Comparison of recruitment tile materials for monitoring coralline algae responses to a changing climate

Emma V. Kennedy1,*, Alexandra Ordoñez1, Bonnie E. Lewis1, Guillermo Diaz-Pulido1,2

1Griffith School of Environment, Australian Rivers Institute – Coasts and Estuaries, Nathan Campus, Griffith University, Nathan, QLD 4111, Australia
2Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4811, Australia

ABSTRACT: Settlement plates are widely used in ecological studies as a simple and effective way to assess recruitment and growth of sessile benthic communities. Plate material and surface complexity, positioning, size, attachment method, placement within the reef and duration of deployment can all influence the communities that recruit. Using different plate types can affect study reproducibility and skew results, yet no standardised guidelines exist for monitoring crustose coralline algae (CCA), a key bioindicator of changing seawater carbonate chemistry. Here, we compare 6 experimental tile materials (including plastic, ceramic and glass tiles) at 2 orientations (horizontal and vertical) for CCA community recruitment and calcification across 3 habitats (back reef, fore reef crest and reef slope). CCA were 20% more abundant, calcified faster and were twice as speciose on fore reef habitats. Community composition also varied with tile material and orientation. Vertically oriented surfaces and plastic and limestone tiles produced the closest approximation of real-world communities. Horizontal surfaces appeared to encourage coralline recruitment, particularly in light-limited environments, but also attracted greater herbivory. PVC tiles were the optimal approach when all factors were considered, as they generated a percent cover representative of adjacent reef communities and produced more consistent calcification estimates (across space and time), as well as being cheaper and easier to work with. Selection of plate type and deployment methods will ultimately depend on the experimental question; here we describe the benefits and costs of each material and/or orientation and suggest the adoption of PVC tiles as an optimal overall approach for monitoring CCA.

KEY WORDS:  Crustose coralline algae · CCA · Substrate · Ocean acidification · Settlement · Recruitment · Calcification · Coral reefs · Great Barrier Reef

INTRODUCTION

Coralline algae

Crustose coralline algae (CCA) are calcifying red algae found in a wide range of marine habitats. In shallow tropical reef environments they play key ecological roles in reef framework building and stabilisation (Littler 1972, Adey 1998). As well as contributing to trophic food webs and coral reef biodiversity, CCA contribute specifically to reef resilience through their roles in (1) resistance, cementing reef framework against storm damage and helping maintain vertical growth in the face of sea level rise (Littler 1972, Adey 1978) and (2) recovery, as several species are known to induce settlement responses in
a range of invertebrate taxa, aiding replenishment of coral (Harrington et al. 2004). With reefs worldwide currently threatened by global climate change, scientific attention has turned to CCA for another valuable feature: their potential as a bioindicator for changing seawater chemistry (Hofmann & Bischof 2014, McCoy & Kamenos 2015).

Many marine calcifiers are expected to be negatively affected by future ocean acidification (Anthony et al. 2008, Jokiel et al. 2008, Kroeker et al. 2010), but the particular sensitivity of CCA high-Mg calcite skeletons to dissolution (Morse et al. 2006) makes them stand out as potentially valuable indicators of changing seawater carbonate chemistry (Martin & Gattuso 2009, Diaz-Pulido et al. 2012). CCA may be responsive to ocean acidification on a number of levels, from individual responses (Semesi et al. 2009, Diaz-Pulido et al. 2012, Egilsdottir et al. 2013, Martin et al. 2013) to community-wide effects (Doropoulos et al. 2012, Price et al. 2012, Ordoñez et al. 2014, Fabricius et al. 2015), exhibiting physiological responses ranging from variations in photosynthetic rates to growth and disease (Anthony et al. 2013, Comeau et al. 2013, Noisette et al. 2013). Mesocosm studies (Anthony et al. 2008, Kuffner et al. 2008, Ordonez et al. 2014) and in situ investigations using natural CO₂ seeps (Hall-Spencer et al. 2008, Fabricius et al. 2011) have demonstrated declines in abundance and calcification rates of CCA communities exposed to end-of-century CO₂ forecasts and to environmental extremes. Sensitivity of calcification has prompted calls for systematic monitoring of CCA net accretion to become an integral part of reef monitoring (Anthony et al. 2013, Comeau et al. 2013, Noisette et al. 2013). Accurate estimates of CCA calcification are also required to determine coral reef carbonate budgets (a measure of reef growth and health proxy; Perry et al. 2008, Kennedy et al. 2013). With the growing focus on CCA as an indicator of ocean acidification and an important reef builder comes a need for more standardised studies in real-world environments. Exploring key biological processes—recruitment and growth—of CCA as a function of other environmental parameters (see McCoy & Kamenos 2015 for a review) will further promote our understanding of global change effects on marine ecology. However, while information exists on optimising settlement tiles for coral recruitment, no such guidance exists for CCA.

**Settlement material**

A variety of artificial tile types are commonly used to assess benthic communities. Slabbed coral (Klumpp & Mckinnon 1992) and local limestone or volcanic rock (Kroeker et al. 2013) resemble the natural reef substratum and therefore are assumed to generate data representative of communities growing on surrounding reef (Carleton & Sammarco 1987). Other studies have proposed the use of rough-sanded polyvinyl chloride (PVC) tiles because they are easily manipulated and support biotic assemblages similar to surrounding substrata (Adey & Vassar 1975, Hixon & Brostoff 1996). Ceramics (primarily terracotta) remain popular—even in CCA studies (Arnold & Steneck 2011, Doropoulos et al. 2012, Mallela 2013, Mallela et al. 2017)—because their surface topography is thought to encourage settlement, a theory supported by a study that demonstrated terracotta had a greater recruitment potential than other materials (Harriott & Fisk 1987). Other forms of artificial substrata thought to encourage CCA growth include cattle tags (Kuffner et al. 2013, Morrison et al. 2013), microscope slides (Roik et al. 2016) and cylindrical PVC piping (Perry et al. 2012, Mallela et al. 2017). However, in most cases little justification is provided for the material used besides the cost and local availability (Field et al. 2007).

