum number of studies needed for analysis (see Methods). This study follows on from a qualitative review on the individual and combined effects of ocean acidification and temperature on early life stages of marine invertebrates (Byrne & Przeslawski, 2013) to adopt a more quantitative and critical approach through the use of meta-analysis.

## Selection criteria and analytical methods

### Preliminary selection criteria

Literature was searched in the ISI Web of Science database using the following search terms, with root words used to expand results: embryo, larva or propagule and combined effect, interaction and multiple stressor. Search results were then confined to the topic of 'marine and freshwater biology' as defined by the database. In addition, we compiled references from reviews that focussed on the effects of environmental factors on early life stages (Pechenik, 1987; Bates, 2005; Byrne, 2011; Wahl *et al.*, 2011). All studies included in our gap analysis review were required to incorporate at least two abiotic stressors on marine embryos or larvae. Studies conforming to these preliminary selection criteria were included in descriptive statistics (see Fig. 1 for preliminary selection criteria).



**Fig. 1** Flow chart showing (1) the preliminary selection criteria used for summary and gap analysis and (2) the refined selection criteria used for meta-analysis of interaction types and generalized linear mixed-effects models. Dashed lines indicate steps in the analytical process, while solid lines indicate tests from which results are presented. Individual tests in the meta-analysis of interaction types include only combined effects, while individual tests in the GLMM include individual and combined effects.

#### *Refined selection criteria/meta-analysis*

Refined selection criteria were then applied to the studies identified by our preliminary selection criteria for consideration in the meta-analysis. To be considered, studies had to incorporate control, individual stress and combined stress treatments, as well as include both the number of individuals (or number of containers, when the response was expressed as a percentage of the individuals in the container) and the standard deviation (or standard error). From these studies, data for individual tests were extracted using published values or from relevant figures using [WEBPLOTDIGITIZER](http://www.arohatgi.info/WebPlotDigitizer) ([www.arohatgi.info/WebPlotDigitizer](http://www.arohatgi.info/WebPlotDigitizer)). Where applicable, authors were contacted directly for values missing from associated publications.

Observations were excluded if they lacked an obvious control. For experiments with three abiotic stressors, responses from each pair of stressors were used at control levels of the third stressor. Among the reported treatment combinations between stressors, we only considered those with a minimum sample size of five individual tests in each covariate level to test for covariate effect of the biological responses to individual or combined stress treatments. Due to the low number of studies in most stressor combinations, only temperature/salinity and temperature/pH combinations were included in the models (Table S1). Figure 1 shows the refined selection criteria for the meta-analysis.

A range of covariates that were related to our hypotheses and consistently available across studies were collated and coded, including the ontogenetic



stage (*Embryo* or *Larva*), the type of biological response to stress (*Sublethal* or *Lethal*), the phylum and whether their larvae calcify. Some studies incorporated multiple developmental stages within our broad ontogenic classification; for example, Arnberg *et al.* (2013) measured feeding and metabolic rates of shrimp at zoeal stages II, III, and IV, all of which encompass the larval stage. In these instances, the most intermediate stage was used (e.g. stage III in the previous example) so as to reduce potential bias in our results from those studies measuring response variables from many contiguous life stages. Where necessary, data were normalized to ensure consistency within each response. For instance, if  $p$  was the proportion of survivors, data were converted to  $1-p$  to obtain an estimate for mortality (which was most commonly reported) (Harvey *et al.*, 2013; Kroeker *et al.*, 2013). Response variables included lethal (survival, mortality, metamorphosis or settlement rates, the latter two only if nonmetamorphosed or nonsettled larvae were inferred to have died) and sublethal (abnormality, growth, development, metabolism, cellular) (Table S1). When several measurements were taken of a similar response variable (i.e. total body length, yolk length, yolk volume), we only used the most inclusive one (Kroeker *et al.*, 2010, 2013). When several observations of the same response were made with the same combination of stressors, species and ontogenies (e.g. abnormality of *Saccostrea glomerata* larvae in Parker *et al.*, 2009, 2010), we included only the most recent.

#### Effect size calculation

We calculated individual, main and interaction effect sizes for each individual test using Hedge's  $d$  (Hedges & Olkin, 1985) and following methods given by Gurevitch *et al.* (2000). We chose Hedge's  $d$  (also called Hedge's  $g$  in sociological and medical research) over other available measures of effect size because (1) it is consistent with the ANOVA model that was used in most of the reviewed studies, in which a significant interaction effect size indicates deviation from the null model of additivity (Gurevitch *et al.*, 2000); (2) it is not affected by unequal sampling variances in the paired groups and includes a correction factor for small sample sizes, thereby working well with as few as five to ten studies (Rosenberg *et al.*, 2013); and (3) it is currently the most common recommended metric for meta-analyses (Rosenberg *et al.*, 2013), thus facilitating comparisons with other studies.

Individual effects represent the response in the presence of a stressor alone relative to the control, while main effects compare the net effect of a stressor in the presence and absence of a second stressor, similar to main effects in ANOVA. Individual effect sizes of

stressors A ( $d_a$ ) and B ( $d_b$ ) were calculated with respect to the control (CT) as:

$$d_a = \frac{Y_A - Y_{CT}}{s} J(m)$$

$$d_b = \frac{Y_B - Y_{CT}}{s} J(m)$$

where  $Y$  is the average performance of the corresponding group,  $s$  is the pooled standard deviation, and  $J(m)$  is a correction term for small samples (Koricheva *et al.*, 2013). For each study, the main effects of stressor A ( $d_A$ ), stressor B ( $d_B$ ) and their interaction AB ( $d_{AB}$ ) were calculated as:

$$d_A = \frac{(Y_A + Y_{AB}) - (Y_B + Y_{CT})}{2s} J(m)$$

$$d_B = \frac{(Y_B + Y_{AB}) - (Y_A + Y_{CT})}{2s} J(m)$$

$$d_{AB} = \frac{(Y_{AB} - Y_B) - (Y_A - Y_{CT})}{2s} J(m)$$

Following Crain *et al.* (2008), we used individual effect sizes to classify studies for the identification of interaction type (synergistic, additive or antagonistic); we also inverted the sign of the interaction effect size when both individual effect sizes were positive, so that a negative interaction effect size indicated a synergy in all situations, that is irrespective of whether each individual stressor induced a positive or negative response (as informed by individual effect sizes).

