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Contribution to the Theme Section 'The ecology of temperate reefs in a changing world'

Little evidence that lowering the pH of concrete supports greater biodiversity on tropical and temperate seawalls

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ABSTRACT: Concrete is one of the most commonly used materials in the construction of coastal and marine infrastructure despite the well known environmental impacts which include a high carbon footprint and high alkalinity (~pH 13). There is an ongoing discussion regarding the potential positive effects of lowered concrete pH on benthic biodiversity, but this has not been investigated rigorously. Here, we designed a manipulative field experiment to test whether carbonated (lowered pH) concrete substrates support greater species richness and abundance, and/or alter community composition, in both temperate and tropical intertidal habitats. We constructed 192 experimental concrete tiles, half of which were carbonated to a lower surface pH of 7–8 (vs. control pH of >9), and affixed them to seawalls in the United Kingdom and Singapore. There were 2 sites per country, and 6 replicate tiles of each treatment were collected at 4 time points over a year. Overall, we found no significant effect of lowered pH on the abundance, richness, or community assemblage in both countries. Separate site- and month-specific generalised linear models (GLMs) showed only sporadic effects: i.e. lowered pH tiles had a small positive effect on early benthic colonisation in the tropics but this was later succeeded by similar species assemblages regardless of treatment. Thus, while it is worth considering the modification of concrete from an environmental/emissions standpoint, lowered pH may not be a suitable technique for enhancing biodiversity in the marine built environment.

KEY WORDS: Coastal defences · Concrete carbonation · Eco-engineering · Materials · pH · Biodiversity · Concrete

1. INTRODUCTION

Coastal marine ecosystems have experienced dramatic changes during the last century, often driven by urbanisation and exemplified by the proliferation of man-made structures such as seawalls, breakwaters, and groynes (Heery et al. 2017, Todd et al. 2019). In major coastal cities, including Sydney, Hong Kong, and Singapore, these artificial structures can comprise over 50% of shorelines (Chapman & Bulleri 2003, Lam et al. 2009, Lai et al. 2015). Designed to prevent erosion and provide flood protection (Loke et al. 2019a), sea defences are likely to become more prevalent with growing coastal populations, rising sea levels

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and increasing storm frequencies (Nicholls et al. 2007, Temmerman et al. 2013). Concomitantly, there has been growing research interest in the ecological functioning of these man-made structures (Bulleri & Chapman 2010, Dafforn et al. 2015, Firth et al. 2016a). However, compared to natural rocky shores, artificial structures tend to support lower species diversity and/or abundances (e.g. Moschella et al. 2005, Lai et al. 2018), different ecological communities (e.g. Chapman & Bulleri 2003, Lam et al. 2009), and higher numbers of non-native species and/or homogenised species assemblages (e.g. Bulleri & Airoldi 2005, Glasby et al. 2007).

Concrete, a composite material comprising Portland cement, water, and a mixture of coarse and fine aggregates, is one of the most commonly used building materials in coastal and marine infrastructure (Dugan et al. 2011). While the physical characteristics of concrete (e.g. durability, strength, and workability) have made it a ubiquitous component of the modern built environment (Dyer 2014), the production process of concrete has a high carbon footprint (Waters & Zalasiewicz 2018). It has also been suggested that concrete has a negative effect on the recruitment of marine biota due to its high surface alkalinity (pH ~13) (Lukens & Selberg 2004, Perkol-Finkel & Sella 2014), reducing initial rates of species colonisation (Nandakumar et al. 2003) and favouring alkotolerant taxa such as barnacles and serpulids over algae (Hatcher 1998, Dooley et al. 1999). This high surface alkalinity potentially compounds the known negative effects of hard coastal defences on intertidal organisms such as the loss of habitat area (Lai et al. 2015), compression of the intertidal zone due to steep gradients (Firth et al. 2014, Loke et al. 2019b), low structural complexity (Chapman & Bulleri 2003, Moreira et al. 2007), and higher risk of desiccation (Tan et al. 2018, Zhao et al. 2019) and temperature stress (Aguilera et al. 2019). With such changes in material and physical structure, seawalls have been considered sub-optimal intertidal habitats and there is a general consensus that the expansion of hard coastal defences at a global scale presents a huge threat to coastal and marine biodiversity (Bishop et al. 2017, Heery et al. 2017).

In response to these threats, ecological engineering — the integration between engineering principles and maximised ecological value — has been increasingly adopted in the marine environment (Chapman et al. 2018, Strain et al. 2018). The aim is to alleviate the negative impacts associated with artificial structures and to increase their ecological functioning (Morris et al. 2019). In particular, 'hard' engineering, the physical modification of existing seawalls or use of habitat enhancement units (Chapman & Underwood 2011), has been experimented with in several countries, both temperate and tropical (Dafforn et al. 2015, Firth et al. 2016b, Loke et al. 2019c). However, ecological engineering techniques applied to seawalls have generally targeted the physical (topographical) differences between natural rocky shores and artificial structures. Therefore, habitat enhancement units tend to focus on manipulating the surface complexity of substrates to incorporate water-retaining features and/or increase structural complexity, via the creation of cavities and the retrofitting of tiles with varying surface topography (Firth et al. 2013, 2014, Loke et al. 2017, Strain et al. 2018). Nevertheless, even with ecological engineering efforts, concrete is often used, as it fulfils industry building and construction safety standards and is easily moulded into various shapes and designs (Waltham & Dafforn 2018).