**Settlement plates**

Previous studies comparing different experimental settlement substrata have focused on optimising plate materials to maximise coral recruitment (Carleton & Sammarco 1987, Harriott & Fisk 1987, Mundy 2000, Petersen et al. 2005); no study has specifically set out to examine the effect of artificial settlement materials on CCA. Consequently, methodologies vary between CCA studies, potentially affecting reproducibility. Positioning (Birkeland et al. 1981, Carleton & Sammarco 1987), plate size (Field et al. 2007), tile material (Harriott & Fisk 1987), surface complexity (Carleton & Sammarco 1987, Diaz-Pulido & McCook 2004, Whalan et al. 2015), attachment method (Mundy 2000), location within the reef (Tomascik 1991) and timing and duration of deployment (Field et al. 2007) can all influence the communities that recruit to settlement tiles. There is a need to understand the consequences (in terms of altered recruitment potential, calcification measurements and community composition) of settlement place choice and standardise settlement plate techniques for field studies (Petersen et al. 2005, Kuffner et al. 2013).
Habitat

Some studies suggest the importance of tile material type is diminished compared with factors such as deployment location and attachment method (Mundy 2000, Field et al. 2007, Burt et al. 2009). Spatial variability in recruitment and growth of CCA may reflect local populations or be subject to environmental variation, with differences between deployment sites often more important than differences between tile orientation and material type (Mundy 2000, Field et al. 2007). Red algae, which include CCA, produce less motile spores that lack flagella, which might limit recruitment (Maggs & Callow 2001). Differential grazing pressure, for example, may affect CCA recruitment and growth; low levels of herbivory from limpets, sea urchins, chitons and fish (Steneck 1983, 1986) can help remove epiphytes and may even stimulate productivity of crusts (Wai & Williams 2005) and community diversity.

Orientation

The orientation angle of settlement surfaces will affect light conditions, sediment accumulation and grazing intensity, which may in turn influence recruitment (Mallela 2007). For example, downward-facing surfaces harbour communities dominated by bryozoans and barnacles rather than CCA (Martin-dale 1992, Mallela 2013). The attachment method can also affect recruitment, with plates fixed directly to the sea floor attracting more CCA than those attached to racks (Field et al. 2007).

Time

CCA communities take time to establish and will peak (in terms of percent cover) from 6 wk (Kroeker et al. 2013) to 8 mo (Arnold & Steneck 2011) following an experimental substratum becoming available. This time period is dependent on the type of settlement material and orientation, as well as the species, competition and natural succession (Arnold & Steneck 2011). For this reason, plate deployment duration may affect study outcomes in terms of CCA recruitment and growth.

Other factors for consideration

CCA calcification estimates may be confounded by the growth of other calcifiers, competitors and/or levels of herbivory. Developing an experimental setup that controls for or minimises these confounding effects may be useful for CCA calcification studies and monitoring efforts. Consequently, we included these factors (grazing marks, percent cover of other calcifiers) in our experimental design.

Aims

We set out to compare and contrast the performance of different artificial tile materials (PVC, polycarbonate, terracotta, limestone, glass and porcelain tiles) for monitoring coralline algae recruitment and growth. In addition to tile material, we explored effects of tile orientation (horizontal versus vertical aspect) and experimental time period (90 versus 180 d) to determine the optimal overall method for monitoring CCA. Finally, we compared the interaction between these factors and deployment location (back reef, fore reef crest, reef slope), as habitat type is well documented to affect the CCA community composition and abundance.

As response variables measured may depend on the underlying scientific question, a number of relevant CCA responses were recorded. These included (1) CCA percent cover (total percent cover living corallines, number of crusts), (2) CCA calcification rate (calcium carbonate accreted, based on changes in buoyant weight) and (3) CCA population characteristics, including mean crust size, and community characteristics including species richness and diversity. Factors such as the number of grazing marks and percent cover of other calcifiers—which may confound estimates of calcification and were likely to be of consequence to studies on CCA—were also measured.

We evaluated the performance of each tile type, orientation and habitat on each of the 3 main metrics (CCA percent cover, CCA calcification rate and CCA community composition), taking into account the additional factors such as grazing. A criticism of artificial tile use is that communities recruiting to plates do not reflect natural populations. Exploring CCA community composition in detail allowed us to identify the materials and orientations that best represented the natural communities found in the 3 reef settings (assessed using standard benthic survey techniques at the same time). Finally, we compared the properties of tile materials measured in this study alongside other key considerations (e.g. cost, ease of use) to provide specific guidance for future CCA studies.
MATERIALS AND METHODS

Experimental design

The study was conducted on the coral reefs around Heron Island, in the southern Great Barrier Reef, Australia (23° 26' 00'' S, 151° 55' 41'' E; Fig. 1), an area known to be favourable to CCA growth (Fabricius & De'ath 2001). Twelve experimental cube-shaped blocks (‘calcification stations’, modified from Kuffner et al. 2013 and Morrison et al. 2013; Fig. 1) were deployed between 5 and 7 March 2014: 4 on the exposed fore reef slope (4.7−6.5 m deep), 4 on the fore reef crest (<4 m, ~3−4 m from the crest) and 4 on the back reef lagoon (1.4 m, ~200 m from shore). Blocks were attached to the reef using decking spikes and L-shaped brackets and spaced 4−6 m apart, facing outward so that vertical tiles fronted the direction of prevailing current flow. This design allowed us to explore differences across reef habitats. Experiments were all performed at the same reef site (Tenements I), as variability in environmental parameters such as light, slope and sedimentation are known to affect CCA calcification rates (Fabricius & De’ath 2001, Mallela 2013).

