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REPORT

Patch size drives settlement success and spatial distribution of coral larvae under space limitation

E. M. Sampayo[1,2] · G. Roff[2,3] · C. A. Sims[1,2] · P. G. Rachello-Dolmen[1,4] ·
J. M. Pandolfi[1,2]

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Abstract Space availability is a key factor linked to the settlement success of marine invertebrates. Settlement space on coral reefs is predicted to become increasingly fragmented and occupied by competitors under future disturbance regimes, yet how this impacts coral settlement remains largely unknown. We test the effects of space limitation on larval settlement in three common Indo-Pacific corals (*Acropora valida*, *Acropora digitifera* and *Anacropora spinosa*) by manipulating substrate area while maintaining a constant larval supply. Settlement success was highly variable among coral species, with reduced space leading to an up to four-fold increase in settlement of *A. valida* larvae, a two-fold decrease in settlement of *An. spinosa* larvae and no significant effect for *A. digitifera*. All species displayed similar spatial settlement patterns, whereby larvae settled gregariously irrespective of how much space was available. At the same time, settlers were found to increasingly occur in aggregates (in direct contact with each other) as space decreased. We propose that increased settler aggregations, coupled with settlement intensification for some species, facilitates the formation of chimeras as space becomes limiting. In colonial organisms, the formation of aggregates and particularly chimeric individuals may offset the negative effects of increased competition for space by allowing settlers to rapidly exceed size-escape thresholds, thereby increasing the likelihood of survival.

Keywords Coral · Recruitment · Settlement · Patch size · Space limitation · Gregarious · Chimera

Introduction

The successful initial colonization of benthic habitats by sessile marine invertebrates relies on the influx and settlement of dispersive larvae. The number of settlers that become reproductive adults is defined as recruitment, which is determined by post-settlement processes that drive settler mortality rates (Connell 1985; Roughgarden et al. 1985). Recruitment is a crucial process in the maintenance of marine populations as well as their renewal following disturbances (Gaines and Roughgarden 1985; Pawlik 1992). Theoretically, settlement of marine invertebrate larvae is directly correlated with larval influx and the availability of free space, whereby settlement is expected to increase as larval supply or suitable substrate increases (Gaines and Roughgarden 1985). However, marine larvae are not passive particles that are only subject to ocean currents, and the relationship between the number of incoming larvae and subsequent settler densities can be modified by a range of factors, including macro- and

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✉ E. M. Sampayo
e.sampayo@uq.edu.au; eugeniadesampayo@gmail.com

[1] Marine Palaeoecology Laboratory, School of Biological Sciences, The University of Queensland, St. Lucia, QLD 4072, Australia

[2] ARC Centre of Excellence for Coral Reef Studies, The University of Queensland, St. Lucia, QLD 4072, Australia

[3] Marine Spatial Ecology Laboratory, School of Biological Sciences, The University of Queensland, St. Lucia QLD4072, Australia

[4] Present Address: Elsevier Publishing, The Hague, The Netherlands

micro-scale hydrodynamics, larval competency, benthic settlement cues, and pre-emption of space (reviewed in Pawlik 1992; Pineda et al. 2010). Of these factors, the availability of free space (also referred to as patch size) in regulating settlement of marine invertebrates has remained comparatively understudied (Gaines and Roughgarden 1985; Bertness et al. 1992; Minchinton and Scheibling 1993; Pineda et al. 2010).

In marine environments, disturbance frequency plays a key role in influencing population turnover as it reduces reproductive output and changes available space for settlement (Connell 1978). Coral reefs are an example of disturbance-driven ecosystems (Graham et al. 2006), and increases in the intensity and frequency of disturbances (e.g. storms, coral bleaching, disease outbreaks) over multiple decades have led to widespread losses of corals and shifts to non-coral dominated states (Pandolfi et al. 2003, 2011; Norstrom et al. 2009; Roff and Mumby 2012; Hughes et al. 2018). Broad-scale loss of coral cover and declines in colony density following disturbances can lead to a net reduction in larval output at local to regional reef scales (e.g. Gilmour et al. 2013), while increases in algae and other heterotrophs result in a decrease of suitable substrate for settling coral larvae (Fairfull and Harriott 1999; Box and Mumby 2007). The ecological implications of such reductions in larval influx and/or settlement space are not well understood, which is largely due to a lack of studies on the biotic and abiotic factors that limit recruitment in reef-building corals.

