Effects of outplant size on *Acropora palmata* fragment survivorship, growth, and condition

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Introduction

Outplanting nursery-reared corals has become an important tool for reef restoration. Though improved performance by larger coral fragments seems likely, the decision to outplant smaller or larger fragments is often not a clear choice, given the additional investment in production for larger sizes. Much effort in the past few years has addressed such questions for *Acropora cervicornis* restocking, but *Acropora palmata* culture at similar scales has lagged significantly. In order to better understand differences in growth and condition of *A. palmata* outplants, we utilized "small" and "large" nursery-reared fragments across three reefs in the upper Florida Keys (Figure 1). This report documents growth, predation, bleaching status, and mortality between two size treatments of outplants between June and November of 2014. Our study sites experienced an intense bleaching event during the summer of 2014[1] (Williams et al. 2015), and also provided a comparison of bleaching resiliency and resistance between size treatments of outplants.

Methods

We outplanted 126 pairs of "large" and "small" size treatments of *A. palmata* fragments across three replicate fore-reef sites in May 2014 (from here "outplants" and "fragments" are used interchangeably). These size treatments were based on standard nursery practices for fragmenting size and season for in-nursery propagation. Fragments of a single genet (originally collected from Snapper Ledge) were propagated in Coral Restoration Foundation offshore nurseries on nursery PVC "trees," and as a result fragments were free of lesions at the time of outplanting. Blocked replicates were outplanted (small and large paired fragments, Figure 2) in order to standardize environmental variation within sites. Large outplants ranged from 76.0 - 210.3 cm^2 [107.7 cm^2 ± 2.4 (mean ± standard error) skeletal area index, see below], and small outplants from 13.4-75.5 cm^2 (51.3 ± 1.3 cm^2 skeletal area index) (Figure 3).

Depths of outplants at the three sites ranged from 19-24 ft at French reef, 14-19 ft at Molasses reef, and 12-17 ft at Pickles reef (Figure 4). The depth of each fragment was measured, as it was expected to be an important covariate to growth. An initial survey to measure starting size and condition was completed in June (survey 01), and two intermediate condition check surveys were conducted in August and September. An additional final survey was carried out in November 2014 (survey 04) to measure size and condition. Size was measured in length (longest dimension), width (axis perpendicular to length) and height with a ruler *in situ*. Condition was evaluated at all four surveys and included a visual estimate of percent live tissue cover, degree of bleaching and counts of the corallivorous snail *Coralliophila abbreviata.* Snails found feeding on outplants were removed at each survey and hence, these four surveys are considered independent replicates for the purposes of statistical analysis (2-way ANOVA on ranks). Bleaching status was scored as None, Partial Paling (part of the fragment had pale coloration), Pale (all of the fragment had pale coloration), Part Bleached (part of the

[1] Williams DE, Miller MW, Bright AJ, Pausch RE (2015) Quick Look Report: 2014 *Acropora palmata* bleaching event in the upper Florida Keys. Protected Resources and Biodiversity Division Report PRBD-2015-02, NOAA SEFSC, Miami, FL, p 22.

fragment had white but alive tissue), or Bleached (all of the live tissue of the fragment was white).

When comparing growth between size treatments, only fragments that maintained at least 95% live tissue cover at all surveys were included. Of the initial 252 fragments, 110 were utilized for growth analysis (exclusions included 10 missing, 75 dead, and 57 with less than 95% live tissue cover). Growth was calculated in two ways: summed linear extension (LE) and change in skeletal area index (SAI). Summed LE was calculated as the sum of change in length, width, and height (cm) of skeleton, and expresses summed growth in each single dimension. Skeletal area index (cm^2) was calculated as the average of the length, width, and height of skeleton, squared, estimating growth in two dimensions (i.e. projected area, but utilizing a mean dimension including height). In this exploratory report, the change in these two growth parameters (summed LE and SAI) between June and November is reported in three ways:

1) **Percent change** is the percent change from the original size of fragments, and is a common way to measure growth.

2) **Absolute change** is the simple increment observed (cm^2 for SAI or cm for summed LE) from survey 01 to survey 04.

3) For large fragments only: **Adjusted change** is the large fragment's absolute change multiplied by the initial percentage of SAI of the corresponding small fragment. That is, if a small fragment's initial size was half of its paired large fragment, the large fragment's absolute change in SAI or summed LE was multiplied by 50%. This adjusted metric is intended to scale growth in comparing between the two size treatments to account for the greater initial investment involved in producing the larger fragment.

