Aquarius Coral Restoration/Resilience Experiment (ACRRE): Interim Report

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Background :

Coral rescue and transplantation are commonly undertaken in cases where they have been damaged or dislodged by human activities or natural events. However, very little is known about the underlying biological reasons why one coral may survive and grow beautifully when transplanted to a reef while another may sicken and/or die. These variations in performance between different source corals are particularly important to understand in the current context of rapid environmental changes in reef environments and our continuing observations of rapid coral loss. The Aquarius Coral Restoration/Resilience Experiments (ACRREs) are designed to increase our understanding of why and how some corals may perform much better as transplants than others. Coral fragments from different sources, including healthy wild colonies from nearby reefs, rescued corals from far-away reefs, and corals that have been cultured in aquaria or field nurseries have been transplanted together to a single location, a 'common garden', at the Aquarius Reef Base. Each transplant is being evaluated across ecological, physiological, and genetic performance measures to understand mechanisms that may determine their ability to thrive in a new home. The results of this study will help scientists and reef managers to plan, permit, and execute coral rescue and transplantation/restoration project more effectively. We will learn what sources of corals can be most successful in enhancing depleted reef populations both in the short term by transplantation, and in the longer term by understanding better what genetic or other biological conditions of the coral aid in their resilience to the changing reef environment.

Activities Summary:

After a nine month planning and permitting process, the ACRRE project was initiated in early June 2008 to test the relative performance of corals transplanted from various source populations, including field nursery and lab-culture, to a 'common garden' at the Aquarius site (Conch reef, 55ft) and a smaller group to a nearby shallow site at Conch reef. Three source populations of *Montastraea faveolata* and four source populations of *Acropora cervicornis* were initially sampled at their origin and transplanted in June 2008 (see Table 1). Each source coral population was visited and sampled (PAM, genotyped, mucous) within two weeks prior to transplant. Many partners including FKNMS, BNP, UM, CRF, and Pennekamp State Park were involved in the actual coral collection and transport process. Four aquanauts undertook the actual epoxying of the coral fragments into their new home during a 7-day Aquarius saturation mission (10-16 June 2008). All fragments were sampled (PAM, size, mucous, photos) after securing in their new home. Transplants were visited/assessed approximately bi-weekly for the first two months and longer intervals thereafter. Major sampling (growth measurements, tissue samples for stress gene expression, mucous samples for microbial profiles, PAM) have been undertaken approximately three times per year Oct/Nov 08, Mar 09, July 09, Oct/Nov 09.

Two subsequent/supplementary activities have been undertaken during 2009. Based on observations of snail (*Coralliophila abbreviata*) predation as a mortality factor in the main experiment and the apparent improved performance of Acerv fragments transplanted into mixed-species plots, an additional coral transplant was conducted at the Conch Shallow site to explicitly examine the dynamics of colonization and impact by snails on coral transplants in different 'neighborhoods' (i.e. plots with differing density and species of coral neighbors). This transplant was set up in March 2009, high density of snails was introduced in July 09 and this experiment was completed in Dec 2009.

Lastly, transplants from two additional Acerv source populations were planned during the NURC mission in July 2009 (a field nursery in BNP, and a wild population at Grassy Key that was observed to be particularly robust). During this mission, we observed a disease outbreak affecting both Acerv and Mfav transplants at the Aquarius site and we debated the merits of continuing with the intended transplants. After consultation with FKNMS permitters and other experts, we decided to proceed with the transplants in an area a bit shallower at Aquarius (~45ft depth) and to 'spread the risk' by transplanting half of the fragments to an alternative site (~45ft at Molasses), as well as a contingent at the Conch Shallow site (analogous to the 2008 transplant). Additional analyses/sampling were focused on the disease event (e.g., samples of affected and healthy tissue for histology in addition to our regular mucous samples).

We noticed a high abundance of cyanobacteria as a component of the benthic community throughout the ARB/ACRRE transplant areas. We made a collection of this material in July 2009 and forwarded it to Dr. Valerie Paul (Smithsonian Marine Station) for analysis of potential natural products/toxicity that might be involved in coral transplant mortality.

All transplanted fragments have been genotyped, while several other laboratory analyses are underway (zoox types for all transplants over time, surface microbial characterization, stress gene expression).

Interim results and observations:

Fragment survivorship

Overall mortality of the transplants at Aquarius has been high for both species, but substantial variation among groups (origins) and amongst genets has occurred. At Aquarius, Acerv survivorship was highest for the transplants from the local CRF field nursery (Fig 1a). Wild transplants from local (Key Largo) or more distant (BNP) wild populations have survived similarly, and transplants cultured in the land-based aquarium have survived poorest at the Aquarius site. Survivorship patterns are somewhat different at the shallow site with the aquarium-cultured and BNP transplants surviving much better at the shallow site (Table 1). Similarly, Mfav survivorship at Aquarius was similar for Key West and Ocean Reef sourced fragments and substantially lower for the aquarium-cultured fragments (Fig 2a).

Substantial variation in the survivorship of different genets within sources was also found in both species (Figs 1b and 2b). Different Acerv genets from BNP ranged in survivorship from 0% to almost 40% and different Mfav genets from Ocean Reef ranged from zero to ~75% survivorship. Interestingly, there appears to be variation in the susceptibility to the July 09 disease event amongst different genets as well. For example, Mfav genet M1116 (from Key West) displayed good survivorship prior to the disease event with drastic mortality during in contrast to genet M1109 which did not suffer any mortality during the disease event. These distinct genet characteristics generate distinct hypotheses regarding molecular characteristics or symbiotic associations that might provide mechanisms for this genotypic variation (e.g., H1:

genet M1115 and M1109 have distinct patterns of zoox association, microbial association, and/or RNA expression), that we will attempt to test as more of the micro-array, microbial and zoox data become available.

There was also marked contrast in survivorship of groups among transplant sites (See Table 1). Most groups had lowest survivorship at Aquarius, compared with the Conch Shallow site or Molasses (for those groups transplanted to Molasses). The only exceptions were the KL Nursery Acerv (very poor survivorship at Conch Shallow, relatively high at Aquarius) and the Ocean Reef Mfav. The UM Hatchery Mfav were transplanted only at Aquarius due to limited supply. Overall, depth changes from source to transplant site likely have something to do with differential survivorship, but the fairly consistent lower survivorship at Aquarius (especially for the two source groups transplanted in 2009 to three sites including the Molasses site at similar depth) is suspicious.