Some studies have suggested that the material of habitat enhancement units should also be manipulated to increase their ecological benefits (Dennis et al. 2018). Partial replacement of cement or coarse aggregates with more environmentally friendly materials, such as granulated blast-furnace slag and pulverised fly ash, has been shown to improve the live cover of benthic organisms on concrete substrates (Dennis et al. 2018, McManus et al. 2018). Altering the concrete matrices has also resulted in higher live cover and primary productivity of pre-fabricated habitat units (Perkol-Finkel & Sella 2014, Sella & Perkol-Finkel 2015). In addition to enhancing species diversity, using natural materials in concrete can reduce its environmental footprint (Dennis et al. 2018). Many of these studies postulated that the reduced pH from such modifications may be beneficial for biotic recruitment (Perkol-Finkel & Sella 2014, Sella & Perkol-Finkel 2015, McManus et al. 2018). pH can influence the colonisation of algae and barnacles at early stages (Guilbeau et al. 2003), which can, in turn, result in different succession patterns (Almeida & Vasconcelos 2015). With contrasting effects of pH on different taxa (Guilbeau et al. 2003), regions with different benthic community assemblages could also be influenced to varying degrees. One of the major criticisms of ecological engineering is that examples are often limited in their scope, and a recent review by Firth et al. (2020) suggested revisiting, repeating, and expanding on experiments to test responses over broader spatio-temporal scales to improve the evidence base (Evans et al. 2019).

One straightforward technique for lowering concrete pH for experimental work is through concrete carbonation. Carbonating concrete *ex situ*, also known

as accelerated carbonation, has traditionally been used to simulate the carbonation process that occurs naturally when concrete is exposed to air (De Ceukelaire & Van Nieuwenburg 1993). This is often performed to test for the effects of long-term carbonation on the metal leaching abilities (Bin-Shafique et al. 1998), compressive strength (De Ceukelaire & Van Nieuwenburg 1993, Chi et al. 2002), and durability (Roy et al. 1999) of concrete, as carbonation can alter its physical properties by densifying the concrete surface (Chi et al. 2002, Fernández Bertos et al. 2004). However, to our knowledge, no previous studies have tested the effects of this approach on benthic diversity and composition.

Whether changes in concrete pH alone (i.e. while keeping structure texture and composition constant) affect the overall species recruitment on habitat enhancement units is unknown. To determine this, we fabricated topographically complex concrete tiles and carbonated half of them to obtain lower surface alkalinity, from here on referred to as 'carbonated tiles'. Given that successional patterns and processes driving intertidal systems can differ with latitude (Sousa et al. 1981), the experiment was conducted in a temperate country (United Kingdom) and a tropical country (Singapore). Specifically, we tested the following hypotheses: (1) carbonated tiles will support higher macrofaunal abundance and species richness than standard non-carbonated tiles, and (2) carbonated tiles will support different biological communities from standard non-carbonated tiles, and these differences will be consistent across time and sites with different community assemblages.

2. MATERIALS AND METHODS

2.1. Tile design and fabrication

A total of 192 experimental tiles were constructed for this study using a single tile design. The face of each tile measured 14 × 10 cm (Fig. 1) and had a smooth and pitted façade (on the left and right hand side, respectively). The smooth surface was designed for photographic analysis of epibenthic percentage cover, while the pitted side was designed to create water-retaining features that would act as refugia for colonising macrofauna (Loke & Todd 2016); this was achieved using the software CASU (Loke et al. 2014). After measuring the angle of seawalls at the chosen study sites, we then adapted all tiles so that the resultant slope of the front-facing façade after installation was standardised at 60° (Fig. 1C,D).

Masters of the tiles were created following Loke & Todd's (2016) protocol, using silicone rubber moulds (Freeman Bluesil™ V-340). Tiles were then cast from the moulds using ratios of 1:3 cement:aggregate and 3:5 water:cement. Pre-drilled

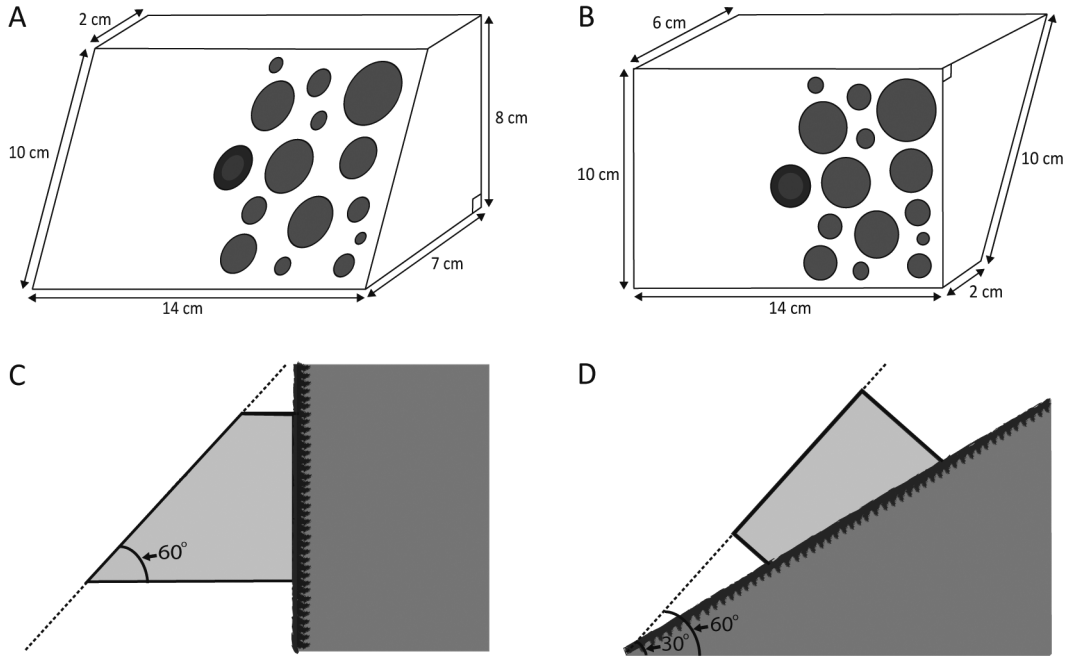

Fig. 1. Dimensions of tiles for (A) vertical and (B) sloping seawalls, with schematics of the tiles when installed on the seawalls (C and D, respectively)

holes were set in the centre of the concrete tiles for installation on seawalls.

