Coral Reefs (2010) 29:729–735
DOI 10.1007/s00338-010-0621-6

REPORT

Propagation of the threatened staghorn coral *Acropora cervicornis*: methods to minimize the impacts of fragment collection and maximize production

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Received: 10 January 2010 / Accepted: 5 April 2010 / Published online: 20 April 2010

Abstract Coral reef restoration methods such as coral gardening are becoming increasingly considered as viable options to mitigate reef degradation and enhance recovery of depleted coral populations. In this study, we describe several aspects of the coral gardening approach that demonstrate this methodology is an effective way of propagating the threatened Caribbean staghorn coral *Acropora cervicornis*: (1) the growth of colonies within the nursery exceeded the growth rates of wild staghorn colonies in the same region; (2) the collection of branch tips did not result in any further mortality to the donor colonies beyond the coral removed for transplantation; (3) decreases in linear extension of the donor branches were only temporary and donor branches grew faster than control branches after an initial recovery period of approximately 3–6 weeks; (4) fragmentation did not affect the growth rates of non-donor branches within the same colony; (5) small branch tips experienced initial mortality due to handling and transportation but surviving tips grew well over time; and (6) when the growth of the branch tips is added to the regrowth of the fragmented donor branches, the new coral produced was 1.4–1.8 times more than new growth in undisturbed colonies. Based on these results, the collection of small (2.5–3.5 cm) branch tips was an effective propagation method for this branching coral species resulting in increased biomass accumulation and limited damage to parental stocks.

Keywords *Acropora cervicornis* · Coral gardening · Propagation · Fragmentation

Communicated by Biology Editor Dr. Andrew Baird

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Introduction

The recent worldwide decline in coral abundance and condition along with associated losses in the key ecosystem services has prompted a myriad of efforts to mitigate further coral losses and restore lost reef function and structure (Edwards and Clark 1998; Edwards and Gomez 2007). One approach gaining acceptance is the use of coral gardening or silviculture techniques for coral propagation and the production of coral colonies for active reef restoration as described by Rinkevich (1995). The coral gardening methodology involves three stages: (1) the initial collection of a limited amount of coral biomass (i.e., nubbins, fragments) from wild or propagated populations; (2) the grow out of the coral fragments within a nursery setting; and (3) the outplanting of nursery-reared corals to depleted or damaged reefs (e.g., Shafir et al. 2001; Epstein et al. 2003; Rinkevich 2006; Shafir and Rinkevich 2008).

While recently gaining wider acceptance, the science of coral reef restoration is still in its infancy, and science-based guidelines for reef restoration methods are needed to evaluate the impacts, efficacy, and efficiency of propagation and restoration approaches (Precht 2006 and references therein). One of the largest knowledge gaps and a source of criticism for reef restoration programs is the potential negative effect of collecting whole colonies on the donor coral populations and/or the potential negative effect of fragmentation on parent colonies (Edwards and

Clark 1998; Epstein et al. 2003; Forsman et al. 2006). Considering the depleted state of many coral populations, this source of concern has often emerged as a roadblock for the procurement of permits to conduct reef restoration activities. This is especially true for the use of the genus *Acropora* in restoration activities in the territorial waters of the United States. The drastic decline of both *Acropora palmata* and *A. cervicornis* in the region and the 2006 listing of this genus as threatened under the US Endangered Species Act (NMFS 2006) highlight the need to develop science-based collection, propagation, and restoration methodologies that limit immediate negative impacts and maximize future gains in coral productivity.

The collection of coral fragments for reef restoration will always cause an immediate negative impact on the fragmented donor colonies due to the metabolic costs associated with the repair of newly formed lesions, regrowth of axial polyps, and competition with fast-colonizing organisms like macroalgae that can establish quickly on the exposed skeleton (Lirman 2000a). Additionally, metabolic costs will be incurred by the fragments extracted, which will need to invest energy in regeneration, potentially resulting in increased mortality and reduced growth and reproduction (Lirman and Fong 1997; Lirman 2000b; Okubo et al. 2007). Thus, for a coral propagation and restoration program to be successful, it clearly needs to minimize the impacts of collection on the parent stock and, after some period of recovery, result in a net increase in tissue and skeleton production through the aggregate growth of the donor colonies and the coral propagules extracted from the parent stock.

