

REVIEW

Effects of ocean acidification on the settlement and metamorphosis of marine invertebrate and fish larvae: a review

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ABSTRACT: Most marine organisms present an indirect lifecycle where a planktonic larval stage reaches competency before settling to the substrate and metamorphosing. Despite the critical importance of these early life history stages, little is known about how global change-related stressors, in particular ocean acidification (OA), affect marine larval settlement and metamorphosis. To date, 48 studies have investigated the effects of OA on larval settlement, focussing mostly on tropical corals (16), echinoderms (11) and fish (8). Most studies show negative effects of OA during settlement and post-settlement processes. For instance, reduced settlement is typically seen along natural pH gradients and in experimentally lowered pH treatments. This generally results in reduced settlement selectivity and metamorphosis and poorer post-settlement fitness. Carryover effects of OA exposure can also occur, with larval environmental history influencing early post-settlement performance. We conclude that OA may (1) alter larval supply for settlement by altering horizontal swimming behaviour or vertical migration; (2) directly influence settlement success through changes in the nature and distribution of suitable settlement substrates (e.g. biofilm, crustose coralline algae); and (3) mediate carryover effects at settlement by altering larval development or larval energy budgets. In contrast to fish larvae, there is little evidence for most invertebrate larvae that their perception of settlement cues is directly influenced by reduced pH. A summation of how OA affects the settlement and metamorphosis of marine invertebrates is timely, since altered settlement rates will influence the future distributions, abundances and ecology of marine benthic communities.

KEY WORDS: Ocean acidification · Settlement · Metamorphosis · Larvae · Recruitment

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INTRODUCTION

Since the start of the industrial revolution, an additional 555 Pg of carbon have been released into the

atmosphere, of which an estimated 155 Pg C (~30%) have entered the ocean (Stocker et al. 2013). The rise in atmospheric CO₂ and other greenhouse gases has resulted in a global increase in average sea surface

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temperatures and a reduction in seawater pH (Caldeira & Wickett 2005). This process, termed ocean acidification (OA), is predicted to further decrease average surface ocean pH by 0.2 to 0.4 pH units by the year 2100 (Stocker et al. 2013).

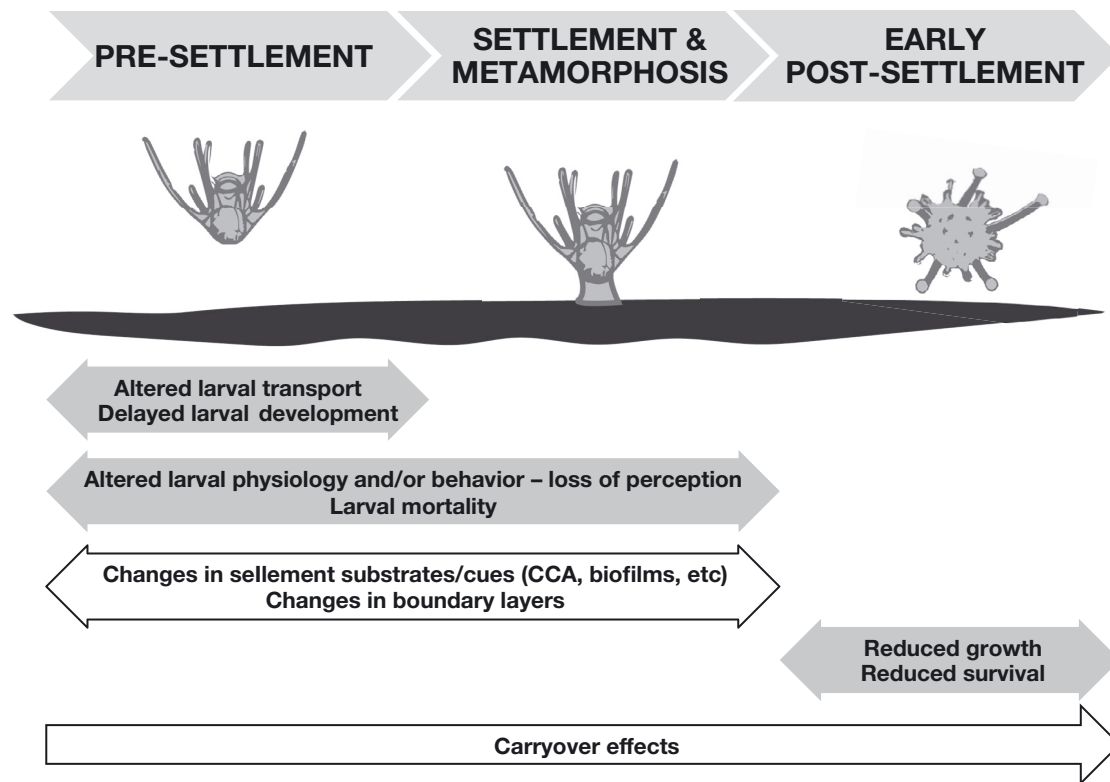
Acidification is predicted to have negative consequences for most marine organisms (see reviews by Kroeker et al. 2010, 2013, Wittmann & Pörtner 2013), and a number of published reviews have discussed the deleterious impacts on key invertebrate groups such as molluscs (Byrne 2010, Gazeau et al. 2013), echinoderms (Byrne 2010, 2011, Dupont et al. 2010b, Byrne et al. 2013, Dupont & Thorndyke 2013) and corals (Hoegh-Guldberg et al. 2007, Kleypas & Yates 2009). These reviews have primarily drawn upon laboratory studies examining the response of marine species at the physiological and organismal level, with observations often used to predict responses to global change at the population and community levels. In addition, field studies at CO₂-vent sites have shown responses consistent with those seen in the laboratory (e.g. Hall-Spencer et al. 2008, Fabricius et al. 2015, Lamare et al. 2016, Foo et al. 2018). However, to fully understand and predict the response of marine species to global change, it is also necessary to identify the most vulnerable life stages and/or life-history processes and bottlenecks (Hofmann et al. 2010). This is especially important for marine organisms that have a complex lifecycle involving a longer-lived benthic adult stage and a shorter-lived planktonic larval stage, as these early life history stages are often thought to be the most sensitive to OA and warming (Hofmann et al. 2010, Byrne 2011, Przeslawski et al. 2015, Byrne et al. 2017).

The transformation from a planktonic larva to a benthic juvenile is a complex process involving progressive steps from the pre-settlement phase (processes that bring larvae to the site of settlement), to the settlement (the exploration, selection, attachment and metamorphosis) and post-settlement stage (early juvenile growth and survival) (Gosselin & Qian 1997, Hunt & Scheibling 1997). Most marine species (at least 70% of all marine invertebrates) broadcast-spawn their gametes into the water column for external fertilisation, with subsequent planktonic development of a free-living larval stage (indirect development) followed by settlement (Thorson 1950). Despite the fact that altered settlement rates can shape future distributions, abundances and ecology of marine benthic communities, little is known about how acidification impacts this transitional process and the degree to which effects on larval development influence their settlement behaviour and success. Hence, in this re-

view, we identify the most likely impacts of OA on larval settlement and metamorphosis (Fig. 1) and give a perspective on the key responses of the settlement stage to this environmental change.

As prior reviews have examined the effects of OA on the early development and settlement (Ross et al. 2011), early life stages (Kurihara 2008, Byrne et al. 2011b) and sensory function of marine organisms (Ashur et al. 2017), here we specifically focus on outcomes for settlement success. We define settlement as the moment when larvae attach to the substrate (both reversible and irreversible) and metamorphosis as the irreversible change from the larval form to the juvenile form that includes a 'set of events and morphogenetic changes which allow the attached larvae to acquire features appropriate to its new life [sic]' (Rodriguez et al. 1993, p. 194). With respect to early post-settlement processes, we refer to juvenile stages immediately after metamorphosis (i.e. within days of settlement).

A search of all databases in Web of Science (up to March 2018) using the key words 'ocean acidification', 'settlement', 'larvae', 'invertebrates' and 'fish', connected with 'OR' and 'AND', identified 48 publications from which we extracted data on taxa, life-stage(s), measured parameters, levels of *p*CO₂ tested (or reported pH levels; the pH scale—NBS scale or total scale—up to 2 decimals is indicated throughout the manuscript unless unreported in the original study), responses observed and biogeographic region. To date, most published research has focussed on settlement (39 studies), with fewer studies assessing early post-settlement processes (22 studies) or carryover effects (4 studies) (Fig. 2). Most studies have examined responses in Cnidaria (only tropical corals), with the settlement response of many key benthic taxa being significantly understudied (e.g. molluscs, crustaceans) or largely overlooked (e.g. sponges). Within the broad-scale phases of pre-settlement, settlement and early post-settlement, we examined a number of key mechanisms that could be altered by OA (Figs. 1 & 3) and discuss implications for future population dynamics. We also identify future research priorities needed to enhance our understanding of how OA will affect settlement processes in the marine environment. Tables S1–S3 in the Supplement at www.int-res.com/articles/suppl/m606p237_supp.pdf, summarise the studies that explore aspects of the effects of elevated *p*CO₂ on settlement (or recruitment) of marine organisms for different taxonomic groups. Table S1 presents an overview of field studies at CO₂ vents, and Tables S2 and S3 gather information on single-stressor or multiple-stressor laboratory studies, respectively.



