Effects of Habitat Variation on Life History Traits and Genetic Structure in the Corkscrew Sea Anemone *Bartholomea annulata* on Caribbean Coral Reefs

by

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A thesis submitted to the Graduate Faculty of
Auburn University
In partial fulfillment of the
requirements of the Degree of
Master of Science

Auburn, Alabama
August 6, 2011

Keywords: sea anemone, phenotypic plasticity, life history, Caribbean, asexual reproduction, coral reef, ISSRs, genetic structure

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Abstract

Phenotypically plastic life history strategies have important implications for how species colonize local habitats and disperse to distant environments, yet few data exist for tropical sea anemones. Here, I use field and laboratory experiments, coupled with molecular analyses, to determine how habitat heterogeneity influences the life history strategies of the corkscrew sea anemone *Bartholomea annulata* on Caribbean coral reefs. Current habitat, rather than site of origin, appears to drive patterns of growth and recruitment in natural populations, while lifespan may be controlled by genetically induced senescence (Chapter 2). Food availability influences growth and reproductive strategies, in that regularly fed individuals grow more rapidly but pedal lacerate less than do starved individuals (Chapter 3). Molecular analyses of natural aggregations of *B. annulata* indicate that clonal proliferation occurs in the field, but is uncommon and plays a minor role in the genetic structure of these populations (Chapter 4). I conclude that *B. annulata* exhibits phenotypically plastic growth and reproduction, but not lifespan, and that variation in these life history strategies is driven in part by current environmental conditions.

Acknowledgements

I would first like to thank my graduate advisor Dr. Nanette Chadwick for providing me with this opportunity at Auburn University. Thank you for molding me into a competent researcher and ecologist. I'd also like to thank Dr. Scott Santos for opening my eyes to molecular ecology, and all the hours spent training me in the molecular techniques I used for Chapter 4. Dr. Ray Henry for serving as a committee member and providing valuable discussion and suggestions to the thesis. Dr. Stephen Ratchford of the University of the Virgin Islands, for all the assistance with fieldwork, sample collection, and opening up your home to me on St. Thomas. Dr. Hal Lescinsky of Otterbein University for introducing me to coral reef ecology as an undergraduate, and providing funding for my travel to Belize. I'd like to thank all of my dive buddies who assisted with this research especially Ashley Isbell, Lindsay Huebner, and Kathy Morrow. Thanks go to other Chadwick lab members Joey Szczebak, Stanton Belford, and Joe Krieger for encouragement and meaningful discussion, as well as members the Santos/Halanych/Fielman Lab for their accommodation and help around the lab. Also to my parents and sisters for their constant support and advice. Last, but certainly not least, my wife Jill for the constant encouragement, patience, and love, as well as being a wonderful dive buddy and lab helper. Finally, I thank the funding sources, a NOAA Puerto Rico SeaGrant #R-I01-1-08 awarded to Dr. Chadwick, and a PADI Foundation grant #4124 awarded to myself and Ashley Isbell.

Table of Contents

List of Tables

List of Figures

CHAPTER 1

Phenotypic plasticity of life history strategies in sea anemones

Throughout an individual's life history, energetic investment tradeoffs occur, such as growth verses reproduction. These tradeoffs are commonly induced by change in environmental conditions, and over evolutionary time, natural selection occurs for phenotypes that involve successful tradeoffs. The degree that a phenotypic trait can be adjusted in response to environmental conditions is called phenotypic plasticity (Via and Lande 1985), and has important implications for an organism's life history (patterns of growth, reproduction, and mortality), in that it may confer broad adaptability to multiple habitats (Bradshaw 1965). Phenotypically plastic life history strategies significantly influence how the members of a species colonize local habitats and disperse to distant environments (Via and Lande 1985). Seasonality (i.e. day-length and temperature), disturbance events (i.e. storms), and other types of environmental change often stimulate these plastic responses.

Many partially clonal animals are plastic with respect to reproductive mode (i.e. sexual vs. asexual reproduction). In these organisms, growth can be intimately linked to sexual and asexual reproduction in that animals may stunt growth, or allocate resources to growth depending on reproductive mode (reviewed by Shick 1991). Furthermore, asexual reproduction of genetically identical individuals can lead to extensive populations of locally adapted individuals. These populations are often dominated by few genotypes and are at risk of local extinction

because change in environmental conditions usually leads to uniform species reactions. On the other hand, extensive sexual reproduction leads to populations with increased genetic variation that are less susceptible to local extinctions, but may not be able to exploit favorable habitats as rapidly as can clonal animals.

In the marine environment, sea anemones are a model system for researchers to examine the phenotypic plasticity of life history strategies. Patterns of growth, reproductive mode, and life span in sea anemones all may vary widely with environmental heterogeneity and thus can be highly plastic (Sebens, 1982; Bucklin, 1987a; b; Shick 1991; Anthony and Svane, 1994; 1995). Detailed field, laboratory, and molecular studies of these plastic life history strategies have revealed important information about the population dynamics, local adaptation, dispersal potential, and genetic population structure of these partially clonal organisms (Shick et al, 1979; Sebens, 1982; Bucklin, 1987a; b; Anthony and Svane, 1994; 1995; Ting and Geller, 2000; Sherman and Ayre, 2008).

Sea anemones grow indeterminately, in that they may not reach a genetically predetermined maximal size, or may shrink after reaching large size (Shick 1991). Growth and shrinkage in body-size can occur rapidly and at any life stage. Annual anemone growth rates vary, but small individuals generally grow significantly more rapidly than do large individuals. Large anemones may show no appreciable change in body size for years, while small anemones may more than double in size each year (Shick 1991). Patterns of growth in anemones are highly influenced by habitat type, and body size is considered a function of current environmental conditions, as well as an indication of the reproductive potential of individuals (Shick 1991).

Along with variable growth patterns, many sea anemones can both broadcast spawn sexually and proliferate asexually. Thus, reproduction in these organisms also may be highly plastic, and involves two major types of trade-off strategies between these reproductive modes. Chia (1976) suggested that asexual reproduction incurs less energetic cost than sex, and thus, under stressful environmental conditions is the optimal method of reproduction. A contradictory theory (the Strawberry-Coral Model) proposed by Williams (1975) states that for sessile plants and animals under optimal environmental conditions, asexual reproduction can maintain populations of local successful genotypes. Sexual reproduction, while potentially decreasing fitness due to meiosis, serves as a means to widely disperse larvae to new habitats and to enhance the evolutionary potential of a species. This model predicts that sexual reproduction will occur in unpredictable, variable periods and habitats, and that this type of reproduction during optimal environmental conditions is a risk because of the low survivorship of the dispersed larvae (Williams 1975).

These conflicting theories have spurred investigations of Williams' model in cnidarians, and have satisfied many of its predictions (Sebens, 1982; Ayre, 1984). However, other studies have shown that stress (high water flow, lack of food, or substratum instability) stimulates asexual reproduction (Shick et al., 1979; Shick and Hoffmann, 1980; Bucklin, 1987a; Anthony and Svane 1994; 1995), and therefore they agree with the contrasting ideas proposed by Chia (1976). It is therefore difficult to characterize sea anemone reproduction and proliferation without a detailed study of the patterns expressed in each species.

Currently, little is known about anemone life span or whether mortality rates naturally vary with habitat type. While individuals belonging to some species of sea anemones can live for decades, most information on anemone longevity comes from studies in captivity (reviewed in

Ottaway, 1980), with limited evidence from field populations (Ottaway, 1980; Sebens, 1983). Data are lacking on rates of natural senescence in field populations, and on variation in mortality rates among field sites, and a long held belief is that these animas may never naturally senesce (Shick 1991). However, some stony corals have been shown to senesce naturally after only a few years of growth, and to halt their sexual reproduction and growth well before they do so (Rinkevich and Loya, 1984). Thus, it is likely that anemones go through a similar process, but no studies have yet documented this process in anemones.

Most data on the phenotypic plasticity of life history strategies in sea anemones come from temperate species. Given the diversity and conspicuous occurrence of sea anemones on some coral reefs, surprisingly little data are available for tropical species. Tropical coral reef sea anemones are hosts in well-known symbioses, such as the anemone-anemonefish mutualism, as well as hosts to obligate cleaner shrimps that remove parasites from reef fish (Chadwick et al., 2008). These animals are also among the most in-demand species in the ornamental aquarium trade, reaching prices of up to $200 per individual (http://www.liveaquaria.com). This high demand for sea anemones and other coral reef organisms has made the aquarium trade a lucrative industry ($US200-300 million annually, Wabnitz et al. 2003), with few scientifically based recommendations for the management of sustainable harvesting.

On Caribbean coral reefs, large sea anemones fill several types of ecological niches; probably most importantly, they serve as symbiotic hosts to a variety of cleaner shrimps (Colin 1978; Knowlton and Keller 1985) that remove parasites from reef fishes (Sargent and Wagenbach 1975; Nizinski 1989). The corkscrew anemone *Bartholomea annulata* is a tropical zooplanktivore and hosts several species of obligate crustacean symbionts (Mahnken, 1972). *B. annulata* also harbors endosymbiotic dinoflagelletes, thus limiting its habitat to shallow, well-lit

coral reefs. *B*. *annulata* has been shown to occupy virtually every type of reef habitat (fore-reef, back-reef rubble, deep fore reef, patch reef, rock-sand interface, and sea grass beds), and is reported to have the most broad habitat use of all Florida reef anemones (N. E. Chadwick, pers. comm.). Only one type of microhabitat is characteristic for *B*. *annulata*. It is a hole/sand pocket dweller (Sebens 1976; Jennison 1981). These anemones live in cracks, holes, crevices, and sand pockets interspersed throughout coral reefs.

While *B. annulata* exhibits broad habitat use on coral reefs, wave energy may limit its habitat suitability. Because individuals of this species possess long delicate tentacles and a hydrostatic skeleton, strong currents and storm surges may limit prey capture ability, increase mortality, and influence the mode of reproduction in this species, similar to patterns observed for several other types of reef cnidarians (Shick, 1991; Johnson and Sebens, 1993; Sebens, 1998). *B. annulata* reproduces sexually and asexually through broadcast spawning and pedal laceration, respectively (Cary 1911). In Florida, asexual reproduction occurs year round (Cary 1911), while sexual reproduction occurs twice a year (Jennison 1981). Only one study to date has examined sexual reproduction in the corkscrew anemone (Jennison 1981), and no studies have examined the rates of sexual verses asexual proliferation in this species in terms of habitat suitability and population maintenance.

Preliminary field studies at St. Thomas, U.S.V.I. (Nelsen 2008) indicate that *B. annulata* possess highly dynamic populations, and suggest that asexual reproduction may be the primary means of local reproduction for this species. However, no quantitative data have been collected on these modes of reproduction. Given the importance of coral reef sea anemones, both ecologically and economically, studies on the plasticity of life history strategies are important to

understand how these organisms proliferate throughout a habitat, and respond to environmental changes.

In this thesis, I address the following questions concerning the ecology and phenotypic plasticity of life history strategies in the corkscrew sea anemone *Bartholomea annulata*.

1. How phenotypicaly plastic are life history strategies in the corkscrew anemone *Bartholomea annulata*?
    a. How plastic are anemone responses in terms of growth, recruitment, and mortality to reciprocal field transplantation at St. Thomas, USVI? (Chapter 2)
    b. How does the feeding regime impact growth, asexual reproduction, and mortality of *B. annulata* in a laboratory setting? (Chapter 3)
2. To what extent do aggregations of *B. annulata* vary in their distribution and abundance among coral reef habitats? What is the population genetic structure of these aggregations?
    a. Molecular investigation using Inter-Simple-Sequence Repeats (ISSRs) of *B. annulata* tentacle clippings (Chapter 4).

CHAPTER 2

Field Transplantation Experiment: Effects of habitat variation on growth, recruitment, and mortality in *Bartholomea annulata*

SUMMARY

Corkscrew sea anemones *Bartholomea annulata* are conspicuous members of Caribbean coral reefs, and are ecologically important as hosts to obligate cleaner shrimps that may impact reef fish diversity. They also are targets of the ornamental aquarium trade, but limited understanding of their life history prevents development of a scientific basis for sustainable harvest. At St. Thomas, USVI, I reciprocally transplanted corkscrew anemones between coral reef habitats that differed significantly in environmental characteristics, to examine effects of habitat variation on their life history traits. Transplanted individuals that were moved between sites, and control individuals that remained within each site, both grew larger and asexually reproduced more rapidly at an inshore than offshore site. Significantly higher levels of suspended particulate matter and/or lower water motion that occurred on the inshore than offshore reefs possibly caused the life history patterns to vary with these environmental conditions, rather than with polyp origin. At both sites, mortality rates were high after 1 month (25-31%) and 4.5 months (47-53%), possibly due to genetically-controlled senescence, and did not differ significantly between the sites. Clonal replication of polyps through pedal laceration offset mortality and stabilized the population at the inshore site, while the relatively low numbers of recruits to the

offshore population did not fully mitigate the high mortality. I conclude that these sea anemones experience high rates of population turnover and strong impacts of habitat variation on some life history traits, with consequences for associated cleaner shrimp. Based on these results, I recommend limitation of commercial harvest to the types of coral reef habitats that are able to support rapid growth and clonal replication of this ecologically important sea anemone.

1. INTRODUCTION

In benthic marine invertebrates that lack the ability to emigrate from poor habitats, phenotypically plastic life history responses to environmental variation can have important impacts on population structure, and lead to population persistence. Phenotypic plasticity may be especially important for organisms that have limited genetic diversity due to indeterminate growth, frequent clonal replication, and intermittent sexual reproduction, such as sea anemones and corals (reviewed by Bruno and Edmunds, 1997). However, little is known about how life history variation impacts populations of tropical sea anemones. In temperate nearshore environments, sea anemones often dominate hard substrate and exhibit highly plastic growth and reproduction in response to changing environmental conditions, as observed in *Metridium senile* (Shick et al., 1979; Shick, 1980; Bucklin, 1987b; Anthony and Svane, 1994; 1995), *M. exilis* (Bucklin, 1987a) *Haliplanella luciae* (Minasian, 1976; Minasian and Mariscal, 1979; Minasian, 1982), *Anthopleura elegantissima* (Sebens, 1980a; Sebens, 1982), and *Actinia tenebrosa* (Ayre, 1984). Only one tropical sea anemone, the weedy species *Aiptasia pulchella* (Hunter, 1984), has been examined for life history plasticity, and was shown to clonally replicate at enhanced rates when exposed to continuous darkness. The few other published studies on life history traits in tropical anemones have focussed on the clownfish anemones and their variation in body size

(Porat and Chadwick, 2004; Roopin and Chadwick, 2009) and clonal replication (Holbrook and Schmitt, 2005) with the presence of fish symbionts, and have presented basic information on their patterns of sexual reproduction (Scott and Harrison, 2007; 2009).

An effective method to determine phenotypic plasticity in sea anemone life histories is through reciprocal field transplants between sites that differ in physical and/or biological characteristics. These "common garden experiments" were developed originally for flowering plants, but have been applied to studies on many types of organisms including fishes (Vollset et al., 2009), birds (Ballentine and Greenberg, 2010), and snakes (Palacios et al 2010). Reciprocal transplant experiments on temperate sea anemones have demonstrated that their life history patterns are highly plastic, but also that patterns of variation are species-specific (Shick et al., 1979; Shick, 1980; Ayre, 1987; Bucklin, 1987b; Anthony and Svane, 1994**)**.

