A comparison of epibenthic reef communities settling on commonly used experimental substrates: PVC versus ceramic tiles.

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Abstract:

Artificial substrates are routinely used in coral reef research to model the recruitment and growth responses of benthic organisms (e.g. coral recruitment and encrusting organisms) to environmental change. Two commonly used, but structurally different, artificial substrates include cylindrical PVC pipes and flat ceramic tiles. Various ecosystem based models extrapolate data from these substrates interchangeably based on the assumption that results are directly comparable. In order to test this assumption we deployed these commonly used artificial substrate materials, PVC poles and ceramic tiles, in shallow patch reefs for 34 months at One Tree Island, Great Barrier Reef. Tiles were positioned to mimic upwards facing, well-lit substrates (exposed), and downwards facing, shaded (cryptic) substrates. Multivariate analyses demonstrated that the community composition differed significantly between all three treatments. The majority of artificial substrate, coral reef experiments focus on key groups of calcifying organisms, primarily: coralline algae, scleractinian coral and/or total calcareous encruster cover. Interestingly, significant differences in the recruitment, colonisation and community composition of these organisms were detected for our three treatments. When compared to ceramic tiles, PVC poles had greater coverage of crustose coralline algae but reduced levels of coral recruits (<1mm diameter) and turf algae. We suggest that comparisons between studies that utilise data from different substrate types should be used with caution. Additionally, large scale modelling and forecasting exercises utilising these data sets should adjust for the inherent biases of each method.

Key words: crustose coralline algae, encruster, turf, coral, recruitment, artificial substrate

1. Introduction:

Real world, *in situ* data on the life histories, growth and development of different coral reef organisms are critical if we are to be able to model reef development and forecast how reefs in the future will respond to changing environmental conditions (Stearn et al. 1977; Mallela and Perry 2007; Kennedy et al. 2013; Hepburn et al. 2015; Jones et al. 2015). The deployment of artificial substrates on the reef, such as PVC pipes and ceramic tiles, provide us with a valuable, potentially non-destructive tool to collect quantitative reef growth data. In reef research, artificial substrates are increasingly being used to assess reef development and assess the impacts of changing environmental conditions (Kennedy et al. 2013; Mallela 2013). In particular, the use of ceramic tiles to study coral settlement and life history traits has been widely adopted (e.g. English et al. 1997; Mundy 2000). In contrast, studies focusing on the growth and development of other epibenthic organisms, in particular calcareous reef building organisms, are characterised by a diverse array of experimental materials which include natural materials such as slices of dead coral (Harriott and Fisk 1987; Klumpp 1992; Pari et al. 1998), and commonly available manmade products including concrete, ceramic tiles, PVC poles, cattle ear tags and glassware (Adey and Vassar 1975; Bak 1976; Martindale 1992; Field et al. 2007; Mallela 2007; Kuffner et al. 2013; Hepburn et al. 2015; Roik et al. 2016). Unsurprisingly, experimental substrates were often selected according to their affordability and local availability (Field et al. 2007).

The diverse range of methods detailed in the literature highlighted a need for a standardised method to be introduced for the analysis of encruster assemblages and resulting coral reef carbonate budget models. Mallela in 2004 suggested a low impact (non-destructive) carbonate budget method which incorporated the use of ceramic tiles to assess encruster assemblages and their rates of carbonate production (Mallela 2004). This built on comprehensive methods conducted in Barbados in the 1970's (Stearn et al. 1977) which also used settlement plate data. This low impact method was successfully trialled and ground truthed in Jamaica (Mallela 2004,2007; Mallela and Perry 2007). Subsequently, a rapid assessment method for assessing encruster assemblages and coral reef carbonate budgets has been proposed using PVC poles. This alternate method builds on pioneering work developed in Curacao (Bak 1976). Known as ReefBudget, this method relies on multiple PVC poles being inserted into the reef and subsequently lifted for analysis (e.g. Morgan and Kench 2014; Perry et al. online resource). Interestingly, data resulting from these two artificial substrate methods have never been directly compared. There has also been some debate about whether or not experimental substrates provide real world information that can be extrapolated to naturally occurring marine assemblages (Glasby and Connell 2001; Perkol-Finkel et al. 2006; Mallela 2007; Burt et al. 2009). If we are to be able to extrapolate from artificial substrate data in a meaningful manner we need to know their caveats. This includes assessing if inter-substrate data sets are comparable, if they simulate real-world (reef-scape) data, and if not, what their inherent biases are.