Transplant Growth Rates:

Growth data is reported here for Acerv Aquarius transplants. Photo analysis of fragment area and lesion healing for the tissue cores collected from the Mfav transplants are still underway. For Acerv, three individual branches on each transplant were benchmarked with a cable tie in March 09 and extension measurements collected from Mar-Oct 09 and were standardized to mm extension per branch tip per month. Branches that displayed breakage or mortality were excluded from this analysis so the n's dwindled in some groups. Overall, there was significant variation in mean branch extension by source-group (Fig 3) with the field nursery group on the high side and the Pennekamp Aquarium group with the lowest. Interestingly, within each source group, individual replicated genets also showed substantial variation (Fig 3)

Microbial associates (excerpted from preliminary report by Woodley & Higgins; NCCOS Charleston, SC):

Mucous samples were collected from each fragment prior to transplant, after transplant, and periodically thereafter. Unfortunately, BNP fragments were not sampled prior to transplant due to logistical challenges and so they are not included in the following preliminary analyses. Samples were quick-frozen in a dry shipper and sent to the Woodley lab in Charleston. DNA was extracted from the samples, amplified via PCR and Denaturing gradient gel electrophoresis (DGGE) of 16S bacterial rDNA was used to profile the mucus-associated coral microbial communities of each sample. Preliminary analyses have focused on Acerv and addressing the following questions:

1. How do the bacterial profiles differ prior to being transplanted?
    a. Within the original site, from colony to colony?
    b. Differences across original sites?

As seen in Figure 4, there is little variation in bacterial profiles associated with each colony within the Key Largo wild site and the Nedimyer/CRF field nursery. There is also little variation across these two sites. In contrast, the captive samples from the Pennekamp aquarium showed variation both among colonies within this site, as well as from the patterns seen in the profiles from the other two sites.

2. Do the bacterial profiles change after they are moved?
    a. Does this depend on "who" they were placed with?

There seems to be only slight changes in the bacterial profiles after transplantation from the Key Largo wild site and the Nursery site. The only major difference seen pre- and post-transplant in most colonies is the disappearance of 3 prominent bands, and the appearance of one prominent band, indicated by arrows (Figure 5). Even with these changes, most colonies seem to display very similar bacterial profiles to one another.

In contrast, the DGGE profiles of samples taken from the Pennekamp aquarium (W) changed markedly after being moved to the Aquarius site. Interestingly, the bacterial profiles of these colonies after transplant bear a strong resemblance to those from the Key Largo Wild site (O) and the Nedimyer Nursery (Y) after transplant. In other words, the fragments cultured in the land-based aquaria appeared to converge in their microbial profiles to resemble those of both field-sourced groups. To our knowledge this has not been documented before.

For samples from all sites, it appears that the placement of the transplants in plots surrounded by those from the same site or from other sites had little effect on the bacterial profiles.

3. Do the bacterial profiles for the surviving colonies change over the course of the study?

Very few changes in DGGE banding profiles are seen over time in the healthy colonies (Figure 6). In contrast, fragments transplanted from land-based culture (as discussed above) show a shift following the initial transplant, but the pattern seen with these samples changes very little over the remainder of the study and resembles those seen in the profiles associated with the other colonies (see Fig 5).

More interestingly, samples taken from a fragment which displayed disease and then recovered (Fig 6, fragment O27). The distinctly different banding pattern in the O27 sequence corresponds to a diseased sample from June 14, 2008 (time of transplant). This colony apparently recovered, as the later sampling of O27 was noted as visually healthy and the DGGE banding pattern returns to that which was observed for the pre-transplant sampling. It is also interesting to note that the sample from an area of apparently unaffected tissue (i.e. apparently healthy) on the same colony taken on June 14, 2008 also has a pattern that resembles the other healthy samples, suggesting that the lesion-associated bacterial assemblage is in strict proximity to the 'disease line'. The bands excised in these samples are of particular interest because the identity of these bands may help elucidate a causative disease agent(s).

Ongoing analyses are focusing on samples from additional disease-affected fragments in an attempt to discern if the distinct band changes corresponding with a disease condition in O27 may be a general characteristic of diseased colonies.

Photosynthetic performance:

Pulse-Amplitude Modulated Fluorometry was used to assess photosynthetic efficiency in the Aquarius transplants and the in-situ Mfav colonies. Measurements were made in dark conditions, but logistical challenges with regard to being on site in the nighttime unfortunately have introduced some variation in these measurements from time to time (e.g., some sample bouts taken after sunset, whereas others taken pre-dawn). Hence, it is best to use these data to compare among source-groups WITHIN each sample bout, and not to try to compare changes over time.

Overall, the PAM measurements are quite variable and strong patterns are difficult to discern in the group averaged data (Fig 7 and 8), especially for the Acerv (different rank-order of groups at different survey dates). One fairly consistent pattern is that the in situ Mfav colonies

native to the Aquarius site have persistently higher photosynthetic yield than the transplants. These colonies are markedly bigger than the transplanted fragments. During Oct 09, substantial bleaching was observed in both transplants and in situ Mfav. We took PAM measurements from both bleached and normally pigmented regions on the in situ colonies, while the smaller transplants were categorized as either bleached or normal. Interestingly, photosynthetic yield was lower (and more variable) in the bleached portions of the in situ colonies, while there was no apparent difference between bleached vs. normal fragments.

In future analyses, we will attempt to standardize the PAM data to zoox type and/or host genotype (per fragment).

Effects of Jan 2010 Cold Snap:

Figure 9 shows temperature records we collected with HOBO temp loggers at the Aquarius and Molasses transplant sites from Oct 09 to Feb 10, encompassing the Jan 2010 cold snap. Molasses displayed markedly colder excursions, down to 17oC but no adverse effects on any coral transplants have been observed, including the Conch Shallow site where we had not deployed a temperature logger.

Transplant 'Neighborhood' Experiment (Collaborator: Lyza Johnston, UM/RSMAS):

Predation by the gastropod, *Coralliophila abbreviata,* was observed to be a primary source of mortality for A.cerv trasnplatns in the early phases of the ACRRE study. Predation pressure on a focal population may be mitigated by the prevailing prey neighborhood composition and structure. In the case of rare or threatened species, such as A.cervicornis, these interactions can potentially impact the persistence and recovery of local populations. Thus, to directly test the effects of coral neighborhood on the impact of the corallivorous gastropod, on the threatened coral species, *Acropora cervicornis*, we conducted an ancillary transplant experiment at Conch Shallow (March-Dec 2009) in which the density and composition of neighboring corals surrounding focal *A. cervicornis* colonies were manipulated, as well as the density of *C. abbreviata* at the study site. Focal *A. cervicornis* colonies either had no neighbors (solitary; control), conspecific neighbors, alternative prey (*Montastraea faveolata*) neighbors, or non-prey (*Porites asteroides*) neighbors within a $1m^2$ plot. One hundred and fifteen individually tagged snails were added to the study system and monitored for five months to assess patterns of movement and resource use.