In this study, we test whether coral production is ultimately enhanced or hindered by the removal of branch tips from parent colonies of the staghorn coral *Acropora cervicornis* by documenting: (1) the role of branch-tip removal on the growth and survivorship of donor colonies; (2) the role of size and handling methods on the growth and survivorship of branch tips; and (3) the role of colony size on the growth rates of donor and control branches. By tracking the growth and survivorship of both donor colonies and the extracted branch tips, we are able to evaluate the value of coral gardening methods for the propagation of *A. cervicornis.*

Materials and methods

All activities for this project were conducted at the staghorn coral nursery established by the University of Miami within Biscayne National Park, Florida in 2007 (25° 21.753′ N, 80° 09.985′ W, 5.5 m of depth). The nursery consists of cinder blocks and cement platforms deployed on a sand patch onto which staghorn fragments collected from nearby reefs were cemented using underwater epoxy (Herlan and Lirman 2009). The staghorn colonies used in this study had been growing in the nursery for at least 2 years and ranged in size from 30 to 295 linear cm. For each donor colony, two branch tips were clipped using pliers to extract one 2.5 cm (small tip) and one 3.5 cm (large tip) fragment. Each of the donor branches was marked with a plastic tie placed 2 cm from the lesion (Fig. 1). A third, unfragmented control branch, was chosen from each donor colony and marked at 2 cm from the intact branch tip (Fig. 1). In addition to the donor colonies, a subset of colonies was chosen haphazardly as unfragmented controls. Three branches were marked within each of these undisturbed colonies at 2 cm from intact branch tips using plastic ties (Fig. 1). The branch tips collected were attached individually to ceramic disks using epoxy and secured to PVC frames in the nursery. The linear extension of the branch tips and the distance between the cable ties and the terminal ends of branches on the donor and control colonies were measured over time using plastic rulers to evaluate linear growth rates as previously described for this species in the Florida Keys by Shinn (1966).

The fragmentation experiment was repeated twice using similar methods to assess the potential negative effects of transport on branch-tip survivorship. In the first experiment (initiated on June 25, 2009 with 15 donor colonies and 7 control colonies), the branch tips collected were transported back to the boat anchored at the site and glued to the ceramic disks while on board. The branch tips were always kept submerged, and they were returned back to the nursery within 1 h of collection. This was done to simulate a scenario in which staghorn fragments are collected at one site and out planted to another. In the second experiment (initiated on July 15, 2009 with 12 donor colonies and 6 control colonies), the branch tips were epoxied to the ceramic disks and deployed onto the PVC frames without ever leaving the water.

Results

The effects of the handling stress of removing the branch tips from the nursery (and onto the boat) to simulate the impacts of transportation were clearly influenced by the size of the branch tips. While 13 out of 15 of the small tips (2.5 cm) were dead after 20 days (87% mortality), only 2 out of 15 of the larger tips (3.5 cm) experienced complete mortality (13%). In the second experiment, where the tips were epoxied underwater and were not removed from the water, mortality was only recorded for 1 out of 12 of the small tips (8% mortality) and none of the large tips after 24 days. No subsequent tissue mortality was observed for the surviving tips over 3–4 months of observation, but

