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Evaluating Acropora cervicornis Growth and Survivorship in a Line Nursery

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EVALUATING *ACROPORA CERVICORNIS* GROWTH AND SURVIVORSHIP IN A LINE NURSERY

By

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Abstract:

Acropora cervicornis and *A. palmata* were once dominant, reef-building corals of Caribbean reefs. Over the last several decades, population declines of Caribbean *Acropora* have been dramatic, and both species are now listed as "Threatened" under the United States Endangered Species Act. Numerous restoration efforts now utilize coral gardening techniques to cultivate these species for transplantation, in which *A. cervicornis* is primarily cultivated both on fixed structures and in line nurseries. This study evaluates growth and survivorship of multiple *A. cervicornis* genotypes grown via two line nursery techniques, and compares the efficacy of each against the conventional method of fixed nursery puck-mounted culture. Suspended nursery culture resulted in higher post-fragmentation survivorship of corals than puck culture, especially in warmer conditions. Disease incidence was significantly reduced by suspended culture, which also prevented predation from fireworms (*Hermodice carunculata*) prevalent in puck corals at the same nursery. Genotypic growth rate differences persisted among techniques, and suspended coral growth was comparable to puck culture. Suspended colonies may need more frequent pruning to avoid branch abrasion and breakage, but the technique is an effective means to reduce disease, predation, and post-fragmentation mortality in *A. cervicornis* nursery culture.

Keywords: *staghorn coral, Acropora cervicornis, restoration, conservation, line nursery, aquaculture, mariculture, coral gardening, fragmentation, propagation*

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Finally I must thank all my friends and family, whose unending encouragement and support I can not undervalue. Thank you all.

Foreward:

This thesis includes two experiments. For the ease of publication, the two are written separately. Figures are prepared in formats conducive to journal submission, and some information is repeated between sections to eliminate interdependency.

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I. Introduction

Coral reefs are diverse ecosystems of immeasurable value that provide a wealth of ecological and economic goods and services (Jameson, 1995; Moberg and Folk, 1999; Brander *et al.*, 2007). Unfortunately, coral reefs are facing worldwide decline brought about by natural and anthropogenic impacts (Hoegh-Guldberg, 1999; Hughes and Connell, 1999; Nyström *et al.,* 2000; Hughes *et al.*, 2003; Pandolfi *et al.*, 2003). Near the turn of the century, it was estimated that as much as 70% of the world's coral reefs were directly threatened by human activities (Goreau, 1992; Sebens, 1994; Wilkinson, 1999), and approximately one in three reef-building corals faced elevated extinction risk (Carpenter *et al.,* 2008). Prominent stresses to corals include climate change, disease proliferation, coastal eutrophication and sedimentation, ocean acidification, and destructive fishing practices (Sebens, 1994; Hughes and Connell, 1999; Hoegh-Guldberg *et al.,* 2007). Impacted by these stressors, coral reefs have been subject to varied, and in many cases dramatic degradation (Bruno and Selig, 2007; Mora, 2007). Caribbean reefs have fared comparatively worse than those elsewhere, experiencing over 80% loss of hard coral cover from 1977 to 2001 (Gardner *et al*., 2003; Fig. 1); an average rate of decline approximately four times greater than in the Indo-Pacific (Mumby and Steneck, 2008).

Of the substantial decreases in hard coral cover in the Caribbean, those of the *Acropora* genus have been some of the most severe (Aronson and Precht, 2001a; Bruckner, 2003), experiencing losses up to 98% in some locations (Miller *et al*., 2002). Historically, *Acropora* were the most prominent corals on Caribbean reef crests. Elkhorn coral, *Acropora palmata,* would dominate the top five meters of a reef, and the staghorn coral, *Acropora cervicornis,* would colonize much of the fore reef at depths of about eight to 15 meters (Goreau, 1959; Goreau and Wells, 1967; Woodley and Robinson, 1977; Fig. 2). Regardless of their past robust populations, both *A. palmata* and *A. cervicornis* are now listed as "Threatened" under the United States Endangered Species Act (NMFS, 2006).

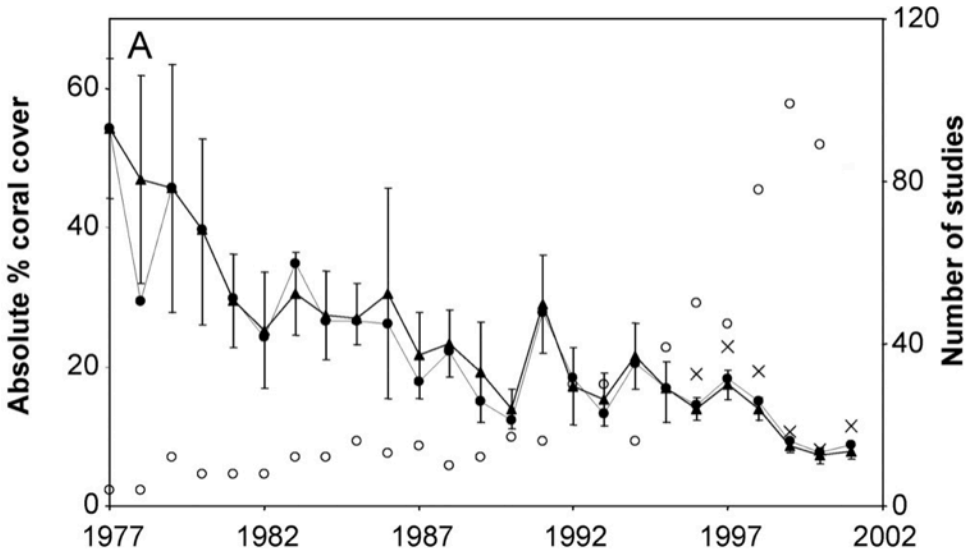

Figure 1: Observed coral percent cover decline in the greater Caribbean from 1977 to 2001 (from Gardner *et al*., 2003). Triangles and closed circles represent weighted and un-weighted absolute mean coral cover respectively, open circles represent number of studies, and 'X' represent data omissions.

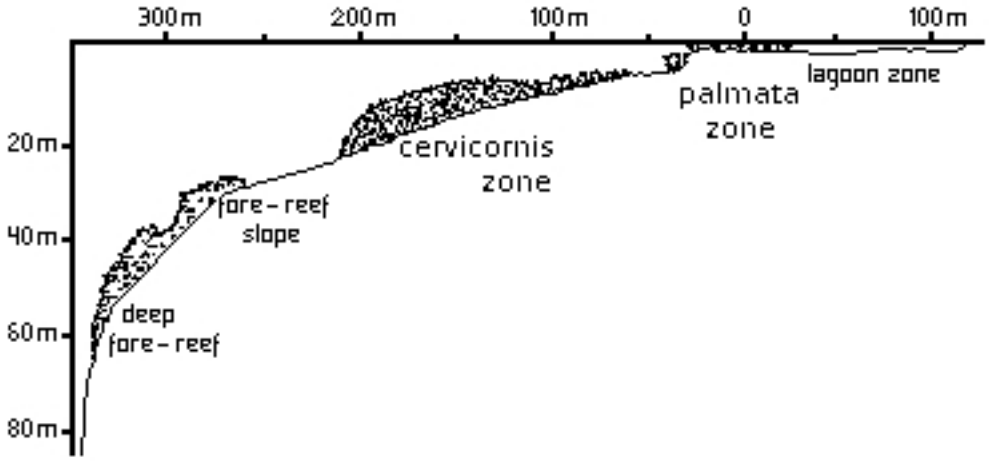

Figure 2: Cross section diagram of Discovery Bay, Jamaica exhibiting classic *Acropora* zonation (adapted from Woodley and Robinson, 1977).

The population collapse of Caribbean *Acropora* is a result of many stresses. Their susceptibility to disease and bleaching is suspected to be higher than other Caribbean coral species (Goreau *et al*., 1998; Hoegh-Guldberg, 1999; Aronson and Precht, 2001b; Williams and Miller, 2005). With the decline of these and numerous other reef-building coral species, many Caribbean reefs are shifting from coral-dominated ecosystems to those dominated by other functional groups such as macroalgal communities (Done,

1992; Knowlton, 1992; Porter, 1992; Hughes, 1994; McClanahan, 2002; Pandolfi *et al.*, 2005; Norström *et al.,* 2009).

Though Caribbean-wide coral cover on reefs has remained relatively stable in recent years compared to the acute decline between the 1970's and 1990's (Bruno *et al.,* 2009; Schutte *et al.,* 2010), present day coral reefs could reach a threshold of irreversible change if such degradation continues (Knowlton, 1992; Mumby *et al.*, 2007). As such, a great variety of measures are being undertaken in attempts to halt or reverse this trend (Jaap, 2000; Rinkevich, 2005). Reaction to the decline of reef ecosystems has brought about many theories on how to best conserve and restore them. Some current conservation methods include: establishing marine protected areas to limit fisheries, the reduction of terrestrial nutrient and sediment runoff, and the installation of mooring buoys at dive sites to mitigate anchor damage. Some current restoration (sometimes referred to as "rehabilitation") methods include: the reattachment of coral colonies and stabilization of reef structures following ship groundings and other physical impacts, whole colony and fragment transplantation from healthy reefs to denuded reefs, and the cultivation and targeted transplantation of coral colonies via nurseries. Additionally, the creation of artificial reefs using materials of opportunity (tires, sunken ships, building debris, rock boulders, etc.) or purpose-built structures (reef balls, EcoReefs®) provides physical habitat for fish, corals, and other reef organisms in attempts to augment existing reef habitat (Abelson, 2006). However, the effectiveness of artificial reefs as a restoration tool is debated. Alternative methods including nursery cultivation of coral species such as *A. cervicornis* have been increasingly adopted in recent years (Young *et al.,* 2012).

II. Species Profile

II.1 *Acropora cervicornis*

The staghorn coral, *Acropora cervicornis* (Fig. 3), is a scleractinian species with a geographic range limited to shallow waters of the greater Caribbean. Fixing calcium and carbonate from seawater, colonies contribute to reef growth and act to fortify and

stabilize reefs by binding reef rubble as they spread and encrust onto new substrates (Gillmore and Hall, 1976). Generally inhabiting fore reefs from five to 25m of depth, they have also been observed as shallow as one meter and as deep as 50m (Lewis, 1960; Goreau and Wells, 1967; Logan, 1969). Their shallow depth range is limited by wave action and their maximum depth by light availability. Additionally, small populations are observed in the patch reef habitats of sheltered back reef and lagoonal areas.

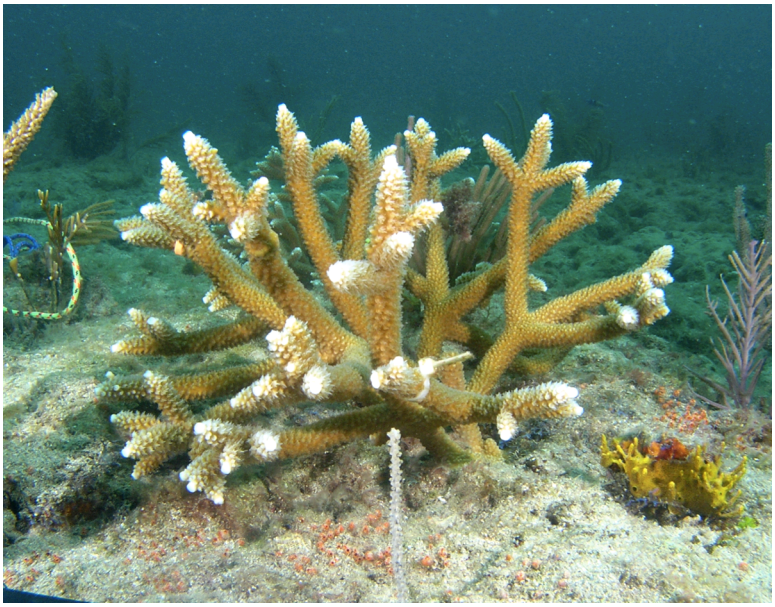

Figure 3: A colony of the study species, *Acropora cervicornis*.

Colonies of *A. cervicornis* are branching, and typically light brown in color. Branches are approximately two centimeters thick and tapered towards their growing tips, with secondary and tertiary branches commonly budding off of parent branches at nearly 45º angles. Branches are covered in small, distinctly protruding polyps (radial polys), and terminate in a single axial polyp. Colonies grow in ramose "bush-like" forms, the nature of which (branch density, branch angle, etc.) can be influenced by varying hydrological conditions (Bottjer, 1980). Growth of *Acropora* species is an important contributor to the physical complexity (also termed "rugosity" or "architectural complexity") of a reef, which positively correlates with fish and reef organism abundance and species richness (Luckhurst and Luckhurst, 1978; Gratwicke and Speight, 2005).

In contrast with recent, dramatic population declines, *A. cervicornis* deposits of Pleistocene and Holocene Caribbean reefs show a robust presence, with some records showing uninterrupted deposition spanning thousands of years (Greenstein *et al.*, 1998; Greer *at al.*, 2009). Such deposits transcend large-scale environmental change including sea temperature and salinity fluctuation, sea level rise and hurricanes, and suggest that recent declines of the species are a product of unnatural ecosystem change (Greer *et al.*, 2009). Once a dominant reef builder (Jackson, 1992), today only a few populations growing in the dense, sprawling aggregations of the past have been described, including populations in Honduras (Keck *et al.,* 2005), the Dominican Republic (Lirman *et al.,* 2010), and Southeast Florida, United States (Vargas-Ángel *et al.*, 2003; Walker *et al.*, 2012).

II.2 *Reproduction*

Acropora cervicornis, like numerous other branching scleractinian corals, reproduces both sexually and asexually. A hermaphroditic species, *A. cervicornis* colonies produce both oocytes and spermatocytes (Szmant, 1986), with gametogenesis beginning in the fall of the year prior to spawning (Vargas-Ángel *et al.*, 2006). Spawning is generally synchronous in nature, with all colonies of a reef spawning together (generally within one hour) within seven nights after the full moon of July or August (Szmant, 1986; Steiner, 1995; Vargas-Ángel *et al.*, 2006). Asynchronous spawning has also been observed, though rarely, and it is suspected to occur as a result of the full moon falling too early in the month of July, and/or the effects of local environmental variability on the rate of gamete development (Vargas-Ángel *et al.*, 2006). The planktonic products of spawning events are larval coral "planulae." An individual planula can drift in the water column for many months before settling onto a reef (Graham *et al., 2008* estimated larval longevity of over 100 days in several broadcast spawning scleractinia), metamorphosing into a polyp, and beginning the process of binary fission and skeletal deposition as it grows into a new colony.

Asexual reproduction is very common in *A. cervicornis* and is promoted by its branching structure. If subjected to a strong force, often storm-driven wave action or collision with

animal life, the rigid branches of *A. cervicornis* can break off, falling to the substrate. If a fragment settles into a stable position of favorable environmental conditions (e.g. substrate type, light availability, etc.), it can attach and grow into a new colony. It is primarily in this way that *A. cervicornis* colonies spread on a reef, and it is speculated that asexual, not sexual reproduction is the primary means by which *A. cervicornis* grows and expands its population (Highsmith, 1982; Vargas-Ángel *et al.*, 2006).

The propensity for *A. cervicornis* to spread by fragmentation allows for the formation of large, dense, monospecific stands (also called "thickets" or "patches"). Though coral species diversity within thicket areas can be reduced, such areas provide a complex physical habitat for a wide range of marine species. Also, as thicket-forming coral species have characteristically high growth rates, their dominance of an area may result in relatively high rates of reef development (Highsmith, 1982).

Though *A. cervicornis* can form robust populations on local scales, its ability to colonize new areas beyond local transportation of fragments is wholly dependent on sexual reproduction. Self-fertilization success is very low in *A. cervicornis*, which may result in a proportionately weak contribution of planulae in areas dominated by a single genet (Fogarty, 2010). Compounding this impediment, fertilization success in broadcast spawning anthozoans has been demonstrated to decrease dramatically within a distance of only a few meters (a product of diminishing sperm concentrations and the longevity of gamete viability) (Brazeau and Lasker, 1992; Oliver and Babcock, 1992). It has thus been suggested that in regards to *A. cervicornis* in the Caribbean, gametes produced by monotypic stands (in which colony density is high, but genetic variability is low to non-existent) may be largely wasted (Kojis and Quinn, 1994). Since population dynamics (percent live coral cover) of *A. cervicornis* thickets can vary greatly over short timescales (Davis, 1982; Gilliam *et al.*, 2006; Gilliam *et al.*, 2011; Walker *et al.,* 2012), the persistent success of sexual reproduction and larval recruitment is likely essential to the continued existence of the species (Szmant, 1986).

To promote sexual reproduction, a suggested practice of *A. cervicornis* restoration efforts is to create "patches" of closely aggregated, genetically diverse individuals (Quinn and

Kojis, 2006; Johnson *et al.*, 2011) and/or artificially increase genetic diversity within existing monotypic patches (Bowden-Kerby, 2008). The propensity of *A. cervicornis* to asexual reproduction makes cultivating colonies via artificial fragmentation an attractive means of supplying restoration efforts.

II.3 *Threats*

Caribbean *Acropora* face numerous and varied stressors. On local scales, staghorn coral can be preyed upon by the annelid *Hermodice carunculata* (bearded fireworm; Pallas, 1766) and the corallivorous snail *Coralliophila abbreviata* (Lamark, 1816). *Hermodice* typically ingests *A. cervicornis* tissue by engulfing up to ~10cm of a branch tip and digesting the tissue directly off the skeleton (leaving the branch connected to the colony) (Shinn, 1976). The corallivorous snail *Coralliophila abbreviata* feeds by scraping tissue off the host (Brawley and Adey, 1982; Baums *et al.,* 2003). Also, the threespot damselfish (*Stegastes planifrons*) will seek refuge in larger colonies of *A. cervicornis,* defending territory in which they clear away areas of coral tissue to promote the growth of turf algae gardens (Precht *et al.,* 2010). In large, healthy populations of *A. cervicornis* that can rapidly regenerate lost tissue, the impact of predation may be minimal (Ott and Lewis, 1972). However in small, struggling populations the impacts of chronic predation can compound the effects of existing stressors.