We tested for publication bias (i.e. underreporting of studies showing no effects) (Jennions *et al.*, 2013) using (1) funnel plots in which the effect sizes of the temperature/salinity and temperature/pH interaction terms were plotted against the standard error (SE) and (2) a correlation (Spearman's  $\rho$ ) test between effect sizes and standard errors. No publication bias was observed in either funnel plot, as they both symmetrically showed larger variation in effect size as SE increased with no evidence for a correlation between effect size and SE (Fig. S1).

#### Generalized linear mixed-effects models (GLMMs)

We used generalized linear mixed-effect models to test the different hypotheses and predict Hedge's  $d$  as a function of individual or combined stress treatments, as well as relevant covariates. Model sets included individual and interactive stressor effects in addition to, where possible, interactions among covariates and between covariates and stressors. Models included a random effect coding for the study to account for the nonindependence of individual tests within a same study; this explicitly incorporates any heterogeneity

between within-study and among-study variances in effect sizes (Mengersen *et al.*, 2013). We assumed a Gaussian distribution and checked the normal distribution of model residuals using the normal scores of standardized residual deviance (Breslow, 1996). Model performance indices included the per cent deviance (De) in Hedge's  $d$  explained by the model, which provided an index of the model's goodness of fit, and the Akaike's information criterion (AIC<sub>c</sub>) to provide an index of Kullback–Leibler information loss that we used to assign relative strengths of evidence to the different competing models. We used the AIC<sub>c</sub> weights (wAIC<sub>c</sub>) to generate weighted-average model predictions of main and interaction effect sizes under different covariate levels. A total of 54 studies representing 55 species relating to temperature/salinity and temperature/pH stress were included in the GLMMs.

### Gap analysis

A total of 128 studies representing 119 species met our preliminary selection criteria (Table S1). Of these, temperature was the most common factor, with 93% of studies including temperature treatments in their experimental designs. The most common combination of factors was temperature and salinity (64% of studies, 70% of species), followed by temperature and CO<sub>2</sub>/pH (23% of studies, 21% of species). Despite the ubiquity of many natural stressors (e.g. UV, predator cues) and prevalence of anthropogenic ones (e.g. pollutants), the interactions between these and temperature, salinity and pH on early life stages remain unknown for most taxa.

Since 2010, there has been a pronounced shift in multiple stressor studies towards temperature and pH (Fig. 2a), whereas studies conducted earlier focussed more on temperature and salinity, often in the context of aquaculture research instead of global change (Fig. 2b). This trend reflects emerging realization of climate change and ocean acidification as the main global threats to marine ecosystems (Wernberg *et al.*, 2012; Kroeker *et al.*, 2013). Nevertheless, the shift away from other stressors, some of which are also linked to climate change (e.g. salinity and hypoxia, see IPCC, 2014), may indicate research or publication bias with research focussed on popular and obvious stressors (e.g. temperature and pH).

Very few studies (14%) included more than two abiotic stressors, although several included biotic factors such as nutrients or parental history (e.g. His *et al.*, 1989; Li & Brawley, 2004). The inclusion of three or more stressors in an experiment can be difficult due to experimental logistics; for example, a fully orthogonal design with three replicates and three stressors involves experimental set-up and monitoring of 27

containers (Przeslawski *et al.*, 2005). In addition, three-way interactions can be extremely difficult to describe and interpret; even two-way interactions can be 'dauntingly complex' (Crain *et al.*, 2008). Despite these challenges, incorporation of three or more stressors provides more ecologically useful information to understand responses of embryos and larvae to current and predicted environmental conditions.

Most studies were undertaken using continuous stressor conditions (Table S1), but seven studies periodically exposed organisms to at least one stressor, usually to more closely reflect tidal conditions (e.g. Russell & Phillips, 2009a,b). All studies were conducted in the laboratory although four studies included complementary field experiments. Of these four studies, three found similar overall effects of stressors between field and laboratory experiments (Preston, 1985; Russell & Phillips, 2009a,b). However, Przeslawski (2005) found that desiccation in the field caused significant mortality to embryos of two gastropod species, while desiccation showed no effect on these species in the laboratory.

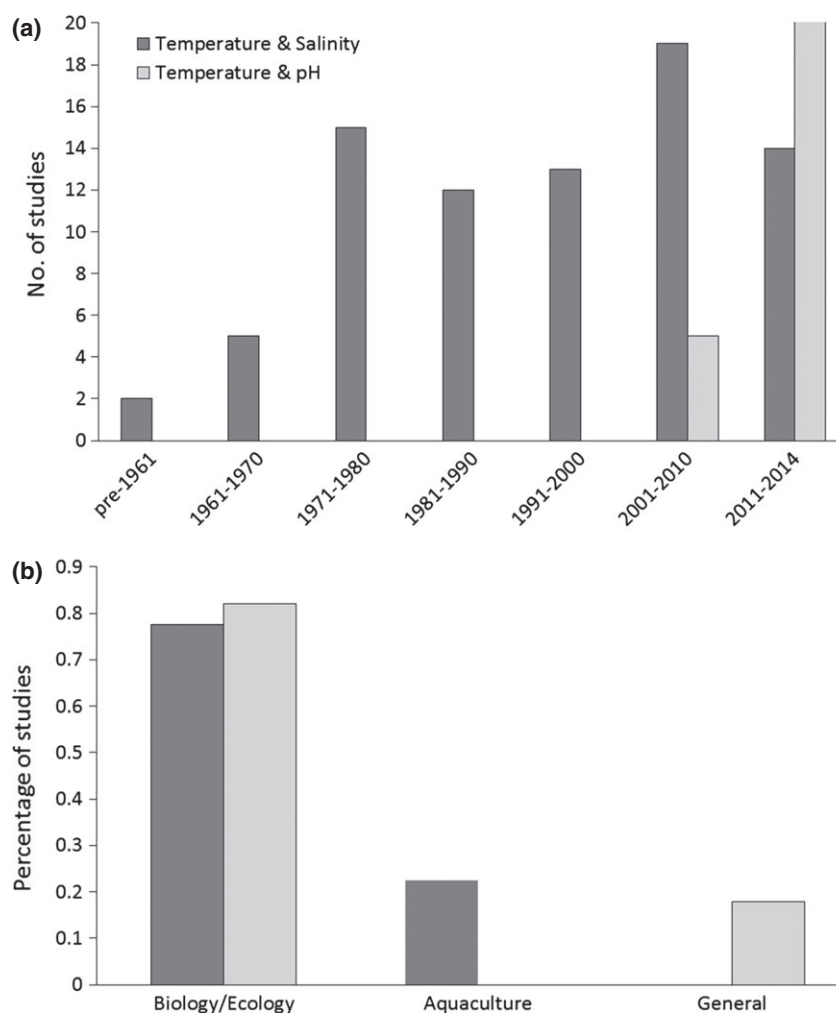
Most studies measured several dependent variables, with survival or mortality being the most common (Table S1). Molluscs were the most common species considered (42 species), followed by arthropods (34 species) and echinoderms (14 species). However, these phyla were not equally represented among stressor combinations, with molluscs and arthropods dominating temperature and salinity experiments, and molluscs and echinoderms dominating temperature and pH studies (Fig. 3a). Most study species occur in shallow waters (estuarine, intertidal, subtidal, reef), with only one known deep-sea species represented (Fig. 3b).