The results indicate that both the density and identity of neighboring corals are important in determining levels of corallivore damage to focal *A. cervicornis* colonies. The observed patterns appear to be due to a combination of a density dependent numerical response to prey and subsequent resource use within neighborhood plots by *C. abbreviata*. Snails exhibited a strong feeding preference for *A. cervicornis* throughout the study period, based on calculated selection indices (α; from Manly et al 1972). Conspecific plots experienced the highest rate of colonization as well as a significantly higher number of snails per plot through time (Fig. 10A). Alternative prey plots experienced an intermediate rate of colonization and density of snails. However, the impact of predation, measured as the colony mortality rate due to predation, of the focal *A. cervicornis* colonies in alternative prey plots was similar to that in conspecific plots (Fig. 10B), indicating that the snails in the alternative prey plots were focusing on the focal *A. cervicornis* colonies, whereas the snails in the conspecific plots were distributed amongst the five

A. cervicornis colonies in those plots. *P. asteroides* did not seem to confer any resistance to predation compared to solitary *A. cervicornis*.

Acknowledgements:

This project was funded by the NOAA-Coral Reef Conservation Program and was conducted under permits from the Florida Keys National Marine Sanctuary (with concurrence/consultation of Florida Fish and Wildlife Conservation Commission and NMFS Southeast Regional Office), Biscayne National Park, and Pennekamp State Park. A host of partners and collaborators enabled the instigation of the project including those who contributed ideas (Aug 2007 ACRRE Workshop Participants), corals (Tom Capo, Tony Emtiaz) collaboration (IBaums, CWoodley, SEdge, MDurako, LMacLaughlin, KNedimyer, LJohnston, DWilliams, AValdivia, RWilborn, DLirman, ABright, KErickson) and logistical support (ABourque, IBerzins, DRothan, KHeim, CJaboly, PGillette, JHerlan, and likely others). A.Valdivia and R.Wilborn have been instrumental in the data analyses. Huge thanks go to the NURC-UNCW team for initiating and supporting the project with their usual stellar operation.

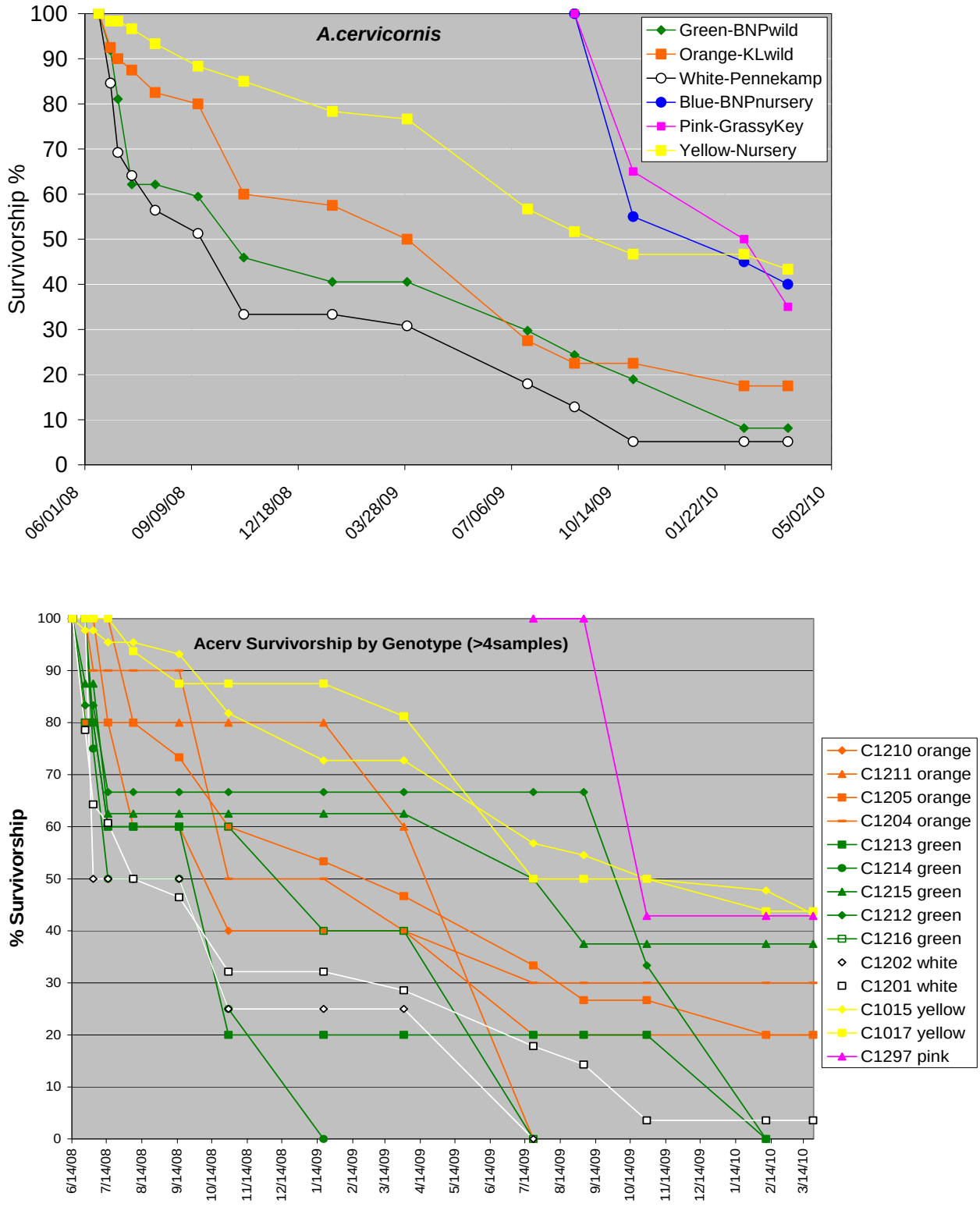


Fig 1: Survivorship curves for A.cervicornis transplants at Aquarius site by source group (top panel) and by genotype (bottom; for those genotypes with at least 4 replicates).

