Effects of substratum on the growth and survivorship of *Montipora capitata* and *Porites lobata* transplants

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**Abstract**

Artificial transplantation of corals is a common method used to restore damaged or unhealthy coral assemblages. Though a number of studies have successfully transplanted coral fragments, there is no general consensus on the type of substratum to be used. The present study focused on the growth and survivorship of *Montipora capitata* (rice coral) and *Porites lobata* (lobe coral) fragments, which were transplanted onto different natural and synthetic substrata. No significant differences in coenosarc tissue growth or survivorship were observed between the species. Measurements after 184 days of growth, found transplant growth to be significantly higher on rhyolite breccia and amorphous coral skeletons than on black 'A'a lava. Nevertheless, no significant differences were observed between any of the other substrata. After 365 days of growth, survivorship was also observed to not be significantly different between substrata; with the only exception of being lower on glass substratum. It is hypothesized that success in a coral reef restoration project is largely determined by the actual coral fragmentation and transplantation process; as no distinct substratum affinity was observed for *M. capitata* and *P. lobata* transplants.

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**1. Introduction**

Coral reef deterioration has been reported worldwide (Birkeland 2004). The observed degradation in coral assemblages is a result of natural causes, anthropogenic effects, or a combination of the two. In terms of natural causes, extreme atmospheric disturbances can dramatically alter the structure of the assemblage by destroying large sections of the reef in a short period of time (Foster et al. 2011; Osborne et al. 2011). Large spikes in ultra violet radiation can also have a negative impact on coral assemblages, by directly damaging the DNA of zooxanthellae and corals (Anderson et al. 2001). Excessive solar radiation also raises the ambient temperature of the water, which frequently leads to the mortality of planulae (Aranda et al. 2011) and the expulsion of zooxanthellae in colonies (i.e. bleaching) (Lough and van Oppen 2009). In addition to damage caused by solar radiation, different forms of infection can also have a dramatic impact. Bacterial, fungal, or protozoan infections can lead to rapid tissue deterioration of colonies, which depending on the virulence of the pathogen, can also lead to significant deterioration of the whole community (Aeby et al. 2011).

In terms of anthropogenic effects, terrestrial runoff is one of the most detrimental effects. Terrestrial runoff, which can be a result of urbanization or changes in land use, often leads to sedimentation (Lee et al. 2006). Sedimentation leads to an increase in turbidity, which inhibits photosynthesis rates of zooxanthellae (Hunte and Wittenberg 1992). Furthermore, high levels of sedimentation can directly abrade and smother coral tissues (Jordan et al. 2010). This often leads to increased energy expenditure of the corals, which is a result of mucous production and using ciliary action to clear the sediment. In addition to causing sedimentation, anthropogenic pollutants and terrestrial runoff can also lead to hypernutritiation (Jessen et al. 2014). This often leads to inhibited coral growth that is the result of an imbalance in the exchange of nutrients between the zooxanthellae and the host coral (Dubinsky and Stambler 1996). Hypernutritiation also reduces light penetration due to nutrient-stimulated phytoplankton growth, which also reduces photosynthesis rates of corals (Woodward 2013). Most importantly, hypernutritiation also brings about a proliferation of seaweeds; which rapidly outgrow, smother, and eventually replace the slower growing corals (Vermeij et al. 2010). The effects of hypernutritiation on algal growth are further magnified, when there is a decline in herbivores due to overfishing (Stuhldreier et al. 2015). Due to all of these negative natural and anthropogenic factors, the slow natural-recovery process, and the high socio-economic value of coral reefs, various kinds of restoration efforts have been conducted.

