Assemblages on limestone and siltstone boulders diverge over six years in a primary-succession transplant experiment

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ABSTRACT: Transplant experiments have been used to test for associations between different rock types and developing assemblages during succession in intertidal boulderfields, with all previous experiments of this type concluding within 1 yr. Therefore, differences in colonisation and recruitment on different rocks have been well established but any late-successional associations remain unknown. To remedy this, a previous transplant experiment contrasting limestone and siltstone was extended so that the assemblages that developed on transplanted bare boulders were investigated over 6 yr. The structure and richness of the later-successional assemblages differed between rocks, with limestone assemblages on both upper and lower surfaces being characterised by more species, and higher abundances of some species, than siltstone. These assemblage differences were most strongly correlated with rock-related differences in surface rugosity and microhabitat density. The later-successional assemblages sampled after 5 and 6 yr were generally similar to earlier-successional assemblages sampled after 11 mo. However, the subtle successional changes that transpired were such that all rock-related differences after 5 and 6 yr were unique to later-successional assemblages only. These rock-related differences, that appear to take multi-year time scales to manifest, would have remained unknown had this experiment concluded within the typical timeframe of <1 yr.

KEY WORDS: Algae · Community · Invertebrates · Mobile · Sessile · Upper versus lower surfaces

INTRODUCTION

Successional change occurs in biological assemblages following a perturbation that opens up a bare space (Connell & Slatyer 1977, Connell 1987). In intertidal boulderfields, the intermittent overturning of boulders by wave action often opens up such spaces, subjecting the algal and invertebrate assemblages on boulder surfaces to successional change (Sousa 1979a, 1980, 1984). Succession may be cut short and started again on frequently disturbed boulders, while progressing to later stages on boulders that are disturbed less often (Connell & Slatyer 1977, Sousa 1979a). As a result, boulder assemblages may be viewed as a highly variable mosaic of successional stages (Sousa 1979a, Connell 1985).

Successional processes in intertidal boulderfields are not solely influenced by physical disturbances initiating change. Rather, succession may also be associated with a suite of environmental variables, including desiccation stress at low tide (Sousa 1979b), boulder size (Sousa 1979a), and lithology of the rock comprising the boulders (often referred to as 'rock type' in the published literature; McGuinness & Underwood 1986, McGuinness 1988, Liversage et al. 2014). Presently, successional patterns on different types of rocks remain under-examined, with all published experiments targeting colonisation and re-

cruitment onto initially bare boulders and concluding within 12 mo (see McGuinness & Underwood 1986, James & Underwood 1994, Green et al. 2012, Liversage et al. 2014). Therefore, rock-related differences have only been established for boulder assemblages early in succession. These include differences in the space occupancy of algae and tubeworms after 4 mo (McGuinness & Underwood 1986), spirorbid worm recruitment after 3 mo (James & Underwood 1994), the space occupancy of algae and oysters over 10 mo (Green et al. 2012), and limpet abundance over 11 mo (Liversage et al. 2014). However, other longer-term successional studies on a single rock type in intertidal reef systems do provide evidence of community changes well beyond 12 mo. Some species increased, whereas others continued to decline for over 3 yr after experimental clearing on boulders (Sousa 1979b) and rock platforms (Turner 1983, Petraitis & Dudgeon 2005), or after natural ice scour events (McCook & Chapman 1997). Divergent successional pathways after disturbance on sheltered intertidal reefs can lead to alternative-state communities (Petraitis & Dudgeon 2005, Petraitis et al. 2009). There is also evidence to suggest that different assemblages can form on intertidal reefs of different rock type (McGuinness & Underwood 1986, Davidson & Chadderton 1994, Savoya & Schwindt 2010, Green et al. 2012, Liversage & Benkendorff 2013, Bavestrello et al. 2018). However, longer-term successional studies (i.e. >1 yr) are required to establish whether the assemblages diverge as a result of rock type or other factors that may vary at the reef scale.

Each rock type possesses a specific set of physical attributes as substratum, and rock-related differences in physical attributes may be associated with some of the biotic patterns identified. Some of these physical attributes include boulder size and hence surface area (Chapman 2002b, Liversage et al. 2014), substratum colour (James & Underwood 1994), microhabitat density (McGuinness & Underwood 1986, Chapman 2000, Moreira et al. 2007, Chapman & Underwood 2011), rugosity (Underwood & Chapman 1989, Chapman & Underwood 1994, Herbert & Hawkins 2006), mineralogy (Bavestrello et al. 2000, Cerrano et al. 2007, Bavestrello et al. 2018), and hardness (Liversage & Benkendorff 2013, Liversage et al. 2014). Generally, associations between assemblages of intertidal biota and rock physical attributes have been assessed in studies of a single rock only, mostly for platform areas on rocky seashores. Furthermore, most biotic associations with rock-related differences in physical attributes have been identified through laboratory- or field-based experimental manipulations that have used

small, modified pieces (i.e. tiles) of either the tested rocks or anthropogenic materials (e.g. asbestos). With the exception of Liversage et al. (2014), very few published studies have evaluated multiple physical attributes of naturally occurring intertidal boulders of differing rock, and examined how multiple physical attributes may be associated with assemblages during succession.