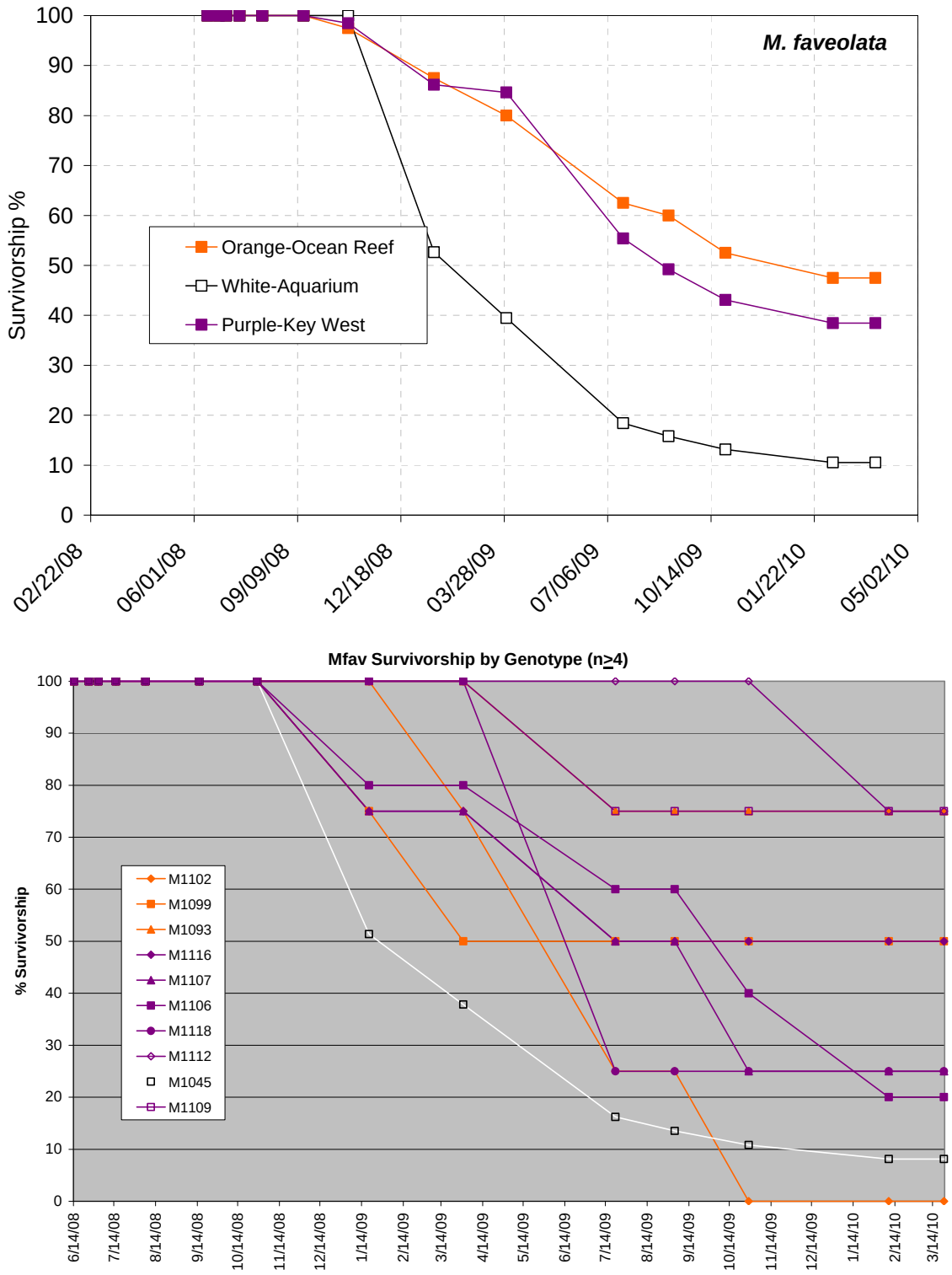


Fig 2: Survivorship curves for M.faveolata transplants at Aquarius site by source-group (top panel) and by genotype (bottom; for those with at least 4 replicate fragments)

