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

Amanda R. Hsiung[1¶], Wen Ting Tan[1¶], Lynette H.L. Loke[1]*, Louise B. Firth[2], Eliza C. Heery[1], James Ducker[2], Victoria Clark[2], Y. Shona Pek[3], William R. Birch[3], Ambert C.F. Ang[1], Rania S. Hartanto[1], Tiffany M.F. Chai[2], Peter A. Todd[1]

[1] Experimental Marine Ecology Laboratory, Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Block S3, Singapore 117557
[2] School of Biological and Marine Sciences, University of Plymouth, Plymouth PL4 8AA, United Kingdom
[3] Institute of Materials Research & Engineering, 2 Fusionopolis Way, Innovis, Singapore 138634

¶*These authors contributed equally to this work*
*Corresponding author, lynetteloke@gmail.com

Concrete is one of the most commonly used materials in the construction of coastal and marine infrastructure despite well-known environmental impacts, including 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 two sites per country and six replicate tiles of each treatment were collected at four 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 generalized 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 factor for enhancing biodiversity in the marine built environment.

Key words: pH, eco-engineering, 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 (Chapman 2003, Todd et al. 2019), sea defences are likely to become more prevalent with growing coastal populations, rising sea levels 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. 2016b). 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 colonization (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 such as the loss of habitat area (Lai et al 2015), compression of the intertidal zone due to its steep gradient (Firth et al. 2014, Loke et al. 2019a), low structural complexity (Chapman & Bulleri 2003, Moreira et al. 2007), and higher desiccation (Tan et al. 2018, Zhao et al. 2019) and temperature risk (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 (Strain et al. 2018, Chapman 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 in several countries, both temperate and tropical (Dafforn et al. 2015, Firth et al. 2016a, Loke et al. 2019b). 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 also resulted in higher live cover and primary productivity of pre-fabricated habitat units (Perkol-Finkel & Sella 2014, Sella & Perkol-Finkel 2015). On top of increasing 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 these 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), sites with different benthic community assemblages could also be influenced to varying degrees.

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, Neves et al. 2013). This is often performed to test for the effects of long-term carbonation on concrete’s metal leaching abilities (Sazzad bin Shafique et al. 1998), compressive strength (de Ceukelaire & van Nieuwenburg 1993, Chi et al. 2002), and durability (Roy et al. 1999) as carbonation can alter the physical properties of concrete by densifying the concrete surface (Chi et al. 2002, Fernandez Bertoz 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) affects 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”. To test for generality, the experiment was conducted in a temperate country (United Kingdom) and a tropical country (Singapore). Specifically, we tested the following hypotheses: (1) carbonated tiles would support higher macrofaunal abundance and species richness than standard non-carbonated tiles, and (2) carbonated tiles would support different biological communities from standard non-carbonated tiles, and these differences would be consistent across time and sites with different community assemblages.

## 2. MATERIALS & 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 cm × 10 cm (Fig. 1) and had a smooth and pitted façade (on 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 and 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 and Todd's (2016) protocol, using silicone rubber moulds (Freeman Bluesil™ V-340). Tiles were then cast from the moulds using cement/aggregate ratio = 1/3 and water/cement ratio = 3/5. Pre-drilled 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 (Sanjuán et al. 2003, 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 $CO_2$ chamber was created using a large cooler box and dry ice (Short et al. 2001, Venhuis & Reardon 2003).

Trials were conducted using concrete coupons (5 cm × 5 cm × 2 cm) to determine the best carbonation conditions (wet or dry), and the duration of curing (2, 6, 12, 20 days) and carbonation (7, 22, 29 days) required to reduce the pH of the tiles. Concrete coupons were split in half using a tile saw and the surface and cross section of the split tiles were stained with two 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 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 days), however, was the most important variable to achieve a pH of less than 8 (Fig. 2A). A sub-sample of the final batch of tiles were assessed using the indicator dyes, which showed that the surface of the carbonated concrete tiles was no more than than pH 8.

Attempts were also made to quantify the pH of the concrete tiles 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, while the method is often used to test for internal concrete pH, it does not give an accurate measurement of surface pH. Therefore, this method was only used to confirm the differences in internal pH between treatments at the 6-month time point (Fig. S1, Table S1). All tiles were prepared in Singapore before half were sent to the UK.

### *2.3. Study sites*

Tiles were deployed in two locations, one temperate and one tropical climate, with two seawall sites at each location. Plymouth (United Kingdom) was chosen as the temperate location and Singapore was chosen as the tropical location.