Corals are characterized by an initial dispersive larval stage followed by a sessile benthic stage, which can lead to a decoupling between propagule output (brood stock) and recruitment at local scales. A decoupled stock-recruitment relationship can influence the capacity of damaged populations to recover following a disturbance (Jones et al. 2009; Gilmour et al. 2013). Studies following local catastrophic mortality events suggest that a stock-recruitment relationship exists, to some degree, for spawning corals (Gilmour et al. 2013; Lukoschek et al. 2013), but is more pronounced in brooding corals (Doropoulos et al. 2015). Whereas brooders release fully competent larvae that settle rapidly and in high frequencies to natal reefs, broadcast spawning corals release eggs that undergo development into larvae during a pelagic stage and this leads to a decoupling of larval sources and sinks (reviewed in Jones et al. 2009). Few experimental studies have directly tested the relationship between larval supply and either short- or long-term settlement success. Prior to the occurrence of post-settlement bottlenecks and during the initial settlement phase, some coral species are unaffected by larval densities (Doropoulos et al. 2017) while others display positive density dependence with increasing settlement as larval densities increase (Heyward et al. 2002; Suzuki et al. 2012; Edwards et al. 2015; dela Cruz and Harrison 2017; Doropoulos et al. 2018). Across longer time-scales (months), initial settlement success is modulated by post-settlement mortality and studies show that a saturation of larval supply either increases recruitment success (dela Cruz and Harrison 2017) or, alternatively, has no overall net benefit (Heyward et al. 2002; Suzuki et al. 2012). Collectively, these inconsistent findings highlight the complexity of the processes occurring before, during and after settlement, and that these processes may be highly variable among species, genera, habitats and regions.

Even if larval supply remained unaffected following environmental disturbances, most marine invertebrate populations are space limited and a change in available settlement space is expected to affect settlement dynamics. Field and laboratory research on sessile marine invertebrates, such as barnacles and mussels, indicates that settlement success is positively correlated to patch size (Gaines and Roughgarden 1985). Counter-intuitively, settlement success can also intensify when less substrate is present (Bertness et al. 1992; Pineda 1994). Reduced substrate availability may interact with gregarious settlement behaviour (a common trait in marine invertebrates) to produce an increase in settler numbers, a phenomenon termed the 'reduced substrate settlement intensification' hypothesis (Pineda and Caswell 1997; Pineda et al. 2010). As coral reefs continue to decline and exhibit persistent shifts to non-coral dominated states (Roff and Mumby 2012), free space for settlement is likely to become fragmented and increasingly occupied by heterotrophic and phototrophic competitors (Fairfull and Harriott 1999; Box and Mumby 2007) that exert strong negative competitive interactions (Kuffner et al. 2006). While it is clear that space availability is a key factor linked to the settlement success of marine invertebrates, the influence of space limitation on coral settlement has remained unstudied.

Here, we test how patch size influences settlement in three common Indo-Pacific coral species: *Acropora valida*, *Acropora digitifera* and *Anacropora spinosa*. We manipulate substrate area by providing settling larvae with a range of tile sizes while maintaining a constant larval supply. Our results indicate that settlement success can be either positively, or negatively correlated to, or unaffected by the amount of available space, depending on the coral species. Overall, coral settlers of all species displayed gregarious behaviour, and reduced space availability caused settlers to be in closer proximity to each other, leading to an increase in settler aggregations.

Materials and methods

Coral larval settlement assays were conducted in two separate experiments. The first experiment was carried out on the southern Great Barrier Reef in November 2013. A total of five *Acropora valida* (Fig. 1) colonies of approximately 30 cm diameter and containing mature gametes (confirmed by in situ visual inspection) were collected from the upper reef slope between 2 and 5 m depth at Harry's Bommie, Heron Island. Colonies with mature gametes were transferred to flow-through aquaria that were set up at Heron Island Research Station. All colonies released positively buoyant egg-sperm bundles simultaneously 6 days after the November full moon, 235–260 min after sunset (November 23 between 22:15 and 22:40, sunset at 18:19). The second experiment was conducted in Palau (Micronesia) during the coral spawning season in April 2017. A total of nine whole colonies of *Acropora digitifera* of approximately 20–30 cm diameter and 10 large branches (30 to 50 cm in length) of *Anacropora spinosa* colonies (Fig. 1) were collected from the Airai province back reef between 2 and 5 m depth. Colonies and branches with mature gametes were transferred to flow-through aquaria at Biota Marine Life Nursery (Palau). All *A. digitifera* colonies released positively buoyant egg-sperm bundles 1 day after the April full moon, 110–200 min after sunset (April 12 between 20:00 and 21:30, sunset at 18:10) and colonies of *An. spinosa* released neutrally buoyant egg-sperm bundles 2 days after the April full moon, 50–95 min after sunset (April 13 between 19:00 and 19:45). The egg-sperm bundles were removed from the water surface of the aquaria using a turkey baster and transferred to 5 L bowls containing filtered seawater (FSW; 0.45 µm). The gametes were gently transferred and mixed between bowls to break up bundles and were left to cross-fertilize. Once the eggs underwent the first cell divisions (thus confirming fertilization had occurred), or at most 2 hours after fertilization was initiated, eggs were gently