Credit: J.C Espinel

Fig. 1. Potential effects of ocean acidification (OA) on pre-settlement, settlement and post-settlement processes in marine species with complex lifecycles (i.e. those with a free-living larval phase). OA can cause direct effects (grey arrows), indirect effects (white arrow in the middle) or carryover effects (white arrow at the bottom). Direct effects influence larval physiology or behaviour in such a way that settlement success will be altered either during pre-settlement processes (i.e. changes in swimming ability or substrate recognition) or during the settlement processes themselves (i.e. changes in larval physiology or behaviour, e.g. larvae not being able to attach). Altered neurosensory structures and altered neurotransmitters or signalling pathways are some of the mechanisms that could be directly altered by OA. Ontogenetic mechanisms that induce larval behavioural changes necessary to migrate to specific settlement areas (e.g. phototaxis, geotaxis) might also be affected. Indirect effects caused by changes in settlement substrates or their associated chemical cues (i.e. changes in community composition in marine biofilms or crustose coralline algae [CCA] crusts) will impede or delay the settlement process itself. Carryover effects influence the settlement success by altering the energy reserves of the larvae due to the larvae being exposed to elevated $p\text{CO}_2$ during their larval development. Altered larval energy levels at the settlement stage might alter or delay settlement, while low energy levels at the time of metamorphosis could compromise the survival and fitness of newly settled individuals

PRESENT-DAY OBSERVED EFFECTS OF ELEVATED $p\text{CO}_2$ /REDUCED pH ON NATURAL SETTLEMENT (AS MEASURED BY RECRUITMENT PATTERNS)

Naturally occurring CO_2 vents are valuable systems for understanding the effects of long-term exposure to elevated $p\text{CO}_2$ on marine ecosystems (Hall-Spencer et al. 2008, Brinkman & Smith 2015, Foo et al. 2018), including potential effects on settlement and recruitment of marine invertebrates (Table S1). Recruitment studies at CO_2 vents provide insight into the potential effects of OA on settlement along natural pH gradients and integrate a range of key processes that occur prior to their

measurement, such as larval supply and post-settlement survival. Studies along these naturally occurring pH gradients show a decrease in settlement with reduced pH in many taxa, although the response appears species-specific. For example, Cigliano et al. (2010) examined settlement in 79 taxa on artificial substrates that were placed for 1 mo along a pH gradient occurring at the vents off Ischia (Italy) and found that calcifying groups (foraminiferans, polychaetes and molluscs) recruited in significantly lower numbers in reduced pH_{NBS} (7.08–7.79) compared with high pH_{NBS} (8.09–8.15). Vermetid mollusc recruitment at CO_2 vents located in Vulcano Island (Italy) was also reduced at low-pH sites (Milazzo et al. 2014).

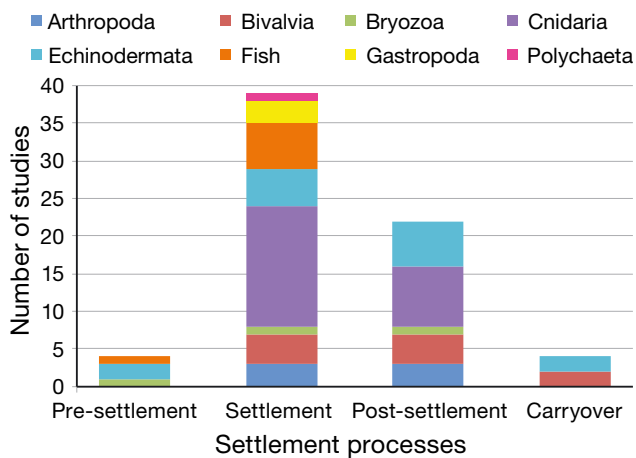


Fig. 2. Studies published examining the effects of ocean acidification on the pre-settlement, settlement, post-settlement and carryover processes in marine invertebrates and fish. Some studies focus on more than 1 species, and/or on more than 1 process associated with settlement. This analysis does not include studies that were carried out in naturally occurring CO_2 vents, since these studies focussed on recruitment (often on artificial substrates) that do not differentiate different settlement processes. A total of 48 published studies are represented

In one of the more extensive experiments on coral settlement associated with natural $p\text{CO}_2$ vents, Fabricius et al. (2017) out-planted artificial substrates for 9 mo at 2 sites with a median and ambient (control) pH_T of 7.8 and 8.0, respectively. After 9 mo, substrates showed differences in *in situ* recruitment, with a greater density of larger coral recruits on control substrates compared with low-pH substrates. The majority of corals had settled on specific crustose coralline algae (CCA) species, with differences in CCA cover (i.e. smaller percentage cover on slates grown at the low-pH site) being the strongest predictor of recruitment density. The research also included experimental choice experiments, which showed significantly more coral larvae choosing to settle on substrates from the control site. In most cases, there was no direct effect of seawater pH on larval substrate choice *in vitro*. Importantly, the study concluded that altered settlement (as opposed to pre- or post-settlement factors) may become the dominant driver of altered coral reefs under OA.

While the recruitment of some species on vents can be adversely affected by OA, other taxa appear unaffected or show greater recruitment under low pH. Ricevuto et al. (2012) working along pH gradients off Ischia measured settlement on artificial substrates, and reported that apart from molluscs, most studied taxa (polychaetes), and especially crustaceans (amphipods, tanaids and isopods), recruited successfully

at both low-pH sites ($\text{pH}_T \sim 7.26$). At the same site, Ricevuto et al. (2014) studied the colonization of 3 polychaete species and noted that all were significantly more abundant at the areas presenting higher $p\text{CO}_2$ levels.

Pre-settlement processes and settlement

Settlement in marine species is directly dependent on pre-settlement processes (i.e. competent larval supply), and includes those factors that affect embryonic and larval development, survival and the immigration of larvae to the site of settlement (Fig. 3A). Much research has focussed on the response of larval stages to the effects of global change (see reviews by Dupont et al. 2010b, Byrne 2011, Kroeker et al. 2013, Przeslawski et al. 2015, Byrne et al. 2017), with acidification and warming both being deleterious to development.

Embryonic and larval development

Many studies show that OA can impact planktonic larval duration (PLD). In the face of naturally high mortality of larvae experienced in the plankton, these changes are likely to result in a significant change in the numbers of larvae reaching settlement, and such indirect effects are often discussed (i.e. echinoderms: Lamare & Barker 2001, Uthicke et al. 2013). Less often considered, and yet to be experimentally tested, is whether OA-driven larval physiological stress affects substrate selectivity of larvae. For example, the 'desperate larvae hypothesis', originally proposed by Knight-Jones (1951) and Wilson (1953), states that larvae which have a longer PLD will choose to settle more readily (i.e. reduce substrate selectivity) in order to avoid further delays in moving out of the water column, although early settlement can also be deleterious (Mos & Dworjanyn 2016). A recent study on larvae of the eastern oyster *Crassostrea virginica* demonstrated a correlation between larval age and a loss in settlement substrate selectivity (Meyer et al. 2018), showing a relationship between the desperate larval hypothesis and a longer PLD. While this phenomenon has been reported for a number of larval groups (i.e. polychaetes) where a longer PLD is due to reduced food availability (Qian & Pechenik 1998), potential alteration in substrate selectivity is yet to be investigated in studies that show OA-induced longer PLDs. Such processes could be important if larval substrate selectiv-