Each calcification station hosted an array of thirty-two 45 × 45 × 5 mm settlement tiles (Fig. 1F): 16 oriented horizontally and 16 oriented vertically, allowing us to test for the role of tile orientation on CCA. Tiles were secured flush to the block surface using a stainless steel screw, leaving no underside exposed as this is known to attract significantly different communities dominated by bryozoans and tube-forming worms (Field et al. 2007, Mallela 2013). To test the effect of material type on CCA, we tested tiles made from PVC, polycarbonate, terracotta, limestone, porcelain and glass (Fig. 1D), materials used in published CCA settlement experiments. In addition to the tile array, a PVC tube (15 mm diameter × 250 mm length) and plastic cards were attached alongside each block (Fig. 1E) as these methods have been proposed for monitoring CCA.

Fig. 1. Location of experimental field sites (A−C), where arrays of different tile materials (D) were deployed onto experimental blocks (E). Tiles were arranged in a specific pattern (F) to ensure that each block was identical and replicate tiles were found both on the inside and edge of each vertical and horizontal panel arrangement.
calcification rates *in situ* (Perry et al. 2012 and Kuffner et al. 2013, respectively). To test for the influence of deployment time on CCA attributes, half the tiles from each block were collected after 3 mo (6–8 June 2014) and the remainder collected at 6 mo (2–6 September 2014). Tiles were returned to the laboratory in fresh seawater, photographed, buoyant weighed and frozen for transport, storage and later microscope analysis.

Benthic habitat surveys were also performed during the same season (autumn) to record percent cover of adult CCA at each site. Five 50 cm² quadrats were placed randomly around the site, and corallines were visually identified to genus level, with unidentified samples collected for confirmation under a dissecting microscope. This allowed us to explore similarities between the CCA communities developed on the tiles and those in the natural environment.

A total of 384 tiles were collected as part of the experiment and used to generate estimates of CCA calcification rate using buoyant weight techniques. A subset of 72 tiles was then inspected to explore CCA percent cover, community metrics and other measures including cover of other calcifiers and herbivory (Fig. 2).

**Response variables**

CCA percent cover

Settlement tiles were photographed underwater and the CCA total percent cover was measured using imaging software Vidana (MSEL, University of Exeter). The abundance of other benthic calcifying organisms, including peyssonnelids, serpulids and bryozoans, were calculated on each plate. Other

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**Fig. 2.** Representative tiles collected in the study, showing coralline algae growth on 6 different materials. Percent cover of corallines did not vary significantly between tile types in this study, although the community composition varied, particularly on glass tiles.
metrics of interest, including number of individual grazing scars and total percent tile area scarred, juvenile corals and other fleshy algae and turfs, were also collected.

CCA calcification rate

Buoyant weight techniques were used to estimate net calcification rates (mg CaCO$_3$ cm$^{-2}$ d$^{-1}$) for each tile. Buoyant weight of every individual tile was measured in seawater (seawater density was determined prior to each weighing session and ceramic tiles were pre-soaked for 24 hours) prior to deployment and immediately after collection. Net calcification rates were calculated by converting initial and final buoyant weight values (in g) to dry weight based on the seawater density recorded during weighing, using an assumed density of calcite of 2.71 g cm$^{-3}$ (following Davies 1989). This was then normalised to surface area (per cm$^2$) and time (per day) for conversion to bulk mass of CaCO$_3$ (mg cm$^{-2}$ d$^{-1}$) for comparison with PVC cards and pipes. This technique relies on the assumption that the method is insensitive to the weight of soft tissue because its density is very similar to that of seawater (Davies 1989). Any tiles with more than 5% cover of other calcifying organisms were excluded from the analysis to avoid confounding CCA calcification estimates. The exceptions to this rule were peysonnelids, which were found on the 93% of tiles (contributing 7 ± 0.9% [mean ± SE] cover) and were included in mass estimates.

CCA community composition

A subset of 72 tiles was examined in greater detail under a dissecting microscope (Fig. 2), using standard identification techniques (e.g. examination of crust thickness, colour, surface appearance, hypothallus and reproductive structures, decalcification and preparation of slides for examination of cell connections under a compound microscope) to identify individual crusts to the lowest possible taxonomic level (Adey & MacIntyre 1973, Ringeltaube & Harvey 2000). Community metrics—percent cover and counts of the number of crusts of each individual species (or genera)—were recorded. Species richness and diversity could be derived from these measurements. Mean thallus size was estimated by dividing total number of individual CCA crusts by total cover (cm$^2$) for each identified taxon.

Statistical analyses

Univariate comparison of CCA attributes (i.e. percent cover and calcification) were performed using ANOVA, t-tests or non-parametric equivalents (Mann-Whitney U or Wilcoxon signed rank tests), based on an assessment of normality of distribution and homogeneity of variance using Shapiro-Wilk and Bartlett’s tests, respectively, in R (R Core Development Team 2008). Two-way interactions among main factors were included in ANOVA models, with Tukey multiple comparisons of means tests performed to further explore relationships. Variability in CCA community assemblage was explored using PERMANOVA in PRIMER. The 3 predictors were tile material (polycarbonate, PVC, terracotta, limestone, porcelain and glass), orientation (horizontal versus vertical) and habitat (fore reef crest, reef slope and back reef). Multidimensional scaling (MDS) ordinations were generated to visualise differences in community assemblage between predictors. Similarity matrices and distance based linear modelling (DISTLM) on transformed coraline species percent cover data were used to identify tile communities that best resembled adult communities surveyed on each reef. All means are presented ±SE unless otherwise indicated.