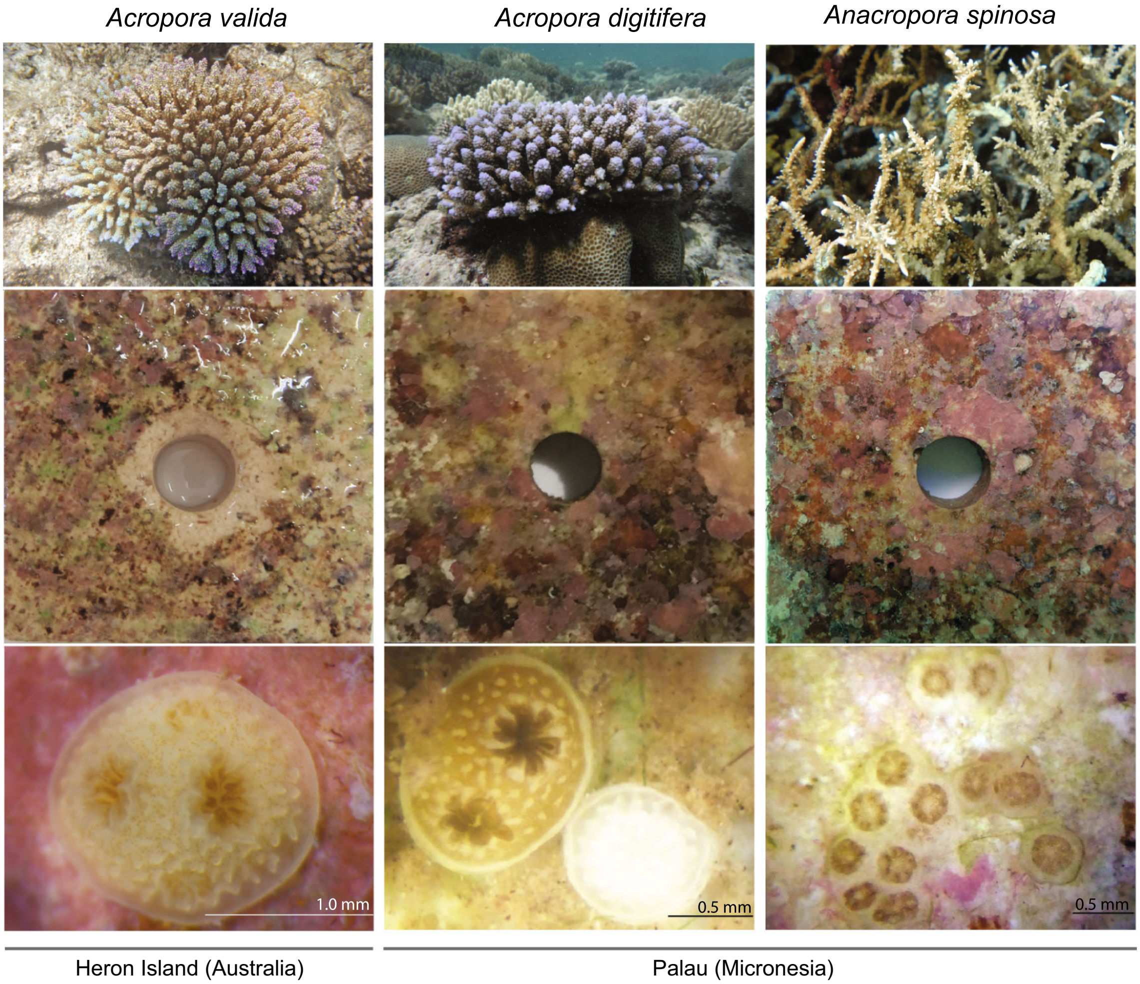

Fig. 1 Adult colonies and settlers of *Acropora valida* (Heron Island, Australia), and *Acropora digitifera* and *Anacropora spinosa* (Palau, Micronesia), and representative photographs of tiles prior to settlement experiment. Fused tissues and merged skeletal features between settlers are visible at 7 to 10 days post-settlement in all species

rinsed with FSW through a 120 µm filter and transferred into clean 5 L bowls containing FSW (filled to 1 cm from the rim). Water in the bowls was kept at temperatures close to field values of between 25 and 26 °C at Heron Island (air-conditioned room) and 27–29 °C in Palau (outdoor area), without additional circulation or aeration. Embryos were left to develop into swimming planula larvae, which took 3 to 4 days for the *Acropora* species and 1to 2 days for *An. spinosa*. During larval development, water changes with FSW were done 1 to 2 times a day.

