



Reef Restoration Foundation

Fitzroy Island Coral Nursery Monitoring:

Methods and Results

April - October 2020



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Key messages

This document outlines the methods, analysis and results that answer one of the key questions for the Reef Restoration Foundation: How do different species perform in the nursery?

Data was collected in April, July and October 2020 to answer these questions in the Fitzroy Island nursery.

For *Acropora* species, net and relative growth was significantly different between 3 and 6 months in the nursery, but no one species was a stand out in terms of growth.

For *Pocillopora* species, growth after 3 months was similar, but after 6 months net growth of *P. damicornis* was approximately double that of *P. meandrina*. Relative growth of fragments was 54 – 61% after 3 months in the nursery and 193 – 288% after 6 months.

Predation and disease were rare and recorded on only a few fragments.

Bleaching affected a low proportion of fragments; <3% in April and July which was significantly less than 6% in October.

Percent tissue mortality of fragments differed among species, with *A. millepora* mortality significantly greater than other species. This species should be monitored carefully to see if better handling can improve *A. millepora* fragment survivorship rates or else discontinue the propagation of this species.

Slow initial growth rates in the Fitzroy Island nursery mimic patterns found at the Hastings Reef nursery, indicating fragment recovery from propagation and handling occurs before growth rates increase significantly.

Acropora species did not vary significantly in growth rates and therefore a broad assemblage of species from this genus should continue to be used in the nursery to enhance diversity during outplanting.

Temperature loggers added to the nursery should assist with interpretation of growth rates related to seasonal effects.

While differences were observed in *Pocillopora* growth among species in the nursery, other factors have led us to recommend discontinuing the propagation of corals from this genus.

We recommend the focus at the Fitzroy Island site is shifted to outplant monitoring, ensuring suitable substrate is available, and developing techniques for securing corals on the reef following the nursery stage, to ensure greatest benefit to reef restoration.

1 Objectives

The objective of this analysis was to determine whether different coral species perform better within the Fitzroy Island nursery. Coral fragments of different species were monitored in the nursery at the time of attachment to the nursery tree in April 2020, and again after 3 months (July) and 6 months (October).

Species performance within the nursery was assessed by comparing fragment mortality, fragment health (proportion of colonies with bleaching, predation, or disease recorded), and fragment growth (linear extension for *Acropora* species or surface area for *Pocillopora* species). Nine species were included in the analysis: *A. intermedia*, *A. millepora*, *A. muricata*, *A. nasuta*, *A. robusta*, *A. tenuis*, *A. yongei*, *P. damicornis* and *P. meandrina* (Figure 1).



Figure 1 The performance of *Acropora* and *Pocillopora* fragments were tested in the Fitzroy Island nursery: (a) *A. intermedia*, (b) *A. millepora*, (c) *A. muricata*, (d) *A. nasuta*, (e) *A. robusta*, (f) *A. tenuis*, (g) *A. yongei*, (h) *P. damicornis* and (i) *P. meandrina*.

2 Coral growth

2.1 Method

Coral growth was estimated for each species by measuring total linear extension (TLE; mm) for *Acropora* species or surface area (SA; mm) for *Pocillopora* species. Measurements were made for 5 fragments per genotype per species from nursery trees 1-10 and 25 fragments per genotype for nursery trees 11-20using Image J software. Photos used in growth analysis were taken at the time of attachment to the nursery, and again after 3 and 6 months resident time. To ensure consistency in measurements over time, lines were traced on the photos as a reference (Figure 2).

Initial fragment size varied among and within species; therefore the units of growth were standardized to allow comparisons among species and times into net growth and relative growth. Net growth was calculated as the difference in SA or TLE after 3 and 6 months in the nursery for each fragment. Relative growth was calculated as the mean percent change in SA or TLE after 3 and 6 months in the nursery for each fragment.



Figure 2 Acropora fragment photographed at (a) time of attachment (b) after 3 months, and (c) after 6 months in the nursery. Yellow highlighted portion is the measurement reference for total linear extension. *Pocillopora* fragment photographed at (a) time of attachment (b) after 3 months, and (c) after 6 months in the nursery.