Acute, climatological events also impact *A. cervicornis*. Specifically, hurricanes have the potential to completely devastate local populations (Knowlton *et al.,* 1981, 1990). Though they have the capacity to enhance *Acropora* populations through fragmentation (Fong and Lirman, 1995), including the enhancement of genetic dispersal beyond the normal range of asexual propagation, the deleterious effects of hurricanes on Caribbean reefs have become magnified by the inability of many reefs to recover following perturbation (Gardner *et al.,* 2005). In light of this, localized populations of *A. cervicornis* are particularly vulnerable, as they would be dependent on weakened (or non-existent) external sources of larval recruitment to recover following destruction (Vollmer and Palumbi, 2007).

Less frequent than hurricanes, prolonged hypothermal events can severely impact *A. cervicornis* populations, especially those within shallow water habitats in more northern latitudes (Porter *et al.,* 1982; Roberts *et al.,* 1982; Lirman *et al.,* 2011). The irregularity and infrequency of such hypothermal or "cold-water" events, combined with the acclimation of corals to warmer conditions rather than colder via seasonal bleaching, intensifies their effect (Lirman *et al.,* 2011). Even though the range of *A. cervicornis* and *A. palmata* has been hypothesized to be expanding northward with increasing sea surface temperatures (Precht and Aronson, 2004), it is suspected that the impacts of cold-water events will persist into the future (Kodra *et al.,* 2011). As such, *A. cervicornis* populations at or near the species' latitudinal limit may face thermally volatile conditions, resulting in regional population instability. This is concurrent with the notion that reef community composition is only stable on average over millennial timescales (Jackson, 1992).

At first, climate change (resulting in habitat range expansion) may seem beneficial to *A. cervicornis*. However, bleaching stress, which can result in colony mortality or reduced fertilization success (through decreased gamete production and sperm motility – Omori *et al.,* 1999), is becoming increasingly prevalent as annual sea temperatures rise (McWilliams *et al.,* 2005). Even though *A. cervicornis* populations flourished through the Holocene Thermal Maximum (at temperatures greater than those of today), it is suspected that the current rate of warming has exceeded or will exceed the capacity for acclimation by corals (Hoegh-Guldberg, 1999). Additionally, associated increases in dissolved carbon dioxide have been shown to negatively impact *A. cervicornis* growth (Renegar and Riegl, 2005) and compromise *Acropora* recruitment success (Albright *et al.,* 2010), which would correspondingly affect the species' capacity to recover.

Rising sea temperatures and associated coral bleaching have at times been accompanied by increases in the number and occurrence of coral diseases (Goreau *et al.,* 1998; Richardson, 1998). It is likely that stresses such as bleaching increase the susceptibility of corals to disease (Harvell *et al.,* 1999). In recent decades, the greatest and most widespread declines in *A. cervicornis* have been brought about by disease, prominently white band disease (Aronson and Precht, 2001b). Whether independent or manifestations

of varied stressors, diseases have become a persistent and significant source of mortality in *A. cervicornis* and other Caribbean reef corals (Goreau *et al.*, 1998; Richardson, 1998; Weil, 2001; Williams and Miller, 2005; Weil *et al.,* 2006). If these afflictions aren't overcome, either naturally or with assistance, *A. cervicornis* populations may never fully recover.

III. Artificial Coral Culture and Transplantation

Coral transplantation has many uses (Table 1), and while whole colonies (wild-sourced) have been used in the past, recent methodologies have adopted the use of cultured specimens for restoration (Bowden-Kerby, 1997, 1999; Edwards and Clark, 1998; Rinkevich, 2000; Lindahl, 2003; Herlan and Lirman, 2008; Grablow, 2010; Larson, 2010). The life histories and characteristics (fast-growing, branching morphologies) of *Acropora* species such as *A. cervicornis* lend them well to such culture.

Table 1 – Examples of Coral Transplantation Efforts

(Edwards and Clark, 1998 - adapted)

Aid recovery of dynamite fishing
Replace corals killed by thermal effluent
Save corals threatened by pollution, construction, dredging, etc.
Reintroduce species into previously polluted areas
Accelerate reef recovery following ship groundings
Enhance attractiveness of tourism areas
Rehabilitate tourist-damaged reefs; create artificial reefs to relieve diving pressure
Rehabilitate reefs impacted by natural events (El Niño, Crown-of-Thorns sea star, etc.)

Nursery cultivation of coral species such as *Acropora* takes advantage of such corals' predisposition to asexual reproduction by fragmentation (Tunnicliffe, 1981; Highsmith, 1982; Clark and Edwards, 1995). Essentially, fragments of a desired species are secured to a selected substrate, and left to grow as if they had undergone fragmentation in a natural setting. It is commonly performed in the aquarium trade by hobbyists and entrepreneurs seeking to cultivate (for sale and trade) a great variety of coral species.

Hobbyists typically use limestone rubble, cement and/or plastic plugs for convenient and transportable growth substrates, whereas in the larger scale of coral farming numerous materials are used such as cement blocks and pedestals, metal and cement frames, and even suspended networks made of wire, mesh, PVC, fishing line, etc. (Fig. 4) (Bowden-Kerby, 1997; Thorton *et al.*, 2000; Soong and Chen, 2003; Okubo *et al.*, 2005; Quinn and Kojis, 2006; Shafir *et al.,* 2006; Putchim *et al.*, 2008; Shaish *et al.*, 2008; Levy *et al.*, 2010; Nedimyer *et al.*, 2011). Utilizing cultured corals for restoration also avoids many of the negative attributes associated with whole colony collection and transplantation between reefs (Table 2).

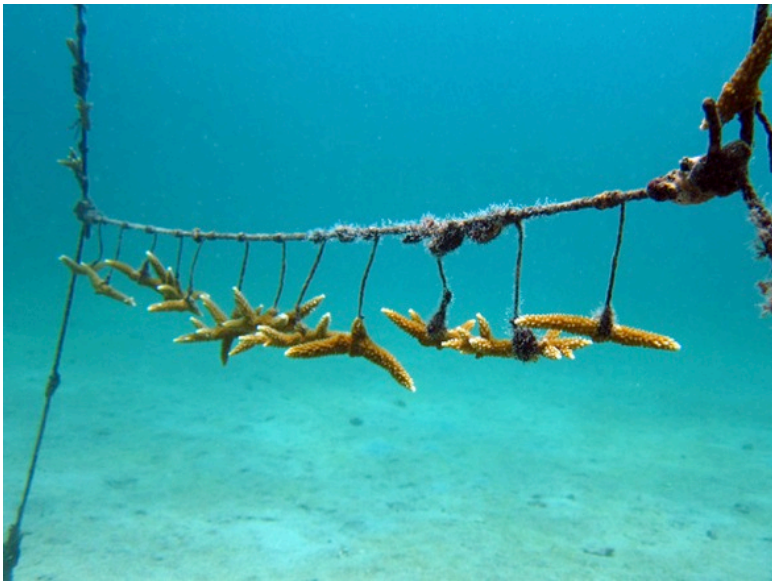

Figure 4: Cultured specimens of *Acropora cervicornis* growing in a suspended nursery (a.k.a. "line nursery") constructed of nautical rigging line and wire.

Table 2 – Potential Drawbacks of Whole Colony Transplantation

(Edwards and Clark, 1998 - adapted)

Loss of coral colonies from donor reef areas
Higher mortality rates of transplanted corals
Reduced growth rates of transplanted corals
Loss of transplanted colonies due to attachment failure
Reduced fecundity of transplanted colonies due to transplantation stress

Admittedly, restoration by transplantation has limitations. Current methods favor the production of fast-growing, branching species when it has been suggested that the introduction of slower-growing, massive species may be more effective in the long term for restoring damaged reefs (Edwards and Clark, 1998). In addition, restoration efforts using current methodologies can only affect small areas, and can not be expected to act as a cure-all for any single species or whole reefs in general. It is unrealistic to assume that with transplantation alone one could maintain the health of the world's reefs. However, what can be achieved by targeted transplantation has further-reaching effects than initial objectives of site-specific regeneration, beautification, etc. (Table 3).

Table 3 – Potential Benefits of Coral Transplantation

(Edwards and Clark, 1998 - adapted)

Immediate increase in coral cover and diversity
Increased recruitment of coral larvae as a result of presence of transplants
Survival of locally rare and/or endangered species when habitat is destroyed
Reintroduction of corals to areas which are larval supply-limited or have post-settlement mortality
Improved aesthetics of areas frequented by tourists
Increased rugosity and shelter for herbivores in bare areas

Transplantation-based restoration may be able to create and/or maintain "hot spots" of reproductive viability for a species, maintaining its presence in an area where it might be subject to local extinction. For species such as *A. cervicornis*, hot spots of sexual reproduction could act as consistent sources of new individuals, accelerating the natural process of recovery - one that is currently hindered by sharp declines in recruitment and gene flow. This has been a product of both population decreases resulting in decreased spawning volume, and the increasing difficulty of planulae to settle amongst heightening algal abundance on reefs (Kuffner *et al.*, 2007; Vollmer and Palumbi, 2007).

IV. Purpose and Objectives

Broadening the knowledge of physiological responses in corals undergoing fragmentation-based reproduction is essential in this age of reef degradation. More specifically, advancing our understanding of the asexual reproduction and growth of nursery-raised corals can only benefit the relatively young practice of coral culture. In the case of *Acropora cervicornis*, continuing the research of restoration methods is paramount. *Acropora cervicornis* and similar coral species are essential to the formation, growth, and continued existence of Caribbean coral reefs.

Investigating differences among *A. cervicornis* colonies grown via two common techniques (line and substrate nursery culture) can further develop our understanding of how *A. cervicornis*, and possibly other *Acropora*, respond to each technique. This is advantageous to coral nurseries, providing valuable knowledge useful to nursery installation and development, specifically for when and where it may be more appropriate to use one technique in lieu of the other. Such enhancements to the efficiency and success of coral nurseries, resulting in a greater production of coral, will ultimately assist efforts to conserve and restore reef habitat. Additionally, potential differences in survival, bleaching stress and disease occurrence between line colonies and those grown on substrate may provide insights into the influence water flow and/or interactions with the benthic community have on coral physiology. This could prove invaluable as restoration efforts begin concentrating on transplanting nursery-generated corals out to natural reefs (commonly termed "outplanting").

This study has three main objectives, all of which aim to elucidate various effects of line nursery culture on *A. cervicornis*:

1. Measure growth and survivorship of *A. cervicornis* colonies grown in a line nursery, and how the quantity of fragments generated for nursery expansion and outplanting is affected. With recent adoption of *A. cervicornis* line nursery culture, it is important to assess its performance against traditional techniques. Doing so can help determine whether investments of nursery resources are justly

allocated to the new technique, rather than adopting line nursery culture simply because it is novel.

2. Determine whether significant differences in growth and survivorship previously observed among genotypes on substrate nurseries persist in line nurseries. Although most genotypes of *A. cervicornis* are easily cultivated in nurseries, some do not fare as well as others, growing more slowly and/or perishing in greater proportion due to various causes. Whether a natural condition or something artificially produced or amplified by nursery culture, increases of growth rate in slower-growing genotypes and/or decreases in mortality as a product of line nursery culture would be beneficial. Additionally, understanding how well specific genotypes will grow in relation to one another is helpful in nursery planning, maintenance, and outplant design (e.g. how much of genotype 'X' can be expected to have grown from nursery stocks within a set period of time).

3. Investigate whether line nursery culture significantly increases *A. cervicornis* fragment survivorship in warm temperature conditions known to cause high mortality rates in fragments (Larson, 2010) (Table 4). Increased tolerance to temperature-associated stress (to the effect of decreased mortality) has been observed, but not quantified by other facilities conducting line nursery culture of *A. cervicornis* (Ken Nedimyer and Katie Grablow of Coral Restoration Foundation, pers. comm.). Currently, it is recommended that fragmentation and transplantation of *A. cervicornis* be limited to the cooler months of October - May (Johnson *et al.,* 2011) when water temperatures do not exceed ~27ºC (Larson, 2010) to avoid heightened mortality. If greater survivorship is observed in line nursery fragmentations conducted during warmer conditions, it would allow for the expansion of the fragmentation season for *A. cervicornis* nurseries. Relieving some of this limitation might assist nursery operations, especially those located in lower latitudes where increased water temperatures occupy a greater proportion of the year.

To accomplish these objectives, two experiments were conducted. The first, fragmenting and growing multiple genotypes of *A. cervicornis* in a line nursery provided colony growth and survivorship to compare against those grown via substrate culture (fragments mounted to cement pucks - Larson, 2010). The second, conducting multiple fragmentation trials of both line and puck-mounted fragments during a period of decreasing water temperatures (Table 4), allowed for direct comparisons of survival to assess whether nursery technique affects mortality rates in fragments.

Table 4 –Acropora cervicornis Nursery Fragment Mortality by Temperature

(Larson, 2010)

Fragmentation Month	Temperature*	Mortality
September	30.3ºC	56%
October	28.6ºC	42%
December	25.2ºC	22%

*Temperature taken as the average of in situ temperatures logged the first week following fragmentation.

EXPERIMENT 1 – EVALUATING GROWTH AND SURVIVORSHIP OF *ACROPORA CERVICORNIS* GROWN VIA THREE NURSERY TECHNIQUES

V. Procedure and Methods

Six freestanding line nursery units, hereafter referred to as "lines," were installed at an existing *Acropora cervicornis* nursery off Ft. Lauderdale, Florida at a depth of approximately seven meters. The nursery, less than one kilometer from shore and located in the sand channel between the nearshore ridge complex and inner reef of the region, is composed of concrete modules (1 m^3) on which hundreds of *A. cervicornis* colonies grow affixed to cement pucks. Modules are positioned 7.5m apart in two 100m parallel rows separated by 30m, and run perpendicular to shore from west to east. Lines were installed between concrete modules in the northern row with a separation of 15m (Fig. 5).

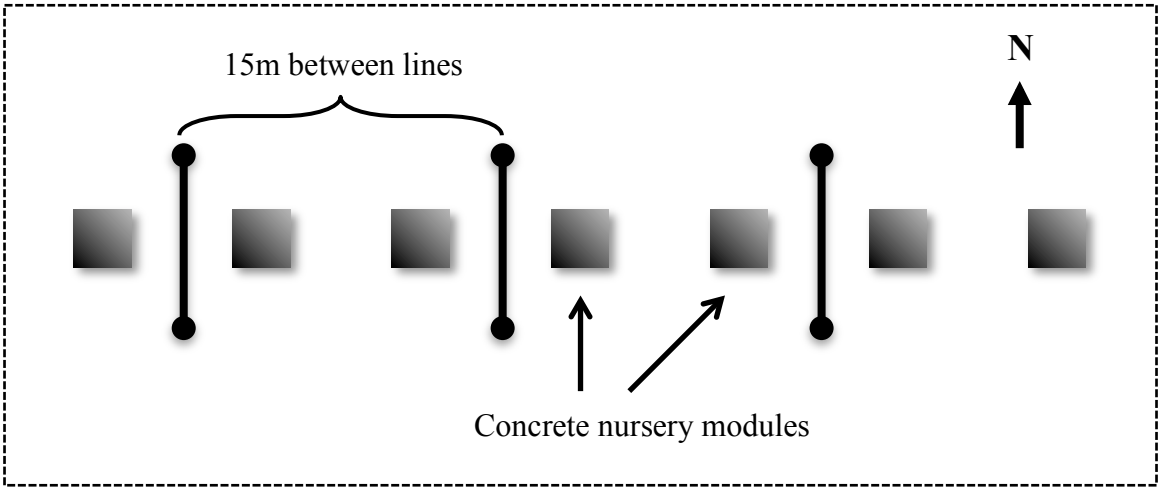

Figure 5. Subsection of the *A. cervicornis* nursery showing positioning of lines and modules.

Six lines each with 24 *A. cervicornis* fragments (of approximately 3cm) were installed; for a planned total of 144 fragments[1]. Fragments of three genotypes (designated "4," "8," and "10" - predefined via microsatellite DNA markers; Larson, 2010) were harvested from existing colonies in a nearby nursery in equal number (48 per genotype).

[1] A miscount during fragment collection resulted in the installation of 143 total fragments. One fragment (genotype 4, vertical orientation) was omitted from the study.

Genotypes were selected if adequate amounts of healthy tissue were available in the source nursery, and if significant differences in growth were previously documented (Larson, 2010). Fragments were not collected from colonies exhibiting disease, bleaching, or severe predation, and were cut to remove existing apical polyps to remove a source of potential variability among fragments. Separated by genotype, fragments were then transported in plastic jars (water-filled) placed inside a cooler to maintain a steady temperature. Transport between nurseries was brief, lasting no longer than two hours from collection to placement in the destination nursery.

To all lines, fragments of each genotype were attached with shielded wire (copper, 20 gauge, malleable plastic shielding). Line nursery operations culturing *A. cervicornis* have also utilized monofilament fishing line instead of shielded wire, however due to a population of (somewhat inquisitive) grey triggerfish (*Balistes capriscus)* at the nursery site, there was concern that monofilament lines could have been bitten through.

Fragments were attached to nursery lines via two techniques, "suspended" and "vertical" (detailed below). Combined with puck-grown colony data from Larson (2010), three growth techniques, each including the same three genotypes (4, 8, and 10) were compared (Table 5). Comparing the same known genotypes grown in the same region removes variation from geographic factors that could be falsely attributed to differences among techniques.

Table 5 – Quantity of Fragments per Genotype and Technique

Technique	Genotype 4	Genotype 8	Genotype 10	*Total*
Suspended	24	24	24	*72*
Vertical	23	24	24	*71*
Puck (Larson, 2010)	9	9	9	*27*

Suspended technique fragments were hung horizontally from nursery lines, with 10 – 15cm of separation between fragments and 10cm of separation from the nursery line (Fig.

6). Wire was first affixed to each fragment (on board the research vessel), followed by winding (and crimping to prevent lateral shifting) the free end of each wire to nursery lines in situ. Vertical fragments were directly attached to vertical nursery lines with polyp apertures facing upward, with 10 – 15cm of separation between fragments, to nursery lines (3/8" polyester nautical rigging) (Fig. 7). Though existing line nurseries typically employ only the suspended technique; this experiment's vertical attachment technique was created to utilize unused space on nursery lines. Acceptable growth and survivorship of these fragments could prompt the use of unutilized space in other line nurseries, providing more options for existing and future line nurseries.