Approximately 300 limestone tiles (5 × 5 cm) for settlement were strung onto ropes with a 0.5 cm plastic spacer between each tile. Strings of tiles were conditioned in the field at each location for 6 weeks prior to spawning to accumulate crustose coralline algae (CCA) and bacterial biofilms, both of which act as larval settlement cues (e.g. Harrington et al. 2004). Tiles were conditioned in locations where the study coral species naturally occurred to ensure that the substrate offered in our settlement experiments contained benthic cover that matched local species-specific substrate preferences/cues. Coral larvae are known to respond differently to substrate types and positive as well as negative settlement cues can come from specific CCA species, algal turfs, benthic invertebrates or biofilms (Harrington et al. 2004; Price 2010; Elmer et al. 2018). To avoid any effects of differences in settlement cues, tiles were haphazardly subsampled from the total available pool of conditioned tiles. Tiles containing large heterotrophs (i.e. sponges, tunicates) or macroalgae were not used to minimize competitive effects (allelopathy, pre-emption of space). Aside from bare substrate, benthos on the tiles consisted mostly of thin CCA crusts, macroalgal settlers and short turfs with few tube worms and small patches of bryozoans (Fig. 1). For our experiments, we produced a continuous range of substrate area, from approximately 2–57 cm^2, by fragmenting tiles using a hammer and chisel. A total of 30 tile fragments for *A. valida*, 36 for *A. digitifera*, and 13 for *An. spinosa* were haphazardly placed into settlement containers (individual white plastic bowls, ∼ 15 × 8 cm) containing approximately 500 ml FSW. Then 100 larvae of each of the *Acropora* species, and 50 larvae of *An. spinosa* were added and left to settle for approximately 48 hours (all larvae were between 3 and 4 days old). The overall replication for *An. spinosa* was lower compared to *Acropora* because less larvae were available. The time allowed for settlement to occur was fixed so that measurements were standardized and comparable across species.

All tiles were photographed to obtain each of their surface area using Image J (National Institutes of Health, Bethesda, Maryland, USA). The total surface area excluded bottom surfaces that were placed flat on the container to prevent larvae from settling underneath. All settlers were visually counted with a dissecting microscope and their position mapped onto a separate digital image layer overlayed onto the tile photograph (Adobe Illustrator CS6; 2012 Adobe Systems Incorporated, USA). Settlement success was defined as the relative proportion of larvae settled within the fixed timeframe allowed for settlement to occur. The probability of settlement success as a function of substrate area was calculated using binomial generalized linear models (GLM; settled = 1, not settled = 0). Despite tiles being haphazardly assigned to settlement containers, we ensured that there were no a priori differences in CCA cover as a function of substrate area since this had the potential to influence larval settlement success in our experiments. The percentage CCA cover on tile fragments was calculated using CPCe after the allocation of 20 random points onto each of the tile photographs (Kohler and Gill 2006). Using a linear model with the ‘stats’ package in R (R Core Team 2019), we then tested whether percentage CCA cover was correlated to substrate area, and if percentage CCA cover influenced larval settlement success and CCA electivity (i.e. settler in contact with CCA).

We calculated three metrics to describe spatial settlement patterns: (1) nearest neighbour distance (NND), (2) Clarke and Evans distance (R) and (3) formation of aggregations (settlers touching). First, settler size was calculated for each species using photographs taken under a microscope with a size reference ($n = 20$ per species). We obtained average diameters (± SD) of 0.65 ± 0.11, 0.74 ± 0.12 and 0.45 ± 0.09 mm for *A. valida*, *A. digitifera* and *An. spinosa* respectively. Settler size was used to calculate the observed versus predicted available space for each settler as well as the percentage unoccupied area on each tile. For the spatial analyses, we only included settlement trials with five or more settlers (*A. valida* n = 26; *A. digitifera* n = 35, *An. spinosa* n = 11). The digital layer created during visual scoring (see above) was imported into ImageJ Software, which was used to generate the X–Y coordinates of each settler on the surface of each tile and to calculate the NND between settlers. The Clarke and Evans metric (R, calculated as the observed mean NND over the expected mean NND) was used to determine whether the spatial distribution of settlers was random, over- or under-dispersed (cf. Aguirre et al. 2013) using the ‘spatstat’ package in R software (R Core Team 2019). Maximum over-dispersion occurs when settlers are equidistant from one another. In contrast, under-dispersion occurs when settlers are closer to one another than expected from a random distribution and is indicative of gregarious behaviour. Significant differences from an under-dispersed pattern for each tile ($R < 1$) were tested using a Monte Carlo-based simulation approach in ‘spatstat’ based on 999 iterations. Linear regressions for NND and R were analysed using the ‘stats’ package in R (R Core Team 2019).

While the Clarke and Evens metric (R) quantifies the spatial distribution of settlers, significant under-dispersion does not mean that settlers are in contact with one another. As such, we defined an additional aggregation metric, where each settler was scored in a binary format as either in direct contact with another settler (aggregated = 1) or not (single = 0). The probability of settlers being found as aggregates or single settlers as a function of substrate area was then analysed using binomial GLMs using the 'stats' package in R. All binomial GLMs were plotted with a 95% confidence interval using the 'ggplot2' package (Wickham 2009).