Contemporary restoration efforts can be broadly classified as the artificial recruitment or artificial transplantation of corals (Ferse et al. 2013). Artificial recruitment of corals is promoted by planting an artificial object into a reef environment. This artificial object acts as a suitable substratum for the settlement, metamorphosis, and growth of planulae (Babcock and Mundy 1996; Petersen et al. 2005). Using a wide-range of
methods, the artificial recruitment rate and survival of planulæ have been studied on a number of natural (Norström et al. 2007) and synthetic substrata (Creed and De Paula 2007; Segal et al. 2012). Nevertheless, the results of these previous studies have shown a lot of variability in the rates of recruitment, density, and survival of planulæ on different substrata (Harriott and Fisk 1987). Due to the large variability in results, there does not appear to be a general consensus on a substratum to be used for the artificial recruitment of corals.

In the process of coral transplantation, a donor colony is first fragmented into smaller pieces. The resulting fragments are then artificially transplanted onto either a natural or synthetic substratum, and then moved into a suitable location in the marine environment for further growth (Edwards and Clark 1999). In previous studies, coral fragments have been transplanted onto substrata that can be commonly found in the reef environment, such as pieces of rubble (Tunnicliffe 1981; Smith and Hughes 1999), consolidated rock (Bowden-Kerby 2001), sand (Bowden-Kerby 2001), and dead coral colonies (Bruckner and Bruckner 2001; Yap 2004). A few studies have transplanted corals onto natural substrata that are less commonly found in the reef environment, such as the valves of dead Tridacna spp. (Cabaitan et al. 2008; Guest et al. 2011). Other studies have transplanted corals onto synthetic substrata such as concrete (Okubo et al. 2005; Herlan & Lirman 2008; Ferse 2010), cultured marble (Schlacher et al. 2007), steel (Romatzki 2014), and plastic (Shafir et al. 2006). In addition, a number of studies have even managed to grow fragments by suspending them in the water column, using wires instead of a solid substratum (Lindahl 2003; Soong and Chen 2003). Though a lot of previous studies have succeeded in managing to transplant juvenile corals, the survivorship of corals was seen to be variable among the different substratum (Table 1). As with substrata used for artificial recruitment, there does not appear to be a general consensus on a substratum to be used for the artificial transplantation of corals. This is due to the observed variability in the survivorship and growth of juvenile corals, which are important criteria in the evaluation of restoration efforts (Guest et al. 2011).

The purpose of this study is to evaluate the growth of coenosarc tissue and survival of Porites lobata and Montipora capitata fragments transplanted onto a range of natural and synthetic substrata. These two species were chosen because they are morphologically quite distinct; where M. capitata has a branched morphology, while P. lobata has a massive morphology. The growth of coenosarc tissue was measured, to determine if a substratum is favored for growth by either species; since the coenosarc is the polyp’s dermal tissue that connects it to the substratum and other polyps (Fig. 1), which tends to deteriorate if conditions for growth are poor (Mortensen 2001). The survivorship of transplants was also evaluated (Oren and Benayahu 1997), to determine whether any substratum is more favorable for survival than another. If transplants of a particular species exhibit a positive change in coenosarc tissue area (>50%), and have a high proportion of transplants surviving (>50%), then it will be deemed that the coral transplants have an affinity for the substratum type (i.e. substratum affinity); and that substratum will be deemed suitable for use in future coral assemblage restoration efforts.

2. Methods

2.1. Study site

The study was carried out in the coral nursery of Ñu‘enue Fisheries Research Center, Honolulu, Hawai‘i. This facility housed large circular tanks (12,000 L) that were built with a flow-through system, in which untreated seawater was pumped from the adjacent water body (Honolulu Harbor) into the tanks. Though using untreated seawater posed the risk of importing contaminants, the facility continued to operate using a flow-through system, in which healthy coral colonies were observed growing just a few meters away from the intake pipe of the tanks. Water...
circulated clock-wise in the tanks before exiting through a drain built into the center. Gas exchange rates at the surface of the tank mimicked a tide pool environment, as fresh seawater was pumped in at irregular intervals. Illumination of corals was variable, but was directly proportional to the rates of insolation reaching the location (21°18′14″ N, 157°52′15″ W) at any given time of the year, as no cover was provided for the tanks.