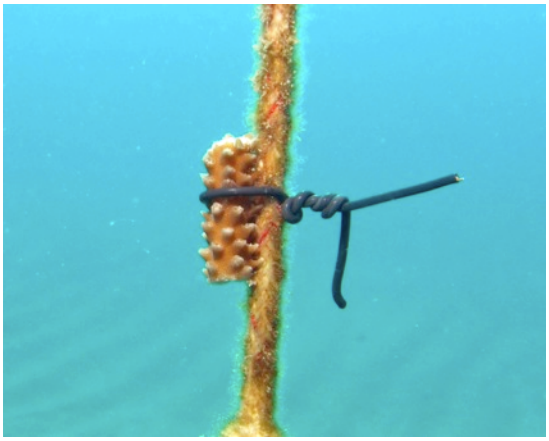

Figure 6 (left): Example of *A. cervicornis* fragments suspended from a nursery line.

Figure 7 (right): Example of an *A. cervicornis* fragment directly attached (vertical method) to a nursery line.

Each nursery line consisted of a single two meter horizontal run supporting 12 suspended fragments, and two vertical runs supporting six vertical fragments each (Fig. 8). Suspended fragments were approximately one meter above the substrate (sand), and vertical fragments were positioned from approximately one to two meters above the substrate. Lines were secured to the substrate with two 75cm long, 10cm diameter screw-in ground anchors, and held upright by two, 15cm diameter styrofoam support buoys. To lessen possible shading effects support buoys on the uppermost vertical fragments, 30cm of buffer space was incorporated.

For both techniques, genotype fragment position was divided evenly among lines and between attachment techniques. This method of placement was chosen to mitigate the

potential severity of losses to any one genotype in the event of line failure or loss. Additionally, by distributing fragments of each technique evenly across the nursery and in equal quantity per line, differing environmental conditions among lines (i.e. currents and wave energy) would not disproportionately affect techniques or genotypes.

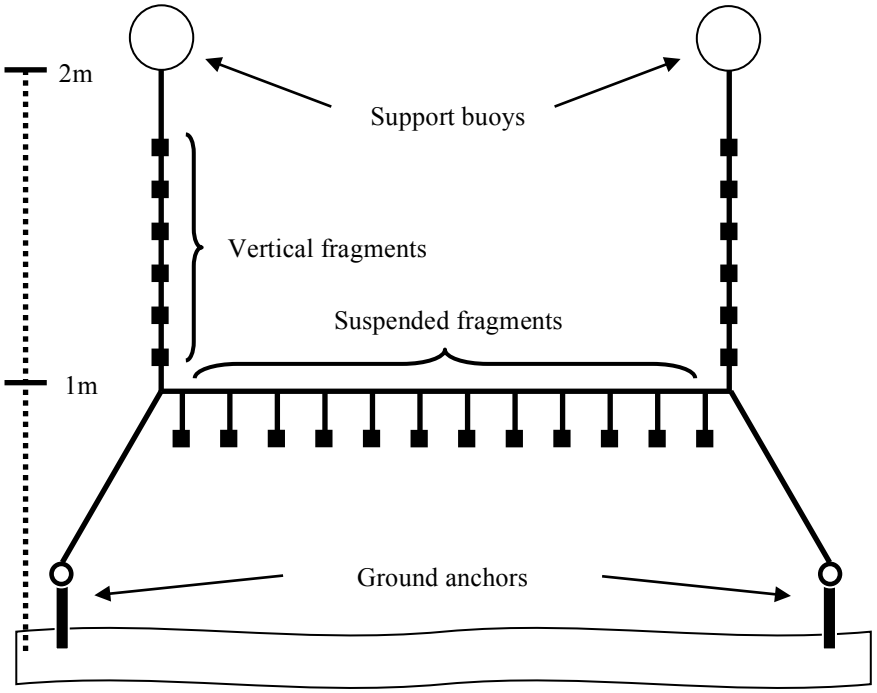

Figure 8: Diagram of a nursery line.

Upon initial fragmentation, and at monthly intervals for a period of one year[2], multiple characteristics of coral growth and condition were recorded. Tissue extension and branch number parameters were selected to replicate those of previous *A. cervicornis* growth and survivorship study (Larson, 2010) to allow for direct comparison of growth data. All length measurements were taken to the nearest millimeter using calipers. The following parameters were measured at each monitoring month:

1. Tissue Mortality – estimated in increments of five percent of the total colony.
2. Tissue Extension - the sum length of all branches. Branches were measured only if ≥ 0.5cm in length (measurement taken from apical tip of new branch to outer surface of parent branch – not to core of parent branch - Fig. 9).

[2] Monitoring during the December 2011 period was not performed due to persistent inclement weather.

3. Branch Number – the summed number of branch measurements taken for tissue extension provided the number of branches for each colony at each recording interval.

4. Attachment – fragments were considered "attached" once tissue grew over attachment wire (and/or nursery line for vertical fragments) such that at any point, coral tissue completely enveloped attachment wire (Fig. 10).

5. Bleaching, predation, general stressors – general fragment condition was assessed with respect to bleaching, disease, suspected branch breakage and/or predation, and overgrowth by competing organisms such as macroalgae, hydroids, etc. (Fig. 11). Each condition (except branch breakage) was recorded as an estimated percentage of the colony affected. For disease and predation events, lengths of dead branch area were also measured.

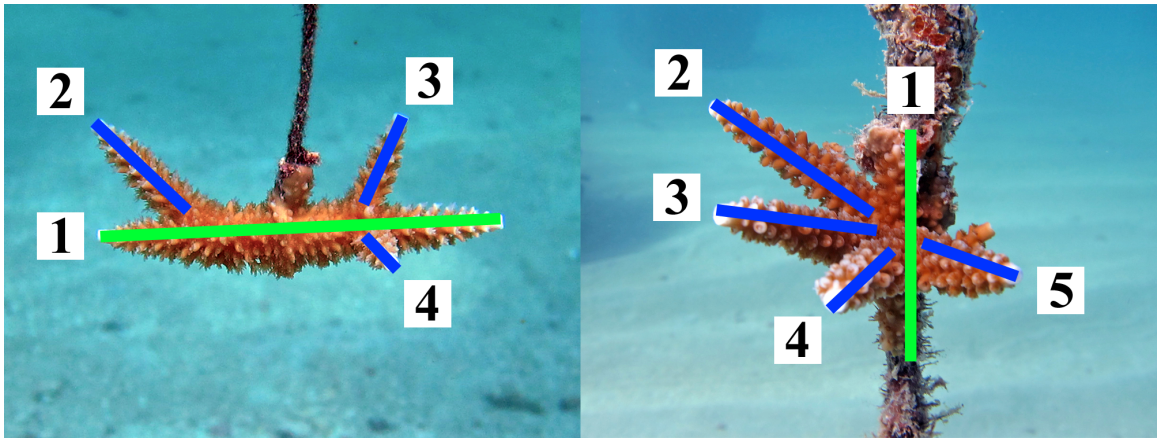

Figure 9: Growth measurements of suspended (left) and vertical (right) colonies. Tissue extension would be taken as the sum of all numbered measurements for each colony. Initial measurements (#1 for each colony) may encompass two growth ends.

During monitoring events, moderate effort was taken to clear nursery lines and colonies of fouling organisms. Example maintenance included: manual removal of hydroids overgrowing colonies (Fig. 11), manual removal of fouling organisms from lines (macroalgae, sponges, bivalves, etc.), and the rotation of suspended colonies to minimize competitive interaction. Maintenance was not exhaustive, and when performed equal effort was applied to all lines.

Figure 10 (left): Example of fragment attachment success, note coral tissue enveloping attachment wire.

Figure 11 (right): Example of hydroid overgrowth, a recorded stressor.

VI. Hypotheses

A. Survivorship:

H_{a01}: There are no significant differences in initial (approximately one month post-fragmentation) or overall fragment survivorship (assessed at year-end) among suspended, vertical, and puck culture techniques.

H_{a02}: There are no significant differences in initial or overall fragment survivorship among genotypes within suspended, vertical, and puck culture techniques.

B. Tissue Extension:

H_{b0}: There are no significant differences in fragment tissue extension among suspended, vertical, and puck culture techniques.

H_{b1}: Gentoype 4 fragment tissue extension significantly exceeds that of genotype 10 fragments within suspended and vertical culture techniques; this relationship among genotypes has already been shown for puck-mounted fragments (Larson, 2010).

C. Branching:

H_{c0}: There are no significant differences in fragment branch number among suspended, vertical, and puck culture techniques.

H_{c1}: Genotype 4 fragment branch number significantly exceeds both genotype 8 and 10 fragment branch number within suspended, vertical, and puck culture techniques.

Based upon the findings of Larson (2010) and observations of existing line nursery operations (Ken Nedimyer and Katie Grablow, pers. comm.), certain characteristics of *Acropora cervicornis* growth are suspected to remain consistent (i.e. genotypic growth rate ratios). Any uniform amplification of growth by line nursery culture would maintain growth rate ratios among genotypes, and reproduce the tendency of genotype 4 to significantly outgrow genotype 10 (H_{b1}). Enhanced growth could also result in significantly greater tissue extension in both suspended and vertical colonies (H_{b0}). Colony branching frequency, as it is directly related to tissue extension, is expected to behave similarly (H_{c1}). The directional freedom that suspended colonies have to grow could lead to significantly greater branching than vertical colonies, which may be limited by the direct adjacency of nursery lines (H_{c0}).

In addition to increased growth, other *A. cervicornis* nurseries employing suspended culture (Coral Restoration Foundation, Ken Nedimyer and Katie Grablow, pers. comm.) have observed increased survivability of suspended fragments compared to puck-mounted fragments, which may be reproduced in this experiment (H_{a01}). The three genotypes in this study were mildly affected by mortality in the previous study (11% for genotypes 4 and 8, n = 1 of 9, 0% for genotype 10; Larson, 2010), and did not significantly differ. Only with increased mortality rates could a significant difference occur among genotypes for either technique. (H_{a02}).

VII. Results

VII.1 *Survival - Initial*

One month following fragmentation (February 2011; 35 days in situ), four vertical fragments and zero suspended fragments had died (Table 6). Initial fragment survivorship did not significantly differ among suspended, vertical, and puck fragments (38 days post-fragmentation; Larson, 2010) ($\chi^2 = 4.750$, df = 2, $p > 0.05$, JMP Pro 9.0.2). Within techniques, survival only significantly differed among genotypes for vertical fragments, in which significantly more genotype 10 fragments died than the other two genotypes ($\chi^2 = 8.301$, df = 2, $p = 0.016$, JMP Pro).

Table 6 – Fragment Mortality One Month Post-Fragmentation

Technique	Genotype 4	Genotype 8	Genotype 10	*Combined*
Suspended (n = 0/24, 0/24, 0/24)	0%	0%	0%	*0%*
Vertical (n = 0/23, 0/24, 4/24)	0%	0%	17%	*6%*
Puck (Larson, 2010) (n = 1/9, 1/9, 0/9)	11%	11%	0%	*7%*

VII.2 *Survival - Overall*

At the conclusion of the monitoring effort (January 2012; 350 days after fragmentation), all suspended colonies had survived. Over 50% of vertical colonies had died (Table 7), significantly more than both suspended and puck-mounted colonies ($\chi^2 = 81.31$, df = 2, $p < 0.01$, JMP Pro). Of the vertical colonies that died, a significant majority were genotype 10 (Fig. 12) ($\chi^2 = 10.531$, df = 2, $p = 0.005$, JMP Pro).

Table 7 – Year-end Complete Colony Mortality

Technique	Genotype 4	Genotype 8	Genotype 10	*Combined*
Suspended (n = 0/24, 0/24, 0/24)	0%	0%	0%	*0%*
Vertical (n = 13/23, 10/24, 18/24)	57%	42%	75%	*57%*
Puck (Larson, 2010) (n = 1/9, 1/9, 0/9)	11%	11%	0%	*7%*
Combined	*25%*	*19%*	*32%*	*25%*

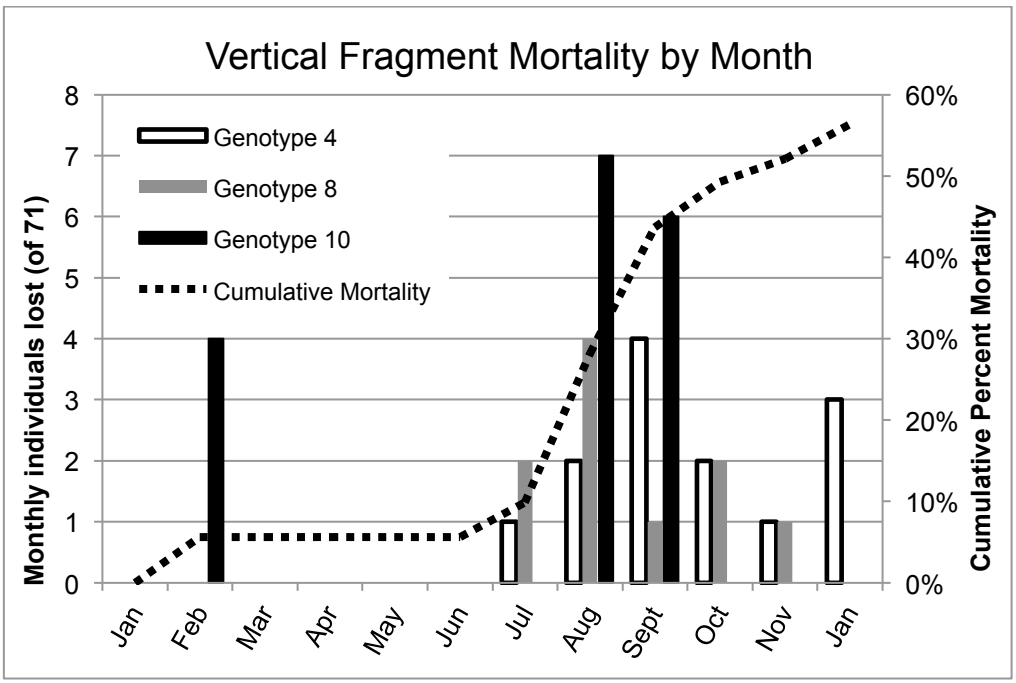

Figure 12: Monthly individual and cumulative percent mortality of vertical fragments. No suspended corals perished during the timeframe of the study. Cumulative percent mortality calculated against total vertical fragments (n = 71).

VII.3 *Partial Mortality - Incidence*

Partial mortality was not recorded by Larson (2010) as in this experiment. As such, partial mortality (sections VII.3, VII.4) includes assessments of only suspended and vertical colonies. Incidences of partial mortality (any recorded mortality less than 100%) in vertical colonies exceeded suspended colonies in every monitoring period, and differed significantly for the first three and last three monitoring months following fragmentation (February, March, April, October, November 2011, January 2012; Fisher's Exact Test, two-tailed, $p < 0.05$, JMP Pro). Among genotypes (techniques combined), significant differences in the frequency of partial mortality were observed for most post-fragmentation monitoring months (Table 8, significantly high mortality highlighted) (Fisher's Exact Test, two-tailed, $p < 0.05$, JMP Pro). The majority of colonies affected were genotype 10 in earlier months (February to June), and genotype 8 in latter months (October and November).

Table 8 – Line Fragment Partial Mortality – Percent Incidence by Monitoring Month

(Suspended and Vertical Techniques Combined; calculated against surviving colonies)

Monitoring Month	Genotype 4	Genotype 8	Genotype 10	Fisher's Exact (two-tailed) p
February, 2011 (n = 0/47, 2/48, 9/44)	0%	4%	20%	0.005
March (n = 0/47, 1/48, 9/44)	0%	2%	20%	0.001
April (n = 0/47, 2/48, 9/44)	0%	4%	20%	0.005
May (n = 0/47, 2/48, 8/44)	0%	4%	18%	0.014
June (n = 0/47, 3/48, 8/44)	0%	6%	18%	0.028
July (n = 2/46, 3/46, 8/44)	4%	7%	18%	0.148
August (n = 3/44, 6/42, 6/37)	7%	14%	16%	0.631
September (n = 6/40, 15/41, 7/31)	15%	37%	23%	0.081
October (n = 9/38, 23/39, 9/31)	24%	59%	29%	0.003
November (n = 16/37, 23/38, 9/30)	43%	61%	30%	0.040
January, 2012 (n = 15/34, 23/38, 11/30)	44%	61%	37%	0.131

VII.4 *Partial Mortality - Severity*

Severity of partial mortality, measured as percent mortality for each fragment (Fig. 13), was assessed by monitoring month, technique, and genotype. Mean colony percent mortality was significantly greater (Wilcoxon pairs test, $p < 0.05$, JMP Pro) in vertical colonies for all months following fragmentation except July and August 2011 ($p = 0.225$, 0.964 respectively). Greater mean percent mortality was observed, and increased consistently, from August 2011 (4% ±15% S.D.) to January 2012 (39% ±35% S.D.) for vertical colonies. Suspended colonies also experienced increasing partial mortality during the same time period, but this was less severe (1% ±5% S.D. to 3% ±10%).

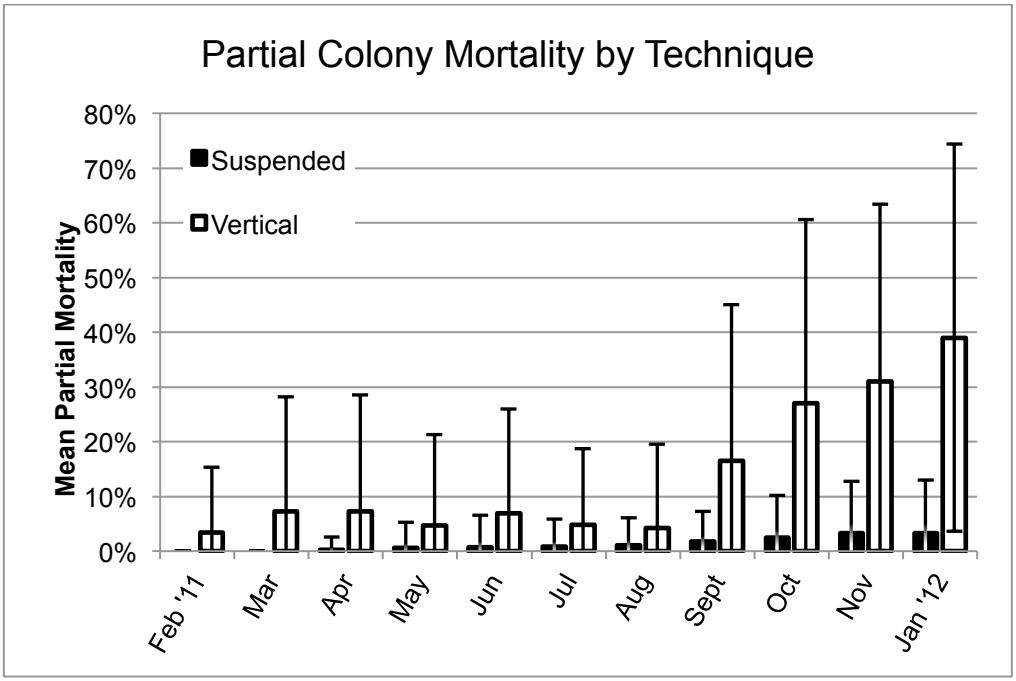

Figure 13: Average partial mortality by monitoring month for colonies in each attachment technique (average calculated against number of surviving colonies in each month, error bars denote standard deviation). Wide standard deviations a product of many individuals with zero recorded partial mortality in both groups.