The effects of different artificial substrates on coral recruitment have been widely documented (Harriott and Fisk 1987; Petersen et al. 2005; Burt et al. 2009; Miller et al. 2009). Currently, we know less about their impacts on calcareous encrusting organisms (sensu Taylor 1990) and the wider epibenthic reef community structure (Field et al. 2007). Substrate orientation (Taylor 1990; Mallela 2007; Hepburn et al. 2015) and morphology (Martindale 1992) are known to influence encruster settlement. For instance, nodular colonies show a preference for convex reef surfaces whilst laminar colonies tend to colonise concave or planar surfaces (Martindale 1976,1992). Whilst many epibenthic organisms also display rugophilic behaviour preferentially settling in cracks, crevices and shaded habitats on the reef (Taylor 1990) some deployment methods omit this important reef parameter (Adey and Vassar 1975; Bak 1976; Nozawa et al. 2011). Caribbean reef comparisons of experimental reef substrates deployed in different orientations over one to two years also note clear differences in recruitment and community composition between vertical and horizontal substrates, and well-lit and shaded substrates (Mallela 2013;

Hepburn et al. 2015). Interestingly, Mallela's (2013) fore-reef study in Tobago found total encruster cover was greatest on exposed-horizontal substrates when compared to vertical or horizontal low-light substrates. In contrast, Hepburn's 2015 Mexico study across a range of reef sites, at various depths and orientations, observed both inter-site variability and especially high encruster cover in cryptic/vertical habitats at some locations. Such findings highlight, irrespective of substrate type, a degree of inter-site variability occurring in the early stages (e.g. initial 24 months) of benthic recruitment and encruster development (Burt et al. 2009).

Two of the most commonly deployed artificial substrates used in reef research to study the growth and development of encrusters and epibenthic organisms include PVC poles and ceramic tiles. Few comparisons of these substrates with natural reef habitats exist (see Table 1 for a summary of these studies). Adey (Adey and Vassar 1975) found that when PVC poles were positioned on the reef to mimic dead branching coral substrates coralline communities were faster to develop on the PVC when compared to the natural dead substrate. Additionally, PVC poles positioned in the reef pavement zone did not attract some of the less dominant coralline algae species observed naturally in the shallow pavement zone. Studies in St Croix (Adey and Vassar 1975) and Curacao (Bak 1976) also noted that PVC poles, including their shaded undersides if orientated accordingly, did not attract certain sclerobionts (e.g. encrusting forams, bryozoans, and specific coralline algae species) typical of shaded/cryptic environments (e.g. the undersides of corals or cryptic pavement areas). A comparison of settlement plates with naturally occurring reef substrates in Barbados provided anecdotal evidence stating that no evidence was seen for a marked difference in the crusts between natural and artificial substrates (Martindale 1976).

Whilst a study in Jamaica that compared assemblages on experimental, cryptically orientated ceramic tiles with the undersides of adjacent platy corals, found no significant difference with regards to community composition and benthic cover (Mallela 2007). The Jamaican study concluded that cryptically orientated ceramic tiles were a good proxy for naturally cryptic (e.g. shaded) reef substrates.

Carbonate budget models, which predict reef accretion, also utilise encruster growth data from a variety of artificial substrates, typically PVC or ceramic settlement plates are used to collect site specific data (Stearn et al. 1977; Mallela and Perry 2007; Morgan and Kench 2014; Perry et al. online resource). In the absence of site specific, *in situ* measurements additional data are often extrapolated from experiments in other regions of the world using various methods and different experimental substrates (e.g. Hart and Kench 2007; Kennedy et al. 2013). While direct comparisons are then made between these different studies data are rarely cross checked or validated between these different approaches. The effects of artificial substrate type, and the deployment method used, on encruster and non-calcareous community development still remains generally overlooked and poorly understood. In addition, the implications of upscaling data sets gleaned from different methods to input into reef scale models (e.g. carbonate budgets) has largely been ignored.

Based on a review of the literature, two of the most common methods used for generating epibenthic data that is then extrapolated for use in reef accretion models utilise data gleaned from two physically different, but readily available, artificial substrates types:1) flat, ceramic (terracotta) tiles, (also called settlement plates) and 2) hollow, white PVC poles. In order to determine if these two approaches are comparable, the variation in community composition and the abundance (% cover) of epibenthic communities (both calcareous and non-calcareous) recruiting to these different, commonly used, artificial substrates were assessed. Horizontally orientated PVC poles and ceramic tiles in a horizontal, downwards facing orientation (cryptic) and ceramic tiles in a horizontal, upwards facing (exposed) orientation were compared and apparent biases examined.