2.2. Preparation of substrata and coral transplants

To investigate whether a substratum affinity would be exhibited by transplants of Montipora capitata and Porites lobata, both natural and synthetic substrata were chosen for further study. Natural substrata chosen were ones that can be commonly found in the reefs around Hawai’i. Most of the natural substrata used were volcanic in origin and included: black ‘A‘alava, red ‘A‘alava, gray amygdaloidal basalt with large (>1.0 cm) calcite vesicles, gray amygdaloidal basalt with small (<1.0 cm) calcite vesicles, gray ignimbrite, brown rhyolite breccia, and white amorphous coral skeletons. Synthetic substrata chosen for further study included some of the more common and inexpensive types used in previous studies. The synthetic substrata selected included white ceramic, translucent glass, white cultured marble, and white porcelain tiles. The size of each substratum was variable, but ranged from approximately 10 × 10 cm to 18 × 18 cm in area. A total of four replicates were allocated.

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2.3. Data collection & analysis

Coral growth and survivorship was monitored using an underwater camera. Photographs of the coral transplants were taken upon initiating the experiment and 78, 184, and 365 days later. Once raw data was collected, the coenosarc surface area was measured using image processing software (ImageJ). Photographs were taken with a metric scale in view each time, to allow calibration of the measurement tool. Furthermore, each of the photographs were taken at a perpendicular angle to the substratum, so as to minimize parallax errors in the measurement of coenosarc tissue.

Changes in coenosarc tissue area were measured after 78 and 184 days of growth. It was not possible to measure coenosarc tissue area after 365 days of growth, because of the fusion of many transplants with adjacent ones of the same species. To account for pseudo-replication, measurements of individual transplants (N = 264) were averaged over their respective substratum before being analyzed. The measured changes in coenosarc surface area on different substrata were analyzed for homogeneity of variance after 78 days (Bartlett’s Test; p = 0.36) and 184 days (Bartlett’s Test; p < 0.05). Differences in coenosarc growth were compared between substratum and species using statistical software (R). A linear model was fit to the 78 day data; where the changes in coenosarc tissue area were explained by the 11 different substrata. A generalized least squares model was fit to the 184 day data (Pinheiro and Bates, 2016); where the changes in coenosarc tissue area were explained by the 11 different substrata, with the model variance being adjusted to account for inter-substratum differences in variance. Survivorship of P. lobata and M. capitata fragments were also measured by analyzing the photographs after 78, 184, and 365 days of transplantation. Survivorship data was fit to a binomial generalized linear model, and differences in mean survivorship were compared between substratum and species (ANOVA). If any models showed significant relationships initially, then they were subject to Tukey’s HSD test. A significance level of 5% was used for all statistical tests.

3. Results

3.1. Coenosarc growth of transplants

Upon initiating the study, the coenosarc tissue area for all of the coral transplants was relatively similar, with a mean and standard error of 1.61 ± 0.07 cm². 78 days after the transplantation of fragments, changes in coenosarc tissue area were observed to not be significantly different between species (ANOVA; F[1,63] = 1.04 × 10⁻³; p = 0.97). The largest mean change in coenosarc tissue surface area was 60.1 ± 8.0%; which was measured for transplants grown on amygdaloidal basalt with small calcite vesicles (Fig. 3, Panel A). In comparison, the smallest
mean change in coenosarc tissue surface area was 33.2 ± 6.5%; which was observed for transplants grown on porcelain tiles. Initial comparisons of coenosarc tissue growth on different substrata showed a significant difference between the means of the variables (ANOVA; \( F_{[10, 54]} = 2.05; \ p = 0.05 \)). Nevertheless, post hoc tests showed the results to not be significantly different between any of the substrata (Tukey’s HSD; \( p > 0.05 \)).