### Effects of multiple stressors on early life stages

The meta-analysis allowed us to investigate several hypotheses related to stress ecology and global change biology of early marine life stages. Of our five hypotheses, two were clearly supported by our results (Hypotheses A, E). The others were dependent on ontogeny or phylum or were not supported by both stressor combinations (temperature/salinity, temperature/pH). This highlights knowledge gaps on responses of embryos and larvae to stress or how responses may be species- or even cohort specific among early life stages of many taxa. Our results are presented below for each hypothesis, in relation to previous findings.

Hypothesis A: Synergistic interactions are the most common.

This hypothesis was supported by our analysis which showed that cumulative effects of any two stressors



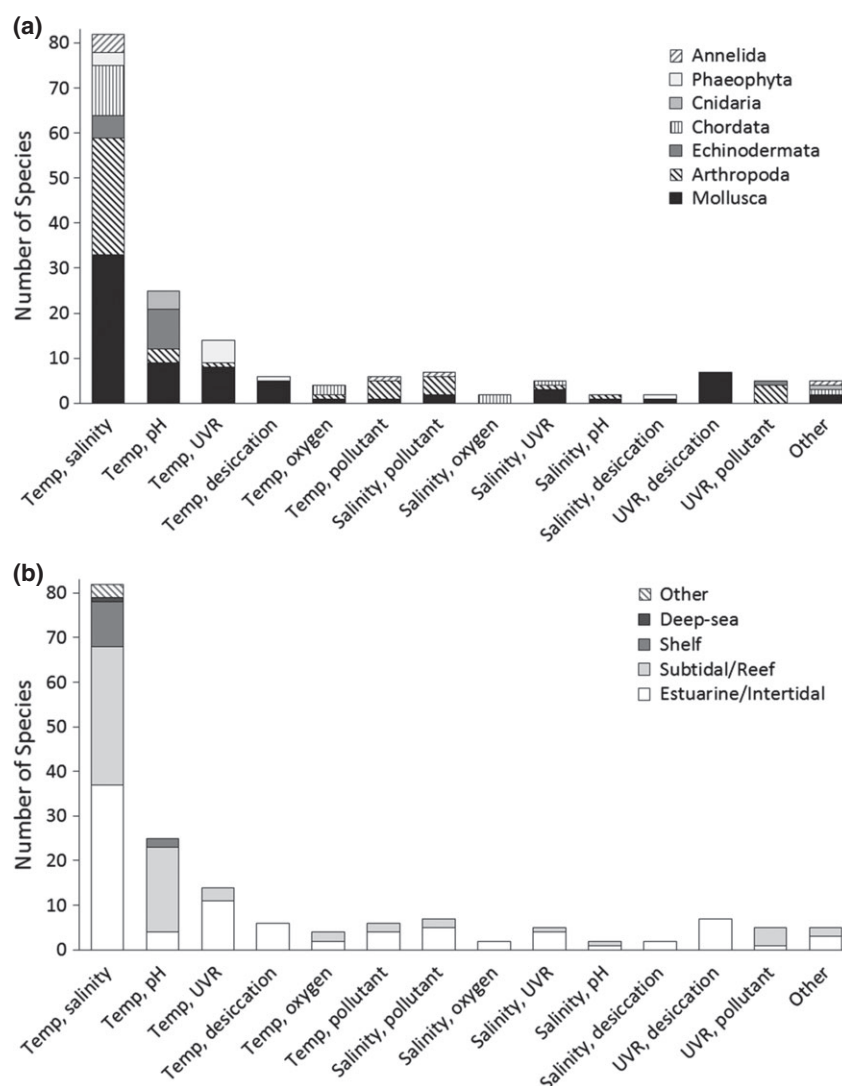
**Fig. 2** Summary of published studies on marine embryos or larvae that incorporate temperature and pH or temperature and salinity over (a) time and (b) journal focus.  $n = 25$  studies for temp/pH;  $n = 79$  studies for temp/salinity. Journal categorized as 'General' includes those not associated with a particular discipline (e.g. PLoS One).

included more synergistic interactions (65%) than additive (17%) or antagonistic (17%) interactions. Interactions between temperature and pH produced more synergisms (76%) than those between temperature and salinity (58%) (Table 2). As mentioned above, the representation of phyla was not equal between these stressor combinations (Fig. 3a). Furthermore, while temperature and pH were only represented by four phyla (cnidarian, arthropod, echinoderm, mollusc), temperature and salinity were represented by six phyla (annelid, arthropod, chordate, echinoderm, mollusc, phaeophyte). As such, it remains uncertain whether synergistic interactions are more common with temperature and pH or whether our result reflected phyla-specific interaction types.

Our results support previous results from a meta-analysis by Harvey *et al.* (2013) who found that synergistic interactions between ocean acidification and

temperature prevail in marine ecosystems. However, other meta-analyses on marine systems contradict this, with no synergies between these stressors on calcification (Kroeker *et al.*, 2013), insufficient evidence in coral reef ecosystems to suggest synergisms are the most common (Ban *et al.*, 2014) and additive effects accounting for the majority of interactions on macroalgae (Strain *et al.*, 2014). We also focussed solely on early life history stages from studies that incorporated multiple stressors, while these other meta-analyses considered all available data, including single-stressor study data and data for adults. It may be that early life stages are more likely than adults to be affected by synergistic interactions between two stressors. Disparities between the prevalence of synergistic interactions among the meta-analyses in Table 1 are likely due to differences in selection criteria, experimental protocols (selection criteria, stressor types and magnitudes, measured





**Fig. 3** Summary of stressor combinations (a) across phyla and (b) across habitat from studies compiled after preliminary selection criteria were applied.

