

Review

The Impact of Ocean Acidification on Reproduction, Early Development and Settlement of Marine Organisms

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Abstract: Predicting the impact of warming and acidifying on oceans on the early development life history stages of invertebrates although difficult, is essential in order to anticipate the severity and consequences of future climate change. This review summarises the current literature and meta-analyses on the early life-history stages of invertebrates including fertilisation, larval development and the implications for dispersal and settlement of populations. Although fertilisation appears robust to near future predictions of ocean acidification, larval development is much more vulnerable and across invertebrate groups, evidence indicates that the impacts may be severe. This is especially for those many marine organisms which start to calcify in their larval and/or juvenile stages. Species-specificity and variability in responses and current gaps in the literature are highlighted, including the need for studies to investigate the total effects of climate change including the synergistic impact of temperature, and the need for long-term multigenerational experiments to determine whether vulnerable invertebrate species have the capacity to adapt to elevations in atmospheric CO₂ over the next century.

Keywords: ocean acidification; temperature; reproduction; *larvae*; settlement; review; echinoderms; molluscs; crustaceans; fish; corals

1. Introduction

It has been suggested that ocean acidification will occur, as a consequence of sequestration of increasing atmospheric CO₂ into the ocean threatening the biodiversity and survival of marine organisms and ecosystems that may be unable to adapt to the current rate of CO₂ absorption by the oceans which exceeds that of any other time on the planet [1-3]. The surface of our oceans will experience a decrease in pH from a level of 8.1–8.2 by 0.3–0.5 units and 0.7–0.77 units by the years 2100 (pH 7.6–7.9) and 2300 (pH 7.33–7.5) respectively [1,4,5]. Such decreases in pH may most affect the sensitive and vulnerable early developmental stages of marine organisms because these life histories have specific environmental needs [6-8]. Yet supporting or rejecting predictions on the impact of ocean acidification induced by climate change is difficult [9,10] because studies investigating the response of marine organisms to ocean acidification are in their infancy [3,11,12] and seemingly authoritative meta-analyses are limited by a paucity of data and evidence from a small sample size of studies and organisms [13-16].

Although studies dating back to the 1960's set the scene for current predictions that ocean acidification may have negative impacts on fertilisation and larval development of marine organisms (oyster *Crassostrea virginica* and clam *Mercenaria mercenaria* [17]; oyster *Saccostrea glomerata* [18]; sea urchins *Paracentrotus lividus* [19,20]; *Sphaerechinus granularis* [21]; Pismo clam *Tivela stultorum* [22] and giant scallop *Placopecten magellanicus* [23]); fish *Pagrus major*, *Sillago japonica* [24] they manipulated pH by acid mineralisation (e.g., sulphuric or hydrochloric), increased alkalinity through the addition of sodium hydroxide for a range of teleosts [25], or used levels of elevated CO₂ often far outside those predicted [26] or where for short time periods [25]. Although these methodologies are not those considered best practice today, these studies, in general, measured negative impacts on bivalve molluscs [22], cleavage in echinoids (*Sphaerechinus granularis* [21]; *Paracentrotus lividus* [19,20]), morphological development in bivalve molluscs (*Saccostrea glomerata* [18]) and increased mortality, decreased growth and skeletal malformations in bivalve molluscs (*Crassostrea virginica* and *Mercenaria mercenaria* [17]). Most recent studies, using best practise methodologies, however, manipulate pH either by directly aerating seawater with CO₂, or saturating seawater with CO₂ prior to adding to the culture media [27] at CO₂ levels predicted for the end of the century and beyond [1,28-30].

Even with these methodologies now in place, determining which hypothesis to support or reject regarding the fate of marine organisms and their *larvae* in a climate changed future ocean continues to be problematic. Overall the major model explaining variation in sensitivity of marine organisms to ocean acidification concerns those organisms which calcify. In general it is suggested that marine organisms with calcium carbonate structures will be most sensitive to ocean acidification [16]. Any variation in sensitivity among such marine organisms is dependent on the mineral form of CaCO₃; aragonite being more soluble than calcite, magnesium-calcite being more soluble than aragonite and with amorphous calcium carbonate (ACC) being most soluble. Higher solubility of CaCO₃ polymorphs is predicted for marine organisms at higher latitudes such as polar regions because high latitude regions where cold waters increase the uptake of CO₂ cause a greater shallowing of the CaCO₃ saturation horizon making calcification energetically more difficult and thus these regions may be the regions where effects of ocean acidification are first be detected [31]. During the early larval stage

many marine organisms synthesise and deposit CaCO_3 through a series of pathways starting with depositing an amorphous calcium carbonate (ACC) crystal skeleton, known to be 30 times more soluble than stable forms of aragonite and calcite secreted later in larval life [31,32]. *Larvae* of marine organisms which often begin the deposition of their shells and skeletons with ACC are thus likely to be more susceptible than adults (Table 1) [7] and depending on their distribution particularly susceptible in the polar regions because they deposit a more soluble form of calcite (ACC) in a region where this is energetically difficult to do so.

Table 1. Calcium carbonate polymorphs deposited by marine *larvae*.

Organism	Skeleton type *	Mineralogy
Echinoderms	Endoskeleton	ACC, High-Magnesium Calcite
Molluscs	Exoskeleton	ACC, Aragonite, Calcite ^, High- or Low-Magnesium Calcite
Crustaceans	Exoskeleton	ACC, High-Magnesium Calcite

* Primary skeleton type but not limited to; ^ usually after settlement.

Not only has it been suggested that the impact of ocean acidification will be more significant for *larvae* than adults [14], but it will be most significant for the earlier sensitive life history stages; including egg and sperm production, fertilisation, cleavage, than the later life history stages of larval development and dispersal, settlement and post-settlement survival, especially for molluscs and echinoderms [7,14,33-36] (Figure 1). Indeed it has been suggested that the sensitivity of *larvae* is hierarchical, being most sensitive when embryos and least sensitive as pediveligers and metamorphs, following a linear sequence; embryos>veligers (D *larvae*)> pediveligers>metamorphs>adults [37]. Such sensitivity may vary among marine groups. For calcifying corals and algae the photosynthetic action of zooxanthellae may ameliorate the negative impact of ocean acidification, but broadcast spawning invertebrates are particularly vulnerable because fertilisation of eggs and sperm occurs in the water column followed by development of planktotrophic *larvae* with direct contact with acidifying and warming waters. There may also be profound “carry-over” consequences from one life history stage to then next. For example, any impact on fertilisation may also have carry-over consequences for larval development [7,35,38,39], larval dispersal and subsequently directly influence the distribution and abundance of settlers and post settlement survival. Such “carry-over” effects may also create cascades and “bottlenecks” for populations resulting in sub lethal or lethal dead-ends [14]. Any sub lethal reductions in rate of development and larval size may also have significant consequences for the survival of marine *larvae* because prolonged larval life phase and delayed settlement may lead to a concomitant increase in the likelihood of predation. Such sub lethal effects may also impact significantly on the energetics of metamorphosis, settlement and post settlement mortality shaping the structure and function of marine ecosystems and adult marine populations (Figure 2)[7,40,41].