2.2. Tile carbonation

Carbonation is often performed by diffusing high concentrations of carbon dioxide into a sealed chamber containing the concrete (Chang & Chen 2006). Carbon dioxide reacts with calcium hydroxide and calcium-silicate-hydrate in concrete to form calcium carbonate and water, reducing the alkaline content in the tiles and lowering its pH (Fernández Bertos et al. 2004). In this experiment, a chamber was created using a large cooler box and dry ice, thus incubating the concrete tiles in quasi-saturated CO_2 at atmospheric pressure and room temperature. This method is readily implemented, without requiring specialized equipment for supercritical and high-pressure CO_2 (Venhuis & Reardon 2003).

Trials were conducted using concrete coupons (5 × 5 × 2 cm) to determine the best carbonation conditions (wet or dry), and the duration of curing (2, 6, 12, 20 d) and carbonation (7, 22, 29 d) required to reduce the pH of the tiles. Concrete coupons were cut in half using a tile saw, and the surface and cross section of the cut tiles were stained with 2 pH indicator dyes: (1) phenolphthalein and (2) bromothymol blue to test the effectiveness of carbonation. Phenolphthalein, a pH indicator which transitions from colourless to light pink around pH 8, becoming a dark pink when the pH value exceeds 9, is typically used to assess the extent of carbonation in concrete (Fig. 2B; Chang & Chen 2006, Thiery et al. 2007). Bromothymol blue, which is less commonly used to test concrete pH, transitions from yellow to light blue from pH 6 to 7, becoming dark blue for pH values above 8 (Guilbeau et al. 2003). When the stained carbonated tiles were colourless (phenolphthalein) and light blue (bromothymol blue), it indicated that the external front-facing surface of the carbonated tiles had a pH estimated to be between 7 and 8 (Fig. 2A).

After several trials were conducted, it was found that the tiles were more rapidly carbonated when dry as opposed to wet, and when they were left to cure for longer before being exposed to CO_2. Carbonation duration (>28 d), however, was the most important variable for achieving a pH of less than 8 (Fig. 2A). A subsample of the final batch of tiles was assessed using the indicator dyes, which showed that the surface of the carbonated concrete tiles was no more than pH 8.

Attempts were also made to quantify the pH of the concrete tiles by measuring concrete powder using a pH meter, but there has been a longstanding lack of a standardised protocol for measuring the pH of pore fluid in concrete (Alonso et al. 2012). Additionally, quantifying pH using concrete powder would be dependent on the point location of the sample and would not give a representative measurement of surface pH. Therefore, this approach was only used to confirm the differences in internal pH between treatments at the 6 mo time point (see Fig. S1 & Table S1 in the Supplement at www.int-res.com/articles/suppl/m656p193_supp.pdf). All tiles were prepared in Singapore, and half of them were subsequently sent to the UK.

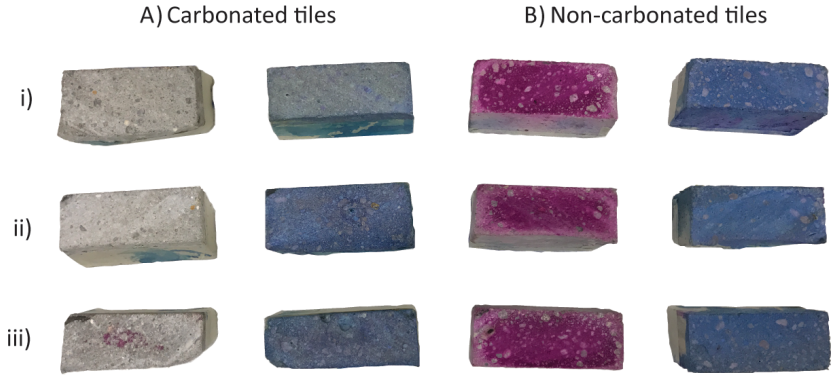

Fig. 2. Images of (A) carbonated tiles stained with phenolphthalein (left) and bromothymol blue (right) after undergoing: (i) 29 d of carbonation and 12 d of drying, (ii) 22 d of carbonation and 20 d of drying, and (iii) 22 d of carbonation and 6 d of drying, with (B) non-carbonated tiles that had been dried for the same amount of time (control) stained with phenolphthalein (left) and bromothymol blue (right)

2.3. Study sites

Tiles were deployed in 2 locations, 1 in a temperate and 1 in a tropical climate, with 2 seawall sites at each location. Plymouth (UK) was chosen as the temperate location and Singapore as the tropical location.

2.3.1. Plymouth, UK

Plymouth is a port city located on the south-west coast of England, UK, where the English Channel broadens into the Atlantic Ocean. Within Plymouth Sound, 33% of the coastline is artificial (mostly seawalls) (Knights et al. 2016). The tiles were installed in

Plymouth in February 2018 onto 2 vertical seawalls at (1) Turnchapel (50.359° N, 4.1178° W) and (2) Cremyll (50.3648° N, 4.1633° W).

