

**Environmental tolerance and reproduction of
Florida false corals *Ricordea florida* (Anthozoa: Corallimorpharia):
Implications for ornamental fisheries management**

by

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Abstract

In the aquarium trade, corallimorpharians are commonly known as false corals or mushroom corals, but little is known about the biology of most species. Some species of corallimorpharians are dominant competitors for space on marine benthos, and they have become increasingly popular organisms in the ornamental aquarium trade due to high demand among home aquarists, thus representing a high value fishery. In the Florida Keys, USA, the most common corallimorpharian is the Florida false coral *Ricordea florida*, which has experienced a sharp decline in abundance since ~ 2010. Major goals of this dissertation were to elucidate aspects of the biology and ecology of this species, in order to enhance scientific understanding about potential causes of the declining abundance, and to provide recommendations to support the management of a sustainable fishery on this species.

Under controlled laboratory conditions, I determined environmental tolerances of *R. florida* to solar irradiance and temperature. The experimental results indicate that individuals of this species thrive when exposed to levels of photosynthetically active radiation (PAR) that match those in shaded microhabitats on shallow coral reefs (~ 40-60 $\mu\text{E m}^{-2} \text{s}^{-1}$). Field observations revealed that polyps of *R. florida* may depend on their proximity to large upright benthic organisms such as gorgonians and sea fans, to provide adequately shaded habitats on shallow reefs in the Florida Keys. Polyps of *R. florida* exhibited various signs of physiological stress when exposed to relatively high PAR levels corresponding to those in unshaded shallow reef habitats. Individuals also thrived at fairly wide range of seawater temperatures (~ 22 -31°C), with more extreme temperatures (both cooler and warmer) causing rapid decline in polyp physiological condition including bleaching and followed in some cases by death. Field

observations showed that the upper bound of this range (31°C) was exceeded during the hottest months of both study years (2017 and 2018), and that the lower bound of this range (22°C) was exceeded during a cold event in 2010 that killed many Florida Keys reef organisms. I conclude that increasingly wide seawater temperature fluctuations likely have contributed, and will continue to contribute, to the decline of *R. florida* populations throughout the shallow Florida Keys.

Field monitoring indicated that populations of *R. florida* have declined significantly since 2010 on some shallow reefs in the middle Florida Keys, but that populations at some sites remained stable during the present study (June 2017 – June 2019). A major physical disturbance in the form of Hurricane Irma (category 4) during September 2017 caused a large decline in monitored populations, which then required ~ 1 year to recover in abundance.

Analysis of reproductive processes in *R. florida* showed that polyps of this species reproduce both sexually and asexually. The sexes are separate, with both male and female polyps spawning gametes for external fertilization in the water column, annually during late June to early July, shortly after the summer solstice, in a similar sexual reproductive pattern to that known for some other corallimorpharian species. Females are larger than males, indicating that they potentially exhibit hermaphroditic protandry. Polyps also are able to replicate clonally to produce aggregations via 3 distinct mechanisms: longitudinal fission, inverse budding, and pedal laceration. During a long-term removal experiment in the middle Florida Keys, aggregations of polyps clonally replicated to complete recover in abundance less than 1 year after the partial removal of 33 to 66% of polyps in each aggregation, such as could be caused by a marine life collector or a natural predator. However, they did not recover in polyp abundance when entire aggregations were removed from the reef, indicating that replenishment solely via recruitment by

sexually-produced larvae or planktonic inverse buds is not rapid enough to replenish entire aggregations over the time scale examined here. It is concluded that a sustainable fishery on this species is potentially possible in the Florida Keys, but only if best management practices are observed, including limitation of collection to $\leq \sim 66\%$ of polyps in each aggregation. Populations of *R. florida* are expected to face increasing threats from other anthropogenic stressors including extreme temperature fluctuations as part of global climate change.

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List of Abbreviations

Aggr.	Aggregation
AU	Auburn University
BCI	body color index
BV	body volume
CCA	chlorophyll concentration per algal cell
CRS	clonal replication stage
DM	dry mass
ER	ecological reserve
MCA	microalgal cell abundance
MTI	mitotic index
NP	Nicholas Parr
ODA	oral disk area
PAR	photosynthetically active radiation
PDA	pedal disk area
ROP	ratio of oral to pedal disk area
SPA	special protection area
SUA	special use area (research only)
UF	University of Florida
WM	wet mass

Chapter 1

Ecology and biology of tropical corallimorpharians: A review

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1 Introduction

Corallimorpharians are commonly known in the aquarium trade as false corals or mushroom corals, and sometimes are misidentified as sea anemones. They are cnidarians belonging to the Class Anthozoa and Subclass Hexacorallia, and comprise an order on the same taxonomic level as other orders in this subclass, such as the scleractinian corals, black corals, actinian sea anemones, ceriantharians, and zooanthids. Corallimorpharians occur in a wide variety of marine environments around the world, from the intertidal zone to the deep sea, and from the tropics to the poles (Carlgren 1949).

There are two distinct types of body morphology in corallimorpharians (Kuguru et al. 2007). Most species live in shallow, tropical marine waters, and possess flat, wide oral discs, many short and often branched tentacles, a short column tapered to a narrow pedal disk, and contain endosymbiotic microalgae (Family Symbiodiniaceae) inside their endodermal cells. However, other types of corallimorpharians inhabit temperate seas, often at considerable depth, and possess oral disks that are the same diameter as their pedal disks, relatively tall columns that also are nearly as wide as their oral discs, and have relatively few, long, unbranching tentacles with no Symbiodiniaceae microalgae (Fautin & Lowenstein 1994).

Although many species of corallimorpharians are not well-studied, these organisms may constitute substantial biomass in some marine ecosystems (Chadwick 1991, Work et al. 2008). Some species also are important in the ornamental fisheries trade (Cato & Brown 2008). Better understanding of their ecophysiology and population ecology is needed to provide a more scientific basis for the conservation management of sustainable fisheries on corallimorpharians, and to improve knowledge about their importance in marine ecosystems. This review describes

the published information available about the biology of corallimorpharians, including their community-level interactions, and also highlights areas for future study.

2 Morphology and physiology

Corallimorpharians are similar morphologically to scleractinians corals, except for the lack of a hard calcium carbonate skeleton. Instead, they have only a hydrostatic skeleton that supports their soft tissues. Their bodies are coated with a lubricous mucus, and depending on the development of their gelatinous mesogleal layer, their body structure can be soft to very rigid. The column is smooth and devoid of specialized organs (den Hartog 1980). Corallimorpharians have a single gut cavity lined with multiples of 6 pairs of mesenteries, the inner edges of which possess unilobed mesenterial filaments , similar to scleractinian corals but in contrast to actinian sea anemones which have tri-lobed mesenterial filaments (Daly et al. 2003). In shallow-water species, the basal pedal disk is irregular in outline and is attached to hard substrate, while deep-sea species often occur on soft substrate and so are not attached firmly to the substrate (Daly et al. 2003).

Corallimorpharians' cnidocysts (stinging organelles; also termed nematocysts) are similar to those of scleractinian corals, made up of spirocysts, homotrichs, spirulae, penicilli D and pencilli E (den Hartog 1980). The sizes of their cnidocysts differ from those of actinian sea anemones, although this variation among orders is not uncommon within the Subclass Hexacorallia (Garese et al. 2016). Unfortunately, the cnidocysts of only a few species have been examined in detail (Miles 1991, Langmead & Chadwick-Furman 1999, Acuña & Griffiths 2004, Garese et al. 2016).

Additionally, the information provided by den Hartog (1980), which has been cited in all works thus far studying the cnidocysts of corallimorpharians, recently has been shown to be incorrect about the absence of spirocysts in species belonging to the Family Discosomatidae (Miles 1991). This discrepancy highlights the need for more studies, including confirmatory studies, about the cnidom composition of corallimorpharians, especially since this can be an important taxonomic trait.

Spirocysts are the most common cnidocysts in polyps of the temperate corallimorpharian *Corynactis carnea*, and are the only cnidocysts that occur in the tentacles. The column in this species contains microbasic p-mastigophores, two types of microbasic b-mastigophores, and holotrichs, while mesenterial filaments have microbasic p-mastigophores and two types of holotrichs, and the pharynx features holotrichs and microbasic b-mastigophores (Acuña & Griffiths 2004, Garese et al. 2016).

In the congeneric tropical species *Discosoma carlgreni* and *D. sanctithomae*, spirocysts, b-rhabdoids, p-rhabdoids D, holotrichs I, and holotrichs II are present. Spirocysts are sparse but typical in the marginal tentacles along with b-rhabdoids, p-rhabdoids D, holotrichs I, and holotrichs II. In the tentacles and column, b-rhabdoids, p-rhabdoids D, small holotrichs I are present, with holotrichs II being present on the column. The stomodaeum and mesenterial filaments possess only p-rhabdoids D and holotrichs I (Pinto & Belem 2000). A study that examined the changes in cnidocyst types during competition with other cnidarians, concluded that the marginal tentacles of Red Sea corallimorpharians *Rhodactis rhodostoma* change their dominant nematocysts from microbasic-b-mastigophores to Type 1 holotrichs over 4 weeks, when exposed to contact with competing stony corals (Langmead & Chadwick-Furman 1999).

The few species which have been examined therefore contain a wide variety of cnidocysts, and the composition of cnidocysts may change during competition or possibly for other reasons.

It is generally accepted that the nervous system of corallimorpharians is similar to that of the closely-related and better studied scleractinian corals, in that they likely possess a diffuse nerve net, however there are no publications available on this subject. This assumption of a complex nerve net capable of directed responses, is supported by some of the observed behavioral responses in this order. The oral disc of corallimorpharians usually can be withdrawn, with the tentacles are fully retractile in many species (den Hartog 1980). This type of contraction can occur in response to feeding or changes in irradiance (Fautin & Hamner 1980, Elliott & Cook 1989, see Chapter 2), and is chemically controlled during feeding (Elliott & Cook 1989).

The internal mesenterial filaments that line the gastrovascular cavity of the polyp likewise can be extruded as a directional response to specific stimuli, such as contact with certain types of competitors, as known for *Corynactis californica* and *Rhodactis rhodostoma* (Chadwick 1987, Langmead & Chadwick-Furman 1999). This extrusion response is different from that in response to food items, in that filaments extruded onto food are released from the polyp mouth, while those in response to competitors exit the polyp via small holes in the column or tentacles, and directionally extend toward certain competing cnidarians to externally kill and digest them (Chadwick 1987).

Additionally, directed locomotion during clonal replication, while very limited (< 10 mm per month), has been recorded. Corallimorpharians have much less developed basilar muscles on the pedal disk, compared to the more mobile actinian sea anemones, which likely accounts for this difference in locomotory speed (Chadwick & Adams 1991, N. Parr pers. obs.).

Another interesting behavior has been recorded by Bos et al. (2011), in which polyps of *Paracorynactis hoplites* feed on echinoderms by stretching their tentacles toward the prey. In terms of neural development, the most interesting part of this feeding is that some polyps extend tentacles towards the prey even before making initial tissue contact, suggesting some sort of nervous control stimulated by possible water-borne chemical cues from the prey, versus random chance tentacle extension (Bos et al. 2011).

A remarkable publication on 3 species of large tropical corallimorpharians has described their ability to rapidly contract the edges of their oral discs, thus capturing and enveloping small fishes that stray onto the oral discs (Fautin & Hamner 1980a, see also Chapter 1, Section 5 below). Caribbean corallimorpharians *R. sanctithomae* also exhibit complex feeding behavior, with nocturnal expansion of the oral disk to envelope and capture prey (Elliott & Cook 1989, see Chapter 1, Section 5).

Thus, although some publications have described interesting behaviors of corallimorpharians in relation to competition, feeding, and locomotion, there is a void in publications on the structure of their nervous system, as well as about other aspects of their anatomy and physiology, including aging and lifespan.

3 Evolution and phylogeny

Corallimorpharians lack the hard calcium carbonate skeleton of scleractinian corals and therefore are not reef structure-building. They are often confused with actinarians (sea anemones), however phylogenetic analyses show that they are actually more closely related to scleractinian corals, having undergone loss of a hard skeleton sometime during the late- to mid-

Cretaceous period, about 110-70 million years ago, just before the K-T boundary 65 million years ago when many marine species including dinosaurs became extinct (Fautin & Lowenstein 1994, Cappola & Fautin 2000, Medina et al. 2006). Corallimorpharians survived that boundary as a relatively new marine group, and have diversified since then. While corallimorpharians and actinarians share superficial characteristics such as lacking a hard skeleton and having similar tentacle arrangement, corallimorpharians have many more similarities with scleractinians, such as cnidae distribution, unilobed mesenterial filaments, lack of basilar muscles, and in most corallimorpharians, lack of a marginal sphincter muscle (den Hartog 1980). Genetic analysis has found substitutions in only two genes between scleractinians and corallimorpharians, suggesting that the similarities between corallimorpharians and sea anemones are parallelisms (Daly et al. 2003). It is likely that these genes function somehow in calcium carbonate skeletal production, since that is the only major character difference between scleractinians and corallimorpharians.

The divergence between these latter 2 groups is believed to have occurred due to slowly rising seawater temperature, relatively low pH (i.e. acidification of the oceans), and high Mg/Ca ratios in the oceans as a result of global climate change during the Cretaceous Period (110-132 million years ago). These conditions are remarkably similar to those developing in the world's oceans today due to anthropogenic climate change. During the Cretaceous, these environmental conditions damaged the abilities of scleractinian corals to fix calcium and build their skeletons, resulting in the mass extinction of many species, but may have actually increased the fitness of soft-bodied corallimorpharians relative to calcareous corals (Kuguru et al. 2008). This process appears to be occurring again during the current era of anthropogenic climate change, in that corallimorpharians are gaining a competitive advantage on some shallow reefs, after the loss of

Symbiodiniaceae microalgae has caused a “bleaching” event leading to mortality of the more vulnerable scleractinian corals (Kuguru et al. 2007).

In terms of diversity within the Order Corallimorpharia, there are four families: Corallimorphidae (Fig. 1-1a; Hertwig 1882), Discosomatidae (Fig. 1-1b; Verrill 1869), Ricordeidae (Fig. 1-1c; Watzl 1922), and Sideractiidae (Danielssen 1890). Within those four families are 10 valid genera and 47 validly identified species (Fautin 2016). Additionally, three other families had been identified previously but are no longer considered valid: Actinodiscidae (Carlgren 1949), Discosomatidae (de Fonbressin & Michelotti 1864; accepted as Discosomidae, Verrill 1869), and Corynactinidae (Andres 1883; now within Corallimorphidae). For a comprehensive recent catalog of species in the order Corallimorpharia, see Fautin (2016).

4 Biogeography and environmental tolerances

Corallimorpharians occur as either solitary polyps or in aggregations of polyps that may form extensive carpets on temperate and tropical reefs (Chadwick-Furman & Spiegel 2000, Torres-Pratts et al. 2011). They have been found in a wide variety of environments from polar to tropical, and from the intertidal zone to 5000 m below sea surface in the deep sea (reviewed in Fautin et al. 2009). Tropical shallow-water species usually host symbiotic microalgal Symbiodiniaceae, while the colder or deeper species are strictly heterotrophic (Carlgren 1949, den Hartog 1980, Fautin et al. 2002).

Given their wide range of marine habitats, corallimorpharians as an order are able to tolerate an extreme range of seawater temperatures. However, individual species have demonstrated high specificity to habitats with narrow temperature ranges. Among the tropical

Symbiodiniaceae-hosting species and much like the tropical scleractinian corals, bleaching is a common response of corallimorpharians to thermal stress (Fitt et al. 2001, Kuguru et al. 2016). For example, an increase in temperature of only 6°C above ambient, or 4°C above local summer maximums (to 31°C) causes the Red Sea reef species *R. rhodostoma* to bleach after only 2 weeks, but these effects are reversible when the temperature is lowered back to ambient (Kuguru et al. 2007). In contrast, prolonged exposure to temperatures 5°C above their optimum temperature appear to be fatal for some scleractinian corals (Coles & Jokiel 1978).

Both the Red Sea corallimorpharians *R. rhodostoma* and *D. unguja* demonstrate some resistance to bleaching during temperature stress, and this resistance is higher than would be expected for scleractinian corals (Kuguru et al. 2008). This finding could have conservation implications, if Symbiodiniaceae microalgae find refuge within the protective tissues of some tropical corallimorpharians and then are able to repopulate the tissues of nearby bleached scleractinian corals after the acute stress is removed.

In calcifying corals, it is expected that lowering pH (i.e. ocean acidification) negatively affects their health due to their consequently inhibited ability to create a CaCO₃ skeleton (Anthony et al. 2008). Similarly, even though corallimorpharians don't calcify, they also exhibit negative effects from lowered pH, and similar to corals that effect is compounded at high temperatures (Kuguru et al. 2010, 2016). Additionally, Kuguru et al. (2007) found a temperature – light interaction, in that polyps of *R. rhodostoma* exhibit less stress from extremely high light levels when temperature remains low.

Two studies have documented an increase in the abundance of corallimorpharians during periods of dissolved nutrient enhancement in the surrounding seawater (eutrophication). Nutrient-laden terrestrial runoff along the coastline of Tanzania appears to have increased populations of 5

species of corallimorpharians (*Rhodactis rhodostoma*, *R. mussoides*, *Ricordea yuma*, *Actinodiscus unguja* and *A. nummiforme*), giving them a competitive advantage over the previously dominant scleractinian corals on some reefs in that country (Muhando et al. 2002). Likewise, at Palmyra Atoll in the central Pacific Ocean, which is an open ocean area with historically low levels of dissolved iron, a shipwreck that introduced an influx of iron caused a phase shift from a diverse reef habitat to one completely dominated by polyps of *Rhodactis howesii* (Work et al. 2008). Finally, on coral reefs in the northern Red Sea, a reef flat exposed to nutrient pollution and other disturbances from nearby hotels along the shoreline experienced a phase shift to dominance by corallimorpharians *R. rhodostoma* which killed surrounding corals (Chadwick et al. 2000).

For Symbiodiniaceae-hosting corallimorpharians, light has been documented as a major factor affecting their distributional patterns (Kuguru et al. 2007, 2008). Some species when transplanted can acclimatize to irradiance levels that differ from those in their original habitats, and clades of their symbiotic Symbiodiniaceae can alter their relative abundances within the host (Kuguru et al. 2007, 2010). Corallimorpharians also may have a competitive edge over corals under conditions of decreased irradiance (Muhando et al. 2002). Interestingly though, Symbiodiniaceae traits might not be the only factor driving the intolerance of some corallimorpharians to high irradiance. Corallimorpharians also compete directly with macroalgae that thrive in high light, as demonstrated for the cold-water species *Corynactus californica* which may occur at high abundance in shaded kelp forests, but not in unshaded shallow environments with dense benthic macroalgae in the northeastern Pacific (reviewed in Chadwick & Morrow 2011).

Most corallimorpharians are strong competitors for space with other cnidarians, and also with some other benthic invertebrates (Chadwick 1991). Given a favorable change in environmental conditions, they can often form large aggregations and take over area of hard substrate (Chadwick 1991, Chadwick-Furman & Spiegel 2000, Work et al. 2008, Carter 2014, Work et al. 2018). Dominance during competition has not been considered as a major driver of their larger biogeographical patterns (Hennessey & Sammarco 2014), but needs to be examined further because these organisms are becoming major space occupiers in an expanding variety of reef systems.

5 Nutrition

The feeding tentacles of corallimorpharians contain cnidocysts (as discussed above in Section 2), which function to capture prey by everting a thread that then wraps around the prey or penetrates its body, immobilizing or killing the prey, after which folding of the oral disc and/or retraction of the tentacles brings the prey into the mouth (Sebens 1976, Fautin & Hamner 1980). A notable exception to this feeding method occurs in 3 Australian species of the Family Actinodiscidae, in which the large polyps lack ectodermal cnidocysts on their discal tentacles, and instead consume fish and other large organisms by entrapping them in their oral discs and pushing them through their mouths, where cnidocysts kill and digest the fish prey (Fautin & Hamner 1980). A similar but less spectacular feeding mechanism is exhibited by relatively small polyps of the Caribbean species *Discosoma sanctithomae*, which at night fold their oral discs to envelop crustaceans, polychaetes, and small fishes (Elliott & Cook 1989).

Another notable exception to reliance on waiting for prey to come into contact with cnidocyst-bearing tentacles, is in the large species *Paracorynactis hoplites* (polyp diameter up to 170 mm), which feeds on echinoderms. Polyps of this species partially digest echinoderms up to twice the polyp size, including crown-of-thorns sea stars (*Acanthaster planci*), by extending a tentacle toward the prey (possibly detected through water-borne chemical cues) and then adhering to it; the polyp subsequently directs other tentacles to adhere to the prey before stretching toward it and partially engulfing the prey. For larger echinoderms, only partial digestion has been recorded, with some prey escaping. However, smaller echinoderms are completely engulfed and digested (Bos et al. 2011). Additional studies into the feeding mechanisms of corallimorpharians are needed to potentially reveal further exceptions to the standard anthozoan feeding mode of waiting for prey to contact cnidocyst-laden tentacles.

In addition to heterotrophic nutrition via prey capture, most shallow tropical corallimorpharians engage in autotrophy via nutrients received from their endosymbiotic microalgae (Family Symbiodiniaceae). In species which possess microalgae, this is an obligate relationship, in that the Symbiodiniaceae and their host cannot survive for long without each other (Douglas 2010). In at least one species examined, the corallimorpharians' gametes do not contain Symbiodiniaceae, so they must be acquired horizontally from the environment rather than vertically from the mother polyp (Kuguru et al. 2007). Corallimorpharians acquire important additional nutrition from their Symbiodiniaceae, and in turn the Symbiodiniaceae receive protection due to being incorporated into the corallimorpharians' tissue (Douglas 2010).

Corallimorpharians that host Symbiodiniaceae demonstrate a variety of adaptations that enhance the protection of their endosymbionts. One of the main dangers to Symbiodiniaceae is the extremely high solar irradiance that they receive in shallow tropical waters. At least one

species of corallimorpharian protects these algae from harmful UV radiation by thickening the host epidermis and moving the algal cells away from the mesoglea when under high light. This mechanism is not universal among all Symbiodiniaceae-hosting organisms, and it has not been observed in scleractinian corals. However, it has been observed in some actinian sea anemones in response to heat (Kuguru et al. 2007). Some corallimorpharians further protect their Symbiodiniaceae by producing low-cost fluorescent proteins and UV light (295 – 400 nm) absorbing compounds, most likely mycosporine-like amino acids. The concentration of these compounds increases as the intensity of UV light is experimentally increased (Kuguru et al. 2008, Krishna & Ingole 2009). In addition to protecting their Symbiodiniaceae from high irradiance, corallimorpharians provide direct protection from predation by culturing the Symbiodiniaceae inside of their tissues which contain cnidocysts and/or anti-predatory chemical compounds (Hines & Pawlik 2012).

In exchange for this protection from both high intensity light and predation, the Symbiodiniaceae provide their host corallimorpharians with photosynthates, organic compounds formed through photosynthesis. In the better-studied scleractinian corals, the main components transferred are glycerol and water-soluble compounds like glucose. These photosynthates account for 50-95% of the coral's energy budget, however this has not been directly measured in corallimorpharians (Baker 2011). The dependence of corallimorpharians on their endosymbionts can be inferred, because their ability to occupy a particular depth niche is directly dependent on the types of Symbiodiniaceae they host, due to the specific abilities of the algae to photosynthesize at certain light levels (Kuguru et al. 2008).

A few adaptations of the Symbiodiniaceae themselves allow them to respond to variation in light intensity, in addition to the host corallimorpharians' responses (Kuguru et al. 2007). If

the light intensity decreases (such as when polyps occur in relatively deep habitats on reef slopes), chlorophyll concentrations within the Symbiodiniaceae cells increase, as well as the abundances of Symbiodiniaceae cells within the host tissue (Kuguru et al. 2008). Additionally, the types of Symbiodiniaceae clades that occur inside polyps vary with depth (Finney et al. 2010). The entire assemblage of Symbiodiniaceae clades can change within corallimorpharians as an adaptation to environmental change in light intensity. This adaptation allows the host to rapidly acclimate to environmental stress caused by disturbances, and they can then change back to the original microalgal types if the original conditions return (Kuguru et al. 2008). Adaptations in the symbiosis between corallimorpharians and Symbiodiniaceae could have implications for their ability to respond to climate change. For example, Symbiodiniaceae are adapted to a certain set of environmental conditions, and corallimorpharians can host only certain clades of Symbiodiniaceae. In the Caribbean, 3 of the most common corallimorpharians all associate with Clade C *Cladocopium* (LaJeunesse 2002, Finney et al. 2010), so their ability to adapt to climate change may be limited by their narrow range of associated microalgal types.

6 Asexual and sexual reproduction

All species of corallimorpharians examined to date can reproduce both sexually and asexually (e.g. clonally; Chadwick-Furman et al. 2000, Kuguru et al. 2007). Corallimorpharians engage in asexual replication via various mechanisms that include polyp fission, pedal laceration, and inverse budding (Chen et al. 1995a, Chadwick-Furman & Spiegel 2000, Edmunds 2007). Fission occurs in many types of anthozoans; in corallimorpharians, it comprises longitudinal fission in which two or more near-equal sized daughter polyps are produced from the 2- or 3-way

splitting of a single parent polyp (Chadwick & Adams 1991). Pedal laceration also occurs in both actinian sea anemones and corallimorpharians, when a small bud forms as a tissue outgrowth near the pedal disc. The pedal lacerate then locomotes away from the parent polyp, the tissue connecting the two is severed, and a relatively small daughter polyp is produced (Chadwick-Furman & Spiegel 2000). Inverse budding is a mechanism of asexual replication that is unique to corallimorpharians; in this mode, a peripheral area of the pedal disc lifts up from the substrate, and folds in toward the oral disc. The edge of the oral disc then folds around the uplifted pedal disc area, to create a rounded bud attached to the top of the oral disc, in which the pedal disc portion faces upwards and the oral disc, tentacles, and even sometimes a small mouth on the bud are oriented downwards (inverted) facing the oral disk of the parent polyp. The tissue area that connects the bud to the parent narrows to form a stalk, and then eventually may constrict to sever the stalk and release a detached bud which floats away in the water column before eventually settling and attaching to hard substrate at a potentially distant reef area (Chen et al. 1995a). Inverse budding therefore produces planktonic buds with the potential for dispersal of polyps, and may contribute to the rapid spread of corallimorpharians on some reefs (Chadwick-Furman & Spiegel 2000, Work et al. 2008).

Rates of asexual reproduction vary widely among individuals both within and between species, with some individuals not cloning at all over several months or years, while other polyps of corallimorpharians are able to produce up to ~ 100 clonally-identical daughter polyps within a single year (Chadwick & Adams 1991, Chen et al. 1995a, Chadwick-Furman & Spiegel 2000). Both of the shallow tropical species that have been studied, *Rhodactis indosinensis* and *R. rhodostoma*, perform all three types of asexual replication, and favor longitudinal fission as the most common mode of replication (Chen et al. 1995a, Chadwick-Furman & Spiegel 2000). The

only temperate species studied, *Corynactis californica*, performs both fission and pedal laceration, at more rapid rates than known for tropical species (Chadwick & Adams 1991). Research is needed to identify mechanisms and rates of asexual reproduction in a wider variety of corallimorpharians, including deep-water species.

The sexual reproduction of corallimorpharians has been investigated in only 3 species from the Indo-Pacific region. Two studies have examined the timing of sexual reproduction; one found that in the temperate non-symbiotic species *Corynactis californica* off the coast of California, oocytes developed for 5 months and spermatophores developed for 1 month, both being released synchronously in early December for external fertilization when zooplankton concentrations were increasing in the surrounding waters (Holts & Beauchamp 1993). In contrast, in the tropical symbiotic corallimorpharian *Rhodactis rhodostoma*, spawning of sexually-produced gametes occurs during late June to early July in the northern Red Sea, when the level of solar irradiance and therefore photosynthesis rates reach their annual maxima. Eggs develop similarly to those in *C. californica*, however sperm develop earlier (~ 6 months before spawning) and remained within polyps until their release (Chadwick-Furman et al. 2000). Similarly, polyps of *R. indosinensis* in Taiwan release gametes during May and June (Chen et al. 1995a). Broadcast spawning in all 3 species examined thus far may result in the long-distance dispersal of many sexual propagules that can colonize new space rapidly.

In all three studies, polyps were found to be gendered, with larger females located in the centers of polyp aggregations, and smaller males on the fringes of each aggregation, suggesting that the polyps could be protandric (Holts & Beauchamp 1993, Chen et al. 1995b, Chadwick-Furman et al. 2000). This idea was further supported by the observation that when small immature polyps of *Rhodactis indosinensis* were experimentally transplanted into the center of

aggregations, they all developed initially into males and then continued to grow until eventually they transformed into females (Chen et al. 1995a). The segregation of polyps by gender and size within aggregations, with large egg-bearing polyps limited to the aggregation centers, is similar to the pattern known for some actinian sea anemones that also form large aggregations of polyps (Francis 1973). Sexual reproduction, including the timing of spawning or the extent of segregation among males and females within aggregations, has not previously been studied in Caribbean corallimorpharians.

7 Competitive interactions

The most well-understood types of ecological interactions involving corallimorpharians are with other benthic space-occupiers on hard substrata, in which the corallimorpharians may engage in interference competition via specialized mechanisms of aggression to monopolize the limited available space for attachment (Chadwick 1987, Langmead & Chadwick-Furman 1999). Competition on coral reefs for limited substratum space can be intense, and many anthozoans have developed competitive mechanisms (reviewed in Chadwick & Morrow 2011). Some species of corallimorpharian in all habitats examined thus far, including the Red Sea (Langmead & Chadwick-Furman 1999), Indian Ocean (Kuguru et al. 2004), Caribbean Sea (Sebens 1976, Miles 1991), and northeastern Pacific Ocean (Chadwick 1987, Chadwick & Adams 1991), appear to be aggressive competitors. Certain species develop marginal tentacles filled with large nematocysts to attack neighboring cnidarians, while others may extrude mesenterial filaments to externally digest their neighbors (Chadwick 1987, Miles 1991, Langmead & Chadwick-Furman 1999). Competition with Caribbean scleractinian corals has been demonstrated by Sebens

(1976), in which polyps of the corallimorpharian *Paradiscosoma neglecta* kill all hard corals that are placed into contact with them, as well engaging in competition with other species of corallimorpharians. While he also observed *Ricordea florida* growing on recently-damaged corals, it was not clear whether the *R. florida* polyps had directly killed the coral tissue, or had opportunistically grown over the bare coral skeletons.

Corallimorpharians also engage in exploitation competition, in which they form thick carpets of aggregated polyps on reefs, inhibiting the growth of other species and outcompeting all other sessile organisms (Langmead & Chadwick-Furman 1999). These aggregations are the result of asexual reproduction via longitudinal fission, pedal laceration (den Hartog 1980), and possibly inverse budding (Chadwick-Furman & Spiegel 2000). The formation of large dense aggregations allows corallimorpharians to prevent the recruitment of other benthic species, and to create an effective monoculture. These carpets may be especially common on shallow tropical coral reefs, and consist of a single species of corallimorpharian that covers an area of several square meters or more, with no gaps between adjacent individuals (den Hartog 1980, Chadwick-Furman & Spiegel 2000, Work et al. 2008).

Multiple species of corallimorpharians, both tropical and temperate, have been demonstrated to develop inducible aggressive structures for space competition (Chadwick 1987, Miles 1991). In *Corynactis californica*, prolonged interspecific contact leads to polyps extruding mesenterial filaments onto neighboring organisms and killing both actinian anemones and scleractinian corals. These mesenterial filaments are extruded out of the mouth and through holes in the column or tentacles, and the feeding tentacles also adhere to neighboring organisms (Chadwick 1987). In *Discosoma sanctithomae*, both modified marginal tentacles and mesenterial filaments are used to attack neighboring scleractinians. In this species, the highly competitive

scleractinian *Meandrina meandrites* initially causes damage to the corallimorpharian, but after ~ 1-5 months, polyps of *D. sanctithomae* are able to fully recover and ultimately to damage the coral. Another less aggressive coral, *Agaricia agaricites*, is immediately injured when placed next to *D. sanctithomae* (Miles 1991).

The competitive edge of some corallimorpharians over scleractinian corals may be enhanced in areas exposed to localized anthropogenic influences, and they often are found growing on dead corals (den Hartog 1980). In the Indian Ocean, corallimorpharians outcompete scleractinian corals especially in areas close to human settlements that have high water turbidity and dissolved nutrient levels (Kuguru et al. 2004). At Palmyra Atoll in the Pacific Ocean, an observational study found that *Rhodactis howesii* overgrew a coral reef only 4 years after a shipwreck introduced high concentrations of iron, a common limiting nutrient in marine ecosystems (Work et al. 2008). However, dominance by *R. howesii* was not reversed after removal of the shipwreck (Work et al. 2018). Disturbance by predators (especially sea turtles *Eretmochelys imbricata*) may reduce the abundance of some Caribbean corallimorpharians and therefore allow scleractinian corals to maintain a competitive advantage in certain reef areas (León & Bjorndal 2002). Given the limited number of corallimorpharian species in which competition has been studied (Miles 1991, Torres-Pratts et al. 2011), more research is needed on the dynamics of competition in this important group.

8 Predation

Corallimorpharians are not a common prey item, potentially due to their low protein content compared to other foods in the marine environment, and/or their protective cnidocysts

and chemical defenses (Hines & Pawlik 2012). Hawksbill Turtles *Eretmochelys imbricata* are among their few known predators; these turtles not only consume corallimorpharians, but in the Caribbean have high selectivity for consumption of *Ricordea florida* (León & Bjorndal 2002). Sea turtles also consume reef corallimorpharians in the Indo-Pacific region (Tkachenko 2014), and are one of the few known predators of actinian sea anemones on coral reefs in the Red Sea (C. Tkachenko, pers. comm.). Anemonefishes actively defend their hosts from sea turtles, in that they swim up to attack turtles as the turtles swim over coral reef areas near host sea anemones (Huebner et al. 2012; L. Huebner, pers. comm.).

Sea turtles such as *E. imbricata* appear to search for and consume some corallimorpharians selectively. Originally, *R. florida* was thought to be a food of opportunity for these turtles, being reported in high abundance at sites where it was part of *E. imbricata*'s diet (León & Bjorndal 2002). However, even where *R. florida* are rare, individuals of *E. imbricata* have shown selectivity, with this corallimorpharian still constituting a significant portion of the turtle's diet (Rincon-Diaz et al. 2011). Populations of *E. imbricata* however are not higher in areas of high *R. florida* concentration, suggesting that this food item does not affect *E. imbricata*'s habitat choice (Rincon-Diaz et al. 2011).

One reason for *E. imbricata*'s selective consumption of *R. florida* could be the mucous produced by the corallimorpharians (León & Bjorndal 2002). *E. imbricata* also are spongivores and need to protect their alimentary canal from the needle-like spicules located within sponges. Consuming cnidarians that produce this mucous, including corallimorpharians, could provide the turtles with this necessary protection to their digestive tracts. Indeed, *E. imbricata*'s diet in the Red Sea, Indian, and Pacific Oceans includes such cnidarians (Brandis et al. 2015). If this important food source were removed from the turtles' diet by overfishing or habitat destruction,

it is possible that this could have a negative effect on their already stressed numbers. Further evidence of this dietary selection is fluorescence that has recently been recorded from the head, flippers, and carapace of *E. imbricata*, likely the result of their consumption of the slowly decaying (~20 days) fluorescent proteins produced by the cnidarians (Krishna & Ingole 2009, Gruber & Sparks 2015).

In addition to predation by *E. imbricata*, consumption of corallimorpharians by corallivorous sea snails also has been documented in the Red Sea, where the gastropods *Drupella* sp. may prey on polyps of *Rhodactis rhodostoma* (Langmead & Chadwick-Furman 1999). This demonstrates that generalist corallivores could also be predators of corallimorpharians, but this has not yet been documented in the Caribbean. Surprisingly, no fishes are known to consume corallimorpharians, even though some coral reef fishes, especially butterflyfishes (Family Chaetodontidae) are highly specialized predators on certain soft-bodied reef cnidarians, including actinian sea anemones (Porat & Chadwick-Furman 2004).

While all corallimorpharians contain cnidocysts with potent toxins, these are not necessarily their only mechanisms for defense from predation, and in many species the cnidocysts do not play an active role in predatory defense (Paul et al. 2006, Hines & Pawlik 2012). Hines and Pawlik (2012) obtained extracts from the tissues of a variety of cnidarians including corallimorpharians, and found that in many reef-inhabiting species, this extract served as a feeding repellent to fishes. Some cnidarian defensive chemicals outside of the cnidocysts include neurotoxins in anemones, antifouling chemicals in gorgonian corals, renillenoic acids (analogs to eicosapentaenoic acid and arachadonic acid and deterrents to generalist feeding fish) in sea pens, and toxic hydrocarbons, fatty esters, and sterols in cup corals (Paul et al. 2006). However, some corallimorpharians, including those not located on shallow reefs, do not appear

to contain strong defensive chemicals, possibly as a result of only limited predatory pressure in some marine environments (Hines & Pawlik 2012).

9 Ectosymbionts

The Caribbean corallimorpharians *Ricordea florida* and *Discosoma sanctithomae* have been observed occasionally to host Pederson cleaner shrimp *Ancylomenes petersoni* which perch on the host tentacles. When *Ricordea florida* was first described in 1860, it was noted that cleaner shrimp occasionally live on the oral disk (den Hartog 1980). This association, though rare, was confirmed in 1982 when a *Periclimenes yucatanicus* cleaner shrimp was observed to associate with a polyp of *Rhodactis sanctithomae* in the wild over a period of at least 19 months in the U.S. Virgin Islands (Williams and Williams 1982). In the controlled environment of an aquarium, Williams & Bunkley-Williams (2000) repeatedly observed cleaner shrimp to associate with almost any sea anemone or false coral.

However, cleaner shrimp associations with Caribbean corallimorpharians in the wild seem to be exceptionally rare. The spotted cleaner shrimp *Periclimenes yucatanicus* and the Pederson cleaner shrimp *P. pedersoni* are selective of their hosts, almost always associating with actinian sea anemones *Condylactis gigantea* when available, and select the actinian anemones *Bartholomea annulata* if *C. gigantea* are not available (Williams & Bunkley-Williams 2000). Despite their success in aquaria, Williams and Bunkley-Williams's (2000) attempts to move *P. yucatanicus* from anemones to corallimorpharians in the wild repeatedly failed. It is believed that cleaner shrimp will form lasting symbioses with corallimorpharians only if the association is

made when the shrimp first leaves the larval state, and due to their selection preferences, this occurs only rarely in the absence of another suitable host (Williams & Bunkley-Williams 2000).

Pederson cleaner shrimp *Ancylomenes pedersoni* are one of the major cleaner organisms in the Caribbean Sea, in that they remove fish ectoparasites which can damage fish health (Bauer 2004). While ecological effects of the removal of cleaner shrimp in the Caribbean are not well studied, long-term field experiments involving the removal of cleaner fish from Egyptian reefs showed a decline in fish diversity (Bshary 2003). If corallimorpharians serve even occasionally as a habitat for cleaner shrimp, they could function as signals to fishes for the presence of cleaning stations (Huebner & Chadwick 2012). Their presence thus could indirectly enhance fish diversity on Caribbean reefs, by serving as a habitat for cleaner shrimp in the absence of the preferred hosts *C. gigantea* and *B. annulata*.

Symbiosis of anemonefish with large corallimorpharians was reported to be rare for all 3 species of the latter in Australia, likely because these species feed on fishes (see above Section 5), so any association could result in consumption of the fish (Fautin & Hamner 1980).

10 Anthropogenic impacts on corallimorpharians

Corallimorpharians and soft corals have become increasingly popular organisms in the ornamental aquarium trade, reflecting an increasing demand for these marine species among home aquarists (Cato & Brown 2008). In Florida, *Ricordea florida* was not collected in significant numbers until 2002, but by 2007 it had climbed to the 12th most collected species in the ornamental fishery (Rhyne et al. 2009). Unfortunately, to date no studies have been conducted to quantify the impacts of removal of corallimorpharians by fisheries on their

population dynamics. However, the effects of moderate removal are likely to be similar to the impacts of consumption of corallimorpharians by predators (see above Section 8).

Corallimorpharians also may be sensitive to anthropogenic climate change, similar to other coral reef cnidarians. During previous global climate change events in Earth's geologic history, Symbiodiniaceae and their corallimorpharian hosts apparently have been able to evolve and persist in response to changing oceanic conditions (Medina et al. 2006). These changes have included increases in water temperature and decreases in pH resulting from an increase in dissolved CO₂ (Douglas, 2010). However, rates of natural global climate change have been dramatically slower in the past than during the current period of human-induced climate change, and as such gradual organismal evolution has been able to keep up with the natural environmental changes. Their slow rate of natural evolution reduces the ability of reef cnidarians to keep pace with the rapid anthropogenic climate change that is occurring now on planet Earth (Finney et al. 2010).