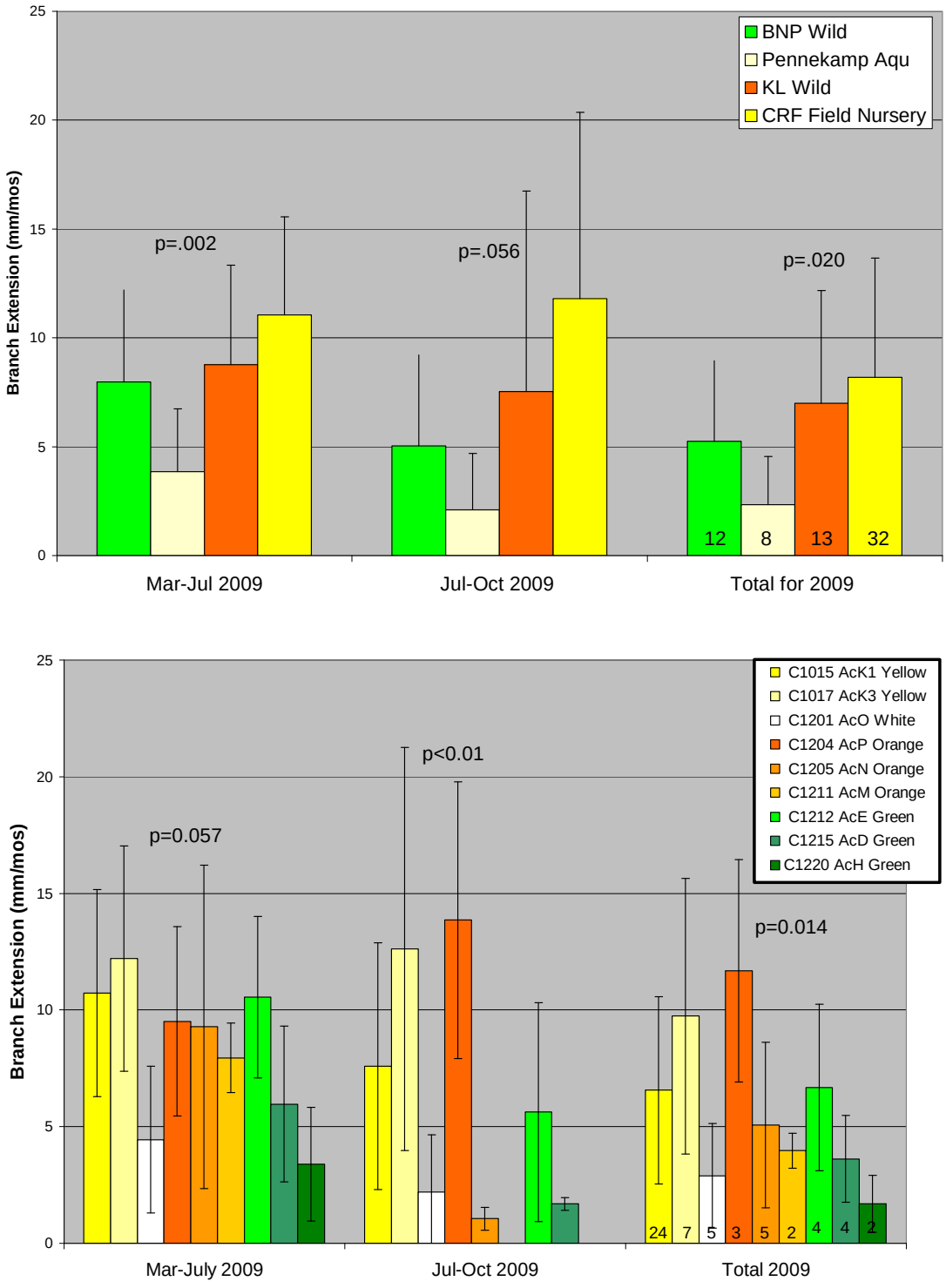


Fig 3: Mean extension rates measured on individual branches (three per transplant) for Aquarius 2008 transplants by source group (top panel) and by genotype (bottom panel; for those starting with at least 4 replicates) between Mar-Oct 2009, and two sub-intervals. Due to mortality and branch breakage over time, the n's for these measurements dwindled over time; ending n's given in last set of bars.

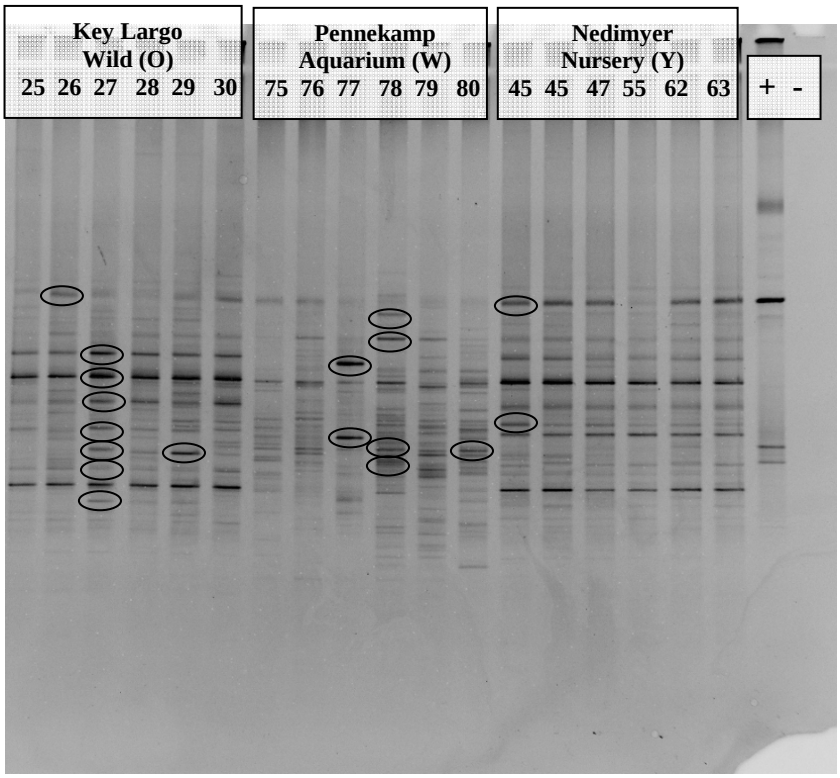


Fig 4. Representative sets of gels for three Acerv fragment source treatments sampled prior to transplant (ie. at source; prior samples were not collected for the BNP group due to logistical problems). Each lane represents a sample from a single fragment. Key Largo Wild (Orange) and Nursery (Yellow) fragments show some degree of similarity in their banding patterns. However, the Pennekamp aquarium fragments show a different and more variable pattern between individual fragments. Note that the presence/absence of the band is meaningful but the darkness of the band may not be.

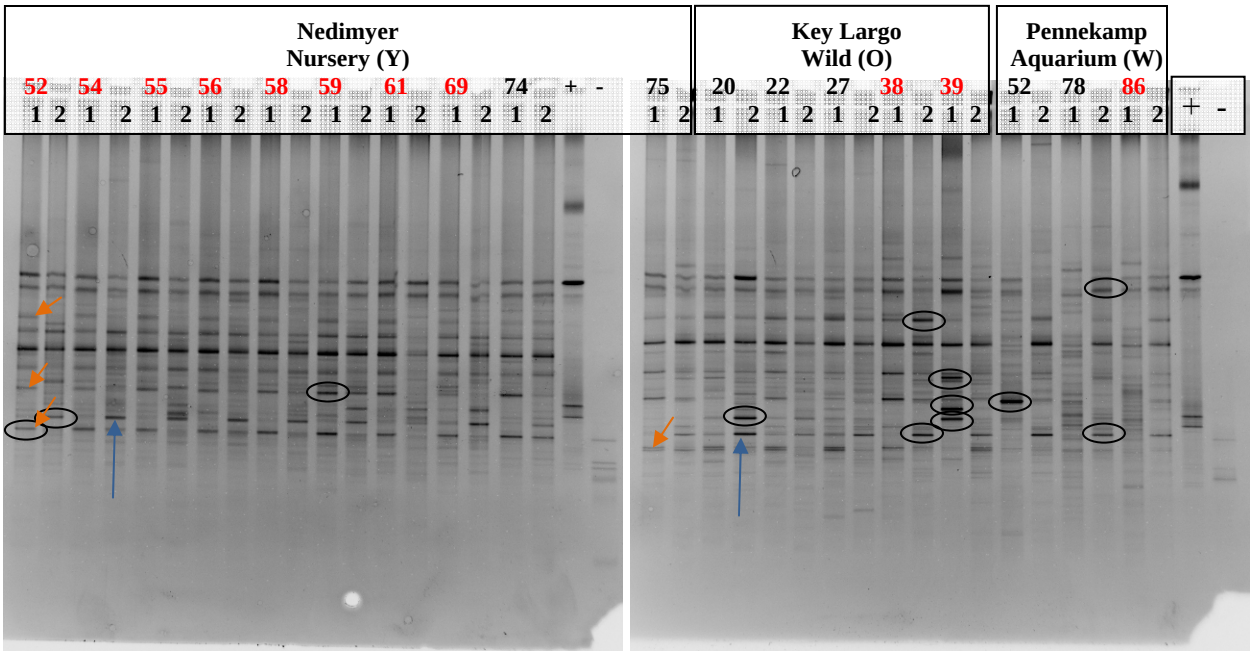


Fig 5: DGGE gels showing bacterial profiles for surviving fragments before and after transplant. Top numbers correspond to tags assigned to each colony, while bottom numbers indicate the following: 1) Pre-transplant; 2) June 14, 2008; **Red** colonies were transplanted into plots surrounded by fragments from other sources. **Black** colonies were transplanted into plots surrounded by their 'neighbors of origin'. Orange arrows point to bands that are no longer seen post-transplant, and blue arrows are used here to point out the appearance of new bands (bacteria) in most samples after the colonies were moved.