2.3.2. Singapore

Singapore is a tropical city-state located just over 1° north of the Equator, separated from Peninsular Malaysia by the Straits of Johor in the north and from Indonesia by the Singapore Strait in the south. Over 63% of Singapore's coastline is made up of seawalls (Lai et al. 2015). In Singapore, tiles were carbonated from January to February 2018 and were installed in late February and early March 2018 at 2 southern islands: (1) grouted granite rip-rap seawall at Pulau Hantu (1.22611° N, 103.75222° E) and (2) vertical seawall at Pulau Seringat (1.23° N, 103.85056° E).

2.4. Field experimental design, sampling, and laboratory procedures

At each site, 24 tiles from each tile treatment (carbonated and non-carbonated) were installed along seawalls at mid-shore height, approximately 1.5 m above chart datum, and spaced at least 0.5 m apart. Six replicates of carbonated and non-carbonated tiles were removed randomly at 3, 6, 9, and 12 mo. However, due to unforeseen temporary restricted access to Pulau Hantu, collection for the 9 mo time point could not be carried out, hence we included a 15 mo time point instead for that site.

Prior to removal of the tiles, fast-moving organisms were picked and placed into self-sealing plastic bags. The tiles were then photographed (for subsequent algal cover analysis) before being removed from the seawall and placed into larger self-sealing plastic bags. Algal cover was quantified using CPCe image analysis software (Kohler & Gill 2006), with percentage cover tabulated from 40 random point intercepts on the smooth surface of the tile. Four common functional groups were used to categorise the algae composition in both countries following Loke et al. (2016) (Table 1).

After algal removal from the smooth surface, the tiles were placed into the freezer (−20°C) for subsequent sorting, counting, and identification using a dissecting microscope. All specimens were identified to species or morphospecies level except for polychaetes, which were identified to family level (Loke & Todd 2016, Loke et al. 2017, 2019a).

2.5. Statistical analysis

As tiles were lost due to wave action, there was an unequal number of replicates for some sites and treatments (Table S2), but there were at least 4 replicates per treatment per site per time point. Data were first examined for the presence of outliers, heterogeneity, non-normality, and overdispersion (Zuur et al. 2010). We then tested for differences in total abundance and species richness using generalised linear models (GLMs). Models with Poisson error were first constructed separately for the 2 countries with treatment, site, and month (categorical) as fixed effects, but models with negative binomial error were subsequently used to analyse abundance due to overdispersed data.

After assessing generalisable patterns in the response to treatment via GLMs, we examined site- and month-specific treatment responses in finer detail in separate models of abundance and richness for each site and month, with treatment as the sole predictor (overarching interaction terms from the general models are also provided in Table S3). Site- and month-specific models of richness tended to be underdispersed, and were therefore fit with Conway-Maxwell-Poisson (COM-Poisson) regressions (Sellers & Shmueli 2010). Negative binomial error structure was maintained for site- and month-specific models of abundance. All univariate tests were performed in R v3.6.0 (R Core Team 2019). COM-Poisson models

Table 1. Functional categories used for classifying algae in this study, adapted from Loke et al. (2016). Examples of taxa and species are from Singapore

Functional group	Dominant component taxa
Microalgae/biofilm	Unidentified cyanobacteria and diatoms, bare surfaces were also classified in this group due to difficulty in differentiating visually
Encrusting algae	Ralfsiaceae and/or Neoralfsiaceae
Ephemeral green turfs	*Ulva* spp.
Red/brown turfs	*Parviphycus antipae*, *Gelidiopsis variabilis*, *Dictyota* spp., and Ceramiales

were constructed and evaluated using the 'COMPoissonReg' package (Sellers et al. 2017), while negative binomial regression was performed using the 'glm.nb' function in the 'MASS' package (Venables & Ripley 2002).

We used permutational distance-based multivariate analysis of variance (PERMANOVA; Anderson 2001) to test for differences in community composition between treatments (we removed 15th month data as they were unreplicated in time; please see Section 2.4. for more information). As there was no overlap in species identities between the 2 countries, analyses were conducted separately for temperate and tropical systems. The abundances were $\log(x+1)$ transformed and the full resemblance matrix was calculated on Bray-Curtis similarities and p-values were generated using 9999 unrestricted random permutations of residuals. PERMANOVA revealed significant differences in community composition among months, but did not reveal significant differences among treatments. All multivariate analyses were performed using PRIMER v7 with the PERMANOVA add-on (Anderson et al. 2008).

Table 2. Total number of species and unique species found on each tile treatment at each site across all time points

Sites	Total number of species		Total number of unique species	
	Carbonated	Non-carbonated	Carbonated	Non-carbonated
Cremyll	11	8	4	1
Turnchapel	8	7	2	1
Pulau Hantu	19	21	4	6
Pulau Seringat	41	41	5	5

3. RESULTS

3.1. Abundance and species richness

A total of 78 114 individuals of 68 species/morphospecies were collected and identified from experimental tiles across both countries. Of these, 13 were temperate species from Plymouth and 55 were tropical species from Singapore. Although there were more unique species found on carbonated tiles than non-carbonated tiles at both sites in Plymouth, this was not observed in Singapore (Table 2; further details in Tables S4 & S5 in the Supplement). Additionally, all species found in both countries were native, with the exception of the non-native barnacle *Austrominius modestus* in Plymouth and the non-native limpet *Siphonaria guamensis* in Singapore (Gallagher et al. 2015, Tan et al. 2018), both of which were found on both treatments at both sites in their respective countries.