RESULTS

CCA percent cover

Total CCA cover (Fig. 3, Table S1 in the Supplement at www.int-res.com/articles/suppl/m569p129_supp.pdf) was unrelated to tile material (3-way ANOVA, $F = 1.7$, $p = 0.16$; Table S2). Orientation was also unimportant as a main effect, although a significant interaction existed between orientation and habitat (3-way ANOVA, $F = 5.7$, $p = 0.007$; Table S2, Fig. 3). This was manifested as horizontally oriented tiles attracting greater CCA cover on the deeper fore reef slope site (41% compared with 28% on vertical tiles), but not on the reef crest. On the back reef the opposite was true (vertical > horizontal, 3-way ANOVA, $F = 11.69$, $p = 0.006$).

Habitat was a significant driver of CCA cover (3-way ANOVA, $F = 28.7$, $p = <0.001$, Table S2). Tiles deployed on the back reef had 20–22% fewer corallines growing on them (mean = 12%) compared with fore reef crest and slope sites (33 and 34%, respectively), but the tiles were not different in terms of percent cover (Tukey’s, $p > 0.05$).
CCA calcification rate

Coralline calcification rates

Tile material ($F = 241.2, p < 0.001$), time ($F = 52.1, p < 0.001$) and to a lesser extent habitat ($F = 3.69, p < 0.035$) were important in determining calcification rates (4-way ANOVA; Table S3, Fig. 4). Terracotta tiles accrued 3 times the mass of other tiles ($0.98 \pm 0.04 \text{ mg cm}^{-2} \text{ d}^{-1}, n = 91$), while limestone tiles only recruited a third of the mass ($0.12 \pm 0.01 \text{ mg cm}^{-2} \text{ d}^{-1}, n = 95$). Polycarbonate, PVC, porcelain and glass tiles all gave comparable estimates of CCA calcification ($0.24 \pm 0.01 \text{ mg cm}^{-2} \text{ d}^{-1}, n = 182$, Tukey’s, $p > 0.05$), and considerably less variable estimates than terracotta tiles (Fig. 4). Inclusion of the PVC pipes and plastic cards in the analysis revealed no difference between the PVC pipe method and polycarbonate, porcelain, glass and PVC tiles (Tukey’s, $p < 0.001$). However, like limestone, the plastic card method accrued less calcified material than other tile types. Interactions were identified between tile material and time ($F = 12.5, p < 0.001$) and tile material and habitat ($F = 2.1, p = 0.027$). Post hoc analyses revealed that these were driven by terracotta tiles showing larger declines in calcification during the second half of the experiment (Tukey’s, $p = 0.003$), while glass tiles exhibited more variable calcification between habitats than other tiles, calcifying more rapidly on the back reef (Tukey’s, $p = 0.05$).

Tile orientation was not important (Fig. 4, Table S3). Habitat differences depended on time: tiles calcified slightly more rapidly ($0.48 \pm 0.04 \text{ mg cm}^{-2} \text{ d}^{-1}, n = 181$) over 3 mo compared with 6 mo ($0.35 \pm 0.02 \text{ mg cm}^{-2} \text{ d}^{-1}, n = 187$), particularly in the shallower back and fore reef crest sites, suggesting a rapid initial colonisation stage followed by a general slowing of growth (4-way ANOVA, $F = 4.89, p = 0.033$; Table S3, Fig. 4). This effect was only significant on back reef habitats, where tiles in the 6 mo experiment experienced a decline of $0.18 \text{ mg cm}^{-2}$ difference daily (Tukey = $p = 0.0004$), but not on the reef crest or slope slopes (differences of 0.10 and 0.04 mg cm$^{-2}$, respectively, $p > 0.9$, Tukey’s test; Fig. 4). There were no other significant interactions between factors (Table S3).

Other calcifying organisms

Calcifiers were responsible for $7.2 \pm 0.9\%$ cover per tile (Fig. S1) and were dominated by the alga *Peyssonnelia*, responsible for 94.4% of calcifiers (excluding CCA). The remaining 5.6% of calcifiers (found on only 43% of tiles) consisted of small invertebrates, predominately calcified tube worms, *Spirorbis* (41%), and cheilostome bryozoans (23%) along with oysters, calcifying green algae and occasionally coral spat (combined 36%). Removal of *Peyssonnelia* from analyses led to all factors becoming non-significant. Tile material (3-way ANOVA, $F = 4.67, p = 0.002$), orientation ($F = 13.75, p < 0.001$) and habitat ($F = 20.93, p < 0.001$) were all important in determining the total number of calcifiers found on tiles (Table S4, Fig. S1). Habitat had the strongest effect, with the back reef attracting fewer calcifiers (2.36%) than the fore reef (slope = 11.22%, crest = 7.97%). Vertical tiles

![Graphs showing CCA cover on tiles](https://example.com/graph.png)
attracted substantially more calcifiers (9.84% compared with 4.52% on horizontal tiles). Glass attracted fewer calcifiers (around 2.5%) compared with PVC and limestone (9.95% and 9.93%, respectively, Tukey’s, p < 0.001; Fig. S1).

CCA community composition

Species richness

Up to 12 different CCA taxa were recorded per tile (5 ± 2 SD taxa; Table S5). Both tile material (3-way ANOVA, $F = 6.17$, $p < 0.001$) and habitat ($F = 26.8$, $p < 0.001$) were key in determining the number of species recorded, but no interactions were observed (Table S6). Glass tiles hosted fewer species than terracotta, limestone and PVC tiles (Fig. 5), with an average of 3 taxa identified compared with a mean of 6 on every other tile type (Tukey’s, $p < 0.001$). Back reef tiles had fewer species (around 3) compared with fore reef (5.8 crest, 6.0 slope).