**Table 2** Per cent of interaction types across temperature/salinity and temperature/pH

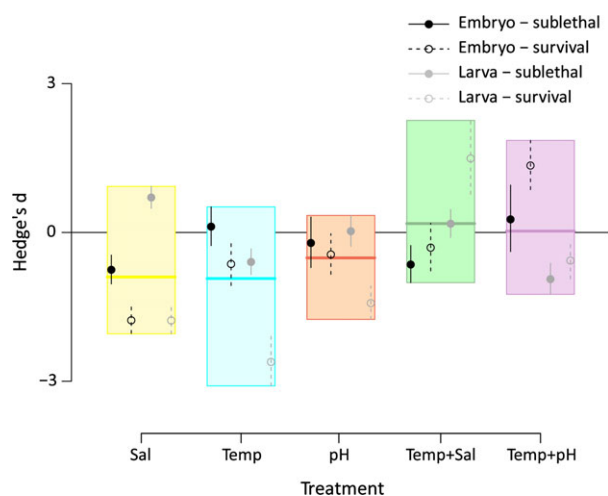
Stressor pair	Number of individual tests	% Synergistic	% Additive	% Antagonistic
Temperature–Salinity	60	58.3	18.3	23.3
Temperature–pH	71	76.1	15.5	8.5

Interaction types are based on Hedges *d* and defined by Crain *et al.* (2008)

response) as well as biological and environmental variables (ecosystem, species, ontogeny).

According to this study, elevated temperatures ameliorate negative effects of salinity on larvae and negative effects of pH on embryos, as evidenced by antagonistic interactions (Fig. 4). This finding has been noted in several studies for temperature and pH (Byrne & Przeslawski, 2013) and may be due to faster growth

and progression through developmental stages at higher temperatures thereby reducing the time in vulnerable planktonic life phase (Havenhand, 1993). In contrast, a previous meta-analysis found negative synergisms between temperature and pH on survival of early life-history stages but not sublethal responses (Harvey *et al.*, 2013). This difference may be due to species-specific differences or low sample size ( $n = 3$



**Fig. 4** Effect size (Hedge's  $d$ ) of sublethal and lethal responses of embryos and larvae based on the partial effects predicted by generalized linear mixed-effects models. Vertical lines indicate 95% confidence intervals, and horizontal lines indicate mean effect sizes for all ontogenies and responses in each treatment. For individual stress treatments, confidence intervals overlapping zero indicate no effects; those  $>0$  indicate positive effects, and those  $<0$  indicate negative effects. For combined stress treatments, confidence intervals overlapping 0 indicate additive effects; those  $>0$  indicate antagonisms, and those  $<0$  indicate synergisms.

studies of combined stressor effects for larval survival and none for embryos in Harvey *et al.* (2013)).

**Hypothesis B:** Early embryonic life stages are less vulnerable to stress treatments than later larval stages.

This hypothesis was partially supported by this study which revealed that larvae were more vulnerable than embryos to individual thermal and pH stress, but not to salinity stress (Fig. 4). Although adults may often be more resilient to stressors than premetamorphic stages (Kroeker *et al.*, 2013), our analysis did not indicate an overall increasing resilience to stress with more advanced development (prior to metamorphosis). Other studies have found that very early echinoderm embryos are more resilient to thermal and acidification stress than later embryonic stages (Byrne *et al.*, 2009; Ericson *et al.*, 2012), possibly due to the presence of protective factors loaded into eggs (Hamdoun & Epel, 2007). We were not able to incorporate the temporal progression through ontogenic stages (e.g. cleavage, blastula, gastrula) due to the paucity of studies that met our refined selection criteria. It appears that the vulnerability of early (prelarval) stages varies across taxa, cohorts and even individuals in part because maternal investment (e.g. nutrient provisions, stress protein

loading) varies across these parameters (e.g. Przeslawski & Webb, 2009; Marshall *et al.*, 2012).

**Hypothesis C:** Increased  $pCO_2$ /decreased pH is more likely to result in sublethal effects during experimental duration than temperature and salinity treatments which are more likely to result in lethal effects.

This hypothesis was partially supported by our results, with survival more likely than sublethal responses to be affected by thermal and salinity stress. In contrast to our hypothesis, lethal responses were also more likely than sublethal responses with pH stress. There were no obvious patterns in the combined treatments (Fig. 4). This may be due to the underlying variation in organism physiology and mechanisms underlying the responses to pH, temperature and salinity stress (Wittmann & Pörtner, 2013). Kroeker *et al.* (2013) also found that acidification equally affected lethal and sublethal responses of molluscan larvae.

**Hypothesis D:** Interaction types between stressors differ among phyla, ontogenies and biological responses.

This hypothesis was partially supported by our results. There were very few consistencies in interaction types among ontogenies or biological response. For example, temperature and pH negatively and synergistically interacted on larval responses but showed additive or antagonistic effects on embryos, depending on response type (Fig. 4). In contrast, most phyla showed consistent interaction types. Consistent synergistic effects were detected between temperature and salinity on molluscs and echinoderms, as well as additive effects between temperature and pH on all phyla considered (Fig. 5). Each biological response is associated with particular physiological mechanisms that can differ across developmental stages (e.g. the onset of calcification/feeding in larvae), so different interaction types across responses and ontogenies are expected (Harvey *et al.*, 2013).

**Hypothesis E:** Ocean acidification is a greater stressor to calcifying than noncalcifying larvae.

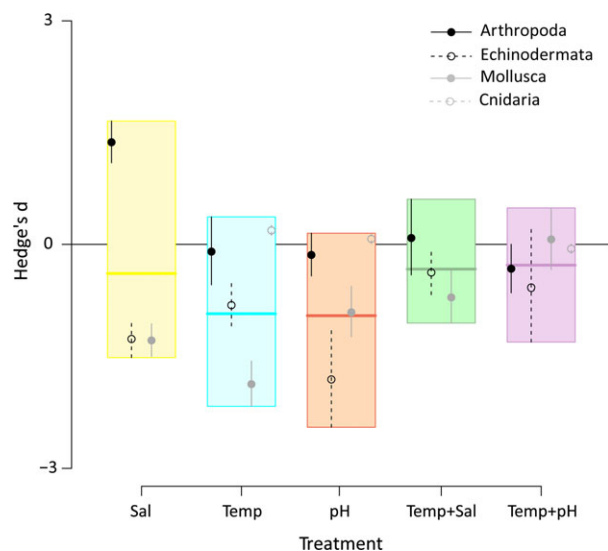
This hypothesis was supported by our meta-analysis, with calcifying larvae more vulnerable to ocean acidification than noncalcifying larvae (Fig. 6). In contrast, both embryos from species with calcifying larvae and those from species with noncalcifying larvae showed equivalent negative effects of ocean acidification, likely due to physiological effects unrelated to calcification

(Fig. 6). Negative synergistic interactions between temperature and pH occurred among noncalcifying embryos and calcifying larvae (Fig. 6).