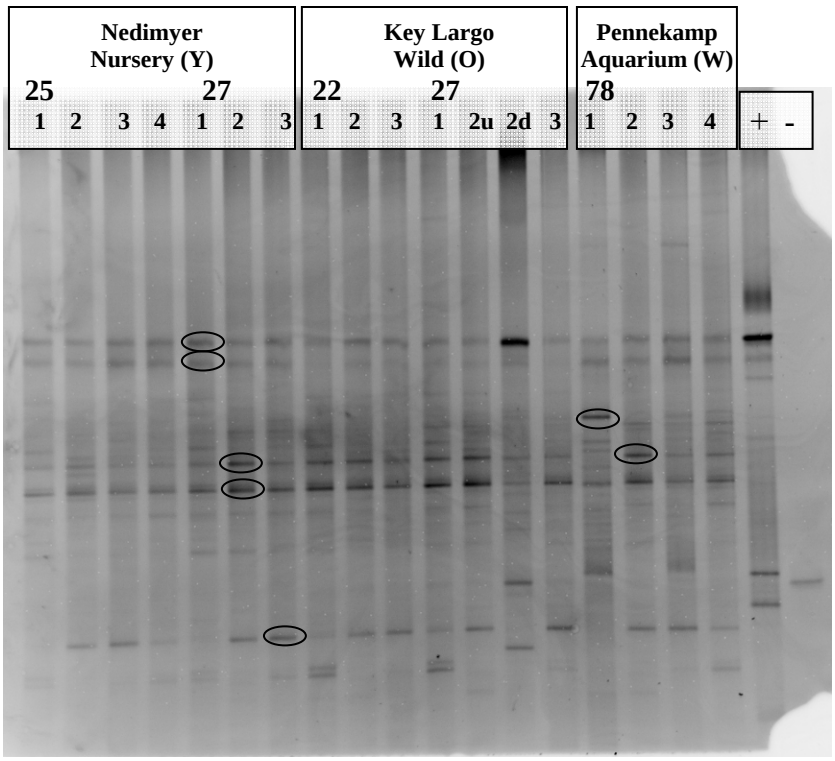
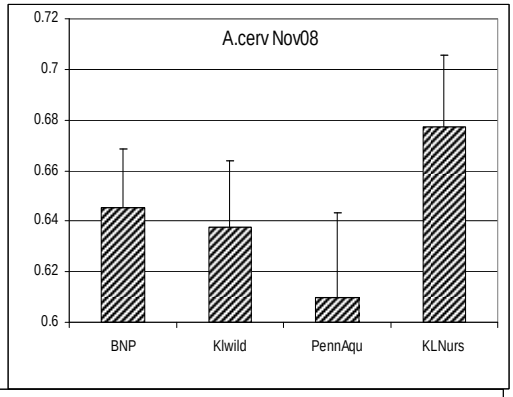
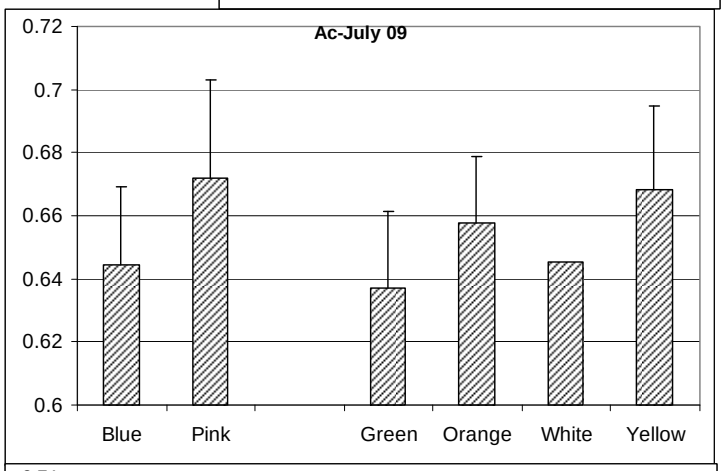
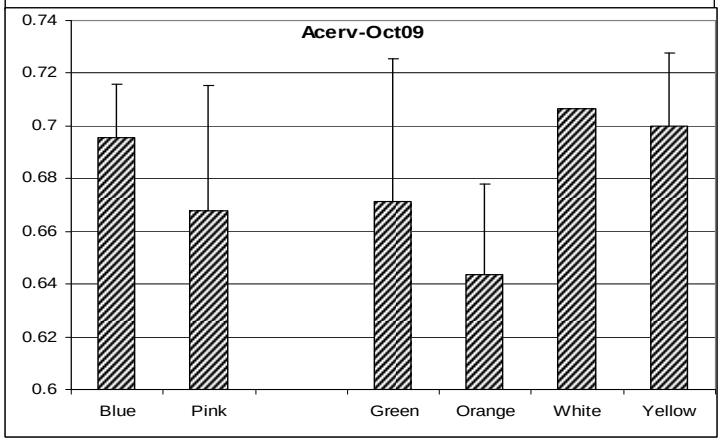


Figure 6:
Top numbers correspond to tags assigned to each colony, while bottom numbers indicate the following: 1) Pre-transplant; 2) June 14, 2008; 3) October 28, 2008; 4) July 9, 2009.