Community composition

Community species data revealed that tile material (PERMANOVA, pseudo-$F = 4.49$, $p = 0.002$), orientation (PERMANOVA, pseudo-$F = 4.93$, $p = 0.001$) and habitat (PERMANOVA, pseudo-$F = 3.14$, $p = 0.001$) were all important in explaining community composition (Table S7, Fig. S2, Figs. 5 & 6). A significant interaction between orientation and site was also identified (PERMANOVA, pseudo-$F = 3.01$, $p = 0.003$). Porolithon spp. dominated the tiles (Fig. S2), accounting for 41–46% relative cover of fore reef CCA community composition and 33% of back reef corallines (Fig. 5). The exceptions were glass and porcelain tiles, and some back reef communities, which were instead dominated by Hydrolithon spp. Neogoniolithon and Pneophyllum spp. made up 3–6% of fore reef and 11–18% back reef of total coralline cover, respectively. Titanoderma and Lithophyllum spp. were less common, each accounting for 1–3% of total CCA cover (Fig. 5, Fig. S3). Four percent of corallines, rising to 18% on the back reef, could not be confidently identified (Fig. 5).

Fig. 4. Mean (±SE) calcified material accrued on tiles (standardised for unit area and unit time) for all experiments. This includes 6 tile types (plus 2 additional methods, PVC pipes and cards), 2 tile orientations, 3 habitats and 2 time periods. Calcification rate differed between tile types, with terracotta tiles accruing significantly more calcified material, and limestone tiles accruing significantly less. Calcification rate was also different between habitats, with fore reef crest tiles accruing marginally more calcified material. Different lowercase letters denote significant differences highlighted by post-hoc tests.
Crust size

Despite glass tiles developing larger crusts ($27.2 \pm 20.1 \text{ mm}^2$) than other tiles ($17.2 \pm 10.4 \text{ mm}^2$), analyses showed that individual crust size was unrelated to tile material (3-way ANOVA, $F = 2.29, p = 0.06$; Table S8). Habitat was important (3-way ANOVA, $F = 10.18, p = 0.00003$), with the back reef tiles producing smaller crusts (mean size = $13 \pm 10.2 \text{ mm}^2$) compared with the fore reef slope ($18 \pm 5.5 \text{ mm}^2$) and crest ($21 \pm 12.6 \text{ mm}^2$). An interaction between orientation and habitat showed crusts grew larger on vertically oriented (16 mm$^2$) than horizontal tiles (10 mm$^2$) on the back reef, but the opposite effect was found in the fore reef sites (vertical versus horizontal on slope: 15 versus 20 mm$^2$ and crest vs 21 versus 22 mm$^2$).

This pattern could be related to different communities growing on different tiles. Glass tiles attracted more *Hydrolithon* spp., which produced the most expansive (but also thinner) spreading crusts (Table S9). *Hydrolithon* spp. also grew almost 5 times larger on glass tiles compared with any other material ($58 \pm 73$...
vs. 12 ± 2 mm²; $F = 5.713, p = 0.00159, \text{df} = 5$) and grew larger on horizontal tiles. In comparison, *Porolithon* spp., the most commonly occurring CCA crust, tended to be reasonably uniform in size (18 ± 2 mm², but up to 81 mm²) regardless of where it was growing.

Resemblance of tile communities to adult reef communities

*Porolithon* spp. was the most abundant CCA observed in the reef surveys (Fig. S2, Fig. 5), dominating communities on the reef crest (51% of CCA community), slope (29%) and back reef (40%). Survey abundances were comparable to tile observations (Fig. 6). *Hydrolithon* spp., the second most common species identified on tiles, were uncommon in surveys (6–8% of corallines) compared with on tiles (26–27%) in the fore reef. This was also true on the back reef (34% on tiles, 1% in surveys). *Lithophyllum* spp. were abundant in surveys (9–23%) but less common on tiles (1–3%). *Neogoniolithon* spp. were found in similar abundances (4–9% on tiles compared with 3–6% of tile communities), as were *Titanoderma*, which made up 0–1% of both tile and reef communities (Fig. 5).

None of the tiles gave a perfect approximation of community composition; however, on the deeper fore reef slope, vertically oriented PVC and limestone tiles were able to explain some of the observed reef data (35% and 37%, respectively, DISTLM models, $p < 0.05$). PVC tiles (horizontally oriented) and limestone (vertical) produced the closest community match on the back reef (22%, $p = 0.087$; 21% $p = 0.14$), and polycarbonate and PVC (horizontal 20% and 21%; vertical 25 and 21%, $p < 0.300$) and vertically oriented limestone tiles (23%, $p = 0.230$) on the fore reef. Analyses using Bray-Curtis coefficients as a similarity measure produced similar patterns: polycarbonate tiles best mirrored the fore reef crest communities, PVC and limestone on the reef slope, and PVC tiles and limestone on the back reef (Fig. 6). Glass tiles consistently gave the poorest approximation of real-world communities, except on back reef sites.

**DISCUSSION**

Artificial settlement plates are a simple and effective way to assess recruitment and growth of sessile benthic marine communities. As early as 1928, experiments using clamshells, drainpipes and logs to attract coral ‘settlers’ and monitor growth rates were being conducted on the Great Barrier Reef (Yonge 1930, Fig. 6. Multidimensional scaling (MDS) ordination of Bray-Curtis similarities between CCA communities recruiting to (A) 6 artificial tile materials, (B) tiles at 2 orientations and (C) across 3 reef habitats. The MDS is based on square-root transformed benthic cover (%) CCA community data. A 2D stress value of 0.17 indicates that the plot is a fair representation of multidimensional community similarity
McCalman 2014). Tiles have been successfully used in in situ CCA studies to address topics as diverse as assessing natural variability in recruitment and life history on coral reefs (Adey & Vassar 1975, Villas-Bôas et al. 2005), to explore the responses of communities under elevated CO2 conditions (Fabricius et al. 2011, Fabricius et al. 2015), and in studies specifically targeting calcification rates and the contribution of CCA to reef growth (e.g. Mallela 2013).