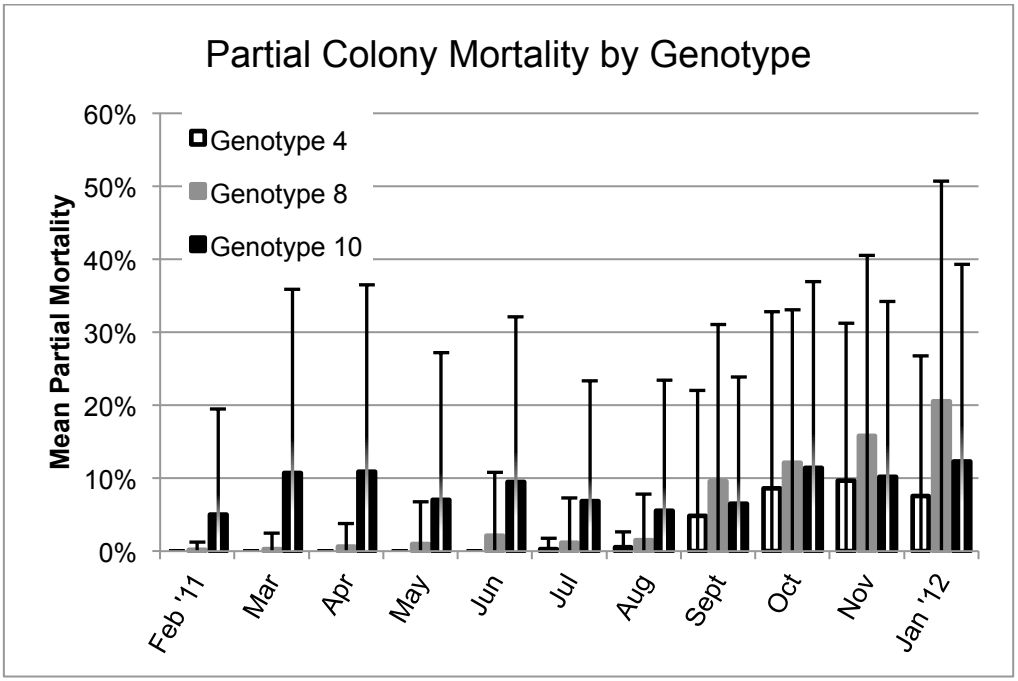

Figure 14: Average partial mortality by monitoring month for each genotype (average calculated against number of surviving colonies in each month, error bars denote standard deviation). Wide standard deviations a product of many individuals with zero recorded partial mortality in all groups.

Among genotypes (Fig. 14), genotype 10 colonies exhibited significantly more partial mortality from February to July ($p < 0.05$, genotype 10 > genotypes 4, 8 for February – May; genotype 10 > genotype 4 for June – July; Wilcoxon Multiple Pairs test – Kruskal-Wallis, JMP Pro). Genotype 8 exhibited significantly greater partial mortality for September and October ($p < 0.05$, genotype 8 > genotype 4, Kruskal-Wallis, JMP Pro).

VII.5 *Stressors*

Recorded stressors included: hydroids, macroalgal overgrowth, sponge overgrowth, bivalve overgrowth, jellyfish entanglement (*Aurelia aurita*), and fire coral (*Millepora sp.*) growth. Except for jellyfish interaction (section VII.6), only qualitative observational data are reported. Possibly due to greater surface area of contact with nursery lines, vertical colony condition data suggested greater, more consistent impacts by fouling communities, particularly from hydroid and macroalgal overgrowth (Figs. 15 and 16).

Figures 15 (left): A vertical colony experiencing tissue loss, presumably from stresses associated with adjacent hydroids.

Figure 16 (right): A recently dead vertical colony, enveloped in macroalgae growing on nursery lines.

Suspended colonies were also affected by hydroids and macroalgal growth, but less frequently (Figs. 17 and 18). A few, small encrusting colonies of fire coral (*Millepora sp.*) also grew in contact with suspended nursery colonies (none observed near vertical colonies), but their presence was ephemeral and it appeared that *A. cervicornis* was the more aggressive species (Fig. 19). For suspended colonies, the greatest observed contributor to partial mortality was bivalve overgrowth (Fig. 20). Molluscs would settle

on available wire substrate directly above a colony, and grow to smother a portion of the upper colony surface (commonly 3 – 4cm of tissue).

Figure 17 (upper left): Hydroids growing down attachment wires resulted in localized tissue death for some suspended colonies.

Figure 18 (upper right): Macroalgae also grew on some colony attachment wires.

Figure 19 (lower left): *A. cervicornis* extruding mesenterial filaments to combat *Millepora sp.* fire coral growing on attachment wire.

Figure 20 (lower right): Molluscan overgrowth of a suspended nursery colony.

VII.6 *Stressors – Jellyfish*

During the August and September 2011 monitoring periods, numerous individuals of moon jellyfish (*Aurelia aurita*) were found entangled on nursery lines (Fig. 21). Seventy two colonies were impacted in August (58% of surviving colonies), and 17 were noted at the September monitoring (15% of surviving colonies) (Table 9). Many colonies that had recently died (clean skeletons with occasional remnants of necrotic tissue) were found enveloped by jellyfish in August and September (Fig. 22), and it is suspected that *A. aurita* was a primary cause of *A. cervicornis* colony mortality in those months (Table 9).

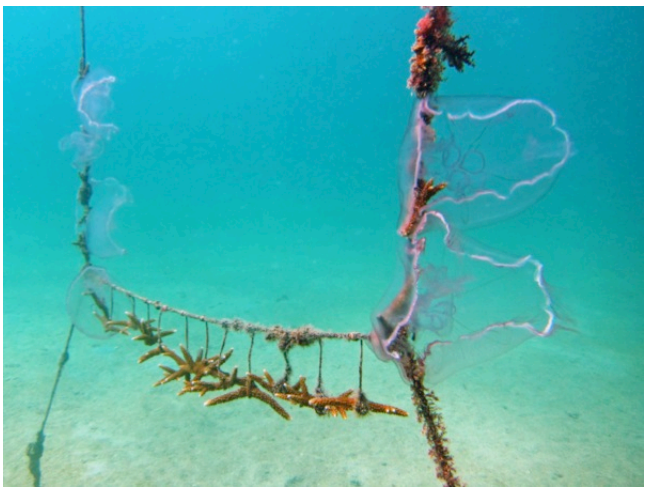

Figure 21 (left): Multiple *Aurelia sp.* jellyfish entangled in nursery lines. Relatively consistent, laminar flow at the nursery location may have contributed to *Aurelia* individuals' inability to free themselves once entangled.

Figure 22 (right): Barren skeleton of a recently dead *A. cervicornis* colony visible through the body of the *Aurelia sp.* jellyfish in which it is enveloped.

In subsequent months (September and October), seven of the 15 colonies that perished had been previously impacted by *A. aurita* (Table 9). Other colonies affected by entangled *A. aurita* exhibited varying degrees of tissue damage (Fig. 23). Suspended colonies appeared to be more resistant to tissue damage by *A. aurita,* possibly because of their larger size, which appeared to keep jellyfish from enveloping them as completely as vertical colonies were (Fig. 24).

Table 9 – Line Nursery Colonies Affected by Jellyfish

Technique	Number of Colonies Affected by Jellyfish		August and September Mortality	Mortality Attributed to Jellyfish
	August	September		
Suspended	42	7	0	0
Vertical	30	10	24	12 (50%)
Combined	72	17	24	12 (50%)

Figure 23 (left): Close-up of vertical *A. cervicornis* with tissue damage from *A. aurita* entanglement. Pale branch tips and corallite ridges show dead areas; dark brown regions indicate remaining healthy tissue.

Figure 24 (right): *Aurelia aurita* jellyfish caught on suspended nursery colonies. The larger size of suspended colonies prevented complete envelopment and the subsequent mortality that many vertical colonies experienced.

When observed, entangled *A. aurita* individuals and tissue fragments were manually removed to limit further impacts to nursery colonies. Impacts from jellyfish entanglement were not observed to the same degree in the adjacent fixed nursery, although significant *Aurelia*-associated mortality was observed by other suspended *A. cervicornis* nursery operations in the south Florida region during the same season (Coral Restoration Foundation, Ken Nedimyer and Stephanie Roach, pers. comm.).

VII.7 *Predation*

No predation of line nursery colonies was observed during the monitoring effort. Predation by fireworms, *Hermodice carunculata,* was observed in the adjacent fixed nursery modules (Fig. 25); structures of which were less than three meters from nursery lines. It is possible that *H. carunculata* did not travel to or inhabit nursery lines because of inadequate refuge. Nursery colonies may also have been far enough from the substrate to be beyond *H. carunculata* detection.

Figure 25: Fireworm (*H. carunculata*) predation of *A. cervicornis* on fixed nursery modules adjacent to the line nursery. Turf algae can be seen growing on the exposed skeleton of dead branch tips.

VII.8 *Disease Incidence*

In early August, four vertical colonies (two of genotype 8, one each of genotypes 4 and 10) were afflicted by rapid tissue necrosis (RTN) (Fig. 26), a condition in which tissue rapidly wastes from one or more regions of a colony (Williams and Miller, 2005). The following month, one possible incidence of a band-type disease (possibly white band disease - unconfirmed) was observed on a suspended colony (genotype 10) (Fig. 27). All four vertical colonies with recorded disease died, while the suspended colony survived with minimal tissue loss (approximately 3cm). Due to the rapid nature in which RTN propagates across a colony, it is possible that more incidences of RTN occurred, but were not observed due to the length of time between monthly monitoring events.

Significant difference in disease incidence was only observed between suspended (n = 1 of 72) and puck-grown colonies (n = 4 of 25[3]) ($p < 0.05$, Fisher's Exact Test, two-tailed, JMP Pro). Vertical colony disease incidence (n = 4 of 55[3]) did not significantly differ from either suspended or puck colonies ($p > 0.05$, Fisher's Exact Test, two-tailed, JMP

[3] Number of diseased colonies assessed against total surviving colonies at month of disease occurrence.

Pro). For each technique, no significant difference in disease incidence was observed among genotypes ($p > 0.05$, Pearson's Chi-sqaure, JMP Pro).

Figure 26 (left): Rapid tissue necrosis on a vertical colony. Progression of tissue loss, if observed, often proceeded outwards from colony regions closest to nursery lines.

Figure 27 (right): A region of mortality on a suspended colony. The degree of algal growth on exposed skeleton suggested a progression of tissue necrosis towards the branch tip characteristic of white band disease.

VII.9 *Growth – Attachment*

Growth-based attachment of fragments to attachment wires was nearly complete at approximately three months (April 2011 monitoring, 96 days in situ), with 97% of suspended colonies and 79% of vertical fragments completely enveloping their attachment wires. As only one colony was lost during the experiment (vertical, ten months into experiment), the methods by which both suspended and vertical fragments were attached appear reliable. Attachment growth of puck fragments (growth of encrusting tissue onto pucks) was not documented for comparison (Larson, 2010).

VII.10 *Growth – Tissue Extension*

Over the 350 day monitoring period, suspended colonies grew to a mean of 62.9 ±33.9cm S.D. (Table 10) (Fig. 28); the maximum colony size attained was 133.3cm. Vertical colonies grew to significantly smaller average size than suspended colonies (Mann-Whitney $U = 27$, $p < 0.001$, StatSoft Statistica software package 6.1), from 3.2 ±0.4cm to 12.1 ±7.1cm (Fig. 29); the maximum colony size attained was 31.1cm. Puck-mounted

corals grew to similar sizes as suspended colonies (68.0 ± 43.4cm S.D.), and also significantly exceeded vertical colony growth (t-test, $p < 0.01$, Statsoft Statistica).

Table 10 – Mean Colony Size (Tissue Extension) at Final Monitoring

Technique	Genotype 4	Genotype 8	Genotype 10	*Technique Overall*
Suspended	106.4 ±16.7cm	40.7 ±13.9cm	42.6 ±13.3cm	62.9 ±33.9cm
Vertical	48.3 ±8.6cm	9.9 ±3.2cm	7.8 ±4.1cm	12.4 ±7.0cm
Puck* (Larson, 2010)	115.4 ±33.5cm	50.8 ±7.0cm	30.0 ±12.7cm	68.0 ± 43.4cm

*Puck growth values extrapolated to a 350 day growth period.

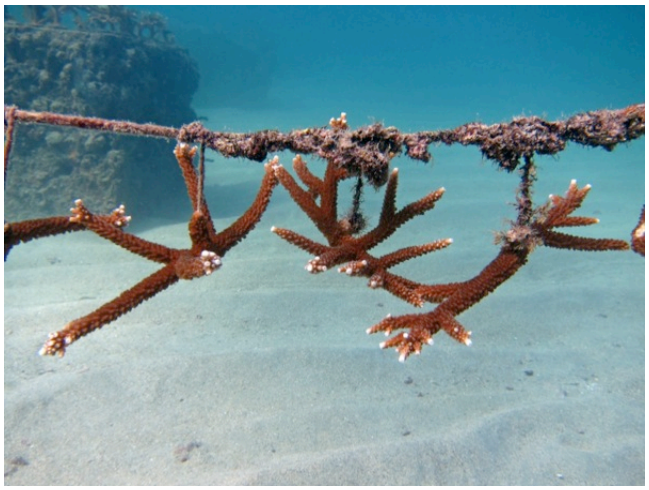

Figure 28 (left): Many suspended colonies achieved summed branch lengths (tissue extension) of over 100cm at the time of final monitoring. Colonies pictured range from 63 to 104cm.

Figure 29 (right): Vertical colonies did not grow as successfully as suspended colonies. Colony pictured 20cm (tissue extension).

Daily mean colony growth rate (Fig. 30; grouped by monitoring period) increased in suspended colonies from fragmentation (0.008 ±0.007cm day^{-1} from January to February 2011) to a maximum in August (0.274 ±0.239cm day^{-1} from July to August 2011). Suspended colony growth averaged 0.247 ±0.149cm day^{-1} for the remainder of the study (August to January, 2012). Colonies grown on pucks (Larson, 2010) did not exhibit a summer growth peak (Fig. 30), and averaged 0.315 ±0.233cm day^{-1} over an equivalent

period[4], though this was not significantly more (Mann-Whitney U = 567, p = 0.414, Statsoft Statistica). Initially, vertical colonies grew similarly (0.004 ±0.007cm day^{-1} from January to February 2011), but only reached a maximum rate of 0.047 ±0.039cm day^{-1} (April to May 2011). Within every culture technique, genotype 4 colonies significantly outgrew genotypes 8 and 10 (ANOVA-Tukey HSD, $p < 0.05$, StatSoft Statistica) (Fig. 31). Per genotype, vertical colonies grew significantly slower than both suspended and puck-mounted colonies (ANOVA-Tukey HSD, $p < 0.05$, StatSoft Statistica).

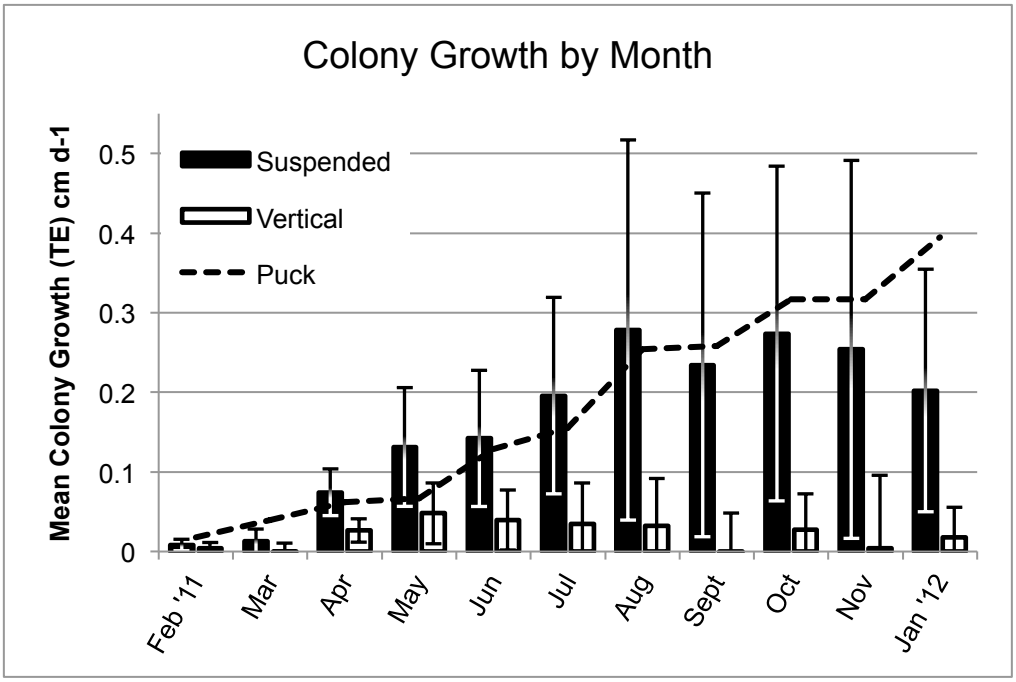

Figure 30: Mean change in colony size (tissue extension; ±S.D.) by monitoring period for suspended and surviving vertical colonies. Dashed line indicates mean puck colony growth over similar time periods.

[4] Growth period taken as July to November, 2008 to standardize the time period relative to initial fragmentation (colony growth from approximately seven months to the conclusion of monitoring).