Figure 1. Life cycle stages of benthic broadcast spawners and impacts of ocean acidification on fertilisation and larval development [7].

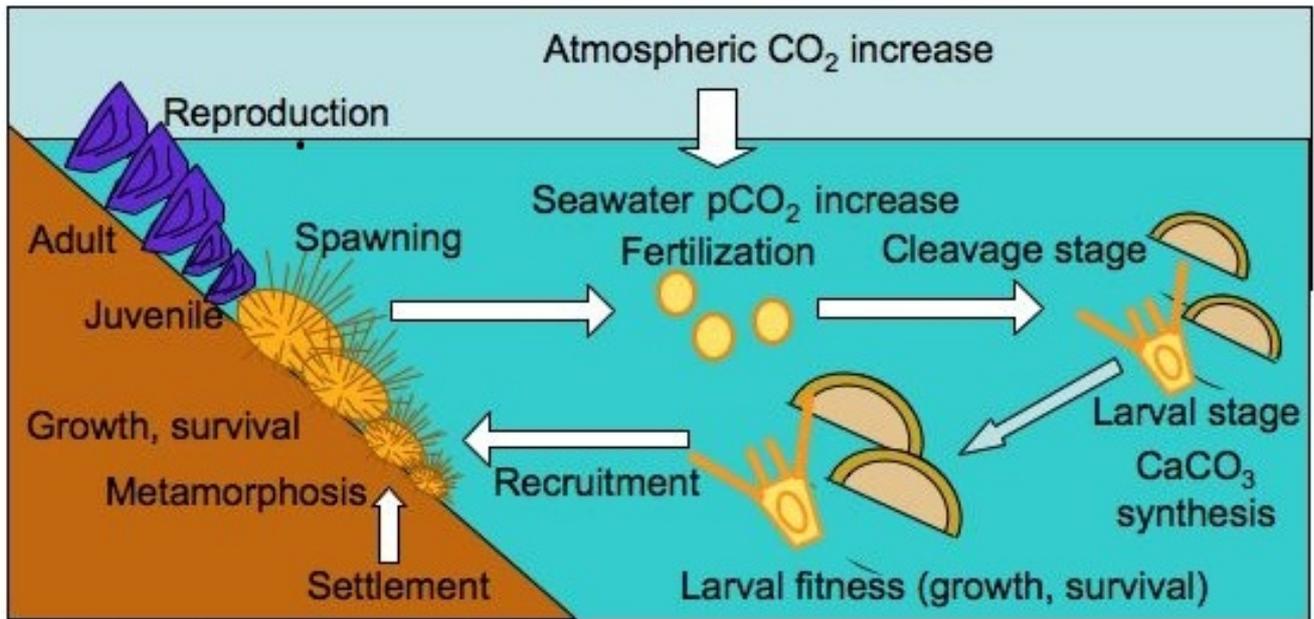
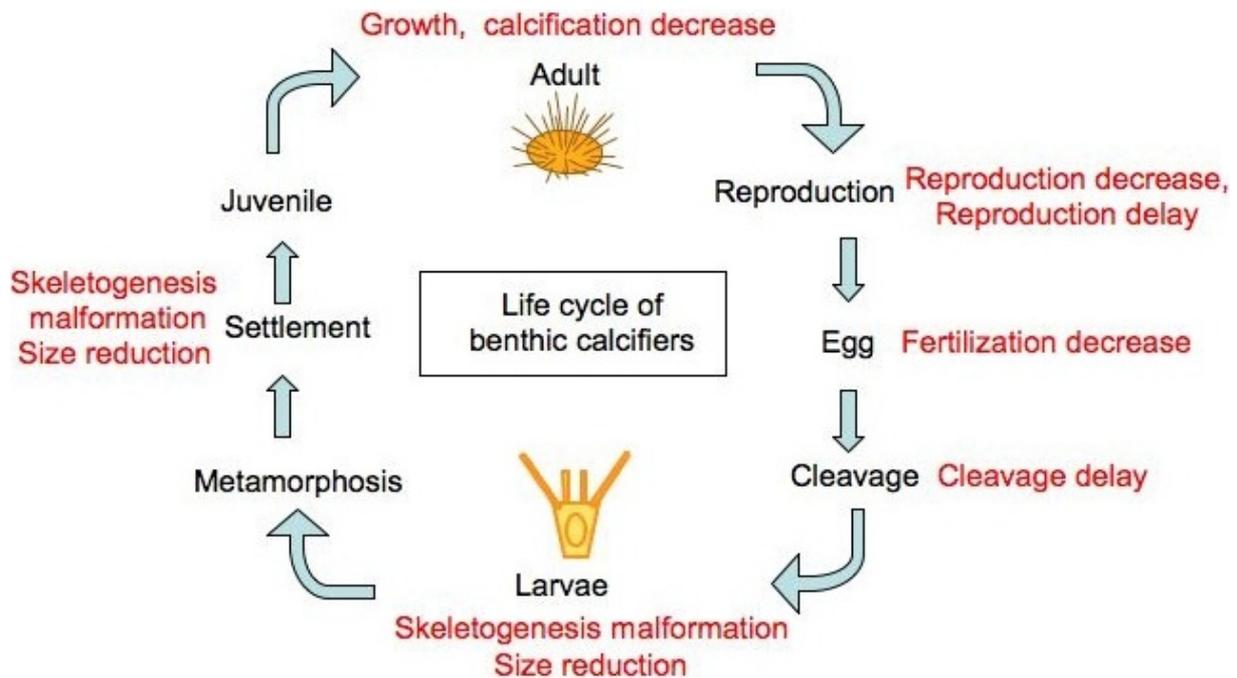


Figure 2. Summary of the major impacts of ocean acidification on the fertilisation, larval development and settlement of broadcast spawning benthic invertebrates [7].



Initially it was anticipated that closely related species may have more similar responses and organisms which have evolved from environments with historically higher levels of CO₂ or fluctuating levels of CO₂ may create a greater capacity in an organism to acclimatise and be more resilient to lowered pH [42]. Variations in responses within and between species populations and the role in resilience and adaptation remain virtually unknown [43,44], especially amongst geographically isolated populations. Also as oceans acidify it is anticipated that they will also warm (by 4 °C [1]) and that the

dual stress of elevated CO₂ and temperature may either act in combination to exacerbate impacts or the positive effects of temperature may ameliorate any negative effects of elevated CO₂ [34,45]. It is essential to investigate if such interactive effects exist [35], to determine how widespread synergistic impacts are on the early development of marine organisms. Most recently the neutral or negative response of *larvae* to ocean acidification in marine organisms has been questioned [32] with suggestions that lecithotrophic *larvae*, produced by approximately 10% of marine benthic invertebrates, may be better competitors and less affected than approximately 60–90% of marine organisms which produce planktotrophic *larvae* and feed on exogenous sources. It may well be that lower pH impacts planktotrophic *larvae* by reducing feeding rate or efficiency [43,46].