Our study set out to evaluate different settlement tile types for monitoring of CCA responses to environmental change. Our results suggest that the choice of settlement tile material, orientation and deployment location (habitat) directly affect different aspects of the individuals, populations and communities of CCA that develop on tiles in a Pacific tropical reef setting. Choice of habitat significantly affected all attributes measured. Settlement material dictated the make-up of the CCA community and influenced rates of calcification, but had little effect on the overall total percent CCA cover. Tile orientation affected community composition but had a minor influence on other variables. Limestone tiles produced lower calcification rates, while terracotta tiles amassed a disproportionate amount of weight and showed a large amount of variability in calcification estimates, making both materials unreliable for studies that set out to determine calcification rates. Plastic (PVC and polycarbonate) tiles gave the most consistent values for calcification and have an added advantage of being suitable for acid washing for more accurate determination of calcification.

Plastic and limestone tiles attracted communities that closest resembled adult coralline communities found on the surrounding reef. Glass tiles attracted large, thin Hydrolithon spp. crusts that were not representative of neighbouring real-world assemblages. Overall, and considering practicality and costs, PVC stood out as being affordable and lightweight, approximated diverse real-world communities well in both orientations, and was useful in calculating all 3 metrics—CCA abundance, calcification rate and community composition. Understanding how CCA differentially recruit to and grow on different artificial materials is important, particularly given the growing number of CCA studies as their important role in reef ecology and potential as a bioindicator for ocean acidification is realised.

CCA percent cover

The abundance of CCA found on experimental tiles in this study (27 ± 15%, mean ± SD) was comparable to the percent cover of mature crusts in our own benthic reef surveys (17 ± 8.1%, mean ± SD) and overlapped with other tile studies from the central Great Barrier Reef (10–40%; Diaz-Pulido & McCook 2004) and Papua New Guinea (23.1–43.3% cover at control sites; Fabricius et al. 2015). Strong effects of habitat (with CCA abundances lower in turbid back reef areas) and orientation (greater CCA abundance on upward facing tiles in the fore reef slope) were clear, but notably, total cover was unrelated to tile material. This contrasts with a previous study that suggests that some materials—such as PVC—actively encourage CCA settlement (Adey & Vassar 1975). It agrees with other studies that propose physical (e.g. hydrodynamics, irradiance) and/or biological processes (e.g. spore supply, competition, predation and grazing) might be stronger drivers of CCA cover than settlement material (e.g. Littler 1973). For example, Field et al. (2007) found no difference in CCA cover on unglazed terracotta and glazed (glass) brick, while Burt et al. (2009) similarly identified strong differences in benthic communities between experimental sites but not between sandstone, gabbro, terracotta, concrete and granite experimental substrata. The lack of a main effect of tile material or any interactions is encouraging for CCA monitoring studies, as it suggests percent cover estimates will be comparable across studies regardless of tile material choice.

Orientation of tiles proved significant in 2 of the reef habitats. Percent cover of CCA was higher on upward facing surfaces at deeper slope sites, but not on the shallow reef crest, suggesting an influence of light availability on successful recruitment (Fig. 3). This complements the findings of Mallela (2013), who reported greater total encruster cover on horizontally oriented (99% cover) fore reef slope experimental tiles (at 10 m), compared with vertically oriented tiles (81% cover), and who also hypothesised the important effect of irradiance on determining CCA cover, but did not examine shallower tiles. The increase in efficacy of horizontal tiles for recruiting CCA with depth could help explain why studies conducted in shallow waters suggest vertical tiles are better at recruiting coral spat (Tomascik 1991), while this effect is reversed with deeper deployments (Birkeland et al. 1981). CCA cover was greater on vertical tiles in the back reef, suggesting smothering by sediment could have inhibited growth on horizontally oriented tiles (Steneck 1997). A number of studies acknowledge the role that tile orientation has on recruitment, settlement and growth of benthic communities (Carleton & Sammarco 1987, Tomascik 1991, Petersen et al. 2005, Strader et al. 2015), but...
overall, the effect of orientation here was minor compared with habitat effects, supporting Mundy (2000), who showed that the effect of plate angle on coral spat recruitment, while detectable, was small.

Habitat was the strongest driver of CCA cover, agreeing with studies that demonstrate the importance of habitat on CCA abundance (Littler 1973, Steneck 1986, Figueiredo & Steneck 2000, Fabricius & De’ath 2001). Tiles placed on reef crest environments were found to attract greater CCA cover, both in this study and in studies focusing on CCA (Villas-Bôas et al. 2005) and coral recruitment (Harriott & Fisk 1987, Burt et al. 2009). Reduced CCA cover on the back reef sites could be explained by differences in propagule supply (there were fewer corallines on the back reef) and survivorship, sedimentation or grazing. Herbivory has been shown to positively affect CCA cover (Steneck 1983), and was greater in our fore reef habitats (Table S1, Fig. S3).

CCA calcification rates

Tile type was a key driver of CCA calcification rate, with terracotta tiles appearing to accrete more calcified mass, and limestone (and plastic cards) generating lower calcification rate estimates. Because percent cover of CCA did not differ between experimental materials, this suggests either that CCA crusts are growing thicker or denser on terracotta tiles, or possibly that the mass of the ceramic tile itself changed over the duration of the experiment. Terracotta (and other ceramics) can undergo a curing process underwater, where saltwater washing through pores in the tile causes secondary lithification. Rapid curing over the first 3 mo may explain the larger difference in buoyant weight seen between the 3 and 6 mo experiments in terracotta compared with other tiles (material x time interaction). Night-time dissolution of carbonates or micro-bioerosion might explain the lower calcification rates of the limestone tiles. Habitat was identified as another driver of CCA calcification (Fig. 4), with higher calcification rates recorded on the reef crest (0.42 ± 0.04 mg cm−2 d−1, n = 118) compared with fore reef slope and back reef sites, although the effect was not as dramatic as between tile types and was reduced with time (Fig. 4). This fits the general pattern of the higher abundances and productivity of CCA in areas of higher light availability and greater water motion (Adey 1978, 1998).