Individual corallimorpharians, similar to many other sessile anthozoans, are not able to escape from climate change stressors by migrating away from stressed habitats. While one species of temperate Pacific Ocean corallimorpharian exhibits limited mobility, an ability that scleractinian corals lack, their locomotion is very slow, at < 15 mm moved per month. Thus, adult polyps are unlikely to be able to migrate away from the equator as the oceans warm (Chadwick & Adams 1991, Torres-Pratts et al. 2011). However, various reef organisms have shown increased thermal tolerance through the responses of their symbiotic Symbiodiniaceae, so this process could increase the chance of avoiding extinction for corallimorpharians (Douglas 2010). Research about the environmental tolerances of Caribbean corallimorpharians is not

available to make any such predictions in terms of their responses to changes in temperature or irradiance.

As stated in Section 4, some research indicates that nutrient loading provides corallimorpharians with a competitive advantage over corals on reefs, resulting in a phase shift in which corallimorpharians eventually dominate some reef areas (Muhando et al. 2002, Work et al. 2008). At one of these sites, attempts to reverse the phase shift have proven difficult (Work et al. 2018). Since most studied species of corallimorpharians are strong competitors for space (see Section 7), this means that even a short-term influx of nutrients could result in an irreversible shift from a reef dominated by diverse reef-building corals, to one dominated by a single species of corallimorpharian.

11 Conclusions and research topics examined in the present thesis

As this review makes apparent, there is a need for further research on corallimorpharians, especially on species with economic or ecological importance, including those collected for ornamental marine life fisheries.

This dissertation investigates aspects of the biology of an economically important corallimorpharian, the Florida false coral *Ricordea florida*, which is a component of the ornamental marine life fishery as well as being common on coral reefs throughout the Caribbean Sea. Despite this prominence, no studies have investigated the ecophysiology, reproductive biology, or population ecology of this species. This dissertation aims to elucidate the environmental tolerances of polyps of *R. florida* for variation in solar irradiance (Chapter 2) and seawater temperature (Chapter 3), spatial and temporal patterns of distribution and abundance in

the Florida Keys (Chapter 4), and aspects of both clonal and sexual reproduction including the ability of aggregations to recover from the removal of polyps in the field (Chapter 5). Based on conclusions from these studies, a recommendation for species management is included here (Chapter 6) and has been submitted to the Florida Fish and Wildlife Commission.

12 References

- Acuña FH, Griffiths CL (2004) Species richness, endemism and distribution patterns of South African sea anemones (Cnidaria: Actiniaria & Corallimorpharia). *African Zoology* 39:193–200.
- Andres A (1883) Le attinie. *Atti dell' Accademia de Lincei*:211–673.
- Anthony KR, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proceedings of the National Academy of Sciences* 105:17442–17446.
- Baker AC (2011) Zooxanthellae. In: *Encyclopedia of Modern Coral Reefs*. Encyclopedia of Earth Sciences Series, Hopley D (ed) Springer Netherlands, p 1189–1192.
- Bauer RT (2004) Remarkable Shrimps: Adaptations and Natural History of the Carideans. Oklahoma City, OK, USA: University of Oklahoma Press.
- Bos AR, Mueller B, Gumanao GS (2011) Feeding biology and symbiotic relationships of the corallimorpharian *Paracorynactis hoplites* (Anthozoa: Hexacorallia). *Raffles Bull Zool* 59:245–250.
- von Brandis RG, Mortimer JA, Reilly BK, van Soest RWM, Branch GM (2015) Taxonomic composition of the diet of hawksbill turtles (*Eretmochelys imbricata*) in the Republic of Seychelles. *Western Indian Ocean Journal of Marine Science* 13:81–91.
- Bshary R (2003) The cleaner wrasse, *Labroides dimidiatus*, is a key organism for reef fish diversity at Ras Mohammed National Park, Egypt. *Journal of Animal Ecology* 72:169–176.

- Cappola VA, Fautin DG (2000) The status of Corallimorpharia (Cnidaria, Anthozoa) based on molecular data from mitochondrial 16s rDNA and nuclear 28s DNA. *American Zoologist* 40:961.
- Carlgren O (1949) A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *K svenska VtenskAkad Handl* 1:1–121.
- Carter AL (2014) Invasion mechanisms of the corallimorph, *Rhodactis howesii*, at Palmyra Atoll. MS Thesis, UC San Diego.
- Cato JC, Brown CL (2008) *Marine Ornamental Species: Collection, Culture and Conservation*. Wiley. Hoboken, NJ, USA: John Wiley & Sons.
- Chadwick NE (1987) Interspecific aggressive behavior of the corallimorpharian *Corynactis californica* (Cnidaria: Anthozoa): effects on sympatric corals and sea anemones. *The Biological Bulletin* 173:110–125.
- Chadwick NE (1991) Spatial distribution and the effects of competition on some temperate Scleractinia and Corallimorpharia. *Marine Ecology Progress Series* 70:39–48.
- Chadwick NE, Adams C (1991) Locomotion, asexual reproduction, and killing of corals by the corallimorpharian *Corynactis californica*. *Hydrobiologia* 216:263–269.
- Chadwick NE, Morrow KM (2011) Competition among sessile organisms on coral reefs. In: *Coral Reefs: An Ecosystem in Transition*. Dubinsky Z, Stambler N (eds) Dordrecht, Netherlands: Springer, p 347–371.
- Chadwick-Furman NE, Spiegel M (2000) Abundance and clonal replication in the tropical corallimorpharian *Rhodactis rhodostoma*. *Invertebrate Biology* 119:351–360.
- Chadwick-Furman NE, Spiegel M, Nir I (2000) Sexual reproduction in the tropical corallimorpharian *Rhodactis rhodostoma*. *Invertebrate Biology* 119:361–369.

- Chen C-LA, Chen C-P, Chen I-M (1995a) Sexual and asexual reproduction of the tropical corallimorpharian *Rhodactis* (= *Discosoma*) *indosinensis* (Cnidaria: Corallimorpharia) in Taiwan. *Zool Stud* 34:29–40.
- Chen CLA, Chen C-P, Chen I-M (1995b) Spatial variability of size and sex in the tropical corallimorpharian *Rhodactis* (= *Discosoma*) *indosinensis* (Cnidaria: Corallimorpharia) in Taiwan. *Zool Stud* 34:82–87.
- Coles SL, Jokiel PL (1978) Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. *Marine Biology* 49:187–195.
- Daly M, Fautin DG, Cappola VA (2003) Systematics of the hexacorallia (Cnidaria: Anthozoa). *Zoological Journal of the Linnean Society* 139:419–437.
- Danielssen DC (1890) Actinida. Kristiania, Norway: Grøndahl & søns bogtrykkeri.
- Douglas AE (2010) *The Symbiotic Habit*. Princeton, NJ, USA: Princeton University Press.
- Edmunds PJ (2007) Physiological ecology of the clonal corallimorpharian *Corynactis californica*. *Marine Biology* 150:783–796.
- Elliott J, Cook CB (1989) Diel variation in prey capture behavior by the corallimorpharian *Discosoma sanctithomae*: mechanical and chemical activation of feeding. *The Biological Bulletin* 176:218–228.
- Fautin D, Guinotte J, Orr J (2009) Comparative depth distribution of corallimorpharians and scleractinians (Cnidaria: Anthozoa). *Marine Ecology Progress Series* 397:63–70.
- Fautin DG (2016) Catalog to families, genera, and species of orders Actiniaria and Corallimorpharia (Cnidaria: Anthozoa). *Zootaxa* 4145:1.
- Fautin DG, Hamner WM (1980) Tropical corallimorpharia (Coelenterata: Anthozoa): feeding by envelopment. *Micronesica* 16(1):37-41.

- Fautin DG, Lowenstein JM (1994) Phylogenetic relationships among Scleractinians, Actiniarians, and Corallimorpharians (Coelenterata: Anthozoa). *Proceedings of the Seventh International Coral Reef Symposium* 2:665-670.
- Fautin DG, White TR, Pearson KE (2002) Two new species of deep-water Corallimorpharia (Cnidaria: Anthozoa) from the Northeast Pacific, *Corallimorphus denhartogi* and *C. pilatus*. *Pacific Science* 56:113–124.
- Finney JC, Pettay DT, Sampayo EM, Warner ME, Oxenford HA, LaJeunesse TC (2010) The relative significance of host–habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus *Symbiodinium*. *Microb Ecol* 60:250–263.
- Fitt W, Brown B, Warner M, Dunne R (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* 20:51–65.
- de Fonbressin PD, Michelotti G (1864) *Spongiaires de la Mer Caraïbe. Les héritiers Loosjes.*
- Francis L (1973) Clone specific segregation in the sea anemone *Anthropleura elegantissima*. *The Biological Bulletin* 144:64–72.
- Garese A, Carrizo S, Acuña FH (2016) Biometry of sea anemone and corallimorpharian cnidae: statistical distribution and suitable tools for analysis. *Zoomorphology* 135:395–404.
- Gruber DF, Sparks JS (2015) First observation of fluorescence in marine turtles. *American Museum Novitates*:1–8.
- den Hartog JC (1980) Caribbean shallow water Corallimorpharia. *Zoologische Verhandelingen* 176:1–83.

- Hennessey SM, Sammarco PW (2014) Competition for space in two invasive Indo-Pacific corals — *Tubastraea micranthus* and *Tubastraea coccinea*: Laboratory experimentation. *Journal of Experimental Marine Biology and Ecology* 459:144–150.
- Hertwig R (1882) *Die Actinien der Challenger Expedition*. Jena, Germany: G. Fischer.
- Hines DE, Pawlik JR (2012) Assessing the antipredatory defensive strategies of Caribbean non-scleractinian zoantharians (Cnidaria): is the sting the only thing? *Marine Biology* 159:389–398.
- Holts L, Beauchamp K (1993) Sexual reproduction in the corallimorpharian sea-anemone *Corynactis californica* in a Central California kelp forest. *Mar Biol* 116:129–136.
- Huebner LK, Chadwick NE (2012) Reef fishes use sea anemones as visual cues for cleaning interactions with shrimp. *Journal of Experimental Marine Biology and Ecology* 416–417:237–242.
- Huebner L, Dailey B, Titus B, Khalaf M, Chadwick N (2012) Host preference and habitat segregation among Red Sea anemonefish: effects of sea anemone traits and fish life stages. *Mar Ecol Prog Ser* 464:1–15.
- Krishna K, Ingole BS (2009) Evolving trends in biosciences: Multi-purpose proteins-GFP and GFP-like proteins. *Current Science* 97(7): 1022-1032.
- Kuguru B, Achituv Y, Gruber DF, Tchernov D (2010) Photoacclimation mechanisms of corallimorpharians on coral reefs: Photosynthetic parameters of zooxanthellae and host cellular responses to variation in irradiance. *Journal of Experimental Marine Biology and Ecology* 394:53–62.

- Kuguru B, Chadwick N, Achituv Y, Zandbank K, Tchernov D (2008) Mechanisms of habitat segregation between corallimorpharians: photosynthetic parameters and *Symbiodinium* types. *Marine Ecology Progress Series* 369:115–129.
- Kuguru B, Fine M, Tchernov D (2016) The photo-physiological response of symbionts to combined effects of temperature and pH in a non-calcifying corallimorpharian, *Rhodactis rhodostoma*. *UOM Research Journal* 18A:122-144.
- Kuguru B, Winters G, Beer S, Santos SR, Chadwick NE (2007) Adaptation strategies of the corallimorpharian *Rhodactis rhodostoma* to irradiance and temperature. *Marine Biology* 151:1287–1298.
- Kuguru BL, Mgaya YD, Ohman MC, Wagner GM (2004) The reef environment and competitive success in the Corallimorpharia. *Marine Biology* 145:875–884.
- LaJeunesse T (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology* 141:387–400.
- Langmead O, Chadwick-Furman NE (1999) Marginal tentacles of the corallimorpharian *Rhodactis rhodostoma*. 1. Role in competition for space. *Marine Biology* 134:479–489.
- León YM, Bjorndal KA (2002) Selective feeding in the hawksbill turtle, an important predator in coral reef ecosystems. *Marine Ecology Progress Series* 245:249–258.
- Medina M, Collins AG, Takaoka TL, Kuehl JV, Boore JL (2006) Naked corals: skeleton loss in Scleractinia. *Proceedings of the National Academy of Sciences* 103:9096–9100.
- Miles JS (1991) Inducible agonistic structures in the tropical corallimorpharian, *Discosoma sanctithomae*. *The Biological Bulletin* 180:406–415.

- Muhando CA, Kuguru BL, Wagner GM, Mbije NE, Öhman MC (2002) Environmental effects on the distribution of corallimorpharians in Tanzania. *AMBIO: A Journal of the Human Environment* 31:558–561.
- Paul VJ, Puglisi MP, Ritson-Williams R (2006) Marine chemical ecology. *Natural Product Reports* 23:153.
- Pinto SM, Belem MJD (2000) Cnidae of two species of Discosomatidae (Cnidaria: Anthozoa: Corallimorpharia) from Brazil. *Proc Biol Soc Wash* 113:129–144.
- Porat D, Chadwick-Furman NE (2004) Effects of anemonefish on giant sea anemones: expansion behavior, growth, and survival. In: *Coelenterate Biology 2003*. Springer, p 513–520.
- Rhyne A, Rotjan R, Bruckner A, Tlusty M (2009) Crawling to collapse: ecologically unsound ornamental invertebrate fisheries. *PLOS ONE* 4:e8413.
- Rincon-Diaz MP, Diez CE, van Dam RP, Sabat AM (2011) Effect of food availability on the abundance of juvenile hawksbill sea turtles (*Eretmochelys imbricata*) in inshore aggregation areas of the Culebra Archipelago, Puerto Rico. *Chelonian Conservation and Biology* 10:213–221.
- Sebens KP (1976) The ecology of Caribbean Sea anemones in Panama: Utilization of space on a coral reef. In: *Coelenterate ecology and behavior*. New York, NY, USA: Springer, 67–77.
- Tkachenko KS (2014) Coral reefs in the face of ecological threats of 21st century. *Zhurnal obshchei biologii* 76:390–414.
- Torres-Pratts H, Lado-Insua T, Rhyne AL, Rodríguez-Matos L, Schizas NV (2011) Two distinct, geographically overlapping lineages of the corallimorpharian *Ricordea florida* (Cnidaria: Hexacorallia: Ricordeidae). *Coral Reefs* 30:391–396.

- Verrill AE (1869) Review of the corals and polyps of the west coast of America. Trans Conn Acad Arts Sci 1:377–567.
- Watzl O (1922) Die actiniarien der bahamainseln, auf grund der sammlung des Hrn. Dr. N. Rósen (1908-09). Arkiv für Zoologi 14:1–89.
- Williams EH, Bunkley-Williams L (2000) Experimental refutation of the ease of associate change by the spotted cleaner shrimp, *Periclimenes yucatanicus* (Decapoda, Palaemonidae), new false-coral associates for the Pederson cleaner shrimp, *P. pedersoni*, and general abundance of associations for both shrimps. Crustaceana 73:503–511.
- Williams Jr EH, Williams LB (1982) First report of *Periclimenes yucatanicus* (Ives) (Decapoda, Palaemonidae) in association with a corallimorpharian anemone. Crustaceana 42:318–319.
- Work TM, Aeby GS, Maragos JE (2008) Phase shift from a coral to a corallimorph-dominated reef associated with a shipwreck on Palmyra Atoll. PLoS ONE 3:e2989.
- Work TM, Aeby GS, Neal BP, Price NN, Conklin E, Pollock A (2018) Managing an invasive corallimorph at Palmyra Atoll National Wildlife Refuge, Line Islands, Central Pacific. Biol Invasions 20:2197–2208.

13 Figures



Figure 1-1. Examples of corallimorpharians belonging to 3 families: Corallimorphidae (*Corynactis californica*, top left), Discosomatidae (*Discosoma nummiforme*, top right), and Ricordeidae (*Ricordea florida*, bottom left). All photos from Wikipedia.org.

Chapter 2

Ecophysiology of Florida false corals *Ricordea florida* (Corallimorpharia: Recordeidae).

1. Shade-loving reef organisms and their responses to photosynthetically active radiation

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1 Introduction

Corallimorpharians are soft-bodied anthozoans that are ecologically important in some benthic marine communities (Carlgren 1949). They appear superficially to be actinian sea anemones, but commonly are known as false corals, mushroom corals, or corallimorphs (Anthozoa: Corallimorpharia; den Hartog 1980; Fautin 2016). Many species occur in shallow tropical marine environments, where their polyps possess short columns and broad oral discs covered by many short tentacles that contain endosymbiotic microalgae (Family Symbiodiniaceae; den Hartog 1980; Douglas 2010). In contrast to scleractinian corals, they lack a calcareous skeleton and therefore do not build reefs, but morphologically corallimorpharians are more similar to scleractinians than to other soft-bodied anthozoans such as actinian sea anemones. Phylogenetic analyses reveal that corallimorpharians arose ~ 110 - 132 million years ago by evolution from a group of scleractinian corals that no longer accreted calcium carbonate skeletons, during a period of rising sea temperatures and relatively low pH and Mg/Ca ratios in the oceans, as a result of global climate change (Fautin and Lowenstein 1994; Cappola and Fautin 2000; Medina et al. 2006). These environmental conditions are similar to those currently developing rapidly in the world's oceans due to anthropogenic climate change (Anthony et al. 2008).

All known corallimorpharians are able to reproduce both asexually (clonally) and sexually, and therefore can occur either as solitary polyps or form large clonal aggregations which may create extensive carpets on shallow reefs in both temperate and tropical regions (Carlgren 1949; Chadwick 1991; Chadwick-Furman et al. 2000; Kuguru et al. 2007). They can function as dominant space competitors that exhibit specialized aggressive behaviors to kill and

overgrow scleractinian corals and other benthic organisms (Langmead and Chadwick-Furman 1999; Chadwick-Furman et al. 2000; Kuguru et al. 2007; Torres-Pratts et al. 2011). Due to their competitive advantage under certain conditions, corallimorpharians may become “winners” during global climate change, in that they are able to monopolize space on shallow tropical reefs after temperature extremes or other stressors cause bleaching and mortality of scleractinian corals (Chadwick-Furman and Spiegel 2000; Kuguru et al. 2007; Work et al. 2008). As dominance by corallimorpharians may create phase shifts on coral reefs away from scleractinian corals during the Anthropocene, it is important to understand their biology and ecology (Work et al. 2008; Norström et al. 2009; Chadwick and Morrow 2011).

The Florida false coral *Ricordea florida* (Fig. 2-1, Family Ricordeidae) occurs on coral reefs throughout the Florida Keys and Western Atlantic Ocean, and is the most common species of corallimorpharian in the Florida Keys (de Fonbressin and Michelotti 1860; den Hartog 1980; Fautin 2016). Like most tropical corallimorpharians, polyps of *R. florida* possess endosymbiotic microalgae in the Family Symbiodiniaceae, and therefore are limited to the shallow photic zone (LaJeunesse 2002). Polyps can reach a diameter of ~ 7.5 cm (although they usually are smaller), and are wide and short, with a broad oral disc covered with many short, unbranched tentacles (den Hartog 1980). While few types of invertivores on coral reefs consume corallimorpharians, likely due to their low protein content compared to other foods and their protective nematocysts and chemical defenses, they are important prey for hawksbill sea turtles (*Eretmochelys imbricate*) which exhibit high selectivity toward *R. florida* as a prey item (León and Bjorndal 2002; Hines and Pawlik 2012).

Individuals of *R. florida*, along with other soft bodied coral-like organisms, are important economically in the ornamental aquarium trade (marine life fishery) due to increasing demand

among home aquarists for these visually aesthetic reef organisms (Cato and Brown 2008). Polyps of *R. florida* were not collected in significant numbers until 2002; however by 2007 they had climbed to the 12th most collected ornamental marine organisms in Florida, with > 10,000 individuals harvested to support a \$500,000 annual industry (Rhyne et al. 2009; Torres-Pratts et al. 2011). Despite their economic importance, only two scientific publications have focused on the biology of this species, and minimal information is available concerning their ecophysiology (den Hartog 1980; Torres-Pratts et al. 2011). The lack of scientific knowledge about the biology of *R. florida* (Williams and Bunkley-Williams 2000; Torres-Pratts et al. 2011) is especially alarming because in the Florida Keys, populations of this species recently declined to only ~10% of pre-2010 abundances, based on estimates by both marine scientists (pers. comm., C. Foord, Coral Morphologic, Inc.; D. Vaughan, Mote Marine Laboratory; A. Rhyne, Roger Williams University) and professional marine life collectors (pers. comm., F. Young, Dynasty Marine; R. Herndon, Sea Kritters of the Florida Keys; L. Gould, Florida Keys Marine Life). The observed decline of *R. florida* in the Florida Keys may be due in part to over-collecting, and/or to environmental stressors such temperature extremes in the form of a cold-weather event in 2010 followed by extremely hot summers during 2014-2015, each of which caused widespread bleaching and mortality of reef cnidarians in the Keys (Kuffner et al. 2015; Manzello 2015; Kemp et al. 2016; Banon et al. 2018).

Solar irradiance is another major environmental factor that controls the abundance and distribution of microalgal-containing cnidarians on reefs, due to their dependence on photosynthesis (Fitt et al. 2001). Impacts of irradiance level on the biology of tropical corallimorpharians have been investigated in the northern Red Sea, where Kuguru et al. (2008) found that crevice-inhabiting polyps of *Discosoma unguja* were more sensitive to high light than

were polyps of *Rhodactis rhodostoma* which occurred in exposed habitats on the intertidal reef flat. In the Indian Ocean, Muhando et al. (2002) found that sites with the highest levels of anthropogenic sedimentation and therefore decreased irradiance contained the highest concentrations of 5 species of corallimorpharians (*Rhodactis rhodostoma*, *R. mussoides*, *Ricordea yuma*, *Actinodiscus unguja* and *A. nummiforme*). They attributed this variation to a competitive advantage by the corallimorpharians over resident scleractinian corals which potentially were stressed by the sedimentation and/or the accompanying low light or high nutrient concentrations in the altered water column. Nothing is known about effects of solar irradiance level on corallimorpharians in the Caribbean region, or how their ranges of tolerance for irradiance may contribute to controlling their distributional patterns. According to Shelford's Law of Tolerance (Shelford 1931), *R. florida* and other corallimorpharians are expected to exhibit a physiologically optimal level of photosynthetically active radiation (PAR), and to show signs of stress and eventual mortality (intolerance) as PAR levels deviate from this optimum.

In the present study, we describe the effects of PAR level on whole polyp and microalgal characteristics of *R. florida*, as well as the upper and lower ranges of environmental tolerance for irradiance in this species under experimental laboratory conditions. We also present correlations among body size measures in this species, to facilitate prediction of body size based on measures that do not require sacrificing polyps (after Zamer 1992; Chadwick-Furman *et al.* 2000). We discuss the implications of our results in terms of the ecology of *R. florida* on coral reefs, as well as the optimal conditions for commercial aquaculture of this species to reduce the ornamental fishery pressure on natural populations.

2 Methods

2.1 *Field observations*

To determine the relative levels of irradiance in reef microhabitats occupied by *R. florida* versus levels on the exposed open reef surface, small HOBO® Pendant Temperature/Light Data Loggers (Onset Computer Corporation, Bourne, MA) were deployed at 6 m depth on the inner reef near American Shoal (Cudjoe Key, FL). This area of high *R. florida* abundance (~ 6 polyps m⁻²; Chapter 4) was selected based on a previous study (Miller et al. (2009) and personal communications from local Marine Life collectors. Six data loggers were initially deployed in August 2017, but in September 2017, Hurricane Irma passed directly over the research site and all but 1 logger was lost. Data from the single remaining logger were not used to indicate absolute irradiance, because they recorded light levels in lux which could not be converted accurately to PAR. Instead, 2 more loggers were deployed in June 2018, 1 in a reef microhabitat occupied by a large *R. florida* aggregation (within 3 cm of a randomly-selected aggregation that contained > 10 healthy-looking, dark green polyps) and 1 on open reef substrate (the nearest bare, unshaded reef substrate to that aggregation, within 1 m on the open exposed reef), to allow recording of relative irradiance levels on the open reef versus in *R. florida* microhabitat. These loggers were collected in July 2018, and then redeployed by placing 1 near a different aggregation of polyps exhibiting the rare orange coloration, and 1 on the nearest bare, unshaded reef substrate to that aggregation (within 1 m on the open exposed reef; again, for relative irradiance levels between the 2 areas). These then were collected in August 2018. Because the loggers became rapidly fouled with encrusting organisms, and thus the data collected by their

light sensors became rapidly altered following deployment, only the first 3 days of light data after each deployment were used (n = 6 days total).

2.2 *Collection and maintenance of organisms for laboratory experiments*

Individuals of *Ricordea florida* were collected during October 2017 and March 2018 from coral reefs at 6 m depth near American Shoal, Lower Florida Keys (24.5°N, 81.5°W; same reef area as the site described above and in Chapters 3 & 4, at least 25 m distant from the deployed data loggers). Individual polyps were collected from distinct aggregations, with 1-5 polyps collected from each aggregation (75 polyps collected per trip x 2 trips = 150 polyps total). An aggregation was defined as a group of polyps (with up to 20 polyps per aggregation observed in the field) that were either in contact or < 15 cm distant from each other, which was the maximal distance observed between morphologically similar polyps. Aggregations were sampled that each contained individuals that were green, blue, or a combination of these colors, with possible additional orange coloration. Polyps exhibiting the rare color morph of completely orange (< 1% of polyps observed in the field) were excluded from collection due to their limited availability. Multiple aggregations of each major color morph were selected for collecting, in order to enhance the potential genetic diversity of polyps collected. Care was taken to avoid collecting polyps that showed signs of tissue damage or bleaching (< 5% of polyps observed in the field). Collection was done by hand, using a hammer and chisel to remove a thin layer (< 0.5 cm thick) of reef substrate with each polyp attached; this was done because the pedal disc of each polyp was thin, delicate, and easily ruptured (Carlgren 1949). Collected polyps were transferred in buckets to the seashore, where they were held in an outdoor commercial aquarium

system with partial shading over the tanks for up to 2 days. Then the polyps were packed into 1 L plastic bags (~ 10 polyps per bag) and filled with seawater and pure oxygen for overnight shipping to the laboratory at Auburn University.

Upon arrival to the laboratory, polyps were placed into one of three culture tanks with ~ 25 polyps per tank (~ 75 polyps from each of the 2 collection periods; ~150 polyps total). No mortality occurred during transport or laboratory acclimation. Polyps then were subjected to a regular culture routine for 14-21 days before use in experiments. The culture system consisted of three 78 L culture tanks attached to the same 156 L sump tank that contained a protein skimmer for mechanical filtration, bio-filter balls and a gravel substrate for organic filtration, and a return pump for water circulation. All three tanks shared the same water supply to increase the total water volume in each culture system and to avoid variation in water quality among tanks that could cause a tank effect during experiments (Hurlbert 1984). Light was provided to each culture tank by overhanging LED light fixtures (Lightimetunnel 165W Full-Spectrum High-output Fixtures; Shenzhen, Guangdong, China). Light output was adjusted so that the level of photosynthetically active radiation (PAR) at the polyp upper surfaces was ~ 80 $\mu\text{E m}^{-2} \text{s}^{-1}$ ($\pm 10\%$), which represented ~ 33% of the irradiance level at 10 m depth on Florida coral reefs (see Results). This irradiance level corresponded to conditions on the shaded reef surfaces where these organisms were observed to occur in the field, and these conditions appeared to support polyp growth during preliminary observations in the laboratory. All PAR measurements were performed using a Biospherical Instruments QSL-2100 light meter (San Diego, CA). Conditions in the culture tanks were maintained to mimic ambient seawater conditions on reefs in the lower Florida Keys: 12:12 hour light cycle, $28 \pm 1^\circ\text{C}$ seawater temperature, salinity 33 ± 2 ppt, pH 8.2 ± 0.2 , low levels of dissolved nutrients (< 0.25 ppm of NH_4^+ , NO_2^- , NO_3^- , and PO_4^{3-}), and $380 \pm$

50 ppm dissolved Ca^{+2} (Southeast Environmental Research Center SERC Water Quality Monitoring Network, <http://serc.fiu.edu/wqmnetwork/>). Polyps were broadcast fed once per week to satiation using Marine Cuisine™ and frozen brine shrimp (San Francisco Bay Brand, Neward, CA). See Roopin and Chadwick (2009) and Cantrell et al. (2015) for details of similar methods used for long-term culture of reef cnidarians in the same laboratory.

After initial acclimation on their original reef substrates for 7 days, each polyp was carefully removed from its calcareous rock substrate and mounted individually onto a clear Plexiglas plate (2 x 2 cm). Removal was done by cutting a minimal amount of tissue (< 0.1 cm thick) from sections of the pedal disc. Each polyp was placed in the middle of a plate, and a piece of standard window screen was secured loosely over the entire plate and polyp, using rubber bands around the plate edges. The polyp on its new plate then was returned to its culture tank. After 7 additional days of regular culture, the screen was removed from each plate, by which time most polyps had regenerated their pedal discs and attached to the plates (~ 90% of polyps). If the polyp was not yet attached, it remained another 7 days beneath the screen; if not attached within 14 days, it was excluded from the study (14 polyps either died or failed to attach to plates, < 10% of 150 individuals). Each plate was numbered on the underside using a paint marker, to aid in polyp identification. The culture of polyps on clear plates ensured that the oral disk of each polyp was oriented facing up toward the light source, and that the pedal disc area was visible and could be measured through the undersurface of the transparent plate. Plated polyps were cultured for at least 2 weeks before use in experiments, during which time they expanded their oral discs, fed normally, and did not change color.

2.3 Irradiance manipulation

Sixty polyps were used for each of two irradiance experiments ($n = 120$ total), with the remaining 26 polyps held in a reserve culture tank (120 used + 14 died/not attached, see above, + 26 held in reserve = 150 total). The first experiment ran during November 2017 to February 2018, using polyps that had been collected in October 2017. The second experiment ran during March to July 2018, using polyps that had been collected in March 2018. For the first experiment, three irradiance treatments were assigned randomly to six sections in the culture tanks (2 sections per tank x 3 tanks), so that each half of a tank was exposed to a different irradiance level. Twenty polyps were selected randomly for assignment to each of the 3 treatments, with 10 polyps in each of the 6 half-tank sections ($n = 60$ polyps total). The irradiance level over each tank section was adjusted by altering the output of the overhanging light fixture to the higher treatment irradiance level, and then placing a shade filter over the other half of the tank to lower the irradiance. The three treatments consisted of PAR levels of 240, 80, and $20 \mu\text{E m}^{-2} \text{s}^{-1}$. These were based on the median irradiance level known for unshaded areas at 10 m depth where these organisms commonly occur on Florida reefs ($240 \mu\text{E m}^{-2} \text{s}^{-1}$), 66% shading at this depth or in unshaded areas at the deepest extend these organisms are found (30 m; $20 \mu\text{E m}^{-2} \text{s}^{-1}$), and very low-light conditions representing highly shaded polyps at a considerable depth ($20 \mu\text{E m}^{-2} \text{s}^{-1}$), respectively (Lesser 2000). Based on the results of the first experiment, a narrower range of three irradiance treatment levels was employed in the second experiment: 10, 40, and $60 \mu\text{E m}^{-2} \text{s}^{-1}$ PAR, in order to more precisely determine the optimal irradiance conditions for this species.

Each of the 2 experiments ran for 8 wk at irradiance treatment conditions, followed by 8 wk of recovery, during which irradiance levels in the tanks were returned to the standard culture level of $80 \mu\text{E m}^{-2} \text{s}^{-1}$. These periods were selected because they are long enough for symbiotic cnidarians to exhibit physiological responses to changes in environmental parameters (Godinot and Chadwick 2009; Roopin and Chadwick 2009).

2.4 Measurement of polyp parameters

Seven parameters were measured during both the treatment and recovery period for each polyp: 4 whole-polyp characteristics consisting of oral disc surface area (OSA), pedal disc surface area (PSA), body color (BCI), and clonal replication status (CRS); and 3 characteristics of microalgae within the polyp tissues, consisting of microalgal cell abundance (MCA), mitotic index (percent cells dividing; MTI), and chlorophyll concentration per algal cell (CCA; after Dunn et al. 2000). The 4 whole-polyp characteristics were measured for all polyps at 0, 4, 8, 12, and 16 weeks (8 weeks of irradiance treatments plus 8 weeks of recovery). The 3 microalgal characteristics (MCA, MTI, CCA) were assessed only 3 times (0, 8, and 16 weeks), for only 24 polyps each time (2 polyps per treatment per tank x 2 tanks = 4 polyps total per treatment x 6 treatments; 20% of polyps), because they required the removal of some polyp tissue. The body size parameters examined here were considered to be a fairly direct measure of polyp fitness, because the number of oocytes produced by each corallimorpharian is known to increase linearly with body size (Chadwick-Furman et al. 2000).

To measure whole-polyp characteristics, the plate containing each polyp was removed from its tank and placed on a piece of rigid plastic grating in air. A digital photograph was taken

of the polyp from above, with a ruler and a color chart inside the photo frame. A modified version of the Coral Watch Coral Health Chart was used, changing the hues to account for those observed in this species while saturation and brightness values remained consistent with those used for the standard color chart (Fig. 2-2; Siebeck et al. 2006). Then the plate was turned over to suspend the polyp in a hole in the grating, and another photograph was taken showing the polyp identification number and pedal disk through the clear plexiglass. The polyp then was immediately returned to its tank. The entire process took < 30 sec per polyp, and did not appear stress the polyps, because they produced only minimal mucous and their slow contraction rates caused them to not contract much during this brief process (only ~ < 5% contraction of the oral disk). ODA measured in air using the methods described here did not appear to differ substantially from that in seawater measured during preliminary studies. Each digital photograph of a polyp then was viewed using ImageJ (Schneider et al. 2012), the oral and pedal disc area of each was outlined and calculated, and all measurements were calibrated using a ruler scale in the photograph. The body color of each polyp was categorized using the modified color chart, as Blue (1-6) or Green (1-6; Fig. 2-2). Body color was measured to detect changes in overall coloration, including signs of bleaching (Siebeck et al. 2006). The CRS of each polyp was assessed to determine the extent to which polyps progressed toward clonal replication. Based on preliminary observations, individual polyps could both advance toward and regress from stages of clonal replication, so each polyp's CRS was classified as one of 3 categories: 1 (initiated or progressed in clonal replication; positive replication), 0 (no change in clonal replication status), or -1 (regressed in clonal replication status; negative replication).

The 3 microalgal characteristics (MCA, MTI, and CCA) were measured less frequently and in fewer polyps than were whole-polyp characteristics, because their assessment required

removal of a relatively large proportion of tissue from these small-bodied polyps, in order to obtain enough tissue for microalgal assessment (~ 10% of polyp wet mass, or 0.02-0.20 g wet mass; similar to wet mass used for measurement of microalgal characteristics in past studies on photosynthetic corallimorpharians; Kuguru et al. 2016). The polyps did not contain long tentacles for sampling, so a wedge of tissue was cut from the edge of the oral disc. Tissue samples were homogenized in artificial seawater using a sonicator, and centrifuged five times (10,000G for 10 min), removing the supernatant and resuspending the pellet in artificial seawater each time (Dunn et al. 2000). A sonicator was used rather than a micro-homogenizer as usually done for cnidarian tissue processing, because of a high mucous content (Roopin and Chadwick 2009). The samples were then resuspended and divided evenly into two containers and centrifuged one last time. One of these samples was resuspended in 90% acetone and left in dark conditions overnight to extract chlorophyll. CCA was then assessed using a spectrophotometer measuring optical density at the wavelengths of 630 μ m, 664 μ m and 750 μ m with 750 μ m used as a reference. The formula used for CCA was from Jeffrey & Humphrey (1975):

$$\mu\text{gr chl-a ml}^{-1} = 11.43(\text{OD}_{664} - \text{OD}_{750}) - 0.40 (\text{OD}_{630} - \text{OD}_{750}) \times \text{acetone vol. (ml)}$$

The other half of the sample was resuspended in 1 mL of seawater, and MCA was determined using a hematocrit (hemocytometer) and normalized to wet mass of polyp tissue. The number of microalgal cells that were observed to be dividing (in couplets) was recorded to calculate MI.

Three additional measures of polyp body size (BV, WM, and DM) were assessed at the end of the recovery period, because the polyps had to be sacrificed in order to obtain these measures. They were used to determine relationships among measures of body size for this species. Each polyp was removed from its plexiglass plate using a razor blade to ensure removal of the entire pedal disk. Then the polyp was gently placed into a 25-mL graduated cylinder

partially filled with 10 mL of sterile seawater, and the polyp's BV was determined to the nearest 0.25 mL by water displacement. For WM, the polyp was gently blotted with a clean KimWipe to remove excess water and placed into a plastic weighing boat of known mass. It was weighed in air on a calibrated electronic scale to determine WM to the nearest 0.0001g. The polyps in their weigh boats then were dried in a 60°C drying oven for five days, removed, and the DM of each was determined using the same electronic scale. A total of 62 polyps were selected randomly from both experiments, for analysis of relationships among these body size parameters.

2.5 *Data analysis*

To determine the extent to which the 4 measures of live body size (ODA, PDA, BV, and WM) predict the dry mass of sacrificed polyps (DM), simple linear regressions were run with DM as the independent variable and all intercepts set to zero.

Effects of irradiance levels on polyp and microalgal characteristics were analyzed in R (R Foundation for Statistical Computing, Vienna, Austria), using the “nlme” package. For analysis of changes in ODA and PDA, percent change between the beginning of each trial and the end of the trail or the end of the recovery period was used. Tank was included as a random effect. The same statistical test was used for CRS, BCI, ODA:PDA, and microalgal characteristics. Statistical outliers were excluded from analysis using Tukey's fences. A t-test was performed before starting trials to detect any systematic variation in polyp size between treatments, and during trials between tanks within each treatment, to detect any tank effects. Data are presented as means \pm SE unless otherwise indicated.

3 Results

3.1 *Field observations*

Data from the field loggers revealed that levels of downwelling irradiance peaked at ~ 14:00 to 16:00 each day (Fig. 2-3). Before 08:00 and after 20:00 each day, irradiance levels were at zero. During the initial 3-day period of irradiance data analysis on 30 June to 2 July 2018 (see Methods for details), the irradiance level adjacent to the examined aggregation of *Ricordea florida* was much lower than on the nearby open unshaded reef surface, leading to a $63.5\% \pm 2.7\%$ reduction in irradiance in the shaded habitat at the aggregation compared to habitat on the open reef (N = 12 hours examined each day x 3 days = 30 total datapoints examined for irradiance levels, Fig. 2-3A). In contrast, during the second 3-day period of irradiance analysis on 19 July 2018 to 21 July 2018, the irradiance level adjacent to the aggregation that had recently had most of the shading organisms around it removed, was only slightly lower than that on the nearby open reef surface (Fig. 2-3B). This led to only a $29.2\% \pm 2.7\%$ reduction in irradiance in the shaded habitat at the aggregation compared to on the open reef. Calibration of these irradiance levels recorded in units of Lux with those presented as PAR measured in microEinsteins by Lesser (2000), on coral reef at a similar depth in the Florida Keys, indicated that the former aggregation (in shaded habitat) received a median irradiance level of $\sim 88 \mu\text{E m}^{-2} \text{ s}^{-1}$ PAR, and the latter aggregation (in recently unshaded habitat due to a storm) received $\sim 170 \mu\text{E m}^{-2} \text{ s}^{-1}$ (calculations based on full sunlight days, not accounting for overcast or stormy conditions).

3.2 *Effects of irradiance under laboratory conditions*

At the beginning of the experiment, polyps did not differ significantly among the 6 irradiance treatment groups in terms of any of the 5 whole-polyp characteristics examined (pedal disk area [PDA], oral disk area [ODA], ratio of oral to pedal disk [ROP], clonal replication stage [CRS], or body color index [BCI]; $p > 0.225$ for all, $n = 20$ polyps per treatment).

After 8 weeks under the treatment conditions, oral disk area (ODA) differed significantly among the 6 treatments ($p < 0.001$, Figs. 2-4 & 2-5). Pairwise comparisons revealed that polyps exposed to the 3 lowest irradiance levels (10, 20, 40 $\mu\text{E m}^{-2} \text{s}^{-1}$) all increased in ODA by ~ 40-60%, at rates that did not differ significantly among the 3 treatments. The percent increase in ODA was significantly less in polyps exposed to the 3 highest irradiance levels (60, 80, and 240 $\mu\text{E m}^{-2} \text{s}^{-1}$), which likewise did not differ significantly from each other (Fig. 2-4). Polyps exposed to both 60 and 80 $\mu\text{E m}^{-2} \text{s}^{-1}$, which were similar to the regular culture conditions, remained static in terms of ODA over 8 weeks (neither shrank nor grew), but those receiving the highest irradiance (240 $\mu\text{E m}^{-2} \text{s}^{-1}$) shrank in ODA by -22% on average (Fig. 2-4).