#### *2.3.1. Plymouth, United Kingdom*

Plymouth is a port city located on the south-west coast of England, United Kingdom, where the English Channel broadens into the Atlantic Ocean. 33% of the coastline within Plymouth Sound is artificial (mostly seawalls) (Knights et al. 2016). In Plymouth, the tiles were installed in February 2018 onto two vertical seawalls at: (i) Turnchapel (50.359, 4.1178) and (ii) Cremyll (50.3648, 4.1633).

*2.3.2. Singapore*

Singapore is a tropical city-state located just over one degree north of the equator, separated from Peninsular Malaysia by the Straits of Johor in the north and from Indonesia by the Straits of Singapore 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 two southern islands: (i) grouted granite rip-rap seawall at Pulau Hantu (1.22611, 103.75222) and (ii) vertical seawall at Pulau Seringat (1.23, 103.85056).

*2.4. Field experimental design, sampling and laboratory procedures*

At each site, 24 of 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 months. However, due to unforeseen temporary restricted access to Pulau Hantu, collection for the 9-month time point could not be carried out, hence we included a 15-month 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 four 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 two countries with treatment, site, and month (categorical) as fixed effects, but models with negative binomial error were subsequently used to analyse abundance due to over-dispersed data.

With differences in sample numbers between sites at some time points (described above) and significant differences in abundance and species richness between months and sites, we removed interaction terms (Table S3) and evaluated whether treatment effects differed by subsequently modelling the abundance and richness data separately for each site and month with treatment as the sole predictor. Site- and month-specific models of richness tended to be under-dispersed, 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. Univariate tests were performed in R v3.6.0 (R Core Team 2019). COM-Poisson models 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 15$^{th}$ month data as they were un-replicated in time; please see the Methods section for more information). As both countries hosted no overlapping species, 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; canonical analysis of principal coordinates (CAP) plots were then used to examine these temporal differences. All multivariate analyses were performed using the PRIMER v7 with the PERMANOVA add-on (Anderson et al. 2008).

## 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 Table S5, S6). Additionally, all species found from both countries were native, with the exception of the non-native *Austrominius modestus* in Plymouth and *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 (Table 3, S7).

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-month, time point (Table 4; further details in Table S8, S9). Carbonated tiles had greater total abundance than non-carbonated tiles at Cremyll at the 9-month time point, and at Pulau Hantu at the 6-month time point (Table 4). In Singapore, species richness was greater on carbonated tiles than non-carbonated tiles at the 3-month time point at Pulau Seringat, and at the 6-month 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 (SS = 124360; Pseudo-$F_{3,70}$ = 39.06; p = <0.001, SS = 38734; Pseudo-$F_{3,67}$ = 8.6198; p = <0.001, for Plymouth and Singapore respectively; Table 5) and sites (SS = 3309.5; Pseudo-$F_{1,70}$ = 3.1183; p = <0.05, SS = 60739; Pseudo-$F_{1,67}$ = 40.55; p = <0.001, for Plymouth and Singapore respectively; 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, 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-month time point, there was no observed difference at the final 12-month time point. In Singapore, biofilm which dominated at 3-month and 6-month time points was succeeded by barnacles and encrusting algae by the 9-month time point (Fig. 4). However, mean barnacle cover fell from 31% to 18% between 9-month and 12-month time points (Fig. 4). Although there appears to be marginal differences between treatments at the 9-month time point, with higher barnacle percentage cover than algae on non-carbonated tiles, this was not observed at the final 12-month time point (Fig. 4).

## 4. DISCUSSION

Findings from our bilateral one-year 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 support. Concrete is generally considered damaging to the environment, yet it remains one of the most utilised 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. 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 (3–4 weeks; Guilbeau et al. 2003, Nandakumar et al. 2003), in subtidal areas with little/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-month at Pulau Seringat and 6-month at Pulau Hantu; Fig. 3) could be related to greater biofilm (e.g., cyanobacteria, diatoms) and microalgal development. pH has been regarded 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, higher abundance of individuals found on carbonated concrete tiles from Pulau Hantu at the 6-month 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 be some early differences in abundance and species richness between tile treatments in Singapore, the effects of carbonation 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 six months 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 <8 by month 6 (Figure S1). This is in line with findings by Dooley et al. (1999) who suggested that the pH of concrete surface will approach seawater pH after three to six months in marine environments. 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 (Sazzad bin Shafique et al. 1998, Fernandez Bertoz 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 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. 2016a, Loke et al. 2019b). This adds to the growing evidence that habitat structure may have a larger influence on community assemblages than substratum material (Anderson & Underwood 1994, Coombes et al. 2015).