CCA calcification rates obtained in this study are similar to those reported in the literature. Kuffner et al. (2013) report CCA calcification rates of 0.13 mg cm−2 d−1 for the Florida Keys (540 g m−2 yr−1) on vertically oriented plastic tiles, which was slightly lower than our own data on vertically oriented plastic tiles (0.27 ± 0.03 mg cm−2 d−1, n = 27). Meanwhile, Mallela (2013) reported vertical CCA calcification rates of 56.3 g m−2 yr−1 (n = 30) compared with horizontal rates of 105.3 g m−2 yr−1 (n = 30) in Tobago, and horizontal rates of 128 and 159 g m−2 yr−1 in Jamaica (Mallela 2007). These were considerably higher than equivalent rates generated here (13.6 ± 0.95 for vertical and 15.1 ± 1.2 g m−2 yr−1 for horizontal tiles), but both Caribbean studies used ceramic tiles (terracotta tile values, present study: 35.7 ± 1.5 g m−2 yr−1, n = 91) over longer time periods (12 mo, compared with 3–6 mo in our study) and also reported significantly higher percent CCA cover.

Role of other calcifiers and grazing in estimates of CCA calcification rates

Our experimental design minimised the growth of calcifying organisms other than CCA, and consequently we found that the contribution of calcifiers to net tile calcification was negligible. Analyses of grazing pressure revealed that PVC and polycarbonate tiles experienced more grazing than other materials, particularly on the fore reef, where grazing pressure tends to be higher (Table S1, Fig. S3). This could be a consequence of herbivore preference (perhaps due to differential turf communities, or comparative softness of the material), or of grazing scars being more visible on plastic (a reason given for selecting plastic tiles in a study targeting the relationship between algae settlement and grazing; Hixon & Brostoff 1996). At fore reef sites, herbivory was greater on horizontal tiles (Fig. S3), indicating that horizontal orientations may be more useful for capturing herbivory processes, while vertically oriented tiles may underestimate them.

CCA community composition

Tile material was important in determining CCA assemblage. Glass tiles attracted large, thin Hydro lithon spp., having the largest dissimilarity compared with the adjacent reef community (Fig. 6A, pink triangle). No tile provided a perfect approximation of adult communities, but some—glass, and terracotta when vertically oriented—were significantly poorer than others. Plastic (both PVC and polycarbonate) and limestone produced the closest match to reef.
CCA assemblages. While we might have expected limestone tiles to provide a good approximation of communities growing on the reef (because it simulates natural reef benthos), it was interesting to note that both plastic tile types frequently gave as good or better approximations of the natural reef community.

Orientation was also important, with vertically oriented tiles providing more reliable estimates of the naturally occurring reef community than vertical tiles on the fore reef as well as hosting more diverse communities (Fig. 6B). The effect of orientation could be tile specific: in each habitat limestone tiles were significantly better at explaining the community composition when they were vertically oriented, while plastic tiles tended to be just as effective in both orientations.

Habitat was also important, with community composition in this study varying between fore reef and back reef habitats (Fig. 6C). This supports work by Dean et al. (2015) that showed marked differences in CCA assemblages between Great Barrier Reef reef slope, crest and back reef habitats. Discrepancies between adult reef communities and the CCA assemblages growing on the tiles could reflect differences in settlement, survivorship or succession.

Other considerations

Time

A 3 mo period was sufficient to generate a calcification rate estimate comparable with other published estimates (Mallela 2007, Kuffner et al. 2013, Mallela 2013). This time frame agrees with the results of Fabricius et al. (2015), who observed similar CCA cover at control sites after 5 mo (38%) and 13 mo (29.6%), and also reported a slight decline in cover following initial establishment. However, tiles deployed for 3 mo would have experienced slightly warmer temperatures (24.9 ± 1.2°C, compared with an average of 23.2 ± 2.1°C for the 6 mo exposure), possibly explaining the observed decline in calcification (although see work by Kuffner et al. 2013 that shows no effect of season on Caribbean CCA growth). Caribbean studies suggest that CCA peaks in abundance between 100 d (Adey & Vassar 1975) and 1 yr (Arnold & Steneck 2011), which might explain higher reported percent covers in Caribbean studies compared to the present study (e.g. Mallela 2007, Mallela 2013). With the majority of monitoring programs occurring annually or biannually, further work would be needed to confirm that rates reported here were not confounded by season.

Practicality

Design of any experiment necessarily involves some practical considerations (Table 1). Glass tiles had a smoother surface, from which CCA detached more easily, something previously noted by Adey & Vassar (1975). Limestone and terracotta tiles were heavy when wet and more awkward to transport and store than the lightweight plastic tiles (e.g. polycarbonate tiles 12.5 ± 0.3 g, terracotta 58.6 ± 0.3 g). We also found that both ceramic tiles had to be thicker to prevent smashing when drilling attachment holes. PVC tiles were less variable in weight and size (16.9 ± 0.04 g), limestone most variable (39.9 ± 1.15 g), creating more consistent experimental substrata, as well as being the easiest to cut, drill and wire into place (Adey & Vassar 1975), and lightweight for storage for long-term archiving. All tile types could be successfully recycled and reused with careful washing, but chemically inert plastics were useful for acid washing for calcification estimates (although it should be noted that there is significant value in using less destructive techniques so that tiles can be archived for future work). Recent work suggests that ceramic tiles may alter the surrounding water chemistry—with some ceramic oven-baked tiles altering seawater concentrations of lithium (104 times higher) and certain heavy metals under experimental conditions, while others were associated with increased calcium and a decrease in pH and alkalinity (Petersen et al. 2005).