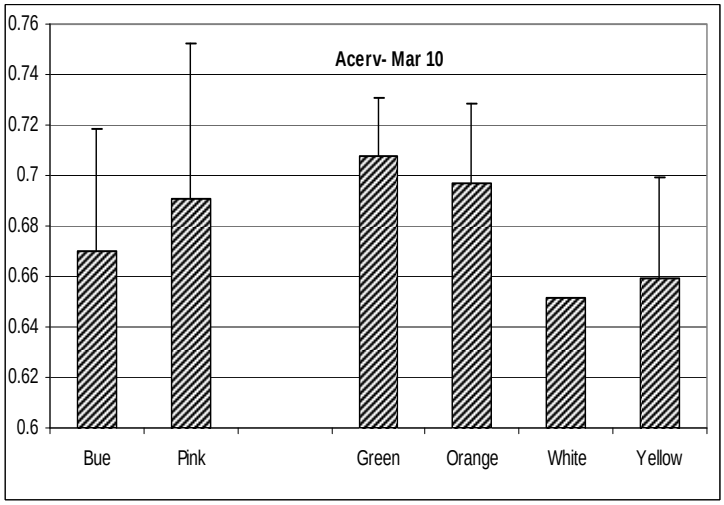
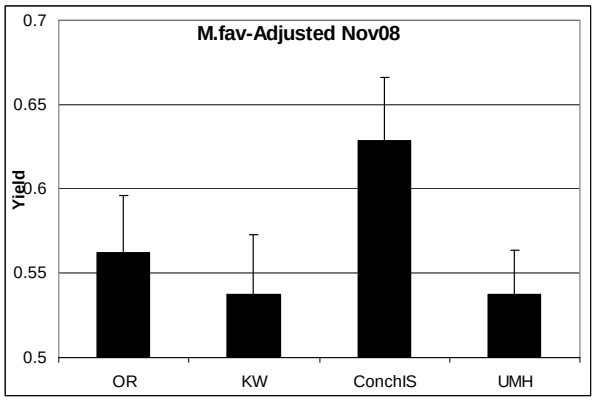
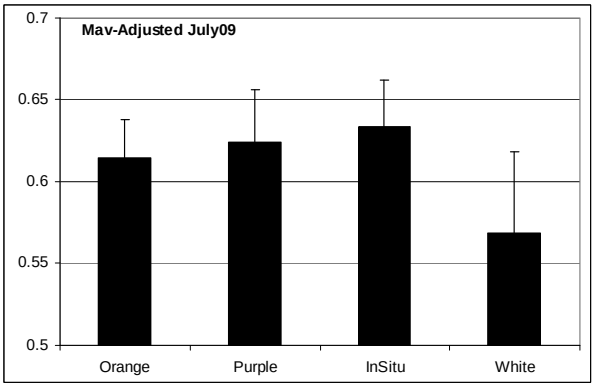
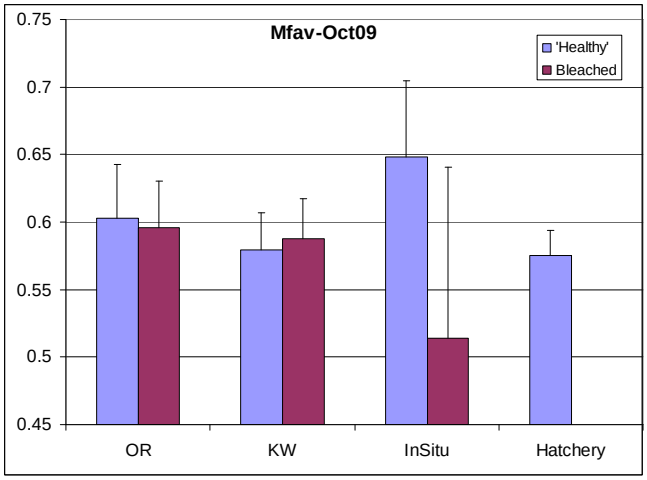


Fig 7. Mean (+1 SD) photosynthetic yield for each source group of A.cervicornis transplants at Aquarius on different dates as measured by PAM fluorometry. Measurements taken in the dark, at least one hour after sunset. See Table 1 for tag colors.







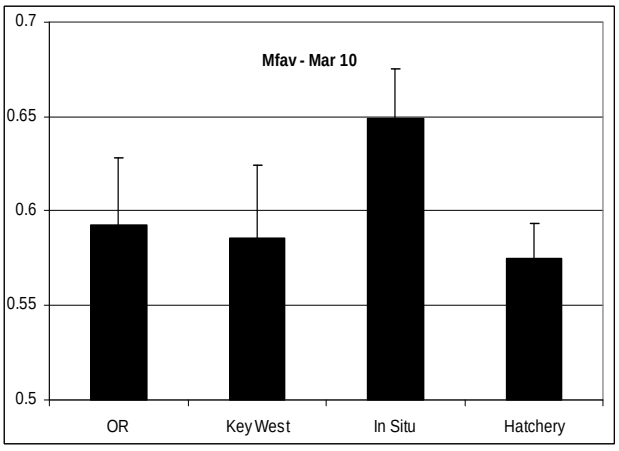


Fig 8: Mean (+1 SD) photosynthetic yield for each source group of M.faveolata transplants at Aquarius and a set of in situ, 'native' colonies resident on the reef near Aquarius as measured by PAM fluorometry.. Different survey dates depicted in different panels. Measurements taken in the dark, at least one hour after sunset. Substantial bleaching was observed during the Oct 09 survey. Transplants were characterized as either 'bleached' or 'normally pigmented'. In situ colonies were mostly mottled so 'bleached' and 'normally pigmented' regions were measured within these much larger colonies. See Table 1 for tag colors.