On Caribbean coral reefs, individuals of the corkscrew anemone *Bartholomea annulata* fill an important ecological niche, in that they serve as symbiotic hosts to obligate cleaner shrimps (Colin, 1978; Knowlton and Keller, 1985) that remove parasites from reef fishes (Sargent and Wagenbach, 1975; Nizinski, 1989). Without regular cleaning interactions, heavy parasite loads can develop on reef fishes and cause tissue damage, disease transmission, and even death (Sikkel et al., 2006). The effects of this complex mutualistic network (microalgae, sea anemones, cleaner shrimps, parasitic trematodes and isopods, reef fishes) radiate out to influence overall coral reef health because cleaner organisms positively impact reef fish diversity (Bshary, 2003; Grutter et al., 2003). As hosts of obligate cleaner shrimps, and thus sites of cleaning interactions, the population dynamics and life history strategies of *B. annulata* play an important role in where fish cleaning takes place, and how fishes locate cleaner shrimps (Huebner, 2010).

As a conspicuous member of Caribbean coral reef communities, *Bartholomea annulata* is also a target of the marine ornamental aquarium trade, and is heavily collected in Puerto Rico (LeGore, 2005) and Florida (Nelsen, 2008). The largely unregulated industry of trade in ornamental reef organisms is valued between US$200-300 million annually, and can have devastating effects on coral reef ecosystems (Chiappone et al., 2001; Wabnitz et al., 2003; Rhyne et al., 2009). Few collecting regulations exist for anemone harvesting (Chiappone et al., 2001), and with the increasing ornamental trade in the Caribbean (Bruckner, 2005), there is a need for scientifically based recommendations to develop sustainable harvesting practices (Chiappone et al., 2001; Hardin and LeGore, 2005; LeGore et al., 2005; Rhyne et al., 2009).

Due to the potential plasticity and ecological importance of corkscrew anemones on coral reefs, members of this species are ideal reef organisms for examination of how habitat variation influences life history traits. They reproduce both sexually through broadcast spawning and asexually via pedal laceration (Cary, 1911), and so may trade off between these 2 strategies depending on seasonality and disturbance regimes. Asexual reproduction occurs year round (Cary, 1911), while sexual reproduction occurs twice each year (Jennison, 1981). Only one study has examined sexual reproduction in the corkscrew anemone (Jennison, 1981), and no studies have examined how asexual proliferation relates to reef habitat suitability and population maintenance. Long-term population studies at St. Thomas, USVI (Nelsen, 2008) suggest that clonal replication may be the primary means of local reproduction for this species, yet no quantitative data have been collected on this mode of reproduction. A laboratory experiment (Chapter 3) revealed that these anemones rapidly alter their growth and clonal replication in response to variation in food supply, and examination of these types of life history responses in field populations is needed to complement this laboratory evidence.

Here I report the results of a reciprocal field transplant between 2 coral reef sites at St. Thomas, USVI, to determine how habitat heterogeneity affects the phenotypic plasticity of recruitment, growth, and mortality in natural populations of *B. annulata*. I also provide scientifically-based recommendations for management of the marine ornamental aquarium trade in these anemones on Caribbean coral reefs.

2. METHODS

On March 3, 2009 a transplant experiment was carried out between 2 coral reef sites at St. Thomas, U.S.V.I., that differed in habitat characteristics: Brewers Bay (BB; 18°20' N, 64°58' W), a nearshore site, and Flat Cay (FC; 18°19' N, 64°59' W), an offshore site (Fig. 2.1). BB had significantly higher levels of sedimentation, smaller sediment grain size, lower water motion, lower live coral cover, and lower water clarity than FC (Fig. 2.2; see Nelsen, 2008 for detailed description of study sites).

Prior to the experiment, 24 individuals of the corkscrew sea anemone *Bartholomea annulata* that were attached to small movable reef substrates (rock fragments, empty conch shells, etc.) were selected haphazardly at BB, and 22 at FC (N= 46 total). Each substrate was tagged with an engraved aluminum tag, the resident anemone was measured (tentacle crown length and width), and the location of each substrate at the site was mapped. The tentacle crown surface area (TCSA) of each anemone was calculated using the equation $((L/2)*(W/2)*\pi)$, where L = length of tentacle crown, and W = width of tentacle crown, because the tentacle crown may be oblong in some anemones (Hirose, 1985; Hattori, 2002; Chadwick and Arvedlund, 2005).

Ten tagged anemones at each site were assigned randomly to a control treatment of no movement between sites, and the remainder to an experimental treatment of reciprocal

transplantation between the 2 sites. There was no significant difference in the initial TCSA of anemones between the control and treatment groups at BB or FC (Mann-Whitney U-Tests, U= 101, p= 0.20 at BB, U= 76.5, p= 0.14 at FC). The anemones at BB were initially significantly larger than those at FC, because anemones in general were larger at the inner reef site (BB) than at the outer reef site (FC, Nelsen 2008, U= 128.5, $p < 0.01$). The anemones initially at each site ranged in size from 5-295 cm^2 TCSA at BB (68.3 ± 68.4 cm^2), and 3-63 cm^2 TCSA at FC (21.3 ± 16.9 cm^2).

The substrates containing anemones in the treatment group at each site were collected in mesh bags, placed in buckets of seawater on small boats, and transported to the other site. The control anemones also were collected and then replaced in their original locations at each site, to control for effects of disturbance alone on the anemones. All tagged anemones were surveyed after 1 mo and 4.5 mo for their patterns of growth, recruitment, and mortality. A recruit was defined as a new anemone that appeared on the same movable reef patch (substrate) as an original experimental anemone. New recruits that appeared during the experiments were not factored into mortality rates, so that mortality indicated the fates of the original experimental polyps only. Also, to avoid over-estimating mortality, tagged substrates that could not be located after 1 mo and 4.5 mo were not included in mortality calculations due to the uncertainty of anemone survival. Tags may become dislodged and buried in the sediment, as well as become completely overgrown by algae and other encrusting organisms. Thus, the anemone may still be present at the site, even if the tag is not.

After final data collection in the first experiment, a second field experiment was set up on July 21, 2009, using 24 different anemones at BB and 22 at FC, employing the same methods. There were no significant differences in mortality, growth, or recruitment between the first and

second experiments, with the exception of growth in BB to FC transplants after 1mo ($U = 30$, $p < 0.01$). Therefore, all data were pooled between the 2 experiments for statistical analyses, which were performed using Systat 13 on a PC platform.

3. RESULTS

3.1. Growth

The percent change in anemone body size after both 1 mo and 4.5 mo decreased significantly with initial body size (Spearman's rank-order correlation, $r = -0.43$, $p < 0.003$ after 1 mo, $r = -0.41$, $p < 0.02$ after 4.5 mo). Small anemones grew significantly more and shrank less than did large individuals (Fig. 2.3a and 2.3b).

In terms of between-site comparisons, anemones at BB grew significantly more and shrank less after 1 mo than did anemones at FC (Mann-Whitney U-Test, $U = 128$, $p < 0.001$). Eleven of 24 surviving anemones (46%) exhibited positive growth at BB while only 3 of 25 surviving anemones (12%) exhibited positive growth at FC after 1 mo (Chi-squared test of independence, $\chi^2 = 6.86$, $p < 0.01$). However, significant differences did not persist after 4.5 mo in terms of either percent growth ($U = 129.5$, $p = 0.31$) or positive versus negative growth ($\chi^2 = 2.73$, $p = 0.098$), possibly due to high rates of anemone mortality at both sites after 4.5 mo (see below).

Percent changes in body size also varied significantly between the 2 treatments (transplanted and control) after 1 mo (Kruskal Wallis Test, $H = 12.34$, $p < 0.01$, Fig. 2.4a). Regardless of site of origin, anemones in BB grew significantly more and shrank less than did anemones at FC (Table 2.1a). However, this pattern did not persist after 4.5 mo ($H = 1.55$, $p =$

0.67, Fig. 2.4b, Table 2.1b), again possibly due high rates of anemone disappearance (mortality) after 4.5 mo (see below).

3.2. Recruitment

After 1 mo, 7 new recruits appeared on experimental substrates at BB, while only 1 new recruit appeared at FC. By 4.5 mo, this number had grown to a total of 12 recruits at BB, and still only 1 at FC. These new anemones formed small aggregations of 2-4 individuals adjacent to the original anemone on each reef substrate. In some cases, the aggregated anemones intertwined their several tentacle crowns. At BB, both control anemones and anemones transplanted from FC received new recruits on their substrates. In contrast, at FC, only 1 anemone (control) received a new recruit. New recruits ranged in size from 7.85 to 61.26 TCSA cm^2 ($x \pm SD = 26.31 \pm 14.55$ cm^2).

3.3. Mortality

There was no significant difference in the mortality rates of anemones at BB versus FC after 1 mo ($\chi^2 = 0.39$, $p = 0.53$) or 4.5 mo ($\chi^2 = 0.72$, $p = 0.40$). Of the 48 anemones tagged at BB, after 1 mo 20 disappeared (42% mortality); in 14 cases, the tagged substrate remained present but the anemone disappeared, and in 6 cases the tagged substrate disappeared and could not be located (Table 2.2a). At FC, of the 44 anemones tagged, after 1 mo 15 disappeared (34% mortality); in 12 cases, only the anemone disappeared, and in 3 cases the tagged substrate was missing (Table 2.2a). After 4.5 mo at BB, 30 anemones disappeared (62% mortality); in 25 cases, only the anemone disappeared, and in 5 cases the tagged substrate disappeared (Table 2.2b). At FC, 25 anemones disappeared (57% mortality); in 18 cases only the anemone

disappeared, while in 7 cases the substrate disappeared (Table 2.2b). Tagged substrates that disappeared and could not be located were excluded from mortality calculations (see above). Thus, N = 42 tagged substrates at BB, and N = 37 tagged substrates at FC were confirmed present throughout the duration of both transplants, and these were the final sample sizes used to calculate mortality.

Mortality rates did not differ significantly between FC control anemones and anemones transplanted from FC to BB after 1 mo ($\chi^2 = 2.64$, $p = 0.10$) or 4.5 mo ($\chi^2 = 3.80$, $p = 0.054$), or between BB control anemones and anemones transplanted from BB to FC after 1 mo ($\chi^2 = 0.32$, $p = 0.57$) or 4.5 mo ($\chi^2 = 0.87$, $p = 0.35$), indicating that site had little influence on anemone mortality rates over these times scales, regardless of origin or treatment.

In terms of changes in the total size of the experimental anemone population at each site, the 42 anemones at BB decreased to 34 after 1 mo (81% of original population size, 27 original anemones plus 7 recruits), and to 27 anemones after 4.5 mo (64% of original size, 18 original anemones plus 9 recruits). Thus, recruitment (N =12 total recruits) mitigated about half of the losses that occurred due to mortality at BB. In contrast, anemone numbers declined from 37 to 30 to 24 over the same period at FC (81% and 64% of original population size, 29 and 23 original anemones at 1 mo and 4.5 mo respectively, plus 1 recruit).

4. DISCUSSION

Here I demonstrate that some of the life history traits of a Caribbean Sea anemone are extremely plastic, in that local environment rather than site of origin appears to determine their patterns of growth and clonal replication over several months. Specifically, anemones transplanted to an inshore reef site shrank less and recruited more than did anemones

transplanted to an offshore site. In contrast, mortality rates were uniform among treatments regardless of site of origin, indicating high anemone mortality occurred overall, possibly due to genetically-determined short lifespans in this species (Nelsen, 2008).

4.1. Growth

The results of my transplant experiments support the idea that BB provides a habitat more conducive to anemone growth than does FC. The lack of continuation of this trend after 4.5mo likely occurred in part due to high mortality rates that reduced my sample sizes. Through increased growth and lack of shrinkage, individuals reached larger body sizes at BB than FC, confirming population model projections for this species at each of these 2 sites (Nelsen, 2008). Increases in body size also likely enhanced the fecundity of individuals, because body size and fecundity are correlated in sea anemones (Chadwick et al., 2000). Higher rates of production of sexual propagules could partially contribute to the greater abundances of anemones at BB than FC (Nelsen, 2008), even though both sites have similar levels of mortality.

The pattern of substantial shrinkage in anemones at FC in both treatment and control groups is somewhat surprising, because FC appears to be a healthier coral reef than BB, in that it has lower sedimentation, and higher coral cover, water clarity, and water motion (Nelsen, 2008). However, relatively high levels of water motion at this offshore site may in fact limit the growth, reproduction, and abundance of individuals of *Bartholomea annulata*. Members of this species have long thin tentacles that easily fragment and contract when disturbed (B. Titus, pers. observation). During periods of high water motion such as storms, individuals at the more exposed field site (FC) completely contract into holes and then re-expand later, while they remain constantly expanded at the protected inshore site (BB, N.E. Chadwick and S. Ratchford,

unpublished data). Thus, high water motion appears to be detrimental to the soft tissues of this anemone, and may cause the tentacles to deform or become severely damaged. In comparison, other Caribbean Sea anemones such as *Condylactis gigantea* and *Stichodactlya helianthus* may be more physically robust and thus able to withstand environments with higher water energy, as they have large thick tentacles (*C. gigantea*) or short stubby tentacles with a broad-flat oral disc and short column (*S. helianthus*; Humann and DeLoach, 2002). Patterns of *B. annulata* size and abundance on patch reefs in Belize also support this idea, in that anemone abundance increases with distance from the outer barrier reef crest, and anemone size and abundance is greatest on the leeward sides of mangrove cays where water motion is low (B. Titus, unpublished data).

Water motion also may influence the prey capture ability of these anemones, because they are suspension feeders that rely on capturing zooplankton in addition to acquiring fixed carbon produced by their microalgae. As such, high water motion may limit tentacle extension and reduce their prey capture abilities (reviewed in Shick, 1991). In calm environments at St. Thomas and Belize, polyps of *B. annulata* tend to have a greater portion of their tentacles, oral disc, and column exposed to the water column than in higher energy habitats (B. Titus, pers. obs.). While water motion is potentially a major factor influencing the body size and abundance of this anemone, differences in predation, sedimentation, and nutrient availability, among other factors that differ between the study sites also may contribute to this pattern. In particular, the nearshore site had lower light penetration and visibility than did the offshore site (Nelsen, 2008), indicating a higher load of suspended particulate matter that might provide more nutrition to the anemones than at the offshore site. My laboratory experiments on the impacts of feeding rate on growth in this anemone indicate that higher food levels stimulate rapid growth, especially in smaller individuals (Chapter 3).

My data indicate that *Bartholomea annulata* is a phenotypically plastic species with respect to growth, so habitat variation contributes significantly to the ability of individuals to attain large body size. Thus, current environmental conditions rather than site of origin determine patterns of growth in this species. In other sea anemones, growth and body size also are plastic; individuals of the plumose anemone *Metridium senile* attain larger body size when transplanted from intertidal to submerged habitats in California (Bucklin, 1987), as well as when transplanted from habitats with high water flow to low flow in Sweden (Anthony and Svane, 1994) and Maine (Shick and Hoffman, 1980). Individuals of *M. senile* respond rapidly (over 1 to 3 months) to transplantation in terms of growth and body size, similar to patterns recorded here for *B. annulata*. The temperate intertidal anemones *Actinia tenebrosa* and *Anthopleura xanthogrammica* may require 5-10 years to become reproductively mature, and decades to reach maximum body size (reviewed in Shick, 1991), but even in these species, body size is in large part a function of current environmental conditions rather than of pre-determined limits imposed by the genotype (Sebens, 1981). The rates of percent change in body size observed here were slightly higher those seen in other sea anemones, in terms of both positive growth (B. annulata up to ~ 200% change over 4.5 months, *A. tenebrosa* up to ~150% change over 24 mo; *A. xanthogrammica* up to ~ 25% change over 6 mo; M. *senile* up to ~ 125% change over 6 mo), and negative growth (B. annulata shrank by up to 90% over 4.5 mo; *A. tenebrosa* shrank by 70% in 12 mo; *A. xanthogrammica* shrank by 50% over 6 mo; *M. senile* shrank by ~ 20% in 1 mo; Ottaway, 1980; Sebens, 1982; Bucklin, 1987b). The relatively slow growth and shrinkage rates in *A. tenebrosa* and *A. xanthogrammica* may be due to their life history strategy (long-lived individuals with slow maturation rates) in contrast to *M. senile* which appears to be a more dynamic species. Rates of growth in B. annulata under laboratory conditions were generally

more rapid than those recorded here in the field, but the ranges in percent change in body size overlapped between the studies (-90% to 215% in the field vs. -43% to 567% change in body size in the laboratory, Chapter 3).