To extend time frames, an earlier boulder-transplant experiment (Liversage et al. 2014) was re-sampled after several more years to determine whether later-successional invertebrate-dominated assemblages diverged on limestone and siltstone boulders. By re-sampling an existing experiment, comparisons between earlier- and later-successional assemblages were possible, thus enabling us to investigate rock-type associations across all stages of succession. When the experiment by Liversage et al. (2014) concluded after 11 mo, the early-successional assemblages on both the upper and lower surfaces of limestone and siltstone had a similar mobile-invertebrate assemblage structure and abundances of common invertebrate species. The only exception was for abundances of limpets belonging to the genus *Notoacmea*, which were consistently denser on siltstone than limestone (Liversage et al. 2014). Due to their consistently sparse space occupancies, sessile invertebrates were not evaluated for these early successional assemblages. Our research builds upon these short-term results (<1 yr) by investigating assemblage structure, species richness and the abundances of individual taxa for the upper and lower surfaces of boulders transplanted over 6 yr. These biotic-dependent variables were investigated for both mobile and sessile assemblages later in succession. As Liversage et al. (2014) reported significant differences in the rugosity and microhabitat density of limestone and siltstone boulders, here we also determined whether these physical attributes, along with boulder surface area (to account for possible species–area relationships), were associated with rock-related biotic patterns. To our knowledge, this is the first boulder-transplant experiment with a duration exceeding 1 yr, presenting a unique opportunity to consider the following 4 null hypotheses (H_1 to H_4) for later-successional stages on boulders of differing rock:

H_1: Assemblages will not differ between transplanted limestone and siltstone; we predict that the assemblages on different rocks will continue to diverge as the communities develop over 5 or 6 yr since initial transplantation;

H_2: Assemblages will not differ between native and transplanted boulders of the same rock; we predict

that the assemblages on transplanted boulders that are of the same rock type as the reef substratum will converge on the native boulders over time;

H_3: Assemblages will not differ between the earlier (11 mo) and later (5 or 6 yr) stages of succession; we predict that the communities that establish on transplanted boulders after 5 or 6 yr will differ to those that had developed after 11 mo;

H_4: Patterns of association between later-successional assemblages and different rocks are not correlated with rock physical attributes; we predict differences in the assemblages will be correlated with the greater surface complexity of limestone compared to siltstone boulders (Liversage et al. 2014).

MATERIALS AND METHODS

Experimental locations and rock types

Boulder translocations were completed at 4 rocky seashores along South Australia's geologically diverse Fleurieu Peninsula (Fig. 1, Liversage et al. 2014). All 4 seashores have a westerly aspect and are subjected to similar oceanic and environmental conditions. Each seashore was located along an 80 km stretch of coast on the southeastern side of a large inverse estuary, Gulf St Vincent, which provides protection from the Southern Ocean's large waves and swell. Two seashores were dominated by siltstone, Myponga Beach (35° 22′ S, 138° 23′ E) and Marino Rocks (35° 02′ S, 138° 30′ E), while 2 seashores were dominated by fossiliferous limestone, Blanche Point (35° 14′ S, 138° 27′ E) and Southport (35° 10′ S, 138° 27′ E) (Fig. 1). Transplants were completed using representative bare boulders sourced from above the tide line at Southport for limestone and Marino Rocks for siltstone.

Fossiliferous limestone boulders were light grey in colour (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m604p021_supp.pdf) and had a softer scratch hardness of 4 on Mohs' scale, while siltstone boulders were a greyish yellow-brown in colour and had a higher scratch hardness of 8 (Table S1 in the Supplement). Both rocks had a mineralogy dominated by silicon dioxide (SiO_2) although limestone contained a higher percentage (71.6%) of SiO_2 than siltstone (55.4%; Table S1). Siltstone had a higher content of several major and trace metals than limestone including aluminium oxide, iron oxide, magnesium oxide, barium, manganese, and zirconium (Table S1).

Experimental design

Bare boulder translocations were completed between November 2009 and February 2010. All boulders (length or width up to 30 cm) were collected from above the high tide mark at source seashores, and thus lacked any established marine assemblages on their surfaces including visible biofilms (see Liversage et al. 2014 for full description). Transplanted boulders (60 rock^{-1}) were tagged and randomly allocated on each shore with alternative rock types placed 10 m apart along mid-low shore parallel transects. Independent boulders were sampled by Liversage et al. (2014) after 1, 4, and 11 mo. We then resampled as many as could be found at each seashore (Table 1) during suitable daytime low tides (predicted low tide ≤0.60 m Australian Height Datum) during the austral summer 5 yr later in February 2015, and again in February–March 2016 after 6 yr had elapsed. Controls, consisting of non-transplanted and untagged boulders native to each seashore (hereafter referred to as 'native' boulders), were also sampled (Table 1). At Myponga Beach and Marino Rocks native boulders were grey siltstone, while at Blanche Point and Southport native boulders were fossiliferous limestone. Native boulders were sampled to determine whether the species composition on transplanted boulders of the same rock had converged with the assemblages naturally present over a timeframe of 5 or 6 yr.
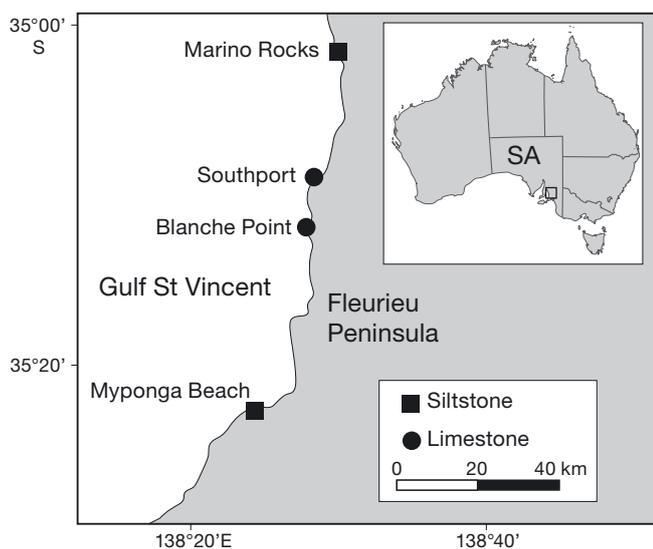
Fig. 1. Rocky seashores used during experimental boulder translocations along South Australia's Fleurieu Peninsula. Inset map shows the location of the study region in Australia