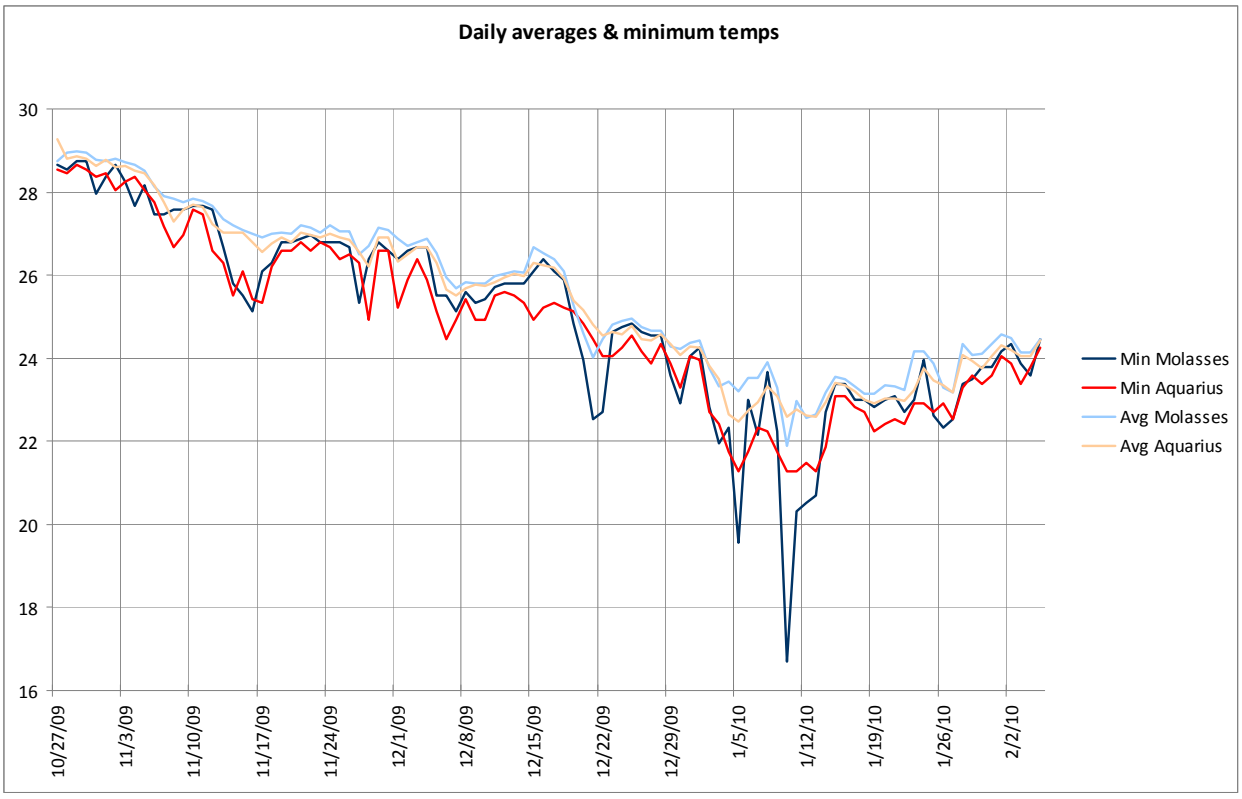


Fig 9: Reef temperatures recorded from Oct 2009 to Feb 2010 at Aquarius and Molasses transplant sites, both ~ 45 ft depth.

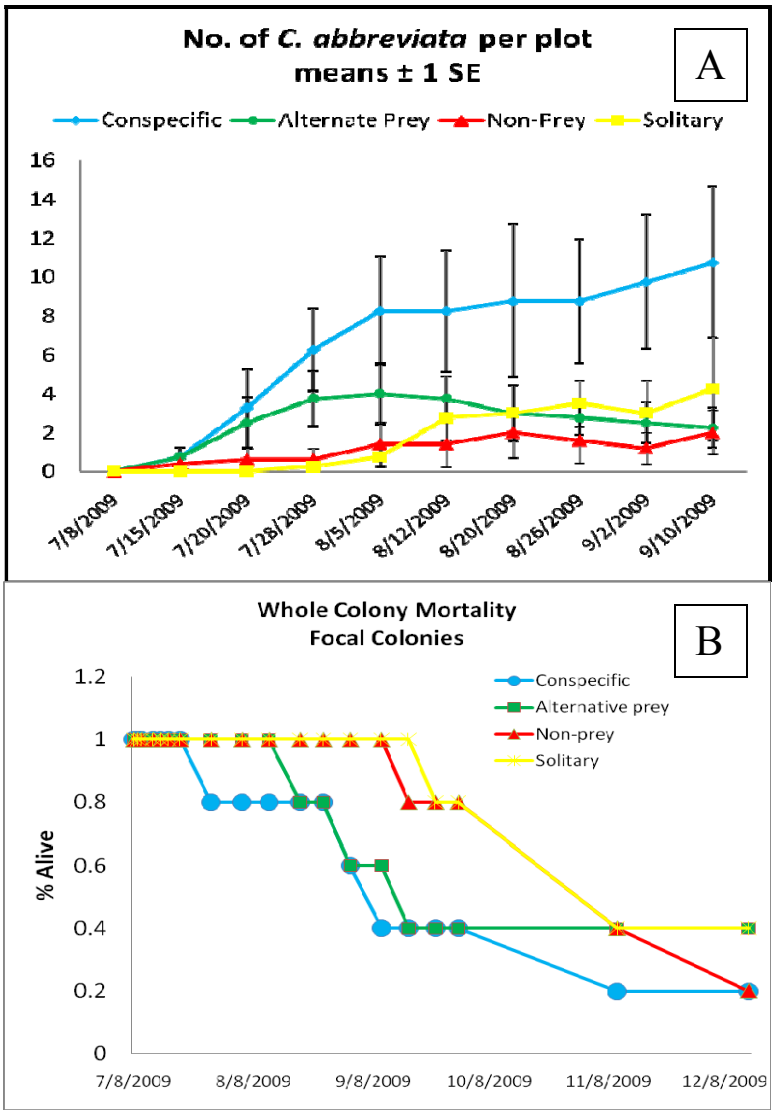


Fig 10**.** A**)** Number of *C. abbreviata* per plot through time. There were significant effects of time and neighborhood treatment, with conspecific plots having significantly more snails per plot through time (repeated measures ANOVA and Tukey HSD post hoc; $p < 0.01$). B) Mortality rates of focal *A. cervicornis* colonies in conspecific plots, alternative prey plots, non-prey plots, and solitary plots.

Table 1: Summary of number of fragments transplanted to Aquarius Reef Base (ARB), Conch Shallow (CS), and Molasses Reef (Mol); and final survivors as observed Mar 2010

Spp	Tag Color	Source	June08	July09		Mar 10	% Survivorship
Acerv		Biscayne Nat Park (BNP)	ARB 38 CS 10			3 5	8 50
		Key Largo patch reefs (KL)	ARB 40 CS 10			7 10	18 100
		Pennekamp Aquarium (PA)	ARB 39 CS 10			2 8	5 80
		Coral Restoration Foundation/ Nedimyer nursery (CRF)	ARB 56 CS 10			26 1	46 10
		BNP Field Nursery		ARB 20 Mol 20 CS 6		8 13 6	40 65 100
		Grassy Key		ARB 20 Mol 20 CS 10		7 12 10	35 60 100
Mfav		Ocean Reef (Key Largo) seawall (OR)	ARB 41 CS 10			19 7	46 70
		Key West seawall/nursery (KW)	ARB 59 CS 10			25 2	42 20
		UM Hatchery (UMH)	ARB 39			4	10