GLMs showed a significant effect of month on abundance and species richness in both Plymouth and Singapore. There was also a significant effect of site on abundance and species richness in Singapore (Table 3), with lower rates of colonisation at Pulau Hantu (Fig. 3). There was, however, no significant effect of treatment in either country (Tables 3 & S6).

Site- and month-specific GLMs revealed that there were significant effects of carbonation at some months and sites, but they were not ubiquitous and none occurred in the final 12 mo time point (Table 4; further details in Tables S7 & S8). Carbonated tiles had greater total abundance than non-carbonated tiles at Cremyll at the 9 mo time point, and at Pulau Hantu at the 6 mo time point (Table 4). In Singapore,

Table 3. Analysis of deviance results for negative binomial (Neg. Bin.) and Poisson GLMs for total abundance and species richness in Plymouth, UK, and Singapore. Significant p-values ($p < 0.05$), as determined by likelihood ratio tests, are shown in **bold**

Source	Plymouth					Singapore				
	df	Dev	Res df	Res Dev	p	df	Dev	Res df	Res Dev	p
Abundance (Neg. Bin. GLM)										
Model			94	164.5				88	448.1	
Site	1	0.9	93	163.6	0.3388	1	253.9	87	194.2	**<0.0001**
Treatment	1	0.9	92	162.7	0.3411	1	2.5	86	191.6	0.1107
Month	1	48.7	91	114.0	**<0.0001**	1	83.0	85	108.6	**<0.0001**
Richness (Poisson GLM)										
Model			94	50.8				88	350.9	
Site	1	0.1	93	50.8	0.8150	1	211.3	87	169.7	**<0.0001**
Treatment	1	0.4	92	50.3	0.5478	1	2.7	86	167.0	0.1008
Month	1	19.1	91	31.2	**<0.0001**	1	117.6	85	49.7	**<0.0001**

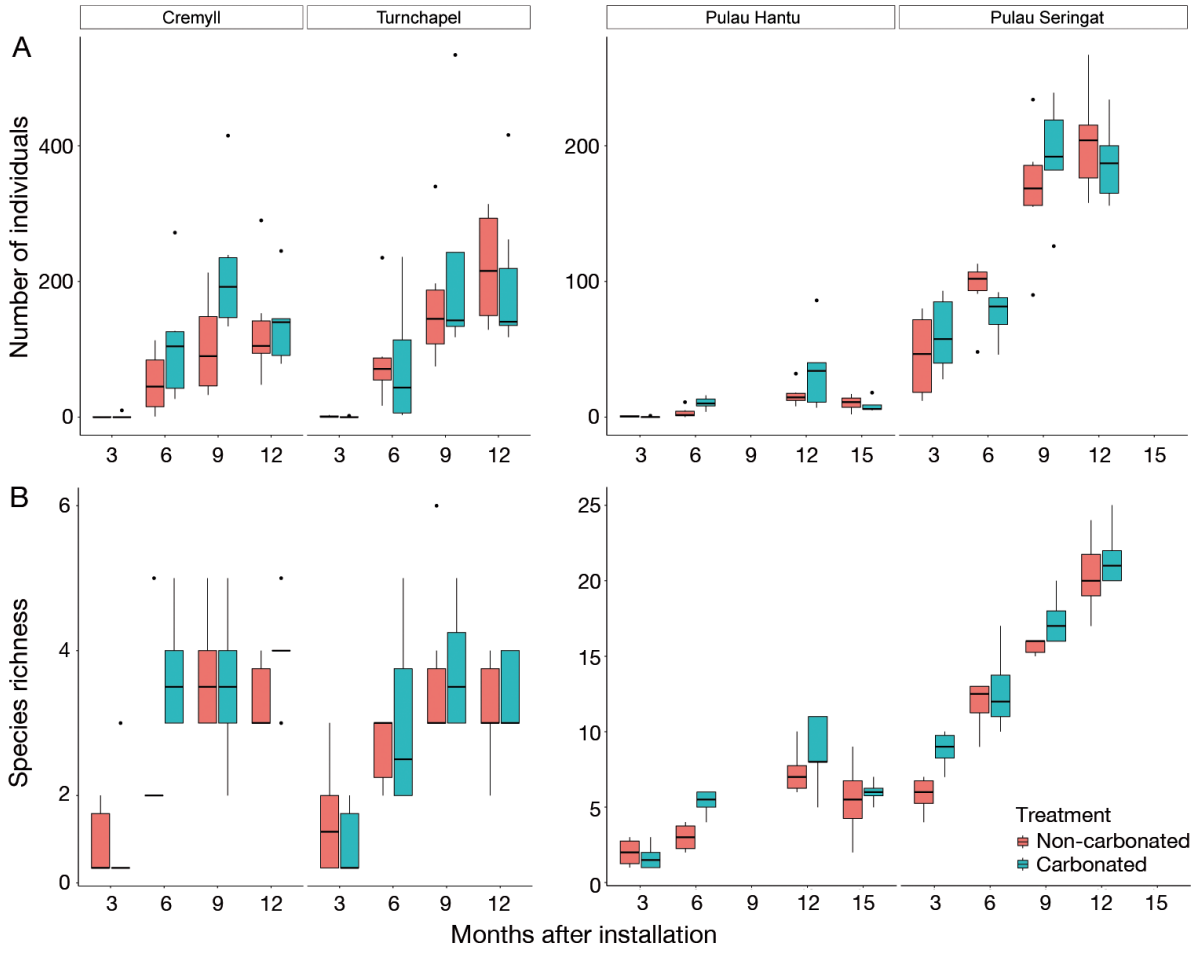

Fig. 3. (A) Abundance (number of individuals) and (B) species richness on tile treatments (non-carbonated and carbonated) across 4 time points (3, 6, 9, 12 mo for Cremyll, Turnchapel, and Pulau Seringat; 3, 6, 12, 15 mo for Pulau Hantu). Boxplot middle lines indicate the median; hinges indicate 75 and 25% quantiles (top and bottom, respectively); whiskers indicate highest and lowest values within 1.5 times the interquartile range from top and bottom hinges, respectively; dots indicate outliers