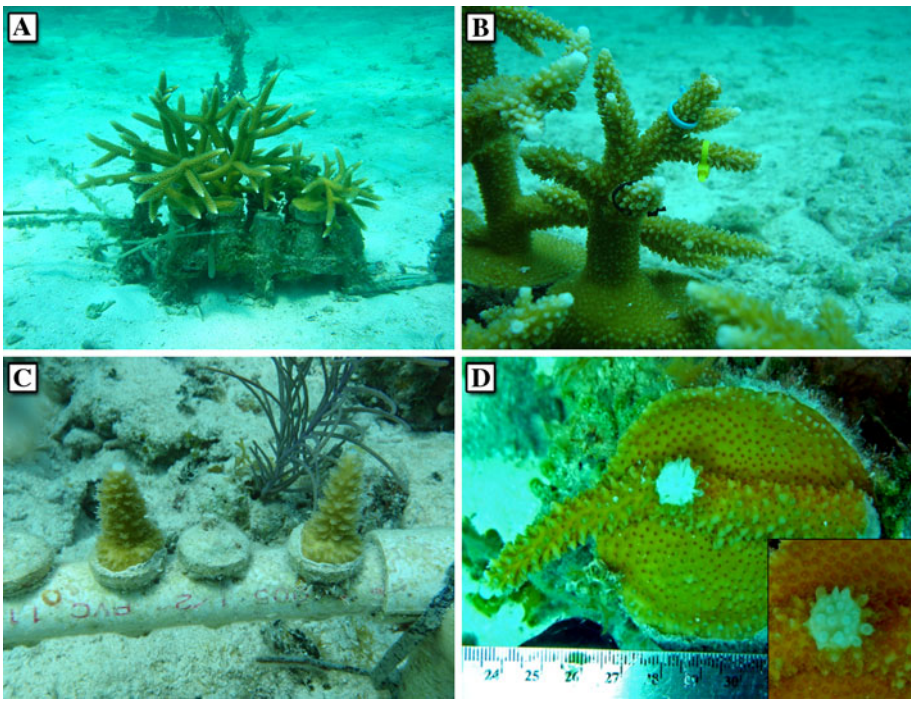

Fig. 1 Photographs of **a** the cinderblocks used as platforms to grow colonies of *Acropora cervicornis* in the Florida nursery; **b** staghorn colony fragmented in this study showing the color plastic ties used to measure linear extension rates; **c** branch tips collected from staghorn colonies (the large tips show healthy growth while the small tip in the middle is completely dead); **d** fragmented staghorn colony showing a skeletal lesion and the recovery of the same lesion 3 weeks later (inset)

some of the tips were broken probably due to predation or physical impacts.

For the first experiment, total length of coral collected was 90 cm (15 × 2.5 cm, 15 × 3.5 cm). Total aggregate length of surviving tips was 55 cm after 39 days, 59 cm after 63 days, and 70 cm after 129 days (Fig. 2a). For the second experiment, total length collected was 72 cm (12 × 2.5 cm, 12 × 3.5 cm). Total aggregate length of surviving tips was 91 cm after 24 days and 110 cm after 90 days (Fig. 2b). The mean annual growth rate was 7.6 cm yr^{-1} (3.5) for large tips and 5.4 cm yr^{-1} (2.3) for small tips. However, when the amount produced was normalized by initial length of fragments, small tips produced a significantly larger amount of new tissue and skeleton (mean production = 1.4 cm of new length per cm of original length, S.D. = ± 0.6) compared to the larger tips (0.9 cm (0.4)) over 90 days (Wilkoxon test, $df = 1$, $x^2 = 4.8$, $P = 0.028$).

When the initial amount of coral collected is used as a recovery benchmark for donor colonies, full recovery was achieved by the regrowth of donor branches within 3–4 months (Fig. 2). The same amount of time was required for control colonies to grow a similar amount of coral (based on the growth of the marked undisturbed branches). When the growth of the surviving transplanted branch tips is added to the regrowth of donor branches, the new coral produced is 1.4–1.8 times more than the amount produced by the same number of branches in control colonies in 3–4 months (Fig. 2). Thus, coral productivity is clearly enhanced by pruning activities, even when some mortality of branch tips is experienced.

To evaluate the immediate effect of fragmentation on branch recovery and growth, the linear growth of the donor and undisturbed branches and colonies was analyzed separately for the first measurement period (i.e., 24–39 days after fragmentation) and the complete growth period (i.e., 90–129 days after fragmentation) for both experiments combined (Fig. 3). For the first measurement period, the daily growth of donor branches (small- and large-tip donor branches combined, 0.028 cm d^{-1}, S.D. = ± 0.013) was significantly slower than that of control branches within donor colonies (0.041 cm d^{-1}, S.D. = ± 0.015) as well as branches within undisturbed colonies (0.040 cm d^{-1}, S.D. = ± 0.01) (Mann–Whitney test, $df = 3$, $x^2 = 18.9$, $P = 0.003$) (Fig. 3). No significant differences in the regrowth of donor branches were found based on the size of the branch tips removed (Wilkoxon test, $df = 1$, $x^2 = 0.7$, $P = 0.78$) (Fig. 3). However, the slow down in growth of the donor branches recorded during the first sampling period of 24–39 days after fragmentation (compared to control branches) was not evident over the extended time period of 90–129 days after fragmentation. In this case, no significant difference in the growth of any of the control or donor branches was detected (Mann–Whitney test, $df = 3$, $x^2 = 1.62$, $P = 0.65$) (Fig. 3), highlighting enhanced growth of donor branches after the initial slow-down period. The annual growth rates calculated in this study were 15.5 cm yr^{-1} (3.9) for branches in control colonies, 13.3 cm yr^{-1} (4.2) for unfragmented branches in donor colonies, 13.8 cm yr^{-1} (5.9) for donor branches (small tips), and 13.5 cm yr^{-1} (6.2) for donor branches (large tips).