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 micro-habitat (<5 mm) structures (Chalmer 1982, Dean & Connell 1987). In this study, many barnacles died in Singapore after initial colonisation, which then served as microhabitats for smaller organisms such as the crab *Nanosesarma minitum*, snails *Zafra* spp. and polynoids (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, 1985), and 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 may 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, Tejada-Martinez et al. 2016) and artificial (Chalmer 1982, Coombes et al. 2017), during early successional phases. While carbonated concrete had previously reduced the settlement of “alkotolerant organisms” (Dooley et al. 1999, Huang et al. 2016) and promoted algal growth (Guilbeau et al. 2003), this effect was not evident in the current experiment. In fact, there were significantly more barnacles on carbonated tiles than non-carbonated tiles at Cremyll at the 9-month sampling point (Table 3, Fig. 3).

To gain a more comprehensive understanding on the effects of concrete pH, future studies can take regular measurements of the tile pH as well as the seawater pH in the water-retaining pits of the tiles. There is also a lack in standardised protocol for testing the pH of other hard substrates such as granite, limestone and other naturally occurring rocks (Aho &

Weaver 2006), which would be useful for investigating the role of substrate pH in influencing marine biodiversity. Nevertheless, this study provides some insight to 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 artificial structures. 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 is 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 and species diversity on seawalls.

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# Tables

**Table 1.** Functional categories used for classifying algae in this study, adapted from Loke et al. (2016).

| Functional group | Dominant Component Taxa (examples from Singapore) |
|---|---|
| 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 |

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

| | Total number of species | | Total number of unique species | |
|---|---|---|---|---|
| **Sites** | 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 |

**Table 3.** Analysis of deviance results for negative binomial and poisson GLMs for total abundance and species richness in Plymouth (left) and Singapore (right). Significant p-values, as determined by likelihood ratio tests, are shown in bold.

| | Plymouth, UK | | | | | Singapore | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Source** | 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** |

**Table 4.** Results from site- and month- specific GLMs for total abundance and species richness. Models for N used a negative binomial error distribution, while Conway-Maxwell-Poisson error was used in models for S. All contained treatment as the sole predictor. The table shows "--" when there was no difference between pH treatments, "C > NC" where carbonated tile treatments had higher abundance or species richness than the non-carbonated pH treatment, and "na" where no data were available. Complete coefficient summaries from each model are provided in Appendix A.

| 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 57 (Plymouth and Singapore, respectively) taxa in response to site, pH treatment and duration since deployment as fixed factors and their interactions.

| Source | df | SS | Pseudo-F | P(perm) | Unique perms |
|---|---|---|---|---|---|
| *Plymouth, UK* | | | | | |
| Site | 1 | 3309.5 | 3.12 | **0.0379** | 9942 |
| Treatment | 1 | 1343.4 | 1.27 | 0.2568 | 9940 |
| Month | 3 | 124360.0 | 39.06 | **<0.0001** | 9914 |
| Site x Treatment | 1 | 1944.1 | 1.83 | 0.1297 | 9941 |
| Site x Month | 3 | 3828.2 | 1.20 | 0.2841 | 9938 |
| Treatment x Month | 3 | 4979.9 | 1.56 | 0.1303 | 9936 |
| Site x Treatment x Month | 3 | 1703.4 | 0.54 | 0.8541 | 9951 |
| Residual | 70 | 83844 | | | |
| | | | | | |
| *Singapore* | | | | | |
| Site | 1 | 60739.0 | 40.55 | **<0.0001** | 9949 |
| Treatment | 1 | 1748.7 | 1.17 | 0.2903 | 9930 |
| Month | 3 | 38734.0 | 8.62 | **<0.0001** | 9910 |
| Site x Treatment | 1 | 519.0 | 0.35 | 0.9628 | 9936 |
| Site x Month | 2 | 27654.0 | 9.23 | **<0.0001** | 9927 |
| Treatment x Month | 3 | 5119.7 | 1.14 | 0.2892 | 9904 |
| Site x Treatment x Month | 2 | 5327.8 | 1.78 | **0.0454** | 9904 |
| Residual | 67 | 100360 | | | |

Pre-proof verion of Hsiung, A.R., Tan, W.T., Loke, L.H., Firth, L.B., Heery, E.C., Ducker, J., Clark, V., Pek, Y.S., Birch, W.R., Ang, A.C. and Hartanto, R.S., 2020. Little evidence that lowering the pH of concrete supports greater biodiversity on tropical and temperate seawalls. Marine Ecology Progress Series, 656, 193-205.