4.2. Recruitment

I hypothesize that the recruits observed in this study resulted at least in part from pedal laceration, because all of them appeared near the original anemones, in some cases with intertwined tentacles, and they appeared throughout the 10-month duration of both experiments. If these recruits were sexually produced, I would expect them all to settle within a short period due to the seasonal gamete cycle of these anemones (Jennison, 1981). While clonal replication may be seasonal in some anemone species such as *Anthopleura elagantissima* (Sebens, 1982) and possibly *Haliplanella luciae* (Minasian, 1982), individuals of *B. annulata* produce pedal lacerates year round (Cary, 1911). In contrast, sexual reproduction occurs twice per year during spring (April) and winter (November; Jennison, 1981). In addition, pedal lacerates with partially overlapping size ranges observed here, also appeared near parent anemones at similar rates in parallel laboratory experiments (7 recruits at BB after 1mo; 10 recruits in laboratory after 6wks; size range from 7 to 24 cm^2 at BB, and 1 to 8.24 cm^2 TCSA in the lab, Chapter 3). Furthermore, environment and not site of origin appears to affect recruitment rate, in that my experimental substrates moved into BB from FC produced more recruits than did those moved into FC from BB.

The factors that stimulate asexual reproduction vary among sea anemone species: high water flow in *Metridium senile* (Shick et al., 1979; Shick and Hoffmann, 1980; Bucklin, 1987; Anthony and Svane, 1994), increased temperature and food availability in *Haliplanella luciae,*

(Minasian, 1982), and seasonally-induced starvation in *Anthopleura elagantissima*, (Sebens, 1982). I did not determine the specific environmental factors that enhance clonal replication in *B. annulata*, but I speculate that low water motion and/or high particulate food availability (see Chapter 3) may be important in natural populations. Laboratory experiments indicate that low food availability can stimulate asexual reproduction in small individuals of this species (Chapter 3). Interestingly, even though clonal replication was most rapid at my inshore site, this rate (0.17 new polyps produced per individual per month) was much slower than under laboratory conditions (0.55 new polyps per individual per month, Chapter 3), and for the sea anemones *M. senile* (up to 15 new polyps per individual per 2 wks in the field, Anthony and Svane, 1994), and *H. luciae* (estimated population doubling time = 8.7 days in lab, Minasian, 1982), but higher than for *A. elegantissima* (0.08 divisions per month in the field, Sebens, 1980a).

While the clonal replication rates of B. annulata observed here were slower than of some temperate anemone species above, they appear to allow members of this species to maintain stable population sizes. Anemones at FC may rely mainly on successful sexual recruitment to maintain or increase anemone population size, as they produced relatively few clonal replicates during my study. Thus, between the known annual spawning times of April and November (Jennison, 1981), anemone populations may continually decrease at FC while potentially remaining more stable at BB due to cloning. While my data indicate that asexual reproduction is occurring at higher rates at BB relative to FC, molecular analysis of *B. annulata* aggregations was done to determine the relative contribution of asexual reproduction to the population structure (Chapter 4).

4.3. Mortality

My observations that rates of mortality did not differ significantly between the 2 study sites, and in all cases were high, are similar to those of Nelsen (2008), who found mortality to be about 10-54% every 3 months during field surveys. He concluded that *B. annulata* have highly dynamic populations, and that high rates of mortality do not appear to be site-specific, but are part of the life history of this species. I estimate that most individuals survive for only a year, and the longest-lived individuals possibly 2 years. The idea that these anemones have short, internally-controlled lifespans is supported by observations of high mortality rates also in laboratory aquaria, where individuals are well-fed and protected from predation and other disturbances (Chapter 3). While individuals belonging to some species of sea anemones can live for decades, most information on anemone longevity comes from studies in captivity (reviewed in Ottaway, 1980), with limited evidence from field populations (Ottaway, 1980; Sebens, 1983). Thus, mortality rates of *B. annulata* under both laboratory and field conditions indicate members of this species may be some of the shortest-lived sea anemones on record.

Some stony corals appear to undergo senescence, with loss of sexual reproduction and cessation of growth prior to internally-programmed death of individuals (Rinkevich and Loya, 1984). Symbionts such as crabs and fishes desert senescing corals prior to death (B. Rinkevich, pers. comm.), and if the same process occurs in *B. annulata*, may explain why some anemones do not possess shrimp symbionts in the field (N.E. Chadwick and A. Isbell, unpublished data).

High turnover rates of *B. annulata* populations indicate that this species should be amenable to sustainable collection if managed properly. Recent declines in the number of *B. annulata* collected in Florida (Nelsen, 2008) show that collection rates do not appear to be currently sustainable. However with the ability to asexually proliferate rapidly, abundances of this anemone potentially could increase rapidly in suitable reef habitats if managed correctly.

The lack of variation in mortality rates among my field sites does not explain the significantly higher abundance of anemones at BB versus FC. Instead, enhanced growth and asexual reproduction of individuals appear in part to cause these differences, and to maintain local population sizes.

4.4. Aquarium Trade

These results have important implications for the marine ornamental aquarium trade, in that the highly dynamic nature of *Bartholomea annulata* populations indicates that they potentially are amenable to sustainable collection. However, the population dynamics of *B. annulata* directly influence their assemblage of obligate crustaceans, which impact reef fishes (Sikkel et al., 2006; McCammon et al., 2010), so it is important to implement careful collecting practices. A greater percentage of anemones at FC have crustacean symbionts than at BB (A. Isbell, pers. comm.), and these cleaners effectively remove parasites from reef fishes (Bunkley-Williams and Williams, 1998; Huebner, 2010; McCammon et al., 2010). Thus, each individual anemone at this offshore site may play a more important role in maintaining reef fish health than do those at inshore sites with higher anemone abundances. Therefore, these anemones should not be collected at offshore sites where they are rare. Likewise, if asexual reproduction maintains anemone populations in suitable habitats such as BB, there may be less impact from collecting at these types of sites (inshore, protected bay), than at offshore reefs with high water motion.

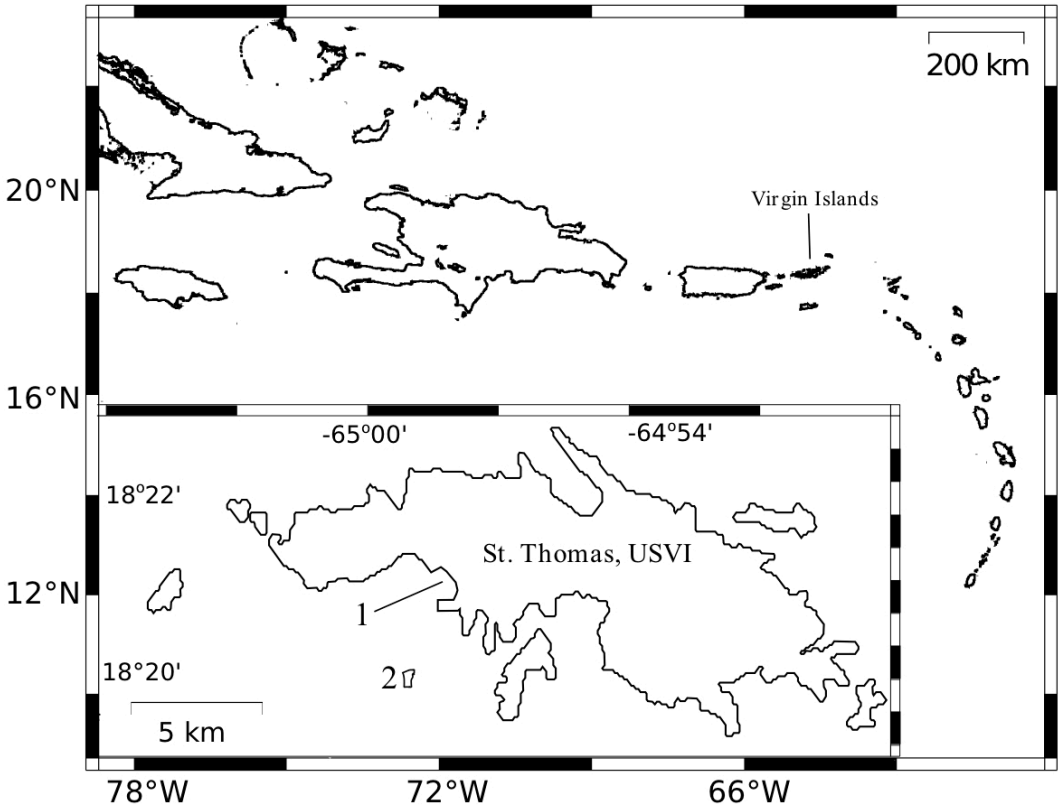


Figure 2.1. Map of coral reef field sites at St. Thomas, USVI: Brewers Bay (1) and Flat Cay (2).

A.

B.

Figure 2.2. Representative images of field transplant reef sites at St. Thomas, USVI. A) Brewers Bay = BB, and B) Flat Cay = FC (Photo by Lindsay Huebner). BB has lower coral cover, higher sedimentation, and lower water clarity relative to FC.

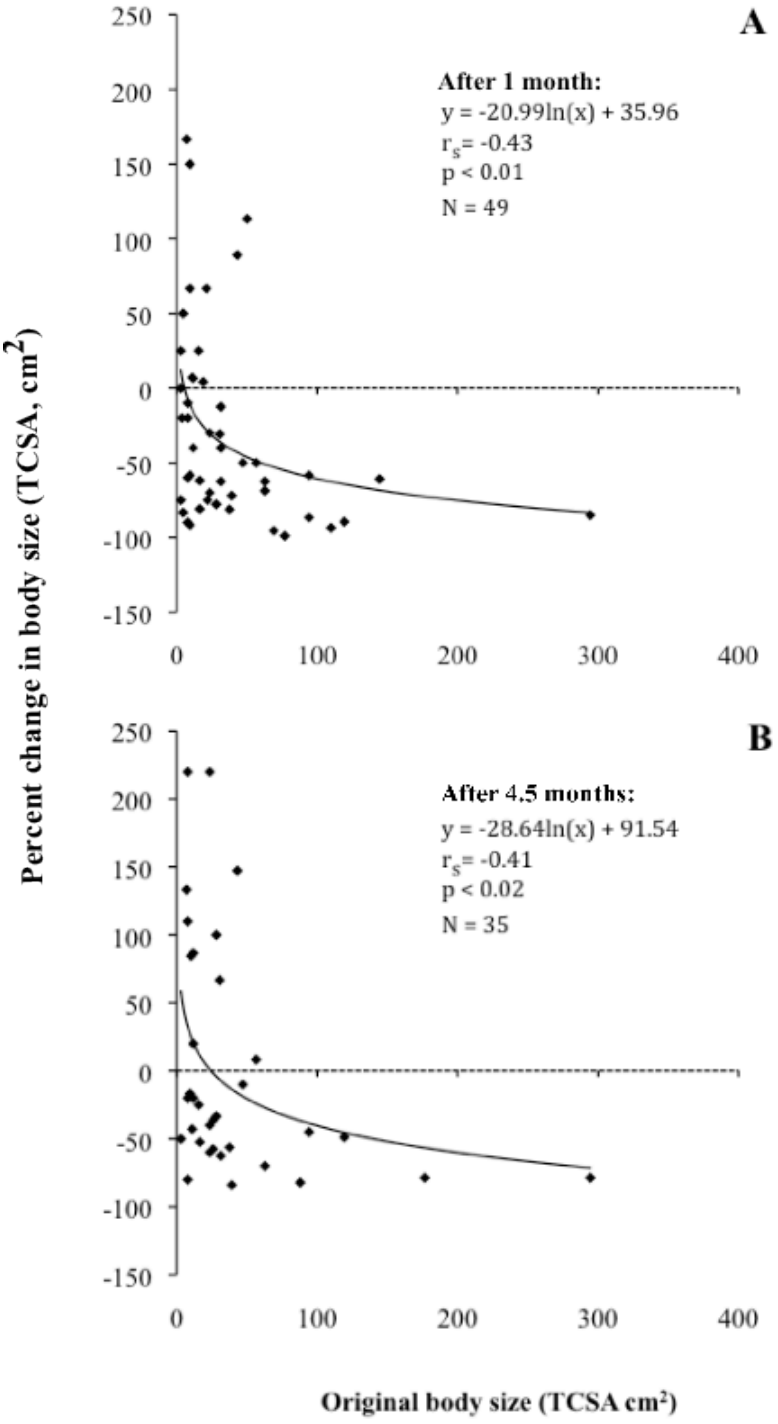


Figure 2.3. Variation in percent change in body size (TCSA = tentacle crown surface area in cm^2) with original body size, after A) 1 mo and B) 4.5 mo in corkscrew sea anemones *Bartholomea annulata* at St. Thomas, USVI.

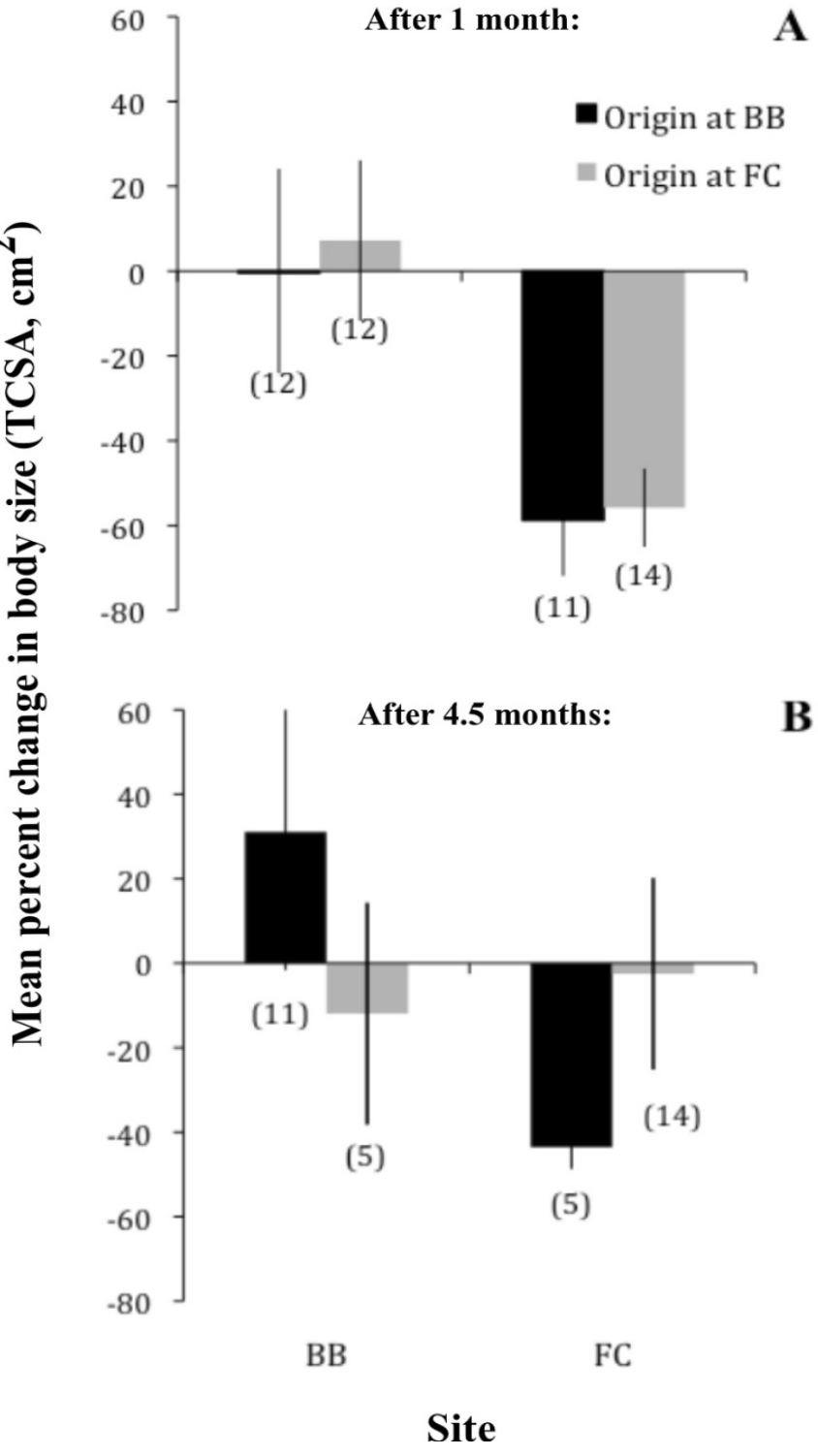


Figure 2.4. Variation in mean percent change in body size (TCSA = tentacle crown surface area cm^2) plus standard deviation bars, of corkscrew anemones *Bartholomea annulata* with site and polyp origin after A) 1 mo and B) 4.5 mo. BB = Brewers Bay and FC = Flat Cay.