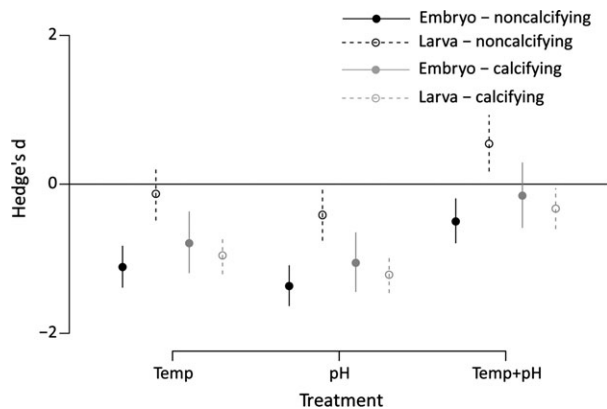
The greater vulnerability of calcifying larvae and adults compared to noncalcifying larvae has been identified in previous reviews (Byrne & Przeslawski, 2013). This is also evident in closely related sea urchins with contrasting calcifying/feeding and noncalcifying/non-feeding larvae (Hardy & Byrne, 2014). However, some calcifying larvae seem resilient to ocean acidification during larval and juvenile stages, potentially due to adaptation to naturally acidic environments (e.g. low pH sediments) (Talmage & Gobler, 2011) or due to the high buffering capacity (very high total alkalinity) of local waters (Range *et al.*, 2012).

### Predicting impacts – limitations and considerations

One of the most significant outcomes of the application of the data from multifactorial stressor experiments to meta-analyses on early life-history responses may be to inform predictions and modelling of future impacts



**Fig. 5** Effect size (Hedge's *d*) of responses of phyla based on the partial effects predicted by generalized linear mixed-effects models. Vertical lines indicate 95% confidence intervals, and horizontal lines indicate mean effect sizes for all phyla in each treatment. For individual stress treatments, confidence intervals overlapping zero indicate no effects; those >0 indicate positive effects, and those <0 indicate negative effects. For combined stress treatments, confidence intervals overlapping 0 indicate additive effects; those >0 indicate antagonisms, and those <0 indicate synergisms. Other phyla were analysed in the model but had too few observations to confidently test hypotheses related to phyla across either salinity/temperature or pH/temperature (Annelida, Chordata, Phaeophyta), while Cnidaria only had sufficient numbers to test across temperature/pH.



**Fig. 6** Effect size (Hedge's *d*) of responses of embryos and larvae from calcifying and noncalcifying species based on the partial effects predicted by generalized linear mixed-effects models. 'Embryo – calcifying' refers to embryos from species with calcifying larvae. Vertical lines indicate 95% confidence intervals. For individual stress treatments, confidence intervals overlapping zero indicate no effects; those >0 indicate positive effects, and those <0 indicate negative effects. For combined stress treatments, confidence intervals overlapping 0 indicate additive effects; those >0 indicate antagonisms, and those <0 indicate synergisms.

to marine ecosystems, particularly regarding vulnerable or resilient species. In this section, we use our results to identify potentially vulnerable and resilient taxa, compare with previous research outcomes and consider limitations with this approach.

### Vulnerable and resilient taxa

Resilient taxa will be able to cope locally or take advantage of new opportunities provided by a changing ocean (e.g. by migration, changes in competition). Previous meta-analyses (Harvey *et al.*, 2013; Kroeker *et al.*, 2013, Ban *et al.*, 2014), as well as this study, indicate it is possible to predict organisms that are resilient to temperature, salinity or pH at early life stages, albeit at a low taxonomic resolution. Arthropods are the most robust taxa as they showed no overall negative effects for individual or combined stressors (temperature, pH, salinity), and early life stages of cnidarians also seem resilient to temperature and pH stress, although we could not test salinity stress (Fig. 5). Molluscs and echinoderms are the most vulnerable to all stressors (Fig. 5). Acidification caused the most negative effects across a wider range of phyla and life stages than temperature (Table 3). This study showed that larvae that calcify are vulnerable to individual thermal and acidification stress (Fig. 6), as is the case for calcifying adults (Kroeker *et al.*, 2013). Noncalcifying early life stages are also vulnerable to temperature and acidification, but

limited research suggests that noncalcifying adults may be resilient to thermal stress (Table 3) (Harvey *et al.*, 2013).

In general, our identification of vulnerable and resilient taxa based on early life stages is supported by previous meta-analyses derived from later life stages (Table 3), as well as field observations and physiological research. Overall, both molluscs and echinoderms seem equally vulnerable to ocean acidification at premetamorphic stages (Fig. 6) (Parker *et al.*, 2010; Byrne *et al.*, 2011), although intertidal and infaunal species may show increased resilience due to adaptation to pH fluctuations in rock pools (Davis *et al.*, 2013) or low pH in sediments (Talmage & Gobler, 2011; Styf *et al.*, 2013; Nguyen & Byrne, 2014). The impacts of acidification on calcification on echinoderms appear largely due to metabolic change (Byrne *et al.*, 2013a; Evans *et al.*, 2014) and less so to their vulnerable high magnesium calcite skeleton (Andersson *et al.*, 2008). The echinoderm skeleton is also protected by an epithelial cover. In contrast, shells of molluscs that lack a conchiolin-like extracellular cover are directly exposed to water chemistry, and thus, ocean acidification has a more direct dissolution impact on the skeleton (Byrne *et al.*, 2009; Tunnicliffe *et al.*, 2009). In response to salinity and temperature stress, molluscan and echinoderm larvae can show increased mortality, delayed development, reduced growth and reduced metabolic rate (Roller &

Stickle, 1989, 1993; Bressen *et al.*, 1995, Przeslawski, 2005), possibly owing to oxidative stress, lysosomal destabilization and increased lipid peroxidation (Deschaseaux *et al.*, 2010, 2011).