ANCOVAs tested for significant effects of depth (covariate) and size treatment on the dependent growth metrics.

Results

Growth

Growth in terms of both change in SAI and summed LE was highly correlated with depth, which had a significant effect on every growth metric examined (Table 1). When examining percent change in both SAI and summed LE, there was no statistically significant difference between size treatments, but small fragments showed a pattern of higher values across sites (Figure 5). There was no significant difference between treatments in change in SAI nor summed LE (Figures 6 & 7); however, small fragments tended to have higher values of change when compared to large fragments' adjusted values. Overall, outplants grew more at Pickles and Molasses reef, which were shallower than French (Figure 4). Because both change in SAI and summed LE demonstrated similar response patterns, only SAI is used from this point on when discussing size of corals.

*Table 1. ANCOVA results for different growth metrics using SAI and summed LE values. *=p<0. 01, and ns denotes no significance. Resulting values are shown in Figs 5-7.*

Growth metric	SAI Factors			Summed LE Factors		
	Depth	Size	Interaction	Depth	Size	Interaction
% change	*	ns	ns	*	ns	ns
absolute change	*	ns	ns	*	ns	ns
adjusted change	*	ns	ns	*	ns	ns

Mortality

Initial outplant size did not have a significant effect on mortality. Over the 6 month monitoring period there was no difference in the number of large (42) and small (33) fragments that died (Table 2; X_1=0.260, p=0.610). Additionally, initial size distribution of surviving fragments (76.5 ± 32.7 cm^2, mean SAI ± standard deviation) did not differ from that of the dead (87.5 ± 40.6 cm^2) fragments (K-S two sample, p=0.10). That is, initial size treatment, regardless of size range within size treatments, did not affect mortality counts. The most mortality at Molasses occurred in late August and early September, earlier in the bleaching event than Pickles, where the majority of dead fragments were observed after the September 19th survey (Figure 8). However, cause of death could not always be linked to bleaching.

Table 2. Percent of outplants with complete mortality from May-Nov 2014, classified by size treatment and site.

	French	Molasses	Pickles
Large	16.2	46.5	35.6
Small	22.4	31.6	26.3

Prevalence of snails

A two-way ANOVA compared the percentage of fragments with snails (i.e., prevalence) between size treatment and sites (Figure 9), with surveys considered as independent replicates, given that all observed snails were removed from each fragment at every survey. These data conformed to parametric statistics assumptions after transformation to ranks. Both size and reef site factors were significant (p=0.004 and 0.002, respectively) in this analysis, with Pickles having significantly higher prevalence than the other sites, and large fragments having significantly higher prevalence than small (p<0.05, Holm-Sidak post-hoc pairwise tests). Interaction between site and treatment was not significant (p=0.676).

Bleaching

All outplants started with 100% unbleached, live tissue. Bleaching status ranged from completely healthy ("None") to completely "Bleached." Small and large fragments displayed similar patterns of bleaching within sites (Figure 10), and showed no significant difference between size treatments across all surveys (X_4=5.582, p=0.233). Bleaching intensity varied with time (Figure 11) and paling was most prevalent during the September survey (Figure 12).

Conclusions

Our original expectations of improved performance by large fragments were not met. Growth, mortality, and bleaching were similar between size classes (with a non-significant suggestion of poorer performance by large fragments). The effects of snail corallivores were significantly worse on large fragments (prevalence and mean snails per fragment).

Overall, coral growth, whether in terms of summed linear extension or skeletal area index, had similar absolute increase between the large and small *A. palmata* fragments of this study. This could be because in this study some coral tended to grow only along a single main axis, so even though larger outplants had a greater circumference with more apical tips, growth rates were almost equal between treatments. The higher initial surface area of large fragments would explain why smaller fragments tended to have larger percent increases; a similar increment of growth appears greater when divided by a smaller initial denominator.

Not surprisingly, growth was strongly related to depth. Fragments at French were planted deeper and grew significantly less than fragments at Molasses or Pickles, which exhibited similar amounts of growth. Since there was little overlap with the depths of fragments at French as compared to the other sites, it is also possible some sort of site-specific effect slowed outplant growth at French.