Table 1. Number of transplanted limestone, transplanted siltstone, native limestone, and native siltstone boulders sampled at each seashore, for each sampling time (yr; values in parentheses). Initially, 60 limestone and 60 siltstone boulders were transplanted onto each seashore between November 2009 and February 2010. The tabulated numbers refer to the number of boulders that could be relocated and sampled 5 or 6 yr later. –: not applicable, as no native rocks of that type occurred at that seashore

Seashore	Transplanted				Native			
	Limestone		Siltstone		Limestone		Siltstone	
	(5)	(6)	(5)	(6)	(5)	(6)	(5)	(6)
Marino Rocks	10	7	10	7	–	–	10	7
Myponga Beach	10	7	10	7	–	–	10	7
Southport	10	4	10	6	10	6	–	–
Blanche Point	7	7	10	7	10	7	–	–

Sampling procedures

Lower surfaces are defined as the underside of the boulder that was in contact with the substratum, while upper surfaces are defined as all remaining surfaces that were not in contact with the substratum. Consequently, upper surfaces generally encompassed both the top and sides of boulders and thus were larger (Fig. S2). Upper and lower surfaces were sampled separately as they often support different assemblages (McGuinness & Underwood 1986). We therefore hypothesised that associations between rock type and later-successional assemblages would be surface-specific. All invertebrate and algal species >5 mm in size were identified and recorded *in situ* wherever possible (see Table S2). Digital photographs were used to subsequently identify any ambiguous specimens that could not be accurately identified in the field. Sampling was restricted to macroscopic species that could be observed *in situ*. Species richness was estimated as the number of species per boulder. The space occupancy of sessile species (algae, tubeworms, and mussels) was quantified using a flexible but inelastic quadrat marked in cm^2. Sessile assemblage coverage was recorded as the total percentage of the boulder surface occupied by all sessile species. Barnacles did not form dense aggregations and hence their space occupancies were often zero, so they were counted and analysed separately. The abundance of all mobile invertebrate species was counted per boulder surface and recorded as the 'mobile assemblage'. Boulder surface area, microhabitat density (i.e. surface features >13 mm in size sensu Liversage et al. 2014), and surface rugosity were all measured using the methods described in Liversage et al. (2014).

Statistical analyses

All analyses were completed using PRIMER v7/PERMANOVA+ software. All tests of permutational analysis of variance (PERMANOVA) were completed using permutations of residuals with 9999 permutations as described in Anderson et al. (2008). A Monte Carlo (MC) p-value replaced a permuted p-value when the number of available unique permutations was <100 (Anderson et al. 2008). Differences (α = 0.05) among rocks (limestone, siltstone), seashores (Myponga Beach, Blanche Point, Southport, Marino Rocks) and sampling times (specific to each analysis) were assumed to be fixed factors or analysed separately. Separate PERMANOVAs were completed for upper versus lower surfaces and for the 5 and 6 yr transplant times to ensure data independence, as the upper and lower surfaces of the same boulders were sampled, and we could not be certain whether the same subset of boulders had been sampled after 5 and 6 yr. Bray-Curtis resemblances were used for species assemblage data and Euclidean distances were used for univariate data, including physical variables. For analyses based on multivariate assemblage data, a dummy variable of 1 was added because of the sparsity of non-zero values among samples (Anderson et al. 2008). Multi-dimensional scaling (MDS) bootstrapped-averages ordination plots were used to visualise significant assemblage differences. Similarity percentages (SIMPER) were used to calculate the average dissimilarity between rocks, and to identify any consistent (i.e. SD/similarity ratio > 1) indicators associated with rock-related differences.

Biotic differences among native and transplanted boulders were tested using a 1-factor PERMANOVA with *a priori* planned comparisons among 4 levels of the factor boulder history (i.e. levels were transplanted limestone, transplanted siltstone, native limestone, and native siltstone; a fixed factor). This approach was employed because we predicted that each contrast between a pair of boulder histories and at each seashore could yield results specific to that pairing. A planned comparison between transplanted limestone versus transplanted siltstone was used to test the first hypothesis (H_1), while a comparison between transplanted and native boulders of the same rock was used to test the second hypothesis (H_2). For the PERMANOVA, all univariate biodiversity measurements were square-root transformed. To overcome limitations with the hierarchical experi-

mental design (i.e. whilst transplanted boulders were orthogonal with relation to shores, native boulders could only be nested within seashores), separate planned comparisons were completed for data from each seashore, for each surface and sampling time.

To assess assemblage development during succession (H_3), comparisons were made between later-successional assemblages and the 11 mo early successional assemblages as reported by Liversage et al. (2014). Square-root transformations were applied to all biodiversity measurements. A 3-factor PERM-ANOVA tested for differences between rocks, sampling times (11 mo, 5 yr and 6 yr) and seashores. The interaction term seashore × rock × sampling time was excluded from analyses as the same subset of boulders may have been sampled after 5 and 6 yr. PERM-ANOVA pair-wise tests were completed for the interaction term rock × sampling time to determine significantly different sampling times for each rock.