2. Methods:

Study sites: Artificial substrates were placed at three patch reef sites (microatolls) at One Tree Island in the Southern Great Barrier Reef. These shallow microatolls form part of an extensive patch reef system within the lagoon at One Tree Island. Microatolls in this study were characterised by having a fully enclosed perimeter of living reef composed primarily of coral and coralline algae. Inside the perimeter, the microatolls were characterised by sand, rock, coralline algae and small coral colonies (depth at low tide < 2m), the outer walls of the microatolls fall steeply to lagoon floor (depth: 2-5 m adjacent to microatoll). At low tide the inner ‘pond’ of the microatolls were isolated from the rest of the lagoon by their circular reef walls. At high tide the water rises above the living walls (common name: piecrust) of the microatolls (≤ 1.5 m) enabling water exchange and free movement of reef organisms These microatolls have been described in great detail in earlier work and previously used site names will be used here for continuity: microatoll 1) Kinsey (described in Kinsey and Domm 1974), microatoll 2) ENCORE 4, and microatoll 3) ENCORE 11 (ENCORE sites decribed in Larkum and Steven 1994; Steven and Atkinson 2003).

Experiment design: Two common artificial substrate types were used in this experiment: 1) unglazed, square, ceramic tiles (13 x 13 cm) and standard, white PVC poles (smooth surface, hollow, 50 cm long, 7cm in circumference). In order to remove the confounding effects of orientation (e.g. horizontal versus vertical substrate effects (e.g. Glasby and Connell 2001; Mallela 2013; Hepburn et al. 2015) all substrates were positioned horizontally and randomly, without touching, inside the three microatolls at depths of 1 - 2 m at low tide. All artificial substrates were positioned to reflect the natural gradient of the lagoon floor (angle of slope) on which it had been placed, typically 0^o to 10^o in slope. All substrates were secured to PVC frames attached to the floor of the microatolls using cable ties threaded through pre-drilled holes in the artificial substrates. Ceramic tiles were attached horizontally in close fitting, overlying pairs leaving only the two outer faces available to recruitment and subsequent colonisation. Each pair of ceramic tiles had one upwards facing, well-lit surface, from here-in referred to as “exposed” (TE) and one downwards facing, shaded, surface from herein referred to as “cryptic” (TC). PVC substrates were also positioned horizontally with the circumference (outer surface) of the PVC tube available for subsequent colonisation.

Substrates remained *in situ* for a period of almost three years in order to be representative of established (multi-year) encruster assemblages. All substrates were deployed in May 2012 and lifted in March 2015 after a total underwater deployment of 34 months. The number of intact replicates analysed at the end of the experiment were: Tiles-Exposed: 30; Tiles-Cryptic: 31; PVC poles: 30.

On collection, artificial substrates were labelled and air dried. A 100 point grid was superimposed over each substrate (TE, TC and PVC), each data point was ≥ 1cm apart. For the square tiles we used a square 10 x 10 grid, for the PVC tubes we used a rectangular 50 x 2 grid. Using a dissection microscope all organisms under each point were identified to taxonomic group (e.g. crustose coralline algae, coral recruit, calcareous worm, turf) and counted (Mallela 2013), see supplementary Table 1 which details all identification categories.

Statistical analysis: Community data were analysed using PRIMER 6 statistical software (Clarke and Gorley 2006). Multivariate analyses were used to test for differences in community composition between the treatments. Multivariate data were square root transformed and the Bray-Curtis similarity coefficient employed to construct a similarity matrix for the percentage cover (%) of colonising epibenthic communities. Non-metric multidimensional scaling (MDS) ordinations were used to assess assemblages between microatolls and across different substrate types. No significant microatoll (reef site) impact was found (supplementary Fig. 1) so data sets were pooled in order to further investigate substrate impacts. One-way analyses of similarities (ANOSIM) tests were used to look for differences in epibenthic communities between exposed tiles, cryptic tiles and PVC. The R-statistic indicated the extent of significant differences, R-statistic values <0.1 were considered negligible (Clarke 1993). If ANOSIM indicated a significant difference between substrate types (R>0.1), Similarity Percentages Analyses (SIMPER), using 1-way analysis on Bray-Curtis similarities for substrate groups using a 90% dissimilarity threshold, was used to indicate which epibenthic groups were responsible for these observed differences.

Key epibenthic groups of interest to the wider reef research community using PVC and settlement plate artificial substrates methods were identified during our literature review. Subsequent analysis focused on these key groups: total epibenthic cover, coralline algae, hard (scleractinian) coral cover and total calcareous encruster cover. The abundance, and substrate preferences, of these groups were further explored using IBM-SPSS 22 statistical software. Normality of distribution and homogeneity of variance were tested using Kolmogorov-Smirnov and Levene's test, respectively. As a significant microatoll effect was not found to influence the community composition of these key epibenthic groups (see supplementary Fig. 1 MDS plot of microatoll community composition and supplementary table 2 detailing Scheirer-Ray-Hare test results) data from the three micro-atoll sites were pooled. To test for differences among the three substrate types the Kruskal-Wallis (KW) test was used due to non-normal data distributions. If the KW test revealed a significant difference between the three substrate types, the Mann-Whitney *U* Test was subsequently used for pairwise comparisons to assess which pair was responsible for the difference. This approach has the same logic as an ANOVA *posthoc* LSD test if it is only applied when the KW test reveals a significant result (Dytham 2003). To account for multiple comparisons a Bonferroni correction of alpha was applied where $p \leq 0.01$ was considered significant. Data transformations were not required to meet the assumptions of these tests.