Despite lower growth rates, greater depth may convey other benefits to outplants. Damage from physical disturbance is certainly expected to be worse at shallow depths, though no significant physical damage was observed in this study. Depth may have helped the Molasses and French fragments avoid total bleaching during the 2014 summer bleaching event, although other observations of *A. palmata* bleaching during this time indicated that depth was not a factor[1] (Williams et al. 2015). However, Pickles, the shallowest site (and expectedly the warmest), experienced the most severe bleaching overall, though there seemed to be little difference between small and large fragments. Both size treatments resisted bleaching and recovered in similar proportions at each site. Since only one genet was outplanted at all sites, differences in bleaching states across the sites can be attributed to environmental, rather than genetic factors.

In addition to larger fragments having no significant advantage in growth, survival, nor bleaching resistance, large fragments exhibited a higher prevalence of snails than smaller outplants at the times of our surveys. The observations for the 6 month time scale presented here suggest that while outplanting larger fragments may give slightly more absolute growth, the difference is not significant. This minimal difference paired with the extra time and resources needed to rear larger corals in a nursery indicates that smaller fragments may be the more efficient choice. The small size treatment tested in this experiment provided a similar or greater yield of coral area (depending on metric used) for lesser initial investment in production, and is thus recommended for outplants under similar conditions.

Acknowledgments

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Figures

Figure 1. Map of three outplant sites, each with ≈42 of "small" and "large" Acropora palmata *fragments. Source: 25˚02'48.71"N, 80˚22'59.29"W. Google Earth. December 16, 2014. June 17, 2015.*

Figure 2. Acropora palmata *fragments were outplanted in pairs as shown here with a "small" fragment (left; 11.0 cm long) and a "large" fragment (right; 19.5 cm long).*

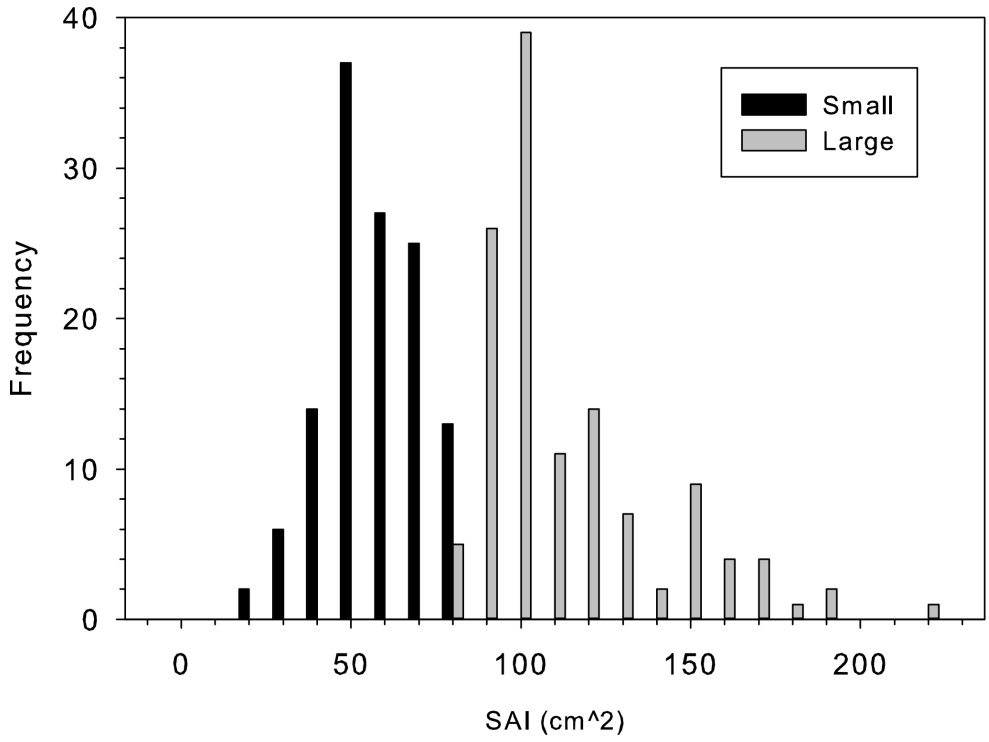

Figure 3. Histogram with frequencies of "small" [<75.5 cm², 51.3 ± 1.3 cm² (mean ± standard error)] and "large" outplanted Acropora palmata *fragments (>75.5 cm², 107.7 ± 2.4 cm²). Skeletal area index (SAI) is an estimate of projected area of the fragment (see methods for explanation).*