Univariate PERMANOVAs confirmed that limestone had significantly more microhabitats and a lower surface rugosity than siltstone boulders (rugosity index closer to 1) (Fig. S2) and, as expected, the upper surfaces of boulders had a greater surface area than lower surfaces for both rocks, and some surface- and year-specific differences in the surface area were found between rocks (Fig. S2). Thus, to determine whether rock physical attributes were correlated with assemblage differences (H_4), a BIOENV analysis was completed separately for each surface. The BEST (BIOENV) procedure searches for a combination of environmental variables whose inter-sample resemblances best match those arising from multivariate biological data (Anderson et al. 2008). Physical attribute data (rugosity, microhabitat density, and surface area) were normalised, with square-root transformations applied to assemblage data. A BEST analysis tested the significance and correlation strength be-

tween the best combination of normalised physical attributes and Bray-Curtis resemblances of mobile or sessile assemblage structure. A RELATE analysis between Euclidean distances of physical attributes and Bray-Curtis resemblances of assemblage structure tested for a significant relationship between biological and physical variables.

RESULTS

H_1: assemblages will not differ between transplanted limestone and siltstone

Later-successional assemblages were often different between transplanted limestone and siltstone (Table 2, Table S3). For upper surfaces, 16 of 32 analyses detected significant assemblage differences, while 10 of 32 analyses detected assemblage differences for lower surfaces (Table 2). These significant assemblage differences for both surfaces were highly variable among seashores and sampling times (Table S3).

There was strong evidence of rock-related differences for later-successional assemblages on siltstone seashores (Table S3, Figs. S3–S5). At Marino Rocks, rock-related differences in species richness were evident on both surfaces after 5 and 6 yr, and the mobile invertebrate assemblage was different on upper surfaces in both years and lower surfaces after 6 yr (Fig. 2, Figs. S3–S5, Table S3). At Myponga Beach, only upper surfaces appeared to develop significantly divergent assemblages, with most assemblage and barnacle abundance differences specific to boulders sampled after 5 yr (Table S3, Figs. S3–S5). On lower surfaces at Myponga Beach, only sessile assemblage structure was significantly different (Table S3). At the 2 limestone seashores there was less evidence of rock-related assemblage differences (Table S3), with the exception of barnacle abundance differing on upper and lower surfaces at Southport and upper surfaces at Blanche Point after 5 yr. Generally, the few differences detected between rocks were unique to a specific surface and sampling time (Table S3, Figs. S3–S5).

For mobile assemblage structure, a greater number of rock-related differences were detected

Table 2. Tally of significant (S) versus non-significant (NS) PERMANOVA results out of 8 tests surface^{-1} (from 4 seashores each sampled twice after 5 and 6 yr) for each biotic variable comparing between transplanted limestone versus siltstone, and between transplanted and native boulders of the same rock. See Tables S3 & S5 in the Supplement for PERMANOVA tests

| Biotic variable | Limestone vs. siltstone | | | | Transplant vs. native | | | |
| | Upper | | Lower | | Upper | | Lower | |
	S	NS	S	NS	S	NS	S	NS
Mobile assemblage structure	4	4	1	7	1	7	1	7
Sessile assemblage structure	4	4	5	3	0	8	0	8
Species richness	5	3	3	5	1	7	0	8
Barnacle abundance	3	5	1	7	1	7	1	7
Totals	16	16	10	22	3	29	2	30
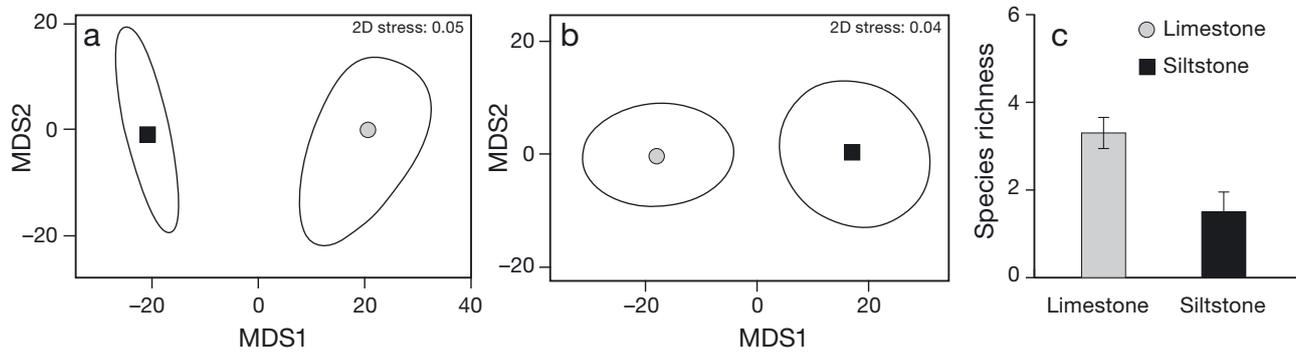
Fig. 2. Significantly divergent assemblages that developed over 5 yr on the upper surfaces of transplanted limestone versus siltstone at Marino Rocks. Multi-dimensional scaling (MDS) bootstrapped-averages ordination plots depicting rock differences for (a) mobile assemblage structure and (b) sessile assemblage structure, and (c) mean (±SE) differences between rocks for species richness (number of species boulder^{-1}). See Figs. S3–S5 in the Supplement for complete summary plots of all significant differences detected

on upper than lower surfaces (Table 2). These structural differences were associated with a small number of consistent indicator species (Table S4). The snail *Bembicium nanum* was always more abundant on the upper surfaces of transplanted limestone. *Notoacmea* spp. were typically more abundant on siltstone, although after 6 yr at Marino Rocks, these limpets were more abundant on limestone. The rock-related differences in sessile assemblage structure were consistently associated with a higher space occupancy of the tubeworm *Galeolaria caespitosa* and a visible biofilm on limestone (Table S4).