184 days after the transplantation of fragments, no significant differences in growth were observed between species (ANOVA; \( F_{[1, 43]} = 1.12; \ p = 0.29 \)). Yet, the change in coenosarc tissue area was observed to be variable among substrata (Fig. 3, Panel B). The largest change in coenosarc tissue surface area was 98.9 ± 6.1%; which was measured for transplants grown on rhyolite breccia. In contrast, the smallest measured growth was 53.2 ± 4.3%, which was observed for transplants grown on black ‘A’a lava. Results of statistical tests showed a significant difference in coenosarc growth between substrata (ANOVA; \( F_{[10, 54]} = 3.32; \ p < 0.05 \)). Results of Tukey’s HSD test showed significant differences in the mean growth of transplants on: amorphous coral skeletons and black ‘A’a lava (\( p < 0.05 \)); and rhyolite breccia and black ‘A’a lava (\( p < 0.05 \)). Nevertheless, no significant differences in the growth of transplants were observed in the other 53 substratum-substratum comparisons (Tukey’s HSD; \( p > 0.05 \)).

3.2. Survivorship of transplants

Upon initiating the experiment, all of the \textit{M. capitata} and \textit{P. lobata} fragments were alive; however, the survivorship of fragments on most substrata began to decline with time (Fig. 4). 365 days after transplantation, the survivorship of fragments was observed to not be significantly different between species (ANOVA; \( F_{[10, 54]} = 3.11; \ p = 0.08 \)). The highest mean survivorship was 100 ± 0%, which was observed for transplants grown on cultured marble tiles. Conversely, the lowest mean survivorship was 50 ± 17%, which was observed for transplants grown on glass tiles. Analysis of survivorship between different substrata showed a significant difference between the lower survivorship of transplants on marble and that on glass (ANOVA; \( p < 0.05 \)); though no significant difference was observed between the other substrata (\( F_{[10, 75]} = 3.11; \ p = 0.12 \)). Furthermore, results of post hoc testing showed no significant difference in survivorship between any of the substrata (Tukey’s HSD; \( p > 0.05 \)).

4. Discussion

4.1. Coenosarc growth of transplants

Throughout the duration of the experiment, growth of coenosarc tissue was observed to not be significantly different between \textit{M. capitata} and \textit{P. lobata}. This is an interesting observation, because these two species are morphologically quite distinct, but the relative change in coenosarc tissue of transplants was noted to be similar. In terms of substratum affinity, results of post hoc testing showed that no significant differences in coenosarc growth were observed after 78 days. After 184 days, coenosarc tissue growth was observed to be significantly higher on both rhyolite breccia and amorphous coral skeletons, than on black ‘A’a lava. The primary difference between these substrata is the relative surface rugosity. The rhyolite breccia and coral skeletons used in this study were relatively flat, while the ‘A’a lava had a complex surface. Thus, it is hypothesized that coral transplants have an affinity for flat surfaces; which are likely to support less sediment than highly rugose substrata. Nevertheless, no significant differences in coenosarc growth were observed between the other substrata, when subjected to a turn-wise comparison. Though not many empirical studies exist which measure the growth rates of coral transplants on different substrata, a number of coral recruitment studies can provide useful insight. Specifically, previous studies have shown that planulae will settle and metamorphose on a wide range of natural and artificial substrata, without exhibiting a strong substratum affinity (Harriott and Fisk 1987; Creed and De Paula 2007; Hata et al. 2013). The results of this study corroborate these observations; as coenosarc tissue growth was measured to be relatively large (>50%) on all substrata, without one substratum being distinctly favored above all others. Thus, it can be inferred that \textit{M. capitata} and \textit{P. lobata} are opportunistic organisms, which will settle and grow on a range of natural and synthetic substrata.

4.2. Survivorship of transplants

After the initial transplantation of \textit{M. capitata} and \textit{P. lobata} fragments, survivorship was observed to be 100%. Approximately 2.5 months following transplantation, it was observed that the mean survivorship of transplants had declined in 10 out of 11 substrata. Interestingly, analysis of field measurements showed that most of the decline in survivorship was a result of transplants detaching, and not algal overgrowth or tissue necrosis. In fact, out of all the transplant mortalities recorded, only 3 fragments (2 \textit{M. capitata}, 1 \textit{P. lobata}) were observed to be deceased and still attached to their respective substratum. Upon further investigation, it was noted that the living tissue of these fragments were subject to contact with marine epoxy, during the initial transplantation, which led to rapid tissue necrosis of the coral fragments. In terms of sources of mortality, Highsmith (1982) and Guest et al. (2011) also noted similar observations; where a significant number of their recorded mortalities were due to transplants detaching, and not due to microbial infection or algal overgrowth. Therefore, it appears that there is a tradeoff between using enough epoxy to prevent detachment of fragments, which is a major source of mortality, and using epoxy, which