Table 2.1. Conover-Inman test for all pairwise comparisons between mean percent changes in the body size (TCSA in cm2) of corkscrew sea anemones *Bartholomea annulata* (a) after 1 mo, and (b) after 4.5 mo in field transplant experiments (see text for details). BB to BB = Brewers Bay Controls, FC to BB = Flat Cay to Brewers Bay transplants, FC to FC = Flat Cay Controls, BB to FC = Brewers Bay to Flat Cay transplants. * Denotes significant p-values ($p < 0.05$).

a.

Treatment	*BB to BB*	*FC to BB*	*FC to FC*
BB to BB	---		
FC to BB	0.534	---	
FC to FC	0.028*	0.006*	---
BB to FC	0.012*	0.002*	0.630

b.

Treatment	*BB to BB*	*FC to BB*	*FC to FC*
BB to BB	---		
FC to BB	0.754	---	
FC to FC	0.644	0.481	---
BB to FC	0.345	0.271	0.481

Table 2.2. Pooled mortality rates of *Bartholomea annulata* anemones at Brewers Bay (BB) and Flat Cay (FC) (a) after 1 mo, and (b) after 4.5 mo in field transplant experiments (See text for details). BB to BB = Brewers Bay Controls, FC to BB = Flat Cay to Brewers Bay transplants, FC to FC = Flat Cay Controls, BB to FC = Brewers Bay to Flat Cay transplants. Pedal lacerate recruits were not factored into mortality.

a.

Treatment	*Survived*	*Died*	*% Mortality*
BB to BB	15	7	32%
FC to BB	13	7	35%
FC to FCC	19	3	14%
BB to FC	10	9	47%

b.

Treatment	*Survived*	*Died*	*% Mortality*
BB to BB	8	14	64%
FC to BB	10	11	52%
FC to FC	14	6	30%
BB to FC	5	12	71%

CHAPTER 3

Laboratory experiment: Effects of feeding regime on life history traits of *Bartholomea annulata*

SUMMARY

Individuals of the corkscrew sea anemone *Bartholomea annulata* are common on Caribbean coral reefs as hosts to obligate crustacean symbionts, some of which are major cleaners of parasites from reef fishes. Despite the ecological importance of these anemones, little is known about their life history strategies or phenotypic plasticity. I determined patterns of growth, clonal replication, locomotion, and mortality in individuals of *B. annulata*, in a laboratory experiment in which I either starved the anemones, or fed them twice each week for 6 weeks. Small fed individuals grew significantly more rapidly (327% increase in body size on average, measured as tentacle crown surface area) than did large fed ones (48% increase), and small starved anemones (103% increase) also grew more than did large starved ones (55% increase). The growth of starved anemones likely was fueled by photosynthetic products from their endosymbiotic microalgae. Only small starved anemones replicated clonally, producing on average 1.43 new anemones per individual via pedal laceration. Feeding regime did not significantly affect locomotion rates, but small anemones locomoted further and more frequently than did large anemones. Individuals in both feeding groups experienced high mortality rates that were similar to those in the field, likely due to senescence. I conclude that small individuals of *B. annulata* respond rapidly to patterns of heterotrophic food input in terms of growth and clonal replication,

while large individuals appear to be less dynamic, and individuals in all size classes have short life spans. Thus, some life history characteristics of this anemone are highly plastic, especially patterns of growth and clonal replication. Environmental conditions such as food availability are to likely have strong impacts on these characteristics in natural populations.

1. INTRODUCTION

Coral reefs worldwide remain under unprecedented anthropogenic influence, and environmental conditions on reefs appear to be changing rapidly in terms of human-induced alterations of ambient water temperature, nutrient levels, sedimentation, and terrestrial runoff (Hughes, 1994; Jackson, 1997; Fabricius, 2005; Pandolfi et al., 2005). Many individuals of reef organisms may not be able to survive such changes in their local habitats, especially benthic invertebrates that often lack the ability to emigrate away from altered habitats. The ability of sessile reef organisms to adjust their life history strategies, such as patterns of growth and reproduction, to quickly changing environmental conditions is important because it contributes to population persistence. Rapid responses may not be possible in some slow-growing organisms that secrete skeletons, such as stony corals that often bleach in response to extreme changes in habitat characteristics. While bleaching has been viewed as an adaptive or stress response (Douglas, 2003), bleached corals experience higher mortality, lower growth rates, and lower fecundity than do unbleached individuals (Hughes et al., 2003).

Other types of reef anthozoans that lack the constraints of a stony skeleton, such as sea anemones and soft corals, may be more dynamic than reef-building corals in terms of their growth and reproduction, and thus more able to respond rapidly to environmental changes on reefs. Soft corals and corallimorpharians are often among the first colonizers after disturbance

events, and may spread rapidly across exposed substrate (reviewed by Norström et al., 2009). For example, in the northern Red Sea multiple photo-acclimation strategies allowed the corallimorpharian *Rhodactis rodostoma* to rapidly colonize reef flats after mass scleractinian mortality (Chadwick-Furman and Spiegel, 2000; Kuguru et al., 2007; 2008). Similarly, soft corals have come to rapidly dominate some reefs after blast fishing and mortality events (reviewed by Norström et al., 2009), and an actinian sea anemone (*Condylactis nanwanesis*) in Taiwan increased its benthic cover from 24% to 42% over 1 year, likely as a result of eutrophication and sedimentation (Tkachenko et al., 2007). Disturbance and phase shifts also are thought to promote generalist species of reef fishes (Nystrom et al., 2008), yet to what extent this is true for anthozoans is unclear.

In temperate shallow marine habitats that experience large seasonal variation in environmental conditions, sea anemones often have phenotypically plastic life histories (Shick, 1980; Sebens, 1982; Bucklin, 1987b; Anthony and Svane, 1994; Chomsky et al., 2004a, b, 2009) and may tradeoff growth with sexual and asexual reproduction depending on habitat and season. In contrast, tropical anemones potentially have less plastic life history strategies because many coral reef habitats do not experience strong seasonal changes in conditions. Only 2 published studies exist on life history plasticity in tropical sea anemones, both on members of the genus *Aiptasia* (Hunter, 1984; Schlesinger et al., 2010), which are cryptic anemones not found exclusively on reefs or in the tropics. Hunter (1984) found that the frequency of pedal laceration increased when anemones were left in continuous darkness but feeding regime did not have an effect, while Schlesinger et al. (2009) found that pedal laceration had a seasonal component and increased during summer months. Furthermore, *Aiptasia* clonemates were shown to have sexual

plasticity, in that a male or female founder could produce male, female, and hermaphroditic clonemates (Schlesinger et al., 2010).

Individuals of the corkscrew anemone *Bartholomea annulata* attain large body sizes (tentacle crown diameter up to 30 cm; Humann and DeLoach, 2002) and commonly occur on Caribbean coral reefs. They serve as hosts to obligate cleaner shrimps (Colin, 1978; Knowlton and Keller, 1985; McCammon et al., 2010) that remove ectoparasites from reef fishes (Sargent, and Wagenbach, 1975; Nizinski, 1989), and thus positively impact coral reef health. The presence of *B. annulata* on Caribbean coral reefs is important in that its distributional patterns determine where cleaning interactions occur and how fish locate cleaners (Huebner, 2010).

Individuals of *B. annulata* also are economically important as major contributors to the Caribbean ornamental aquarium trade (LeGore et al., 2005). Coral reef invertebrates have the most poorly understood life histories of all animals in the aquarium trade (Wabnitz et al., 2003), and over collection especially of sea anemones may lead to local extinctions (Chiappone et al., 2001). Tracking of anemone collection rates in Florida reveals that the numbers of animals collected per year steadily declined through the late 1990's (Chiappone et al., 2001), and reached an all-time low in 2007 (Nelsen, 2008). Few life history data exist for *B. annulata*, and it is unclear how members of this species respond to changes in environmental conditions, or whether they are amenable to sustainable collection.

Here, I investigate how food availability affects patterns of growth, clonal replication, locomotion, and mortality in *B. annulata* under laboratory conditions. I then discuss the plasticity of life history strategies in this anemone, how individuals are expected to respond to environmental heterogeneity, and their suitability for intensive collection for the aquarium trade.

In a companion paper, I demonstrate that transplantation between coral reef habitats strongly impacts the life history patterns of this common coral reef anemone (Chapter 2).

2. METHODS

This study was conducted during June-July 2009 at Auburn University, in closed-system aquarium tanks that each contained approximately 160 L of artificial seawater which circulated between an upper tank and a lower sump (80 L, 71 cm x 33 cm x 35cm each), and were illuminated by a 6-bulb TEK-LIGHT™ suspended above the upper tank. Temperature and day length (illumination time) were controlled by electronic timers, and these along with salinity and nutrient levels in each tank were adjusted on a daily basis to mimic coral reef conditions (culture details in Roopin et al., 2008; Roopin and Chadwick, 2009). Individuals of *Bartholomea annulata* were collected from shallow coral reefs at St. Thomas, USVI, and the Florida Keys, and maintained in these tanks under a regime of feeding to repletion 2x wk^{-1} on a diet of mysid, brine, and chopped cocktail shrimp, for several months, during which time they grew and replicated clonally. About 2 wk prior to the start of the feeding experiment, all anemones were starved to create equal nutritional state in all individuals (Roberts et al., 1999; Chomsky et al., 2004).

At the beginning of the experiment, individuals were assigned randomly to 2 groups: (1) Fed (N = 19 anemones), and (2) Starved (N = 18 anemones). Due to space constraints, the individuals in each treatment were grouped into 2 tanks (10 and 8 anemones in starved tanks, and 10 and 9 anemones in fed tanks, N = 4 tanks total). While this grouping could lead to pseudoreplication due to variation in conditions other than feeding regime between the tanks, practical considerations prevented housing each anemone in a separate tank, and past feeding

studies on anemones have effectively used this type of design (Chomsky et al., 2004a). Anemone body sizes did not differ significantly between the two treatments at the start of the experiment, in terms of either tentacle crown surface area (TCSA, Mann-Whitney U-Test, U=167, p = 0.91, range = 3.1-239.1 cm^2 Fed, N = 19, and 1.2-234.8 cm^2 Starved, N = 18), or oral disc surface area (ODSA, Mann-Whitney U-Test, U= 167.5 p = 0.93, range = 0.79-38.48 cm^2 Fed, N = 19, and 0.19-50.26 cm^2 Starved, N = 18). Anemones in the fed treatment were fed 2x wk^{-1}, because previous studies showed that anemones grew most rapidly under this feeding regime, and more frequent feeding did not increase growth rates (reviewed in Chomsky et al., 2004a).

The location of each anemone in the tanks was recorded on a map, and each was identified by placing a metal pin with a numbered tag in the sand adjacent to the base of the anemone. The relative body size, location, and body color pattern of each anemone (banding patterns on oral disk and tentacles) also were used to identify individuals. To determine whether anemones could be followed clearly during weekly censes, their locations were monitored daily during the first week. They then were monitored weekly, after initial observations indicated that they did not move large distances each day.

Each week, I measured on each anemone the tentacle crown length and width, oral disc length and width, column height (CH), and column diameter (CD). Tentacle crown surface area (TCSA) and oral disc surface area (ODSA) were calculated using the equation $((L/2)*(W/2)*\pi)$ after Nelsen (2008) where L = length of tentacle crown or oral disc, and W = width of tentacle crown or oral disc. This formula was used because the oral disc and tentacle crown both may be oblong in some anemones (Hirose, 1985; Hattori, 2002; Chadwick and Arvedlund, 2005). Pedal disc dimensions were not measured, because the anemones attached their bases to the tank walls beneath a layer of sand at the bottom of the tanks, so the pedal discs were buried and not easily

accessible for measurements. Mortality (disappearance), the appearance of asexually-produced pedal lacerates, and the identity of adjacent parent anemones were recorded as well. Anemone disappearance indicated mortality, because these delicate invertebrates decomposed rapidly upon death and their remains dissolved in the tank, thus their bodies quickly disappeared. A pedal lacerate was defined as a small new anemone that appeared suddenly at or near the base of a larger anemone. Because a layer of sand at the bottom of each tank obstructed the view of anemone pedal discs, polyp buds were not observed prior to the appearance of each pedal lacerate. The process of pedal laceration in .*B. annulata* was described in detailed by Cary (1911, identified as *Aiptasia annulata*).

Rates of anemone locomotion were determined by each week measuring the distance from the pin at the base of the anemone (see above, previous location) to the current anemone location. Each week, this numbered pin then was moved to the anemone's current location, if the animal had moved.

All statistical analyses were performed using the computer program SYSTAT version 13.0 (2010) on a PC platform. Non-parametric correlations (Spearman's rank-order), t-tests (Mann-Whitney U-test), and ANOVAs (Kruskal-Wallis) were used because data were not normally distributed. Results are presented as means +/- SD unless otherwise noted.

3. RESULTS

3.1. Growth

All 3 non-tentacular measures of body size in this sea anemone (oral disk surface area [ODSA], column diameter [CD], and column height [CH]) correlated significantly with tentacle crown surface area (TCSA), so I used TCSA as the main parameter to assess sea anemone size

changes (Spearman rank-order correlations: TCSA vs. CD, $r_s = 0.91$, $p < 0.001$. TCSA vs. CH, $r_s = 0.85$, $p < 0.001$. TCSA vs. ODSA, $r_s = 0.92$, $p < 0.001$, Fig. 3.1). Percent changes in TCSA over 6 wk ranged from –42.9 to 567.0% in fed anemones, and -23.1 to 289.6% in starved anemones, and for all the anemones as a group, they did not differ significantly between the 2 treatments (Mann-Whitney U-Test, U= p = 0.14). The mean percent increase in TCSA in the surviving fed anemones was 210.60% while starved anemones increased an average of 83.35%. However, percent change in TCSA varied significantly among anemones when they were grouped by both size class and feeding regime (Kruskal-Wallis one-way analysis of variance, $H = 10.522$, $p < 0.02$). Small (TCSA < 50cm^2) fed anemones grew significantly more than those in all other groups, which did not differ from each other (Table 3.1; Fig. 3.2).