Results

The percentage cover of crustose coralline algae (CCA) on settlement tiles offered to *Acropora valida* larvae at Heron Island averaged 34.1% ± 3.0 SE. In Palau, CCA cover was 46.9% ± 2.5 SE and 47.7% ± 3.3 SE on tiles offered to larvae of *Acropora digitifera* and *Anacropora spinosa*, respectively (Figure S1). In the settlement trials of all three study species, the percentage CCA cover was unrelated to substrate area and did not influence larval settlement success (the proportion of larvae settled) or electivity to CCA (Table S1). Overall, the majority of *A. digitifera* (91.8% ± 1.3 SE) and *A. valida* (84.7% ± 4.0 SE) larvae settled in contact with CCA, in contrast to 60.8% (± 7.9 SE) of *An. spinosa* larvae (Figure S1). Given the lack of correlation between CCA cover and the abovementioned variables, we are confident that substrate area was the main parameter driving the settlement patterns observed in our experiments, as described below.

The mean settlement success of larvae differed significantly among the three study coral species. Larvae of *A. valida* had the lowest mean settlement success of 16.2% ± 2.1 SE compared to *A. digitifera* with 33.6% ± 2.4 SE and *An. spinosa* with 47.2% ± 6.6 SE (*A. valida* < *A. digitifera*, $Z = -8.739$, $p < 0.001$; *A. valida* < *An. spinosa*, $Z = -10.162$, $p < 0.001$; *A. digitifera* < *An. spinosa*, $Z = -4.389$, $p < 0.001$). The settlement probability of *A. valida* larvae was inversely related to substrate area (Fig. 2a; $Z = -9.416$, $p < 0.0001$), with the probability of settlement increasing up to 4.5-fold (0.05–0.23) as space became scarcer (from 47.1 to 2.2 cm^2). In contrast, *A. digitifera* larvae showed no significant relationship between settlement probability and substrate area (Fig. 2b; $Z = 0.99$, $p = 0.322$), and the settlement probability of *An. spinosa* larvae was positively correlated with substrate area (Fig. 2c; $Z = 5.311$, $p < 0.0001$), increasing up to 1.8-fold (from 0.38 to 0.68) as more space became available (from 56.9 to 4.9 cm^2).

A reduction in space caused larvae of the two *Acropora* species to settle closer together (smaller NND), while a change in space had no influence on the distance between *An. spinosa* settlers (Fig. 2d–f; *A. valida,* $F = 5.676$, $p = 0.025$; *A. digitifera*, $F = 87.20$, $p < 0.0001$; *An. spinosa*, $F = 0.785$, $p = 0.3986$). Settlers of all three study species displayed an under-dispersed spatial settlement pattern, with all but one replicate significantly non-random (Clark and Evans R; Fig. 2g–i; $R < 1$, $p < 0.001$). This indicates that larvae of all species settled closer together than expected from a random distribution and that the larvae exhibited gregarious settlement. While *An. spinosa* and *A. valida* maintained a similar dispersion pattern regardless of available space, the R-value of *A. digitifera* settlers showed a weak, but significant, positive trend with increasing substrate area, indicating that settlement is less under-dispersed (gregarious) as substrate area increases (Fig. 2h; *A. digitifera*, $F = 13.68$, $p = 0.0008$). In addition to the Clark and Evans metric (Fig. 2g–i), we established an aggregation metric to specify whether settlers were in direct contact with each other and thus formed aggregates (Fig. 2j–l). While the Clark and Evens metric remained fairly stable in relation to substrate area, it was evident that settlers of all study species were much more likely to form aggregates as the available area decreased (*A.* valida, $Z = -6.077$, $p < 0.0001$; *A digitifera*, $Z = -4.566$, $p < 0.0001$; *An. spinosa*, $Z = -10.29$, $p < 0.0001$). Finally, we note that, irrespective of the substrate size, a large proportion of the provided substrate remained unoccupied for all three coral species (Figure S2).

Discussion

The aim of our study was to determine the effects of reduced settlement substrate availability or 'patch size' on marine larval settlement success and spatial settlement patterns. We studied three common Indo-Pacific coral species, *Acropora valida*, *Acropora digitifera*, and *Anacropora spinosa,* and found that patch size influenced settlement success but the response was highly species-specific. Conversely, the effect of patch size on spatial settlement patterns was relatively consistent among species. The settlers of all three study species exhibited gregarious settlement behaviour, forming more settler aggregates as available substrate area decreased. As the experiments were conducted in different locations (*A. valida* at Heron Island, Great Barrier Reef; *A. digitifera*, *An. spinosa* in Palau, Micronesia), the differences in settlement success may reflect geographic differences related to latitude and/or sea surface temperatures, differences in crustose coralline algae (CCA) community or cover (Heron < Palau) as well as inherent species-specific