2.2 Analysis

Variation in TLE and SA net growth and relative growth among species was assessed using a Gamma GLMM with log-link and fragment ID as the random effect, which accounted for its repeated use. The global models were defined as: Net growth (or Relative growth) ~ Species x Time in nursery + (1 | Fragment ID) and run using the glmer function in the *Ime4* package in R (Bates et al., 2015). The global models were then compared against all possible subsets of the model using the dredge function in the *MuMIn* package in R (Barton, 2020). The best model was considered that with the lowest AIC. The

Acropora relative growth model had convergence errors, likely due to small sample size for many species. Therefore, the best Acropora relative growth model was the simplest error-free model. Assumptions were verified by plotting residuals versus fitted values. Analysis of deviance was used to determine significance levels of main effects and Wald chi-square statistics are presented for GLMMs. We conducted post hoc Tukey-adjusted pairwise comparisons using the *emmeans* package in R (Lenth, 2020).

Some *Acropora* fragments experienced negative growth between sampling due to breakage, therefore a constant was added to the response variable in both analyses (88 in net TLE growth analysis, 46 in relative TLE growth analysis) to allow for negative net and relative growth and the need to transform the raw data to meet the assumptions of the analysis. Fragments were only included in the analysis if growth was measured at each of the three time points: n=53 for *P. damicornis*, n=25 for *P. meandrina*, n=11 for *A. intermedia*, n=103 for *A. muricata*, n=15 for *A. robusta*, n=24 for *A. tenuis*, and n=13 for *A. yongei*. *A. nasuta* (n=9) and *A. millepora* (n=2) were not included in statistical analysis due to low sample size.

2.3 Results

Pocillopora species

All *Pocillopora* species fragments experienced an average increase in growth after 3 and 6 months in the nursery (Figure 3).



Figure 3 Comparison of mean surface area (<u>+</u> standard error) in April (attachment to nursery), July (3 months in nursery) and October (6 months in nursery) 2020 for two *Pocillopora* species.

For *Pocillopora* net growth, the best fit model included a significant interaction between species and time spent in the nursery (Table 1; Figure 4). Net growth after 3 months in the nursery did not differ among the two species (range: $751 - 1099 \text{ mm}^2$; Tukey post hoc, p>0.05). Between 3 and 6 months in the nursery was a period of significant growth for both species, particularly for *P. damicornis* where net growth was approximately double that of *P. meandrina* (Figure 4; Tukey post hoc test p<0.05).

 Chi-square
 DF
 Pr(Chi-sq)

 Species
 14.0
 2
 <0.001</td>

 Time in nursery
 432.9
 1
 <0.001</td>

 Time in nursery x Species
 5.6
 2
 <0.05</td>

 Table 1 Gamma GLMM analysis of deviance table (Wald Type II Chi-square tests) for best-fit model predicting surface area net growth of *Pocillopora* coral fragments in the nursery.



Figure 4 Comparison of mean surface area net growth (<u>+</u> standard error) after 3 months and 6 months in the nursery for two *Pocillopora* species.

For relative growth, the best fit model included a significant interaction between species and time spent in the nursery (Table 2). Both species had similar relative growth (54 – 61% increase) after 3 months in the nursery (Figure 5). After 6 months in the nursery, relative growth was significantly greater for *P. damicornis* (288 \pm 18%) than *P. meandrina* (193 \pm 29%; Tukey post hoc test p<0.05; Figure 5).

р	edicting surface area relative	growth of Pocil	<i>lopora</i> coral	fragment	s in the nursery	•

Table 2 Gamma GLMM analysis of deviance table (Wald Type II Chi-square tests) for best-fit model

	Chi-square	DF	Pr(Chi-sq)
Species	1.9	1	>0.05
Time in nursery	423.7	1	<0.001
Time in nursery x Species	7.1	1	<0.01



Figure 5 Comparison of *Pocillopora* mean relative growth (% change <u>+</u> standard error) after 3 months and 6 months in the nursery for two *Pocillopora* species.