The percent change in pedal disk area (PDA) of polyps also differed significantly among the 6 treatments ($p < 0.001$, Figs. 2-4 & 2-5). Polyps exposed to the 2 lowest irradiance levels (10 and 20 $\mu\text{E m}^{-2} \text{s}^{-1}$) shrank in PDA by ~ 10% on average (not significantly different from each other, pairwise comparisons, $p = 0.778$), while those at medium irradiance (40 and 60 $\mu\text{E m}^{-2} \text{s}^{-1}$) both grew (by 53% and 33% on average; also not significantly different from each other; Fig. 2-4). Polyps at higher irradiance (80 $\mu\text{E m}^{-2} \text{s}^{-1}$) shrank by ~ 10% on average (not significantly different than at 10 or 20 $\mu\text{E m}^{-2} \text{s}^{-1}$), and at the highest irradiance exposure, they lost almost half their pedal disk area (shrank by - 41% on average; Fig. 2-4).

Due to contrasting effects of irradiance on PDA versus ODA (compare Figs. 2-4A,B), after 8 weeks the ratio of oral to pedal disc surface areas (ROP) was highest (~ 6:1 OSA:PSA) at the 2 lowest irradiance levels (10 & 20 $\mu\text{E m}^{-2} \text{ s}^{-1}$; Fig. 2-4C; not significantly different from each other, but significantly larger than in all 4 other treatments, $p < 0.001$ for all pair-wise comparisons). Polyps exposed to the 4 higher irradiance levels exhibited ODA:PDA ratios of only ~ 3:1 on average, that did not differ significantly from each other (Fig. 2-4C). Overall these patterns indicated that while polyps at the lowest irradiance shrank in their pedal disk areas, they expanded their extensile oral disks; at medium irradiance, both their pedal and oral disks grew, and at high irradiance, both shrank.

In terms of the clonal replication stages (CRS) exhibited by polyps, this parameter also varied significantly among treatments at 8 weeks (Fig. 2-6, $p = 0.0125$). None of the polyps completed clonal replication (i.e., produced new daughter polyps) during the 16-week experiment (8 week treatment + 8 week recovery; see other recovery patterns below), but many of the polyps advanced and/or regressed in expressing stages of partial (not complete) clonal replication during this period. Polyps exhibited neutral to positive CRS in all treatments except for at very high irradiance (240 $\mu\text{E m}^{-2} \text{ s}^{-1}$), where they had a negative index indicating more regression than advance in clonal replication stages (Fig. 2-6). The CRS of polyps was almost zero at the lowest irradiance levels, peaked at 60 $\mu\text{E m}^{-2} \text{ s}^{-1}$, and became negative at the highest irradiance, similar to patterns exhibited for polyp body size in terms of PSA (compare Figs. 2-4 & 2-6). Pairwise comparisons revealed that CRS was significantly lower at 240 $\mu\text{E m}^{-2} \text{ s}^{-1}$ compared to 40, 60, and 80 $\mu\text{E m}^{-2} \text{ s}^{-1}$ ($p < 0.05$). At 60 $\mu\text{E m}^{-2} \text{ s}^{-1}$ CRS was significantly higher than 10, 20, or 240 $\mu\text{E m}^{-2} \text{ s}^{-1}$. An additional pairwise comparison showed that the $39 \pm 12\%$ of polyps that initiated cloning during the treatment at 60 $\mu\text{E m}^{-2} \text{ s}^{-1}$ was significantly

greater than the $6 \pm 6\%$ in the $80 \mu\text{E m}^{-2} \text{s}^{-1}$ treatment ($p = 0.012$) and from the $0 \pm 0\%$ in the $244 \mu\text{E m}^{-2} \text{s}^{-1}$ treatment ($p = 0.003$). All other treatments were between 17% and 24% and did not vary significantly.

Finally, polyp body color index (BCI) likewise did not differ significantly among the 6 treatments at the start of the experiment (Fig. 2-6, $p = 0.33$). After 8 weeks of irradiance treatments, body color index differed significantly among the treatments ($p < 0.0001$). Polyps became significantly darker (an average of 0.2 steps on the color chart scale) only at low irradiance ($20 \mu\text{E m}^{-2} \text{s}^{-1}$; $p < 0.0001$, Fig. 2-6). At all other irradiance levels, body color became lighter, especially at the highest irradiance level ($240 \mu\text{E m}^{-2} \text{s}^{-1}$) where color lightened by 0.5 steps on the color chart (significantly lighter than in all other irradiance conditions except for $60 \mu\text{E m}^{-2} \text{s}^{-1}$). No other pair-wise comparisons showed significant differences in BCI change.

After another 8 weeks under recovery conditions (all polyps returned to culture irradiance level of $80 \mu\text{E m}^{-2} \text{s}^{-1}$), the polyps retained some residual effects of the irradiance treatments in terms of most whole-polyp parameters. Both the ODA and PDA of polyps that had been exposed to moderate irradiance ($40\text{-}60 \mu\text{E m}^{-2} \text{s}^{-1}$) during treatments remained significantly larger than for polyps recovering from all 4 other treatments, which did not differ from each other (Fig. 2-5). This pattern held for all groups except for polyps recovering from the lowest irradiance treatment of only $10 \mu\text{E m}^{-2} \text{s}^{-1}$, which remained at significantly smaller PDA than for all other groups, even 8 weeks after returning to the culture irradiance level. In terms of polyp expansion index or the ratio of oral to pedal disk area (ROP), $80 \mu\text{E m}^{-2} \text{s}^{-1}$ remained significantly more expanded after recovery, however all other conditions no longer had significant differences ($p < 0.0001$, Fig. 2-5). The clonal replication index (CRI) after 8 weeks recovery still showed some slightly significant differences ($p = 0.045$), however the pair-wise analysis showed no patterns. Finally,

the polyp body color index (BCI) during recovery exhibited a maximum difference ($10\mu\text{E m}^{-2} \text{ s}^{-1}$ versus $60\mu\text{E m}^{-2} \text{ s}^{-1}$) of only 1.0 steps which was significant ($p = 0.026$).

3.3 *Microalgal characteristics*

The 3 examined microalgal characteristics varied widely among the few individuals sampled within each irradiance treatment, but none of them varied significantly among treatments after 8 weeks ($p = 0.326, 0.797,$ and 0.121 for cell abundance [MCA], mitotic index [MTI], and Chl a concentration per algal cell [CCA], respectively; Fig. 2-7). They likewise did not differ among treatment groups after the 8-week recovery period ($p = 0.446, 0.449,$ and 0.702 for MCA, MTI, and CCA, respectively; Fig. 2-7). Due to this lack of differences among treatments in response to the initially wide range of irradiance levels tested, and the high level of potential stress to polyps caused by removing tissues for analysis, the responses of microalgal characteristics were not measured subsequently, in terms of their responses to the narrower range of irradiance levels tested during the second set of treatments.

4 Discussion

We demonstrate here that irradiance levels in a shaded coral reef microhabitat occupied by Florida false corals *Ricordea florida* are substantially lower than those in nearby open unshaded reef habitats, and appear similar to irradiance levels experimentally demonstrated here to optimize fitness-related characteristics of this species ($\sim 40\text{-}60 \mu\text{E m}^{-2} \text{ s}^{-1}$). Conversely, irradiance levels in a coral reef microhabitat that recently lost shading due to a storm, and is

occupied by apparently stressed polyps of this species, are similar to those that our laboratory experiments revealed to induce physiological stress and suboptimal levels of fitness-related traits in polyps ($\sim 170\text{-}250+ \mu\text{E m}^{-2} \text{s}^{-1}$). Both major parameters of whole-polyp fitness examined here (pedal disk area as a measure of body size, and clonal replication status) reach maximal values when experimentally exposed to $\sim 40\text{-}60 \mu\text{E m}^{-2} \text{s}^{-1}$, and polyps appear to compensate for lower irradiance by expanding their oral disks, and becoming measurably darker in pigmentation, both of which are mechanisms known to enhance photon capture by cnidarians in low light (Siebeck et al. 2006). These 2 types of observational and experimental evidence together indicate that members of this species exhibit a classic light tolerance curve, with optimal irradiance levels at $\sim 40\text{-}60 \mu\text{E m}^{-2} \text{s}^{-1}$, suboptimal levels indicating polyp stress (including compensatory mechanisms) at levels much lower ($< 20 \mu\text{E m}^{-2} \text{s}^{-1}$) or higher than that range ($> 80 \mu\text{E m}^{-2} \text{s}^{-1}$), and likely lethal effects at very low ($< 5\text{-}10 \mu\text{E m}^{-2} \text{s}^{-1}$) or relatively very high irradiance ($250+ \mu\text{E m}^{-2} \text{s}^{-1}$). As such, this corallimorpharian appears to be adapted for occupying shaded shallow reef microhabitats and/or deeper slopes on Caribbean coral reefs, in contrast to related species which thrive on exposed shallow reef flats (Chadwick-Furman and Spiegel 2000).

During our field studies, we frequently observed *R. florida* polyps to occur near the bases of sea fans, soft corals, and other upright reef organisms, indicating that they may either select and/or differentially survive in shaded habitats on shallow reefs. Other types of soft-bodied reef anthozoans have been shown to occur in shaded microhabitats that receive significantly less light than the open reef surface, including giant actinian sea anemones (Dixon et al. 2014). This conclusion is supported by the observed high percent shading adjacent to a healthy-looking aggregation of polyps, and in contrast the relatively low percent shading next to an apparently stressed aggregation. This stress appears to have been caused by the removal of nearby shading

organisms during Hurricane Irma the previous year, followed by a lack of recovery of the shading organisms, to the detriment of the corallimorpharian polyps.

Our laboratory results indicate that polyp body size in particular, as measured in PSA, follows Shelford's (1931) Law of Tolerance for irradiance levels, in that polyp size peaks at moderate levels, is reduced at more marginal levels indicating stressed zones on either side of the optimum, and is extremely reduced at extremely high or low levels of irradiance, suggesting intolerance. These experimental responses in combination with our field observations suggest that shallow unshaded habitats on coral reefs, which receive high solar irradiance, are not tolerated well by *R. florida*.

Our laboratory observations also indicate that clonal replication in this species may be a slow process even under optimal conditions, with none of the polyps undergoing an entire clonal replication process (from initiating to completing clonal replication) during the 16 weeks of the study. However, 4 polyps that had begun clonal replication before the study began, completed cloning during the 8 weeks of temperature treatments (1 at $240 \mu\text{E m}^{-2} \text{ s}^{-1}$, 2 at $80 \mu\text{E m}^{-2} \text{ s}^{-1}$, and 1 at $40 \mu\text{E m}^{-2} \text{ s}^{-1}$) and another 1 completed cloning during the 8 week recovery period (at $40 \mu\text{E m}^{-2} \text{ s}^{-1}$). Interestingly, several polyps that were exposed to low to moderate irradiance levels initiated cloning (3 polyps each at 10 and $20 \mu\text{E m}^{-2} \text{ s}^{-1}$, 4 at $40 \mu\text{E m}^{-2} \text{ s}^{-1}$, and 7 at $60 \mu\text{E m}^{-2} \text{ s}^{-1}$), but almost none at relatively high irradiance (1 at $80 \mu\text{E m}^{-2} \text{ s}^{-1}$, and none at $240 \mu\text{E m}^{-2} \text{ s}^{-1}$). This pattern closely follows the overall trend in the clonal replication stages (CRS) exhibited by these polyps after several weeks in the temperature treatments, in that CRS also peaked at low to moderate irradiance levels. Variation in the rates of initiation of cloning thus further support our conclusion that $40 - 60 \mu\text{E m}^{-2} \text{ s}^{-1}$ appears to be the optimum irradiance level for *R. florida*.

The lack of significant changes observed in characteristics of the microalgal endosymbionts within polyp tissues in response to irradiance level is in contrast to clear microalgal responses to irradiance observed for some other corallimorpharian species (Kuguru et al. 2007). The lack of pattern detected in the present study may have been due in part to the small sample sizes (number of polyps) and limited sample dates examined here for microalgal traits. These sampling limitations were caused in by our having only a limited number of polyps available for each treatment, and the need to remove substantial amounts of tissue from only a few of those polyps for microalgal sampling, in order to avoid stressing the polyps due to tissue loss. The lack of microalgal pattern may also have been due to this species using instead whole-polyp behavioral responses to acclimatize to different irradiance levels, by varying the expansion level of the oral disc and/or tentacles. This type of behavioral response to low light has been observed also in actinian sea anemones (Holte 2018), and appears to be mediated by signals provided by the endosymbiotic Symbiodiniaceae (reviewed in Shick 2012). Future studies that involve repeat sampling of microalgal traits in polyps of *R. florida* would benefit from larger sample sizes, so that several whole polyps could be sacrificed each sample period to obtain more accurate microalgal data. Alternately, methods would need to be developed to enhance the accuracy of repeat-sampling only small amounts of tentacle mass collected from all polyps in the study, which will be challenging due to the small body size and short tentacles possessed by polyps of this species.

The color hue changes observed here in the corallimorpharian polyps varied significantly with irradiance level but represented only slight changes of ~ 0.7 steps on the modified Reef Check color chart. Differences of at least 2 steps on this type of color chart are necessary to reflect actual changes in microalgal abundances or chlorophyll pigment concentrations in other

types of cnidarians (Siebeck et al. 2006). Therefore, these slight color changes may indicate a lack of microalgal changes in the polyps examined here, or it is possible that in these corallimorpharians, only slight color changes are needed to reveal altered microalgal abundances or chlorophyll levels. It is also possible that the observed significant changes in color hue of the corallimorpharians may indicate changes in the host animal pigmentation with the highest animal pigment level at only $20 \mu\text{E m}^{-2} \text{ s}^{-1}$. However, it has been demonstrated that corals use increased pigmentation to protect endosymbionts from damaging UV radiation and this highest pigment production at the lowest tolerable light level would not fit into this model (Dove et al. 2001; Salih et al. 2001).

Our demonstration of strong correlations among all examined body size parameters of *R. florida* indicates that only one of those parameters need be measured on polyps in order to fairly accurately infer body size (Fig. 2-8). Although wet mass and body volume both strongly correlate with dry biomass, the methods to obtain both of these live polyp measures involve stressing the polyps by manually manipulating them and exposing them to air, and they also require the polyps to be removed from and then later remounted onto culture plates during long-term studies. As such, neither of these 2 measures are preferable for use as indicators of body size in live experimental studies, unless obtained only at the end of the study. In contrast, measurement of ODA at a constant irradiance level seems to be the best method to estimate polyp biomass without stressing the polyp, in part because it can be measured without removing the polyp from water, or even touching the polyp, and does not require culturing polyps on transparent plates. In contrast, PDA varies increasingly with polyp size in relation to DW and can be measured only through clear plates. The tight correlations among body size measures examined here are similar to those obtained for Red Sea corallimorpharians *Rhodactis*

rhodostoma (Chadwick-Furman et al. 2000), and for other cnidarians (Chadwick et al. 2000, Cantrell et al. 2015). Based on the findings of León & Bjorndal (2002), the dry biomass composition of *Ricordea florida* can be further broken down into 53.3% organic matter, including 26.5% carbon and 3.5% nitrogen, and energy content of 12.1 kJ g⁻¹ which increases to 22.8 kJ g⁻¹ for ash-free dry mass (León and Bjorndal 2002).

We conclude that on shallow coral reefs, polyps of *Ricordea florida* depend on the presence of shaded microhabitats, and require relatively low levels of solar irradiance in those habitats in order to optimize their fitness-related traits. It is likely that polyps in relatively deep coral reef areas such as on lower reef slopes may alter their microhabitat use, to occur instead in open unshaded habitats due to low downwelling light levels on the deep slope, as known for some other reef anthozoans (Dixon et al. 2014). Shading on shallow Indo-Pacific reefs may be provided by reef holes and crevices due to high rugosity of the hard reef surface (Dixon et al. 2014), but our field observations in Florida suggest that shaded habitats on potentially less rugose Caribbean reefs may more frequently occur under large upright sessile organisms such as octocorals. A large decrease in *R. florida* abundance in the Lower Florida Keys following Hurricane Irma in September 2017, as reported in our related study (Chapter 4) therefore may have been at least partly caused by extensive damage to these large upright reef organisms, many of which were sheared off the reef by the force of the storm. This relationship also indicates that any future disturbances to large octocorals or sponges, including disease or removal, could have devastating consequences for populations of *Ricordea florida* on shallow reefs in Florida and elsewhere in the Caribbean Sea. The depth limit for *R. florida* is at ~ 30 m below sea level, indicating additionally that deep reef slopes potentially could serve as a refuge for this species in case of extensive damage to populations in shallow waters (den Hartog 1980).

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6 References

- Anthony KR, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proc Natl Acad Sci* 105:17442–17446.
- Banon Y, McDonald KC, Eakin CM, Devotta DA, Liu G (2018) Thermal Stress and Bleaching in coral reef communities during the 2014-2016 Caribbean Bleaching Event. American Geophysical Union Fall Meeting Abstracts.
- Cappola VA, Fautin DG (2000) The status of Corallimorpharia (Cnidaria, Anthozoa) based on molecular data from mitochondrial 16s rDNA and nuclear 28s DNA. *Am Zool* 40:961–961.
- Carlgren O (1949) A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *K svenska VtenskAkad Handl* 1:1–121.
- Cato JC, Brown CL (2008) *Marine Ornamental Species: Collection, Culture and Conservation*. John Wiley & Sons, Hoboken, NJ, USA.
- Chadwick NE (1991) Spatial distribution and the effects of competition on some temperate Scleractinia and Corallimorpharia. *Mar Ecol Prog Ser Oldendorf* 70:39–48.
- Chadwick NE, Morrow KM (2011) Competition among sessile organisms on coral reefs. In: Dubinsky Z., Stambler N. (eds) *Coral Reefs: An Ecosystem in Transition*. Dordrecht, Netherlands: Springer 347–371.
- Chadwick-Furman NE, Spiegel M (2000) Abundance and clonal replication in the tropical corallimorpharian *Rhodactis rhodostoma*. *Invertebr Biol* 119:351–360.

- Chadwick-Furman NE, Spiegel M, Nir I (2000) Sexual reproduction in the tropical corallimorpharian *Rhodactis rhodostoma*. *Invertebr Biol* 361–369.
- den Hartog JC (1980) Caribbean shallow water Corallimorpharia. *Zool Verh* 176:1–83.
- Dixon AK, Needham D, Al-Horani FA, Chadwick NE (2014) Microhabitat use and photoacclimation in the clownfish sea anemone *Entacmaea quadricolor*. *J Mar Biol Assoc U K* 94:473–480.
- Douglas AE (2010) *The Symbiotic Habit*. Princeton University Press, Princeton, NJ, USA.
- Dove SG, Hoegh-Guldberg O, Ranganathan S (2001) Major colour patterns of reef-building corals are due to a family of GFP-like proteins: *Coral Reefs* 19:197–204.
- Dunn SR, Thomason JC, Le Tissier MD, Bythell JC, Moosa MK, others (2000) Detection of cell death activity during experimentally induced bleaching of the symbiotic sea anemone *Aiptasia* sp. 1:145–153.
- Fautin DG (2016) Catalog to families, genera, and species of orders Actiniaria and Corallimorpharia (Cnidaria: Anthozoa). *Zootaxa* 4145:1.
- Fautin DG, Lowenstein JM (1994) Phylogenetic relationships among scleractinians, actinarians, and corallimorpharians (Coelenterata: Anthozoa). 2:665–670.
- Fitt W, Brown B, Warner M, Dunne R (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* 20:51–65.
- Godinot C, Chadwick NE (2009) Phosphate excretion by anemonefish and uptake by giant sea anemones: demand outstrips supply. *Bull Mar Sci* 85:1–9.
- den Hartog JC (1980) Caribbean shallow water Corallimorpharia. *Zool Verh* 176:1–83.
- Hines DE, Pawlik JR (2012) Assessing the antipredatory defensive strategies of Caribbean non-scleractinian zoantharians (Cnidaria): Is the sting the only thing? *Mar Biol* 159:389–398.

- Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecol Monogr* 54:187–211.
- Jeffrey ST, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem Physiol Pflanz* 167:191–194.
- Kemp DW, Colella MA, Bartlett LA, Ruzicka RR, Porter JW, Fitt WK (2016) Life after cold death: reef coral and coral reef responses to the 2010 cold water anomaly in the Florida Keys. *Ecosphere* 7:e01373.
- Kuffner IB, Lidz BH, Hudson JH, Anderson JS (2015) A century of ocean warming on Florida Keys coral reefs: Historic in situ observations. *Estuaries Coasts* 38:1085–1096
- Kuguru B, Chadwick N, Achituv Y, Zandbank K, Tchernov D (2008) Mechanisms of habitat segregation between corallimorpharians: photosynthetic parameters and *Symbiodinium* types. *Mar Ecol Prog Ser* 369:115–129.
- Kuguru B, Fine M, Tchernov D (2016) The photo-physiological response of symbionts to combined effects of temperature and pH in a non-calcifying corallimorpharian, *Rhodactis Rhodostoma*. *UOM Research Journal* 18A:122–144.
- Kuguru B, Winters G, Beer S, Santos SR, Chadwick NE (2007) Adaptation strategies of the corallimorpharian *Rhodactis rhodostoma* to irradiance and temperature. *Mar Biol* 151:1287–1298.
- LaJeunesse T (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Mar Biol* 141:387–400.
- Langmead O, Chadwick-Furman NE (1999) Marginal tentacles of the corallimorpharian *Rhodactis rhodostoma*. 1. Role in competition for space. *Mar Biol* 134:479–489.

- León YM, Bjorndal KA (2002) Selective feeding in the hawksbill turtle, an important predator in coral reef ecosystems. *Mar Ecol Prog Ser* 245:249–258.
- Lesser MP (2000) Depth-dependent photoacclimatization to solar ultraviolet radiation in the Caribbean coral *Montastraea faveolata*. *Mar Ecol Prog Ser* 192:137–151.
- Manzello DP (2015) Rapid Recent Warming of Coral Reefs in the Florida Keys. *Sci Rep* 5:16762.
- Medina M, Collins AG, Takaoka TL, Kuehl JV, Boore JL (2006) Naked corals: skeleton loss in Scleractinia. *Proc Natl Acad Sci* 103:9096–9100.
- Miller SL, Chiappone M, Rutten LM (2009) Large-scale assessment of the abundance, distribution, and condition of benthic coral reef organisms in the Florida Keys National Marine Sanctuary – 2009 Quick look report and data summary. Key Largo, FL, USA: CMS/UNCW.
- Muhando CA, Kuguru BL, Wagner GM, Mbije NE, Öhman MC (2002) Environmental effects on the distribution of corallimorpharians in Tanzania. *AMBIO J Hum Environ* 31:558–561.
- Norström A, Nyström M, Lokrantz J, Folke C (2009) Alternative states on coral reefs: Beyond coral–macroalgal phase shifts. *Mar Ecol Prog Ser* 376:295–306.
- Rhyne A, Rotjan R, Bruckner A, Tlusty M (2009) Crawling to collapse: Ecologically unsound ornamental invertebrate fisheries. *PLOS ONE* 4:e8413.
- Roopin M, Chadwick NE (2009) Benefits to host sea anemones from ammonia contributions of resident anemonefish. *J Exp Mar Biol Ecol* 370:27–34.
- Salih A, Larkum A, Cox G (2001) Photoprotection from photoinhibition of symbiotic algae in corals by fluorescent pigments. *Sci Access* 3(1):1-4.

- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671.
- Shelford VE (1931) Some concepts of bioecology. *Ecology* 12:455–467.
- Shick JM (2012) *A Functional Biology of Sea Anemones*. Berlin, Germany: Springer Science & Business Media.
- Siebeck UE, Marshall NJ, Klüter A, Hoegh-Guldberg O (2006) Monitoring coral bleaching using a colour reference card. *Coral Reefs* 25:453–460.
- Torres-Pratts H, Lado-Insua T, Rhyne AL, Rodríguez-Matos L, Schizas NV (2011) Two distinct, geographically overlapping lineages of the corallimorpharian *Ricordea florida* (Cnidaria: Hexacorallia: Ricordeidae). *Coral Reefs* 30:391–396.
- Williams EH, Bunkley-Williams L (2000) Experimental refutation of the ease of associate change by the spotted cleaner shrimp, *Periclimenes yucatanicus* (Decapoda, Palaemonidae), new false-coral associates for the Pederson cleaner shrimp, *P. pedersoni*, and general abundance of associations for both shrimps. *Crustaceana* 73:503–511.
- Work TM, Aeby GS, Maragos JE (2008) Phase shift from a coral to a corallimorph-dominated reef associated with a shipwreck on Palmyra Atoll. *PLoS ONE* 3:e2989.
- Zamer WE (1992) Physiological energetics of the intertidal sea anemone *Anthopleura elegantissima* I. Prey capture, absorption efficiency and growth. *Marine Biology* 92:299–314.

7 Figures

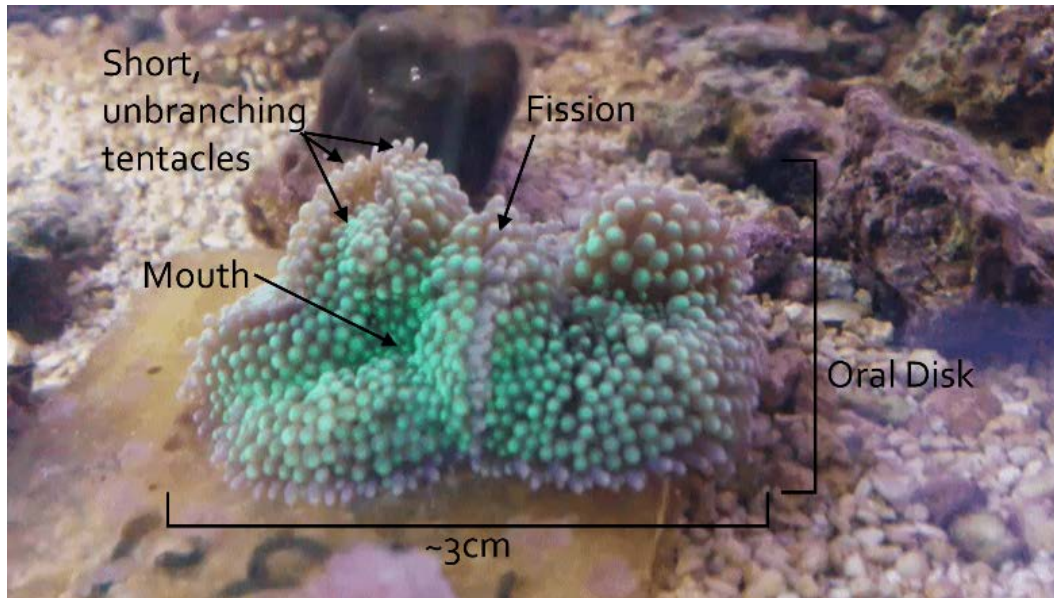
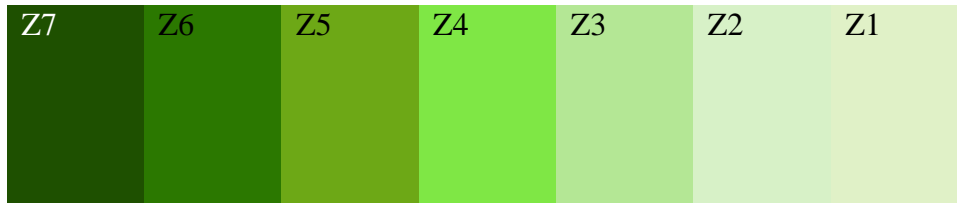


Figure 2-1. Polyp of *Ricordea florida* in the process of clonal replication via longitudinal fission in a laboratory culture tank. The polyp's pedal disk is attached to a Plexiglass plate, and the polyp identification number is visible through the clear plexiglass. Note the arrows indicating the short unbranched tentacles on the oral disk, the 2 mouths at the centers of each of the 2 halves of the dividing polyp, and the line of fission at the center. Scale bar = 3 cm. An ultraviolet (UV) light source at left is causing the polyp especially on that side to exhibit green fluorescent pigmentation.



Ricordea florida color chart

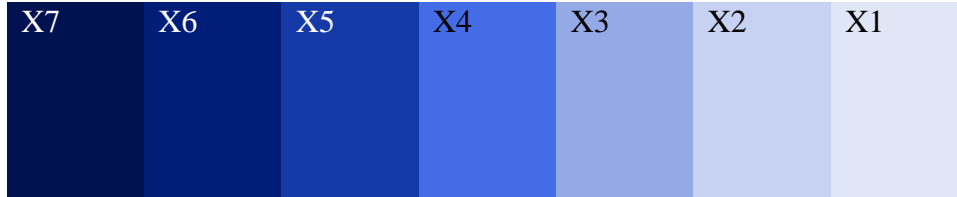


Figure 2-2. Color chart used to quantify levels of body color index (BCI) in polyps of *Ricordea florida*. This is a modified version of the Coral Watch color chart Siebeck et al. (2006), based on the major color patterns of green and blue pigmentation in this species. Note that hue was set to 60 for the green chart, and to 160 for the blue chart, based on hues found in *R. florida*. Saturation and brightness remain the same as in the original Coral Watch chart, but a seventh level was added here (in contrast to only 6 saturation levels in the Coral Watch chart), with a saturation of 255 and a brightness of 40 to account for some very dark polyps in this species.

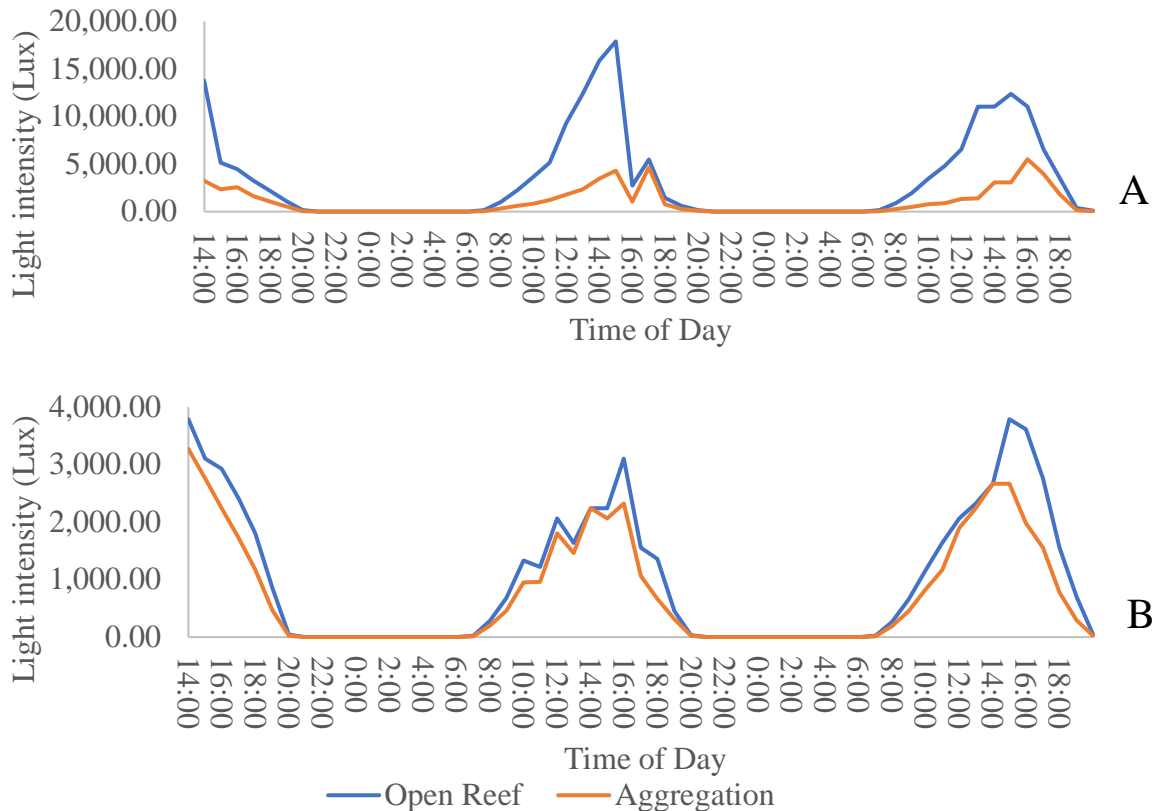


Figure 2-3. Temporal (over 3 days) and spatial (between reef microhabitats) variation in levels of downwelling irradiance on a coral reef at 6 m depth, on the back reef near American Shoal in the lower Florida Keys, where polyps of *Ricordea florida* occurred at high abundance. A. Variation between irradiance on the open exposed (unshaded) reef surface ~ 3 cm distant from an aggregation of polyps, and in shaded reef habitat adjacent to an aggregation of healthy-looking dark green polyps. Data shown for 30 June to 2 July 2018. B. Variation between irradiance on the open exposed (unshaded) reef surface ~ 3 cm distant from an aggregation of polyps, and in recently unshaded habitat adjacent to an aggregation of orange polyps that appeared to be in stressed condition, after an unknown disturbance removed most of the shading organisms near this aggregation. Data shown for 19 July to 21 July 2018. Data are shown as hourly recordings of lux, and thus are for comparative purposes only, and cannot be directly converted to photosynthetically active radiation (PAR).

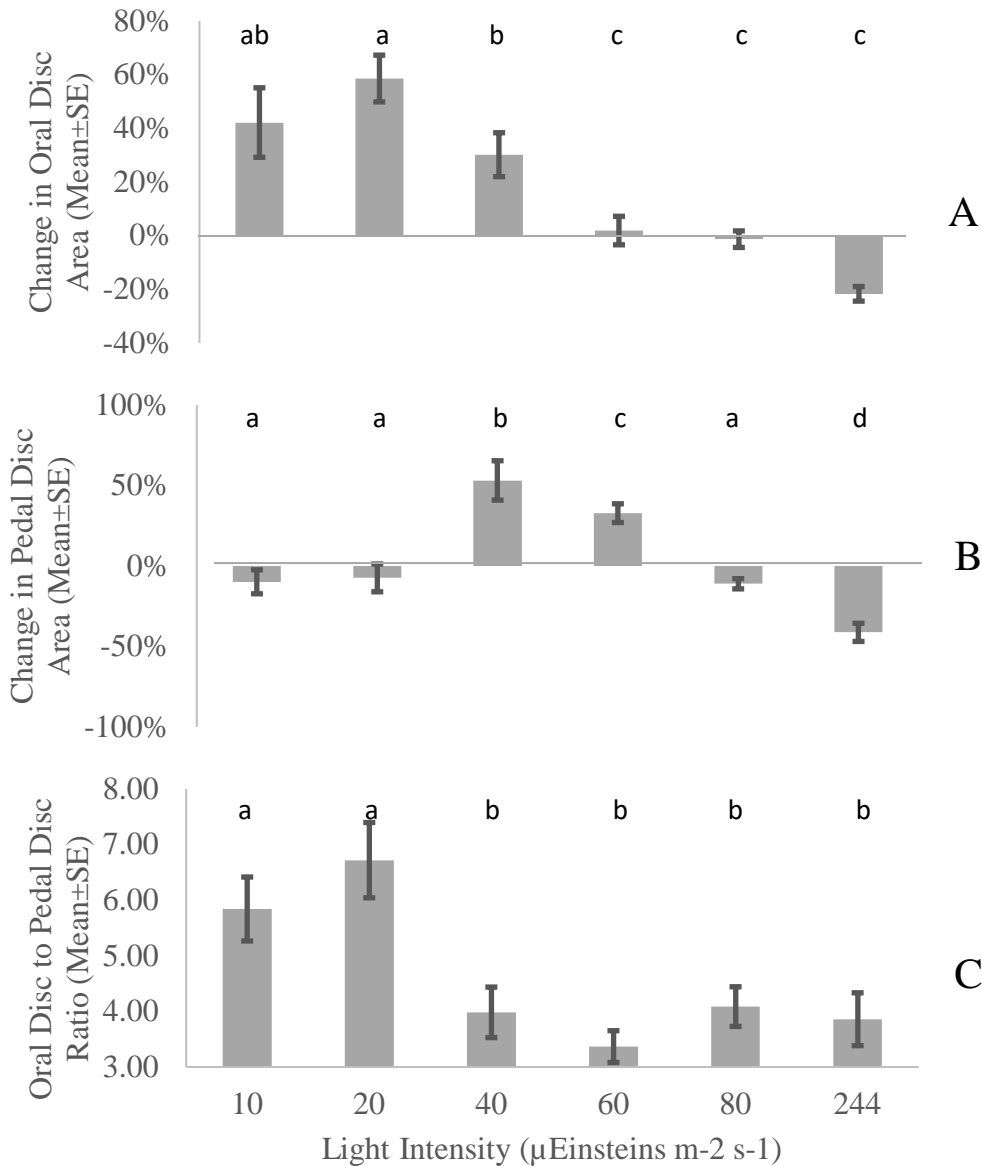


Figure 2-4. Variation in body size characteristics of *Ricordea florida* in response to experimental irradiance laboratory treatments. Shown are values after 8 weeks under treatment conditions at each level of photosynthetically active radiation (PAR). A. Percent change in oral disk area (ODA). B. Percent change in pedal disk area (PDA). C. Ratio of oral to pedal disk area (ROP). The same letters above bars indicate treatments that did not differ significantly from each other. Note that ODA and ROP are maximal at low irradiance level ($20 \mu\text{E m}^{-2} \text{ s}^{-1}$), and that PDA is maximal at slightly higher irradiance level (at $40 \mu\text{E m}^{-2} \text{ s}^{-1}$). See text for details.

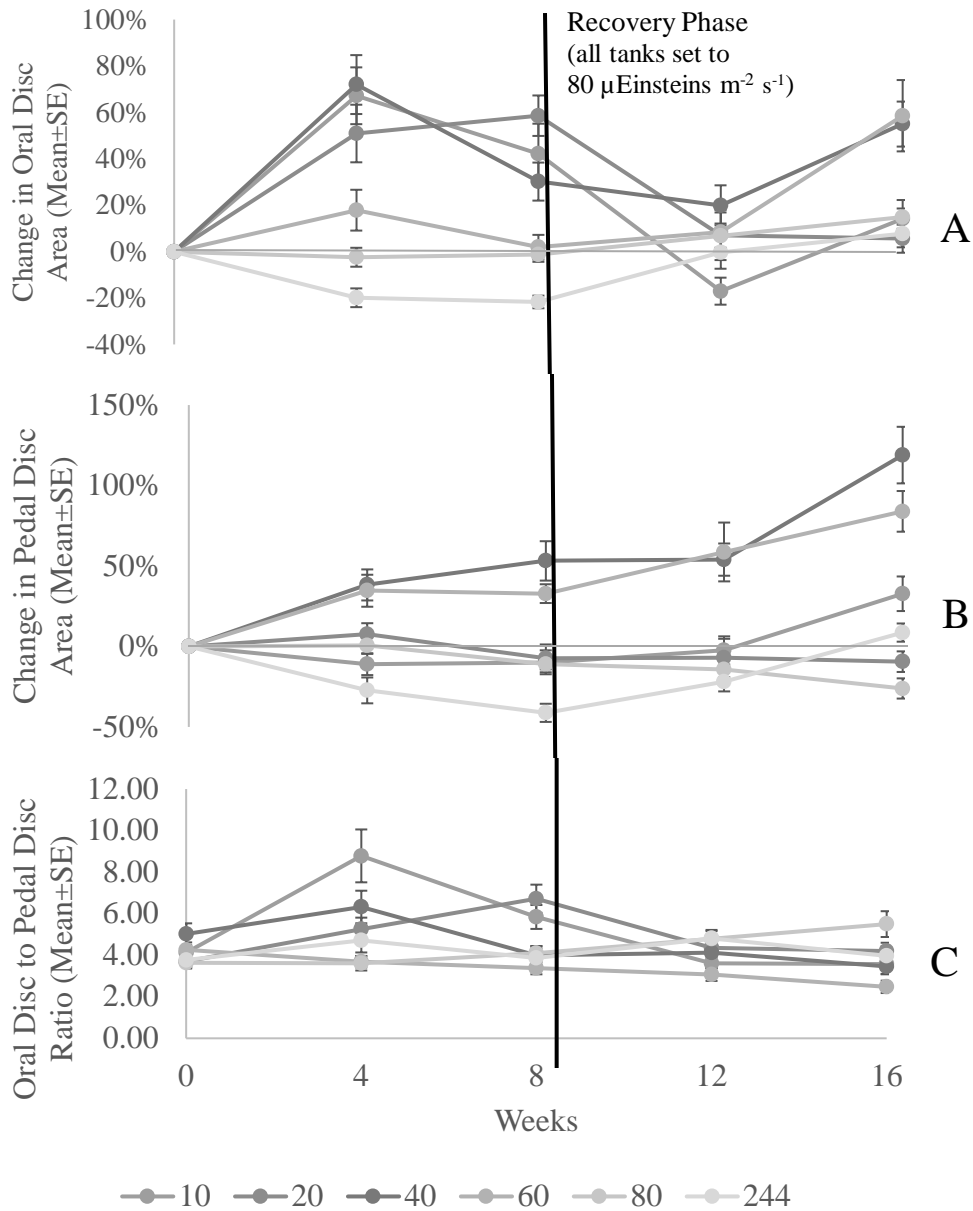


Figure 2-5. Variation over time in the body size characteristics of *Ricordea florida* in response to experimental irradiance laboratory treatments. Shown are values during 8 weeks under treatment conditions, and then 8 weeks of recovery (16 weeks total) when irradiance was returned to 80 $\mu\text{Einsteins m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR). A. Percent change in oral disk area (ODA). B. Percent change in pedal disk area (PDA). C. Ratio of oral to pedal disk area (ROP). See text for details.