The species richness of later-successional assemblages on transplanted boulders was significantly different between rocks, with a greater number of differences on upper than lower surfaces (Table 2, Fig. S5). Species richness differences were consistent across surfaces and sampling times, with the assemblages on limestone generally having a higher species richness than siltstone (Fig. S5). The abundance of barnacles was generally similar between rocks, with rock-related differences recorded on just 4 occasions overall and no obvious rock-related trend apparent (Table 2, Fig. S5).

H2: assemblages will not differ between native and transplanted boulders of the same rock

Assemblage structure and species richness were generally similar between native and transplanted boulders of the same rock for both sampling times (Tables 2 & S5). For upper surfaces, just 3 of 32 analyses detected significant assemblage differences (Table 2). No significant differences were detected between transplanted and native boulders, for either surface, for any of the variables measured after 5 yr

(Table S5); 12 mo later, mobile-invertebrate assemblage differences were detected for siltstone upper surfaces at Marino Rocks and limestone lower surfaces at Southport (Table S5). Species richness was different on lower surfaces at both limestone seashores, whilst barnacle abundance was different on upper surfaces at Southport (Table S5).

H3: assemblages will not differ between the earlier (11 mo) and later (5 or 6 yr) stages of succession

For the upper and lower surfaces of both rocks, there was limited evidence for a change in species composition between earlier- and later-successional assemblages (Table 3). Later-successional assemblages shared 12 to 16 species in common with assemblages sampled after 11 mo, predominantly abundant limpet and snail species (Table 3). However, there was an exchange of rarer species, which were generally recorded as singletons, between earlier- and later-successional assemblages, with the greatest changes recorded on limestone lower surfaces (Table 3). Nevertheless, the following later successional species were only recorded after 5 and 6 yr on both rock types: spirorbid sp., *Actinia tenebrosa*, *Austrocochlea constricta*, *Zuzara venosa*, with all of these except the spirorbid only occurring on the underside of boulders (Table 3). Two small gastropods (*Cantharidella balteata* and *Rissoina* sp.) were detected on both rocks after 11 mo, but not on any transplanted boulders after 5 and 6 yr.

For both surfaces, the mean species richness of early-successional assemblages were lower than in native assemblages on the same rock after 1 and 4 mo (Figs. 3 & 4). At 11 mo, these parameters had converged between transplanted and native boul-

Table 3. Species compositions on the upper and lower surfaces of transplanted limestone and siltstone for assemblages sampled after 11 mo, 5 yr, and 6 yr (values given in parentheses). Changes in algal species composition could not be evaluated because no data were collected for algae on boulders transplanted for 11 mo. M: mobile; S: sessile; MS: mostly sessile, limited pedal locomotion; C: clonal reproduction; B: brooding; E: benthic egg mass, IF: internal fertilisation; FS: free spawning; PL: pelagic larvae

Species	Life history	Limestone Upper (11)	(5)	(6)	Limestone Lower (11)	(5)	(6)	Siltstone Upper (11)	(5)	(6)	Siltstone Lower (11)	(5)	(6)
Notopsilus sp.	M, FS								x				
Galeolaria caespitosa	S, FS	x	x	x	x	x	x	x	x	x	x	x	x
Spirorbid sp.	S, FS		x	x		x	x	x	x			x	x
Notoplana australis	M, IF, PL		x						x		x	x	x
Actinia tenebrosa	MS, C, FS					x	x					x	x
Isanemonia australis	S, C, FS					x	x	x				x	x
Ischnochiton elongatus	M, FS				x	x	x				x	x	x
Ischnochiton smaragdinus	M, FS										x		
Montfortula rugosa	M, IF, PL		x	x	x								
Notoacmaea spp.	M, IF, PL	x	x	x	x	x	x	x	x	x	x	x	x
Bembicium nanum	M, E, PL	x	x	x	x	x	x		x	x		x	x
Bembicium vittatum	M, E, PL	x	x	x	x					x		x	
Cellana tramoserica	M, IF, PL	x	x	x	x	x	x	x	x	x	x	x	
Nerita atramentosa	M, E, PL	x	x	x	x	x	x	x		x	x	x	x
Siphonaria diemenensis	M, E, PL	x	x	x	x	x	x	x	x	x	x	x	x
Siphonaria zelandica	M, E, PL	x	x		x	x		x			x		
Austrocochlea constricta	M, IF, PL					x	x					x	x
Austrocochlea porcata	M, IF, PL				x						x	x	
Diloma concamerata	M, IF, PL	x	x	x	x	x	x	x		x	x	x	x
Cantharidella balteata	M, IF, PL	x			x			x			x		
Rissoina sp.	M, IF, PL				x						x		
Haustrum vinosum	M, E, PL				x								
Brachidontes rostratus	S, FS		x									x	
Xenostrobus pulex	S, FS	x	x		x	x	x	x	x		x	x	x
Halicarcinus ovatus	M, B, PL	x											
Ozius truncatus	M, B, PL				x	x	x				x	x	x
Cyclograpsus granulosus	M, B, PL						x						
Euidotea bakeri	M, B, PL						x						x
Zuzara venosa	M, B, PL					x	x						x
Chthamalus antennatus	S, IF, PL	x	x	x	x	x	x	x	x	x	x	x	x
Tetraclitella purpurascens	S, IF, PL	x		x	x	x	x				x	x	x
Total species		14	15	12	16	21	19	11	10	11	17	20	18

ders (see also Liversage et al. 2014). This convergence of species richness was maintained between later-successional and native assemblages on the same rock, as evidenced on limestone (Fig. 3a,c) and siltstone (Fig. 4a,c) after 5 and 6 yr for both surfaces.