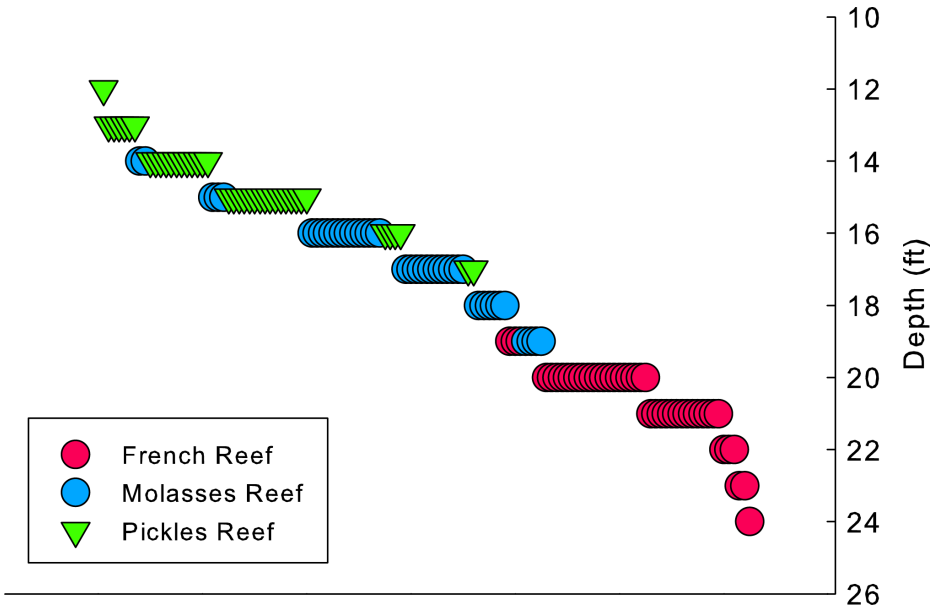

Figure 4. Distribution of depths of outplanted Acropora palmata *fragments at three reef sites. Horizontal axis is unitless; symbols are offset to indicate individual fragments.*

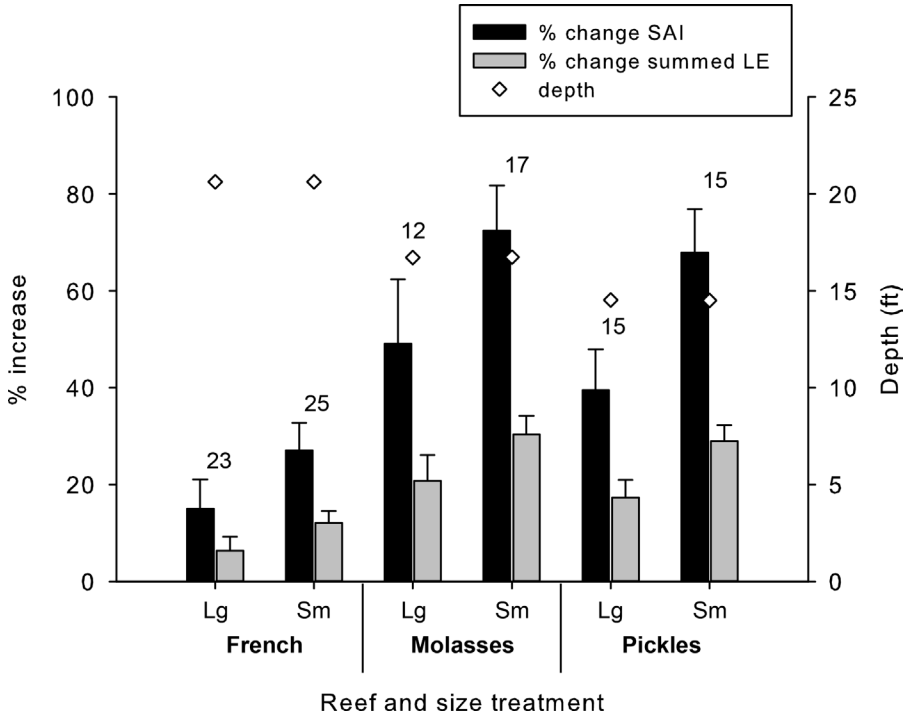

Figure 5. The average percent change in skeletal area index (SAI; black) and summed linear extension (LE; gray) from original fragment size. Average depth for each group is shown (diamonds; right axis); number of samples included in analysis given over bars. Error bars represent one standard error.