3. Results:

Multivariate analyses were used to compare the community composition of epibenthic assemblages colonising the different artificial substrate types after 34 months of deployment. MDS ordinations gave a good representation of community assemblage (2D Stress: 0.08) and depicted differences in epibenthic assemblages between the three test substrates (Fig. 1). The one-way ANOSIM test comparing benthic composition between artificial substrates indicated a significant difference: ANOSIM, Global R of 0.457 ($p = 0.001$). ANOSIM Pairwise Tests also indicated differences between pairs of substrate types: exposed tile v PVC: R = 0.5 ($p < 0.001$), cryptic tiles v PVC: R = 0.6 (p < 0.001), and exposed v cryptic tiles: R = 0.3 ($p < 0.001$). One-way Similarity Percentages (SIMPER) indicated that the categories primarily responsible for these differences were: turf, total non-calcareous cover, total calcareous cover, crustose coralline algae and uncolonised (bare) substrate (see supplementary material table 3).

Total epibenthic cover by all organisms was significantly different between substrates (Kruskall-Wallis: $H = 47.6$. df = 2, $p = 0.000$) with PVC having significantly greater cover compared to exposed tiles (Mann-Whitney U test: PVC median = 94%, exposed median =71%, U = 38, $p = 0.000$), Fig. 2a. No difference was observed between PVC and cryptic tiles, whilst cryptic tiles had significantly higher epibenthic cover than exposed tiles (Mann-Whitney U test: cryptic median = 94%, exposed median =71%, U = 62.5, $p = 0.000$), Fig. 2 a. PVC poles displayed 29 and 1 % more total epibenthic cover than exposed and cryptic tiles respectively. Whilst mean total calcareous cover (%) was also 39 and 27 % greater on PVC compared to exposed and cryptic tiles respectively (supplementary material table 1).

The percentage cover of crustose coralline algae (CCA) was significantly different between the three substrates (Kruskall-Wallis: $H = 51.9$, df = 2, $p = 0.000$). CCA cover was significantly different when PVC was compared with cryptic (Mann-Whitney U test: PVC median = 85.5 %, cryptic median = 61 %, U = 67.5, $p = 0.000$) and exposed substrates (Mann-Whitney U test: PVC median =85.5% %, exposed median = 55%, U =13.0, $p = 0.000$), Fig. 2 b. On average, PVC poles displayed 37 & 26 % more CCA than exposed and cryptic tiles respectively (supplementary material table 1).

The percentage cover of coral recruits (scleractinian corals < 1cm in diameter) was also found to be significantly different between substrates types due to coral recruits only being observed on grids superimposed over cryptic settlement plates (Kruskall-Wallis: $H = 8.0$, df = 2, $p = 0.018$). Due to the low numbers of coral colonies > 1cm in diameter being recorded, no significant differences were observed for coral colonies (scleractinian coral > 1cm in diameter) or total coral cover (recruits + colonies), Fig. 2 c.

PVC poles had significantly more calcareous cover than both cryptic and exposed tiles, (Kruskall-Wallis: $H = 54.512$, df = 2, $p = 0.000$), Fig. 2d. The percentage cover of all combined non-calcareous organisms (e.g. sponges, turf, macro algae, ascidians) was not significantly different between substrates. However, the cover of turf algae (defined as algal assemblages < 1mm in height) was significantly different (Kruskall-Wallis: $H = 49.2$, df = 2, $p = 0.000$) as was the proportion of non-colonised substrate (Kruskall-Wallis: $H = 45.9$, df = 2, $p = 0.000$). PVC substrates had significantly less turf colonising them when compared to cryptic and exposed settlement plates**.** Levels of bare substrate were similar between PVC and cryptic plates, whilst exposed plates displayed significantly elevated levels of bare substrate. See supplementary Tables 1 for full data set.

4. Discussion:

Our multi-year data set demonstrates that two of the most commonly used experimental substrates, unglazed ceramic tiles (both cryptic and exposed) and PVC poles, are colonised by different epibenthic assemblages. Consequently, results from studies using different substrates are unlikely to be directly comparable. In particular, we found that recruitment and growth by sclerobionts, which include key calcareous reef building organisms (e.g. scleractinian coral recruits and coralline algae), were significantly different between substrate types. PVC substrates were characterised by greater coverage of photophilic, encrusting, coralline algae, but unlike cryptic tiles, coral recruits were not observed on their 100 point grids. In contrast, settlement plates were characterised by higher turf algae cover. We therefore suggest the choice of artificial substrate and method (e.g. orientation) of deployment be driven by the research aim and suggest caution when selecting and extrapolating data sets for real world models (e.g. predicting ecosystem growth and development).