At limestone seashores, a similar convergence over time was observed on upper and lower surfaces for the mean sessile-assemblage space occupancy on transplanted versus native limestone boulders (Fig. 3b,d). Minimal recruitment was recorded during the first 4 mo (mean space occupancy <0.1% per boulder surface), but by 11 mo, the sessile assemblage that had recruited to transplanted limestone had a similar space occupancy to native limestone assemblages (Fig. 3b,d). At siltstone seashores total space occupancy was consistently low, with the sessile space occupancy on transplanted siltstone after 11 mo (mean space occupancy <0.1% per boulder surface) markedly lower than on native siltstone, especially on lower surfaces (Fig. 4b). By 5 yr, sessile space occupancy on transplanted boulders converged with that on native siltstone (Fig. 4b,d).

On upper and lower boulder surfaces, the later-successional assemblages sampled after 5 and 6 yr were generally similar to the earlier-successional assemblages sampled after 11 mo described by Liversage et al. (2014) (Table S6). However, we detected significant sampling time × rock interactions for sessile assemblage structure on both surfaces and species richness on lower surfaces (Table S6). Post hoc tests detected differences in the sessile assemblages on lower surfaces of limestone after 11 mo
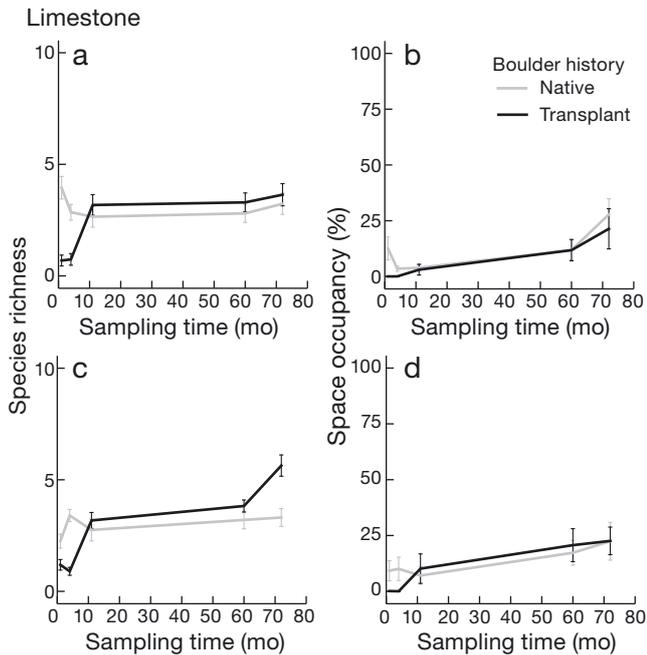
Limestone

Fig. 3. Mean (±SE) trajectories over time, averaged across limestone seashores, showing convergence of assemblages on transplanted limestone with native boulders for upper (a,b) and lower (c,d) surfaces for species richness (a,c) and space occupancy (b,d). Each *y*-axis extends to encompass the full range of the raw data
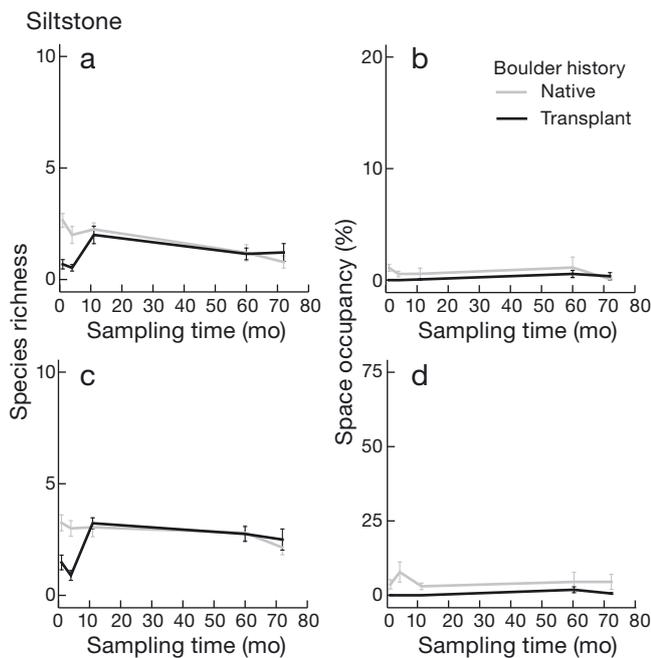
Siltstone

Fig. 4. Mean (±SE) trajectories over time, averaged across siltstone seashores, showing convergence of assemblages on transplanted siltstone with native boulders for upper (a,b) and lower (c,d) surfaces for species richness (a,c) and space occupancy (d,e). Each *y*-axis extends to encompass the full range of the raw data

compared to 6 yr, and siltstone after 11 mo compared to both later-successional times (PERMANOVA pairwise permuted p-values <0.05). These structural differences were associated with *G. caespitosa* space occupancy (SIMPER SD/sim ratio >1), which more than doubled on both rocks between earlier (11 mo grand mean = 3.8 ± 1.8 %) and later (5 yr grand mean = 10.2 ± 2.0 %, 6 yr grand mean = 8.6 ± 2.1 %) times.