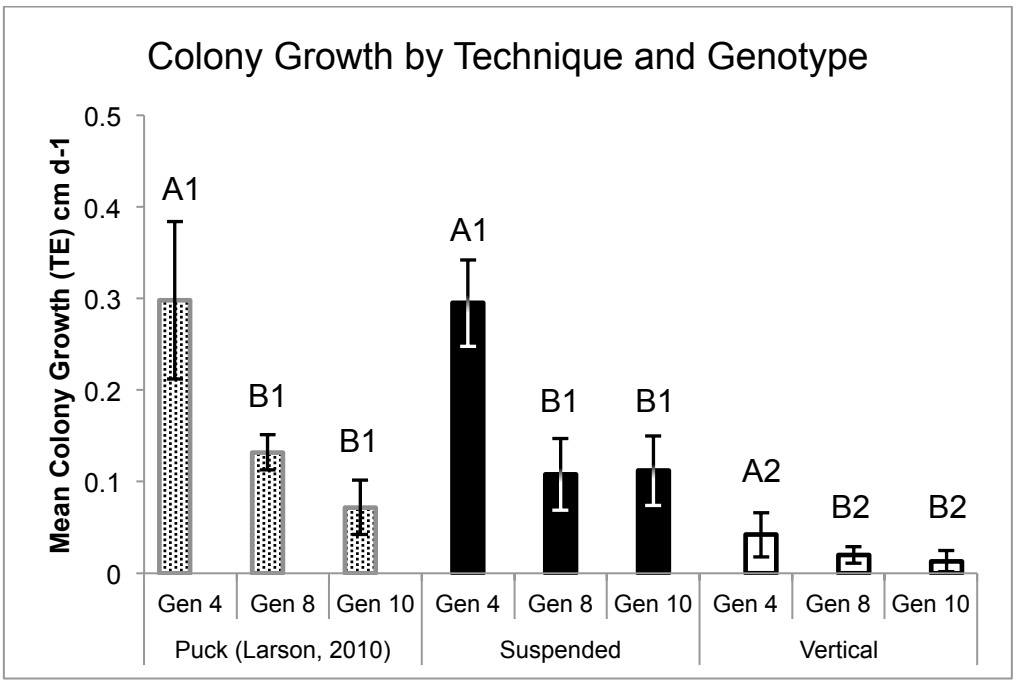

Figure 31: Mean daily colony growth (tissue extension; ±S.D.) over the course of each study (approx. one year) by technique and genotype. Like-letter groups indicate statistical similarity within techniques; like number groups indicate similarity within genotypes (all comparisons ANOVA-Tukey HSD, $\alpha = 0.05$, StatSoft Statistica 6.1).

Suspended colonies did not grow significantly more than their like-genotype counterparts grown on pucks (Fig. 31). However, many colonies exhibited notable abrasion (Fig. 32) with increasing frequency during the latter half of the study (August 2011 – January 2012). This may have contributed to artificially hindered growth and branch generation in affected colonies. Analyzing colony growth at a time before most abrasion (six months, Fig. 33) revealed significantly higher growth for suspended genotype 10 colonies compared to puck-grown colonies (Kruskal-Wallis one-way analysis of variance, $p < 0.05$, StatSoft Statistica).

Figure 32: Abrasion in two suspended colonies. Instances of abrasion increased as colonies grew larger. The presence of many stunted branch tips at areas of colony contact may indicate hindrances to growth.

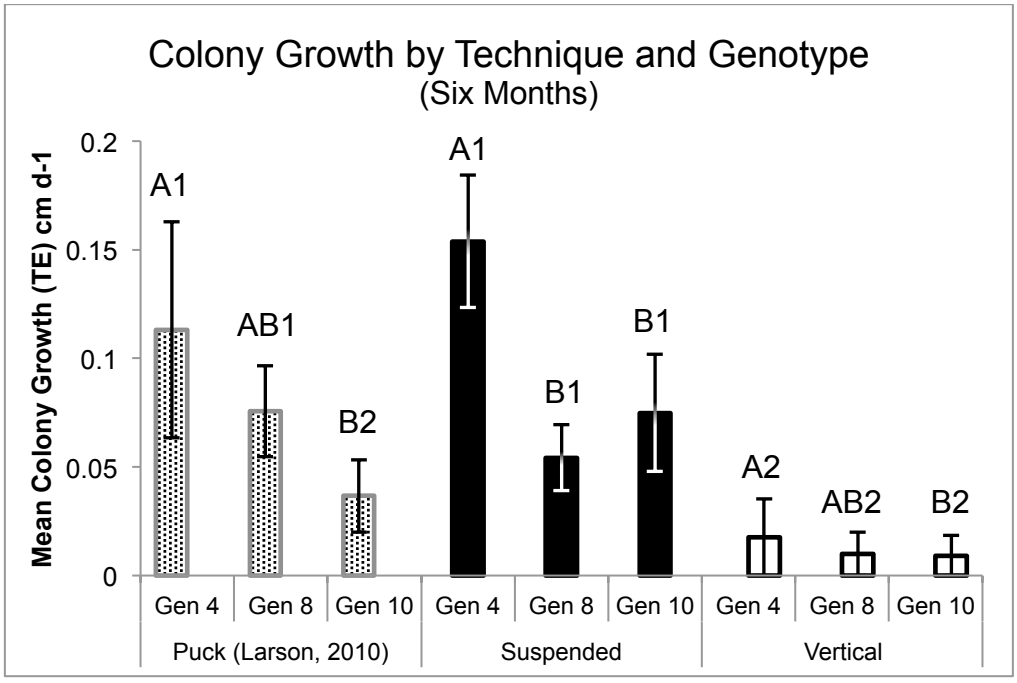

Figure 33: Mean daily colony growth (tissue extension; ±S.D.) over the first six months of each study by technique and genotype. Like-letter groups indicate statistical similarity within techniques; like number groups indicate similarity within genotypes (all comparisons ANOVA-Tukey HSD, $\alpha = 0.05$, StatSoft Statistica 6.1 - except for those involving suspended genotype 10 corals, Kruskal-Wallis, $\alpha = 0.05$).

VII.11 *Growth – Branching*

The distribution of mean branches per colony (Fig. 34) closely followed that of mean colony tissue extension (Fig. 31). Within every technique, genotype 4 corals produced significantly more branches (branches counted at ≥ 0.5cm) than both genotype 8 and 10 corals, averaging three times as many branches (301% ±125% S.D.). Per genotype, both puck-grown and suspended colonies grew significantly more branches than vertical colonies.

Analyzing colony branch number before prominent abrasion in suspended colonies (six months, Fig. 35) produced several differences. For puck-grown corals, genotype 8 colonies did not significantly differ in branch number from genotype 4, but did grow significantly more branches than genotype 10 colonies. For suspended genotype 8 and 10 colonies, the opposite trend occurred, in which they did not significantly differ in branch number from vertical colonies of the same genotype.

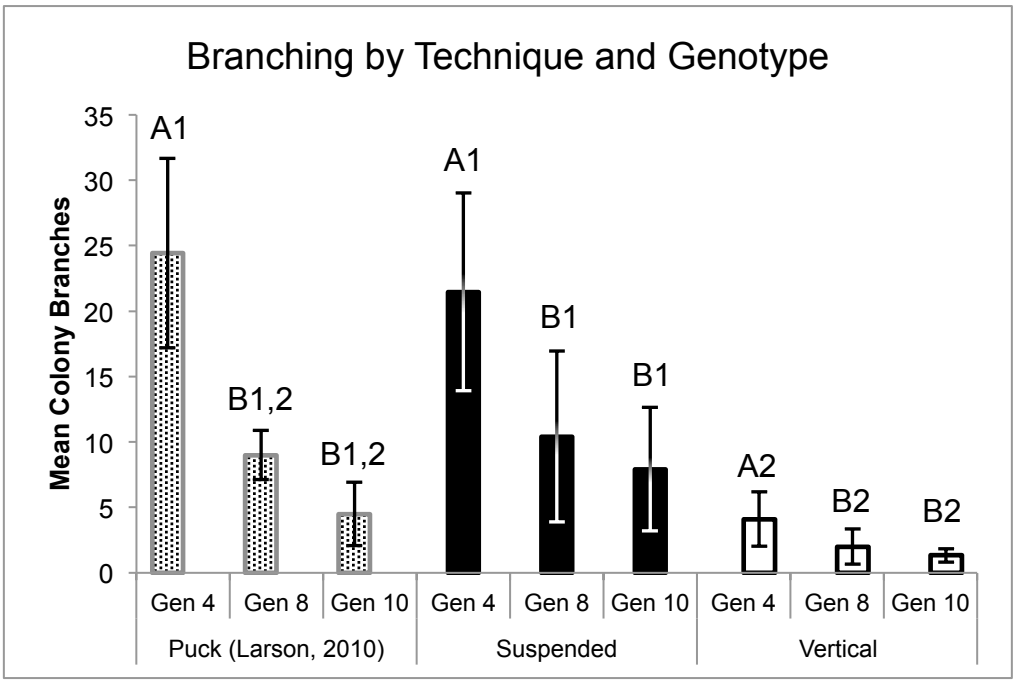

Figure 34: Mean number of branches per colony (±S.D.) at the conclusion of each study (approx. one year) by technique and genotype. Like-letter groups indicate statistical similarity within techniques; like-number groups indicate similarity within genotypes (all comparisons ANOVA-Tukey HSD, $\alpha = 0.05$, StatSoft Statistica 6.1 - except for those involving vertical genotype 10 corals, Kruskal-Wallis, $\alpha = 0.05$).

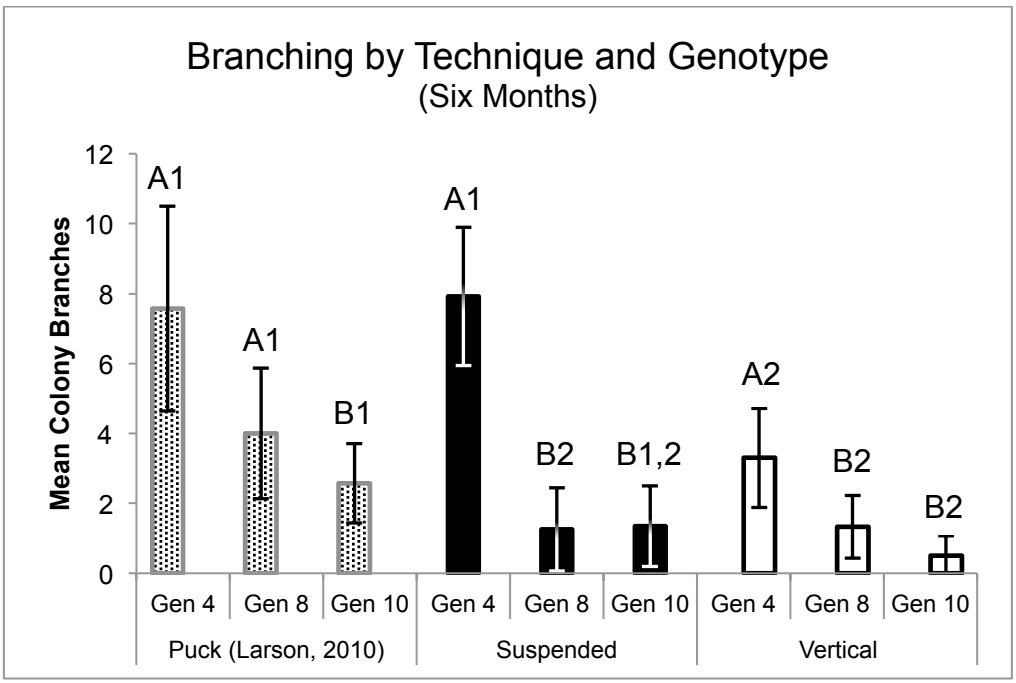

Figure 35: Mean number of branches per colony (±S.D.) over the first six months of each study by technique and genotype. Like-letter groups indicate statistical similarity within techniques; like-number groups indicate similarity within genotype (all comparisons Kruskal-Wallis, $\alpha = 0.05$, StatSoft Statistica 6.1 – except puck and genotype 4 colonies, ANOVA-Tukey HSD, $\alpha = 0.05$).

VII.12 *Growth – Fragment Generation*

Nursery corals are commonly grown for the purpose of generating fragments, either to expand a nursery or to outplant for population restoration. Fragment production (Table 11) combines both colony mortality and growth into a metric that represents actual nursery production. To maximize the efficiency of material used, fragments are generally cut at approximately 3cm; which results in sufficient survivorship without wasting tissue. Percent return values, generated from the calculated fragments grown (also 3cm) vs. invested at the onset of each study, varied with the success of both fragment survivability and subsequent growth. Exemplifying the effects of mortality to fragment production, mean puck colony growth exceeded suspended colonies by a small margin (Table 10), yet the percent return of suspended colonies greatly exceeded that of pucks.

Table 11 – Fragment Production (3cm) by Technique

Technique	Fragments Invested	Fragments Grown*	Percent Return
Suspended	72	1043	1449%
Vertical	71	75	106%
Puck** (Larson, 2010)	27	291	1078%

*Values for number of 3cm fragments grown calculated by dividing every colony branch length by three, rounding down to the nearest whole integer, and summing the products. **Values for Larson study limited to the three genotypes shared by both studies. Fragments grown and percent return of puck colonies comparable within five percent, as monitored growth period (334 days) was exceeded by suspended and vertical colonies (350 days).

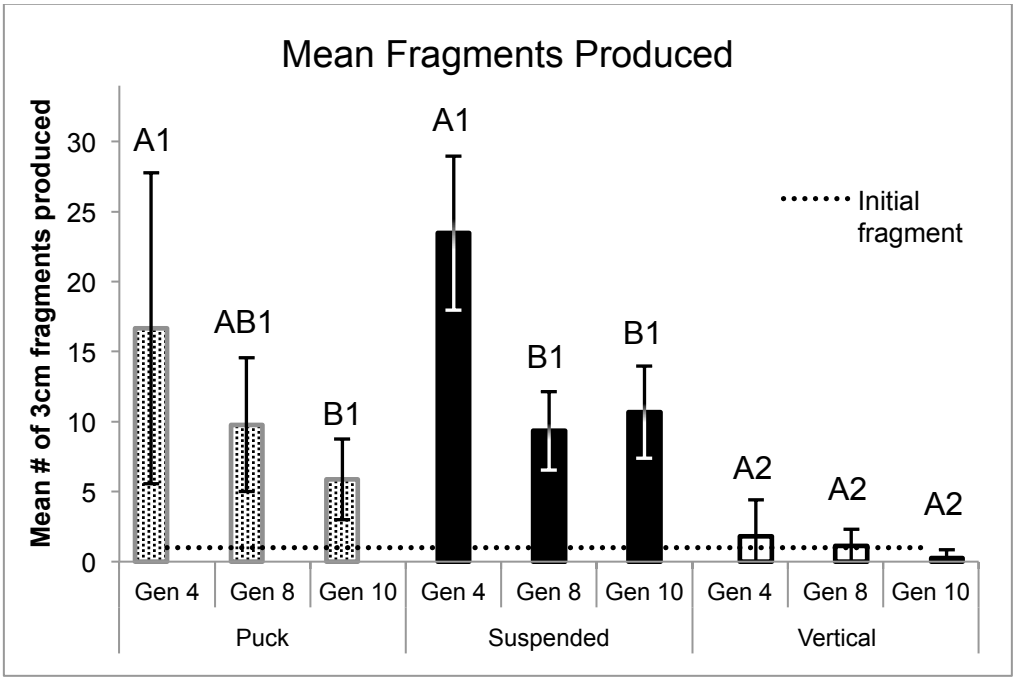

Figure 36: Mean number of fragments produced (±S.D.) per colony by technique and genotype. Colonies that perished (producing zero fragments) included in analysis to represent true fragment return. Dotted line at y = 3cm indicates approximate initial fragment tissue investment. Like-letter groups indicate statistical similarity within techniques; like-number groups indicate similarity within genotypes (all comparisons Kruskal-Wallis, $\alpha = 0.05$, StatSoft Statistica 6.1).

The distribution of the mean number of 3cm fragments produced per colony (Fig. 36) is similar to the growth distribution (Fig. 31). For every genotype, vertical colonies

produced significantly fewer fragments than suspended and puck-mounted colonies; in one instance the amount of living tissue had actually decreased at the end of the experimental period (genotype 10). Distributions of puck and suspended colony fragment production were similar, and only differed in that puck-grown genotype 8 fragment production did not significantly differ from genotype 4, while this difference was significant for suspended colonies. This was not due to decreased production by suspended genotype 8 colonies (9.3 ±2.8 S.D. fragments) compared to genotype 8 puck colonies (9.8 ±4.8 S.D. fragments), but likely due to the slight increase in production by suspended genotype 4 colonies (23.5 ±5.5 S.D. fragments) compared to genotype 4 puck colonies (16.7 ±11.1 S.D. fragments).

VIII. Discussion

VIII.1 *Survivorship*

Fragment survivorship one month following fragmentation was strong (94% for vertical fragments, 100% for suspended, 93% for puck). Initial post-fragmentation mortality (complete) for line nursery fragments was only observed in genotype 10 (vertical), which was the only genotype to exhibit no complete mortality when grown on pucks (all eleven months monitored - Larson, 2010).

The survival of all suspended colonies over the course of this experiment is promising; typical survival rates of suspended *Acropora cervicornis* nursery corals range from 86% - 97.5% (Young *et al.,* 2012). Though this result can not be expected to reoccur for all nurseries and *A. cervicornis* genotypes, any reduction in mortality is beneficial to nursery operations, increasing the ratio of fragments grown versus invested.

Vertical colonies (57% complete mortality at one year) did not exhibit the strong survivorship of suspended colonies. Considering this significantly higher mortality, the technique should not be adopted by *A. cervicornis* nursery operations. Increased mortality in latter months (Fig. 12), when no bleaching or prominent disease outbreaks were observed, suggests the influence of fouling organisms on colony health (discussed

in section VIII.2). For colonies of genotype 10, which exhibited the greatest complete mortality, it is possible that the genotype's propensity to slower growth contributed to its inability to outgrow competing organisms. In contrast, genotype 10 corals exhibited the lowest complete mortality when grown by Larson (2010). It appears that the robustness of individual genotypes may not be persistent among locations and/or culturing techniques.