Most recently there have been a series of meta analyses on the effects of ocean acidification on marine organisms, especially on fertilisation and larval development, which have created an effect size weighted across a number of hypotheses. The aim of these meta-analyses has been to more powerfully estimate the overall effect compared to single studies. [13-16]. We do need meta-analyses and syntheses they provide [13-16], but interpretation needs to be coupled carefully with the details of investigations and experimental design used to determine which major hypotheses can be supported. Hypotheses proposed and tested so far show a series of variable responses of marine organisms to elevated CO₂ [16] which make general conclusions difficult. The purpose of this review is to summarise the responses to ocean acidification in fertilisation and larval development in a range of invertebrates including echinoderms, molluscs, crustaceans, corals and fish against the leading hypotheses, implications for survival and settlement and the potential consequences for marine populations.

2. Fertilisation

Across a broad range of broadcast spawners with planktotrophic *larvae* dependent on an exogenous food supply, it is thought that fertilisation compared to larval development is relatively robust to ocean acidification and decreased pH at levels predicted for the year 2100 ([14]; echinoderms [34,47,48]; molluscs [49]; copepod *Acartia tsuensis*, [50]; fish Baltic cod, *Gadhus morhua*, [51]), but may be negatively impacted at pH levels predicted for 2300 ([1,52,53] Table S2 in supplementary), especially if sperm concentration is low and limiting [47,54,55] (Table S2).

Such conclusions are against a background of contrasting results, however, even for the same species from the same (*Heliocidaris erythrogramma* [34,56]) and different geographic regions (*Crassostrea gigas* [33,39,49]). Havenhand *et al.* [56] found significant reductions in sperm swimming speed (a reduction of 11.7%), motility (16.3% reduction) and fertilisation success (24.9% reduction) in the sea urchin, *Heliocidaris erythrogramma* (Table S2). Byrne *et al.* [34,47,48], however, found no significant effect of elevated CO₂ on fertilisation success, cleavage and gastrulation for the same and different sea urchin species sourced from the same geographical region even with the additional stressor of elevated temperature (Table S2). Similarly, there was no effect of elevated CO₂ on sperm speed, sperm motility or fertilisation in the Pacific oyster, *Crassostrea gigas* [33,49], located in Japan and Sweden, but there was reduced fertilisation in Australia [39]. Such contrasting results, even for identical species, could be due to differences in experimental methodology arising from single male and female crosses in Havenhand *et al.* [56] versus multiple males and females in Byrne *et al.* [34,47,48] and high fertilisation success in controls [56] 62% versus [47,48] 80–90%). It

remains unclear whether intra and inter specific differences are real or due to differences in experimental techniques [47,54,55] by the pooling of gametes from males and females [34,47,48], creating arguments about polyandry *versus* single crosses and their influence on results and interpretation and or misinterpretations and measurement in fertilisation due to polyspermy. Whether polyandry or single crosses, even recent studies are based on small sample sizes of males and females ([34] 2 males: 2 females; [47] 3 males: 3 females; [54], 1 male: 1 female) and additionally may suffer from pseudoreplication where containers are not factored as a source of variation in the analysis. Earliest studies on echinoderms found no effect on fertilisation of *Hemicentrotus pulcherrimus* and *Echinometra mathaei*, at pH 7.6 and 7.7 but at pH 6.8 for *Hemicentrotus pulcherrimus* and 6.8–7.1 *Echinometra mathaei* (–0.5 to –0.2 less than that predicted for 2300), there were significant effects with reduced fertilisation success and reduced cleavage speed in both species of sea urchins [52,53] and a trend for decreasing fertilisation with decreasing pH between 7.3–7.4 (Table S2). There were also no significant differences in fertilisation of the mollusc, *Mytilus galloprovincialis*, between controls and elevated CO₂ [57] and at pH 6.8–7.1 for *Echinometra mathaei* [53]. There was also decreased fertilisation success with increasing CO₂ concentrations for the coral, *Acropora palmata* [58]. As sperm concentration declined, the reduction in fertilisation success was exacerbated at elevated CO₂.

Although fertilisation appears robust to elevated CO₂, when synergistic factors such as temperature is combined with elevated CO₂ [47,48] it is temperature, rather than elevated CO₂ which either has or has no effect on fertilisation in echinoderms (*Heliocidaris erythrogramma*, [34,48]). Although elevated CO₂ was not found to reduce fertilisation, nor temperature to enhance fertilisation in a range of echinoderms including once again the echinoid, *Heliocidaris erythrogramma* and the closely related species, *Heliocidaris tuberculata*, *Tripneustes gratilla*, *Centrostephanus rodgersii* and the asteroid, *Patiriella regularis* from a range of regions along the east coast of Australia ranging from (42°50'S, 147°15'E) to (30°12'S, 153°16'E) [48]. Nor was there a synergistic interaction between elevated CO₂ and temperature and no trend for reduced fertilisation with increased concentration of CO₂ over a range of sperm concentrations [47,54,55] Sperm concentration appears to be a significant factor for organisms when other conditions are suboptimal. Ericson *et al.* [54] found no effect on fertilisation of elevated CO₂ and decreased pH at levels predicted for the year 2100, for the echinoid, *Sterechinus neumayeri*, but did find an effect of elevated CO₂ when sperm concentration was suboptimal. Reuter *et al.* [55] also found maximum fertilisation rates in the sea urchin, *Strongylocentrotus franciscanis*, were only maintained at increased sperm concentrations. Overall elevated CO₂, decreased the range of sperm concentrations over which high fertilization success was likely [55]. In molluscs, in contrast when the additional stressor of suboptimal or elevated temperature was combined with elevated CO₂ there was a synergistic impact and correspondingly significantly reduced fertilisation (reduction in fertilisation of up to 26% and 51% for the Pacific oyster, *Crassostrea gigas* and the Sydney Rock oyster, *Saccostrea glomerata*, respectively at suboptimal temperature [39]).

The few studies done between closely related species do not as yet provide enough evidence for supporting a similarity or difference in response. There were significant differences in fertilisation when responses of *Saccostrea glomerata* and *Crassostrea gigas* were compared; a significantly greater reduction in fertilisation in *Saccostrea glomerata* compared to the perhaps more robust

Crassostrea gigas [39], but when Byrne *et al.* [48] compared the fertilisation response among the echinoids, *Heliocidaris erythrogramma* and the closely related species *Heliocidaris tuberculata*, *Tripneustes gratilla* and *Centrostephanus rodgersii* there was no difference.