# Figures

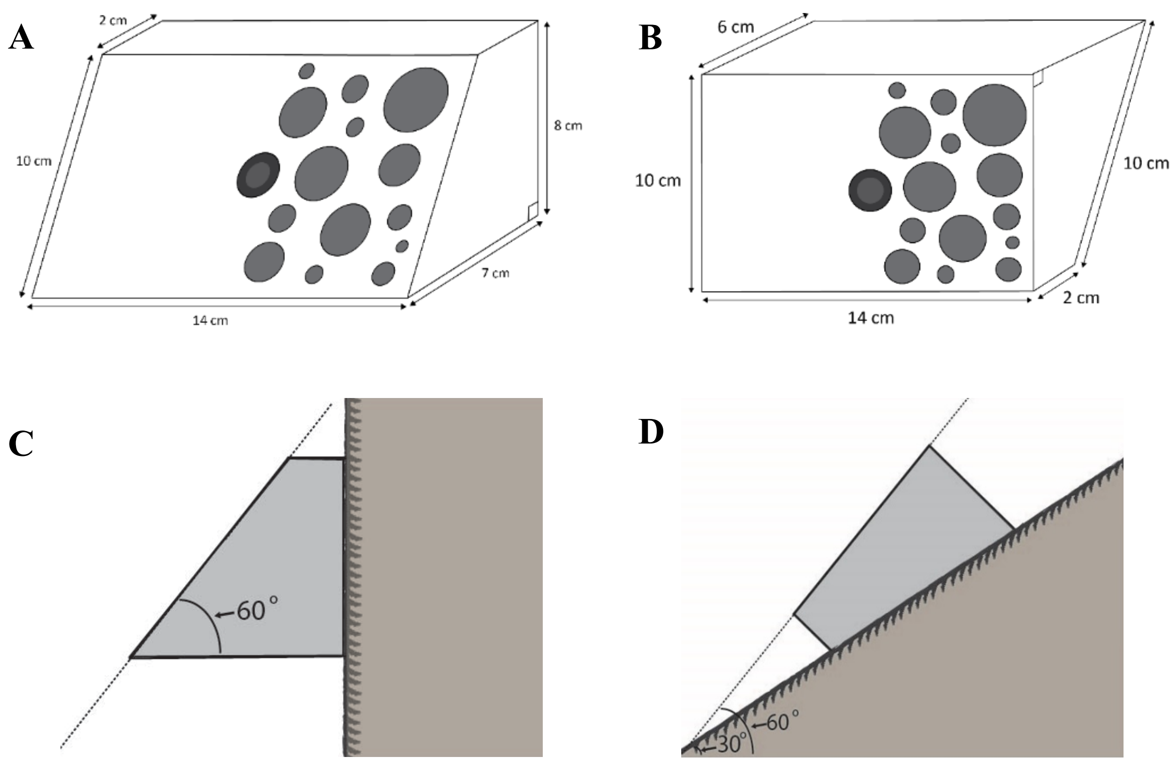


**Figure 1.** Dimensions of tiles for (A) vertical and (B) sloping seawalls, with schematics of the tiles when installed on the seawalls (C and D, respectively).