The percent change in body size decreased exponentially with initial body size in both feeding groups (Fed: $r_s = -0.84$, $p < 0.001$, Starved: $r_s = -0.69$, $p < 0.02$, Fig. 3.3). Some small fed anemones more than quadrupled their size, while large ones grew little if at all (Fig. 3.3a). In contrast, starved small anemones only doubled their body size (Fig. 3.3b). Thus, anemones with small body size experienced a large percent increase in TCSA when fed, but not as much when starved.

3.2. Clonal replication

A significantly higher proportion of anemones produced pedal lacerates in the starved group (7/18) than in the fed group, which did not produce any (0/19, χ^2 test of independence, $\chi^2 = 9.11$, $p < 0.01$). Also, the starved anemones that produced pedal lacerate clonemates were significantly smaller (10.32 ± 8.61 cm^2 TCSA, N= 7) than those that did not (98.46 ± 101.56 cm^2 TCSA, N = 11; Mann-Whitney U-Test, U = 65.5, $p < 0.02$). Pedal lacerates were produced only

by anemones <28 cm^2 TCSA, and the smallest individuals (<10cm^2 TCSA) produced the most clonemates, up to 3 per individual (total = 10 pedal lacerates produced by all anemones).

3.3 Locomotion

The proportion of anemones that locomoted during the 6-wk experiment did not differ significantly between the fed (6/19 = 32% of anemones) and starved treatments (6/18 = 33% of anemones, χ^2-test, $\chi^2 = 0.013$, $p = 0.91$). However, the anemones that locomoted were significantly smaller (19 ± 21.14 cm^2 TCSA, N = 13) than those that did not (72.06 ± 90.1 cm^2 TCSA, N = 24, Mann-Whitney U-Test, U= 220.5, $p < 0.05$). Also, the distances that individuals moved over 6 wk correlated significantly with anemone body size ($r_s = -0.33$, $p < 0.05$; Fig. 3.4).

3.4. Mortality

About a third of the anemones died by the end of the 6-week experiment (6/19 = 32% of fed anemones, and 6/18 = 33% of starved anemones), and this proportion did not differ significantly between the treatments (χ^2 test of independence, $\chi^2 = 0.013$, $p = 0.91$). In addition, the body sizes of anemones that died did not differ significantly from those that survived, in either treatment (Fed: U = 29.5, $p = 0.43$; Unfed: U = 35, $p = 0.96$). The anemones appeared to die due to senescence, and did not appear diseased or damaged prior to death, indicating that anemones under both treatments here had short lifespans, similar to patterns in natural field populations (Nelsen, 2008).

4. DISCUSSION

I demonstrate here that individuals of the corkscrew sea anemone *Bartholomea annulata* are phenotypically plastic in terms of their life history strategies, and respond to differences in feeding regime by altering their rates of growth and clonal replication. Small individuals appear to respond the most, in that they grow rapidly when fed, and initiate clonal replication when starved, while larger individuals are relatively static. These findings have important implications for anemone populations on coral reefs, and show that variation in environmental factors such as food availability can cause rapid alteration of both the body sizes and clonal reproductive strategies of these common sea anemones. The patterns observed here indicate that when populations of this anemone experience elevated food concentrations, they may produce larger individuals, while they may produce more abundant, smaller individuals when food levels are low. Phenotypic plasticity in *B. annulata* also was observed in a companion study in which anemones were reciprocally transplanted between coral reef sites in St. Thomas, USVI (Chapter 2). Interestingly, the anemones became larger, were more abundant, and appeared to clonally replicate more frequently on a near-shore reef exposed to more suspended particulate matter than on an offshore reef (Nelsen, 2008, Chapter 2).

4.1. Growth

The areal measure of body size in these anemones (TCSA) varied exponentially with the 2 linear measures (CD and CH), because it is a two-dimensional versus one-dimensional measure of body size. In contrast, TCSA varied in a linear fashion with ODSA, because both are two-dimensional measures of body size. Similar relationships between one-dimensional (length) and two- (surface area) or three-dimensional measures (wet or dry mass) of body size were reported

also in other studies on sea anemones (Chadwick-Furman et al., 2000), stony corals (Chadwick-Furman et al., 2000) and anemonefish (Godinot and Chadwick, 2009).

I demonstrate here that individuals of *B. annulata* can survive and thrive with little heterotrophic input, in that some small anemones can more than double their body size within 6 wk even when starved. Thus, these anemones appear to meet a major portion of their energy needs by utilizing photosynthetic leachate from their endosymbiotic microalgae. The few studies that have examined effects of feeding on symbiotic sea anemones, indicate that when starved, some anemones in both tropical and temperate habitats lose body mass (Sebens, 1980b; Hunter, 1984; Tsuchida and Potts, 1994), however a temperate species, *Anemonia viridis,* was observed to grow when starved (Roberts et al., 1999). The CZAR (contribution of zooxanthellae to animal respiration) of tropical anemones can approach 100%, but is significantly lower in temperate species, indicating that tropical anemones have a much higher potential than do temperate anemones for autotrophy than heterotrophy (Muller-Parker and Davy, 2001). Thus, starved individuals of *B. annulata* anemones exhibit expected patterns of positive growth for a tropical symbiotic sea anemone. This pattern contrasts with that of non-symbiotic anemones, which universally shrink when starved (reviewed in Chomsky et al., 2004).

In addition to the nutrition supplied autotrophically by algal symbionts, heterotrophic food input appears to be important to these anemones, in that juvenile anemones grow significantly more rapidly when fed than when starved. In natural populations, the rapid growth of small individuals of *B. annulata* may lead to faster acquisition of crustacean symbionts. Possession of more resident shrimps in turn can enhance inorganic nutrient acquisition by the anemones (Spotte, 1996) and protection from predation by fireworms (McCammon, 2010), leading to a positive feedback loop of anemone growth and anemoneshrimp abundance. Large

body size correlates with high survival rate and sexual reproduction in many organisms with indeterminate growth (Hughes, 1984), so food input is likely to significantly enhance individual fitness in this anemone. While algal-translocated materials alone may be able sustain individuals of *B. annulata* for limited periods (up to 6 wk in the present study), prey capture may be required to attain the minimum body size for successful sexual reproduction.

The pattern that I observed here of smaller anemones growing significantly faster than large anemones in terms of percent change in body size is similar to growth patterns observed in other anemone species (Sebens, 1980b; Chomsky et al., 2004a). These results indicate that, similar to other organisms, small individuals of these anemones allocate substantial energy to somatic growth, and large anemones may allocate their energy elsewhere, possibly toward gonad development. Jennison (1981), conducted a histological study of reproduction in several species of anemones from the Caribbean, and noted that small *B. annulata* anemones (<0.75 cm basal diameter**)** did not posses gonads, indicating a minimum size for gonad development.

4.2. Clonal replication and locomotion

In contrast to rapid growth by small individuals when food resources are abundant, small unfed anemones of this species appear to stunt their individual body size and to produce numerous clonemates instead. This strategy may increase the likelihood that a given genotype will survive in highly variable periods or habitats, and is similar to patterns observed for some other species of anemones, in which stressful environmental conditions, such as lack of food, also stimulate pedal laceration (Sebens, 1979; Anthony and Svane, 1994; 1995). Asexual reproduction through pedal laceration appears to incur a low energetic cost (Hunter, 1984) relative to sexual reproduction, and may be expressed in some *B. annulata* anemones when they

are unable to regularly capture prey. However, results from a companion study in which these anemones were transplanted between field habitats indicate that some reef habitats allow individuals to both grow rapidly and to produce many pedal lacerates (Chapter 2).

My observation that small anemones locomote more frequently than do large individuals is similar to patterns known for mobile stony corals (Chadwick-Furman and Loya, 1991) and for the sea anemone *Epiactis prolifera* (Dunn, 1977). Young juveniles of *Anthopleura elegantissima* also can move rapidly, but only after they contact genetically unique conspecifics (Francis, 1973b). Conversely, in the sea anemone *Actinia tenebrosa*, adults move more frequently than do juveniles (Ottaway, 1978). However, individuals of *A. tenebrosa* attain small body sizes relative to most other anemone species, indicating that locomotory rates in some anemones may be a function of absolute body size rather than life stage (juvenile vs. adult).

Many studies have investigated the types of stimuli that initiate locomotion in sea anemones, which usually behave as sessile (immobile) organisms relative to other marine invertebrates. Most have shown that anemones move to escape from harmful stimuli, such as intraspecific and interspecific competitors (Francis, 1973b; Sebens, 1976; Ottaway, 1978), predators (Edmunds et al., 1976; Dunn, 1977; Ottaway, 1977), and physical disturbances (Ottaway, 1978; Anthony and Svane, 1994). Escape from competitors is unlikely to have caused the locomotion of anemones in the present study, because individuals of *B. annulata* do not exhibit aggressive behavior towards conspecifics (Sebens, 1976), and were not crowded in my experimental tanks. Conversely, these anemones often aggregate under both laboratory and field conditions (B. Titus, pers. obs.). Predation, physical disturbance, and interspecific competition can also be ruled out as there were no predators present, the anemones were not physically disturbed, and there were no other species of cnidarians present in the experimental tanks.

Feeding regime also did not influence locomotion rates in the present study, thus these anemones do not appear to locomote to better position themselves for prey capture. However due to the importance of microalgae to the nutrition of tropical symbiotic anemones (reviewed by Muller-Parker and Davy, 2001), individuals may locomote if they do not receive adequate irradiance (Pearse, 1974). Sebens and DeRiemer (1977) suggested that individuals of *B. annulata* orient to maximize exposure to light of their tentacles and oral discs, where the majority of algal symbionts reside. Positive phototaxis occurs in other symbiotic sea anemones, including the temperate Pacific anemone *Anthopleura elegantissima* (Pearse, 1974) and the tropical Caribbean anemone *Lebrunia coralligens* (Gladfelter, 1975). Some free-living fungiid corals also locomote toward light (Yamshiro and Nishihira, 1995). However, not all phototaxis is positive. The giant Caribbean anemone *Condylactis gigantea* moves away from direct sunlight into shaded environments (Zahl and McLaughlin, 1959). Pearse (1974) concluded that phototaxis can vary even among individuals within each species, in that symbiotic anemones from shaded habitats prefer less intense light, while conspecifics from exposed habitats prefer direct sunlight. The levels of irradiance in my tanks were intermediate (94 to 127 $\mu E\ m^{-2}\ s^{-1}$) to the range of irradiance levels in field habitats occupied by *B. annulata* (59 to 195 $\mu E\ m^{-2}\ s^{-1}$, Nelsen 2008), so it is not clear why the small individuals in my study locomoted frequently. Locomotion by some small anemones may simply be a strategy to disperse and explore new habitats when young and small, in contrast to the more sedentary lifestyle of larger, often older individuals.

4.3. Mortality

The mortality rates observed here in both fed and starved anemones are similar to those known for individuals of this species in both field experiments (Chapter 2) and long-term field

observations (Nelsen, 2008). The 32-33% mortality over 6 wk observed here and the 33-29% over 5 wk in field experiments are a bit higher than the 10-54% over about 13 wk (3 mo) in field observations (Nelsen, 2008). However, all 3 studies conducted under different conditions (laboratory experiment, field observations, and field experiment) indicate a high rate of population turnover and thus a short natural life span in these anemones. This limited lifespan appears due to genetically-triggered senescence rather than to environmental factors, because it is similar in different environments. Senescence and short life spans are known to occur also in some stony corals that form small, compact colonies (Rinkevich and Loya, 1984), and in solitary mushroom corals (Goffredo and Chadwick-Furman, 2003).

Nelsen (2008) also documented high recruitment rates at a coral reef site and estimated a population turnover rate of about 2 years. Thus, individuals of this species are highly dynamic and may recover rapidly from disturbances such as removal for the aquarium trade, and thus be suitable for development of a sustainable fishery. However, any substantial reductions in abundances of these anemones on coral reefs may negatively impact fish populations, because they are hosts to obligate cleaner shrimps (Knowlton and Keller, 1985; McCammon et al., 2010). Further information is needed on the population dynamics of both *B. annulata* and its assemblage of crustacean symbionts, in order to develop a sustainable fishery for the ornamental trade in these important reef organisms.

4.4. Conclusions

This study shows that small individuals of the sea anemone *B. annulata* rapidly adjust their life history strategies, allowing them to occupy a wide variety of coral reef habitats, and to potentially recover quickly from disturbance events. My findings are consistent with those from

studies on temperate anemones and some tropical corals, and provide the first evidence of highly plastic life history strategies in a tropical coral reef anemone. While this study focused on the effects of feeding regime, understanding anemone responses to temperature anomalies also will be critical in the future, as rising sea surface temperatures pose a severe threat to coral reef organisms (Hough-Guldberg, 1999).

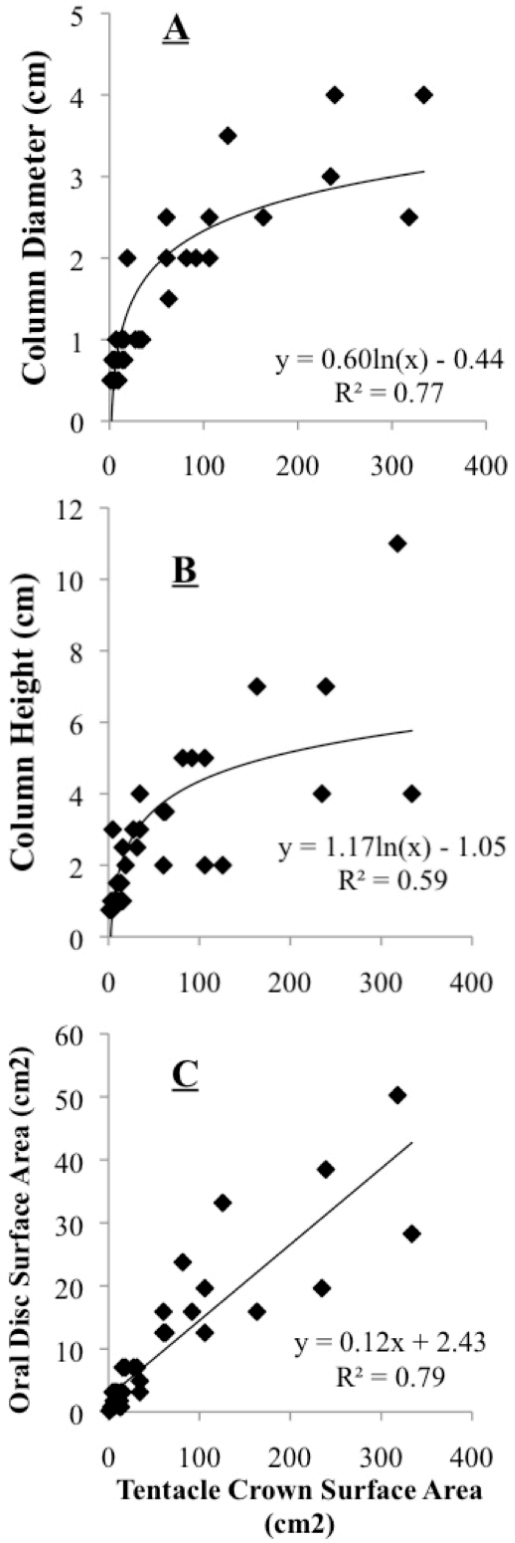


Figure 3.1. Variation in (A) column diameter (CD), (B) column height CH), and (C) oral disc surface area (ODSA) with tentacle crown surface area (TCSA) of the sea anemone *Bartholomea annulata*.