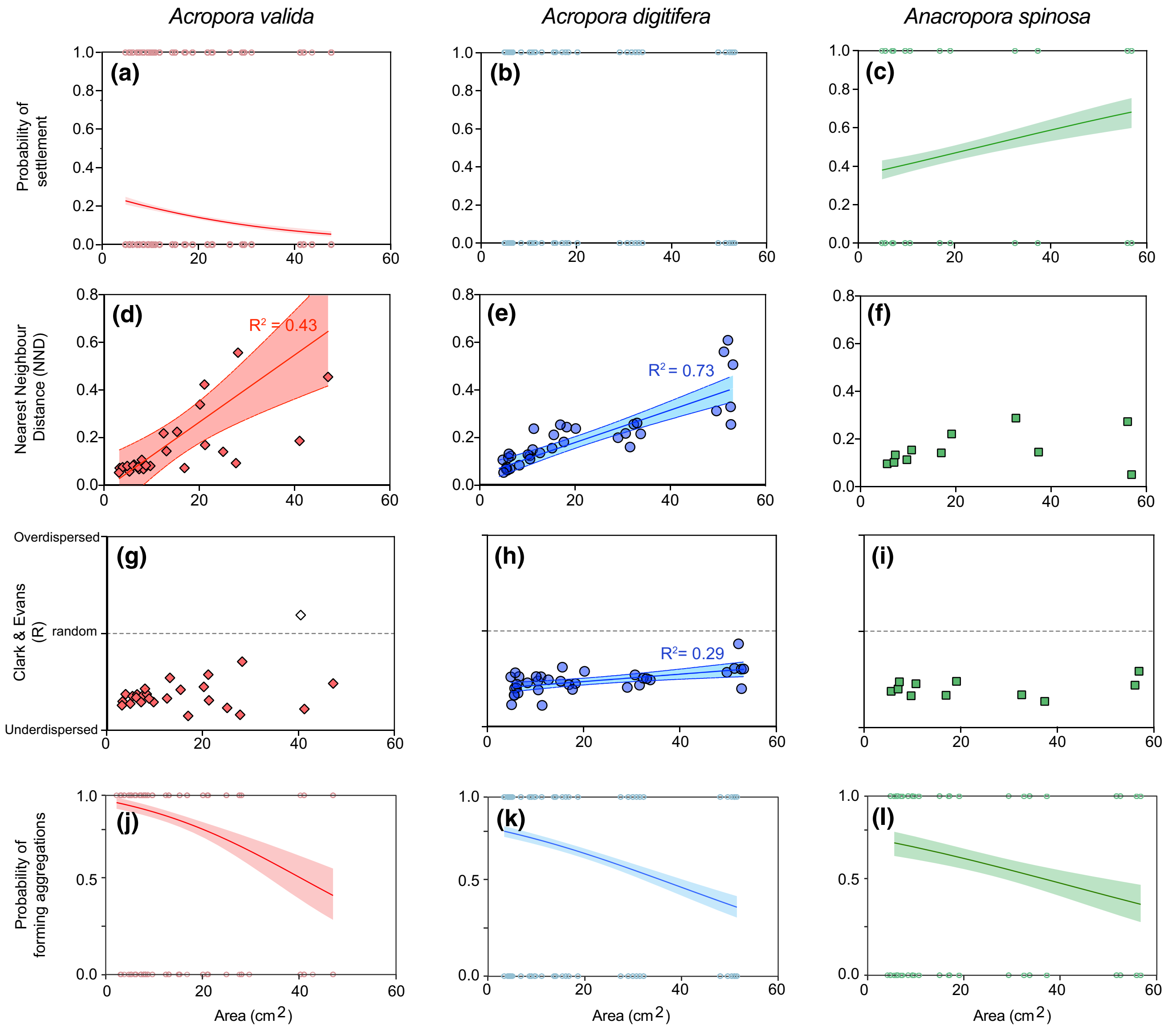

Fig. 2 For three experimental coral species, *Acropora valida*, *Acropora digitifera* and *Anacropora spinosa,* the effects of reduced settlement space was tested in relation to: **a–c** the probability of settlement (1 = settled, 0 = not settled), **d–f** average nearest neighbour distance (NND), **g–i**, Clarke and Evans distance (*R*) (closed symbols significantly differ from random; open symbols are non-significant), and **j–l** the probability of aggregation (single settler = 0, settlers touching = 1). Significant relationships are shown as a line with the 95% CI, and all model fits shown were significant at $p < 0.001$. Note that **a–c**, and **j–l** are binomial GLM fits, and **d–i** are GLM fits (see methods). The absence of a plotted line indicates there was no significant correlation

settlement responses that can differ among closely related species. Nonetheless, our data highlights an important role of patch size in defining settlement success and spatial settlement patterns of coral larvae. Our finding that there is a correlation between patch size and settlement processes is likely to have long-term effects on coral recruitment success into reef communities and will be particularly relevant as settlement space is increasingly pre-empted by marine hetero- and autotrophs (i.e. algae, sponges, tunicates) in disturbed or degraded marine habitats (Roff and Mumby 2012).