H_4: patterns of association between later-successional assemblages and different rocks are not correlated with rock physical attributes

A significant relationship was detected between ordinations of mobile invertebrate assemblage structure and physical attributes for upper (RELATE Spearman Rho; $\rho = 0.102$, p = 0.0013) but not lower surfaces (RELATE Spearman Rho; $\rho = -0.019$, p = 0.6846). For upper surfaces, mobile-assemblage structure was weakly, but significantly, correlated with rock differences in microhabitat density combined with surface area (BEST; $\rho = 0.106$, p = 0.0040). Likewise, significant relationships were detected between ordinations of sessile assemblage structure and rock physical attributes for both upper (RELATE Spearman Rho $\rho = 0.118$, p = 0.0082) and lower surfaces (RELATE Spearman Rho; $\rho = 0.059$, p = 0.0459). Rock-related patterns of sessile assemblage structure for upper surfaces were weakly, but significantly, correlated with rock differences in microhabitat density combined with surface rugosity (BEST; $\rho = 0.127$, p = 0.0170) but no physical attributes were correlated with sessile invertebrate structure on lower surfaces (BEST; $\rho = 0.059$, p = 0.1330).

The higher abundances of *B. nanum* on upper limestone surfaces may be associated with their greater surface area and complexity compared to siltstone. Statistically significant weak-to-moderate correlations were detected between *B. nanum* abundance and surface area (r = 0.348, p < 0.01), rugosity (r = −0.281, p < 0.01) and microhabitat density (r = 0.560, p < 0.01). However, the r^2 values for each of these correlations explained less than one-third of the total variation between *B. nanum* abundance and each physical attribute. The higher space occupancy of *G. caespitosa* on limestone was only correlated with rock differences in microhabitat density on both upper (r = −0.230, p = 0.02) and lower surfaces (r = 0.270, p < 0.01). The r^2 values for these correlations explained <10 % of the total variation between *G. caespitosa* abundance and microhabitat density.

DISCUSSION

By re-sampling an earlier transplant experiment (Liversage et al. 2014) after 5 and then 6 yr, the present study tracked assemblage development on different bare rocks from earliest recruitment through to later stages of succession. Before this work, transplant experiments addressing hypotheses about the effects of rock on succession were completed within 1 yr, so only early differences in colonisation were known (McGuinness & Underwood 1986, James & Underwood 1994, Green et al. 2012). Our study overall identified some early-successional rock-type differences (Liversage et al. 2014), then later confirmed distinct rock differences in the structure and richness of later-successional assemblages (see Table 2).

The assemblages that developed on transplanted boulders were dominated by sparse mobile or sessile invertebrates (see Tables 3 & S2). This composition contrasts strongly with the algal dominance on boulders manipulated along the California coast where hypotheses about succession in intertidal boulderfields were first tested (Sousa 1979a,b, 1980). Those pioneering experiments identified a highly variable mosaic of successional stages on boulders, with species richness peaking earlier in succession and declining through later stages (Sousa 1979a,b, 1980, Connell & Keough 1985). The assemblages on our transplanted boulders appeared to follow a similar pattern of succession (see Figs. 3 & 4). Transplanted boulders supported a highly variable mosaic of later-successional assemblages, differing between seashores and sampling times (see Table S7). Moreover, siltstone assemblages peaked in species richness earlier in succession (i.e. by 11 mo), before declining thereafter (Fig. 4). In contrast, limestone assemblages peaked in species richness later during the overall succession (i.e. by 5 yr) (Fig. 3), highlighting how rock as an environmental variable can influence succession.

Our results showed that later-successional assemblages were in many ways similar to earlier-successional assemblages sampled after 11 mo. The only consistent rock difference identified by 11 mo was higher abundance of the limpets *Notoacmea* spp. on siltstone (Liversage et al. 2014). This difference appeared to persist after 5 yr but not after 6 yr, where abundances were often higher on limestone. At 11 mo, there was also a trend (albeit not significant) for higher species richness and abundances of *Bembicium nanum* and *Galeolaria caespitosa* on limestone versus siltstone. These rock-related trends ultimately manifested into significant differences late in succession. Similarly, in previous long-term successional studies on intertidal rock platforms, depending on the patch size, some changes in community structure took over 4 yr to manifest (McCook & Chapman 1997, Petraitis & Dudgeon 2005, Petraitis et al. 2009). Our results indicate that the later-successional assemblages that developed on different rocks were subtly different from early assemblages, such that early trends became later differences. Consequently, had this experiment concluded within the typical timeframe of <1 yr, successional differences between limestone and siltstone that took multi-year timescales to manifest would never have been identified.

The species richness and space occupancy of earlier-successional assemblages generally converged with assemblages on native boulders within 11 mo (Liversage et al. 2014), although sessile-taxa space occupancy on siltstone took longer (see Figs. 3 & 4). This 11 mo convergence was consistent with other transplant experiments, which had reported rapid recruitment onto initially bare boulders over several weeks to months (McGuinness & Underwood 1986, James & Underwood 1994, Chapman 2002a). Despite no major changes in assemblage structure from 11 mo to 5 yr, there was some evidence of species turnover, and several species were only found on transplanted boulders after 5 or 6 yr. Of these, spirorbids are *r*-selected free spawners that have been found to recruit to panels within 6 mo in other Australian studies (Anderson & Underwood 1997). Therefore, the late arrival of this species may simply reflect the timing of a settlement event, followed by sufficient growth for detection with the naked eye. Conversely, sea anemones may be later successional species, as a study of intertidal succession in South Africa found no *Actinia equina* after 8 or 9 yr across 3 locations (Dye 1992). Some mobile species like the brooding isopod *Zuzara venosa* and gastropod *Austrocochlea constricta* were also only detected after 5 or 6 yr on transplants, whereas 2 small gastropods were only detected within the first year (see Table 3). However, as all these species were uncommon in our study, we cannot confirm successional patterns over shorter temporal variation. Unlike previous studies on algal communities (Sousa 1980), we did not detect any successional patterns that aligned with specific life-history traits.