Arthropods and cnidarians were identified as the most resilient phyla to temperature and pH stress. (and salinity, for arthropods only). Early life stages of lobsters are resilient to individual and combined effects of temperature and acidification (Styf *et al.*, 2013), and ocean acidification does not have a pronounced effect on the adult (Wittmann & Pörtner, 2013) or early life stages (Egilsdottir *et al.*, 2009; Pansch *et al.*, 2012; Arnberg *et al.*, 2013) of several arthropod species. This supports field observations at CO<sub>2</sub> vent sites where communities at gas release points are dominated by amphipods and noncalcifying polychaetes (Kroeker *et al.*, 2011). Importantly, later life stages of some arthropods may be vulnerable to environmental stress; for example, many larvae of the subtropical barnacle *Balanus trigonus* developed to cyprids after exposure to warm brackish waters but failed to attach in these conditions (Thiyagarajan *et al.*, 2003).

In contrast to our results on the stress tolerance of the early life stages of cnidarians, this phylum is often cited as one of the most vulnerable to ocean acidification and other stressors (Byrne & Przeslawski, 2013; Harvey *et al.*, 2013; Kroeker *et al.*, 2013, Wittmann & Pörtner, 2013) most likely due to the higher sensitivity of

**Table 3** Summary of individual and combined effects on survival based on phylum and ability to calcify

	Temp	Sal	pH	Temp × sal	Temp × pH
Phylum (embryos/larvae)					
Arthropod	0	+	0	0	0
Echinoderm	—	—	—	—S	0
Mollusc	—	—	—*,†	—S	0
Cnidarian	+		0		0
Phylum (juveniles/adults)					
Arthropod	0‡		0‡		—S‡
Echinoderm	0‡		0‡, —‡		
Mollusc	—‡		—†‡		—S‡
Cnidarian	0‡		0‡, —‡		
Calcification (embryos/larvae)					
Calcifying	—		—		0
Noncalcifying	—		—		0
Calcification (juveniles/adults)					
Calcifying	—‡		—‡		—S‡
Noncalcifying	0‡				

Survival and sublethal responses are pooled for this study, while survival was used for all other studies. Unless otherwise listed, responses are from this study.

0, no significant effect or no interaction; —, negative effect; +, positive effects; S, synergism; A, antagonism. Empty cells denote no data available from published meta-analyses.

\*This study.

†Kroeker *et al.* (2013).

‡Harvey *et al.* (2013).

juvenile and adult stages than their noncalcifying larvae (Anlauf *et al.*, 2011). Previous studies on coral larvae have found negligible or no effects of temperature and pH on development (Chua *et al.*, 2013a,b; Putman *et al.*, 2013), although Cumbo *et al.* (2013) found that lethal effects were not evident until after more than 5 days of stress exposure. Furthermore, Rivest & Hofmann (2014) showed that the metabolic phenotypes of coral larval among cohorts released from the same coral colony varied among day of release, thus affecting their metabolic response to warming and acidification. Unsurprisingly with phylum-level generalizations, there are numerous exceptions where species in phyla that are identified as resilient in meta-analyses are actually highly sensitive to stress (e.g. crab in Walther *et al.*, 2010) and vice versa (e.g. bivalves in Thiagarajan & Ko, 2012).

#### *Limitations and considerations*

Although phylum-level patterns are useful to identify vulnerable taxa, we are unlikely to be able to identify patterns at high taxonomic resolutions (e.g. genus or species level) due to the large variation in intrinsic and extrinsic characteristics (e.g. phenotype/genotype, epigenetic flexibility, local plasticity), as well as low level of replication across studies for a given species or genus. In fact, there may be even larger differences between populations of the same species, with variations in parental history, maternal investment and biotic variables (e.g. food availability) influencing stress response of larvae and juveniles (e.g. barnacles, Pansch *et al.*, 2014; echinoderms, Kelly *et al.*, 2013). Meta-analyses may be useful to reduce this error by facilitating the identification of trends and broad patterns, strengthened using multiple species from numerous cohorts and regions (Nakagawa & Poulin, 2012). With regard to fine scale (e.g. species level, seasonal variation, latitudinal variation), meta-analyses can only be used to identify patterns if there are sufficient comparable studies within and among species, seasons or latitudes to reduce error to an acceptable level. This is a significant challenge with regard to the vast majority of marine invertebrate species. Although there are too few multifactorial studies on marine early life stages to identify fine-scale patterns, our focus on broadscale patterns in context with low-resolution taxonomy (e.g. phyla) or habitats (e.g. shallow vs. deep) yielded valuable insights and information at the scales used for marine management (Thrush & Dayton, 2010). As more multifactorial studies on early life stages are conducted using more complex designs (see recommendations below and Dupont & Pörtner, 2013), fine-scale patterns will be more reliably identified for more confident predictions.

Our study focussed on interactions between abiotic stressors, but these can also interact with biotic stressors (Strain *et al.*, 2014), thereby further complicating already frustratingly intricate and unpredictable responses. Other factors related to nutrition, genetics or disturbance history may cause substantial variation in organisms' responses (Hughes & Connell, 1999; Vehmaa *et al.*, 2012; Kroeker *et al.*, 2013). Therefore, the impact of climate change and ocean acidification on benthic invertebrates will vary according to the types, magnitudes and durations of existing and past natural and anthropogenic stressors.

Although we compiled a large body of literature for inclusion in our gap analysis and meta-analysis, the broad range of experimental variables used (e.g. response variables, stressor magnitude, experimental duration) precluded detailed assessment of individual and interactive effects due to methodological variation among studies, as noted elsewhere (Darling & Cote, 2008; Kroeker *et al.*, 2013; Ban *et al.*, 2014). As the number of studies related to multiple stressor effects on marine embryos and larvae increases, it may be possible to incorporate graduated ranges in stress (e.g.  $p\text{CO}_2$  ppm, temperature degrees) into meta-analyses models where each response variable is separately analysed (e.g. Harvey *et al.*, 2013; Kroeker *et al.*, 2013).

Multifactorial studies incorporating just one or two stressors and meta-analyses of the data from these studies have been criticized because they oversimplify complex systems (Dupont & Pörtner, 2013; Wittmann & Pörtner, 2013; Sett *et al.*, 2014). For instance, multifactorial studies typically examine individuals' responses in static conditions within a short timeframe, use abrupt treatment exposures and do not incorporate gradients or flux (e.g. tidal change). In addition, variation due to maternal provisioning, source habitat conditions and food provisioning has been shown to determine the tolerance of larval and juvenile barnacles to ocean acidification (Pansch *et al.*, 2014), and the response of coccolithophores to increasing  $p\text{CO}_2$  is dependent on the thermal environment (Sett *et al.*, 2014). Thus, multifactorial experiments provide an indication of the boundaries around stressor level effects and potential threshold trigger values, but they do not necessarily reflect what will occur in the field with different cohorts, habitats, parental history or environmental complexity; this is generally better represented by performance curves (e.g. Sett *et al.*, 2014).