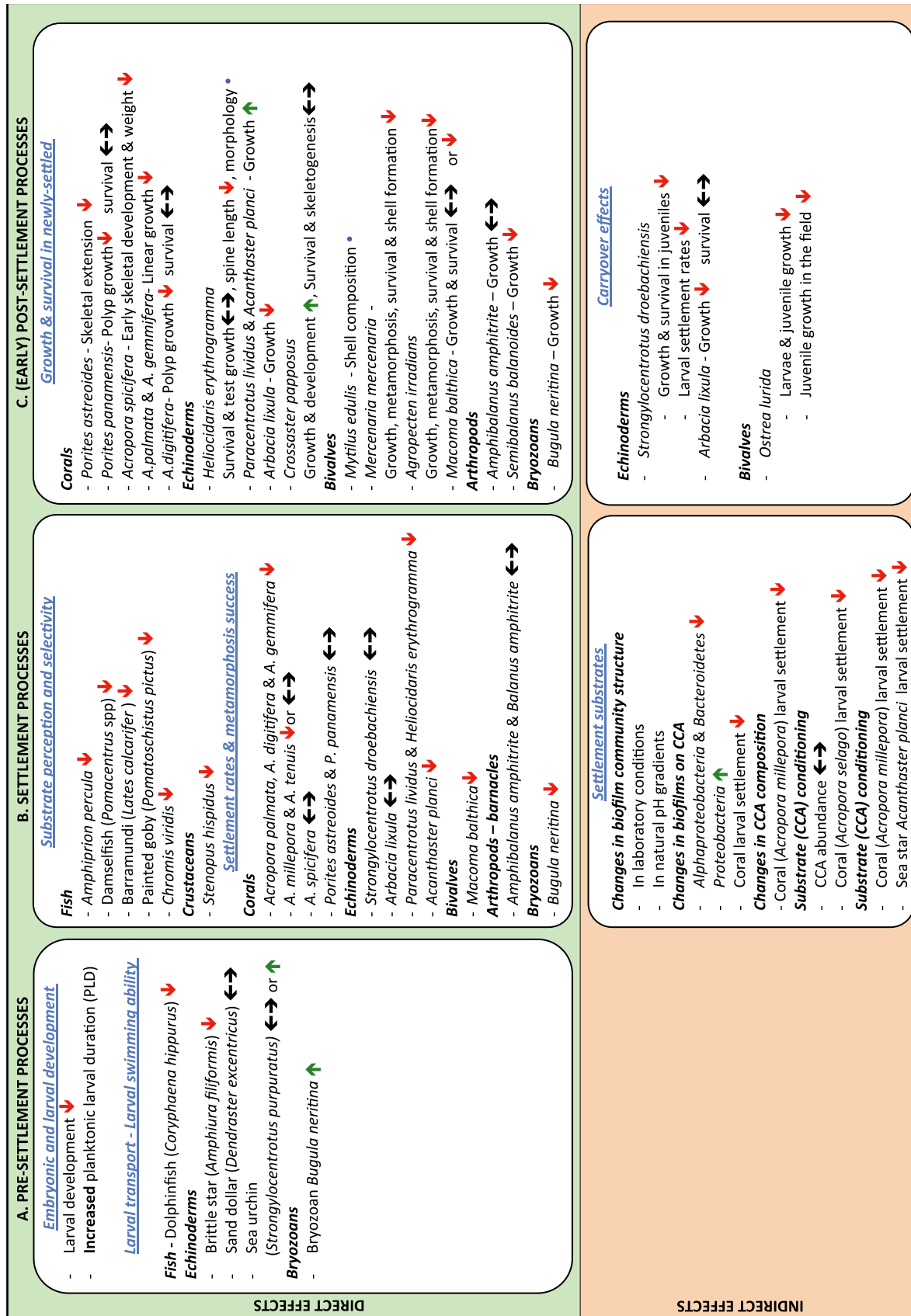


Fig. 3. Summary of the information currently available in the literature on the key direct and indirect responses associated with settlement in marine invertebrate and fish larvae to ocean acidification conditions. Responses were determined to be direct or indirect for (A) pre-settlement, (B) settlement and (C) post-settlement processes. Arrows indicate the effect caused by exposure to reduced pH or increased pCO₂: red downward arrows indicate negative effects, green upward arrows indicate positive effects, double-sided black arrows indicate no effects observed, and a blue dot indicates that changes were observed, but it was not reported whether these were positive or negative

ity at settlement is strongly influenced by PLD, in which case OA may indirectly affect settlement patterns.

Larval transport

Whether it is passive transport due to water movement or active transport associated with larval swimming behaviour, larval flux is a key process determining larval supply for settlement. As already reported, climate change-driven altered ocean currents and ocean warming are presently influencing larval distributions (Richardson 2008) and thereby settlement patterns on larger spatial scales. In addition, smaller-scale changes in larval transport and movement may be important, especially associated with active larval swimming behaviour. This is seen in swimming fish larvae that show important changes in their behaviour under elevated $p\text{CO}_2$ and which can affect their horizontal movement and settlement. Examples include the work by Munday et al. (2009), who found that tropical clownfish *Amphiprion percula* larvae under elevated $p\text{CO}_2$ have impaired olfactory ability to detect homing signals important for swimming to suitable settlement sites. In addition, laboratory exposure of dolphinfish *Coryphaena hippurus* larvae to elevated $p\text{CO}_2$ (~1600 $\mu\text{atm CO}_2$) caused a reduction in swimming duration and orientation that may have implications for dispersal success and recruitment (Pimentel et al. 2014). OA may not have the same direct effects on invertebrate larvae; however, there may be more subtle effects that alter horizontal transport. For example, larval stages often alter vertical distributions through swimming in order to influence their larger-scale horizontal transport, such as cross-shelf transport (Morgan et al. 2014). Of relevance here are the observations that reduced seawater pH can alter larval shape (Byrne et al. 2013, Kamyra et al. 2014) and thereby alter larval swimming ability and behaviour, such as shown for echinoderms (Chan et al. 2016). This has implications for the capacity of larvae to maintain their position in the water column or to reach their preferred settlement sites. For example, Chan et al. (2016) found that horizontal swimming speeds in brittle star *Amphiura filiformis* larvae were slower in reduced seawater pH at levels commensurate with near-future projected OA, with morphological changes thought to be the cause (i.e. reduced arm lengths involved in propulsion).

Interestingly, however, not all taxa show a loss of swimming capacity. Chan et al. (2011) found no dif-

ferences in swimming speeds of sand dollar *Dendraster excentricus* larvae in response to elevated $p\text{CO}_2$ (1000 μatm , $\text{pH}_{\text{NBS}} \sim 7.75$), despite changes in morphology, while Chan et al. (2016) found either no effect or positive effects of reduced pH (7.7) on the swimming speeds of larval sea urchins *Strongylocentrotus purpuratus*. Larvae of the bryozoan *Bugula neritina* also showed enhanced swimming (32% faster) and swam for longer periods at pH_T 7.6 compared with larvae reared in control pH_T 7.9, with the larvae growing to a larger size at low pH (Pecquet et al. 2017). A direct effect of increased H^+ concentrations in lower pH was proposed to alter action potentials and ciliary beating that stimulated swimming, while longer swimming durations may reflect a loss of settlement cues. To quantify the potential effects of behavioural changes on settlement and recruitment among marine invertebrate taxa, it is important to understand the interaction of changes in swimming ability and larval duration with water movement. This will require physical transport modelling of dispersal that incorporates OA-induced changes in larval behaviour and physiology (Chan et al. 2018).

Settlement processes

Settlement rates in elevated $p\text{CO}_2$

A number of laboratory studies have reported the effects of OA on settlement rates and patterns (Table S2). For tropical corals, Doropoulos et al. (2012) noted significant reductions in settlement of *Acropora millepora* larvae under elevated $p\text{CO}_2$ (800 and 1300 μatm , pH_T 7.79 and 7.60, respectively), Webster et al. (2013b) reported reduced metamorphosis success in *A. millepora* and *A. tenuis* under OA conditions (pH_T 7.51, 1638 $\mu\text{atm CO}_2$), and Albright et al. (2010) noted a linear decline in *A. palmata* settlement rate across a 491 to 876 $\mu\text{atm } p\text{CO}_2$ range ($\text{pH}_T \sim 7.96$ to 7.75), with a reduction of 69% between the lowest and highest $p\text{CO}_2$ tested. Metamorphosis in *A. digitifera* declined from 98 to 83% in larvae exposed for 2 h to ambient pH_T of 8.05 (~331 $\mu\text{atm CO}_2$) and reduced pH_T of 7.33 (~2100 $\mu\text{atm CO}_2$), respectively (Nakamura et al. 2011). In the same experiments, when the larvae were exposed to reduced pH for 7 d, a similar trend in metamorphosis rate was observed, and metamorphosis levels were much lower (i.e. <25%). Yuan et al. (2018) found reduced settlement in *A. gemmifera* larvae when exposed to reduced pH_{NBS} (7.8 and 7.5) at the time of settlement. In echinoderms, Dupont et al. (2013) noted delayed settlement in

S. droebachiensis, while in the sea urchin *Paracentrotus lividus*, García et al. (2015) observed delayed settlement by 8 d in pH_{NBS} 7.7 compared to pH_{NBS} 8.1, and no settlement at pH_{NBS} 7.4. For *Arbacia lixula* and *Heliocidaris erythrogramma*, however, there was no delay in settlement at pH_{T} 7.69 and pH_{NBS} 7.8 and 7.6, respectively (Byrne et al. 2011a, Wangensteen et al. 2013). For the crown-of-thorns sea star (CoTS) *Acanthaster cf. solaris*, the settlement rate on pH-conditioned substrates declined with decreasing seawater pH and was significantly lower at pH_{NBS} 8.04 and 7.95 (~609 and 783 $\mu\text{atm CO}_2$) compared with ambient and pre-industrial seawater pH_{NBS} (8.14 and 8.25, respectively) (Uthicke et al. 2013).

Less is known about the effects of settlement and metamorphosis for other invertebrate taxa. Settlement in the bivalve *Macoma balthica* was examined in OA mesocosm experiments across a range of $p\text{CO}_2$ levels (319–1347 $\mu\text{atm CO}_2$, pH 7.94–7.43), with peak settlement being delayed (857–1347 $\mu\text{atm CO}_2$, pH_{T} 7.59–7.43) and lower (1072 $\mu\text{atm CO}_2$, pH_{T} 7.51) at elevated $p\text{CO}_2$ (Jansson et al. 2016). Guo et al. (2015) found reduced survival and metamorphosis in larval abalone (*Haliotis diversicolor* and *H. discus hannai*) that had been reared under control CO_2 conditions (447 μatm , pH_{NBS} ~8.15) when exposed to high $p\text{CO}_2$ concentrations (1500, 2000 and 3000 μatm , pH_{NBS} ~7.7, 7.6 and 7.4, respectively). Dooley & Pires (2015) tested the effects of pH on the settlement and metamorphosis of the gastropod *Crepidula fornicata* and interestingly found significantly higher settlement and metamorphosis at pH_{T} 7.51 and 7.71 than at 7.96.