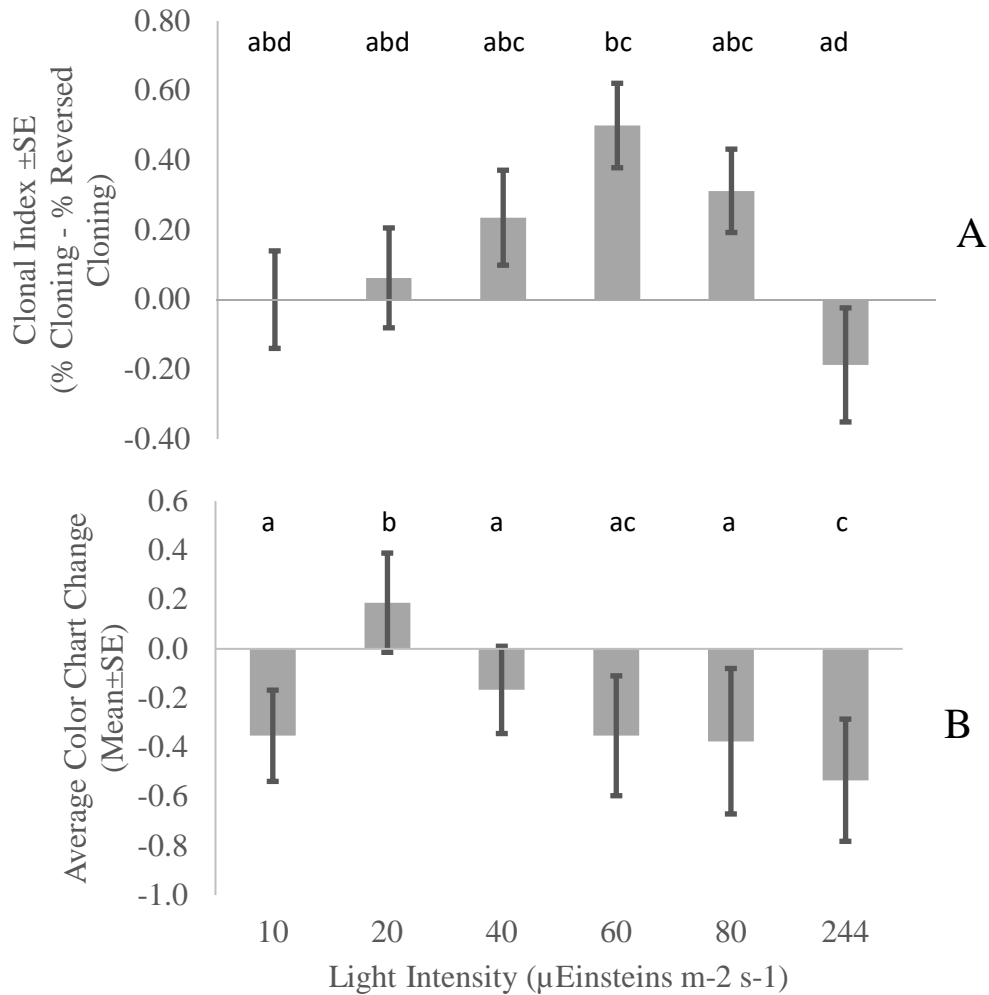


Figure 2-6. Variation in whole polyp characteristics of *Ricordea florida* in response to experimental irradiance laboratory treatments. Shown are values after 8 weeks under treatment conditions at each level of photosynthetically active radiation (PAR). A. Clonal replication stage (CRS). B. Body color index (BCI). The same letters above bars indicate treatments that did not differ significantly from each other. Note that CRS was maximal at the relatively high irradiance level of $60 \mu\text{E m}^{-2} \text{ s}^{-1}$, but that BCI was maximal at lower irradiance of only $20 \mu\text{E m}^{-2} \text{ s}^{-1}$. See text for details.

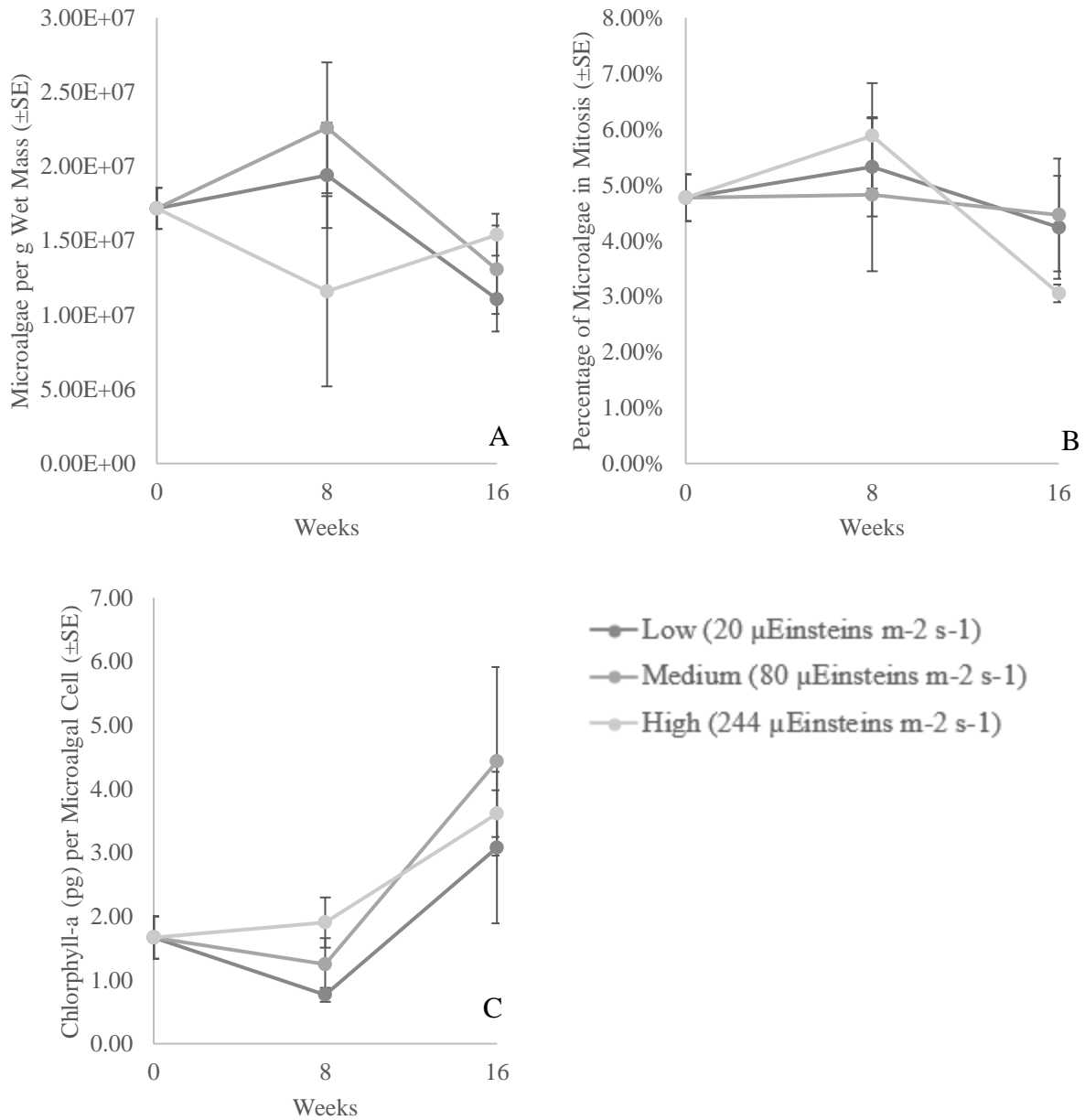


Figure 2-7. Variation over time in the microalgal characteristics of *Ricordea florida* in response to experimental irradiance laboratory treatments. Shown are values after 8 weeks under treatment conditions, and then 8 weeks of recovery (16 weeks total) when irradiance was returned to 80 $\mu\text{Einstein m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR). A. Percent change in microalgal cell abundance (MCA). B. Percent change in mitotic cell index (MCI). C. Percent change in chlorophyll concentration per microalgal cell (CCA). See text for details.

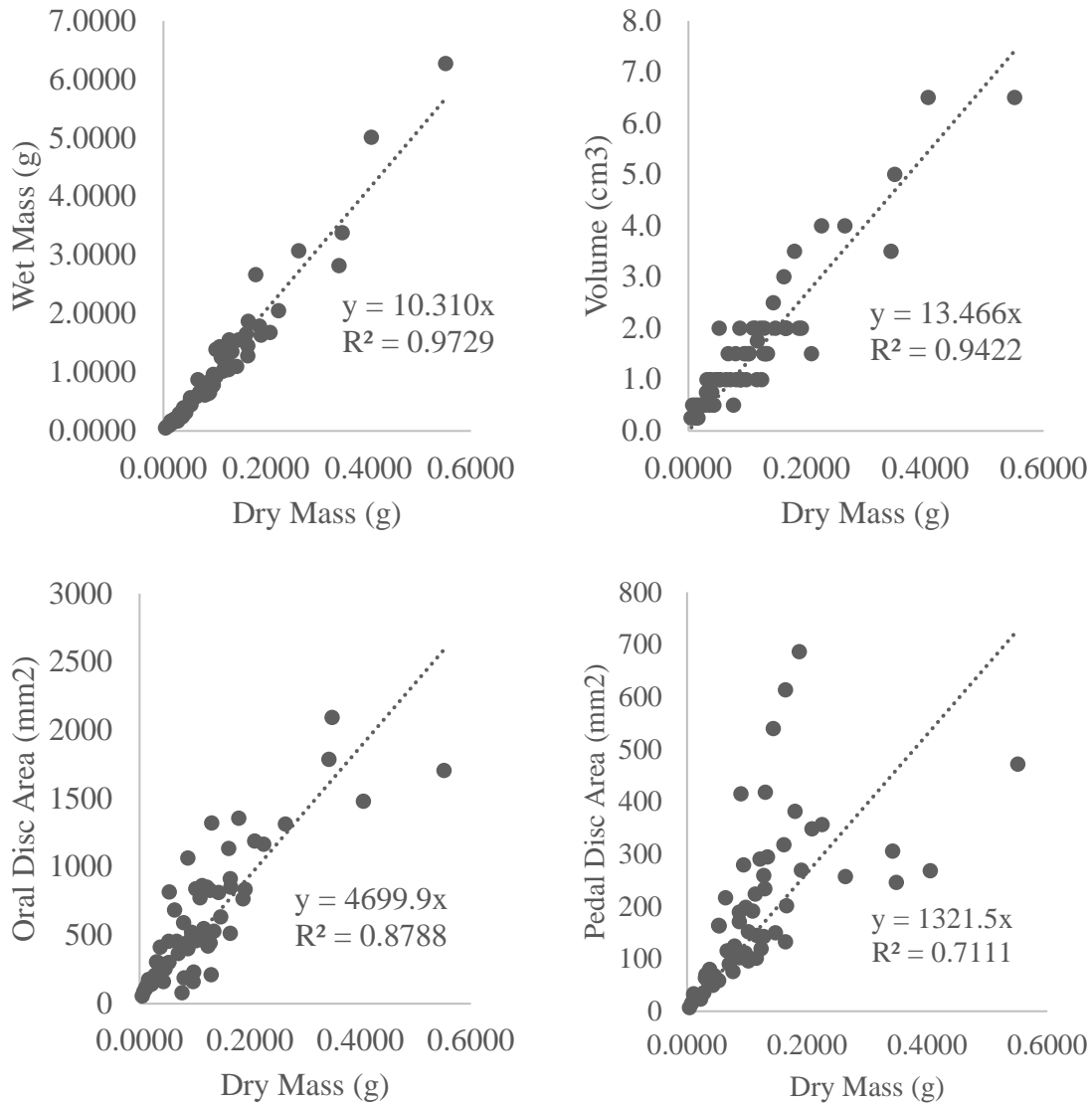


Figure 2-8. Correlations between 4 measures of body size in live polyps of *Ricordea florida* (wet mass [WM], volume [BV], oral disk area [ODA], pedal disk area [PDA]) and 1 measure of body size in sacrificed polyps (dry mass [DM]). Note the fairly wide spread in the data at large polyp sizes, but high correlation coefficient values (R^2) due to the tight correlations at small to medium polyp sizes (N = 112 polyps). All correlations were highly significant ($p < 0.0001$).

Chapter 3

Ecophysiology of Florida false corals *Ricordea florida* (Corallimorpharia: Ricordeidae).

2. Effects of sea water temperature and determination of thermal limits

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1 Introduction

Understanding of the effects of temperature stress on coral reef cnidarians is becoming increasingly important during the current period of anthropogenic climate change, which is causing wide temperature fluctuations on tropical reefs (Fitt et al. 2001; Siebeck et al. 2006; Kuguru et al. 2016). Most types of soft-bodied reef cnidarians, including actinian sea anemones, corallimorpharians, and octocorals, contain endosymbiotic microalgae (Family Symbiodiniaceae) and therefore are susceptible to temperature-induced bleaching, similar to the reef-building scleractinian corals (den Hartog 1980; Fitt et al. 2001; LaJeunesse 2002). The taxonomic group of soft-bodied cnidarians that is most similar to scleractinian corals is the corallimorpharians, which lack a calcareous skeleton and therefore do not build reefs, but otherwise appear to be so similar that some of the latter are referred to colloquially as false corals. Phylogenetic analyses reveal that corallimorpharians arose ~ 110 - 132 million years ago by evolving from a group of scleractinian corals that no longer accreted calcium carbonate skeletons, during a period of rising sea temperatures, relatively low pH and Mg/Ca ratios in the oceans as a result of global climate change (Fautin and Lowenstein 1994; Cappola and Fautin 2000; Medina et al. 2006). These environmental conditions are similar to those that are developing rapidly in the world's oceans today due to anthropogenic climate change, and so deserve investigation in terms of their impacts on extant species of corallimorpharians (Anthony et al. 2008).

All corallimorpharians replicate clonally to produce aggregations of polyps, which may create extensive carpets on shallow reefs in both temperate and tropical regions (Carlgren 1949; Chadwick 1991; Chadwick-Furman et al. 2000; Kuguru et al. 2007). They function as dominant

space competitors with other sessile organisms, especially other cnidarians including stony corals (Langmead and Chadwick-Furman 1999; Chadwick-Furman et al. 2000; Kuguru et al. 2007; Torres-Pratts et al. 2011). Due to their competitive advantage under certain environmental conditions, corallimorpharians may become “winners” during global climate change, in that they are able to monopolize space on shallow tropical reefs after temperature extremes, seawater acidification, or other stressors cause bleaching and mortality of scleractinian corals (Chadwick-Furman and Spiegel 2000; Kuguru et al. 2007; Work et al. 2008). Dominance by corallimorpharians therefore may create phase shifts on coral reefs away from the reef-building scleractinian corals during the Anthropocene, so it is important to understand the ecophysiological factors that may enhance their dominant status (Work et al. 2008; Norström et al. 2009; Chadwick and Morrow 2011). However, no published studies have quantified the temperature tolerances of any of the Caribbean species of corallimorpharians.

Florida false corals *Ricordea florida* (Corallimorpharia: Family Ricordeidae; see Fig. 3-1 in Chapter 2) occur on coral reefs throughout the Florida Keys and Western Atlantic Ocean, and are the most common corallimorpharians in the Florida Keys (de Fonbressin and Michelotti 1860; den Hartog 1980; Fautin 2016). Polyps can reach a diameter of ~ 7.5 cm (although they usually are smaller), and are wide and short, with a broad oral disc covered with many short, unbranched tentacles (den Hartog 1980). Our related studies have revealed that members of this species prefer shaded, low-light habitats on shallow reefs, and that several of their fitness-related traits decline when they are exposed to high levels of irradiance such as found on open exposed reef substrate (Chapter 2).

Individuals of *R. florida* are collected heavily for the ornamental aquarium (Cato and Brown 2008; Rhyne et al. 2009; Torres-Pratts et al. 2011), and in the Florida Keys, recently have

declined to only ~ 10% of pre-2010 abundances (reviewed in Chapter 4). This decline may be due to a combination of over-collecting, environmental stressors such as Hurricane Irma in 2017 (pers. obs.), and temperature extremes in the form of a cold-weather event in 2010 followed by extremely hot summers during 2014 and 2015, which caused widespread bleaching and mortality of reef cnidarians in the Keys. In order to understand the effects of these past temperature extremes and predict impacts of future thermal stress on *R. florida*, experimental studies are needed.

The goals of the present study were to characterize the temperature regime on coral reefs where Florida false corals *R. florida* occur, and to experimentally determine effects of seawater temperature on fitness-related traits in this species. We aimed to quantify the temperature responses of this species in terms of the characteristics of entire polyps (body size, clonal replication rate) and their endosymbiotic microalgae (abundance, mitotic index, chlorophyll a concentration). We also quantified changes in whole-body color (hue) as a potential proxy for estimation of microalgal (Symbiodinaceae) abundance, in an attempt to establish a non-invasive, objective measure of symbiosis health for these corallimorpharians (Siebeck et al. 2006). We hypothesized that (1) the fitness of polyps of *R. florida* is maximized at intermediate coral reef temperatures, and (2) polyps bleach and die at extreme temperatures which represent the upper and lower thermal limits for this species.

2 Methods

2.1 *Field observations*

To characterize variation in seawater temperature on Florida coral reefs where *Ricordea florida* occur, we conducted field observations over 2 years (15 August 2017 to June 2019). We selected a field site at 6m depth on the inner coral reef near American Shoal (Cudjoe Key, Florida, USA; 24.5°N, 81.5°W; more specific coordinates not provided to prevent overcollection of polyps), because this site contained high abundance of polyps of *R. florida* (~ 6 polyps m⁻²; high relative to several other sites in the Florida Keys, Miller et al. 2009; pers. obs.; same field site as described in Chapter 2).

To determine the relative levels of irradiance in reef microhabitats occupied by *R. florida* versus levels on the exposed open reef surface, small HOBO® Pendant Temperature/Light Data Loggers (Onset Computer Corporation, Bourne, MA) were deployed at 6 m depth on the inner reef near American Shoal (Cudjoe Key, FL). This area of high *R. florida* abundance (~6 polyps m⁻²; Chapter 4) was selected based on reports from local Marine Life collectors and Miller et al. (2009). Six data loggers were initially deployed in August 2017, but in September 2017, Hurricane Irma passed directly over the research site and all but one logger was lost. On 30 June 2018, a second logger was deployed. Therefore, 2 loggers were deployed at the field site as of 30 June 2018: (1) in shaded habitat, adjacent to (< 5 cm distance from) a large, healthy aggregation of *R. florida* (randomly selected at the field site that each contained at least 10 healthy-looking, dark green polyps) which was shaded by large octocorals, and (2) in exposed habitat, on haphazardly-selected open reef substrate < 1 m from the selected aggregation. The 2 loggers were each deployed in different reef micro-habitats, to detect potential effects of downwelling light level on seawater temperature near the reef substrate (bottom temperature, Bahr et al. 2016). The field site continued to be revisited each 2-3 months (19 July 2018, 9 August 2018, 1 January 2019, 16 March 2019, 12 June 2019).

Thus, bottom seawater temperature was recorded at the field site every 60 min by at least one logger for the period of 15 August 2017 through September 2019. There are two gaps were from Dec 29, 2017 until Mar 2, 2018 and from Jan 1, 2019 to Jan 13, 2019. The first gap was the result of a flooded logger and the second gap was the result of the devices memory storage being filled due adverse weather conditions delaying collection. These field methods resulted in our obtaining information on variation in seawater temperature on the reef surface adjacent to polyps of *R. florida*, over extended periods in the field.

2.2 *Collection and maintenance of organisms*

To provide individuals of *R. florida* for laboratory experiments, we collected polyps during July 2018 and January 2019, from the coral reef near the above field site (same field site as described in Chapter 2). More polyps were collected for the temperature experiments described here (100 polyps collected total per trip x 2 trips = 200 polyps total) than for those used in irradiance experiments (Chapter 2), because more treatments were applied. Collection and transport methods were the same as those described in Chapter 2, as were the culture conditions for these polyps in the laboratory. However, for the present study, polyps were allowed to attach to unglazed ceramic tiles (ceramic bath tiles each 2.5 x 2.5 cm) instead of to clear Plexiglass plates, because we did not need to view the polyp pedal disks for the present study. Polyps readily attached to the tiles, and grew and fed normally under laboratory culture conditions, indicating healthy condition. Seawater temperature in the culture tanks was maintained at $28 \pm 1^\circ\text{C}$, because preliminary observations indicated that the polyps appeared

healthy at this temperature, and this is within the range of temperatures observed at our field site (see Results).

2.3 *Laboratory experiments*

To test our hypotheses that (1) the fitness of polyps of *R. florida* is maximized at intermediate coral reef temperatures, and (2) polyps bleach and die at extreme temperatures which represent the upper and lower thermal limits for this species, we conducted laboratory experiments. Experimental temperature trials (each ~ 12 week duration) were run between September 2018 and May 2019, with each polyp subjected to one of 8 treatments: 16, 19, 22, 25, 28, 31, 32.5 or 34°C. Treatment temperatures were selected to span the natural range of seawater temperatures recorded in the field (see Sections 2.1 and 3.1), plus a few levels outside that range to determine the upper and lower lethal limits for this species. The 8 treatments were run in batches during the year (2-3 treatments per trial x 3 trials) rather than all simultaneously, due to space constraints involving the number of separate replicate tanks (each with its own sump tank filter system) needed during each trial. To initiate a temperature trial, we randomly selected 2-3 of the 8 treatment temperatures (ranging 16-34°C, see above), and then randomly assigned 20-24 polyps to each treatment (8 treatments x 20-24 polyps per treatment = 179 total treatment polyps). We cultured the remaining 31 polyps in a reserve tank (179 treatment polyps + 31 reserve polyps = 200 total polyps collected). Each group of 20-24 treatment polyps then was assigned randomly to 1 of 2 treatment tanks at a designated temperature (10-12 polyps per tank), to provide tank replication within treatment (2 treatment tanks x 2-3 treatments per trial = 4-6 total tanks used per trial).

To begin each trial, the seawater temperature in each tank was adjusted gradually over 1 wk. A Bayite Temperature Controller (Model# BTC201, Shenzhen, Guangdong, China) attached to submerged aquarium heater(s) was used to raise seawater temperatures in the tanks that were assigned to treatments above the normal culture temperature of 28°C (see Section 2.2). A small pump that sent water from the sump tank through coiled tubing in a chilled water bath, also connected to a Bayite Temperature Controller, was used to lower the temperatures in tanks assigned to treatments below normal culture temperature. Then treatment temperatures were maintained for 5 weeks, slowly returned to normal culture temperature (28°C) over 1 week, and maintained at this recovery temperature for 5 more weeks (total trial period = 1 week adjustment + 5 weeks treatment + 1 week adjustment + 5 weeks recovery = 12 weeks per trial). All other environmental conditions in each tank (irradiance, water flow, dissolved nutrients, feeding regime) remained the same as under regular culture conditions (see Chapter 2 for details).

Throughout each 12-week trial, we measured 6 fitness-related characteristics of the experimental polyps: 3 whole-polyp characteristics (oral disc area [ODA], body color index [BCI], clonal replication status [CRS]), and 3 microalgal characteristics (microalgal cell abundance [MCA], mitotic index [percent cells dividing; MTI], chlorophyll concentration per algal cell [CCA] after Dunn et al. 2000). The 3 whole-polyp characteristics were measured 7 times during each trial for all treatment polyps; at weeks 0 (prior to trial), 1 (after treatment temperature had been reached), 3, 6 (end of treatment), 7 (after return to culture temperature of 28°C), 9, and 12 (end of recovery period). The 3 microalgal characteristics were assessed only 3 times (at weeks 0, 6, and 12) for only 4 polyps per treatment each time (2 polyps per tank x 2 tanks per treatment = 4 polyps total per treatment; < 20% of polyps), because they involved

removal of polyp tissue (similar to methods for measurement of characteristics in response to variation in irradiance levels, Chapter 2).

To measure whole-polyp characteristics, we photographed each tank from above, with a scale bar and color chart placed inside the tank on the same plane as the polyps for reference. We used a modified version of the Coral Watch Coral Health Chart (Siebeck et al. 2006; for details see Chapter 2, especially Fig. 2-2). We added a scale bar, and slightly changed the hues used in our chart, to account for the range of hues that we observed to occur naturally in this species, while maintaining saturation and brightness values consistent with those in the standard coral chart (Fig. 3-2; Siebeck et al. 2006). All pumps and lights were turned off for ~ 30 sec prior to photographing the tank, to ensure no distortion or glare in the photograph.

We analyzed the photographs using ImageJ (Schneider et al. 2012), and characterized BCI using the modified coral chart, to quantify changes in whole-body coloration and relate them to characteristics measured for endosymbiotic microalgae within the polyps (see details below and in Chapter 2). We also outlined the oral disc of each polyp and calculated ODA as a measure of body size. We used ODA as the measure of body size for the present study, because our related studies showed that ODA correlates tightly with pedal disk area and all other body size measures, and therefore is an accurate measure of body size in this species (Chapter 2).

The CRS of each polyp also was assigned 1 of 3 categories: 1 (initiated or progressed in clonal replication; positive replication), 0 (no change in clonal replication status), or -1 (regressed in clonal replication status; negative replication; details in Chapter 2).

To measure the 3 microalgal characteristics (MCA, MTI, and CCA), fewer polyps were sampled less frequently than for whole-polyp characteristics, because microalgal examination required the removal of a relatively large proportion of tissue from these small-bodied polyps, to

obtain enough tissue for assessment (~ 10% of polyp wet mass = 0.02-0.20 g wet mass, similar to the wet mass removed to measure microalgal characteristics in past studies on corallimorpharians, Kuguru et al. 2016). We processed tissue samples from polyps and analyzed microalgal characteristics using standard methods (see Chapter 2 for details).

2.4 *Data analysis*

Effects of experimental temperatures on whole-polyp and microalgal characteristics were analyzed in R (R Foundation for Statistical Computing, Vienna, Austria) using the “nlme” package. For analysis of changes in ODA, percent change between from beginning of the trials (week 0) was used. Tank was included as a random effect. The same statistical test was used for CRS, BCI, and microalgal characteristics. A t-test was also performed before starting trials between treatments to detect any systematic variation in polyp size between treatments and during trials between tanks within a treatment to detect any tank effects.

Potential variation in microalgal abundance (MCA) with the body color index (BCI) was examined using simple linear regression, with the dataset including polyps from the present study and some from our related study on effects of photosynthetically active radiation on *R. florida* (n= 113 polyps; Chapter 2). The subset of polyps from each of these 2 studies was selected for this regression analysis haphazardly, until a sample size of > 100 polyps total was reached.

3 Results

3.1 *Field observations*

Bottom seawater temperature at the field site (6 m depth) varied over the 2-yr study period from a minimum of 20.7°C to a maximum of 32.4°C (Fig. 3-3). The maximum field temperature of 32.4°C was recorded for > 1 hour during each of 3 days in a row between August 17-19, 2017. Temperatures also exceeded 32.0°C during 4 d in a row between August 16-20, 2017, then on 1 day the next summer (Jul 15, 2018), and during another 3 day in a row between July 28-31, 2018.

In contrast, during winter the temperature dropped to a minimum of 20.7°C, on Jan 29 - 30, 2019, and also was < 21.0°C throughout much of the day on January 23, 2019. Interestingly, throughout those relatively cool winter days, the temperature was ~1°C higher overnight than during the day (Fig. 3-3). Throughout the 2 years of field data collection, the mean bottom seawater temperature on the reef surface was 27.6°C and the median was 27.5°C.

Of particular interest was the temperature variation during Sep 2017, when Hurricane Irma (category 4) passed over the field site. The eye of the storm passed over the site early during the morning of Sep 10, and the one logger that was recovered afterwards had collected data before, during, and after the storm (Fig. 3-3). The week before the arrival of the storm, daily temperatures were > 30.6°C, but between 20:00 on Sep 9 and 18:00 on Sep 10, the temperature dropped 2.9°C to only 27.7°C.

Comparison of bottom temperatures between the shaded and exposed reef habitats revealed only a very small difference of < 0.1°C during all periods, over the 15 months when 2 loggers were present on the reef (June 2018 to September 2019).

3.2 *Laboratory experiments*

At the beginning of the temperature trials (week 0), polyps did not differ significantly among the 8 treatment temperature groups, in terms of most of the 3 whole-polyp characteristics examined (oral disk area [ODA], clonal replication stage [CRS], or body color index [BCI]; $p > 0.05$, $n = 20-24$ polyps per treatment). There were 4 exceptions in this pattern, among the 8 treatment groups x 3 whole-polyp characteristics measured for each group (24 datasets total). At week 0 the polyps assigned to the 16°C treatment exhibited significantly larger ODA and lighter color (BCI) than did those at the start of all other treatments ($p < 0.05$), possibly because these polyps had been cultured in the laboratory setting for a longer period of time than most other polyps, as this was one of the last temperature trials run. Also, slightly but significantly fewer polyps assigned to the 31°C treatment exhibited the same clonal replication stage (CRS) at week 0, than did polyps in the other treatments ($p < 0.05$), and the polyps assigned to the 22°C and 32.5°C treatments exhibited significantly paler hue (on average ~ 0.2 steps less color in BCI) at week 0 than did the polyps in the other treatments ($p < 0.05$).

All polyps exposed to the two most extreme temperatures (16 and 34°C) died before the end of the 5-week treatment period, and therefore were excluded from statistical analyses. At the lowest temperature (16°C), no polyps died during the first week, but ~50% died by 3 weeks, and all had expired by 4 weeks. Even more rapid mortality was observed for polyps exposed to the highest temperature (34°C), in which 8% of polyps died by week 1, then 88% died as of week 2, and all were gone by week 3. At the less extremely high temperature (32.5°C), only 1 polyp had died by week 6, and in all of the other 5 temperature treatments, no mortality was observed.

By week 5, polyp body size (oral disk area, ODA) differed significantly among the 6 non-lethal treatments (19-32.5°C, $p < 0.001$, Fig. 3-4). Pairwise comparisons revealed that polyps exposed to the highest non-lethal temperature of 32.5°C shrank significantly more than in all other temperatures ($p < 0.05$). In contrast, the polyps exposed to a relatively high temperature of 31°C grew significantly more than did those at both the lowest (19°C) or and highest non-lethal temperature (32.5°C, $p < 0.05$). Polyps at all of the more moderate temperatures (22-31°C) did not differ from each other in body size and grew significantly more than did those at 19°C ($p < 0.05$).

Polyps exposed to medium-to-high temperatures (25-31°C) all exhibited positive clonal indexes (clonal replication stage, CRS), which indicated active clonal replication; those at the moderate temperatures of 25 and 28°C had significantly higher CRS than those at 31°C (both $p < 0.05$, Fig. 3-4). Interestingly, at the very low, lethal temperature of 16°C, almost half the polyps (11/24) progressed in clonal replication before they died with all 11 rapidly completing fission. Then both of the newly-fissioned daughter polyps produced by each of the 11 replicating polyps died at the same time. This process caused the CRS at 16°C to be significantly higher than for those of polyps in any other temperature treatment ($p < 0.01$). None of the 34°C polyps completed clonal replication before they all died. Polyps in all of the other temperature treatments did not differ significantly from each other in the proportion that completed clonal replication, during either the treatment or recovery periods (Fig. 3-5; $p < 0.05$ for both periods).

Body Color Index (BCI) measurements indicated that polyps at the moderately high temperature of 28°C became significantly darker in hue than those in all 5 other non-lethal treatments ($p < 0.05$, Fig. 3-4). In contrast, polyps at the highest non-lethal temperature (32.5°C) exhibited substantial bleaching, in that they became lighter in hue (by 3.2 color steps during the

6-wk treatment period), rendering them significantly lighter in BCI than the polyps in all other non-lethal temperature treatments ($p < 0.05$).

After polyps in the temperature treatments all were returned their normal culture temperature of 28°C, some of them exhibited recovery of fitness-related traits, especially those that had been exposed previously to extreme but not lethal temperatures. For most groups, whole body size (ODA) did not appear to change substantially after 5 weeks under recovery conditions, with no significant difference in ODA among polyps in 5 of the groups (those exposed to 19-31°C, $p > 0.05$). In addition, the already-shrunken polyps that had been exposed to the highest non-lethal temperature (32.5°C) and were bleached (Fig. 3-4) continued to decline in body size during the recovery period (Fig. 3-4). They did not continue to replicate clonally, in that their CRS remained significantly lower compared to that of polyps that had received all other treatments, except those at 25°C and 19°C. None of the other groups (those at 19-31°C) differed significantly in clonal replication status (CRS) by week 5 of the recovery period (Fig. 3-3). Polyps that had bleached during the high temperature (32.5°C) treatment also did not recover from bleaching, in that they did not significantly increase their body hue (BCI) by the end of the 5-week recover period ($p < 0.05$). Polyps from the 31°C treatment likewise continued to lose pigment, in that they became slightly but significantly lighter in hue during recovery, than did those exposed to more moderate temperatures (22°C or 28°C, Fig. 3-4). In contrast, polyps from the 22°C treatment became significantly darker during recovery than did those exposed to lower temperature (19°C, $p < 0.05$). None of the other groups differed significantly in hue by the end of recovery.

The 3 examined microalgal characteristics varied widely among the few individuals sampled within each temperature treatment. However, some trends were significant and matched

those of the whole-polyp characteristics. Similar to the whole-body bleaching and shrinkage observed in polyps exposed to the highest non-lethal temperature (32.5°C), the microalgal cell abundance (MCA) quantified in their tissues also was significantly lower than in polyps at some of the more moderate temperatures (22°C and 28°C, $p < 0.05$), and remained so during the recovery period (Fig. 3-5A). After 6 weeks in treatments, the polyps at 32.5°C also exhibited significantly lower division rates of their microalgae (mitotic index, MTI) than in polyps at slightly lower temperature (31°C, $p < 0.05$), but then increased their MTI during recovery such that no significant difference remained by the end of the trial ($p = 0.86$; Fig. 3-5B). Chlorophyll concentrations in the microalgae (CCA) varied widely within the treatment groups and not significantly among them, during any stage of the trials ($p > 0.05$; Fig. 3-5C). The body hue of whole polyps (Body Color Index, BCI) correlated loosely but significantly with the abundance of microalgal cells inside their tissues ($n = 112$, $r^2 = 0.134$, $p < 0.001$, Fig. 3-6). Wide scatter in the data as indicated by the low correlation coefficient revealed a large amount of variation in cell abundance (MCA) with polyp color (BCI), and thus a fairly weak relationship between these two indicators of polyp health and bleaching status.

All treatment results were examined for tank effects, by comparing the responses of polyps from 1 tank to those in the other tank exposed to the same treatment conditions. Both before and during trials, the 2 tanks in each treatment did not differ significantly from each other in any of the whole-polyp or microalgal characteristics ($p > 0.17$ for all comparisons), with one exception. Polyps in the 2 tanks exposed to 25°C differed significantly from each other at the end of the 6-wk treatment period, in terms of their ODA ($p < 0.05$). No obvious cause for this tank effect was observed, in terms of water quality parameters, level of macroalgal growth, or any other detectable differences between these 2 tanks.

4 Discussion

We demonstrate here that seawater bottom temperatures vary widely on a seasonal basis on a coral reef in the Florida Keys where Florida false corals *Ricordea florida* are abundant, and that annual average temperatures observed in the field (~ 26-28° C) are the same as those observed in the laboratory to cause maximal levels of fitness-related traits in this species. Our experimental results indicate that optimal temperature for this species appears to be ~ 28°C, that 19° and 31°C are the most extreme sublethal temperatures these polyps can tolerate without mortality, and that more extreme temperatures (16° and 34°) are lethal. These sublethal laboratory temperatures also closely match the observed annual minimum and maximum temperatures observed at our field site (~ 19-32°C). This species appears to follow Shelford's Law of Tolerance (Shelford 1931), in that polyp body size (as measured in ODA), clonal replication status (CRS), and body hue (body color index, BCI) all remain high throughout a broad optimal temperature range of 22 - 31°C, with maxima at ~ 25-28°C, and a rapid transition to intolerance from even short exposures (< 3 weeks) at only 3-6°C outside of this range. The recorded annual natural temperature extremes of ~ 19 and 32.5°C recorded in our field observations thus appear to represent stressful environmental conditions for these polyps, in that they shrink and cease or reverse clonal replication, with the polyps at the higher temperature (32.5°C) also losing color beyond the 2-step bleaching threshold established by the Coral Watch Color Chart (Siebeck et al. 2006).

No differences in temperature were recorded based on level of shading. This is different than the findings of Bahr et al. (2016) and is likely because their loggers were deployed at a

depth of only 35cm and ours were at a depth of ~ 6m. The deeper depth and therefore weaker irradiance and more convective mixing likely buffered against these effects.

In terms of microalgal characteristics examined here, the observed wide variation within treatments and only limited treatment effects likely are due in part to the limited number of samples we obtained for these analyses. We were unable to collect additional microalgal samples during our experiments, because adequate tissue mass for microalgal analysis could not be obtained from these small, short-tentacled polyps without causing extensive polyp damage during the trials. A future study could remedy this limitation by using a much larger number of polyps, so that whole polyps could be sacrificed each week for microalgal analysis. Alternately, methods could be developed to obtain more accurate data from very small tentacle tissue samples collected repeatedly from all polyps in the study, which will be difficult to do based on the overall small body size and short tentacles in this species (see also discussion of this issue in Chapter 2).

Despite the wide variation among our samples, we observed a significant decrease in microalgal cell abundance at 32.5°C compared to more moderate temperatures, which correlated significantly with the observed loss of whole-polyp color at this temperature. This pattern indicates that *R. florida* polyps outside of their optimal temperature range (22 - 31°C, with maxima at ~ 25-28°C), will experience a significant decrease in their concentrations of endosymbiotic microalgae, and will become visibly bleached, similar to the pattern known for scleractinian corals in Florida (reviewed in Fitt et al. 2001).

The tank effect that we observed in the 25°C treatment caused a depression in polyp coloration and microalgal concentration even at this moderate temperature, due a difference in the results from the two experimental tanks used. As such, results from the 25°C trial cannot not

on their own be used to draw conclusions about temperature tolerance in this species. However, polyps incubated at temperatures both slightly below (22°C) and above (28°C) this value exhibited high microalgal concentrations and no loss of coloration, so we conclude that 25°C is within the optimal temperature range for *R. florida*.

Due to the weak correlation between microalgal concentration and polyp body color, only ~ 13% of changes in microalgal concentration can be accounted for by body color level. Therefore, while body color may be a partial indicator of polyp health and bleaching status, it should not be used alone to predict or quantify microalgal concentrations in these corallimorpharians, unlike the conclusions of Siebeck et al. (2006) for scleractinian corals.

We conclude that polyps of *R. florida* can adapt to a wide range of temperatures, 19-31°C, with an optimal range broadly between 22 and 28°C, but maximal values of some fitness traits at the upper end of this range, at ~26-28°C. When comparing this range to the conditions recorded on the reef Lower Florida Keys over the past 2 years, it appears that *R. florida* in their natural habitat have recently experienced low temperatures near the limit of their optimal range, and high temperatures that result in polyp stress. Examination of a 30-year data set of subsurface water temperatures in the Upper Florida Keys at Molasses Reef reveals that during the cold event of 2010 in the Keys, seawater temperatures dropped below 16°C, representing the zone of intolerance for this species (Fig. 3-7; NOAA NBDC Station# MLRF1). Therefore, we conclude that extremely cold temperatures during the cold-water event likely were at least partially responsible for the rapid drop in populations of this species reported by marine life collectors around this time (reviewed in Chapter 4). We also conclude that during the past several summers, members of this important ornamental species have increasingly been exposed to their upper thermal limits of tolerance, in that the temperatures we recorded in the field adjacent to

aggregations exceeded 32°C for several days during each of the 3 summers in 2017, 2018, and 2019. As such, potential future increases in bottom seawater temperature on Caribbean coral reefs during the summer, of even ~ 1.5°C above these recently recorded values (i.e., to > 33°C), and conversely any winter drops in temperature of only ~ 4°C below these values (to 16°C, such as may occur during cold snaps or El Niño events), if persisting for ~ 3 weeks or even possibly shorter, could lead to the eradication of this species from the Caribbean Sea.