We contend that multifactorial experiments and meta-analyses incorporating their resulting data play an important role in providing insights into general patterns, particularly for early life stages which are often difficult or intractable to investigate in field conditions. One of the main advantages of multifactorial



experiments is that they incorporate more realistic conditions as found in the natural environment facilitating identification of synergistic or antagonistic interactions between stressors. For example, arthropods seem resilient to effects associated with pH across their life stages, but when also exposed to elevated temperature as adults they show a negative response due to synergistic interactions between pH and temperature (Table 3) (Harvey *et al.*, 2013). The goal to attain ecological realism must be tempered with the caveat that the natural environment is far more complex than two or three stressors can reflect due to the numerous natural and anthropogenic stressors in the field, as well as intrinsic factors such as maternal provisioning. However, this certainly does not diminish the value of such experiments. Multifactorial experiments provide a crucial framework within which to assess stress tolerance levels and fine tune exploration of the range of sensitivities to identify thresholds. Although there is a paucity of research to identify impacts based on spatial (e.g. habitat, region), temporal (e.g. periods of stress exposure) or gradient (e.g. magnitude of stress exposure) patterns, meta-analyses incorporating multifactorial experimental data have the capacity to broadly identify impacts based on taxa (e.g. vulnerable or resilient). They can also inform future physiological research on target species and responses to more accurately predict impacts of changing environmental conditions.

### Future directions and recommendations

Understanding the mechanisms underlying differences in sensitivities to stressors requires attention to the cellular, molecular and genetic controls of physiological processes such as calcification, cellular respiration and DNA and protein repair rates (Melzner *et al.*, 2009; Todgham & Hofmann, 2009; Hofmann & Todgham, 2010; Evans & Watson-Wynn, 2014). Recent syntheses for echinoderm larvae confirmed that regardless of phylogeny, latitude or habitat, acidification has a negative impact on calcification due to hypercapnia-driven alteration in metabolism (Byrne *et al.*, 2013a) and expression of metabolic genes (Stumpp *et al.*, 2012; Pespini *et al.*, 2013; Evans & Watson-Wynn, 2014). An understanding of physiology may help explain complex responses to multiple stressors (e.g. Davis *et al.*, 2013), as has been shown in the variation in the metabolic responses of amphipods (Calosi *et al.*, 2013). In addition, physiological investigations will help increase the taxonomic resolution of predictions of impact. For example, our study identified the vulnerability of early life stages from noncalcifying species to reduced pH, and future targeted physiological studies may reveal the mechanisms behind these effects and facilitate

predictions focussed on particular species where physiological pathways are known (e.g. Basile *et al.*, 2005). While mechanistic/response-curve type studies are essential to understand the physiological underpinning of performance in a changing environment (e.g. Wittmann & Pörtner, 2013; Sett *et al.*, 2014), it will be some time before comprehensive data are available across taxa and habitats for early life stages for identification of broad patterns via meta-analysis.

Although several trends have emerged from this review and meta-analysis, the challenge now is to translate these results to the field. There is a dearth of information about *in situ* responses of embryos and larvae to multiple stressors, largely due to the challenges of working with marine larvae in the field (e.g. difficulty of applying appropriate stressors and tracking larvae). In comparison with our study, Ban *et al.* (2014) used a much higher proportion of field studies in their meta-analysis, most of which focussed on juveniles or adults. There is nonetheless an urgent need for field experiments or observations on marine larvae related to climate change impacts (see in Wernberg *et al.* (2012), particularly due to local- and regional-scale variations in environmental changes which are crucial to consider for ecological realism (Byrne & Przeslawski, 2013). Although not focussed on early life stages, field sites at which environmental conditions have changed at a local scale (e.g. CO<sub>2</sub> vents, upwelling zones) can help us to infer recruitment patterns related to climate change and ocean acidification, as well as revealing wider community and ecosystem impacts. The tax-specific responses of early life history stages to pH are reflected in the divergent ecosystem responses of benthic communities at CO<sub>2</sub> vent sites, used as a proxy for future ocean conditions (Cigliano *et al.*, 2010; Kroeker *et al.*, 2011; Fabricius *et al.*, 2011). However, the presence of elevated trace elements (e.g. Mn<sup>2+</sup>, Zn<sup>2+</sup>, Sr<sup>2+</sup>) recorded in the skeleton of urchins living near vent sites (Bray *et al.*, 2014) and a range of trace elements in sediments and sea grasses (Vizzini *et al.*, 2013) indicates that distribution of biota at some of these sites may be influenced by factors in addition to pure CO<sub>2</sub> injection.

Most studies included in our meta-analysis have been future-shock experiments where propagules generated from parents living in present day conditions are placed in future conditions. While this approach provides a good assessment of stress tolerance, it cannot account for phenotypic plasticity and genetic adaptation to an ocean that has been gradually changing for decades and will continue to do so over coming decades. Acclimatization of adults to changing environmental conditions can determine the thermal tolerance of offspring (Byrne *et al.*, 2011; Parker *et al.*, 2012 but

see Roller & Stickle, 1993), as well as their resilience to reduced pH (Hettinger *et al.*, 2012; Parker *et al.*, 2012), indicating phenotypic plasticity (nongenetic) in gamete development where parent environmental history, particularly that of the female during egg development (= maternal imprinting), influences stress tolerance of progeny. For some species, phenotypic plasticity may help mitigate the effects of changing climate on fitness over generations potentially providing a temporal window for evolutionary (genetic) adaptation to occur. Quantitative genetics studies indicate the presence of genetic traits to facilitate resilience and adaptation to climate change for sea urchin embryos and larvae (Foo *et al.*, 2012; Kelly *et al.*, 2013), but not for mussel larvae (Sunday *et al.*, 2011). However, as might be expected from the current pace of environmental change, evidence for evolutionary adaptation to contemporary global change is rare (Gienapp *et al.*, 2008). Questions on the potential for adjustment and adaptation to climate change will require long-term, transgenerational experimental studies (Sunday *et al.*, 2011; Kelly *et al.*, 2013b; a, Munday *et al.*, 2013; Sunday *et al.*, 2014).