Some studies on aquaculture species have demonstrated a correlation between OA (reduced pH) and spat settlement in natural environments. For example, Barton et al. (2012) showed a decrease in Pacific oyster *Magallana gigas* (formerly *Crassostrea gigas*) larval recruitment if spawning coincided with naturally occurring low-pH upwelling events along the coast of Oregon, USA. Similarly, Kripa et al. (2014) found a strong correlation between low pH values in a tropical estuary along the southwest coast of India and poor spat density (low settlement) of Indian backwater oysters *Crassostrea madrasensis*. For the Sydney rock oyster *Saccostrea commercialis*, these problems may be obviated by the attraction of these larvae to concrete settlement substrata where the surface pH_{NBS} of 9.24 may provide a buffering effect (Anderson 1996).

The influence of pH on settlement is likely to be species-specific, with some studies reporting no differences in settlement rate among pH levels. In corals, for example, Foster et al. (2015) observed

that *Acropora spicifera* settlement was not significantly different in treatments that included elevated $p\text{CO}_2$ (872–976 μatm , pH_{T} 7.77–7.75), and in *A. tenuis*, settlement of larvae reared in 1000 $\mu\text{atm CO}_2$ (nominal pH treatment: 7.6) was unaffected (Kurihara 2008). Chua et al. (2013) also found no effects of elevated $p\text{CO}_2$ (700 μatm) on metamorphosis in *A. millepora* and *A. tenuis*. Similarly, Albright et al. (2008) found that the settlement of *Porites astreoides* was not significantly different across a range of $p\text{CO}_2$ levels (380 to 720 $\mu\text{atm CO}_2$, pH_{T} 7.95 to 7.80), while Olsen et al. (2015) found no difference in settlement at ambient (nominal pH 8.1) and reduced seawater pH (nominal pH 7.6). Campbell et al. (2017) found no effect of reduced pH (pH_{NBS} 7.85) on the settlement of the same species of coral. Anlauf et al. (2011) observed no effect of reduced seawater pH (pH_{NBS} 7.85) on the settlement of *P. panamensis* larvae. Echinoderm settlement also exhibits a range of responses to OA. Both Dupont et al. (2013) and Wangensteen et al. (2013) reported no change in settlement of the sea urchins *Strongylocentrotus droebachiensis* and *Arbacia lixula* respectively, at pH_{T} 7.69. The presence of smaller *A. lixula* did not translate to a longer PLD, as settlers appeared at the same time as in controls (Wangensteen et al. 2013), although the settling *A. lixula* larvae were smaller than the juveniles generated in control conditions. For *Heliocidaris erythrogramma*, those larvae that completed development in OA conditions (pH_{NBS} 7.6 and 7.8) settled at the same time as controls and responded normally to the coralline algal settlement cue and metamorphosed into juveniles, although there was a decrease in the percentage of normal juveniles (Byrne et al. 2011a). Settlement and metamorphosis of the polychaete tubeworm *Hydroides elegans* larvae was not different when reared under control (8.17) and reduced (7.56) pH_{NBS} conditions (Lane et al. 2013).

Barnacle larvae appear to be relatively unaffected by reduced pH during settlement and metamorphosis. For example, McDonald et al. (2009) followed the percentage of settlement in *Amphibalanus amphitrite* at ambient pH_{NBS} (8.2) and reduced pH_{NBS} (7.4) over a 46 h period, during which time there was no significant difference in the cumulative increase in settlers between pH treatments. Similarly, the percentage of settlement in *Balanus amphitrite* in the presence of a conspecific settlement cue did not decline under a reduced pH_{NBS} of 7.61 compared with pH_{NBS} 8.20 (Campanati 2016), although settlement was lower in reduced pH in the absence of the specific cue.

Substrate perception and selectivity

Of the species studied, the majority have shown a decrease in settlement under elevated $p\text{CO}_2$ /reduced pH (Fig. 3B; Tables S2 & S3). As settlement in marine invertebrate larvae typically involves a series of specific steps (exploration, attachment, metamorphosis), altered settlement under changing ocean conditions could be influenced by a range of processes, both direct and indirect (Fig. 1). Larvae detect the proximity of, search for and explore suitable substrates using a range of senses including water-borne chemicals (Hadfield & Paul 2001, Gerlach et al. 2007, Swanson et al. 2012). A reduction in settlement could therefore be due to a direct loss of sensory capacity from altered seawater pH. Larvae of the tropical fish *Amphirion percula* show a reduction in homing behaviour under reduced pH as a result of the loss of olfactory capacity, attributed to impaired neurotransmission (Munday et al. 2009). Devine et al. (2012) found that elevated $p\text{CO}_2$ caused impairment of olfactory discrimination of settlement cues in larval damselfish *Pomacentrus amboinensis*; however, the larvae showed an ability to compensate by means of alternative senses (i.e. visual cues). For larvae of barramundi *Lates calcarifer* reared under end-of-century levels of elevated CO_2 (~1675 μatm), elevated $p\text{CO}_2$ negatively influenced the auditory preference at the time of settlement (Rossi et al. 2015). In the same species, Pistevos et al. (2017) observed that the attraction of larvae towards physicochemical cues such as salinity or temperature was altered when pre-settlement larvae were exposed to extreme levels of OA (~1400 μatm CO_2), possibly affecting future recruitment of this species. Painted goby *Pomatoschistus pictus* larvae that were reared in similarly high $p\text{CO}_2$ conditions (1503 μatm , pH_{NBS} 7.66) presented altered auditory responses important for the settlement of this species to the preferred coastal reef habitat (Castro et al. 2017). In the presence of elevated $p\text{CO}_2$ water (700 and 1100 ppm CO_2), larval blue-green damselfish *Chromis viridis* and crustaceans (banded coral shrimp *Stenopus hispidus*) both presented altered ability to chemically recognize conspecific cues (Lecchini et al. 2017). These observations are noteworthy, as the chemical information associated with offspring is important for habitat selection at settlement in these species.

For invertebrate larvae, studies that specifically examined the direct effects of seawater pH on larval settlement reported contrasting effects on sensory perception under reduced pH. Doropoulos & Diaz-Pulido (2013) found a significant decline in settlement in the coral *Acropora selago* on CCA when lar-

vae were settled under elevated $p\text{CO}_2$ levels (447, 705 and 1214 μatm CO_2 ; pH_{T} 7.98, 7.81, 7.60). Similarly, settlement rates significantly declined in *Pocillopora damicornis* larvae settled in reduced seawater pH (7.9 and 7.6), compared with rates of settlement in controls (pH 8.1) (Viyakarn et al. 2015). For a non-feeding (lecithotropic) bryozoan larva (*Bugula neritina*), settlement was affected by pH, but in a time-dependent manner (Pecquet et al. 2017). In this respect, settlement in *B. neritina* was delayed but not inhibited by reduced pH. Specifically, settlement of larvae decreased linearly (from ~90 to ~0%) across a pH_{T} range (8.17–6.46) after 1 and 2 h, but over time, differences in settlement rate among pH levels lessened so that by 6 h, settlement was <75% across all pH levels. Pecquet et al. (2017) speculated that the longer larval duration and the shorter-term decrease in settlement rate of bryozoan larvae was potentially the result of elevated $p\text{CO}_2$ blocking specific signalling pathways (i.e. Wnt signalling) associated with bryozoan settlement, inhibiting the ability of the larvae to perceive settlement cues.

There were no differences in settlement rates of barnacles (*Balanus amphitrite*) among pH treatments (pH_{NBS} 8.20 and 7.61), both when conspecific settlement cues were present or absent (Campanati 2016), indicating that reduced pH did not alter the sensory capacity of the competent cyprids. For CoTS larvae (*Acanthaster cf. solaris*), Uthicke et al. (2013) showed no significant difference in settlement on CCA in larvae settled under 4 seawater pH_{NBS} levels, ranging from 8.25 to 7.95. The authors concluded that the seawater pH did not directly influence the ability of the larvae to respond to settlement cues. Webster et al. (2013b) drew the same conclusion for *Acropora millepora* larvae using the same technique, where larvae settled on the same CCA extracts under 4 seawater pH_{T} treatments (7.57 to 8.09) showed no difference in settlement.

Changes in settlement substrates

Indirect effects of reduced pH on larval settlement would include the modification of settlement substrates that alter settlement cues (Figs. 1 & 3B). This is important, as OA can change the nature of substrates that larvae are able to perceive, which can lead to altered settlement patterns (e.g. Webster et al. 2013b). Amongst the best known settlement substrates are CCA and marine biofilms, the latter comprised of heterotrophic bacteria, cyanobacteria, protozoa, fungi and benthic diatoms in a matrix of extracellular poly-

mers (Harder et al. 2002, Lam et al. 2003, Sneed et al. 2015). While it is well established that CCA and their associated microbes play a significant role in the induction of larval settlement (Negri et al. 2001, Harrington et al. 2004, Webster et al. 2004), microbial biofilms alone can also induce larval settlement (Webster et al. 2004, Whalan & Webster 2014).