species richness was greater on carbonated tiles than non-carbonated tiles at the 3 mo time point at Pulau Seringat, and at the 6 mo time point at Pulau Hantu. There were no other significant effects of carbonation detected from site- and month-specific GLMs.

3.2. Community composition

PERMANOVA revealed significant differences in colonising assemblages among months (Plymouth: SS = 124360, Pseudo-$F_{3,70}$ = 39.06, p < 0.0001; Singapore: SS = 38734, Pseudo-$F_{3,67}$ = 8.6198, p <

Table 4. Results from site- and month-specific GLMs for total abundance and species richness. Models for abundance used a negative binomial (Neg. Bin.) error distribution, while Conway-Maxwell-Poisson error was used in models for richness. All contained treatment as the sole predictor. –: no difference between pH treatments; C > NC: carbonated tile treatments had higher abundance or species richness than the non-carbonated pH treatment; na: not available. Complete coefficient summaries from each model are provided in the Supplement

Country	Site	3-month	6-month	9-month	12-month	15-month
Abundance (Neg. Bin. GLM)						
Plymouth, UK	Cremyll	–	–	C > NC	–	na
	Turnchapel	–	–	–	–	na
Singapore	P. Hantu	–	C > NC	na	–	–
	P. Seringat	–	–	–	–	na
Richness (Conway-Maxwell-Poisson GLM)						
Plymouth, UK	Cremyll	–	–	–	–	na
	Turnchapel	–	–	–	–	na
Singapore	P. Hantu	–	C > NC	na	–	–
	P. Seringat	C > NC	–	–	–	na

Table 5. Permutational distance-based multivariate analysis of variance (PERMANOVA) results based on Bray-Curtis dissimilarities of the relative abundances (log-transformed) of 13 and 55 (Plymouth, UK, and Singapore, respectively) taxa in response to site, pH treatment, and duration since deployment as fixed factors and their interactions. Significant p-values (p < 0.05) are shown in **bold**

Source	df	SS	Pseudo-*F*	p(perm)	Unique perms
Plymouth					
Site	1	3309.5	3.12	**0.0379**	9942
Treatment	1	1343.4	1.27	0.2568	9940
Month	3	124 360.0	39.06	**< 0.0001**	9914
Site × Treatment	1	1944.1	1.83	0.1297	9941
Site × Month	3	3828.2	1.20	0.2841	9938
Treatment × Month	3	4979.9	1.56	0.1303	9936
Site × Treatment × Month	3	1703.4	0.54	0.8541	9951
Residual	70	83 844			
Singapore					
Site	1	60 739.0	40.55	**< 0.0001**	9949
Treatment	1	1748.7	1.17	0.2903	9930
Month	3	38 734.0	8.62	**< 0.0001**	9910
Site × Treatment	1	519.0	0.35	0.9628	9936
Site × Month	2	27 654.0	9.23	**< 0.0001**	9927
Treatment × Month	3	5119.7	1.14	0.2892	9904
Site × Treatment × Month	2	5327.8	1.78	**0.0454**	9904
Residual	67	100 360			

0.0001; Table 5) and sites (Plymouth: SS = 3309.5, Pseudo-$F_{1,70}$ = 3.1183, p < 0.05; Singapore: SS = 60739, Pseudo-$F_{1,67}$ = 40.55, p < 0.0001; Table 5), but none between treatments, regardless of country or month (Table 5). Despite significant results for the interaction term (site × treatment × month) in Singapore, no significant differences were detected when pair-wise comparisons were conducted between treatments within sites and months.

In Plymouth, the non-native barnacle *A. modestus* dominated the surfaces of all tiles (Fig. 4). Despite having higher percentage cover on carbonated tiles than non-carbonated tiles at the 3 mo time point, there was no observed difference at the final 12 mo time point. In Singapore, biofilm which dominated at the 3 and 6 mo time points was succeeded by barnacles and encrusting algae by the 9 mo time point (Fig. 4). However, mean