One of the main hindrances to synthesizing data from multifactorial experiments is the lack of standard response variables. Responses such as survival, growth and developmental stage are relatively easy and quick to measure and can help build larger and comparable data sets to facilitate identification of patterns. The benefits of refined analyses on larger data sets are evident in comparison with two meta-analyses on ocean acidification effects, the first in which no effects on molluscs were detected (Kroeker *et al.*, 2010) and the second incorporating 39 new studies showing that acidification caused significant negative effects in molluscs (Kroeker *et al.*, 2013).

Most multifactorial studies on early life stages have involved temperature and tropical intertidal and subtidal species, and we know almost nothing about the effects of multiple stressors on species from deeper or polar waters. Whereas many marine taxa are showing poleward range expansion as the ocean warms (Poloczanska *et al.*, 2013; Sorte *et al.*, 2010; Sunday *et al.*, 2012 but see Przeslawski *et al.*, 2012), deep-sea and polar organisms may be particularly vulnerable in a changing ocean because they have limited or no options to migrate and may already be living close to their maximum thermal tolerance levels (Whiteley, 2011). The high sensitivity of Antarctic marine invertebrates to increasing temperature is considered to be due to cold adaptation through millions of years in stable low temperature conditions (Peck, 2005; Pörtner *et al.*, 2007). Early life stages of some Antarctic species such as *Sterichinus neumayeri* may be able to survive exposure to single stressors but not multiple stressors (Kapsenberg & Hofmann, 2014), or

they may show sublethal effects (Ericson *et al.*, 2012; Byrne *et al.*, 2013b).

Based on our study, we recommend the following considerations for future studies incorporating multiple stressors, particularly those focussed on early life stages of marine species:

- To provide a holistic assessment of potential impacts, multiple life stages for a given species should be examined, including fertilization, embryos, larvae, juveniles and reproductive adults (e.g. Byrne *et al.*, 2009; Parker *et al.*, 2009).
- Researchers conducting multifactorial studies should publish their data (e.g. mean, error, sample size for each treatment) such that effect sizes can be calculated for potential inclusion in future meta-analyses (Nakagawa & Poulin, 2012).
- Studies incorporating potential stressors should employ realistic treatment levels incorporating normal environmental conditions experienced by source populations, as well as extreme conditions as measured in the field or predicted in appropriate models (e.g. Przeslawski *et al.*, 2005; Kapsenberg & Hofmann, 2014; Nguyen & Byrne, 2014).
- A greater understanding of physiology and genetics is needed to increase our understanding of the processes and natural variability behind biological responses (mortality, growth, developmental rate) (e.g. Walther *et al.*, 2011; Foo *et al.*, 2012; Rivest & Hofmann, 2014) and mechanisms involved (Evans & Watson-Wynn, 2014) which will in turn facilitate more accurate predictions of impacts when environmental conditions change (Wittmann & Pörtner, 2013).
- Multifactorial studies should incorporate a field component, although we acknowledge the great difficulty in experimenting on larvae *in situ*. Practical ways to achieve this may be to examine brooded or encapsulated embryos (e.g. Przeslawski, 2005; Davis *et al.*, 2013), to couple manipulative laboratory experiments with field observations over environmental gradients (e.g. Preston, 1985; Russell & Phillips, 2009a) or to use naturally occurring field proxies (e.g. CO<sub>2</sub> vent sites in Kroeker *et al.*, 2011).
- To increase ecological realism, long-term experiments with a designated acclimation period, as opposed to “future-shock” type exposures, will provide a more realistic framework for global change studies (e.g. Roller & Stickle, 1993).
- To account for adaptive capacity, multigenerational experiments should be conducted where time and resources allow (e.g. Qiu & Qian, 1999; Parker *et al.*, 2012; Fischer and Phillips, 2014). Researchers can then investigate the potential for organisms to acclimate or adapt to changing environmental conditions.

- Standard response variables should be routinely measured in all multistressor studies on marine embryos and larvae: survival, developmental rate, growth, abnormality rate and calcification (the latter only for studies including pH). These response variables are prevalent in the literature, relatively easy to measure, and are underpinned by various physiological mechanisms (e.g. Harvey *et al.*, 2013; Kroeker *et al.*, 2013).
- Research should explore multiple stressors in addition to salinity, temperature and pH to provide a more integrated and realistic perception of global change impacts. This should include other climate change-related stressors (especially hypoxia. See Frieder *et al.*, 2014), local anthropogenic stressors (e.g. pollutants) and natural stressors (e.g. UV, desiccation).
- Research effort should focus on potentially vulnerable taxa (e.g. calcifying species, molluscs, echinoderms for temperature and pH stress) including exploited species, as well as species from habitats or regions with little or no data (e.g. deep-sea taxa) or those regions for which conditions are changing quickly or at a large magnitude (e.g. Antarctic Peninsula, south-eastern Australia).

## Conclusion

This study was a critical assessment of the effects of multiple stressors and their interactions on early life history stages of marine invertebrates. In this, we availed of a long history of research on multiple stressors (e.g. temperature and salinity in Kinne & Kinne, 1962; Pechenik, 1987), as well as renewed momentum in the context of global change (e.g. temperature and acidification, Byrne & Przeslawski, 2013; Russell & Phillips, 2009b; Wernberg *et al.*, 2012). Results from this study investigate several hypotheses regarding early life stages and abiotic stressors, highlighting the importance of understanding stress responses and ecology of early life stages, and provide context for future experiments. It is hoped that this study further informs research on multiple stressors to strengthen links between patterns observed in the field and early life history processes and responses. Our results will facilitate interpretation of complex responses of species to environmental change to discern potentially vulnerable taxa of interest to marine management (e.g. monitoring programmes, food security).

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** List of studies and species included in the gap analysis (after preliminary selection criteria applied) and in the meta-analysis (highlighted, after refined selection criteria applied).

**Table S2.** Summary of results from generalized linear mixed-effects models with Hedge's *d* as a function of stressor combinations (temperature  $\times$  salinity, temperature  $\times$  pH) using a range of covariates related to hypotheses.

**Figure S1.** Funnel plots of the temperature/salinity (left) and temperature/pH (right) interactions showing no evidence of publication bias. The vertical dashed line shows the mean effect size. The solid line indicates the null value. The absence of asymmetry in the funnel plots [as evidenced by an absence of correlation between effect size and the inverse standard error; Spearman's  $\rho = 0.17$ ,  $P = 0.26$  (left) and  $\rho = 0.37$ ,  $P = 0.05$  (right)] indicates no likelihood of underreporting the lack of synergistic or antagonistic effects.

**Data S4.** Reference list based on preliminary and refined selection criteria.