The recruitment of sessile marine invertebrates into local communities relies on the supply of planktonic propagules and their ability to settle, metamorphose and survive (Connell 1985). Settlement success of larvae relies on the density of incoming propagules, the availability of suitable substrate and active larval behaviour (Gaines and Roughgarden 1985; Roughgarden et al. 1985; Pawlik 1992; Minchinton and Scheibling 1993; Pineda et al. 2010). Suitable substrate is defined by its quality as well as patch size, which is usually considered to be bare or free space. In our study, patch size is not defined as free or bare substrate but instead the area of suitable substrate provided for settlement, which for coral larvae represents a solid substrate with biotic cover. Various biotic cues trigger coral settlement and metamorphoses, and include specific CCA species or bacterial biofilms (Harrington et al. 2004; Dixon et al. 2014). Conspecifics can also act as natural settlement inducers in a range of sessile benthic taxa including barnacles, polychaetes, oysters (Pineda et al. 2010) and corals (Vermeij 2005; Vermeij and Sandin 2008). Interestingly, closely related heterospecific adults also appear to increase settlement of coral larvae (Birkeland et al. 1981; Dixon et al. 2014). It is clear that a range of biogenic cues as well as larval influx (density dependence) can influence coral larval settlement success (Suzuki et al. 2012; Edwards et al. 2015; dela Cruz and Harrison 2017; Doropoulos et al. 2018). Here, we provide the first study (to our knowledge) that investigates the direct influence of patch size on settlement success prior to post-settlement bottlenecks.

As benthic substrate availability on coral reefs becomes more fragmented and increasingly occupied by other organisms, suitable settlement substrate for coral larvae is reduced (e.g. Fairfull and Harriott 1999; Norstrom et al. 2009; Doropoulos et al. 2016). Our results indicate that reductions in substrate area may lead to a decrease (e.g. *An. spinosa*) and, counter-intuitively, also an increase in overall settlement success (e.g. *A. valida*) of coral larvae. A single previous study that investigated the connection between patch size and settlement success in corals found no effect (Birkeland et al. 1981) and this is similar to our findings for *A. digitifera*. However, it is likely that, in their study, any relationship between settlement success and substrate area would have been modulated by post-settlement processes (e.g. competition, predation, growth) because settlement was monitored between 2.5 to 6 months after substrates were deployed. To isolate the influence of patch size on initial coral larval settlement, our experiments were carried out in the absence of predators or large heterotrophic or autotrophic competitors and settlement was recorded before any post-settlement bottlenecks could occur. The early life stages of corals are subject to significant demographic bottlenecks (Vermeij and Sandin 2008), and increased settlement rates may directly translate to increased survivorship and recruitment into the local community. Within disturbed coral reef environments, increased pre-emption of substrate or direct competition from macroalgae and heterotrophs (i.e. sponges, tunicates, bryozoans) (Fairfull and Harriott 1999; Box and Mumby 2007), coupled with increased allelopathic effects (e.g. Kuffner et al. 2006), may offset increases (i.e. *A.valida*) or amplify reductions (i.e. *An. spinosa*) in coral settlement success correlated to patch size. Our study highlights that patch size is an important substrate characteristic that can modulate settlement success prior to the occurrence of post-settlement bottlenecks and, along with propagule influx and substrate quality, acts to define recruitment into local communities.

The diversity in responses to reduced space for settlement corresponds to findings in other marine invertebrates. Many species of barnacles and mussels experience an increase in settlement success with increasing patch size (Gaines and Roughgarden 1985; Roughgarden et al. 1985), similar to our finding for *An. spinosa* larvae. Interestingly, our finding for *A. valida* is consistent with the ‘reduced substrate settlement intensification’ hypothesis (Pineda and Caswell 1997), where settlement of some barnacles and bivalves increases with decreasing substrate availability. This counter-intuitive settlement response has been attributed to hydrodynamic patterns, increased unsuitable substrate or behavioural settlement cues (Bertness et al. 1992; Pineda 1994). In our experiments, there were no differences in larval influx or hydrodynamics that would lead to the concentration of larvae into the same settlement area. There were also no differences in substrate suitability as settlement cues were unlikely different across our size treatments, and we found no correlation between CCA cover and settlement success (similar to findings of Elmer et al. 2018). As such, we conclude that the most likely driver underlying the observed differences in settlement success as a function of substrate area is the gregarious behaviour of coral larvae. Here, settlement intensification under reduced space as observed for *A. valida* larvae could be caused by gregarious larval behaviour because it has the potential to inflate invertebrate settler numbers (Pawlik 1992; Pineda et al. 2010). Settlement cues from ‘founding’ larvae more readily reach other larvae when less substrate is available (Pineda et al. 2010), leading to settlement clumps and a further cascading amplification of the settlement cue. In contrast, larvae of *An. spinosa* did not show settlement intensification despite their gregarious behaviour, and settlement success decreased as substrate area decreased. It is plausible that settlement cues from founding larvae may also have deleterious effects when aggregations reach certain densities, and this may then suppress settlement success. Overall, larval behaviour leading to gregarious settlement appears to be a factor involved in initial settlement success that is inherently different among corals, even for corals of the same genus (e.g. *A. valida* and *A. digitifera*).