The use of ceramic (e.g. terracotta) settlement plates for scleractinian coral recruitment studies have been widely endorsed (e.g. English et al. 1997; Burt et al. 2009; Mallela and Crabbe 2009; Humanes and Bastidas 2015). Our results found that in the shallow patch reefs of One Tree Island coral recruits were primarily attracted to cryptically orientated ceramic tiles in contrast to PVC poles. Other studies assessing the recruitment patterns of scleractinian corals to different substrate types (e.g. petri dishes, sliced coral skeletons and ceramic tiles) also note substrate specific results and suggest ceramic tiles as the best substrate for coral recruitment studies based on the premise that they attract the most coral recruits (Harriott and Fisk 1987). Possibly due to their initial small size (e.g. <1mm) and preference for cryptic habitats, which makes *in situ*, underwater observations difficult, we were unable to find any studies that validate this widely accepted approach with naturally occurring, reef substrate data.

Research focusing on other key reef building organisms such crustose coralline algae and other calcareous encrusters also uses a wide range of readily available substrates including PVC poles, ceramic tiles, cattle ear tags and glass to assess reef growth and calcification questions (Bak 1976; Field et al. 2007; Mallela 2007; Kuffner et al. 2013; Mallela 2013; Hepburn et al. 2015; Roik et al. 2015). Whilst many of these studies claim to measure the 'natural range and variability' of growth and calcification there is very little literature available to validate or ground-truth these findings. The few validation studies and observations we found were based on Caribbean reefs. Early studies using experimental PVC substrates on reefs to investigate the growth of encrusters noted that PVC, if deployed vertically on the reef surface, or at angles mimicking dead branching coral (*Acropora palmata),* favoured recruitment by crustose coralline algae (Adey and Vassar 1975; Bak 1976). Adey and Vassar (1975) observed how coralline overgrowth occurred more slowly on naturally occurring dead coral branches, when compared to PVC. This was attributed to the more uniform PVC surface being more conducive to coralline settling while not initially providing a good holding surface for mobile reef organisms (e.g. crab and worms), organisms that presumably could hinder early settlement and growth. Both studies (Adey and Vassar

1975; Bak 1976) noted how PVC substrates were devoid of shaded (cryptic) reef elements, in particular crustose coralline algae species (e.g. *Neogoniolithon accretum*), encrusting foraminifera and bryozoa. Organisms commonly occurring on the cryptic, dead bases of reef building coral colonies (*Montastrea annularis)*, accounted for 12 and 8 % of encruster cover respectively but were not observed on vertical PVC (Bak 1976). Another 12 month study at 10m on Jamaican fore-reefs ground truthed findings and found that cryptically orientated, unglazed, ceramic tiles had similar encruster assemblages when compared to the underside of adjacent platy corals (Mallela 2007). With cryptic (shaded) habitats on the reef estimated to account for up to two-thirds of the reef volume and 75% of total available reef space (Jackson et al. 1971; de Goeij and Van Duyl 2007) our results suggest that studies that rely on growth and calcification data sourced only from the outside surface of PVC poles overlook epibenthic communities that are characteristic of cryptic reef habitats (e.g. shaded overhangs and crevices) and indeed can make up the greater portion of the reef (Buss and Jackson 1979; Gischler and Ginsburg 1996). Such findings could result in misleading or biased reef growth interpretations.

Many experiments that utilise artificial substrates only deploy the substrates for a short period of time (e.g. < 1 year). As a result whilst providing data on settlement and initial growth rates they may not provide data on established or mature communities which are indicative of a large portion of *in situ* reef growth. Observations in St Croix, Caribbean (Adey and Vassar 1975) using PVC substrates at shallow depths (< 3m) noted that a one year deployment period is probably suitable to reach a climax state on substrates positioned on exposed algal ridges. In contrast, in shallow, cryptic habitats (e.g. 1-2 m reef pavement) several years growth on PVC may be required to reach a climax community. We suggest that studies should also consider what stage of growth and development they are measuring and note this in their comparisons, data extrapolations and interpretations.

Our findings add to a growing number of studies that demonstrate how encruster recruitment and benthic cover varies according to substrate type. However, another potential source of error in reef scape accretion models is when models upscale percentage cover data into reef accretion data (also known as calcification or calcium carbonate production). In order to determine how much calcium carbonate ($CaCO_3$) is being added to the reef the percentage cover of calcifying organisms is combined with organism specific calcification rates (linear extension and skeletal density) in order to calculate rates of $CaCO_3$ production g m^{-2} y^{-1} (Stearn et al. 1977; Hubbard et al. 1990; Mallela and Perry 2007). Due to the paucity of site specific growth rate data a number of encruster carbonate production studies and reef accretion models extrapolate growth rate and skeletal density data from other studies in order to estimate carbonate production (e.g. Pari et al. 1998; Hart and Kench 2007). By combining percentage cover data gleaned from natural or artificial substrates with encruster growth rate data extrapolated from other locations another level of error is potentially introduced into reef scape accretion models (Mallela 2013).