5 Acknowledgements

Specimens were collected under FWC Permit #SAL-17-1907-SR and NOAA Permit #FKNMS-2017-031. Funding was provided by a Lerner-Gray Grant for Marine Research from the American Museum of Natural History to NP, and by an Intramural Grant from Auburn University to NEC. We thank Captain Roy Herndon and his daughter Dayanara for discussions about Marine Life Fisheries and for providing boating support and access to our field site. Special thanks to Dr. Donald Behringer (University of Florida) and Bennett Paradis for assisting in the project design. We thank members of the Chadwick laboratory group at Auburn University for assistance with the daily tank care that supported our laboratory culture and experiments on these corallimorpharians.

6 References

- Anthony KR, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proc Natl Acad Sci* 105:17442–17446.
- Bahr KD, Jokiel PL, Rodgers KS (2016) Influence of solar irradiance on underwater temperature recorded by temperature loggers on coral reefs: Evaluation of underwater temperature loggers. *Limnol Oceanogr Methods* 14:338–342.
- Cappola VA, Fautin DG (2000) The status of Corallimorpharia (Cnidaria, Anthozoa) based on molecular data from mitochondrial 16s rDNA and nuclear 28s DNA. *Am Zool* 40:961–961.
- Carlgren O (1949) A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *K svenska VtenskAkad Handl* 1:1–121.
- Cato JC, Brown CL (2008) *Marine Ornamental Species: Collection, Culture and Conservation*. Hoboken, NJ, USA: John Wiley & Sons.
- Chadwick NE (1991) Spatial distribution and the effects of competition on some temperate Scleractinia and Corallimorpharia. *Mar Ecol Prog Ser Oldendorf* 70:39–48.
- Chadwick NE, Morrow KM (2011) Competition among sessile organisms on coral reefs. In: Dubinsky Z., Stambler N. (eds) *Coral Reefs: An Ecosystem in Transition*. Dordrecht, Netherlands: Springer, pp 347–371.
- Chadwick-Furman NE, Spiegel M (2000) Abundance and clonal replication in the tropical corallimorpharian *Rhodactis rhodostoma*. *Invertebr Biol* 119:351–360.

- Chadwick-Furman NE, Spiegel M, Nir I (2000) Sexual reproduction in the tropical corallimorpharian *Rhodactis rhodostoma*. *Invertebr Biol* 361–369.
- Den Hartog JC (1980) Caribbean shallow water Corallimorpharia. *Zool Verh* 176:1–83.
- Dunn SR, Thomason JC, Le Tissier MD, Bythell JC, Moosa MK, others (2000) Detection of cell death activity during experimentally induced bleaching of the symbiotic sea anemone *Aiptasia* sp. 1:145–153.
- Fautin DG (2016) Catalog to families, genera, and species of orders Actiniaria and Corallimorpharia (Cnidaria: Anthozoa). *Zootaxa* 4145:1.
- Fautin DG, Lowenstein JM (1994) Phylogenetic relationships among scleractinians, actinarians, and corallimorpharians (Coelenterata: Anthozoa). 2:665–670.
- Fitt W, Brown B, Warner M, Dunne R (2001) Coral bleaching: Interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* 20:51–65.
- de Fonbressin PD, Michelotti G (1860) Mémoire sur les coralliaires des Antilles. De l'imprimerie royale, Roma, Italy: Sapienza University of Rome.
- den Hartog JC (1980) Caribbean shallow water Corallimorpharia. *Zool Verh* 176:1–83.
- Kuguru B, Fine M, Tchernov D (2016) The photo-physiological response of symbionts to combined effects of temperature and pH in a non-calcifying corallimorpharian, *Rhodactis Rhodostoma*. *UOM Research Journal* 18A:122–144.
- Kuguru B, Winters G, Beer S, Santos SR, Chadwick NE (2007) Adaptation strategies of the corallimorpharian *Rhodactis rhodostoma* to irradiance and temperature. *Mar Biol* 151:1287–1298.
- LaJeunesse T (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Mar Biol* 141:387–400.

- Langmead O, Chadwick-Furman NE (1999) Marginal tentacles of the corallimorpharian *Rhodactis rhodostoma*. 1. Role in competition for space. *Mar Biol* 134:479–489.
- Medina M, Collins AG, Takaoka TL, Kuehl JV, Boore JL (2006) Naked corals: skeleton loss in Scleractinia. *Proc Natl Acad Sci* 103:9096–9100.
- Miller SL, Chiappone M, Rutten LM (2009) Large-scale assessment of the abundance, distribution, and condition of benthic coral reef organisms in the Florida Keys National Marine Sanctuary – 2009 Quick look report and data summary. Key Largo, FL: CMS/UNCW.
- Norström A, Nyström M, Lokrantz J, Folke C (2009) Alternative states on coral reefs: beyond coral–macroalgal phase shifts. *Mar Ecol Prog Ser* 376:295–306.
- Rhyne A, Rotjan R, Bruckner A, Tlusty M (2009) Crawling to collapse: Ecologically unsound ornamental invertebrate fisheries. *PLOS ONE* 4:e8413.
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671.
- Shelford VE (1931) Some concepts of bioecology. *Ecology* 12:455–467.
- Siebeck UE, Marshall NJ, Klüter A, Hoegh-Guldberg O (2006) Monitoring coral bleaching using a colour reference card. *Coral Reefs* 25:453–460.
- Torres-Pratts H, Lado-Insua T, Rhyne AL, Rodríguez-Matos L, Schizas NV (2011) Two distinct, geographically overlapping lineages of the corallimorpharian *Ricordea florida* (Cnidaria: Hexacorallia: Ricordeidae). *Coral Reefs* 30:391–396.
- Work TM, Aeby GS, Maragos JE (2008) Phase shift from a coral to a corallimorph-dominated reef associated with a shipwreck on Palmyra Atoll. *PLoS ONE* 3:e2989.

7 Figures

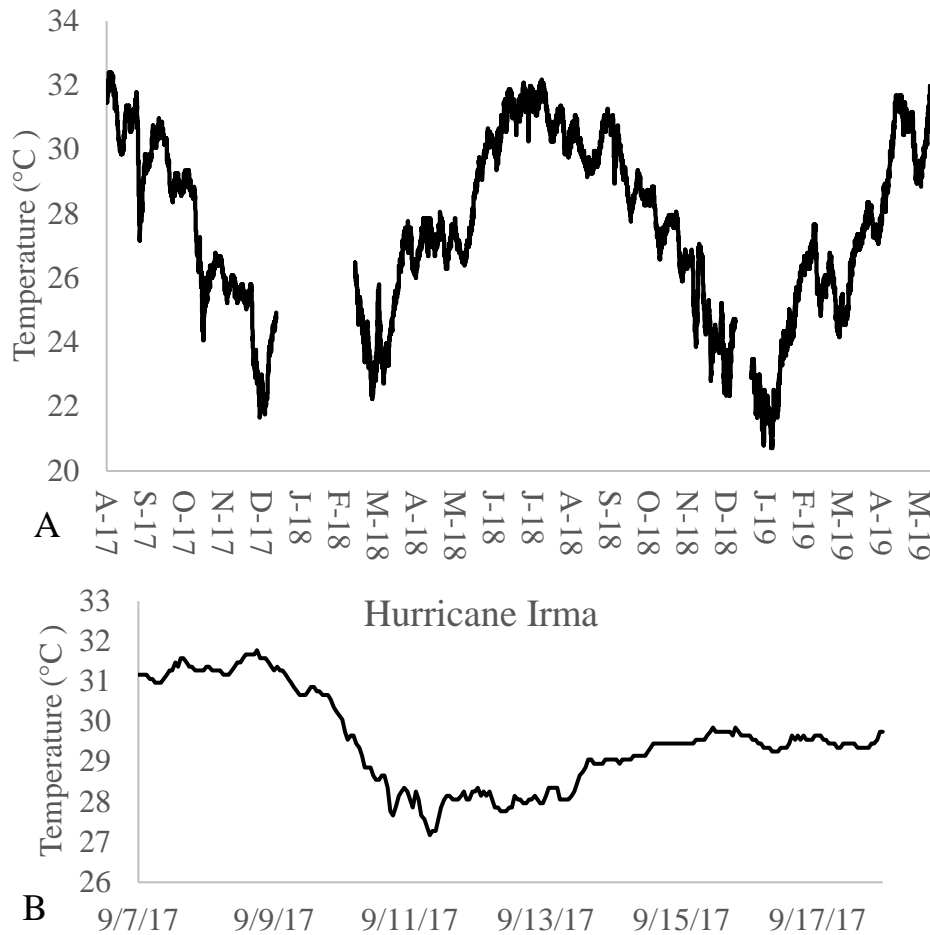


Figure 3-1. Temporal variation in bottom seawater temperature at 6 m depth on a back reef near American Shoal, Cudjoe Key, Florida, USA, as recorded by a HOBO® Pendant Temperature/Light Data Loggers (Onset Computer Corporation, Bourne, MA) attached to the reef surface. A. Variation over ~ 2 yr (August 2017 – May 2019). The 2 gaps in data were caused by the logger becoming flooded during January to February 2018, and the memory card becoming filled before recovery in December 2018. B. Variation over 10 days in September 2017, around the time that Hurricane Irma (category 4) passed directly over the study site on September 10. Note the drop in temperature as the storm arrives and after.

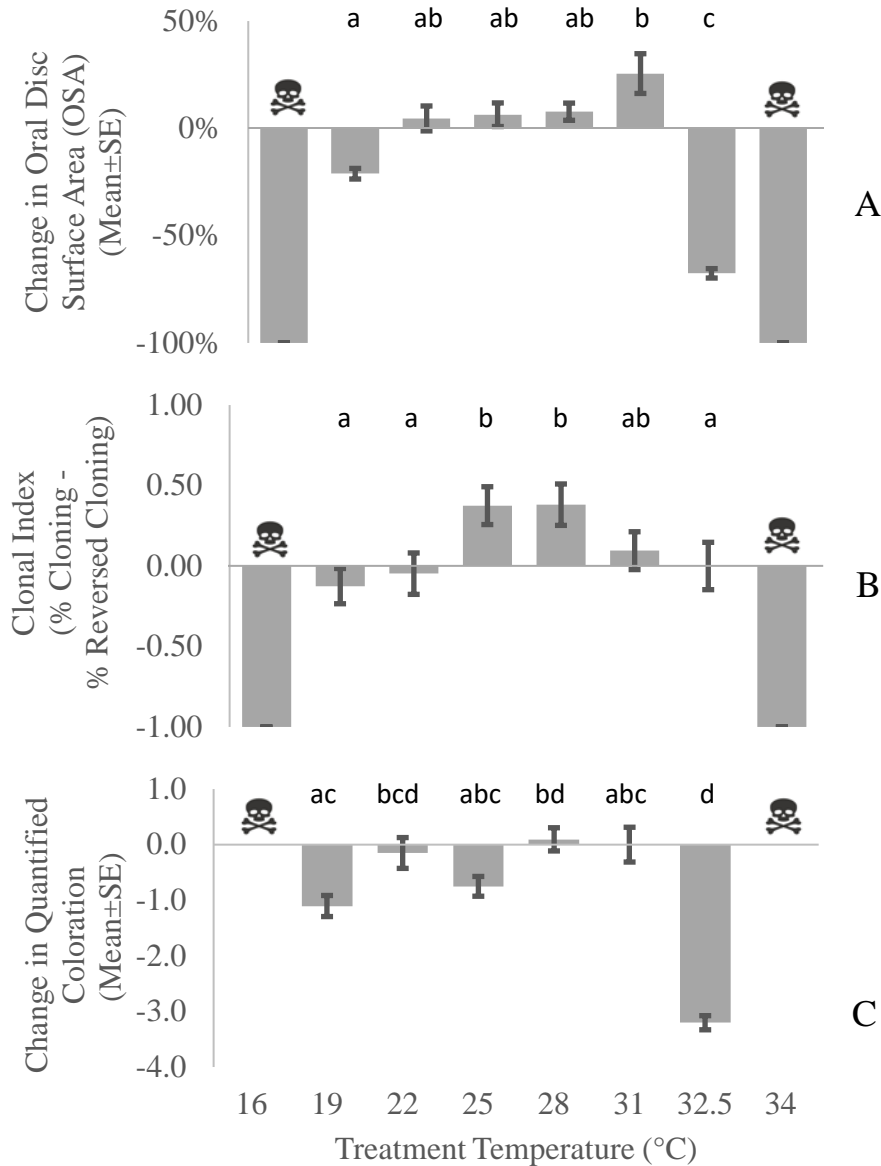


Figure 3-2. Variation in whole polyp characteristics of *Ricordea florida* in response to experimental temperature laboratory treatments. Shown are values after 5 weeks under treatment conditions. A. Percent change in body size (oral disk area, ODA). B. Clonal replication stage (CRS). C. Body color index (BCI). The same letters above bars indicate treatments that did not differ significantly from each other. Note that at 16° and 32°C, all polyps died and thus exhibited 100% decrease in traits except for BCI, where polyps lightened some but not completely before dying; the skull and crossbones symbols indicate polyp death. See text for details.

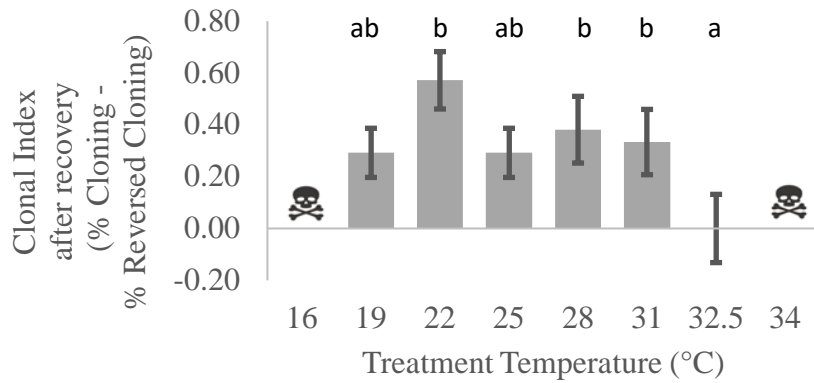


Figure 3-3. Variation in Clonal replication stage (CRS) of *Ricordea florida* during recovery after experimental temperature laboratory treatments. Shown are values after 5 weeks of recovery when polyps were returned to moderate culture temperature of 28°C, after an initial 6 weeks under the 8 treatment temperature conditions. Clonal index could not be calculated for 16°C or 34°C due to all polyps perishing before the end of the trial and therefore no polyps being present during the recovery phase.

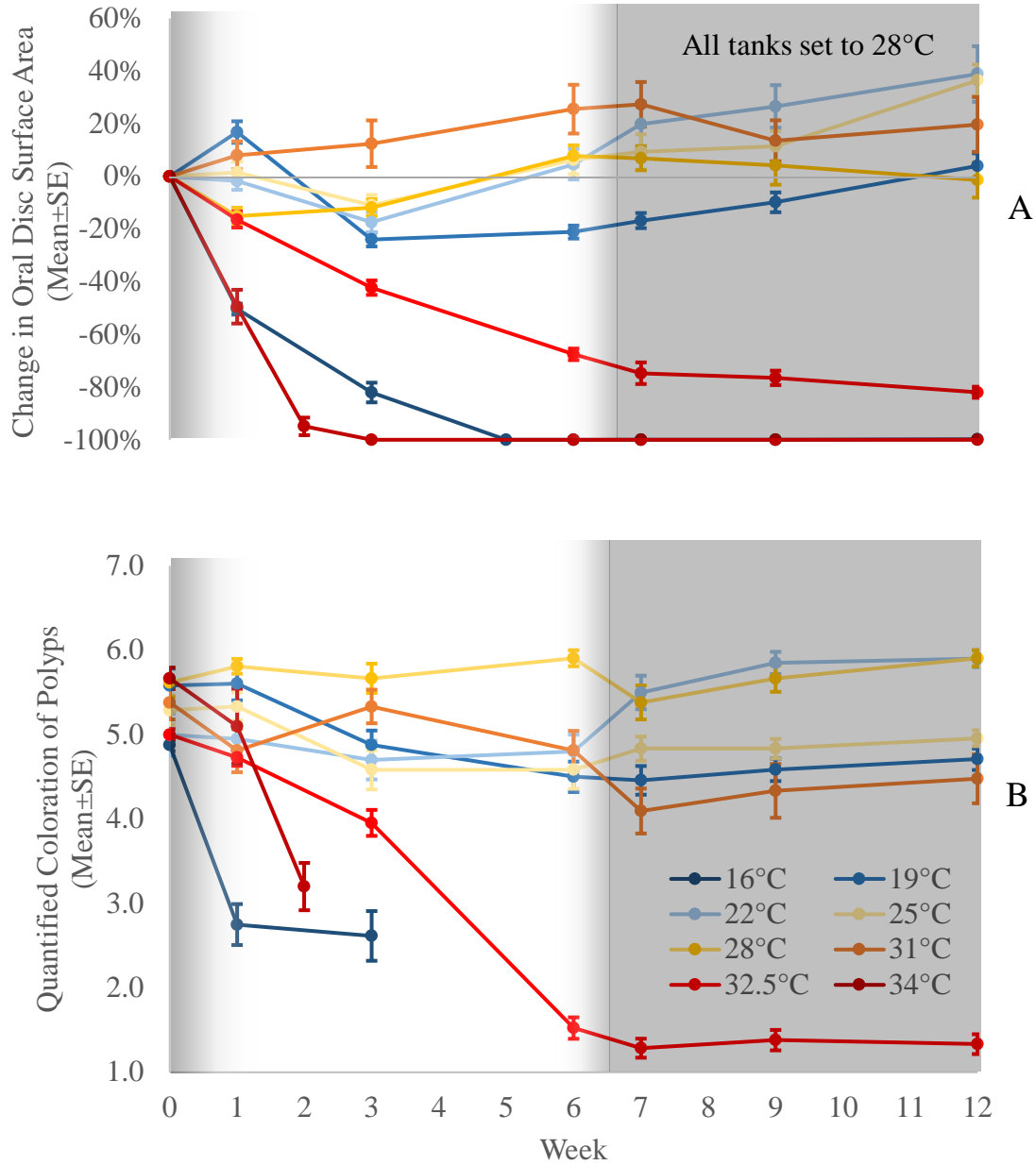


Figure 3-4. Variation over time in whole polyp characteristics of *Ricordea florida* in response to experimental temperature laboratory treatments. Weeks 0-6 represent the 8 temperature treatment conditions, then weeks 7-12 were recovery conditions when all polyps were returned to the moderate culture temperature of 28°C. A. Percent change in body size (oral disk area, ODA). B. Body color index (BCI). Note that at 16° and 32°C, all polyps died within 3-5 weeks. See text for details.

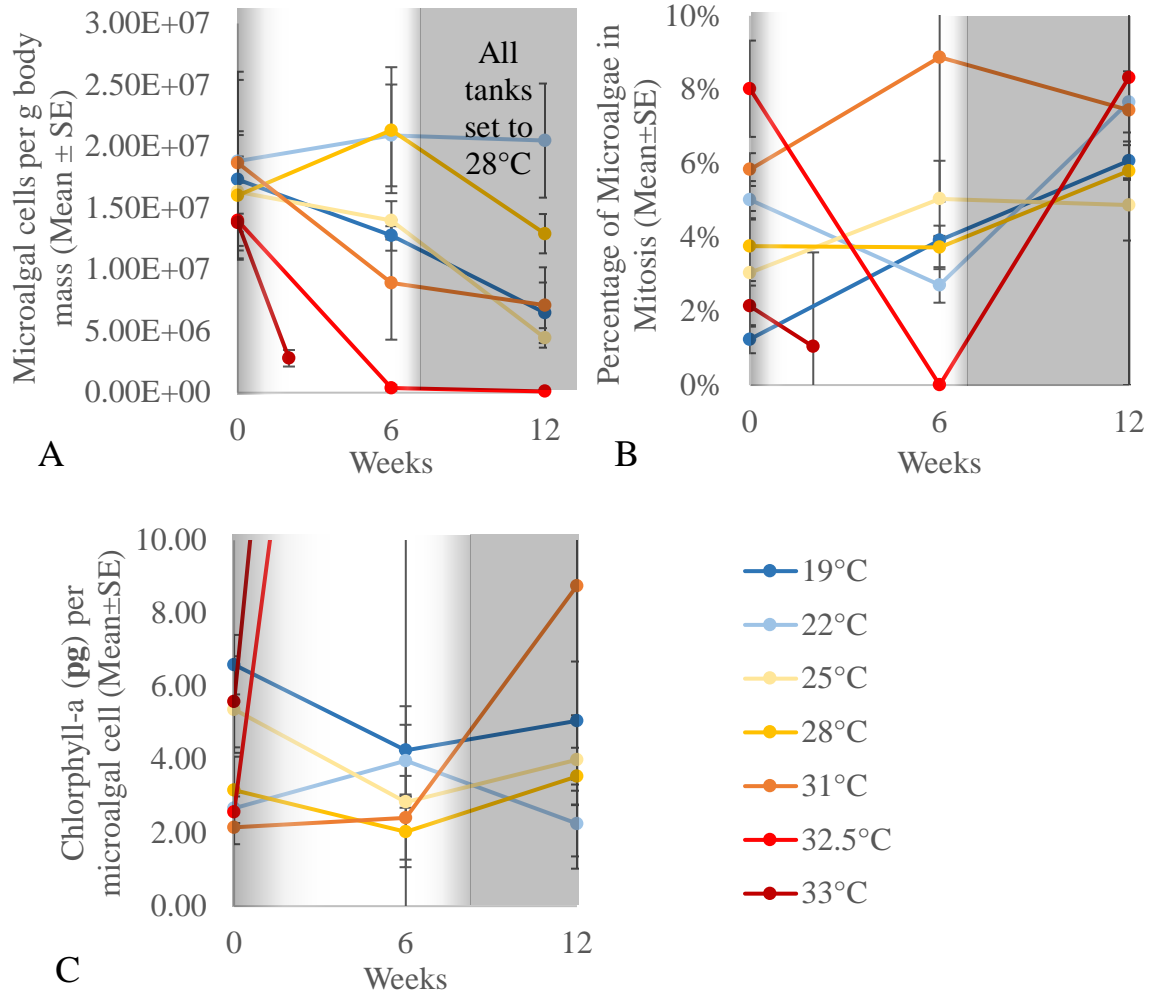


Figure 3-5. Variation over time in microalgal characteristics within polyps of *Ricordea florida* in response to experimental temperature laboratory treatments. Weeks 0-6 represent the 8 temperature treatment conditions, then weeks 7-12 were recovery conditions when all polyps were returned to the moderate culture temperature of 28°C. A. Microalgal cell abundance (MCA). B. Mitotic index (MTI). C. Chlorophyll concentration per microalgal cell (CCA). See text for details.

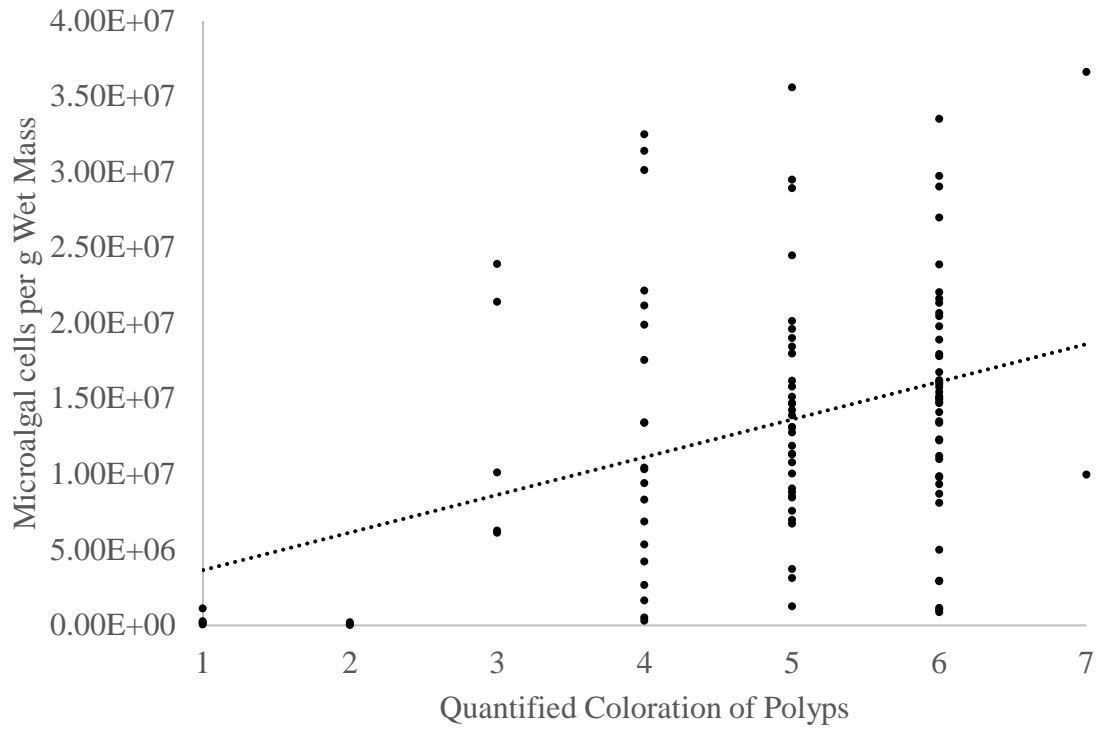


Figure 3-6. Correlation between the quantified color of polyps (body color index; BCI) and microalgal cell abundance (MCA) in laboratory polyps of *Ricordea florida* (n = 112 polyps, y = $2.49e06x+1.16e06$, $r^2 = 0.1336$, $p = 4.38e-05$).

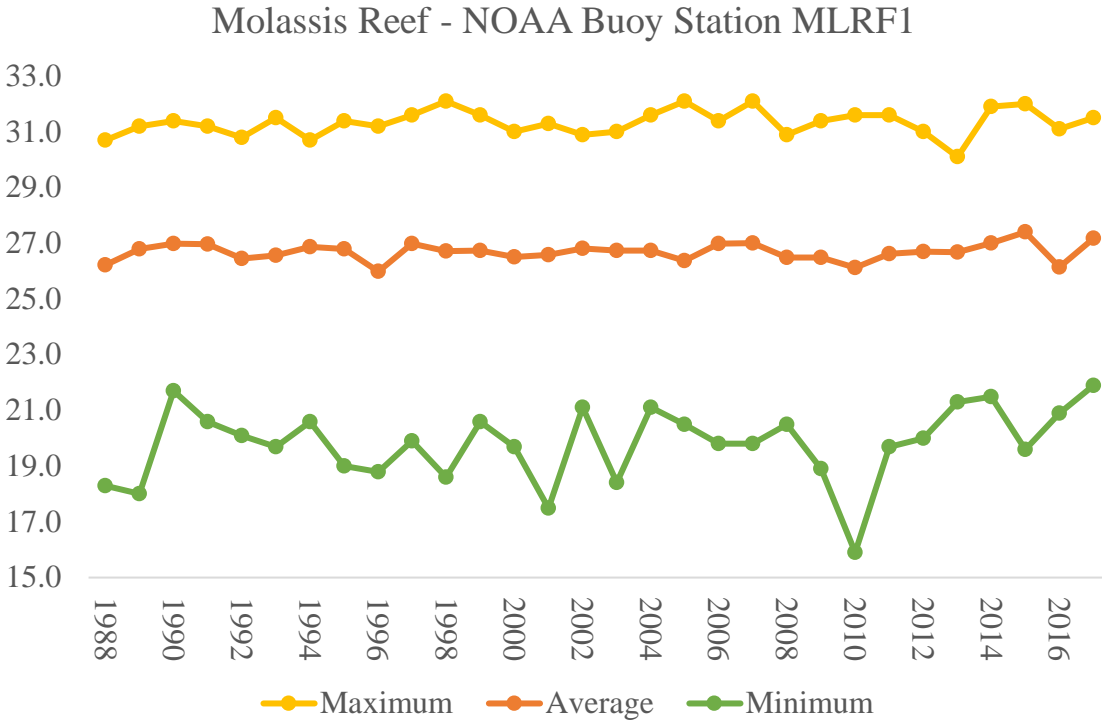


Figure 3-7. Temporal variation in surface seawater temperature at Molasses Reef, Florida Keys, USA, over 30 years (1988-2017). Shown are the annual maximum, average (mean), and minimum temperatures recorded by a surface weather buoy MLRF1, as recorded by the NOAA National Data Buoy Center (ndbc.noaa.gov).

Chapter 4

Spatial and temporal patterns of distribution and abundance of Florida false corals *Ricordea florida* in the Florida Keys

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1 Introduction

Corallimorpharians occur in a wide variety of marine environments around the world, from the intertidal zone to ~ 5000 m depth below sea level in the deep sea, and from the tropics to the poles (Fautin et al. 2009). Tropical shallow-water species usually host symbiotic microalgae belonging to the Family Symbiodiniaceae, from which they obtain photosynthates, while cold-water and deep sea species are strictly heterotrophic (Carlgren 1949, den Hartog 1980, Fautin et al. 2002). Corallimorpharians occur as either solitary polyps or in aggregations of polyps that may form extensive carpets on both temperate and tropical reefs, and many species are dominant competitors for space on benthic hard substrate (Chadwick 1987, Chadwick-Furman & Spiegel 2000, Torres-Pratts et al. 2011).

A common corallimorpharian in the Florida Keys and throughout the Caribbean on shallow coral reefs is the Florida false coral *Ricordea florida* (den Hartog 1980). Polyps of *R. florida* possess endosymbiotic microalgae (Symbiodiniaceae), and therefore are limited to the shallow photic zone due to their requirement of exposure to solar irradiance for photosynthesis of their microalgae (LaJeunesse 2002). Polyps can reach a diameter of ~ 7 cm, although usually they are smaller. The body shape is wide and short, with a broad oral disc covered with many short, unbranched tentacles (den Hartog 1980). Two genetically distinct lineages of this species appear to coexist in the Caribbean region, one limited to the northeastern Caribbean from the Florida Keys to Puerto Rico, and the other occurring throughout the Caribbean Sea from Florida in the north to Curaçao in the south (Torres-Pratts et al. 2011). These two lineages are distinguishable thus far based only on molecular evidence, with no clear morphological or

physiological differences between them. Other aspects of the biogeography of this species, including the extent to which these two lineages interbreed, are not yet understood.

While studies have regularly assessed the distribution and abundance of the dominant scleractinian reef-building corals and octocorals in the Florida Keys, the only Keys reef survey to include corallimorpharians was conducted over a decade ago Coral Reef Evaluation and Monitoring Project [CREMP] [https://myfwc.com/research/habitat/coral/cremp/;](https://myfwc.com/research/habitat/coral/cremp/) Miller et al. (2005-2009), and reported population measures once each year during 2005-2009. Seasonal fluctuations in population size, as well as changes in population structure such as polyp or aggregation sizes of *R. florida*, have never been studied.

The goals of the present study were to: (1) quantify spatial patterns of distribution and abundance of *R. florida* in the Florida Keys, (2) monitor temporal changes over 2 years in population characteristics at a site with high abundance in the Lower Keys, and based on these 2 types of evidence, to (3) create a model that predicts the distribution of this species in Keys reef habitats.

2 Methods

2.1 *Spatial patterns of distribution and abundance*

We selected 5 sites for one-time surveys to quantify spatial patterns of distribution and abundance in Florida false corals *Ricordea florida*, with all sites < 100 km distant from each other in the Middle and Lower Florida Keys, U.S.A. These sites were selected because they were known to contain sufficiently high abundances of this species for quantification of population

characteristics, based on published information (Miller et al. 2009), and based on interviews with marine life collectors (F. Young, R. Herndon, pers. comm.) about known abundance patterns of this species prior to a major cold water event in 2010 that caused mass mortality of Keys marine organisms (Kemp et al. 2016). This species has never been reported north of Biscayne Bay, FL, and the above interviews indicated that over the last few decades it has become rare in the Upper Keys. Florida Keys sites in the area of the Marquesas and Dry Tortugas were not included due to funding constraints, and a lack of previous reports on the populations in those areas. Therefore, the data here represent potentially maximal abundances of this species in the Middle and Lower Florida Keys, at 5 sites ordered from inshore to offshore: (1) an inshore reef on the Atlantic side of Scout Key, (2) a mid-channel reef near Channel Marker 47 located near the city of Marathon, (3) a group of mid-channel patch reefs in the Western Sambo Ecological Reserve (ER), (4) a patch reef in the Looe Key Special Use Area (SUA), and (5) an offshore back reef in the Looe Key Sanctuary Preservation Area (SPA; all sites were 3-8 m depth below sea level; Fig. 4-1).

During July 13 - August 10, 2018, at each site divers entered the water from a boat, and the coral reef surface was searched on SCUBA until the first aggregation of *R. florida* polyps was found. Then a predetermined random compass bearing (created with a random number generator) was used to set the direction of a plastic transect tape that was deployed along the reef surface, with one end located ~ 10 cm from the originally-located aggregation (without including it), and extending 10 m across the reef (transect area = 1 x 10 m). The transect was divided into 10 consecutive 1x1 m² quadrats, by swimming along the left side of the transect tape and using a 1-m long PVC pipe held perpendicular to the transect tape, to designate the dimensions of each quadrat. Inside each quadrat, the following information was collected for all observed polyps of *R. florida*: aggregation size (number of polyps in each aggregation, defined as all polyps < 15 cm

distant from each other, which was the maximal distance observed between morphologically-similar polyps based on body coloration patterns), body sizes of all polyps in the aggregation (oral disc area [ODA] calculated by measuring the oral disc width and length to the nearest 0.1 cm, using a flexible plastic ruler), and clonal replication status of each polyp (cloning or not, defined as containing multiple mouths to indicate a stage of polyp fission, or inverse bud(s) on the oral disk indicating inverse budding; see Chapter 5 for details). After the first transect was surveyed at each site, 2 more transects were deployed haphazardly at least 25 m apart on the reef surface, using the same methods (3 transects x 10 quadrats each = 30 1x1 m² quadrats examined per reef site).

In each of the 3 transects, substrate rugosity was quantified by laying a 10-m-length small-link stainless steel chain (Lowe's Company, Mooresville, NC, USA) along the substrate adjacent to the plastic transect tape, taking care to follow the contours of all protuberances on the reef surface, such as live coral colonies, and all depressions such as reef crevices. The distance of this substrate rugosity transect (the metal chain) relative to the 10-m straight plastic transect tape was recorded. This measurement was used to obtain a comparative measure of reef rugosity, by dividing the linear distance that the convoluted 10-m chain reached along the plastic transect tape by the total length of the 10-m straight plastic tape, then subtracting that from 1 (after Fuad 2010).

Non-parametric ANOVA tests were applied to analyze variation in these environmental and population parameters among the 5 field sites, followed by pair-wise comparisons for differences between each pair of sites. Analysis was applied to each of the 5 quantified population parameters: polyp abundance (number of polyps m⁻²), aggregation size (number of polyps per aggregation), aggregation abundance (number of aggregations m⁻²), polyp body size

(ODA in mm^2), and abundance of polyps undergoing clonal reproduction (number of cloning polyps m^{-2}), as well as the 1 environmental parameter (rugosity index). Non-parametric t-tests were applied to the polyp abundance data at 2 sites (Western Sambo ER and Looe Key SUA), to determine if polyp abundance had changed significantly between 2009 (as reported by Miller et al. 2009) and 2018 (as reported here), because these were the only 2 sites examined during both decades.

2.2 *Temporal patterns of population change*

To measure temporal changes in population characteristics of *R. florida* among seasons, a population was examined every 2-5 months for 2 years (9 sample periods between June 2017 and June 2019), at a long-term study site on a coral reef near American Shoal (24.5°N , 81.5°W ; Fig. 4-1). This site was examined in addition to the 5 sites described above, it and was selected because it contained high polyp abundance to allow for quantification of population changes over time (after Dixon et al. 2018). During the first sample period in June 2017, two $5 \times 1 \text{ m}$ (10 m^2 total) band transects were deployed, which were divided into a total of 10 $1 \times 1 \text{ m}$ quadrats, using the methods described above ($N = 10$ quadrats examined, after Miller et al. 2009). The locations of all transects examined for temporal change were determined by haphazardly deploying them in an area of reef surface that contained highest abundance of *R. florida*. The 2 ends of each transect were marked by hammering iron cut-nails into the reef. Because high water motion generated by Hurricane Irma (category 4; passed directly over the site on September 10, 2017) removed these initial markers from the reef, the locations of the 2 initial transects could no longer be determined during the next sample period in October 2017. As such, a single large

transect then was deployed in the same reef area during October 2017, with dimensions of 10 x 1m (10 m² total), divided into 10 - 1 x 1m quadrats (N = 10 quadrats). The transect area was enlarged in December 2017 by establishing a single permanent transect of 15 x 1m (15 m² total), that was divided into 15 - 1 x 1m quadrats (N = 15 quadrats). This permanent transect was marked by large galvanized steel landscaping stakes at each end of the transect, with a small polystyrene buoy attached to each stake and extending ~1 m up from the sea floor, for ease of transect relocation (see Acknowledgements Section for details of permitting information; NOAA Permit# FKNMS-2017-031).

During each of the 9 sample periods (see above), a plastic transect tape marked in cm was deployed between the permanent marker nails at the end of each transect, and NP swam along the left-hand side of the tape, holding perpendicular to the transect tape a 1-m long PVC tube with marks every 10 cm (as described in the section above for spatial sampling). All polyps of *R. florida* that occurred within the area of 1 m to the left of the tape were mapped, by noting the location of each polyp, both on the transect tape (distance along transect) and on the PCV pipe (distance laterally from the transect tape). For each observed polyp within the quadrats along transects, the same information was collected as described for spatial sampling above. In addition, the percent cover of polyps on the reef surface was estimated within each quadrat. Because the field site for this long-term study on temporal variation was located in a back-reef area that contained a mixture of unconsolidated silt and consolidated limestone substrate, windy conditions sometimes reduced the underwater visibility to < 1 m. Therefore, data collection was not possible during some planned sample periods, such as during an extended period with windy conditions between August and December 2018, when data were not collected despite attempts to dive at the site.

A generalized linear model was applied to analyze variation in the 5 recorded population parameters among the 9 sample periods, using the “lme” function in R (R Foundation for Statistical Computing, Vienna, Austria).

2.3 *Spatial model of habitat occupation*

To develop a spatial model of reef areas likely to contain high abundance of *R. florida*, we examined a detailed map of the Florida Keys (Unified Reef Tract of the Florida Keys, Florida Fish and Wildlife Conservation Commission, <https://myfwc.com/research/gis/regional-projects/unified-reef-map/>). We downloaded a copy of this map onto a desktop computer, and created a layered spatial model using Geographical Information Systems (GIS) via the program ArcGIS ArcMap 10.6.1 (Ersi, Redlands, CA, USA).

We used the following information to create our model: (1) Individuals of *R. florida* occur only on consolidated (hard) marine substrate, so ‘coral reef and hardbottom’ were the only bottom types included as potential habitat in the map. (2) Individuals are most abundant at depths of 5 - 18 m (~ 18 - 60 feet) below sea surface, so only areas within this depth range were included (after Miller et al. 2009, Encyclopedia of Life 2016). (3) Our field observations (Present Chapter and Chapter 5) and laboratory experiments (Chapter 2) indicated that high abundance of *R. florida* occurred only in shaded reef areas, usually under or near large upright octocorals (gorgonians or sea-fans), so we used data from the Coral Reef Evaluation and Monitoring Project (CREMP; <https://myfwc.com/research/habitat/coral/cremp/>) to create on our map a 2-km area around each CREMP field site with at least 15% cover of octocorals. Areas in the Florida Keys where all 3 of these habitat conditions overlapped (hard substrate, ~ 5 - 18 m depth, and > 15%

cover of octocorals) were included in our layered GIS map as habitat areas likely to contain high abundances of *R. florida*.