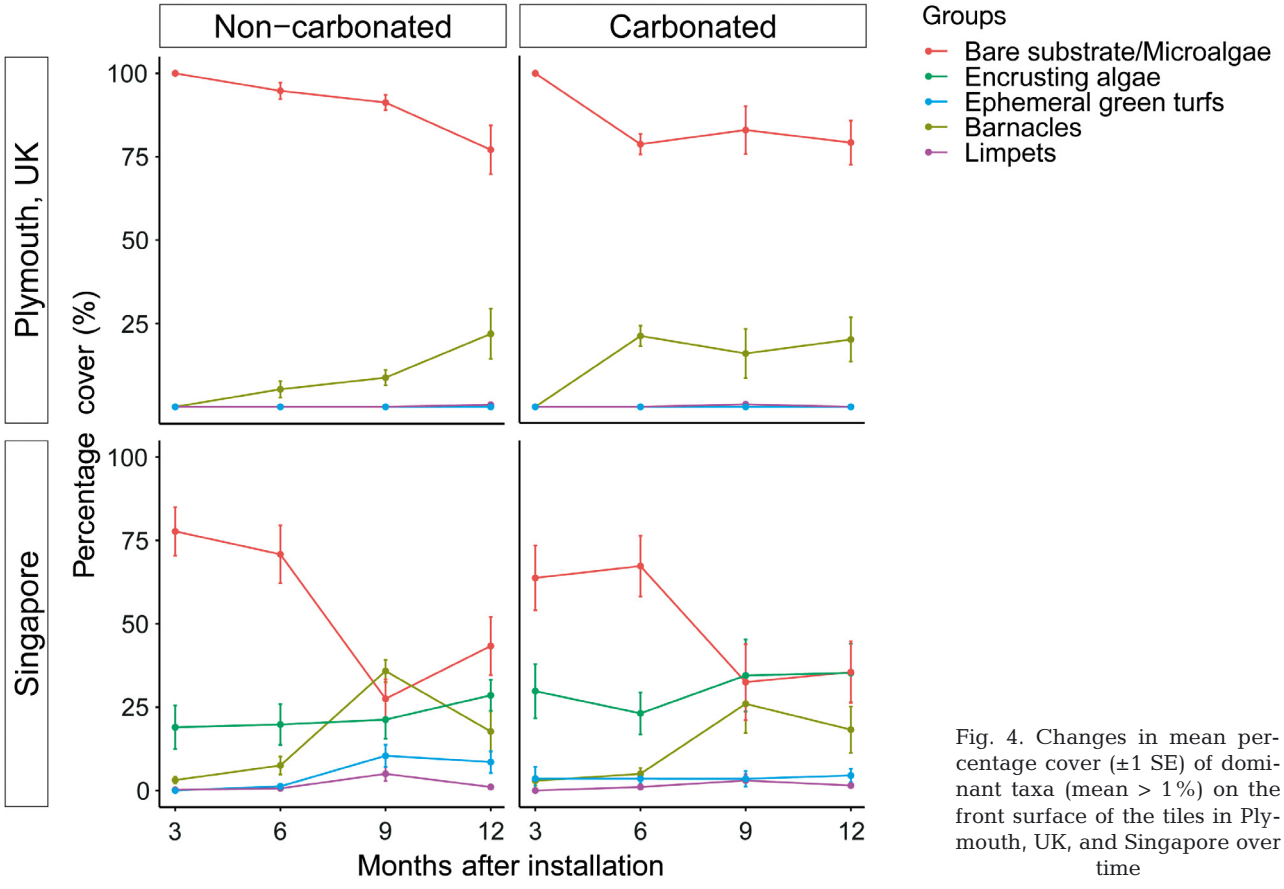

Fig. 4. Changes in mean percentage cover (±1 SE) of dominant taxa (mean > 1%) on the front surface of the tiles in Plymouth, UK, and Singapore over time

barnacle cover fell from 31 to 18% between the 9 and 12 mo time points (Fig. 4). Although there appear to be marginal differences between treatments at the 9 mo time point, with higher barnacle percentage cover than algae on non-carbonated tiles, this was not observed at the final 12 mo time point (Fig. 4).

4. DISCUSSION

Findings from our bilateral 1 yr study indicate that lowering the pH of concrete did not significantly increase the abundance and species richness of intertidal benthic organisms on retro-fitted enhancement tiles, and did not significantly alter the community composition they supported. Concrete is generally considered harmful to the environment, yet it remains one of the most commonly used materials in the world and is prevalent in the construction of marine and coastal infrastructure (Bulleri & Chapman 2010, Waters & Zalasiewicz 2018), including marine biodiversity enhancement units (see O'Shaughnessy et al. 2020 for review). Some researchers have proposed that lowering the pH of concrete would further increase species richness on enhancement units (Perkol-Finkel & Sella 2014, Huang et al. 2016, Reef Ball Foundation 2017). However, previous studies that showed positive effects of lowered concrete pH on benthic diversity were only conducted over short time periods (3 to 4 wk; Guilbeau et al. 2003, Nandakumar et al. 2003), in subtidal areas with little or no emersion (Perkol-Finkel & Sella 2014, Sella & Perkol-Finkel 2015), or had also made additional adjustments to the concrete composition and surface texture (Perkol-Finkel & Sella 2014, Sella & Perkol-Finkel 2015, Dennis et al. 2018) which made it difficult to discern if pH was indeed responsible for the positive effect. Given that the current experiment, which tested the effects of pH alone, found no overall significant differences in species recruitment on the tiles, lowering pH might not be an efficacious ecological engineering technique for increasing intertidal biodiversity on artificial structures.

While the effects of soil pH on plants have been thoroughly studied in the terrestrial environment (Bååth & Arnebrant 1994, Robson 2012), the influence of substrate pH on benthic marine life remains poorly understood (Nandakumar et al. 2003, Sekar et al. 2004). Higher species richness on carbonated concrete at earlier time points in Singapore (3 mo at Pulau Seringat and 6 mo at Pulau Hantu; Fig. 3) could be related to greater biofilm (e.g. cyanobacteria, diatoms) and microalgal development. pH has been regarded as an important factor in the colonisation of natural biofilms (Sekar et al. 2004). Further, carbonating concrete can create smaller pore diameters when calcium is precipitated into carbonate form (Roy et al. 1999) that can also encourage microalgal attachment (Guilbeau et al. 2003). These layers of biofilm and microalgae are food resources which could have provided greater foraging opportunities for grazers (Irigoyen et al. 2011), such as limpets (e.g. *Siphonaria guamensis*, *Patelloida saccharina*) and snails (e.g. *Nerita undata*). For example, the higher abundance of individuals found on carbonated concrete tiles from Pulau Hantu at the 6 mo time point was also mainly due to a single snail species, *N. undata*, a microalgal feeder (Underwood 1984). Concrete carbonation, however, had little or no effect at sites which had low algal growth generally, such as at Cremyll and Turnchapel in the UK (Fig. 4).