It is also far too soon to support resilience related adaptability from acclimatisation by organisms to the low pH conditions experienced in their environments. Moulin *et al.* [42] found fertilisation rates were greater compared to controls when gametes of the temperate sea urchin, *Paracentrotus lividus*, originated from individuals sourced from more acidic tidal pools, but doubt can be raised over whether these differences are representative because of low replication (one acidic pool was compared to one control pool). There was a trend, however, for decreased fertilisation in the subtidal sea urchin, *H. tuberculata*, prompting the comment that species characterising shallow coastal waters are perhaps more robust to pH fluctuations in their environment [47]. In the only study done on a subtidal species of mollusc, however, there was no effect of temperature or elevated CO₂, on fertilisation in the temperate abalone, *Haliotis coccoradiata* using concentrations of CO₂ expected for 2100 [47]. Studies such as Byrne *et al.* [47,48] and more recent findings [42] support the hypothesis that the robustness of fertilisation to ocean acidification may be because the natural process of fertilisation is characterised by low pH conditions, with any inhibitory effect on sperm motility as described by Havenhand *et al.* [56] overridden by jelly peptides on eggs and respiratory effects [47]. Chronic multigenerational tests are yet to be done, however, to test hypotheses related to acclimation and pre adaptive capacity suggested in Parker *et al.* [43,59].

Recent meta-analyses of the overall effects of elevated CO₂ on fertilisation are restricted by the paucity of studies available with reductions in effect size below 2000 ppm pCO₂ for sea urchin embryos (−9%, 3 papers, 2 species; [13]), copepods (−33%, 2 papers 2 species) and yet increased effects for bivalves (+2%, maximum 2 papers 2 species) and a cephalopod (*Sepia officinalis*, +98% [15]), although the ppm range was not specified. With such small databases being employed generalisations on the overall effect on fertilisation leaves our understanding of the impacts of ocean acidification on fertilisation based on species specific studies done in different geographic regions, where population and experimental similarities and differences cloud the conclusions. Authors do agree that differences in experimental methodologies among studies hamper our ability to make robust generalisations [14]. Questions of the impact of pooling of gametes from small numbers of males and females also create arguments about polyandry *versus* single crosses and their influence on results and interpretations of recent studies, whether fertilisation success has been inaccurately estimated from fertilised and polyspermy embryos [55] and/or whether high fertilisation success in controls (62% [56] compared to 80–90% [47,48]) alters interpretations. Despite these unanswered questions, authors are now in agreement that fertilisation may be relatively robust to near future ocean acidification [13-15,36]. There is however still acknowledgement of the paucity of data on the diversity of marine invertebrates [47] and lack of studies which correlate response of organisms with any natural variations of pH in their environment, which may provide organisms with pre adaptive capacity [42,43,60].

3. Larvae

Even if it turns out that fertilisation is robust to near future predictions of ocean acidification, larval development is much more vulnerable [14]. Especially for those many marine organisms which start to calcify in their larval and/or juvenile stages [7,16] (Table S2).

3.1. Echinoderms

Larval development has been shown to be more sensitive than fertilisation to elevated concentrations of atmospheric CO₂, particularly for echinoderm embryos and *larvae*, with delayed development, reduced survival and size and skeletal abnormalities mainly investigated in sea urchin and brittle star planktotrophic *larvae* (*Hemicentrotus pulcherrimus* and *Echinometra mathaei*, [52,53]; *Ophiothrix fragilis*, [60,61]; *Paracetrotus lividus* [42]). Earlier and later studies using a range of pH levels within and outside emission scenarios for the end of the century, ranging from 3 days to 8 days in sea urchins and brittle stars, have reported delayed development and abnormal pluteus morphology. *Larvae* of the sea urchins, *Hemicentrotus pulcherrimus* and *Echinometra mathaei*, exposed to elevated CO₂ were abnormally trapezium shaped, failed to develop arms and spicules or reach pluteus stage [52,53]. *Larvae* of the ecological keystone brittlestar, *Ophiothrix fragilis*, either were abnormal, had altered skeletal proportions and asymmetry during skeletogenesis and there was a delay in development at low pH, with *larvae* at elevated CO₂ treatments taking longer to reach the same developmental stage [61]; for example, 50% of the control *larvae* were 6 armed after 5.42 days compared to 5.73 days at pH 7.9. There was also 100% mortality of experimental *larvae* whereas control *larvae* had 70% survival over the same time [61]. Sea urchin embryos of *Paracetrotus lividus*, failed to develop to reach pluteus stage, displaying an abnormal bowl-shaped morphology, shorter rod length and high proportions of asymmetry [42].

There is little evidence to suggest that a geographic distribution model explains the delayed development and skeletal abnormalities in echinoderm *larvae*. Clark *et al.* [60] tested the prediction that the calcification of *larvae* of polar species would be more negatively impacted than temperate and tropical species and found opposite to that predicted. The Antarctic species, *Sterechinus neumayeri*, was least affected by elevated CO₂ while there were variable yet similar impacts on sea urchin *larvae* distributed from the tropic to Antarctic regions. Further, Ericson *et al.* [54] found no difference in visual abnormality or rate of development in *Sterechinus neumayeri larvae*, among pH treatments (Table S2). Significant differences among pH treatments first appeared at gastrulation (day 6) where embryos were 20% shorter, displayed no normal larval development, had rupturing of the gastrula epithelium and irregular morphology at pH 7, but no difference in abnormality levels or length among the other treatments (although at pH 7.3 some delayed development was apparent). Clark *et al.* [60], found lowering the pH to 7.0, did not significantly affect survival of the polar species, *Sterechinus neumayeri* or the temperate species, *Pseudechinus huttoni* and *Evechinus chloroticus*, but significantly reduced survival of tropical, *Tripneustes gratilla*. The main affect, however, was a reduction in calcification of pluteus *larvae* which was not greater for Antarctic species nor dependent on geographical location. The significant decreases in larval calcification were observed in the temperate, *Evechinus chloroticus* (30.6%) and *Pseudechinus huttoni* (36.9%) with small and

non-significant decreases of 3.9% in polar, *Sterechinus neumayeri*, and 13.8% in tropical, *Tripneustes gratilla*, with a reduction in growth for 3 out of the 4 species, but with no significant difference in growth in the temperate *P. huttoni*. Finally although acidified seawater did not change the morphology of larval skeletons among species, as found in other studies, there were fine scale differences in skeletal structures. There was an apparent loss of integrity of the surfaces of the skeletal rods for temperate, but not for the polar *S. neumayeri*, which along the tropical, *T. gratilla* had smooth skeletal rods contrasting with the temperate *E. cloroticus* which was pitted and *P. huttoni* which was eroded at reduced pH. It was suggested by the authors of this study that polar species, which experience naturally lower CaCO₃ saturation states may be better adapted to ocean acidification than those species from habitats with naturally higher CaCO₃ saturation states, such as the temperate and tropical species [60]. In addition, the greater effects on the temperate and tropical species may have been the result of a synergistic interaction of reduced pH with higher temperature (*S. neumayeri* -1.9 °C, *P. huttoni* 12 °C, *E. cloroticus* 15 °C, *T. gratilla* 26 °C) [60].