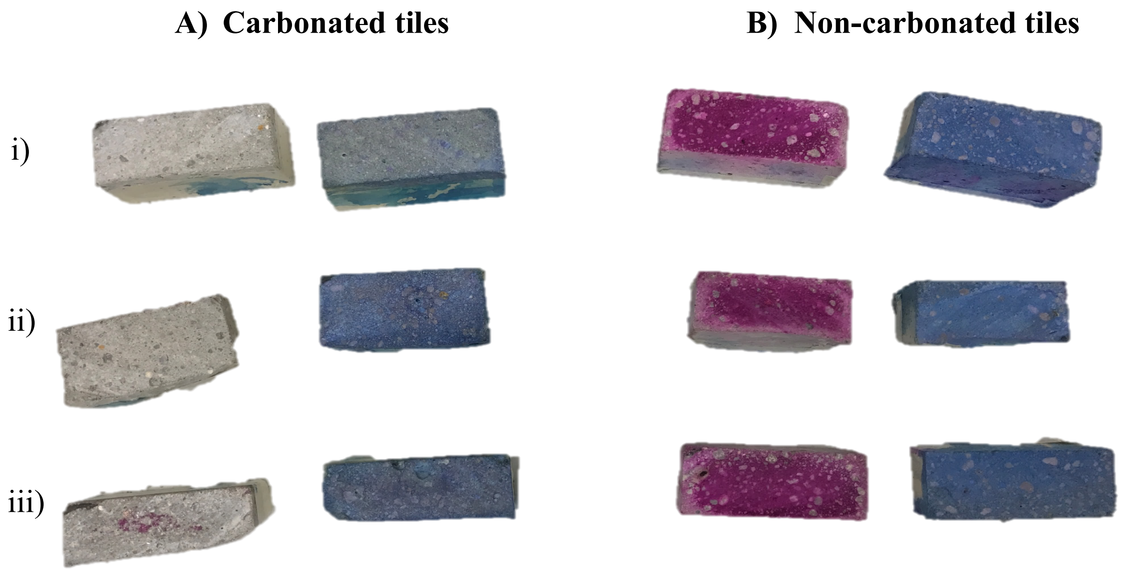


**Figure 2.** Images of (A) carbonated tiles stained with phenolphthalein (left) and bromothymol blue (right) after undergoing: i) 29 days of carbonation and 12 days of drying, ii) 22 days of carbonation and 20 days of drying, and iii) 22 days of carbonation and 6 days of drying, with (B) non-carbonated

tiles that dried for the same amount of time (control) stained with phenolphthalein (left) and bromothymol blue (right).