Acidification could affect marine biofilms in 2 ways: by changing the species composition and/or by inducing differences in the chemical cues emitted by biofilms (Russell et al. 2013). Distinct changes in the community structure of biofilms as a result of seawater pH have been found both experimentally (Witt et al. 2011, Webster et al. 2013b) and across environmental pH gradients (Lidbury et al. 2012, Johnson et al. 2013). Hence, it is likely that larvae settling in future predicted OA conditions will encounter biofilms that differ in composition and function to those found today. Webster et al. (2013b) considered the effects of changes in biofilms associated with CCA grown under reduced seawater pH conditions (pH_T 8.09, 7.91, 7.72 and 7.57) as the mechanism for altering settlement patterns. In that study, decreases in the abundance of dominant microbial groups (*Alpha-proteobacteria* and *Bacteroidetes*) and the appearance of *Proteobacteria* were observed in reduced seawater pH, coinciding with lower settlement of coral larvae, although it was not possible to deduce if altered settlement on CCA is due to changes in the chemical inducer of the algae or changes in the associated microbial community. Certainly, microbial films play an important role in the induction of settlement in marine species (Johnson & Sutton 1994, Hadfield & Paul 2001, Huggett et al. 2006), and changes in their composition, such as related to age, can affect settlement rate in marine invertebrates (i.e. Pearce & Scheibling 1991, Toupoint et al. 2012). The impact of OA-induced changes in biofilm communities on recruitment success warrants further examination.

The coral *Acropora millepora* settles differentially on substrates, depending on the pH in which the substrates were grown (Doropoulos et al. 2012). In this respect, Doropoulos et al. (2012) found that substrates conditioned for 60 d at 800 and 1300 $\mu\text{atm CO}_2$ (pH_T ~7.79 and 7.60, respectively) induced significantly lower settlement than substrates under control conditions (400 $\mu\text{atm CO}_2$, pH ~8.04) and also changed the pattern of settlement selectivity on substrate types. Changes in CCA species composition were observed on the treated substrates, and the settlement preference of *A. millepora* larvae varied for each substrate. Interestingly, this involved a decline in the settlement of larvae on the previously most preferred CCA (*Ti-*

tanoderma spp.) when pCO_2 levels were elevated. Similarly, Doropoulos & Diaz-Pulido (2013) showed direct evidence that rates of coral (*A. selago*) settlement were consistently reduced under OA conditions in the presence of 3 common CCA species, despite the fact that CCA abundance was unaffected. Webster et al. (2013b) observed a similar effect of substrate conditioning on *A. millepora* settlement, with the percentage of settlement significantly lower on pH_T 7.57 and 7.72 (~1638 and 1187 $\mu\text{atm CO}_2$) conditioned substrates compared with control substrates conditioned at pH_T 8.09 (~464 $\mu\text{atm CO}_2$). Larvae of the CoTS *Acanthaster* cf. *solaris* also settled at significantly lower rates on substrata that had been conditioned for 85 d in reduced pH_{NBS} (8.05 and 7.94) compared with those conditioned in seawater pH at present-day (pH_{NBS} 8.15) and pre-industrial (pH_{NBS} 8.23) levels (Uthicke et al. 2013).

Multiple studies have shown that benthic diatoms are very important cues for larval settlement in certain species of marine invertebrates (e.g. some species of abalone, Ding et al. 2017). Previous studies have shown the importance of benthic diatoms in the settlement process of the polychaete *Hydroides elegans* (Harder et al. 2002, Lam et al. 2003) which seems to be induced by heat-stable surface components emitted from the diatoms (Lam et al. 2003). Little is known, however, on how OA affects benthic diatom communities. For instance, a recent study has shown changes in the species composition of benthic diatoms and a significant increase in diatom abundance with increasing pCO_2 at a natural volcanic vent site off the coast of Italy (Johnson et al. 2015). The potential for such changes in diatom communities to occur under future OA conditions and the outcome of such changes on settlement (and early post-settlement growth) on invertebrates is of interest for future research.

Altered larval settlement as a result of OA-induced changes to CCA and microbial/microalgal biofilms could have profound ecological effects. Modification of the nature of the settlement substratum as a result of elevated pCO_2 may cause a loss or reduction in the abundance of encrusting species, as well as changes in the biofilm, both of which have been linked with changes in settlement patterns. For instance, Doropoulos et al. (2012) linked changes in coral settlement to differential responses of CCA under reduced pH and discussed the implication of these findings for the future recruitment of corals. Similarly, Uthicke et al. (2013) suggested that future changes in CCA under OA conditions that reduce settlement in CoTS *A. cf. solaris* could be the most important bottleneck

for future recruitment of the species. These observations have wide implications for settlement in future oceans, given that CCA and biofilms influence the settlement of numerous marine taxa (Pearce & Scheibling 1991, Hadfield & Paul 2001). Findings that CCA communities change in abundance and species composition in response to OA, both experimentally (Kuffner et al. 2008) and along natural pH gradients (Hall-Spencer et al. 2008, Fabricius et al. 2011), has significant implications for the settlement of marine species in future oceans.

Changes in host–symbiont interactions

The role of microbial symbionts in marine invertebrate larvae (particularly in sponges and corals) has received considerable research attention in the past decade (Bourne et al. 2016, Webster & Thomas 2016). In some coral species, phototrophic symbionts (dinoflagellates) supply energy to the larvae during the planktonic life stage (Rivest et al. 2018), and in sponges, microbial symbionts crucial to host health are vertically transmitted from adults to larvae (Webster et al. 2010). Environmentally induced disruption of these microbial symbionts can destabilise the holobiont with adverse consequences for host health across life history stages (Webster et al. 2011a, Fieth et al. 2016, Pita et al. 2018). Given that larval settlement is a crucial step in the life cycle of the larvae, environmental factors affecting the health or performance of the symbiont could jeopardize the outcomes of larval settlement for those larvae strongly dependent on symbiont health. Rivest et al. (2018) tested the effects of OA and warming on host and symbiont of *Pocillopora damicornis* larvae and found that the mechanisms used to tolerate high $p\text{CO}_2$ in coral larvae and the symbiont differ, with greater gene expression changes measured in the symbiont. Similarly, Webster et al. (2013a) found that reduced pH/increased $p\text{CO}_2$ caused microbial shifts in coral and foraminifera over 6 wk, despite no visible signs of host stress being detected over this period. The high sensitivity of host-associated microbes to OA highlights the need for research that assesses the implications of microbial shifts for larval health and recruitment processes.

Early post-settlement processes

Quantifying the effects of OA on early post-settlement processes is key to understanding future

species population persistence/success because (1) measurements of ‘settlement’ in experiments often incorporate some post-settlement period; (2) effects of reduced seawater pH on larvae can carry over to post-settlement fitness; and (3) early post-settlement processes are key to determining recruitment patterns (Rowley 1989, Hunt & Scheibling 1997).

A number of studies have indicated that early post-settlement growth and survival is adversely affected by reduced pH, with corals consistently showing reduced growth in OA experiments (Fig. 3C). For instance, skeletal extension in newly settled *Porites astreoides* is positively correlated with aragonite saturation state, with 50 and 78% reductions in growth occurring at 560 and 720 ppm CO_2 , respectively (Albright et al. 2010). Foster et al. (2015) also found that while survival of newly settled *Acropora spicifera* was unaffected, a significant reduction in early skeletal development and weight (48 to 60%, compared to controls) occurred when $p\text{CO}_2$ levels were elevated to ~900 μatm . Similarly, Albright et al. (2010) found a 39 and 50% decline in the linear growth of *A. palmata* at $p\text{CO}_2$ levels of 560 and 800 μatm , respectively, compared with controls. Reduced polyp growth at pH_T 7.31 and 7.64 has been reported for *A. digitifera* (Suwa et al. 2010); however, Nakamura et al. (2011) found no significant effect on survival on settled *A. digitifera* recruits. Similarly, growth of newly settled *P. panamensis* was only slightly reduced (3%) at pH_{NBS} 7.77–7.92, with no effect on survivorship (Anlauf et al. 2011).

Growth of newly settled bryozoan ancestrula were slowed by 5% after 24 h in reduced pH_T 7.60, compared with controls (Pecquet et al. 2017). Fitzer et al. (2014) found that juvenile *Mytilus edulis* that were spawned and grown under high $p\text{CO}_2$ conditions (1000 μatm) did not produce aragonite, and their shells were entirely calcitic. In the soft-sediment clam *Mercenaria mercenaria* and scallop *Argopecten irradians*, Talmage & Gobler (2010) studied the growth, survival and condition of larvae and juveniles under past, present and future (21st and 22nd centuries) ocean CO_2 concentrations. Higher CO_2 concentrations were related to lower growth and metamorphosis as well as to lower survival and lipid accumulation rates in juveniles. In addition, newly settled bivalves grown under near pre-industrial CO_2 levels displayed thicker, more robust shells than individuals grown at present CO_2 concentrations, whereas bivalves exposed to CO_2 levels expected later this century had shells that were malformed and eroded (Talmage & Gobler 2010).