Table 3.1. Conover-Inman test for all pairwise comparisons between mean percent changes in TCSA in *Bartholomea annulata* anemones during a six-week feeding experiment. Small anemone size class = (TSCA < 50cm^2) and large anemone size class = (TCSA > 50cm^2). * Denotes significant p-value (< 0.05).

Treatment and Size Class	*Small Fed*	*Large Fed*	*Small Unfed*
Small Fed	---		
Large Fed	0.003*	---	
Small Unfed	0.027*	0.242	---
Large Unfed	0.002*	0.911	0.199

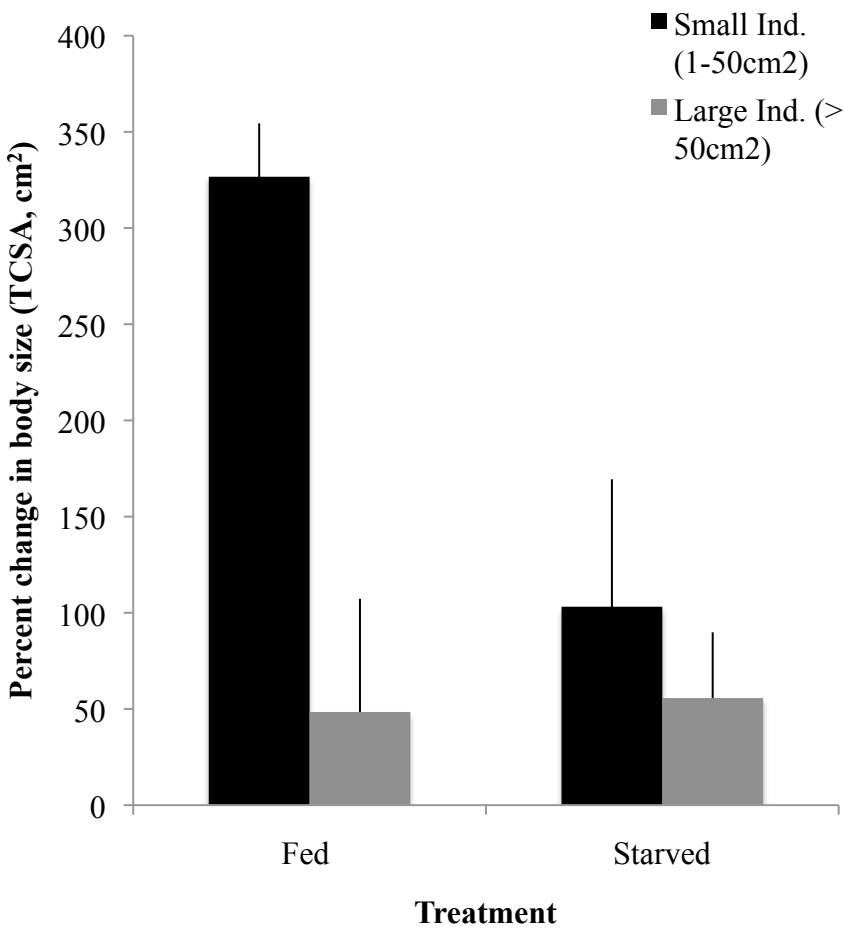


Figure 3.2. Variation in percent change of tentacle crown surface area (TCSA) with feeding treatment and body size, under laboratory conditions in the sea *anemone Bartholomea annulata*. Shown are means plus standard error bars.

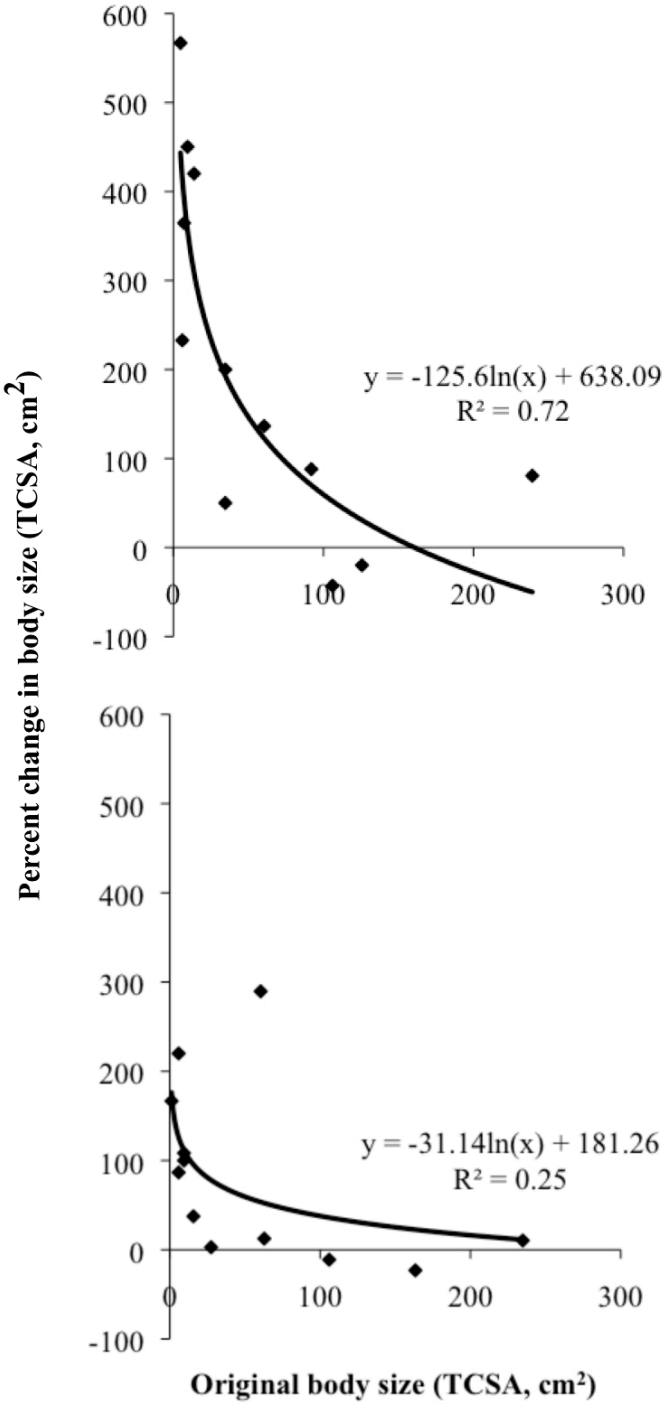


Figure 3.3. Variation in percent change in body size with original size of the sea anemone *Bartholomea annulata* under laboratory conditions after six weeks in either of 2 treatments: (A) Fed 2x wk-1, or (B) Starved.

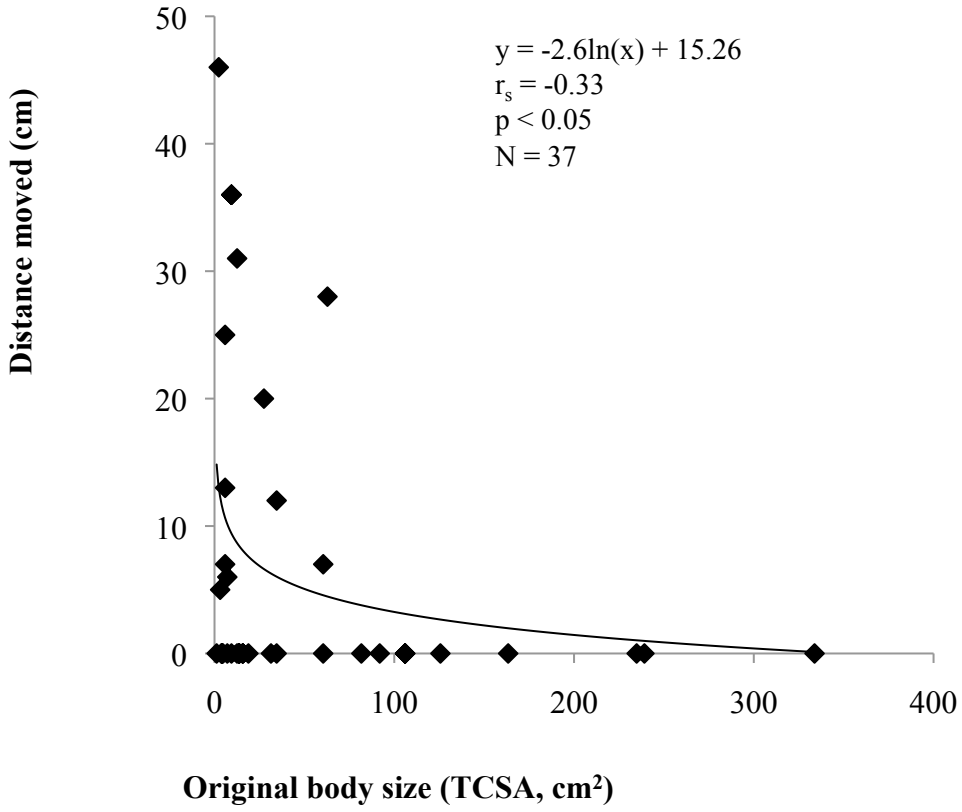


Figure 3.4. Variation in distance moved with body size, for individuals of the sea anemone *Bartholomea annulata* during 6 weeks under laboratory conditions.

CHAPTER 4

Patterns of distribution, abundance, and genetic structure in aggregations of *Bartholomea annulata* at St. Thomas, USVI, and Belize

SUMMARY

Clonal propagation is an important life history strategy for many sessile organisms, and often leads to the formation of large monoclonal aggregations. In the marine environment, the contribution of sexual and asexual reproduction to the genetic structure of temperate sea anemones has been thoroughly studied, yet less attention has been paid to tropical species. The corkscrew anemone *Bartholomea annulata* occurs on tropical coral reefs in the Caribbean and can form small aggregations of 2-4 anemones. Previous laboratory and field studies indicate that this species is phenotypically plastic with respect to reproductive mode, and that some coral reef habitats promote more asexual reproduction than others. Here, I investigated the distribution patterns and the genetic structure of *B. annulata* aggregations in St. Thomas, USVI, and Belize, using field surveys and molecular analyses. *B. annulata* aggregations occurred primarily at shallow field sites with low water motion and high nutrient runoff, and were rarer on exposed offshore reefs. Inter-simple-sequence-repeat (ISSRs) markers revealed that only ~10% of anemone aggregations were clonal, and that genotypic diversity was high (Shannon diversity indices $I = 5.09$). Thus, this species appears to rely primarily on sexual reproduction, and

aggregations consist largely of unrelated individuals. Managers of the ornamental aquarium trade should subsequently establish harvesting limits that reflect the life history of this species.

1. INTRODUCTION

The ability to reproduce asexually is an important life history strategy for many sessile organisms. Clonal proliferation allows organisms to successfully compete for space, circumvent the metabolic costs of meiosis, spread locally adapted genotypes, and ensure the survival of the genotype until sexual reproduction is possible (Shick and Lamb, 1977; Honnay and Bossuyt, 2005; Foster et al., 2007). Frequent asexual reproduction often leads to clumped distributional patterns consisting of monoclonal aggregations, which can lead to reduced sexual reproduction (Honnay and Bossuyt, 2005). The resulting populations may be dominated by one or few genets, which can have considerable consequences for local populations, such as reduced genotypic diversity leading to uniform population reactions to environmental change, and possible local extinctions (Honnay and Bossuyt, 2005).

In the marine environment, many organisms proliferate asexually, creating large aggregations of clonemates, including sponges (Zilberberg et al., 2006; Calderón et al., 2007), seagrasses (Schlueter and Guttman, 1998), tunicates (Chadwick-Furman and Weissman, 1995b), stony corals (Baums et al., 2005), corallimorpharians (Chadwick-Furman and Spiegel, 2000; Edmunds, 2007), gorgonians (Coffroth and Lasker, 1998), soft corals (McFadden et al., 1997) and actinian sea anemones (Francis, 1973a; Purcell and Kitting, 1977; Shick and Lamb, 1977; Ting and Geller, 2000; Sherman and Ayre, 2008). In some species, phenotypic variation may be considerable enough to distinguish between clonal aggregations. For example, the clonal sea anemones *Metridium senile* and *Anthopleura elegantissima* form large aggregations of

clonemates that can be distinguished by color, and by anemone-free zones that mark the boundaries between genets (Francis, 1973a; Hoffman, 1976). For other organisms (i.e. seagrasses) with little phenotypic variation and/or a continuous distribution, it may be virtually impossible to determine where one monoclonal aggregation ends and another begins. In these cases, molecular tools allow researchers to determine the relative contributions of sexual and asexual reproduction to population structure as well as to distinguish between genets (Coffroth and Lasker, 1998; Schluter and Guttman, 1998; Ting and Geller, 2000; Baums et al., 2005; Foster et al., 2007).

Much work has focused on temperate sea anemone aggregations and the contribution of asexual reproduction to these populations (Francis, 1973a; Hoffman, 1976; Purcell and Kitting, 1977; Shick and Lamb, 1977; Fujii, 1987; Francis, 1988; McFadden et al., 1997; Ting and Geller, 2000; Sherman and Ayre, 2008). In contrast, clonality and the formation of aggregations in tropical sea anemones have received less attention. Tropical sea anemones, unlike many temperate species, are not dominant benthic macro-fauna and usually occur as solitary polyps. However, a few species form aggregations on coral reefs. The bulb-tip anemone *Entacmaea quadricolor*, along with *Heteractis magnifica,* are two of the most widespread and common actinian hosts of anemonefishes, and are known to form dense monoclonal aggregations in shallow water in some reef regions (Fautin and Allen, 1997; Fautin and Smith, 1997). In both species, clonality is inferred from identically colored individuals that constitute an aggregation (Fautin and Allen, 1997). Similarly, *Condylactis nanwanensis*, a tropical species in Taiwan, can form extensive aggregations on coral reef crests (Tkachenko et al., 2007). Currently, the sun anemone *Sticholdactyla helianthus* is the only actinian anemone known to form dense, carpet-

like aggregations on Caribbean coral reefs (Humann and DeLoach, 2006), but whether these aggregations are clonal is unknown.

The corkscrew anemone, *Bartholomea annulata*, is an ecologically important sea anemone on Caribbean coral reefs because it hosts the obligate cleaner shrimp *Ancylomenes pedersoni* as well as a variety of other crustacean symbionts (Mahnken, 1972; Knowlton and Keller, 1985). How *B. annulata* are distributed on coral reefs impacts where cleaning takes place, because they appear to function as visual aids to fishes attempting to locate cleaner shrimps (Huebner, 2010). *B. annulata* is also a popular invertebrate species in the marine ornamental aquarium trade in the Caribbean (Legore et al., 2005). Many in-demand coral reef invertebrates have poorly known life histories, which often leads to their over-harvesting (Wabnitz et al. 2003). Previous research has shown that *B. annulata* has highly dynamic populations, with high mortality and population turnover being two characteristics of this species (Nelsen, 2008; Chapters 2 and 3). Thus, individuals of *B. annulata* may be suitable for well-regulated collection, provided their life history strategies lead to rapid recovery and recolonization. Currently, no scientifically based collecting regulations exist for this species

Previous research has shown that *B. annulata* has phenotypically plastic life history strategies, in that habitat variation and not site of origin influences rates of growth and asexual reproduction (Chapters 2 and 3). I observed that individuals of *B. annulata* commonly form small aggregations (2-4 anemones; Figure 4.1) at inner reef sites characterized by low flow and high sedimentation, and are rare on offshore reefs characterized by high flow and low sedimentation. I hypothesize that these aggregations are comprised of clonemates, and that habitat variation (e.g. flow, sedimentation, irradiation etc.) and plastic life history strategies lead to differences in the distribution patterns of *B. annulata* aggregations among coral reef habitats.