Acropora species

All species fragments experienced an average increase in growth after 3 and 6 months in the nursery (Figure 6).





For Acropora net growth, the best fit model included a significant effect of time spent in the nursery but no difference in net growth among species (Table 3; Figure 7). Net growth after 3 months in the nursery was 10 ± 1 mm, significantly less than 39 ± 2 mm after 6 months.

 Table 3 Gamma GLMM analysis of deviance table (Wald Type II Chi-square tests) for best-fit model predicting TLE net growth of Acropora coral fragments in the nursery.

	Chi-square	DF	Pr(Chi-sq)
Time in nursery	133.1	1	<0.0001



Figure 7 Comparison of total linear extensions (TLE) mean net growth (<u>+</u> standard error) of *Acropora* fragments after 3 months and 6 months in the nursery (all species used in analysis combined).

For relative growth, there was a significant effect of time spent in the nursery (Table 4) which did not differ among species (Table 4, Figure 8). After 3 months in the nursery TLE increased $16 \pm 2\%$; after 6 months TLE increased $52 \pm 3\%$ (Figure 8).

 Table 4 Gamma GLMM analysis of deviance table (Wald Type II Chi-square tests) for best-fit model

 predicting TLE relative growth of Acropora fragments in the nursery.

	Chi-square	DF	Pr(Chi-sq)
Time in nursery	374.4	1	<0.0001



Figure 8 Comparison of total linear extension (TLE) mean relative growth (% change <u>+</u> standard error) of *Acropora* species after 3 months and 6 months in the nursery (all species used in analysis combined).

3 Coral health

3.1 Method

Coral health was measured by recording the presence/absence of (1) bleaching (Figure 9), (2) predation, and (3) disease on nursery fragments for each species. Health measurements were made at the time of attachment to the nursery in April 2020, and again after 3 and 6 months.



Figure 9 A bleached fragment from the Fitzroy Island nursery.

3.2 Analysis

No statistical analysis was conducted on predation or disease data because instances of these occurring were very infrequent.

Variation in the proportion of fragments bleached among species and time in the nursery was assessed using a Binomial GLMM with logit-link and fragment ID as the random effect, which accounted for its repeated use. The global model was defined as: Bleached(0/1) ~ Species x Time + (1 | Fragment ID) and run using the glmer function in the *lme4* package in R (Bates et al., 2015). The global model was then compared against all possible subsets of the model using the dredge function in the *MuMIn* package in R (Barton, 2020). The best model was considered that with the lowest AIC; however this model and all others with species included had convergence errors, likely due to small sample size for many species and the large number of zeros in the data. Therefore, the best model was the simplest error-free model. Assumptions were verified by plotting residuals versus fitted values. Analysis of deviance was used to determine significance levels of main effects and Wald chi-square statistics are presented for the GLMM. We conducted post hoc Tukey-adjusted pairwise comparisons using the *emmeans* package in R (Lenth, 2020).

Fragments included in the bleaching analysis varied among some species between time periods, ranging from: n=71 for *P. damicornis*, n=25-26 for *P. meandrina*, n=11 for *A. intermedia*, n=8-9 for *A. millepora*, n=121-122 for *A. muricata*, n=9 for *A. nasuta*, n=19-26 for *A. robusta*, n=24-27 for *A. tenuis*, and n=15 for *A. yongei*.

3.3 Results

Disease was recorded once in July on an *A. muricata* fragment (white band disease), and twice in October on one *A. muricata* and five *P. damicornis* fragments (disease type not identified). No predation was recorded on fragments in April or July. In October four instances of predation were recorded, on two *A. muricata*, one *A. yongei* and one *P. damicornis* fragment.

Bleaching was recorded on some fragments each month but with very low numbers; including *A. muricata* and *A. nasuta* fragments in April, *A. nasuta* and *P. meandrina* fragments in July, and *A. intermedia*, *A. muricata*, *A. robusta*, *P. damicornis* and *P. meandrina* fragments in October. The best model indicated bleaching was significantly greater in October ($6 \pm 1\%$ of fragments bleached; Tukey post hoc p<0.05) compared with April (<3% fragments bleached) and July (<1% fragments bleached) when bleaching was significantly lower (Table 5; Figure 10). There was no difference in bleaching among species.