Once again highlighting the species specific nature of responses to environmental stressors, findings on the synergistic, multiple stresses experienced by *larvae* of echinoderms to the combined effect of elevated CO₂ and temperature are rare and perhaps as a function of this leave us with contrasting conclusions [34,45]. In contrast to the findings in *H. erythrogramma* [34] where development was impaired by raised temperatures, but not lowered pH, Sheppard Brennan *et al.* [45], testing the hypothesis that temperature enhancement would counteract the negative effect of acidification, found elevated CO₂ reduced calcification in the tropical sea urchin, *Tripneustes gratilla* (also used by Clark *et al.*, [60]). Varying temperatures and pH levels, significantly increased larval growth until the thermal limit was reached (30 °C), and decreased as pH decreased. These increased growth rates with +3 °C rise in temperature effectively reduced, but did not negate, adverse effects in *larvae* raised at low pH [45].

It is likely that if an exogenous food supply is limiting, this may interact with elevated CO₂ and determine organism response. There have been very few studies done to determine the effect of elevated CO₂ in relation to mode of feeding and feeding efficiency. In the lecithotrophic cephalopod, *Sepia officinalis*, embryos developed with no impact on calcification in low pH [62], as did the sea star, *Crossaster papposus* over the 38 day duration of the experiment [32]. *Larvae* grew 2.8 times faster and juveniles 2.2 times faster at pH 7.7 compared to controls; 50% of *larvae* had progressed into the juvenile stage by day 28, whilst 95% of controls had developed rudiment, but remained in the preceding settlement stage [32]. In contrast, Havenhand *et al.* [56] found at pH 7.7, identical to Dupont *et al.* [32,61], that the percentage of eggs which developed into swimming *larvae* of the sea urchin, *Heliocidaris erythrogramma*, after 24 hours was 25.9% lower than the control. Although 60–90% of marine organisms produce planktotrophic *larvae* dependent on exogenous food, some studies from lecithotrophic *larvae* (which appear unaffected by elevated CO₂), suggests that an organism's food efficiency and rate of metabolism may impact on their resilience to elevated CO₂ [43] and supports suggestions from Dupont *et al.* [32] that further investigation of the impact of ocean acidification on lecithotrophic *larvae* is needed.

In the absence of adequate adaptation or acclimation, under elevations in CO₂ sub lethal effects on sea urchin, brittlestar and seastar *larvae* from a range of geographical regions may develop more slowly, be smaller, have skeletal abnormalities and impaired physiological responses to environmental stressors which may be either ameliorated or exacerbated by temperature increases predicted for the end of the century. Some authors suggest that this may result in the disappearance of echinoderms from the surface oceans within the next 50–100 years [61]. To date what these studies do consistently show are the many sub lethal effects that may result.

3.2. Molluscs

The vulnerability to elevated CO₂ observed in echinoderms has also been reported in studies on *larvae* of molluscs (Table S2). A number of studies have found a delay in development or less development (the oysters *Crassostrea gigas* and *Saccostrea glomerata* [33,35,39]; the mussel *M. galloprovincialis* [57]; gastropod *Littorina obtusata* [63]), morphological shell abnormalities such as convex hinge and mantle protrusion and impacts on calcification size and growth rate (the oyster *Crassostrea gigas* [33,35,39]; the mussel *M. galloprovincialis* [57]; gastropod, *Littorina obtusata* shorter lateral, but longer spiral shell length [63] and *Saccostrea glomerata* [35,39,64]), decreases in shell length and thickness in the mussel (*Mytilus edulis* [65]), hatching rate (gastropod, *Littorina obtusata* [63], mussel *Mytilus edulis* [65]), degraded shells (mussel *Mytilus edulis* [65], polar pteropod *Limacina helicina* [66]) decreased rate of metamorphosis, shell thickness and loss of hinge integrity (bay scallop *Argopecten irradians* and the hard clam *Mercenaria mercenaria* [67]) and in the Mediterranean pteropod *Cavolinia inflexa* [68] shells were absent after 13 days due to dissolution, yet *larvae* displayed “normal” swimming action (Table S2). In rare instances there have been positive instead of negative impacts of elevated CO₂. For example, there was no effect on calcification rates, size or weight in juveniles of the grooved carpet clam *Ruditapes decussates* raised at *p*CO₂ of 1694 and 4245 ppm (pH 7.84 and 7.46 respectively), but mortality was reduced in acidified treatment (pH 7.46), possibly due to delayed reproductive development of clams preventing spawning in acidified treatments [69] as an energy saving survival strategy. Also in contrast to the other molluscs with mainly planktotrophic *larvae*, the lecithotrophic juvenile European cuttlefish, *Sepia officinalis*, which hypercalcify, similar to some teleost fish and decapod crustaceans, showed no adverse growth or developmental effects with significantly more CaCO₃ accreted into cuttlebones [70] and eggs increased in weight [71] when raised at elevated *p*CO₂. Of the few studies to investigate the synergistic impacts of elevated *p*CO₂ (600, 750, 1000 ppm, mean pH 8.02, 7.95 and 7.83 respectively) and temperature (18, 22, 26 and 30 °C), Parker *et al.* [39] found impacts on larval development including reduced development, size and increased abnormality in D veliger *larvae* and spat for both *Crassostrea gigas* and *Saccostrea glomerata* were exacerbated as did Lischka *et al.* [66] in the polar pteropod, *Limacina helicina*. Shell diameter, degradation and increment were significantly affected by CO₂, but not temperature, whereas the combination of elevated temperature and CO₂ increased mortality, yet with temperature the more dominant factor [66].

The CaCO₃ polymorphs deposited during embryonic and larval development are expected to be more soluble in an acidifying ocean than that which is deposited following settlement and metamorphosis. For example, calcification in bivalve molluscs generally begins in the early trochophore stage with the

deposition of amorphous calcium carbonate, the most soluble polymorph of CaCO_3 . During later larval development CaCO_3 deposition is converted to aragonite and following settlement and metamorphosis, calcite, the most stable CaCO_3 polymorph, is deposited [72,73]. Rather than earlier stages being more sensitive than later [37] there is an absence of evidence of hierarchical sensitivity and more evidence of sensitivity of larval stages being species specific. The pediveligers of *S. glomerata* were more sensitive than spat to changes to CO_2 and temperature, but this reversed in *C. gigas* with newly metamorphosed spat showing greater sensitivity than pediveligers [39]. This result is surprising given that the CaCO_3 polymorph (calcite) deposited by spat is less soluble than that deposited by the preceding pediveliger larval stage. In the lecithotrophic abalone, *Haliotis rufescens* thermal tolerance was impaired at pH 7.87 compared to control (pH 8.05) for pretorsion and late veliger life stages, but not posttorsion and premetamorphic veligers, revealing variable sensitivity of life stages to low pH [74]. Given also that the first effects of elevated CO_2 on the bivalve species are observed during the trochophore and D-veliger stage, corresponding with the first onset of shell mineralisation, evidence does not support a hierarchical level of response.