While seawater pH can reduce early post-settlement growth and survival under laboratory conditions, there are also examples of limited impacts of reduced pH. In one of the most comprehensive studies on early post-settlement responses to reduced pH, Wolfe et al. (2013) examined a range of developmental responses in newly settled sea urchins *Heliocidaris erythrogramma* under a range of pH_T levels (8.13–7.44). Survival and test growth was robust to near-future reduced pH levels, although spine length was reduced and morphology altered under the lowest pH levels. Interestingly, a contrasting study showed that post-settlement sea urchins (*Paracentrotus lividus*) were significantly larger at pH_{NBS} 7.7 compared to 8.1 (García et al. 2015), which was attributed to elevated metabolic rates in individuals reared in reduced pH under non-energy limited conditions. However, juveniles of the sea urchin *Arbacia lixula* generated at pH_T 7.69 were smaller (Wangenstein et al. 2013). In lecithotrophic echinoderm larvae and juveniles of the sea star *Crossaster papposus*, Dupont et al. (2010a) found that, when cultured at low pH (7.7), growth and development were faster with no visible effects on survival or skeletogenesis. Juvenile CoTS *Acanthaster cf. solaris* grown under acidification conditions during their early herbivorous phase grew faster under OA conditions, which was attributed to the greater vulnerability of their CCA food grown under the same conditions, an example of indirect effects of OA on algal-grazer interactions (Kamya et al. 2017, 2018). In addition, while the juveniles reared in OA were larger, they weighed less because they were less calcified (Kamya et al. 2018). The early benthic juveniles of the dwarf cushion sea star *Parvulastra exigua* are also highly tolerant of low pH, with deleterious effects only seen after 4 wk at pH_T 7.24 (Nguyen & Byrne 2014). For newly settled bivalves (*Macoma balthica*), growth in mesocosm experiments was not significantly different up to 17 d post-settlement (Jansson et al. 2016), nor was there a clear effect of increased $p\text{CO}_2$ on juvenile survival. In an earlier study on the same species, Green et al. (2004) found reduced survival of juveniles in marine sediments in very low estuarine pH conditions (pH 7.2).

Changes in early post-settlement barnacle growth under reduced pH appear to be species-specific. McDonald et al. (2009) recorded growth of *Amphibalanus amphitrite* unaffected by pH_{NBS} 7.4 for up to 8 wk, Campanati (2016) found no difference in the size of 15 d old post-settlement juvenile *Balanus amphitrite* between control (pH_{NBS} 8.2; $\sim 400 \mu\text{atm CO}_2$) and low pH_{NBS} (7.61, $\sim 2000 \mu\text{atm CO}_2$) treatments,

while Findlay et al. (2010) found reduced growth of newly settled cyprid larvae of *Semibalanus balanoides* in pH_{NBS} 7.70 ($\sim 1100 \mu\text{atm CO}_2$) over a 20 d period.

While early post-settlement corals appear sensitive to reduced pH, many other newly settled invertebrates appear relatively tolerant. For corals, the direct difficulties of calcifying in reduced pH appears to explain the reduced growth rates, and it is likely that the same mechanism explains the slower growth of newly settled bryozoans, the poorer development of early-juvenile urchin calcareous spines and the dissolution of shells in bivalves. However, for other newly settled calcifying taxa, such as barnacles and some molluscs, calcification is not impaired. It is still unknown if altered post-settlement growth rates are also due to other physiological responses to reduced pH, such as reduced metabolic rates or altered acid–base regulation. Indeed, the studies available on the effects of reduced pH on non-calcifying newly settled invertebrates are scarce, and the wider effects (not associated with calcification) of elevated $p\text{CO}_2$ on newly settled individuals are yet to be determined. Furthermore, laboratory experiments do not account for the wider ecological effects, such as altered food supply (biofilms, CCA) on newly settled individuals. For example, newly settled juveniles, such as sea urchins, will first feed on biofilms, which in turn can change in abundance and composition under elevated $p\text{CO}_2$ (Witt et al. 2011, Lidbury et al. 2012, Webster et al. 2013b, Raulf et al. 2015).

Carryover effects

Larval developmental history may also influence post-settlement development in marine species (Pechenik 2006). Such carryover or non-lethal responses include the effects of larval nutrition and environment on metamorphosis, settler sizes and post-settlement growth (i.e. Phillips 2002). Given the documented effects of reduced seawater pH on larval development (Byrne et al. 2011, 2017) and feeding, metabolism and energy budgets (Stumpp et al. 2013), there is strong potential for OA-induced carryover effects to occur in post-settlement stages. Hettinger et al. (2012) found strong carryover effects on larvae of Olympia oysters *Ostrea lurida* reared in reduced pH_{NBS} (7.73 and 7.86), where reduced pH resulted in smaller larvae and smaller post-settlement juveniles that grew significantly slower even in ambient pH water (growth in 1 wk old juveniles was reduced by 41 % if their larval stage was reared under reduced

pH). This same study showed that carryover effects occurred irrespective of the pH during post-settlement. Carryover effects were also observed when *O. lurida* larvae were reared in laboratory conditions through metamorphosis in both control (400 μatm , $\text{pH}_{\text{NBS}} \sim 8.10$) and elevated $p\text{CO}_2$ concentrations (1000 μatm , $\text{pH}_{\text{NBS}} \sim 7.80$), resulting in a 80% reduction in metamorphosis (Hettinger et al. 2013). Additionally, carryover effects were also observed when the juveniles were out-planted into the field (Hettinger et al. 2013), and these effects can be long-lasting. In particular, while survival rates did not differ, juvenile growth in the field was 15 to 50% lower than in controls for juveniles exposed to elevated $p\text{CO}_2$ (1000 μatm) as larvae. Carryover effects were still evident 4 mo post-settlement, with growth 20–40% lower in juveniles from exposed larvae.

In the Pacific oyster *Magallana gigas*, reduced metamorphosis due to carryover effects in larvae reared under combinations of temperature (24 and 30°C), pH_{NBS} (8.04 and 7.47) and salinity (15 and 25 psu) was evident (Ko et al. 2014). Decreased pH_{NBS} (7.47) significantly delayed pre- and post-settlement growth, alone or in combination with the other stressors. For the sea urchin *S. droebachiensis*, Dupont et al. (2013) found negative carryover effects of exposure to elevated $p\text{CO}_2$ on the post-settlement survival and growth. While settlement success after 28 d was not related to $p\text{CO}_2$ exposure as a larva (i.e. 1200 vs. 941 μatm , pH_T 8.1 and 7.7, respectively), mortality in juveniles was 95% after 3 mo if they had been exposed to elevated $p\text{CO}_2$ as larvae, compared with 46 to 60% in juveniles originating from control larvae. Mortality of juveniles in a second sea urchin, *Arbacia lixula*, generated from OA conditions did not differ from controls, but they were only reared for 3 d post-settlement. The juveniles generated in OA conditions were smaller than the controls (Wangensteen et al. 2013). In corals, Yuan et al. (2018) observed reduced percentage settlement of *Acropora gemmifera* larvae that had been cultured at 3 concentrations of CO_2 (corresponding to pH_{NBS} 8.1, 7.8 and 7.5). After settlement, the recruits showed decreased calcification and growth, changes consistent with the down-regulation of calcification-related genes.

Carryover effects between larvae and juveniles are often attributed to altered energetic states in larvae due to the need to physiologically mitigate the effects of reduced pH (i.e. increased acid/base regulation, longer development times). If that is the case, it is likely that settling larvae have depleted metabolic reserves available for early juvenile development or earlier for the process of metamorphosis into poten-

tially smaller juveniles (Parker et al. 2015). In order to better understand the OA-induced carryover effects on larval settlement, analysis of the effects of OA on the energetic reserves during the larval stage and at the time of settlement are required.

FURTHER CONSIDERATIONS

Multi-stressors and settlement

OA occurs concomitant with other climate change-related stressors, including ocean warming, hypoxia, changes in nutrient concentrations or stratification and altered salinity from freshwater runoff. It is therefore important to consider the interactions among multiple global change-related stressors. While many studies have considered the effects of multiple stressors on marine larvae (reviewed by Przeslawski et al. 2015), few have specifically examined the effects of multiple stressors on the process of settlement and early post-settlement development (Table S3). Campanati (2016) examined the interaction between low oxygen and reduced pH on settlement and metamorphosis of *Balanus amphitrite* and subsequently the growth and condition of newly settled juveniles. Settlement was lower under reduced oxygen and in a reduced pH/O_2 treatment, but not when pH was applied as a single stressor. There were no effects of the stressors, administered singly or in combination, on growth of newly settled juveniles, but the condition index of the juveniles subjected to low O_2 was significantly poorer when pH was added as a co-stressor. Foster et al. (2015) quantified settlement survival, growth and calcification of newly settled *Acropora spicifera* under elevated temperatures (24 and 27°C) and $p\text{CO}_2$ (250 and 900 μatm). There was no significant effect of treatment on settlement, either administered separately or in combination, while the negative effects of elevated $p\text{CO}_2$ on early development were not significantly different under warming.