When the test results from every planned comparison between transplanted limestone versus siltstone were summed for both surfaces, 26 significant differences were detected (Table 2). In contrast, just 5 significant differences were detected from planned comparisons between transplanted and native boul-

ders of the same rock. This provides strong evidence for an association between rocks and later-successional assemblages. Our prediction that such associations may be surface-dependent was not supported. Six more rock-related biotic differences were detected on upper than lower surfaces, but the structural and richness differences identified, and the species associated with those differences, were similar for both surfaces. Ten more (18 versus 8) significant rock-type differences were detected on siltstone versus limestone seashores (see Table S3), with one reef-scale (i.e. between seashores) rock-type difference detected (for mobile invertebrates on lower surfaces after 5 yr, see Fig. S6 and Table S8). This result emphasises the scale dependency of rock-related differences in assemblages, with differences generally being specific to either the reef or boulder scale (McGuinness 1988, Green et al. 2012, Liversage et al. 2014). The recurrence of this outcome from multiple transplant experiments highlights the importance of studying multiple spatial scales to fully assess the associations between lithology and boulder assemblages during succession.

The consistently higher species richness on limestone than siltstone is a finding that is unique to later-successional assemblages, with no significant rock-related richness differences identified earlier during succession (Liversage et al. 2014) or for earlier-successional assemblages on boulders transplanted elsewhere (McGuinness & Underwood 1986, Green et al. 2012). Instead, these results mirror those reported for established assemblages on Italian subtidal reefs, where limestone supported a higher benthic species richness than granite or quartzite (Bavestrello et al. 2000, Guidetti et al. 2004). It has been well established that substrata with the greatest surface complexity often support the highest species richness (Blanchard & Bourget 1999, Le Hir & Hily 2005, Cosentino & Giacobbe 2015, Loke & Todd 2016). Consequently, it is likely that the surface complexity of limestone provides suitable habitat for later-succession species than the relatively flat, featureless siltstone.

Rock-related differences have been reported from earlier-successional studies, associated with higher abundances of one or more species on one of the tested rocks (McGuinness & Underwood 1986, James & Underwood 1994, Green et al. 2012, Liversage et al. 2014). We identified a range of different taxa contributing to the rock-related differences in assemblage structure (see Table S4) but, unlike earlier-successional studies, the identity of taxa associated with abundance differences was inconsistent, often

varying among seashores, sampling times, and surfaces. Only *B. nanum* and *G. caespitosa* displayed a consistent rock association, recorded at higher abundances on limestone than siltstone (see Table S4). For *B. nanum*, abundances were most strongly correlated with the higher microhabitat density on limestone, with this snail frequently recorded within depression microhabitats (N. Janetzki pers. obs.), and thus a moderate correlation detected between their abundance and microhabitat density. It is possible that, by sheltering in depression microhabitats on boulder upper surfaces, *B. nanum* is afforded some protection from the extremes of temperature and desiccation during emersion, which is consistent with this species sheltering in pit microhabitats elsewhere (McGuinness & Underwood 1986). For *G. caespitosa*, abundance differences were also associated with the greater microhabitat density of limestone. Microhabitats provide potential refuges from a range of abiotic stressors and biotic interactions (McGuinness & Underwood 1986) and may facilitate successful establishment of *G. caespitosa* recruits. *G. caespitosa* settlement into disturbed patches shows a behavioural response resulting in gregarious metamorphs (Minchinton 1997). This is consistent with low levels of settlement in the early successional stages of our study, after which conspecific attraction from the successful early recruits could facilitate further accumulation in microhabitats on limestone. It is also possible that rock differences in *G. caespitosa* abundance are associated with the lighter colour of limestone, with higher settlement of these white tubeworms recorded on lighter versus darker substrata (Marsden & Anderson 1981).

Despite detecting rock-related differences for later-successional assemblages (see Table 2), surface area, rugosity, and microhabitat density accounted for little of this biotic variability on either surface. This contrasts strongly with many other studies, which have shown rock differences in microhabitat density (McGuinness & Underwood 1986, Chapman 2000, Moreira et al. 2007, Chapman & Underwood 2011) and rugosity (Raimondi 1990, Chapman & Underwood 1994, Berntsson et al. 2000, Le Hir & Hily 2005, Herbert & Hawkins 2006, Savoya & Schwindt 2010) to be strongly associated with biota. Additional rock-related physical and chemical differences could be included in future models to further examine the factors influencing intertidal succession. For example, limestone has cooler surface temperatures than siltstone (N. Janetzki unpubl. data) and rock-related temperature differences have been associated with biological patterns elsewhere (Raimondi

1988). Furthermore, mineralogy varies between rocks (Table S1) and could influence settlement, recruitment, persistence or competitive abilities of some species. For example, we detected different amounts of quartz (silica) minerals (i.e. the dominant mineral in both rocks; Table S1), and differences in silica content are strongly associated with marine biotic patterns elsewhere (Bavestrello et al. 2000, Cerrano et al. 2007).

In conclusion, this study documents assemblage structure and the abundances of individual taxa for later-successional boulder assemblages, with limestone developing assemblages characterised by a higher species richness and higher abundances of some species than siltstone. These assemblage differences were most strongly correlated with rock-related differences in surface complexity. Assemblages that developed on transplanted boulders were in most ways similar to established communities on native boulders after both 11 mo and 5 or 6 yr, although the subtle changes that transpired allowed early-successional rock-related trends to manifest into significant later-successional differences. These later-successional differences that took multi-year timescales to develop would have remained unknown had this experiment concluded within the historical timeframe of <1 yr. These results show that succession in intertidal boulderfields is not solely influenced by physical disturbance regimes, with rock type influencing both the early and later stages of succession.

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