Genotypic variation may explain differences in larval development responses for the same species in different geographical locations (*Crassostrea gigas* Japan [33]; Sweden [49]; Australia [39]) and provide the potential for climate proofing aquacultural industries. Recently, significant differences in sensitivities to ocean acidification have been found even within the same species [43,44]. Selectively bred lines of the Sydney Rock oyster *Saccostrea glomerata*, when reared at elevated $p\text{CO}_2$, were more resilient with only a 25% reduction compared to a 64% reduction in shell growth of spat of wild populations, perhaps suggesting inherent genetic diversity among populations of species or environmental pre adaptive capacity which may ameliorate the impacts of ocean acidification on oyster populations [43]. Similarly, calcification rate differed between source populations of *Mercenaria spp.* signifying possible genotypic variation [44], while maternal provisioning may safe guard *larvae* from a high CO_2 world and lead to differences in evolutionary responses between species including molluscs and echinoderms [46,75].

3.3. Mechanisms in Echinoderms and Molluscs

The mechanisms associated with the response of marine *larvae* to ocean acidification are poorly understood. Few proteomic and genomic studies have shown that CO_2 -driven acidification may cause changes to various physiological pathways in *larvae* including those associated with biomineralization, skeletogenesis, energy-metabolism, respiration and molecular chaperones. The search to determine the vital physiological mechanisms and processes affected in marine invertebrates by elevated CO_2 during larval development has revealed calcification, protein synthesis, physiology, metabolism and molecular and gene expression are potentially the major processes affected [7,33,76-78] requiring further exploration and prioritising for research [41].

Studies are revealing that significant up-regulation and change in the gene expression profile of the heat stress gene *hsp70* which can be compromised at elevated CO₂ and temperature in the sea urchin, *Strongylocentrotus franciscanus* [79]. There was also down regulation of biomineralization, skeletogenesis and energy metabolism genes, whilst some acid-base and ion regulation genes were up-regulated in *larvae* of the sea urchin *Lytechinus pictus* which were significantly smaller and had more triangular skeletons and shorter arms than controls [80]. In contrast, decreased pH also had no effect on expression of two shell mineralisation genes *ap24* or *engrailed* at any developmental stage in the abalone *Haliotis rufescens* [74], but up and down regulation of proteins was found in *Saccostrea glomerata*, although these are yet to be identified [46]. Up and down regulation of genes associated with various pathways during exposure to ocean acidification suggests an organisms effort to compensate for the effects of elevated pCO₂ [81]. There have also been studies which highlight the variability in the potential ecotoxicological consequences associated with elevated CO₂ accumulation of metals varied; ^{110m}Ag increased and ¹⁰⁹Cd decreased with decreasing pH, whereas ⁶⁵Zn accumulation was highest at pH 7.85, but lower at pH 7.6 than 8.1 (control, [71]) in the cuttlefish *Sepia officinalis*.

In most recent studies [82,83] have found that under elevated CO₂ sea urchin *larvae* of *Strongylocentrotus purpuratus* allocated a smaller percentage of available energy to somatic growth with up regulation of genes such as ATP synthase data suggesting a higher need of ion regulatory activity and down regulation of calcification related genes. Although it remains unclear, which cellular processes impact energy budgets negatively, increased ion regulatory effort for cellular homeostasis is a good candidate (see e.g., [84]). Although many species capable of controlling intracellular pH, they are often incapable of maintaining extracellular pH [85].

3.4. Crustaceans

The studies done on crustaceans have been at CO₂ exposures more than double that which is expected at the end of the century and across a range of groups including copepods [50,86], shrimps [87,88], barnacles [89-91], crabs [92,93] and amphipods [94] (Table S2). The variation in responses to elevated CO₂ in isolation and in combination with temperature or salinity, together with ecotype and geographical origin sensitivity within and between species indicates that a generalised statement on the affect of acidification on crustaceans is not possible on the data available to date (Table S2).

At acute worst case scenario CO₂ exposures ranging between 72 hours to 9 days there was no reduction in hatching success in the copepod *Acartia tsuensis* and little sensitivity to elevated CO₂ during any stage of their early development [50], but there was decreased hatching success and increased naupliar mortality of the copepods *Acartia erythraea* [86] and decreased hatching success of *Calanus finmarchicus* [95] Table S2. The shrimp *Palaemon pacificus* also displayed variable responses at different developmental stages, pH and length of exposure [87]. Following long term (30 weeks) exposure of adults to pH 7.89 and 7.64, survival and egg production in early developmental stages decreased in both treatments. Growth rates, however, were unchanged, although the second antennae was significantly shorter and moulting frequency increased after 21 weeks at pH 7.89 with moulting frequency decreased between weeks 10 and 12 and growth rates decreased at pH 7.64.

McDonald *et al.* [91] found no effect of elevated CO₂ on egg production, naupliar survival or nauplii or cyprid size, attachment or metamorphosis or juvenile growth in the subtidal barnacle *Amphibalanus amphitrite*, reared at pH 7.4, but did find effects on shell resistance to dislodgement in the adults and reduced upper wall shell strength. In contrast, Findlay *et al.* [96], found a slower rate of development of embryos in the common intertidal northern hemisphere barnacle *Semibalanus balanoides* with an estimated 19 days delay in reaching 50% hatching stage at pH 7.7. In further studies on the post larval stages (cyprid through to commencement of feeding) of *Semibalanus balanoides* differences in responses were dependent on the origin of the settlers. There was no effect on growth rate, but reduced calcium content and survival at elevated CO₂ and temperature [90] of metamorphosing post-larvae of temperate origin while growth and development was reduced in post-larvae of Arctic origin [89]. Such geographic differences in responses within the population of *S. balanoides* need to be considered with species specific responses as in the same study there was no effect on calcium content, or survival, but a decrease in growth rate at pH 7.7, for the intertidal barnacle *Elminius modestus* from sheltered rather than rocky shores.