While the tendency of corals to exhibit gregarious settlement was first recorded over a century ago (Duerden 1902; Harrigan 1972; Lewis 1974), subsequent observations have been largely anecdotal. Few studies exist on the extent of gregarious behaviour in corals under natural conditions, how variable it is among species, whether it influences settler survival or if the behaviour is modified by environmental cues. Gregarious settlement of solitary organisms, such as barnacles or bivalves, is associated with energetic costs and leads to the suppression of growth rates, a reduction in maximum size and reduced reproductive output per individual. Nonetheless, gregarious settlement behaviour in colonial organisms such as corals, ascidians, hydrocorals or sponges is common and may have been maintained throughout evolution due to certain benefits related to the formation of chimeras (Rinkevich 2004). Gregarious settlement leading to the fusion of multiple individuals and formation of chimeric aggregates has been described for several hard coral species, e.g. *Acropora millepora, Stylophora pistillata* (Frank et al. 1997b; Amar et al. 2008; Puill-Stephan et al. 2012a) and *Pocillopora damicornis* (Raymundo and Maypa 2004), as well as in various soft coral species (Barki et al. 2002; Rinkevich 2004). The formation of chimeras appears restricted to the early life stages in these taxa and once allorecognition develops, usually between 2 and 12 months (Frank et al. 1997a; Puill-Stephan et al. 2012b), fusion of neighbouring settlers no longer takes place and fusion lines, detachment, or death of all but one chimeric partner occur (Frank et al. 1997b; Barki et al. 2002). All three coral species studied here exhibited gregarious settlement behaviour and a high probability of forming aggregates, which increased significantly as settlement space became smaller. Of the three coral species in our study, *An. spinosa* exhibited the greatest propensity for aggregation among settlers, followed by *A. digitifera* and then *A. valida*. Settlers in direct contact with one another were observed to have fused tissues within 10 days post-settlement (Fig. 1), and we therefore assume that a large proportion of settlers in aggregations form chimeric juveniles.

Despite potential negative trade-offs, chimerism in colonial invertebrates is thought to be promoted by gregarious behaviour coupled with a juvenile immature allorecognition (Barki et al. 2002; Rinkevich 2004). The few studies on chimerism in scleractinian corals report predominantly beneficial outcomes. For example, the formation of chimeric colonies in hard corals has been linked to increases in colony size and/or growth rates as well as increased survival in some coral species (Raymundo and Maypa 2004; Amar et al. 2008; Puill-Stephan et al. 2012a). The persistence of chimerism into adulthood has been documented in 2 to 5% of wild colonies of *A. millepora* on the Great Barrier Reef (Puill-Stephan et al. 2009), suggesting that chimerism may be more common than previously assumed under natural conditions. Gregarious settlement leading to the formation of chimeras or even fused settler pairs that maintain fusion lines, facilitates an immediate rapid size increase that allows settlers to exceed size-escape thresholds (Raymundo and Maypa 2004; Vermeij and Sandin 2008; Doropoulos et al. 2016). Experimental evidence suggests that multi-polyp settlers have a higher probability of survival compared to single polyp settlers when exposed to grazer disturbance (Christiansen et al. 2009), and smaller coral colonies are more susceptible to being outcompeted by macroalgae than larger colonies (Ferrari et al. 2012). Interestingly, fusion of *Agaricia* settlers in the Caribbean may be more frequent under moderate competition for space (Sammarco 1982), which is consistent with our findings of increased aggregate formation under limited space. The rapid increase in settler size, which is critical for survival during the early life-history stages, likely represents the most important evolutionary benefit associated with chimerism in colonial organisms such as corals.

Settlement of marine invertebrate larvae is primarily regulated by larval influx and availability of suitable substrate (substrate quality and patch size). Previous studies indicate that settlement may be either positively (Gaines and Roughgarden 1985) or negatively correlated to patch size for a range of marine invertebrates (Pineda and Caswell 1997). While all marine invertebrates are susceptible to high-rates of mortality during the early life-history stages, corals are particularly susceptible to post-settlement bottlenecks in response to environmental factors such as thermal stress and macroalgal competition (Randall and Szmant 2009; Doropoulos et al. 2016). Our findings have implications for predicting future patterns of recruitment and coral recovery under a changing climate that facilitates habitat fragmentation. We show that the impacts of reductions in settlement substrate availability are coral species-specific and predict this may lead to differential shifts in recruitment success among species followed by changes in community composition. In addition, the increased formation of aggregates under limiting space availability may offset some of the negative effects of increased competition for space by allowing settlers to rapidly exceed size-escape thresholds.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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