Results from this shallow microatoll study demonstrate that PVC poles and ceramic tiles (exposed and cryptic orientations) are characterised by different suites of epibenthic assemblages. Unfortunately, we were unable to validate our findings with data from natural reef settings. Our analysis of epibenthic reef communities on artificial substrates was fine-scale and required the use of a dissection microscope for the identification of small organisms (<1mm in diameter). We were unable to get this level of resolution from underwater, *in situ* observation during this study. Clearly if we are to extrapolate and upscale data from artificial substrates to answer ecosystem-development questions we need to know if our data is comparable to natural reef substrates. Ground-truthing of data in order to calibrate data sets obtained using different approaches and enable the extrapolation of inter-substrate data in a meaningful manner will be our next challenge.

In conclusion, the results presented here add to small, but growing body of settlement plate literature, indicating that settlement and growth of marine epibenthic communities on different artificial substrates may not be directly comparable (Harriott and Fisk 1987; Field et al. 2007). Key to these findings are that a specific substrate type, positioned in a certain orientation, may bias, promote or deter recruitment of specific organisms (see summary table 1). We know for example, that the outside of PVC poles and upwards facing ceramic tiles attached to the surface of the reef are unlikely to have epibenthic assemblages characteristic of cryptic reef habitats (e.g. foraminifera and bryozoan that overgrow the dead bases and undersides of coral colonies (Bak 1976; Mallela 2007). In spite of this, and due to a paucity of site specific information, such data sets are being extrapolated for use in reef development models without always noting their limitations. We suggests that data sets should be interpreted and extrapolated with care and any caveats made clear. The choice of substrate clearly depends on the research question. If the aim of the study is to use a substrate that promotes recruitment, growth and subsequent survival of your target organism, the ‘more is better’ approach, then this and other studies suggest PVC poles are ideal for initiating and propagating crustose coralline algae communities whilst ceramic tiles facilitate coral recruitment. However, without validation and ground truthing, such findings may be misleading if they are subsequently used as proxy for naturally occurring reef habitats and used to assess reef growth and/or carbonate accretion. Clearly artificial substrates are a useful, non-destructive and affordable tool in coral reef research. However, if they are to be to be used to model ecosystem development (e.g. sclerobiont growth and calcification) the choice of substrate and its orientation needs to be justified, methods should also be validated, and limitations noted.

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Figure Legends:

Fig. 1 Multidimensional scaling (MDS) ordination of Bray-Curtis similarities between epibenthic organisms recruiting to three artificial substrate types: cryptic tiles (C), exposed (E) tiles and PVC. The MDS is based on square root transformed benthic cover (%) data. The 2D stress value indicates that the plot is a good representation of multidimensional community similarity.

Fig. 2 Box-whisker plots detailing total percentage cover on cryptic ceramic tiles (TC), exposed ceramic tiles (TE) and PVC poles. The thick bar represents the median value, the interquartile range is represented by the box, and the full range as the whiskers, raw values given in Supplementary table 1. *Significant differences noted on graphs, to account for multiple comparisons a Bonferroni correction of alpha was applied where $p \leq 0.01$ was considered significant.

Table 1. A summary of studies that ground truth data on artificial substrates (PVC and ceramic tiles) with natural, in situ reef substrates

Artificial Substrate	Compared to natural reefs substrate	Findings	Location	Reference
PVC poles mimicking dead coral branches	Dead branching coral substrates	Coralline communities develop faster on PVC	St Croix	Adey & Vassar 1975
PVC poles in reef pavement	Reef pavement	PVC did not attract less dominant coralline algae species	St Croix	Adey & Vassar 1975
PVC poles attached to reef	Cryptic reef areas (e.g. undersides of corals/shaded locations)	PVC did not attract certain sclerobionts typical of cryptic reef substrates	Curacao	Adey & Vassar 1975,Bak 1976
Settlement plates	Natural reef substrate	Anecdotal evidence: no difference in crusts observed	Barbados	Martindale 1976
Cryptic settlement plates on reef	The underside of adjacent platy corals	No significant difference in community composition or benthic cover	Jamaica	Mallela 2007

Supplementary Material:

Supplementary Fig. 1 Multidimensional scaling (MDS) ordinations of matrices constructed from percentage cover of all benthic organisms recruiting to artificial substrates: cryptic tiles (C), exposed tiles (e) and PVC at each microatoll site (E11, E4 and K). Multivariate analyses does not find a site affect.