Unlike many temperate species that form large aggregations on continuous stretches of hard substrata, *B. annulata* attach their pedal discs to crevices at the rock-sand interface and are frequently found along the margins of coral reefs, where continuous reef substrate gives way to a mixture of rubble, coral, and sandy substrate. In many habitats, large expanses of sand frequently separate individuals (Titus, pers. obs.) and may act as a barrier to the dispersal of clonemates. Thus, I further hypothesize that each aggregation represents a unique genotype, and that sexual reproduction is important in *B. annulata* populations.

Here, I study the patterns of distribution and genetic structure in the corkscrew sea anemone *Bartholomea annulata* through field surveys and molecular analyses using Inter-Simple Sequence Repeats (ISSRs). While phenotypic variation in the spiraling pattern of tentacle nematocysts and tentacle color exists, these variations are slight and difficult to discern with an untrained eye (See Figure 4.1b). Thus, a molecular approach was employed to determine clonality. By assessing the genetic relatedness of *B. annulata* aggregations, I am able to correlate variation in coral reef habitats with reproductive strategies, and estimate the probability that *B. annulata* aggregations are clonemates. Using aggregation frequency as an indicator of asexual reproduction will eliminate the need for extensive molecular investigation of all *B. annulata* populations, and will contribute to management plans for the sustainable harvest of this species.

2. METHODS

2.1. Study Sites

2.1.1. St. Thomas, USVI

Three coral reefs were used in this study at St. Thomas, USVI: Flat Cay (FC), Ratchford Reef (RR), and Range Cay (RC; Figure 4.2a). FC (18°19’ N, 64°59’ W) is an offshore fringing

reef 2.2 km south of the island. FC is between 6-10m in depth and is characterized by higher coral cover, lower sedimentation rates, higher water motion, and greater light penetration than RR and RC (Nelsen 2008). RR and RC are inshore patch reefs located 150 m apart within Brewers Bay (18°20' N, 64°58' W), a partially enclosed bay adjacent to the Cyril E. King airport. Both RR and RC reefs are between 3-7 m depth and are characterized by high algal cover, low coral cover, low water motion, high sedimentation rates, and lower irradiance relative to FC (Nelsen, 2008). FC and RR were previously mapped field sites established by Nelsen (2008), and RC was previously established for anemone transplant experiments (Chapter 2) and Huebner (2010) for fish cleaning observations.

2.1.2. Belize

In Belize, eight coral reef sites were used that occurred in the back-reef lagoon in the south-central region of the Meso-American Barrier Reef (6 patch-reefs and 2 mangrove cays; Wee Wee Cay, WWC, and Peter Douglas Cay, PD). Here, patch reefs and mangrove cays dominate the back-reef lagoon and have formed on top of submerged karst (limestone) hills since the last ice age (18,000 ybp). The tops of these patch reefs are generally small (<100 m diameter) and shallow (<2 m), and quickly slope to a depth of 16-17 m where the benthos is covered in fine carbonate mud. Reef sites centered on Wee Wee Cay (Figure 4.2b) were selected prior to the trip using Google Earth™.

2.2. Field surveys

In St. Thomas, previously established reef sites FC and RR (Nelsen, 2008) were used to gather abundance and aggregation data from November 8th-13th, 2009 on *Bartholomea annulata*

populations. At FC, two transects (70 x 11 m, and 73 x 5 m) for a total area of 1,155 m^2 were surveyed, while at RR a 47 x 6 m (282 m^2) area was surveyed as established by Nelsen (2008). For the purpose of this study, an aggregation is defined as two or more *B. annulata* anemones that are physically touching, often with intertwining tentacles. Using SCUBA, each individual anemone within an aggregation was measured (tentacle crown surface area, cm^2), tagged, and mapped at FC and RR.

Anemone abundance and aggregation data were also gathered on SCUBA from 6 patch-reefs as well as 2 mangrove cays in Belize from December 1st-6th 2009. At each patch reef two transects, each 25 x 2 m (50 m^2) were surveyed. Each transect was placed in 3-6 m depth on patch reef slopes, where continuous reef substrate gave way to a mixture of rubble, coral, and sandy substrate to maximize *Bartholomea annulata* abundance. Two 25 x 2 m (50 m^2) transects were also surveyed along the leeward margins of two mangrove cays (WWC and PD). Transects at WWC and PD were placed at 1 m depth and done on snorkel among the mangrove roots where *B. annulata* were most numerous. Transects were not surveyed on the windward sides along the mangroves as it was too shallow and inaccessible due to fringing reefs that reached the surface.

2.3. Tentacle clippings

Bartholomea annulata tentacle clippings were collected from RR and RC at St. Thomas, USVI August 22nd-29th, 2010. At each reef site, 25 *B. annulata* aggregations (2-4 anemones per aggregation) were sampled haphazardly using forceps. Each clipping was placed in a separately labeled tube, transported back to the University of the Virgin Islands MacLean Marine Science Center, and preserved in 0.5mL of RNAlater. Tentacle clippings were also obtained from 2 *B.*

annulata aggregations at Belize from leeward mangroves at PD cay and used as comparison samples.

2.4. DNA extraction, Inter-Simple-Sequence-Repeats, and PCR

Template DNA was extracted from *Bartholomea annulata* tentacle clippings using the 2X CTAB protocol after Coffroth et al. (1992). DNA concentrations were quantified using a Nanodrop ND-1000 spectrophotometer, and diluted with ddH_20 to a concentration of ~20 ng/µL. Genomic DNA was amplified with the polymerase chain reaction (PCR) using Inter-Simple-Sequence-Repeat (ISSRs) primers. ISSRs are a fragment-based analysis that uses short (16-25 bp) microsatellite repeats as primer sites to amplify regions of DNA that are potentially variable in length between the primer sites. ISSRs can be highly polymorphic and have been shown to be reliable, reproducible genetic markers (Reddy et al., 2002) and were chosen over mitochondrial DNA since the latter evolves slowly in most cnidarians (Shearer et al., 2002).

The PCRs were carried out in a total volume of 25µL with final reagent concentrations of 1 x PCR buffer, 1.5 mM $MgCl_2$, 0.2mM dNTPs, 0.06 U *Taq* polymerase, 1 µM ISSR primer, and 20ng/µL template DNA. PCR amplification was performed using one cycle of 94 ºC for 1.5 min, 35 cycles of 94 ºC for 45 s, 45 ºC for 50s, 72 ºC for 1:40 s, and one cycle of 94 ºC for 50 s, 45 ºC for 50 s, and 70 ºC for 5 min. Samples were then held at 4 ºC for ~10-15 min. PCR products were separated by electrophoresis on 1% SB agarose gels for 70 min at 135 volts. Four unique primers were screened, and three selected for further analysis (Table 4.1).

2.5. Defining clonality

As *Bartholomea annulata* anemones are hosts to endosymbiotic dinoflagellates belonging to the genus *Symbiodinium*, it makes generating molecular markers specifically targeting the animal DNA difficult. Currently, there are no nuclear markers specific for this species. Here, I circumvent this issue by investigating clonality of the holobiont (host DNA + dinoflagellate DNA), as neither host nor symbiont can survive extended periods of time without the other. Thus, the total ISSR banding patterns are hence generated from both symbiotic partners, and variation between individuals may be attributed to host DNA, dinoflagellate DNA, or both. Clones are defined as individuals exhibiting the same ISSR fingerprints across the three primers that were utilized.

2.6. Data analysis

ISSR fingerprints were generated from 41 anemone aggregations (N = 21 from RR, N = 18 from RC, 2 from Belize) and a total of N = 84 individuals. Individual bands in a fingerprint were scored as present (1) and absent (0) for each anemone, and a data matrix was created to make comparisons across all samples for each primer. A group of samples with homologous fingerprints across all primer sets were considered to belong to the same genet. The program Fingerprint Analysis with Missing Data (FAMD; Schlüter and Harris, 2006) was used to calculate pairwise band similarities based on Jaccard's coefficient, as well as to conduct a cluster analysis with the Unweighted Pair Group Methods using Arithmetic Averages (UPGMA). Gentotypic diversity was calculated using two methods: (1) *G*, the number of unique genotypes detected, and (2) *PD*, the proportion of distinguishable genets ($PD = G/N$), where N represents the number of individual anemones. Genetic diversity was measured with percent polymorphic loci (*PPL*), and the Shannon diversity index (*I*), which was calculated using FAMD. Genotypic

and genetic diversity data were calculated for only samples from St. Thomas, USVI, while Belize samples were added to the UPGMA tree for comparison. The program FIGTree v1.3.1 was used to visualize the dendrogram (Morariu et al., 2008).

3. RESULTS

3.1. Field Surveys

At St. Thomas in the USVI, a total of 223 *Bartholomea annulata* individuals were surveyed at RR (n = 121) and FC (n = 102). There were 21 *B. annulata* aggregations at RR but only six at FC. The average aggregation size was 2.47 ± 0.68 anemones at RR and 2.5 ± 0.84 anemones at FC, and they were not significantly different between these two sites (Mann-Whitney U-Test, U = 62, p = 0.98). At RR, 52 anemones occurred in aggregations (43% of population, Table 4.2), while at FC only 15 anemones occurred in aggregations (15% of population, Table 4.2). There were significantly more anemones in aggregations at RR than at FC (Chi-squared test of independence; $\chi^2 = 21.04$, $p < 0.00001$).

At Belize, 128 *Bartholomea annulata* anemones were surveyed on six patch reefs and 61 among leeward mangrove roots at WWC and PD. There were 11 total aggregations on the six patch reefs and 10 total aggregations at WWC and the PD mangroves. The average aggregation size found on patch reefs was 2.4 ± 0.70 anemones, and 2.3 ± 0.48 anemones among mangroves. Patch reefs had a total of 26 anemones occurring within aggregations (20% of population, Table 4.2), while WWC and PD had a total of 23 anemones occur in aggregations (38% of population, Table 4.2). WWC and PD had a significantly higher proportion of anemones occur in aggregations than on patch reefs ($\chi^2 = 6.51$, $p < 0.02$).

3.2. Clonal and genetic diversity

A total of 57 scorable bands were obtained across the three ISSR primer utilized, with 49 of these being polymorphic (*PPL* = 86%; Table 4.1). At St. Thomas, USVI, 4 of 39 (~10%) *Bartholomea annulata* aggregations were clonal across the three primers: 2 from RR and 2 from RC. Of the 80 individual anemones sampled, 77 unique genotypes (*G*) were detected (PD = 92%), and the Shannon diversity indices (*I*) = 5.09, indicating this population highly diverse, and that genotypes have a high degree of evenness. The UPGMA dendrogram revealed that individuals did not group by reef site in St. Thomas. Also, all clonal aggregations were shown to represent unique genotypes at St. Thomas. On the other hand, ISSR analysis on 2 aggregations taken from the same site at Belize revealed samples to be clonal within and between aggregations (Figure 4.3). UPGMA cluster analysis showed these samples to be approximately 26% different from all samples in St. Thomas (Figure 3).

4. DISCUSSION

Here I show that there are low levels of clonality within *Bartholomea annulata* aggregations (10%) and thus I reject my original hypothesis that all aggregations are comprised of clonemates. These findings are also in contrast to assumptions made by Jennison (1981) that small infertile anemones frequently encountered in his study were the result of recent pedal lacerations. While the occurrence of anemone aggregations is often an indication of high levels of asexual reproduction in other temperate and tropical species (see Introduction), this does not appear to be the case with *B. annulata*. Instead, the examined individuals and populations were highly diverse and apparently derived via sexual reproduction. Thus, in determining the extent of clonality for the entire population, I estimate that ~5% of all *B. annulata* anemones are derived

asexually, because approximately half of the RR population occurs in aggregations (Table 4.2), and the remaining individuals occur as solitary polyps. This is surprising, in that *B. annulata* is closely related to members of the genus *Aiptasia* (Daly et al., 2008), an aquarium pest that can form extensive aggregations of monoclonal individuals (Schlesinger et al., 2010). However, *B. annulata* individuals are much larger than those of all *Aiptasia* anemones and it has been suggested that large body-size can reduce clonal proliferation in some species (Shick, 1991).

I do, however, fail to reject my second hypothesis that clonal *Bartholomea annulata* are genetically unique among aggregations. Here, no clonal aggregation in St. Thomas exhibited the same fingerprint among aggregations. This suggests that clonemates do not locomote large distances from the parent anemone. Here, even anemones sampled only 10 cm apart (i.e. RC6a and RC6b) had unique genotypes (Figure 4.3).

Low levels of clonality may be due in part to the heterogeneous substrata along the edges of patchy coral reefs. Ayre (1984) demonstrated that stable homogeneous substrata (continuous and consolidated rocky shores) promote populations dominated by asexual reproduction in *Actinia tenebrosa*, while heterogeneous environments (patchy habitats) promoted populations that were predominately sexually reproducing. Zilberberg et al. (2006) saw a similar pattern in the sponge genus *Chondrilla*, in that extensive asexual reproduction was common in homogenous habitats (39% clonal), while substrata characterized by boulders interspersed with sand patches and algal turf had low levels of clonality (7% clonal). At St. Thomas, *Bartholomea annulata* aggregations attached to reef rocks were often completely surrounded by sand, making it unlikely that any individual could locomote across hard substrate to another patch of reef just meters away. In contrast, *B. annulata* aggregations from Peter Douglas Cay, Belize showed clonality within and between aggregations. These aggregations were separated by ~7 m and

attached to a homogeneous bed of peat along the margin of the island. In such a mangrove habitat, fewer barriers to clonemate dispersal are apparent, and the spread of clonemates could theoretically produce monoclonal populations of *B. annulata*.