VIII.2 *Partial Mortality and Stressors*

Though colonies subject to partial mortality (PM) still grow and contribute tissue for fragment production, PM should not be ignored. For nurseries growing colonies for fragment production (rather than whole colony transplantation), the PM within a nursery is comparable in its effect to the same amount of whole colony loss. For instance, an average of 10% PM within a nursery is a 10% reduction in available tissue to fragment; approximately the same reduction would occur if 10% of nursery colonies were to die.

Vertical colonies exhibited far greater PM than suspended colonies (Fig. 13). Competition by fouling organisms growing on nursery lines (bivalves, macroalgae, sponges, hydroids, etc.) was likely the primary contributor to vertical colony mortality (whole and partial). The greater contact area between vertical colonies and nursery lines (and thus line-fouling biota) resulted in consistent stress to these colonies. In contrast to natural conditions, upward growth only increased colony tissue contact with competing organisms. Vertical colonies that appeared healthiest following the conclusion of the experiment were found with outward branching morphologies, rather than upward.

For most vertical colonies, interaction between colony tissue and competing organisms unnaturally occurred at branches and branch tips, in addition to encrusting basal tissue (which normally serves as the main barrier between *A. cervicornis* colonies and the benthos). It is also possible that the flexibility of nursery lines and/or the foreign nature of the substrate (polyester) hindered the growth of encrusting tissue for vertical colonies. Decreased mortality and PM for colonies directly affixed to nursery lines may be possible with increased cleaning effort, or if nurseries are constructed of a material that resists

fouling; however, resources may be better allocated to increasing capacity for suspended colonies.

In contrast to vertical colonies, the interface between suspended colony tissue and competing organisms occurred only on attachment wires. In this narrow area, encrusting coral tissue grew up attachment wires while fouling organisms grew downwards. The significant reduction of physical space in which competing organisms could grow against *A. cervicornis* colonies likely decreased associated stresses, resulting in increased colony health.

The largest contribution to PM in suspended colonies came from bivalve overgrowth (Fig. 20). Losses of *A. cervicornis* colony tissue, caused by physical smothering by bivalve shells, likely resulted from the inability of coral colonies to defend against bivalve growth. Bivalve individuals did not appear to settle on or directly adjacent to suspended *A. cervicornis*, but rather began growth on colony attachment wires several centimeters away, eventually growing into contact with nursery colonies. Bivalves, protected from coral defenses by their shells, appeared to grow unhindered by contact with *A. cervicornis*. Additionally, bivalve growth onto nursery colonies added additional stress by bringing other fouling organisms, such as hydroids, into contact with *A. cervicornis*. Suspended colony PM due to bivalves could be easily remedied with regular maintenance, such as the simple pruning of these organisms with pliers or similar tools.

VIII.3 *Stressors - Jellyfish*

Dense aggregations of jellyfish (*Aurelia aurita*) brought an unanticipated stressor to line nursery corals (Figs. 21, 22, 23, 24). Although similar numbers of vertical and suspended colonies were affected by jellyfish entanglement (35 and 43 unique colonies, respectively), suspended colonies were affected to a lesser degree than vertical colonies (no suspended colonies died after jellyfish exposure).

The increased resistance of suspended colonies to jellyfish-related stresses, confirmed by a lack of mortality and the reversal of observed tissue damage could be a product of multiple factors, including: the greater size of suspended colonies during jellyfish blooms

(proportionately less colony tissue affected), the suspended attachment technique allowing for greater colony movement and water exchange, and the decreased interaction with competing organisms (fouling biota) resulting in more available energy for tissue repair.

Although unlikely, if sufficient numbers of jellyfish became entangled in overcrowded, over-weighted nursery lines, the integrity of nursery structure could be compromised in high current or wave energy conditions. Such an event could result in numerous losses of colonies and the expenditure of nursery resources for repairs. With jellyfish populations increasing in recent decades (Mills, 2001; Brotz *et al.*, 2012), such blooms may become a common occurrence at *A. cervicornis* nurseries. As such, more frequent monitoring of line nursery condition is prudent in the summer months.

VIII.4 *Predation*

The lack of predation to vertical and suspended colonies through the duration of the experiment demonstrates another positive quality of line nurseries. For the concrete nursery modules occupying the same location, predation by the polychaete *Hermodice carunculata* (fireworm), was a consistent source of tissue loss in *A. cervicornis* colonies (Fig. 25) (pers. obs., not recorded in this experiment). Prey species selection by *H. carunculata* varies with prey species abundance (Berkle, 2004), and as *A. cervicornis* density increases with growth at nursery locations, it is probable that predation by *H. carunculata* would also increase. *Hermodice carunculata* typically consume *A. cervicornis* branch tips, which immediately reduces colony growth rate by the removal of apical polyps. The elimination of unpredictable predation can in turn remove a degree of uncertainty for nursery operators, allowing for more reliable projections of stock growth. For nurseries that do experience significant predation, transitioning to suspended culture is advisable. Additionally, predation by *H. carunculata* on *A. cervicornis* has been correlated (albeit weakly) with white band disease occurrence in natural populations (Vargas-Ángel *et al.*, 2003), the increase of which would further hinder nursery production.

VIII.5 *Disease*

The significant reduction of disease in suspended colonies is advantageous to nurseries, decreasing tissue loss and increasing colony health. Suspended culture may allow *A. cervicornis* colonies to better resist the onset of disease in stressful conditions such as prolonged warm water events (suspended and vertical colony disease observed in summer months). The separation of colonies from benthic biota may also decrease the likelihood of disease contraction, as benthic organisms have been shown to carry bacterial coral pathogens (*Coralliophila abbreviata*, carrying *Serratia marcescens* - Sutherland *et al.,* 2010).

Due to the relative speed of complete colony death via rapid tissue necrosis (RTN) compared to the time between monitoring months, it is possible that other occurrences of RTN went unrecorded for vertical colonies (which experienced significantly greater mortality than suspended or puck colonies). Though vertical colonies did not experience significantly higher disease rates than suspended colonies, it is possible that disease, specifically RTN, was a more significant source of vertical colony mortality than observed.

VIII.6 *Growth – Attachment*

While puck-mounted fragments are firmly affixed with epoxy putty with reliable success, it was unknown whether fragment loss (via detachment) from the line nursery would occur. Active attachment by fragments to nursery lines via tissue growth was utilized as a metric by which the relative "risk" of fragment detachment could be assessed. This was based on the presumption that detachment risk decreased once fragments actively grew onto nursery lines.

Successful growth of colony tissue over wires confirms the suitability of shielded wire as an attachment medium for suspended culture of *A. cervicornis*. Envelopment of attachment wire by coral tissue is important to prevent growth of competing organisms, and ensures that colonies remain firmly attached as their mass increases with growth. Of note, it is important that colonies attached by wire or similar mechanism be firmly

wound/tied, for loose attachment may allow for fragment loss, or the hindrance of fragment growth onto attachment media because of repeated loss of contact.

The 20 gauge shielded wire utilized in this experiment held colonies well beyond the duration of monitoring (a few suspended colonies became detached from nursery lines between 15 and 18 months post-fragmentation). Thicker wire may not significantly reduce colony detachment, and if used could increase the likelihood of breaking coral fragments when being wound (thicker wire would require more forceful twisting of fragments). Thinner wire, while easier to wind, should also be avoided as its longevity may be inadequate (the use of thinner gauge wire, 24 gauge, resulted in unacceptable suspended colony detachment over time, Ken Nedimyer, pers. comm.).

VIII.7 *Growth – Tissue Extension*

Statistically similar growth rates of like-genotype suspended and puck-grown *A. cervicornis* indicates that suspended culture does not inherently increase growth. However, the greater growth of suspended genotype 10 corals compared to puck-grown colonies (growth analysis at six months to remove possible influences of colony abrasion) suggests that slower growing genotypes of *A. cervicornis* may significantly benefit from suspended culture. This study only incorporated three genotypes, however, it is possible that other genotypes of *A. cervicornis* may exhibit significant differences in growth between these two techniques. If true, the culturing of such genotypes via their "technique of preference" should increase nursery efficiency.

Regardless of the perceived benefits of line nursery culture to *A. cervicornis* (separation from benthic biota, decreased sedimentation, potentially increased water flow, etc.), vertical colonies did not perform well, growing significantly less than both suspended and puck corals. This is attributed to tissue loss due to PM, and the possible diversion of colony resources to defense and/or wound healing rather than growth.

A drawback unique to suspended culture is tissue abrasion, made possible by the mobility of nursery colonies. Tissue abrasion between adjacent colonies (Fig. 32) may have inhibited growth, and consequently limited the detection of significant growth branching

differences among treatments. Before noticeable abrasion began to occur, mean growth of genotype 4 colonies in suspended culture exceeded that of puck-grown colonies by 36% (at six months). The observed summer growth rate peak for suspended colonies may illustrate this encumbrance to suspended colony growth, as puck-grown colonies did not exhibit a peak, but continually increased in growth rate (Fig. 30). If allowed to grow unrestricted, this difference could have widened over the remainder of the study. More separation between suspended colonies and/or more regular fragmentation could alleviate this hindrance.

A possible alternative to wider spacing and/or more frequent fragmentation is to utilize branch grafting. Branches of like-genotype colonies of *A. cervicornis* can fuse together if they grow in direct contact with one another (Gillmore and Hall, 1976; Tunnicliffe, 1981; Johnson *et al.*, 2011), and could be fastened together to promote fusion, reducing the mobility of neighboring colonies thus reducing abrasion. This practice may be valuable in circumstances when colony fragmentation is not practical, such as during warmer water temperatures adverse to fragment survival.

VIII.8 *Growth – Branching*

Suspended colony branching significantly exceeded that of vertical colonies. This was expected, but rather than from the greater directional freedom of growth in suspended colonies, it came from significantly higher partial mortality and growth impediments to vertical colonies. If greater investment was made to clean nursery lines of fouling organisms, stresses to vertical colonies could have been reduced. If true, growth by vertical colonies (both tissue extension and branch number) may have more closely matched that of suspended colonies.

Within every technique, genotype 4 corals had significantly more branches than both genotype 8 and 10 corals, reflecting higher growth rates. This further confirms that genotypic growth rate relationships persist regardless of the culturing technique utilized. Suspended colony branching did not exceed puck-grown colonies, although this may be a product of growth hindrance from abrasion (previously discussed). This may have disproportionately affected the larger genotype 4 colonies, as suspended genotype 8 and

10 colony branch number increased in relation to others between six and twelve months (Fig. 34).

Interestingly, suspended genotype 8 colonies had grown significantly fewer branches at six months than puck-grown colonies of the same genotype, while this difference was not observed in genotype 10 corals, the slowest-growing genotype. This may demonstrate another parameter of variability among genotypes – apical polyp generation. Significantly slower generation of apical polyps by some genotypes could reflect differences in hormonal regulation of growth, processes which are not yet well-understood (Tarrant, 2005).

VIII.9 *Growth – Fragment Production*

Fragment production comparisons, incorporating colony mortality, provide a more realistic assessment of nursery production than growth alone. The number of fragments produced compared to the number initially invested is an appropriate metric to assess nursery efficiency. Though no significant differences in fragment production (3cm, calculated) were observed between puck and suspended culture techniques, factors that affect production (i.e. disease and predation) can vary dramatically among nurseries (pers. obs.). For *A. cervicornis* nurseries that employ both fixed and suspended culture, line space may be reserved or added for slower-growing genotypes, while existing benthic nursery substrate can be allocated to faster-growing corals. In this way, the majority of losses from fragmentation mortality and/or predation could be incurred by fast-growing genotypes, whose increased growth and tissue regeneration could make up for such losses more easily.

The retention of fragment production differences among genotypes is also useful to nursery operators. To prevent artificially-induced imbalances to genetic distribution, restoration efforts aim to outplant genetically diverse fragment assortments. If source nursery genotype stocks become imbalanced (likely given that some genotypes can grow more than twice as fast as others), nurseries might transplant more corals of certain genotypes, or be forced to leave material in a nursery indeterminately. To prevent this potential waste of product, nurseries could start with unequal numbers of fragments

among genotypes with known growth differences, such that the genetic makeup of fragments produced would be more uniform.

Though selecting faster-growing genotypes of coral (i.e. genotype 4) could allow nurseries to produce more fragments faster, *A. cervicornis* cultured for restoration should not be chosen based upon growth alone. Nurseries are designed to provide ideal growing conditions for coral colonies; the healthiest genotypes in a nursery may not remain so when outplanted. Though disease resistance in genotypes is a desirable trait to propagate, this experiment revealed that what was previously considered a more robust genotype (genotype 10), succumbed to the greatest mortality among genotypes (Table 7). Other important characteristics, such as sexual reproductive effort, are not yet evaluated. Many other genetic parameters may yet be described, and it would be unfortunate if coral nurseries unknowingly selected against important traits yet to be discovered.

IX. Conclusions and Recommendations for Nursery Managers

Suspended growth of *Acropora cervicornis* is effective, and results in enhanced survivorship and growth rates similar to those seen in identical genotypes grown via traditional puck-mounted culture. The benefits associated with suspended nurseries (design versatility, efficient use of physical space, separation of nursery colonies from predators, etc.) make their use attractive to existing and future *A. cervicornis* nursery operations. The following findings of this study should prove valuable:

i. Direct attachment of fragments to nursery lines (vertical attachment in this experiment) results in significant mortality and decreased growth, and should be avoided.

ii. Partial mortality can be mitigated by regular (every one to two months) maintenance of nursery lines, with particular effort taken to removing bivalves, hydroids, and other fouling organisms growing on attachment wires near nursery colonies. Partial mortality decreases the amount of coral tissue

useable for nursery expansion and outplanting, and may compromise the health of nursery colonies.

iii. Predation, especially by *Hermodice carunculata* (fireworm), may be significantly reduced or completely eliminated by suspended culture, and would allow for more predictable estimation of nursery production.

iv. Suspended culture significantly reduces disease occurrence in nursery corals compared to puck culture.

v. More frequent nursery visitation should be performed during jellyfish blooms, as they may become entangled on nursery colonies and cause unnecessary stress and/or mortality.

vi. Suspended colonies need more frequent fragmentation than puck-grown colonies to reduce branch abrasion, which can hinder growth. Puck colonies can theoretically be left to grow indefinitely, while line nursery techniques can not support indefinite growth (excessive colony weight, detrimental colony interaction). If adequate fragmentation is not feasible, extra spacing between colonies should be planned compared to puck culture. Based upon the findings of this study, a minimum of 15cm spacing between fragments of 3cm is adequate for one year of growth.

vii. Genotypic growth differences persist among culturing techniques. Nursery managers may elect to adjust starting ratios among genotypes to produce more desirable and balanced distributions of fragment genotypes for outplanting. Alternatively, faster-growing genotypes of *A. cervicornis* may be grown on existing benthic nursery structures, whereby losses from predation or mortality can be more easily overcome by their increased growth.

EXPERIMENT 2 – INVESTIGATING EFFECTS WATER TEMPERATURE AND NURSERY TECHNIQUE ON *ACROPORA CERVICORNIS* FRAGMENT MORTALITY

X. Project Description

Observations by nursery collaborators lead to this experiment. Larson (2010) and the Coral Restoration Foundation (Ken Nedimyer and Katie Grablow, pers. comm.) observed that there were differences in *Acropora cervicornis* fragment survival among genotypes grown on pucks. The Coral Restoration Foundation also observed differences in survival between puck and suspended culture methods. Larson (2010) found that post-fragmentation mortality was greater in warmer water conditions for puck-mounted corals, while the Coral Restoration Foundation did not observe a notable difference utilizing suspended nursery techniques. This experiment was designed to compare fragment survival of three *A. cervicornis* genotypes using both puck and suspended nursery techniques between the months of August and January. This period was chosen to capture the temperature range in which significant differences in fragment survivorship have been documented (September – December, 2007; Larson, 2010).

By removing temporal and geographical variation, this experiment clarifies the extent of fragment survivorship difference between puck and suspended techniques. The inclusion of three genotypes with varying post-fragmentation survivorship (Larson, 2010) allowed for the relationship between techniques to be evaluated with a range of genetic hardiness representative of nursery operations. This also allowed for the survivorship among genotypes to be assessed in each technique across temperatures. These investigations are valuable to nursery operations, providing data useful for scheduling fragmentations, and what effects to fragment survival can be expected depending on the nursery technique utilized.

XI. Procedure and Methods

To test the effects of nursery technique on *Acropora cervicornis* fragment mortality during a period of decreasing water temperature, four fragmentation trials were conducted. For each, two common nursery techniques were compared: puck-mounted culture, in which fragments are fixed to cement pucks with epoxy putty (Figure 37), and suspended culture, where fragments are freely suspended in the water column (line nurseries, Figure 38).

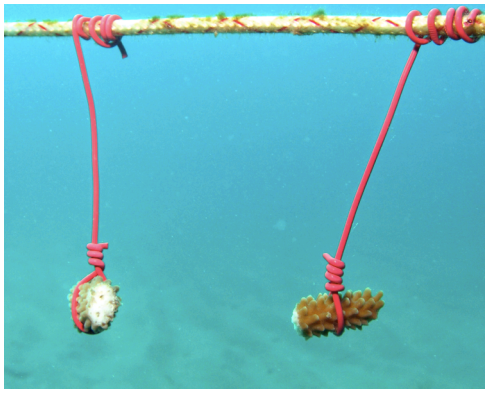

Figure 37 (left): *A. cervicornis* fragments affixed to cement pucks with epoxy putty.

Figure 38 (right): Line nursery *A. cervicornis* fragments suspended in the water column.

For all trials, *A. cervicornis* colonies of three genotypes were fragmented using both techniques (genotypes predefined via microsatellite DNA markers; Larson, 2010). Fragments were supplied by *A. cervicornis* stock in an existing nursery near the experimental location, in which colonies had been grown on cement pucks attached to concrete blocks. The same three genotypes (designated "4", "6," and "10") were used in all trials. Genotypes were selected based upon several factors, primarily if sufficient nursery material was available for fragmentation, and if significant differences in mortality following fragmentation were previously observed (Table 12, puck-mounted mortality 38 days after fragmentation). Documented variation in genotypic mortality was an important inclusion for this experiment; doing so incorporated a range of genotype hardiness that more appropriately reflects the variety of genotypic composition in existing nurseries.