Juvenile *Gammarus locusta* amphipods growth rate and survival at elevated pCO₂ [88] was not significantly impacted by a decrease in sea water pH of up to 0.5 units. Although, there was a significant increase in the expression of *hsp70* and *gapdh* gene at a pH of 7.5, which may indicate metabolic changes in response to acidification [88]. While larvae of the European lobster *Homarus gammarus* as measured by carapace length, but there was a reduction in carapace mass with a reduction in exoskeleton material (calcium and magnesium) content of the carapace although there was no effect on survival [92]. When elevated CO₂ was combined with the additional stressor of salinity, there was no effect on the number of hatchlings or calcium deposition in the amphipod *Echinogammarus marinus* reared under elevated CO₂, but there was an observed trend (albeit a non significant interaction) for an increase in the developmental time to hatching when lowered pH was combined with the additional stressor of lowered salinity [94]. Larvae of two populations of the spider crab *Hyas araneus* raised at 710 and 3000 ppm pCO₂, however, displayed delayed development, reduced growth and fitness with zoeal and megalopa stages being most sensitive in the northernmost (79°N) and southernmost (54°N) populations respectively [93]. Further significant differences in responses were found between the two populations sampled when the additional stressor of temperature was added, with the megalopae of the southernmost population exhibiting high sensitivity to enhanced CO₂ and the northernmost exhibiting high sensitivity to increased temperature [93,97]. The Helgoland southern megalopae accumulated more calcium than the northernmost Svalbard megalopae, with higher calcium contents at the highest elevations in CO₂. There was also a lower capacity for calcium incorporation in crab larvae at the cold end of their distribution suggesting they may be more sensitive to ocean acidification than temperate regions. This suggests that possibly enzymes and transporters of calcium ions and their assembly in the chitinous layer in a temperature dependent mechanism.

3.5. Corals

Different developmental stages and species of corals exhibit similarly variable and complex responses in acidification studies (Table S2). Survival of planular *larvae* of the intertidal *A. digitifera* was not affected at pH 7.6 or 7.3, whereas survival of the subtidal western Pacific *Acropora tenuis* was greater at pH 7.3 than at 7.6 [98]. In contrast, the survival and settlement of planular *larvae* of the intertidal tropical eastern Pacific coral *Porites panamensis* was unaffected at pH 7.85 and similarly unaffected by the synergistic effect of elevated CO₂ and temperature [99]. This difference in survival response to acidification between even closely related species (*A. digitifera* and *A. tenuis*) from different tidal zones warrants further investigation to establish whether species of intertidal origin are more robust to fluctuations in pH due to inherent variability in their environment, and whether a difference in tidal origin can explain differences in species responses for other invertebrates such as echinoderms [47].

Suwa *et al.* [98] also found that primary polyp size of *A. tenuis* reduced as pH decreased. Furthermore, establishment of symbiosis with zooxanthellae was delayed. Zooxanthellae levels were, however, not affected in *P. panamensis* primary polyps raised at lowered pH whereas elevated temperatures resulted in a reduction of almost 60%, although zooxanthellae levels were greater at elevated temperature and CO₂ combined than at elevated temperature alone [99], suggesting that reduced pH may moderate the effect of temperature rise on zooxanthellae levels. Also biomass of *P. panamensis* primary polyps was slightly lower when reared at elevated CO₂ or elevated temperature separately, but biomass was 45% lower and skeletal mass and dry weight (calcification) reduced by almost one third when the two stressors were combined indicating a synergistic interaction between temperature and pH. In the most recent studies, Albright *et al.* [58] and Albright and Langdon [100] found that in the Caribbean corals *Acropora palmata* and *Porites astreoides* that settlement and growth were all negatively impacted by increasing CO₂, settlement being reduced by 42–60% relative to controls with results indicating that settlement cues were altered and post settlement growth also was reduced.

3.6. Fish

Finally the effects of ocean acidification on the early developmental stages are not solely confined to invertebrates (Table S2). Gametes embryos and *larvae* of vertebrates such as fish are also vulnerable to changes in ocean chemistry. When embryos and *larvae* of the Japanese whiting, *Sillago japonica* and the silver seabream, *Pagrus major*, were exposed to extreme pCO₂ concentrations, the percentage hatching and survival rates decreased significantly with increased CO₂ and length of exposure [101]. These concentrations of CO₂ however, were much greater than those expected to occur as a result of anthropogenic inputs of atmospheric CO₂. In a series of more recent studies, Munday *et al.* [102–104] investigated the impact of more realistic pCO₂ concentrations on the eggs and *larvae* of the orange clown fish *Amphiprion percula* finding no significant effect of elevated CO₂ on the embryonic development, egg survival and hatching size of *A. percula*. In fact, in general the clown fish *larvae* that were reared at elevated CO₂ had an increased growth rate compared to the controls [104]. These positive effects, however, were reversed during settlement as the *larvae* reared at elevated CO₂ showed

impaired olfactory discrimination and homing ability [103], although there were no physical abnormalities in the structure of their nasal cavities. Olfactory cues that prompted avoidance or neutral behaviour in controls (pH 8.15) stimulated strong preference behaviour in *larvae* raised at pH 7.8 in addition to significant reduction in response to usually positive preferences. *Larvae* raised in pH 7.6 remained unresponsive to all cues, positive or negative whether tested at pH 7.6 or control level, whereas responses of *larvae* raised in control pH, but tested at pH 7.6 were unchanged from those raised and tested at the control level. This confusion of olfactory cues could potentially disrupt suitable site selection at settlement. More recent studies by Munday [105,106] on the marine clown fish *Amphiprion percula* reared at ambient (pH 8.15) and elevated CO₂ (pH 7.6–7.8) found no effect on otolith size, shape, symmetry or otolith chemistry at pH 7.8 and slightly greater otolith area at pH 7.6. It may be that fish will be able to regulate acid-base status to compensate for acidosis [105,106]. This is different to many marine organisms which being osmo-conformers have a low capacity for ionic regulation and a lesser ability to acid–base regulate through the process of ionic exchange [77].