3 Results

3.1 *Spatial patterns of distribution and abundance*

The abundance of polyps of *Ricordea florida* varied significantly among the 5 reef sites examined, from ~ 0.3 – 2.2 polyps m⁻² (mean values per site; Table 4-1; p = 0.009). The 3 sites that were protected from commercial collection (Western Sambo ER, Looe Key SUA, and Looe Key SPA) did not differ significantly in polyp abundance from the 2 sites that were not protected (Scout Key and Marker 47; p = 0.421). Polyp abundance at Marker 47 (2.23 ± 0.84 polyps m⁻²) was significantly higher than at all 4 other sites except Looe Key SPA (p < 0.04), which also contained relatively high abundance (1.73 ± 0.53 polyps m⁻²). Abundance at those 2 sites was significantly higher than at the 2 sites with the lowest observed abundance, Western Sambo ER and Scout Key (p < 0.04); no other statistically significant differences occurred among the 5 sites.

At all sites, the most common aggregation size was small (< 4 polyps per aggregation; Fig. 4-2), with no significant difference among sites in aggregation size (Table 4-1). At most sites, a substantial proportion of polyps were solitary (not in aggregations; Fig. 4-2). At Western Sambo ER, only 1 aggregation of 5 polyps was observed inside the transects, and qualitative observations outside the transects revealed a total of only 2 additional aggregations of *R. florida*

on the entire reef surface at that site, despite extensive searching. The largest aggregation observed was at the Marker 47 site (21 polyps in 1 aggregation; Fig. 4-2).

Similar to the pattern for polyp abundance, the abundance of aggregations was significantly higher at the Marker 47 site (0.97 ± 0.40 aggregations m^{-2}) than at all other sites except Looe Key SPA ($p < 0.0008$; 0.50 ± 0.16 aggregations m^{-2}). Aggregation abundance likewise was significantly higher at Looe Key SPA than at Western Sambo ER ($p = 0.008$; Table 4-1) but did not differ significantly from that at the other 3 sites. No other significant differences were observed in pairwise comparisons among sights, including in comparisons between protected versus unprotected sites.

The individual polyp characteristic of body size varied widely within each site (Fig. 4-3). Most polyps were small, with the 301-600 mm^2 ODA size class containing a higher percent of individuals than all other size classes, at all 5 sites examined ($42.9\% \pm 5.5\%$ of polyps), and large individuals were rare. The 5 sites did not differ significantly in the body sizes of polyps. (Table 4-1).

The abundance of cloning polyps was highest at the Looe Key SPA (0.60 ± 0.53 polyps m^{-2} ; significantly higher than at all other sites, $p < 0.002$). Cloning polyp abundance did differ significantly among any of the other sites, including between protected versus unprotected areas.

The environmental parameter of reef surface rugosity varied significantly among the 5 sites, with similarly high rugosity at both Western Sambo ER (0.250 ± 0.011) and at Looe Key SUA (0.247 ± 0.035). Rugosity did not differ significantly between those 2 sites, but was significantly higher than at the other 3 sites examined ($p < 0.04$ and < 0.03 , respectively), which did not differ significantly from each other. The level of reef rugosity did not appear to relate directly to any of the 5 population parameters examined for *R. florida* at each site (Table 4-1).

Qualitatively, it was observed that the Looe Key SUA site contained the most complex reef community and highest species richness of benthic reef organisms (hard and soft corals, sponges, etc.), as well as the highest percent cover of scleractinian corals. In contrast, the abundance of *R. florida* was relatively low at this site (Table 4-1).

Both sites with previously published data on *R. florida* polyp abundance (Western Sambo ER and Looe Key SUA) exhibited a drastic decrease in abundance over the last decade (Table 4-1; $p < 0.05$), even though both sites were inside areas protected from fisheries collection. Most notable was the > 100 -fold drop in polyp abundance at the site which previously had exhibited the highest known abundance of this species (Western Sambo ER), from > 30 polyps/m² in 2009, to only 0.25 polyps m⁻² in 2018. The other 3 sites were not protected from collection activities, and had previously hosted sustainable fisheries, but were abandoned over the last decade by collectors due to declines in polyp abundance (according to interviews with marine life collectors, see Methods), and all 3 sites were observed to contain low polyp abundances in 2019 (< 3 polyps m⁻²; Table 4-1).

In terms of fine scale distributional patterns, the dispersion of aggregations on the reef surface was patchy, with most 1-m² areas containing no aggregations, while others contained up to 7 aggregations m⁻².

3.2 Temporal patterns of population change

The abundance of polyps at the long-term study site on the back reef near American Shoal (6.0 ± 0.2 polyps m⁻², range = 0 - 24, N = 9 sample periods, Fig. 4-4A) was $> 2x$ higher than at any of the one-time survey sites examined above for spatial variation (Table

4-1). Even at this relatively high abundance level, the polyps covered < 1% of the reef surface (Fig. 4-4B). High variability in polyp abundance among the quadrats examined (e.g. the number of individuals per quadrat) indicated that polyps were significantly clumped ($\chi^2 = 1.458$, $df = 8$, $p = 0.993$), and that percent cover also was highly patchy ($\chi^2 = 0.0053$, $df = 8$, $p > 0.999$).

Statistically significant increases in the number of cloning polyps ($p = 0.01$) and in polyp body size ($p = 0.01$) occurred initially after Hurricane Irma, between October 2017 and March 2018. However, both stabilized starting in May 2018 and did not change significantly after that ($p > 0.5$). There were no statistically significant temporal trends in any of the other population characteristics examined (polyp abundance, aggregation size or abundance, or percent cover of polyps; $p > 0.3$ for all; Fig. 4-4).

Population size structure at the long-term site was similar to that observed at the other 5 sites examined for spatial variation, in that during most sample periods, most of the polyps were small, with a large proportion in the 301-600 mm² size class (36.0% \pm 1.1%, $N = 9$ sample periods, Fig. 4-5). However, in contrast to the other sites, more polyps at the American Shoals site reached relatively large body sizes (up to 6,635 mm²). Hurricane Irma, which passed over the long-term site on 10 September 2017, appeared to cause loss of polyps, especially of small individuals (Figs. 4-4A & 4-5) that were solitary or in small aggregations (Fig. 4-6), resulting in ~ 30% drop in polyp abundance (from 6.2 to 4.2 polyps m⁻²). Polyp abundance then did not recover to pre-hurricane levels until 6 months later in March 2018 (7.1 polyps m⁻²), and it did not stabilize until May 2018 (5.6 polyps m⁻², Fig. 4-4A & 4-5). A similar trend occurred in percent cover after the hurricane (Fig. 4-4B).

No polyps were observed to be undergoing asexual replication during December 2019, a few months after the hurricane (when this parameter began to be measured; see Methods). Then

for the rest of the study (March 2018 – June 2019), ~ 2-3 polyps m⁻² were observed to be undergoing asexual replication during each sample period (2.5 ± 0.2 , N = 6 sample periods between March 2018 - June 2019, Fig. 4-4A). This steady rate of asexual replication likely contributed to the stable population size and structure observed during most of the study (Figs. 4-4, 4-5, and 4-6). The proportion of polyps that were cloning appeared to be spread fairly evenly over the sampled reef surface (i.e., among the quadrats examined), in that cloning polyps did not appear to be clumped when the number of polyps per quadrat was controlled for ($\chi^2 = 2.124$, df = 6, p = 0.117).

Aggregation sizes at the long-term site spanned a similarly wide range as at the 5 other sites examined (up to 18 polyps per aggregation), but at the long-term site a higher proportion of polyps occurred in large aggregations than at the other sites (compare Figs. 4-2 and 4-6). Even so, at the long-term site, the most common aggregation size was only 2-3 polyps per aggregation ($40.0\% \pm 2.2\%$ of all polyps, N = 9 sample periods). Immediately after the hurricane, solitary polyps completely disappeared, as well as most of those in aggregations of only 2-3 polyps, leaving only relatively large aggregations at this site (Fig. 4-6). Then in December 2017, the population was dominated by relatively large solitary polyps (Figs. 4-5 and 4-6).

Following damage to the study site by the September 2017 hurricane, all 6 examined population characteristics (polyp abundance, aggregation abundance, aggregation size, population size structure [polyp body sizes], percent clonal replication, and percent cover) appeared to rapidly recover (within 3-8 months), and then remained stable for the rest of the study period (May 2018 - June 2019; Figs. 4-4, 4-5, 4-6).

3.3 *Spatial model of habitat occupation*

The GIS modelling created a map of habitats in the Middle and Lower Florida Keys that were likely to contain high abundances of *R. florida*, based on the 3 criteria of benthic substrate type, depth below sea level, and presence of dense upright soft corals as shading organisms (Fig. 4-7). This spatial model revealed that mid-channel patch reefs in the Middle to Lower Keys on the Atlantic side comprise habitats that potentially are able to support high abundances of this species. Comparison of this spatial model with the locations of the 6 sites where populations of *R. florida* were examined for the present study (Figs. 4-1 & 4-7) revealed that all of the study sites with relatively high abundances of *R. florida* (> 2 polyps m^{-2} ; Table 4-1) were within the modeled habitat areas. The spatially modeled map also was compared to 3 currently active collecting sites that supported commercial fisheries on *R. florida* during 2018-2019 (as determined by interviews with commercial fishers, see Methods), and included them all. Note that the scale of the modeled habitat map was intentionally left large, with the boundaries shown only at large scale (e.g. coarse rather than fine spatial scale), in order to protect the propriety information of commercial collectors, and to prevent use of the map information for engaging in overfishing of this species at the potentially high-abundance sites.

4 Discussion

Our results reveal that current populations of *R. florida* in the Middle and Lower Florida Keys remain much lower than they were historically before 2010. This pattern suggests that this species cannot tolerate extremely cold seawater events such as occurred during 2010, or hot

water events such as occurred during 2014-2015 in the Florida Keys (see Chapter 3 for details). Our results also indicate that this succession of extreme temperature events may have removed enough entire aggregations of *R. florida* in the Keys, that long-term population processes have not been able to restore this species to pre-2010 levels of abundance. This lack of observed recovery may have occurred due to an inadequate number of polyps remaining on the reefs for replenishment of these populations through the asexual reproduction of existing polyps (Chapter 5). Our field monitoring results also reveal that populations of this species are capable of recovering rapidly from a high-water motion event such as a hurricane, and they may then exhibit stable characteristics over at least 1 year. These corallimorpharians occupy only a small percentage of the reef surface even when abundant. They appear to recover from population loss at least partially via asexual reproduction of the remaining polyps following a disturbance that removes some polyps from the population on a reef, but also are capable of annual sexual reproduction (Chapter 5).

Both of the scientific studies that have been conducted to date (Miller et al. 2009, present study) and interviews conducted with marine life collectors (present study) indicate that overall, populations of *R. florida* have been declining over the past few decades in the Florida Keys. During interviews, marine life fishers reported that they initially observed an increasing scarcity of this species in the Upper Keys over several decades since the 1980's, and then later a pattern of declining abundance that extended into the Middle and Lower Keys since the 2000's. At present, only a few professional marine life fishers still collect polyps of *R. florida* in the Florida Keys, due to the limited number of reef sites that remain with high abundance. The present quantitative study supports these qualitative patterns, in that a comparison of 2 sites which were surveyed during both 2009 and 2018 revealed significant declines. The other 3 one-time survey

sites examined here supported such low abundances of *R. florida* that regular collection of polyps for the fishery, which previously occurred in these areas, would have been economically unviable. The Looe Key SPA site examined here currently is protected from collection, but lies near the edge of the SPA zone, and collection of *R. florida* has occurred previously just outside of the SPA near this site (within 300 m at nearby sites with consistent bottom composition). In contrast, the area within the SPA that was examined here (Looe Key SPA), also supported a historically higher abundance of this species in 2009 than was recorded here in 2018. As such, the observed decline in abundance over the past 10 years at this protected site does not appear to be solely due to collecting pressure, and may thus be related mostly to the severe temperature events mentioned above (severe cold event in 2010 and high El Niño temperatures in 2014-15).

We conclude that at some reef sites in Florida, protection from collection does not appear to have allowed this species to recover from the cold-water event of 2010, in that abundances within the 3 protected areas examined here (SPA, SUA, and ER) were not higher than at the 2 sites examine outside of the protected areas. Notably, the highest abundance of *R. florida* was observed on a reef at Marker 47 and at the back reef near American Shoal, both of which are outside of protected areas, and also have historically been commercial collection sites. However, in order to definitively assess the effectiveness of protection zones for this species, a much larger number of sites, both inside and outside of marine protected areas, needs to be examined for populations of this species. The currently protected areas in the Florida Keys focus on spur-and-groove fore reefs with high percent coral cover, which are not ideal environments for *R. florida*. In the spatial model developed here, only a small proportion of the potentially ideal habitat area for this corallimorpharian in the Keys overlaps with areas where these animals are protected from collection.

Due to the geographical orientation of the Middle and Lower Florida Keys, there is only < 20 km difference in latitude between the northernmost site examined here (Marker 47) and the southernmost site (Western Sambo ER). Therefore, latitude is not likely to a major factor in causing differences among the 5 examined populations, and the highest abundance was recorded at the northernmost patch reef (Marker 47, at ~ 2 polyps m⁻²). The Upper Florida Keys are oriented in a more north/south direction than are the Middle and Lower Keys, so future surveys extending to the Upper Keys are needed to reveal latitudinal variation in characteristics of this species, similar to recent field studies on corkscrew sea anemones *Bartholomea annulata* that were conducted throughout the Keys (O'Reilly & Chadwick 2017).

The changes observed here in a population of *R. florida* over 2 years reveal interesting effects of Hurricane Irma. Because polyps of *R. florida* are dependent on large organisms such as gorgonians or sea fans for shade (Chapter 2), impacts on this species from the hurricane may have been mainly in terms of removing shade and in shearing off or silting over vulnerable polyps that were small or solitary. The eye of Hurricane Irma passed directly over our research site as a devastating category 4, and destroyed or damaged most of the large shade organisms on this reef. Because polyps of *R. florida* are relatively flat and tightly attached to the substrate, it is likely that only some of them were physically removed by the storm, but the population size dropped markedly and then took ~ 8 months to recover. This time lag to full recovery likely occurred in part because the shade organisms upon which *R. florida* depended were destroyed by the storm, and the population could not fully recover until the shade organisms also recovered. The observed lag in recovery of cloning rates by *R. florida* after the storm, with values near zero in December 2017, likewise may have been caused in part by a lag in the recovery of shading organisms for this species.

While we have determined that *R. florida* spawn during mid-summer each year (Chapter 5), it is not known how long the planktonic planula larvae of this species remain in the water column after fertilization of the spawned gametes. However, a spike in the benthic population abundance between December 2017 and March 2018, with a large increase in the abundance of very small polyps, could represent a recruitment event of planula settling on the reef. A jump of the same magnitude might not have appeared the following year during early spring, because without the disturbance of a hurricane the amount of available reef substrate for settlement was much lower. Even so, a modest increase in the number of very small individuals on the reef surface was also recorded between March 2018 and June 2018.

Our spatial model indicated that the areas of highest known current abundance of this species in the Florida Keys all occurred within the identified areas of prime habitat for this species. However, it is possible to publish only a large-scale, spatially vague version of this spatially modeled habitat map at present, due to concerns that publication of a detailed habitat map could be used by the ornamental industry to increase fishing pressure on this species beyond sustainable limits. If more sustainable fishing practices are established in the future, a finer scale spatial model could be produced from this type of analysis. This type of map could aid collectors in locating areas of high population abundance, and thus to avoid overfishing areas with relatively low abundance, and thereby avoid causing local extinctions. Further refinement of this model will also be possible when more information is available about habitat selection preferences of *R. florida*, including for depth below sea surface and other factors.

We present here the first monitoring study of a corallimorpharian species that is of high fisheries importance, and one of the first that includes long-term monitoring data for a major tropical invertebrate species in the marine life fisheries trade. In contrast, commercial collection

of rose-tipped sea anemones *Condylactus gigantia* was halted by the Florida Fish and Wildlife Conservation Commission, not based primarily on scientifically quantified evidence, but instead based on reports from fishers and other stakeholders that the species was declining (Sheridan et al. 2015). Hopefully, the type of quantitative field data presented here will be used increasingly in the future to assist managers in making scientifically based decisions about how to manage marine life fisheries on tropical marine invertebrates.

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6 References

- Carlgren O (1949) A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. K svenska VtenskAkad Handl 1:1–121.
- Chadwick NE (1987) Interspecific aggressive behavior of the corallimorpharian *Corynactis californica* (Cnidaria: Anthozoa): effects on sympatric corals and sea anemones. Biol Bull 173:110–125.
- Chadwick-Furman NE, Spiegel M (2000) Abundance and clonal replication in the tropical corallimorpharian *Rhodactis rhodostoma*. Invertebr Biol 119:351–360.
- Encyclopedia of Life (2016) Florida Corallimorpharian - *Ricordea florida* - Overview. <http://eol.org/pages/1006431/overview> (accessed April 11, 2016).
- Fautin D, Guinotte J, Orr J (2009) Comparative depth distribution of corallimorpharians and scleractinians (Cnidaria: Anthozoa). Mar Ecol Prog Ser 397:63–70.
- Fautin DG, White TR, Pearson KE (2002) Two new species of deep-water Corallimorpharia (Cnidaria: Anthozoa) from the Northeast Pacific, *Corallimorphus denhartogi* and *C. pilatus*. Pac Sci 56:113–124
- Fuad MAZ (2010) Coral reef rugosity and coral biodiversity. MS Thesis, International Institute for Geo-informational Science and Earth Observation, Bunaken National Park, North Sulawesi, Indonesia.
- den Hartog JC (1980) Caribbean shallow water Corallimorpharia. Zool Verh 176:1–83.
- Kemp DW, Colella MA, Bartlett LA, Ruzicka RR, Porter JW, Fitt WK (2016) Life after cold death: reef coral and coral reef responses to the 2010 cold water anomaly in the Florida Keys. Ecosphere 7:e01373.

- LaJeunesse T (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Mar Biol* 141:387–400.
- Miller SL, Chiappone M, Rutten LM (2009) Large-scale assessment of the abundance, distribution, and condition of benthic coral reef organisms in the Florida Keys National Marine Sanctuary – 2009 Quick look report and data summary. Center for Marine Science / University of North Carolina at Wilmington (CMS/UNCW).
- O’Reilly E, Chadwick N (2017) Population dynamics of corkscrew sea anemones *Bartholomea annulata* in the Florida Keys. *Mar Ecol Prog Ser*. 567:109-123.
- Sheridan NE, Fautin DG, Garrett MJ (2015) Gametogenesis and reproductive periodicity of the “biologically vulnerable” giant Caribbean sea anemone, *Condylactis gigantea*, in Florida. *Invertebr Biol* 134:116–128.
- Torres-Pratts H, Lado-Insua T, Rhyne AL, Rodríguez-Matos L, Schizas NV (2011) Two distinct, geographically overlapping lineages of the corallimorpharian *Ricordea florida* (Cnidaria: Hexacorallia: Ricordeidae). *Coral Reefs* 30:391–396.

8 Tables

Table 4-1. Variation in 5 population characteristics of Florida false corals *Ricordea florida* and 2 environmental characteristics among 5 coral reef sites examined during 2018 in the Middle and Lower Florida Keys (ordered from inshore to offshore), with N = 20 – 1 m² quadrats examined at each site. Data are shown as mean values, with SE in parentheses. Note that for polyp abundances, values are presented at 2 of the sites for both 2009 (from N = 15 1 m² quadrats examined by Miller et al. 2009) and 2018 (present study). Note also that polyp abundances dropped more than 4-fold at both sites between the years examined (* = significant difference between years, p < 0.05). The bottom 2 rows show the statistical test result (ANOVA) for variation in each characteristic among the 5 sites, and the level of significance. See text for details.

Reef site	Polyp abundance (polyps/m ²)	Aggr. size (polyps/aggr.)	Aggr. abundance (aggr./m ²)	Polyp body size (OSA, mm ²)	Polyps cloning (polyps/m ²)	Reef surface rugosity	Depth (m)
Scout Key Inshore	0.27 (0.17)	1.33 (0.21)	0.07 (0.12)	415 (55)	0.17 (0.14)	0.103 (0.009)	3.0
Marker 47, Marathon Mid-channel Patch Reef	2.23 (0.84)	2.17 (0.56)	0.97 (0.40)	440 (60)	0.53 (0.19)	0.087 (0.047)	5.2
Western Sambo ER Mid-channel Patch Reef	2009: 31.60 (2.89) 2018: 0.25 (0.25)*	5.00 (0.00)	0.05 (0.04)	633 (219)	0.15 (0.12)	0.250 (0.011)	5.2
Looe Key SUA Offshore Patch Reef	2009: 2.25 (0.47) 2018: 0.73 (0.40)*	3.67 (1.54)	0.13 (0.07)	417 (62)	0.03 (0.03)	0.247 (0.035)	7.9
Looe Key SPA Low-relief Back Reef	1.73 (0.53)	2.74 (0.40)	0.50 (0.16)	525 (46)	0.60 (0.53)	0.123 (0.384)	7.0
ANOVA p-value	0.009	0.323	0.0002	0.615	< 0.0001	0.019	---

7 Figures



Figure 4-1. Satellite image of the locations of 5 coral reef sites each examined once during July-August 2018 in the Middle and Lower Florida Keys, U. S. A., for spatial patterns of distribution and abundance of Florida false corals *Ricordea florida*, and of 1 site examined repeatedly (every 2-5 months) during June 2017 – May 2019, for temporal patterns (back reef near American Shoal). Source: satellite image downloaded from Google Earth Pro (Mountain View, CA), imagery date 10/2019.

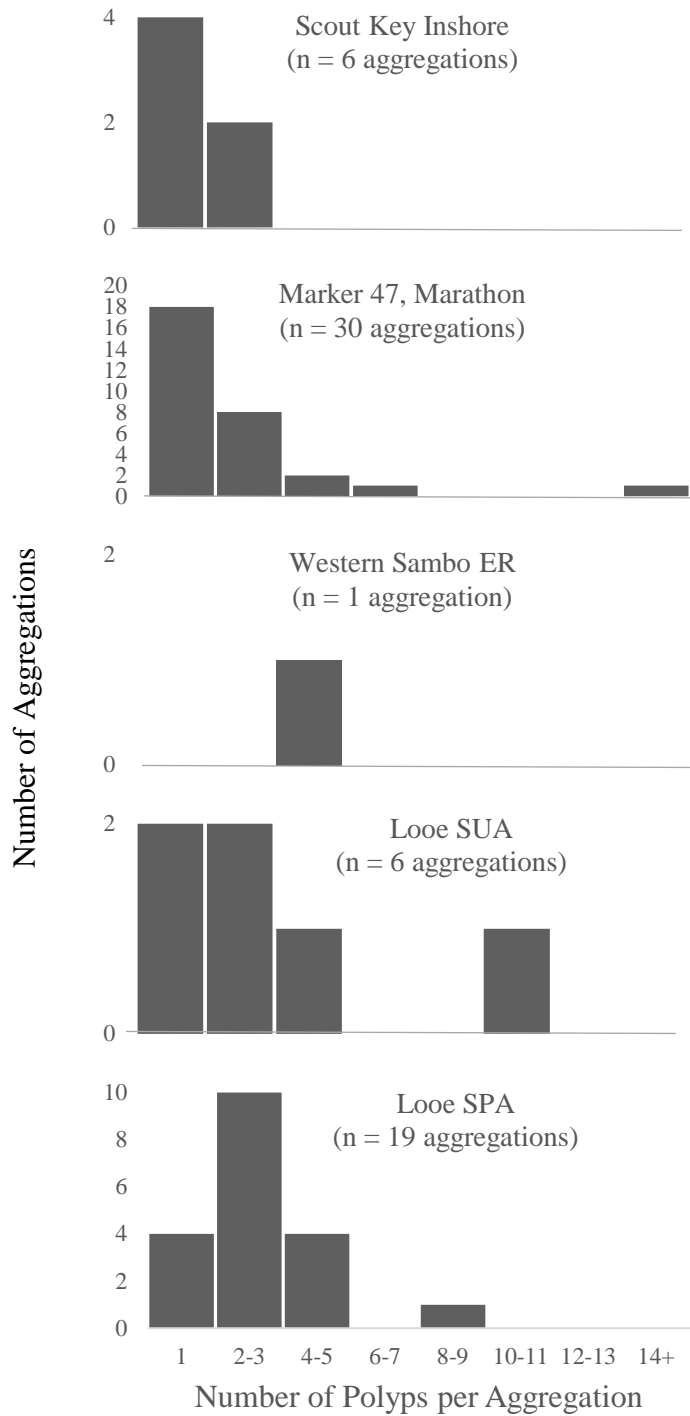


Figure 4-2: Variation in the size structure of aggregations of Florida false corals *Ricordea florida* among 5 reef sites in the Middle Florida Keys, ordered from inshore to offshore. See map Fig. 4-1 for exact site locations. Note that the y-axes vary in scale.

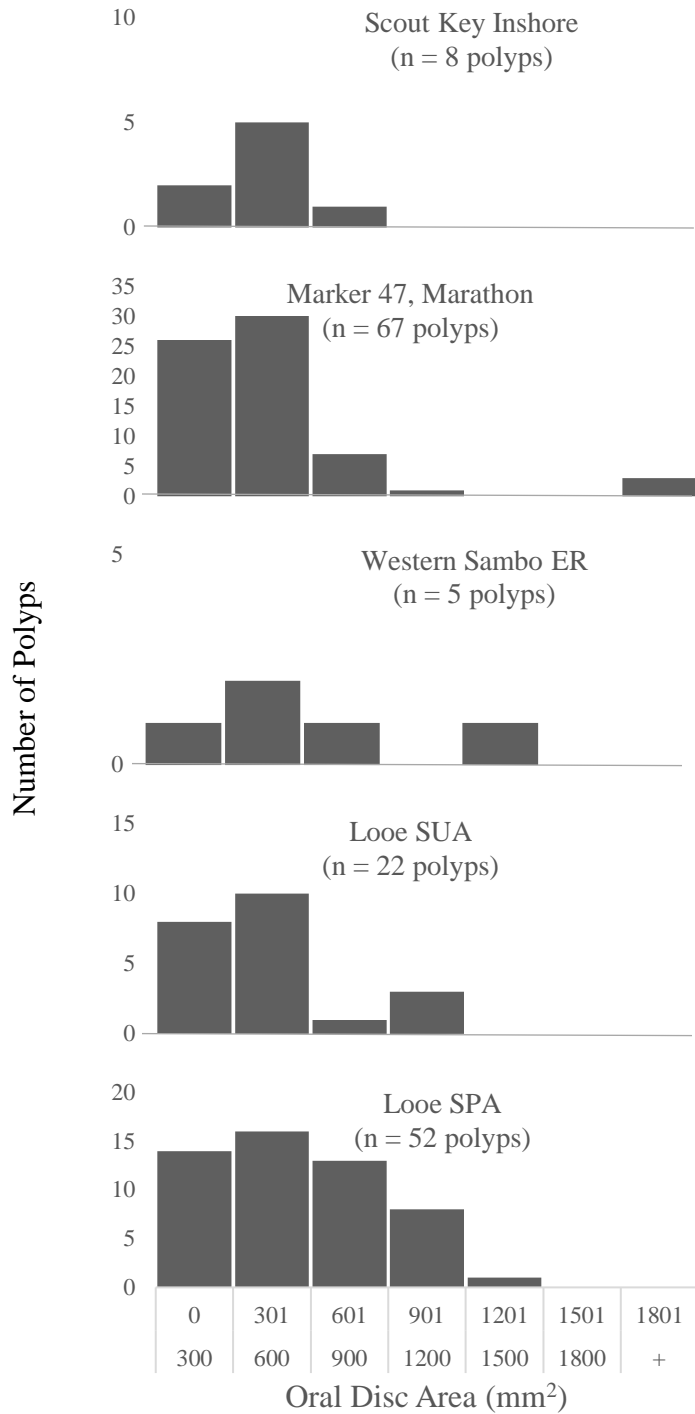


Figure 4-3. Variation in population size structure of Florida false corals *Ricordea florida*, in terms of polyp body sizes, among 5 reef sites in the Middle and Lower Florida Keys, ordered from inshore to offshore. See map Fig. 4-1 for site locations. Note that the y-axes vary in scale.

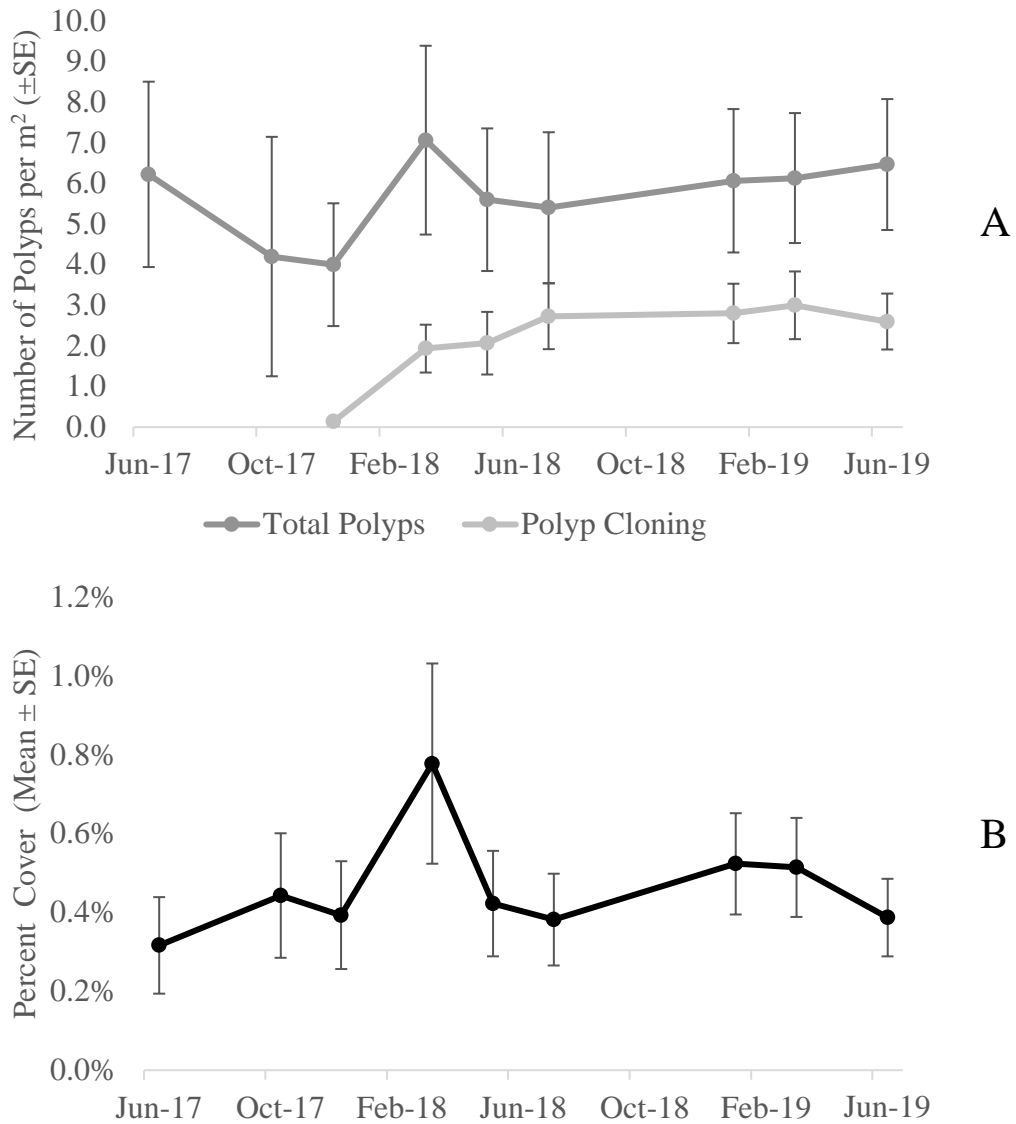


Figure 4-4: Temporal variation over 2 years in characteristics of a population of Florida false corals *Ricordea florida* at American Shoal back reef in the Lower Florida Keys. A. Abundance in total number of polyps, and in number of polyps undergoing clonal replication (data collection for cloning started in December 2017). B. Percent cover on reef substrate (N = 10 to 15 quadrats examined per sample date).

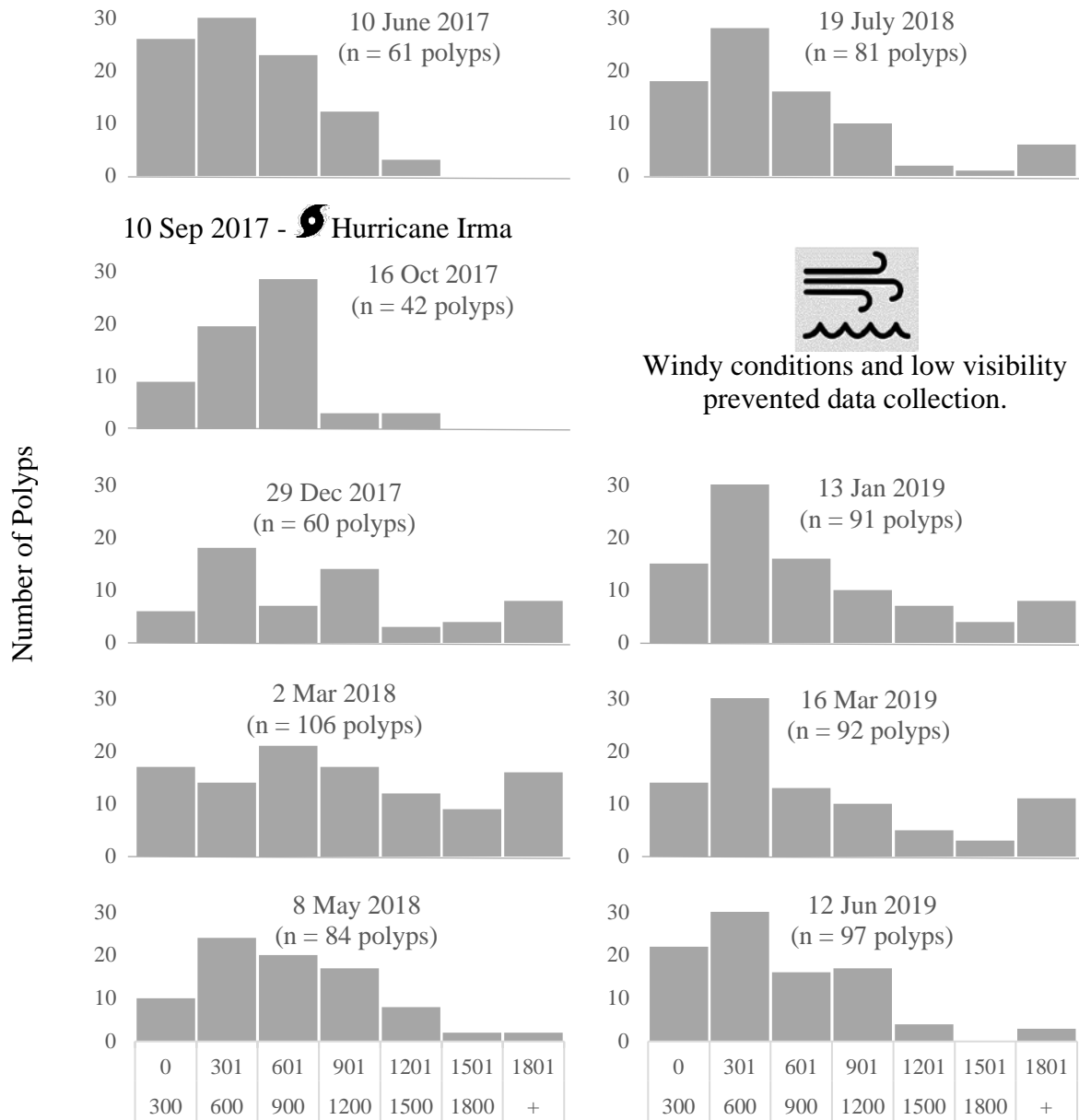


Figure 4-5: Variation over 2 years in the population size structure of Florida false corals

Ricordea florida at American Shoal back reef in the Lower Florida Keys. Note that Hurricane

Irma (category 4) occurred on 10 Sept 2017 date, and that high winds prevented sampling

between Jul 18 and Jan 19. Note, values recorded in the field during June and October 2017 were

multiplied by 1.5 to scale them up to the larger transect areas (15m² per transect) measured

starting in December 2017 (see text for details).

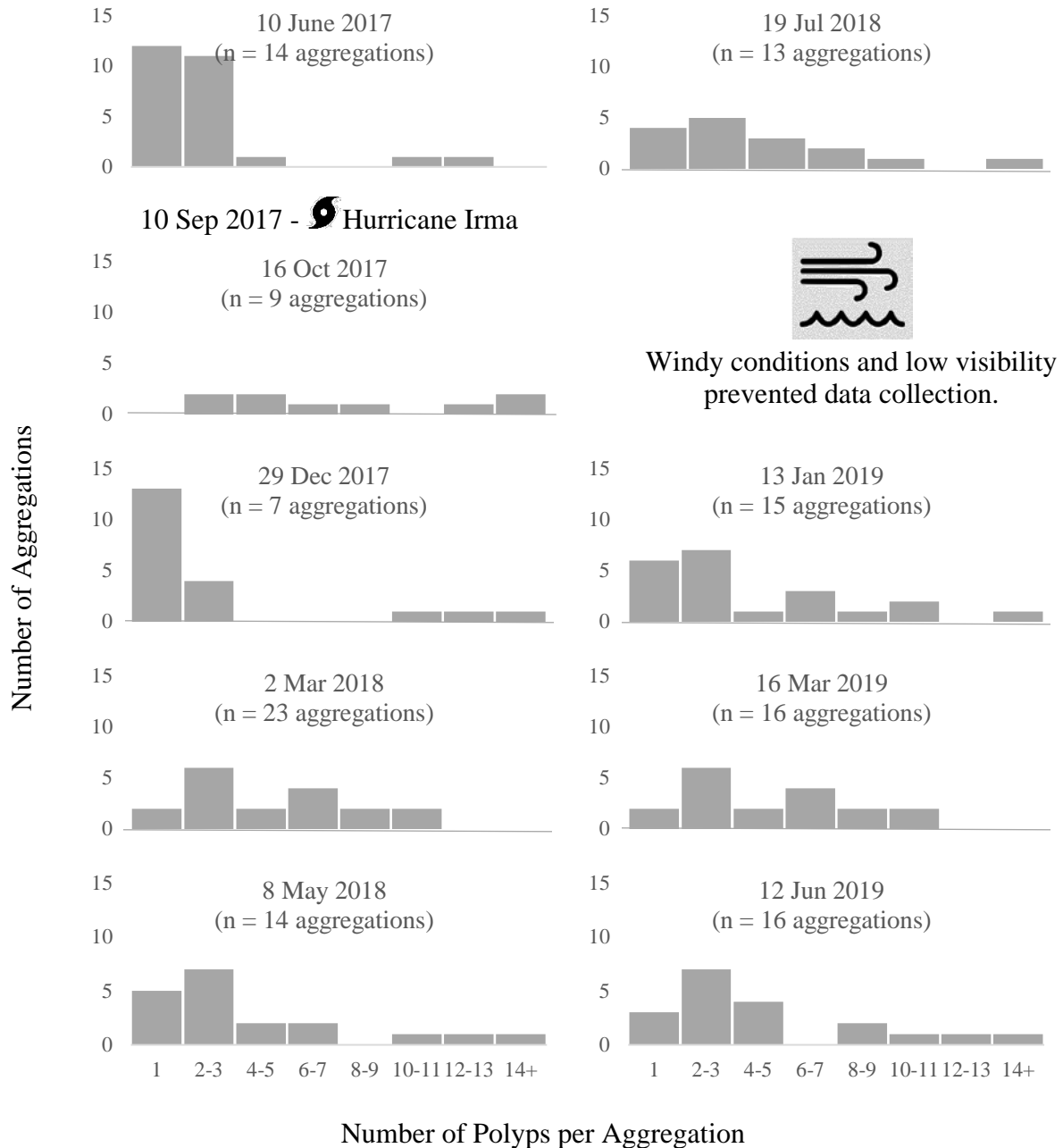


Figure 4-6: Variation over 2 years in the aggregation sizes (number of polyps per aggregation) of Florida false corals *Ricordea florida* at American Shoal back reef in the Lower Florida Keys.

Note that Hurricane Irma (category 4) occurred on 10 Sept 2017 date, and that high winds prevented sampling between Jul 18 and Jan 19. Note, values recorded in the field during June and October 2017 were multiplied by 1.5 to scale them up to the larger transect areas (15m² per transect) measured starting in December 2017 (see text for details).