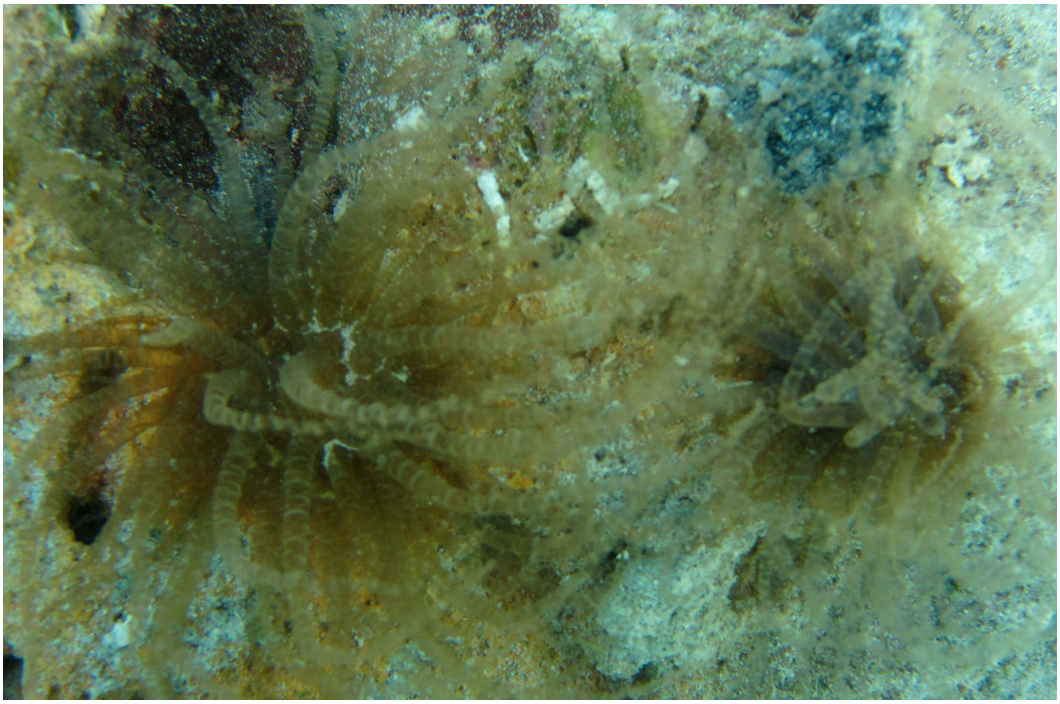
Because the majority of aggregations are apparently not derived via clonal propagation, the distribution and persistence of *B. annulata* aggregations requires another explanation. Interestingly, at both St Thomas and Belize, the reef habitats with the lowest water motion (BB at St. Thomas, Nelsen, 2008; leeward mangrove roots at PD and WWC, Belize) had the highest abundances of *B. annulata* aggregations. This low water motion may possibly limit larval dispersal and lead to high rates of self-recruitment of sexually produced larvae within reef sites. As a pocket dweller, *B. annulata* does not aggressively compete for space with corals (Sebens, 1976), and on low coral cover habitats (i.e. Brewers Bay and leeward mangrove cays), these anemones may thrive. *B. annulata* also does not compete or show aggression toward con-specifics (Sebens, 1976), and continuous self-recruitment may increase the probability that anemones encounter each other, colonize the same microhabitat, and form aggregations. A targeted study designed to determine levels of genetic connectivity among populations is warranted, to further infer patterns of larval dispersal and successful recruitment, as well as what biological or physical factors may facilitate aggregation in this anemone species.

Mainly sexually reproducing populations of *Bartholomea annulata* also have important implications for species conservation and the ornamental aquarium trade. While these animals have highly dynamic populations (Nelsen, 2008), over-exploitation could potentially reduce anemone abundance, and thus, the frequency that spawned gametes will encounter one another and result in fertilization. Such a scenario is currently unfolding for the giant Caribbean anemone *Condylactis gigantea,* an exclusively sexually reproducing species that is now

considered to be overharvested (Chiappone et al., 2001; Rhyne et al., 2009). *C. gigantea* is the most sought-after Caribbean anemone species, and the decrease in the number of individuals harvested between 1994 and 2007 is thought to be more a function of its increasing rarity, rather than decreasing demand (Rhyne et al., 2009). As *B. annulata* hosts a diverse assemblage of crustacean symbionts, the ecological impacts to reef communities of overharvesting may radiate through multiple trophic levels. While I detected clonality within *B. annulata* populations, these levels were low, and managers of the aquarium trade should establish harvest quotas to appropriately reflect the life history of the species.

While I defined clonality as the holobiont (host DNA + *Symbiodinium* DNA), I did not determine whether the DNA from the host, *Symbiodinium*, or both, is responsible for the variation in banding patterns. While tentacle samples were taken from one bay at St. Thomas, *Symbiodinium* clades can be partitioned over fine scales (between reefs), and even within the same coral colony (Santos et al., 2003; Kirk et al., 2005; 2009; Thornhill et al., 2009). Also, it is unclear how *Symbiodinium* are acquired by *Bartholomea annulata* during asexual reproduction. *B. annulata* attaches its pedal disc in holes and crevices and is not exposed to much direct sunlight. Thus, *Symbiodinium* is partitioned unevenly throughout the host body, with fewer algal cells in the column than the tentacles (Sebens and DeRiemer, 1977). It is unclear whether algal cells are partitioned to the pedal disc itself, and as *B. annulata* proliferates asexually through pedal laceration, an understanding of whether *Symbiodinium* are acquired from the parent, or externally would help with interpreting our results. Without host specific markers, we are unable to determine clonality in the anemone itself. Thus, our findings should be considered a conservative estimate of clonality in *B. annulata* populations.

A.



B.

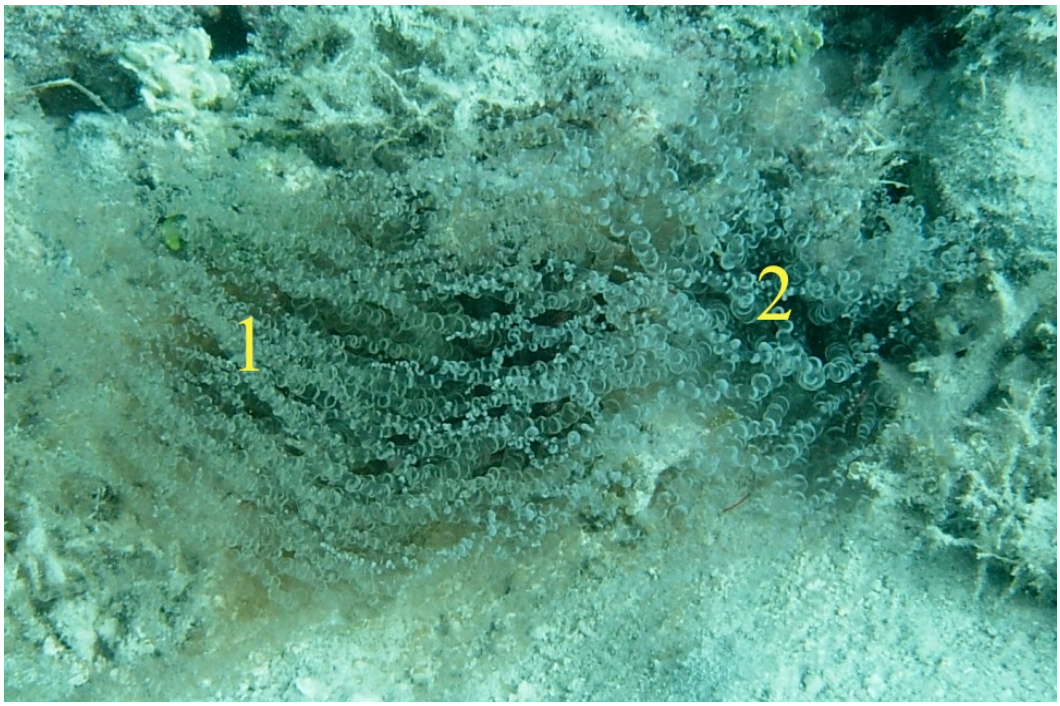
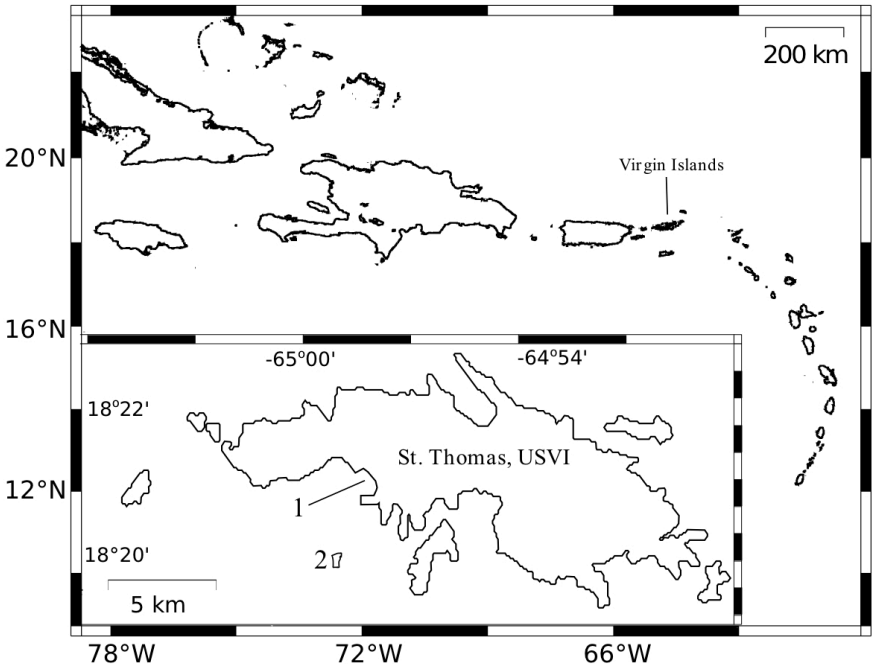


Figure 4.1. Representative images of aggregating *Bartholomea annulata* anemones at St. Thomas, USVI. (A) Anemones with exposed oral discs attached to the underside of a flipped over reef rock. (B) Two aggregating anemones (numbered 1 & 2) with slight variation in color and nematocyst spiraling.

A.



B.

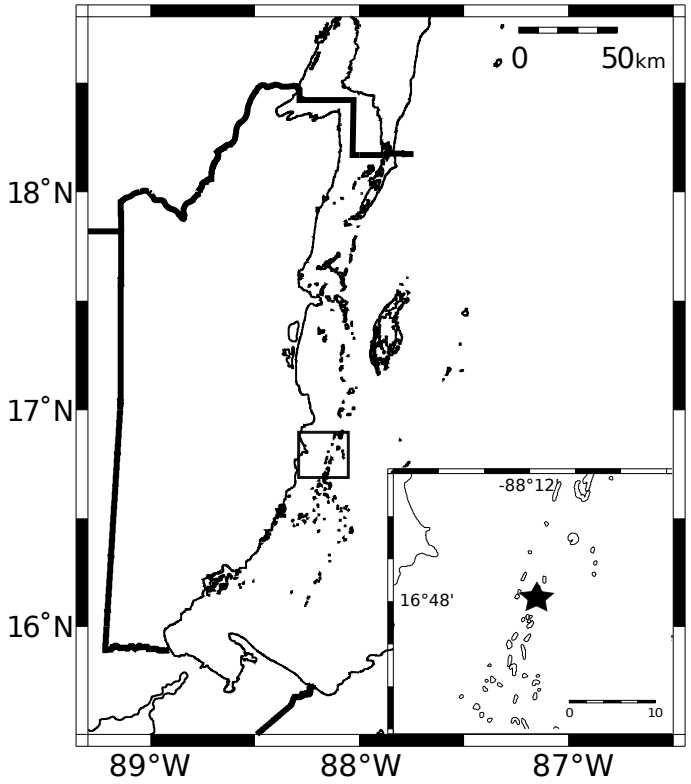


Figure 4.2. Maps of field survey and tentacle sampling locations in (A) St. Thomas, USVI (1 = Brewers Bay, BB; 2 = Flat Cay, FC), and (B) Belize (Star = Wee Wee Cay).

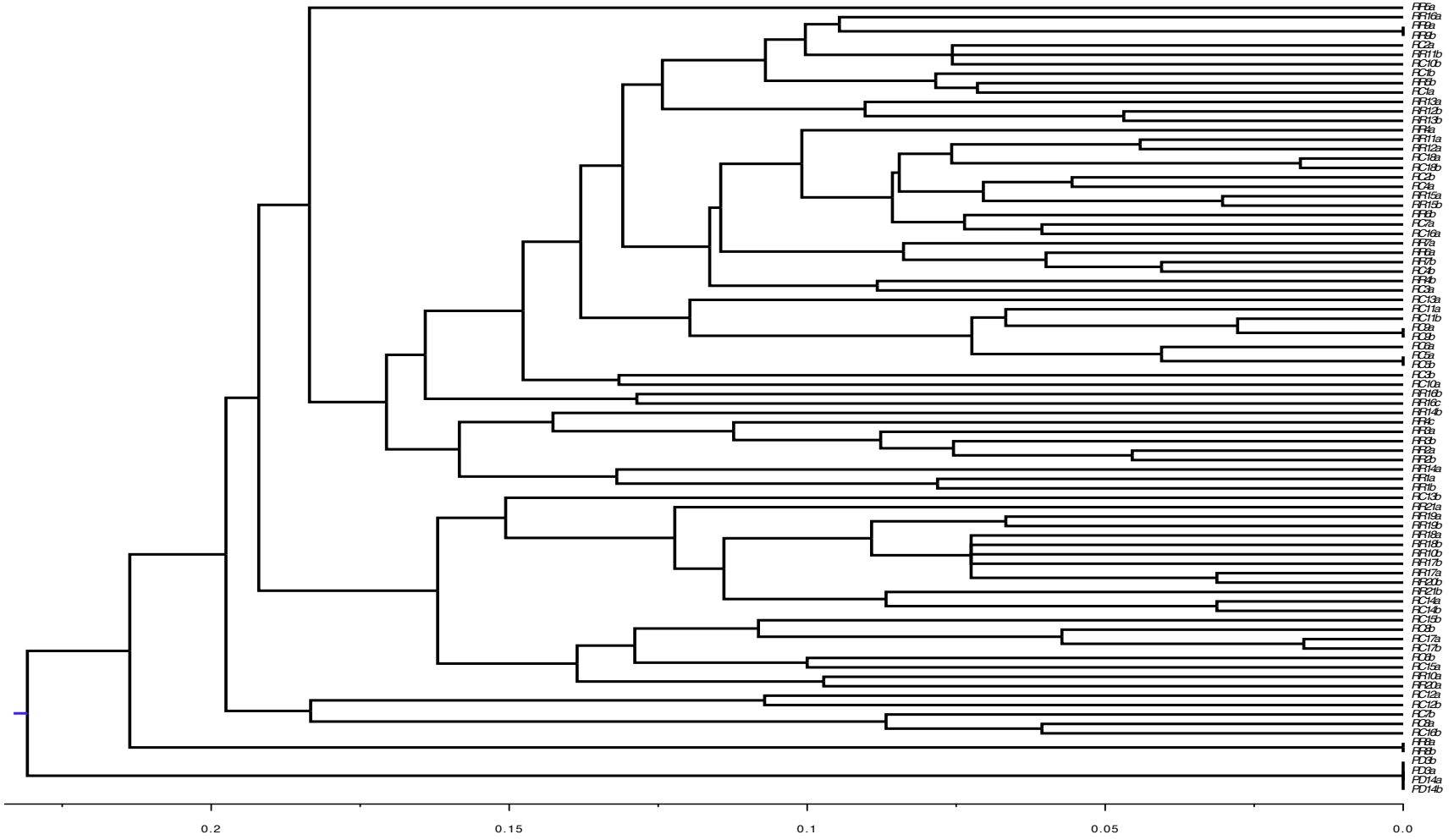
Table 4.1. ISSR primers with primer sequence, the number of scorable bands, and the number of polymorphic bands.

Primer	Sequence	Scorable bands	Polymorphic bands
901	$(GT)_6YR$	21	21
Becky	$(CA)_7YC$	17	13
DAT	$(GA)_7RG$	19	15

Table 4.2. *Bartholomea annulata* aggregation data from St. Thomas, USVI and Belize.

Study Site	*Reef*	*Total B. annulata surveyed*	*# of aggregations*	*# of B. annulata in aggregations*	*% of total population in aggregations*	*Aggregation abundance/m^2*
St. Thomas	RR	121	21	52	43%	$0.07/m^2$
	FC	102	6	15	15%	$0.005/m^2$
Belize	Patch reefs	128	11	26	20%	$0.04/m^2$
	Mangroves	61	10	23	38%	$0.1/m^2$

Figure 4.3. UPGMA Dendrogram of 43 *Bartholomea annulata* aggregations from populations at St. Thomas, USVI (RR and RC samples), and Belize (PD samples). Scale bar represents Jaccard's coefficient.



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