Table 12 – Documented Fragment Mortality Rates of Selected Genotypes

(December 2007 fragmentation - Larson, 2010)

Genotype ID	4	6	10
Post-Fragmentation Mortality	11%	50%	0%

Fragmentation trials were conducted at an existing *A. cervicornis* nursery site off Ft. Lauderdale, Florida at a depth of approximately seven meters. The nursery, less than one kilometer from shore and located in the sand channel between the nearshore ridge complex and inner reef of the region, is composed of both concrete modules (one cubic meter in size) and line nursery units ("lines") on which hundreds of *A. cervicornis* colonies are grown. To compare fragment mortality between techniques and temperatures, trials were conducted in conditions conducive to fragment survivorship, and in conditions where significantly higher fragment mortality has been observed (temperatures exceeding ~27ºC – Larson, 2010) (Table 13). Each of the four trials consisted of 36 fragments, 12 of each genotype, which were evenly distributed between puck and suspended nursery techniques (Figure 39).

Table 13 – Mortality Rates of Acropora cervicornis Nursery Fragments

(Larson, 2010)

Month of Fragmentation	Percent Mortality (time after fragmentation)	Temperatures*
September (2007)**	56% (35 days)	30.5ºC
October (2007)**	42% (21 days)	28.5ºC
December (2007)	19% (38 days)	25ºC

*Mean nursery water temperature during the first week following fragmentation.

**September and October trials included some genotypes not investigated in this experiment.

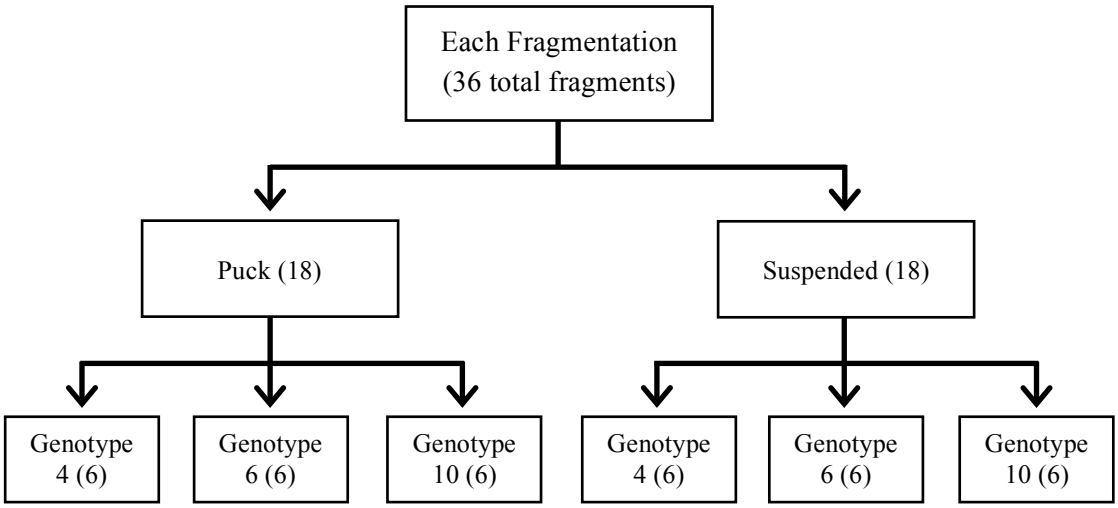

Figure 39: Fragment distribution among techniques and genotypes for all fragmentation trials.

On the day of each trial, all *A. cervicornis* fragments were harvested at lengths of approximately three centimeters from colonies grown in the source nursery. To reduce the possibility of donor colony condition negatively influencing fragment survival, only colonies that did not exhibit disease, bleaching, or noticeable predation were utilized. Fragments, separated by genotype, were transported in plastic jars (water-filled) placed inside a cooler to maintain a steady temperature. Transport between nurseries was brief, lasting no longer than two hours from collection to placement in the destination nursery.

Fragments were affixed onto pucks using two-part epoxy putty. Pucks (~8cm in diameter, made with cement and fine carbonate sand), were secured to nursery modules with epoxy putty (Figure 40). Following established techniques (Herlan and Lirman, 2008; Larson, 2010; Johnson *et al.*, 2011), fragments were then attached to pucks in vertical orientation using a conservative amount of pre-mixed epoxy putty, such that polyp apertures were facing upward, and that a minimal amount of fragment tissue was covered by epoxy.

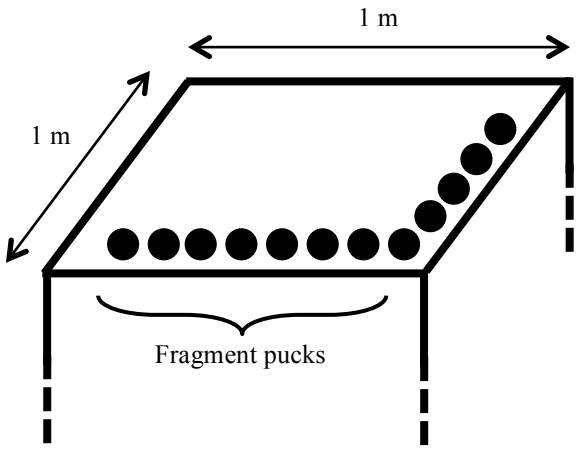

Figure 40: Diagram of a concrete nursery module and the placement of pucks. Not to scale; actual puck capacity 36 (module surface perimeter able to accommodate four rows of nine pucks).

Fragments attached using the suspended culture technique were hung horizontally from nursery lines approximately 1.5m from the substrate (sand) (Fig. 41), with 10 – 15cm of separation between fragments and 10cm of separation from the nursery line (Fig. 38). Fragments were attached with shielded wire (copper, 20 gauge, malleable plastic shielding). Wire was first affixed to each fragment (on board the research vessel), followed by winding (and crimping to prevent lateral shifting) the free end of each wire to nursery lines in situ. Fragment position on each line was randomly determined, such that genotypes were distributed evenly among modules, but randomly ordered on each module. Nursery lines were constructed of 3/8" polyester nautical rigging line, secured to the substrate with two 75cm long, 10cm diameter screw-in ground anchors, and held upright by two, 15cm diameter styrofoam support buoys.

Four fragmentation trials were conducted. One trial was completed in each of the following months: August, September, and November 2011, and January 2012. The first two trials were conducted when water temperatures were expected to be warmer than what has been recommended for fragmentation (maximum ~27ºC - Larson, 2010); cooler temperatures were expected in the latter trials. If fragments were physically lost from the nursery, percent survivorship for a treatment was calculated based only upon the fragments remaining.

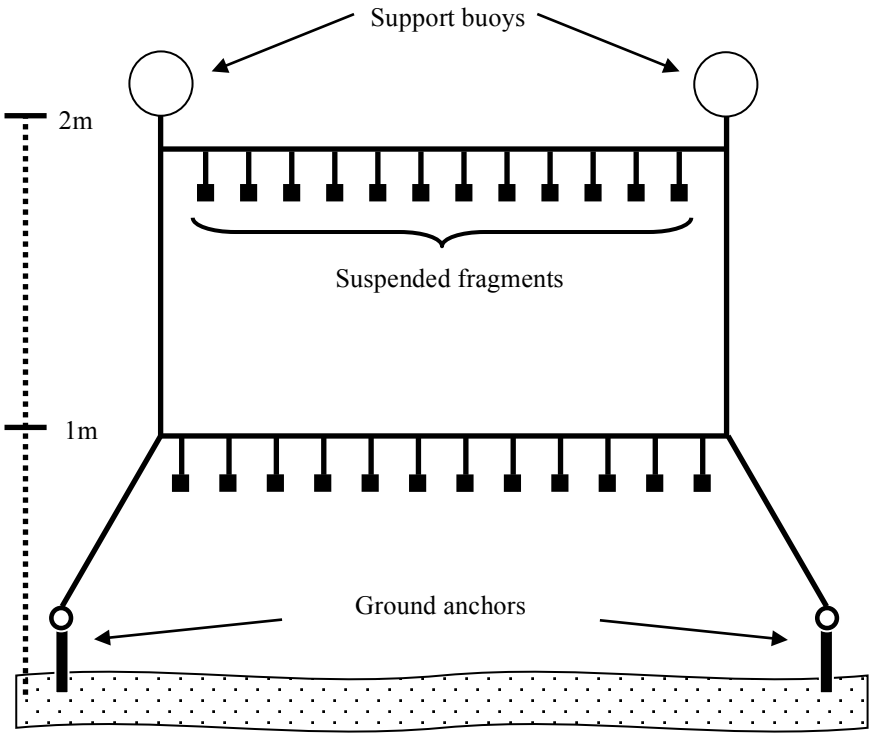

Figure 41. Diagram of suspended nursery module showing the position of suspended fragments in this study.

At approximately one month following each trial (Table 14), fragment tissue mortality was assessed. Tissue mortality was estimated to the nearest ten percent, and fragments were only considered dead if no live tissue remained. An in situ temperature logger (Onset® TidbiT™ v2) sampling at two-hour intervals, was deployed at the nursery. Percent mortality was assessed against the average temperature logged during the first week following fragmentation (Table 14). As fragmentation-related mortality is considered to occur within the first few weeks, additional mortality observed after the initial monitoring interval was not attributed to fragmentation stress (Clark and Edwards, 1995; Bowden-Kerby, 2001; Herlan and Lirman, 2008).

Table 14 – Fragmentation dates, time to assessments, and temperatures

Fragmentation Trials: Order and Date	Time Between Fragmentation and Assessment	Temperatures (week average post-fragmentation)
(1) – August 3, 2011	36 days	30.4ºC
(2) – September 8, 2011	50 days	30.6ºC
(3) – November 17, 2011	49 days	25.8ºC
(4) – January 5, 2012	43 days	22.4ºC

XII. Results

Though fragment survivorship for all trials, genotypes, and techniques is compared in multiple ways within following sections, this comprehensive table (Table 15) of survivorship is provided for ease of reference:

Table 15 – Comprehensive Fragmentation Trial Survivorship

Fragmenation (Temperature)	Technique	Genotype 4	Genotype 6	Genotype 10	*overall*
August, 2011 (30.4ºC)	Suspended	100%	100%	100%	*100%*
	Puck	83%	33%	83%	*67%*
	overall	*92%*	*64%*	*92%*	*83%*
September, 2011 (30.6ºC)	Suspended	83%	100%	50%	*76%*
	Puck	50%	67%	0%	*39%*
	overall	*67%*	*82%*	*25%*	*58%*
November, 2011 (25.8ºC)	Suspended	83%	83%	67%	*78%*
	Puck	100%	80%	33%	*71%*
	overall	*92%*	*82%*	*50%*	*74%*
January, 2012 (22.4ºC)	Suspended	100%	100%	100%	*100%*
	Puck	100%	100%	50%	*83%*
	overall	*100%*	*100%*	*75%*	*92%*
All Suspended		**92%**	**96%**	**79%**	***89%***
All Puck		**83%**	**70%**	**42%**	***65%***

XII.1 *Survivorship - Technique*

In all trials, suspended fragment survivorship exceeded puck-mounted fragments (pooled survivorship of all genotypes, Fig. 42). These differences were significant for August and September fragmentations, where suspended fragment survivorship exceeded that of puck fragments by 33% and 37% respectively (Pearson's Chi-square, $p < 0.05$, JMP Pro 9.0.2). Suspended fragment survivorship was highest for August and January trials (100%), and matched survivorship observed in suspended fragments for Experiment 1 of this study (conducted January 2011). Suspended fragment survivorship for September and November trials (76% and 78% respectively), which did not match January 2011 rates, were significantly lower than August and January 2012 rates (Pearson's Chi-square, $p < 0.05$, JMP Pro).

Considering the September and August trials were only separated by 36 days, suspended fragment survivorship in August (100%) was unexpectedly high. This disparity is surprising, for changes in fragment survivorship as a result of seasonal progression would likely be more gradual. The same difference was observed in puck fragments, though survivorship was not as high (67% and 39% for August and September, respectively).

Puck fragment survivorship was significantly low for the September trial (39%) compared to the maximum of 83% in the January trial (Pearson's Chi-square, $p < 0.05$, JMP Pro). September puck survivorship was also significantly lower than previously documented rates (September 2007 fragmentation - Larson, 2010) (Pearson's Chi-square, $p < 0.05$, JMP Pro).

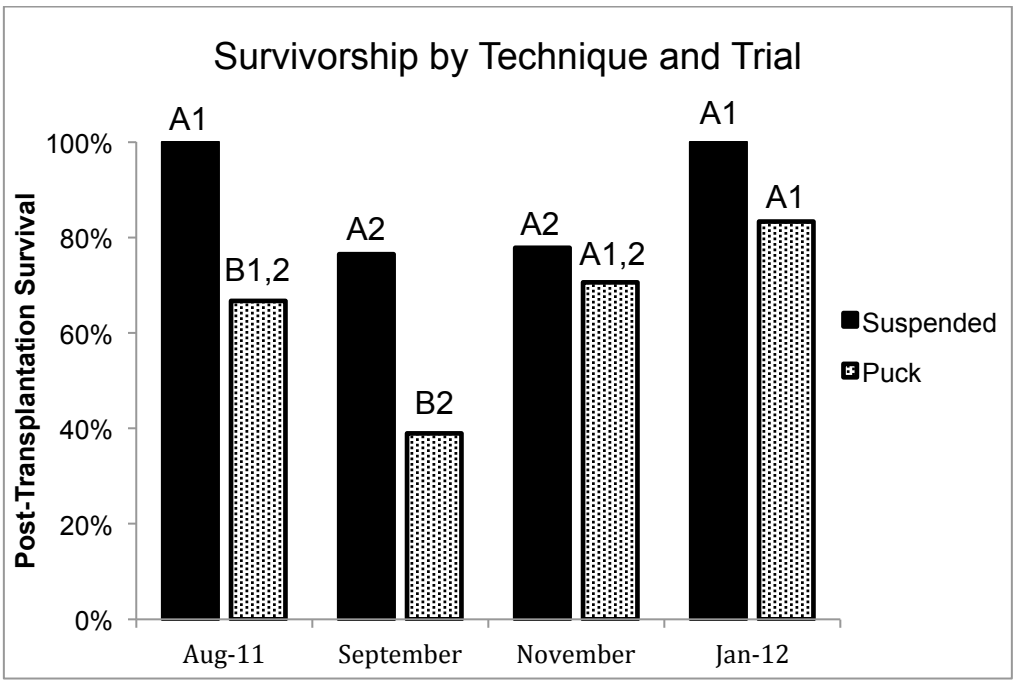

Figure 42: Percent fragment survivorship by technique and fragmentation month. Survivorship values pooled from all genotypes. Like-letter groups indicate statistical similarity between techniques within months, and like-number groups indicate similarity among months within techniques (paired comparison, Pearson's Chi-square, $\alpha = 0.05$, JMP Pro 9.0.2).

XII.2 *Survivorship – Genotype*

For suspended fragments, 100% survivorship occurred in over half of all treatments (by genotype, Fig. 43). Full survivorship was observed for all genotypes in August and January, and also for genotype 6 in September. November was the only fragmentation trial in which no single genotype maintained 100% survival. Significant variation in survivorship among genotypes was only observed in the September trial, in which genotype 10 had the lowest rate (Pearson's Chi-square, $p < 0.05$, JMP Pro). This difference was also observed for genotype 10 fragments in the "vertical" line nursery technique in Experiment 1 (but not for suspended fragments). Genotype 6, which had the lowest documented survivorship among these three genotypes when grown on pucks (50% - Larson, 2010), exhibited the highest overall survivorship in the suspended technique (Table 15), though this was not significant. This departure from past survivorship rates also occurred in September, in which genotype 6 survivorship significantly exceeded that of genotype 10 (Pearson's Chi-square, $p < 0.05$, JMP Pro).

Comparing survivorship of suspended fragments within genotypes among trials, only genotype 10 fragment survivorship significantly differed, with the lowest rate occurring during September (Fig. 43). Survivorship rates for genotype 4 and 6 fragments also decreased in September and November, though not significantly.

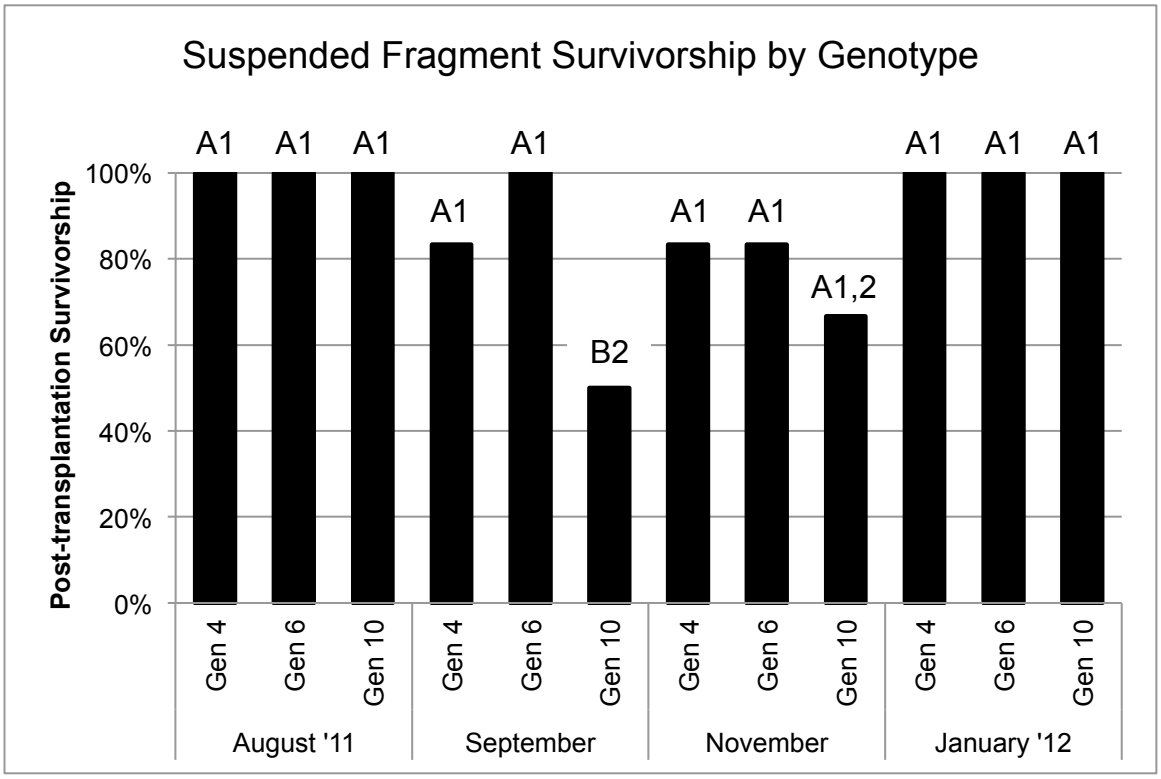

Figure 43: Suspended fragment survivorship for each genotype in each fragmentation trial. Like-letter groups indicate statistical similarity within months among genotypes, like number groups indicate similarity within genotypes among months (paired comparison, Pearson's Chi-square, $\alpha = 0.05$, JMP Pro 9.0.2).