4. Summary

With such a range of responses across life history stages, phyla, species and geographic regions, recent meta-analyses have once again attempted to draw conclusions on the sensitivity to elevated CO₂ of different developmental stages of marine organisms [13–16] with conflicting conclusions. In the most extensive meta-analysis [16] when all taxonomic groups (calcifying algae, corals, coccolithophores, molluscs, echinoderms, crustaceans, fishes, fleshy algae and seagrasses) were combined, survival (42 results—R, 32 species—S), growth (86R, 38S), calcification (63R, 37S) and reproduction (12R, 8S) were all negatively affected by acidification, whereas photosynthesis (45R, 20S) was positively affected. When life stages for all groups were examined relative to survival there was no difference amongst taxonomic groups, *larvae* had the most negative mean effect, although the result was not significant (8R), whereas adult survival had a smaller mean effect, yet was significant (25R) [16]. Although the effect of elevated CO₂ and associated decrease in pH on larval development of both echinoderms and molluscs is highly variable, dependent on the life stages, early feeding strategies, latitudinal distribution, previous acclimation due to habitat and robustness of gene expression processes, there is a generally negative impact. There are conflicting views on whether earlier rather than later life history stages are more sensitive. In a meta-analysis by Kroeker *et al.* [16], based on 73 studies with 251 experimental results there was no variation in mean effect among life stages for different biological variables concluding “this synthesis did not support all the leading hypotheses for variation in response to ocean acidification” “instead found the explanatory power of these hypotheses was specific to organisms within taxonomic groups”. Further meta-analyses [13,15,16] do not support the hypothesis that early life history stages are more sensitive than adults to the effect of ocean acidification. Hendriks *et al.* [13] found reduced larval growth (–16%) in sea urchins, but found adult growth significantly more sensitive (–62%), yet data was limited with only 2 papers and 2 species contributing to the sea urchin embryo and 1 paper and 2 species contributing to the adult data. A later exercise by Hendriks and Duarte [15] consolidated this position concluding there was no difference in effect size of different life stages by pooled groups of organisms using a dataset restricted to 16 studies species in 2 studies, yet in contrast Dupont *et al.* [14] found gametes and early

developmental stages of echinoderms appeared “far more impacted” than adults relative to calcification, growth and survival although the number of experimental responses was heavily weighted towards *larvae* with 47, 10 and 17 responses for *larvae*, juveniles and adults respectively. Calcification and growth affects across all life stages for all groups was not significant, but growth was significantly negative in calcifying algae and corals, yet significantly positive in fish and fleshy algae. When life stages were analysed for groups with regard to growth, echinoderm juveniles (5R, 4S) had a larger negative effect than *larvae* (10R, 6S). When survival was examined mollusc *larvae* (5R, 4S) had a larger negative effect than adults (10R, 10S) and crustacean adults (5R, 5S) had a larger negative effect than juveniles (6R, 3S) indicating the most sensitive life stage varies among taxonomic groups and life stage sensitivity variation is small compared to life history. However, it can be seen that data sets are becoming smaller and consequently less powerful as indicators as the interrogation becomes more targeted. Further analysis of the dataset revealed low-magnesium calcite and aragonite calcifiers were negatively affected by ocean acidification, yet high magnesium-calcite calcifiers were not significantly affected [16]. Reviews and meta-analyses of numerous studies reveal that extraction of statistical information subject to various criteria (life stage/taxonomic group/energy pathways/species *etc.*) are far from consistent regarding the potential impact of ocean acidification at biological levels (e.g., [13-16,36]), suggesting that the complexity of biological systems confounds simplistic conclusions. Such studies highlight the need to study multiple species from different geographical locations to differentiate species-specific responses and differentiate between which hypotheses are supported and rejected by the evidence.

5. Conclusions

The common negative impact of ocean acidification on the early developmental stages of most species studied to date is a reduction in the rate of larval development, a reduction in larval size and alteration to shell integrity (Table S2), both of which may potentially affect survival and prolong larval life. Prolonging the length of larval life is generally considered negatively because it increases the chance of predation in the water column, particularly in the absence of properly calcified shells and skeletons and reduces the time available to settle. Furthermore, reduced larval size can reduce the feeding efficiency of *larvae* [33,67], and smaller *larvae* are more susceptible to starvation because they encounter comparatively less food [33,107-109]. There are also implications for energetics of *larvae* and larval dispersal. Altered energetics may influence metamorphosis as found in bivalves [67] with impaired metamorphosis [67,91] and flow on effects [10] to post settlement mortality. Therefore, even sub-lethal effects of elevated CO₂ can severely alter the composition and fitness of *larvae* and given the high mortality rates of *larvae* in the water column [6] and during the transition to benthic settlers, small perturbations to *larvae* potentially may have large alterations to settlement dynamics, post-settlement mortality, recruitment and ultimately adult populations.

Evidence from field studies on the impact of ocean acidification on the settlement of invertebrates utilising the natural pH gradient of pH 8.17 (control)–6.57 (336–5148 ppm) produced by volcanic CO₂ vents in shallow coastal waters off Ischia in Italy found differences in invertebrate groups depending on the pH [110]. Calcifiers, including foraminifera, bivalvia, the gastropod *Osilinus turbinatus* and the isopod *Cymodoce truncata* were primarily restricted to control sites, whereas most polychaetes, the

gastropod *Rissoa variabilis* and the amphipod *Caprella acanthifera* were present in sites across the full pH gradient. The polychaete *Syllis prolifera* and the tanaid *Leptochelia dubia* were more abundant at low and intermediate pH respectively. Copepods were generally absent from low pH zones and the isopod *Dynamene cf bifida* was most abundant at the most acidic site. A previous study in the same area by Hall-Spencer *et al.* [111] along a pH gradient of 8.2 (control) to 7.4 found that sea urchin abundance was significantly reduced below pH 7.5, whereas abundance of the barnacle *Chthamalus stellatus* was only impacted at a mean of pH 6.6. Natural carbon dioxide seeps in Milne Bay, Papua New Guinea also provide insights into the future community structure of coral reefs under elevated CO₂ concentrations [112] with a 40% reduction in biodiversity of corals and dominance by *Porites* [112].

Over the coming decades, it is likely that a rising surface ocean CO₂ will also be accompanied by rising surface ocean temperatures. Despite our collective research, there are still large unknowns of the biological consequences of the dual impact of acidifying and warming oceans on fertilisation, larval development and settlement on marine organisms. Increasingly studies are considering the synergistic or antagonistic impacts of other environmental variables (*i.e.*, temperature, nutrients, hypoxia salinity; [12,34,35,39,102,113-116]). Those that have, are finding that differences do exist [43,45], but there is more to be done.

If we are to move forward we need chronic experiments which allow the potential for species to acclimate over long term perturbations a measure of variability in responses of organisms within and between populations and an assessment of adaptive capacity associated environmentally induced plasticity [117] and focus on identifying the underlying mechanisms [43,44,46,77]. Current models remain constrained by acute experiments where in a period of days there is a sudden drop in pH of 0.4 units which does not mimic well the longer time frame over which this will occur (0.0044 pH/yr [15]). Negative results from short term studies on *larvae* make it difficult to extrapolate to long term impacts on marine environments absolutely, especially when these potentially could couple with positive effects on adults (increased production and robustness of propagules, [15]) and greater capacity to acclimate [42,43,46] giving further support for an entire life cycle environmental approach to measuring the impact of ocean acidification. Extrapolation is also difficult because results from the laboratory are not necessarily replicable in the field and caution is needed with translating between these two contexts because of unforeseen consequences [38,118,119].

Many of the organisms discussed in this review contribute significant ecological roles to the marine environment and negative impacts, even if sub lethal may result in ecological and economic consequences. If we are to create the authoritative and robust meta-analyses and generalisations that we need to manage our marine environments [16] then we need significantly more research.

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