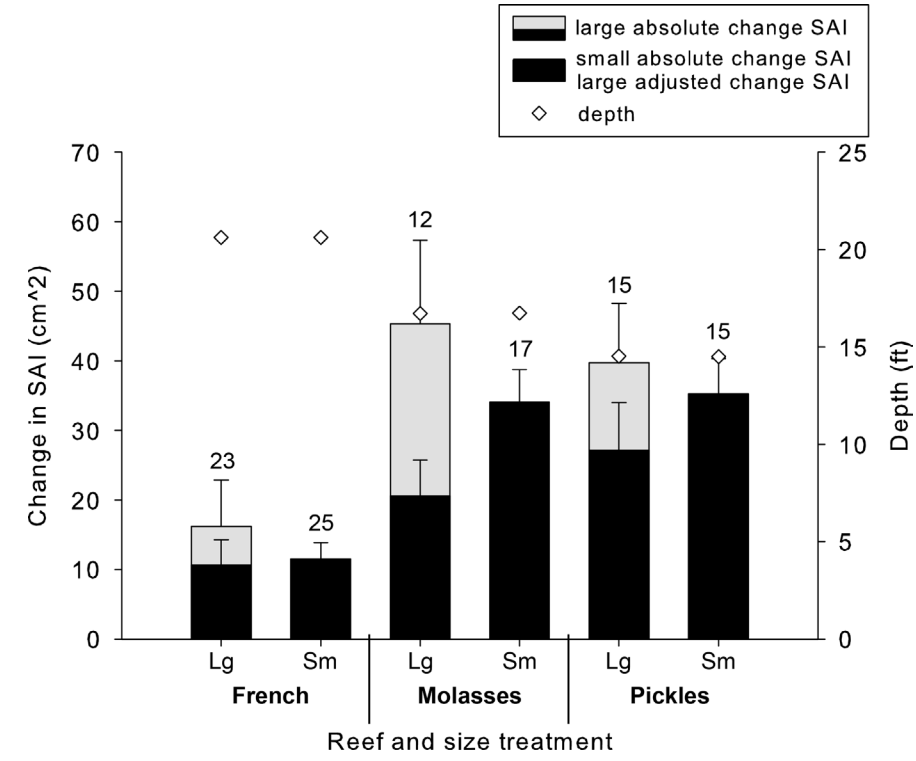

Figure 6. Average absolute change in skeletal area index (SAI), shown as black bars for small fragments. For large fragments, absolute change shown as the sum of gray and black bars, with black bars depicting percentage of absolute change scaled to the area of corresponding small fragment (for "adjusted change" definition, see Methods). Error bars represent one standard error, number of fragments included in analysis given above bars, and depth (diamonds) on right axis.