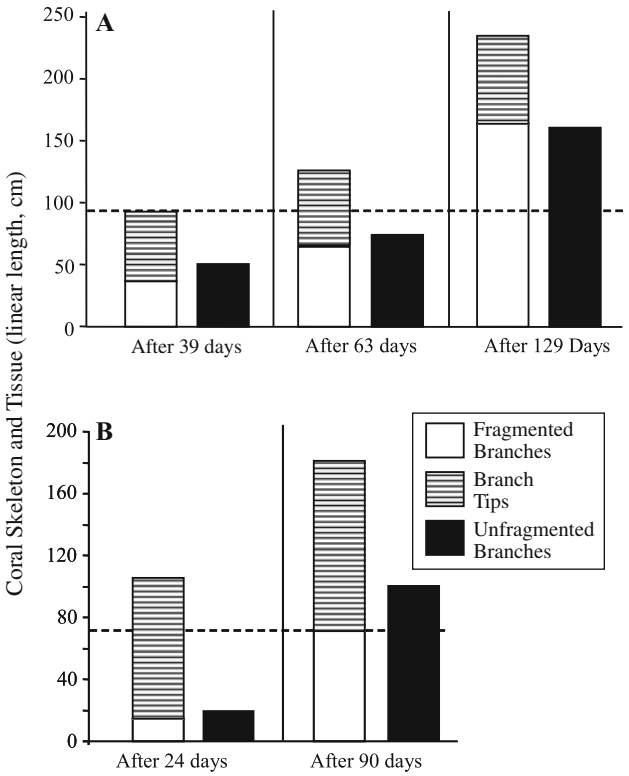

Fig. 2 Amount of tissue and skeleton (linear length) of staghorn branch tips and branches used in experiment 1 (**a**) and 2 (**b**). The white bars represent the total amount of new coral produced by the fragmented donor branches ($n = 30$ branches for experiment 1 and 24 for experiment 2). The striped bars represent the total linear length of all surviving branch tips in each experiment (pooled for 2.5- and 3.5-cm branch tips). The black bars represent the total amount of new coral produced by unfragmented branches in control colonies. The dashed horizontal lines represent the total amount of coral collected from colonies at the start of the experiments (30 branch tips in experiment 1 and 24 branch tips in experiment 2)

The effect of branch-tip collection on the donor colonies was limited to the creation of skeletal lesions, and no partial or total tissue mortality was observed on either the donor or control colonies. The lesions healed quickly, and new apical polyps were observed after 3 weeks (Fig. 1). When the size of the donor colonies (30–295 linear cm) was regressed against the short-term (24–39 days after fragmentation) linear extension rates of the donor branches, no significant relationships were found (linear regression, $r^2 = 0.12$ $P = 0.086$). When the regressions between colony size and linear extension rates were repeated with data for the longer observation period (90–129 days after fragmentation), the linear extension rates of the donor branches did show a significant positive relationship with colony size (linear regression, $r^2 = 0.28$ $P = 0.001$). No relationships between linear extension rates and size of control colonies were found for either the initial period (linear regression, $r^2 = 0.08$, $P = 0.63$) or the final period (linear regression, $r^2 = 0.02$, $P = 0.79$).

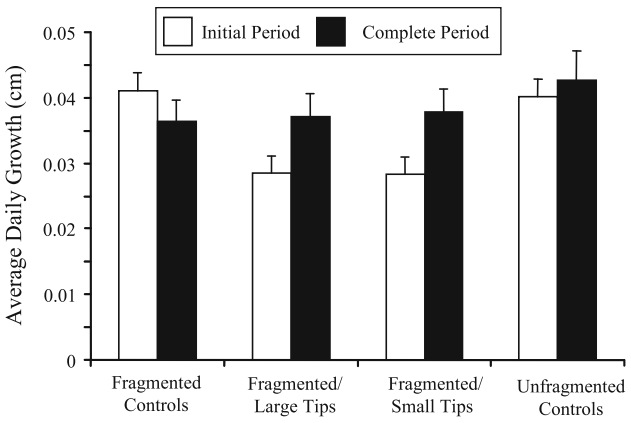

Fig. 3 Mean daily growth (cm, S.E.) of branches from fragmented and unfragmented staghorn colonies. Fragmented Controls = unfragmented branches from colonies that were fragmented, Fragmented/Large Tips = donor branches from which larger tips (3.5 cm) were collected, Fragmented/Small Tips = donor branches from which smaller tips (2.5 cm) were collected, Unfragmented Controls = unfragmented branches from colonies that did not experience any fragmentation. White bars represent growth from the initial sampling period (24–39 d after fragmentation), and black bars represent growth from the complete sampling period (90–129 d after fragmentation). Data from both experiments were pooled for analysis