Even though there might have been some early differences in abundance and species richness between tile treatments in Singapore, these did not persist. Biofilm formation can strongly influence the settlement of macrofouling taxa such as barnacles, serpulids, and mussels (reviewed by Almeida & Vasconcelos 2015), but the lack of significant differences between treatments beyond 6 mo suggests that, even if there were differences in initial microalgal attachment, it was not enough to influence subsequent successional species. Additionally, the surface pH of non-carbonated tiles in Singapore appeared to have reduced to <9 by 6 mo (see Fig. S1 in the Supplement). This is in line with the findings of Dooley et al. (1999), who suggested that the pH of a concrete surface will approach seawater pH after 3 to 6 mo in a marine environment. As such, colonisers may not experience major differences in concrete pH between tiles of different treatments after a few months of seawater exposure.

Substrate alkalinity is also unlikely to affect primary or secondary consumers during low tide, since leaching occurs when concrete is submerged in water (Li et al. 2005). Calcium oxide (CaO) in Portland cement reacts with water to form calcium hydroxide (CaOH), contributing to the high pH of the substrate. Lowering concrete pH via carbonation can also influence the solubility of metals, where copper, cadmium, and cobalt are increasingly mobilised, and calcium and strontium become more tightly bound (Bin-Shafique et al. 1998, Fernández Bertos et al. 2004), but this mostly occurs during submersion. Nevertheless, the water-retaining pits of the non-carbonated concrete tiles still accommodated a higher abundance and

Fig. 5. Example of a non-carbonated tile at 12 mo from Pulau Seringat, Singapore, with several empty barnacle shells that acted as microhabitats for smaller organisms

richness of benthic organisms than the flat surfaces of the tiles. Water-retaining features of habitat enhancement units, even non-carbonated concrete ones, provide organisms with shelter from desiccation and thermal stresses (Firth et al. 2016b, Loke et al. 2019c). This adds to the growing evidence that habitat structure may have a larger influence on community assemblages than substratum material (Anderson & Underwood 1994, MacArthur et al. 2019).

At small scales, the presence of motile fauna (i.e. gastropods, non-encrusting polychaetes, decapods) is often highly influenced by the availability of refugia and foraging opportunities in habitats (Schmidt & Scheibling 2007, Irigoyen et al. 2011). The empty shells of dead barnacles provide additional complex microhabitat (<5 mm) structures (Chalmer 1982, Dean & Connell 1987). In the present study, many barnacles died in Singapore after initial colonisation, and their shells then served as microhabitats for smaller organisms such as the crab *Nanosesarma minitum*, snails *Zafra* spp., and polynoid worms (Fig. 5). At a larger scale, seawall design and location can affect benthic colonisation (Jackson 2014). For instance, slope differences can affect the susceptibility of seawalls to extreme surface temperatures, with sloping seawalls absorbing more solar radiation compared to vertical ones (Zhao et al. 2019). Additionally, Pulau Hantu is a particularly sheltered site compared to Pulau Seringat (Loke et al. 2016). Both temperature and wave exposure can affect hard-shore communities (McQuaid & Branch 1984, Heery et al. 2020), and the lower abundance and species richness at Pulau Hantu (sloping) compared to Pulau Seringat (vertical) at all time points is likely due to their very different gradients. These biotic and abiotic influences on the succession of the tiles could play a greater role in controlling community patterns compared to the pH of the concrete tiles.

Furthermore, barnacles and serpulids often settle on new intertidal substrate surfaces, both natural (Dean & Connell 1987) and artificial (Chalmer 1982), during early successional phases. While carbonated concrete has previously been reported to reduce the settlement of 'alkotolerant organisms' (Dooley et al. 1999, Huang et al. 2016) and promote algal growth (Guilbeau et al. 2003), this effect was not evident in the current experiment. In fact, there were more barnacles on carbonated tiles than non-carbonated tiles at Cremyll at the 9 mo sampling point (Fig. 3).

To gain a more comprehensive understanding of the effects of concrete pH, future studies could take regular measurements of the tile pH as well as the seawater pH in the water-retaining pits. There is a lack of standardised protocols for testing the pH of other hard substrates such as natural stone (Aho & Weaver 2006), which would be useful for investigating the role of substrate pH in influencing marine biodiversity more generally. Nevertheless, this study provides some insight into the potential effects of pH on marine benthic colonisation from an ecological engineering perspective.

As the demand for urban coastal development rises in response to the threats of sea level rise and increasing coastal populations, it is important to consider engineering solutions that can maximise the ecological functioning of the marine built environment (Airoldi et al. in press). However, the influence of substrate pH on benthic colonisation is relatively understudied, with little evidence to support the hypothesis that lowering concrete pH can increase species richness or abundance of organisms. Our experiment indicates that the effects of pH on benthic colonisation are non-significant and we suggest that manipulation of the physical structure of habitat enhancement units, such as increasing topographical complexity and adding water-retaining features, is a more effective eco-engineering approach to enhancing the ecological value of and species diversity on seawalls.

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