By contrast, Chua et al. (2013) found that elevated temperature was the more important stressor in the metamorphosis of *A. millepora* and *A. tenuis*. For *Porites panamensis* planulae settlement, no significant differences were found when exposed to OA, temperature or a combination of both (Anlauf et al. 2011). However, significant reduction in growth of primary polyps appeared when subjected to a combination of high temperature and low pH. No interaction between temperature and pH on development of newly settled sea urchins was seen by Wolfe et al. (2013), with the exception of reduced spine length,

where there was a stronger effect of temperature at ambient pH levels. Wangensteen et al. (2013) tested both temperature and pH on larval development, settlement and juvenile survival of a Mediterranean population of the sea urchin *Arbacia lixula*, reporting that elevated temperature resulted in increased larval survival and size. Acidification resulted in higher survival rates, but in smaller larvae with marginal effects on larval morphology. No carry-over effects between larvae and juveniles were detected in early settler survival, although settlers from larvae reared at pH_T 7.69 were significantly smaller than their counterparts developed at pH_T 8.09.

A complex experiment tested the interactive effects of biotic (exposure to macroalgae) and abiotic (temperature and pH) variables on the settlement of the coral *P. astreoides* (Olsen et al. 2015). While the rate of settlement was significantly lower when competing with the algae *Dictyota* spp., there was no significant effect of either pH or temperature on coral settlement, nor any interaction among the biotic and abiotic factors on the process. Huggett et al. (2018) examined larval settlement of the sea urchin *Heliocidaris erythrogramma* on the coralline alga *Amphiroa anceps* that had been preconditioned in a combination of elevated temperature (23 vs. 19°C) and reduced pH (0.3 pH unit reduction) treatments during 21 d. The surface microbial community on *A. anceps* was altered by both temperature and pH treatment, although sea urchin settlement only decreased on algae from the temperature treatments, alone or in combination with reduced pH, while no effect was seen on larval settlement when presented with algae conditioned under reduced pH.

These observations point to the potentially complex interactions of environmental change with macroalgae and associated biofilm responses, whereby settlement is influenced by specific components of the microbial community that differentially respond to warming and acidification, and not simply by broad-scale microbial shifts. Ko et al. (2014) reported carry-over effects (reduced metamorphosis) in Pacific oyster larvae reared under combinations of temperature (24 and 30°C), pH_{NBS} (8.04 and 7.47) and salinity (15 and 25 psu). The combination of decreased pH_{NBS} (7.47), elevated temperature (30°C) and reduced salinity (15 psu) significantly delayed pre- and post-settlement growth.

While too few studies have been conducted to allow a generalisation on the direct effects of multiple stressors on settlement and early post-settlement development, it is clear that important interactions will likely occur (Przeslawski et al. 2015). In addi-

tion, indirect effects on settlement from interactions of multiple stressors are yet to be considered, not only for carryover effects from the larval phase, but also related to settlement substrates. In this respect, substrates used to test for changes in settlement are typically subjected to altered pH, but not in combination with temperature (i.e. Uthicke et al. 2013). This is despite the fact that temperature in combination with pH has been shown to alter the settlement substrate characteristics of coralline algae (Martin & Gattuso 2009, Diaz-Pulido et al. 2012, Johnson & Carpenter 2012) and their associated benthic microbial communities (Webster et al. 2011b, Russell et al. 2013).

Microhabitats — diffuse boundary layers

Larval settlement typically involves movement into the surface boundary layer of the substrate (Koehl & Hadfield 2010). The importance of this is that seawater pH can change across small distances from the overlying water column into the diffuse boundary layer. For example, measurements of seawater pH in boundary layers can vary from the overlying water column by up to 0.35 units lower (Hurd et al. 2011) and up to 0.5 units higher (Hurd et al. 2011, Cornwall et al. 2013, 2014). Changes in pH in boundary layers are generally associated with biological activity (respiration, calcification and photosynthesis), and their magnitude can vary as a function of physical processes (e.g. water flow, temperature), day vs. night variability (e.g. photosynthesis vs. respiration) and biological variability (Hurd et al. 2011, Cornwall et al. 2014). In addition, pH variation in boundary layers is more likely to occur on short time scales. Diel variation in photosynthesis of coralline algae, for example, resulted in a boundary layer pH 0.5 units higher than the overlying water column under illumination, and 0.35 units lower in the dark (Hurd et al. 2011). These observations suggest that settlement and early post-settlement development may be occurring in pH conditions that are not the same as the water column where the larvae develop, both in terms of pH level and the degree of variability.

Diffuse boundary layers also exist around slow-moving or sessile marine invertebrates. For example, Hurd et al. (2011) showed that pH on the surface of sea urchins was 0.35 units lower than that of the overlying seawater. The relevance for this is that many highly gregarious species (i.e. barnacles and tubeworms) settle directly onto adult conspecifics (Hadfield & Paul 2001), where pH levels are po-

tentially lower than the water column and which may influence processes such as cue perception and early post-settlement development. Similarly, a number of species that settle specifically onto their prey (i.e. *Phestilla* nudibranchs on *Porites* corals, Miller & Hadfield 1986) may encounter pH values associated with diffuse boundary layers around the prey animal surface. The boundary layer associated with building environmental structures is also an important consideration as seen where oyster larvae are attracted to the calcium hydroxide cue emanating from these structures as well as the likely buffering influence of the high pH of this habitat (pH 9.24) (Anderson 1996).

To date, however, there are no examples of research where the effects of boundary layer seawater pH on settlement have been tested directly, although experiments that incorporate pH treatments beyond that predicted for future water column levels may provide some insight. A key step in understanding the process would be to quantify pH at the substrate level during experiments. Experiments where the boundary layer is manipulated (i.e. through altered water flow or biological activity on the substrate) may also provide greater insight into the future effects of OA on larval settlement.

CONCLUSIONS

A review of the literature shows that, to date, 48 studies have focussed on how the key processes of larval settlement and metamorphosis are impacted by OA. Most research (39 studies) has focussed on the processes of settlement (Figs. 2 & 4), with fewer studies investigating the pre- (4) and post-settlement (22) processes.

The bulk of research assessing effects of OA on larval settlement has examined the settlement process of (tropical) corals (Cnidaria), echinoderms (pre-, post- and settlement processes) and fish (pre-settlement processes), generally revealing a negative effect on settlement and post-settlement processes. In other taxa (arthropods, bivalves, sponges and bryozoans), most of the effects caused by exposure to reduced seawater pH were also negative.

Recruitment of marine species with indirect life-cycles involves a series of discrete and relatively independent processes, with settlement linking these through its association with the larval phase and post-settlement juveniles (Fig. 1). Indeed, settlement and metamorphosis are arguably the most important processes in a marine species' lifecycle, as they

determine the final location where the organism will spend its life, especially in sessile and benthic taxa. Selective settlement maximises the potential for optimal future developmental conditions. However, despite the importance of settlement, most research into OA effects on recruitment has focussed on pre-settlement processes and effects on post-settlement juveniles.

Hypotheses for future testing

From this comprehensive review of the literature, we have identified 8 hypotheses to prioritise for experimental testing:

(1) Larval survival, growth and development rates, which can be reduced by elevated $p\text{CO}_2$, will have direct effects on settlement by determining the number of larvae reaching competency.

(2) Larval supply for settlement will be altered through elevated $p\text{CO}_2$, affecting horizontal swimming behaviour or vertical migration that will, in turn, influence horizontal advection.

(3) Longer PLD under elevated $p\text{CO}_2$ will alter settlement through decreased larval substrate selectivity (i.e. the desperate larval hypothesis).

(4) Perception of settlement cues in marine invertebrate larvae will not be influenced by elevated $p\text{CO}_2$ directly, as opposed to fish larvae.

(5) Larval settlement will be altered via elevated $p\text{CO}_2$ effects on the composition and distribution of suitable settlement substrates.

(6) Elevated $p\text{CO}_2$ will interact with other environmental stressors to have additive or synergistic impacts on the settlement of marine larvae.

(7) OA will alter diffusive boundary layers in such a way that it will negatively affect larval settlement and recruitment.

(8) Carryover effects of larval development under elevated $p\text{CO}_2$ will occur at settlement due to delayed larval development, smaller larval size or altered energy budgets that reduce fitness at metamorphosis and during post-settlement development.