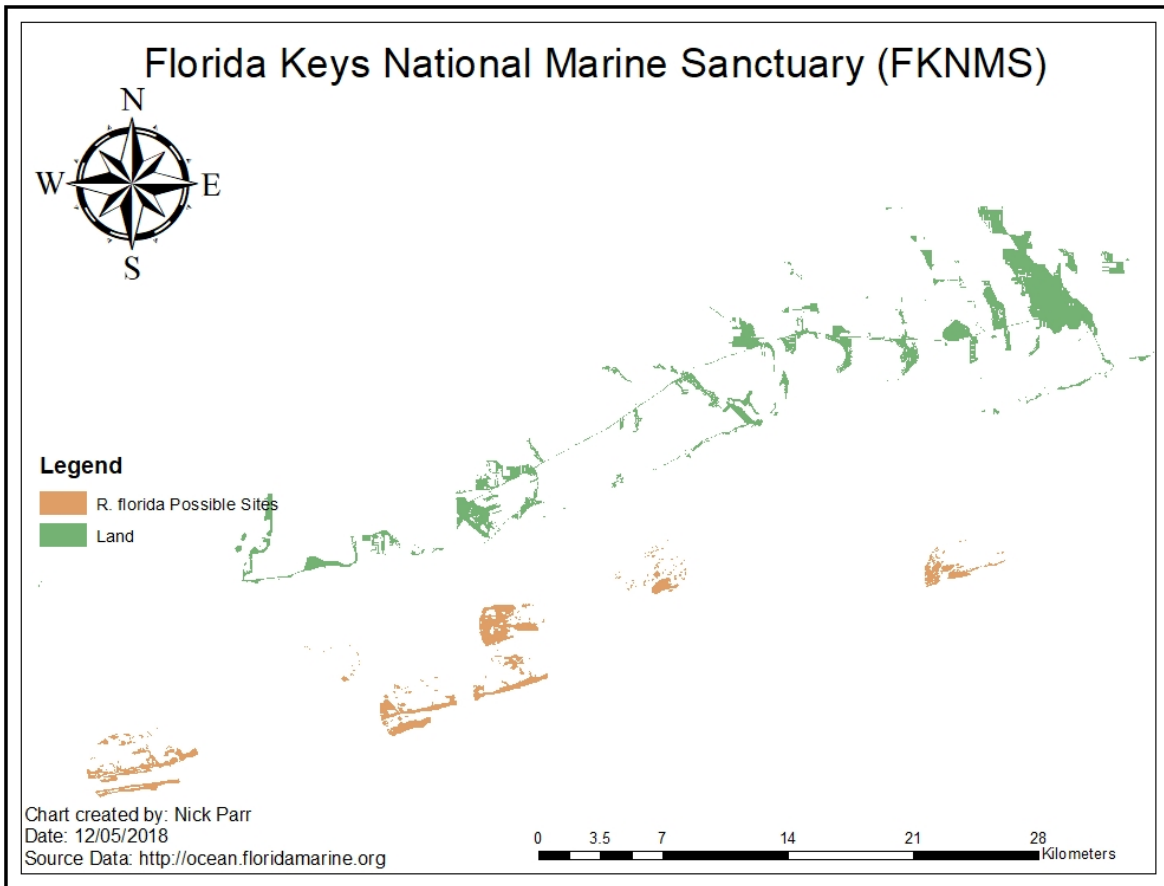


Figure 4-7: Spatial model of habitats predicted to contain abundant Florida false corals *Ricordea florida* in the Florida Keys, USA. Green areas represent *R. florida* habitat, based on 3 main factors: type of benthic substrate (hard bottom), depth below sea level (5.5 to 18 m depth), and the presence of dense upright octocorals for shading of *R. florida* polyps. See text for details.

Chapter 5

Sexual and asexual reproduction of Florida false corals *Ricordea florida*: Recovery following removal of aggregations in the Lower Florida Keys

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1 Introduction

Over the past several decades, the world's coral reefs have come under increasing pressure from a combination of natural disturbances (e.g. hurricanes, crown-of-thorns sea star outbreaks) and anthropogenic stressors which are both global in scope (e.g. warming seas, ocean acidification) and localized to certain areas (e.g. land based sources of pollution, coral breakage by divers; Anthony et al. 2008, Kuffner et al. 2015, Sweet & Brown 2016, Hughes et al. 2017, Paradis et al. 2019). This suite of stressors has led to massive die-offs of reef-building corals and related cnidarians (Fitt et al. 2001, Hughes et al. 2017). The populations of some reef species also are being depleted by high rates of collection for the marine life aquarium trade (Rhyne et al. 2009, Dee et al. 2014).

Tropical corallimorpharians (Anthozoa: Hexacorallia) are known in the aquarium trade as 'false corals' or 'mushroom corals', and are frequent target organisms for the marine life trade due to their often bright colors and ability to fluoresce (Rhyne et al. 2009, Dee et al. 2014). They are close relatives to scleractinian reef-building corals, but do not produce a calcium carbonate skeleton and so are soft-bodied, often forming aggregations of small, flat polyps on reef surfaces in both the Indo-Pacific and Caribbean regions (Fautin 2016). Little is known about the biology of most species of tropical corallimorpharians (reviewed in Chapter 1). To understand how their populations will change in the future, and to design restoration strategies for over-collected species, it is important to understand how they reproduce, as well as the extent of their recovery capabilities following collection disturbance.

All species of corallimorpharians examined thus far are able to reproduce both sexually and asexually (Chen et al. 1995a b, Chadwick-Furman et al. 2000, Edmunds 2007, Kuguru et al.

2007). They engage in asexual reproduction (clonal replication) through 3 major mechanisms that include longitudinal fission (which produces approximately equal-sized daughter polyps), and budding (which produces small daughter polyps relative to the parent polyp; Edmunds 2007). Two types of bud formation may occur depending on the species: pedal laceration of buds from the polyp base, and inverse budding from outgrowths on the oral disc (Chen et al. 1995a, Chadwick-Furman & Spiegel 2000). Longitudinal fission occurs via 2- or 3-way splitting of a single parent polyp (Chadwick & Adams 1991). In pedal laceration, a small tissue outgrowth near the pedal disc develops into a bud with tentacles and a mouth, which then locomotes across the substrate away from the parent to 'lacerate' or split from the pedal disk and form a new individual (Chadwick-Furman & Spiegel 2000). Inverse budding thus far is thought to be unique to corallimorpharians, and is known for only 2 Indo-Pacific species (Chen et al. 1995a, Chadwick-Furman & Spiegel 2000). In this mode of clonal replication, an area along the edge of the pedal disc lifts up from the substrate and folds in toward the oral disc. The edge of the oral disc then folds around the uplifted pedal disc area, to create a rounded bud attached to the top of the oral disc, in which the aboral surface faces upward and the oral disc, tentacles, and even sometimes a small bud mouth are oriented downwards (inverted) facing the oral disk of the parent polyp. The tissue that connects the rounded bud to the parent narrows to form a stalk, and then eventually constricts to sever the stalk and release a detached bud which floats away in the water column before settling and attaching to hard substrate elsewhere on the reef (Chen et al. 1995a).

Rates of clonal replication have been quantified in only 3 species of corallimorpharians, and appear to vary widely among individual polyps, with some individuals not cloning at all over several months or years, while other polyps may produce up to ~ 100 clonally-identical daughter

polyps per year (Chadwick & Adams 1991, Chen et al. 1995a, Chadwick-Furman & Spiegel 2000). The 2 tropical species that have been examined (*Rhodactis indosinensis* and *R. rhodostoma*) use all 3 clonal mechanisms described above, with longitudinal fission being the most common (Chen et al. 1995a, Chadwick-Furman & Spiegel 2000). The one temperate species examined, *Corynactus californica*, exhibits both fission and pedal laceration, at more rapid rates than have been documented in the 2 tropical species (Chadwick & Adams 1991). However, in a case study where a source of iron was introduced to Palmyra Atoll in the Pacific Ocean via a shipwreck, another species (*R. howesii*) appeared to clone so rapidly that within 1 year the aggregations almost completely covered $\sim 16 \times 10^6$ m² of shallow coral reef surface that previously had supported $\sim 30\%$ hard coral cover (Work et al. 2008). Clonal replication in corallimorpharians allows them to form large aggregations of genetically-identical polyps, and this process combined with their aggressive damage to other cnidarians allows these aggregations become dominant space occupiers on some temperate (Chadwick 1991) and tropical reefs (Chadwick-Furman & Spiegel 2000, Kuguru et al. 2004, Work et al. 2008).

Quantification of sexual reproductive processes in corallimorpharians also has been limited to the same 3 species examined for clonal replication. Separate female and male polyps in *C. californica* release oocytes and sperm respectively, in a synchronized spawning event during early December each year; gametes then are fertilized externally and planktonic larvae develop during the season when zooplankton concentrations are increasing in the surrounding waters, in late winter to early spring (Holts & Beauchamp 1993). In contrast, in the tropical species *R. rhodostoma* that hosts endosymbiotic microalgae, male and female polyps spawn gametes during late June to early July in the northern Red Sea, during the season when solar irradiance and therefore rates of microalgal photosynthesis are near their annual maxima

(Chadwick-Furman et al. 2000). Similarly, in Taiwan the male and female polyps of *R. indosinensis*, which also host microalgae, release gametes synchronously between May and June each year (Chen et al. 1995a). Broadcast spawning in all 3 species likely is followed by long-distance dispersal of planktonic larvae, contributing to their wide geographical distributions. In addition, the production of many floating sexual propagules each year (plus the floating clonal buds produced by tropical species, see above) allows them to colonize new space rapidly both locally and on distant reefs.

Polyps in large aggregations are spatially segregated by gender, with relatively large females located in the aggregation center, and small males occurring around the periphery (Holts & Beauchamp 1993, Chen et al. 1995b, Chadwick-Furman et al. 2000). This pattern also occurs in some cold-water actinian sea anemones, in which small warrior males or non-reproductives along the aggregation margins develop specialized aggressive organs that they use to repel members of neighboring aggregations, while large females at the clonal center instead produce abundant eggs (Francis 1976). Small immature polyps of *R. indosinensis* that were experimentally transplanted into the center of aggregations developed initially into males and then into females as they became larger, indicating potential protandric hermaphroditism (Chen et al. 1995a).

The Florida false coral *Ricordea florida* is a common corallimorpharian throughout the Caribbean Sea, and is economically important in the marine life fisheries of the Florida Keys (Rhyne et al. 2009), but no published information exists about the rates and patterns of either sexual or asexual reproduction in this species. Many of the commercial collectors who target this species recognize the need to conserve the populations, and therefore when they collect, they remove only some of the polyps from each aggregation, allowing clonal reproduction to

replenish their fishing areas (L. Gould, R. Herndon, F. Young, pers. comm.). This collecting practice may cause effects that are similar to those resulting from grazing behavior by hawksbill sea turtles *Eretmochelys imbricata* which are the only known predators on this species (Brandis et al. 2015), and which may engage in partial predation of aggregations.

Better understanding of reproductive patterns in Florida false corals *R. florida* is needed to provide information both for comparison with other corallimorpharian species, and to support sustainable fisheries management practices on this species. The goals of the present study therefore were to determine: (1) the annual spawning cycle for release of sexually-produced propagules, (2) the modes and rates of asexual reproduction (clonal replication), and (3) potential rates of recovery via both sexual and asexual mechanisms, after simulated disturbance in the form of partial or complete removal of polyps from aggregations.

2 Methods

2.1 *Sexual reproduction*

To determine the annual spawning cycle for release of sexually-produced propagules in *Ricordea florida*, polyps were collected from a back reef in the Lower Florida Keys near American Shoal (Cudjoe Key, Florida, USA; 24.5°N, 81.5°W, 6m depth) every 2-4 months for 1 year (6 sample periods during August 2017 - July 2018). This site was selected because it supported a stable population of abundant polyps, and also was visited frequently to collect data for a separate study on long-term population monitoring (Chapter 4). During each of the 6 sample periods, a large aggregation (defined as a group of > 4 polyps that all were < 15 cm

distant from each other) was selected haphazardly, and 2 polyps were collected from the aggregation: 1 relatively large (~ 20 to 30 mm oral disc diameter and 1 relatively small polyp (~ 10 to 20 mm oral disc diameter; see Chapter 4 and below for details). Then a second large aggregation was selected at least 1 m distant from the first one, and 2 more polyps collected in the same manner. Finally, 2 solitary polyps which were not in an aggregation (e.g. with all other polyps > 15 cm distance) were selected haphazardly and collected (6 polyps collected per sample period x 6 sample periods = 36 polyps total). Polyps were selected for collection in each of these types of groupings and body sizes, because previous research indicated that gender and gonadal development vary with these factors in related species (Chen et al. 1995b, Chadwick-Furman et al. 2000). Collection was performed by hand, using a hammer and blunt diving knife to remove a thin layer (< 0.5 cm thick) of reef substrate with each polyp attached (after Chapter 2). Care was taken to collect only polyps that were at least 1 m distant from long-term quadrats established at the same study site for assessment of long-term population changes (Chapter 4) and for quantification of responses to experimental removal (see Section 2.3 below).

After collection, all polyps were transferred into buckets of seawater on a support boat and transported back to shore (after Chapter 2). On shore, they were anesthetized in 7% MgCl₂ for ~ 1 min to relax the polyp tissues and reduce tentacle contraction, before being placed into individual 60-mL plastic vials filled with 20 mL of 10% formalin, in order to preserve their tissues for gonadal analysis (after Kuguru et al. 2007).

All preserved polyps were transported to Auburn University, where each was removed from its vial under a fume hood, and the pedal disc along with any attached reef substrate was cut off carefully using a scalpel or dissection scissors. This process exposed the gastrovascular cavity and internal mesenteries which are the sites of gonadal development in corallimorpharians

(Holts & Beauchamp 1993, Chen et al. 1995b, Chadwick-Furman et al. 2000). Each polyp then was examined for the presence of ovaries (containing oocytes) or testes (containing spermaries), at low magnification (10x) under a dissecting microscope. Tissues that appeared to be ovaries or testes were photographed and then carefully removed from the polyps, rinsed in ethanol, and stained with 0.1 $\mu\text{g}/\text{mL}$ DAPI for 60 min to stain for genetic material, before being rinsed again with ethanol. The excised gonadal tissues were placed onto glass slides. Cover slips were placed on top of the ovaries without flattening them or pressed gently on top of testes to slightly flatten them for viewing at high magnification. The slides were examined under a light microscope with fluorescence and phase contrast to visualize morphological details of the whole gonads and to view genetic material stained by DAPI.

Based on results of this repeated sampling during August 2017 – July 2018, 42 additional polyps were collected from the same field site on 12 June 2019, which was shortly before the expected annual spawning period (see Results section). This more extensive one-time sample was collected to enhance quantification of the relationship between body size and gender. For this sample, polyps were selected haphazardly that belonged to the 3 same grouping categories as described above (large polyps in aggregations, small polyps in aggregations, and solitary polyps), taking care to include polyps in a wide range of body sizes (~ 7 to 25 cm^2 ODA, 0.19 - 4.39 g wet mass), from ~ 30 different aggregations or solitary polyp groupings. Processing for these polyps was the same as described above, except that the wet mass of each was determined before it was examined for gonad presence and gender. Each polyp was removed from its vial of formalin, dabbed dry with a Kimwipe®, and any fragments of reef substrate removed, being careful to leave the pedal disk intact. The polyp then was weighed on a calibrated electronic scale to an accuracy of 0.0001g, its pedal disk was removed, and its internal mesenteries were

examined for gonads as described above. Wet mass was used here as a measure of polyp body size because it correlates tightly with all other measures of body size in this species (Chapter 2). The wet mass of these preserved polyps likely was smaller than their wet mass when alive, due to the dehydrating effects of formalin on animal tissues, and the water retention abilities of live polyps. Variation in gender (female, male, or juvenile [e.g. no gonads; non-reproductive]) with body size was analyzed using non-parametric ANOVA followed by posthoc pairwise comparisons.

2.2 *Asexual reproduction*

To determine the modes and rates of asexual reproduction (clonal replication) in *R. florida*, 20 polyps were monitored weekly during February - July 2018 (19 weeks) in laboratory tanks at Auburn University. These processes were assessed in cultured laboratory polyps in order to quantify maximal rates of asexual reproduction under ideal laboratory conditions, to support the development of aquaculture methods for this species, and also because it was possible to observe polyps weekly in the laboratory but not at the remote field site. Culture conditions were the same as those used during our previous laboratory studies on this species (see details in Chapters 2 and 3), which indicated that the optimal level of photosynthetically active irradiance (PAR) was $\sim 60 \pm 6 \mu\text{E m}^{-2} \text{ s}^{-1}$, optimal seawater temperature was $28 \pm 1^\circ\text{C}$, and optimal feeding rate was $\sim 1\text{x}$ per week of broadcast feeding to satiation, with Marine Cuisine™ and frozen brine shrimp (San Francisco Bay Brand, Newark, CA). These culture conditions allowed polyps of this species to actively grow and replicate, indicating healthy physiological condition.

Each week, the following data were collected for each monitored laboratory polyp: body size and dimensions (oral disk length and width, for calculation of ODA; see Chapter 2 for details), number of mouths, distance between mouths if more than one present (indicating longitudinal fission), presence of any small buds visible near the pedal disk and extending out from the polyp margin under the oral disc (indicating pedal laceration), or presence of any folds in the oral disc or growths on top of it (indicating inverse budding; see details below).

Less frequent, qualitative observations of asexual reproductive modes and rates were made on polyps that were used for other types of experiments in the laboratory (Chapters 2 and 3), and on polyps that we monitored for temporal changes in a field population in the Florida Keys (Chapter 4 and below).

2.3 *Recovery from disturbance*

To quantify potential rates of recovery of aggregations of *R. florida* via both sexual and asexual mechanisms after a disturbance in the form of partial or complete removal of polyps from aggregations, a field experiment was performed at the same study site used for polyp collection and long-term monitoring (see above). This site was selected for the field experiment because it contained abundant polyps and was visited frequently for monitoring (as described above), and also because it supported moderate percent cover of scleractinian corals (mostly large bouldering corals) but was dominated by medium-sized octocorals (sea fans and gorgonians, Order Alcyonacea, each ~ 1-2 m tall). These upright organisms provided shading from high solar irradiance levels and thus created optimal irradiance conditions for polyps of *R. florida* (Chapter 2). At this site, individuals of *R. florida* thus occurred mostly in healthy-looking

(non-bleached) aggregations near the bases of the upright reef organisms (Chapter 4), and were expected to exhibit frequent sexual and asexual reproduction.

To set up the field experiment, a central site marker was placed by hammering a 10" galvanized steel landscaping stake into the hard substrate of the reef using a 1.8 kg sledgehammer, and then attaching a polystyrene buoy to the spike using 1m of polyester line, and recording precise GPS coordinates for the marker. The location for the experiment site marker was selected haphazardly in the middle of an area that contained dense aggregations of *R. florida*, avoiding spatial overlap with permanent quadrats that were already established at the same site for long-term abundance surveys (Chapter 4). A plastic tape measure was extended for 10 m in each of the 4 main compass directions (north, east, south, and west), and another marker was placed at the end of each 10-m distance from the central point. We used 10" galvanized steel landscaping spikes for all markers, because preliminary trials revealed that 8" spiral landscaping spikes bent easily when pounding them into the reef surface, and regular cut nails were so small that they were difficult to relocate later, and they also often detached from the reef surface. We did not paint the markers because bright orange paint sprayed on the marker spikes during preliminary trials flaked off and created marine debris.

Around each of the 4 peripheral markers for experimental quadrat locations, a circular search pattern was used to locate 4 large (at least 10 polyps) healthy-appearing aggregations of *R. florida* (no signs of bleaching or recent removal of polyps, as evidenced by disturbed reef substrate) that each occurred < 6 m distant from the peripheral marker (N = 4 quadrat locations x 4 quadrats in each location = 16 peripheral quadrats). For each aggregation found near a peripheral marker, a rigid 0.5m x 0.5m PVC quadrat frame was centered on the aggregation. The two diagonal corners of each quadrat were marked with spikes and plastic surveying tape. Two

additional quadrats were established near the central site marker (16 peripheral quadrats + 2 central quadrats = 18 quadrats total). The compass heading and distance of each quadrat from the closest quadrat marker spike was recorded as an aid for repeated relocation of all experimental quadrats (Fig. 5-1).

All 18 quadrats were established on 15 August 2017, however Hurricane Irma (category 4) passed directly over the site on 10 September 2017 and removed many of the nails that served as quadrat markers, making it impossible to relocate each quadrat during the next survey in October 2017. A new series of quadrats using the same methods thus was re-established at the same site in June 2018, after a delay of several months to allow for recovery of populations, based on evidence that ~ 6 months was required for population recovery of *R. florida* (Chapter 4).

On 30 June 2018 after quadrat re-establishment, the following information was collected for all polyps within each of the 18 quadrats: polyp body size (oral disc width and length for calculation of ODA [see details in Chapter 4], measured to the nearest 0.1 cm using a small flexible plastic ruler) and clonal replication status (cloning or not, see details below). The percent cover of all polyps in each quadrat also was estimated. Then, using a random number generator, 1 of 4 experimental treatments was assigned randomly to each of the 4 quadrats within each peripheral cluster: removal of 0%, 33%, 66%, or 100% of the polyps from the aggregation within the quadrat. Each treatment was applied to 1 quadrat in each cluster, creating a randomized block design (Hurlbert 1984; Fig. 4-1). Additionally, the 2 most extreme treatments (0% and 100% removal of polyps) were applied to the 2 central quadrats to enhance the sample size for those 2 treatments, resulting in N = 4 quadrats total for the 33% and 66% removal treatments, and N = 5 quadrats for the 0% and 100% removal treatments. To simulate the population effects of polyp

removal methods used by commercial marine life collectors in the Florida Keys, polyp removal was performed by an experienced collector of this species. Standard industry collection methods were used, in which the diver scraped the reef surface with a blunt-tipped knife to remove each polyp, taking care to minimize damage to the polyp's pedal disc, and to minimize removal of reef substrate. Due to time limitations for each dive underwater, the treatments were applied to 3 of the quadrat clusters (North, West, South) on 30 June 2018, and to the remaining 2 clusters (East, Central) on 19 July 2018.

The site then was revisited 5 times (every 1-4 months) for almost 1 year (19 July 2018, 9 August 2018, 13 January 2019, 16 March 2019, and 12 June 2019), and the same 2 types of data were recorded for each of the remaining polyps in each quadrat. The total percent cover of polyps inside the quadrat also was estimated. Due to persistent windy conditions creating low underwater visibility (< 1 m) during 11 Oct 2018, the site visit for that date was aborted without data collection.

ANOVA tests were applied to analyze variation among the 4 treatments over time in the number of polyps per quadrat, percent remaining of the original number of polyps per quadrat, polyp body sizes, percent of polyps that were cloning, and the percent cover of polyps on the reef surface.

3 Results

3.1 *Sexual reproduction*

Examination under a dissecting microscope (10x magnification) of the 6 preserved polyps that were collected on each of 5 dates during the first study year (15 August 2017, 16 October 2017, 29 December 2017, 2 March 2018, and 8 May 2018) did not reveal any obvious structures that appeared to be developing gonads. When the polyps were examined which had been collected on the sixth collecting date of the first year (30 June 2018), the 2 largest polyps (out of 6 total) each contained many rounded oocytes that were loosely attached to mesenteries in the central gastrovascular cavity of each polyp, and thus were categorized as female polyps (Fig. 5-2). The fully developed oocytes filled a large portion of the volume of each gastric cavity. In addition, the next largest polyp (1 of the 6 total collected) contained oblong, light-colored testes that were located along the periphery of the gastrovascular cavity near the body wall, loosely attached to the mesenteries near where they intersected with the polyp wall (Fig. 5-3). This polyp thus was categorized as a male. The other 3 polyps collected on that date all were relatively small and did not appear to contain any gonads when viewed under the dissecting microscope, so were categorized as juveniles.

Histological sections that were prepared from excised gonads of the 2 female polyps collected on 30 June 2018 revealed large, waxy oocytes attached to the polyp mesenteries (Fig. 5-4). Each oocyte had a single rounded indentation on its surface, but otherwise was spherical. Staining revealed no genetic material on the outside of the oocytes. A flattened, excised testes of the 1 male polyp revealed round spermaries with many sperm. The sperm heads were oriented toward the center of each spermary packet (Fig. 5-5). Each testis was comprised of numerous spherical spermaries which contained fully-developed sperm with their tails oriented outwards from the spermary, suggesting that spawning was imminent.

Of the 6 polyps that were collected during the seventh and last sample date during the initial year of study (19 July 2018), none appeared to contain any oocytes or testes. The largest 2 polyps from that sample date contained some mesenteries that appeared to be pushed together, and others with large empty, open spaces between them inside the gastrovascular cavity, while in contrast the relatively small polyps from that collection date contained more evenly-spaced mesenteries with no large empty spaces between them (Fig. 5-6). It was therefore inferred that at this study site the polyps of *R. florida* had separate sexes, developed fully formed gonads by the end of June, and both males and females spawned their gametes for external fertilization sometime during early July. The smaller polyps collected in July 2018 appeared to be males or juveniles, while the largest polyp with open spaces where numerous oocytes appeared to have been previously located therefore likely was female.

Most of the polyps that were collected during the following summer, shortly prior to the presumed annual spawning period (on 12 June 2019), contained ripe gonads: 35.7% contained ovaries with oocytes (female), 35.7% contained testes (male), and the remaining 28.6% contained neither ovaries nor testes (juvenile; N = 42 polyps total). Gender varied significantly with body size (ANOVA, $p < 0.001$). Posthoc pairwise comparisons revealed that females (2.34 ± 0.21 g wet mass) were significantly larger than males (1.39 ± 0.17 g), which were significantly larger than juveniles (Fig. 5-7; 0.53 ± 0.08 g; $p < 0.002$ for each pairwise test).

During field collection, individuals of this species were observed to form loose aggregations, with polyps often several cm distant from each other within a given aggregation. The largest observed polyps were not located consistently in the centers versus the edges of aggregations, and some of the largest polyps were solitary (not in aggregations). Therefore, the

locations of polyps in relation to each aggregation did not appear qualitatively to relate to either body size or gender.

3.2 *Asexual reproduction*

In laboratory cultured polyps, the most common mode of asexual reproduction (clonal replication) was longitudinal fission, with 60% of the monitored individuals (12 of the monitored 20 polyps) exhibiting at least some stages of fission during the 6 months of observation. Fission was slow, with only 10% of all polyps (2 the 20 polyps) completing fission in 6 months and no polyp beginning and finishing fission within that time period. One polyp fissioned to produce a total of 3 daughter polyps, and the other fissioned to produce 2 daughter polyps.

Six distinct stages of fission were observed (Fig. 5-8): Stage 1= no fission, in which the polyp did not appear to be engaged in fission; Stage 2 = early fission, in which the polyp developed a second mouth near the first one; Stage 3 = mid-stage fission, in which the polyp began to elongate laterally and the 2 or 3 mouths moved further apart; Stage 4 = advanced fission in which the polyp tissue pinched together or narrowed between 2 of the mouths, creating an hourglass shape to the elongated polyp body (some polyps passed through this stage so quickly that it was not observed in all fissioning polyps); Stage 5 = final stage in which the 2 newly-forming daughter polyps began to separate by locomoting away from each other, but remained attached via a narrow tissue bridge; and Stage 6 = completed fission, in which the 2 daughter polyps were completely separated. In some cases during Stage 6, one of the daughter polyps had already developed a second mouth and begun to fission again. A doubling time for polyp fission

could not be established, because no polyps both began and completed fission during the study period.

Inverse budding was the second most commonly-observed type of cloning in laboratory cultured polyps, with 40% of individuals (8 of the 20 monitored polyps) exhibiting at least some stages of this asexual mode. Similar to the pattern for longitudinal fission, inverse budding was slow, with none of the polyps completing the process of releasing an inverse bud during the 6 months of observation.

Five distinct phases of inverse budding were observed (Fig. 5-9): Stage 1 = no budding, in which the polyp did not appear to be engaged in inverse budding; Stage 2 = early budding, in which small folds appeared along the edge of the oral disc (in many cases, the polyp regressed from this stage back into Stage 1); Stage 3 = mid-stage inverse budding, in which the edges of at least 1 folded tissue area fused together, and this raised fused region of tissue elongated toward the mouth at the center of the polyp's oral disc; Stage 4 = advanced inverse budding, in which the raised bud moved away from the oral disc edge, became spherical, and in some cases developed a mouth on the side facing the oral disc of the parent polyp; and Stage 5 = pre-detachment phase, in which the tissue area connecting the bud to the parent polyp constricted to form a stalk. A final phase of this budding process, Stage 6 (completed inverse budding, in which the stalk severed and the spherical bud detached and floated away in the water column) was not observed here, but has been described from past studies of inverse budding in corallimorpharians (Chen et al. 1995a, Chadwick-Furman & Spiegel 2000).

The third mode of asexual reproduction observed in laboratory polyps, pedal laceration, was rare. It was not recorded in the 20 polyps monitored here for 6 months, but was observed to

be completed by a polyp that was cultured in the laboratory as part of a separate experiment (Fig. 5-10; Chapter 2).

Field observations and those made on other polyps cultured in the laboratory confirmed that these 3 modes of asexual reproduction occurred in all groups of polyps that were observed for this and related studies (Chapters 2-4), with longitudinal being most commonly observed, inverse budding being less common, and pedal laceration being rarely observed.

3.3 *Recovery from disturbance*

At the beginning of the field experiment that was initiated during 30 June – 19 July 2018, the 18 selected large aggregations (1 per quadrat x 18 quadrats examined) contained 20.3 ± 1.6 polyps per quadrat (range = 10 - 31). After the 4 experimental treatments were applied, aggregation sizes in some of the quadrats were greatly reduced (Fig. 5-11). The abundance of polyps in the control aggregations in which no polyps had been removed (0% removal) did not differ significantly from those in which only a third of polyps had been removed (33% removal), at any point during the study year ($p > 0.09$), and remained high at ~ 10-21 polyps per aggregation (Fig. 5-11A). In contrast, the percent of remaining polyps in the 33% removal aggregations remained significantly lower ($p < 0.05$) than that in the unmanipulated aggregations (0% removal, ie: 100% of polyps remaining), until week 50 (12 June 2019; $p = 0.24$), when they did not differ because polyps in the 33% removal had replicated enough to regain their original numbers. Percent cover differed between these 2 treatments during only some of the sample periods: it was significantly lower initially (week 0, $p = 0.02$) and at week 6 ($p = 0.04$), but not at week 3 ($p = 0.24$) or after week 28 ($p > 0.07$; Fig. 5-11B). The percent of polyps that were

cloning within each aggregation also did not differ significantly between the control and the one-third removal (33%) treatments, at any time during the study year ($p > 0.08$). Cloning percent remained high in both treatments during most of the year, at ~30-40% of polyps, but then varied more at the end of the year, at ~ 20-60% of polyps (Fig. 5-11C).

Aggregations from which most polyps (66%) had been removed contained a significantly lower abundance of polyps than in the control unmanipulated group ($p < 0.01$), until week 6 (9 August 2018) when they recovered enough that they did not differ significantly from the control group ($p > 0.12$; Fig. 5-11A). In contrast, the percent of the original number of polyps within each aggregation remained significantly lower than in the control group ($p < 0.05$) until late during the study year (week 50; $p = 0.84$). Percent cover in the 66% removal treatment also remained significantly lower than in the control treatment ($p < 0.02$), until halfway through the study year (week 28; 13 Jan 2019; $p > 0.08$; Fig. 5-11B). Conversely, the percent of polyps cloning remained high throughout, and at no point was significantly different from that in the control group ($p > 0.09$; Fig. 5-11C).

Aggregations in the 100% removal treatment showed very little recovery, with few polyps and low percent cover of any polyps that had immigrated into those quadrats, throughout the study year. Compared to the control group in which no polyps were removed, polyp abundance in the 100% removal group remained significantly lower throughout the entire study ($p < 0.03$; Fig. 5-11A), as did the percent of the original number of polyps remaining within the quadrat ($p < 0.02$), and the percent cover of polyps on the reef surface ($p < 0.007$). In contrast, the percent of polyps cloning was significantly lower until week 50 ($p < 0.009$), at which point the few polyps that had immigrated into these 100% removal quadrats increased their cloning rate, so that it did not differ significantly from that in the unmanipulated control aggregations (p

= 0.074). The total number of polyps that appeared in all 5 quadrats to which the 100% removal treatment had been applied, never exceeded 13 during the study year, making the sample size for assessing percent cloning very small, and indicating that many of these 13 immigrated polyps engaged in clonal replication.

Due to the staggered starting point for the east and central cluster, no week 6 sampling was done for 1 plot in each the 33% and 66% removal treatment ($n = 3$) and 2 plots in the 0% and 100% removal treatments ($n = 3$). Another note is that due to the loss of a data sheet while surfacing from the dive at week 50, and inability to re-collect the lost data because the spikes marking the quadrats just been removed at the end of the experimental study, the sample sizes at week 50 were slightly decreased: for the 33% and 66% removal treatments, sample size was reduced by 2 ($n = 2$), and for the no removal control treatment, sample size was reduced by 1 ($n = 4$). No data were lost for the 100% removal treatment.

4 Discussion

We show here that Florida false corals *Ricordea florida* reproduce sexually on an annual cycle, with small males and large females both broadcast spawning their gametes during late June to early July each year, near the period of maximal daylength and seawater temperature in the Florida Keys. They also employ 3 clear mechanisms of asexual reproduction, with fission being the most common, and clonal replication occurring at fairly slow rates over a 6-month period. Finally, aggregations of this species appear able to recover fully from removal of ~ 33-66% of polyps, within < 12 months in a field population in the Lower Florida Keys. In contrast,

if all polyps are removed from each aggregation, full recovery via the recruitment of potentially planktonic propagules may require several years if recovery occurs at all.

The ability of *R. florida* to reproduce both sexually and asexually is similar to the pattern observed in the 3 other corallimorpharian species examined thus far for reproductive abilities (Holts & Beauchamp 1993, Chen et al. 1995a b, Chadwick-Furman et al. 2000, Edmunds 2007, Kuguru et al. 2007). The sperm and oocytes of *R. florida* also appeared to be similar to those of other members of the anthozoan Subclass Hexacorallia (stony corals and sea anemones; Fadlallah & Pearse 1982, Wedi & Dunn 1983, Chadwick-Furman et al. 2000, Al-Hashmi 2012). In the oocytes, the single indentation likely led to a sperm duct located within a waxy ball. As in scleractinian corals, this waxy ball provides buoyancy to the oocyte to aid in dispersal, and also provides necessary energy storage; after the fertilized planula consumes enough of the buoyant lipids, it then becomes negatively buoyant and can potentially settle on the benthos (Tsai et al. 2016). Based on the increased abundance of very small polyps of this species that we observed at our long-term study site in the Florida Keys between December and March 2018, it is possible that the planula larvae of this species remain in the water column for a lengthy period of up to six months (~ July to December / March each year) before settling and rapidly growing (Chapter 3), however further research is needed to confirm this pattern across multiple years. The spermary packets in this species were oriented with the sperm tails facing outwards, which suggests that spawning of the sperm was likely to occur very soon after the late June period of observation in the present study.

Our observation of no gonads present in the polyps collected during mid-July demonstrates that this species spawned some time between 30 June and 19 July 2018. This period would correspond to the first new moon after the summer solstice, and did not include a

period with a full moon. This timing also coincides with the longest daylength during the year, and therefore the highest daily energy availability via photosynthesis of the endosymbiotic microalgae in shallow tropical corallimorpharians (Chen et al. 1995a). In addition, the precise timing of likely nocturnal spawning as known for other anthozoans corresponds to the lowest nocturnal light (new moon) during the evening spawn, to reduce predation by nocturnally active planktivorous fishes (Ward 1992, Schlöder & Guzman 2008). Polyps collected the following summer during June 2019 were sampled prior to the summer solstice, and many of them contained abundant gonads, further indicating that spawning occurs after the solstice each year.

The largest polyps examined were all female, the medium sized polyps were male, and the smallest polyps were juvenile, indicating clear segregation of genders among size classes similar to the pattern known for all other species of corallimorpharians examined to date, and also suggesting potential hermaphroditic protandry (Holts & Beauchamp 1993, Chen et al. 1995b, Chadwick-Furman et al. 2000). Once reaching a minimum body size for sexual maturity, the polyps of *R. florida* therefore appear to initially become males, and then if they grow further, they could then transform into females. The process of sex change could enhance polyp fitness, because producing large, lipid-dense oocytes is more energy intensive than is sperm production, so after polyps become large in body size they are more able to switch energy allocation from inexpensive sperm production to the more energy-intensive production of a large number of waxy eggs. It should be noted that the wet masses of the preserved polyps examined here likely were smaller than those of the same polyps when they were alive (see details in Methods section). Therefore, this potential reduction in body mass after preservation should be taken into account when attempting to predict the wet mass or other body dimensions of live polyps from preserved ones (see correlation graphs of the body size dimensions of live polyps, Chapter 3).

The 3 types of asexual reproduction observed here for *R. florida* are the same as those documented for the other 2 species tropical corallimorpharians examined thus far, at the same relative rates of fission being most common, then inverse budding, and then pedal laceration being rare (Chen et al. 1995a, Chadwick-Furman & Spiegel 2000). However, absolute rates of asexual reproduction differ among these species (Chen et al. 1995a, Chadwick-Furman & Spiegel 2000). Longitudinal fission likely contributes the most to creating large aggregations of *R. florida* on coral reefs, while inverse budding may allow planktonic dispersal of asexual propagules to establish new aggregations on the reef throughout the year, in addition to those established via sexual propagules from the annual spawning event. The rareness of pedal laceration explains in part why monitoring of wild populations reveals only rare individuals of the smallest size class (oral disc area < 300mm²) of polyps (Chapter 4). Overall, the relative rates of the three types of asexual reproduction are fairly similar to the patterns known for other corallimorpharians (Chen et al. 1995a, Chadwick-Furman & Spiegel 2000, Edmunds 2007).

Based on the field experimental results, complete removal (100%) of an aggregation drastically diminishes its chances for recovery. Of the 5 plots where the entire aggregation was removed, none recovered to near the number of original polyps, despite the fact that the reef substrate from which they were removed remained bare, and the shading organisms that they were under remained healthy. By the end of the study, 4 of the 5 quadrats in the 100% removal treatment had no polyps at all in them. None of the quadrats in the other 3 treatments ever dropped to 0 polyps, including the plots that became disturbed by natural processes (potential predation by sea turtles; N. Parr, pers. obs.) during the study.

A few localized disturbances were noted at quadrats during the course of this study. Between weeks 3 and 6, one of the control quadrats experienced a drop in number of polyps

from 23 to 11, and the shading gorgonian appeared to have been damaged. Since marine life collectors avoid damaging any benthic organisms and this area was marked as a research area for no removal, it is unlikely that this disturbance was anthropogenic. Therefore, it is possible that a Hawksbill turtle *Eretmochelys imbricata* fed on the polyps within that quadrat and inadvertently damaged the shade-providing gorgonian nearby. Other evidence of turtles visiting the reef site include a beak-like bite out of the polystyrene buoy ball that marked the site, and the polyp abundance in another control quadrat dropping from 15 polyps to 1 polyp between weeks 37 and 50, once again with damage to the shading organism. Another disturbance during week 37 in two other quadrats was observed, in which the two aggregations of *R. florida* appeared undisturbed, but the shading organisms were broken at their bases. Then during the sample period immediately afterwards, within one of the 66% removal quadrats the number of polyps dropped from 6 to 3, and in one of the control quadrats the number of polyps dropped from 17 to 11. These patterns of change provide support for our conclusion based on separate experiments, that polyps of *R. florida* on shallow reefs are shade-dependent (Chapter 2), in that after shading organisms are damaged, the nearby *R. florida* polyps may reduce in numbers. These patterns however also could have been caused by sea turtle predation or other factors.

Our concurrent study on observed natural changes in the population of *R. florida* throughout the year, at the same study site as that examined here, indicated that in the absence of natural disturbances, this reef site supports a stable population structure of this species (Chapter 4). However, this species also is near its thermal tolerance limit during the hottest months of the year, which coincided with the first 6 weeks of the present study (Chapter 3). Therefore, it is possible that a more rapid initial recovery would have been possible if collection had occurred at a cooler time of the year in the absence of natural disasters.

We conclude that the current practices used by marine life collectors, of removing only part of each aggregation, supports the regular recovery of *R. florida* aggregations after removal. While aggregations where only 33% polyps were removed recovered quicker, both the 33% and 66% removals were able to recover within one year. The use of this removal method, combined with limitation of overall collection pressure within each reef area, and allowing populations to recover after natural disasters or stressors (e.g. hurricanes or El Niño events), may allow this fishery to remain sustainable indefinitely, or until overall environmental conditions change.

5 Acknowledgements

We thank Captain Roy Herndon and his daughter Dayanara for discussions about Marine Life Fisheries and for providing boating support and access to our field site. Thanks to Dr. Donald Behringer (University of Florida) for assisting in the project design. Special thanks to Emily Hutchinson and Kristine Fisher for help with logistics and Shannon Duffy for assistance with field work. Field work was done under FWC Permit #SAL-17-1907-SR and NOAA Permit #FKNMS-2017-031. Funding was provided by a Lerner-Gray Grant for Marine Research from the American Museum of Natural History to NP, and by an Intramural Grant from Auburn University to NEC.