Less than half as many instances of 100% survivorship were observed for puck fragments (Fig. 44). Out of 12 total treatments, only three (genotype 4 in November, and genotypes 4 and 6 in January) experienced no mortality compared to seven of twelve in the suspended technique. Survivorship significantly varied among genotypes in every trial except August. Genotype 10 exhibited significantly lower survivorship in September, November, and January (Pearson's Chi-square, $p < 0.05$, JMP Pro). Genotype 6 fragments exhibited the lowest survivorship in August, although this was not significant. Genotype 6, as in suspended fragments, did not exhibit poor survivorship as previously

observed (Larson, 2010) (Table 12), and significantly exceeded genotype 10 survivorship in September and January trials.

Unlike suspended fragments, for which generally higher survivorship rates resulted in few significant differences within genotypes among months, puck fragments regularly differed (Fig. 44). In the August trial, genotype 6 fragment survival was significantly lower than the January trial (Pearson's Chi-square, $p < 0.05$, JMP Pro). September genotype 4 fragment survival was significantly lower than in November and January, and the survival rate of genotype 10 fragments from the same trial was significantly lower than in August and January (Pearson's Chi-sqaure, $p < 0.05$, JMP Pro).

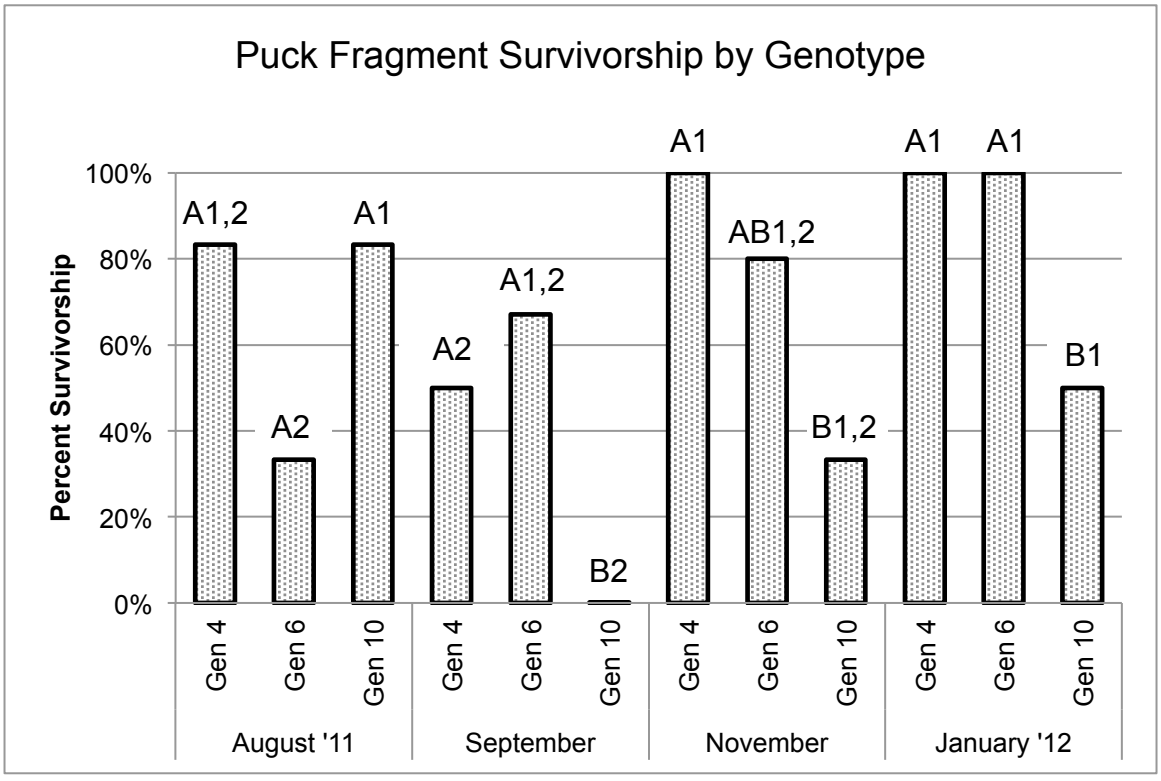

Figure 44: Puck fragment survivorship for each genotype in each fragmentation trial. Like-letter groups indicate statistical similarity within months among genotypes, like number groups indicate similarity within genotypes among months (paired comparison, Pearson's Chi-square, $\alpha = 0.05$, JMP Pro 9.0.2).

VII_b.3 *Survivorship - Temperature*

To assess whether temperature significantly correlated to fragment survival rates, logistic regressions were performed for each genotype and technique (Table 16). For treatments that produced significant results ($p < 0.05$, highlighted), temperature differences significantly affected fragment survivorship. Suspended fragment survival rates were not significantly affected by temperature, while puck fragment genotypes 4 and 6 were affected (Logistic regression, $p < 0.05$, JMP Pro). Puck genotype 10 fragments were not significantly affected by temperature conditions.

Table 16 – Analysis of Fragment Survivorship Against Temperature

Technique	Genotype	-LogLikelihood	DF	χ^2	p
Suspended	4	0.078	1	0.157	0.692
	6	0.087	1	0.174	0.667
	10	0.553	1	1.107	0.293
	overall	0.362	1	0.724	0.395
Puck	4	3.951	1	7.902	0.005
	6	2.875	1	5.751	0.017
	10	0.040	1	0.080	0.777
	overall	2.859	1	5.718	0.017

The relative effects of temperature (a continuous, numerical dataset) to fragment survivorship (a binary dataset) can be further compared graphically with point-biserial correlations (Fig. 45). The correlation coefficient (r_{pb}) values calculated are equivalent to Pearson product-moment correlation (r) values, and though they cannot determine significance between correlations, they can illustrate the strength of a correlation relative to another. Suspended fragment survivorship, which did not significantly vary with temperature, did not correlate strongly with temperature ($r_{pb} = -0.098$). Puck fragments, which did significantly vary with temperature (overall, Table 16), exhibited a stronger correlation ($r_{pb} = -0.275$). This illustrates that recommendations that fragmentation be conducted in cooler temperatures is more applicable to puck than suspended culture.

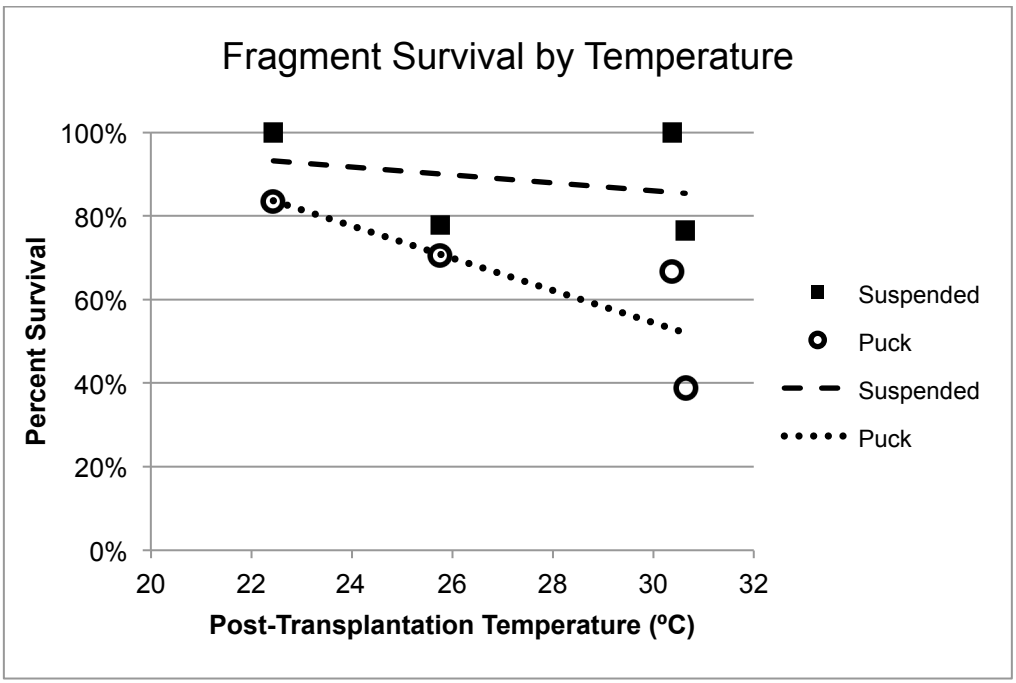

Figure 45. Fragment survivorship by temperature for both techniques. Each point represents either suspended or puck survivorship in an individual trial. Survival rates from pooled genotype survival. Linear fit (point-biserial) represented by dashed and dotted lines.

Fragment survivability was also assessed against temperature variability (variability defined as the standard deviation of temperature readings during the first week following fragmentation) (Fig. 46). Temperature variability, though not a direct indicator of other environmental parameters which may affect survival, may be related to conditions (currents, wave energy, etc.) which can drive variations in temperature. Both suspended and puck fragment survival rates positively correlated with temperature variability (r_{pb} = 0.262, 0.304 respectively), indicating that survivorship increased as temperature variability increased. Compared to correlations with temperature alone, both suspended and puck fragment survivorship correlated more strongly with temperature variability. This may show that environmental conditions related to temperature variability (currents, wave energy, etc.) were more influential to fragment survivability than temperature itself.

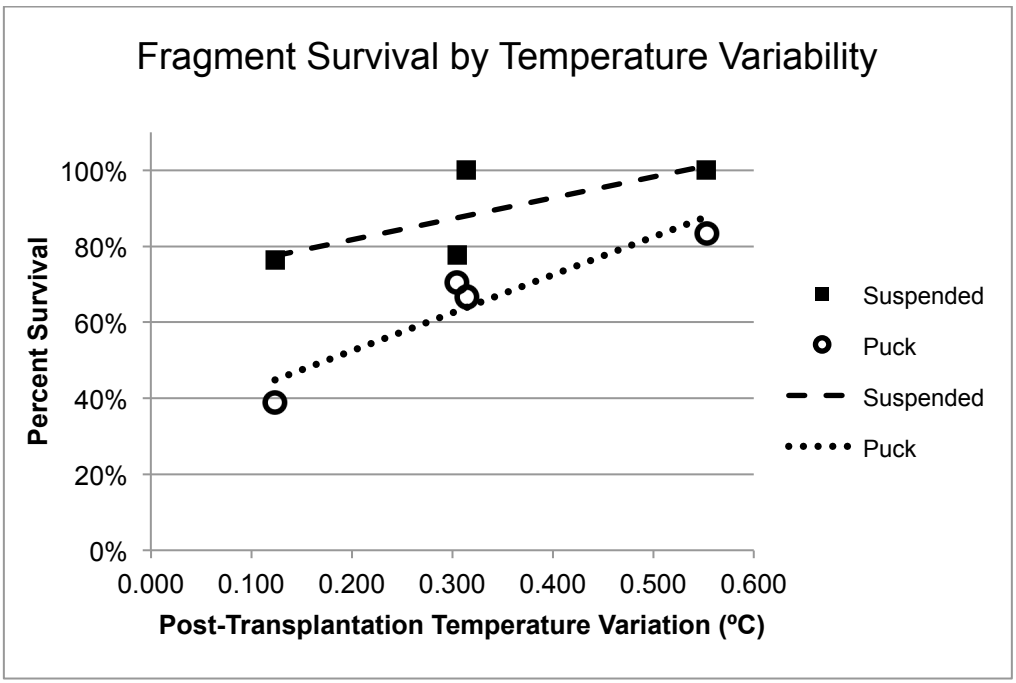

Figure 46. Fragment survivorship by temperature variability for both techniques. Survival rates from pooled genotype survival. Linear fit represented by dashed and dotted lines.

XIII. Discussion

XIII.1 *Survivorship - Technique*

The increased survivorship of suspended colonies in every fragmentation trial strongly indicates that the technique results in less stress to fragments. Significant differences between puck and suspended fragment survival were seen in the August and September trials, suggesting that the benefits of suspended culture are greater in warmer conditions. The exact reason for increased fragment survivorship in suspended culture is not known, but it is possible that the partial mortality incurred by fragment attachment to pucks (regions of a fragment smothered by epoxy) could cause additional stress.

Another possible contributor to stress reduction in suspended fragments is water flow. For corals undergoing bleaching and temperature-related stresses, increased water flow has been documented to increase survivorship (via enhanced passive diffusion of gases and metabolites - Nakamura and Woesik, 2001) and decrease oxidative stress on the photosynthetic pathway in zooxanthellae (via increased oxygen efflux from coral tissues -

Finelli *et al.*, 2006). Due to frictional resistance to water flow near the substrate, and decreasing wave energy with depth, water movement can be greater with distance from the substrate. In this way suspended fragments may receive more water flow compared to puck fragments on or near the substrate. This difference may be minimal, but there could be acute instances (hours or days) of weak flow and/or wave action that could exacerbate existing thermal stress to recently fragmented corals. This was likely not a factor in this experiment, as puck fragments were also a good distance above the substrate (~1m), but for nurseries where puck fragments are grown much closer to the substrate, conditions may significantly differ compared to suspended colonies.

XIII.2 *Survivorship - Genotype*

Increased fragment survivorship by utilizing suspended culture (Fig. 42) is beneficial, and can be particularly useful for genotypes with lower survival rates. For nurseries utilizing both suspended and fixed culture techniques, placement of genotypes could be influenced by the relative strength or weakness of their post-fragmentation mortality (if known). In this experiment, the relative benefit to each genotype varied, and is expected to vary for other genotypes not tested. The continued culture of *Acropora cervicornis* at nursery operations will likely elucidate more of these differences.

Curiously, the lowest survivorship was observed in genotype 10, which was the only genotype to exhibit no mortality when previously grown on pucks (Larson, 2010). It is unknown why this genotype, which was previously the best survivor among genotypes grown by Larson (2010), became the poorest survivor in this study (both techniques - Table 15). Survivorship differences among genotypes may not be persistent over time and/or between locations. In addition to temperature, other unmeasured conditions (e.g. increased/decreased turbidity, nutrient influx from upwelling, current and wave conditions, etc.) could have varied enough to elicit changes in survivorship between this experiment and Larson's.

Changes in donor nursery colony condition within those four years could also have been responsible for changes in fragment survivorship rates, specifically the bacterial

communities associated with coral colony mucus. Theorized to contribute to disease resistance (Reshef *et al*., 2006; Ritchie, 2006; Rosenberg *et al.,* 2007), those bacterial assemblages are extremely diverse, and have been documented to differ with location and time (Beal *et al*., 2012; Morrow *et al*., 2012). It is possible, then, that the bacterial assemblage originally associated with nursery fragments in 2007 could significantly differ from the assemblages of 2011. If true, fragments cut from wild donor colonies in 2007 would not be equivalent to like-genotype fragments cut from nursery colonies in 2011. Should the bacterial community associated with coral colonies play a significant role in survivorship as speculated, such differences could have contributed to the shifts in both genotype 6 and genotype 10 fragment survivorship observed between this study and Larson's.

XIII.3 *Survivorship - Temperature*

The significant effect of temperature (Table 16) on puck fragment survival was expected, and reconfirms recommendations to restrict such fragmentation to cooler conditions. The lack of correlation between temperature and suspended fragment mortality suggests that the technique is not as temperature-restricted as puck culture. Fragmentations in temperatures approximately 30ºC and higher, though no mortality in suspended fragments was observed at 30.4ºC, is not advised as significant variation in survivorship was observed in similar conditions (76% at 30.6ºC).

High mortality rates observed in the September trial (at 30.6ºC) followed the predicted influence of high temperatures on fragment survivability. Significantly higher survivorship of August fragments, even though temperature conditions were similar (30.4ºC), was unexpected. Other conditions, such as currents and/or wave action, may have varied enough between August and September to influence these changes in fragment survivorship. If temperature variation can be considered a proxy for current speed and/or wave energy, the importance of these parameters to fragment survival in warmer conditions may be evident (Fig. 46). This would help explain why fragment survivorship was significantly higher in August compared to September for some treatments, even though temperatures were similar.

For nurseries incorporating both suspended and fixed culture, slower-growing genotypes, or genotypes of which nursery operators wish to increase their stock, could be grown on lines to allow for near year-round fragmentation. Increasing production of certain genotypes can be helpful for outplanting efforts, in which a uniform transplantation ratio among numerous genotypes is desired to promote genetic variability, sexual reproduction success, and to decrease the probability of negative, inadvertent effects such as genetic swamping, inbreeding, and outbreeding depression (Baums, 2008).

XIV. Conclusions and Recommendations for Nursery Managers

Growth of *Acropora cervicornis* via suspension in the water column appears to reduce stress to fragments, increasing survivorship compared to the traditional puck-epoxy technique. This benefit intensifies in warmer temperatures at which *A. cervicornis* fragmentation was previously considered inadvisable. Though these benefits do not extend to outplanting efforts (in which fragments must still be secured to reef substrate), the following findings of this study should prove valuable to nursery culture of *A. cervicornis*:

i. Suspended culture of *A. cervicornis* reduces initial post-fragmentation mortality compared to puck culture. This difference is greater with increasing temperature.

ii. Within the experimental temperature range (22.4 – 30.6ºC), suspended fragment survivorship was not significantly affected by temperature, whereas puck fragment survivorship was. This suggests that suspended fragments are less susceptible to temperature-related mortality, although fragmentation in conditions above 30ºC is not recommended, as significant variation in survivorship was observed.

iii. Greater increases to fragment survivorship were observed in genotypes exhibiting the highest mortality when grown via the traditional puck-epoxy

technique. Nurseries employing both suspended and fixed culture techniques may wish to reserve suspended nursery space for such genotypes.

iv. Documented rates of genotype-specific survivorship were not replicated in all trials. Genotypes that are considered poor or strong survivors may not be so in different locations or years. This is especially important considering the transport of corals among many reef areas for outplanting. Nurseries should not disregard certain genotypes because of repeatedly low survival, as such trends are not necessarily permanent.

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