CONCLUSIONS

The choice of experimental set-up and settlement plate material will depend on the research questions being addressed, but results presented here may provide guidance, especially for researchers studying coralline algae in tropical coral reef environments (Table 1). For example, a study looking to understand net calcification rates accounting for material removed by grazing may want to orientate tiles horizontally to maximise grazing pressure. Depending on the study site, horizontal tiles may maximise coralline cover—particularly in light-limited environments (>5 m), while vertically oriented tiles may encourage growth of more diverse communities as well as growth of a small number of calcifiers. Studies looking to obtain estimates of CCA community composition representative of the surrounding reef community should look to avoid glass and porcelain tiles. While limestone tiles, often selected for
Table 1. Evaluation of different tile types and orientations. ✓: factor maximized and/or well-represented by tile type in this study; x: tile unsuitable; ns: non-significant; na: not applicable

<table>
<thead>
<tr>
<th>Experimental tile type</th>
<th>Abundance of CCA (% cover)</th>
<th>Species richness</th>
<th>CCA community Resemblance to reef biota</th>
<th>Herbivory (% tile grazed)</th>
<th>Calcification estimates</th>
<th>Other calcifying organisms</th>
<th>Suitability for buoyant weight dissolution techniques</th>
<th>Suitability for dissolution techniques</th>
<th>Ease of use</th>
<th>Average cost/tile (AUD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
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</tr>
<tr>
<td>Polycarbonate</td>
<td>ns (27%)</td>
<td>Average</td>
<td>Good match</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Lightest tile (3 g cm⁻²) but more expensive than PVC</td>
<td>$1.17</td>
</tr>
<tr>
<td>PVC</td>
<td>ns (26%)</td>
<td>✓ More speciose</td>
<td>Good match</td>
<td>✓</td>
<td>✓ More grazed (7%)</td>
<td>✓ Fair</td>
<td>✓</td>
<td>✓ Ideal</td>
<td>Lightweight (4 g cm⁻²), performs well under microscope</td>
<td>Cheapest ($0.92)</td>
</tr>
<tr>
<td>Terracotta</td>
<td>ns (34%)</td>
<td>✓ More speciose</td>
<td>Average (dominated by Porolithon)</td>
<td>✓ More grazed (6%)</td>
<td>✓ Fair</td>
<td>✓ Attract more Peyssonnelia</td>
<td>✓ Ideal</td>
<td>Ideal</td>
<td>Heavy to transport (13 cm⁻²) and store</td>
<td>$1.15</td>
</tr>
<tr>
<td>Limestone</td>
<td>ns (24%)</td>
<td>✓ More speciose</td>
<td>Average (2%)</td>
<td>✓ High and variable</td>
<td>✓ Low estimates</td>
<td>✓ Attract more Peyssonnelia</td>
<td>✓ Unsuitable</td>
<td>Unsuitable</td>
<td>Heavy to transport (9 cm⁻²)</td>
<td>$2.90</td>
</tr>
<tr>
<td>Glass</td>
<td>ns (24%)</td>
<td>X Fewer</td>
<td>X Thin, large Hydroolithons</td>
<td>X Poor match</td>
<td>✓ Fair</td>
<td>✓ Attract less Peyssonnelia</td>
<td>✓ Ideal</td>
<td>Ideal</td>
<td>Medium weight (7 g cm⁻²)</td>
<td>$0.95</td>
</tr>
<tr>
<td>Porcelain</td>
<td>ns (24%)</td>
<td>Average</td>
<td>Average (4%)</td>
<td>✓ Fair</td>
<td>✓ Average</td>
<td>✓ Unsuitable</td>
<td>Unsuitable</td>
<td>Unsuitable</td>
<td>Medium weight (6 g cm⁻²); made ID difficult</td>
<td>$3.15</td>
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<tr>
<td>Orientation</td>
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<tr>
<td>Horizontally oriented</td>
<td>✓ Interaction</td>
<td>× Less</td>
<td>Interaction larger crusts (depends on material)</td>
<td>Average (more heavily grazed)</td>
<td>ns</td>
<td>✓ Fewer</td>
<td>na</td>
<td>na</td>
<td>na</td>
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<td></td>
<td>× Declines on the back reef</td>
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<tr>
<td>Vertically oriented</td>
<td>× Interaction</td>
<td></td>
<td>Interaction larger crusts on reef</td>
<td>Average (depends on material)</td>
<td>ns</td>
<td>× Attract more Peyssonnelia</td>
<td>na</td>
<td>na</td>
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<td></td>
<td>× Declines with depth</td>
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<td>Habitat</td>
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</tr>
<tr>
<td>Deployed on fore reef</td>
<td>✓ Higher cover (34%)</td>
<td>✓ More speciose</td>
<td>Average na</td>
<td>(more heavily grazed on horizontal tiles)</td>
<td>✓ Higher rates</td>
<td>× Attract more Peyssonnelia</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Deployed on back reef</td>
<td>X Lower cover (12%)</td>
<td>X Less speciose</td>
<td>X Dominated by Hydrolithon spp.</td>
<td>Average (marginally less heavily grazed)</td>
<td>X Lower rates</td>
<td>✓ Attract fewer calcifiers</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>
their similarity to real reef substrata, approximated real communities well, they were no better than plastic tiles and did not function as well when oriented horizontally. Terracotta tiles, widely adopted because of their microstructure and similarity to real reef substrata, did not approximate community composition or abundance any better than plastic tiles. They were also found (along with limestone tiles) to be unsuitable for exploring calcification rates—either using buoyant weight techniques (because of inconsistent results and curing) or dry weight/acid wash techniques—due to their solubility. In terms of practicality and costs, PVC stood out as being affordable and lightweight, approximated real-world communities well in both orientations, and was suitable for calculating all 3 metrics—CCA abundance, calcification rate and community composition.

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