6 References

- Al-Hashmi A (2012) Coral reef reproduction and its features in the Arabian Gulf (Jebel Ali, UAE). *International Journal of Environment and Sustainability* 1(3):12-21.
- Anthony KR, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proceedings of the National Academy of Sciences* 105:17442–17446.
- Brandis RG von, Mortimer JA, Reilly BK, Soest RWM van, Branch GM (2015) Diet of hawksbill turtles (*Eretmochelys imbricata*) in the Republic of Seychelles. *Western Indian Ocean Journal of Marine Science* 13:81–91.
- Chadwick NE (1991) Spatial distribution and the effects of competition on some temperate Scleractinia and Corallimorpharia. *Marine ecology progress series Oldendorf* 70:39–48.
- Chadwick NE, Adams C (1991) Locomotion, asexual reproduction, and killing of corals by the corallimorpharian *Corynactis californica*. *Hydrobiologia* 216:263–269.
- Chadwick-Furman NE, Spiegel M (2000) Abundance and clonal replication in the tropical corallimorpharian *Rhodactis rhodostoma*. *Invertebrate Biology* 119:351–360.
- Chadwick-Furman NE, Spiegel M, Nir I (2000) Sexual reproduction in the tropical corallimorpharian *Rhodactis rhodostoma*. *Invertebrate Biology*:361–369.
- Chen C-LA, Chen C-P, Chen I-M (1995a) Sexual and asexual reproduction of the tropical corallimorpharian *Rhodactis* (= *Discosoma*) *indosinensis* (Cnidaria: Corallimorpharia) in Taiwan. *Zool Stud* 34:29–40.

- Chen CLA, Chen C-P, Chen I-M (1995b) Spatial variability of size and sex in the tropical corallimorpharian *Rhodactis* (= *Discosoma*) *indosinensis* (Cnidaria: Corallimorpharia) in Taiwan. *Zool Stud* 34:82–87.
- Dee LE, Horii SS, Thornhill DJ (2014) Conservation and management of ornamental coral reef wildlife: Successes, shortcomings, and future directions. *Biological Conservation* 169:225–237.
- Edmunds PJ (2007) Physiological ecology of the clonal corallimorpharian *Corynactis californica*. *Marine Biology* 150:783–796.
- Fadlallah YH, Pearse JS (1982) Sexual reproduction in solitary corals: Synchronous gametogenesis and broadcast spawning in *Paracyathus stearnsii*. *Mar Biol* 71:233–239.
- Fautin DG (2016) Catalog to families, genera, and species of orders Actiniaria and Corallimorpharia (Cnidaria: Anthozoa). *Zootaxa* 4145:1.
- Fitt W, Brown B, Warner M, Dunne R (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* 20:51–65.
- Francis L (1976) Social organization within clones of the sea anemone *Anthopleura elegantissima*. *The Biological Bulletin* 150:361–376.
- Holts L, Beauchamp K (1993) Sexual Reproduction in The Corallimorpharian Sea-Anemone *Corynactis californica* in a Central California Kelp Forest. *Mar Biol* 116:129–136.
- Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, Babcock RC, Beger M, Bellwood DR, Berkelmans R, Bridge TC, Butler IR, Byrne M, Cantin NE, Comeau S, Connolly SR, Cumming GS, Dalton SJ, Diaz-Pulido G, Eakin CM, Figueira WF, Gilmour JP, Harrison HB, Heron SF, Hoey AS, Hobbs J-PA, Hoogenboom MO, Kennedy EV, Kuo C, Lough JM, Lowe RJ, Liu G, McCulloch MT,

- Malcolm HA, McWilliam MJ, Pandolfi JM, Pears RJ, Pratchett MS, Schoepf V, Simpson T, Skirving WJ, Sommer B, Torda G, Wachenfeld DR, Willis BL, Wilson SK (2017) Global warming and recurrent mass bleaching of corals. *Nature* 543:373.
- Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecological monographs* 54:187–211.
- Kuffner IB, Lidz BH, Hudson JH, Anderson JS (2015) A century of ocean warming on Florida Keys coral reefs: Historic in situ observations. *Estuaries and Coasts* 38:1085–1096.
- Kuguru B, Winters G, Beer S, Santos SR, Chadwick NE (2007) Adaptation strategies of the corallimorpharian *Rhodactis rhodostoma* to irradiance and temperature. *Marine Biology* 151:1287–1298.
- Kuguru BL, Mgaya YD, Ohman MC, Wagner GM (2004) The reef environment and competitive success in the Corallimorpharia. *Marine Biology* 145:875–884.
- Paradis BT, Henry RP, Chadwick NE (2019) Compound effects of thermal stress and tissue abrasion on photosynthesis and respiration in the reef-building coral *Acropora cervicornis* (Lamarck, 1816). *Journal of Experimental Marine Biology and Ecology* 521:151222.
- Rhyne A, Rotjan R, Bruckner A, Tlusty M (2009) Crawling to collapse: Ecologically unsound ornamental invertebrate fisheries. *PLOS ONE* 4:e8413.
- Schlöder C, Guzman HM (2008) Reproductive patterns of the Caribbean coral *Porites furcata* (Anthozoa, Scleractinia, Poritidae) in Panama. *Bulletin of marine science* 82:107–117.
- Sweet M, Brown B (2016) Coral responses to anthropogenic stress in the twenty-first century: An ecophysiological perspective. *Oceanography and Marine Biology: An Annual Review* 54:271–314.

- Tsai S, Chang W-C, Chavanich S, Viyakarn V, Lin C (2016) Ultrastructural observation of oocytes in six types of stony corals. *Tissue and Cell* 48:349–355.
- Ward S (1992) Evidence for broadcast spawning as well as brooding in the scleractinian coral *Pocillopora damicornis*. *Marine Biology* 112:641–646.
- Wedi SE, Dunn DF (1983) Gametogenesis and reproductive periodicity of the subtidal sea anemone *Urticina lofotensis* (Coelenterata: Actiniaria) in California. *The Biological Bulletin* 165:458–472.
- Work TM, Aeby GS, Maragos JE (2008) Phase shift from a coral to a corallimorph-dominated reef associated with a shipwreck on Palmyra Atoll. *PLoS ONE* 3:e2989.

7 Figures

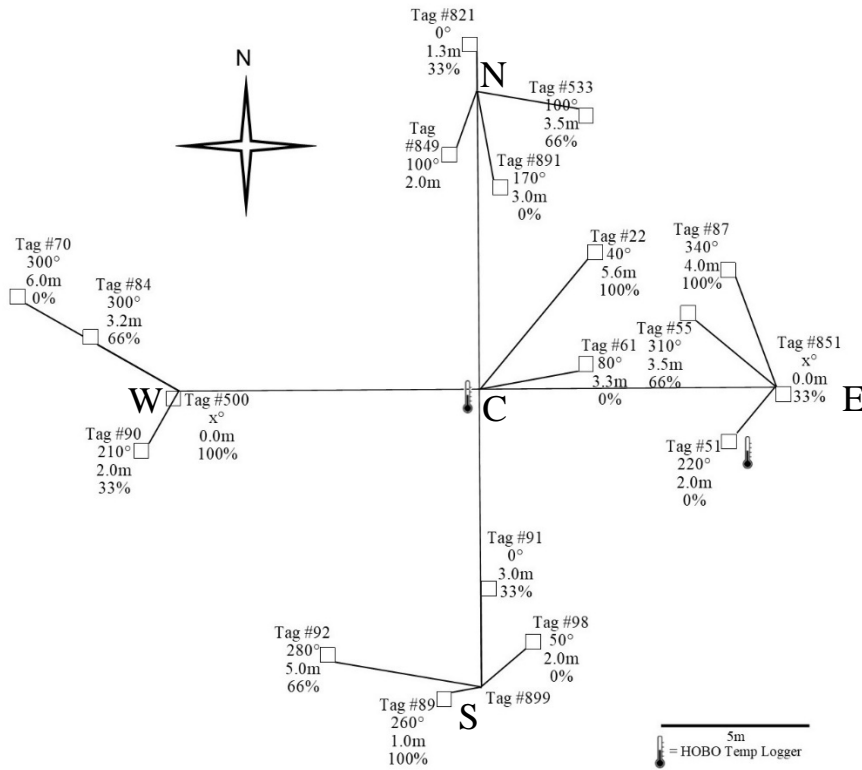


Figure 5-1. Map of field site for experimental manipulation of Florida false corals *Ricordea florida* at the back-reef near American Shoal in the Lower Florida Keys, to determine recovery rates of aggregations from removal disturbance. Shown are the central site marker (C) and 4 peripheral site markers each located 10 m from the central marker, at the 4 cardinal compass headings (N, E, S, W). Near each peripheral marker, 4 quadrat locations were selected that each contained an aggregation of *R. florida*, and experimental treatments were assigned randomly as 0, 33, 66, or 100% of polyps removed from each aggregation, plus 2 quadrats at the central marker were assigned 0 and 100% removal treatments. Each quadrat was 0.5 x 0.5 m in area (squares; N = 18 quadrats total). Two temperature and light HOBO loggers also were placed at the site (see Chapters 2 and 3). Map is drawn to scale. See text for details.

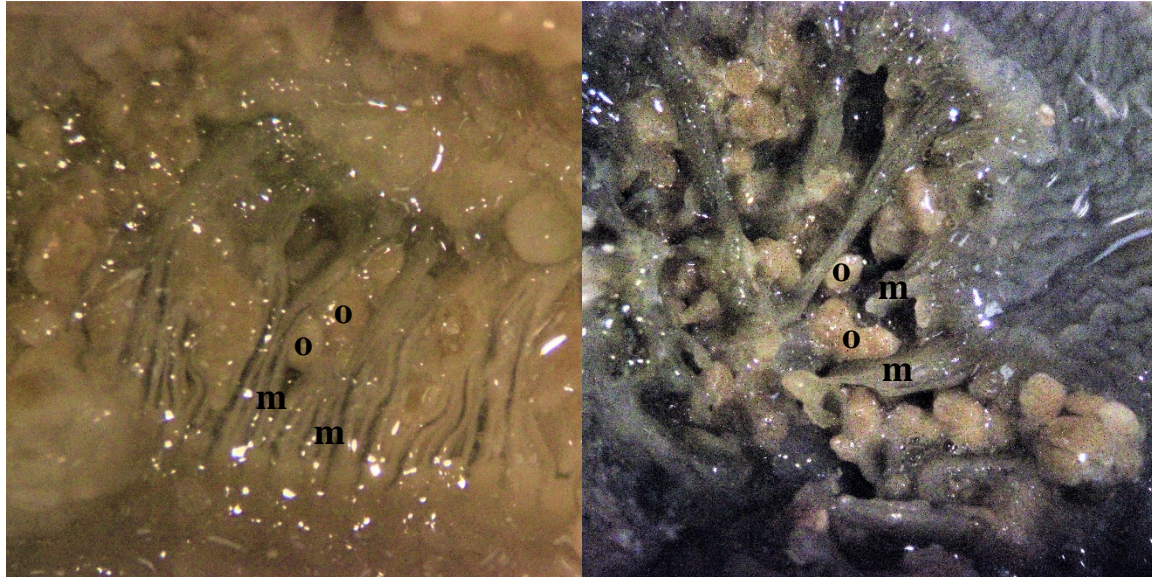


Figure 5-2. Photographs of ovaries in polyps of the Florida false coral *Ricordea florida* that were collected on 30 June 2018 from a coral reef in the Lower Florida Keys. Left: View of ovaries containing oocytes, located on the polyp mesenteries, as seen in a transverse view of a polyp that has been cut in half laterally. The oral disk of the polyp is at upper right, and the basal area is at lower left. Right: Aboral view of abundant ovaries with mature oocytes, as seen from the bottom of a different polyp, with the pedal disc removed. o = ovary, m = mesentery.

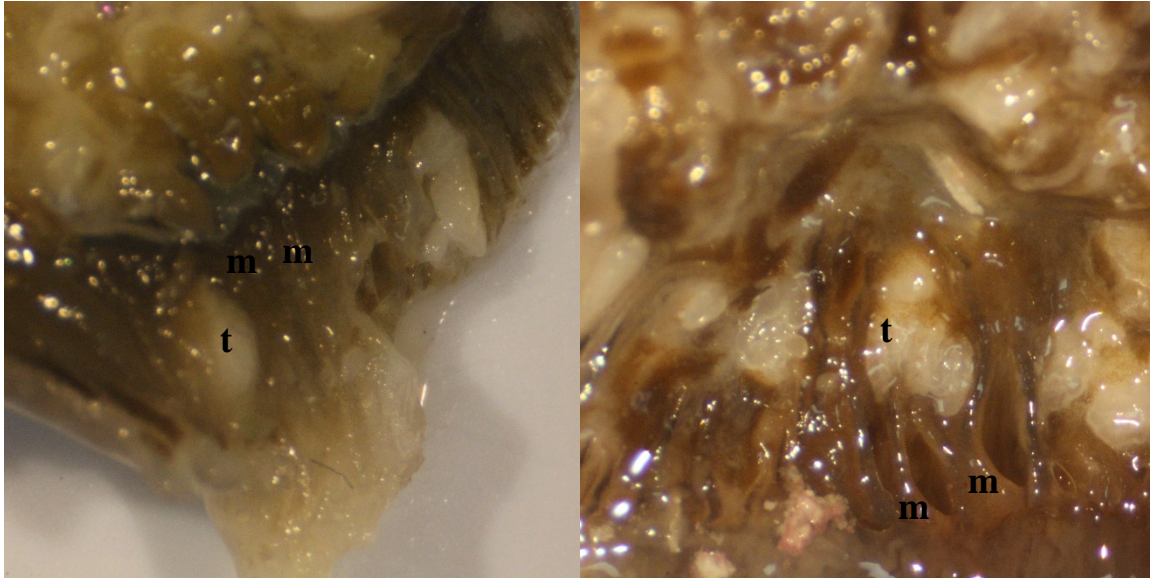


Figure 5-3. Photographs of testes in polyps of the Florida false coral *Ricordea florida* that were collected on 30 June 2018 (left) and 12 June 2019 (right) from a coral reef in the Lower Florida Keys. Left: View of testes, as seen in a transverse view with the body wall removed. At the lower right is the lateral edge of the polyp, and at upper left is the central area of the polyp, looking down from the top area of the polyp. Right: Aboral view of abundant testes as seen from the bottom of a different polyp, with the pedal disc removed. At the top is the central mouth area of the polyp, with the mesenteries radiating outward from the center. t = testis, m = mesentery.

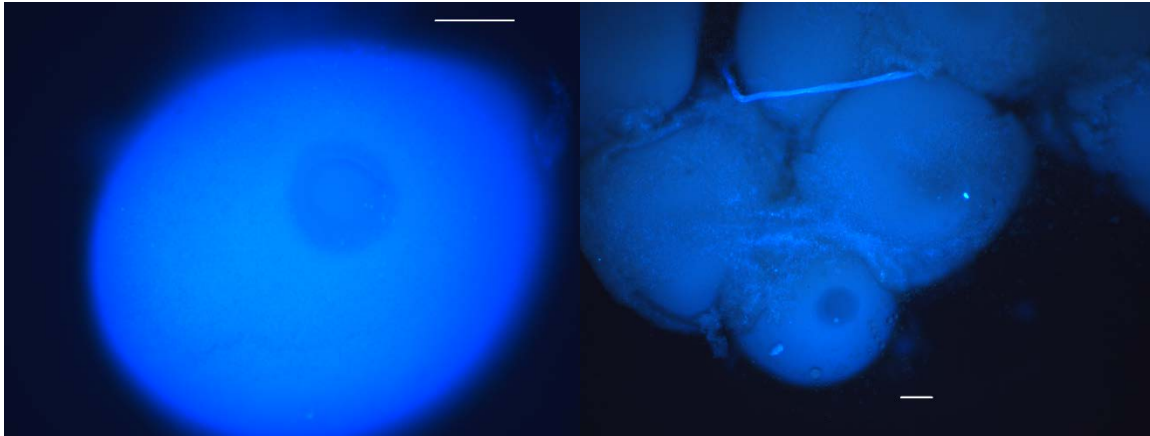


Figure 5-4. Photographs of oocytes extracted from a preserved polyp of *Ricordea florida* that was collected from a coral reef in the Lower Florida Keys on 30 June 2018. Shown are images of whole oocytes under high-power fluorescence microscopy, with a DAPI stain for genetic material. Left: A single oocyte. Right: A cluster of oocytes forming part of an ovary, in which the surrounding ovarian tissue is visible. Note the large circular depression visible on the surface of some oocytes. Scale bars = 0.1 mm.

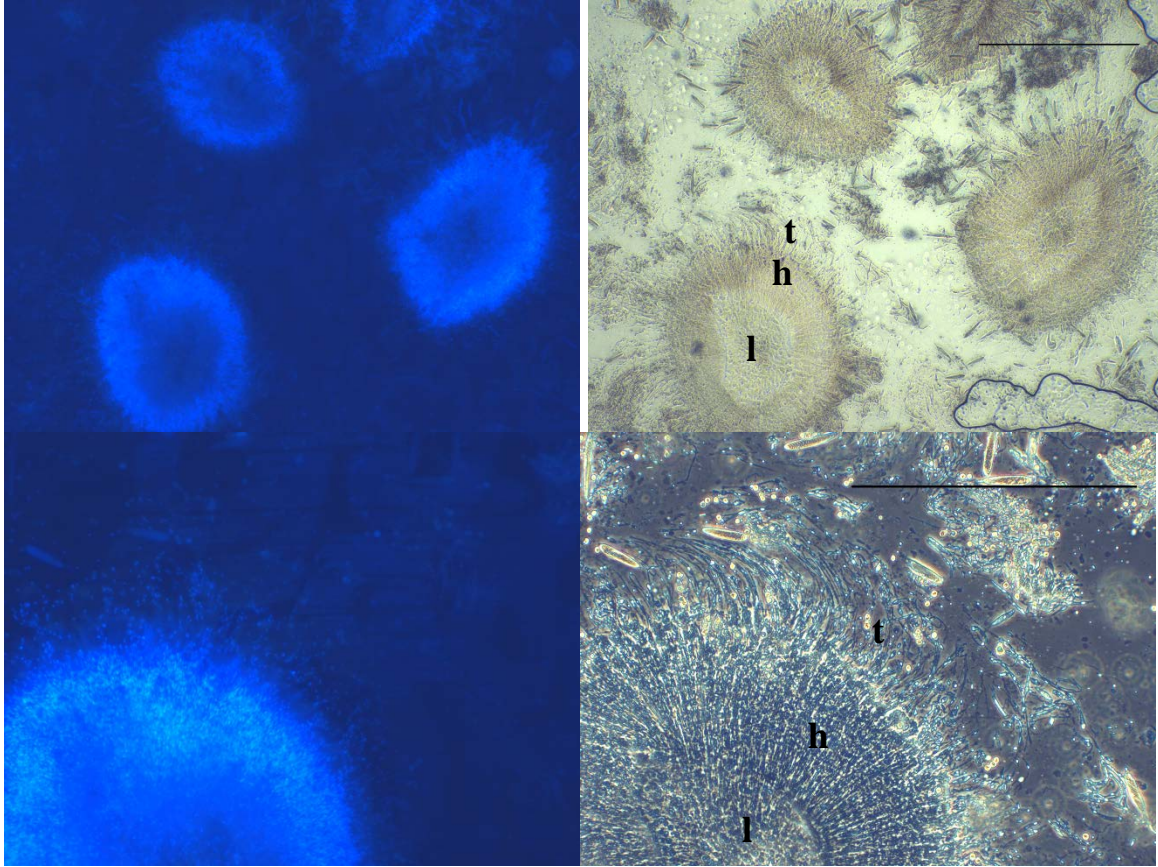


Figure 5-5. Photographs of testes as viewed in histological thin sections of tissue from a preserved polyp of *Ricordea florida* that was collected from a coral reef in the Lower Florida Keys on 30 June 2018. Shown are 2 levels of magnification, with lower power above and higher power below. Left: Testes under fluorescence microscopy with a DAPI stain for genetic material. Right: the same testes under phase contrast. t = sperm tails, h = sperm heads, l = testes lumen. Note the strong genetic material staining around the periphery of each circular testis, indicating DNA in the sperm heads. Also note the numerous sperm tails visible extending outward from the testis, especially in the right photographs. Scale bars = 0.1 mm.

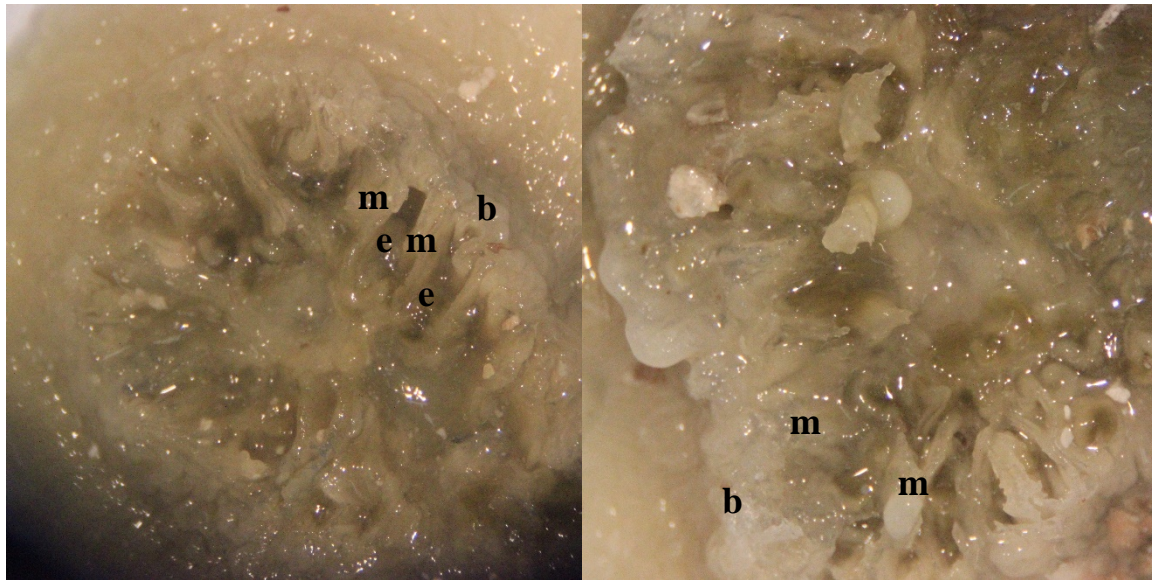


Figure 5-6. Photographs of 2 recently spawned female polyp of the Florida false coral *Ricordea florida* that were collected on 19 July 2018, from a coral reef in the Lower Florida Keys. Left: Whole polyp in aboral view with the pedal disk removed showing large empty spaces that may have been filled with ovaries the month before (compare with Fig. 5-2). At the center is the mouth area, with mesenteries radiating outward from the mouth. Right: Whole polyp in aboral view with the pedal disk removed, showing relatively close mesenterial spacing in a different polyp that may not have previously contained ovaries (i.e. potentially a juvenile). e = empty space between mesenteries, m = mesentery, b = body wall.

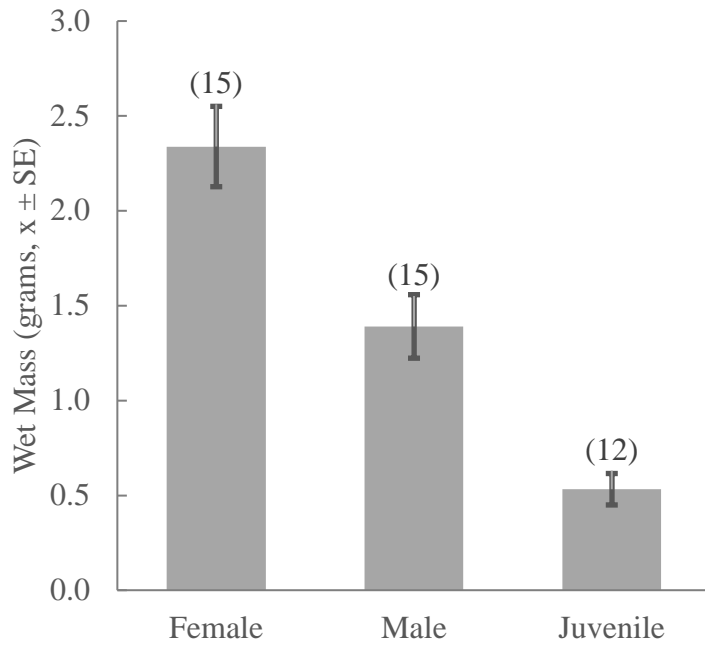


Figure 5-7. Variation in body size with gender in polyps of Florida false corals *Ricordea florida* collected from a coral reef in the Lower Florida Keys on 12 June 2019. The 3 genders differed significantly from each other in body size ($p < 0.001$; see text for details). Sample size in number of polyps examined of each gender is shown in parentheses above each bar.

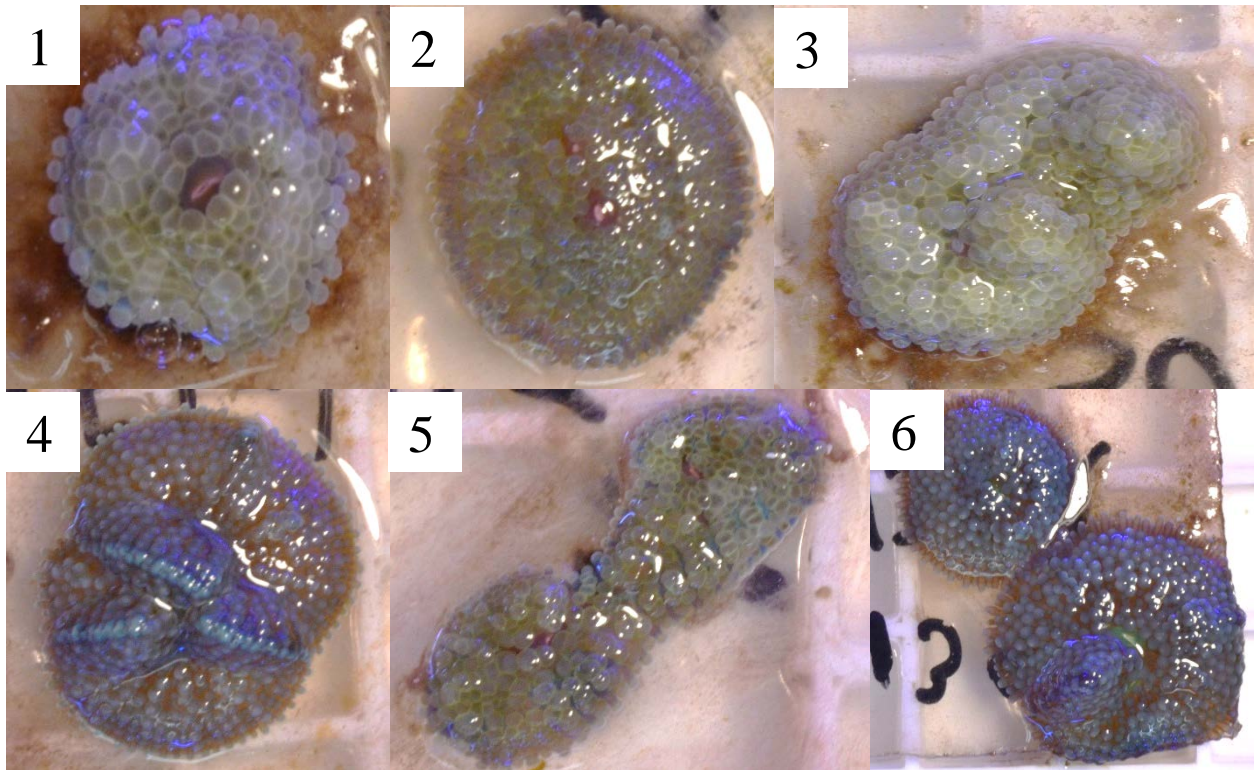


Figure 5-8. Six stages of longitudinal fission in live polyps of Florida false corals *Ricordea florida* that were cultured under laboratory conditions. Photographs were taken of live polyps on clear Plexiglas culture plates, which had been temporarily removed from culture tanks and photographed in air, hence the light reflections on the moist polyps. **Stage 1:** No fission; polyp is not actively engaged in clonal reproduction via fission. **Stage 2:** Early fission of polyp with 2 mouths. **Stage 3:** Mid fission in which polyp begins to elongate and mouths move apart. **Stage 4:** Late fission in which a fold of tissue forms between the 2 mouths (does not occur in all fission events). **Stage 5:** Near-complete fission, in which tissue narrows between 2 daughter polyps as they move away from each other and begin to separate. **Stage 6:** Completed fission, with 2 distinct separated polyps.

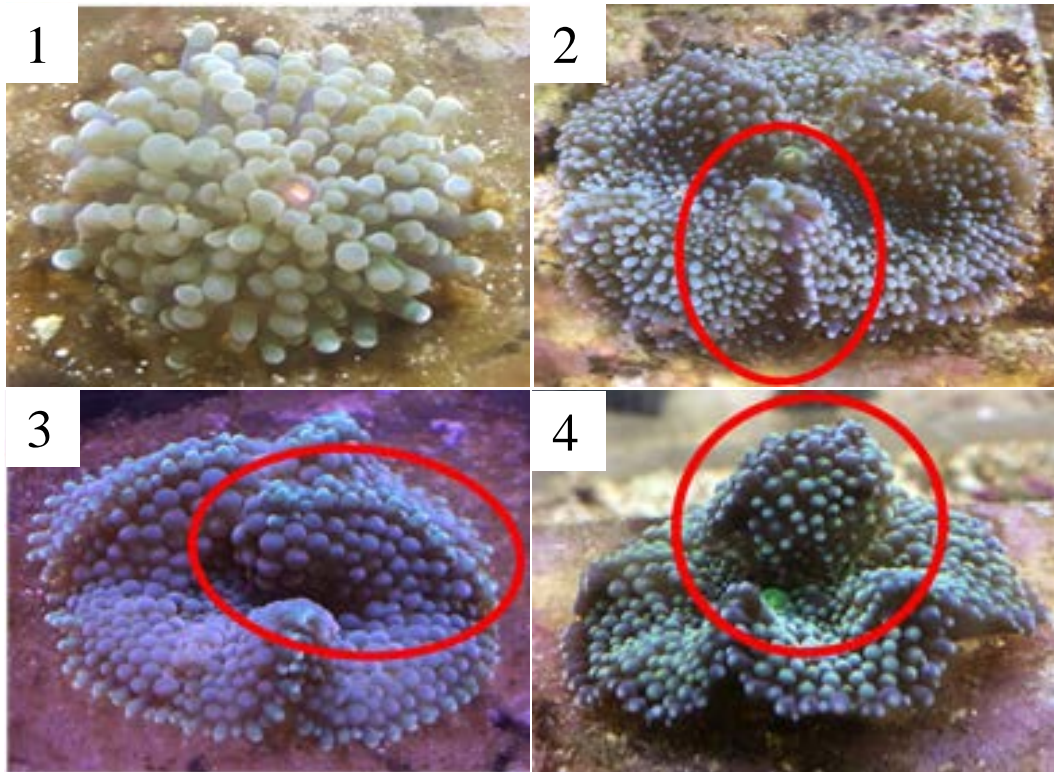


Figure 5-9. Four stages of inverse budding in polyps of Florida false corals *Ricordea florida* that were cultured under laboratory conditions. Photographs were taken of live polyps underwater inside their culture tanks. **Stage 1:** No inverse budding; polyp is not actively engaged in clonal reproduction via inverse budding. **Stage 2:** Early budding; one or more folds form in the edge tissue of the oral disc. **Stage 3:** Mid budding; edges of tissue fold fuse together and elongate toward the polyp center. **Stage 4:** Late budding; a spherical bud raises up from the oral disc surface but is still attached to the parent polyp. Note that Stage 5: Bud detachment, was not observed in the laboratory. Red circles indicate the location of each bud stage on the polyp. Photographs by Sarah Austin.

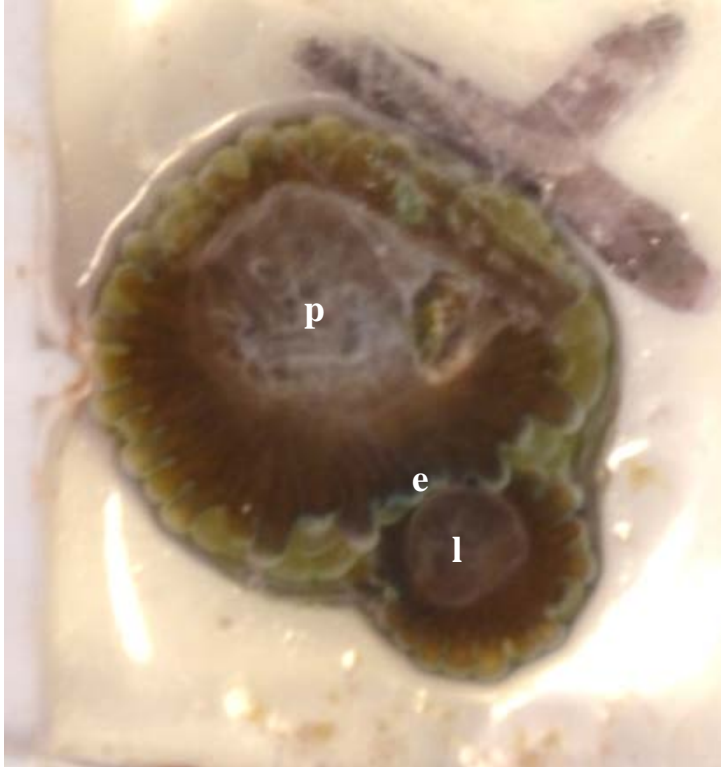


Figure 5-10. Pedal laceration in a polyp of the Florida false coral *Ricordea florida*, as viewed aborally through the clear plexiglass of a culture plate to which the polyp was attached in the laboratory. p = pedal disk of parent polyp, l = pedal lacerate, e = edge of oral disk of parent polyp.

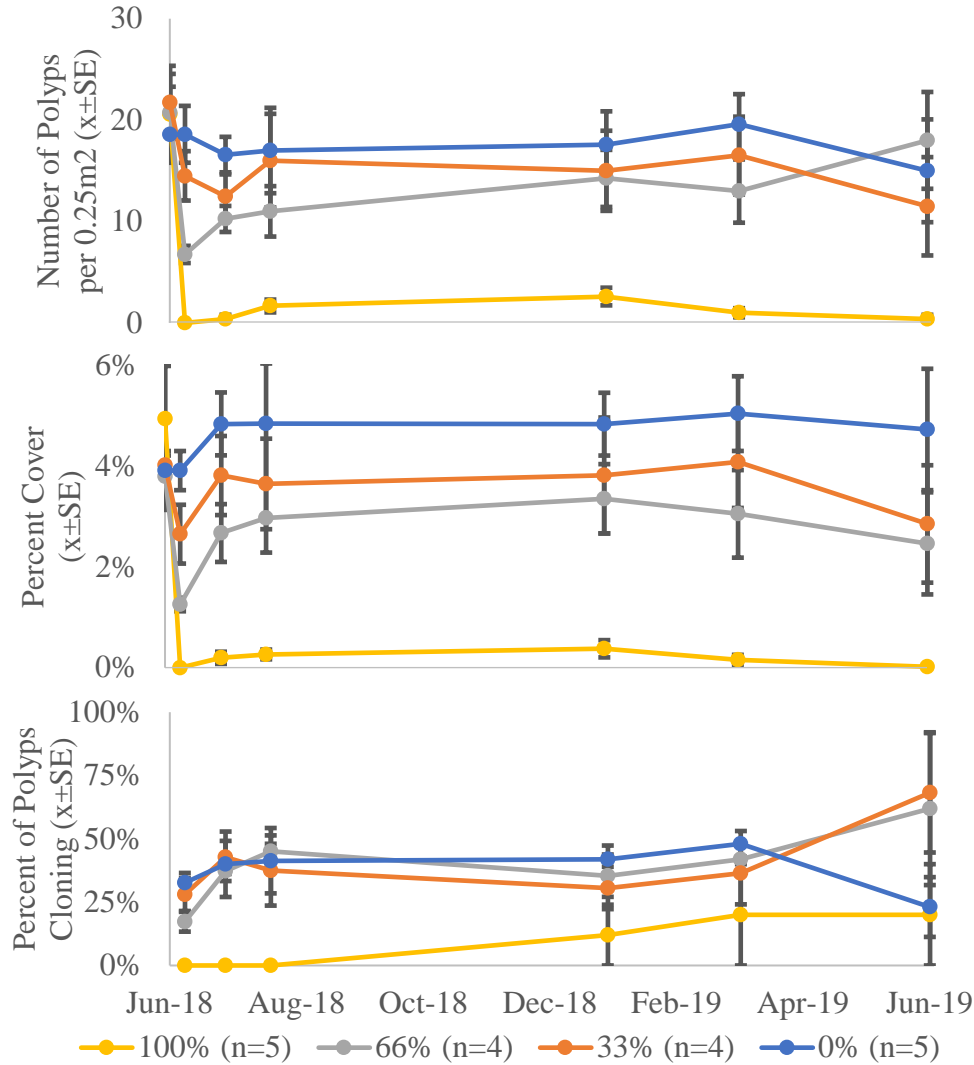


Figure 5-11. Variation in characteristics of aggregations of Florida false corals *Ricordea florida* over 1 year, in response to experimental treatments applied on a coral reef near American Shoal, Cudjoe Key, Lower Florida Keys. Treatments = 100% of polyps removed from each aggregation (N = 5 aggregations), 66% removed (N = 4), 33% removed (N = 4), and 0% removed (N = 5; control group). Treatments were initiated between 30 June and 19 July 2018, and then the recovery of aggregations from the removal experiment were followed for 1 year. Note that both the 33% and 66% removal treatments fully recovered within < 1 year, but that the 100% removal treatment did not. See text for details.

Chapter 6

Recommendations for fishery management of Florida false corals *Ricordea florida*

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1 Introduction

This brief chapter is written in the form of a letter to fisheries managers at the Florida Fish and Wildlife Conservation Commission. It briefly summarizes the relevant evidence from this thesis in terms of application to the fisheries management of this species. The length was intentionally limited to ~ 1 page in single-spaced format, at the request of the fisheries managers. The content of this letter both forms a component of this thesis, and also is being submitted separately to the Florida Fish and Wildlife Conservation Commission, at their request.

2 Content of the fisheries recommendation letter

Florida Fish and Wildlife Conservation Commission

Farris Bryant Building

620 S. Meridian St.

Tallahassee, FL 32399

Dear Fisheries Managers,

In 2016, during an informal stake-holder assessment with collectors and scientists working in the Florida Keys, the Florida false coral *Ricordea florida* was identified as a species of concern due to perceived declining populations. Over the last four years, I undertook an ambitious project as part of my doctoral dissertation at Auburn University to investigate the environmental tolerances, distribution, abundance, and reproductive capabilities of this species in the Florida Keys.

My findings document that Florida Keys populations of *R. florida* for which we have long-term data have declined significantly since pre-2010 levels. While during 2018 individuals of *R. florida* were found in a variety of hardbottom reef types, in all cases their abundances were less than known historic values. This pattern was quantified throughout a variety of sites from Marathon to Western Sambo, both within and outside of protected areas.

Based on my experimental studies, it appears that this decline in population abundance can be attributed at least partially to environmental conditions. Individuals of *R. florida* have a thermal tolerance range of 22-31°C (72-88°F), exceeding which leads to rapid decline in health followed by polyp death within days. In the Keys, the lower level of this temperature range was exceeded in 2010 during the cold event, and the upper level was exceeded during the hottest months of the 2014-15 El Niño, as well as during both years in which I recorded temperatures (2017-18) on the back reef where this species is found. Individuals of *R. florida* also require shading from high light, and so are dependent on upright shading organisms such as gorgonians and sea fans, which likely led to an observed temporary decline in *R. florida* numbers following the effects of Hurricane Irma in September 2017, which removed many of the upright soft corals from the reef.

Relevant conclusions about the reproduction of this species are that individuals of *R. florida* broadcast spawn annually during late June to early July. This species also clonally replicates and can recover within < 1 year from the removal of some of the polyps from each aggregation, but not if entire aggregations are removed.

Based on these findings, I recommend that the fishery should be closed from June to August to allow for the annual spawn and to avoid further stressing the populations during the hottest months each year when they are already at the edge of their upper thermal tolerance limit. Collectors also should be encouraged to adopt best management practices of collecting < 66% of polyps within each aggregation, and to avoid collecting from the same aggregation twice within a span of several months, to allow for full recovery of polyp abundances through clonal reproduction.

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