Supplementary Table 1. Descriptive statistics detailing epibenthic percentage cover (%) of key groups of benthic cover (raw data available on request).

Supplementary Table 2. Scheirer-Ray-Hare (SRH) test results

Supplementary Table 3. SIMPER analysis for comparison between epibenthic communities recruiting to three artificial substrate types: exposed tiles (e), cryptic tiles (c) and PVC poles. Analyses based on Bray-Curtis similarities, calculated from square-root transformed benthic cover data and the benthic categories listed in supplementary table 1. The low contributions cut-off was 90%. Abbreviations: Av.Diss: average Bray–Curtis dissimilarity between substrates; Diss/SD: average dissimilarity divided by its standard deviation; Contrib%: percentage contribution to average dissimilarity; Cum.%: cumulative percentage contribution to dissimilarity.

Figure 1

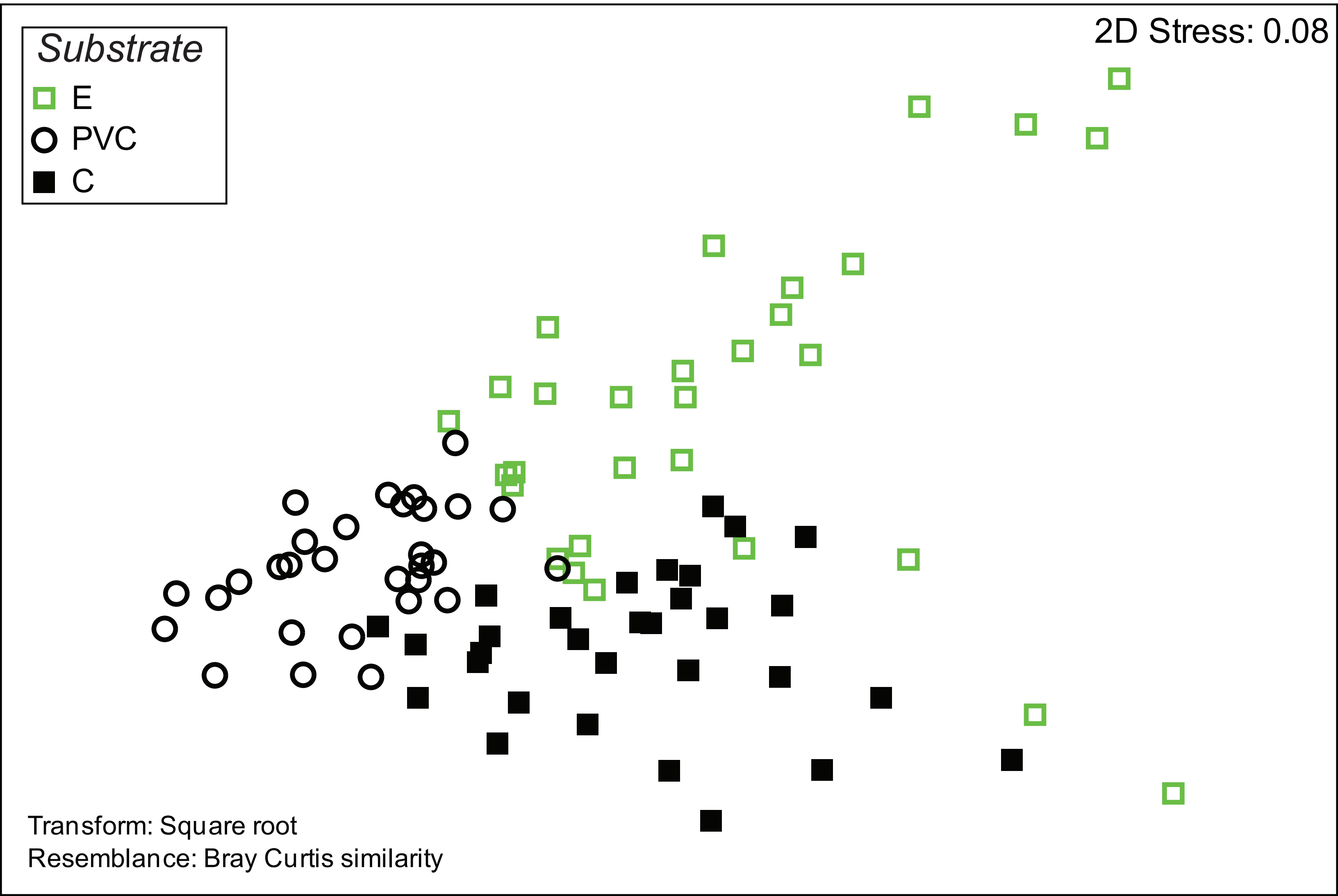
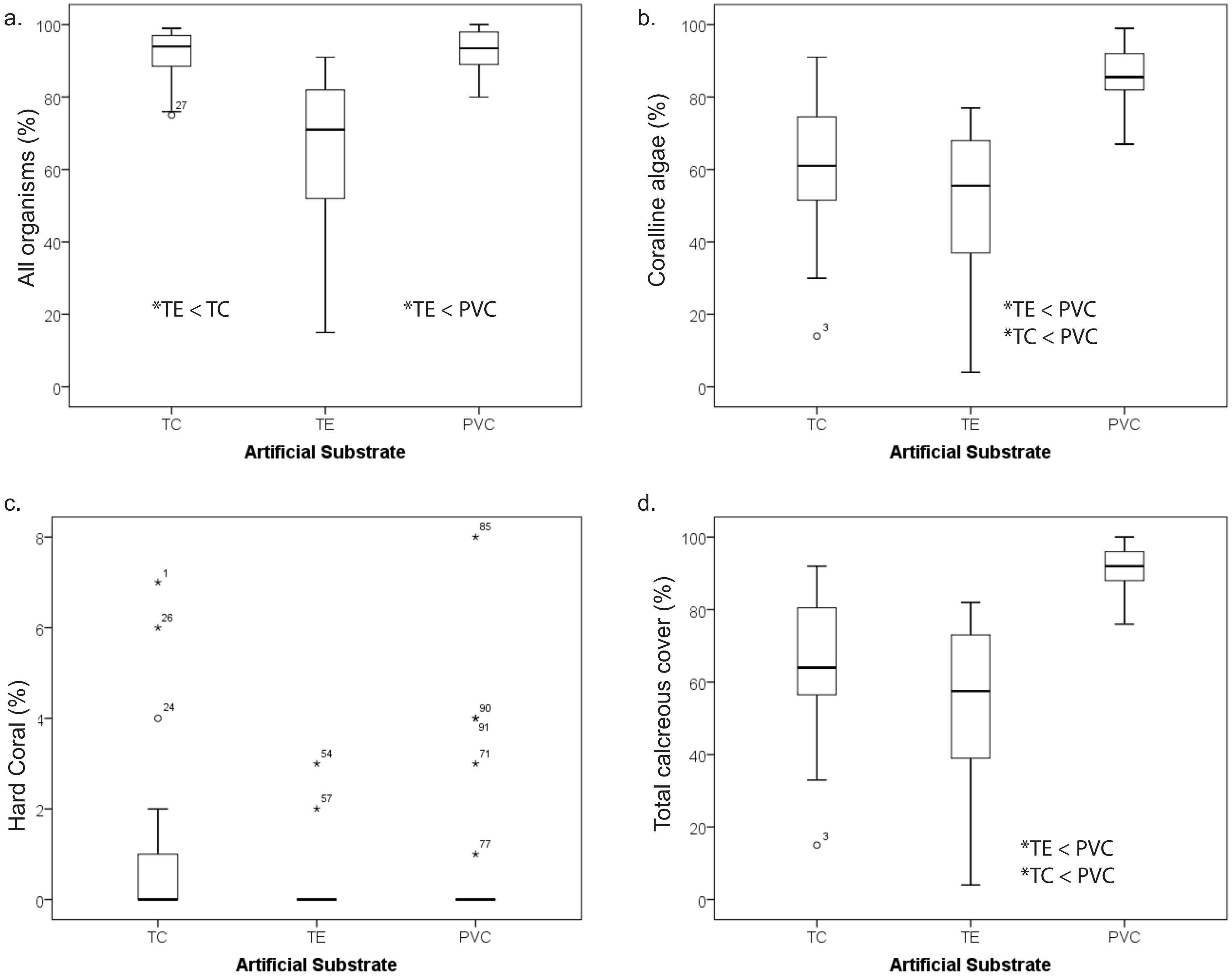

Substrate
E
PVC
C
2D Stress: 0.08
Transform: Square root
Resemblance: Bray Curtis similarity

Figure 2


a.
All organisms (%)
100
80
60
40
20
0
27
*TE < TC
*TE < PVC
TC
TE
PVC
Artificial Substrate
b.
Coralline algae (%)
100
80
60
40
20
0
3
*TE < PVC
*TC < PVC
TC
TE
PVC
Artificial Substrate
c.
Hard Coral (%)
8
6
4
2
0
1
26
24
54
57
85
90
91
71
77
TC
TE
PVC
Artificial Substrate
d.
Total calcreous cover (%)
100
80
60
40
20
0
3
*TE < PVC
*TC < PVC
TC
TE
PVC
Artificial Substrate

Supplementary Material Tables

Supplementary Material Fig 1