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Fig. 3. Panel A shows the change in coenosarc tissue area of transplanted coral fragments, from day 0 to day 78. Panel B shows the same relationship, but from day 78 to day 184. Both panels depict the mean and standard error in coenosarc growth. The substrata labelling is as follows: “A” = ‘A’a lava (black); “B” = ‘A’a lava (red); “C” = amygdaloidal basalt (calcite vescicles > 1.0 cm); “D” = amygdaloidal basalt (calcite vescicles < 1.0 cm); “E” = ceramic tile; “F” = coral skeleton (amorphous); “G” = glass tile; “H” = ignimbrite; “I” = marble tile; “J” = porcelain tile; “K” = rhyolite breccia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
can be a source of mortality by itself. Nevertheless, alternative methods of attachment (e.g., steel/plastic wires) have been shown to cause significant tissue damage to transplants initially, which often leads to their mortality (Bruckner and Bruckner 2001; Forrester et al. 2013). Until a novel methodology for attaching coral transplants to substrata is created, it is argued that non-toxic marine epoxy resin is more suitable for coral assemblage restoration efforts than wires.

In terms of survivorship over time, the results of this study showed that the largest number of mortalities were recorded 78 days after transplantation; after which survivorship rates became more steady. Herlan & Lirman (2008) also noted the majority of their mortalities to occur within the first 8 weeks after transplantation, and then dramatically decline with time. The likely explanation for these observations is that after fragmentation and transplantation, the coral transplants need to adapt to their new environment. If the environment is suitable, then the fragments will start to grow coenosarc tissue to self-attach to the substratum. During this time, the coral transplants are subject to a range of metabolic stress; as they heal the injuries caused by the process of fragmentation, as well as growing extra tissue for self-attachment. When the coral transplants have fully self-attached, it can be interpreted that they have recovered from their initial injuries and adapted to their new environment; which is seen as a steady pattern in survivorship over time. The results of Herren et al. (2006) and Herlan & Lirman (2008), which this study corroborates, have shown that this event occurs approximately 2–12 weeks after transplantation.

The results of this study also showed that 365 days after transplantation, there were relatively few differences in survivorship of coral fragments grown on different substrata. The only statistically significant difference in survivorship was observed for transplants grown on glass and cultured marble. At the end of the study, all fragments attached onto glass had detached. Yap et al. (1998) also observed poor survivorship in coral transplants attached onto glass, when compared to other substrata. Though, given the fact that both of these substrata had a low surface rugosity, and no significant difference in coenosarc tissue growth was observed between them, the most likely explanation for the observed difference in survivorship is the amount of epoxy used. In other words, inadequate amounts of marine epoxy were used to attach fragments onto glass tiles, which resulted in a large number transplants detaching. Conversely, an optimal amount of epoxy was used for transplants attached onto marble tiles, which resulted in perfect survivorship. Nonetheless, 365 days after transplantation, no other significant differences in survivorship were observed between substrata. With the exception of fragments attached onto glass, the survivorship of transplants attached onto the other substrata was ≥ 70%. This is an important finding, because it also highlights the opportunistic nature of corals. In other words, the results of this study and the combined data of other studies (Table 1) have shown that corals do not seem to exhibit a distinct substratum affinity. Rather, most hermatypic corals seem to be opportunistic organisms, which will settle and grow on any solid substrata; as long as no other factors are acting upon it. Given the fact that fragments were obtained from only one colony of each species, and this study was limited to two species, this hypothesis needs to be further investigated in future studies.

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