Knowledge gaps and new research direction in larval settlement research

Lack of knowledge about the impacts of OA on larval settlement in most marine species currently limits our ability to predict the broader ecological outcomes of altered settlement for future recruitment. Here we have highlighted a need for further research on a

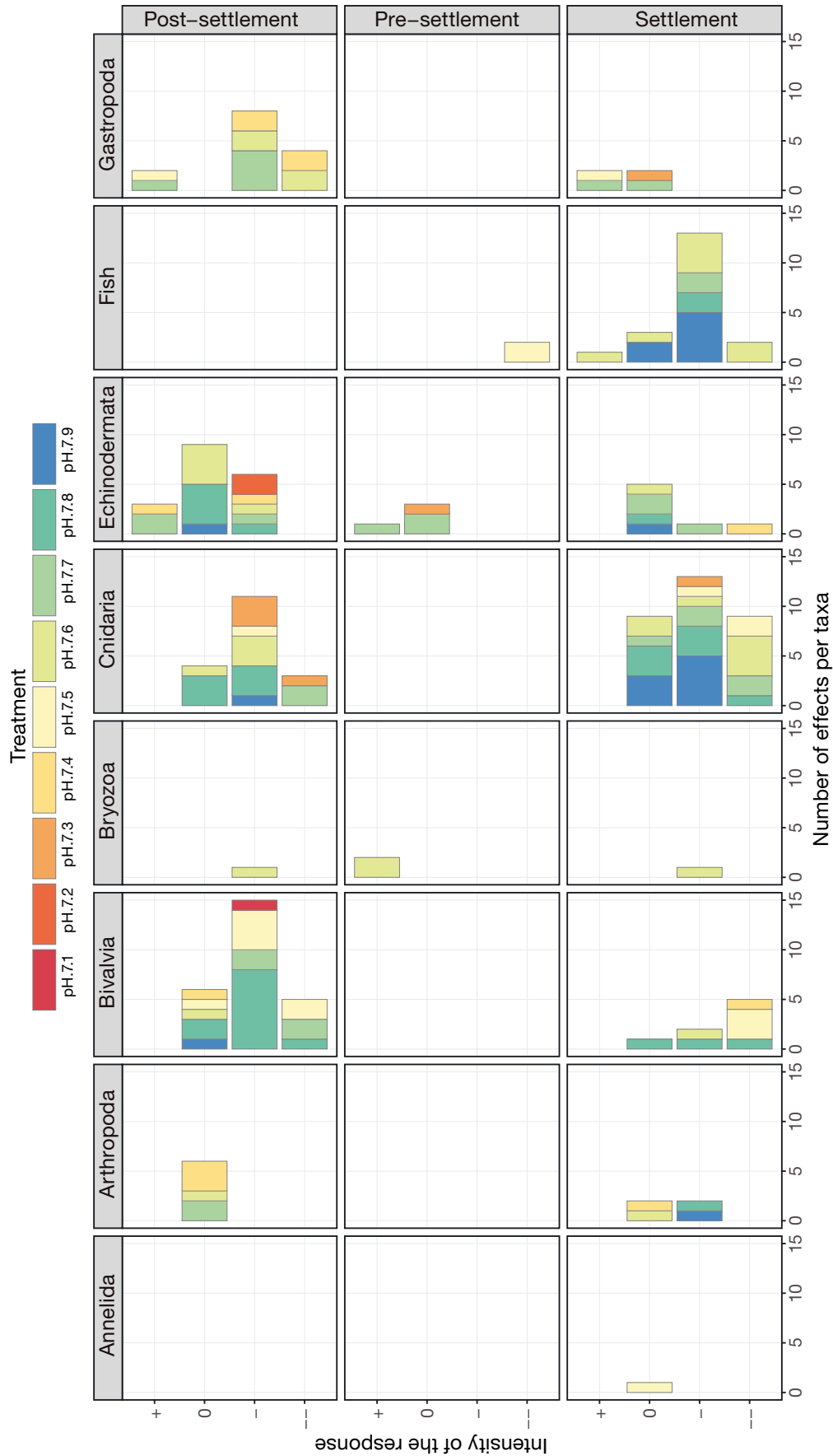


Fig. 4. Summary of results from all studies currently available on the response of different marine taxa to reduced seawater pH during the processes of pre-settlement, settlement and early post-settlement. Each unit in the histogram represents 1 response of 1 species related to the controls for that particular experiment. The gradation in intensity of the response is as follows: + is an increase up to 50%; 0 is no effect; - is a decrease up to 50%; -- is a decrease in the response by more than 50%. The colour code shows the different nominal pH treatments in each published experiment

Table 1. Knowledge gaps in larval settlement research and experimental strategies to address those gaps. OA: ocean acidification, PLD: planktonic larval duration, CCA: crustose coralline algae

Knowledge gaps	Experimental strategies on larval settlement research
1. How will elevated pCO ₂ (hypercapnia) or reduced pH affect the number and quality of larvae reaching competency (through altered larval survival, growth and development rates)?	<p>Studies focussing on:</p> <ul style="list-style-type: none"> • Diverse taxa • Elucidating the mechanisms underpinning reduced larval survival and development rates that in turn influence the number of larvae reaching settlement; e.g. altered ion transportation (e.g. membrane potentials), enzyme activity, gene expression, metabolic rates or altered acid base regulation • Evolution of larval energy reserves when exposed to different pH treatments, and determining how the amount of energy left at the time of settlement influences settlement success • Modelling the interaction between reduced larval survival and development on numbers of larvae reaching settlement
2. How will elevated pCO ₂ or reduced pH influence larval swimming behaviour in such a way that it will alter larval supply for settlement?	<p>Studies focussing on:</p> <ul style="list-style-type: none"> • Swimming ability and mechanisms of marine larvae in large numbers of taxa • Interaction of changes in swimming ability, changes in vertical distributions and larval swimming duration with water movement — studies in flumes and how these interactions relate to successful recruitment • Evolution of larval energy reserves when exposed to different pH treatments and effects on active larval swimming • Physical transport modelling studies combining larval dispersal with OA-induced changes in larval behaviour
3. Does elevated pCO ₂ or reduced pH decrease substrate selectivity in such a way that it would alter settlement success (i.e. the 'desperate larva hypothesis')?	<p>Studies focussing on:</p> <ul style="list-style-type: none"> • Understanding the interaction between OA and the desperate larval hypothesis and how a loss in substrate selectivity might affect settlement success and early post-settlement survival • Effects of longer PLDs on settlement behaviour — do larvae alter their substrate selectivity? • Consequences of changes in settlement selectivity sufficient on settlement patterns and recruitment to suboptimal conditions?
4. Does elevated pCO ₂ or reduced pH influence larval settlement through direct pathways, namely by directly altering the larval behaviour or physiology and subsequently changing the larval sensory capacity or attachment mechanisms?	<p>Studies focussing on:</p> <ul style="list-style-type: none"> • Direct effects of OA on larval settlement in large numbers of taxa • Physiological mechanisms by which OA directly impairs settlement: is it through sensory alteration or attachment structures in the larvae? • Understanding the mechanisms responsible for the sensory recognition of substrates by larvae, e.g. neurosensory signalling pathways (e.g. Wnt signalling) at cellular/molecular levels
5. How will elevated pCO ₂ or reduced pH alter the composition and distribution of suitable settlement substrates in such a way that it will indirectly affect larval settlement?	<p>Studies focussing on key changes that occur on settlement substrates under OA conditions that may affect settlement success and settlement patterns</p> <ul style="list-style-type: none"> • Distribution/composition of settlement substrates (e.g. metagenomics) • Changes in specific settlement-inducing chemical signals associated with settlement substrates (waterborne or adsorbed) (e.g. composition of cue and molecular changes responsible for changes)
6. How will elevated pCO ₂ or reduced pH interact with other environmental stressors to have impacts on the settlement of marine larvae?	<p>Studies focussing on:</p> <ul style="list-style-type: none"> • Interaction with other global stressors, e.g. temperature • Interaction with local stressors, e.g. nutrients, sedimentation, salinity, pollution • Single-stressor vs. multi-stressor experiments
7. Will elevated pCO ₂ or reduced pH alter the boundary layers in such a way that it will negatively affect larval settlement and recruitment?	<p>Studies focussing on:</p> <ul style="list-style-type: none"> • Understanding the relationship between boundary layers and settlement/early post-settlement: how important are boundary layers for settlement success and for survival of newly settled individuals? • Elucidating the effects of OA on boundary layers on biofilms, CCA and living organisms under different conditions, e.g. light vs. dark, flow vs. no flow

Table 1 (continued)

Knowledge gaps	Experimental strategies on larval settlement research
8. Carryover effects of larval development under elevated pCO ₂ will occur at settlement due to delayed larval development or altered energy budgets that reduce fitness at metamorphosis and during post-settlement development.	<p>Studies focussing on:</p> <ul style="list-style-type: none"> • Elucidating the molecular mechanisms behind the carryover effects (e.g. changes in genetic make up of the population due to genetic bottlenecks or selection of specific traits, epigenetics, etc.) • Energy reserves responsible for carryover effects at time of settlement (e.g. reduced larval size and lipid levels at time of settlement)
9. General	<ul style="list-style-type: none"> • Most studies on invertebrate settlement have taken place under laboratory conditions. Mesocosm experiments that reflect natural environments and which include different trophic levels and their interactions would improve our understanding of settlement success under natural conditions • Future settlement studies need to take into consideration local variability and ecologically relevant pH levels, as well as the organisms' tolerance range

broader range of taxa that focuses on both the direct and indirect effects of OA. This research should explore the pH environment at the scale relevant to the settling of small larval stages (boundary layers, variability) and incorporate the multiple stressors that occur in conjunction with OA at time of settlement, both at present and in the future. Table 1 presents a summary of knowledge gaps in larval settlement research based on the hypotheses presented above. For each of the knowledge gaps, research suggestions have been formulated in order to address those gaps.

Addressing these knowledge gaps will provide a more comprehensive understanding of the complex processes and interactions that affect and steer larval settlement success in marine invertebrates. It will provide a greater understanding in the relative importance of settlement responses to OA compared with other important life-history processes and finally allow a more generalised understanding by examining a greater range of taxa and larval forms. Moreover, this knowledge would support policy makers in decisions pertaining to the conservation and management of marine ecosystems under future OA scenarios.

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