Table 5 Binomial GLMM analysis of deviance table (Wald Type III Chi-square tests) for best-fit model predicting presence of bleaching of coral fragments in the nursery.

	Chi-square	DF	Pr(Chi-sq)
Intercept	58.7	1	<0.001
Time	16.4	2	<0.001



Figure 10 Proportion of fragments bleached (<u>+</u> standard error) (a) in April (attachment to nursery), July (3 months in nursery) and October (6 months in nursery) 2020 for seven *Acropora* and two *Pocillopora* species (all species used in analysis combined).

4 Coral mortality

4.1 Method

Coral mortality was measured by estimating percent tissue mortality (0–100%) on each fragment for each species (Figure 11). Mortality estimates were made at the time of attachment to the nursery, and again after 3 and 6 months.



Figure 11 Nursery fragment with 10% mortality.

4.2 Analysis

All statistical analysis was conducted in R version 4.0.2 (R Core Team, 2020). Variation in mortality among species and over time was assessed using a zero inflated beta-regression using the *gamlss* package in R (Rigby & Stasinopoulos, 2005). Percent mortality data was converted to proportions (range: 0-1) to meet the response variable structure required for beta regression. The global model was defined as: Mortality ~ Species x Time in nursery. Assumptions were verified by plotting residuals versus fitted values. Post hoc tests are not available in the *gamlss* package.

The number of fragments included in the mortality analysis varied among species but were consistent across all three times: n=57 for *P. damicornis*, n=25 for *P. meandrina*, n=11 for *A. intermedia*, *A. millepora* (n=8), *A. nasuta* (n=9), n=117 for *A. muricata*, n=19 for *A. robusta*, n=25 for *A. tenuis*, and n=15 for *A. yongei*.

4.3 Results

Overall % mortality of fragments differed among species but not survey times. Mortality of *A. millepora* fragments was $18.5 \pm 7.7\%$, which was significantly greater than all other species where mean mortality ranged between 0.4 and 7.7% (Figure 12).

Table 6 Parameter estimates and goodness of fit statistics for the zero inflated beta-regression best-
fit model predicting % mortality of coral fragments in the nursery. P-values represent significance for
each species compared to reference species A. millepora.

	Estimate	SE	t-value	p-value
Intercept	0.49	0.43	1.14	0.26
A. intermedia	-1.81	0.57	-3.16	<0.01
A. muricata	-1.45	0.45	-3.22	<0.01
A. nasuta	-1.68	0.65	-2.60	<0.01
A. robusta	-1.98	0.50	-3.97	<0.0001
A. tenuis	-1.61	0.50	-3.20	<0.01
A. yongei	-1.61	0.51	-3.17	<0.01
P. damicornis	-1.76	0.54	-3.27	<0.01
P. meandrina	-1.85	0.57	-3.24	<0.01



Figure 12 Percent mortality of seven *Acropora* and two *Pocillopora* species fragments (all survey times combined).

5 Lessons learnt

Analysis of the first 6 months of data from the Fitzroy Island nursery provide valuable lessons in how to improve monitoring and data collection within nurseries, and where future effort should be focused. The following outcomes and lessons should be considered:

- The slower growth rates during the initial 3 months for all species in the Fitzroy Island nursery was also a pattern found in in the Hastings Reef nursery. The similarity between sites in growth rates occurred despite a shift in time of year of the study, which suggests the slower growth rates may not be related to water temperatures. Temperature loggers added to the nurseries at both locations will assist in the interpretation of growth and fragment condition linked to seasonal effects.
- The consequences of fragment handling and manipulation when fragments are placed in the nursery have also been raised as potentially impacting early growth rates. Other studies into restoration and coral propagation have noted delayed growth during a 'recovery phase' following fragmentation stress which usually dissipates after 6 weeks (Goergen et al., 2018; Lirman et al., 2010). Ongoing guidance from new RRF staff with coral husbandry expertise should help further curb these impacts. The ongoing study at Fitzroy Island into the growth rates and fragment condition between growing tips and basal branches of *Acropora* species will also provide valuable insights into whether targeting certain portions of a coral colony will enhance growth rates, especially reducing the lag in growth over the initial weeks in the nursery.
- We recommend discontinuing the propagation of *Pocillopora* corals at the Fitzroy Island location based on a number of factors including the results found in this study. *P. damicornis* broods and broadcast spawns as a mode of reproduction over monthly cycles (Schmidt-Roach et al., 2013). This species may therefore have a greater chance to re-populate open reef space if a local population is still present in the area. Focus on other important reef building species from the genus *Acropora* will provide greater ecological value as well as faster returns in the nursery. *P. meandrina*, a broadcast spawning species, may require assistance if this species has been lost from a disturbance. However, this study found *P. meandrina* was slower growing in the nursery which makes it less favourable for propagation where high growth rates are considered an important factor in the nursery. The slower growth rates of *P. meandrina* reflect its adaptation to exposed reef fronts with high wave energy where it takes on a flattened and compact form and only commonly found (C. Veron pers. comm.).

Assessing the historical local coral assemblage for a reef prior to a major disturbance should drive the species considered appropriate for local propagation to assist in reef recovery. At Fitzroy Island, corals from the family Pocilloporidae were not a significant portion of the local coral assemblage, further suggesting these species should no longer be added to the nursery in order to free up space for more appropriate species for the outplanting site.

• No significant differences in growth rates were detected among the *Acropora* species in the nursery. We recommend continuing with a broad selection of *Acropora* growth forms and species in order to provide a diverse coral assemblage at outplanting in the area. It would be advantageous to maintain some of the same species studied here in order to compare growth rates, mortality and bleaching/disease over multiple years and varying times of year if measurements in the nursery are to continue.

- The significantly higher mortality of *A. millepora* does suggest it may be more sensitive to stress of fragmentation or handling when placed in the nursery. This species may need to be avoided when propagating but current work to reduce fragment handling stress could mitigate these losses. We recommend continuing to collect *A. millepora* for the time being with due care when handling and continued monitoring to assess if mortality rates decline.
- Overall, we recommend a greater focus on resources be shifted to outplant monitoring at the Fitzroy Island site, ensuring suitable substrate is available, and the technique for securing corals on the reef following the nursery stage to ensure greatest benefit to reef condition.

References

- Barton, K. (2020). *MuMIn: Multi-Model Inference. R package version 1.43.17.* <u>https://CRAN.R-project.org/package=MuMIn</u>.
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using Ime4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01
- Goergen, E. A., Ostroff, Z., & Gilliam, D. S. (2018). Genotype and attachment technique influence the growth and survival of line nursery corals. *RESTORATION ECOLOGY*, 26(4), 622-628.
- Lenth, R. (2020). *emmeans: Estimated Marginal Means, aka Least-Squares Means. R* package version 1.5.0. <u>https://CRAN.R-project.org/package=emmeans</u>.
- Lirman, D., Thyberg, T., Herlan, J., Hill, C., Young-Lahiff, C., Schopmeyer, S., Huntington, B., Santos, R., & Drury, C. (2010). Propagation of the threatened staghorn coral Acropora cervicornis: methods to minimize the impacts of fragment collection and maximize production. *Coral Reefs*, 29(3), 729-735.
- R Core Team. (2020). *R: A language and environment for statistical computing. R Foundation for Statistical Computing.* Vienna, Austria. Retrieved from <u>https://www.R-project.org/</u>
- Rigby, R. A., & Stasinopoulos, D. M. (2005). Generalized additive models for location, scale and shape, (with discussion). *Applied Statistics*, 54(3), 507-554.
- Schmidt-Roach, S., Lundgren, P., Miller, K. J., Gerlach, G., Noreen, A. M., & Andreakis, N. (2013). Assessing hidden species diversity in the coral Pocillopora damicornis from Eastern Australia. *Coral Reefs*, 32(1), 161-172.