Discussion

In this study, we conducted manipulative experiments with colonies of *Acropora cervicornis* to evaluate whether the coral gardening approach fulfills two key tenets of coral restoration: "do not cause irreversible harm to parent/donor populations" and "maximize coral productivity through vegetative growth". We found that (1) the growth of colonies within the nursery exceeded the growth of wild staghorn colonies in the same region; (2) the removal of branch tips did not result in any mortality to the donor colonies (beyond the initial losses due to fragmentation); (3) decreases in linear extension of the fragmented donor branches were only temporary, and donor branches grew faster than control branches after the initial recovery period of 3–6 weeks, indicating a shift of resources toward recovery; (4) fragmentation did not affect the growth rates of control branches in the same colony; (5) small branch tips experienced initial mortality due to handling but surviving tips grew well over time, creating new colonies; and (6) when the growth of the recovering fragmented donor branches was added to the growth of the surviving tips, the overall production exceeded that of control branches on colonies that were not fragmented, making a strong case for the benefits of sequential pruning of branching colonies as a mechanism to maximize coral productivity.

Coral gardening and nursery activities are commonly initiated with the collection of fragments or colonies from wild coral stocks (Rinkevich 1995). In cases where donor

coral populations are severely depleted or highly protected, the amount of coral to be collected is often restricted. One of the first choices researchers encounter is whether to collect a single or few large fragments or several small fragments (Rinkevich 2000). The results from this study provide important insights into this trade-off. From a fragment-survivorship perspective, it is clearly better to collect larger fragments to minimize fragment mortality (Bowden-Kerby 1997, 2001; Raymundo and Maypa 2004; Okubo et al. 2005; Forsman et al. 2006). However, productivity (i.e., growth normalized by initial amount of coral collected) in this study was related negatively to fragment size and smaller fragments produced a larger amount of biomass per unit collected, thus increasing recovery rates. Other researchers have shown also that relative growth or productivity decreases with increasing ramet size in corals (Yap et al. 1998; Okubo et al. 2005) and other clonal organisms (Karlson 1988). Thus, the collection of fragments > 3.5 cm is unlikely to provide any added benefits in terms of branch or colony growth. Moreover, Herlan and Lirman (2009) showed that the mortality of *A. cervicornis* fragments in the staghorn nursery is not related to size beyond a 3–4 cm threshold, effectively diminishing the benefits of reduced mortality with increasing size beyond this threshold. Considering the trade-off between size-based productivity and mortality documented here, the collection of branch tips 3.5 cm in length appears to be a good compromise between the lower productivity of larger tips (compared to smaller tips) and the higher survivorship associated with larger fragments when fragments need to be transported to locations away from the collection site. In contrast, in cases where in situ propagation is desired (i.e., nursery expansion, thicket formation), the collection of a larger number of smaller branch tips can maximize vegetative productivity without the costs of higher mortality of smaller fragments caused by transportation.

Lesions on donor colonies recovered quickly, and new axial corallites were formed in 3 weeks. The initial slow down in branch growth caused by lesion recovery was compensated by the enhanced growth of damaged branches after axial corallites were developed. In fact, 3–4 months after fragmentation, the mean growth of donor branches exceeded that of control branches (Fig. 3). This pattern suggests a potential shift in resources within the colonies toward damaged branches. This is consistent with observations by Castanaro and Lasker (2003) who reported that branches formed after fragmentation grew faster than unfragmented branches already present in colonies of the Caribbean octocoral *Pseudopterogorgia elisabethae*.