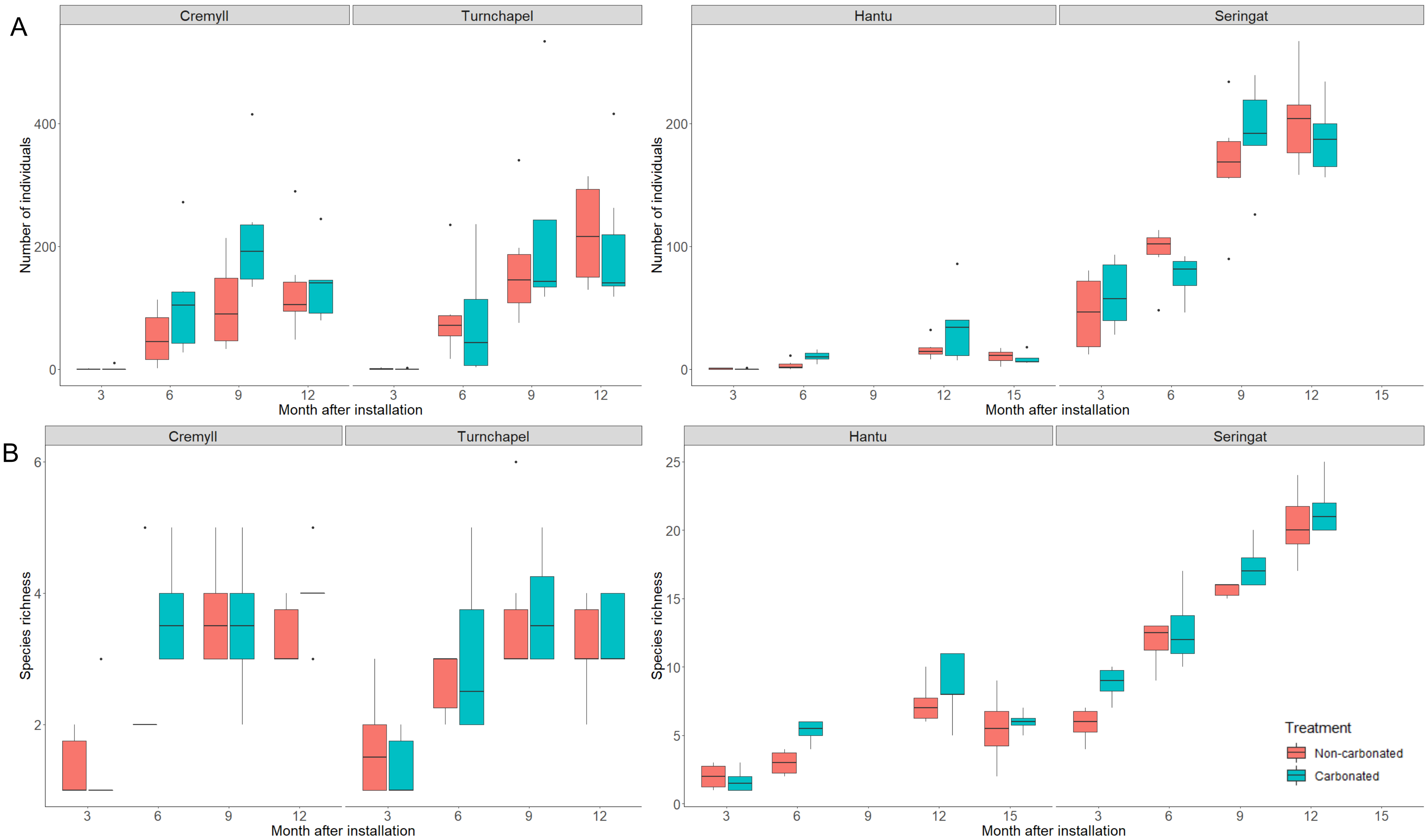


**Figure 3.** (A) Abundance (number of individuals) and (B) species richness on tile treatments (non-carbonated and carbonated) across four time points (3-month, 6-, 9-, 12- for Cremyll, Turnchapel and Pulau Seringat, 3-month, 6-, 12-, 15- for Pulau Hantu). Boxplot middle lines indicate the median; hinges 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.

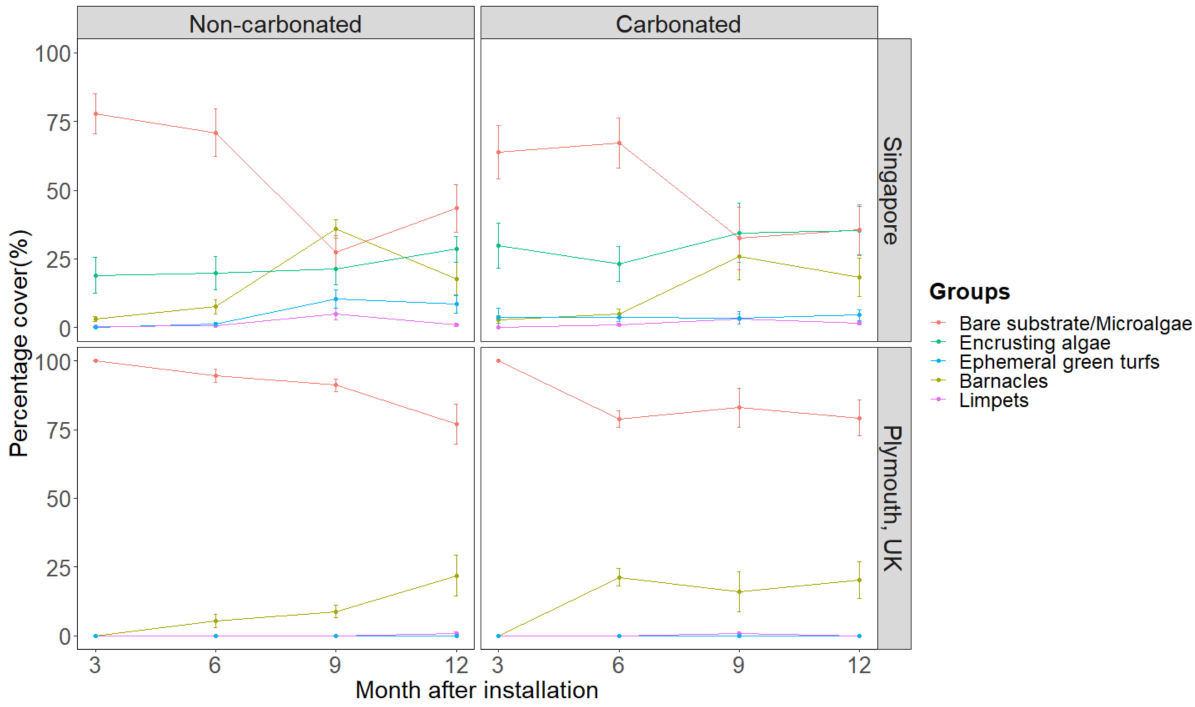


**Figure 4.** Changes in mean percentage cover (±1SE) of dominant taxa (mean > 1%) on the front surface of the tiles in Plymouth and Singapore over time.

**Figure 5.** Example of a non-carbonated tile at 12-month from Pulau Seringat, Singapore, with several empty barnacle shells that contributed to microhabitats for smaller organisms.