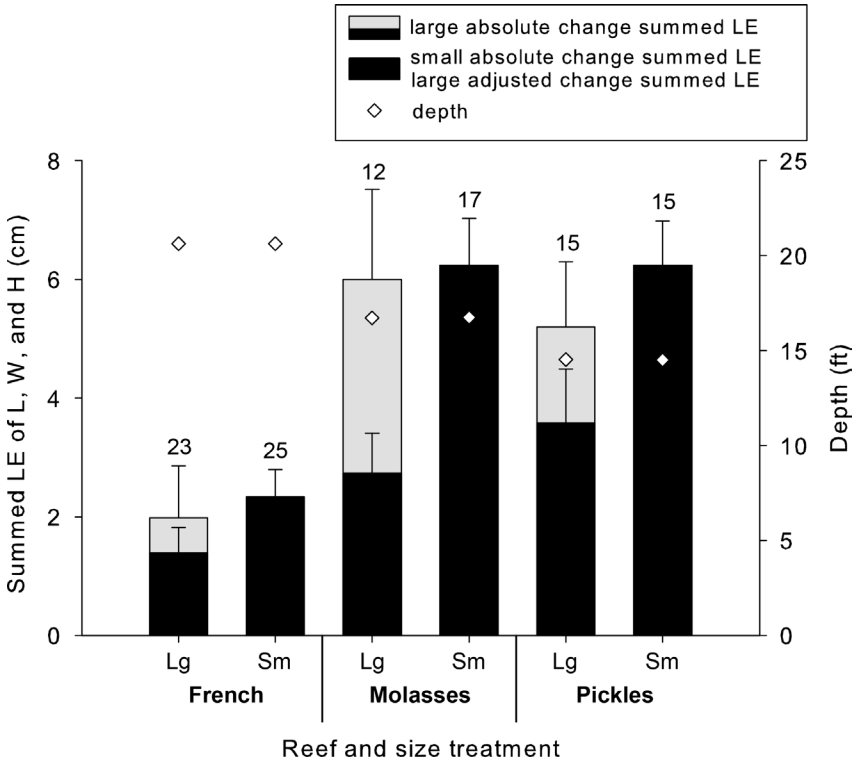

Figure 7. Average absolute change in summed linear extension (LE), given as black bars for small fragments. For large fragments, absolute change shown as the sum of gray and black bars, with black bars depicting adjusted change, or percentage of absolute change scaled to the area of corresponding small fragment (for "adjusted change" definition, see Methods). Error bars represent standard error, number of fragments included in analysis given above bars, and depth (diamonds) on right axis.

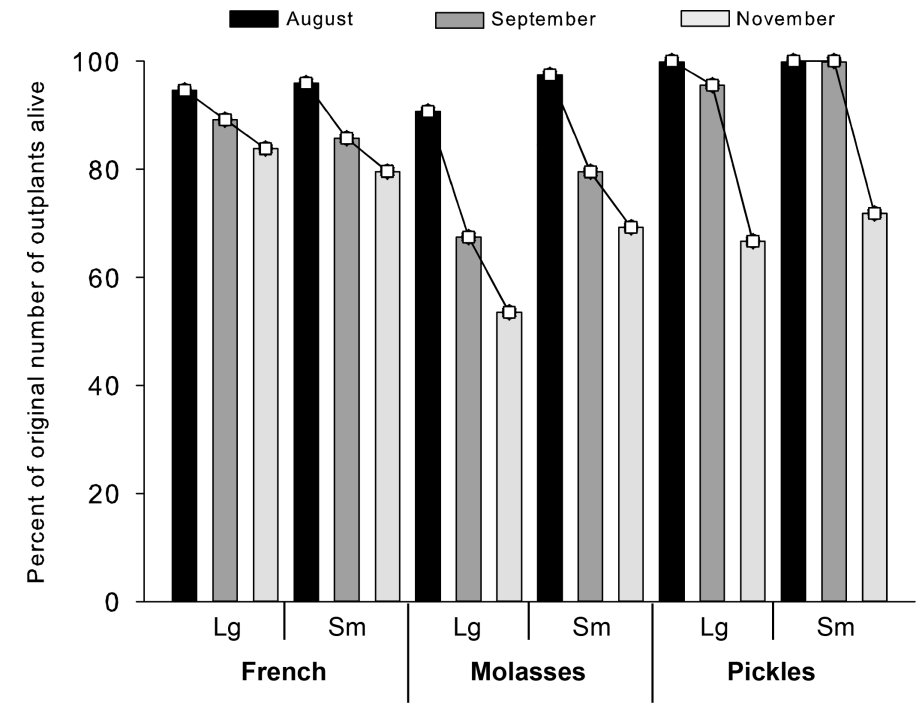

Figure 8. Survivorship expressed as the percentage of original number of outplanted fragments. Different colors denote time of individual survey, not mortality; line graph follows change over time. Size treatment and site on x axis.