The enhanced linear growth exhibited by damaged coral branches is similar to the increased growth of *Acacia* shoots documented under heavy grazing pressure from ungulates in the African savanna (du Toit et al. 1990). Thus, pruning activities can lead to enhanced clonal production of the parent stock, and sequential pruning (fragment collection followed by a period of recovery) can be beneficial for stock enhancement in *A. cervicornis* and other clonal taxa like gorgonians that are harvested commercially (Lasker et al. 2003). The benefits of pruning are especially evident when taking into account the growth of the branch tips collected. In this context, and provided that branch mortality is not extremely high, the separation of ramets from the parent colonies is a viable strategy for sustaining vigorous production and lends support to the coral gardening approach as a successful restoration approach. While the removal of a limited number of branch tips per colony has been shown to enhance productivity, no information is yet available on the maximum amount that can be extracted from colonies of various sizes before causing long-term negative effects on parent-colony growth or other vital functions like reproduction, which are commonly influenced by size and disturbance history in clonal organisms (e.g., Hughes and Connell 1987; Smith and Hughes 1999; Beiring and Lasker 2000; Lirman 2000b; Epstein et al. 2001; Okubo et al. 2005). Further manipulative studies are needed to determine both the maximum amount that can be collected from donor colonies as well as the optimal time required for recovery between pruning activities, and trade-offs between clonal propagation and reproduction.

The recovery of lesions resulted in an initial decrease in the growth of fragmented branches; the impact within the colonies was limited to the damaged branches. The control branches within donor colonies showed growth rates similar to those of branches in control colonies. This shows that, at the minimal level of fragmentation used in this study, the negative impacts are limited only to the fragmented branches and that resources can be directed toward recovery without affecting the continued growth of undamaged branches. Also, while the size of the donor colony did not influence early recovery patterns, the continued growth of damaged branches was related to colony size, with larger colonies exhibiting faster growth rates of donor branches. Therefore, for initial and subsequent pruning activities, it would be highly desirable to collect branch tips from the largest colonies available. Collecting from larger colonies in the field would ensure that a smaller fraction of the colony biomass is collected at a given time, that faster, sustained growth of the fragmented donor branches would occur, and that trade-offs with respect to sexual reproduction would be minimized.

One of the common criticisms leveled against reef restoration is that, in the continued presence of the stressors that caused the reef decline in the first place, these efforts, which are often costly, will not reverse declining trends. While this is clearly true in most cases and restoration

cannot be considered a viable option when faced with continued disturbances, the natural recovery of the threatened genus *Acropora* in the Caribbean faces a particular challenge even if stressors are removed. The drastic reduction that this genus has experienced throughout its range has resulted in a present condition where the depleted adult populations are facing reduced reproductive potential due to low adult density, limited genotypic diversity, and potential lack of connectivity (Bruckner 2002). Thus, the establishment of reproductive coral thickets through active restoration appears to be a potentially viable option for supplementing the natural recovery of this genus. To date, successful coral gardening projects have focused on the propagation of fast-growing branching coral species, benefiting from the life-history characteristics of these taxa that include fast growth and regeneration rates and naturally effective asexual propagation via fragmentation. These characteristics, shared by Caribbean acroporids (Highsmith 1982; Lirman and Fong 1997; Lirman 2000b), make this taxon a good candidate for coral gardening projects.

Acropora cervicornis has proven to be an excellent choice for nursery propagation. The growth of nursery-reared staghorn colonies' (i.e., control colonies in this study) fragments [15.5 cm yr^{-1} (S.D. = ±3.9)] within the Florida nursery exceeded the growth recorded for wild *A. cervicornis* in the Florida Keys (10.9 cm yr^{-1}) using similar methods as documented previously by Shinn (1966). Moreover, the collection of small branch tips appears to be a viable option for the propagation of staghorn coral. The fast recovery of lesions and the linear extension rates of fragmented branches indicate that minimum damage is inflicted on the donor colonies and that these damaged colonies are able to shift resources toward rapid regrowth.

Finally, while this study has identified branch-tip collection as a viable propagation methodology, it is not yet known whether the extraction of >2 branch tips from colonies may indeed result in a significant source of mortality and reduced growth and fecundity to the parent colonies. It is expected that thresholds exist for the maximum extraction of biomass from parent colonies before irreversible damage is caused, but this is a knowledge gap that needs to be filled before comprehensive collection guidelines for *Acropora cervicornis* are developed by management agencies charged with the protection and expansion of these keystone and threatened coral resources.

Acknowledgments This research was supported by Counterpart International, the Frohring Foundation, the Nature Conservancy, and the NOAA Restoration Center, U.S. Department of Commerce, with funding from the American Recovery and Reinvestment Act (Award #NA09NFF4630332). The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of Counterpart International, the Frohring Foundation, the Nature Conservancy, the NOAA Restoration Center or the U.S. Department of Commerce.

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