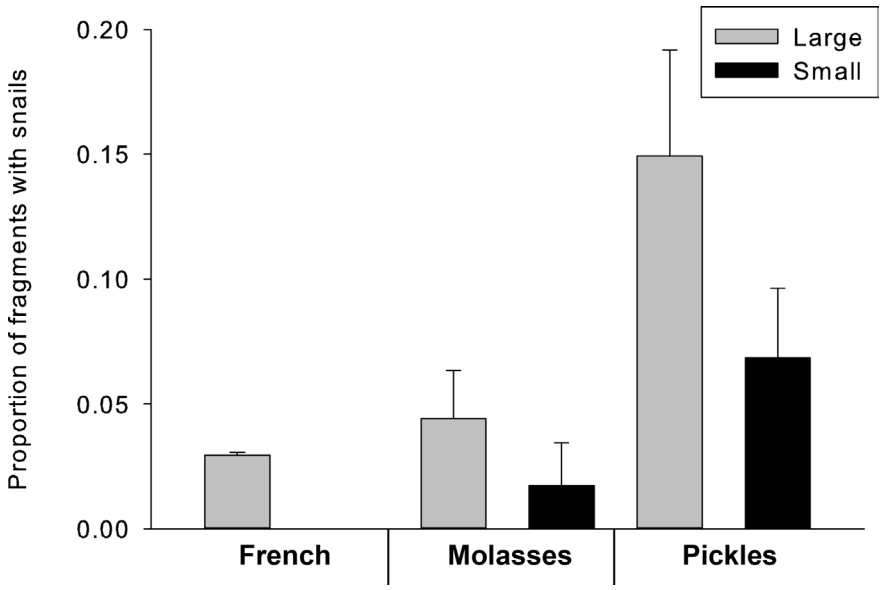

*Figure 9. Mean snail (*Coralliophila abbreviata*) prevalence (percent live fragments with snails; + 1SE). Both site and size had significant effects on snail prevalence.*

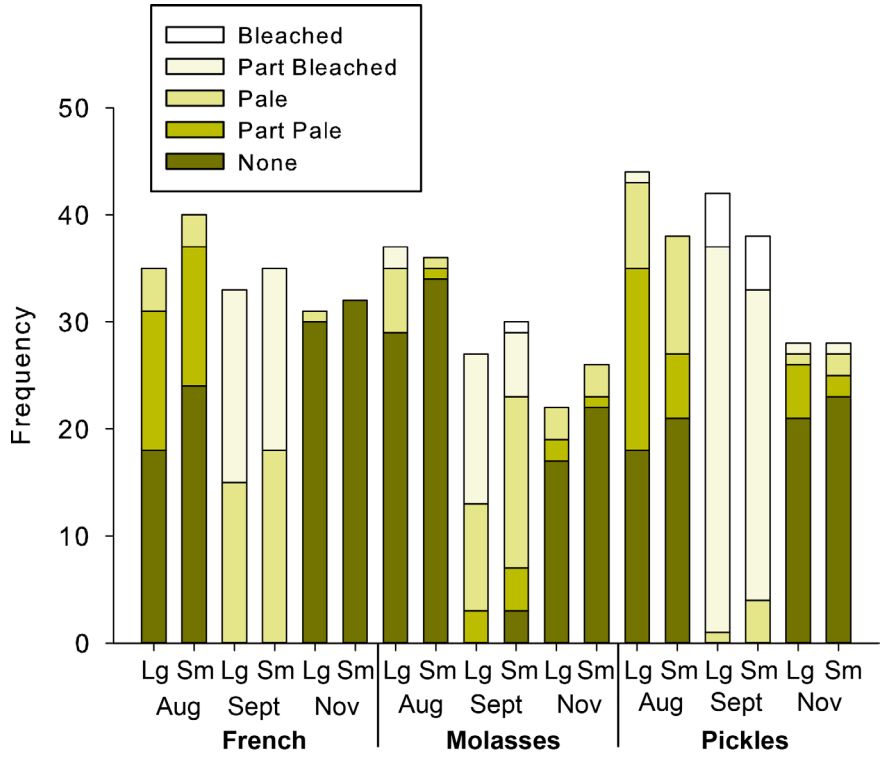

Figure 10. Frequency of bleaching condition occurrence among live Acropora palmata *fragments at the three sites, with size treatments and survey months listed. No fragments displayed paling or bleaching initially (May 2014; Fig 11).*

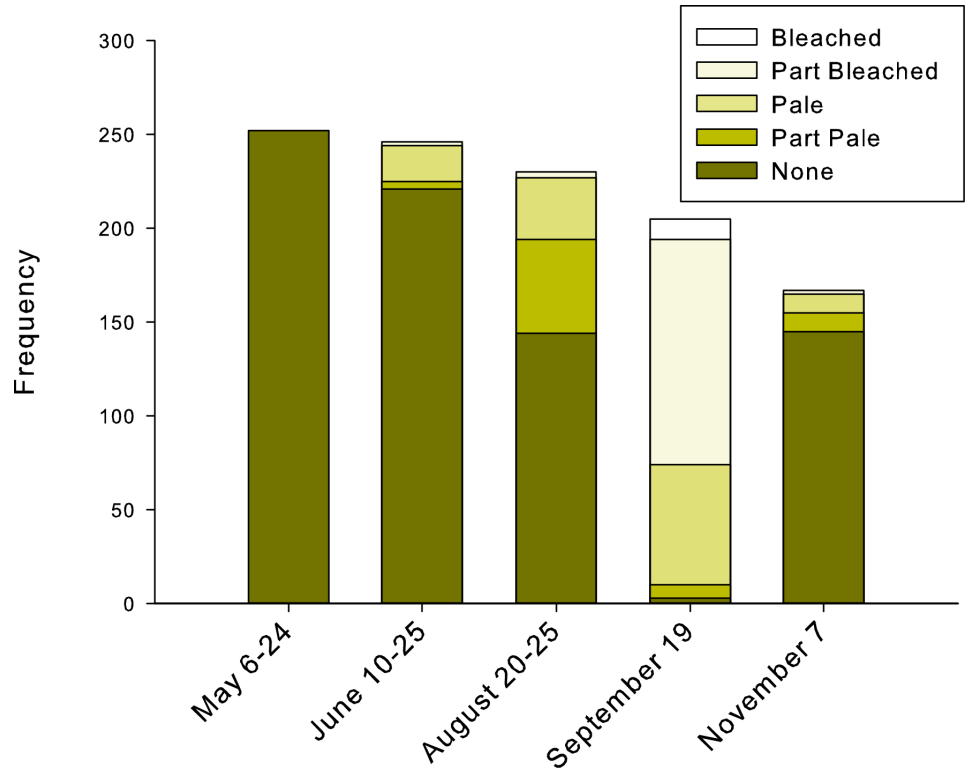

Figure 11. Time series of frequencies of bleaching condition among live outplanted Acropora palmata *fragments, with all sites and size treatments pooled.*

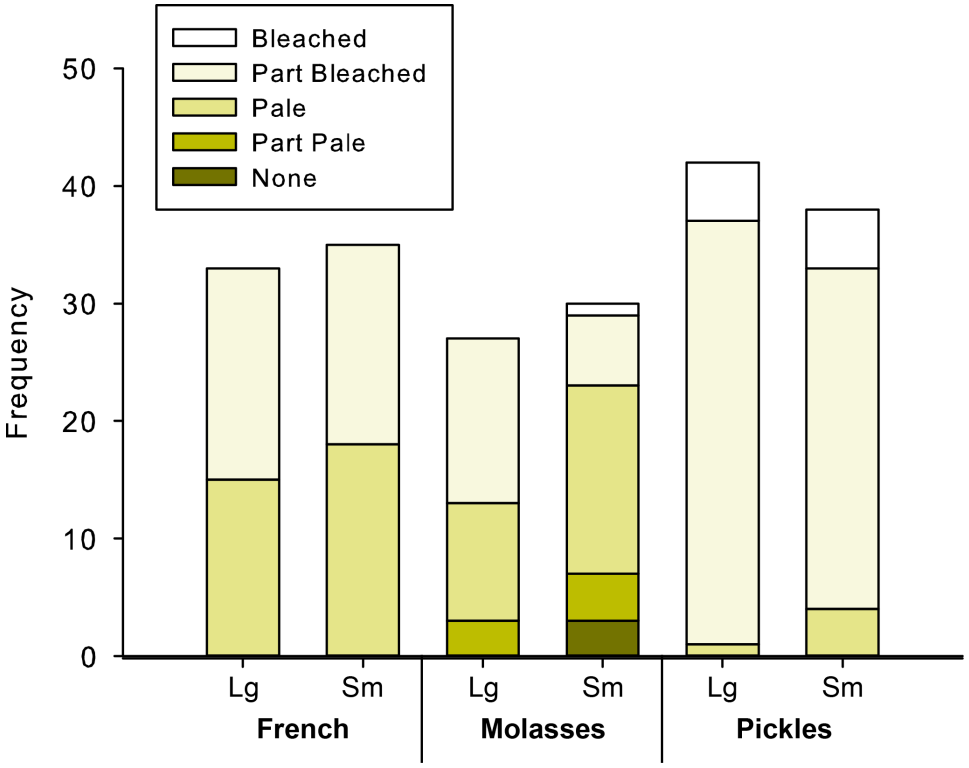

Figure 12. Frequency of peak bleaching condition (September 19th survey) among live Acropora palmata *outplants by site and size treatment.*